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MEAT QUALITY OF DARK-CUTTING CATTLE

by

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Submitted in partial fulfilment for the degree
Magister Scientiae Agriculturae

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Pretoria

June 2000

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I declare that the thesis herewith submitted for the M.Sc. (Agric) Food Science degree at the University of Pretoria had not been previously submitted by me for a degree at any other University.

"I can do all things through Christ which strengtheneth me."

Philippians 4: 13

Acknowledgements

I wish to thank the following people and institutions for their contributions to the completion of this research:

The board of directors of the abattoir concerned for granting me the opportunity to conduct this research, for their financial contributions towards travelling and tests expenses involved.

The management and staff of the abattoir for their supportive assistance, keen willingness in assisting to co-ordinate the various projects and materials used in the research.

The management and staff of the various feedlots for their support and dedicated assistance in co-ordinating their part of the projects.

Prof. J. J. Roberts for his advice, support and leadership in the initial stages of the research.

Ms. H. L. de Kock , of the Department of Food Science, University of Pretoria, for her valuable advice and contributions during the preparation of the thesis.

Dr. E. C. Webb, of the Department of Animal and Wildlife Science, University of Pretoria, for his enthusiasm, encouragement and advice, especially with the physiology part of the thesis.

Prof. J. G. van der Walt and personnel, of Department of Physiology, Onderstepoort University of Pretoria, for their valuable assistance with regard to blood sampling and muscle biopsies taken at the feedlots and the analyses of these samples.

Ms. R. Owen and Mr. J. Grimbeeck, of Statomet, Department of Statistics, University of Pretoria, for invaluable advice concerning the statistical analysis of data.

Dr. P. V. Tarrant, of The National Food Centre, Dublin, Ireland, for valuable information supplied.

The consumer panellists who provided valuable information on the hedonic character of the meat samples.

My husband, Hannes for his love, keen support and inspiration.

My children, Otto, Waldi, Christoph, Marzanne and Erich Krogmann, for their love, support and understanding.

My Mother for her love and encouragement..

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ABSTRACT

Meat quality of dark-cutting cattle

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The dark, firm and dry (DFD) or dark-cutting condition causes large losses to both the cattle feedlot and meat industry due to its unattractive, dark red colour and its limited shelf life. Dark-cutting, is a direct consequence of low muscle glycogen at slaughter and results in a lack of normal acidification of meat during rigor development causing high pH (>5.8). Glycogen breakdown in muscle may be rapidly triggered by increased circulating adrenaline or by strenuous muscular activity. Several other factors have also been found to lead to the DFD condition in cattle.

The aims of this investigation were to determine the incidence of DFD in cattle slaughtered at a South African abattoir and to identify potential causative factors of this syndrome. It was furthermore also important to evaluate the sensory attributes, microbiological quality and hence, shelf life of DFD meat. The incidence of DFD carcasses, based on pH measurements (threshold $\geq$ 5.8) within 1 h *post mortem* on 22 178 cattle in the *M. longissimus dorsi,* was 11.8%. The estimated overall incidence measured over a 5 –day period, testing only cattle supplied by the four main feedlots, was 7.83% (N= 5 659), measuring pH_{24} ($\geq$ 5.8), confirmed by colour grades (values of 7 or 8 were classified as DFD). The incidence of dark-cutting beef, also seemed to be

feedlot related with the highest being 12.37% and the lowest 1.75%. It is suspected that the incidence of DFD carcasses is higher in extremely hot or cold weather or large fluctuations, and should decrease during the months with milder temperatures. Transport distances did not affect the DFD incidence. In experiment 2, cattle from Feedlot C, transported over a short distance (45 km) had a higher incidence of DFD (9%) than those of Feedlot A (1.7%), transported over the longer distance of 290 km.

Long holding periods in the holding pens at the abattoir (> 24 h and even as high as 54 h), appeared not to have a significant influence on the occurrence of DFD. There was no correlation between bruised carcasses and DFD carcasses (the respective means of bruising were 6.7% and 3%, and respective mean incidences of DFD were 1.7% and 9%). Using Scheffe's test to compare the effect of gender on the incidence of DFD, significantly more bulls and steers were affected by dark-cutting than heifers ($P < 0.0001$). Mounting behaviour among young bulls, and social regrouping of steers during the pre-slaughter period, have been shown to increase the incidence of dark-cutting. Avoidance of mixing unfamiliar bulls and steers during transport and holding periods has been recommended to minimise stress. The low incidence of dark-cutters ($P > 0.05$) in heifers could possibly indicate a non-oestrus situation in the majority of the heifers, since a significant association between the presence of oestrus and DFD was shown previously by other researchers. The effect of gender on the incidence of dark-cutting, related to feedlot, was also significant. Higher incidences of DFD were found with steers of Feedlots B, C and D (14.5%, 11.6% and 13.7% respectively) than in Feedlot A (1.9%). The lowest overall incidence of dark-cutters was found in Feedlot A (1.7%).

It appeared as if the breed type of the cattle had an effect on the dark-cutting beef. The *Bos indicus* (Brahman-type) animals had a significant effect ($P < 0.001$), on the colour of the beef when compared with European breeds.

Cattle of two feedlots, fasted for periods of 40 h and 60 h, showed that a shorter fasting period had a significant effect ($P < 0.001$) on the pH. It

appeared as if pre-slaughter stress, induced dark-cutting more readily in fasted than in fed cattle. Fasting also inhibited muscle glycogen resynthesis during recovery of stress.

The effect of pre-slaughter handling on the blood composition and muscle glycogen content of cattle of three different feedlots was studied. After transport (phase 1), the glycogen depletion was significant for all three feedlots ($P < 0.05$). Further glycogen depletion was found after slaughter (phase 2) ($P < 0.01$). The hematocrit increased after transport, which indicated that animals of Feedlot B ($P < 0.0001$) experienced stress. During the second phase, a significant decrease in hematocrit for Feedlot B was observed ($P < 0.05$). For Feedlot A, a non-significant increase was found in phase 1, with a significant ($P < 0.05$) increase in phase 2. The hematocrit of cattle of Feedlot C, however, showed a significant decrease during both phases. The blood glucose concentration for all three feedlots increased during phase 1, but was not significant. However, the blood glucose concentrations for all three feedlots increased significantly during phase 2 which probably indicated that the animals experienced stress during this period. The increase in creatine kinase was significant ($P < 0.01$) during both phases 1 and 2 for Feedlot A. Fluctuations were found in the creatine kinase concentrations of Feedlot C. Feedlot B, however, showed a significant decrease in the creatine kinase concentrations. Free fatty acid concentrations for all feedlots increased linearly over the different stages. The high free fatty acid concentrations in blood samples of phase 2, of Feedlots B and C confirmed that these animals experienced stress, especially from the period being held in the pens to after slaughter ($P < 0.01$ for both). The free fatty acid concentration, appear to be the best blood parameter to relate to meat quality. The DFD incidence during this experiment, for Feedlots B and C, is 35%. For Feedlot A, with the lowest DFD incidence (16%), the increase of free fatty acid concentration was not significant. Although the effect of growth promoters was not tested, the use of beta-agonists as growth promoters may increase the incidence of dark-cutting meat unless a sufficient withdrawal period before slaughter is observed. Feedlots A and C implanted Revelor in 9

month old cattle, while Feedlot B implanted both Synovex S (at 6 weeks) and Revelor (at 4 months).

Consumer sensory evaluations indicated that the general appearance ($P < 0.05$), colour ($P < 0.001$) and acceptability ($P < 0.01$) of raw normal steak were preferred significantly to those of raw DFD steak. Twice as many panellists preferred the raw normal steak than raw DFD because of the more attractive red colour, compared to the almost black colour of raw DFD steak. The hedonic ratings of the sensory attributes of fried normal steak versus fried DFD steak were non-significant for all attributes, although it was expected that consumers would find the DFD steak more tender and less flavoursome than normal steak.

DFD meat samples (N= 84) were tested over a 7-week period for total aerobic count, *Pseudomonas*, coliforms and *Eschericia coli*. Normal beef samples were expected to be acceptable, both on microbiological grounds and sensory evaluations until at least the seventh week. Microbiological spoilage of vacuum-packaged DFD meat took place during the second week and deterioration was more evident during the fourth week, due to the presence of cheesy odours. During the fifth week, green discolouration of the meat was observed.

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CHAPTER 1

INTRODUCTION AND MOTIVATION

Dark cutting beef is unattractive because of its dark red colour; it is discriminated against by the consumer and therefore has been a problem to the beef industry for many years, particularly when the consumer can exercise a choice at purchase, based on visual preference.

As early as 1965, Hedrick reported that the consumer discriminated against dark-cutting beef in its fresh form and that there was no clear evidence whether the palatability characteristics of dark-cutting beef differed from normal beef.

It was observed over several years at the abattoir concerned, that a dark-cutting syndrome existed amongst the cattle. This problem was never quantified, nor qualified. The potential causes were not identified either, but only left to speculation.

Although incidences of dark-cutters in other countries have been reported, statistics of the South African meat industry was not available at the time of writing this report. In the USA, Kreikemeier, Unruh & Eck (1998) reported that, in more than 8 000 heifers at a commercial slaughter plant, 1.7% were dark cutters.

A survey was also conducted by Brown, Bevis & Warriss (1990) to estimate the incidence of dark cutting beef in the United Kingdom. The overall incidence was 4.1%. Increased incidences were associated with both short and long transport distances. Slaughter on the day of arrival, rather than overnight lairage also increased the incidence. Bulls had the highest incidence and heifers the lowest. A seasonal effect was also recorded.

The results, however, indicated that other factors in addition to those examined were also important. The 1995 National Beef Quality Audit (NBQA) of Smith *et al.* reported that DFD beef carcasses resulted in a loss of $6.08 per animal harvested in the United States (Scanga, Belk, Tatum, Grandin & Smith, 1998).

The terminology, DFD (dark, firm and dry) and DC (dark cutting) are used to describe meat which has a value of ultimate pH which is higher than normal (threshold of 5.8) and in addition to this, also has a dark colour. The dark cutting conditions worsens with increasing value of ultimate pH (Hedrick & Stringer, 1964; Hedrick, 1981; Kenny & Tarrant, 1988; Tarrant, 1981; 1989). According to Tarrant (1981) the actual value at which meat is defined as DFD is determined by the particular muscle, type of animal (i.e. young or old) and also by the nature of the product and marketing circumstances.

Dark cutters result from pre-slaughter stress, which depletes muscle glycogen needed to produce the lactic acid that reduces the pH of *post mortem* muscle (McVeigh & Tarrant, 1982; Cockram & Corley, 1991; Schaefer, Jones & Stanley, 1997; Scanga *et al.*, 1998).

The need to minimise pre-slaughter stress in particular, including transport and handling stress, are suggested as key factors in reducing the incidence of dark cutting (Kenny & Tarrant, 1988; Grandin, 1988; Bartos, Franc, Rehak & Stipkova, 1993; Schaefer *et al.,* 1997; Kreikemeier *et al.,* 1998). It was therefore important to determine the incidence of dark-cutters at this particular abattoir and to investigate the possible causes of this problem before preventative measures could be taken to reduce the incidence of dark-cutting. In addition, a consumer panel was used to assess the sensory attributes of both the DFD and normal beef in both the raw and fried state. The microbiological quality and shelf life of the DFD meat, also had to be established.

CHAPTER 2

LITERATURE REVIEW

2.1 DEFINITION / DESCRIPTION OF THE DARK, FIRM, DRY (DFD) CONDITION OCCURING IN BEEF

The term DFD is used to describe meat which has a value of ultimate pH which is higher than normal. There is general agreement on the relation between meat pH and intrinsic characteristics such as darkness, keeping quality and consistency. Values below pH 5.8 are considered normal. At values above pH 5.8, beef may be rejected on the basis of lower keeping quality and the dark colour of the meat (Lawrie, 1958; Hedrick, Boillot, Brady, & Naumann, 1959; Tarrant, 1981). Although the DFD conditions worsens progressively with increasing value of ultimate pH (Tarrant, 1981), consumers associate the dark colour of the beef with meat from old animals or meat that has deteriorated (Hedrick, 1958). Tarrant (1981) is of the opinion that the actual pH value at which meat is defined as DFD, is determined by the particular muscle, type of animal (i.e. young or old) and also by the nature of the product and the marketing circumstances.

Other terms often used to describe this condition are dark-cutting (Lawrie, 1958), dark-cutters (Hedrick, 1958), DCB or dark-cutting beef (Brown *et al.*, 1990) and "black beef"(Tarrant, 1989).

2.2 THE INCIDENCE OF DFD

The incidence of DFD reported in other countries varies from 0.77% to 15%. Overall results reported in the United Kingdom was 4.1% (Brown *et al.*, 1990);

the occurrence of commercial feedlot heifers classified as dark-cutters varied from 0.77% to 4.5% and in pregnant heifers there was no incidence compared to 1.7% in non-pregnant heifers (Kreikemeier & Unruh, 1993). The US National Beef Quality Audit revealed that 5% of federally inspected steer and heifer carcasses were dark-cutting (Shackelford, Koohmarie, Wheeler, Cundiff & Dikeman, 1994). Tarrant (1981) reported incidence rates that ranged from 1 to 5% for steers and heifers, 6 to10% for cows and 11 to15% for young bulls.

2.3 CAUSES OF DFD MEAT

Dark cutters result from pre-slaughter stress, which depletes muscle glycogen stores and thus reduces the glycogen needed to produce the lactic acid, that is responsible for the decrease of the pH of *post mortem* muscle (Hedrick, 1981; McVeigh & Tarrant, 1982; Tarrant, 1989). The abnormally high pH increases the light-absorption and water-holding abilities of *post mortem* muscle and results in an undesirable, dark, firm and dry, cut lean surface (Lister, 1988). Even though this is understood at the clinical level (Cockram & Corley, 1991), the stress factors that induce the condition are not as clear. Various factors have been reported by various researchers to play a role in creating the dark-cutting condition: weather, growth promotants, gender, genetics, disposition and handling practices before slaughter as well as transport (Hedrick *et al.*, 1959; Voisinet, Grandin, O'Connor, Tatum & Deesing, 1997 and Scanga *et al.*, 1998).

2.3.1 Handling

The effect of pre-slaughter handling on the behaviour and blood composition was studied by Kenny & Tarrant (1987a; 1987b) and also by Cockram and Corley (1991). The main problems that Cockram & Corley (1991) identified were the routine use of driving instruments and delays caused by stoppages

in the slaughterline. The plasma concentration of cortisol at the time of slaughter was positively correlated with the time spent standing still and with the time spent in the pre-stun pen. Cattle kept overnight in the lairage had a greater concentration of free fatty acids at the time of slaughter than those slaughtered on the day of arrival. Some of the handling problems observed, were caused by incorrect design of the handling facilities (Kenny *et al.*, 1987a) and high stock densities (Tarrant, Kenny, Harrington & Murphy, 1992).

2.3.2 Long distance transportation

Evidence of dehydration and fatigue after road transport for 24h showed (Tarrant *et al.*, 1992) that any extension of journey time or deterioration in transport conditions would be detrimental to the welfare of the animals. Puolanne & Aalto (1981) also reported that the incidence of dark-cutting beef increased with distance and duration of transport and when the rest period immediately before slaughter was increased the incidence of DFD meat first decreased, but increased to an inexplicable high value at 8-11 h of rest, and then decreased again after further rest. However, Brown *et al.* (1990) found that increased incidence of dark cutting was associated with short and long distances. Cattle adapted better with long transport distances than with short distances, which was indicated by lower heart-rates and cortisol levels (Honkavaara, Leppävuori, Rintasalo, Eloranta & Ylünen, 1999). These authors also concluded that the farm, transportation and the slaughterhouse are important links in the production chain and meat quality is determined by the poorest link in the chain. It seemed that loading and unloading are the major critical points (Van Logtestijn & Romme, 1981) and it did not make much difference if the transport duration-distance varied within a certain limit. In a study to establish whether there was any relationship between concentrations of stress hormones at exsanguination and meat quality measurements, it seems likely that travel sickness has little or no effect on meat quality (Bradshaw, Randalf, Forsling, Rodway, Goode, Brown & Broom, 1999).

2.3.3 Muscular activity

Glycogenolysis can occur during heavy exercise or in response to catecholamine secretion in the absence of exercise. The activity that accompanies the mixing of unfamiliar bulls or heifers in oestrus elicits *ante mortem* glycogen depletion and results in dark-cutting meat (McVeigh and Tarrant, 1983; Kenny and Tarrant, 1988; Schaefer, Jones, Tong & Young, 1990). Between different muscles, there are characteristic differences in glycogen content. Struggling at death can cause the breakdown of considerable quantities of glycogen (Lawrie, 1958), but since the circulation stops at about this time, the lactic acid formed is not appreciably lost from the muscle and thus still contributes to the ultimate pH. Wether lambs were used by Apple, Minton, Parsons, Dikeman, & Leith (1994) to evaluate the influence of treadmill exercise (TME) on physiological responses and meat quality. TME caused dramatic changes in blood constituents but had minimal influences on meat quality.

2.3.4 Weather

Grandin (1992) reported that the occurrence of dark cutting beef is highest during very cold weather combined with precipitation, which increases the rate of body-heat loss and elicits shivering. The incidence of dark cutting beef is also high in very warm weather or when large fluctuations in temperature occur over short periods of time (Scanga *et al.*, 1998).

2.3.5 Feeding and Fasting

Howard & Lawrie reported in 1956 that fasting, exhausting exercise and training are among the factors known to determine the quantity of glycogen available at death for the formation of lactic acid (Lawrie, 1958). They concluded that the stresses of fasting and enforced exercise, although relatively ineffective separately, might significantly raise the ultimate pH if combined. On the other hand, Lawrie reported (1958) that it is evident that in natural circumstances neither fasting, enforced exercise nor a combination of both bring about a marked raising of ultimate pH and these factors must, therefore, be largely discounted in explaining the occurrence of dark-cutting beef.

An intensive study was conducted by McVeigh & Tarrant (1982) to determine the effect of feeding and fasting on the glycogen content of the muscle. The glycogen content of the muscle of meat animals at slaughter has an important influence on the *post mortem* biochemical reactions that determine the quality of meat. Dark cutting beef is a direct result of low muscle glycogen stores at the time of slaughter. Glycogen deficiency, in turn, may result from physiological stress caused by physical activity or emotional excitement. Both of these conditions are commonly encountered by meat animals in the immediate pre-slaughter period (McVeigh & Tarrant, 1982). The main finding was that resting muscle glycogen content and repletion rates were influenced by type of feeding (high vs low energy intake) and by fasting in beef heifers. Repletion rates for fed heifers were similar to that reported for barley-fed Friesian bulls recovering after behavioural stress (McVeigh & Tarrant, 1982). In relation to the dark-cutting problem in beef, fasting *per se* will have very little effect on meat quality due to its limited influence on resting muscle glycogen content, which is in agreement with the view of Lawrie (1958). However, fasting substantially lowered the rate of glycogen recovery to such an extent that a fasted, glycogen-depleted animal could not recover sufficient glycogen to ensure normal meat quality (McVeigh & Tarrant, 1982).

2.3.6 Breed type of cattle

The glycogen muscle content at slaughter of unstressed and stressed animals in two different cattle breeds and the effect of breed on the incidence of dark cutting was investigated by Sanz, Verde, Saez & Sanudo (1996). Observations have shown relevant differences in the behaviour of Brown Swiss bulls and Pirenaico bulls. The results however, indicated that in the *M. longissimus dorsi* and *M. sternomandibularis,* glycogen concentration are similar in both Brown Swiss and Pirenaico breeds and that in spite of the apparent difference in temperament of these breeds, there was no influence on the incidence of dark-cutting. The main cause of dark-cutting was due to physical and emotional stress of mixing unfamiliar bulls overnight.

The effect of breed of cattle on the incidence of the dark, firm and dry condition in the longissimus muscle was also studied by Shackelford *et al.,* (1994). Chianina crosses had darker-coloured lean than all breed groups except Tarentaise and Simmental crosses ($P< 0,05$). Moreover, a higher percentage ($P< 0,05$) of Chianina crosses than of all other breed groups had unacceptably dark-coloured lean. Lean colour and texture were lowly heritable, whereas lean firmness was moderately heritable. This experiment demonstrated that there is genetic variation in the incidence of the DFD condition; however genetic variation was small relative to environmental variation.

Random matings of 10 Charolais sires and eight Limousin sires to crossbred cows produced 392 steers and heifers that were used to evaluate genetic influences on beef palatability (Jeremiah, Aalhus, Robertson & Gibson, 1997). Very few antagonistic genetic relationships existed between production/ carcass traits and palatability traits but these authors found that carcasses with the very darkest lean were tender, but when those carcasses were excluded a negative relationship existed between lean darkness and tenderness among steer and heifer carcasses.

2.3.7 Temperament

Previous studies have shown that there is a relationship between the dark-cutting condition and animal behaviour. Several studies have shown that mixing strange cattle together results in fighting, mounting and other physical activity that increases the incidence of dark cutting (Grandin, 1980; Price & Tennessen, 1981 and Kenny & Tarrant, 1987a). Temperament, which has been shown to be moderately heritable in cattle (Hearnshaw & Morris, according to Voisinet *et al.,* 1997) generally means the excitability or the tendency of an animal to become agitated when it is handled. Temperament has been shown to be related to various aspects of livestock production, such as milk production and daily weight gain (Voisinet *et al.*, 1997). The same authors also reported that heifers had a more excitable temperament than steers and that the animals with the most excitable temperament exhibited more borderline dark cutting and tougher meat. Voisinet *et al.* (1997) reasoned that those animals with the most excitable temperaments might be more susceptible to stress generated by routine handling practices, such as handling and transport, which occur just prior to slaughter. In an experiment by McVeigh & Tarrant (1982) the effect of propanolol on muscle glycogen metabolism during social regrouping of young bulls was tested. Propanolol was ineffective in preventing muscle glycogen depletion, suggesting that muscle glycogen depletion during social regrouping of cattle is not mediated predominantly by catecholamines.

2.3.8 Gender

Tarrant (1981) reported that dark cutting is much more relevant (11 to 15%) in young bulls. Although circulating testosterone may play a role in determination of fibre types (Ashmore according to Tarrant, 1981) the increased incidence of dark cutting is predominately attributed to enhanced muscle glycogen depletion. Bulls are known for increased aggressive and

sexual activity culminating in considerably more mounting behaviours and fighting when co-mingled, than normally seen on other gender classes (steers and heifers).

