The Effect of Selecting for "Robustness" on Temperament in Dairy Cows

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To my mother and father for their love and support

Declaration

I declare that I have composed this thesis, and that the work described is my own. All assistance received is acknowledged. The work has been submitted for no other qualification.

Jenny Gibbons

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Publications

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Abstract

Increased rates of involuntary culling as a consequence of poorer health and fertility had led to the conclusion that dairy cows appear to be less "robust" or adaptable than in the past. A way to address these concerns in breeding programs could be to select for health and welfare by including appropriate traits in a broader breeding index. However, it is important to consider any consequences that such breeding goals may have on dairy cow temperament and welfare. There were two phases to this study. The main objective of phase I was to develop tests for measuring responsiveness to humans and novelty, aggression at the feedface and sociability in dairy cows for use on commercial farms. To allow these tests to be used on commercial farm, they must be short in duration, non-invasive and not disruptive to the daily farming routine, while at the same time allowing comparisons between an individual cow's responses in a number of similar situations.

Results from this study suggested that a standardised human approach test and a stationary visual object are reliable tests for measuring responsiveness of dairy cows to changes in their environment. Measuring behaviour at the feedface proved to be an effective measure of between cow aggression. Inter-animal distance, position in relation to the herd, behavioural synchrony and presence at the feedface proved accurate measures of sociability. The remaining part of the study (Phase II) focussed on assessing how the implantation of a breeding index can affect the temperament of dairy cows on commercial farms. The tests developed were then recorded on 402 first lactation Holstein-Friesian dairy cows selected from sires that scored high (HI) and low (LO) for robustness (health, fertility and longevity traits) to produce two treatment groups on 33 commercial farms. For the purpose of this thesis, only the results from the assessment of aggressiveness are presented. Continuous focal sampling was used to record aggressive behaviour during feeding of the HI and LO cows within the herd. Cows from the HI group were involved in more aggressive interactions, initiated more aggression and received more aggression than cows from the LO group. There was a strong influence of management factors influencing aggression such as the quality of stockmanship, feedface design and nutrition.

In conclusion, daughters from sires scoring high for robustness may be expressing a greater ability to maintain position at the feedface during an aggressive interaction. This highlights the importance of assessing the correlated effects of selective breeding, in this case for robustness, on behavioural traits.

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CHAPTER 1

General Introduction

1.1.Introduction

Traditionally, animal breeding was mainly directed at improving production. Recently, there has been increased attention on the 'undesirable' side effects of genetic change in production traits on animal welfare (Rauw *et al.,* 1998). Public concerns about the effect of breeding primarily for production traits on farm animal welfare has led to the view that there should be a greater focus on breeding for what are described as 'robust' animals (Star *et al.*, 2008). As a result attention has been shifted from production traits to focus more on improving health and welfare of production animals.

In many countries, selection pressure in the dairy industry has largely focused on milk production over the last 50 years. There is concern that unfavourable genetic relationships between health traits and milk production have contributed to an increased incidence of health problems, a reduction in ability to reproduce and a decline in longevity in modern dairy cows (Royal *et al.,* 2000; Veerkamp *et al.,* 2003). This has led the UK's Farm Animal Welfare Council to suggest that 'breeding companies should devote their selection to health traits to reduce lameness, mastitis and improve fertility' (FAWC, 1997). Dairy breeding companies have recognised these problems and are enhancing their breeding indices to include welfare traits to improve cow fertility, calving ease, survival and to reduce lameness and mastitis (Cassell, 2001; Wall *et al.,* 2007).

While balanced breeding should improve the animal's robustness through improving disease resistance, health, fertility and longevity, it can be questioned whether selection for these traits fully addresses the welfare of dairy cows in the wider sense. It is important to investigate the desirability of selecting for robust dairy cows. Of particular concern and interest is to understand whether selection for robust dairy cows would have undesirable outcomes on behaviour and cow temperament. More specifically, in this thesis, I was interested in temperament both in terms of its contribution to robustness and also any possible undesirable consequences of selecting for robustness, and whether if cows are bred to be more robust, will their temperament and welfare change? However, before this question can be answered, I needed to identify what aspects of temperament were appropriate to study in the context of robustness. Once identified, appropriate tests of temperament needed to be deigned and validated. Three major aspects of temperament were identified: 1) It is important to investigate emotional responsiveness of individual cows towards human interaction and towards challenge within the environment. 2) It is equally important to examine the aggressive style of individual cows and their willingness to compete for feed and displace other cows at the feedface. Feeding behaviour is likely one of the most important behaviours because it determines feed intake which comes at the top of an animal's hierarchy of needs. 3) Finally, the last temperament trait of interest was sociability, in terms of a cow's ability to cope in group housing. Modern production systems involve a lot of regrouping so that the group dynamics are constantly changing and a cow's ability to cope and adapt to her social environment with minimum stress is important.

In this chapter, I will introduce the role of genetic selection in the dairy industry, paying particular attention to the importance of expanding selection programmes to include welfare friendly traits, specifically behaviour. The potential usefulness of studying temperament in dairy cattle might not immediately be apparent, so I will examine the importance of studying livestock temperament. Next, I will review the relationship between human psychology and animal temperament research. This will be followed by a detailed review of research on cattle temperament traits, evaluating what methods have been used and what other areas need to be investigated.

1.2. Dairy cattle breeding

During the 20th century the main goal for animal agriculture was to increase production and efficiency to satisfy a consumer market that demanded animal products at low costs. Therefore, it is not surprising that the main aim of dairy cattle breeding for the last 50 years was to improve production through genetic selection to increase milk yields. In many European countries, milk yield per cow has more than doubled in the last 40 years (Oltenacu and Algers, 2005). Up until the 1980's most of the milk yield increases were due to improved feed management and feed quality. Since then, selective breeding has played a major role in increasing milk production, in particular, effective use of artificial insemination, selection of progeny test bulls and world-wide distribution of semen from bulls of high genetic merit for milk production (Pryce and Veerkamp, 2001). Data from the UK national milk records show an increase in average yields of dairy cows of about 200kg/year from 1996 to 2002 and 50% of the progress in milk yield is attributed to genetic selection (Pryce and Veerkamp, 2001). However, increasing milk yield has come at a cost.

There is considerable evidence that selection for milk production traits alone has led to increases in involuntary culling as a result of increased incidences of lameness, mastitis, metabolic disorders and reduced fertility (Esslemont and Kossaibati, 1995; Rauw *et al.,* 1998; Royal *et al.,* 2000; Pryce *et al.,* 2001, 2002; Veerkamp, 2003). Poor fertility is a large problem for the dairy farmer and it is approximated that 20.6% of culls in dairy herd are due to fertility-related reasons (Kingshay Farming Trust, 1999). The fertility of dairy cows has declined worldwide with on-farm conception rates in the UK declining at a rate of 1% per year over the last 20 years. On-farm pregnancy rate to first service decreased from 56% in 1975-1982 to 40% in 1995-1998 (Royal *et al.,* 2000). Furthermore, in a study of 50 herds in England, Esslemont and Kossaibati (1997) found that farmers reported failure to conceive as the predominant reason for culling their cows with 44% of first lactation, 42% of second lactation and 36.5% of cows in total being culled for this reason. This decline in fertility can be explained by management changes within the dairy industry but also due to an unfavourable genetic relationship between milk yield and reproductive traits (van Arendonk *et al.,* 1989; Oltenacu *et al.,* 1991; Hoeskstra *et al.,* 1994; Pryce *et al.,* 1997 and Pryce *et al.,* 1998). Changes in cow behaviour may also have played critical role in the declining reproductive performance of high producing cows. In a study of 17 commercial herds that used electronic oestrus-monitoring systems, Dransfield and colleagues (1998) showed that high producing cows exhibited oestrus with lower intensity and shorter duration relative to lower-producing cows.

The incidence of lameness, mastitis and metabolic disorders has also increased greatly over the last two decades. Ingvartsen *et al.,* (2003) reviewed 14 genetic studies on the relationship between milk performance and health in dairy cattle. These studies showed an unfavourable genetic correlation between milk yield and incidence of ketosis (0.26-0.65), ovarian cysts (0.23-0.42), mastitis (0.15-0.68) and lameness (0.24-0.48). This study strongly suggests that continued selection for higher milk yield alone is likely to increase the prevalence of such production diseases. In a more recent study, Ouweltjes *et al.* (2007) concluded that genetic selection on milk production, without taking udder health into account, will cause increased sensitivity for udder health problems. The main strategy to reduce involuntary culls due to poor health and fertility is to breed healthier animals by including health, longevity and fertility traits into breeding indices.

The process of genetic improvement in dairy cattle is a complex area. For the purpose of this review, I will briefly describe how selection indices are used in dairy cattle breeding, the interaction between the environment and genetics and finally the possible solutions to problems with dairy cattle breeding.

1.2.1 Genetic selection in the dairy industry

In dairy cattle breeding, most of the traits that are of interest are only expressed by cows (e.g. milk yield). However, because fewer bulls than cows are needed for dairy cattle breeding, genetic improvement is greatest through selection of bulls (Simm, 1998). Young bulls are identified for their high predicted genetic merit and the milk production and performance traits are recorded on the daughters of these bulls. Breeding values are then calculated for each bull from the collation of his daughters' and sisters' performance records in commercial herds. Additionally, breeding values are predicted for cows from her own performance but also the performance of her relatives. The top bulls and the top cows are then mated to produce a new batch of young bulls of high predicted genetic merit.

A selection index is a combination of performance traits that creates a single score of genetic merit for each animal for that index. The emphasis on different traits depends on their economic importance and capacity for genetic improvement. Indices combine the predicted transmitting ability (PTAs) for several traits into a single overall score. PTAs are a measure of the genetic merit of a particular animal for a particular trait (e.g. milk yield). PTA is not a measure of performance, but predicts the amount of a trait the offspring will, on average, receive from its parents. PTAs enable comparison between cows sired by the same bull, but who are in different herds and managed under different farming systems. In the UK, dairy farmers have a range of indices available to bring about genetic change in dairy cattle. These indices include:

- The 'Production Index' (PIN) for milk, fat and protein content
- The 'Survival Index' called 'Lifespan'. Lifespan is calculated from a cow's actual survival, however this information is only available once the animal is culled (or dies). Until a cow's actual survival information is available, lifespan is predicted from information on type traits (feet, legs and udder), somatic cell count and records from relatives. There is a strong relationship between milk production and survival as cows with low milk yields are generally culled earlier from the herd. Therefore, lifespan is corrected for milk yield to ensure it is a measure of a cow's ability to survive rather than of her failure to produce milk.
- Index for linear type traits such as stature, udder conformation, foot angle etc.
- The 'Cow fertility index' provides a prediction of cow fertility and is based on a combination of calving interval, non-return rates, body condition score and insemination records (milk yield at time of insemination, days from calving to first insemination, number of inseminations needed to get a cow in calf).
- The 'Management Index' includes milking speed and locomotion traits (Simm, 1998).

These various indices can then be combined into an overall economic index which can help clarify farmers' decisions over bull selection. Traits are economically weighted in indices such as £PIN and £PLI (Profitable Lifetime Index). £PIN is based purely on production traits (milk, fat and protein), however its main limitation is its lack of non-production information relating to health and welfare. These problems with £PIN led to the development of £PLI. £PLI includes health, fertility and longevity traits (somatic cell count, locomotion, udder composite, fertility and lifespan) in addition to production traits (milk yield, fat and protein content). £PLI is continually under development with fertility traits being added in 2007 and currently work is underway to include calving ease.

1.2.2 Genotype by environment interaction (G ^{x} E)

Often an animals' genetic merit is not consistent across all environments. This can cause frustration to a producer who is selecting animals based upon a predicted performance but is not obtaining the expected results. Therefore, understanding the basis of genotype by environment interaction (G ^{x} E) and its influence on the dairy cattle industry will assist in future animal production, particularly with breeding for robustness.

In this section, I will outline the definition and theory behind G ^{x} E interactions and then briefly discuss G ^{x} E interactions in dairy cattle. The performance of an animal depends not only on its genetic makeup but also by the environment. The expression of its genetics is modified by the environment (management system) in which the animal lives. Disentangling genetic and management is difficult. G ^{x} E occurs when different genotypes respond differently to changes in the environment (Falconer and Mackay, 1996). In this case genotype can refer to breeds or to individuals with certain phenotypic or genotypic performance, QTLs or genes. The ability of animals to be affected by the environment is known as phenotypic plasticity or environmental sensitivity (Falconer *et al.*, 1996). Two distinct G ^{x} E interactions can occur, and these are known as scaling and re-ranking. When the differences between genotypes vary between environments without changes in their ranking this is known as a scaling effect. For example, high genetic merit dairy cattle will continue to produce more milk solids than low genetic merit dairy cattle in both low and high concentrate feeding systems (Fulkerson *et al.*, 2000). Generally, G ^{x} E is less important if only scaling effect occurs because the best selected individuals in one environment would still perform the best in other environments. There is mainly a scaling G ^{x} E effect on genotypes when they have been defined on selection for production and where environment are defined based on different diets (Veerkamp *et al.,* 1994; Pryce *et al.,* 1999). However, if the genotypes rank differently in different environments the effect of G ^{x} E is known as re-ranking. For example, Kolver *et al.* (2002) reported a re-ranking of New Zealand and North American Holstein Friesians between grazing- and total mixed ration-based systems (TMR). On a pasture based system New Zealand Holstein Friesians produced more milk solids than North American Holstein Friesian, however on the TMR system, North American Holstein Friesians produced more than the New Zealand Holstein Friesians

The difficulty with G ^{x} E studies lies in measuring the environment. For this reason many studies are carried out on experimental farms with environment defined on differences in feeding levels and system. Published results on the lack of G ^{x} E included milk production (Veerkamp *et al*., 1995; Kolver *et al*., 2002), body condition score (Veerkamp *et al*., 2002), health and fertility (Pryce *et al*., 1999; Ouweljes *et al*., 2007). However, advances in statistical modelling of large datasets have allowed good estimates of correlations and heritability of traits in different environments. The reaction norm model quantifies the phenotype expressed by a certain genotype over a number of different environments and has recently been introduced to study G ^{x} E in animal breeding (de Jong and Bijma, 2002).

In term of breeding for robustness, G ^{x} E plays an important role as some animals are more environmentally sensitive for some traits. The identification of robust sires for both production and functional traits combined, or sires best suited to a particular environment, has the potential to allow producers to improve, or maintain, performance as well as health and welfare within their herds. In order for this to be possible, the effect of G ^{x} E interactions on the various breeding goal traits has to be determined.

1.2.3 Possible solutions

There are a number of procedures that could be used to improve health, fertility and welfare in high-producing dairy cows. Using bulls with high genetic merit for fertility and health will help. Additionally, crossbreeding and improving selective breeding programmes offers a route to improving health, fertility and longevity traits. In New Zealand and Ireland, crossbreeding has been shown to have considerable benefits, particularly in terms of fertility and survival (Harris *et al.,* 2001; Dillon *et al.,* 2003). However, the decline in dairy health and fertility will be reversed only when the genetics for dairy reproduction and health are improved through a balanced genetic selection strategy. Adding traits or altering weighting on different traits in genetic indices can be used to improve health, fertility and production in dairy cattle. As of 2007, £PLI index in the UK was re-weighted with reduced emphasis on production traits and an increased weight on health (somatic cell count, leg and feet traits), fertility and longevity traits. The aim of this re-weighted index is to halt the predicted genetic decline in health and fertility traits. This type of strategy will have the greatest effect on the existing UK population of Holstein Friesian dairy cows.

1.3. Undesirable side-effects of genetic selection on welfare & behaviour

There are numerous examples where a lack of balance in breeding programmes has led to both behavioural and physiological problems in animals. In pig production, selection is primarily based on high growth rate and minimum backfat thickness, i.e. leaner tissue growth rate. The primary aim in poultry meat production is rapid growth and to enhance feed efficiency. However, high levels of selection pressure on production in these cases have had unexpected consequences on welfare traits (Pigs: Geers *et al.,* 1990; Rauw *et al.,* 1998; Chickens: Jones and Hocking, 1999; Jensen and Andersson, 2005). Pigs selected for high lean gain may have more excitable temperaments and are more fearful than fatter genetic lines (Grandin 1993, 1994; Shea-Moore, 1998). Pigs with higher lean growth show an increased stress response to transportation leading to lower meat quality (Grandin, 1997). In cattle, stereotypic licking may be linked to selection for high milk yield (Grandin and Dessing 1998), selection for double-muscled beef cattle results in more calving difficulties (Appleby, 1998) and selection for docility in cattle may indirectly alter maternal defensive aggression (Review: Turner and Lawrence, 2007). In laying hens, increased aggression and propensity to suffer from osteoporosis is related to selection for early sexual maturity and egg production (Craig *et al.,* 1975). The leg problems and disorders in broilers have been attributed to side-effects of selection rather than being a result of the housing conditions and stocking densities in which they are reared (Reiter and Bessei, 1998). However, it is not just selection on production traits that may cause unexpected side effects on welfare as selection on any single trait may cause problems. For example, Belyaev (1979) reported abnormal maternal behaviour and pup-killing as a result of selecting for a behavioural trait (tameness) in foxes.

1.3.1 Breeding for robustness

The concept of robustness has recently become a main interest in animal production and breeding (Pigs: Knap, 2005; Poultry: Star *et al.,* 2008). This thesis is part of a DEFRA-LINK funded project entitled "Identifying and characterising 'robust' dairy cows". At the time that this project started, robustness was a very general term that was poorly defined. For the purpose of the project and this thesis, robustness was defined as the ability of a cow to remain productive, fertile, long-lived and healthy in a range of environments. Now, there are many definitions of robustness (Knap, 2005; Veerkamp *et al.,* 2007; Strandberg, 2007) but the general consensus is that robustness relates to the ability or capacity of an animal to adapt and produce in a wide variety of environmental conditions. However, another concept that may contribute to robustness is behaviour. Behaviour may be one of the traits that the animal can adapt in order to support production in different environments or to cope with challenging situations.

1.3.2 Behavioural traits associated with robustness

Robustness could be achieved by improving functional traits such as health, fertility and longevity as well as investigating the influence of the environment. Even though traditional breeding techniques have had impressive results with respect to animal production, it is also clear that they carried some detrimental effects on animal welfare and behaviour as discussed above (Rauw *et al.*, 1998). It is important to investigate whether the use of broader breeding goals lead to an improved quality of the animal's life. Breeding for robustness is economically important in terms of functional traits such as health, fertility and longevity, and should improve the traits that are included in the index. Breeding for these traits may have a negative effect on other traits such as behaviour, and very little in-depth research has been carried out in this area. Despite the welfare advantages of breeding for functional traits, caution is needed. It can be questioned whether selection for these traits fully addresses the complete issue of welfare of dairy cows, and it must be ensured that selection for these traits does not have unforeseen consequences on behaviour. If there are strong genetic relationships between functional traits and behaviour, then breeding for better functional traits may lead to breeding for an alteration in variation of behavioural traits within the population. For example if breeding for health traits was positively correlated with increased competition during feeding, then selecting for these traits could result in breeding for more competitive cows. The possibilities and practicalities of selective breeding for temperament traits in livestock, together with the ethical and economic consequences of doing so, have been much debated in recent years by researchers and breeders. Many problematic behavioural traits have now been assessed for their likely response to selection including feather pecking and cannibalism in poultry, poor maternal care in sheep, fearfulness during handling in beef cattle, poor responses to milking in dairy cattle and, in pigs, tail-biting, aggression at regrouping and savaging and crushing of piglets by sows. It is enlightening, however, that selection has not yet been implemented in any of these cases apart from beef cattle fearfulness by certain breed societies in certain countries and, more commonly, dairy cattle behaviour during milking. If breeding programs are to select for temperament then proper ethical evaluation and regulation will be necessary on the basis that selecting on temperament may result in major changes to the integrity and sentience of the animals.

Before behaviour or temperament can be incorporated into selection indices, research needs to be undertaken to determine which behavioural traits are desirable or undesirable. Equally, it is important to investigate any undesirable consequences that selective breeding for other goals may have on animal behaviour. One way to achieve this is to investigate the differences in temperament between different genotypes at the commercial level. Furthermore, directed research towards more balanced breeding programmes that take into account temperament as well as production and health traits is required. The ultimate goal of this project is to investigate individual differences in behaviour of high and low robust dairy cows. To utilise behaviour as a robustness trait, it is important that the behaviour traits investigated have a) have direct relevance to robustness, b) are repeatable, i.e. it is an underlying behaviour trait that is being recorded and c) are easily recordable on many animals at the commercial level. In this thesis, a range of temperament traits will be investigated.

1.4. Temperament in livestock

Temperament refers to consistent individual differences in behaviour of animals (more detail in section 1.5.3). People who routinely work with animals notice individual animals have different temperaments. There are a number of different temperament traits which have been reported to have consistent individual differences, for example, aggressiveness (Riehart and Hendrick, 1992), risk-taking (Wilson *et al.,* 1994), fearfulness (Boissy 1995), sociability, exploration (Dingemanse *et al.,* 2002) and activity (Sih *et al.,* 2003). An animal's temperament can reflect how individual animals cope and interact with their environment (Manteca and Deag 1993; Boissy and Bouissou 1995). In farm animals, individuals that are less well adapted to their environment may have reduced welfare which in turn can lead to reduced productivity (Burrow and Dillon, 1997; Voisinet *et al.,* 1997).

Many authors have illustrated the importance of temperament from an economic viewpoint and a number of studies have demonstrated links between temperament and production. Beef cattle with poor temperament have been shown to have lower weight gain (Tulloh, 1961; Burrow and Dillon, 1997; Voisinet *et al.,* 1997; Petherick *et al.,* 2002) and poorer feed conversion efficiencies (Petherick *et al.,* 2002) compared to those with a calm temperament. Dairy cattle with calm temperament had a 25-30% greater milk production volume in comparison to those with poorer temperament (Drugociu *et al.,* 1977). Research carried out by Fell *et al.* (1999) showed that average daily gain was lower in a group of cattle classified as nervous compared to a group classified as calm (1.04kg/day vs. 1.46kg/day). Fell *et al.* (1999) also found a relationship between temperament traits and immunological status. Data from the experiments showed 42% of nervous animals were taken to the hospital pen for varying reasons but none of the calm animals needed removing from the herd during the experimental period. Experiments co-ordinated by Stahringer *et al*. (1990) demonstrated that heifers with poorer temperaments had a delayed onset of the oestrus cycle (i.e. puberty) in comparison to quieter heifers.

Although it would be advisable to investigate selection for temperament traits or assess the effect of selection for other traits on behaviour, progress in this area has been hampered by a number of issues. To date, the difficulty of objective behavioural data collection on large numbers of animals and the lack of heritability information has prevented the inclusion of behavioural traits in selection indices. In addition, for a trait to be included in a breeding program, it must have an important economic value and be easily measured at an acceptable cost (Schutz and Pajor 2001; Lawrence, 2008) and be heritable (Simm, 1998). Despite this, some countries have started to include some behavioural traits. For instance, dairy temperament (generally defined as the animal's response to milking) and milking speed have been included in breeding objectives of some countries. In Norway, the amount of emphasis placed on selection for dairy temperament has increased from 1.9 to 4% over the past 20 years (Heringstad *et al.,* 2001). In Australia, Bowman *et al.* (1996) incorporated both dairy temperament and milking speed in the breeding objective. By including either temperament or milking speed alone, the improvement of efficiency on milk, fat and protein was only 5%. However, by selecting with both traits, efficiency was further improved.

Determining the extent to which animals differ in their temperament will aid our understanding of how individuals respond to potentially stressful challenges (e.g. increase in stocking density due to disease outbreak, changes in routine or stockperson). Then we can start to ask questions about the benefits of different temperament traits to the animal and the owner or keeper and investigate the benefits of breeding for traits that are advantageous in specific environments. Selection for temperament traits is becoming increasingly important in breeding programmes, particularly in the case of pigs (D'Eath *et al.,* 2008; Turner *et al.,* 2008) and laying hens where selection for behavioural traits such as reduced aggression is of ethical importance. Researchers have begun to search for genes that govern the expression of temperament and are attempting to understand how the genes and environment interact with temperament (Chickens: Buitenhuis, 2003; 2004; 2008; Wiren *et al.,* 2008; Dairy Cattle: Gutiérrez-Gil *et al.,* 2008). Irresponsible selection for temperament may have a negative influence on other traits, therefore, genetic and environmental aspects of temperament should be explored further before including them into breeding programmes.

In the next section, I will discuss the concepts of temperament in animals and temperament (or personality) in humans. Personality in humans has been studied a great deal, and a number of studies have investigated the concepts in animals. Various descriptive frameworks and models for measuring human personality have been developed. Attempts have been made to apply these dimensions in animal research. In dogs for example, Gosling and John (1998) have suggested the existence of four dimensions: emotional reactivity (analogous to human neuroticism), energy (analogous to human extraversion), affection (analogous to human agreeableness) and intelligence (analogous to human openness/intellect). The final human dimension of conscientiousness does not map so readily onto animal behavioural traits. Applied research in livestock has usually focused on measuring only certain traits within certain personality dimensions in order to address a particular hypothesis. As such, most livestock research does not purport to measure personality *per se* and use of the term is restricted to occasions where correlations are demonstrated between a wide range of traits measured in diverse contexts and which are representative of a number of broad dimensions. Personality in humans has been studied a great deal, and a number of studies have investigated the concepts in animals. This literature is important to understand when we need to develop tests for temperament in dairy cows so that we can assess the effects of selection on these traits.

1.5 The concepts of temperament and personality in animals

1.5.1 Definitions of temperament or personality

There is controversy about the definition of the two related concepts of personality and temperament, but both terms are often used synonymously. The precise definition of temperament varies in the literature. Many definitions have been put forward over the years, many with the same underlying meaning. Table 1.1 provides examples of the different definitions available. Temperament often has a more restrictive meaning than personality. Temperament is used to describe formal aspects of behaviour such as differences in emotionality or describes traits that are demonstrated early in life in humans (Thomas and Chess 1977; Budaev, 1997; Clarke *et al.,* 2006). Temperament consists of traits an individual is born with. It differs from personality, which is a combination of your temperament and life experiences, although the two terms are often used interchangeably. Temperament is considered to be determined by your unique neurological characteristics and unlike personality, it is more resistant to change (Goldsmith *et al*., 1987). The point of consensus between various approaches is **that consistency over time and across situations** are the major distinguishing features of temperament and personality traits (Buss and Plomin, 1975, 1984; Strelau, 1983; Funder and Colvin 1991; Liebert and Spiegler, 1993; Jensen 1995; Budaev, 1997; Gosling, 2001). It is important to highlight that “consistent” does not mean that trait values cannot change with age or environmental conditions, but that differences between individuals are largely maintained (Réale, 2007).

Additionally, in the literature, there are a multitude of terms used to refer to the different aspects of temperament or personality which is often very confusing. The dimension of anxiousness is a good example, as it has been described as behavioural inhibition, fearfulness, emotionality, neuroticism, shyness, timidity and harm avoidance by various researchers (Kagan *et al*., 1988; Boissy, 1995; Ray and Hansen, 2004; Ley *et al.,* 2008). The same trait can be measured by different methods, and the same methods have been used in different fields to measure different traits (Gosling, 2001). For example, boldness can be associated with the reaction of an animal to a novel object, to a predator or to a conspecific. However, despite the variety of definitions and adjectives used, the underlying principle that animals and humans behave in consistent ways over time and situations is the main defining characteristic of a trait.

Table 1.1 A non-exhaustive list of definitions of temperament, personality and coping style[+]

Definition	***Source***
Temperament: a person's or animal's nature, especially as it permanently affects their behaviour. Personality: the combination of characteristics or qualities that form an individual's distinctive character.	The Oxford English Dictionary (2005)
Temperament: relatively consistent, basic dispositions inherent in the person that underlie and modulate the expression of activity, reactivity, emotionality, and sociability.	Buss *et al.* (1987)
In addition to the notion that temperament reflects biologically based individual differences in emotional responding, modern temperament theories also incorporate Allport's idea that these biological differences are innate and form the foundation upon which mature personality develops.	Clark and Wilson (1999), p. 400
Personality: those characteristics of individuals that describe and account for consistent patterns in feeling, thinking and behaving. Temperament: in human research the inherited, early appearing tendencies that continue throughout life and serve as foundation to personality.	Gosling (2001), p.46
Temperaments and personalities: integrated behavioural phenotypes and stable traits that are consistent over time and across situations; broad and consistent dimensions of individuality.	Budaev (1997), p. 399
Temperament: the individual basic stance towards environmental change and challenge	Mason (1984)
Temperament: biologically rooted individual differences in behaviour tendencies that are present early in life and are relatively stable across various kinds of situations and over the course of time.	Bates (1987)
Coping style: a coherent set of behavioural and physiological stress responses which is consistent over time and which is characteristic to a certain group of individuals. It seems that coping styles have been shaped by evolution and form general adaptive response patterns in reaction to everyday challenges in the natural habitat.	Koolhaas *et al.* (1999)

[+] Adapted from Réale *et al.,* 2007

1.5.2 History of the study of temperament in animals

The extension of the concepts of temperament and personality to animals is not new. Towards the beginning of the 20th century, Pott (1918) crudely described temperament in farm animals as "predisposing tendency in animals to convert its food either into milk or flesh". In the early 1930s, Pavlov (1955) first used dogs to model human temperament types and described individual differences in the conditioning performance of dogs. Hall (1941) defined temperament in rats "as consisting of the emotional nature, the basic-needs structure and the activity of an organism". In the past, personality has been typically used to describe humans, however, nowadays it is more acceptable to use the term personality to describe these characteristics in animals other than humans (Capitanio, 1999; Gosling, 2001). Some authors have regarded personality as incorporating issues such as self concept (Strelau, 1983). In this sense, some researchers have regarded "personality" as not being applicable to animals and for this reason do not like to use the term 'personality' when referring to animal temperament, because using the word 'personality' then becomes a form of anthropomorphism. However, there is a growing body of evidence for self-awareness and consciousness in some non-human species (Griffin, 1993; Dawkins, 1993). Therefore, the term "personality" should have a wider applicability to both humans and animals. It is possible to apply the terms "temperament" and "personality" to the behaviour of animals without any impression of anthropomorphism provided it is defined objectively and precisely.

In more recent years, a range of terms have been used by animal ethologists to describe individual differences in behaviour between animals. Some of these terms include temperament (Lansade *et al.,* 2008 a,b,c; Olmos and Turner, 2008; Lansade and Bouissou, 2008; Hoppe *et al.,* 2008; Ngaio *et al.,* 2008), personality (Gosling, 2001; Philips and Peck, 2007; McGrogan *et al.,* 2008; Lloyd *et al.,* 2008; Ley *et al.,* 2008), behavioural syndromes (Bell, 2005; Martin and Réale, 2008), coping strategies (Koolhaas *et al.,* 1999; Bolhuis *et al.,* 2006; Kristiansen and Fernö, 2007) and constructs or axes (Gosling, 2001; Sih *et al.,* 2004). These terms have been used to refer to temperament traits in a wide variety of species from octopuses to primates and humans (Mather and Anderson, 1993; Boissy, 1995; King and Figueredo, 1997; Koolhaas *et al.,* 1999; Gosling, 2001; Sih *et al.,* 2004). At the present time, there is no clear and meaningful distinction in terminology (Matthews *et al.,* 2003). For the purpose of this paper, I will use the term temperament for the sole reason that it is the preferred term in the dairy industry, except where it is more appropriate to use the term personality, for example in the case of quoting or referring to a study that uses the term 'personality'.

1.5.3 Coping style and temperament

The term 'coping style' (Koolhaas *et al*., 1999) is used to refer to a set of correlated temperament or personality traits. Coping styles and temperament traits can be linked together. For instance, proactive individuals considered to be non-neophobic are exploratory, bold, aggressive, active, non-flexible individuals whereas neophobic individuals are unexploratory, shy, non-aggressive, non-active and highly flexible individuals and can be considered as reactive. Individuals are classified according to their ability to cope with novelty, risk and challenging situations, such as those described in the literature (Engel and Schmale, 1972; Henry and Stephen, 1977; Benus *et al.*, 1991). For example, the "coping style hypothesis" describes the responses of animals to social and non-social challenging or novel situations that are consistent between individuals and plays a major role in applied animal behaviour research (Koolhaas *et al.,* 1997, 1999).

