liveexport

Inanition of Sheep

Literature Review

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Abstract

Inappetence of sheep has been identified as a problem for the live export industry, as a cause of death on its own, and because of its association with the development of salmonella outbreaks and subsequent death of animals. A review of the relevant literature was conducted, with particular reference to voluntary refusal to eat as it may relate to the process for live export of sheep. Regulation of appetite and feed intake occurs through the integration of key areas in the brain sensing the current energy status through signals from the GIT, and via metabolic and stress hormones. Many factors were noted that can influence feed intake, including factors associated with the sheep (age, source, fatness), environment (season and climate), feed (type of feed and prior experience), and pre-embarkation stressors (transport and fasting, mixing, social stress). The effects of inappetence in causing ketosis and hypoglycaemia were discussed. Some information was presented regarding anorexia in other species, such as in disease, aging, depression, and under stress, to highlight possible newer avenues of investigation in sheep. Finally, methods that have been used to alleviate inappetence were reviewed, such as provision of hay or chaff in the troughs during the feedlot phase. From this review, some potential projects to investigate the issue were suggested.

Executive summary

Inappetence of sheep has been identified as a problem for the live export industry, as a cause of death on its own, and because of its association with the development of salmonella outbreaks and subsequent death of animals. A review of the relevant literature was conducted, with particular reference to voluntary refusal to eat as it may relate to the process for live export of sheep.

In references published over the last 15-20 years, inanition and salmonellosis have been considered the most important causes of death in exported sheep, together accounting for more than 60% of deaths in both the assembly and the shipping periods, with feedlot non-feeders more likely to die during the voyage than those that eat during the feedlot period. While the overall mortality rates have declined over this time, it appears that these are still the most important causes of death in exported sheep, with deaths due to the persistent inappetence-salmonellosis-inanition complex (PSI) occurring during the shipment phase of the export process.

Why some sheep refuse to eat has not been determined. Normally, short-term control of feed intake is brought about by the integration of peripheral and central sensory pathways related to hunger and satiation, whilst long-term energy balance is accomplished through a highly integrated system that minimizes the impact of short-term fluctuations in energy balance on metabolic reserves. The key areas in the brain, including hypothalamus and brainstem, can receive and integrate signals about the current energy status and satiety from the GIT, and metabolic reserves via metabolic hormones such as leptin and insulin, to result in a decision whether to eat or not. Other hormones, in particular stress hormones, also affect the central regulation of feed intake.

There are many factors that influence feed intake and inappetence, including aspects about the sheep, environment and season, feed offered, and pre-embarkation management. Older, fatter sheep, especially if transported in the second half of the year, are considered at high risk of becoming persistently inappetent, with resultant high mortality rates. This indicates interaction between adiposity and season, which has been investigated to some extent and appears to be related to the inability of such sheep to continually mobilise fat as an alternative energy source when they do stop eating, compared to those sheep in the first half of the year which have a metabolism more tuned to fat mobilisation. There are many aspects of the pre-shipping management which have been investigated and to date these have not yielded consistent results that could be applied practically as preventative measures. However, further investigation of on-farm factors could yield useful results, given that there is a greater mortality rate of sheep from a small number of farms. Feed type and delivery, and very importantly, the background of animals as to whether they know of pellets as food, can impact on feed intake, and feeding hay or chaff in the initial feedlot period may help to get sheep eating initially.

The interaction of animal factors and the stresses imposed on them is an area that can be further investigated. Mustering, handling, transporting, fasting, mixing with other sheep, altered diet including novel feeds, and shipping itself all constitute many stressors to the sheep, with individual and cumulative effects, that may influence feed intake. While previous work concluded that social dominance did not prevent animals from accessing the feed troughs, the effects of social and other psychological stresses on whether sheep choose to eat remain to be examined. Work on other species indicates that physical and social stresses can reduce feed intake, and in humans there appears to be an interaction between body fatness and hypersensitivity of the hormonal stress axis,

such that with increasing body weight and over-stimulation of the stress axis there is elevated secretion of cortisol, with depression and loss of appetite.

Once a sheep becomes inappetent it must mobilise body reserves to sustain essential processes, and the ability to mobilise reserves can differ depending on season and adiposity of the animals. This may also influence the resultant metabolism of the animal, such that fat sheep may be more likely to develop ketosis and hypoglycaemia, which can in turn contribute to further mental depression and loss of appetite, a further explanation for why fatter sheep may be less likely to regain their appetite. The release of cytokines such as occurs in the sickness response can also inhibit feeding.

It is apparent that the issue of inappetence in sheep in the live export industry continues to have an impact on the industry. It is a multi-factorial problem, and further investigations are warranted, both into on-farm and pre-embarkation management practices that may be associated with increased risk of inappetence, and into the physiology of why some sheep voluntarily refuse to eat. Such work could assist in developing management to reduce the incidence as well as treat practically those animals that do not eat.

Contents

1 Background

Inappetence of sheep has been identified for many years as a problem for the live export industry, as a cause of death in its own right, and also because of its association with the development of salmonella outbreaks and subsequent death of animals (Higgs et al 1993), in the persistent inappetence-salmonellosis-inanition (PSI) complex. Persistent inappetence predisposes sheep to disease such as salmonella, and those that do not die of that disease will eventually die of inanition. In the 1980s and 1990s, inanition and salmonellosis were identified as the primary causes of death for 60-75% of deaths on board ship (Richards et al 1989; Norris et al 1990; Richards et al 1991; Kelly 1996).