Among other kinds of behaviour, mounting was shown to be the most important in the induction of dark-cutting beef (Bartos *et al.*, 1993). An experiment by Mohan Raj, Moss, Rice, Kilpatrick, McCaughey & McLauchlan, (1992) showed that vasectomised bulls were more homosexually hyperactive and thus produced the highest number of dark-cutters, suggesting that the vasectomised bulls are more prone to dark-cutting due to mounting that occurs during pre-slaughter mixing. In a previous experiment by these authors, male cattle were emasculated by total castration, vasectomy or immunocastration and one group was left entire. The effects of the treatments on meat quality related parameters were examined. Dark cutting beef was undetected in the steers and occurred at very low levels in the other male types. Steers had histochemical profiles, significantly different from those of the other male types. Total castration seemed to produce more profound physiological changes than vasectomy and immunocastration. But the problem of using bulls instead of steers includes a more aggressive behaviour and at marketing often resulted in DFD meat. Immunocastration of bulls produced docile behaviour and superiority in production compared with steers (Mohan Raj, Moss, McCaughey, McLauchlan, McCaughey & Kennedy, 1991).

Dark-cutting has also been shown to increase in heifers, which exhibit mounting behaviour near the time of oestrus (Kenny & Tarrant, 1988). However, a study by Voisinet *et al.* (1997) showed that heifers had a higher incidence of borderline dark-cutters than their steer contemporaries. This differs from a study by Jones & Tong (1989) who found a higher incidence of dark cutting in steers than heifers. It does, however, agree with a study by Murray (1989), in which heifers had a higher incidence of dark cutting than steers. Murray (1989) proposed that the higher incidence of dark cutting in heifers was related to more rapid cooling of lighter heifer carcasses and thus,

a slower decline in pH. Another study, by Kreikemeier & Unruh (1993) on heifers showed that pregnant heifers had a lower incidence of dark-cutters than non-pregnant heifers and contributed this to the pregnant heifers' increased carcass fat thickness.

2.3.9 Growth promotants

Implants have been under suspicion for promoting carcass quality defects since their introduction (Grandin, 1992). Scanga *et al.,* (1998) explained that because implants modify growth curves through hormonal changes, added sources of stress to hormonal shifts ultimately could increase the risk of dark cutters. Furthermore, administering combination (androgen and estrogen) implants to steers and estrogen implants to heifers, especially as re-implants before slaughter, seemed to inflate the manifestation of stress and ultimately led to an increase in the incidence of dark cutters. They suggested that re-implants should extend past 100 days to minimise carcass non conformance that results from DFD.

Hedrick *et al.* (1959) and Grandin (1992) identified animal sex, biological type, use of growth promotants, and handling as potential contributors to an increased incidence of DFD. Grandin (1992), used a large commercial database to identify and quantify management (biological type, implant type, and implant administration) and environmental factors that affect the incidence of DFD and to develop decision trees for use in the reduction of losses in carcass value as a result of DFD.

Observations made by Grandin (1992) in several large commercial slaughter plants indicate that the use of two or three trenbolone acetate implants in steers and heifers may increase the incidence of dark cutters.

2.3.10 Preventative measures of DFD

Hedrick *et al.* (1959), Grandin (1992) and Shackelford *et al.* (1994) reported that control of *ante mortem* stress through proper management would be the most effective method to reduce the incidence of dark cutting beef. Feedlot management practices (Scanga *et al.*, 1998) should also incorporate seasonal climatic trends (hot weather and large temperature changes) at the time of administration of implants. In addition, the use of good handling practices, well-designed handling facilities, and proper shipping practices must also be used. By optimally combining these factors, producers can continue to optimise growth performances with the use of moderate growth-promoting implants but at the same time reduce economic losses and carcass non conformance due to DFD.

A practical method to prevent dark-cutting was tested on 2234 bulls slaughtered under commercial conditions by Bartos *et al.* (1993). They found that it is necessary to keep the same social groups from loading to slaughter, strictly avoiding any mixing of unfamiliar bulls.

2.4 ANIMAL PHYSIOLOGY

Transport and handling are reported to evoke an increase in circulating cortisol and in this context, transport and handling are thus viewed as stressors (Cockram & Corley, 1991). These observations are consistent with the reported findings of other coinciding cortisol-driven events in transported cattle such as neutrophile/ lymphocyte responses and evoke responses of other stress hormones such as beta endorphin and thyroxin (Scott, Schaefer, Jones, Mears & Stanley, 1993). In this respect, in addition to the aforementioned endocrine factors, many other physiological insults are documented when animals are transported and handled (Schaefer, Jones &

Stanley, 1997). Plasma epinephrine and cortisol concentrations were also higher in stressed lambs than in unstressed lambs (Apple, Dikeman, Minton, McMurphy, Fedde, Leith & Unruh, 1995).

2.4.1 Muscle Glycogen

It was reported that muscle glycogen deficiency may result from physiological stress caused by physical activity or emotional excitement. Mild to moderate muscular activity activates the sympathetic system, and thereby initiate glycogenolysis (Apple *et al.,* 1994). Glycogenolysis can occur during heavy exercise or in response to catecholamine secretion in the absence of exercise and it would be reasonable to suspect muscular activity as a mechanism leading to glycogen depletion and dark-cutting meat in sheep (Blum & Eichinger, 1988) and in cattle (McVeigh & Tarrant, 1983; Tarrant *et al.,* 1992).

Transport and handling usually oblige cattle to a reduced energy intake as well as to some degree of physical exercise and, occasionally, to mixing, which in turn can precipitate such events as fighting and mounting. Such activities deplete muscle glycogen, especially of the fast twitch fibers (Lacourt and Tarrant, 1985). This situation usually contributes to a lower muscle glycogen content and rate of muscle glycogen repletion (McVeigh and Tarrant, 1982; Yambayamba, Aalhus, Price & Jones, 1996) which in part is one of the causative factors in dark cutting and may also, according to Cole, have implications for immune function (Schaefer *et al.*, 1997).

The events that should occur for the optimum conversion of muscles to meat are quite complex (Swatland, 1984). The pH should be lowered by the formation of lactate from anaerobic glycolysis. Inadequate lactate formation may leave the meat dark, firm and dry, while too much lactate, formed too quickly while muscles are still warm, may leave the meat pale, soft and exudative (PSE). Davey & Graafhuis (1981), found that approximately 100 mol/g lactate is produced in muscle having a normally low ultimate pH (pH

5.2) whereas only 40 mol/g lactate would be expected in a DFD muscle with an ultimate pH of 6.2. In other words the DFD muscle has less than half the available glycogen store of normal meat from which to produce lactic acid.

After an animal is exsanguinated (Swatland, 1984), muscle fibres may survive for some time by anaerobic glycolysis but sooner or later, however, they run out of energy. Either the primary store of carbohydrate, glycogen, is depleted, or else the end product of anaerobic glycolysis, lactate, deactivates biochemical systems by its lactic acid.

Methods of assessing the stress status of cattle at the time of slaughter could be of value in investigations into the specific causes of dark cutting beef. The measurement of concentrations of various plasma or serum constituents in blood samples collected during exsanguinations has been used in several species to assist in investigations into the handling of animals prior to slaughter and its effect on meat quality (Tume & Shaw, 1992).

2.4.2 Hematology

Transport and handling stress have been observed (Schaefer *et al.*, 1997) to alter numerous blood cell components. In general, an increase in packed cell volume is commonly seen and likely reflects both a splenic response to stress and a degree of dehydration.

Leukocyte responses in cattle to transport and handling are frequently reported. Blood from treadmill exercised lambs, also had greater hematocrit percentages and higher total protein and haemoglobin concentrations (Apple *et al.*, 1994).

2.4.3 Electrolytic balance

Although cattle have a substantial buffering capacity, transport and handling can be seen to cause significant changes in electrolyte balances and hydration is clearly a logical result of factors such as time off water, increased respiration rates, urinary and ruminal water loss, and excessive sweating in transported and handled animals (Schaefer *et al.*, 1997).

2.4.4 Additional metabolic changes

In addition to the foregoing, a host of significant metabolic changes have also been observed in transported and handled cattle. These include an increase in blood enzymes such as creatine phosphokinase (Tarrant & McVeigh, 1979; Kenny & Tarrant, 1988 and Cockram & Corley, 1991), lactate dehydrogenase and aminotransferase (Scott *et al.*, 1993). Notable changes in cardiovascular events, particularly an increased heart rate, are likewise seen to occur in transported and handled cattle (Schaefer *et al.*, 1997). Other findings of Cockram & Corley (1991) indicated that the plasma concentration of cortisol at the time of slaughter was positively correlated with the time animals spent standing still and with the time spent in the holding pen. The plasma activity of creatine kinase was positively correlated with the time spent in the race, but no correlations between creatine kinase and physical activity in the race were found. Cattle kept overnight in the lairage had a greater concentration of free fatty acids at the time of slaughter than those slaughtered on the day of arrival. There were no other significant differences in either the blood composition or the handling and behaviour of cattle kept overnight in the lairage compared with those slaughtered on the day of arrival. A positive correlation was also found (Warriss, 1987) between cortisol levels and the degree of carcass damage caused by fighting. Tume & Shaw (1992) indicated that the stresses of transportation and/or of the abattoir

environment, caused an elevation of the plasma cortisol values and the beta-endorphin, although, not sufficient to lead to the production of DFD meat.

2.4.5 The effect of needle biopsy in skeletal muscle on blood constituents and muscle glycogen

Tarrant & McVeigh (1979) assessed the use of the physiological response to biopsy by measuring serum free fatty acid and blood glucose concentrations, plasma creatine kinase activity and heart rate. This is important in relation to the use of the technique for investigating muscle metabolism in beef cattle to demonstrate that the experimental procedure did not cause undue stress. Muscle glycogen concentration was also measured. These parameters were unchanged by the experimental procedure, with the exception of blood glucose and heart rate, which showed significant increases. The experimental animals showed no adverse effects after repeated biopsy and these authors concluded that the technique is suitable for investigating beef muscle metabolism in vivo, and does not cause an unacceptable level of stress.

2.5 BEEF QUALITY

Beef is the product which results from the transformation each muscle undergoes during the different operations of meat processing (Dumont, 1981). These operations are also very variable and the variety of treatments acting on heterogeneous material results in a product of an extremely diversified nature. Beef is thus a simple name, which covers a very complex product. The meaning given (unconsciously) by consumers to quality has a major economic importance because it explains the consumer's attitude toward each product. In fact, the trade is governed by the quality, amount and price of products. In the case of beef, a large number of traits must be considered as quality factors, acting more or less strongly on the consumer to determine

first his acceptance of the meat and then his degree of satisfaction (Dumont, 1981).

The abnormal characteristics of DFD meat make it more difficult to use than normal meat. The dark colour of DFD meat makes it less attractive to the consumer who is apt to confuse the colour with that of very old animals or with meat that has been badly stored. The sticky character of DFD meat, for which an objective method of measurement remains to be found, not only makes it less acceptable but also makes the preparation of the muscles more difficult for cutting, trimming and preparation into portions for retail sale (Sornay, Dumont & Fournaud, 1981).

It is therefore important for the producer and processor to supply beef products to the consumer, which will fulfil in his/her expectations. This guaranteed eating quality of beef is important not only for local supply of beef, but also for the export trade. When bearing in mind that for bovine meat and animals in 2005, the trade is put at 8.3 million tons at the global level, which is some 1.5 million tons more than in the mid 1990's and that the increase reflects larger imports for Europe, amongst others, also by some African countries (Gü kan, 1999).

Quality attributes primarily affected by transport and handling in cattle include pH, colour, texture, and moisture and a degradation of these variables is collectively referred to as dark cutting or dark-firm-dry, high pH, low glycogen meat (Lacourt and Tarrant, 1985; Tarrant, 1989).

2.5.1 Texture

Tenderness is by far the most important criterion of beef quality. It is only if tenderness is satisfactory that any judgement about flavour and juiciness need be performed. Tenderness is without doubt the key; it is the limiting factor in the eating quality of beef (Dumont, 1981). One important criterion of

the acceptabil ty of meat is the tenderness (chewability) which is influenced largely by the ripening and heat treatment of the meat. Many factors, probably contribute to an increased shear force and the reduced meat tenderness, although not of equal importance (Jeremiah, Aalhus, Robertson & Gibson, 1994).

Katsaras & Peetz (1990) reported that tenderness is determined by certain enzymatically controlled morphological changes. The structural changes, in heat-treated (55° to 95° C) DFD meat, which had been stored for 7 days at 2° C were examined under the scanning electron microscope. From 65° C onwards DFD meat shows definite transverse breaks in the L-bands and Z-line connections and signs of granulation or breakdown of the connective tissue fibres. These changes increase as the level of heat treatment is increased. Fragmentation of myofibrils is greater than in the normal meat, but cooking losses are much smaller. Katsaras & Peetz (1990) explained that these differences evidently lead to greater tenderness in DFD meat as compared with normal meat. When the glycogen reserve is reduced prior to slaughter, little or no lactate can be produced resulting in high ultimate pH. Variations in pH, affect protein hydration and water holding capacity of the meat, which could affect toughness and juiciness. Endogenous enzymes damage the myofibrillar proteins in the early *post mortem* period. Proteolysis in the region of the Z disc is thought to be mainly responsible for tenderising during *post mortem* storage and general proteolysis for flavour development in the lean. Enzyme activity is dependent mainly on pH and temperature.

Katsaras & Hamm, according to Katsaras & Peetz (1990) also reported that DFD meat is usually very tender although not in the same way as with normal meat. On the other hand, several researchers found that beef tenderness decreased as ultimate pH increased from pH 5.4 to pH 6.1. (Dransfield, 1981; Wulf, Tatum, Green, Morgan, Golden & Smith, 1996). Thomson & Trout (1999) concluded from a laboratory experiment, that pH by itself, does not appear to be the cause of tough meat in beef with moderately high ultimate pH values. Aalhus, Jones, Tong, Jeramiah, Robertson & Gibson, (1993)

reported that pale, soft and exudative (PSE) beef was more tender than normal beef.

In DFD meat, according to Bouton, the moisture content after cooking, produces a more open structure and more tender meat, although the shear strength of raw DFD meat is unaffected by pH. At high pH, *pre-rigor* stretching or *post mortem* ageing have no effect on tenderness (Dransfield, 1981). Lee & Ashmore (1985) on the other hand, studied the toughening effect of early *post mortem* high temperatures. Regardless of environmental temperature, the grass-fed showed greater toughness than the feedlot-fed and conventionally chilled carcasses.

The effect of gender on the tenderness of meat was also studied by Voisinet *et al.* (1997) who found that heifers had significantly tougher meat and a higher incidence of borderline dark-cutters than the steers. However, Prost *et al.* found no gender based differences in tenderness, according to Voisinet *et al.* (1997).

2.5.2 Juiciness

Although juiciness may be described as two sensory components – an initial impression of wetness during the first few chews and sustained juiciness appreciated during mastication and salivation – such a distinction has not been made in studying DFD beef. Studies at The Meat Research Institute (in the UK) showed that juiciness of roast *M. longissimus dorsi* tended to increase with increase in pH in steer beef but not in bull beef. In steer beef, juiciness is increased, on average, 1 point (on a 5 point scale) from pH 5.5 to 7.0, whilst the variation between animals of similar pH was 1 to 2 points. In consumer trials in England, bull beef of normal pH was equal in juiciness to normal pH steer beef. In roasting joints, only DFD bull beef was slightly drier than steer beef of normal pH. The pH did not affect juiciness in roast and grilled beef joints, in beef muscles nor in roast venison (MacDougall, Shaw,

Nute & Rhodes, 1979). However, dehydrated high pH beef was ranked more juicy than normal pH beef (Dransfield, 1981).

The effect of electrical stimulation and ageing was also tested on lean, fat as well as dark-cutting carcasses (Jeremiah *et al.,* 1997) and they found expected differences, based upon differences in carcass fatness. Electrical stimulation improved overall palatability and flavour, however the juiciness was reduced by the application of electrical stimulation. In addition, *post mortem* treatment showed that striploin steaks from dark-cutting carcasses aged for 3 days were rated less juicy by the laboratory panel than similar steaks of normal carcasses.

2.5.3 Flavour

In contrast to studies on tenderness, little work has been reported on the influence of pH on flavour. Flavour of roast *M. longissimus dorsi* tended to be disliked more as pH increased in steer beef, although there was no noticeable effect in bull beef (Dransfield, 1981). MacDougall *et al.* according to Dransfield (1981), found wide variations in flavour scores at all pH's and on average, flavour scores, based on acceptability, fell only 3 points of the 14 point scale when the pH was raised from 5.5 to 7.0. Similar tests on roast venison *M. semimembranosus* showed no significant relationship between pH and panel mean hedonic flavour scores. However, normally coloured lean received higher ($P < 0.05$) flavour intensity scores than either dark- or pale coloured lean (Wulf *et al.*, 1996).

The acceptability of flavour of DFD beef was also assessed from consumer trials carried out in England (Dransfield, 1981). With normal pH, flavour was equally acceptable in bull and steer beef, but DFD bull beef had a poorer flavour. The overall effect of pH was small – only 0.1 points on a 4 point scale. Summarising, beef of normal pH was more acceptable than DFD beef because people, in general, preferred stronger beef flavour. Lawrie

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suggested in 1979 that the lower flavour might result from the swollen structures of high pH beef interfering with the access of the palate to flavour substances. Ingram noted in 1949, a similar phenomenon in bacon, where normal pH muscles had more 'free' (unbound) saline available for taste than at high pH where the water was bound and the salt unavailable for taste. Although this phenomenon may play some role in reducing the flavour of DFD beef, it is, however not the complete cause since steam volatiles from lamb and beef at pH 6.0 are different from those at pH 5.5 – 5.8 (Park and Murray according to Dransfield 1981).

It seems likely that weak flavour results from the lack (or very low level) of carbohydrate expected in meat of high ultimate pH, producing little interaction with amino acids or proteins (Dransfield, 1981). Flavour profiles indicate an appropriate fatty aroma in a higher proportion ($P < 0.05$) of roasts from normal carcasses than in comparable DFD carcasses (Jeremiah *et al.*, 1991). Katsaras & Hamm confirmed that DFD meat had, compared with normal meat, a stale (flat) off-flavour (according to Katsaras & Peetz, 1990). This is due to the fact that normal meat, because of adenosine triphosphate (ATP) breakdown 24 hours after slaughter, contains not only meat-souring, aroma-enhancing flavour components but also higher concentrations of inosine monophosphate (IMP) than DFD meat. Potthast & Hamm found in 1976, that higher contents of IMP breakdown products, that is, inosine and hypoxanthine, can, however, be detected in DFD meat (according to Katsaras & Peetz, 1990).

Because it has not reddened well and has a flat flavour, DFD meat is unsuitable as shop meat (Katsaras & Hamm, according to Katsaras & Peetz, 1990). Other objections which were mentioned concerning the sensory and technological properties of DFD, were that: it is tasteless or of abnormal taste, is unacceptable for curing or drying, is difficult to brown during frying and is of abnormal appearance and stickiness.

21 156 74 010
b 15096294

2.5.4 pH

Considerable effort has been directed to the reliable monitoring of the DFD condition. Subjective procedures have been developed based on colour and with the additional use of pH indicator strips (Honikel according to Davey & Graafhuis, 1981). Although changes in the adenine-inosine ratio measured spectrophotometrically can be used, monitoring pH with glass electrodes is the more usual objective method. In normal practice the ultimate pH value of *M. longissimus* from beef animals for instance, is determined at the 11^{th}-12^{th} rib, either by surface or probe electrode (Munns and Burrell, 1966). Such investigations reveal considerable variations in ultimate pH values amongst the muscles of beef animals, pigs and sheep (Davey & Graafhuis, 1981).

The acceptability of beef of abnormal ultimate pH is greatly influenced by production and trading circumstances. There is scope for the preparation of a comprehensive pH standard for use in quality control and quality assessment. Measurement of ultimate pH is widely practised and pH standards vary considerably, to meet particular production and marketing circumstances.

A suitable pH standard for use within the European Community would take into account the type of carcass under examination and the process and market for which it is intended. In this context, it is important to emphasise the wholesomeness of dark-cutting beef, despite its technological and sensory disadvantages (Tarrant, 1981).

There is general agreement on the relation between meat pH and intrinsic characteristics such as darkness, keeping quality and consistency. Values below pH of 5.8 are considered normal everywhere. At pH values of 5.8 and 5.9, beef may be rejected on the basis of lower keeping quality and darkening of the fresh meat colour. Colour and spoilage are the main objections to dark-cutting in beef. The consistency of DFD meat is rated as a much less important objection than colour or keeping quality (Tarrant, 1981).

2.5.5 Colour and colour measuring

Colour has been, and still is, a very important factor in beef marketing. The importance of colour as an indicator of freshness is probably overestimated in the consumer's mind, because it has been clearly established, by Billault *et al.*, that there is no clear relation between colour defects and meat spoilage (Dumont, 1981). Even though colour of lean is an irrational basis for consumer preference, it still remains, as pointed out by Hood (1978), that the psychological attractiveness of the bright-red colour makes it a fundamental criterion in the purchasing process.

The depth of oxymyoglobin on the surface of dark-cutting beef is also likely to be diminished because of the unusual structural features of the latter. This is another consequence of high pH. The iso-electric point of the principal muscle protein, actomyosin, is at pH 5.6. Considerable variation may exist in pH, colour and waterholding properties among muscles of a given beef carcass designated as being dark-cutting. Considerable variation may exist for these muscle properties among carcasses (Hedrick, 1981).

Hunt and Hedrick (according to Hedrick, 1981) characterised four quality groups of beef carcasses: dark-cutting, firm and dry (DFD); normal in colour, firmness and exudation (NOR); normal in colour, but soft and exudative (NSE); and pale in colour, and soft and exudative (PSE). Observations made by Hedrick over a 25 year period of research related to beef quality, indicate that short-term stress is conducive to the PSE condition and long-term stress is conducive to the DFD condition. In other words, cattle that are stress-susceptible and if subjected to stress for approximately one hour immediately before slaughter may be NSE or PSE and if subjected to stress for several hours before slaughter may be dark-cutting (Hedrick, 1981).

Although pH readings are taken when determining dark-cutting carcasses, it is mostly used in combination with colour grading, because pH measurements are difficult to standardize across different operators and it requires

considerable skill to obtain consistent results. A colour grading system according to colour scores, are used by many abattoirs and meat processing plants as a standard. An example is the Colour Grade System developed by the Cooperative Extension Service (Iowa State University, 1982). The disadvantage of this system is that it is subjective and depends on the operators' interpretation of the meat colour when he compares it with the standard.

In addition, the use of existing colorimeters for the surface colour measurement is not feasible because the partial ribbing of the carcass at grading time does not permit access. Attempts have been made (MacDougall & Jones, 1981) to measure the scatter coefficient of raw beef with a fibre optic probe, which successfully identified more than 80% of the samples with a pH value of 6.1 and above. However, Truscott *et al.*, (according to Swatland, 1989) reported that measurements obtained with the same probe were poor indicators of visually assessed muscle colour.