The "coping style hypothesis" was originally based on work with wild house mice, and indicated that there is a bimodal distribution with individuals showing very high or very low levels of aggression. Individuals at either extreme may have a selective advantage under different environmental conditions (Benus, 1991). There are many "coping style" terms used in the literature, such as 'manipulators' and 'adjusters' (Benus *et al.,* 1991; Sluyter *et al.*, 2000), proactive and reactive copers (Koolhass *et al.*, 1999), fast and slow attackers and active and passive copers (Benus *et al.*, 1991) that describe the extreme phenotypes within a population. Regardless of which label is used, each type has a closely associated set of behavioural and physiological characteristics. Even though bimodal distributions have been identified in some studies (Verbeek *et al.,* 1994; Budaev, 1997), this is not the case in others (Dellu *et al.*, 1993, 1996; Forkman *et al.,* 1995).

Individuals will often vary along a continuum with two extremes represented as tendencies (shy-bold: Wilson *et al.,* 1994; proactive-reactive: Koolhaas *et al.,* 1999). For example, the shyness-boldness continuum is an essential element of behavioural variation that has been researched in both humans and other species (Wilson *et al.*, 1994). These differences in shyness and boldness of individuals may be due to the tendency of a bold animal to take the most risks while shy animals are more likely to avoid risks (Kagan *et al*., 1988, Wilson *et al*., 1994; Coleman and Wilson 1998). Boldness has been correlated with increased risk taking (Mettler and Shivik, 2006), increased dominance (Mettler and Shivik, 2006) and longer life span (Cavigelli and McClintock, 2008). Shy-bold traits have been shown to be partly heritable (Dingemanse *et al.,* 2002) and partly learned (De Azevedo and Young, 2006). Shy-bold traits are correlated with past experiences with predators, past exposure to risky situations or novel stimuli. Terms such as ‘approach’, ‘avoidance’, and ‘sociability’ are correlated with shy-bold behaviour.

There is a difference in the expression of shy-bold traits based on context (Coleman and Wilson, 1997; Réale *et al.,* 2000). Context specificity occurs when an individual’s expression of a trait varies depending on the context (Coleman and Wilson, 1998; Sih *et al.,* 2004). However, there is much debate as to whether the shy-bold continuum is context specific. It is not within the scope of this review to go into further detail of context specificity, however, context-specific personality traits in human and animal studies have been reported (Kagan *et al.,* 1988; Siegel and Macdonald 1998; Coleman and Wilson, 1998; Réale *et al.,* 2000; Wilson and Stevens 2005).

1.5.4 Measures of human personality and their use in animal research

Various descriptive frameworks and models for measuring human personality have been proposed and used throughout the years. The most influential structural theory of personality is the five-factor model (FFM) and is used to describe the variation in human personalities (Digman, 1989; 1990). The five factor theory, asserts that all personality traits are biologically based (Blatny *et al.,* 2007). The FFM is a hierarchical model with five broad dimensions:

- Neuroticism (associated with anxiety, fearfulness, frustration, negative affectivity)
- Extraversion (sociability, exploration, impulsiveness, novelty seeking, positive affectivity and activity)
- Conscientiousness (competence and self-discipline)
- Agreeableness (trust and compliance) and
- Openness to experience (intellect).

A large number of traits in each of the five broad factors are used to characterise someone's personality, such as outgoing, friendly, reserved, hostile or competitive. However, Extraversion and Neuroticism have a strong physiological and genetic background compared to the other factors.

The five-factor model of human personality provides a useful framework for the study of personality traits and can be adapted to describe many behaviour traits in animals. In dogs, Gosling and John (1998) suggest four dimensions which best represent dog personality traits. These are Energy (analogous to human Extraversion), Affection (analogous to human Agreeableness), Emotional reactivity (analogous to human Neuroticism) and Intelligence (analogous to human Openness/Intellect). The final dimension in the human five factor model is Conscientiousness but there is no evidence of it in any species other than humans and chimpanzees. Similarly, Réale *et al*. (2007) suggests the following categories for animals: activity, shyness or boldness (response to potentially risky situations), exploration (response to novel situations), aggressiveness and sociability.

Among other temperament theorists, Buss and Plomin (1984) theorised that temperament is composed of a set of early-developing personality traits. They distinguished between three basic temperaments referred to as 'EAS' or Emotionality, Activity and Sociability, which break down into more specific components as shown in Table 1.2. This model agrees with the FFM in that Emotionality can be related to Neuroticism while the remaining traits make up Extraversion. Buss and Plomin (1984) argues that these components are heritable, stable, predictive of adult personality, adaptive in an evolutionary sense and that EAS temperament classes are evident in other primates. Thomas and Chess (1977) listed nine dimensions, and Derryberry and Rothbart (1988) proposed nineteen but most of these dimensions appear to relate theoretically to the EAS temperaments. Kagan *et al.* (1988) proposed the use of a shyness-boldness continuum in children which was defined as behavioural inhibition versus boldness. For a more detailed review of the different structures of personality in humans and animals see Budaev (2000).

In human personality studies, traits of temperament are usually measured using questionnaires. In order to minimise subjectivity, researchers often collect data on one individual from numerous questionnaires to obtain a more objective measure (Cavigelli, 2005). However, these are methods that are not adaptable to animal and infant temperament research. Researchers rely on two main methods to measure temperament or personality of animals. The first of which is an individual's behavioural responses to a variety of environmental situations (Matthews *et al.*, 2003). This enables researchers to rely on direct observations, which provide an objective way to identify reliable individual differences between animals. Such methods can be carried out over a reasonably extended period and continual developments in technology allow for longer and more detailed behavioural sampling. Alternatively, researchers can rely on interviews with humans that have observed the animals or infants in various situations (Gosling and John, 1999).

Table 1.2 Components of temperament described by Buss and Plomin (1984)

Temperament	*Component*
Emotionality	
-Fear	Apprehension, worry, fear face, escape, avoidance
-Anger	Transient hostility, angry face, pout, angry, aggression
-Distress	
Activity	Tempo, vigour, endurance
Sociability	Tendency to affiliate, responsiveness when with others

* Adapted from Matthews *et al.*, 2003.

1.6 How to define temperament in cattle

In farm animals, temperament is often described as an individual trait influencing an animal's behavioural response to handling. For instance, in an early study by Tulloh (1961) the term temperament was used to describe the "behaviour of cattle in the bail". However, other authors describe temperament as more than this. Lyons (1989) described temperament as an enduring characteristic of an individual's overall behavioural style, emotional tone, reactivity or responsiveness which is a "dynamic attribute of an individual that modulates environmental influences on behavioural and physiological systems". This means that temperament is described not merely as the response to handling or restraint. The temperament of animals depends not only on their reactions to people but also to social and environmental situations and novelty (Grignard *et al.,* 2000, 2001). Conversely, some other researchers (Kerr and Wood-Gush, 1987) used the term behavioural pattern to describe most behaviours and temperament to indicate exclusively how reactive or docile animals are to challenge by humans.

1.6.1 Temperament tests used in cattle

Many tests have been developed to assess cattle temperament. However, most researchers have developed their own methods to assess temperament depending on the situation. To date, there has been no agreed upon criteria to assess temperament in cattle. The main tests of temperament in cattle are the open-field test (Kilgour, 1975), social separation test (Boissy and Bouissou, 1995; de Passillé *et al.,* 1995; Boissy and Le Neindre, 1997; van Reenan *et al.,* 2004; Müller and Schrader, 2005) the flight tests or docility tests (Le Neindre *et al.,* 1995), the crush test (Tulloh, 1961; Grignard *et al.,* 2001) and the flight speed test (Burrow *et al.,* 1988). These will be discussed in further detail below.

1.6.1.1 Open-field test

The open-field test is designed to measure behavioural responses such as locomotor, activity and exploratory behaviour as reactions to a novel environment. The open-field test has been extensively used in laboratory animals. The open-field test involves placing an animal in a novel arena for a few minutes and then recording some aspects of its behaviour thought to best represent the degree of fear the animal shows. The open-field arena acts as an anxiogenic stimulus and allows for measurement of anxiety induced behaviours. The open-field test has been used to measure response to fear provoking situations in cattle (Kilgour, 1975). Temperament ratings based on scales include; ambulation scores, vocalisation score, elimination score (Kilgour, 1975) and ease of sorting (Boivin *et al.,* 1992b; Kilgour *et al.,* 2006). However, its efficacy and validity has been questioned in farm animal temperament studies (Manteca and Deag, 1993; Walsh and Cummins, 1976). It is likely that the behaviour of cattle in an open-field reflects a number of different motivations (e.g. fear of the handling involved in moving the animal to the open field, distress at being separated from social companions etc.) rather than a single motivation such as fearfulness of open spaces or locomotor motivation.

1.6.1.2 Flight and docility tests

The flight test (also referred to as human approach test or avoidance test) was first established as a means to examine the influence of human behaviour and interaction with a human on the emotional state of an animal, i.e. fearfulness (Hemsworth *et al.,* 1986; Von Borell and Veissier, 2007). Although these tests have been used for over 20 years to assess behaviour, their validity and repeatability have not been evaluated in all farm animal species. Flight test responses by cattle have been measured in a range of different experimental conditions, ranging from testing the animals at pasture (Murphey *et al.,* 1980), in the home pen (Waiblinger *et al.,* 2006) to testing in an open-field (Jago *et al.,* 1999; Kilgour *et al.,* 2006). Flight and human approach tests have more recently been adapted for use on commercial farms as part of on-farm welfare assessments (Waiblinger *et al.,* 2006; Windschnurer *et al.,* 2008, 2009). However, there are some problems with using these tests. Firstly, it can be argued that the avoidance distance can be influenced by habituation. An increase in frequency of people walking past pens may result in the animals avoiding humans less. It is expected that visual contact without aversive experience has a positive effect on the responsiveness of animals (Waiblinger *et al.,* 2006). Additionally, other factors that have been shown to affect the responsiveness of animals to humans are stockperson attitude and milking behaviour (Jago *et al.,* 1999; Breuer *et al.,* 2003; Waiblinger *et al.,* 2003), and the type of calf rearing system the animal was reared in (Raussi *et al.,* 2003). However, in dairy cattle it has been shown that the avoidance distance is not context specific (Waiblinger *et al.,* 2003; Windschnurer *et al.,* 2008).

A related test that assesses the reaction of an animal to a human is the docility test. The docility test involves an experimenter attempting to restrain an animal for 30 seconds in a corner of a testing pen with only his/her arms. A docility score is calculated by combining different behaviours measured during the test. Docility score has a heritability of 0.2 (Le Neindre *et al*., 1995). In France the docility test has been used to select for improved temperament in Limousin cattle since 1992. Limousin bulls that are part of AI breeding programmes are evaluated on the docility test in test stations and in progeny test stations.

1.6.1.3 Crush test & flight speed

The crush test involves restraining an animal in a handling crush and measuring its behavioural response to the restraint. A number of researchers have used a subjective scale to assess the temperament. Grandin (1993) relied on an observer to rank beef cattle (steers) temperament based on their movements in the squeeze chute on a five point scale. Another criteria used by the author to grade temperament was whether they balked when entering the squeeze chute. The movement score assigned to beef cattle by Kilgour *et al.* (2006) was on a subjective 1-7 scale. Based on reactivity during restraint, Ewbank (1961) classified animals as docile, alarmed, greatly alarmed or submissive. Holmes *et al.* (1972) used an observer grading of temperament from 1 to 5 (quiet to unmanageable) during restraint in the squeeze chute. Shrode and Hammack (1971) also used a 1 to 5 scale and termed animals with a score of 5 as being most rebellious. Similarly a 1 to 5 scale of quiet to nervousness was used by Vanderwert *et al.* (1985).

Objective techniques, such as time taken to move a measured distance after release from restraint, have also been used in a limited number of experiments with beef cattle (Burrow *et al.,* 1988; Kilgour *et al.,* 2006; Muller and Von Keyserlingk, 2006; Curley *et al.,* 2006). Kilgour *et al.* (2006) also assessed the distance up to which an animal could tolerate the presence of an observer. In addition to behavioural measures, physiological parameters such as heart rate (Le Neindre, 1989; Gringnard *et al.,* 2001; Kilgour *et al.,* 2006) and cortisol levels (Munksgaard and Simonsen, 1996) have been used to assess reactivity. Manteca and Deag (1993) highlighted the importance of using a variety of tests or measures in assessing temperament as we may miss many facets of temperament if we stick to a single test or measure.

1.6.1.4 Explanatory variables

It is important that explanatory variables such as breed, age and environmental conditions are taken into account in temperament studies. Differences in temperament exist between breeds. Murphey *et al.* (1980) found that he could approach dairy bred animals more closely than beef bred animals. Mullan *et al.* (2001) reported that Dutch Holstein showed greater reactivity in an open-field test than Norwegian dairy cattle. Furthermore, management system had a greater effect on the behaviour of Dutch Holstein cattle compared to Norwegian dairy cattle. The Dutch Holstein cattle showed greater exploratory behaviours when housed indoors compared to those at grass. Boivin *et al.* (1992a) concluded that in beef calves, handling at weaning was more effective in improving temperament than preweaning handling. Le Neindre *et al.* (1995) handled Limousin heifers in a pen, and measured the amount of aggression, the time spent in the corner of the pen as well as escape reactions, in order to assign a 'docility score'. The study found that animals that were maintained indoors were more docile than those reared outside.

1.6.2 Measuring temperament traits

When developing tests to measure temperament, it is important to consider trait repeatability to ensure it is an underlying temperament trait that is being measured rather than a transient response to the environment. Additionally, it is important to measure heritability of temperament traits in order for it to be incorporated into a breeding programme.

1.6.2.1 Repeatability

Repeatability is a concept that measures how consistent individuals are in their behaviour (Lessells and Boag, 1987; Boake, 1989; Falconer and Mackay, 1996) within and between rounds of tests (Kilgour, 1998; Le Neindre *et al.,* 1998; Erhard, 2003) and is a statistic that gives an estimate of the proportion of variation among individuals that is due to individual differences (Falconer and Mackay, 1996). Assessing repeatability to allow behavioural tests to be validated can be difficult. This is because animals can react differently: some may habituate to the test-situation and some others may lower their threshold for expressing behaviour after being subjected to the same test situation several times (Forkman *et al.,* 2007). This is especially the case in tests based on novelty since test situations are not novel from the second exposure.

The variance of behaviour can be analysed into a component within individuals, measuring the differences between the performance of the same individual, and a component between individuals, measuring the permanent differences between individuals (Falconer and Mackay, 1996). The within individual component is entirely environmental, caused by temporary differences of environment between successive repeats. The between-individual component is partly environmental and partly genetic, the environmental part being caused by circumstances that affect the individuals permanently (Falconer and Mackay, 1996). In this analysis, the variance due to temporary environmental circumstances is separated from the rest, and thus can be assessed (Falconer and Mackay, 1996). Repeatability is calculated from the between animal and within animal components of variance as r = variation between animal/(variation between animals + variation within animals).

Repeatability equals heritability in the broad sense plus any environmental effects which persist over the period of observations. Repeatability can therefore be regarded as an upper limit on heritability if no permanent environmental effects exist (Boake, 1989). Therefore, heritability will never be higher than repeatability. In calculations of repeatability, relationships between individuals need not be known, whereas heritabilities are calculated for individuals of known relationships (Boake, 1989). The level of repeatability can be used to indicate whether efforts to measure heritability are likely to be worthwhile (Boake, 1989).

Understanding whether individuals show consistent behaviours in repeated trials is not always easy to ascertain from the repeatability ratio because low repeatability values can indicate either consistent behaviour (low variation between and within individuals) or random behavioural response (high variation between and within individuals) (Widemo and Sæther, 1999; Cummings and Mollaghan, 2006). Repeatability can be low for three reasons: (1) when between animal variation is relatively small. This will occur if individuals are relatively similar and similarity might be attributable to either genetic or environmental effects. Further experimentation or use of a more sensitive test would help understand the influence of each effect. (2) The between animal variation is relatively large. This is a consequence of environmental influences. These influences might occur in conditions that are not suitably controlled. Factors such as temperature, diurnal rhythms or hormonal state could affect an animal's performance and (3) Repeatability can also be low if learning occurred between successive measurements of the behaviour as indicated by changes in within animal scores.

To examine trends in repeatability values calculated from variance components for temperament traits, I conducted a literature survey to summarise the currently published repeatability values. At present, relatively little comparative information is available on repeatability of all aspects of cattle temperament traits as most of the literature covers flight speeds and crush scores. Kadel *et al.* (2006) reported repeatabilities of 0.46 for flight time exiting a crush and 0.36 for a subjective crush score. Halloway and Johnston (2003) reported similar repeatabilities of flight time and crush score (0.31 and 0.44) for Angus cattle measured 73 days apart. However, Petherick *et al.* (2002) reported a higher repeatability for flight speed before and after feedlot entry of 0.68 in 2-3 year old Brahman steers. Kilgour *et al.* (2006) found a repeatability coefficient of 0.19 for flight distance to an approaching human in a socially isolated and novel environment.

Due to the scarcity of literature on repeatability of cattle temperament traits, other livestock species were also reviewed. Wolf *et al.* (2008) examined the repeatability of behaviour in sheep in an arena test. The repeatability of traits for vocalisation (0.58 – 0.71) tended to be higher than for locomotion (0.38-0.40) and for proximity to a human (0.17-0.60). Kilgour and Szantar-Coddington (1995) found similar results with repeatability estimates of 0.48 for locomotion (total distance travelled) and 0.57 for the number of bleats. However, in a similar trial, Kilgour (1998) showed repeatability for distance travelled (0.61) and the number of bleats (0.25). Repeatability estimates for ewe maternal behaviour score have been shown to vary from 0.09 (Everett-Hincks *et al.,* 2005) to 0.32 (Lambe *et al.,* 2001), however the difference in these repeatability values may be due to the different scales used. Everett-Hincks *et al*. (2005) used a 5 point scale whereas Lambe *et al.* (2001) used a 6 point scale. Valros *et al.* (2003) determined if individual sows are stable in their activity, frequency and carefulness of standing-to-lying over the course of lactation. Sow activity level and frequency of standing-to-lying increased and showed high within-sow repeatability (0.51 and 0.50) throughout a 5-week period. The 'carefulness of standing-to-lying' score did not change significantly over lactation and showed low within-sow repeatability (0.27). Collectively these studies show that many important and relevant temperament traits have good repeatabilities.

1.6.2.2 Heritability of temperament traits

It is equally important to assess the genetic component of temperament. A number of studies have shown that temperament has moderate heritabilities (Burrow, 1997). In both the beef and dairy industries, this has promoted interest in genetic selection for or against specific temperament traits in relation to commercial production (Brotherstone, 1995; Donoghue *et al.,* 2006). There is a significant genetic variation for production and specific temperament traits within the cattle population. The heritability (h^2) of a trait is defined as the proportion of variation in a population that is due to the variation in genetics between animals (Simm, 1998). Low heritability values reflect the fact that the variation in a specific trait is primarily due to environmental influences, rather than being due to genetics. As a result the trait is difficult to alter by selection. Heritability can therefore be estimated from the correlation between related animals. There are three types of correlation that are widely used in animal breeding and trait selection: phenotypic, genetic and environmental (Simm, 1998). Simm (1998) provides definition for all three types of correlations. Phenotypic correlations measure the direction and strength of the association between two performance traits, for example, the correlation between live weight and fat depth measured on the same animal. Genetic correlations measure the direction and strength of the association between genetic merit or breeding values for the two characteristics. Whereas, an environmental correlation is a measure of the extent to which environmental conditions that are favourable for one character are favourable or unfavourable for a second.

Few studies have been conducted to estimate the genetic, as opposed to the environmental, effects on behaviour traits. The heritability of feeding behaviour was first studied by Hancock (1950). This work suggested a strong genetic component to grazing behaviour, however, later research has shown weaker heritabilities of grazing behaviour (Macha and Olsarova, 1986). Managing a dairy farm with maximum efficiency relies heavily on the docility of the cows. It is important to ask if dairy sires differ in the behaviour or temperament of their daughters. Depending upon the type of temperament test, researchers have found varying degree of levels of heritability for docility (Table 1.3). Heritability estimates of behavioural traits range from weakly inheritable (0.01) to more heritable (>0.03). To put this in perspective, the heritability of milk yield, for example, ranges from 0.20 to 0.25. Heritability of milking behaviour is lower than milk yield being approximately 0.16. Behavioural responses in the crush have generally been found to have a variable heritability of 0 to 0.4. The heritabilities for other behavioural traits such as responsiveness to novelty or social isolation and sociability have not been estimated and warrant future research.

Table 1.3 Heritability estimates of temperament traits

Authors & Year	**Temperament Trait**	**Breed**	**h^2**
O'Blesness *et al.,* 1960	Temperament	Dairy	0.40
Beilharz *et al.,* 1966	Dominance		0.44
Dickson *et al.,* 1970	Milking temperament	Dairy	0.53
Shode and Hammack, 1971	Crush Score	Beef	0.40
Mishra *et al.,* 1975	Temperament	Dairy	0.19
Wickham, 1979	Temperament	Dairy	0.09-0.12
Strickin *et al.,* 1980	Social behaviour	Beef	0.48
Sato *et al.,* 1981	Temperament	Beef	0.45
Fordyce *et al.,* 1982	Movement in crush Movement in race Movement in crush + restraint	Beef	0.25 0.17 0.67
Fordyce and Goddard, 1984	Temperament in crush Vigour of movement Kicking Bellowing Kneeling down	Beef	0-0.10 0.0 0.0 0.09 0.10
Buddenberg *et al.,* 1986	Maternal behaviour		0.06
Sullivan and Burnside, 1988	Aggression during feeding	Dairy	0.11
Morris *et al.,* 1994	Handling	Beef	0.22
Le Neindre *et al.,* 1995	Docility Score	Beef	0.22
Visscher and Goddard, 1995	Temperament	Dairy	0.18-0.29
Burrow and Corbet, 1999	Flight speed Flight speed score Crush score	Beef	0.38-0.45 0.08 0.30

h^2=Heritability

1.6.2.3 Interpretation and validity

The study of animal temperament/personality is in its infancy and there is much controversy in the areas of the methodology and interpretation. It is also important to assess validity of temperament tests. Validity concerns the extent to which behavioural measurements actually measure those traits the experimenter wishes to measure (Manteca and Deag, 1993). Measures of temperament traits are inferred from behaviour seen during specially designed tests (e.g. flight scores) or in specific contexts (e.g. crush scores). Often the interpretations used have been over-simplistic and the measures taken lack evidence of validity (Rushen, 2000). There are different ways of determining whether a behavioural test gives reliable information. Most studies investigating aspects of temperament used the correlational approach to link behaviour across a range of situations in the search for consistent individual differences (Lawrence *et al.,* 1991; Jensen, 1994; Forkman *et al.,* 1995; Spoolder *et al.,* 1996). The main method for validating behavioural results is to look at whether animals express the same traits in other similar situations (Pervin, 1996) by carrying out a large number of tests in a variety of situations, then using statistical procedures to find out which behaviours in which of the tests are related. If links are found, they are interpreted post-hoc and named (e.g. sociability, activity etc.). Methods often used are principal component analysis and factor analysis (e.g. Forkman *et al.,* 1995; Spoolder *et al.,* 1996). However, Liebert and Spiegler (1993) criticised this approach by pointing out that the analysis of the data entailed many subjective decisions. Another option is to develop separate tests which assess specific temperament traits. For example, assessing a trait of fearfulness in two different situations, one involving novelty and the other involving suddenness. Each test has to be shown to be consistent across time and situations for it to be a meaningful indicator of temperament. The different tests can then be applied to a number of individuals to investigate relationships between the temperament traits and correlated to assess validity.

Correlations found between behaviours in different tests show that the behavioural responses are useful measures, and not purely a result of very specific responses to immediate test environment only (Mendl and Harcourt, 1988). Temperament is thought to be composed of several dimensions. Consistency or lack of it in one of these dimensions is potentially independent of consistency or lack of it in the other (Goldsmith *et al.,* 1987). Therefore, correlations are only expected between behaviours from tests that are thought to measure the same trait.

1.7 Conclusions and objectives of the study

There is an urgent need to determine the level of genetic effects on temperament traits and to understand the interacting environmental effects. Research directed towards more balanced breeding programmes that take into account behavioural as well as physical traits is needed to address welfare concerns. Before temperament traits can be incorporated into selective breeding programmes, it is essential to evaluate the potential consequences of selection for these traits on other functional and production traits. It is necessary, therefore, to increase our knowledge of how temperament traits are influenced by other behaviours as well as the animal's genotype and environment. Identifying characteristics of dairy cattle temperament which relate to robustness and utilising genetic tools to develop suitable breeding programmes with wider goals which incorporate temperament across a range of environments offers scope to develop a more welfare friendly and sustainable industry.

1.8 Thesis aims

This review presents the concept of temperament, discusses the different definitions of temperament and the suitability of developing an integrated concept of temperament. It also focused on the methods used in the past to measure temperament in cattle and contrasts objective and subjective techniques. Past research on heritable aspects of temperament and applications of research on temperament in relation to production was also discussed.

The aim of this project was to investigate the consistency of three different temperament traits in dairy cattle and assess one of these at a commercial level on cows from different extremes of a robustness index. Firstly, temperament traits that may be implicated in robustness needed to be identified and secondly temperament tests needed to be designed and validated for the identified traits. Specifically, the aims were:

1. To design a practical test to measure emotional responsiveness in dairy cattle and assess intra- and inter- test consistency.
2. To investigate the agonistic behavioural reactions of dairy cattle during feeding and to assess whether cow variables (age and lactation stage) and management variables (feedface space per cow) influence the expression of aggressiveness in individual cows.
3. To develop reliable and valid tests to assess sociability of individual dairy cows.
4. To investigate if selection for high and low robustness affects aggression during feeding in cattle on commercial farms.

CHAPTER 2

Responsiveness of Dairy Cows to Human Approach and Novel Stimuli

In this chapter, I was responsible for experimental design, carrying out the experiment, data and statistical analysis and writing the manuscript.

2.1 *Abstract*

This study investigated intra-test and inter-test consistency of dairy cattle temperament to a series of tests involving human approach and exposure to novelty. Thirty-six lactating Holstein-Friesian cows were each subjected to three human approach tests and three novel stimuli tests. Flight response score was assessed by an experimenter approaching cows when they were in the passageway of the home-pen (AP), lying down (AL) and at the feedface (FF). Each human approach test was repeated on each cow three times. The intra-animal repeatability estimates were 0.65, 0.40, and 0.27 for AP, AL and FF tests, respectively. Repeatability evaluates an individual's consistency across tests by comparing it to the variation within the group. Cows showed moderate consistency in their flight response scores to the different approach tests (W_{35}=0.56, P<0.01). Three novel stimuli (water spray, striped boards and flashing light) were individually presented once to each cow. Investigatory and reactivity behavioural responses were assessed. Cows showed the greatest reactivity response to the water spray compared to the striped boards (U_1=56, P<0.001) and flashing light (U_1=66, P<0.001). No statistically significant agreement existed between the novel stimuli reactivity and investigatory responses with the AP flight response scores. In conclusion, consistency over time was demonstrated over a relatively short period for the AP test and consistency between human approach situations was shown, however, consistency between human and novel situations was not found.

2.2 *Introduction*

It is considered that selection for production alone in farm animal species has resulted in numerous undesirable side effects in animal behaviour and physiology (Grandin and Dessing, 1998; Pigs: Geers *et al.,* 1990; Rauw *et al.,* 1998; Chickens: Jones and Hocking, 1999; Jensen and Andersson, 2005; Dairy cattle: Rauw *et al.,* 1998, Royal *et al.,* 2000). Grandin (1993, 1994) observed that breeding for slender body shape and a lean carcass composition has resulted in cattle and pigs with easily excitable temperaments. This leads to increased balking and handling problems. Pigs

selected for high lean gain can have more excitable temperaments and are more fearful than fatter genetic lines (Shea-Moore, 1998). Pigs with higher lean growth show an increased stress response to transportation leading to lower meat quality (Grandin, 1997). On the other hand, over-selection for a single behavioural trait can also cause problems. Belyaev (1979) found that selecting foxes for a calm temperament resulted in negative effects on maternal behaviour, changes to both body shape and coat colour, and neurological problems. Breeding for desirable temperament traits is becoming increasingly important, particularly for good mothering ability in pigs (Grandinson, 2005), reduced aggression in pigs (Turner *et al.*, 2008) and laying hens (Blokhuis and Wiepkema, 1998; Buitenhuis *et al.*, 2003).
In the dairy industry, there is considerable evidence that selecting for production traits alone is associated with a reduction in health and fertility (Rauw *et al.,* 1998; Royal *et al.,* 2000; Pryce *et al.,* 2001, 2002; Veerkamp, 2003). Dairy breeding companies have recognised these problems and are enhancing their breeding indices to include functional traits to improve cow fertility, calving ease, survival and to reduce lameness and mastitis (Cassell, 2001; Wall *et al.*, 2007). While it is valuable to improve functional traits, it is important to determine if there are any contributions or possible undesirable consequences that the use of these breeding programmes may have on dairy cow temperament.

To date, minimal investigation into the effect of selective breeding on dairy cattle temperament has been carried out. It is possible that selection programmes may alter dairy cattle temperament, in order to investigate this, I first need to design tests to measure specific temperament traits in dairy cattle. This paper focuses on two aspects of dairy cow temperament, responsiveness to human and environmental (novelty) challenge. It is accepted that animals react to humans and novelty with a strong inter-individual variability (Cattle: Kilgour *et al*., 2006; Goats: Lyons *et al.,* 1988; Pigs: Lawrence *et al*., 1991). Human handling procedures may elicit stronger responses in some animals than others causing them stress, while animals that are over-reactive in response to novelty may not respond well to changes in their daily routine or environment. An ideal level of responsiveness is one that is adaptive, resulting in functional reactions to challenging situations.

Emotional responsiveness towards humans and environmental challenges (e.g. novel objects) could be considered to be a temperament trait. Temperament is generally defined as a behavioural tendency present early in life and relatively consistent across various kinds of situations and over the course of time (Humans: Bates, 1987). Although, human researchers do not uniformly agree with this definition (McCrae *et al*., 2000), animal researchers agree even less about how to define temperament (Gosling, 2001). The distinction between temperament and personality is unclear and is not consistent in the literature. The point of consensus between the various approaches is that an individual's temperament or personality remains relatively stable across various kinds of situations and over time (Humans: Buss and Plomin, 1975, 1984; Funder and Colvin 1991; Liebert and Spiegler, 1993; Pigs: Jensen 1995). For the purpose of this paper, I will use the term temperament, as it is the preferred term in the dairy industry.

The human literature generally suggests that for a behaviour to be classed as a temperament trait the animal must show consistency in its behaviour over time and across situations. Reactions towards humans have been shown to be stable for a period of several weeks (cattle: Grandin, 1993), several months (Goats: Lyons *et al*., 1988) or even several years (Bighorn sheep: Rèale *et al*., 2000; Horses: Lansade and Bouissou, 2008d). Some experimental studies have shown that reaction to humans remains stable across different situations. In cattle, Grignard *et al*. (2001) found correlations between responses to a docility test and to a crush test.

It has been also been shown that animals response to human handling changes over time (Erhard *et al*., 2006; Wolf *et al*., 2008). However, a stable temperament trait may exist if each individual’s change in response follows a consistent pattern and inter-individual variation still exits at the end of the testing period. A study assessing cattle’s response to human approach at a feedface found a suggestive QTL despite habituation shown in repeated tests (Gutiérrez-Gil *et al,* 2008). This study suggests an underlying genetic basis to this trait and therefore provides evidence for a genetic influence on cattle temperament.

In order for temperament tests to be feasible for use on commercial farms, it is necessary to be able to test the animal in its home environment without removing the animals from its social group. Many researchers have done this by evaluating human approach and avoidance tests in the home environment (Rousing and Waiblinger, 2004), at the feedface (Waiblinger *et al.*, 2003; Winckler *et al.*, 2007) and while lying (Windschnurer *et al.*, 2008). The principle behind these studies is that the amount of avoidance or approach behaviour provides an integrated measure of the fear level in the animals (Hemsworth and Coleman, 1998) as part of on-farm welfare assessments. Additionally, it is necessary for animal based welfare assessments to be short in duration in order to assess large number of animals during a short time frame. However, the human approach test used in the present study is subtly different, as I aimed to allow the animal sufficient time to express its innate temperament as suggested by Marchant *et al*. (1997).