In any group of sheep introduced to an intensive management system, there appears to be a certain number that voluntarily refuse to eat. Sheep exported live are first assembled in feedlots for several days, then trucked and loaded onto the ship, and spend generally two to three weeks on the ship. There are stresses involved at each stage of this process, and the sheep need to adapt to eating the pelleted diet that is fed on ship.

This project will produce a comprehensive review of the relevant scientific literature on appetite and feed intake of sheep, with particular reference to voluntary refusal to eat as it may relate to the process for live export of sheep, to help highlight avenues for experimental research that could identify, manage, and treat animals at risk.

2 Project objectives

This project aims to deliver:

- A comprehensive review of literature on feed intake of sheep with particular reference to voluntary refusal to eat. Current information on hormones and metabolites which affect feed intake will be collated, along with consideration of other factors, such as stress response, which may affect appetite.

- The review will include consideration of the live export of sheep and highlight possible future avenues for experimental research to address inanition.

3 Literature review

3.1 Introduction

Hunger is a term used to describe a physiological and psychological state that results in feeding; **appetite** in animals expresses that degree of hunger as measured by the feed intake (Baile and Forbes 1974; Radostits et al 2000). Thus **inappetence** is the partial absence of appetite (and therefore reduction in feed intake) and **anorexia** is the complete absence of appetite (Radostits et al

2000). In the context of the live export industry, More (2002) described **persistent inappetence** as the complete, but voluntary refusal to eat, and this will lead to **starvation** with mobilisation and use of energy stores, biochemical and physiological changes, and ultimately death from **inanition**. Sheep that fail to eat will die usually within 2-4 weeks (House et al 2006).

In the intensive sheep industries where animals must adapt to novel feeds such as pellets or grain, it is recognised that there are some sheep that do not adapt at all to the feed, voluntarily refusing to eat. These inappetent or anorexic animals are termed '**shy feeders'** (Syme 1986). It includes those sheep that are inhibited from eating due to dominant behaviour of other sheep, fail to eat due to nutritional disorders from disease and fail to adjust to confined conditions (McDonald and Norris 1983).

3.2 Inappetence during live export

Richards et al (1989) found that inanition and salmonellosis were the most important causes of death in exported sheep, together accounting for more than 60% of deaths in both the assembly and the shipping periods (Figure 1). Further evidence for the importance of inanition as a significant cause of mortality (over 60% of deaths) was presented by Richards et al (1991) and Kelly (1996). This differs from results of Gardiner and Craig (1970) who considered that salmonellosis and heat stroke were the main causes of death during live shipment. However, the diagnostic criteria adopted as the cause of deaths may have differed between the two studies. In particular, the primary diagnosis of inanition in studies by Richards et al (1989) was made on animals with acute enteric salmonellosis that had poor gut fill. This was based on the probability that this group of animals was anorectic before salmonella lesions developed (Richards et al 1989).

The number of non-feeders was found to range from 0.2% to 23% at the end of the feedlot period before shipping, with the majority of these eating by day 5 of the voyage (Norris et al 1990). However, feedlot non-feeders are more likely to die during the voyage than those that eat during the feedlot period with the likelihood of death varying from 3.2 to 13.9 times more likely (Norris et al 1989a; Norris et al 1990; Higgs et al 1996). Those sheep that remain inappetent during the voyage are at an even higher risk of dying (Richards et al 1989).

Overall mortality rates have declined over the last 20-30 years, from 4% in the 1970s to less than 1% in the last few years (Norris and Norman 2007). However, recent investigation has shown that inanition is still an important cause of mortality in the live sheep export industry (Makin et al 2006), and its association with another major cause of death, salmonellosis, warrants its continued investigation.

More (2002) described two different syndromes of salmonellosis within the live export trade. One of these was the salmonellosis related to intensive feedlotting, where stressed animals are exposed to a heavy challenge with Salmonella organisms. The other syndrome is related to inappetence and was termed the persistent inappetence-salmonellosis-inanition complex (PSI) (More 2002).

Deaths associated with the PSI complex usually occur during the shipment phase of the export process following a long period of inappetence. During the feedlot phase, there is often a high percentage of non-feeders; however, this percentage decreases over time and most animals are eating following loading onto ship (See Figure 2). Persistently inappetent sheep that are non-feeders

both in the feedlotting and shipping phase are at greatest risk of death from salmonella (circled in Figure 2). Norris et al (1989b) found that sheep which failed to eat late in the feedlot period had a 6.9 times greater risk of death aboard ship due to inanition and a 5.9 times greater risk of death due to salmonellosis than those that ate.

This was supported by Higgs et al (1993) who did a study where 57 non-feeders were selected out of a feedlot along with a control group of 10 feeders and housed in an animal house. Lesions and death from salmonellosis were exclusively in inappetent sheep while lesions did not appear in any of the feeding controls. Lesions of salmonellosis were found only in sheep that were seriously ill or had died, which suggested that under conditions of lot feeding and sea transport, most sheep with enteric lesions are likely to die. Furthermore, adrenal weight, as an indicator of stress duration and severity, was greater in sheep that had died from inanition and even greater in sheep that had enteric lesions of salmonellosis. It was concluded that sheep were predisposed to salmonellosis by inappetence and that stress was involved in lesion development (Higgs et al 1993).