The Colormet (CFOP) is a Canadian fibre optic probe reflectance meter that has been developed for spectrophotometric studies of meat by Swatland (1989). It has the potential to measure pH-related differences in the optical properties of muscle tissue.

The potential of the Colormet to identify dark-cutting carcasses among a larger population of carcasses and comparing the results with objective measurements of meat quality, namely pH and muscle surface colour was assessed by Swatland (1989). The results of his study have shown the superior segregating power of Colormet over both subjective and objective evaluations for dark-cutting. Colormet could demarcate intermediate dark-cutting, and the similar shape of the reflectance spectrum of the three clusters obtained indicated that identification of dark-cutting could rely on the luminosity parameter (L^*). Therefore, a Colormet L^* cut-off point could be determined under controlled *post mortem* conditions and be of value for DFD

identification in commercial operations under standardized *post mortem* grading time (Gariepy, Jones, Tong & Rodrique, 1994).

In a later study by Swatland (1990) to develop and improve methods to detect dark-cutting in intact carcasses, the approach was to invert the problem and to ask why normal beef that becomes acidic after slaughter gradually becomes brighter and to understand the mechanism by which a high ultimate pH causes the meat to appear dark. It was found that the reflectance spectrum of meat originates from the trapping of light at low wavelengths by pH-dependent scattering within myofibrils.

In beef with a high pH, transmittance is high because scattering is low; hence, meat appears dark-cutting. Dark-cutting, therefore, is viewed as a failure to develop normal pH-related brightness (Swatland, 1990).

The feasibility of using a probe with a single optical fibre to detect PSE (pale, soft and exudative) muscle within sides of pork was also examined by Swatland (1998). The single fibre allowed a reduction in probe diameter, which facilitated penetration of carcasses with a hardened outer rind. A full spectrum beam splitter and crossed polarisers were used to separate outgoing illumination from incoming light from the meat. Major differences between PSE and DFD (dark, firm and dry) pork were detected from 440 to 450nm, and from 550 to 560 nm ($P < 0.005$).

In summary, optical differences between dark and pale pork were detectable with a single optical fibre using a dichroic beam splitter, and the differences agreed with results obtained by a variety of other methods. However, the spectrum was a unique interaction between apparatus and samples, and discrimination was based on very small differences in light intensity, which might be difficult to resolve in a practical application (Swatland, 1998).

2.5.6 Waterholding Capacity

In determining waterholding capacity (WHC) of meat, the *post mortem* rate of pH fall is a very important factor. A fast muscle pH decline determines the denaturation of sarcoplasmic, and myofibrillar proteins, increasing the tendency of actomyosin to contract, affecting the amount of fluid free to enter extracellular spaces and the meat structure (paleness) (Bendall according to Roseiro, Santos & Melo, 1994). The high water-holding capacity of DFD meat (Sornay *et al.*, 1981) results from its high ultimate pH; this can be an advantage at the practical level. As fresh meat, it is subject to less weight loss during storage and cooking.

The early assessment of the three waterholding capacity categories of meat (PSE/ Normal/ DFD) in order to assure their optimum utilization by the industry, are time consuming and difficult to apply under field conditions. Because speed is absolutely essential, and damage to the meat, in order to define the overall carcass quality, is often not allowed in practice, subjective methods are usually adopted (Kauffman *et al.*, according to Roseiro *et al.*, 1994). However, the relationships between objective measurements and subjective scores found in different countries have been inconsistent because of the nature of human physiological and psychological responses.

2.6 MICROBIOLOGY AND SHELF-LIFE

Meat quality can reflect differences in rate and/or extent of *post mortem* glycolysis. Muscles in which glycolysis is restricted due to lack of glycogen enter *rigor mortis* at a high pH (Chrystall, Devine, Davey & Kirton, 1981). If meat with a high pH is stored as a vacuum-packaged chilled product, it will have a poor shelf-life due to deterioration and early greening (Gill & Newton, 1979).

Buchter (1981) confirmed that DFD meat causes the meat industry financial losses not only because of the dark and unpleasant colour, but also due to the low keeping ability, especially of vacuum-packed meat.

Studies on the microbiology of DFD meat have shown that the reduced shelf-life of such meat is not only due to a high ultimate pH but also the very low concentration of available glucose, the usual substrate for meat spoilage bacteria (Gill & Newton, 1979). In normal meat, glucose is preferentially utilised by meat bacteria. Aerobic spoilage is delayed until glucose is exhausted and amino acids are attacked. In the absence of glucose, amino acids are attacked without delay and spoilage odours can be detected when bacterial densities are still low. Early aerobic spoilage of DFD meat can be prevented by addition of small quantities of glucose (Gill & Newton, 1981).

During the storage of fresh DFD meat, undesirable odours and green discolourations may develop very rapidly. Bacterial growth is enhanced and the metabolism altered in such a way that there is a more direct attack on muscle proteins with the development of unpleasant odours. Vacuum packaged meat with a high pH (>6.2) spoils rapidly but Taylor & Shaw (1977) reported that if the pH is in the range 5.9-6.2, greening occurs only on meat packaged in film with a relatively high oxygen permeability. Deterioration, such as greening in packaged meat, or the development of objectionable smells and off-flavours then occurs at rather low bacterial counts (10^6

c.f.u./g), substantially reducing acceptable shelf-life (Davey & Graafhuis, 1981).

The high pH of DFD meat allows growth of species inhibited at the normal ultimate pH of meat. One such organism, *Alteromonas putrefaciens,* is responsible for 'greening' of vacuum-packaged meat. This type of spoilage is not prevented by addition of glucose but can be controlled by addition of small quantities of citrate to vacuum-packaged meat (Gill & Newton, 1981).

The microbiological condition of the carcass from a healthy animal is largely related to the standard of carcass dressing. A DFD carcass requires even more hygienic handling than a normal carcass due to its higher pH (Davey & Graafhuis, 1981).

The most common psychrotophs found on chilled meat (Newton & Gill, 1978; Nottingham, 1982) are certain strains of *Pseudomonas, Moraxella, Acinetobacter, Lactobacillus, Brochothrix thermosphacta, Alteromonas,* certain genera of the family Enterobacteriaceae, and *Yersinia enterocolitica*, species not usually found in significant numbers on normal meat.

In the case of vacuum-packaging, all bacterial flora develop at the same time, without the usual inhibition of specific type (Sornay *et al.*, 1981). However, Nottingham (1982) was of the opinion that total counts of vacuum packaged beef, is not as significant as a measure of spoilage as they are with meat stored under aerobic conditions. An initial rapid growth of total counts is normally found with vacuum packaged meat, which then tapers off (Egan & Roberts, 1987; Newton & Gill, 1978; Hayes, 1992).

Should oxygen be more readily available in vacuum-packaged DFD meat, it could lead to abnormal development of aerobic bacteria, such as *Pseudomonas,* due to their ability to grow faster than all other competing species at low temperatures. It was also found that the growth rates and final numbers of *Pseudomonas* species were unaffected by maximum numbers of

competing species due to their inability to compete with *Pseudomonas* for the available oxygen (Gill & Newton, 1977; Newton & Gill, 1978; Nottingham, 1982; Hayes, 1992). When *Pseudomonas* counts reached about 10^6 /cm², off odours were produced (Nottingham,1982).

Gill (1982) and Jay (1992) reported that the microbial flora in the impermeable pack becomes dominated by lactic acid bacteria (mainly lactobacilli and leuconostocs) which represent 50-90% of the total flora at the end of storage.

Faecal organisms in beef carcass dressing procedures are considered as a critical source of contamination (Gill, McGinnis & Badoni, 1996). According to Nottingham (1982), the ratio of *Escherichia coli* to the total aerobic flora can be used as an indicator of whether the major source of carcass contamination is the intestinal tract or the hide or fleece. Newton, Harrison & Smith (1977) found that under conditions where growth of competing organisms is slow, psychrothopic Enterobacteriaceae can become a significant proportion of the flora.

Kaya & Schmidt (1991) showed that DFD meat is unsuitable for storage under vacuum because of the danger of a build up of *Listeria monocytogenes,* but there is no danger of growth of *Listeria* in pieces of vacuum-packed beef with a pH of less than 5.8 and at storage temperatures of about 2°C.

Newton & Rigg (1979), determined that the storage life of vacuum packed normal pH beef cuts ranged from 4 to 15 weeks, and Gill (1982) and Hayes (1992) found that vacuum packaged meat at -1°C has a shelf-life of 12-14 weeks during which time it develops a cheesy or dairy flavour which finally renders it unacceptable due to the short chain fatty acids.

2.7 MUSCLES MOST LIABLE TO BE AFFECTED

A survey, which was conducted by Tarrant (1981), indicated that the *Musculus longissimus dorsi* is the only muscle, which was universally considered to be liable to dark-cutting. In Denmark, *M. longissimus dorsi* was found to be the best indicator muscle for identifying DFD, because it showed a tendency to increased ultimate pH values before other muscles. When the pH in the *M. longissimus dorsi* was approximately 6.5, the whole carcass was usually found to be badly affected, and slightly higher pH values might be found in some of the other muscles.

However, in New Zealand, it was noted that the *M. longissimus dorsi* is of commercial importance; it is most affected by DFD (Tarrant, 1981); it is observed at an early stage at quartering; it is much studied by other researchers; and it is a readily accessible muscle for sampling (Zerouala & Stickland, 1991). It was noted that no single muscle seemed to be most susceptible and certainly measurement on one muscle could not be used to predict for other muscles of the carcass (Tarrant, 1981). The muscles mostly affected by dark-cutting as compiled according to a questionnaire analysed by Tarrant (1981) are listed in Table 2.1

Table 2.1: Muscles most liable to be affected by the dark cutting condition (Tarrant, 1981)

MUSCLE	Percentage
M. longissimus dorsi	92
M. semimembranosus	38
M. biceps femoris	33
M. semitendinosus	21
M. adductor	21
M. gluteus medius	21
M. trapezius	17
M. biceps brachii	13
M. psoas major	8
M. infraspinatus	8
M. supraspinatus	8

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CHAPTER 3

OBJECTIVES

The first objective was to investigate the incidence of dark-cutting at a specific abattoir over a three month period. The following objective was to determine from these results, the main supplying feedlots that manifested the DFD problem and to investigate the possible causes of this problem at these feedlots by using a reasonable large commercial database to identify and/or quantify management and environmental factors. The hypotheses were made that the following aspects were possible causes of DFD: gender, genotype, fasting period, feedlot *per se* and feedlot management. The third objective was to randomly select animals from three feedlots for an experiment to establish the stage of 'stress-experience'. Blood samples and muscle biopsy samples were taken at the feedlot (as control but also to establish if deviations were present already, prior to handling and transportation), after arrival at the abattoir (effect of transportation and handling) and thirdly after slaughter. The following hypotheses were formulated: deviations in the blood parameters should indicate firstly, the stage of stress experienced by the animals and secondly, variations within a feedlot should also be indicative of an effect of management factor(s). An increase in the blood constituents: (a) glucose (b) creatine kinase and (c) total free fatty acids should be indicative of stressful situations occurring. An increase in the hematocrit would also indicate that the animals were under stress. A decrease in the muscle glycogen was indicative of glycogen depletion. An attempt was made to correlate these conditions to DFD meat.

The fourth objective was to compare consumer acceptability of DFD and normal meat, both in the raw and the cooked form. It was anticipated that DFD meat would have a less acceptable raw colour (Dransfield, 1981) but be more tender although the texture might be too soft and mealy. In addition, it was expected

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that the flavour would be less acceptable compared to normal pH meat (Buchter, 1981). The final and fifth objective was to test DFD meat samples, using normal meat as the control, over a 7 week period for the following organisms: Total aerobic count, coliforms, *Escherichia coli* and *Pseudomonas*. During this period other physical quality attributes (general appearance, colour and odour) were also evaluated to establish the shelf-life of the DFD vacuum packaged meat.

CHAPTER 4

MATERIALS AND METHODS

Due to the complexity of this research study and because different disciplines of study were involved, the experiments were carried out over different time periods and using different cattle, carcasses and beef joints as required. Figure 4.1 indicates the overall conceptual framework used for the entire study. Figure 4.2 illustrates an overview of the factors which could play a role in the dark-cutting problem of feedlot cattle.

4.1 EXPERIMENTAL DESIGN TO DETERMINE THE INCIDENCE OF DARK-CUTTING BEEF

4.1.1 An estimate of the incidence of dark-cutting beef based on pH_1

The incidence and possible causes of dark cutters were studied at a local commercial abattoir, which slaughtered approximately 600 cattle per day. The cattle were supplied by various feedlots from different geographical regions and were transported for various distances to the abattoir.

The investigation to determine the incidence of dark cutters was initially carried out over a 3 month period, i.e., December to February. This is summer time in South Africa and day temperatures ranged from 25°C to 33°C.

For the initial part of this study, the pH value (pH threshold $\geq$ 5.8 was regarded as DFD) of all the carcasses (N= 22 178) were measured in the *M. longissimus dorsi* 45 minutes *post mortem* (pH_1) as well as 24 h *post mortem* (pH_{24}). The ultimate pH was reached after 24 h, which was confirmed by previous studies (Fabiansson, Erichsen & Reuterswald, 1984) and therefore, pH_{24} only was measured to estimate the pH of the meat in subsequent data collected.

CHAPTER 4

EXPERIMENT 1:

An estimate of the overall incidence of DFD at a local abattoir based on pH_1 over a 3 month period (N = 22 178)

EXPERIMENT 2:

2.1 An estimate of the incidence of dark-cutting beef based on pH_{24} from four selected feedlots A, B, C and D (N = 5 659) supplying the abattoir

2.2 The effect of gender, bruising (caused by mounting, excitement and rough handling) and holding periods in the pens on the incidence of dark-cutting (Feedlots A, B, C and D; N = 5 659)

EXPERIMENT 3:

The effect of breed type (Brahman and European breeds) and fasting periods (40 h and 60 h) on the incidence of dark-cutters (Feedlot A and B)

EXPERIMENT 4:

Analyses of blood samples and muscle samples from cattle of Feedlots A (N = 20), B (N = 20) and C(N =20) before and after transport and after slaughter to measure stress levels

EXPERIMENT 5:

5.1 Sensory evaluation of raw DFD beef compared with raw normal beef (consumer panel: N = 64)

5.2 Sensory evaluation of fried DFD beef compared with fried normal beef (consumer panel: N = 64)

EXPERIMENT 6:

6.1 Microbiological quality of DFD beef (control: normal beef)

6.2 Shelf life of vacuum packaged DFD beef (control: normal beef)

Fig. 4.1: Conceptual framework for the study of meat quality from dark-cutting cattle

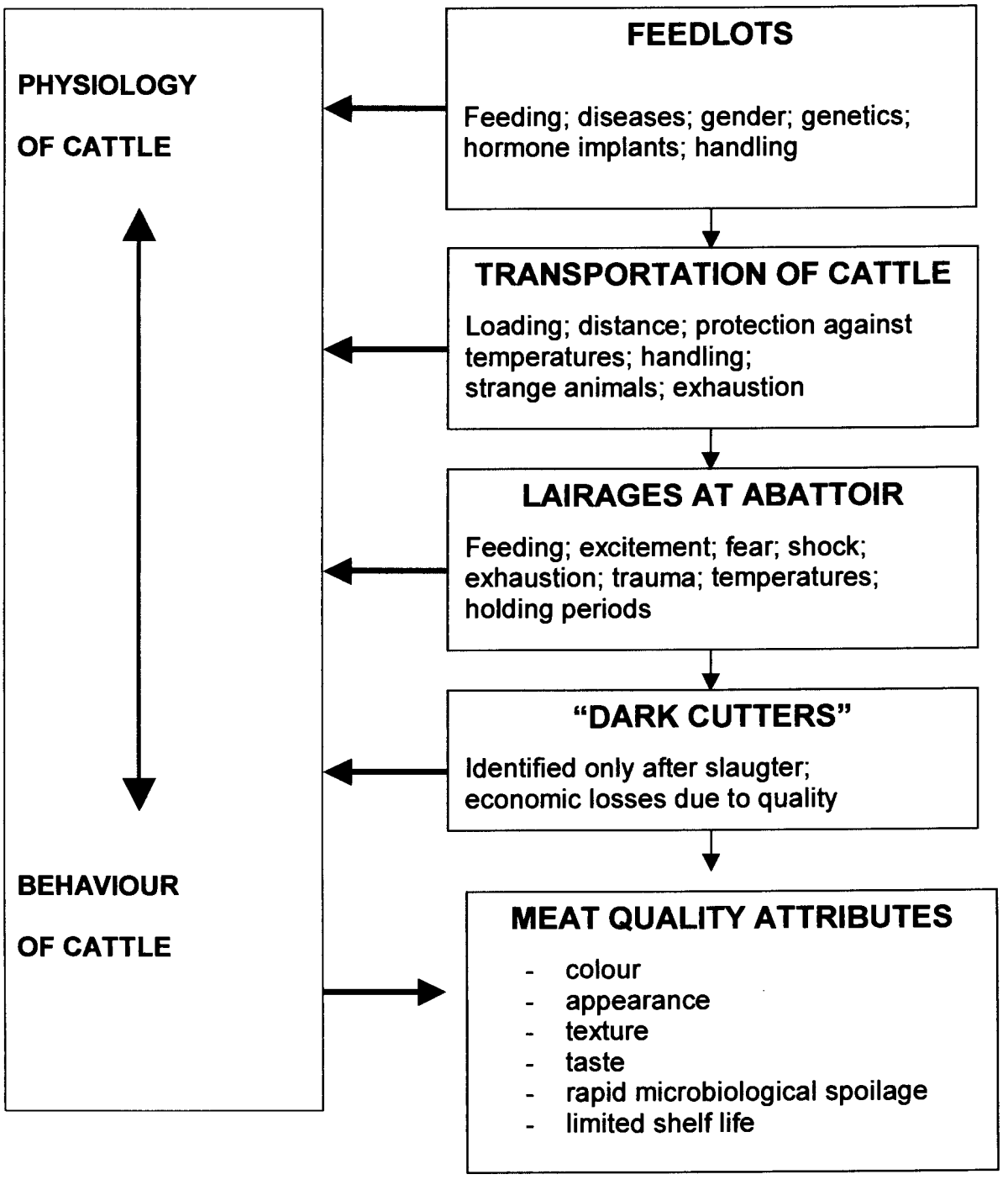

Fig 4.2 An overview of the factors which could play a role in the dark- cutting problem of feedlot cattle

It was daily routine during the slaughter process to record the pH taken approximately 45 to 60 minutes *post mortem* and to downgrade the carcasses with a pH of >5.8.

4.1.2 An estimate of the incidence of dark-cutting beef based on pH_{24} from four selected feedlots supplying the abattoir

It was decided to conduct a controlled study of the DFD phenomenon to ascertain the feedlots involved, incidences per feedlot, transport and handling matters, and other factors which affected the incidence of DFD. Fig. 4.3 shows the experimental design for this experiment.

DATE	FEEDLOT NAME	CARCASS NUMBER	GENDER	pH 24H	COLOUR 24 H	% DFD	% BRUISED	HOURS IN PENS

Fig.4.3 Experimental design to investigate the incidence of DFD of four feedlots

The objective was to obtain the overall mean % incidence of DFD based on the total number of cattle supplied to this abattoir over a 5 day period. From the first data obtained, it was decided to select only the carcasses from the four main feedlots (~90% of the cattle were supplied by them anyway) for the second experiment. This was conducted during July, which is peak winter in South Africa. The animals were grouped into either steers, bulls or heifers. A total of 5 659 carcasses from the normal supply of four feedlots were evaluated.

The feedlots will be named A, B, C and D, for confidentiality reasons and will be referred to as such in the rest of the study (only cattle from feedlots A, B and C were used in further experiments. These feedlots were situated in different geographically areas and sent cattle on a regular basis to the abattoir

concerned. The cattle were slaughtered in the normal process that involved stunning by captive bolt, suspension by the hind leg, and exsanguination. The carcasses were immediately electrically stimulated after exsanguination using contact electrodes delivering 140V for approximately 8s.

4.2 THE POSSIBLE CAUSES OF DFD

4.2.1 The effect of gender, bruising (caused by mounting, excitement and rough handling) and holding periods in the pens, on the incidence of dark-cutting

Discussions were also held with the respective Feedlot Managers to ascertain possible factors, which could contribute to the DFD problem. It was decided to start investigating the possible causes of DFD using the same lot of cattle as in 4.1.2. The following aspects for each feedlot were recorded: (i) number of cattle slaughtered (ii) sex of animals (iii) number of hours in holding pens prior to slaughter (iv) number of carcasses identified and downgraded due to bruising by the Veterinary Meat Inspectors (v) number of carcasses downgraded due to DFD, based on pH $\geq$ 5.8 (vi) colour, evaluated by experienced abattoir staff as well as the researcher.

At 24 h *post mortem*, the chilled carcasses were ribbed, (11^{th}/ 12^{th} rib) and both the left and right *Musculus longissimus dorsi* were evaluated for colour grade by an experienced beef grader and the ultimate pH (pH_{24}) - was measured in *M. longissimus dorsi* by means of penetrating electrodes and a portable electronic pH-meter. A Hanna portable microprocessor pH/mv meter, model HI 9025, waterproof series IP 67, was used and calibrated during tea breaks (every 3 hours) with Merck pH 4 and pH 7 standard solutions. A spear type Ingold 10—404-3041 meat electrode was used. The pH measurements were carried out by trained personnel, including the researcher.

The standards used to identify DFD meat were a pH $\geq$ 5.8 and a colour standard of > 6 (range 1 to 8), compared to the Standards for Beef Colour - Appendix A (Iowa State University, 1982).

4.2.2 The effect of breed type and fasting on DFD

The next phase was to investigate the effect of other factors, i.e. the breed type and the effect of fasting prior to slaughter on the incidence of DFD (Fig. 4.4). The co-ordination and management of this experiment was a huge task and it was therefore decided to use only two feedlots from different regions, for investigation.

Feedlot A, which appeared to have a low incidence of DFD, and another feedlot with a higher incidence of DFD, Feedlot B were included. It was further decided to also include two breed types from each feedlot (each lot: N = 55). Predominantly Brahman crossbreeds and a European crossbreed (Feedlot B: supplied predominantly Angus crossbreeds and Feedlot A: supplied predominantly Hereford crossbreeds) were used. Only steers were used in this experiment.

To exclude or at least minimise the interaction of other factors for e.g. excitement, the steers were in each individual case, drawn from the same feedlot pen. To avoid social regrouping as far as possible, they were neither mixed with strange cattle on the trucks (used to transport them to the abattoir) nor mixed with unfamiliar cattle in the lairages at the abattoir.

The respective feedlot management, withdrew feeding at certain time intervals to achieve the following fasting periods prior to slaughter: 40 hours and 60 hours. The exact same slaughter procedures were followed as previously. The DFD identification procedures as discussed before, were also followed.