In addition to the human approach test, I aimed to develop a test that assessed the animals' response to novelty. Herskin *et al.* (2004) and Schrader (2002) conducted novel object test with dairy cattle with minimal situational novelty in the home- pen. In spite of several researchers measuring animal responses to a variety of novel stimuli, few studies have investigated novelty tests in the home environment on commercial farms. McMullan *et al.* (2006) assessed the reactivity of dairy cattle to a surprise effect test (waterspray) on 22 commercial farms. In the present paper, tests were designed to measure dairy cattle's response to novel stimuli in the exit route from the milking parlour. A criterion of this test was that human handling was minimal to differentiate human approach and novel object tests.

Ultimately, the aim was to design practical tests that will measure emotional responsiveness in dairy cows on commercial farms. I evaluated three tests of human approach and three novel stimuli tests. Each test procedure was designed to provide a challenging situation which drew out aspects of the animal's individual temperament in a familiar environment, in the presence of conspecifics, and without altering the social set-up. I then assessed the three human approach and three novel stimuli tests for intra- and inter-test consistency.

2.3 Materials and methods

2.3.1 Animals and management

The study was conducted at the SAC Dairy Research Centre (Dumfries, Scotland, UK). The experimental animals used were thirty-six healthy lactating Holstein-Friesian cows. There were five primiparous and thirty-one multiparous cows (parity = 3.3 ± 2.1; mean ± S.D.). When necessary, the parity of the animals was balanced across experimental groups. All cows were subjected to the same husbandry procedures and fed a total mixed ration (TMR). The TMR was available *ad libitum* and consisted of 59% grass silage and 41% concentrate on a dry matter basis. The study was carried out during the winter period while the cows were housed indoors. The cows fed from a feedface with a diagonal railed feed barrier design. The housing contained rubber-matted cubicles with saw-dust covering and automatically scraped passageways. The cows were routinely milked three times daily at 04:00, 13:00 and 21:00h. Cows were painted using a standard household paint with their lactation number and a random experimental letter (A-V) on their back for ease of identification. All experimental animals were locomotion scored weekly and cows identified as lame were excluded from the study. Locomotion was scored on a 5-point scale modified from Manson and Leaver (1988).

2.3.2 Behavioural Responsiveness Assessment

Each cow was individually assessed in two behavioural responsiveness assessments designed to measure responses to human approach and to novel stimuli. The design allowed cows to be tested in the home-pen with penmates present. Six animals from the experimental group were excluded from the novel stimuli assessment due to ill health. The assessments were carried out by the same female experimenter who was unfamiliar to the cows at the start of the experiment.

2.3.3 Human Approach Assessment

The human approach assessment consisted of three subtests. In order to test intra-animal repeatability, each subtest was repeated three times per cow over an 11 day period. The subtests were carried out using a Latin square design, to avoid the test order confounding the results. The order of subtest repeats were the same within cow but different between cows. The same subtest was not carried out more than once on each cow per day, with a minimum of 2 days separating a repeat of the same subtest and a minimum of 30 minutes between different subtests. The subtests and behavioural variables are described below.

2.3.3.1 Approaching human test in the Passageway (AP)

The aim of this subtest was to create a situation where the cow was given space to express her response to human approach. Only one subtest repeat was carried out per animal per day with 3.23 ± 0.18 days (mean ± S.E.) between subtest repeats. The criteria for starting this test was that the focal cow had to be standing idle in the passageway of the housing area, with sufficient space to move away from the experimenter, and had no more than 2 cows standing within 1m. If the criteria were fulfilled, the experimenter approached the cow from a distance of 3m in a standardised way. The experimenter approached the focal cow using strides of approximately 0.5m and after every step the observer remained motionless for 10 seconds to allow the cow to respond. The experimenter approached diagonally from the front towards the cow's neck, avoiding eye contact with the cow, looking towards the feet of the cow and keeping arms and hands close to the body. Avoidance was recorded using a flight response score which was defined as the distance at which the cow responded by taking two or more steps in the opposite direction from the approaching experimenter. The distance was measured by eye using cubicle width (approx. 1m) as a guide. In addition, 1m sections were chalked on to the passageway wall to aid the experimenter in measuring the distance to the cow. The flight response score was measured on a 10-point ordinal scale (Table 2.1). If a cow failed to move away from the experimenter then she was allocated the maximum flight response score of 9. On completion of the test, a qualitative assessment was made of the cow's response based on Wemelsfelder *et al.* (2001). The experimenter marked an individual visual analogue scale (VAS) for six qualitative terms (Table 2.2), according to a subjective judgement of whether a cow scored low or high for each term. The VAS consisted of 125mm horizontal line with two vertical lines marking the extreme points of the scale (0 mm: term absent, 125 mm: term present throughout the test). Scores for each term were measured as the distance in millimetres from the 0-point. Several other studies have used similar methods but approached the animal more quickly (Waiblinger *et al*., 2003; Winckler *et al.,* 2007). Pilot studies on non-test cows showed that the 10 second pauses were necessary to allow animals to fully express their emotional response to an approaching human.

Table 2.1 The flight response score used to score the cow's flight response to the AP test.

Score	***Behavioural Response***
0	Cow moves away when experimenter is ≤3m but >2m away
1	Cow moves away when experimenter is ≤2m but >1m away
2	Cow moves away when experimenter is ≤1m but >0m away
3	Cow moves away when experimenter is 0m away
4	Cow does not move away when experimenter is 0m away
5	Cow moves away as experimenter extends arm to touch
6	Cow moves away as experimenter touches the cow's head/shoulder
7	Cow moves away as experimenter touches the cow's body/rump
8	Cow moves away as experimenter touches the cow's udder/legs
9	Cow does not move away within the 5 minutes duration of the test.

Table 2.2 Qualitative terms and descriptions used in the AP Test.

Term	Description
At Ease	A relaxed, confident animal that maybe curious but shows no sign of tension.
Nervous	An animal that is quite restless/wary/uneasy as the experimenter approaches. May avoid experimenter. The animal may quiver/flinch when a hand is placed on her
Attentive	An inquisitive or playful animal that is very alert to the experimenter approaching and/or other events happening around her.
Passive	A docile animal that appears comfortable and/or calm as experimenter approaches. May be shy and quiet.
Aggressive	An animal that appears agitated/irritated or annoyed as experimenter approaches. A dominant animal which may attempt to kick or to butt the experimenter by lowering her head to swing/lunge towards the experimenter.
Social	An animal that interacts positively with the experimenter. Maybe inquisitive and try to sniff/lick/rub against experimenter.

2.3.3.2 Approaching human test at the feedface (FF)

The aim of this test was to assess the animal's response to an approaching human whilst feeding (Ball *et al.,* 2003). Only one subtest repeat was carried out per animal per day with 3.26 ± 0.19 days (mean ± S.E.) between subtest repeats. On the first day of this test, chalk lines marking distances of 0, 0.5, 1, 1.5, 2 and 2.5m from the cow's head whilst feeding (base of silage) at the feedface were marked on the ground. These chalk lines were used to aid the experimenter in measuring the distance to the cow and were not altered for the duration of the testing period. The cows were tested during two 3-h periods (mid-morning and late afternoon) for 11 consecutive days. The test commenced 30 minutes after the feed was delivered at the feedface. The experimenter walked up and down the passageway in front of the feed rail at a distance of 2.5m. When a cow approached the feed rail, the experimenter moved directly to the 2m line in front of the passageway and stood still for 30s while the cow was feeding, to ensure that a feeding bout had started. After the 30s the experimenter walked in a slow and controlled manner towards the cow as described in the AP test. At the 0m line, the experimenter stopped and kept motionless for 10s, extended her arm towards the cow and then remained motionless for 10s. Finally, the experimenter tried to touch the cow's head for a few seconds. Termination of the FF subtest occurred when the cow withdrew its head from beneath the feed rail and did not resume feeding for 10s or if the cow withdrew from her feeding space and took up feeding at another location. The behavioural response of the individual cow was categorised according to a 1-6 point ordinal scale (Table 2.3).

2.3.3.3 Approaching human test while lying (AL)

The aim of this subtest was to assess the cow's response to an approaching human whilst lying down in a cubicle. Only one subtest repeat was carried out per animal per day with 3.22 ± 0.18 days (mean ± S.E.) between subtest repeats. To avoid neighbouring cows affecting the behaviour of the focal cow only cows with free cubicles to the right and left were used. From a distance of two cubicles away (approx. 2.2m) the experimenter approached the individual cow in a standardised way described in the AP test. The experimenter approached the rump of the cow (end of the cubicle). To avoid frightening the cow and to give the cow an opportunity to respond to the experimenter's approach, the experimenter approached the cow from the direction in which the cow's head faced. Generally a cow lying on her left side will have its head facing towards the right and vice versa. On arrival at the end of the cubicle, the experimenter encouraged the cow to rise, by a vocal command ("Up girl") and then kept motionless for 10s. Finally, the experimenter gave two hand slaps to the cow's rump and then kept motionless for 10s. The cow's response was assigned a score on an ordinal scale between 1-6 (Table 2.3).

Table 2.3 The flight response score used to score the cow's flight responses to the FF and AL Test.

Score	Behavioural Response
1	Cow retreats when observer is <2.0m but >1m away
2	Cow retreats when observer is <1m but >0m away
3	Cow retreats when observer is 0m away
4	Cow retreats as observer extends arm to touch (FF) or cow stands when vocal command is given (AL)
5	Cow retreats as observer touches head (FF) or cow stands after receiving mild tactile encouragement to rise (AL)
6	Cow does not move away when touched (FF) or cow does not stand (AL)

2.3.4 Novel Stimuli Assessment

The novel stimuli assessment consisted of three subtests, which were individually presented to each cow in the passageway exiting the parlour. The three novel stimuli tests were only conducted once on each animal as it was considered that these test situations would not be novel on repeated exposure. The passageway (width 1.83m) was a familiar environment to the cows and contained an automatic weigh-crate that held each cow and released them at 20s intervals thereby creating space between each cow and allowing them to freely interact with the stimuli. The experimenter stood out of sight except when cows failed to pass the stimuli within 20s, the experimenter stood behind the animal and vocally encouraged the cow to walk-on whilst walking behind her. If required the experimenter gently moved her arms up and down to encourage the cows to walk-on. For the duration of the tests, prior to milking the cows were divided into three batches of ten cows and milked together so that they exited the parlour together. Each batch experienced the novel stimuli in a Latin square design with 2 days separating each test. All test sessions were recorded by a digital camcorder mounted onto the wall. Behaviour was scored from the videotape and always by the same observer. The three subtests are described below.

2.3.4.1 Stationary visual object (striped boards)

The aim of this test was to assess the cow's response to a bright coloured stationary object. Two boards (30cm x 60 cm) covered in black and yellow striped tape were positioned at approximately cow shoulder height on either side of the passageway. Black and yellow are frequently encountered as a warning colouration in various animal groups (Hutson *et al.*, 2000). The cow's behavioural responses were divided into two categories: investigative and reactive. An ordinal scale was used to measure the cow's level of investigation towards the boards (Table 2.4). An ethogram of four reactive behaviours (stop, avoid, startle, increase pace) was created to record the animal's reaction to each stimuli (Table 2.5). Due to the short duration of this test, the cow's immediate response to the stimuli was assessed. The frequency of all reactive behaviours was low so were summed for each individual animal for each test. The sum total of reactive behaviours equalled the cow's total reactive score. High reactive scores were taken as an indicator of high reactivity.

2.3.4.2 Flashing visual object (flashing light)

The procedure above was repeated with one flashing orange light placed on the right-hand side of the passageway exiting the parlour.

Table 2.4 The ordinal scale used to score the cow's investigatory responses to the striped boards and flashing light novel stimuli.

Score	Behavioural Response
1	Animal passes, no response towards novel stimulus and no change in pace.
2	Animal passes with no change in pace, shows some interest but with a lot of hesitation, extends head towards novel stimulus, no contact or interaction with stimulus.
3	Animal shows interest with a little hesitation, may slow down, pause or stop to look at stimulus but doesn't move closer, no contact or interaction with the stimulus.
4	Animal stops, briefly (< 3s) sniffs, licks or rubs the stimulus and walks on.
5	Animal approaches without hesitation, stops, sniffs, licks or rubs the stimulus (> 3s).
***6**	As above in 5 but animal sniffs **BOTH** boards and walks on.

*Score 6 only applies to Striped Board Test.

Table 2.5 Ethogram of reactive behaviours recorded towards the striped boards and flashing light novel stimuli.

Behaviour	Description
Stop	Stop, with head down (head is below the shoulder height) or with head up (head is raised above the shoulders) on approach to novel stimulus
Avoid – Light	Animal deviates from normal walking path avoiding the light. Animal does not look in the direction of the light.
Avoid – Board	Animal walks down middle of passageway and does not look in the direction of the boards.
Startle	The animal flinches, jumps or bucks in response to stimulus
Increase pace	Increase in pace from a walk to a trot/gambol

2.3.4.3 Startle test (water spray)

The aim was to assess the response of cows to a startle test. The cow's reaction to three gentle squirts of water from a hand-held water pistol on the hindquarters was recorded. The operator of the water gun was out of sight from the cows and obscured by two wooden boards (1.2m x 0.9m) positioned beside the passageway exiting the weigh-crate. The cows were habituated to the presence of the wooden boards, by having them in position 7 days prior to commencing the test. The cow's behavioural responses were measured using an ordinal scale that scored the cow's level of reactivity towards the water spray (Table 2.6). Each level of this scale typically incorporates those below it, forming a series of increasing response magnitude.

Table 2.6 The ordinal scale used to score reactivity responses to the water spray test.

Score	Behavioural Response
1	Animal passes, no change in pace, no response or sign of interest towards water spray.
2	Animal pauses briefly, temporary break in rhythm of stride (3s), shows some interest by an observable movement of the head towards the direction of the water spray.
3	Animal passes, no change in pace, observable movement of tail in response to water spray.
4	Animal increases pace from walk to trot/gambol
5	Animal increases pace, observable movement of tail, deviates away from direction of water spray.
6	Animal shows an intense flight reaction, jumps/flinches/bucks increasing pace from walk to trot/gambol.

2.3.5 Statistical analyses

All statistical tests were run using GenStat® for Windows™ 7th Edition except for repeatability estimates of AP, AL and FF flight response scores which were run in SAS version 9.1. All data were checked for normality using probability distribution plots.

2.3.5.1 Intra-test consistency of the human approach assessment

Three aspects of consistency of the three human approach assessments were investigated: (1) repeatability estimates, (2) Friedman's test to analyse the consistency of the magnitude of AP, AL, FF flight response scores (3) a principal component analysis (PCA) was used to visually evaluate the consistency of the qualitative terms in the AP test.

To assess consistency of behavioural responses, the repeatability estimate (*r*) of each measure was calculated. Due to the ordinal nature of the AP, AL and FF flight response score and the continuous nature of the AP qualitative terms two different statistical procedures were used. Firstly, the AP qualitative terms were log transformed and variance components were computed using Restricted Maximum

Likelihood (REML: Paterson and Thompson 1971). In the REML model, animal ID number and subtest repeat were fitted as random effects. Repeatability then can be estimated using the within and between animal variance components following Lessells and Boag (1987):

$$\text{Repeatability} = \frac{\text{Variation between cows}}{\text{Variation between cows} + \text{Variation within cows}}$$

An ordinal probit model including cow as a random factor was fitted using the NLMIXED procedure in SAS version 9.1 in order to calculate repeatability estimates for AP, AL, FF flight response scores.

Repeatability is an estimate of the proportion of variation among individuals that is due to individual differences (Boake, 1989). A cut-off value of ≥ 0.5 was used to distinguish those flight response scores and qualitative terms that gave the most repeatable results, and indicates that 50% of the variance occurs between cows rather than within individuals (Lessells and Boag, 1987), signifying a level of consistent individual responses across subtest repeats. Repeatability close to 0 would indicate that an animal responds differently to each test repeat and repeatability near 1 would indicate that repeated measurements of the same individuals give identical estimates.

To determine if cows responded significantly different between subtest repeats, a Friedman's test ($S_{degrees\ of\ freedom}$) was used on the un-transformed data. Each of the human approach subtests (AP, AL, FF) was considered alone with the subtest repeat as the treatment and cow as the block.

A PCA was used to analyse and objectively summarise relationships between the qualitative terms. For the three AP test repeats, the cows' response to all six qualitative terms were entered into a spreadsheet with 18 data columns, one for each test repeat and 36 rows, one for each cow. The components were rotated using a varimax rotation to increase the interpretability of the components by maximising the variance of each component. The covariance matrix was used as the terms were all measured on the same scale and therefore did not require standardisation. The first component explains most of the variation, the second component explains most of the remaining variation and so on. The coefficients of the variables, known as the loadings indicate the importance of each of the original variable for the principal component and are graphically represented. In such a graphical representation, the original variables that are correlated to each other will tend to cluster. Pearson's correlations (r) were used to investigate the relationship between qualitative terms.

2.3.5.2 Inter-test consistency

Consistency across situations was investigated using multivariate statistical analysis between parameters measured during different tests.

2.3.5.2.1 Human Approach Assessment

All flight response variables from the different human approach tests were entered in to a PCA using a correlation matrix. A total of nine loadings, one for every test (3 x AP, 3 x AL, 3 x FF) was computed and graphically represented. The correlation matrix was used to standardise variables, as all tests were not measured on the same scale. To examine whether individual responses were consistently ranked the same across the three different human approach subtests, I used Kendall's coefficient of concordance ($W_{degrees\ of\ Freedom}$) (Siegel and Castellan, 1988). The median flight response scores of AP, AL and FF were used. If individuals were consistently ranked the same among tests then the concordance coefficient equals one, whereas if ranks varied randomly from test to test concordance coefficient equal zero. No threshold figure for *W* exists above which a variable maybe considered consistent. Napolitano *et al.* (2005) suggests an interpretation of *W* coefficient of less than 0.4, between 0.4 and 0.6 and greater than 0.6 to indicate low, moderate and high agreement, respectively.

2.3.5.2.2 Novel Stimuli Assessment

Due to the ordinal nature of the reactivity and investigatory responses, non-parametric statistics were used. Friedman's test was used to test for differences in individual cows' reactivity responses to three different stimuli. A Mann-Whitney *U*-test ($U_{degrees\ of\ freedom}$) examined differences in investigatory responses between the board and flashing light tests. Spearman's rank correlation coefficients (r_s) were calculated to investigate the consistency of investigatory response. The agreement between reactivity responses across the three novel stimuli tests were calculated using Kendall's coefficient of concordance.

2.3.5.2.3 Consistency between human approach and novel stimuli behavioural parameters

It is important to examine agreement between different behavioural scores that are considered to measure traits that are thought to be similar. The agreement between the median AP flight response score and the reactivity score from striped boards, flashing light and water spray were calculated using Kendall's coefficient of concordance. Similarly, the agreement between the median AP flight response and the striped board and flashing light investigatory scores were calculated using Kendall's coefficient of concordance. The AP flight response had the highest repeatability (0.65) and was used for this reason.

2.4. Results

2.4.1 Intra-test consistency

The median (Q1-Q3) flight response scores were 2 (1.75-5), 6 (6-6) and 5 (4-5) for AP, AL and FF, respectively. Six of the 36 cows responded with a flight response score of greater than 5 in the three test repeats. Repeatability estimates for AP, AL and FF flight response were 0.65, 0.40 and 0.27, respectively. Repeatability estimates for the qualitative terms of social, passive, and at ease had repeatability estimates above 0.50 indicating that more variation occurs between cows than within cows

(Table 2.7). In contrast, attentive and aggressive terms had low repeatability estimates. The loadings of the first and second component of the qualitative PCA analysis are shown in figure 2.1. The first component accounts for 72.9% of the variation and the second 9.3%, taken together this accounts for 82.1% of the total variation. The variables (at ease, passive, social) with the highest loading on Component 1 are significantly correlated (Table 2.8). Pearson's correlation coefficients of the qualitative terms (Table 2.8) shows that there was a positive correlation between the AP flight response score and the terms 'At ease' (r=0.66, d.f.=35, P<0.001), 'Passive' (r=0.62, d.f.=35, P<0.001) and 'Social' (r=0.73, d.f.=35, P<0.001).

I used Friedman's test to examine whether the flight response scores for each of the three human approach subtests were stable over the three subtest repeats. Cows did not significantly differ in their individual responses to repeats of the human approach subtests (Friedman: AP test S_2=3.02 P=0.22, AL test S_2=3.60, P=0.17, S_2=3.57, P=0.17).

Table 2.7 Variation between cow and within cow estimates and repeatability (r) estimates for all human approach test measures.

Measures	***Test 1,2,3***
Flight response scores[1]	
AP	0.65
AL	0.40
FF	0.27
Qualitative terms[2]	
At Ease	0.52
Nervous	0.50
Attentive	0.02
Passive	0.51
Aggressive	-0.0049
Social	0.62

[1] Calculated using NLMIXED procedure in SAS.

[2] Calculated using REML in Genstat.

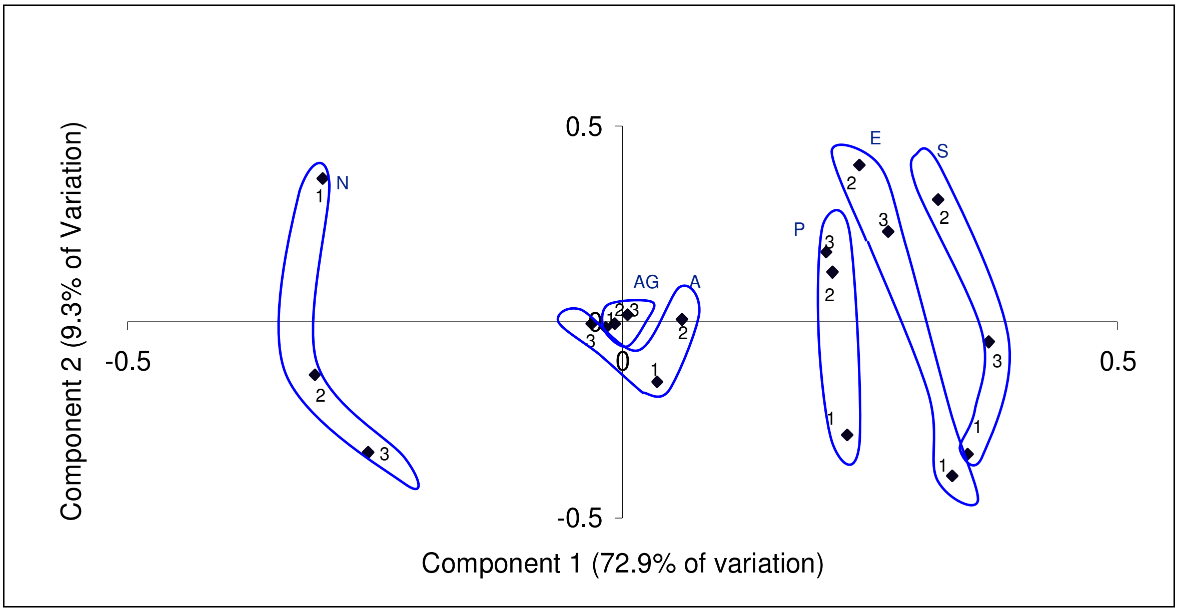


Figure 2.1 Graph showing the loadings for the qualitative terms of the AP test (n=36). The qualitative terms measured are A=Attentive, AG=Aggressive, E=At East, N=Nervous, P=Passive and =Social.

Table 2.8 Pearson Correlation Coefficients between AP test variables

	1	*2*	*3*	*4*	*5*	*6*	*7*
1. Flight Response	-						
2. At Ease	**0.66**						
3. Nervous	**-0.53**	**-0.68**					
4. Attentive	0.17	0.04	0.10				
5. Passive	**0.62**	**0.69**	**-0.68**	0.16			
6. Aggressive	-0.10	-0.14	0.05	-0.04	*-0.21*		
7. Social	**0.73**	**0.73**	**-0.74**	0.18	**0.62**	-0.09	-

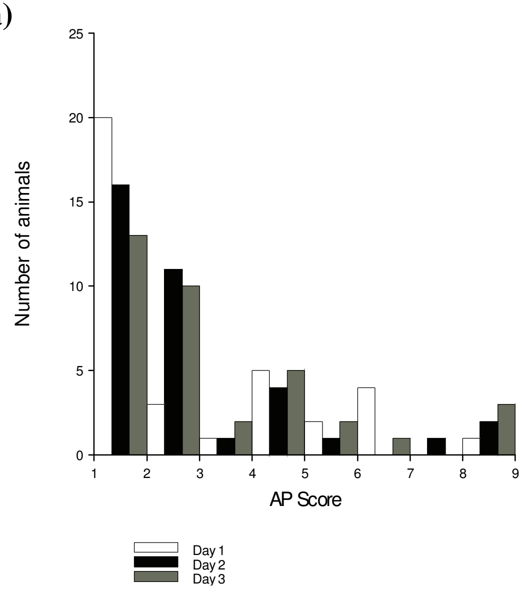
Column numbers in the top row correspond to the numbered variables in the first column. P<0.05 (italicised coefficient) or P<0.001 (bold coefficient)

2.4.2 Inter-test consistency

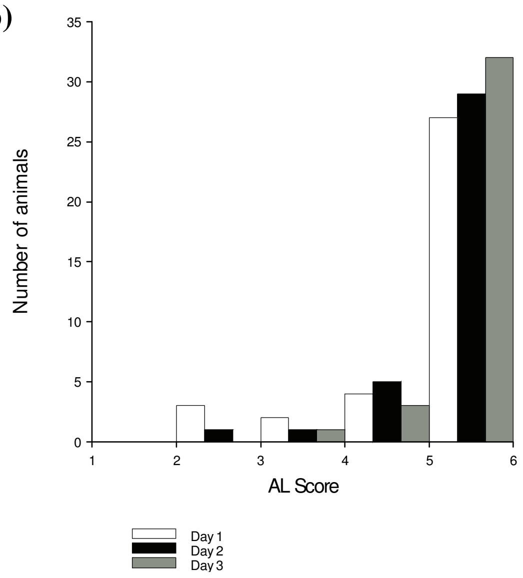
2.4.2.1 Human Approach Assessment

The frequency distribution of the flight response scores in all three human approach subtests are shown in Figure 2.2. A plot of the loadings of the first two components (Figure 2.3), which describe 36.3% and 14.4% of the total variation, respectively and summed together accounts for 50.7% of the total variation. The value of the loadings represents the degree to which the parameter influences the component. Values that are close together in a diagram such as Figure 2.3 are usually well correlated and may have a common motivational background. The variables formed three clear clusters (Figure 2.3). The loadings for the AP subtest are closely clustered together corresponding to high consistency. The loadings of the AL and FF subtests are more disperse and may indicate moderate to low consistency. A moderate level of agreement between the median flight response scores of AP, AL, FF subtests indicated moderate consistency within animals across the three subtests (Kendall's coefficient of concordance: W_{35}=0.56, P<0.01).

a)



b)



c)

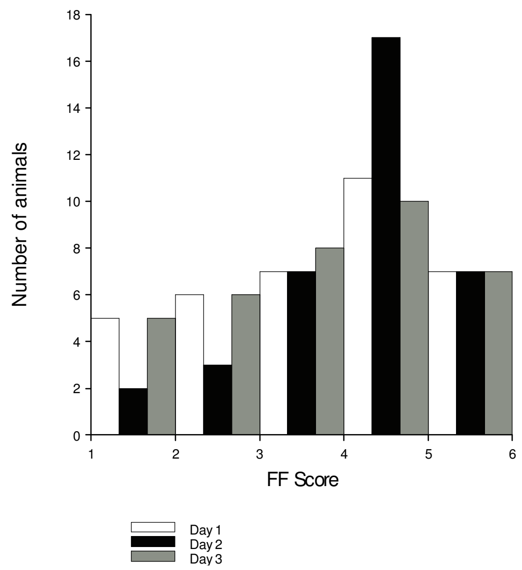


Figure 2.2 Graph showing the frequency distribution of ordinal scale scores for a) Approach passageway (AP) test b) Approach lying (AL) test, c) Flight from feeder (FF) test.

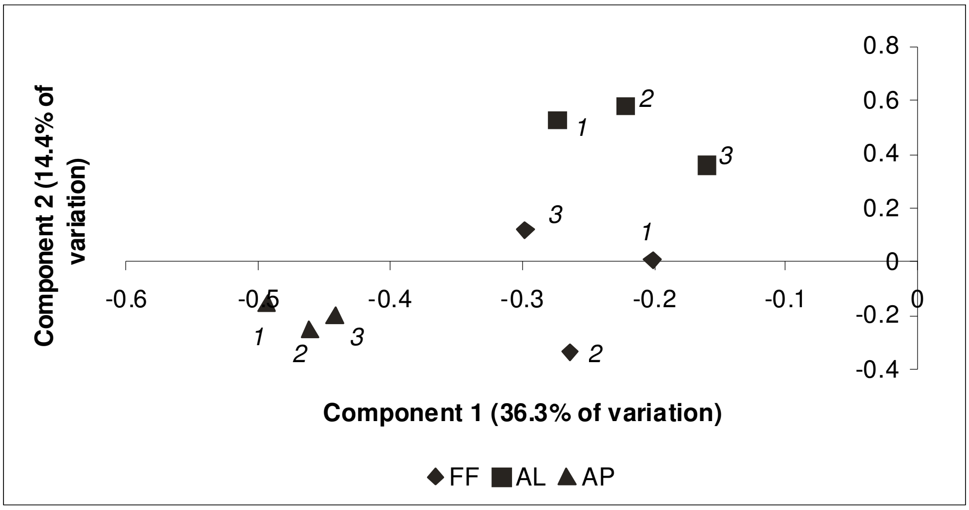


Figure 2.3 Graph showing the loadings for the flight response scores of the three human approach tests (n=36). AP (Approach Passageway), FF (Flight from Feeder), AL (Approach Lying)

2.4.2.2 Novel Stimuli Assessment

The frequency distribution of the investigatory and reactivity responses to the novel stimuli are shown in Figure 2.4. The reactivity response was significantly higher for the "water spray" than for either the "striped boards" (Mann-Whitney U: U_1=56, P<0.001) or the "flashing light" (U_1=66, P<0.001). There was a negative correlation between the animal's investigatory and reactivity responses to the striped boards (r_s=-0.47, d.f.=28, P<0.01) and the flashing light (r_s=-0.4, d.f.=28, P<0.05). There was no difference in overall reactivity responses between the striped boards and the flashing light (U_1=353, P=0.12). Animals displayed significantly higher investigatory responses to the striped boards than to the flashing light (U_1=4.509, P<0.05). Kendall's coefficient of concordance showed a low consistency within animals for reactivity response across the three novel stimuli (W_{29}=0.27, P<0.05).

2.4.2.3 Consistency between human approach and novel stimuli

No significant concordance was found between the median AP flight response score and the novel stimuli reactivity scores (W_{29}=0.27, *P*=0.34) and the investigatory responses (W_{29}=0.40, P=0.21).

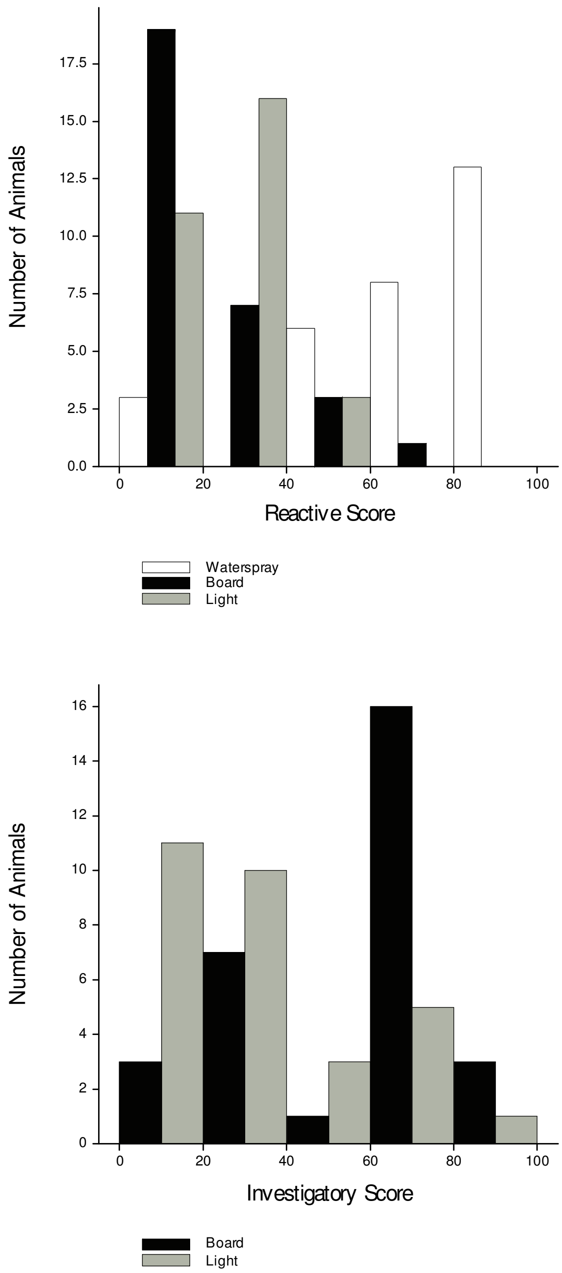


Figure 2.4 Graph showing the frequency distribution of proportion scores for reactivity and investigatory responses to novel stimuli tests.