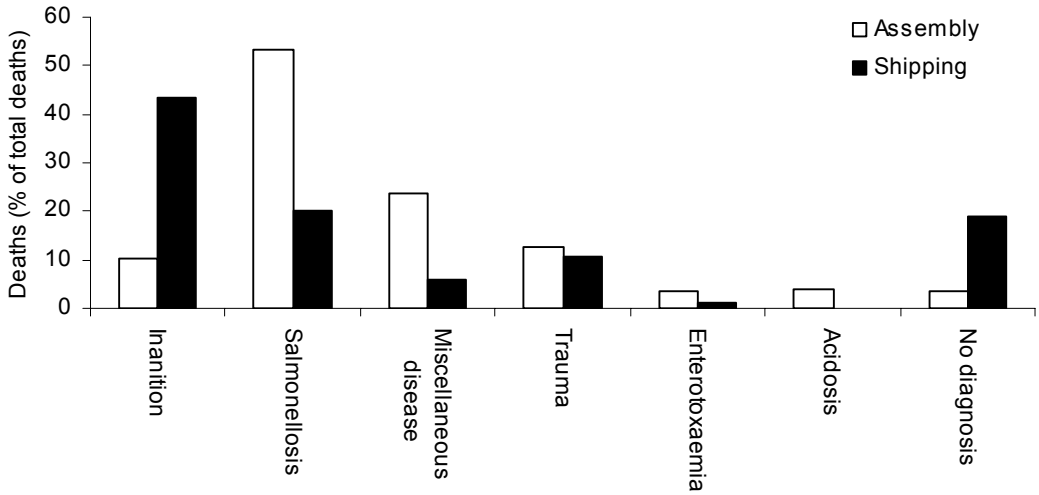

Figure 1. Deaths of sheep during assembly (2 feedlots) and shipping (5 shipments) as a % of total deaths (adapted from Richards et al 1989)

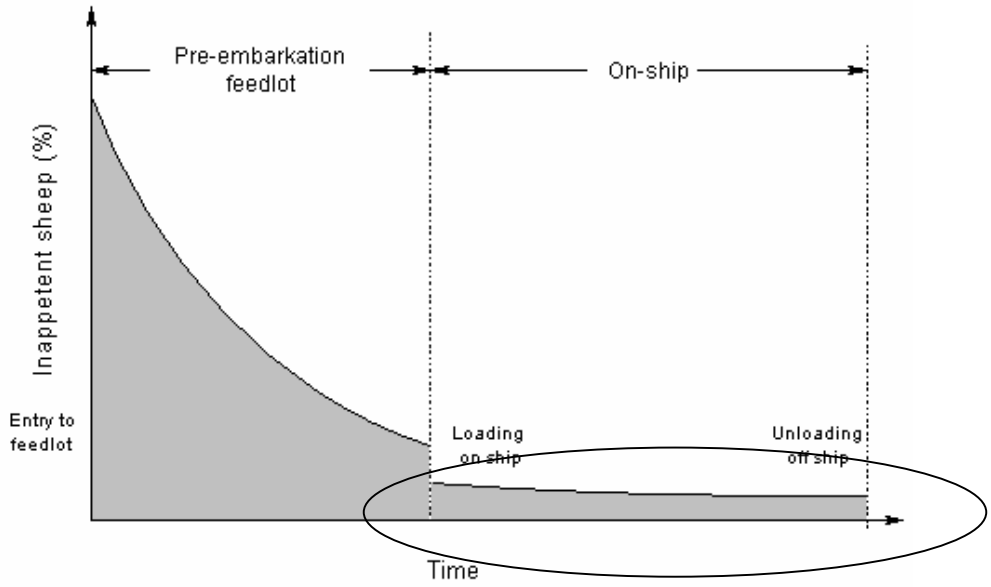

Figure 2 Inappetence in sheep during live export. Death as part of the persistent inappetence – salmonellosis – inanition (PSI) complex is mainly confined to those sheep that remain persistently inappetent after loading on-ship (circled) (More 2002)

3.3 Regulation of appetite and food intake

Food intake regulation can be separated into several phases (Aguilera et al 2000). These include effects via the GIT due to inputs from local stretch and chemical receptors as ingesta distends the organs, and chemicals are released from the food in the digestive process. Subsequently there are effects of circulating compounds such as glucose and amino acids on the liver, and then the central phase when appetite is regulated through peripheral (circulating plasma substances and neurotransmitters) and brain stimuli. In the case of inappetent or shy-feeding sheep, it appears to be a centrally initiated refusal to eat, because these sheep do not appear to even start eating the novel feed. This section describes the central mechanisms for appetite and intake control, followed by examination of external and sheep factors that can influence feed intake, and how those are related to practices in the live export industry.