	FEEDLOT A		FEEDLOT B	
Breed type	Brahman crossbreed	Angus crossbreed	Brahman crossbreed	Hereford crossbreed
N =	55	55	55	55
Temperature at lairages	6-16°C	6-16°C	4-14°C	4-14°C
Fasting period	40 hrs	40 hrs	40 hrs	40 hrs
Fasting period	60 hrs	60 hrs	60 hrs	60 hrs

Fig.4.4 Experimental design to determine the effect of breed type and fasting on DFD

4.3 BLOOD SAMPLES AND MUSCLE BIOPSIES

The aim of this experiment was thus to identify the stage of stress encountered by the cattle by means of measuring specific blood constituents and muscle glycogen content and to correlate these results with the quality parameters of the meat. The three different phases were:1st phase: on the farm prior to loading onto the trucks; 2nd phase: after transportation to the abattoir and off-loading into the holding pens; 3rd phase: directly after slaughter. The experimental design is schematically illustrated in Fig. 4.5.

FEEDLOT A → FEEDLOT B → FEEDLOT C

Phase 1: ***AT FEEDLOT PRIOR TO LOADING-blood samples drawn and muscle biopsies taken***

Bloodparameters	**Muscle**
▪ Hematocrit ▪ Creatine kinase ▪ Free fatty acid ▪ Glucose content	Muscle glycogen

Phase 2: ***ARRIVAL AT ABATTOIR, DIRECTLY AFTER BEING OFF- LOADED-blood samples drawn and muscle biopsies taken***

Bloodparameters	**Muscle**
▪ Hematocrit ▪ Creatine kinase ▪ Free fatty acid ▪ Glucose content	Muscle glycogen

Phase 3: ***AFTER SLAUGHTER; blood collection from jugular vein and muscle samples taken from carcass (post mortem)***

Bloodparameters	**Muscle**
▪ Hematocrit ▪ Creatine kinase ▪ Free fatty acid ▪ Glucose content	Muscle glycogen Muscle colour pH_{24} Microbiology & shelf life

Fig. 4.5 **Experimental design for the blood sampling and muscle biopsies taken from 20 cattle each of 3 different feedlots**

4.3.1 Experimental methods

At each of feedlots A, B and C, twenty steers aged 12 to 16 months, of various crossbreeds were randomly taken during the normal loading procedures prior to being transported to the abattoir. Feedlot D was not included in this experiment due to unwillingness of the Feedlot management. The cattle lot, destined for slaughtering were selected from the various pens for weighing. Each steer was marked with a number from 1-20, respectively, on the left buttock using white paint for easier identification during subsequent handling and also to ensure that there would be no confusion with the feedlot's own numbers.

The numbers on the ear tags were also recorded for later cross checking. Care was taken when handling the animals to avoid exciting them. The results from phase 1 were regarded as the control. Blood samples and muscle biopsies were collected from the randomly selected cattle, while the cattle were restrained in a crush.

The cattle were loaded onto trucks according to normal practice at the feedlots. The cattle were transported from the feedlot to the abattoir, off-loaded according to normal procedures. The distance transported in each case and transportation period is indicated in Table 4.1. The period that the cattle were held in the holding pens at the abattoir before slaughter and the temperature at each holding pen were also recorded. This experiment was conducted during winter. Tarrant & McVeigh (1979), showed that experimental animals had no adverse effects after repeated biopsy and it was concluded that the technique was suitable for investigating beef muscle *in vivo*, and did not cause an acceptable degree of stress.

Discussions were held with the respective Feedlot Management to obtain other important data (Table 4.2) which could contribute to the DFD situation.

Table 4.1: Environmental factors and transportation information relevant to cattle used for experiment 4, for blood parameters analyses and muscle glycogen determination

FEEDLOT	DISTANCE TRANSPORTED	PERIOD (TIME) TRANSPORTED	HOURS FASTED PRIOR TO SLAUGHTER	TEMPERATURE AT ABATTOIR PENS
A	290km	4.5 h	26	22-24°C
B	180km	3 h	24	20-23°C
C	45km	50 min	25	19-22°C

Table 4.2 Feedlot Management Information for the respective Feedlots A, B, C used for blood parameter analyses and muscle glycogen content determination

Climatic condition at feedlot:

FEEDLOT A	FEEDLOT B	FEEDLOT C
Dry, little dust, mild temperatures. Winter months, very cold. Day of blood sampling- very windy.	Dry, little dust, mild temperatures. Winter months, very cold. Day of blood sampling –no wind.	Dry, little dust, mild temperatures. Winter months, very cold. Day of blood sampling- very windy.

Diseases:

None	None	None

Feed:

Hominy chop; maize gluten; cassava; wheaten bran; chicken litter; molasses; milled wheat straw; salt; calcium phosphate	Molasses; maize; silage; soya oilcake/ cottonseed oilcake; soya fat; fish flour; salt; calcium phosphate; ureum	Hominy chop; cottonseed oilcake; ureum; Eragrostis curvula

Hormone implants:

Revelor® at age of ~9 Months (mass~210kg)	Synovex 'S' ® at ~6 weeks (mass< 180kg) and Revelor® at 4 months	Revelor® at age of ~9 months(mass~200kg)

Biological breeds(cattle used in experiment):

Friesian and Friesian crossbreeds	Crossbreeds: Afrikaner; Bonsmara; Brahman; Drakenberger; Fries; Guernsey; Simmentaler	Crossbreeds: Afrikaner; Bonsmara; Brahman; Simmentaler

Geographical areas of animal origin

East- and South Free State	North West Province; Northern Cape; Mpumalanga	South FreeState; North West Province

The second phase of blood samples and muscle biopsies were done exactly as in phase 1, immediately after off-loading at the holding pens at the abattoir. The cattle were again restrained in a crush for the blood and muscle sampling.

The third phase of sampling were done directly after cutting of the jugular vein on the slaughter floor. The muscle samples were taken from the *M. longissimus dorsi* within 20 minutes after killing.

4.3.1.1 Blood collection and analyses

Blood was taken from the jugular vein by venepuncture, using a needle of 1mm internal diameter, and held on ice. Plasma was prepared by centrifugation of the heparinised blood. Serum was prepared by holding the blood at 4° C for about 4 h until clotting and a degree of clot retraction had occurred.

The samples were then centrifuged and the serum was collected and stored at –25° C until assayed for free fatty acid (FFA).

4.3.1.2 Hematocrit

The heparinised blood sample was mixed at room temperature and an aliquot was collected into a microhemotocrit tube, centrifuged for 5 min at 14 000 *g* and the packed cell volume was determined using a hematocrit reader (Hawksley, Lansing).

4.3.1.3 Measurement of creatine kinase

Creatine kinase (CPK) activity was measured in fresh plasma using a Boehringer reagent kit (CK NAC-act., Cat. No.126349). A unit of enzyme activity is defined as 1 mol of substrate used per min at 25° C. This is an optimised standard method conforming to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (Tarrant & McVeigh, 1979).

4.3.1.4 *Measurement of blood glucose*

For the measurement of blood glucose, heparanised blood was deproteinised with uranyl acetate immediately after collection and then centrifuged. The glucose content of the supernatant was measured in duplicate by the enzymic procedure of Bergmeyer, Bernt, Schmidt & Stork (1974).

4.3.1.5 *Determination of free fatty acids (FFA)*

Free fatty acids (FFA) were extracted from serum using the 2-phase system of Kashket and measured in duplicate by the automated colorimetric procedure of Lindqvist *et al.* (according to Tarrant & McVeigh, 1979).

4.3.1.6 *Muscle sampling technique*

A Bergström biopsy needle with an internal diameter of 5 mm was used following the technique as described by Tarrant & McVeigh (1979). The location for sampling was the middle portion of *M. longissimus dorsi* between the levels of the last thoracic and fifth lumbar vertebrae. A small area of skin, approximately 25 mm^2 was shaved, disinfected and made insensitive by freezing with a fine jet of ethyl chloride. The skin and subcutaneous tissues were further anaesthetised with 2.5 ml of 0.5% w/v lignociane hydrochloride. A 10 mm skin incision was made with a sterile scalpel and continued through the connective tissue sheath covering the muscle. The biopsy needle was inserted and muscle samples weighing 100 to 200 mg were obtained. A second sample was taken through the same incision. Aseptic precautions were maintained through the biopsy procedure. The wound was treated with sulphonamide powder and covered with a small piece of sterile cotton and spray plaster.

The muscle samples were quickly inspected and separated from non-muscle tissue where necessary. They were frozen in liquid nitrogen within 60 seconds of excision.

4.3.1.7 Glycogen assay

The frozen muscle biopsies were accurately weighed and the exact weight was recorded (sample size ±100 mg) and then homogenised in 0.03M hydrochloric acid (2 ml) using an homogeniser. A vessel for the homogeniser was especially manufactured for this purpose. The vessel was pulled out and replugged for homogenate distribution and mixing of cells.

The homogenate was transferred to a marked glass tube. The vessel was rinsed with 0.5ml 0.03M HCl . Samples not analysed immediately were stored at –20° C and thawed at 37° C for 10-15 minutes when needed for testing.

The homogenate was heated at 100°C for 10 min in a waterbath and cooled. The homogenate was centrifuged for 20 min at 2 500 rpm in a Vortex centrifuge.

Blanks, standards and samples were centrifuged in duplicate. Using the GOD Perid method (Kunst, Draeger & Ziegerhorn, 1984), the free glucose was measured, i.e. mixed, incubated in a dark cabinet at 20 –25°C and read at 610 nm absorbency on spectrophotometer. Two ml of 2N H_2SO_4 was added to the original homogenate and then vortexed.

The homogenate was then heated at 100° C for 120 min. The glycogen was thus hydrolysed to glucose. Samples were subsequently cooled at room temperature. The homogenate was then neutralised to a pH between 6-7 by adding 2N NaOH, while mixing and then centrifuged again for 20 min at 2 500 rpm. The supernatant was transferred to a clean 10 ml measuring cylinder.

The homogenate was diluted with distilled water to the 10 ml mark. This dilution was transferred to clean glass tubes and centrifuged again for 25 min at 2 500 rpm.

The glucose measurement was again done according to the GOD Perid method. The difference between the free glucose and the total glucose represented the glucose derived from glycogen.

4.3.2 Measurement of pH

The pH measurements were taken within 1 h *post mortem* in the first experiment and 24 h *post mortem* on the chilled meat, which was approximately 2-4°C, in subsequent experiments. Using a clean stainless steel spear, rinsed with distilled water and dried with a clean paper towel, a hole was punched into the left side between the 11^{th} and 12^{th} rib in the *M. longissimus dorsi.* A speartype electrode was probed into the meat and duplicate pH reading taken using a Hanna electronic portable microprocessor pH/mv meter, model HI 9025, waterproof series IP67. This procedure was repeated on the right side and the average pH was calculated.

4.3.3 Meat colour measurements

The colour shades were determined 24 h *post mortem* on the chilled meat. After the *M. longissimus dorsi* was cut through between ribs 11 and 12, the lean colour was scored by experienced meat evaluators using the Standards for Beef Colour, Co-operative Extension Service, Iowa State University, Ames, Iowa, AS-515/reprinted / April 1982 (Appendix A).

The colour standards ranged from 1-8 with the following classification:

1- Bleached red ; 2- Very light cherry red; 3- Moderately light cherry red; 4- Cherry red; 5- Slightly dark red; 6- Moderately dark red; 7- Dark red; 8- Very dark red .

Those carcasses with lean colour scores of 7 and 8 were considered to be unacceptably dark.

4.3.4 *Post mortem* meat sampling techniques

Meat samples used for colour evaluation, pH determination, microbiological tests and shelf life studies were aseptically dissected from the 24 h *post mortem* chilled *M. longissimus dorsi* (7 samples of approximately 1-1,5 kg each, from each of Feedlot A and Feedlot B of colour shade 7 or 8 to represent dark cutting beef and 1 sample of approximately 1,5 kg from each feedlot of colour shade 4 or 5 to represent normal colour beef). The aseptical techniques involved using sterile knives, sterile plastic bags and sterile disposable gloves. A separate sterile knife and new disposable gloves were used for each carcass dissected. The knives were furthermore placed in alcohol flamed to minimise contamination from the surroundings. These samples were subdivided into 8 portions each and were vacuum packed immediately after dissection using *Cryovac* (constructed from a triple co-extrusion of EVA/PVDC/EVA) high density, low oxygen permeable material and kept chilled at a temperature of 2-4°C during the total investigation period. An additional 8 samples of approximately 1,5 - 2 kg each, from Feedlot B of colour shade 7 or 8 to represent dark cutting beef and 8 samples of approximately 1,5 –2 kg of colour shade 4 or 5 to represent normal colour beef, were dissected and vacuum packed for consumer sensory evaluation.

4.4 CONSUMER SENSORY EVALUATION

4.4.1 Consumer panel

A total of 64 beef eating consumers, including equal numbers of males and females were used to compare DFD steaks with normal steaks both in the raw and the fried state. Respondents consisted mainly of students and staff members from the University of Pretoria, which consumed beef on a regular basis (at least once a week). At the first session, raw steaks were evaluated by 16 consumers and the same respondents returned to evaluate the fried steaks. This cycle was repeated three more times.

4.4.2 Evaluation

Evaluation was done in a sensory laboratory fitted with 16 individual sensory booths, following standard sensory practices (Stone & Sidel, 1993). A total of eight evaluation sessions were held (four each for raw meat and fried meat respectively). Four sessions with a 15 minute break between sessions were held per day (08:30, 9:30; 10:30; 11:30) over two successive days. Samples (two) for each respondent were coded with randomly selected 3-digit code numbers. Judgements were recorded by marking a 9-point scale with word anchors (totally unacceptable and very acceptable) at the extreme ends for both raw steaks (Figure 4.6) and fried steaks (Figure 4.7). The panellists were also requested to indicate which one of the two steaks they preferred. The respondents remained anonymous.

4.4.3 Sample preparation

The fresh raw steak samples (*M. longissimus dorsi)* were cut with a sharp stainless steel knife, into slices approximately 50-60mm x 50-60mm, with a thickness of 15mm. All excessive external fat was removed. For the first evaluation one each DFD (colour 7 or 8) and one normal (colour 4 or 5) piece of raw meat was placed onto a rectangular plastic white tray (120mm x 75mm). The presentation order of the meat samples was randomised.

For the second evaluation, fried steaks were similarly presented to the respondents. For the frying process, four electrical frying pans were used, simultaneously. Four pieces of steak were fried per frying pan simultaneously. Twenty ml of fresh sunflower oil was put into the frying pan, pre-heated until the oil started to bubble, and the steaks then fried for 4 minutes on each side. The fried steaks were halved using a sharp stainless steel knife, and one DFD and one normal meat piece were placed on each tray. A white plastic tray was covered with aluminium foil and a loose piece of aluminium foil (150mm x 100mm) was used to cover the samples to be opened by the respondent.

ODOUR

	1				5				9
491									
208									

GENERAL APPEARANCE

	1				5				9
491									
208									

COLOUR

	1				5				9
491									
208									

TASTE/FLAVOUR

	1				5				9
491									
208									

TEXTURE

	1				5				9
491									
208									

JUICINESS

	1				5				9
491									
208									

OVERALL ACCEPTABILITY

	1				5				9
491									
208									

PREFERENCE: sample code:- - -

Fig. 4.7 Score sheets used by the respondents for fried steaks; hedonic rating scales (1= very unacceptable; 9= very acceptable)

4.4 MICROBIOLOGICAL ANALYSES AND SHELF LIFE STUDY

4.5.1 Sampling of meat

Sterile sampling techniques were used to sample the master meat samples (*M. longissimus dorsi*) of 6 DFD and 1 normal carcass from each of two feedlots, 24h *post mortem* (Table 4.3). These 14 master samples were subdivided into 8 portions each and vacuum packaged individually (12 DFD meat samples x 8 and 2 normal meat samples x 8). The shelf life study was conducted over a 7 week period.

The meat samples were stored in a commercial chill room and temperature was monitored and ranged between 2-4°C over that period. One meat sample from each carcass was removed every following week on the same day of the week at approximately the same time of the day and prepared for analyses. The samples were effectively marked on the external of the polyethylene bags on the day of sampling to ensure that no mixing of samples could occur during that period.

The sample sizes used for microbiological tests, were as follows: 50g samples were aseptically taken from each of the 6 DFD identified meat samples vacuum packaged previously and of the 1 normal meat sample of Feedlot A and this was repeated on meat samples from Feedlot B. The meat was also evaluated for odour on opening of the vacuum packaged meat. The remainder of the meat samples were evaluated for colour only after the samples for microbiological tests, were taken.

CHAPTER 4

Table 4.3: Meat samples used to determine microbiological quality and shelf-life of DFD beef

Feedlot A:	***pH**	****Colour score**
Brahman crossbreeds		
	6.40	8
DFD Beef	5.87	7
	6.10	8
	6.25	8
	6.29	8
	6.35	8
Normal beef	5.72	5
(CONTROL)		
Feedlot B:	***pH**	****Colour score**
Friesians		
	6.02	8
DFD Beef	5.93	7
	6.09	8
	6.20	8
	6.34	8
	6.25	8
Normal beef	5.74	5
(CONTROL)		

*pH measured 24 h *post mortem*
**-Colour score based on IOWA State University colour grading chart; 24 h *post mortem*

4.5.2 Microbiological analyses and shelf-life determination

Tests commenced on samples collected 24 h *post mortem.* The tests were repeated after 7 days, 14 days, 21 days, 28 days, 35 days,42 and 49 days. The pH of the 7 master meat samples of each feedlot was measured 24 h *post mortem* as discussed previously.

The colour of the meat (according to scores 1to 8) and odour of the meat were evaluated, by a panel of 3 food scientists, by means of sensory evaluation over the 7 week period to contribute towards the determination of the shelf life of the meat samples.

Equipment, media and reagents used for microbiological tests were according to FDA Analytical manual (AOAC, 1992a, 1992b). The micro-organisms tested for were:

Total aerobic plate count of viable organisms, following AOAC method 966.23 (AOAC, 1992a); presumptive coliform organism count and *Escherichia coli* I, following AOAC method, chapter 4 (AOAC, 1992b)*; Pseudomonas*, following British Pharmacopœia method A266 (British Pharmacopœia, 1988).

Each sample was plated out in triplicate and the mean organism count in each case was calculated.

4.6 STATISTICAL ANALYSIS

The results (variables and their interactions) were statistically analysed by the Statistical Analysis Systems (SAS Institute Inc., 1996) using PROC MEANS (procedure means), GLM (Generalized Linear Model) and PROC REG

(procedure regression) where applicable. Levels of significance were tested by means of the F-test or t-test and multiple range analysis were performed

using the Bonferroni test method. Analysis of variance techniques included one-way analysis of variance as well as multifactor analysis of variance (Type III sums of squares and least square means (LSM). Scheffe's test was used in testing feedlot variables. Kruskal-Wallis analysis of variance techniques were used in the analyses of the consumer study. Pearson's correlation coefficient was used for correlation studies.

Levels of significance are quoted at the $P < 0.01$, $P < 0.05$ and $P < 0.1$ levels.

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CHAPTER 5

RESULTS

5.1 THE INCIDENCE OF DARK-CUTTING BEEF AT AN ABATTOIR IN SOUTH AFRICA

5.1.1 An estimate of incidence of dark-cutting beef based on pH_1

The mean percentage dark-cutters for the 3-month period was reasonably high, i. e., 11.8% (Table 5.1).

Table 5.1: An estimate of the incidence of dark-cutting carcasses ($pH_1 \geq 5.8$) at a South African abattoir

Month	N	Number of dark-cutters based on pH_1	% Dark-cutters
December	8 325	997	11.9
January	6 843	825	12.0
February	7 010	813	11.6
TOTAL	**22 178**	**2 635**	**mean=11.8**

An estimate of the incidence of dark-cutting beef was determined by evaluating the carcasses from the four main feedlots (A, B, C and D) supplying the abattoir (Table 5.2). It was important to conduct the research on these cattle only, as carcasses from these feedlots are used in 90% of the cases to supply the retail stores with vacuum packed primal cuts. The retail stores generally return the meat if it is DFD beef or just appears to be dark in colour, resulting in major losses for both the abattoir and the customer. In addition, the clients loose trust in the abattoir, hence seeking alternative suppliers.

The carcasses, which were downgraded by the veterinarian because of bruising, were also recorded to establish if there was any correlation with the DFD condition. Feedlot A had the lowest incidence of dark-cutters and

Feedlot D the highest. Bruising was more evident on carcasses supplied by Feedlot A and B, while Feedlot C had the lowest percentage of bruised carcasses.

Table 5.2: An estimate of the incidence of dark-cutting cattle (pH_{24}) and bruised carcasses from four feedlots that supply a South African abattoir

FEEDLOT	N =	Number dark-cutting	% Dark-cutting	Number bruised	% Bruised
A	1 707	29	1.7	114	6.68
B	1 265	125	9.88	84	6.64
C	1 305	118	9.04	39	3.0
D	1 382	171	12.73	74	5.36
TOTAL:	**5 659**	**443**	**Mean=7.83**	**311**	**Mean=5.50**

N = total number of cattle tested during this research period

Tables 5.3 to 5.6 provide summaries of the cattle supplied on a daily basis during the monitoring period at Feedlots A to D.

The time that each load of cattle was off-loaded at the holding pens, and time of slaughter were also recorded; thus the holding periods before slaughter.

It appears as if long holding periods prior to slaughter had no effect on the incidence of dark-cutting e.g. at Feedlot A (Table 5.3) on day 2, cattle were held in the pens for 54 h prior to slaughter with an incidence of 1.4% dark-cutting, which was the same as for the shorter holding period of 23 h on day 15. Observations made at the other feedlots were similar, e.g. at Feedlot D (Table 5.6) on days 2, 9, and 13 the incidence of dark-cutters were 30%, 29% and 30% respectively for holding periods of 26 h, 23 h and 20 h. For equivalent holding periods for example on days 5, 10 and 4, the percentage dark cutters were much lower at 9%, 6% and 5% respectively.