2.5 Discussion

2.5.1 Intra-test consistency

The present study investigated the repeatability of individual differences in the responsiveness of dairy cows across three repeats of three different human approach situations. The moderate intra-animal repeatability of AP flight response score (0.65 across three test repeats) indicates that this measure is consistent within cows. This agrees with other avoidance distances of cows retested after 2 days (Lensink *et al.,* 2003), 4-5 days (Rousing and Waiblinger 2004), 2-3 weeks (de Rosa *et al*, 2003) and up to 2 months (Winckler *et al.,* 2007). These tests were considered to be repeatable. Windschnurer *et al.* (2008) reported correlation coefficients of 0.7 for avoidance distance to humans in the barn, this is near to the repeatability estimate in this study (0.65). It is important to highlight the repeatability found in this study does not mean that the responsiveness to humans will never change during the cow's life. Qualitative terms, 'social', 'passive', and 'at ease' all showed moderate to high repeatability estimates. The qualitative terms changed logically with time over repeats of the test. Animals tend to become more social, at ease and passive as test days increased indicating that the first day of the test may have had a slight novel effect but by days 2 and 3 the novel effect had declined. An animal's behaviour is expected to change somewhat over time, but how much change depends on the length of time elapsed and the resulting differences in the animal's physiological state and experiences (Tulloh 1961; Grandin 1993; Miller *et al.,* 2006). Correlations between the AP flight response score and the qualitative terms implies these measures are assessing similar aspects of the animal's response to human approach.

The AL test was not very effective in eliciting standing behaviour and there was a limited range of responses observed. Therefore, the moderate repeatability (0.40) for the AL test is less likely to be a good test of the cow's individual temperament. This may indicate that these cows were habituated to human approach whilst lying and also may be insensitive to mild tactile stimulation.

The flight response score from the FF test showed a very low repeatability (0.27) and is therefore less likely to be a good test of the cow's individual temperament. Contrary to this, Ball *et al.* (2003) using the same scoring system obtained a repeatability score of 0.52 for a group of bull calves and repeatability score of 0.50 for a group of heifer calves. Both groups were approximately 11 months of age. The difference in results may be attributed to a number of different factors: age, differing levels of social agonistic behaviour at the feedface (Waiblinger *et al.*, 2003), human/handling experience (Boissy and Bouissou, 1988; Munksgaard *et al.,* 1997, 2001; Waiblinger *et al.,* 2003). In adult lactating dairy cows the motivation to avoid an approaching human might compete with other motivations such as hunger, such that the motivation to feed greatly out weights the motivation to move away from an approaching person. Similar findings to the results in this study were found by Waiblinger and colleagues (2003). Winckler *et al.* (2007) showed high correlations (0.79 – 0.91) of avoidance distance of dairy cows at the feedface across 5 consecutive farm visits. Similarly, Windschnurer *et al.* (2008) reported correlation coefficients of 0.7 for avoidance distance at the feeding place. In both these studies, the animals were restrained in the feed barrier which is not the case in the present study. This difference in the approach feedface methodology might account for the difference in repeatability found between the present study and these other studies.

Repeatability evaluates an individual's consistency across tests but compares it to the variation across the experimental group. The ability to find a good repeatability of a trait relies on detecting differences between animals in the trait of interest. A difficulty with repeatability estimates is interpreting estimates close to 0.50, the cut-off point used in this study. The easiest way to interpret a repeatability of 0.5 is that behavioural responses are common across the group as both among and within individual responses are similar. With respect to the repeatability level, there is no general threshold figure above which a variable maybe considered repeatable. Therefore, I suggest that less than 0.4 to be low, between 0.4-0.7 to be moderate and greater than 0.7 to be high. The behavioural responses of cows may be similar if the test is insufficiently sensitive to identify unique individual differences. Another difficulty is the context, for example, in the FF subtest where the cows' motivation to respond is outweighed by its motivation not to respond. Although results from this study show a moderate concordance within cows across the human approach tests, this is not consistent with the low repeatability estimates for AL and FF. When all cows share the same or nearly the same behavioural response as in the AL test, repeatability estimates are bound to be low. This may be attributed to the lack of novelty in the AL test. Conversely, the high repeatability estimates of the AP tests can be attributed to the high level of variability between individuals compared with within individuals (Boake, 1989).

At this point in the discussion, the duration of tests needs to be addressed. The tests used in this study are longer in duration than those generally used as part of welfare assessment (e.g. Rousing and Waiblinger 2004) but are feasible in the context of an on-farm temperament study. The duration of the pauses were chosen after extensive pilot work. This work showed that 10s pauses were necessary to allow cows the opportunity to express their emotional response to an approaching human. The long duration of pauses (10s) in the AP test was necessary in order to give the animal an appropriate opportunity to express her response to the approaching human. Primarily, this time frame allowed the experimenter sufficient time to observe the animal's behaviour in order to accurately score the subjective terms used in this test. The range of AP scores (2 (1.75-5); median (Q1-Q3)) suggest a wide variation in how animals responded to the approaching human. Six of the 36 cows responded with a flight response score of greater than 5 in the three test repeats. This fact that animals remained stationary until the experimenter was close enough to extend arm to touch, may suggest that the slow approach worked for these animals. As the main aim was to draw out aspects of the animal's innate temperament and not just to assess the animal's fear of humans, therefore, the method used in this study was appropriate.

The spread of the FF data (Scores 5 (4-5); Median (Q1-Q3)) towards high scores indicating that animals remained at the feedface until the experimenter extends arm to touch. This supports that the slow approach worked, as you would expect that if conflicting motivations caused animals to move away, they would do so in the early stages of the approach and receive a low score.

2.5.2 Inter-test consistency

2.5.2.1 Human Approach Assessment

A principal component analysis (PCA) was used to visually evaluate the relationship between the different human approach subtests and their test repeats, and to indicate if variables changed logically with time. The three AP repeat values heavily influenced the first principal component and all three repeats clumped together. This provides further evidence of consistency within individuals. As the AP test shows the highest intra-animal consistency over time and across situations I assume that this measures a temperament trait in this context.

2.5.2.2 Novel Stimuli Assessment

I investigated individual reactivity and investigatory responses to three different novel stimuli in a familiar environment without changing their social situation. At this stage, it is important to clarify that all novel stimuli were chosen so that they could be adequately disinfected between farms to adhere to biosecurity measures on dairy farms. Kilgour *et al.* (2006) reported that cattle habituated to novel situations and concluded that novel tests are not novel from the second exposure onwards. Therefore, it was sensible to only expose cows once to each novel stimulus to avoid habituation and to maintain a degree of novelty. However, there is a dilemma here, as generally, repeated observations are needed to ensure that an underlying temperament trait is being measured. In addition to this, three very different novel tests were used. The striped boards and flashing light stimuli were both visual objects whereas the water spray was primarily tactile and these different stimuli evoked different responses. The different characteristics of the novel stimuli along with the need to design a test that could be used on commercial farms restricted us to behavioural details that could be observed easily and described simply. A simple subjective assessment of an animal's reactivity and investigatory responses using an ordinal scoring system was applied. However, the use of subjective ordinal scales have their limitations, it is difficult to statistically compare the magnitude of behavioural responses between individuals. For these reasons, qualitative comparisons between the novel tests were carried out using conservative non-parametric statistics.

The overall results of the novel stimuli tests show that when dairy cows were exposed to novel stimuli in a familiar environment the water spray test evoked the greatest reactivity response followed by the light and then the striped boards. The striped boards induced increased behavioural exploration compared with the flashing light. There was a negative intra-test correlation between reactivity and investigatory behaviour towards each of the visual novel stimuli tests. The reactivity to the water spray test showed a low agreement to a cow's response in the other novel stimuli tests. Essentially the subtests did differ in some respects and were found to measure different responses. No correlations were found across novel stimuli tests and therefore, I cannot conclude that a single temperament trait in response to novel stimuli exits in dairy cattle.

As the main aim was to choose a practical test of responsiveness to be used on commercial farms it is useful to consider the overall practicality of each subtest. In the water spray test, there was an increased reactivity shown by the presence of startle behaviours (trotting, gambolling, bucking), particularly in first lactation animals. The response to the water spray was not correlated to a cow's responses in the other tests and would, therefore, imply that this cannot be used as a predictor of how cows may respond to other situations. Such tests have been found to be of use for beef cattle, with Lanier *et al.* (2000) showing that reactivity to sudden, intermittent stimuli at auctions can be used to indicate an excitable temperament. Due to the extreme reactions and to avoid accidents or injury to the animals, it was decided that the water spray test would not be suitable for use on commercial farms.

Responses to novel stimuli can be affected by conflicting emotions such as reactivity and investigatory. Reactivity and investigatory behaviours are connected as an animal may move to get away from an aversive stimulus but it may also move to gather information about that stimulus (Montgomery, 1955; Hemsworth *et al.,* 1996; Hemsworth and Coleman, 1998). In this study, the short duration of the tests only permitted the immediate reaction of the cow to be recorded and may not have allowed sufficient time for both reactivity and investigatory responses to be displayed. The flashing light was visually startling which may have presented more of a threat to the cows than the striped boards. The more sudden a stimulus, the more intense the neural message it initiates, thus causing a heightened fear-related response. The striped board showed a good range of reactivity and investigatory responses and is practical and safe to use in a commercial farm setting.

2.5.3 Consistency between human approach and novel stimuli

This present study is one of few studies to investigate the relationship between the response to a novel stimuli and human approach tests. In this study, there was no significant concordance between the novel stimuli reactivity and investigatory responses with the AP flight response score. This conclusion is supported by Boivin *et al.* (1992) who found no relationship between open-field tests and handling tests, indicating that they do not reflect the same animal characteristics. This has also been found in other studies (e.g. Boissy and Bouissou, 1988). As mentioned previously, the AP test shows the highest intra-animal consistency across time and situations leading us to conclude that this is a good measure of temperament in this context. Behavioural responses in the visual novel stimuli tests were not predictive of the response to a startle test (water spray), nor could they be used to predict response to human approach. This suggests responsiveness to novel stimuli (in this case, reactivity and investigatory responses) are not consistent across situations but may be context specific (Wilson *et al.*, 1994). It can be concluded that response to human approach and novel stimuli are not governed by the same underlying mechanism.

2.6. Conclusion

In summary, a single test of responsiveness is not appropriate to assess both responses to humans and to novelty from the six tests evaluated. This study has shown cows to be consistent in their behavioural responses in a human approach test in the passageway of the home-pen and therefore, this test can be used to assess a core aspect of temperament, which is consistent over time. The results of this study do not support the hypothesis that temperament measured as reaction to human and reaction to novelty are related. It was concluded that dairy cattle vary widely in their responses to human and novel tests, with only the responses to a human approach in the passageway being consistent over time, and therefore, the only type of test which can indicate some core factor of temperament. The tests used in this study are longer in duration then those generally used as part of welfare assessment but are feasible in context of an on-farm temperament study.

CHAPTER 3

Consistency of aggressive feeding behaviour in dairy cows

In this chapter, I was responsible for the experimental design, data collection with help from farm staff. Fritha Langford and Jo Donbavand helped with the video analysis. I was responsible for the statistical analysis and the writing of the manuscript.

3.1 Abstract

This study tests the two main characteristics of a temperament trait, consistency across time and consistency across situations. The temperament trait of interest was aggressiveness during feeding in dairy cattle. In this study, we focused on whether it is possible to infer a trait of aggressiveness from the measurement of behavioural responses expressed by individual cows during feeding. Aggressive behaviour appears in many contexts but this paper focuses on aggressive behaviour in a competitive situation over a feed resource in housed dairy cattle. The aim of this study was to design a method to assess underlying aggressiveness that would be practical for use on individual cows during peak feeding when housed on commercial farms. Ten primiparous and 30 multiparous healthy lactating cows were housed in a group (parity =3.5 ± 2.15; mean ± S.D). To assess individual aggressiveness, cows were observed at different feedface space allowances, 0.6m per cow (standard) and 0.3m per cow (reduced) in the following situations: 1) all cows in the group with access to a standard feedface (ALL 0.6) for five days and replicated three times thereafter at 90 day intervals; 2) all cows with access to a reduced feedface (ALL 0.3) for five days; 3) primiparous cows (lactation 1) were separated from the mulitparous group and given access firstly, to a standard feedface (PRIM 0.6) and secondly, to a reduced feedface (PRIM 0.3). The behaviour of the aggressor and recipient were recorded for each aggressive interaction for 60 minutes after feed arrival. A 'displacement' index (DI), 'aggressiveness' index (AI) and a 'competitive success' index (CSI) were also calculated for each cow. Repeatability estimates and Kendall's coefficient of concordance were both used to assess consistency of aggressor and recipient behaviours across time. 1) The within cow repeatability was highest for CSI (r = 0.61) and lowest for non-response behaviours (r =0.04) across the three repeats. 2) Correlations between individual aggressiveness in the standard and reduced feedface were moderate and all were significant. 3) Primiparous cows received more aggressive interactions and were more frequently displaced when in the multiparous group (ALL 0.6) compared to when in the primiparous only group at both the standard (PRIM 0.6) and reduced (PRIM 0.3) feedface lengths. These results highlight the complexity of aggressive style of cows during feeding and illustrate that

some measures of aggressive feeding behaviour are repeatable within cows, between cows and across stage of lactation.

3.2. Introduction

In terms of animal welfare, it is considered that the focus on artificial selection for production traits in farm animals has resulted in numerous undesirable side effects in animal behaviour, physiology and health (Grandin and Dessing, 1998; Pigs: Geers *et al.,* 1990; Rauw *et al.,* 1998; Chickens: Jones and Hocking, 1999; Jensen and Andersson, 2005). Traditionally, breeding programmes for dairy cattle in many countries have focused mainly on selecting for increased milk yield. Data from the UK national milk records show an increase in average yields of dairy cows of about 200kg/year from 1996 to 2002 and 50% of this progress in milk yield is attributed to genetics (Pryce and Veerkamp, 2001). In dairy cattle, selection pressure on production alone has led to an increase in the frequency of involuntary culling as a result of increased incidences of lameness, mastitis, metabolic disorders and reduced fertility (Rauw *et al.*, 1998; Pryce *et al.*, 1999, 2001, 2002; Royal *et al.*, 2000; Veerkamp *et al.*, 2003).

Selection for production can affect behaviour of farm animals. Results from poultry, suggest that improved egg production may have increased aggression as a correlated trait (Muir, 1996) and similar results may be expected in pig breeding programmes (Muir and Schinckel, 2002; Løvendahl *et al.*, 2005). In dairy cattle, there is a strong genetic correlation between milk yield and feed intake (0.46-0.65) (Veerkamp, 1998). High milk production requires dairy cows to consume more food, therefore, it is conceivable that selection for milk yield may increase resource-defence aggression during feeding. Aggression has consequences on animal welfare. In addition to possibly causing injury and stress to the individuals involved, social stress may cause some cows to alter their feeding times to avoid aggressive interactions (Olofosson, 1999; DeVries and von Keyserlingk, 2006; Huzzey *et al.*, 2006).

Breeding goals used by livestock breeders have been broadened in most farm animal species to include multiple traits. So an opportunity now exists to investigate if the addition of health and fertility traits to breeding goals may have any possible consequences on animal behaviour or temperament. Before this can be achieved, behaviour and temperament tests need to be validated. Temperament traits are inter-individual propensities to behave in certain ways (Matthews et al., 2003) and are stable across time (Uher et al., 2007). An important aspect of characterising temperament traits is to investigate the extent to which they show consistency across time and across situations (Bates, 1986). It is important to highlight that within individual consistency does not mean that trait values cannot change with age or environmental conditions but that differences between individuals are largely maintained (D'Eath, 2004; Réale *et al.*, 2007). Some individuals are consistently more aggressive than others. Consistency has been reported in various species for territory defensive aggression in pigs (D'Eath, 2004; Janczak *et al.*, 2003; Erhard and Mendl, 1997), maternal defensive aggression (Cattle: Hoppe *et al.*, 2008, Morris *et al.*, 1994; Brown, 1974; Buddenberg *et al.*, 1986; Pigs: Marchant-Forde, 2002, Vangen *et al.*, 2005) competitive aggression over feed in pigs (Ruis *et al.*, 2000) and feeding and foraging behaviours (Pigs: Nielsen 1999; Fish: Wilson, 1998).

Aggressive behaviour appears in many contexts but this paper focuses on aggressive behaviour in a competitive situation over a feed resource in dairy cattle. The provision of food to housed cattle is associated with increased activity and aggression between animals (Jezierski and Podluzny, 1984; Philips and Rind, 2001). I was interested in the aggressive approach of individual cows in terms of a cow's willingness to compete for feed or her ability to displace other cows at the feedface.

The aim of the work described here was to measure the behavioural reactions of dairy cattle during peak feeding in a way that can be practically and easily recorded on commercial farms. Additionally, if individual cow aggressiveness is to be assessed on multiple farms, it is necessary to understand how cow variables (age and lactation stage) and management variables (feedface space per cow) influence the expression of aggressiveness in individual cows. In current dairy breeding programmes, many of the individual cow measures such as locomotion and conformation traits are recorded on primiparous cows only. If aggressiveness is to be included in breeding programmes, it is important to fully understand long-term consistency of aggressiveness of primiparous cows in different competitive and social situations. To achieve this the following questions were investigated: 1) are cows consistent in how they express aggressive behaviour during peak feeding? 2) do primiparous cows show different levels of aggression when housed with other primiparous cows only, compared to when they are housed with multiparous cows? 3) do parity, feedface length and social dominance rank influence aggressive behaviour?

3.3. Material & Methods

3.3.1 Animals and management

The study was carried out on the Crichton herd during the winter period while the cows were housed in a cubicle barn at the SAC Dairy Research Centre (Dumfries, Scotland, UK). The Crichton herd contained approximately 60 lactating Holstein-Friesian cows at any given time. The herd structure was dynamic with cows entering and leaving the group depending on calving dates (year round calving), illness and culling. Recording of behaviour at the feedface was carried out three times at 90 day intervals. Drying off and calving dates were calculated for all 60 cows and the three recording times were chosen so that 60 cows would be present during each of the three recording periods. Social stress caused by the addition of new animals into a herd has shown that some cows may alter their feeding times to avoid aggressive interactions (Huzzey *et al.*, 2006). For this reason no new animals were mixed into the experimental herd during the two week period prior to recording feeding behaviour. Over the course of the three observational periods, a total of 20 cows were diagnosed with an illness or were undergoing treatment. Therefore, the remaining forty healthy cows were assigned as the focal cows of this study. There were 10 primiparous and 30 multiparous (parity =3.5 ± 2.15; mean ± S.D.) cows in the group. At the start of the experiment the milk yield, days in milk (DIM) and body weight of the focal group were 30.1 ± 7.26 l/day, 105 ± 91.8 days and 679.43 ± 70.87 Kg (mean ± S.D) respectively. All cows were subjected to the same husbandry procedures and fed a total mixed ration (TMR). The TMR was available *ad libitum* and consisted of 59% grass silage and 41% concentrate on a dry matter basis. The cows fed from a feedface with a diagonal railed feed barrier design. Each individual head-bail measured 0.3m in width and 0.9m in height. Each cow had access to a cubicle with a saw-dust covered mattress. The passageways were concrete with automatic scrapers. The cows were routinely milked three times daily at 05:00, 13:00, and 21:00 h in a herringbone milking parlour. Cows were painted using a standard non-toxic household paint with their lactation number and an allocated experimental letter (A-V) on their back for ease of identification.

3.3.2 Experimental Treatments

3.3.2.1 Behavioural measures across time (ALL 0.6)

The recording of feeding behaviour during the first hour post feed delivery was carried out for five consecutive days three times (total of 15 observation days) with intervals of 90 days between recordings (Fig 3.1). This set-up allowed recording of individual cows during early, mid- and late lactation. Feed was provided at 15:00h, 11:00h and 10:00h for recordings 1, 2 and 3 respectively. It was considered that the different times of food provision in this study would not have an effect on feeding behaviour as a dairy cow's behavioural time budget is focused around feeding with peak feeding activity occurring immediately following feed delivery (Haley *et al.*, 2000). For all three repeats the cows had access to a standard length of two head-bails (0.6m) per cow. Data collection during repeats 1 to 3 began when the cows were 105 ± 91.8 DIM, 194 ± 91.8 DIM, 284 ± 91.8 DIM (Mean ± S.D.) respectively.

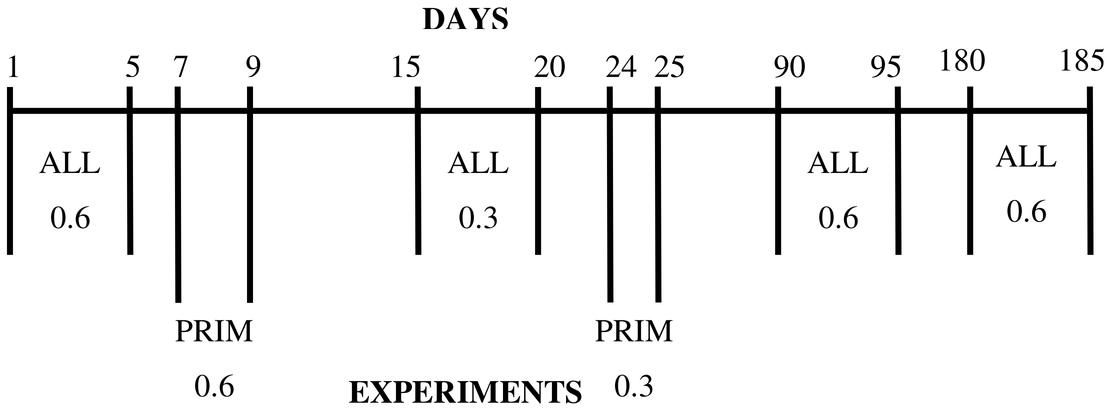


Figure 3.1. Timeline of experiments.

3.3.2.2 Behavioural measures across situations (ALL 0.3)

Ten days after completion of ALL 0.6 above, the herd (including primiparous) were exposed to a reduced feedface for five days (Fig 3.1). The feedface space allowance per cow was reduced from two to one head-bails per cow (0.6 to 0.3m per cow). Feed was delivered at 15:00h. The reduction in feedface space per cow was achieved by attaching a mesh wire barrier to the feedface to reduce the number of head-bails available. On test days, feed was evenly distributed along the accessible feed-space. No habituation period was given for this test.

3.3.2.3 *Primiparous cows at standard (PRIM 0.6) and reduced (PRIM 0.3) feedface*

The primiparous cows (n=10) were observed in three situations: (1) a standard feedface (0.6m per cow) with the multiparous herd (ALL 0.6, as described in 2.2.1 above); (2) two days after the completion of phase 1, the primiparous cows were separated from the herd and had access to a standard feedface (PRIM 0.6); (3) three days after ALL 0.3 the primiparous cows were exposed to a reduced feedface (PRIM 0.3) (Fig 3.1). The multiparous cows were housed together but in a separate and adjacent pen to the primiparous cows. Both the primiparous group and multiparous group were fed at the same time and feed was evenly distributed along the available feed space. The recording of behaviour was carried out for two consecutive days in

PRIM 0.6 and PRIM 0.3 and compared to the first two days of recording from ALL 0.6.

3.3.3 Variables recorded

3.3.3.1 Aggressive Behaviours

The aggressive interactions that occurred between cows at the feedface were continuously monitored during the 60-minute period following feed arrival. Feed was delivered either while the cows were gone for milking or after the cows had returned from milking. In the latter situation, access to the feedface was only permitted once all cows had returned from the parlour. This was to ensure that all cows were present at the recording start point. Recording started 10-minutes after feed arrival or upon access to the feedface.

Twelve Panasonic WV BP140 cameras were positioned 3m above the feed face. The cameras were linked to a Sprite Lite SLS X16 multiplexer and a Panasonic AG-6124 time-lapse video recorder. The analysis of the videotapes was carried out by three observers. From the video recording, each occurrence of each of the behaviours shown in Table 3.1 was recorded along with the identities of the aggressor and recipient cows involved. In order to condense information, these behaviours were pooled into the following behavioural categories for the aggressor (contact (C) and non-contact (NC)) and recipient (active avoidance (AA), non-response (NR) and aggressive response (AR)) (Table 3.1). A cow was determined to be the aggressor of an interaction when she exhibited one of the aggressor behaviours (Table 3.1) either as she approached another cow at the feedface or towards a cow that was already positioned next to her at the feedface. Aggressor and recipient behaviours were averaged per cow over the experimental periods (5 days for ALL 0.6 and ALL 0.3 and 2 days for PRIM 0.6 and PRIM 0.3). tally the total number of recorded aggressor and recipient behaviours each cow performed. For analyses, the following dependent variables were computed: (1) the proportion of contact (C) and non-contact (NC) behaviours that each cow initiated out of the total aggressive interactions and (2) the proportion of active avoidance (AA), non-responsive (NR) and aggressive responsive (AR) behaviours that each cow displayed out of the total aggressive interactions. In addition, three measures reflecting individual displacements, aggressiveness and competitiveness were calculated from the data. These are described below:

3.3.3.2 Displacement index (DI)

Displacement at the feedface was defined as the complete withdrawal of the recipient's head from beneath the feedrail following an aggressive interaction from another cow (aggressor). The number of displacements were used to calculate a 'displacement' index (DI) (Galindo and Broom, 2000) in order to evaluate a cow's ability to displace other cows. The DI is an estimate of a cow's ability to displace other cows relative to itself being displaced: DI = no. of active displacements / no. of active displacements + no. being displaced. The DI ranges from 0 to 1 which corresponds respectively to always being displaced or always successfully displacing others.

3.3.3.3 Aggressive index (AI)

I calculated an aggressive index (AI) in order to evaluate the relative aggressiveness of each cow within the herd. The AI (adapted from Barroso *et al.,* 2000) was used to determine whether animals tended to be an aggressor or a recipient of aggression: AI = no. of times aggressor / total number of interactions. The AI values ranged from 0 to 1 corresponding to whether an individual is always a recipient or always an aggressor.

3.3.3.4 Competitive Success Index (CSI)

Some cows had a high AI score but a low DI score indicating that they might be competitive in their aggressive behaviour but not always successful in displacing other cows. Conversely, some cows had a low AI score but a high DI score indicating that they might not be aggressive but nonetheless successful in displacing other cows. Therefore, for each cow a competitive success index (CSI) was calculated as the average (mean) of the DI and AI (CSI = mean DI + mean AI). This method accounts for the aggressive performance of the individual as well as how successful the cow is in displacing other cows. The CSI values ranged from 0 to 1 corresponding to low and high competitiveness respectively.

Table 3.1 Ethogram of behaviours and behavioural categories recorded for aggressor and recipient of aggressive interactions between cows at the feed face.

Aggressor	Category	Description
Pushing	Contact (C)	The cow uses some part of the body other than the head to displace the recipient.
Butting	Contact (C)	The cow uses head to head, head to neck or head to flank contact to displace the recipient.
Bulldoze	Contact (C)	The cow forcefully enters the front of the feedface displacing more than one individual.
Penetrate Feeder	Contact (C)	The cow pushes with force between two eating cows at the feedface resulting in physical contact with cows on both sides.
Blocking	Non-Contact (NC)	The cow uses the body to physically block the recipient from gaining access into the feedface.
Threatening	Non-Contact (NC)	The cow presents a threat posture by presenting the forehead with inclined head or the cow engages in a threatening swing of the head in the direction of the recipient but no contact occurs between the two individuals.
Recipient		**Description**
No Response	Non-Responsive (NR)	The cow shows no physical response.
Avoids	Active Avoidance (AA))	The cow moves/turns head in opposite direction in order to avoid aggressor.
Withdraws Back	Active Avoidance (AA)	The cow withdraws head from beneath the feedrail and moves straight back into passageway.
Withdraws Side	Active Avoidance (AA)	The cow withdraws and moves along >1 head-bail to the right/left
Retaliates	Aggressive Responsive (AR)	The cow retaliates with an attack (e.g. bunt, push etc) towards the aggressor.
Fight	Aggressive Responsive (AR)	The cow retaliates with an attack towards aggressor and further aggressive interactions follow.

3.3.3.5 Observer Reliability

Inter-observer and intra-observer reliabilities were calculated for all behavioural categories. Inter-observer reliability was estimated from the video recordings by comparing the observations of the three observers for the same 2h period. The average for each behaviour category and index was calculated for each cow across observers. Each observer’s score was then compared to the observer average with the difference expressed as a percentage. To measure intra-observer reliability, each individual observer scored 1h of video footage on two separate occasions, four weeks apart. The reliability of each category of behaviour was then calculated as a correlation between the two separate scores.

3.3.4 *Statistical Analysis*

All statistical tests were run using GenStat® for Windows™ 7th (2004; Lawes Agricultural Trust, Rothamsted Experimental Research Station, Harpenden, Hertfordshire, UK). All data were checked for normality.

3.3.4.1 Intra-test consistency

Two common approaches were used to measure consistency of aggressiveness over time: (1) repeatability estimates and (2) consistency of the rank orders using Kendall's coefficient of concordance.

To allow comparison to other similar behavioural studies, repeatability estimates were calculated to assess consistency of aggressive behaviour. Repeatability estimates can provide a great deal of information about variation among and within individuals within a group when repeated measures are recorded for a specific trait. Repeatability of temperament traits are a challenge to measure, particularly for highly plastic or strongly context specific dependent traits (Dohm, 2002). Additionally, aggressiveness of an individual may change over the course of time for example due to developmental changes. In this study, repeatability quantifies the consistency of aggressiveness within individuals, relative to differences in aggressiveness among individuals (Lessells and Boag, 1987). All behavioural categories (C, NC, AA, NR, AR) and the DI, AI and CSI variables are presented as proportions of total interactions. In order to calculate repeatability estimates (r) they were converted to percentages and then transformed using an angular transformation. This transformation was used since it was considered appropriate to convert these data to a percentage of total interactions for analysis and since the angular transformation is a standard transformation for creating homogeneous error variation with percentage data. To estimate repeatability, variance components were computed with mixed models using Restricted Maximum Likelihood (REML: Paterson and Thompson 1971). In the REML model, animal ID number and test repeat were fitted as random effects. Repeatability then can be estimated using the within and between animal variance components following Lessells and Boag (1987):

$$\text{Repeatability} = \frac{\text{Variation between cows}}{\text{Variation between cows} + \text{Variation within cows}}$$

Repeatability estimates close to 0 would indicate that all the animals respond differently to each test repeat and repeatability close to 1 would indicate that repeated measurements of the same individuals give identical estimates.

Repeatability estimates are known to be sensitive to changes in mean values between trials (Falconer, 1981: van Berkum *et* al., 1989). In case the repeatability estimates were biased, Kendall's coefficient of concordance was also calculated. The simplest way to investigate whether the level of difference between individuals is largely maintained is to calculate the rank order consistency. Kendall's coefficient describes the overall level of agreement in terms of ranks between the three repeated values for each individual's behavioural response (Siegel and Castellan, 1988). If individuals are consistently ranked the same across tests then the concordance coefficient (W) equals one, whereas if ranks vary randomly over time the concordance coefficient equals zero. No threshold figure for W exists above which a variable maybe considered consistent. Napolitano *et al.* (2005) suggests an interpretation of W coefficient of less than 0.4, between 0.4-0.6 and greater than 0.6 to indicate low, moderate and high agreement respectively. Due to difficulties with interpreting repeatability estimates, particularly estimates between 0.2 and 0.4, linear regression to compare repeatability estimates and Kendall's coefficients of the behavioural variables were used.