3.3.1 Central and peripheral homeostatic control of appetite and intake

The central nervous system (CNS) undertakes the role of sensing nutrient intake and body reserves, integrating the information, and regulating energy intake and energy expenditure to balance the internal metabolic environment of the animal, i.e. homeostasis. Short-term control of feed intake is mainly brought about by the integration of peripheral and central sensory pathways related to hunger and satiation, whilst long-term energy balance is accomplished through a highly integrated system that minimizes the impact of short-term fluctuations in energy balance on metabolic reserves. Critical elements of this control system are hormones secreted in proportion to the animal's metabolic reserves, and the CNS targets upon which the hormones act.

A neural network sensitive to energy status signals has been identified as the homeostatic control centre for the regulation of feed intake and energy balance, and has been located to areas of the CNS stretching from the hypothalamus in the midbrain to the lower part of the brainstem. Other regions of the CNS, particularly those associated with stress and learning, also interact with these main control centres regulating intake, and may be particularly important when homeostatic regulation is unbalanced, such as in the case of "shy feeders" or persistent inappetence. The following discussion introduces the main pathways involved in the homeostatic control of appetite and feed intake, and highlights possible mechanisms attributed to disturbed homeostatic energy balance.

3.3.2 Key areas and hormonal pathways within the brain

A major site of feed intake regulation is the hypothalamic arcuate nucleus. The arcuate contains two distinct neuronal populations that express leptin and insulin receptors (Schwartz et al 1996; Elias et al 1999) which, as mentioned above, are key metabolic hormones involved in homeostatic regulation. One is a neuronal population that expresses proopio-melanocortin (POMC). POMC is a precursor peptide hormone that is further processed into multiple hormones, including the anorexigenic hormone alpha-melanocyte-stimulating hormone (α-MSH). Leptin activates POMC-containing neurons resulting in release of α-MSH and therefore decreased feed intake (Schwartz et al 1997; Elias et al 1999). Leptin also interacts with the second arcuate population to inhibit the release of the orexigenic peptide hormones, neuropeptide Y (NPY) and agouti-related protein (AgRP) (Cowley et al 2001; Korner et al 2001). Leptin inhibition of the NPY and AgRP-containing neurons also results in the reduction of gamma-aminobutyric acid (GABA) inhibition of POMC neurons. The end result is that, at times of energy excess and increased fat, increased leptin concentrations directly cause appetite inhibiting (anorexigenic) arcuate pathways to be activated and appetite stimulating (orexigenic) arcuate pathways to be de-activated. Conversely, during times of energy deficit when leptin conentrations are low, there is a reduction in the inhibitory influences of leptin on orexigenic pathways. Leptin is not the only key player in the homeostatic control of feed intake and energy balance, but where commonality exists appears to be related to leptin's interaction with the NPY, AgRP and POMC neuronal targets (Small et al 2002).

The evidence for key roles for NPY and the melanocortins in the control of feed intake and energy balance is increasing. Central injection of NPY stimulates feed intake in animals, including ruminants (Clark et al 1984; Morley et al 1985; Stanley et al 1986; Miner et al 1989). Hypothalamic NPY gene expression and circulating concentrations increase in response to feed restriction (Kalra et al 1991; Archer et al 2002; Kurose et al 2005) and decrease in response to feed excess (Widdowson et al 1997; Archer et al 2002: Kurose et al 2005). There is also evidence for the differential expression of orexigenic neurones, depending on the timeframe of the feed restriction. For example, it has been reported that there are NPY expressing cell bodies in the dorsomedial hypothalamus that do not possess leptin receptors (Chronwall et al 1985; Bi et al 2004) and that NPY expression in these neurons is increased by chronic, and not acute, feed restriction (Bi et al 2003), and by the metabolic demands of lactation (Li et al 1998; Sorensen et al 2002), shown in rodents and ruminants.

Gene expression of POMC in the arcuate is decreased during lactation in sheep (Sorensen et al 2002), possibly facilitating the lactation-associated increase in appetite. In addition, the expression of the endogenous melanocortin antagonist AgRP, which is co-localised with NPY in the arcuate, is up-regulated during feed restriction in sheep (Archer et al 2002) and lactation (Sorensen et al 2002), and central administration of AgRP increases feed intake in mice (Fan et al 1997).

Although NPY-neuronal expression is without question a potent orexigenic stimulus, absence of NPY or its receptors in genetically-modified rodent '*knock-out*' models does not result in the cessation of feed intake (Erickson et al 1996; Pedrazzini et al 1998). This may just indicate that there are multiple systems for stimulating feed intake and the absence of one is not sufficient to block this critical behaviour. As mentioned previously, melanocortins are the other arcuate peptides that play a major role in mediating the effects of circulating metabolic signals on feed intake and energy balance. POMC gene expression and circulating α-MSH levels are positively correlated with feed restriction in rats and sheep (Lincoln and Richardson 1998: Kim et al 2000), and, at least in mice, central administration of the POMC-derivative α-MSH or melanocortin agonists inhibit feed intake (Fan et al 1997). Arcuate nucleus NPY/AgRP- and POMC-containing neurons have primary projections to both the paraventricular nucleus (PVN) (Baker and Herkenham 1995) and to the lateral hypothalamus (Elias et al 1999), both of which are involved in the autonomic regulation of food intake. The PVN plays a major role in anorexigenic signaling, whilst the lateral hypothalamus plays a role in stimulating or maintaining food intake. NPY and melanocortin receptors are found on multiple neuronal cell types within the PVN, and some of the peptides expressed by these PVN neurons, e.g. oxytocin, corticotrophin-releasing hormone (CRH) and gastrin-releasing peptide (GRP), may mediate the downstream anorexigenic actions of NPY and melanocortin signaling (Arletti et al 1989; Levine et al 1983; Ladenheim et al 1996; Blevins et al 2004; Ladenheim et al 2002; Lu et al 2003).