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Table 5.3:The number and % dark-cutting and number and % bruised carcasses of Feedlot A and the respective holding hours at the holding pens prior to slaughter for each load of cattle received

Day	Gender	Total Received	Number Dark Cutting	% Dark Cutting	Number Bruised	% Bruised	Hours in Pens
1	STEERS	144	1	0.7	6	4	25
2	STEERS	144	2	1.4	16	11	54
3	STEERS	108	4	3.7	13	12	24
4	STEERS	144	2	1.4	7	5	23
5	STEERS	36	1	2.8	0	0	17
6	STEERS	188	3	1.6	7	4	21
7	STEERS	40	1	2.5	4	10	23
8	HEIFERS	40	0	0	4	10	23
9	STEERS	108	3	2.8	13	12	23
10	HEIFERS	160	2	1.25	0	0	48
11	STEERS	154	2	1.3	9	6	23
12	STEERS	154	2	1.3	15	10	22
13	STEERS	107	4	3.7	16	15	25
14	STEERS	36	0	0	0	0	22
15	STEERS	144	2	1.4	4	3	23
Total		**1 707**	**29**	**1.7**	**114**	**6.7**	

Table 5.4: The number and % dark-cutting and number and % bruised carcasses of Feedlot B and the respective holding hours at the holding pens prior to slaughter for each load of cattle received

Day	Gender	Total Received	Number Dark Cutting	% Dark Cutting	Number Bruised	% Bruised	Hours in Pens
1	STEERS	55	11	20	6	11	23
2	STEERS	55	5	9	3	6	37
3	STEERS	110	23	21	11	10	22
4	HEIFERS	55	0	0	2	4	21
5	STEERS	110	21	19	11	10	21
6	STEERS	55	8	15	7	13	18
7	STEERS	55	5	27	1	2	21
8	HEIFERS	110	0	0	7	6	21
9	HEIFERS	55	0	0	6	11	21
10	STEERS	55	12	22	0	0	22
11	HEIFERS	55	0	0	0	0	23
12	STEERS	55	6	11	1	2	20
13	HEIFERS	55	0	0	5	9	21
14	HEIFERS	55	2	4	0	0	22
15	HEIFERS	55	4	7	3	6	21
16	HEIFERS	55	0	0	4	7	20
17	STEERS	55	4	7	6	11	21
18	STEERS	110	17	16	5	5	20
19	STEERS	55	9	16	6	11	22
TOTAL		**1265**	**125**	**10**	**84**	**7**	

Table 5.5: The number and % dark-cutting and number and % bruised carcasses of Feedlot C and the respective holding hours at the holding pens prior to slaughter for each load of cattle received

Day	Gender	Number received	Number dark-cutting	%Dark-cutting	Number bruised	% Bruised	Hours in pens
1	STEERS	106	14	13.2	2	1.9	16
2	STEERS	110	17	15.5	3	2.7	17
3	HEIFERS	54	0	0	0	0	15
4	STEERS	54	6	11.1	1	1.8	15
5	STEERS	110	11	10	2	1.8	18
6	STEERS	110	14	12.7	4	3.6	20
7	HEIFERS	108	2	1.8	2	1.8	19
8	STEERS	54	5	9.3	5	9.3	18
9	STEERS	108	12	11.1	3	2.8	21
10	HEIFERS	55	0	0	0	0	20
11	STEERS	108	8	7.4	5	4.6	22
12	STEERS	110	7	6.4	3	2.7	15
13	HEIFERS	110	1	0.9	2	1.8	18
14	STEERS	108	21	19.4	7	6.5	16
TOTAL		**1305**	**118**	**9.0**	**39**	**3**	

Table 5.6: The number and % dark-cutting and number and % bruised carcasses of Feedlot D and the respective holding hours at the holding pens prior to slaughter for each load of cattle received

Day	Gender	Number Received	Number Dark Cutting	% Dark Cutting	Number Bruised	% Bruised	Hours in Pens
1	STEERS	66	5	8	2	3	23
2	STEERS	108	32	30	2	2	26
3	STEERS	85	7	8	1	1	19
4	STEERS	85	4	5	1	1	20
5	STEERS	44	4	9	4	9	24
6	BULLS	154	33	21	18	12	24
7	BULLS	102	13	13	7	7	19
8	BULLS	42	7	17	4	10	19
9	STEERS	89	26	29	7	8	23
10	STEERS	191	11	6	2	1	23
11	HEIFERS	88	7	8	0	0	21
12	HEIFERS	44	0	0	5	11	21
13	STEERS	44	13	30	10	23	20
14	STEERS	68	5	7	5	7	23
15	HEIFERS	86	0	0	1	1	23
16	STEERS	86	4	5	5	6	22
TOTAL		**1382**	**171**	**12**	**74**	**5.4**	

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There is no correlation evident, between the incidence of dark-cutters and the incidence of bruised carcasses either, for example at Feedlot B (Table 5.4) with an incidence of 16% dark-cutters on day 18, the bruised carcasses were 5% but on the next day also with 16% dark-cutting, the incidence of bruising was 11%. At Feedlot A with a comparatively much lower incidence of dark-cutting (mean 1.7%), the incidence of bruising (mean 6.7%) was higher than at Feedlot C (mean 3%) (Table 5.5), although the incidence of dark-cutting was higher (mean 9%) at this feedlot.

5.2 THE EFFECT OF GENDER AND FEEDLOT ON THE INCIDENCE OF DFD

The effect of gender on the incidence of dark-cutters was evaluated and the results are summarised in Table 5.7. The incidence of DFD was significantly higher ($P < 0.001$) in bulls and steers compared to heifers.

Table 5.7: Effect of gender on the incidence of dark-cutting carcasses (Scheffe's test)

GENDER	Least square mean (LSM)[1]	± Standard Error	P
Heifers	1.41^{a}	1.42	0.0001*
Steers	10.52^{b}	0.85	
Bulls	11.05^{b}	1.62	

Comparisons significant at the 0.001 level are indicated by '*'; * ($P< 0.0001$)
$\alpha = 0.05$; Confidence = 0.95; df = 54; MSE = 30.65149; Critical Value of F = 3.16825
^{1ab} - LSM dark-cutting incidence (%) with different superscripts differed significantly

The effect of the feedlot (feedlots A, B, C, D) on the percentage dark-cutting carcasses is summarised in Table 5.8. The incidence of DFD was significantly higher ($P < 0.001$) at Feedlots B, C and D compared to Feedlot A.

Table 5.8: Effect of feedlot (A, B, C, D) on the incidence of dark-cutting carcasses (Scheffe's comparisons)

FEEDLOT	Least square mean (LSM)[1]	± Standard Error	P
A	1.48[a]	2.54	0.0001*
B	8.18[b]	1.82	
C	6.11[b]	2.33	
D	11.05[b]	2.72	

Comparisons significant at the 0.001 level are indicated by '*'; (P< 0.0001); α = 0.05; Confidence = 0.95; df = 54; MSE = 30.65149; Critical Value of F = 2.77576
[1] [ab] - LSM dark-cutting incidence (%) with different superscripts differed significantly

5.3 THE EFFECTS OF BREED, FASTING AND FEEDLOT ON DFD BEEF

The average pH of the breeds (Brahman and Non-Brahman: European breeds, i. e. Angus or Hereford) did not differ significantly (Table 5.9).

The rank average pH-values among the four feedlots did not differ significantly and no interaction effect of breed and feedlot was found. Animals fasted for a period of 40 h prior to slaughter had a significantly higher ultimate pH compared to those fasted for a 60 h period (Table 5.10).

Table 5.9:Analysis of variance for breed, fasting, feedlot with dependent variable, rank average pH

SOURCE	DF	MEAN SQUARE	F Value	P
BREED	1	21.51	0.03	0.871 NS
FEEDLOT	1	31.65	0.04	0.844 NS
BREED*FEEDLOT	3	400.52	0.05	0.483 NS
FASTING	1	35 623.56	44.13	0.0001**
BREED*FASTING	3	1 360.55	1.69	0.197 NS
FEEDLOT*FASTING	3	4 017.55	4.98	0.0280 *

* (P< 0.05), ** (P< 0.001), NS= no significance (P> 0.05)

The interaction of fasting and breed did not influence the pH. However, the effect of fasting within feedlot was significant. Animals of Feedlots A and B, fasted for 40 h had significantly higher ultimate pH-values than cattle from Feedlot B fasted for 60. It appears that longer fasting is related to lower ultimate pH. Cattle from Feedlot A fasted for 60 h had the lowest pH-values (Table 5.11).

Table 5.10: Comparison (Bonferroni-test) of the effect of fasting on the pH of carcasses

FASTING PERIOD	LSM AVE pH	± SE	P	LSM RANK pH	± SE	P
40hrs	5.88	0.04	0.0001*	89.95	4.03	0.0001*
60hrs	5.63	0.04		52.62	3.92	

* (P< 0.001)

Table 5.11: Comparison (Bonferroni-test) of the effect of feedlot on the pH of carcasses

FEEDLOT	FASTING	LSM AVE pH	± SE	LSM RANK pH[1]	±SE	COMPARISON	P
A	**40 h**	5.98	0.05	95.67[a]	5.80	A(40h):A(60h)	0.0001**
A	**60 h**	5.59	0.05	45.79[c]	5.80	A(40h):B(40h)	0.159NS
						A(40h):B(60h)	0.0001**
B	**40 h**	5.79	0.05	84.24[a]	5.58	A(60h):B(40h)	0.0001**
B	**60 h**	5.66	0.05	59.44[b]	5.28	A(60h):B(60h)	0.085NS
						B(40h):B(60h)	0.002 *

* (P< 0.01), ** (P< 0.001), NS= no significance (P> 0.05)

[1abc] - LSM rank pH-values with different superscripts differed significantly

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Breed had a significant effect on the intensity of the colour of the meat (Table 5.12). The colour of the Brahman meat was significantly darker ($P < 0.001$) compared to that from the European breeds (Angus and Hereford) (Table 5.13). Feedlot and fasting period as well as the interaction effects did not significantly affect the colour of the meat.

Table 5.12: Analysis of variance for breed, fasting and feedlot with dependent variable, rank average colour

SOURCE	DF	MEAN SQUARE	F Value	P
BREED	1	28 963.97	30.79	0.0001*
FEEDLOT	1	1 577.84	1.68	0.198 NS
BREED*FEEDLOT	3	357.62	0.38	0.539 NS
FASTING	1	962.12	1.02	0.314 NS
BREED*FASTING	3	1 162.05	1.24	0.269 NS
FEEDLOT*FASTING	3	1 648.09	1.75	0.189 NS

* (P< 0.001), NS= no significance (P> 0.05)

Table 5.13: Effect of breed on the colour of the meat (least square means and standard error) (Bonferroni-comparison)

BREED	LSM AVE COLOUR	± SE	P	LSM RANK COLOUR	± SE	P
Brahman	6.12	0.15	0.0001*	84.76[a]	4.27	0.0001*
Non-Brahman (European breeds	5.03	0.15		51.10[b]	4.31	

* (P< 0.001)
[1] [ab] - LSM rank colour-values with different superscripts differed significantly (P< 0.0001)

5.4 IDENTIFICATION OF POTENTIAL STRESS FACTORS ENCOUNTERED BY CATTLE PRE- AND POST TRANSPORTING TO THE ABATTOIR AND PRIOR TO SLAUGHTER BY ANALYSING BLOOD CONSTITUENTS AND MUSCLE GLYCOGEN

Specific blood constituents and muscle glycogen were tested at the following three stages: stage 1: at the feedlot, prior to loading and transportation; stage 2: after transportation and off-loaded at holding pens at the abattoir; stage 3: after slaughter. Phase 1 is the comparison between stage 1 and stage 2; phase 2 is the comparison between stage 1 and stage 3.

As expected, the glycogen concentration of the muscle was significantly higher for all three feedlots prior to transportation than after (Table 5.14). The glycogen content also decreased further after the cattle were held in the holding pens at the abattoir and slaughtered. The glycogen concentrations decreased linearly for cattle from all three feedlots over the three successive stages as illustrated in Fig. 5.1.

Table 5.14: The mean muscle glycogen content (μ mol g^{-1}) (± standard deviation) of cattle of three feedlots measured at three stages

FEEDLOT	STAGE 1:		STAGE 2:		P	STAGE 3:		P
	MEAN	± SD	MEAN	± SD	(phase 1)	MEAN	±SD	(phase2)
A	80.5 [a]	4.07	74.6 [b]	4.7	0.045*	68.6 [b]	4.7	0.013*
B	79.5 [a]	4.65	71.3 [b]	4.93	0.018*	61.3 [b]	6.47	0.0002**
C	81.4 [a]	4.24	65.5 [b]	4.73	0.001*	60.3 [b]	5.55	0.0002**

[1] [ab] - Mean muscle glycogen values in a row differed significantly with different superscripts
*P ≤ 0.05; ** P ≤ 0.01

It appears that the cattle from Feedlot A were able to sustain the traumatic events from the feedlot to after slaughter, slightly better than the cattle from Feedlots B and C. The mean glycogen content of Feedlot A decreased with 11.9μ mol g^{-1} during phase 2 whereas the glycogen content of Feedlot B and C, decreased with 18.2 and 21.1μ mol g^{-1} respectively during this phase.

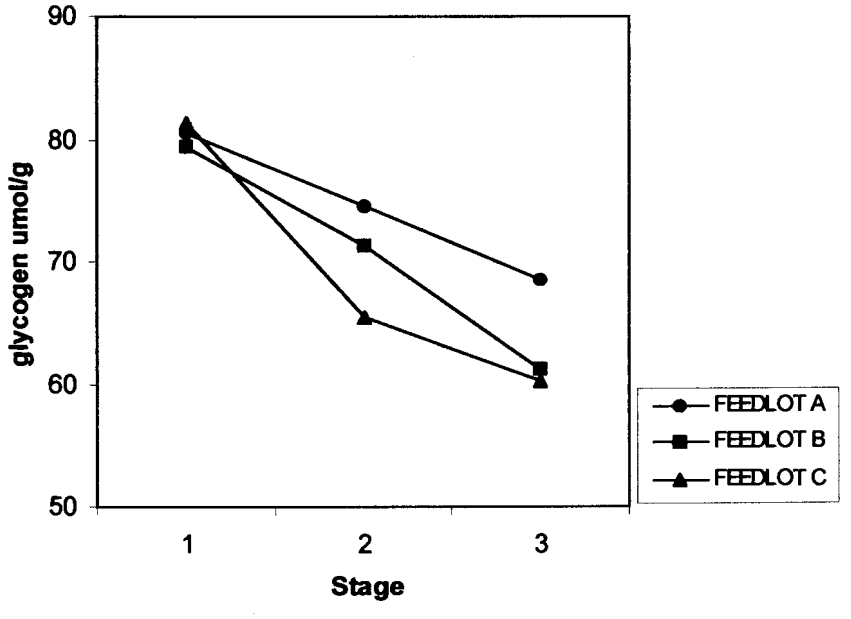

Fig. 5.1: Mean glycogen concentrations (μ mol/g) measured at three successive stages in feedlot cattle

Table 5.15: Repeated Measures Analysis of Variance Univariate Tests of Hypotheses for feedlots' effect for hematocrit over different phases

SOURCE	DF	MEAN SQUARE	F Value	P
Phase	1	164.83	17.22	0.0001*
Phase*Feedlot	5	240.38	25.12	0.0001*

General Linear Models Procedure; * (P< 0.001)

Table 5.16: Analysis of variance of contrast variable, hematocrit, of cattle of three respective feedlots

FEEDLOT	STAGE 1: MEAN[1] ± SD	STAGE 2: MEAN ±SD	P (phase 1)	STAGE 3: MEAN ± SD	P (phase 2)
A	30.76[a] 2.47	31.15[a] 2.22	0.265 NS	32.05[b] 3.35	0.050*
B	30.73[a] 2.47	35.63[b] 3.65	0.0001***	25.97[b] 7.31	0.010**
C	34.68[a] 3.92	30.41[b] 3.14	0.0494*	28.55[b] 3.85	0.0010***

General Linear Models Procedure

[ab] - Mean hematocrit-values in a row with different superscripts differed significantly

* (P≤ 0.05), ** (P≤ 0.01), *** (P≤ 0.001), NS= no significance (P> 0.05)

With the repeated measures ANOVA, the effect of phase was significant and the interaction of feedlot x phase also had a significant effect on the hematocrit (Table 5.15). A significant effect was found between the levels of hematocrit measured at phase 1 for Feedlots B and C and for phase 2 for Feedlots A, B and C respectively (Table 5.16). The results suggest that the hematocrit values of cattle of Feedlots B and C, decreased after they were held at the pens at the abattoir and slaughtered.

The hematocrit values of cattle from Feedlot C decreased linearly during the experiment. For Feedlot A, the hematocrit effect was significant only at phase

2 indicating that no change in the hematocrit took place during transportation and off-loading at the abattoir.

Table 5.17: Repeated Measures Analysis of Variance Univariate Tests of Hypotheses for phase within feedlots' effect for glucose

SOURCE	DF	MEAN SQUARE	F Value	P
Phase	1	35.0192	19.83	0.0001*
Phase*Feedlot	5	1.9800	1.12	0.3418 NS

* (P< 0.001), NS= no significance (P> 0.05)

The interaction between feedlot and phase was not significant for glucose. It follows that there was no feedlot-effect on the concentration of glucose at different stages (Table 5.17).

The concentrations of glucose measured at the feedlots were significantly lower compared to that after slaughter (Table 5.18). The glucose concentrations measured at the feedlots compared to after transportation and off-loading did not differ significantly, but were higher when measured after slaughter.

Table 5.18: Analysis of variance of contrast variable, glucose, of cattle from three respective feedlots

FEEDLOT	STAGE 1: MEAN[1] ± SD		STAGE 2: MEAN ±SD		P (phase 1)	STAGE 3: MEAN ± SD		P (phase 2)
A	4.49[a]	0.47	4.71	0.43	0.117 [NS]	5.60[b]	1.03	0.0003*
B	4.85[a]	0.47	4.90	0.53	0.568 [NS]	6.86[b]	2.50	0.0017*
C	4.72[a]	1.23	4.87	0.43	0.225 [NS]	5.86 [b]	2.40	0.0001**

[1 ab] - Mean glucose values in a row with different superscripts differed significantly

* (P≤ 0.01), ** (P≤ 0.001), NS= no significance (P≥ 0.05)

The interaction between phase and feedlot for the concentration of creatine kinase was significant. This means that feedlot influenced the concentration of creatine kinase at different stages (Table 5.19).

Table 5.19: Repeated Measures Analysis of Variance Univariate Tests of Hypotheses for feedlots' effect for creatine kinase over different stages

SOURCE	DF	MEAN SQUARE	F Value	P
Phase	1	689 996.06	3.20	NS
Phase*Feedlot	5	1 489 380.64	6.90	0.0089*

* (P≤ 0.01), NS= no significance (P≥ 0.05)

Table 5.20: Analysis of variance of contrast variable creatine kinase of cattle of three respective feedlots

FEED-LOT	STAGE 1: MEAN[1]	± SD	STAGE 2: MEAN	±SD	P (phase 1)	STAGE 3: MEAN	± SD	P (phase 2)
A	62.55[a]	22.26	111.58[b]	68.88	0.006 **	226.06[b]	153.83	0.0002**
B	734.37[a]	1064.94	180.35[b]	162.98	0.0439*	144.49[b]	245.77	0.0335*
C	64.92[a]	98.86	246.90[b]	238.27	0.0241*	135.24[b]	106.45	0.006 **

[1] [ab] - Mean creatine kinase-values in a row with different superscripts differed significantly

*(P≤ 0.05), ** (P≤ 0.01)

Significant differences in the concentrations of creatine kinase were observed between the three different stages pre-slaughter (Table 5.20) for each of the three feedlots, A, B and C. Feedlot A had a linear increase in the concentration of creatine kinase from stage 1 to stage 3. At Feedlot C the creatine kinase concentrations increased after transportation and off-loading followed by a decrease after slaughter. At Feedlot B the creatine kinase

concentrations decreased after transportation and off-loading followed by a decrease after slaughter.

Significant interaction was also found between the phase and the feedlot for free fatty acids. This means that feedlot affects the concentrations of free fatty acid as measured between different phases (or at different times) pre-slaughter (Table 5.21).

Table 5.21: Repeated Measures Analysis of Variance Univariate Tests of Hypotheses for feedlots' effect for free fatty acid over different stages

SOURCE	DF	MEAN SQUARE	F Value	P
Phase	2	0.8412	29.36	0.0001*
Phase*Feedlot	4	0.4082	14.25	0.0001*

* ($P \leq 0.001$)

The concentrations of free fatty acids were significantly higher at phase 2 in cattle from Feedlot B and for both phases at Feedlot C (Table 5.22). The free fatty acid concentrations measured at Feedlot A compared to after transportation and off-loading (phase 1), and after slaughter (phase 2), did not differ significantly, but were higher in both instances, when measured.

Table 5.22: Analysis of variance of contrast variable, free fatty acid, of cattle of three respective feedlots

FEEDLOT	STAGE 1: MEAN[1]	± SD	STAGE 2: MEAN	±SD	P (phase 1)	STAGE 3: MEAN	± SD	P (phase 2)
A	0.26	0.10	0.34	0.16	0.128 NS	O.36	0.29	0.126 NS
B	0.27[a]	0.19	0.32	0.14	0.197 NS	0.53[b]	0.14	0.0001*
C	0.18[a]	0.07	0.69[b]	0.28	0.0001*	0.46[b]	0.16	0.0001*

[1] [ab] - Mean Free fatty acid-values in a row with different superscripts differed significantly

* ($P \leq 0.001$), NS= no significance ($P \geq 0.05$)

No significant correlations were found between the colour or pH_{24} (Table 5.23) as measured on *M.longissimus dorsi* and the post slaughter (phase 2) levels of the various blood parameters (hematocrit, glucose, creatine kinase and free fatty acids) at any of the three feedlots.

Table 5.23: Correlation of colour (scale 1-8) and pH(24) of *M. longissimus dorsi* obtained from cattle from three different feedlots with respective blood parameters (glucose, creatine kinase, hematocrit, free fatty acid) *post* slaughter

Blood Parameter	Feedlot	N	COLOUR Pearson's correlation coefficient (R)	COLOUR P	pH(24) Pearson's correlation coefficient (R)	pH(24) P
GLUCOSE	A	19	-0.067	0.784 NS	0.115	0.640 NS
	B	19	-0.249	0.305 NS	-0.231	0.341 NS
	C	19	-0.013	0.959 NS	0.235	0.333 NS
CREATINE KINASE	A	19	0.251	0.300 NS	0.098	0.691 NS
	B	19	-0.078	0.752 NS	-0.008	0.973 NS
	C	19	-0.246	0.136 NS	-0.118	0.479 NS
HEMATOCRIT	A	19	0.318	0.185 NS	0.149	0.542 NS
	B	17	-0.003	0.992 NS	0.276	0.284 NS
	C	19	-0.222	0.194 NS	-0.032	0.851 NS
FREE FATTY ACID	A	19	0.256	0.290 NS	0.258	0.286 NS
	B	19	-0.247	0.309 NS	-0.331	0.166 NS
	C	18	-0.151	0.549 NS	-0.151	0.549 NS

Correlation analysis using Pearson's correlation coefficients/ Prob > [R] under Ho : Rho = 0/ Number of observations; NS= no significance (P>0.05)

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The correlation of muscle $pH_{(24)}$ (*M.longissimus dorsi)* with the colour score (Table 5.24) obtained from Feedlots A, B, and C was significant. The best correlation found at Feedlot C was R= 0.9 and then at Feedlot A (R= 0.7) followed by Feedlot B (R= 0.6). The incidences of dark-cutting carcasses for the three respective feedlots (A, B, and C) are summarised in Table 5.25. Carcasses with pH-values of $\geq$ 5.8, but with colour scores below 7, were not classified as DFD. If the incidence of dark-cutters was based on only the pH-value, it would have been even higher at 5 (26%), 13 (65%) and 7 (35%) for Feedlots A, B and C respectively.