3.3.4.2 Effect of cow characteristics on aggressiveness

The effect of the cow characteristics (parity, stage of lactation and competitive success) on aggressive behaviour was investigated using a Generalized Linear Mixed Model (GLMM). Due to the unbalanced nature of the data, the Genstat REML procedure (Lawes Agricultural Trust, 2004) was used. Each of the cow characteristic variables were individually added as a fixed effect and analysed with each behaviour as the response variable (C, NC, AA, NR, AR, DI, AI) (univariate analysis). For this analysis, the cow characteristic variables were separated into classes thus preventing outlying data from confounding the results. Parity was split into five categories (1, 2, 3, 4, 5+) and the days in milk (DIM) were divided into three categories (1) early lactation <100 DIM, (2) mid lactation between 100 and 200 DIM and (3) late lactation > 200 DIM. Wald tests which use a χ^2 distribution were applied to examine the level of significance. The Wald statistic (W) is presented with the relevant degrees of freedom and probability value for the fixed effects of the GLMM. A binomial error distribution with a logistic link function was used. Additionally, Spearman's rank correlations (r_s) were used to investigate the inter-relationships between behavioural variables and possible determinants of aggressive behaviour.

3.3.4.3 Effect of feedface length

To assess within-cow consistency across different feeding contexts, each behavioural variable for all individuals in the standard (0.6m) and reduced (0.3m) feedfaces were correlated using Spearman rank. Spearman rank correlation assesses the association based on ranks of the data values (Siegel and Castellan, 1988). The Mann-Whitney test was used to test the effect of feedface length on the overall herd level of aggressive behaviour.

3.4.4.4 Primiparous cow group

To assess consistency of the aggressive behaviour of the primiparous cows across the three situations, Spearman rank correlation coefficients were calculated. The

Kruskall-Wallis test was used to test the effect of the three situations on the primiparous group's aggressive behaviour. Where there were significant differences, the Mann-Whitney test was used to test which situation differed from the other.

3.4.4.5 Inter and Intra-Observer Reliability

Inter-observer reliability was tested using a Kendall's Coefficient of Concordance test for each behaviour category. Intra-observer reliability was assessed using a Spearman's rank correlation. The inter- and intra-observer reliability was high for all behaviours. Kendall's coefficient of concordance for inter-observer reliability was above 0.95 for all behaviours ($P<0.001$). For intra-observer reliability, the Spearman rank coefficients were above 0.95 for all behaviours ($P<0.001$).

3.4. Results

3.4.1 Consistency across time

Every cow was observed performing aggression and receiving aggression at least once. The number of observations per animal was 51.32 ± 28.34 (mean ± S.D.) and varied from a minimum of 4 to a maximum of 110. Repeatability estimates between the behavioural categories varied greatly and are presented in Table 3.2. Repeatability estimates were moderately high for the competitive success index (CSI), moderate for four behavioural variables (contact, active avoidance, aggressive index and displacement index), with lower repeatability estimates for the remaining behavioural variables (non-contact, aggressive responsive and non-responsive).

From the Kendall's coefficients calculations, consistency was significant for contact (C: $W=0.54$, $P<0.01$), active avoidance (AA: $W=0.66$, $P<0.001$), displacement index (DI: $W=0.61$, $P<0.001$), aggressive index (AI: $W=0.6$, $P<0.01$) and competitive success index (CSI: $W=0.78$, $P<0.001$) (Table 3.2). Linear regression was used to investigate the relationship between the repeatability estimates and Kendall's coefficient of concordance ($y=1.19x-0.38$, $r^2=0.87$) (Figure 3.1).

Table 3.2 Medians, 1st and 3rd quartiles, repeatability estimates, and estimated variance components between and within cows for aggressive behavioural variables.

	C	*NC*	*AA*	*NR*	*AR*	*DI*	*AI*	*CSI*
Overall								
Median	31.97	5.88	40	6.07	0	43.03	41.04	39.41
Q1	16.67	0	22.22	0	0	17.16	21.36	25.45
Q3	47.72	13.39	58.58	13.33	4.65	66.99	59.76	57.87
Estimated variance components:								
Between cows	79.80	29.30	163	5.70	14.35	158.60	120.10	159.96
Within cow	222.30	116.50	250.40	136.70	64.83	378.00	280.20	259.59
Repeatability[1]	0.26	0.20	0.39	0.04	0.18	0.29	0.31	0.61
Kendall's Coefficient	0.54	0.44	0.66	0.43	0.40	0.61	0.60	0.78
	**	ns	***	ns	ns	***	**	***

[1] Repeatability=variance between cows/variance within cows + between cows

Key to acronyms: C, contact; NC, non-contact; AA, active avoidance; NR, non-responsive; AR, aggressive responsive; DI, displacement index; AI, aggressive index; CSI, competitive success index.

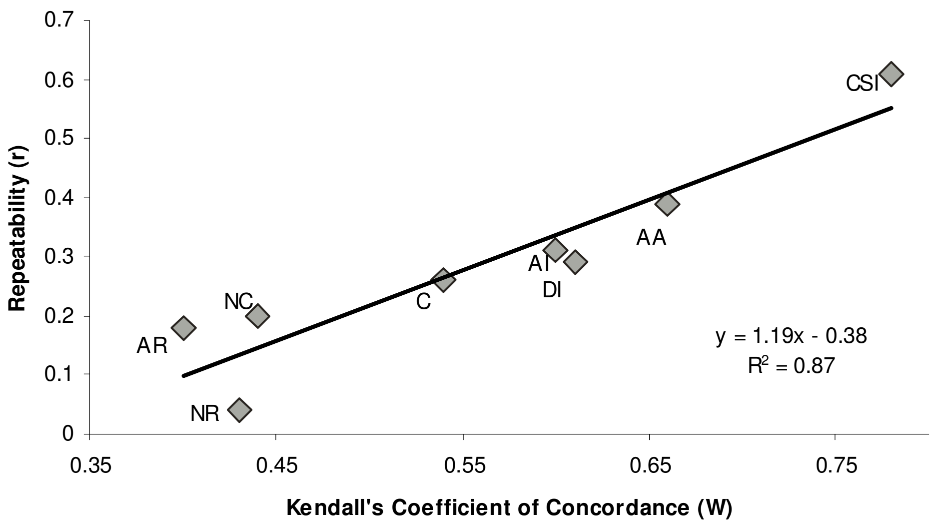


Figure 3.1 Linear regression of repeatability estimates and Kendall's coefficient of concordance of the eight behavioural variables recorded.

Key to acronyms: AR, aggressive responsive; NR, non-responsive; NC, non-contact; C, contact; AI, aggressive index; DI, displacement index; AA, active avoidance; CSI, competitive success index.

3.4.2 Effect of cow characteristics on aggressive behaviour

A correlational matrix for the aggressive behaviours is shown in Table 3.3. The effects of cow characteristics on behaviour are shown in Table 3.4 and Table 3.5. Older animals displayed more aggressive behaviours (C, DI, AI) with the exception of animals in parity 5+. Cows in early lactation (<100 DIM) have higher displacement index scores (DI) (W_2=6.40, P<0.05), show greater active avoidance (AA) (W_2=9.61, P<0.05) and consequently fewer non-responsive (NR) behaviours (W_2=22.18, P<0.001) then cows in mid or late lactation.

Table 3.3 Spearman rank correlation matrix of all variables measured. Only significant results are shown. df=38

	AA	*AI*	*AR*	*C*	*DI*	*NC*	*NR*
1. AA	-	-	-	-	-	-	-
2. AI	**-0.73**	-	-	-	-	-	-
3. AR	-	-	-	-	-	-	-
4. C	**-0.75**	**0.86**	-	-	-	-	-
5. DI	**-0.82**	**0.82**	-	**0.72**	-	-	-
6. NC	**-0.69**	0.46	-	-	0.50	-	-
7. NR	-	-	-	-	-	-	-

Column numbers in the top row correspond to the numbered variables in the first column. *P<0.05* (italicised coefficient), P<0.01 (underline coefficient) or ***P*<0.001** (bold coefficient), - (non-significant).

Key to acronyms: AA, active avoidance; AI, aggressive index; AR, aggressive responsive; C, contact; DI, displacement index; NC, non-contact; NR, non-responsive.

Table 3.4 The effects of parity on the aggressive behaviour of dairy cows during feeding. Mean (SE)[1]

	Parity (df=4)					Wald	
Behav	1	2	3	4	5+	Stat	Sig.
C	0.21 (0.03)	0.33 (0.04)	0.40 (0.04)	0.53 (0.04)	0.33 (0.07)	14.10	**
NC	0.03 (0.01)	0.11 (0.02)	0.10 (0.02)	0.12 (0.03)	0.09 (0.03)	8.61	NS
AA	0.56 (0.05)	0.42 (0.05)	0.65 (0.04)	0.28 (0.05)	0.31 (0.08)	9.86	*
NR	0.10 (0.02)	0.11 (0.02)	0.07 (0.02)	0.06 (0.02)	0.07 (0.04)	3.53	NS
AR	0.03 (0.01)	0.03 (0.01)	0.05 (0.02)	0.02 (0.01)	0.01(0.01)	4.47	NS
DI	0.26 (0.02)	0.47 (0.06)	0.51 (0.05)	0.65 (0.08)	0.42 (0.08)	12.53	*
AI	0.24 (0.03)	0.44 (0.05)	0.50 (0.04)	0.65 (0.05)	0.42 (0.09)	16.61	**
CSI	0.25 (0.03)	0.46 (0.04)	0.50 (0.03)	0.65 (0.06)	0.42 (0.08)	16.25	**

Key to acronyms: C, contact; NC, non-contact; AA, active avoidance; NR, non-responsive; AR, aggressive responsive; DI, displacement index; AI, aggressive index; CSI, competitive success index.

Table 3.5 The effects of lactation stage on the aggressive behaviour of dairy cows during feeding. Mean (SE)[1]

Behaviour	Lactation Stage (df=2)			Wald Stat	Sig.
	Early <100DIM	Mid 100-200 DIM	Late >200 DIM		
C	0.33 (0.05)	0.31 (0.04)	0.36 (0.03)	1.28	NS
NC	0.06 (0.01)	0.08 (0.02)	0.11 (0.02)	4.42	NS
AA	0.48 (0.05)	0.46 (0.05)	0.33 (0.03)	9.61	**
NR	0.03 (0.01)	0.08 (0.02)	0.13 (0.02)	22.18	***
AR	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)	0.48	NS
DI	0.51 (0.05)	0.39 (0.05)	0.42 (0.05)	6.40	*
AI	0.39 (0.05)	0.39 (0.04)	0.47 (0.04)	2.83	NS
CSI	0.40 (0.04)	0.39 (0.04)	0.48 (0.03)	5.75	NS

[1] Mean proportion with their respective standard errors of behaviours as calculated by frequency of behaviour / total frequency of interactions

NS Non Significant, *P<0.05, **P<0.01,***P<0.001

Key to acronyms: C, contact; NC, non-contact; AA, active avoidance; NR, non-responsive; AR, aggressive responsive; DI, displacement index; AI, aggressive index; CSI, competitive success index.

3.4.3 Effect of Feedface length

The group displayed more contact (C) and non-responsive (NR) behaviours and consequently fewer non-contact (NC) and active avoidance (AA) behaviours when provided with 0.3m per cow compared with 0.6m per cow (Table 3.6). There was no effect of feedface length on displacement index (DI, U=705, P=0.36, N=40), aggressive index (AI, U=723, P=0.46, N=40) or competitive success index (CSI, U=703.5, P=0.36, N=40). Animals in the low and mid- CSI groups displayed greater non-responsive (NR) behaviours in aggressive interactions at the reduced feedface (W_2=7.30, P=0.03). At the reduced feedface, 5+ parity cows showed more NC aggressor behaviours (W_2=21.51, P<0.001) and 2nd parity cows displayed more AR behaviours (W_4=16.47, P<0.01) compared to at the standard feedface.

Individual's responses to the standard and reduced feedface in terms of C (r_s=0.34, P<0.05, df=38), NC (r_s=0.41, P<0.01, df=38) and consequently DI (r_s=0.33, P<0.05, df=38) were significantly correlated. None of the remaining variables were significantly correlated across the standard and reduced feedface (AA: r_s=0.26, P=0.10; NR: r_s=-0.06, P=0.70; AR: r_s=0.26, P=0.09; DI: r_s=0.24, P=0.14, AI: r_s=0.27, P=0.09).

Table 3.6 Aggressive feeding behaviours[1,2] with 0.3 and 0.6m of feedface per cow. The median and lower and upper interquartiles for the test of treatment in Mann-Whitney (U_{df}) are provided.

Behaviours	**Feedface Length**		**U_{40}**	**P**
	0.6m	**0.3m**		
C	0.36 (0.25-0.46)	0.45 (0.32 - 0.57)	594	0.047
NC	0.07 (0.04-0.15)	0 (0-0.07)	404	<0.001
AA	0.40 (0.29-0.55)	0.24 (0.2-0.39)	459	<0.001
NR	0.08 (0.04-0.13)	0.18 (0.09 -0.28)	427	<0.001
AR	0.02 (0-0.04)	0 (0-0.05)	614.5	0.054

[1] Data are medians proportion of behaviours for the 5 d per treatment for 40 cows

[2] Proportion of behaviours are calculated as frequency of behaviour / total frequency of interactions

Key to acronyms: C, contact; NC, non-contact; AA, active avoidance; NR, non-responsive; AR, aggressive responsive.

3.4.4 Primiparous cow group

Individual primiparous cows did show highly significant correlations for contact (C, r_s=0.85, P=0.002) and active avoidance (AA, r_s=0.67, P=0.035) behaviour between ALL 0.6 and PRIM 0.6. At the group level, the primiparous cows received more aggressive interactions when housed with the multiparous cows compared to PRIM 0.6 (U=0, P<0.001, N=2) and PRIM 0.3 (U=5, P<0.001, N=2). Consequently, primiparous cows actively avoided aggressive interactions more frequently when in the multiparous group compared to PRIM 0.6 (U=21, P=0.027, N=10) and PRIM 0.3 (U=24, P=0.050, N=10) (Table 3.7). Primiparous cows were also more frequently displaced in the multiparous group than in the PRIM 0.6 group (U=19, P=0.018, N=10) and PRIM 0.3 group (U=2, P<0.001, N=10).

Table 3.7 Aggressive feeding behaviours with all cows at 0.6m and 0.3m feedface per cow and primiparous cows only at 0.6m and 0.3m of feedface per cow.

	All Cows		Primiparous Cows		
Parameter	**0.6m**	**0.3m**	**0.6m**	**0.3m**	H_2
C	4 (3-9)	6 (3-8)	5 (1-8)	2.5 (0-4)	4.16 ns
NC	0.5 (0-1)	0 (0-0)	0 (0-1)	0 (0-0)	3.64 ns
AA	9 (5-14)	4 (2-7)	3 (2-4)	1 (1-3)	9.91 *
NR	2 (1-4)	2.5 (1-4)	1.5 (0-2)	0 (0-2)	4.98 ns
AR	0 (0-1)	0 (0-0)	0 (0-1)	0 (0-0)	3.42 ns
DI	0.42 (0.25-0.52)	0.37 (0.25-0.5)	0.50 (0.25-0.62)	0.23 (0-0.67)	2.66 ns
AI	0.36 (0.17-0.45)	0.53 (0.33-0.57)	0.50 (0.25-0.62)	0.45 (0-0.5)	4.27 ns
CSI	0.31 (0.23-0.51)	0.54 (0.45-0.97)	0.45 (0-1)	0.76 (0.38-0.93)	3.42 ns

[1] Median (lower and upper interquartiles) for the test of treatment in Kruskall-Wallis (H_{df}) are provided.

Key to acronyms: C, contact; NC, non-contact; AA, active avoidance; NR, non-responsive; AR, aggressive responsive; DI, displacement index; AI, aggressive index; CSI, competitive success index.

3.5 Discussion

3.5.1 Intra-test Consistency

The overall pattern of the results suggests that the measures can be grouped into low, intermediate and high consistency classes. The repeatability estimates for non-contact, aggressive-response and non-responsive behaviours were low. These measures were also non-significant when analysed using Kendall's test and were not well correlated across the two analysis approaches. In contrast the repeatability for competitive success index (CSI) was above 0.6, significant by Kendall and was on the trend line of the regression between the analysis approaches. The intermediate group (contact, aggressive index, displacement index and active avoidance) are characterised by repeatability estimates of less than 0.5, significant rank-orders by Kendall and being correlated across the analysis approaches.

The only other published repeatability studies of aggressive behaviour of which I am aware concern pigs, fish, beef cattle and dogs. In the present study, repeatability estimates for aggressiveness at feeding of dairy cattle range between 0.26 and 0.61 and fall within the range of values reported in other species. Repeatability of attack latency during resident intruder tests in pigs vary from 0.18 (Cassady, 2007) to 0.41 (D'Eath 2004). (Note that the repeatability of 0.41 is to be viewed with caution as the data were not normalised before statistical analysis). Aggressive displays in fish have been shown to be repeatable at 0.51 (Bergmuller and Taborsky, 2007). In beef cattle, repeatability of maternal protective behaviour was estimated at 0.33 across two breeds (Hoppe *et* al., 2008). Attacking, guarding and biting in Belgium shepherd dogs were shown to be repeatable with estimates of 0.47, 0.47 and 0.51 respectively (Courreau and Langlois, 2005).

The range of repeatability estimates in the literature would support the conclusion that three traits (non-contact, aggressive-response and non-responsive) have been identified with low individual consistency, and one trait (CSI) with high individual consistency. However, it should be highlighted that the frequency of aggressive-responsive behaviours were very low and therefore the repeatability of this behaviour should be view with caution. The position of the intermediate group is harder to judge given that the repeatability estimates although less than 0.5 are rather similar to those reported in other species and in addition are traits which have consistent ranking between individuals.

Repeatability can be used to indicate whether efforts to measure heritability are likely to be worthwhile (Boake, 1989). Repeatability estimates (r=0.26-0.61) from the current study, highlight that it might be worthwhile calculating heritabilities of these behaviours, however it should be noted that behaviour is sensitive to genetic relationships within the population and the environment in which the animals are kept.

In all behaviour traits measured with the exception of CSI, the repeatability estimates point towards considerable variability in response (e.g. the high within animal values) probably due to temporary environment effects (e.g. lactation physiology, changes in dominance hierarchy and learning). Although the cows displayed some consistency in aggressive response across test repeats, they also maintained a level of flexibility of response to unidentified differences in the repeated tests. The very low estimates of repeatability for recipient behaviours, NR (0.04) and AR (0.18), may be explained by the low observed frequency of these behaviours and additional records would be beneficial. The low level of AR behaviours observed may be due to the diagonal partitions in the feedface design. The use of barriers on feedfaces provides a division between the cows and may reduce the number of head swinging, butting or threatening behaviours and therefore displacements during feeding. Huzzey *et al.* (2006) found fewer displacements at a headlock feed barrier compared to a post and rail barrier. Bouissou (1970) found that feed barriers that completely separated the heads of adjacent cows enabled lower ranking individual's better access to feed.

3.5.2 Effect of cow characteristics on aggression

Individual differences in aggressiveness were also associated with differences in parity and stage of lactation. Compared to older animals, primiparous cows tended to show a lower level of physical aggressive behaviour and a higher level of active avoidance. Increased active avoidance indicates that the feeding pattern of the primiparous cows is frequently interrupted causing them to withdraw from the feedface. This is supported by Mc Phee *et al*. (1964) who reported that lower ranking animals were more often disturbed at feeding.

Previous studies have considered the effect of stage of lactation on dairy cow feeding behaviour (Grant and Albright, 1995; DeVries *et al*. 2003) however this is the first study to investigate stage of lactation on aggressiveness during feeding. In this present experiment, aggressiveness in early, mid and late lactation was measured. The relationship between the individual behavioural measures indicated that aggressive behaviour in dairy cattle alters across the lactation. Cows in early lactation (<100 DIM) showed greater active avoidance and consequently fewer non-responsive recipient behaviours when aggressed upon. These results suggest that cows in early lactation may be withdrawing frequently from the feedface as a result of aggressive interactions, possibly associated with early lactation animals having just entered the stable milking herd and still attempting to establish themselves in the hierarchy. In this study, the experimental group was 'dynamic' with newly calved cows frequently entering and dry cows leaving the herd, as is typical of a commercial dairy farm situation.

3.5.3 Effect of feedface length

When provided with a reduced feedface (0.3m per cow), there was a reduction in the overall level of the group's aggressive interactions and successful displacements compared to standard feedface (0.6m per cow). In this study, this might be explained by subordinate individuals not competiting for access to the feedface during the reduced feedface treatment. In future studies, assessing dominance hierarchy of the herd would provide additional information on the social stability of the group. Additionally, replicating the study on several groups of animals would provide a better representation of aggressive behaviour. This is contradictory to Huzzey *et al*, (2006) who reported increased aggression and displacements in an increased competition situation. However, Collis *et al.* (1980) reported no change in the amount of aggressive behaviour when feedface was reduced from 1.05 to 0.45m per cow.

The within-individual correlation coefficients between the standard and reduced feedfaces were relatively low (0.33-0.43), even for correlations that were significant (C, NC and CSI). This indicates that whilst there was some degree of consistency in aggressor behaviour across situations, these correlations may have relatively little predictive value. At the individual level, no consistency was found for recipient behaviours indicating that cows showed a considerable plasticity of response to changes in feedface length. These results may indicate that individuals adopt a flexible strategy during a competitive situation and readily adjust their behaviour to maintain their position at the feedface.

3.5.4 Primiparous cow group

The primiparous group displayed a lower frequency of aggressor behaviours, fewer successful displacements and a higher frequency of active avoidance behaviours when housed in the multiparous group compared to when they were housed in a primiparous cow only group.

Out of the eight behavioural variables measured across three different situations, only two measures showed within-individual consistency across two out of the three situations. Primiparous cows showed high (r_s>0.8) to medium (r_s>0.6) within-individual consistency for aggressor contact and aggressor active avoidance behaviours respectively at a standard feedface with multiparous cows and primiparous cows only. For the remaining variables, no within-individual consistency was found indicating that cows altered their behaviour to deal with the changing situations, specifically in the primiparous only group at the reduced feedface. This indicates that primiparous cows' aggressive behaviour may be affected by the presence of older or dominant cows within the herd. This is supported by Konggard and Krohn (1978) who showed that when first calf heifers were separated from older cows, eating time increased by 11.4%, meals per day increased by 8.5%, dry matter intake increased by 11.7% and lying periods increased by 19% per day. In support of this, Grant and Albright (2001) suggest that lactating primiparous cows are often the lower ranking animals and benefit from separate grouping. Commercial farmers recognise this fact and some house their primiparous cows in a separate group to the rest of the herd. As aggressiveness is expressed differently in a group with only primiparous cows, it is important that this is taken into account in farm management programmes by grouping primiparous cows separately from the multiparous cows.

Future research could investigate if aggressiveness during feeding is heritable. If aggressiveness is found to be heritable, then it maybe accessible to genetic selection. This may allow us to select against aggressive genotypes (Grandin, 1997; Van Reenan *et al.,* 2002; Boissy *et al*., 2005) and improve feeding and grazing behaviour by reducing competition and aggressiveness and ultimately improve animal well-being. In future studies, it would prove more useful to record both feeding behaviour measures (feeding activity, meal duration, meal frequencies) and detailed social behaviour (aggressive and non-aggressive) at the feedface in order to gain a better insight into dairy cattle behaviour during feeding across stages of lactation.

3.6. Conclusions

The objective of this study was to assess consistency of aggressive feeding behaviour across time and across situations in dairy cattle. These results highlight the complexity of aggressiveness during feeding. This study demonstrated that individual cows showed consistency in contact and active avoidance behaviours across time. Results indicate that some degree of individual consistency in aggressor behaviour exits between different competitive situations.

CHAPTER 4

Measuring Sociability in Dairy Cows

In this chapter, I was responsible for experimental design. I carried out the experiment with contribution from research technician David Bell with animal handling. I was responsible for data and statistical analysis and writing the manuscript.

4.1 Abstract

Sociability is the relative preference of individual animals to seek out close contact with conspecifics. The aim of this study was to develop suitable tests that could be used to measure the sociability of individual cows on commercial farms. A standardised runway test was used as a "gold standard" test of social motivation and was repeated three times on forty-six focal cows. In the runway test, the average latency to reach 5m and 2m from the herd and the time spent in these areas were recorded and analysed for repeatability. Latency to reach the 5m line over the three tests was the most repeatable variable (0.54) and was taken as a measure of social motivation against which to assess other measures of sociability shown by the cows in their home-pen. The home-pen measures were the distance of each cow to the two nearest neighbours, location of the cow in the cow shed, and the level of synchrony based on individual behaviour of each focal cow compared with the rest of the herd's behaviour. Cows that had high latencies to reach the 5m line had fewer recordings with two near neighbours (W_1=5.31, P=0.021), were less synchronised with the herd (W_1=4.82, P=0.028), were not present at the feedface during peak feeding (W_1=4.13, P=0.042) and stood at the periphery of the cow shed (W_1=4.03, P=0.045). This indicates that these measures could be used to assess the sociability of individual dairy cows in on-farm studies.

4.2 Introduction

Traditionally, breeding programmes in dairy cattle have focused mainly on selecting for increased milk yield. However, there is considerable evidence to suggest that selection for milk production traits is associated with an increase in lameness, mastitis, metabolic disorders and reduced fertility (Pryce *et al.*, 1997; 1998; Rauw *et al.*, 1998; Royal *et al.*, 2000; Veerkamp *et al.*, 2003). Dairy breeding companies have recognised these problems and are enhancing their breeding indices to include functional traits like cow fertility, calving ease, survival and resistance to lameness and mastitis (Veerkamp *et al.*, 1995; Stott *et al.*, 2005; Wall *et al.*, 2007). While it is valuable to improve functional traits, it is important to determine if there are any contributions or possible undesirable consequences that the use of these breeding programmes may have on dairy cattle temperament.

To date, minimal investigation into the effect of selective breeding on dairy cattle behaviour traits has been carried out. Sociability is a natural component of farm animal behaviour but can have negative consequences and is an aspect of temperament that requires attention. Sociability is a term that is used to describe the motivation of individuals to remain close to conspecifics (Sibbald *et al.*, 2006). Genetic improvement of functional traits (longevity and fertility) involves breeding dairy cows that can produce a live calf, show observable oestrus, conceive when inseminated, maintain adequate body condition, resist infectious disease, avoid udder and leg injuries, walk and stand comfortably and produce milk of desirable composition (Miglior *et al.*, 2007). Dairy cows selected for functionality may have a reduced ability to cope with stress or adapt to their physical and social environment which may compromise welfare or be ethically undesirable. One strategy an animal may adopt is to increase feed intake. This might lead to animals altering their feeding times to feed outside peak feeding times, thereby showing lower social cohesion with the herd. In order to investigate this, it is necessary, first to design tests to measure specific social behaviour traits in individuals. The social behaviour of cattle is characterised by a tendency to form and maintain cohesive social groups. Modern husbandry practices impose constraints to the environment of cattle including disturbances of their social environment which can induce stress and reduce their production and welfare. Aspects of social behaviour such as social motivation and synchrony may be used in breeding programmes in order to breed animals that can thrive in group housing and cope well with social challenges (e.g. regrouping).

Behaviour traits are inter-individual propensities to behave in certain ways and are consistent within individuals across time (Sih *et al.*, 2004; Réale *et al.*, 2007). It is known that there is considerable individual variation in sociability of domestic chicks (Jones and Mills, 1999; Jones *et al.*, 1999), cattle (Hopster and Blokhuis 1994; Boissy and Le Neindre 1997; Fisher *et al.*, 2000; Ball 2003) and sheep (Sibbald and Hooper, 2004). In previous research, sociability has been assessed at a group level as well as on individual animals. At the group level, sociability measures include behavioural synchrony or social cohesion (Cattle: Benham, 1982, Miller and Woodgush 1991, Rook and Huckle, 1995; Horses: Rifa, 1990; Birds: Webster and Hurnik, 1994) and inter-individual distance of individuals within a group (Hedger 1950, 1963; McBride 1971, Sheep: Sibbald *et al.*, 2005; Cattle: Dudziński *et al.*, 1982). On an individual level, the motivation to be close to social companions has been assessed by measuring how hard animals will work to gain access to conspecifics (Calves: Holm *et al.*, 2002; Mice: Sherwin, 2003; Silver fox: Hovland *et al.*, 2007), and in behavioural responses to isolation (Cattle; Hopster and Blokhuis 1994; Boissy and Le Neindre 1997; Fisher *et al.*, 2000; Ball 2003). A frequently used test is the runway test which measures the distance or speed that animals run towards conspecifics (Birds; Mills and Faure, 1991; Sheep: Sibbald *et al.*, 2000; Cattle: Ball 2003; Horses: Lansade *et al.*, 2008). Runway tests involve moving an animal to one end of a corridor and then measuring the time it takes the subject to approach a small group of conspecifics held at the opposite end. The total time spent by the subject near the group of conspecifics is also measured. For example, both domestic chicks and Japanese quail approached conspecifics more readily when given a choice of an empty goal box or one containing members of same species (Launay *et al.*, 1991; Mills *et al.*, 1995).

The focus of this paper was to develop reliable and valid tests to assess sociability of individual dairy cows that can be practically and easily recorded on commercial farms. In this study, the responses of animals in a runway test were compared to observations of spontaneous behaviours indicative of sociability in the home-pen. A runway test was used as a 'gold standard' as it is a commonly used test that shows consistency across time and is suitable for use in dairy cows (Ball, 2003). The specific aims of the work described here were to: 1) assess intra-test consistency of social motivation measures in a standardised runway test and 2) validate sociability measures by investigating the relationship between social responses of individual cows to a runway test with individual measures of proximity, synchrony and location within the cow shed.

4.3 Materials and methods

4.3.1 Animals and management

The study was carried out during the winter period while the cows were housed in a cubicle shed at the SAC Dairy Research Centre (Dumfries, Scotland, UK). The group structure was dynamic with cows entering and leaving the group depending on calving dates, illness and culling. The experimental herd contained 54 lactating Holstein-Friesian cows. Forty-six of these cows (10 primiparous and 36 multiparous) were used as the focal cows in this study. These forty-six cows were specifically chosen as they were in good health and were not due to enter their non-lactating (dry-period) prior to calving before the end of the study. The cows were (mean ± S.D.) 144.8 ± 94 days in milk (DIM), had an average parity of 3.24 ± 2.09 and produced 31.48 ± 6.7 kg of milk per day.

The housing system was a cubicle system in which cows were able to move about freely. The housing consisted of two feedfaces (25.2m each), adjacent to each feedface was two rows of cubicles facing one another open at the front (‘head-to-head’). Each row contained 14 cubicles. The cubicles (2.13 x 1.19m) were bedded with a saw-dust covered mattress and were provided at a ratio of 1:1. The feed (25.2 x 3.2m) and cubicle (25.5 x 2.10m) passageways were concrete with automatic scrapers. All cows were subjected to the same husbandry procedures and fed a total mixed ration (TMR) composed of 59% grass silage and 41% concentrate on a dry matter basis. There was enough space for all cows to comfortably feed at the feedface. Cows were fed at 10:00h and feed was pushed at 04:30h and 21:00h daily. The cows fed from a diagonal railed feed barrier (50.40m in length) with 108 individual head-bails (0.3m wide x 0.9m high). The cows were routinely milked three times daily at 05:00, 13:00, and 21:00 h in a herringbone milking parlour. Cows were painted using exterior gloss paint in black with their lactation number and an allocated experimental letter (A-V) on their back for ease of identification.

4.3.2 Test procedure

Test days were Tuesday, Wednesday and Thursday for three consecutive weeks and involved making instantaneous behavioural scan sampling of the whole herd including the 46 focal cows in the mornings (10:00-13:00h). On the same days, the runway tests were carried out on the focal cows in the afternoons (15:00-18:00h).

4.3.2.1 Behavioural Scans

On test days, after morning milking, the entire herd was prevented having access to the feedface until feed was delivered. The first of the instantaneous behavioural scan samples was taken once feed had been delivered and cows allowed access to the feedface. One scan was recorded every 20 minutes, the total duration of time required to scan the group was 20 minutes. For each scan, the activity (idle, ruminate, feed, drink, groom, aggression), position (feedface, passageway, and cubicle), posture (standing or lying) for each cow was recorded as well as its two nearest neighbours. A cow was considered occupying the feedface when her head was under the feed barrier. A cow was scored as occupying the cubicle area when at least her two front hooves were in the cubicle bed. A cow was considered occupying the passageways when she was standing or walking in any of the passageways adjacent to the feedface or cubicles. Measures of sociability were calculated from the data as follows:

- *Nearest Neighbour (N):* The distance in meters from each focal cow to the nearest two neighbours was measured by eye using cubicle width (approx. 1m) as a guide. The nearest neighbours were defined as the two cows with the shortest distance to the focal cow at the time of the scan; there was no requirement for either individual to spend a particular period of time in the vicinity of the focal cow. There was no limit to how far each nearest neighbour could be from the focal cow. For each individual focal cow the proportion of the total 81 scan points that the cow had two neighbours greater than 2m away (NN2), two far neighbours greater than 1m away (FN), two near neighbours less than 1m away (NN) and one near neighbour and one far neighbour (NFN) was calculated.