The lateral hypothalamus is the site for neurons containing another two peptides that have been implicated in mediating the downstream orexigenic actions of NPY and melanocortin signaling on intake, namely orexin (Chen et al 1999) and melanin-concentrating hormone (MCH) (Qu et al 1996). The PVN and lateral hypothalamus have projections to the dorsal-vagal complex of the hindbrain (Horst et al 1984; Peyron et al 1998), which receives neural input from the GIT. Therefore, one could justifiably surmise that there is cross-talk between CNS control centres receiving inputs from both neural and circulating signals.

There are additional neurotransmitters which have yet to be incorporated into the known pathways. For instance, histamine is involved in energy homeostasis as a neurotransmitter in the brain, and recently it has been suggested that central histaminergic activity inhibits feeding behaviour in ruminants, with a centrally administered receptor antagonist resulting in decreased feed intake (Kurose and Terashima 2007).

3.3.3 Central nutrient sensing

Central and peripheral signals communicate information about the current state of energy balance to the key brain regions, including the hypothalamus and brainstem. Hunger and satiety represent coordinated responses to these signals, and the decision on whether to eat or not appears to be controlled by multiple factors, with the final decision relying on the 'total signal' reaching the CNS from many types of receptors in many parts of the body. Metabolic hormones, hormones involved in nutrient partitioning, reproductive hormones, and stress hormones are all potentially involved in the regulation of intake, along with signals and peptides released from the gastrointestinal tract (GIT).

A. Signals from the gastrointestinal tract

In ruminants, tension receptors in the muscular wall of the rumen and reticulum provide a measure of distension while epithelial receptors provide information on the fibrousness of the digesta (Forbes and Barrio 1992). The epithelial receptors are also sensitive to the chemical nature of the digesta,

particularly acidity. There are mechano- and chemoreceptors in the abomasum and duodenum, and chemoreceptors in the liver. It has been established that afferent fibres from nerves of the GIT continuously receive information related to a number of mechanical and chemical stimuli. They transmit this neural information to the CNS to exert feedback control of both gastrointestinal muscle contraction and intestinal secretions, and also participate in the control of feed intake. The location of the integration of gastrointestinal neural information is mainly in the hindbrain (Leslie et al 1982; Shapiro and Miselis 1985).

The GIT releases more than 20 different regulatory peptide hormones that influence a number of physiological processes, including appetite. Most of these hormones are sensitive to GIT nutrient content, and short-term feelings of hunger and satiety are believed to be mediated, in part, by coordinated changes in circulating GIT hormone levels (Badman and Flier 2005). GIT hormones have a number of functions, including the regulation of blood glucose levels, gastrointestinal motility and growth, exocrine secretion and adipocyte function (Murphy and Bloom 2006). These functions are often integrated with their actions in the central regulation of appetite circuits, and the GIT hormones themselves interact to stimulate or suppress the release of other hormones. GIT hormones can activate circuits in the hypothalamus and brainstem, the main central nervous system centres responsible for the regulation of energy homeostasis. For many GIT hormones, the precise mechanisms of central action are unknown or contentious, but a lot of evidence indicates signalling to the brainstem via the vagal nerve, and/or interaction with the NPY/AgRP/POMC/CART neuronal systems in the arcuate nucleus of the hypothalamus to affect the feeding centres of the brain. Additionally, the hindbrain contains high concentrations of binding sites for a hunger suppressing humoral substance, cholecystokinin (CCK) (Zarbin et al 1983: Moran et al 1986), so that the hindbrain may be able to detect CCK in the circulation, raising the possibility that these hindbrain regions may be involved in suppression of feed intake by systemic as well as neural signals.

Although the system is complex, by examining the wide-ranging effects of GIT hormones on appetite it is possible to discern simple themes. There seem to be three major roles for GIT hormones in appetite regulation. First, the release of GIT hormones can modulate normal hunger and satiety. For example, circulating ghrelin levels increase before a meal and correspond to meal initiation (Henry 2003). A number of anorectic GIT hormones are released postprandially as satiety signals, e.g. cholecystokinin, peptide YY, pancreatic polypeptide, amylin, glucagon-like peptide, and oxyntomodulin (Murphy and Bloom 2006). However, these increases in circulating concentrations are often small, and it seems likely that satiety might represent the cumulative effects of a number of submaximal GIT hormone responses. GIT hormones might thus have additive effects on appetite. Second, GIT hormones may reduce food intake in animals with specific GIT diseases. A number of anorectic GIT hormones are elevated in GIT disease in humans (Besterman et al 1983; Adrian et al 1986). This might be a specific function of hormones in the distal GIT. Enteroendocrine cells might, for example, release hormones at high levels in response to undigested foodstuffs, the presence of which would suggest that the function of the upper GIT is compromised. Third, very high levels of GIT hormones may be released to generate conditioned taste aversion in response to the ingestion of unpalatable feeds (Murphy and Bloom 2006).