Table 5.24: Mean, standard deviation and Pearson's correlation coefficient (R) of $pH_{(24)}$ of *M. longissimus dorsi* obtained from cattle from three different feedlots with respective colour gradings (scale 1-8)

Feedlot	N	MEAN pH_{24}	±Standard deviation	Mean Colour	±Standard deviation	R
A	19	5.75	0.20	5.21	1.23	0.4*
B	20	5.84	0.10	6.45	0.51	0.6*
C	20	5.74	0.11	6.20	1.06	0.9**

(P < 0.05); ** (P <0.001)

Table 5.25: The DFD incidence for Feedlots A, B and C

FEEDLOT	N	Number of dark-cutters	% Dark-cutters
A	19	3	16
B	20	7	35
C	20	7	35

5.5 CONSUMER SENSORY EVALUATION

5.5.1 Consumer evaluation of raw DFD beef steaks compared to raw, normal beef steaks

The characteristics of raw beef, that consumers are interested in, are those that they can assess when judging the beef on display in the retail store or the carcasses and large meat cuts at the meat wholesaler. These would, to a large extend, be the general appearance and colour. The mean and standard deviation for these sensory attributes were determined without differentiating between male and female consumers (Table 5.26). Table 5.27 indicates the least square means (LSM) and standard error (SE) and ANOVA of the sensory attributes measured.

Table 5.26: Means (± standard deviation) of sensory attributes hedonically rated by panel (N=64 consumers) of DFD raw beef steaks and normal raw beef steaks

	DFD RAW BEEF STEAK (N = 64)		NORMAL RAW BEEF STEAK (N =64)	
ATTRIBUTE	**Mean**	**± SD**	**Mean**	**± SD**
General Appearance	4.97	0.92	6.00	1.63
Colour	4.81	1.62	6.19	1.71
Overall Acceptability	5.03	1.51	6.19	1.76

[1] 1= Very unacceptable; 9= Very acceptable

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Table 5.27: Comparison by ANOVA[1] of DFD raw steaks and normal raw steak (LSM and ± SE)

SENSORY ATTRIBUTE	DFD RAW BEEF LSM[2] and ± SE		NORMAL RAW BEEF LSM and ± SE		P
General Appearance	55.09[a]	4.35	74.75[b]	4.32	0.0017*
Colour	51.59[a]	4.30	78.21[b]	4.27	0.0001**
Overall Acceptability	54.22[a]	4.41	75.62[b]	4.38	0.0008*

[1] Kruskal-Wallis test statistic for multiple comparison using X^2 distribution with 1 degree of freedom. The null hypothesis was rejected if Zstat was > the critical value ZC, where 1 phi (ZC) = α/ (K(K-1)). Phi is the cumulative standard normal distribution function, α is the desired overall significance level, and K is the number of groups compared.
[2] [ab] Respective LSM values in a row differed significantly * ($P \leq 0.01$), ** ($P \leq 0.001$)

It was important to know the preference of the consumer when he/she had a choice between normal beef steaks and DFD beef steaks (Table 5.28). Significantly more consumers preferred the normal raw, steak than the raw, DFD steak. A comparison between male and female consumers was also drawn in this instance, which showed no significant difference in preference of male and female consumers.

Table 5.28: Ratio of preference by consumer panel (N= 64) of raw DFD, beef steaks versus raw normal beef steaks

GENDER	RATIO OF PREFERENCE Raw DFD steak	RATIO OF PREFERENCE Raw normal steak	P
Males	34.38 %[a]	65.63%[b]	0.0166*
Females	32.31% [a]	67.69% [b]	0.0170*
Total	33.33% [a]	66.67% [b]	0.0168 *

Ratio of preference with different superscripts in a row differed significantly

*P< 0.050

By using the binomial distribution test, significantly more panellists preferred the normal raw steaks (P= 0.0168) which could be attributed to the pleasant red cherry colour of these meat samples compared to the dark red (almost black) colour of the DFD meat samples.

Other comments, which were also made by the panellists, were that the DFD meat samples appeared to be old, not fresh, "off", deteriorated and 3 panellists described it to be matured.

5.5.2 Consumer evaluation of fried, DFD beef steaks compared to fried, normal beef steaks

According to Dumont (1981) the consumer often has two faces or is a ' double person'. The first individual is judging quality on display and on cooking, the other is tasting meat on the plate, as a beef-eater. It was, therefore, important to obtain the consumer's opinions on the steaks on consumption. For the second part of this experiment, normal beef steak and DFD beef steak were fried and served immediately to the same consumer panel which evaluated the raw steak.

The ratings of the male consumers (n= 37) and female consumers (n= 27) were also grouped separately and Kruskall-Wallis analysis of variance was conducted on these four groups (1= male; DFD steak; 2 = male; normal steak; 3 = female; DFD steak; 4 = female; normal steak) (Table 5.29). Although there was no significant difference in preference of male consumers, female consumers appeared to prefer fried normal steaks. There was also no significant difference between fried DFD steak and fried normal steak for each sensory attribute evaluated.

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Table 5.29: Mean and ± standard deviation of sensory attributes hedonically ranked[1] by panel (N=64 consumers) of DFD fried beef steaks and normal fried beef steaks

SENSORY ATTRIBUTE	GENDER GROUP		MEAN[1]	± STANDARD DEVIATION	P
Odour	M M F F	1 2 3 4	6.06 5.73 5.83 6.15	0.25 0.24 0.27 0.28	0.68
General Appearance	M M F F	1 2 3 4	6.26 5.57 5.76 5.82	0.25 0.24 0.27 0.28	0.33
Colour	M M F F	1 2 3 4	5.86 5.92 6.00 5.74	0.24 0.23 0.26 0.27	0.96
Taste	M M F F	1 2 3 4	5.97 5.43 5.62 6.00	0.26 0.25 0.29 0.30	0.41
Texture	M M F F	1 2 3 4	5.43 5.65 5.14 5.85	0.29 0.29 0.32 0.33	0.52
Juiciness	M M F F	1 2 3 4	5.80 5.43 6.21 5.74	0.29 0.28 0.31 0.33	0.38
Acceptability	M M F F	1 2 3 4	5.94 5.30 5.79 6.11	0.26 0.25 0.29 0.30	0.26

Kruskal-Wallis test statistic for multiple comparison using X^2 distribution with 3 degrees of freedom. The null hypothesis is rejected if Zstat is > the critical value ZC, where 1 phi (ZC) = α/ (K(K-1)). Phi is the cumulative standard normal distribution function, α is the desired overall significance level, and K is the number of groups compared.
NS = P > 0.05 (No significant difference).
Scale: 1= totally unacceptable; 9= very acceptable

It was important to know the preference of the consumer between fried DFD beef steaks and normal beef steaks (Table 5.30). Overall, it seemed as if there was no significant preference for either the normal fried steak or the fried DFD steak. A comparison between male and female consumers was also drawn in this instance, which showed that significantly more female consumers preferred the normal fried steak, than the DFD fried steak. There was, however, no significant difference in preference of male consumers.

Table 5.30: Ratio of preference by consumer panel (N= 64) of fried DFD, beef steaks versus fried normal beef steaks

GENDER	Fried DFD steak	Fried normal steak	P
Males	54.05 %	45.95 %	0.103 ^{NS}
Females	33.33 %	66.67 %	0.0168*
Total	45.31 %	54.69 %	0.103 ^{NS}

*P< 0.050; NS = P > 0.050

5.6 MICROBIOLOGICAL QUALITY AND SHELF-LIFE OF DFD BEEF

5.6.1 Microbiological quality

Meat samples from two different feedlots, as well as biologically different cattle, were tested over a seven-week period. Feedlot A supplied predominantly Brahman crosses and Feedlot B supplied Friesian cattle during this period.

Six DFD (pH$\geq$ 5.8 and colour score 7 or 8) meat samples were tested in triplicate and for the control, one normal (pH<5.8) meat sample was also tested in triplicate.

CHAPTER 5

The comparison by ANOVA of the total aerobic count (log_{10} c.f.u /g) of all the DFD samples, for both feedlots (N = 14) compared to the total aerobic count (log_{10} c.f.u /g) of the normal meat samples (N = 2), are shown in Table 5.31 and Fig. 5.2. A significantly higher total aerobic count was found in the DFD samples compared to the normal meat samples. The total aerobic count changed significantly, however differently, over seven weeks for the DFD and normal meat samples.

Table 5.31: Total aerobic count (log_{10} c.f.u /g) of DFD (N = 14) and normal meat samples (N = 2) over a seven-week period (ANOVA - repeated measures design)

SOURCE	df	SUM OF SQUARES	MEAN SQUARES	F	P
Samples	1	49.15	3.20	15.35	0.002*
Weeks	6	3.90	0.33	11.97	0.0000**
Samples*weeks	6	1.73	0.33	5.30	0.0001**

$P < 0.05$*; $P \leq 0.0001$**

The total aerobic plate counts of the DFD meat samples compared to the normal meat samples were initially very similar. The total aerobic plate counts of the DFD samples increased rapidly and reached a level of $10^{9.5}$ c.f.u. /g and 10^{9} c.f.u./g at week five for Feedlots A and B respectively, and then started to decline. The bacterial growth of the normal meat samples, however, started to decline from week four after maximum populations of $10^{5.5}$ c.f.u./g and $10^{6.5}$ c.f.u./g were reached for Feedlot A and B respectively.

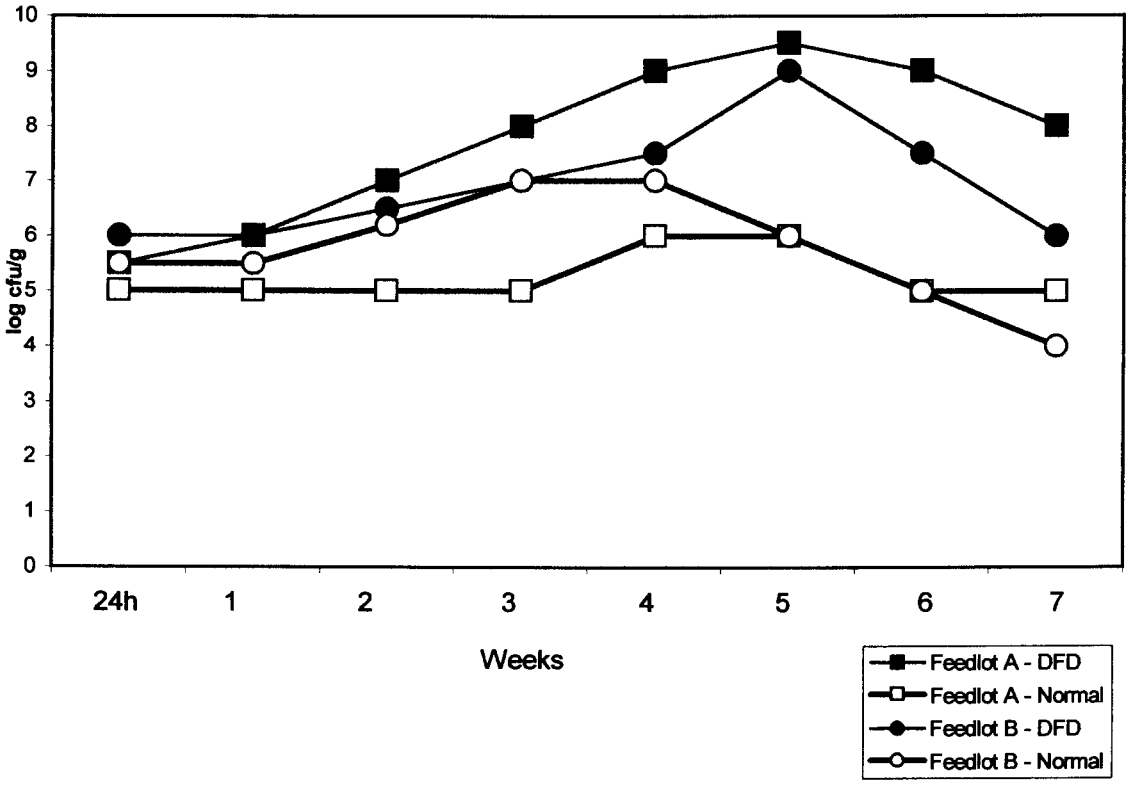

Fig. 5.2 Growth of total aerobic count on vacuum packaged DFD beef and normal beef of two feedlots over a 7 week period at 4°C

CHAPTER 5

The comparison by ANOVA of the *Pseudomonas* population (log_{10} c.f.u /g) of all the DFD samples, for both feedlots (N = 14) compared to the *Pseudomonas* population (log_{10} c.f.u /g) of the normal meat samples (N = 2), are shown in Table 5.32 and Fig. 5.3. A significantly higher *Pseudomonas* population was found in the DFD samples compared to the normal meat samples. In the case of the normal meat samples the population decreased after 4 weeks. The interaction effect of samples x weeks was significant.

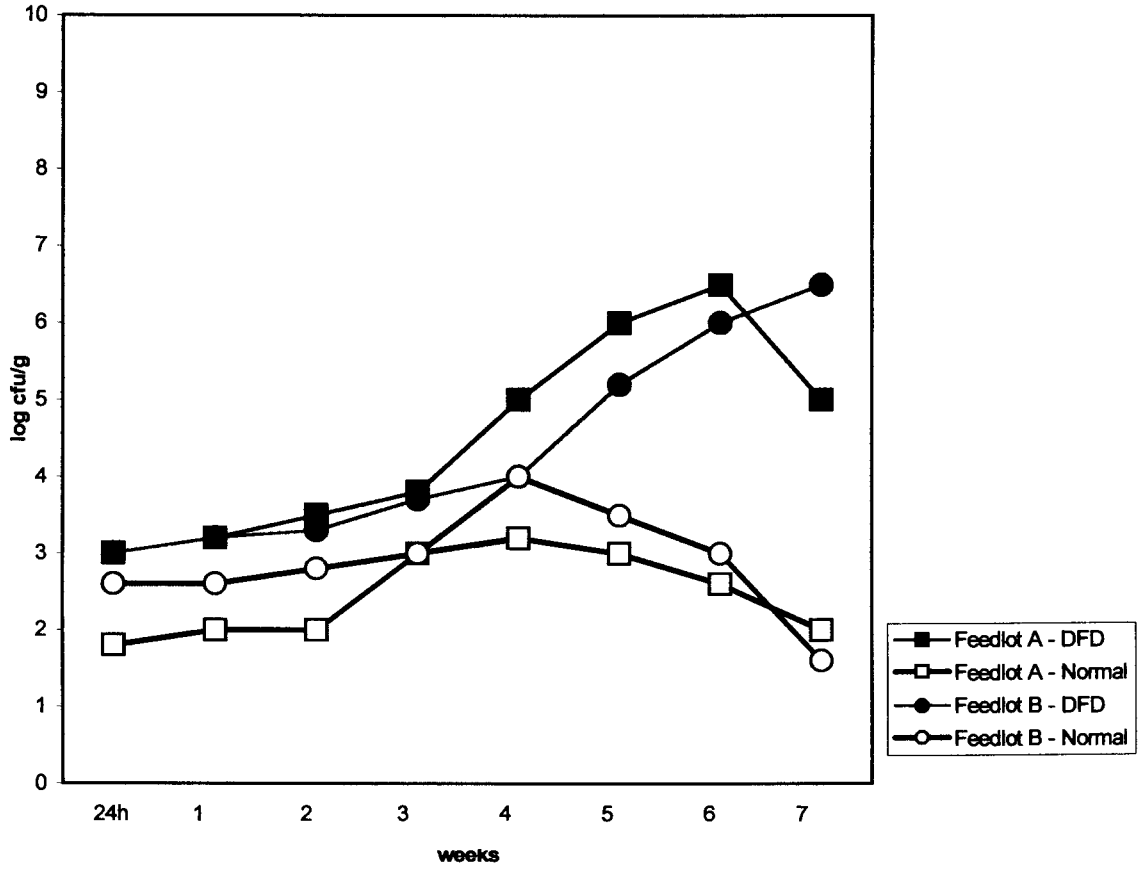

Fig. 5.3 Growth of *Pseudomonas* on vacuum packaged DFD beef and normal beef of two feedlots over a 7 week period at 4°C

Table 5.32: *Pseudomonas* population (log_{10} c.f.u /g) for DFD (N = 14) and normal meat samples (N = 2) over a seven-week period (ANOVA - repeated measures design)

SOURCE	df	SUM OF SQUARES	MEAN SQUARES	F	P
Samples	1	43.21	0.86	50.21	0.00001*
Weeks	6	3.42	0.33	12.17	0.0000*
Sample*weeks	6	3.14	0.33	11.17	0.0000*

P < 0.0001*

The populations of both DFD and normal meat samples (Feedlot A), as well as DFD and normal meat samples (Feedlot B), increased gradually to week four, where after the populations of the normal beef started to decline rapidly. The populations of the DFD samples, however increased even further up to week six and week seven, for Feedlots A and B, respectively.

The comparison by ANOVA of coliforms (log_{10} c.f.u /g) of all the DFD samples, for both feedlots (N = 14) compared to the normal meat samples (N = 2) are shown in Table 5.33 and Fig. 5.4. A significantly higher coliform count was found in the DFD samples compared to the normal meat samples. Coliform counts for both DFD and normal samples differed significantly over the seven week period.There was however, no significant interaction between samples and time.

Table 5.33: Coliform population (log_{10} c.f.u /g) of DFD (N = 14) and normal meat samples (N = 2) over a seven-week period (ANOVA - repeated measures design)

SOURCE	df	SUM OF SQUARES	MEAN SQUARES	F	P
Samples	1	5.72	0.68	8.41	0.013*
Weeks	6	3.90	0.33	11.97	<0.0001**
Samples*weeks	6	1.73	0.33	5.30	0.99^{NS}

P < 0.05*; P ≤ 0.0001** ; NS = No significance

The reasonably low counts of coliforms from Feedlot A and B, for both DFD and normal meat samples, increased gradually until week three, when their growth was retarded and counts started to decrease at week four for all the samples. The maximum count reached for a DFD samples was $10^{4.5}$ c.f.u./g (Feedlot B) and the maximum count reached for a normal beef sample (Feedlot A) was $10^{3.5}$ c.f.u. /g.

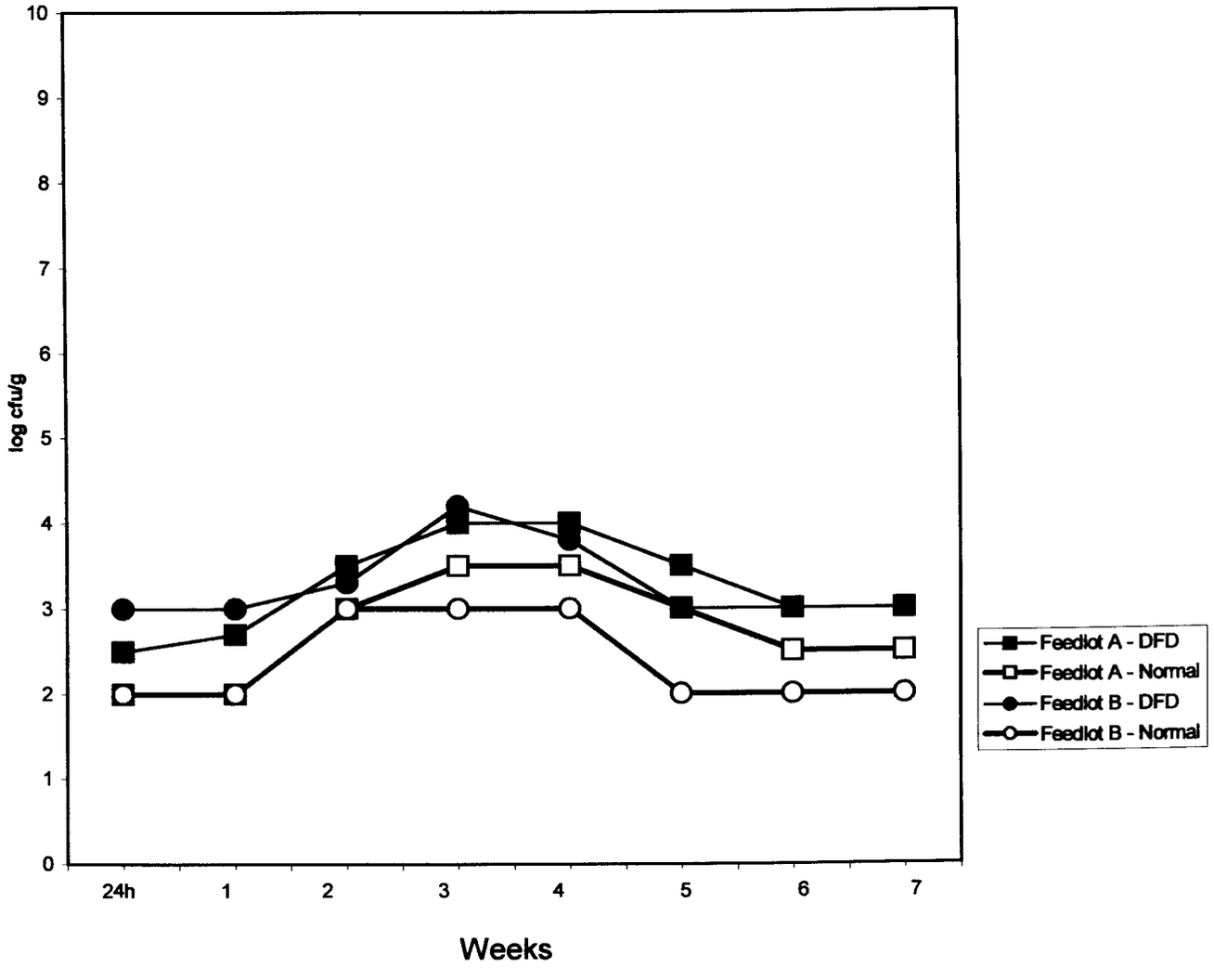

Fig. 5.4 Growth of coliforms on vacuum packaged DFD beef and normal beef of two feedlots over a 7 week period at 4°C

CHAPTER 5

The comparison by ANOVA of *Escherichia coli* populations (log_{10} c.f.u /g) of all the DFD samples, for both feedlots (N = 14) compared to the *Escherichia coli* populations (log_{10} c.f.u /g) of the normal meat samples (N = 2), are shown in Table 5.34 and Fig. 5.5. There was no significant difference in the *Escherichia coli* population of the DFD samples compared to the normal meat samples. The *Escherichia coli* population changed significantly, however differently, over the seven week period for the DFD and normal meat samples, respectively.