However, only four cows fell into the category with two neighbours greater than 2m away (NN2) and therefore, data are not presented.

- *Location (L)*: For each scan, the location of each individual focal cow was categorised as 1-3 according to the cow's proximity to the outside edge of the shed. The location categories are as follows: (L1) within 1 cow length of the outside edge of the shed, (L2) within 2 cow lengths from the outside edge of the shed, (L3) more than 2 cow lengths from the outside edge of the shed. For each individual focal cow the proportion of time spent in each location was calculated from the total of the 81 scan points.
- *Synchrony Index (SI):* The behavioural scan data gave a representation of the herd behavioural activity which showed us how many cows were performing each type of behaviour. This allowed us to identify the dominant herd behaviour and calculate the level of synchrony within the herd. The behavioural scan data were grouped into five categories for analysis: feeding at the feedface, standing in the cubicle, lying in the cubicle, standing half in the cubicle and standing in the passageway. For every scan point the proportion of cows performing each behavioural category was calculated. From this calculation, the behaviour that the majority of cows within the herd were performing at any given scan point was identified as the primary behaviour. The second step was to identify scans where herd synchrony was present (i.e. when the primary behaviour dominated all other behaviours). Across all scans, the mean proportion of cows performing the primary behaviour was 0.63 ± 0.19 (Mean ± S.D.). Herd synchrony was defined as occurring when ≥60% of the herd were performing the same primary behaviour. Analysis was carried out on a total of 40 scans where synchrony (by this definition) occurred. A synchrony index was used to determine whether focal animals displayed herd synchrony or not. The synchrony index was calculated as follows: no. of scans performing dominant herd behaviour/no. of scans performing dominant herd behaviour + no. of scans not performing dominant herd behaviour. The synchrony index ranged from 0 to 1 which corresponds to complete asynchrony to complete synchrony, respectively.
- *Feeding Index (FI):* Presence at the feedface during peak feeding was calculated. Peak feeding was defined as the first hour (first three scans) after delivery of

fresh feed. The mean proportion of cows feeding during peak feeding was 0.81 ± 0.12 (Mean ± S.D.). Figure 1 shows the percentage of cows present feeding, standing and lying during the nine scan points. A feeding index was used to determine whether focal animals were present at peak feeding, and was calculated as the no. of scans the cow was present at peak feeding/ no. of scans present at peak feeding + no. scans absent at peak feeding. The feeding index ranged from 0 to 1, which corresponds to always absent and to always present at peak feeding, respectively.

4.3.2.2 Runway Test

The forty-six focal cows were subjected to a runway test, once per week over a three week period. Each week, the 46 focal cows were randomly assigned to one of three groups (n= 15, 15 and 16). Over the three test weeks, this equated to a total of 9 test groups. One group was tested on each experimental day to coincide with the scan sampling test days (Tuesday, Wednesday and Thursday). The groups were balanced for parity (lactation 1, 2, 3, 4, 5+). This design allowed the group composition to vary from week to week in order to control for effects of social hierarchy. This was done because a subordinate animal's motivation to return to its herd may be affected by the presence of a dominant animal (Beilharz and Zeeb, 1982). By altering the group composition, the influence of dominant cows was randomised across test groups.

The runway (18m x 6.6m) was a concrete floored passageway situated between the cow's home-pen and the milking parlour. This passageway was part of the collecting area for the parlour and the cows walked through the passageway to and from milking three times daily. To ensure the cows were completely habituated to the test area, the cows were held in this area for a further fifteen minutes before each milking for 5 days prior to the start of testing.

On test days, the test group of cows were penned at one end of the runway (Fig 2.1). In turn, each cow was removed from its test group and gently moved up to the start line of the raceway by two familiar experimenters dressed in blue overalls. The cow was held behind a gate in a holding pen, allowed to settle for 30 seconds, and then released, allowing the cow the freedom to move out of the holding pen and move up and down the passageway. The test duration was 300s from when the cow crossed the start line. Following the test, the test animal was then put back into the test group and the next animal selected. Animals were selected in a predetermined random test order. All test sessions were recorded by a digital camcorder (Canon XM2). The latency to reach the 5m and 2m marks from the test group and duration of time spent in the 5m and 2m areas of the runway test were taken from video recordings and used as measurements of social motivation.

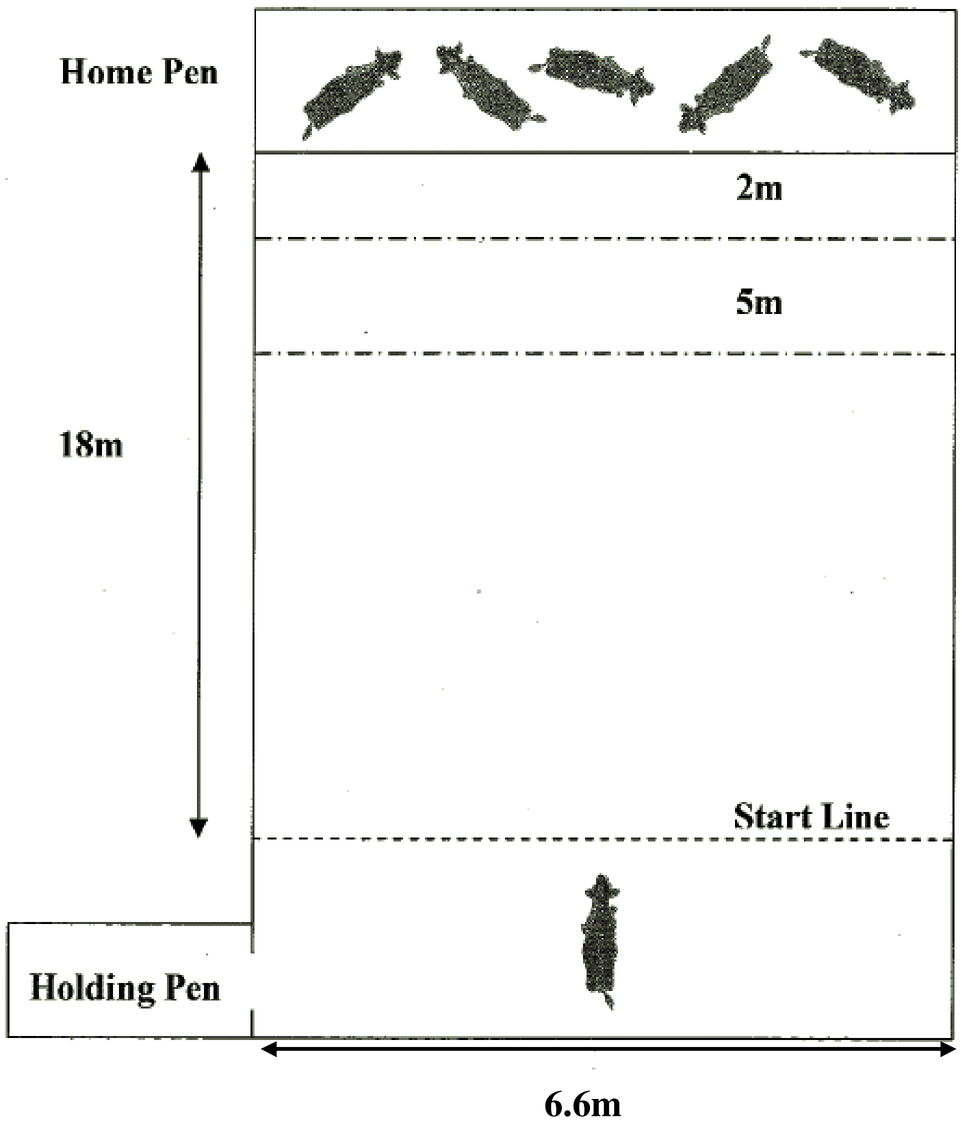


Figure 4.1 Experimental set-up used to study social motivation in cows using a standardised runway test.

4.3.3 Statistical Analysis

All statistical tests were run using GenStat® for Windows™ 7th (2004; Laws Agricultural Trust, Rothamsted Experimental Research Station, Harpenden, Hertfordshire, UK). All data were checked for normality.

4.3.3.1 Intra-test consistency of runway test measures

Two approaches were used to assess the consistency of the sociability measures from the runway test: (1) repeatability estimates, (2) Kendall's coefficient of concordance. To assess consistency of cows' responses to the runway test, repeatability estimates (*r)* for duration and latency across the three test repetitions were calculated. Latencies of 300 seconds (indicating that the cow did not reach the 5m or 2m line within the allocated time (300s)) were treated as censored data. The censored data was replaced by estimated values using the GenStat® CENSOR procedure before calculating repeatability estimates (*r*). The CENSOR procedure assigns a value greater than 300s by estimating the expected value of each censored observation. Latencies to the 5m and 2m lines were transformed by natural logarithm transformation, and durations were transformed by angular transformation to meet assumptions of normality. Repeatability is an estimate of the proportion of variation among individuals that is due to individual differences (Boake, 1989). To estimate repeatability, variance components were computed from a Linear Mixed Model (LMM) using Restricted Maximum Likelihood (REML: Paterson and Thompson 1971). In the LMM, animal ID number and test repeat were fitted as random effects. Repeatability then can be estimated using the within and between animal variance components following Lessells and Boag (1987):

$$\text{Repeatability} = \frac{\text{Variation between cows}}{\text{Variation between cows} + \text{Variation within cows}}$$

A cut-off value of ≥ 0.5 was used to distinguish those social measures that gave the most repeatable results, and indicates that 50% of the variance occurs between cows rather than within individuals (Lessells and Boag, 1987), signifying a level of consistent individual responses across test repeats. Repeatability estimates close to 0 would indicate that all the animals respond differently to each test repeat and a repeatability approaching 1 would indicate that repeated measurements of the same individuals gave identical estimates.

Kendall's coefficient of concordance (*W)* analysis was used on the un-transformed data as a conservative test of consistency as the repeatability estimate (*r*) is very sensitive to the average value of traits (Falconer and Mackay, 1996). The level of concordance (*W)* was used to investigate the within-individual consistency across test repeats using rank orders (Siegel and Castellan, 1988). If individuals were consistently ranked the same among tests then the concordance coefficient equals one, whereas if ranks varied randomly from test to test then the concordance coefficient equals zero. No threshold figure for *W* exists above which a variable maybe considered consistent. Napolitano *et al.* (2005) suggests an interpretation of *W* coefficient of less than 0.4, between 0.4 and 0.6 and greater than 0.6 to indicate low, moderate and high agreement, respectively.

In addition, Friedman's test (*S*) was used on the un-transformed data to determine if there was a significant difference in social motivation between cows in the runway test.

4.3.3.2 Inter-test consistency of sociability measures in behavioural scans and runway test.

The effect of the most repeatable measure from the runway test (latency to 5m line) on eight behavioural scan variables (i.e. NN, FN, NFN, L1, L2, L3, SI, FI) were investigated with Generalized Linear Mixed Models (GLMM) using REML. The behavioural scan variables were each fitted as the response variable with the latency to 5m line as the fixed effect. Runway test repeat and cow were fitted as nested random effects. The behavioural variables were proportion data and a binomial distribution (number of occurrences out of 81 scans) was assumed with a logistic link function added. Five metre latency was transformed using natural log transformation before it was fitted as the fixed effect. Statistical significance of terms in GLMMs was tested using the Wald statistic (*W*).

4.3.3.3 Age Effect

The effect of age on social motivation was also tested. The experimental herd was not entirely balanced for lactation groups (1, 2, 3, 4 and 5+) so GLMM using REML was used to determine whether there were any effects of lactation number on the sociability measures. The behavioural scan variables were fitted with a binomial distribution and the runway measures with a Poisson distribution in the models and logistic and logarithm link functions were used, respectively.

4.3.3.4 Competitive Success Index (CSI)

A competitive success index (CSI) was calculated to investigate if the sociability measures used in this study were influenced by the cow's competitive success at the feedface during peak feeding. From previous work on these cows (Chapter 3), CSI was calculated. However, CSI was only available for 34 of the 46 focal cows used in the present study, as not all of the cows used in the present study were used in the previous one. The CSI method establishes the success of each animal by assessing aggressive interactions in this case at the feedface. This method assesses the number of aggressive actions an individual performs as well as its success in actually displacing other cows. An aggressive index (AI = no. of times aggressor/no. of times recipient + no. of times aggressor) (adapted from Barroso *et al.*, 2000), displacement index (DI = no. of active displacements/no. of active displacements + no. being displaced (Galindo and Broom, 2000) and competitive success index (Mean of DI and AI) were calculated for each cow. The CSI values ranged from 0 to 1 corresponding to low and high competitiveness, respectively. The CSI was fitted as a covariate in the fixed effect of a GLMM to investigate its effect on the sociability measures.

4.4. Results

4.4.1 Intra-test consistency in the runway test

Four measures were recorded in the runway test: latency to reach the 5m and the 2m lines and duration of time spent in 5m and 2m areas. Six of the 46 animals did not cross the 5m line and 11 of the 46 animals did not cross the 2m line in all three test repeats.

Repeatability estimates and Kendall's coefficient of concordance was used to assess the within-cow consistency of the different measures of social motivation in the runway test. There was variation in the repeatability estimates for the social motivation variables. The within-cow repeatability for latency to the 5m line showed a moderate repeatability estimate (0.54) and a highly significant concordance (0.74). The rest of the variables were moderately repeatable (Table 4.1). Cows showed consistency in their response to runway test repeats, despite a slight increase in measures as test days progressed. There were significant differences between cows for all measures as shown by the Friedman's test (Table 4.1).

Table 4.1 Medians, 1st and 3rd quartiles, repeatability estimates, estimated variance components between cows and within-cows for Runway Test Sociability measures.

Statistic	*Test Measure*			
	Latency		Duration	
	5m	2m	5m	2m
Median (s)	116.5	205.5	124	73
Q1 (s)	42	72	0	0
Q3 (s)	300	300	266	204
Repeatability Estimate[1] Across 3 Tests	0.54	0.49	0.42	0.39
Estimated variance component:				
Between cows	0.58	0.44	356.2	314.9
Within-cow	0.50	0.46	499.0	484.3
Kendall's Coefficient (d.f.=45)	0.74 ***	0.71 ***	0.57 **	0.59 ***
Friedman's Test				
Day Effect (d.f.=2)	3.43 NS	1.68 NS	5.52 NS	1.70 NS
Cow Effect (d.f.=45)	100.04 ***	96.12 ***	82.62 **	80.42 **

[1] Repeatability=variance between cows/variance within-cows + between cows

Significant levels: *P<0.05, **P<0.01, ***P<0.001, NS = Non Significant.

(s) = Seconds

4.4.2 Relationship between behavioural scan variables

All eight behavioural scan variables were analysed to determine the relationship between them. Significant correlations for measures recorded during the behavioural scans are shown in Table 4.2. In general, it appears that many of these variables are related.

Table 4.2 Matrix of spearman rank correlations (r_s) between the behavioural scan variables of sociability. Only significant results shown.

Behavioural Scan Variable	*1*	*2*	*3*	*4*	*5*	*6*	*7*
1. 2 neighbours <1m away (NN)	-						
2. 2 neighbours >1m away (NF)	**-0.795**						
3. 1 near and 1 far neighbour (NFN)							
4. Feeding Index (FI)	**0.770**	**-0.622**					
5. Synchrony Index (SI)	**0.742**	**-0.602**		**0.988**			
6. Location 1[†]	<u>-0.419</u>			**-0.507**	**-0.516**		
7. Location 2[†]	*-0.361*	**0.484**		**-0.476**	<u>-0.451</u>		
8. Location 3[†]	**-0.494**	**0.703**		*-0.357*	*-0.350*		

Column numbers in the top row correspond to the numbered variables in the first column. Significant levels: ***P*<0.001**, <u>P<0.01</u>, *P<0.05*

[†]See text for definition of the different locations within the cow shed.

4.4.3 Inter-test consistency of sociability measures in behavioural scans and runway test.

Latency to the 5m line was chosen as the most appropriate measure for comparison to behavioural scan variables because it was the most consistent. All eight behavioural scan variables were analysed to assess the relationships between each of them and latency to the 5m line. Cows that had high latencies to the 5m line had fewer recordings with two near neighbours (W_1=5.31, P=0.021), were less synchronised with the herd (W_1=4.82, P=0.028), were less likely to be present at peak feeding (W_1=4.13, P=0.042) and more likely to stand at the outside edge of the shed (W_1=4.03, P=0.045) (Table 4.3). All other relationships were not significant.

Table 4.3 Table showing the relationship between sociability behavioural variables and latency to 5m line.

Response Variable	***Mean (±S.E)[1]***	***Effect (SE)***	***Wald Statistic***	***P-value***
2 neighbours <1m away (NN)	0.70 (±0.01)	-0.001 (0.0008)	5.31	*
2 neighbours >1m away (NF)	0.21 (± 0.01)	0.0011 (0.0021)	0.29	NS
1 near and 1 far neighbour (NFN)	0.09 (± 0.01)	0.0021 (0.0031)	0.46	NS
Synchrony Index (SI)	0.73 (±0.02)	-0.0024 (0.0010)	4.82	*
Feeding Index (FI)	0.36 (± 0.01)	-0.001 (0.0005)	4.13	*
Location 1[†]	0.13 (± 0.01)	0.0022 (0.0010)	4.03	*
Location 2[†]	0.18 (± 0.01)	-0.0003 (0.0023)	0.02	NS
Location 3[†]	0.01 (± 0.01)	0.0013 (0.0020)	0.23	NS

[1] Means (± S.E) shown as proportion of total scans

[†]See text for definition of the different locations within the cow shed.

Significance levels: **P*<0.05, ***P*<0.01, ****P*<0.001, NS = Non Significant

4.4.4 Age and Dominance Effects

There were no effects of lactation number or competitive success (CSI) on behavioural scan variables, synchrony index or feeding index. A positive significant effect of CSI was found for latency to reach the 5m line (W_{33}=54.59, P=0.01). No effect of CSI was found for latencies to reach 2m lines or the durations in the 5m and 2m areas.

4.5 Discussion

4.5.1 Intra-test consistency of runway test

The first aim of this work was to assess the consistency of individual cows' motivation to reach their herdmates in three repeats of a runway test using two statistical methods (repeatability estimates and Kendall's coefficient of concordance). Responses of dairy cows to the runway test were shown to be low to moderately repeatable. Repeatability estimates evaluate an individual's consistency across tests by comparing it to the variation across the experimental group (Falconer and Mackay, 1996). The repeatability of the Latency to reach the 5m line in the runway test was the most repeatable of the measures recorded, it exceeded 0.5 across all three tests and was significant by Kendall. Findings from the present study are similar to previous work carried out by Hopster and Blokhuis (1994) who examined the repeatability of behavioural responses of dairy cattle to social isolation and found repeatability values of between 0.58 and 0.69 for several behavioural measures. However, Fisher *et al.*, (2000) repeated a test of sociability three times on the same cows at monthly intervals, and found a repeatability estimate of 0.34 for the time taken to join conspecifics. In the present study, repeatability coefficients decreased over successive test repeats for all four runway test measures. Despite this evidence of habituation to the runway test, animals remained consistent in their response over the length of the experiment. This is similar to findings reported by McBride and Wolf (2007) in sheep.

There is often difficulty in interpreting repeatability estimates. Repeatability is computed as a ratio of within-cow to between-cow variation. Understanding whether individuals show consistent behaviours in repeated trials however, is difficult to ascertain from the repeatability ratio. This is because low repeatability values can indicate either a consistent response (low variation between and within-cows) or a random response (high variation between and within-cows; Hayes and Jenkins, 1997; Widemo and Sæther, 1999). To provide further support, both repeatability estimates and Kendall's coefficient of concordance are presented. The highly significant concordance for latency to the 5m and 2m line suggest high rank-order consistency over the three repeats. To date one of the largest difficulties in assessing consistency of behaviour is the lack of clear criteria to decide when consistency is adequate. Finding statistically significant concordance and moderate repeatability estimates for the 5m line instilled confidence that the animal's response was consistent and therefore, their behaviour was indicative of an underlying sociability trait.

4.5.2 Inter-test consistency of latency to 5m line and sociability scan variables

To be considered a behaviour trait, individual differences in behaviour have to be consistent across time and/or across situations (Sibbald *et al.*, 2006; Erhard and Schouten, 2001; Réale *et al.*, 2007). The second aim of this study was to investigate the relationship between measures of sociability taken from behavioural observations of group behaviour and the most consistent measure in the runway test (latency to 5m line). Predictability of behaviour in other situations can be used as an indicator of the 'reliability' of a test, or the consistency of a behaviour trait (Sibbald *et al.*, 2006). The animal's performance in the runway test appears to predict a range of social behaviours occurring spontaneously in the home pen. Given the infeasibility of carrying out runway tests on-farm due to time and logistical constraints, social behaviour observations in the home pen represent a more practical method of recording sociability of individual cows under farm conditions. The presence of neighbours less than 1m away, the extent of behavioural synchrony, the presence at the feed face during peak feeding and the position of the animal within the housing area can be considered more practical measures to assess cattle sociability under commercial conditions.

It is logical to expect the measures of sociability from the behavioural scan observations to significantly correlate with each other as some of these measures are highly related. In particular, the high correlations between measures of synchrony and presence during peak feeding indicate that in future studies recording one of these measures would be sufficient. Location of a cow within its home-pen gives additional information regarding its sociability. This study highlights the fact that animals that remain on the periphery of the pen are more likely to have a lower social response in a standardised runway test and therefore indicative of lower sociability. The analysis showed that the number of observations in which an animal was observed with less than 1m to two neighbours (NN) is a better indicator of an individual's sociability compared to the other nearest neighbour measures taken in this study. The NN measure correlated to all other sociability scan variables recorded and had the highest significant relationship with latency to the 5m line. Distances of less than 1m to nearest neighbours are the most useful measure of an individual cow's sociability within the context of this study.

However, further validation is necessary to fully understand the biological relevance of different levels of sociability and their relationship to welfare and health. Outcomes from other studies can be used to indirectly interpret the biological significance of the sociability measures used in this study. As measures of proximity, both the distance to nearest neighbours and latency to the 5m line are used. These measures give some information on how close a mature cow chooses to be to other cows. Observations of inter-cow distances in more natural settings suggest that most animals keep 1m apart while standing (Sato *et al.*, 1984), 2-3m during lying and 4-10m during grazing (Fraser and Broom, 1997). The distances recorded in this study fall within this range suggesting that cows maybe able to achieve acceptable social distances under housed conditions such as those used in this study.

This study also demonstrated that presence at the feedface during peak feeding is related to social motivation in a runway test. Therefore, presence at the feedface during peak feeding may reflect the sociability of an individual animal. However, animals that choose to feed at the time of greatest feed availability could also be those animals most highly motivated to feed. Presence at the feedface is greatly driven by both behavioural (social facilitation) and physiological factors (milk yield and or stage of lactation). On the other hand, animals that avoid peak feeding times or feed at the end of the feedface are choosing to avoid interactions with their herd-mates. These animals may be adopting a coping strategy that minimises the level of social stress in their daily routine. Further research is required to investigate factors affecting trade-offs made by individuals.

There are many interactions between an individual's sociability and its environment. The level of sociability may be variable as it results from the interactions between genetics and epigenetic factors such as early influence and previous experience. It is well established that social contacts during development influence social behaviour in adulthood. For example, heifer calves housed in groups formed at birth expressed closer associations than those housed in groups formed at the age of six months (Bouissou and Andrieu, 1978). There are many aspects of the farm environment that may influence the expression of sociability such as stocking density, feedface length, cubicle to cow ratio, feed availability, previous experience, calf rearing, social rank and age. Further research is needed for a deeper understanding of the relationship between an individual's sociability and health, production and adaptability to social challenge and change is needed to ensure not only optimal production but also to maximise welfare. There is potential for livestock selection for sociability if simple and validated methods for detecting sociability can be shown to have genetic variation. This could influence the ability of animals to adapt to their environment, therefore enhancing production and maximising animal welfare.

4.6 Conclusion

A runway test was used to assess sociability and it produced results that revealed considerable variation in responses between animals, and good consistency within animals. Latency to reach the 5m line in the runway test was then used to find reliable measures of sociability that are applicable to on-farm conditions. The analysis suggests that reliable and practical behavioural indicators of sociability are a measure of an individual's level of synchrony with the herd, position in the shed, presence at the feedface during peak feeding and frequency of having two near neighbours.

CHAPTER 5

Effect of selection for robustness on aggressiveness during feeding in dairy cows

In this chapter, I was responsible for experimental design and carried out all of the experiment with help from Ramona Donald, Ruth Turl and Lindsey Maggs. I was responsible for data and statistical analysis and writing the manuscript.

5.1 Abstract

Aggressive behaviour during feeding was investigated on 350 primiparous dairy cows from 54 sires, 24 of which were HI robust sires with 195 daughters and 30 of which were LO robust sires with 155 daughters. These animals were located on 33 commercial farms. During feeding, actor and recipient behaviours of focal cows in aggressive interactions were recorded using continuous focal sampling. Three separate analyses were carried out, one for actor behaviours (ACT, n=234; LO=104, HI=130), one for recipient responsive behaviours (RES, n=299; LO=133, HI=166) and one for non-responsive behaviours (NR n=196; LO=91, HI=105). The influence of management factors on aggressiveness was also investigated. Increased actor behaviours were associated with silage ME less than 12.4 Mj/Kg DM (W_3=9.66, P=0.045) and less than 2.63 months at grass (W_3= 10.11, P=0.042). There was no significant effect of robustness with respect to actor behaviours. Cows from the HI group responded to aggression more frequently than cows from the LO group (W_1=7.42, P=0.006). Increased recipient responsive behaviours in cows were associated with being fed a TMR (W_3=10.77, P=0.004), having relaxed stockhandling on the farm (W_3=12.95, P=0.005), and higher number of stockpeople (W_3=8.00, P=0.046). There was no significant effect of robustness with respect to non-responsive behaviours during an aggressive interactions (W_1=1.78, P=0.182). The results from this study imply that daughters from sires scoring high for robustness may be showing stronger motivation to maintain their position at the feedface during an aggressive interaction. This highlights the importance of assessing the correlated effects of selective breeding on behavioural traits.

5.2. Introduction

Until recently, animal breeding in commercial species was mainly directed at improving production with less attention paid to improving health and welfare. In the dairy industry, selection pressure has been largely focused on milk production over the last 50 years. However, it is now recognised that the health and welfare of production animals is important. There is evidence that unfavourable genetic correlations with milk production have contributed to the reduction of cow health and longevity (Rauw *et al.*, 1998; Royal *et al.*, 2000; Pryce *et al.*, 2001, 2002; Berry *et al.*, 2003). In recent years, this has led to the dairy industry enhancing their selection indices to include fertility, survival, lameness and mastitis as a means to effectively prevent the decline in health and longevity (Veerkamp *et al.*, 1995; Stott *et al.*, 2005; Wall *et al.*, 2006, 2007a). This concept of breeding for improved robustness has recently become a main interest in animal production (Pigs: Knap, 2005; Poultry: Star *et al.*, 2008). There are many definitions of robustness available (see Knap, 2005; Veerkamp *et al.*, 2007; Strandberg, 2007; Lawrence *et al.*, 2009). The general consensus is that robustness is a combination of functional traits and other non-productive traits, such as behaviour that provide the cow with the ability to cope with variable environments (Klopčič *et al.*, 2009). The study described in this chapter was part of a larger project that aimed to identify and characterise robustness in dairy cows. A robust cow was defined as one that is long-lived and healthy in a range of environments (Lawrence *et al.*, 2007).

The aggressiveness of individual animals towards group-mates is important to consider in the context of selective breeding. It has been shown that selection for production can adversely affect aggressive behaviour of farmed animals. For example, selection for improved egg production may unintentionally increase aggression in poultry (Muir, 1996) and similar results may be expected in pig breeding programmes (Muir and Schinckel, 2002). However, selection for particular behaviour traits can also affect other behaviours. Several studies have shown that selection for reduced fearfulness in animals increases maternal defensive aggression (Rodents: Maestripieri and D'Amato, 1991; Boccia and Pedersen, 2001; Pigs: Løvendahl *et al.*, 2005; Sheep: Murphy *et al.*, 1998). The focus of this study is on aggressive style of individual dairy cows as one aspect of temperament that is appropriate to study in the context of robustness. Of particular interest is the cow's willingness to compete for feed or to displace other cows at the feedface. If aggressive behaviour is genetically linked to aspects of robustness, it is possible that selecting for robustness may result in breeding for aggressive cows. Selection for high milk production and improved body condition requires dairy cows to consume more food (Veerkamp, 1998). Therefore, it is conceivable that selection for these traits may alter resource-defence aggression during feeding. Cows may become more aggressive trying to gain access to feed particularly during peak feeding times. Aggression has obvious consequences on animal welfare. In addition to possibly causing injury and stress to the individuals involved, social stress may cause some cows to alter their feeding times to avoid aggressive interactions (Olofsson, 1999; DeVries and von Keyserlingk, 2006; Huzzey *et al*., 2006). These cows may have lower access to food resulting in lower feed intakes, lower growth rates (Nakanishi *et al*., 1993) and reduced milk yields (Hasegawa *et al.*, 1997).

The aim of this study was to investigate the possible effects of selection for robustness on feeding aggression in dairy cattle. In order to achieve this, a comparison between the aggressiveness during feeding of daughters from sires which scored high on a robustness index with daughters from sires which scored low on the same index on commercial farms throughout the UK was undertaken. The robustness index is an extension of the £PLI (Profitable Life Index) with increased emphasis on on locomotion, somatic cell count, udder health, fertility and lifespan reflecting both the farming industry and wider consumer demand for higher welfare standards (Wall *et al.,* 2007b).

5.3 Materials and Methods

5.3.1 General Farm Information

The study was carried out during the winter indoor housing period (November 2005 to May 2006) on 33 commercial dairy farms throughout Great Britain. These herds were located in England (26), Wales (1) and Scotland (6) (Fig 5.1). There were a total of four female observers with two observers visiting any one farm. The mean herd size was 288.26 cows (± 21.52 S.E.) with a 305 day average milk yield of 8488.96 ± 143.51 (mean ± S.E.) litres per cow. All herds had predominantly Holstein/Holstein Friesian cows, and all focal cows were Holstein. All farmers fed their cattle a conserved forage ration with a compound feed or blend during the winter housing period. Thirty farmers fed the ration as a total mixed ration (TMR) and 5 farmers fed the ration as forage with additional concentrate fed either in the parlour or in out of parlour feeders. There was a range of different types of feedface on the farms including strap and post, diagonal railed barrier and feed troughs. Six farms housed the cows all year round and the remaining 29 farms had the cows at grass for 4.17 ± 0.38 (mean ± S.E.) months per year.

Figure 5.1 Geographical location of all farms that participated in this study.

5.3.2 Focal cow and farm selection

A robustness index (RI) was calculated for all commonly used pedigree Holstein bulls and included fertility, body condition score, heifer growth rate, locomotion and somatic cell count (Wall *et al.,* 2007b). At the time of selection, the breeding index in the UK was £PLI which includes the production index for milk (l), %fat and %protein along with lifespan, somatic cell count and locomotion. The new robustness traits were added to £PLI scores to create a 'robustness' index score for each bull. The distribution of the robustness index scores for bulls was normal (Figure 5.2). For the purposes of this study, sires were defined as being HI if they had a robustness index of greater than +£9 and LO if they had robustness index of less than -£11. There was a difference of two standard deviations between the LO and HI groups.