B. Metabolic hormones

There are a number of key metabolic hormones that may affect the feeding control centres of the central nervous system. For a circulating signal to have an effect, it must be able to gain access to the brain, through controlled transport across the blood-brain barrier (BBB). Studies of the BBB have

revealed a limitation on the rate of exchange of lipid-insoluble substances, such as metabolic hormones, between blood and nervous tissue (Davson et al 1987). This means that the power to control the composition of the environment of the cells making up the brain tissue is built into the system via carrier-mediated transport, or 'facilitated transport' of lipid-insoluble molecules (Oldendorf 1975).

Metabolic hormones that could signal nutrient status to the brain include insulin and leptin. Insulin has long been recognised as a central homeostatic signal, providing rapid, dose-related information to the brain about short and long term nutritional status, and insulin can inhibit further feeding behaviour (Henry 2003; Niswender and Schwartz 2003; Plum et al 2006).

Leptin is a hormone produced by adipocytes that can act on the brain to induce satiety (Campfield et al 1996). The circulating concentration of leptin is related to the percentage of body fat, and is decreased by fasting, and it has been proposed as a signal of metabolic status. Acute changes in leptin are associated with alterations in feed intake of sheep, such that central leptin administration of leptin reduces feed intake, and fasted-induced reductions in leptin are related to increases in feed intake (Henry et al 1999; Ahima and Flier 2000; Miller et al 2002). However, leptin appears to also have a longer term role in energy homeostasis (Chilliard et al 2005; Delavaud et al 2007), and interactions with other hormones and factors such as sex of the animal and season further complicate the system (Clarke et al 2000).

Glucagon is another hormone involved in the regulation of food intake – peripheral administration reduces food intake in sheep (Deetz and Wangsness 1981), but glucagon is most likely not acting directly on the brain but rather on the liver since the depressive effect of glucagon can be prevented by hepatic vagotomy in rats (Geary and Smith 1983).

C. Stress hormones

Acute stress reactions are coordinated and regulated by the central nervous system, partly via increased secretion of hormones of which corticotrophin-releasing factor (CRF) plays a central role. The CRF is secreted in the brain, primarily from the PVN of the hypothalamus that has also been shown to mediate an effect on feeding (Krahn et al 1984). CRF has also been located in the GIT (Petrusz et al 1992). Centrally administered, CRF reduces feed intake in sheep (Ruckebusch and Malbert 1986). Because CRF released into the pituitary portal system stimulates ACTH, and consequently cortisol, these hormones could mediate the decreased appetite. However, hypophysectomy has no effect on feeding or on the actions of CRF on feeding (Morley et al 1982; Levine et al 1983). This agrees with the finding that exogenous cortisol administration does not influence intake in sheep (Baile and Martin 1971) and cattle (Head et al 1976). Interestingly, in overweight humans, the results of recent studies have suggested that the hormonal stress axis becomes hypersensitive with increasing body fat (Björntorp 1996).

3.4 Factors influencing feed intake and inappetence

3.4.1 Sheep factors

A. Age

Higgs et al (1991a) found that death rates during live shipment of hogget wethers were significantly lower than adult wethers. The differences in the basic appetite patterns of young and adult sheep may explain the lower mortality rate of the hogget wethers (Higgs et al 1991). Younger, growing animals have higher nutrient demands than mature animals and therefore appetite exceeds maintenance requirements despite seasonal changes in voluntary intake (Kay 1979; Blaxter and Boyne 1982; Kay 1985). Consequently, young sheep are not as likely to be candidates for persistent inappetence because their appetites are not subject to the degree of cyclical changes seen in adults (Higgs et al 1991).

B. Source of sheep

There is large variation between lines of sheep and proportion of inappetent animals in pre-embarkation feedlots (McDonald et al 1990) and on ships (Norris et al 1989a; Higgs et al 1999), but the reasons are unclear. The differences may be due to permanent factors such as genotypic differences or differences in permanent learning or imprinting (McDonald et al 1988a) or nutritional and management history (Norris et al 1989a).

Norris et al (1989a) explored these findings further by examining 5 shipments of sheep that carried between 15 and 35 lines (farm sources), with a total of 133 lines examined. During the live export process most deaths of sheep from these lines occurred aboard ship. Half the deaths aboard ship occurred in 25% of the 133 lines of sheep in the 5 shipments. From this study it was concluded that if high mortality lines of sheep could be identified before purchase and managed differently or not exported, the overall mortality would be reduced substantially.

Norris et al (1989b) conducted a study to determine possible risk factors associated with death rates aboard ship in different lines of sheep. The study included sheep from 133 lines (farms) and truck drivers and previous owners of sheep were asked for information on possible risk factors for inappetence during lot feeding and for shipboard mortality. The questions asked are listed below:

- Purchase history: homebred or brought from dealer
- Previous experience in mixing with new sheep
    - Not in previous 6 months
    - In previous 2 months
    - In previous 2 – 6 months
- Previously transported in truck
- Previously yarded (> 3 times)
- Hand fed as unweaned lambs
- Hand fed in past 5 months (diet was specified)
- Age
- Rainfall zone of farm of origin
- Trucking company
- Distance travelled (km)
- Hours on truck

- Hours off feed

Furthermore, 50 sheep per line were systematically selected for frequent observation, where marker bars were used every 48 hours during lot-feeding to determine the number of feeders and non-feeders.