Table 5.34: *Escherichia coli* population (log_{10} c.f.u /g) of DFD (N = 14) and normal meat samples (N = 2) over a seven-week period (ANOVA - repeated measures design)

SOURCE	df	SUM OF SQUARES	MEAN SQUARES	F	P
Samples	1	0.033	0.41	0.08	0.78^{NS}
Weeks	6	1.91	0.14	14.11	0.0000**
Sample*time	6	0.53	0.14	3.94	0.002*

P < 0.05*; P < 0.0001** ; NS = No significance

The *E. coli* counts appeared to increase up to weeks five and started to decline thereafter for Feedlot A, normal meat. The *E. coli* counts for DFD meat, Feedlot A, increased linearly from week one to week seven. The *E. coli* counts for both the DFD and normal meat samples, Feedlot B, increased gradually up to week six. There was no further growth of *E. coli*, on both the DFD and normal meat of Feedlot B after week six.

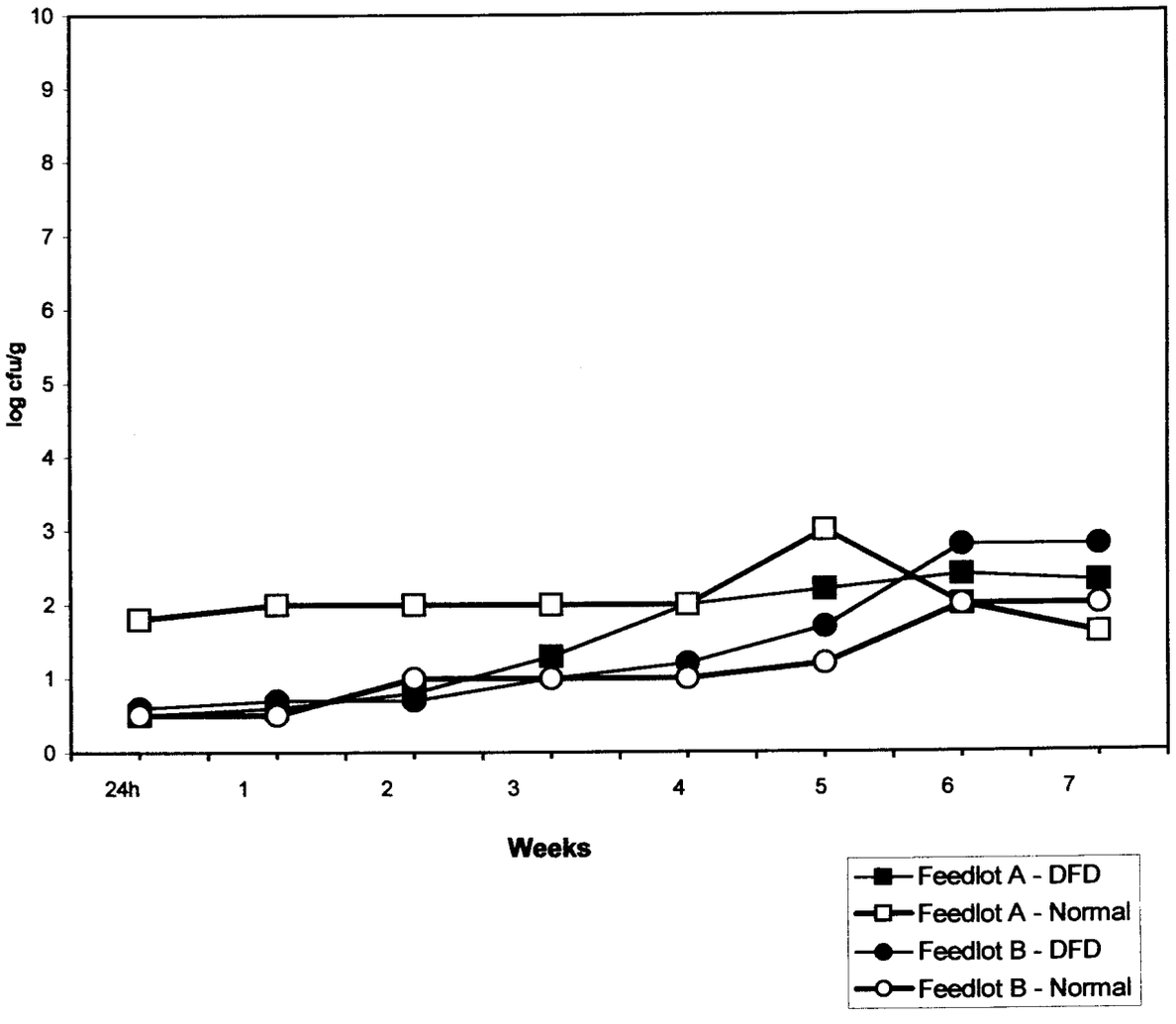

Fig. 5.5 **Growth of *Escherichia coli* on vacuum packaged DFD beef and normal beef of two feedlots over a 7 week period at 4°C**

5.6.2 Shelf-life determination of vacuum packaged DFD meat

Shelf-life determination of vacuum-packaged beef was conducted by evaluating the odour and the colour of the DFD samples over a seven-week period. Changes in the odour and the colour of meat are normally indicators of increases in the microbial populations. Table 5.35 show the odour evaluation of DFD vacuum packaged samples over seven weeks, when the tests were concluded. At the fourth week, cheesy odours were detected in two samples each of both Feedlot A and B. At week six all the DFD meat samples had cheesy odours and some of the samples smelled off. At week seven, most of the DFD samples had offensive odours.

No abnormal odours were detected on the normal meat samples (control samples) during the entire seven-week period.

Table 5.36 indicates the colour evaluation of DFD samples over seven weeks when the tests were concluded. Green discolouration of half of the DFD samples was observed at week five. At week seven, green discolouration was observed in all of the DFD meat samples. The control samples (normal beef) showed no signs of green discolouration during the seven-week storage period.

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Table 5.35: Odour evaluation of vacuum packaged beef cuts

Feedlot A:	*pH_{24}	Colour score 24 hrs	Odour Week 1	Odour Week 2	Odour Week 3	Odour Week 4	Odour Week 5	Odour Week 6	Odour Week 7
	6.40	8	Normal	Normal	Normal	Cheesy	Cheesy	Off	Off
DFD Beef	5.87	7	Normal	Normal	Normal	Normal	Normal	Cheesy	Cheesy
N = 49	6.10	8	Normal	Normal	Normal	Normal	Cheesy	Cheesy	Cheesy
	6.25	8	Normal	Normal	Normal	Normal	Cheesy	Cheesy	Off
	6.29	8	Normal	Normal	Normal	Normal	Cheesy	Cheesy	Off
	6.35	8	Normal	Normal	Normal	Cheesy	Cheesy	Cheesy	Off
Normal beef CONTROL N = 8	5.72	5	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Feedlot B:	*pH	Colour score 24 hrs	Odour Week 1	Odour Week 2	Odour Week 3	Odour Week 4	Odour Week 5	Odour Week 6	Odour Week 7
DFD Beef	6.02	8	Normal	Normal	Normal	Normal	Normal	Cheesy	Cheesy
N = 49	5.93	7	Normal	Normal	Normal	Normal	Normal	Cheesy	Cheesy
	6.09	8	Normal	Normal	Normal	Normal	Normal	Cheesy	Cheesy
	6.20	8	Normal	Normal	Normal	Normal	Cheesy	Cheesy	Off
	6.34	8	Normal	Normal	Normal	Cheesy	Cheesy	Off	Off
	6.25	8	Normal	Normal	Normal	Cheesy	Cheesy	Off	Off
Normal beef CONTROL N = 8	5.74	5	Normal	Normal	Normal	Normal	Normal	Normal	Normal

* -pH measured 24 h *post mortem* **-Colour score based on IOWA State University colour grading chart

CHAPTER 5

Table 5.36: Colour shelf-life of vacuum packaged beef cuts

COLOUR	*pH	**Colour	Colour	Colour	Colour	Colour	Colour	Colour	Colour
Feedlot A:	24 hrs	score 24 hrs	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
	6.40	8	8	8	8	8	green	green	green
DFD	5.87	7	8	8	8	8	8	8	green
Beef	6.10	8	8	8	8	8	8	green	green
N = 49	6.25	8	8	8	8	8	green	green	green
	6.29	8	8	8	8	8	green	green	green
	6.35	8	8	8	8	8	green	green	green
Normal Beef CONTROL N = 8	5.72	5	5	5	5	5	5	5	5
Feedlot B:	*pH 24 hrs	**Colour score 24 hrs	Colour Week 1	Colour Week 2	Colour Week 3	Colour Week 4	Colour Week 5	Colour Week 6	Colour Week 7
	6.02	8	8	8	8	8	8	green	green
DFD	5.93	7	8	8	8	8	8	8	green
Beef	6.09	8	8	8	8	8	8	green	green
N = 49	6.20	8	8	8	8	8	8	green	green
	6.34	8	8	8	8	8	green	green	green
	6.25	8	8	8	8	8	green	green	green
Normal beef CONTROL N = 8	5.74	5	5	5	5	5	5	5	5

* -pH measured 24 h *post mortem;* **-Colour score based on IOWA State University colour grading chart: green = slightly green discolouration

Mild off odours, with a sour, cheesy note, were detected during the fourth and fifth week, on DFD beef of Feedlot A and Feedlot B, respectively, which increased in the subsequent weeks.

The typical dairy or cheesy odours, which develop in vacuum packaged beef has been reported to be produced by *Lactobacillus* species (Newton & Gill, 1978).

The dark red colour of the DFD meat samples (N = 98) persisted during the entire storage period with a slight green discolouration, which developed from week five in 50 % of the samples. No discolouration was noticed in the control samples until week 7 when the experiment was concluded. This was in accordance with Gill & Newton (1979) who found that vacuum packaged DFD meat spoils more rapidly than meat with a normal ultimate pH, with the development of off odours and a characteristic green discolouration.

It has been reported that the greening is caused by *Alteromonas putrefaciens*, which results from the action of H_2S on myoglobin. Although a number of pseudomonads and other bacteria can produce H_2S under oxygen-limited conditions, *Alteromonas putrefaciens* produces much larger quantities than other organisms (Nottingham, 1982). *Alteromonas putrefaciens* is unable to grow on meat of normal pH and under aerobic conditions.

CHAPTER 6

DISCUSSION

6.1 The incidence and factors responsible for DFD

The incidence of dark-cutting cattle (pH_{24}), was 7.8% at the four feedlots, but 11.8% overall, based on pH_1. Although there is not enough direct evidence, the incidence of DFD is probably also affected by the season and should therefore be studied over the various seasons; it is suspected that the incidence of DFD carcasses is higher during the months with extremely hot or cold weather or large temperature fluctuations, and should decrease during the months with milder temperatures. Cold exposure for instance, may deplete muscle glycogen reserves and thereby induce dark-cutting beef.

The incidence of dark-cutters in the present study was very similar to that reported by in other countries. There is a perception worldwide that the incidence of dark-cutting beef is increasing (Kreikemeier *et al.*, 1998). An estimate of the incidence of dark-cutting beef in the United Kingdom (Brown *et al.*, 1990) showed that the overall incidence of dark-cutting (pH $\geq$ 6.0) was 4.1%. According to Zeroula & Stickland (1991), the incidence rates ranged from 1-5% for steers and heifers, 6-10% for cows and 11-15% for young bulls. Other incidences of dark-cutters were reported by Scanga *et al.* (1998) in Colorado of 6%; Bartos *et al.* (1993) in the Czech Republic from 6.6% for socially stable groups of animals, and for the socially unstable groups as high as 40%.

Correlation between the holding hours in pens prior to slaughter, and the incidence of dark-cutting cattle was not significant. However, Feedlot A had the lowest incidence of dark-cutters, the mean holding period and standard deviation for these cattle were the highest, respectively, 26.4h $\pm$ 10.23h. However, Van der Wal, Engel & Hulsegge (1997) showed that longer resting periods before slaughter induced a slightly higher ultimate pH, accompanied by a somewhat darker colour in pork.

Mohan Raj *et al.* (1992) found that overnight lairage of mixed steers, resulted in 17% DFD and lairage of mixed bulls, even up to 1h resulted in 23.55% DFD, suggesting that overnight lairage of mixed steers and holding of mixed bulls in lairage, even for a short period, should be avoided if the incidence of DFD is to be kept low.

There appears to be no correlation between the % bruised carcasses and % dark-cutting cattle at the respective feedlots.

The effect of gender on the incidence of dark-cutting beef was confirmed in the present study; the number of dark-cutting steers and bulls were significantly higher than heifers. This is in agreement with a study of Jones & Tong (1989) which found a higher incidence of dark-cutters in steers than in heifers. On the other hand, Kreikemeier & Unruh (1993), indicated that carcasses from pregnant heifers showed no incidence of dark cutters ($P > 0.05$) compared with a 1.7% incidence of dark cutters in carcasses from non-pregnant heifers. However, this aspect, pregnancy or non-pregnancy, was not tested in the present study. The incidence of dark cutting has been shown to increase in heifers that exhibit mounting behaviour near the time of oestrus (Kenny & Tarrant, 1988). Other researchers reported that the occurrence of commercial feedlot heifers classified as dark-cutters varied from 0.77% (Jones & Tong, 1989) to 4.55% (Kreikemeier & Unruh, 1993).

Zerouala & Stickland (1991) clearly showed that bulls are affected by DFD more than steers and that there was some indication that DFD steers were intermediate in their muscle characteristics between DFD bulls and normal bulls. The highest incidence of dark-cutting amongst bulls and the lowest amongst heifers, was also found by others (Augustini & Fischer, 1979; Buchter, 1981; Tarrant, 1981; Brown *et al.*, 1990; Mohan Raj *et al.*, 1991). A later study by Mohan Raj *et al.* (1992) indicated that vasectomised bulls were found to be more prone to dark-cutting, due to mounting that occurs during pre-slaughter mixing.

Fasting for 40 h led to higher pH-values compared to 60 h. According to a study conducted by McVeigh & Tarrant (1982), fasting substantially lowered the rate of glycogen recovery to such an extent as to render it unlikely that a fasted, glycogen-

depleted animal could recover sufficient glycogen to ensure normal meat quality. This was also confirmed by others (Lawrie, 1958; Hedrick *et al.,* 1959; Hedrick, 1981; Tarrant & McVeigh, 1979). However, a study done by Yambayamba *et al.* (1996) on the metabolites and meat-quality in feed-restricted re-fed beef heifers, found no indication from the longissimus muscle, that feed restriction led to a greater incidence of meat quality defects such as DFD.

It appeared that the Brahman breed had a highly significant effect on the incidence of dark-cutters based on colour identification ($P < 0.001$). This is in agreement with previous studies which suggest that *Bos indicus* breeds are more prone to DFD.

Although the effect of breed on the incidence of dark-cutting has been studied by several researchers, little is known about the effect of biological type on the propensity of cattle to exhibit the DFD condition (Voisinet *et al.,* 1997). These authors, however, showed that cattle with the most excitable temperament ratings, *Bos indicus,* produced a higher incidence of borderline dark cutters than cattle with calm temperament ratings. Tarrant & Sherington reported in 1980 that the tropically adapted *(Bos indicus)* breeds have been suspected to be more susceptible to DFD (Voisinet *et al.,* 1997). Lorenzen, Hale, Griffin, Savell, Miller & Smith (1992), however, reported a slightly lower incidence of DFD in *Bos indicus* than in *Bos taurus* beef. On the other hand, results of a study by Sanz *et al.* (1996) indicated that in spite of apparent differences in temperament of the Brown Swiss (calm temperament) and the Pirenaico breed (more aggressive temperament), there was no influence on the incidence of dark-cutters. In stead, they found that the main cause of dark-cutting, was due to the physical and emotional stress of mixing unfamiliar bulls overnight. An experiment by Shackelford *et al.* (1994) also demonstrated that there is genetic variation in the incidence of the DFD condition.

The much lower incidence of dark-cutting in cattle from Feedlot A than in the other feedlots cannot be explained. It could possibly be feedlot management related. Although the effect of growth promoters were not tested, it is suspected that the possible incorrect administering of these substances, might have side-effects which could lead to an increase in dark-cutting. According to Tarrant (1989) the use of

beta-agonists as growth promoters was shown by Allen *et al.* to increase the incidence of dark-cutting meat unless a sufficient withdrawal period before slaughter is allowed. Similarly, Scanga *et al.* (1998) reported that, administering combination (androgen and estrogen) implants to steers and estrogen implants to heifers, especially as re-implants before harvest, seemed to inflate the manifestation of stress and ultimately lead to an increase in the incidence of dark cutters.

6.2 Physiology

A number of other factors were reported to influence the DFD condition and these were also investigated. These included: (a) the feedlot *per se* (management procedures) (b) the loading, transport and unloading of the animals at the abattoir and (c) pre-slaughter holding conditions, in holding pens (lairages) at the abattoir. Relationships between blood constituents and muscle reaction to stress as related to the DFD condition in carcasses of cattle have been reported (Hedrick & Stringer, 1964; Hedrick 1981; Monin, 1980; McVeigh & Tarrant, 1982).

The muscle glycogen results obtained from the three different stages were used to determine whether and when the animals experienced 'stress'. Significant differences were measured at phase 1 and phase 2 for Feedlots A, B and C. The decrease in the glycogen concentrations suggested that the cattle experienced stress after transportation and off-loading and after being held in the lairages at the abattoir. Blum & Eichinger (1988), McVeigh & Tarrant (1983) and Tarrant *et al.*, (1992) reported that muscular activity is a mechanism leading to glycogen depletion and dark-cutting meat. Transport and handling, mixing of unfamiliar steers and bulls, which in turn can precipitate such events as fighting and mounting, are activities that deplete muscle glycogen (Lacourt and Tarrant, 1985; McVeigh and Tarrant, 1982; Yambayamba *et al.*, 1996 and Schaefer *et al.*, 1997).

The hematocrit results obtained for the three different stages were also used to determine whether and when the animals experienced 'stress'. An increase in the hematocrit for Feedlot A was non-significant for phase 1 but significant for phase 2 ($P < 0.05$) suggesting that the animals experienced stress during the holding period

in the pens to after slaughter and most likely not during transportation to the abattoir. These results suggested that cattle from Feedlot A, were handled carefully during the loading, transportation and off-loading operation. However, cattle from Feedlot B, already experienced stress during phase 1, as indicated by the significant increase in the hematocrit results of blood samples collected at the respective feedlots and compared with blood samples taken after transport and off-loading at the abattoir. A significant decrease in the hematocrit was obtained during phase 2, which represents the phase from the feedlot to after slaughter ($P < 0.050$). A significant decrease in the hematocrit was obtained for phase 1 and phase 2 for cattle from Feedlot C.

The increase in the hematocrit for these results is in line with previous findings that the haematological data is indicative of a stress response (Tarrant *et al.*, 1992). Lambooy and Hulsegge (1988), found slightly increased hematocrit and haemoglobin values in pregnant heifers transported by road for 24hrs. Tarrant *et al.* (1992) also reported that the white blood cell count and neutrophil numbers increased ($P < 0.001$) and the numbers of lymphocytes and eosinophils decreased ($P < 0.001$) when steers were transported over long distances. The packed cell volume and red blood cell count increased ($P< 0.001$), as did the concentration of total protein, haemoglobin and fibrinogen ($P < 0.001$). Apple *et al.* (1994) showed that exercised lambs also had greater ($P < 0.001$) hematocrit percentages, total protein and haemoglobin in blood samples. The elevation in total white blood cell count and neutrophil numbers, and the reduction in lymphocyte and eosinophil numbers are expected in situations of acute stress.

Increased glucose concentrations in the blood have also been correlated positively with stressful situations (Tarrant & McVeigh, 1979; Tarrant *et al.*, 1992; Apple *et al.*, 1994; 1995). Similar results were obtained in this study where significant increased glucose concentrations were obtained at the holding pens at the abattoir and after the cattle were slaughtered. There was an increase in the glucose concentrations during the period of transportation and off-loading, but it was not significant. When evaluating these findings, it appears that the animals actually experienced trauma during the period being held in the holding pens at the abattoir

to after slaughter and not during the first phase. The plasma concentration of glucose was also positively correlated with the time spent trotting and the number of times struggling occurred (Cockram & Corley, 1991). These results were confirmed in a study by Schaefer *et al.* (1997) who made consistent observations that transport and handling affected blood pH, glucose concentration and interstitial water space and suggested the use of electrolyte therapy for reducing transport stress.

A significant phase x feedlot interaction effect was found for creatine kinase concentrations. Feedlot A showed an increase in creatine kinase concentrations, during transportation and off-loading, as well as in holding pens and on slaughter, while fluctuations were found for Feedlot C. Feedlot B, however, showed a decrease in creatine kinase concentrations during phase 1 and phase 2. Cockram & Corley (1991) showed that the plasma activity of creatine kinase could be positively correlated with the time spent in the race, but no correlation between creatine kinase and physical activity in the race was found. Holmes *et al.* (according to Cockram & Corley, 1991) reported that an increase in the activity of creatine kinase in the plasma of heifers may also be caused by fasting, exercise and the administration of adrenaline. Similarly, highly significant increases above resting levels were observed in body temperatures, heart rate, serum non-esterified fatty acid concentration and plasma creatine kinase activity (McVeigh & Tarrant, 1983) and elevated concentrations of creatine kinase were found when unfamiliar animals were mixed (Warriss *et al.*, 1984).

The free fatty acid concentrations measured over the different stages showed no significant increase for Feedlot A. However, for Feedlot C, the free fatty acid concentrations increased significantly for phase 1 and phase 2. For Feedlot B, the increase was not significant for phase 1, but significant for phase 2. These results, the increase in the free fatty acid concentrations, agree with that of an experiment to determine the effect of pre-slaughter handling on the behaviour and composition of blood (Cockram & Corley, 1991). These researchers found that cattle kept overnight in the lairage, had a greater concentration of free fatty acids at the time of slaughter than those slaughtered on the day of arrival and this also agrees

with the results reported by Tarrant & McVeigh (1979); McVeigh & Tarrant (1983) and Warriss *et al.* (1984).

Other blood constituents which have not been tested in this study, have also been tested by several researchers and also showed to be indicative of stress experienced by animals. Examples are, cortisol and beta-endorphin levels, by Warriss *et al.* (1984) and Kenny & Tarrant (1987a) who found a positive correlation between elevated levels of cortisol and beta-endorphin and degree of stress prior to slaughter. However, Tume & Shaw (1992) found in their experiment that the cortisol and beta-endorphin levels were insufficient to produce dark-cutting meat.