To compare the behaviours of daughters of HI and LO robust sires on farms, the national milk recorder databases were interrogated to identify farms that had at least eight daughters of HI robust sires and eight daughters of LO robust sires. Primiparous cows were used as the subjects of this study as young animals would be less likely to have their behaviour modified by the environment, and using only primiparous animals would avoid the potential bias created by involuntary culling of LO robust animals as they get older. The initial search identified 46,897 primiparous cows in 6,379 herds of which 23,723 were from HI sires and 23,174 were from LO sires. There were a total of 549 HI and 402 LO sires. However, only 179 herds had 8 or more daughters from each of the HI and LO groups of sires. In the first instance, all of these 179 herds were contacted to enquire if they were interested in participating in this study. A total of 102 farms responded, however, 53 of these replied to say that they were not interested in participating in the study and a further 14 did not meet the farm management selection criteria. From this point forward, daughters of low robustness bulls will be referred to as LO cows while the daughters of high robustness bulls will be referred to as HI cows. A total of 402 primiparous cows were identified, however, behaviour data at the feedface was successfully collected and analysed on 350 daughters from 54 of the identified sires, 24 of which were HI robust sires with 195 daughters and 30 of which were LO robust sires with 155 daughters. Mean (±S.E.) number of daughter per sire was 6.36 (±1.11) with a range of 1-36 (min-max).

Dairy farms vary greatly in management practices. In order to reduce the effect of varying environments in this study, farms that were organic, farms that used straw courts or farms housing primiparous cows separately from the multiparous cows were excluded from this study as these factors are known to affect their behaviour and health (Haskell *et al.*, 2006, Bach *et al.*, 2006; Langford *et al.*, 2006; Rutherford *et al.*, 2009). Only 35 farms that responded met the farm management criteria and detailed communications with the farmer prior to the visit were carried out to determine if focal animals were lactating and healthy.

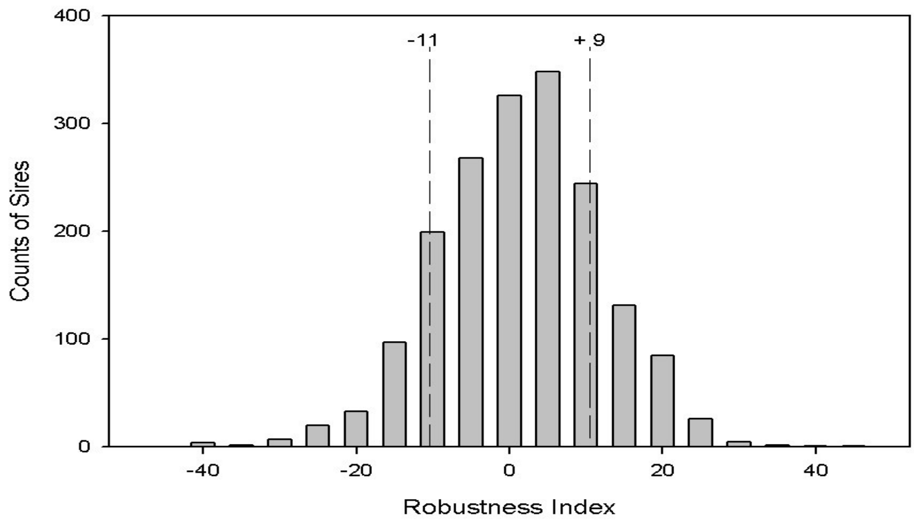


Figure 5.2 A histogram of bulls score on the robustness index (RI). High robust bulls had a RI score of greater than +£9 and LO bulls had a RI score of less than -£11, the groups were separated by two standard deviations.

5.3.3 Behavioural Observations

The main aim was to record the aggressive interactions of each individual focal cow for a 15-minute period during peak feeding. On arrival to each farm, focal cows were individually marked during milking with a spot on their rump using marker spray (Ritchey Super Sprayline Stock Marker) and a unique colour-coded tail tape (Scapa) for ease of identification. Peak feeding was defined as the first two hours following arrival of fresh feed when all cows were present in the home pen or on return of 60% cows from milking where fresh feed was delivered while the cows were gone for milking.

In order to habituate the cows to the presence of the observer, the observer walked up and down the feed passage prior to feeding with the camera and tripod for a half hour. This procedure was carried out on all farms on the day of arrival and always immediately prior to video recording individual cows at the feedface.

The same recording procedure was carried out for each focal cow after feed had been delivered. Focal cows were located at random at the feedface. The observer set up the camera (a Canon XM2 digital camcorder) and tripod in the feed passage at a minimum distance of 1.5m from the focal cow. At the start of recording, the identity of the focal cow as shown by her unique colour coded tail tape or eartag number was recorded. The behaviour of the cow was video-recorded for 15 minutes. If a focal cow withdrew completely from the feedface before 15 minutes were recorded, the remainder was collected at a later point. Fifteen minutes was considered an appropriate recording time as it is a substantial proportion of peak feeding for any one cow. Feeding bouts of cattle range between 2 to more than 40 minutes (Tolkamp and Kyriazakis, 1999). Analysis of previous work indicated that sufficient interactions occur within a fifteen minute time frame (Chapter 3). Additionally, using fifteen minute recordings allowed us time to locate and record all focal animals during the 3.5 day visits to each farm.

On the majority of farms, peak feeding was in the early morning, however, due to time constraints on some farms, it was necessary to also use the peak feeding period after the cows returned from afternoon or evening milking to allow observations on all focal animals. In this situation, peak feeding was defined as when >60% of the cows were feeding at the feedface. De Vries *et al.* (2003) reported that the return from milking stimulates feeding behaviour in housed dairy cows.

As behaviour is greatly affected by illness, each farmer filled out a post-visit health check form, recording whether or not the focal cows suffered any health condition (mastitis, reproductive disorder, lameness) in the two weeks after our visit. Twelve focal animals developed a health condition within this time frame and were dropped from the analysis. After parturition, primiparous cows are introduced to a new social environment (milking herd) and milk facilities for the first time. This can be a stressful and challenging time affecting natural behaviour so cows that had calved less than two weeks prior to our visit were not included in the study.

The analysis of the videotapes was carried out by two observers. Observers were blind to the treatment groups of HI and LO robustness. From the video recording, each occurrence of the behaviours shown in Table 5.1 were recorded along with the identities of the actor and recipient cows involved. A cow was designated as the actor in an interaction when she exhibited one of the actor behaviours (Table 5.1) either as she approached another cow at the feedface or directed this behaviour towards a cow that was already positioned next to her at the feedface. The animal receiving this behaviour was designated as the ‘recipient’.

Table 5.1 Ethogram of behaviours and behavioural categories recorded for actor and recipient of aggressive interactions between cows at the feed face.

Actor	**Category**[1]	**Description**
Pushing	C	The actor uses some part of the body other than the head to displace the recipient.
Butting	C	The actor uses head to head, head to neck or head to flank contact in an attempt to physically displace the recipient.
Bulldoze	C	The actor forcefully enters the front of the feedface displacing more than one individual.
Penetrate Feeder	C	The actor pushes with a lot force between two eating cows at the feedface resulting in physical contact with cows on both sides.
Blocking	NC	The actor uses the body to physically block the recipient from gaining access into the feedface.
Threatening	NC	The actor presents a threat posture by presenting the forehead with inclined head or the actor engages in a threatening swing of the head in the direction of the recipient, no contact occurs between the two individuals.
Recipient		**Description**
No Response	NR	The recipient shows no physical response.
Avoids	AA	The recipient moves/turns head in opposite direction in order to avoid actor.
Withdraws Back	AA	The recipient withdraws head from beneath the feed rail or strap and moves straight back into passageway.
Withdraws Side	AA	The recipient withdraws and moves along one cow space at the feedface
Retaliates	AR	The recipient retaliates with an attack (e.g. bunt, push etc) towards the actor.
Fight	AR	Two individuals push and butt each other repeatedly.

[1] Key to acronyms: C, Contact; NC, Non-contact; NR, Non-responsive; AA, Active Avoidance; AR, Aggressive responsive

5.3.3 Explanatory Variables

5.3.3.1 Farm assessment

A detailed building audit was carried out to identify any potential factors influencing aggressive behaviour at the feedface during peak feeding (Table 5.2). A farmer interview gathered general herd and management information on herd size (HS), number of months grazed at grass (MG), number of people involved in the day to day care of the herd (P), gender of stock carers that were primarily responsible for rearing calves (G) and housing type of calves during first six weeks after birth (single pens or group housed). More detailed information was collected on nutrition provided to the dairy cattle. Nutritional information gathered included whether a total mixed ration (TMR) was fed, quantity of concentrate fed (CTCY) (tonnes/cow/year), quantity of concentrate fed in TMR (kg/cow/day), quantity of forage fed (F) (kg/cow/day), metabolisable energy (ME) of concentrate (CME) (Mj/Kg) and silage (SME) (Mj/Kg).

Table 5.2 A summary of building audit measures recorded.

General Area	Descriptor	General Area	Descriptor
Building Design	Building dimensions	**Water**	Trough number
	Ventilation type		Trough dimensions
	Cubicle number	**Feedface**	Feed space allowance per cow
	Cubicle design		Feedface design
	Cubicle dimensions		Feedface dimensions
	Step height	**Parlour**	Parlour design
	Bedding material		Total milking stations
Passageways	Passage dimensions		Milking station dimensions
	Flooring type		Collecting yard dimensions
	Scraping method		Walking condition of collecting yard
	Scraping frequency		
	Grooming equipment		
	Walking condition		

5.3.3.2 Stockhandling (SH) assessment

As it was thought that the quality of the interactions between the stockhandlers and the cows could affect the expression of aggressive behaviour, a qualitative assessment was made of the stockperson's level of interaction with the cows and also the cows' reaction to the stockperson. The stockperson was observed during three situations: 1. moving the cows from the cubicles through the holding yard into the parlour, 2. cleaning the udders and attaching the milking machine clusters and 3. moving the cows out of the parlour. The stockperson's level of interaction with the cows was scored in each of these three situations using qualitative visual analogue scales (VAS) based on Wemelsfelder *et al.* (2001). The three VAS ranged from relaxed to harsh. Relaxed indicated positive interactions towards the cows (pats, strokes, hand resting on the back, leg or flank of the cow, gentle verbal encouragement towards the cows consisting of whistling, talking or clapping hands). Harsh indicated mild to severe negative interactions towards the cows (forceful slaps/hits, negative use of plastic pipe (or other objects) towards cows, twisting cow's tail to force forward, loud, harsh shouting or the use of an object to hit against metal bars to create a loud noise) (Rennie *et al*., 2003).

Additionally, the observers marked four VAS to assess the cow's reaction to the stockhandler. The scoring of the VAS was carried out according to a subjective judgement of the herd's reaction to the stockhandler moving the cows in and out of the parlour. The terms were set out in pairs: relaxed to nervous, placid to aggressive, eager to reluctant and slow to fast. The first three pairs of terms (relaxed to nervous, placid to aggressive, eager to reluctant) were scored as the cows entered and exited the parlour. The final pair of terms qualitatively measured the speed (slow to fast) at which the cows entered and exited the parlour. The seven VAS were recorded twice by two different observers. The VAS consisted of 125mm horizontal line with two vertical lines marking the extreme points of the scale, for example, 0mm indicates relaxed and 125mm indicates nervous. Scores for each term on the VAS scale were measured as the distance in millimetres from the 0-point. The scores were converted to a percentage (0%=relaxed; 100%=harsh). A mean stockhandling percentage was calculated for each farm and was used as an explanatory factor in the statistical analysis (see below).

5.3.4 Data Analysis

402 focal cows (out of the 420 focal cows identified) were observed feeding at the feedface for the allocated 15 minutes. Eighteen cows were not observed feeding at the feedface during the visit. A total of 52 of these individuals (HI=33, LO=19 across 21 farms) did not receive aggression or perform an aggressive act during the recorded 15 minutes, and so had a zero score for interactions. Initial analysis showed that there was no effect of robustness on whether or not a cow was involved in an aggressive interaction (W_1=2.29, P=0.130). It was considered that by having no behaviour data on these individuals, no knowledge was gained regarding these individual's aggressive style at the feedface and they were subsequently removed from the analysis.

The proportion of cows displaying behaviours were 0.65, 0.24, 0.53, 0.36, 0.13 for contact (C), non-contact (NC) active avoidance (AA), non-responsive (NR) and aggressive responsive (AR) respectively. For analyses, the data were pooled for individual cows to derive total actor (ACT) behaviours (pooled C and NC), total recipient responsive (RES) behaviours (pooled AA, AR) and recipient non-responsive behaviour (NR). The proportion of all cows displaying ACT, RES and NR behaviours were 0.67, 0.85 and 0.56 respectively.

Three separate analyses were carried out, one for actor behaviours (ACT, n=234; LO=104, HI=130), one for recipient responsive behaviours (RES, n=299; LO=133, HI=166) and one for non-responsive behaviours (NR n=196; LO=91, HI=105). A summary of responses to the farmer's management questionnaire is shown in Table 5.3. A total of 420 animals were successfully identified on-farm. Reasons for failure to collect data on cows are identified in Table 5.4. The intention of collecting data on cows from sires at extreme ends of this novel breeding index was achieved (Robustness Index: W_1=4.73, P=0.030; LO= –17.95 (±0.41), HI=13.74 (±0.26)).

Additionally, there were no significant differences in PIN (Production Index) (W_1=0.05, P0.831) or £PLI (W_1=2.21, P=0.139) between the HI and LO of sires used or days in milk (W_1=3.13, P=0.078) between HI and LO cows.

Table 5.3 Summary of responses by herd managers (n = 35) to questions related to herd management along with corresponding means ± SE (continuous variables) or counts (binary or categorical variables)

General Information			
Herd Size: Milking cows	288.26 ± 21.52		
No. stockpeople	3.29 ± 0.20		
Calf housing type 1st six weeks after birth	Individual (18)	Group (13)	Unknown (3)
Nutrition			
TMR fed?	Yes (30)	No (5)	
Quantity of concentrate fed? (tonnes/cow/year)	2.77 ± 0.17		
TMR concentrate (Kg/cow/day)	8.56 ± 0.59		
Total concentrate (Kg/cow/day)	11.06 ± 0.71		
Concentrate ME (Mj/Kg)	12.87 ± 0.14		
Fertility			
Average Calving Interval (days)	420.72 ± 3.0		
Mating age of primiparous cows (months)	15.33 ± 0.43		
Average age at 1st calving (months)	25.62 ± 0.77		
No. days after calving cows are re-served	54.97 ± 2.44		
Average no. of serves to conception	2.47 ± 0.09		
Lameness			
% cows treated for lameness in year previous to visit (n=28)+	41.09 ± 4.89		
Frequency of foot bathing (per week)	2.08 ± 0.36		
Mastitis			
No. of mastitic cows at time of visit (under treatment/withdrawal)	3.86 ± 0.47		
No. treated clinical cases in year previous to visit +	138.73 ± 16.24		

+ for year 2005

Table 5.4 A summary of total number of animals on which data was both successfully and unsuccessfully collected.

Data Collection	*Low*	*High*	*Total*
Successful	**182**	**238**	**420**
Unsuccessful			
Management[1]	92	148	241
Mastitis	3	1	4
Lame	43	43	86
Misc[2]	15	23	38
Unsuccessful Total	**154**	**215**	**369**
Grand Total	**337**	**453**	**789**

[1] includes being housed on straw at time of visit, not yet calved, freshly calved, housed with multiparous cows, out at grass, dry, incorrectly identified as 1st lactation cows (were actually in 2nd lactation) and identification problems.

[2] includes other illness, dead or reason unknown.

5.3.5 Observer Reliability

Inter-observer reliabilities for all behavioural categories were estimated from the video recordings by comparing the observations for the two observers. A total of 440 interactions were scored across 45 cows on three farms to determine inter-observer agreement. A strong significant positive correlation was found between observers (r_s=0.78, P<0.001). The mean (± s.e) for observer 1 and 2 were 2.6932 (± 0.0357) and 2.6545 (± 0.0384) respectively for all behaviours scored. To measure intra-observer reliability, each individual observer scored the same thirty cows on two separate occasions, four weeks apart. Intra-observer agreement was strong for both observers (Observer 1: r_s=0.83, P<0.001, df=168; Observer 2: r_s=0.82, P<0.001, df=114). Martin and Bateson (2001) suggest a correlation of 0.7 is desirable with respect to behavioural measures.

A mean score of stockhandler quality was calculated for each observer. The data were non-normal so non-parametric correlations were used. Spearman rank correlation was used to assess the observer reliability between stockhandler quality and the cows' response to stockhandling on each farm. A significant positive correlation was found between observers (r_s=0.64, P<0.001, df=33). Moderate correlations were found for the qualitative assessment of the cows' reaction to the stockhandlers' handling methods across observers (Table 5.5).

Table 5.5 Spearman rank correlation coefficients (r_s) and significance values for observer reliability of the qualitative assessment of cow's reaction to stockhandler on 35 farms visited.

	r_s	*P-value*[1]
Relaxed/Nervous	0.561	<0.001
Placid/Aggressive	0.431	0.014
Eager/Reluctant	0.551	<0.001
Slow/Fast	0.516	0.002

[1] df=33

5.3.6 Statistical Analysis

All data were checked for data entry errors and missing values were amended using the paper records or by a follow up communication with the farmer. All analyses were made using Genstat 8 statistical software (Genstat 11th edition, Lawes Agricultural Trust, VSN International Ltd, Oxford, UK). Generalized Linear Mixed Models (GLMM) were used to investigate factors affecting aggressive behaviour. Details are shown below. Spearman's rank correlations were used to identify relationships between explanatory variables.

5.3.6.1 GLMM

Statistical analysis involved both uni-variate and multi-variate analyses using Generalized Linear Mixed Models (GLMM) with Restricted Maximum Likelihood (REML), which allow for unbalanced data sets. All models took the same format with ACT, RES or NR fitted as the response variable, with farm and focal cow identity fitted as random terms and the dispersion parameter fixed at 1. Explanatory variables were fitted into the models as fixed effects. Non-linear explanatory variables were converted to factors (f) using interquartile ranges creating four levels of each factor. This method prevented outlying data from confounding the results. GLMMs were fitted with a Poisson distribution (count of aggressive behaviour) and a logarithim link function was used (McCullagh and Nelder, 1989). A Poisson distribution is appropriate when the data are counts of events occurring randomly. The logarithm is the link function for a poisson distrubition (McCullagh and Nelder, 1989). For all models, the Wald statistic and corresponding degrees of freedom are reported as W_{df} and the probability value (compared to a X^2 distribution) for the fixed effect. The Wald test assesses the contributions of individual terms in the fixed effects component of the models.

5.3.6.2 Uni-variable analysis

To reduce the number of explanatory variables in the final models, all explanatory variables were screened. To do this, GLMMs were carried out in a uni-variable basis where each explanatory variable was fitted as the sole explanatory variable (fixed effect) in the model to identify its importance with respect to behaviour. ACT, RES and NR results are presented from these uni-variate analyses. Continuous variables were checked for linearity against the response variable. Linear explanatory variables were included in the multi-variable analysis as covariates (c). All explanatory variables with a statistical association of P<0.250 were then incorporated into a multi-variable model.

5.3.6.3 Multi-variable analysis

All explanatory variables (P<0.25) were included in a multi-variable model using forward step-wise selection with the most significant variables from the uni-variable model being added first. In the case of explanatory variables having the same P-value in the uni-variable analyses, these variables were added in order of the highest Wald-statistic value. Variables within the final model were chosen based on their additional significance when all other explanatory variables in the model had been fitted. Variables that had confounding effects between each other were tested by running the model with and without each variable. Any variable showing a significant effect was retained and the other removed. This process eventually led to models that remained stable regardless of variable order. Significance was attributed at $P<0.05$.

5.4. Results

5.4.1 Relationship between explanatory variables

All explanatory variables were analysed to assess the relationship between them. Significant correlations between the explanatory variables measured are shown in Table 5.6. The most important explanatory variables in relation to aggressive behaviours as shown by the multi-variate analysis were herd size, months at grass, whether a total mixed ration was fed and stockhandling score.

Table 5.6 Matrix of rank correlations between the significant explanatory variables recorded during farm visits. Significant correlations shown.

	HS	P	G	SH	CME	DIM	PSW	MG	FFW	SME	AYH	TMR	ODL
HS													
P	0.168												
G	**-**	-											
SH	**0.248**	0.080	**0.230**										
CME		**-**	**-0.186**										
DIM			*0.122*										
PSW		**0.193**	**-0.240**	-0.165	**0.252**								
MG			**-0.175**	**-0.409**		-0.170	**0.249**						
FFW	**0.195**	**0.265**			*-0.115*								
SME	-	*0.126*	*0.119*	**0.286**	-0.172								
AYH	**-**		**-0.208**	**-0.303**		**-0.205**	0.153	**0.648**	**-0.221**	-0.156			
TMR	**-**	**-**			*-0.113*			0.166	**-0.489**	**0.279**	**0.186**		
ODL					**0.293**	-0.130	**0.263**	**0.317**	**-0.232**	**-0.178**	0.154	*0.134*	
CTCY		*0.108*	**0.170**	*0.123*		*0.129*	0.162	-	**0.626**	**0.229**	**-0.195**	**-0.268**	**-0.219**

Significance levels: **P<0.001**, P<0.01, *P<0.05*.

Key to acronyms: HS, herd size; P, no. of people that care for animals daily; G, gender of calf rearers; SH, stockhandling score; CME, Concentrate ME; DIM, Days in Milk; PSW, passageway space allowance per cow (m^2/cow); MG, months at silage; FFW, feedface width per cow; SME, Silage metabolisable energy; AYH, all year housed; TMR, total mixed ration fed or not; ODL, presence of outdoor loafing area, CTCY, concentrate fed in tonnes per cow per year.

5.4.2 Behavioural Observations

5.4.2.1 Recipient responsive behaviour uni-variate analysis

Robustness index category (RI), metabolisable energy of the concentrate (CME) and silage (SME), feeding a total mixed ration (TMR), stockhandling (SH) and the number of people caring for cows (P) were all significant at the <0.250 cut-off level (Table 5.7). The robustness index was the most significant explanatory variable (W_1=5.15, P=0.018). Cows from HI robust sires responded more when involved in an aggressive interaction than LO robust cows. Cow with higher numbers of responsive behaviours were fed concentrate with a ME content of between 13.05 and 13.49 Mj/kg DM (W_3=10.97, P=0.026) and a silage ME less than 12.4 (Mj/Kg) (W_3=8.13, P=0.064), fed a TMR (W_1=3.52, P=0.069) and cared for by more than 4 relaxed stockpeople (W_3=7.27, P=0.088).

Table 5.7 Means (± S.E.M) from the uni-variable analyses showing the main explanatory variables associated with recipient responsive behaviours during aggressive interactions at feeding (n=299)

Explanatory Variable	*Covariate (c) or Factor (f)*	*Wald*	*P-value*	*Categories Mean (±S.E.) for each category*			
RI				**LO**	**HI**		
				n=133	n=166		
	f	W_1=5.15	0.018	3.50 (± 0.22)	4.27 (± 0.31)		
CME				**<12.5**	**12.6-13.04**	**13.05-13.49**	**>13.5**
				n=58	n=64	n=98	n=79
	f	W_3=10.97	0.026	3.52 (± 0.30)	3.00 (± 0.27)	4.96 (± 0.48)	3.68 (± 0.27)
SME				**<12.4**	**12.5-13.03**	**13.04-13.49**	**>13.5**
				n=94	n=87	n=49	n=69
	f	W_3=8.13	0.064	4.40 (± 0.29)	3.56 (± 0.32)	3.50 (± 0.25)	3.24 (± 0.31)
TMR				**Yes**	**No**		
				n=254	n=45		
	f	W_1=3.52	0.069	4.11 (± 0.22)	2.89(± 0.3)		
SH				**<8.42**	**8.43-17.82**	**17.83-32.74**	**>32.75**
				n=78	n=95	n=54	n=72
	f	W_3=7.27	0.088	4.90 (± 0.56)	3.81 (± 0.28)	3.89 (± 0.39)	3.04 (± 0.24)
P				**<2.125**	**2.126-2.9**	**3-3.9**	**>4**
				n=68	n=143	n=46	n=42
	f	W_3=5.72	0.149	3.66 (± 0.29)	4.05 (± 0.24)	2.96 (± 0.35)	4.97 (± 0.94)

Raw data means are shown with their respective standard errors.

Key to acronyms: RI, robustness index; CME, Concentrate ME; SME, Silage metabolisable energy; TMR, Total mixed ration; SH, stockhandling; P, no. of people that care for animals daily.

5.4.2.2 Recipient responsive behaviours multi-variable model

The factors that were significant in the uni-variable model were put into the multi-variable model. In the final model, four explanatory variables were associated with an increase in animals responding to aggressive interactions. Increased responsive behaviours in cows were associated with being fed a TMR (W_3=10.77, P=0.004), having relaxed stockhandling on the farm (W_3=12.95, P=0.005), HI robustness (W_1=7.42, P=0.006) and higher number of stockpeople (W_3=8.00, P=0.046) (Table 5.8).

Table 5.8 Mean (S.E.M) from the multi-variable analyses showing the main effects of explanatory factors on actor (ACT) and recipient responsive (RES) behaviours during aggressive interactions at the feedface.

Response Variable (model)	***Wald***	***P-value***	***Explanatory Variables***			
			Robustness			
			LO	HI		
RES	W_1=7.42	0.006	3.50 (± 0.22)	4.27 (± 0.31)		
			Mean months at grass			
			<2.63	2.64-4.9	5-5.9	>6
ACT	W_1=10.11	0.042	4.85 (± 0.54)	2.78 (± 0.26)	4.60 (± 0.70)	3.45 (± 0.22)
			No. of people			
			<2.125	2.126-2.9	3-3.9	>4
RES	W_3=8.00	0.046	4.90 (± 0.56)	3.81 (± 0.28)	3.89 (± 0.39)	3.04 (± 0.24)
			Stockhandling Quality			
			<8.42	8.43-17.82	17.83-32.74	>32.75
RES	W_3=12.95	0.005	4.90 (± 0.56)	3.81 (± 0.28)	3.89 (± 0.39)	3.04 (± 0.24)
			Use of a TMR			
			Yes	No		
RES	W_1=10.77	0.004	4.11 (± 0.22)	2.89(± 0.3)		
			Silage ME (mj/kg DM)			
			<12.4	12.5-13.03	13.04-13.49	>13.5
ACT	W_3=9.66	0.045	4.51 (± 0.43)	4.20 (± 0.60)	2.76 (± 0.29)	2.82 (± 0.28)

Raw data means are shown with their respective standard errors.

5.4.2.3 Recipient non-responsive behaviour uni-variable analysis

Herd size (HS), months at grass (MG) and robustness index (RI) were all significant at the <0.250 cut-off level (Table 5.9). Cows with higher number of non-responsive behaviours were more likely to be in a herd with 219- 264 cows (compared to higher and lower herd sizes) (W_3=10.87, P=0.032), and be turned out to grass for less than 2.63 months a year (W_3=7.84, P=0.077). There was no significant effect of robustness with respect to animals not responding to an aggressive interaction (W_1=1.78, P=0.182).

Table 5.9 Means (± S.E.) from the uni-variable analyses showing the main explanatory variables associated with recipient non-responsive behaviours during aggressive interactions at feeding (n=196)

Explanatory Variable	*Covariate (c) or Factor (f)*	*Wald*	*P-value*	*Mean (±S.E.)*			
HS				**<218**	**219-264**	**265-316**	**>317**
				n=76	n=61	n=35	n=24
	f	W_3=10.87	0.032	1.93 (±0.14)	2.36 (±0.21)	3.14 (±0.29)	2.42 (±0.40)
MG				**<2.63**	**2.64-4.9**	**5-5.9**	**>6**
				n=52	n=59	n=54	n=31
	f	W_3=7.84	0.077	2.39 (±0.21)	1.81 (±0.17)	2.76 (±0.25)	2.55 (±0.29)
RI				**LO**	**HI**		
				n=91	n=105		
	f	W_1=1.78	0.182	2.20 (±0.16)	2.46 (±0.16)		

5.4.2.4 Recipient non-responsive behaviour multi-variate analysis

Herdsize was the only significant term in the multi-variate analysis (W_3=9.19, P=0.027).

5.4.2.5 Actor uni-variable analysis

Feeding a TMR diet, mean months out at grass (MG), metabolisable energy of the silage (SME), herd size (HS), all year housing (AYH), robustness index (RI), number of people caring for cows (P), outdoor loafing area (ODL) and stockhandling quality (SH) were all significant at the <0.250 cut-off level (Table 5.10). Cows with higher numbers of actor behaviours were fed a TMR, grazed at pasture for less than 2.63 months per year, were fed a silage ration with a ME less than 12.4 Mj/Kg DM, had a herd size between 265-319 milking cows, were housed all year round, had 2.13-2.9 stockpeople on the farm, had access to an outdoor loafing area and experienced harsh stockhandling. Feeding a TMR was the most significant explanatory variable (W_1=5.87, P=0.020).

Table 5.10 Means (± S.E.) from the uni-variable analyses showing the main explanatory variables associated with total actor interactions at feeding (n=234)

Explanatory Variable	*Covariate (c) or Factor (f)*	*Wald*	*P-value*	*Mean (±S.E.)*			
TMR				**Yes**	**No**		
				n=201	n=33		
	f	W_1=5.87	0.020	3.92 (± 0.25)	2.24 (± 0.31)		
MG				**<2.63**	**2.64-4.9**	**5-5.9**	**>6**
				n=72	n=66	n=58	n=38
	f	W_3=8.86	0.053	4.85 (± 0.54)	2.78 (± 0.26)	4.60 (± 0.70)	3.45 (± 0.22)
SME				**<12.4**	**12.5-13.03**	**13.04-13.49**	**>13.5**
				n=73	n=59	n=42	n=60
	f	W_3=8.39	0.062	4.51 (± 0.43)	4.20 (± 0.60)	2.76 (± 0.29)	2.82 (± 0.28)
HS				**<218**	**219-264**	**265-316**	**>317**
				n=86	n=66	n=38	n=44
	f	W_3=8.79	0.063	2.77 (± 0.23)	4.52 (± 0.57)	4.76 (± 0.65)	3.30 (± 0.31)
AYH				**Yes**	**No**		
				n=47	n=187		
	f	W_1=2.88	0.102	4.60 (± 0.69)	3.45 (± 0.22)		
RI				**LO**	**HI**		
				n=104	n=130		
	f	W_1=2.61	0.106	3.27 (± 0.25)	4.02 (± 0.35)		
P				**<2.125**	**2.126-2.9**	**3-3.9**	**>4**
				n=48	n=119	n=32	n=35
	f	W_3=5.90	0.139	2.92 (± 0.37)	4.27 (± 0.37)	2.78 (± 0.40)	3.57 (± 0.48)
ODL				**Yes**	**No**		
				n=160	n=74		
	f	W_1=3.33	0.068	3.84 (± 0.30)	3.35 (± 0.30)		
SH				**<8.42**	**8.43-17.82**	**17.83-32.74**	**>32.75**
				n=63	n=64	n=53	n=54
	f	W_3=4.81	0.186	3.97 (± 0.44)	3.64 (± 0.39)	4.17 (± 0.63)	2.93 (± 0.29)

Raw data means are shown with their respective standard errors.

Key to acronyms: TMR, total mixed ration; MG, months at grass; SME, Silage metabolisable energy; HS, herd size; AYH, all year housed; RI, robustness index; P, no. of people that care for animals daily; ODL, presence of outdoor loafing area; SH, stockhandling.

5.4.2.6 Actor multi-variable model

In the final model, two explanatory variables were associated with an increase in animals initiating aggressive interactions (Table 5.8). All year housing (AYH) was rejected in favour of months out at grass (MG) as they are highly correlated and MG is a more sensitive measure. Increased actor behaviours were associated with silage ME less than 12.4 Mj/Kg DM (W_3=9.66, P=0.045) and less than 2.63 months at grass (W_3= 10.11, P=0.042). There was no significant effect of robustness with respect to actor behaviours.

5.4.3 Stockhandling (SH) assessment

The mean (min-max) stockhandling score was 24.05 (1.50 – 98.33) across the 35 farms. The stockperson's level of interaction with the herd was correlated with three out of the four terms used to assess the cow's reaction to handling. Stockperson handling had a significant positive correlation with the relaxed – nervous (r_s=0.65, P<0.001), placid – aggressive (r_s=0.482, P=0.003) and eager – reluctant (r_s=0.60, P<0.001) terms. This suggests that harsh stockhandling practices were associated with cows that are more nervous, more aggressive and more reluctant to enter the milking parlour. Quality of handling was not significantly correlated with the speed with which cows entered and exited the parlour (r_s= - 0.24, P=0.16).