This study found that there was no consistent association between the factors described above and inappetence during lot feeding and mortality aboard the ship. It was therefore concluded that none of the farm or transport factors examined in this study predisposed significantly to inappetence during lot-feeding or mortality aboard ship.

Norris et al (1989b) suggested that the reasons for this may be that the factors examined had a level of risk lower than could be detected by their methods, that none of the factors in the study contributed significantly to mortality, or that other possible risk factors not examined could be involved. The study did not examine pasture type, growth rate, prevalence of nematodes with anthelmintic resistance, and change in body condition of wethers before sale, all factors that may be associated with between-line feeding behaviour and survival (Norris et al 1989b).

Further support for variations between farm sources comes from Bailey and Fortune (1992). In this study a cohort of 1600 merino wethers were followed over a 21 day period which included an 8 day assembly phase in a feedlot and a 13 day journey by ship. Although the purpose of the study was to monitor patterns of feed intake and liveweight change, it was noted that of the 21 animals which died on this voyage, 11 of them came from a single farm source and most of these appeared to die from starvation.

Higgs et al (1999) suggests that the explanation may lie in the location of farms, with animals coming from an area of a longer pasture growing season being more susceptible to mortality due to inappetence. In this study it was found that farms that produce sheep with a greater risk of high mortality rates during export can be identified using results of mortality rates from previous years.

C. Breed

It is not well understood if breed of sheep influences incidence of inappetence. All of the studies undertaken in the pre-embarkation feedlot and/or ship trials have concentrated on the Merino.

D. Sex

Research into incidence and prevalence of inappetence on board live stock ships and pre-embarkation feedlots has focused primarily on merino wethers (Richards et al 1986; Norris et al 1989a; Richards et al 1989). More recently researchers have considered sex as a contributor to differences in voluntary feed intake, through the influence of the hormone leptin, with a seasonal pattern of responsiveness to leptin that is more pronounced in females than in males (Clarke et al 2000). However, given this finding, there is no evidence to suggest that ewes would be more susceptible to inappetence compared to either rams or wethers.

Feed intake can be influenced by testosterone and/or photoperiod treatment, through effects on NPY and POMC gene expressions (Clarke et al 2003; Dobbins et al 2004). Subsets of both NPY and POMC-producing cells in the sheep brain express oestrogen receptors (Skinner and Herbison

1997), at least in the ewe, so expression of the relevant genes can be influenced by gonadal steroids. Whether these cells also express androgen receptors is not known (Scott et al 2004).

E. Fatness

There is evidence to suggest that fatness may play a role in predisposing sheep to inappetence during live export (Higgs et al 1991; Richards et al 1991). Richards et al (1989) found that sheep which died from inanition had greater reserves of body fat than controls and sheep that died from other causes. This finding was further explored by Higgs et al (1991) who found that adiposity is a central factor leading to persistent inappetence and subsequent death from inanition. They found that there was a significant correlation between condition score and shipboard death rates in two of the three voyages studied and individual fat sheep had twice the risk of death from inanition. A relationship between voluntary feed refusal and adiposity has been observed in several pen experiments (Schinckel 1960; Panaretto 1964). Schinckel (1960) reported a syndrome in extremely fat sheep closely resembled the findings of Higgs et al (1991). The syndrome was characterized by a complete refusal to eat and subsequent death within 2 to 4 weeks; the sheep had substantial quantities of omental and mesenteric fat at death. It is not known what factor precipitates a complete refusal to eat. Higgs et al (1991) postulated that the syndrome may be linked to interference with the seasonal control of appetite and metabolism.

Richards et al (1991) notes that it is important to assess the phase of lipid metabolism (utilisation versus accretion) when assessing adiposity and inappetent sheep. It has been shown that fasted, thin sheep have a markedly decreased blood insulin concentration, whereas in fat sheep the insulin concentration is maintained (McNiven 1984). The high blood insulin values in fat sheep would inhibit lipolysis and promote lipogenesis, whereas the low values in thin sheep would enable lipolysis to continue.

The level of fatness of sheep affects the transport of key metabolic signals into the brain, a phenomenon that has now been seen in a number of animal species. NPY-producing cells express receptors for the metabolic hormones, leptin, insulin (Hahn et al 1998; Hillebrand et al 2002), and ghrelin (Butler and Cole 2001; Willeson et al 1999), allowing reception of peripheral metabolic information, and the ability for leptin and insulin to reduce NPY expression (Schwartz et al 1992; Hillebrand et al 2002). POMC-expressing cells also possess leptin (Benoit et al 2002; Hillebrand et al 2002) and insulin receptors Benoit et al 2002), and these circulating factors increase POMC expression. Accordingly, these two cell types are well placed to act as “first-order” neurons in the central recognition of peripheral metabolic status (Schwartz et al 2000).

The interaction of adiposity and season will be explored below, and the metabolic effects of inappetence of fat versus thin sheep discussed in a subsequent section.