Correlation analysis using Pearson's correlation coefficient was done to correlate the colour (scale 1-8) and the pH_{24} of the *M. longissimus dorsi* with the respective blood parameters (glucose, creatine kinase, hematocrit, free fatty acid) as obtained from results of the final stage, i.e., *post* slaughter. No-significant correlations were found. However, significant correlations, with Pearson's correlation coefficient, were found between the colour (scale 1-8) and the pH_{24} of the *M. longissimus dorsi* of all three feedlots, which is in agreement with Tarrant (1989); Yambayamba *et al.* (1996) and Lahucky, Palanska, Mojto, Zaujec & Huba (1998).

6.3 Consumer sensory evaluation

The colour and general appearance of normal steaks were preferred over DFD steaks. The overall acceptability of the normal steak was also significantly higher. This is in accordance with conclusions made by researchers with regards to the importance of an acceptable colour for the consumer. Hood (1978) said that the psychological attractiveness of the bright-red colour makes it a fundamental criterion. However, Dumont (1981) stated that the importance of colour as an indicator of 'freshness' is probably overestimated in the consumer's mind. The effects of lean colour on palatability were studied by Wulf *et al.*, (1996) who found that lean colour was significant ($P < 0.05$) for taste panel tenderness score; darker coloured meat was considered to be less tender compared to normal and pale lean.

On the other hand, normally coloured lean received higher ($P < 0.05$) flavour intensity scores than either dark- or pale coloured lean.

Although it was expected that a significant difference would be found in the acceptability of tenderness and flavour of fried DFD versus fried normal beef, no significant differences were found in consumer acceptability for any of the sensory attributes. The tenderness and flavour differences may have been too slight to have a significant effect on consumer acceptability.

Increases in tenderness associated with high pH have been shown in beef (Fredeen, Martin & Weiss, 1974; Barnier, Geesink, Smulders & Van Logtestijn, 1992). Dransfield (1981) also assessed consumers reactions to test the relationship between tenderness and pH and found that DFD beef is, on average, only marginally more tender than normal pH meat. Jeremiah, Tong & Gibson (1991) found that carcasses with the very darkest lean were tender but when those carcasses were excluded, a negative relationship existed between lean darkness and tenderness among steer and heifer carcasses. A possible explanation for the more tender DFD beef was made by Katsaras & Peetz (1990) who studied morphological changes in dark cutting beef when heated and found that fragmentation of myofibrils was greater in DFD meat than in normal meat, and cooking losses were much smaller. These differences evidently led to greater tenderness in DFD meat as compared with normal meat. The effect of electrical stimulation on the tenderness and pH of meat was studied by several people (Dutson, Savell & Smith, 1981; Paleari, Beretta, Gigni, Parini, Rasi, Crivelli & Bertelo, 1993). This method proofed to have great value in reducing the pH, and making the meat more tender.

As part of the EEC beef co-ordination programme, quality assessments were compared between 5 institutes, each using their standard methods for grilled sirloin steaks and it was found that juiciness of steaks was not significantly related to pH in any of the 5 laboratories (Dransfield, 1981). Wulf *et al.* (1996) also reported that lean colour was not a significant ($P > 0.05$) source of variation for taste panel juiciness.

Katsaras & Peetz (1990) reported that DFD meat had, compared with normal meat, a stale "off" flavour. In this study no significant differences in consumer acceptability in any of these mentioned sensory attributes (tenderness, juiciness, flavour, odour, colour) were found when comparing fried DFD beef steak with fried normal beef steak, although the variances in the raw condition were significant probably due to the dark colour which made the meat unattractive.

6.4 Microbiological Quality And Shelf-Life

Whilst the microbiological analyses represented the type and number of micro-organisms present in the meat samples, sensory evaluations, i.e. odour and colour, were done to determine the shelf-life of the respective meat samples.

The total aerobic counts of the respective samples showed a gradual increase up to week five and then started to decrease gradually. This was in accordance with previous tests by Newton & Gill (1978) and Hayes (1992). However, Nottingham (1982) was of the opinion that total aerobic counts of vacuum packaged beef, do not have the same significance as a measure of spoilage as they do with meat stored under aerobic conditions. Generally, total counts increase rapidly at first, then levelled off at about 10^6 to 10^7/ cm². Egan & Roberts (1987) found that subsequent growth is slow so that a storage life of 10-12 weeks is possible at 0-1°C; by that time the final total count of *ca* 10^7/ cm² will be reached which is only about 1% of that obtained with permeable films. On the other hand, Sutherland found in 1975 that the aerobic gram negative bacteria did increase along with lactic acid bacteria in vacuum-packed beef stored at 0-2°C for up to 9 weeks (Jay, 1992). The most significant microbial effect of vacuum packaging is the restriction of growth of aerobic bacteria so that lactic acid bacteria become dominant, as shown by Gill (1976) and Jay (1992).

Qualitatively the microbial flora in the impermeable pack becomes dominated by lactic acid bacteria (mainly lactobacilli and leuconostocs) which represent 50-90% of the total flora at the end of storage; *Brochotorix thermosphacta* and pseudomonads are typically only a minor part of the flora (Gill, 1982).

Sensory evaluation of the DFD beef cuts, showed a slight sour, cheesy odour at the fourth week. The odour only worsened in the subsequent weeks. This could have been due to spoilage associated with lactobacilli and leuconostos, which produce sour or dairy odours due to the formation of fatty acids, these principally being acetic or butyric acids (Gill, 1982; Hayes, 1992) and the later obnoxious odours could have been caused by *Enterobacter liquefaciens* (Gill, 1982).

Both authors also reported that the spoilage of anaerobically stored meat developed slowly after the maximum density of bacteria had been reached. It was further claimed that vacuum packaged meat at -1°C has a shelf-life of 12-14 weeks during which time it develops a cheesy or dairy flavour which finally renders it unacceptable due to the short chain fatty acids. There is also evidence of amines in vacuum packaged meat due to bacterial activity. Both groups of substances appear to be derived from breakdown of amino acids (Gill, 1982).

The green discolouration observed in 50% of the DFD meat samples at week five, could have been caused by *Alteromonas putrefaciens*. This bacteria forms hydrogen sulphide which combines with the muscle pigment to form green sulphmyoglobin (; Gill, 1977; Gill, 1982; Gill & Newton, 1979 and Nottingham, 1982).

The growth of micro-organisms on the normal meat samples, i.e. for total viable count, coliforms, *E. coli* and *Pseudomonas,* appeared to be retarded during the first 3-4 weeks. Similar results were reported by Gill (1982) in that for vacuum-packed fresh meats, stored at 0-5°C, a lag phase of *ca* 4 days to 4 weeks (at 0°C) was usually observed.

The same researcher also reported that there appeared to be no important differences in the composition of the aerobic spoilage flora of normal and DFD meat. In DFD meat, the onset of spoilage was determined by the time bacteria took to grow to a sufficient density for the products of the degradation of amino acids to become detectable.

With normal meat carrying a spoilage flora dominated by pseudomonads, the presence of glucose prevented formation of these spoilage substances until the bacterial density was two orders of magnitude greater.

Pseudomonas have been reported to dominate the spoilage flora in the later stages due to their ability to grow faster than all other competing species at these low temperatures. It was further found that the growth rates and final numbers of *Pseudomonas* species were unaffected by maximum numbers of competing species due to their inability to compete with *Pseudomonas* for the available oxygen (Gill & Newton, 1977; Newton & Gill, 1978; Nottingham, 1982 and Hayes, 1992).

The gradual decrease of populations could be attributed to the presence of competing anaerobic organisms, like the lactobacilli and leuconostos discussed elsewhere.

It was also essential to determine the coliform population and more specifically the *E. coli* population as indicative of the hygienic conditions of the abattoir and also to determine its effect on DFD beef. Faecal organisms in beef carcass dressing procedures are considered as a critical source of contamination (Gill *et al.,* 1996).

The starting populations were relatively low and had a lag phase for the normal meat samples of both feedlots with an increase at week four. At week five the *E. coli* population of the normal meat samples started to decline. The DFD meat samples of both feedlots showed a linear increase in the *E. coli* population until week six when it started to decline probably due to competing organisms which suppressed their growth and also due to the lack of oxygen and the low storage temperature (4°C). However, Newton *et al.* (1977) reported that upon prolonged storage at refrigerated temperatures, spoilage could begin and under conditions where growth of competing organisms is slow, psychrothopic Enterobacteriaceae could become a significant proportion of the flora.

In this experiment, the spoilage of the DFD beef cuts already started to take place during the second week when the total aerobic counts for the DFD samples were

> 10^6 /g. At the fourth week, cheesy odours were detected. The deterioration of samples were also more evident during week 5 when green discolourations of the meat samples, were observed. These results were in agreement with Davey & Graafhuis (1981) who reported that deterioration, such as greening in packaged meat, or the development of objectionable smells and off-flavours, occurs at rather low bacterial counts (10^6/g) and reduces acceptable shelf-life substantially.

The normal beef samples were acceptable, both on microbiological grounds and sensory evaluations, until the seventh week when the tests were concluded.

These findings are in accordance with Newton & Rigg (1979) who determined that the storage life of vacuum packed beef cuts ranged from 4 to 15 weeks, depending on the oxygen permeability of the plastic films.

DFD meat spoils rapidly because of a deficiency of lactic acid but also due to a deficiency in glucose (Newton & Gill, 1978; Gill 1982). Thus, it is rather the absence of glucose than the high pH which is the determining factor for the onset of early spoilage, and glucose may be absent from meat with a pH as low as 6.0.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Various intrinsic or extrinsic factors, or the combination of these factors, could trigger muscle glycogen breakdown, which may result in dark-cutting meat, if the animals are exposed to stress for long enough. To minimise the DFD syndrome in cattle, from the feedlot to slaughter, the challenge is to identify the specific contributing factor(s). The factor(s) can then be eliminated or adjustments made to reduce the incidence. Various factors, which could cause the DFD syndrome, were researched in this study.

The estimated overall incidence of dark-cutting beef (pH_1) at a South African abattoir, in the hot summer months, is 11.8%. The incidence of dark-cutting beef (pH_{24}) , also seems to be feedlot related with the highest being 12.7% and the lowest 1.8%.

Long holding periods (> 24 h) in the holding pens at the abattoir, appear not to have a significant influence on the occurrence of DFD. Bulls and steers are affected more by dark-cutting than heifers. Mounting behaviour among young bulls, and social regrouping of steers during the pre-slaughter period, have long been associated with an increase in the incidence of dark-cutting. It would, therefore, be advisable not to mix unfamiliar bulls and steers during transport and holding periods. The low incidence of dark-cutters in heifers could possibly indicate a non-oestrus situation in the majority of the heifers, since a significant association between the presence of oestrus and DFD was shown previously by other researchers. The effect of gender on the incidence of dark-cutting, related to feedlot, is also significant.

Fasting has a significant effect on the incidence of dark-cutting beef. Cattle fasted for 40 h had a higher incidence of dark-cutting compared to cattle fasted for 60 h. The effect of fasting on the incidence of DFD is also feedlot-related. It seems as if pre-slaughter stress may induce dark-cutting more readily in fasted than in fed cattle.

CHAPTER 7

Fasting causes a gradual decline of muscle glycogen in resting cattle and sheep although the mechanism of muscle glycogen depletion during starvation is unclear.

It appears as if the breed type of the cattle has an effect on dark-cutting beef. *Bos indicus* (Brahman-type) animals have a higher susceptibility to dark-cutting beef when compared with European breeds. During the entire research period, there was a tendency that DFD beef manifested more when this type of cattle was used. This aspect requires further research.

The effect of pre-slaughter handling on the muscle glycogen and blood composition of cattle of three different feedlots was studied. The decrease in muscle glycogen concentration during transportation and handling over the different stages, suggests that glycogen depletion is the cause of elevated ultimate pH and DFD meat.

The hemotocrit increase from the feedlot to after transport, suggests that animals of Feedlot A and B, experience fatigue and dehydration. During the second phase, i. e., the period after being off-loaded at the pens at the abattoir until after slaughter, a further increase in hematocrit for Feedlot A was observed, suggesting an increase in the stress response. A decrease in the hematocrit for Feedlot B was observed during this period. A linear decrease in the hematocrit was observed at Feedlot C.

The blood glucose concentrations for all three feedlots increased significantly after they were held at the pens at the abattoir and slaughtered, which probably indicates that the animals experienced stress during this period. Transportation caused an increase in the plasma concentrations of glucose, although not significantly.

Significant increases in creatine kinase concentrations were observed in Feedlots A and C after transportation. This may reflect a stress response. Animals of Feedlot A also showed an increase in creatine kinase after they were held in the pens at the abattoir and slaughtered. The sudden significant decrease in the creatine kinase concentration for Feedlot C cannot be explained. The significant decrease in the creatine kinase concentrations during transportation, handling and being held at the pens for Feedlot B, was also not expected.

CHAPTER 7

The free fatty acid concentration is probably the best blood parameter to relate to meat quality. The free fatty acid concentrations for Feedlots B and C show a significant increase from the feedlot to after being held at the abattoir and slaughtered. These results suggest that these animals experienced stress during the holding period in the pens at the abattoir. The DFD incidence for Feedlots B and C, was 35%. For Feedlot A, with the lowest DFD incidence (16%), the increase in the free fatty acid concentration was not significant.

It appears as if it is not so much the handling, before transportation and the transportation *per se,* which contribute to the variance in the free fatty acid concentration and the increase in the glucose concentrations.

Although the effect of growth promoters was not tested, it is suspected that the possible incorrect administering of these substances, might have side-effects which could lead to an increase in dark-cutting. This aspect needs to be investigated at the feedlots concerned.

Consumer sensory evaluations indicate that the colour and acceptability of raw normal steak is preferred to those of raw DFD steak. Twice as many panellists prefer the raw normal steak than raw DFD because of the more attractive red colour, compared to the almost black colour of raw DFD steak.

Although it was expected that consumers would find the fried DFD steak more tender and less flavoursome than normal steak, no significant differences were found in the acceptability of the steaks.

Microbiological spoilage of vacuum-packaged DFD meat appears to take place during the second week of storage. During the fourth week cheesy odours develop and deterioration is more evident during the fifth week due to green discolouration of the meat.

CHAPTER 7

The normal beef samples are expected to be acceptable, both on, microbiological grounds and sensory evaluations, until at least the seventh week. DFD meat spoils rapidly because of a deficiency of lactic acid but also due to a deficiency in glucose and it is rather the absence of glucose than the high pH, which is the determining factor for the onset of early spoilage.

CHAPTER 8

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APPENDIX A

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Standards for Beef Colour

Cooperative Extension Service

IOWA STATE UNIVERSITY

Ames, IOWA 50011

AS-515/ Reprinted/ April 1982

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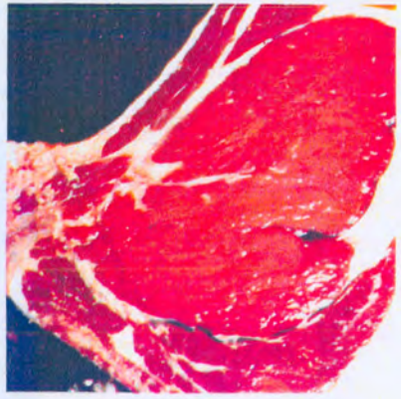
Beef Color Standards



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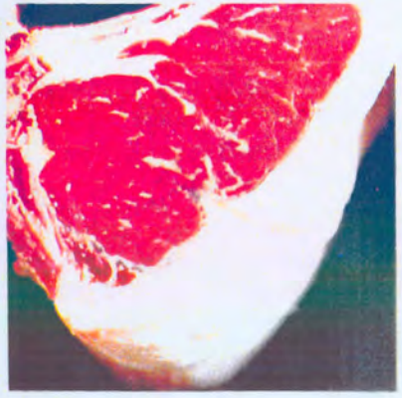

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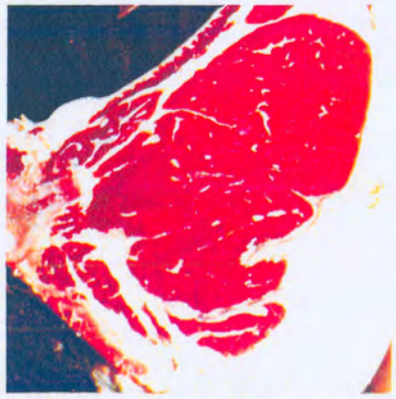

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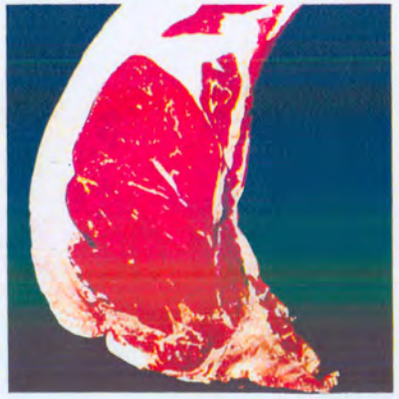

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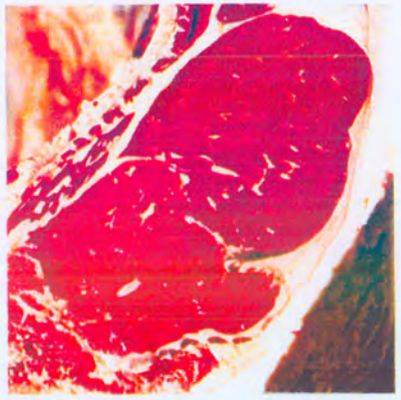

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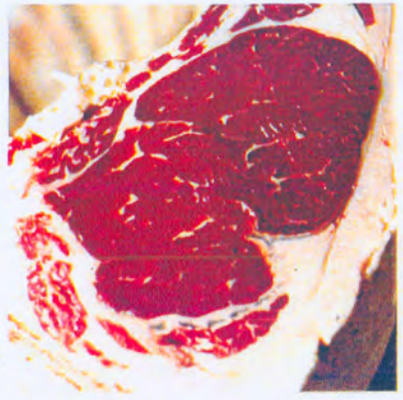

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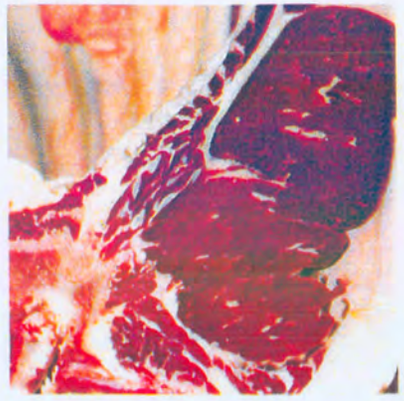

8 Very Dark Red

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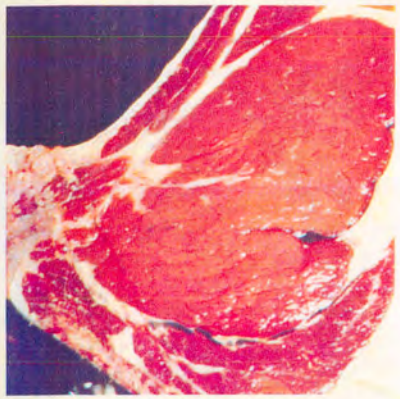
YUNIBESITHI YA PRETORIA

Beef Color Standards



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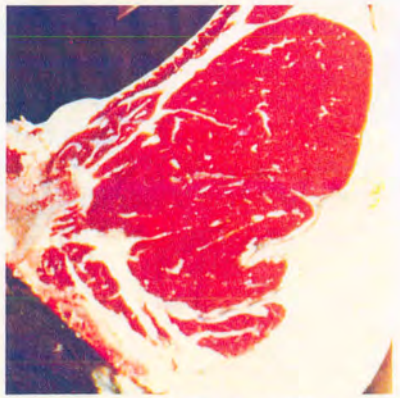

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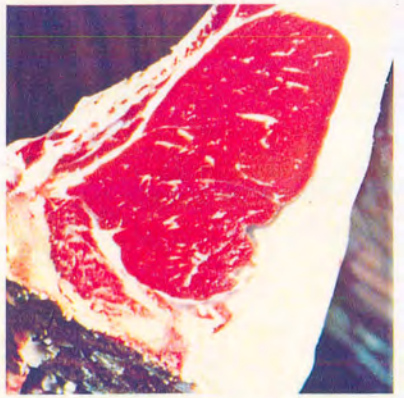

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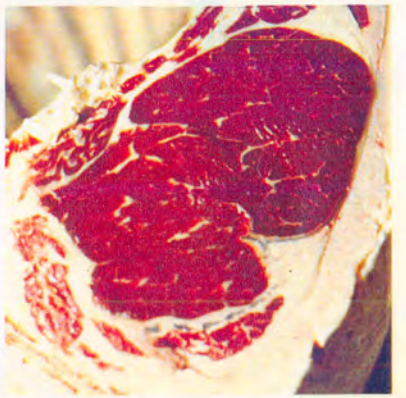

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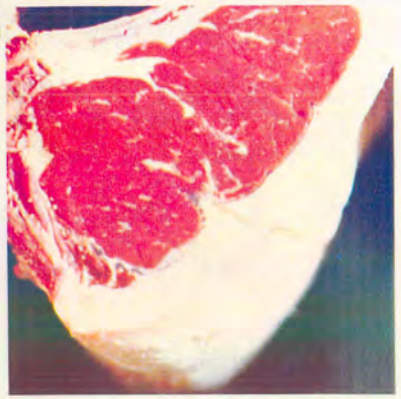
2 Very Light Cherry Red



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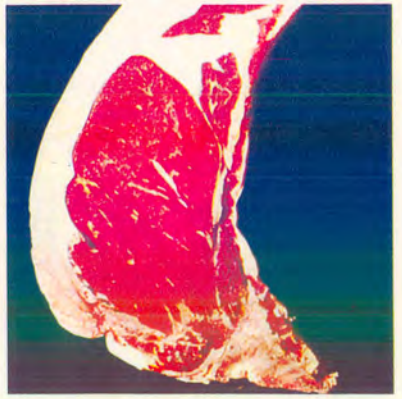

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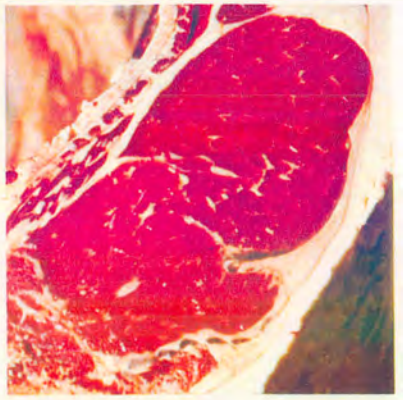

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