5.5 Discussion

5.5.1 Effect of robustness on aggression and its implications

This study was a preliminary investigation into the effect of selection for improved health and welfare on aggression during feeding in dairy cattle. The results show that HI robust cows responded more when involved in an aggressive interaction compared to LO robust cows. This suggests that robust primiparous cows are more defensive over feed and are bolder in defending their position at the feedface. It could be speculated that this is due to the fact that robust cows need to eat more to maintain robustness.

Primiparous cows are often smaller in body size, more timid and shyer in performing aggressive acts and have a lower ranking in a group's dominance hierarchy than older cows (Harris *et al.*, 2007). Therefore, primiparous cows are more susceptible to increased aggression from older cows at the feedface. Studies have shown that competition from older cows resulted in lower dry matter intake and milk production (Phelps, 1992) and decreased feeding time in primiparous cows housed with older cows compared with primiparous cows grouped separately (Konggaard and Krohn, 1978). Additionally, Gibbons *et al.* (Submitted, Chapter 3) reported that primiparous cows received more aggressive interactions and were more frequently displaced when housed with multiparous cows. With this in mind, it is very relevant that selection for robustness resulted in primiparous cows being more defensive in their aggressive style at the feedface. Self-defence in an aggressive situation at the feedface may reduce the frequency of displacements of primiparous cows. This may in turn increase her time at the feedface and her feed intake leading to a positive influence on her welfare, health and productivity. However, it would be very important to monitor the behaviour of these cows as they get older to determine whether a self-defence strategy results in positive welfare of these cows. Additionally, it is important to investigate if defensive heifers are aggressive as older animals.

Even though there are clear benefits for a robust cow to be defensive over a feed resource, it is also important to consider this further. For example, is it possible that defensive individuals that do well in situations where aggressiveness is called for (e.g., competing for feed at the feedface) might be unsuitably defensive in other situations (e.g. in the parlour or during a routine handling procedure)?. Future work is necessary to look at the genetic and phenotypic relationship between defensive aggression at the feedface with aggression in other contexts.

5.5.2 Effect of explanatory variables on aggressive behaviour during feeding

A large number of different parameters relating to farm management practices were investigated as possible explanatory variables for levels of aggression in the focal cows. More actor behaviours at the feedface were observed where access to grazing pasture was minimal. It is widely accepted that reduced incidences of aggressive behaviour at pasture is due to increased social space and an increased opportunity to avoid dominant individuals, which are not options available to housed cows (Miller and Wood-Gush, 1991; Boe and Faerevik, 2003). However, all of the cows in this study were housed, and had been for some time when the observations were made, and yet differences in their summer management were still evident. It is unknown how all year round housing, or short, medium and long-term access to pasture may affect the relationship between animals, social dominance, synchrony or feeding behaviour within the herd. There is a need for further detailed studies of dairy cow feeding aggression as presently there is a trend to move towards all year round housing in the UK dairy industry.

Farms that fed a TMR had primiparous cows (of both HI and LO robustness) that displayed higher levels of actor behaviours and were more defensive when aggressed upon compared to farms that did not feed a TMR. Feeding a TMR is known to increase competition at the feedface which can negatively affect behaviour causing subordinate cows to alter their feeding times to avoid aggressive interactions (Miller and Wood-Gush, 1991). Additionally, increased actor behaviours were observed on farms feeding silage with an ME less than 12.4 Mj/Kg DM. This increase in actor behaviours might be as a direct result of the poor energy quality of the silage with primiparous cows being hungrier and therefore potentially more competitive over this resource.

This study showed that the negative stockhandling practices directed at the cows was significantly associated with animals that responded less when involved in an aggressive interaction at the feedface. Additionally, farms which had a low number of people involved in the day to day handling of the herd were associated with animals that responded more when involved in an aggressive interaction. As this was an observational study and did not directly manipulate levels of stockhandler quality or number of stockhandlers, the results do not necessarily indicate strong causal connections between stockhandling quality or number of stockpeople and cow behaviour. There may be an indirect relationship via a third correlated variable that was not quantified in this study. However, the fact that these stockhandler variables are explaining any degree of variation in aggressive behaviour at the feedface is noteworthy. A few studies have related the stockperson's attitude and behaviour with milk yield (Breuer *et al.*, 2000; Waiblinger *et al.*, 2002) and to behavioural response in the presence of humans (Breuer *et al.*, 2003) in dairy cattle. To our knowledge no study has investigated the potential relationship between stockhandling quality and animal-to-animal interaction. There is a need for further work into the biological basis of the effect of stockhandling procedures on the behaviour and emotions of animals.

It is important to note that these different farm management practices may not necessarily directly relate to aggressive style, as this type of analysis is only capable of detecting relationships between the variables, rather than cause and effect. In order to address issues of causality, more controlled and longer experiments would need to be used but by practical necessity this would be on one or a few farms.

5.5.3 Consequences of breeding on behaviour

The possibilities and practicalities of selective breeding for temperament traits in livestock, together with the ethical and economic consequences of doing so, have been much debated in recent years by researchers and breeders. Many problematic behavioural traits have now been assessed for their likely response to selection, however, selection has not yet been implemented in any of these cases apart from beef cattle fearfulness by certain breed societies in certain countries and, more commonly, dairy cattle behaviour during milking (Heringstad *et al.,* 2001). This is not simply because these issues have only recently reached the attention of breeders, or because the heritabilities have only recently been estimated. Where selection has occurred, the trait has frequently not been integrated into existing multi-trait indexes but remains stand-alone.

There is a risk that behavioural traits may be antagonistically genetically correlated with existing economic traits, meaning that selection for an improvement in one will directly result in an opposite and undesirable response in the other. Traits in a selection index are weighted based on their economic value. To be incorporated into an index requires that the economic value of a temperament trait must also be estimated. Some economic consequences of a trait like aggression ought to be quantifiable, such as its impact on growth rate or milk yield. It is extremely important to understand the correlated response in behavioural traits, even where strong economic and ethical arguments can be made favouring selection.

5.6 Conclusion

The results of this study have illustrated the potential of applying practical measures to score a wider range of temperament traits on farms that what is currently measured. There was a significant positive association between animals from sires with high scores for robustness and defensive aggressive behaviour. In terms of aggressive behaviour, there is a need for continual monitoring of the effects of selection policies on aggressive behaviour given the potentially adverse effects this may have on welfare and production.

CHAPTER 6

General Discussion

6.1 Introduction

Milk yield has always been the major component of the breeding goal for dairy cattle, as milk production has a direct impact on the income of dairy farmers. However, selection focused mainly on milk yield may have led to an increase in the risk of some health and fertility disorders (Pryce *et al.*, 1998; Rauw *et al.*, 1998). This had directed breeding companies to breed for more 'robust' dairy cattle to ensure an appropriate level of performance, health, and welfare of their cattle. In association with the dairy industry and DEFRA, the "Identifying and characterising 'robust' dairy cows" project was started in 2004. The aim of the project was to investigate the feasibility of increasing robustness by using animal breeding. The experiments described in this thesis investigated behaviours that could be related to robustness of dairy cattle. Behaviour may be an important aspect of the animal's ability to maintain production, health and fertility in a range of environments. One of the questions addressed is whether breeding for robustness has any detrimental side-effects on dairy cow temperament or behaviour. Responsiveness to humans and the environment, aggression during feeding, and sociability were behaviours that were deemed appropriate to investigate in relation to robustness.

I needed to determine whether it is possible to quantify temperament traits of responsiveness, aggressiveness and sociability from the measurement of behaviour expressed by individual animals in three specifically designed experiments. Temperament characteristics are inferred from behaviour. An animal's behaviour is assumed to reflect underlying temperament if it is consistent across time and across situations. To do this for my chosen traits, I tested two important characteristics of a trait: consistency across time and across situation. This allowed me to identify which behaviours showed the best consistency across time and across situation.

The purpose of this chapter is firstly to discuss the main findings of the first three experimental (chapters 2,3,4) in terms of their overall significance and to highlight some questions raised by the results that may be interesting topics for future research. Using the results from chapter 5, the constraints of measuring aggressive behaviour during feeding on individuals on commercial farms will be discussed. Secondly, I aim to highlight the methodological constraints of temperament testing. Thirdly, the main aim of the general discussion is to discuss the concept of breeding for robustness in dairy cows with reflection on results from chapter 5.

6.2 Interpretation of results

6.2.1 Responsiveness to human approach and novel stimuli

In Chapter 2, clearly the response to human approach in the passageway (AP) showed repeatability over time. From this it would be fair to assume that some degree of temperament stability exits and this is not just random variation in behaviour. A recent study by Fulwider *et al*. (2008) reported dairies with higher percentages of cows that either approached or touched the experimenter in an approach test had lower somatic cell counts. Future work could investigate the relationship between an individual cow's responses to human approach with other aspects of its health (e.g. lameness, metabolic and reproductive conditions as well as fertility).

The low repeatability found in the flight-from-feed (FF) test is a result of high variability within individuals over time compared to low between cow variation Boake (1989), suggested that this is likely to lead to low repeatability. This result can be interpreted in a variety of ways. Test conditions may have varied in a fashion that was not predictable or the response to the test may have varied because the behaviour was measured in a situation that was insufficiently controlled and day-to-day variations in the environment had more of an influence than the cow's temperament. Factors such as the satiation level of the animal could affect an animal's performance in the FF subtest. It could be concluded that in lactating dairy cows the motivation to avoid an approaching human might compete with other motivations such as hunger. The motivation to feed may outweigh the motivation to move away from an approaching person. This effect could be controlled in future studies by ensuring that the animals were all at the same level of hunger, perhaps by more careful measurements of the time of day, time since feeding, length of time the animal was or had been feeding, along with the quantity of feed consumed by the animal.

If distinct temperament types exist and the three novel object tests (NOT) have similar stimulus value, it is expected to find agreement between the tests. The significant but low correlations within the animal's reactivity response across the three NOT indicate that individual animals are responding similarly to each NOT. The fact that these correlations are present despite potentially confounding factors confirms the presence of behavioural phenotypes related to reactivity to novelty in dairy cattle and indicates a stability of the tests. Potential confounds include the cows' previous experience with novelty, variation in genetics and early experience as well as the hormonal status of the cows.

Chapter 2 is one of few studies to investigate the relationship between the response of dairy cattle to novel objects and also to human approach. In this study, there was no significant agreement between the reactivity and investigatory responses to novel stimuli with the AP flight response score to human stimuli. In a recent study by Brown *et al.* (2009) a similar result was presented. These authors found no correlation between latency to first contact in a human approach test (HAT) and NOT in pigs. It is possible that an animal's response to human approach and novel stimuli are not governed by the same underlying mechanism. Behaviour is highly variable and each test may be measuring more than one trait. Additionally, the scoring systems used in the present study may not provide a reliable measure of the animal's temperament. Future studies could focus on more objective measures of behavioural responsiveness to HAT and NOT such as latency to withdraw from human approach, latency to first contact in HAT, latency to first contact and latency to pass novel stimulus in NOT. An important finding in this study is certainly the fact that humans do not elicit the same internal state (responsiveness) as novel inanimate objects.

6.2.2 Aggressive feeding behaviour

Chapter 3 focused on assessing individual consistency of aggressive feeding behaviours over time and across situations. As the results of this study are discussed in-depth in Chapter 3, this section will focus primarily on the results that deal with measuring consistency of aggressive feeding behaviour. In this experiment the differences between individuals were largely maintained for contact and active avoidance behaviours over a relatively long time frame indicating that individuals do have distinct behavioural phenotypes or temperaments. However, despite this, cows were more likely to actively avoid and not respond to aggressors when in early lactation compared to mid- or late lactation. Early lactation cows are more likely to actively avoid as they are new to the herd and will not readily respond to aggressors until they have established themselves in the social hierarchy. At the beginning of their lactation, cows have a higher requirement for nutrients and may be more likely not to respond to aggressors in order to maintain their position at the feedface. It is worth remembering that in humans at least, personality is not totally a rigid entity but is changeable with time, development and experience (Roberts and DelVecchio, 2000). Temperament traits are inferred from behaviour but an animal's behaviour changes substantially with development. This makes studying consistency or change in temperament a difficult task.

The repeatability results highlight a relatively high within-cow variation for all aggressive behaviours measured. As previously discussed regarding responsiveness to humans and novelty, this within-cow variability could be attributed to test conditions varying in a fashion that was not predicted or factors such as the satiation level. These may influence the way an animal initiates or responds to aggression. As before, controlling these factors associated with satiation would benefit future studies and may clarify some results.

A number of studies have shown that temperament traits may provide a basis for predicting some later behaviour, for example in pigs (Erhard *et al.*, 1999) and in goats (Lyons *et al.*, 1988). Recording feeding behaviour in primiparous cows might be an indicator of dominance ability later in life. In dairy cattle, a future dominant cow might be considered to be aggressive, receive fewer attacks and be less prone to withdrawals and often does not react when attacked. The ability to be able to predict dominance at an early age may help understand social behaviour development.

There are many unanswered questions regarding cow to cow aggression within dairy cattle, particularly why and when it occurs and what the contributing factors are. Future work could involve collecting more measures of social and non-social challenges that may contribute to the aggressiveness of the individual cow. This could give us a better understanding of aggressiveness within the wider context of temperament in cattle. Aggressive behaviours may change in their functional meaning. In particular, behaviours that characterise aggressiveness at one life stage may not be typical of a cow at another stage of life. Other influences on the motivation to perform an aggressive behaviour such as fear or stress (Jensen, 1994), will alter those aspects of temperament that are expressed at any one point. In humans there are reported to be different types of aggressiveness, such as hostile (reactive and impulsive) versus instrumental aggression (proactive, premeditated and controlled) (Ramírez, 2006). Studies of this nature might be difficult to achieve in dairy cattle, but may be worth future investigation to help understand why some animals are aggressive without any obvious benefits.

6.2.3 Measuring sociability

Chapter 4 provides novel information regarding practical experimental and on-farm measures of social behaviours in dairy cattle. Results showed within individual consistency in social motivation in a runway indicating that individual cows do have distinct social behavioural phenotypes. Social motivation as expressed in a runway test was correlated with other measures of social behaviour that could be practically and easily recorded on-farm. Social motivation is a particularly influential behaviour characteristic of cattle. Its underlying levels are likely to influence aspects of social cognition and interaction, including synchrony, aggression, stability of the social hierarchy as well as behavioural and physiological response to disruption of the social environment. Investigating variation in social motivation is relevant from both an animal welfare and genetic selection perspective. Measuring aspects of sociability, such as synchrony, can be a useful welfare measure on farm. For example, it is important to know if housing conditions allow animals to stay synchronised whilst maintaining preferred cow-cow distances.

The overall objective of this experiment was to devise a practical method for measuring social behaviour of housed dairy cattle on commercial farms. I had to determine a scan starting point, scan interval and scan duration which were easily applicable to commercial farms with different management systems. During housing, dairy cattle structure their daily routine around feeding so the provision of fresh feed was chosen as the start time. This allowed for consistency of start time across farms regardless of the difference in management systems between farms. Some researchers have carried out studies to investigate the effect of different scan-sampling intervals. Some studies have used scan sampling intervals ranging from 20s to 30 minutes in length but how interval length is determined is not always clear (Sato et al., 1993; Durrell *et al*., 2004; deVries *et al.* 2004; Sibbald et al., 2006). Using transponders, Neisen *et al.* (2009) recommended that intervals of every 2, 8, and 17 minutes are used for observation of housed dairy cattle. Mitlohner *et al.* (2001) used video recording to show that intervals of 10 minutes or less effectively represented behaviour of feedlot cattle. I chose a scan interval of 20 minutes over a 3 hour time period. The 20 minute scan interval was chosen using guidance from published literature but also for practical reasons based on the number of animals in the study.

It could be questioned whether I can draw any conclusions regarding synchrony of the herd when the results are clearly influenced by feeding behaviour at the start of the observation period. A more suitable measure for synchrony would be to record lying and standing behaviour over a longer period of time than 3 hours. This could have been achieved by recording scans for a longer period of time. Alternatively, video-recording could have been used. Video-recording is both time consuming and impractical on commercial farms. Future work could involve investigating different starting points, scan intervals, scan duration and different methods for neighbour sampling of live behavioural observations of housed dairy cattle.

A better knowledge and understanding of social relationships can generate specific tools to alleviate problems due to social tension. Modern husbandry practices impose constraints to the environment of cattle including disturbances of their social environment (e.g. regrouping) which can induce stress and reduce their production and welfare. The expression of the dominance relationships can be aggravated by farming conditions that lead to negative influences on the subordinates. In addition, a better management of the relationships among the group should also provide useful means for increasing the adaptation of animals to their non-social environment and social facilitations. A better respect of the social needs and the social abilities of cattle, ultimately, will help to ensure not only optimal production but also to maximise animal welfare.

6.2.4 Effect of selection for robustness on aggressiveness in dairy cows

To my knowledge, chapter 5 is the first study to attempt to measure aggressiveness during feeding at the individual level on commercial dairy farms. For this thesis, I focused on aggression as it is one temperament trait that has considerable cross species consistency, appearing several times in a review by Gosling and John (1999) and in numerous other studies (See Gosling, 2001). Techniques refined in experiment 2, (Chapter 3) were then implemented in the larger study of experiment 4. Aggression was recorded during feeding of dairy cattle from HI and LO robustness on commercial farms. The results of this study provide evidence of a significant association between robustness and defensive aggressive behaviour. It may be argued that a more controlled experiment would be needed to confirm the result of this study. The strength of this study design is in the fact that HI and LO cows were observed on each farm and confounding variables considered to effect feeding behaviour were accounted for in the analysis. In terms of aggressive behaviour, there is a need for continual monitoring of the effects of selection policies on aggressive behaviour given the potentially adverse effects this may have on welfare and production.

Measuring aggression, or indeed any behavioural information, at the individual level on commercial farm is no easy task. Initial challenges included sourcing farms, finding sufficient numbers of focal cows on each farm and developing an appropriate method to identify focal animals on-farm as not all farms brand their cows. Once on-farm, constraints included dealing with different management practices. Two farms changed their management practice and started housing their primiparous cows separately from the multiparous herd days prior to visiting. Focal cows were regularly absent from the milking herd despite confirmation from the farmer that they were in the milking herd. Despite these problems, substantial amounts of data were gathered.

There are a few weaknesses in the methodology which could be refined in future work. For example, the presence of the experimenter during recording may have caused a disturbance to the behaviour of the cows during feeding. This disturbance was minimised by habituating the cows to the experimenter's presence. However, I accept that within the short time on each farm some cows may not have been totally habituated. The problems encountered in this study highlight the difficulties involved with working on commercial dairy farms in the UK. On the other hand, results from this study are commercially relevant.

6.3 The challenges involved in temperament testing

Temperament is biologically determined and arises from the interactions of genetics, development and experience. Temperament is also relatively consistent. That is, the individual differences in temperament characteristics are stable. For example, a cow that is dispositionally more exploratory than its conspecifics as a calf should still be more exploratory at an older age. It is important not to expect temperament characteristics to be rigidly stable in animals but expect them to show some degree of consistency. The study of temperament is not without controversy. Studying the consistency of temperament poses theoretical and methodological challenges. Theoretically, the most probing question is: under what conditions or situations is temperament likely to be stable or unstable? Methodologically, from a development perspective, change is essential, yet some degree of stability or consistency is necessary for the maintenance of individual distinctiveness. If it is biologically sensible to expect that traits change with time, decisions need to be made regarding what level of variation can be defined as stable. This is necessary in order to decide what behavioural traits are part of the individual's personality or which are heavily influenced by the environment. This can make the study of temperament consistency a very difficult task.

As with all studies attempting to investigate underlying temperament traits, we as behavioural scientists are confronted with the challenge of designing tests that specifically look at certain temperament traits. It is important to design tests to investigate the expression of one temperament trait without the expression of others influencing it. The expression of other traits can affect the expression of the temperament trait of interest. For instance, tests are generally carried out on single animals isolated from their conspecifics and in order to standardise such tests, subjects are usually separated in a test arena. By using this approach, the reaction of the animals towards the experimental stimuli may also be influenced by their reaction towards the test situation itself (Manteca and Deag, 1993). For this reason, the studies in this thesis involved tests that were carried out in the home environment, thereby reducing the effect of a novel test arena on the trait of interest. However, behaviour, especially in a group setting, is highly variable and each of the test situations used may have measured more than one trait.

As behavioural responses are so easily affected by a range of factors other than the reactivity of the animal (it's actual psychobiological state at the time of testing, and the conditions of the particular test), it is essential to check whether responses being measured as traits meet the criteria of repeatability. A huge number of studies have taken measures of temperament using a wide range of different test situations. However, many studies fail to either test for repeatability of the test measures or give any details of whether the measures have previously been examined for consistency of response. If behaviour is not consistent over time, behavioural tests are of little use as a tool to assess temperament. Repeatability evaluates an individual's consistency across tests but compares it to the variation across the experimental group. The ability to find a good repeatability of a trait relies on detecting differences between animals and assessing within animal variation in the trait of interest.

The importance of testing for repeatability is increasingly being recognised. Fisher *et al.* (2000) tested Limousin x Jersy cross animals, some of which were reared artificially as dairy calves, and some of which ran with their dams until six months of age. Each animal was placed next to a pen of six or more of its penmates in a yard, and approached by an observer from a distance. The test was repeated three times at monthly intervals, and showed a high repeatability of 0.51 ± 0.03. Purcell *et al.* (1988) also repeated a flight test three times on their study animals, but results concerning repeatability were not presented or discussed. Hopster and Blokhuis (1993) repeated a social isolation procedure within a week and found a high repeatability value of between 0.58 and 0.69 for several behavioural measures. Fisher *et al.* (2000) repeated a test of sociability three times on the same animals at monthly intervals and found the measure to be only moderately repeatable (0.34 ± 0.04).

In this thesis, repeatability estimates were used to assess statistically the consistency of the traits of interest. A difficulty with repeatability estimates is interpreting estimates close to 0.50, the cut-off point used in this study. The easiest way to interpret a repeatability of 0.50 is that behavioural responses is similar across the group as both among and within individual responses are similar. With respect to the repeatability level, there is no general threshold figure above which a variable may be considered repeatable. The behavioural responses of cows may appear to be similar if the test is insufficiently sensitive to identify unique individual differences. In the future, investigating the use of different statistical techniques to assess consistency of individual differences in temperament is required, along with assessment of appropriate cut-off point at which a trait is deemed repeatable. In general, a deeper understanding of what is meant by trait consistency is necessary.

The concern of repeating behavioural tests is the possibility of habituation. In Chapter 2, cows were consistent in their behaviour to the AP tests, when tests were carried out on average every 3 days. This is in agreement with Spoolder *et al*. (1996) who found that pigs were consistent in their behaviour when tests were carried out every 3 days, suggesting that this time scale was sufficient to avoid the occurrence of habituation. Consistency in behaviour was also found by Jones (1988) when hens were tested at 3 day intervals. However, tests of responsiveness to novely are different. Kilgour *et al.* (2006) reported that cattle habituated to novel situations and concluded that novel tests are not novel from the second exposure onwards. Therefore, it was sensible to only expose cows once to each novel stimulus to avoid habituation and to maintain a degree of novelty. However, there is a dilemma here, as generally, repeated observations are needed to ensure that an underlying temperament trait is being measured. In Chapter 4, the interpretation of the runway results may be confounded by habituation of the animals to the test situation. It could be hypothesised that the first time the cows experience the runway it is novel and potentially fear inducing and with time both of these factors are reduced thereby decreasing the cow's motivation to reach conspecifics.

An added difficulty was the need to design a test that could be used on commercial farms. This restricted me to behavioural details that could be observed easily and described simply. A simple subjective assessment of an animal's reactivity and investigatory responses using an ordinal scoring system was applied to the HAT and NOT. However, the uses of subjective ordinal scales have their limitations; it is difficult to statistically compare the magnitude of behavioural responses between individuals. For these reasons, qualitative comparison between the novel tests was carried out using conservative non-parametric statistics.

6.4 Robustness of dairy cows

Robustness is a term that has rapidly become a main interest in animal production (Knap, 2005). The concept of robustness incorporates many functional traits but also concepts such as behaviour (Lawrence *et al.*, 2009) and behavioural integrity (Star *et al.*, 2008). These concepts of behaviour and integrity are not yet part of breeding programs because further development is required for practical recording. In this next section, I will discuss the definition of robustness and the concept of behaviour and robustness as well as the ethical implication of robustness as a breeding goal.

6.4.1 Definition of Robustness

Breeding for robustness through selective breeding programmes has the potential to increase the animal's ability to interact, and adapt successfully within its environment leading to improved welfare and productivity. I would like to consider exactly what is meant by robustness. In the Oxford Dictionary (2008) robust is defined as 'sturdy or resilient, strong and healthy, uncompromising and forceful'. In terms of animal production, robustness is a term that can be defined in different ways and the definitions vary greatly in the literature. The definition of robustness varies depending on whether researchers are interested in robustness of the individual cow in a given environment or in variation among cows within a population across environments. There appears to be some consensus that robustness is the ability of a cow or breed to be able to function and cope with varying environments. The combination of traits required by a robust cow also varies from author to author. So to put the Oxford Dictionary definition of robust in production terms, a robust cow is strong, healthy, resilient but uncompromising in terms of performance (milk, health, fertility and longevity) across varying environments. However, it is also important to consider non-performance traits such as behaviour. From a behavioural viewpoint, robust cows are not easily affected by changes in their environment i.e., a robust animal is flexible in its behaviour, remains healthy, long-lived and productive with the minimal amount of stress, and adaptable across environments.

6.4.2 Behaviour and Robustness

Breeding for robustness is thought to be a means to improve health, fertility and longevity in livestock species. The relationship between temperament and traits which improve health, fertility and longevity is an interesting area of research. This thesis provided the opportunity to attempt to investigate the effect of breeding for fitness on dairy cattle behaviour. Personality was the theme in an issue of the Journal of Behaviour (volume 142, 2005), in which biologists and ecologists researched personality in a number of animal species ranging from fish to birds. In these papers, inquiry focussed on possible fitness benefits of various personality types. Instead of attention focussing on how many traits animals might have, focus was put on the fact that within any species there is a spread of variation in traits affecting fitness. This leads to so many questions relating to dairy cattle. For example, do dairy cattle with higher exploratory behaviours live longer, are they better at adapting to changes and challenges within their housing system, do they have greater ability to be healthy, are they more capable of dealing with physiological stress Additionally, breeding for robustness may have an effect on behaviour at the group level, for example, competition with feeding or social stress. Breeding for behaviourally robust cows is feasible but would require substantial investment in data and technology. DNA markers would provide a useful tool to support selection. This would require good association studies and ongoing multiple marker development.

6.4.3 Ethical concern

There are ethical issues surrounding the issue of breeding for genetic improvement. It is ethically important to consider any undesirable side effects or consequences that robust breeding goals may have on dairy cow temperament and welfare. The results of this study highlight the fact that robustness could be directly linked to cows that are more capable of maintaining their position at the feedface. However, if farmers were to select for robust dairy cattle there is a concern this would lead towards breeding for more defensive individuals. In the long term, this could reduce the natural behavioural variation within the population. From an ethical perspective it is important that selective breeding for fitness traits does not result in breeding for animals that are inflexible in their behaviour. Estevez *et al.* (2003) reported that some birds were more rigid in their aggressive behaviour, and directed aggression to all individuals. Individuals that are not able to adapt their behavioural strategies could be problematic causing stress and injury to themselves and others as well as being more difficult to manage. It is very important that variation in behavioural traits such as aggression is retained within the population. I can only speculate that if the variation in behaviour is accidentally altered in the population through selection on production or functional traits this could cause disruption to the natural dominance hierarchy of the herd.

Let us consider the importance of aggression in dairy cattle. Aggressive individuals that do well in situations where aggressiveness is called for (e.g. in competition for food or mates) might be unsuitably aggressive in situations where caution or care is more appropriate (e.g., handling or milking by humans). Conversely, less aggressive individuals should do well in situations where low aggression is appropriate. However, these individuals might fare poorly in situations where aggression is favoured. For example, during peak feeding individuals with low aggression might avoid these feeding times and alter their behaviour to feed when the feeding area is quieter. This may result in these individuals receiving less nutrition from their feed especially in total mixed ration (TMR) based systems. When a TMR is available, cows sort the TMR eating the most nutritional concentrate first resulting in the nutritional quality of the TMR decreasing throughout the day (De Vries *et al.*, 2005). In such a situation, low aggressive cows would be at a nutritional disadvantage to the aggressive individuals. However, this suggestion is fraught with ethical dilemmas because incorporating competitiveness into breeding goals would increase the number of already aggressive or competitive individuals. Alternatively, breeders could just monitor the level of aggressiveness from particular sires.

Future work should involve more controlled studies investigating heritability of temperament traits and genetic correlations between different temperament traits as well as between temperament traits and production, health and fertility parameters. This would provide greater insight into which behaviour traits are desirable or undesirable. Once this research has been carried out then designing selection programmes to reduce competitive interactions might be of interest to dairy cattle producers for example to improve feeding and grazing behaviour as well as to improve animal well-being (Bijma *et al.*, 2007).

6.5 Future Directions

Unfortunately, it was not the objective of this study to investigate the relationship between all temperament traits studied. There were insufficient numbers (n=20) of animals exposed to all four tests (HAT, NOT, Runway test, competition at the feedface) to test the relationship between all temperament traits recorded. Future work could investigate the relationship between different temperament traits. In dairy cattle, humans make up a large part of their social environment and are in some sense relatively dominant. It would be interesting to investigate if the socially sensitive animals are more agreeable towards human approach. In future research, a number of avenues of approach are open such as the improvement of methods to measure temperament traits and the development of selection methods for more appropriate temperament.

The genetic relatedness of traits also requires investigation. Studies of the behaviour of domestic animals have revealed a genetic influence on docility in cattle (Bovin *et al.,* 1994; Grignard *et al.* 2001). Le Neindre reported heritabilities of 0.22 and 0.28 while Morris *et al.* (1994) found estimates ranging from 0.22-0.32. Gauly *et al.* (2001) reported that the degree of heritability differed according to test (separation/restraint) and breed (German Angus/ Simmental). These researchers reported heritabilites for a range of behavioural variables between 0.0-0.61 for German Angus cattle and 0.0-0.59 for Simmental cattle. In wild sheep docility is reported to have a heritability of 0.21 (Réale *et al*., 2000). Maternal behaviour has a heritability of 0.14 in German Angus and 0.42 in Simmental cattle (Hoppe *et al*., 2008). Lambe *et al*. (2001) showed maternal behaviour to have a heritability of 0.13 in sheep. Heritability of behaviour has also been reported in other species. In foxes, confident behaviours have heritabilities ranging from 0.12-0.20 (Kenttamies *et al*., 2002). Exploratory behaviour in wild great tits ranges from 0.22 to 0.41 (Dingemanse *et al*., 2002). Until recently studies of dairy cattle temperament have been scarce, which shows that the study of the genetics of behaviour in dairy cattle is still in its infancy. No selection on the sole basis of behaviour, such as those performed in quail (Mills *et al*. 1995), mink (Hansen, 1996) and foxes (Belyaev & Trut, 1979) have been preformed in dairy cattle. The effect of sire influence and the interaction between genetics and the environment on behaviours such as learning (heifers learning to use cubicles etc), emotional reactiveness and the tendency to develop stereotypic behaviour are largely unexplored. There is a need to investigate the genetic basis of behaviour in dairy cattle. Additionally, it is necessary to determine which environmental factors may interact with each other or with genetics.

On a basic research level it would also seem to me that there is great merit in further strengthening our understanding of such concepts as temperament and personality. I am not alone in considering temperament and personality to be extremely complex involving multiple dimensions, each of which may be more important in some situations than in other and each of which affects the expression of others (Cavigelli, 2005; Reale *et al*., 2007). Additionally, it is important to investigate observer reliability and repeatability of subjective temperament measures. However, validating the subjective measures with an objective measure of temperament would eliminate observer bias and may offer a more accurate tool for temperament selection.

6.6 Conclusions

It has long been assumed that cattle, as other animals, show individual differences in temperament traits. The experiments in this thesis have confirmed the existence of repeatable, independent behavioural traits in cattle that are consistent over time. This project has highlighted the significant challenges facing research into consistency of temperament traits. The work contained in this thesis provided additional information relevant to temperament research in livestock species. This thesis has demonstrated that a measure of consistency is vital in showing that personality/temperament traits actually exist. Rather than discussing and speculating on the possible implication of robustness on dairy cattle temperament, this study involved measuring aggression on cow from HI and LO robustness on commercial farms. The information provided from this thesis is relevant to farm animal ethology and dairy cattle breeding and management.

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