3.4.2 Environmental factors

A. Season

Many mammals show seasonal cycles in food intake, body weight, and energy metabolism and this is true of the sheep (Clarke 2001). The annual cycle in day length (photoperiod) is utilized as a time cue to synchronize this physiology in a range of species, including sheep, where increasing daily exposure to light results in an increase in feed intake (Tucker et al 1984), in part apparently due to

the increased expression of NPY (Clarke 2001). Expression of NPY in the sheep brain (Clarke et al 2000; 2003) is high under long photoperiods and is closely linked to the time of year when feed intake is highest. In Soay rams, POMC expression is higher under short photoperiods, a condition when testosterone levels are high and feed intake is low (Clarke et al 2003).

Studies have found that there is an association between season and shipboard death rates due to inanition; death rates associated with inanition were higher in the second half of the year (Higgs et al 1991; Higgs et al 1993). The explanation for high death rates in mature sheep in the second half of the year is likely related to interference with the long term mechanisms controlling appetite and energy metabolism. Appetite and basal metabolism follow annual cycles with a low point in winter and a high point in summer (Blaxter and Boyne 1982; Kay 1985). In the first half of the year, sheep in the south west of Australia are in a period of live weight loss while in the second half of the year there should be weight gain while those animals are on the better feed, and also while the photoperiod effects on their appetite should contribute to increased feed intake. It was suggested that adipose tissue is the labile fraction of seasonal weight fluctuation (Burton and Reid 1969; Butterfield 1988); thus sheep in the second half of the year are fatter.

There also are differences in the metabolic response to inappetence, depending on the time of year. Richards et al (1991) found that sheep in May responded to inappetence by continuing to mobilize fat (as seen by elevated concentrations of non-estrified fatty acids (NEFA) and glycerol) and generate ketones as an alternative source of energy. However, in August, fatty acid mobilization failed to persist; circulating NEFA, glycerol and ketones fell sharply to concentrations similar to those of feeding sheep. Coincident with this there were increased plasma protein and urea concentrations, suggesting an endogenous protein source was supplying alternative energy.

Therefore it was concluded that the higher death rate in sheep exported by sea in August was due to the failure of inappetent sheep to continually mobilize depot fat as an alternative energy source, while sheep showing voluntary feed refusal in the first half of the year have a metabolism tuned to fat mobilization and therefore more likely to survive long periods of inappetence, assuming depot fat is not limiting.

The mechanism behind this seasonal effect is still being unravelled, but leptin appears to have a role. Leptin is produced by adipose tissue, so the circulating concentrations of leptin correlate with body fatness (Blache et al 2000). Additionally, in seasonal animals such as sheep, the increased feed intake and weight gain in long days are associated with high circulating concentrations of leptin, and decreased intake and weight loss during short days with low leptin concentrations (Adam and Mercer 2004). While this would seem to be counter-intuitive, given the usual role of leptin to decrease feed intake, there is a change in leptin sensitivity at these times, so that in short days there is high sensitivity and in long days there is relative insensitivity (Adam and Mercer 2004). Primary hypothalamic appetite regulating targets for leptin respond differently to changes in circulating leptin and nutritional status induced by photoperiod, compared to changes induced by food restriction; for instance plasma leptin concentration is reduced in sheep fed below maintenance requirements (Bocquier et al 1998; Delavaud et al 2000). Both underfeeding and a 48 hour fast can reduce leptin gene expression (Bocquier et al 1998; Kumar et al 1998) but Blache et al (2000) found no acute diurnal or meal related fluctuations in rams.

B. Climate

Increases in temperature and humidity can cause reductions in feed intake in ruminants, and these are the conditions most likely to be encountered by sheep exported to the Middle East. A reduction in feed intake is an immediate response to heat stress (Conrad 1985), and this is followed by a fall in metabolic rate which helps to balance heat production with heat loss (Clarke et al 2001). The causes of reduced voluntary feed intake of cattle due to rising environmental temperatures have been reviewed by Bianca (1965), and similar mechanisms are expected for sheep. In this review it was proposed that the hypothalamus acted as an integrator for regulating food intake and other functions involving energy balance. This was supported by Andersson and Larsson (1961) who found that warming the pre-optic area and rostral hypothalamus of goats with thermodes caused the hungry animals, which had just begun to eat with good appetite, to stop eating within 1 minute.

The extent to which feed intake is reduced due to heat stress varies in the literature. In the live export of sheep to the Middle East, it is possible for animals to encounter periods of prolonged and continuous high heat and humidity during the northern hemisphere summer months (Beatty et al 2006). Given these conditions, extreme reductions in feed intake in cattle have been observed (Beatty et al 2006). Little has been published regarding the effects that these extreme conditions may have on sheep feed intake, although anecdotal evidence suggests that prolonged periods of heat stress will have similar effects on sheep.

3.4.3 Feed factors

A. Feedlotting and pelleted feed

For most of the sheep sourced for live export, their previous management would have been as grazing animals on pasture or stubble; supplementary feeding may have occurred when the animal was a juvenile, but is less common after that stage (Purser 1980). Once entering the live export process there is a considerable change in management, and the animals are lot fed for a short period before shipping.

The main objective of this lot feeding is for adaptation to the novel pelleted feed. It may take time to train the animals to accept the pelleted diet which will be used during the voyage. Livestock exposed to new feeds can exhibit neophobia to that feed. Neophobia is characterised by a period of low feed intake, followed by increased consumption leading to a relatively stable level of feed intake (Bowman and Sowell 1997). It is also important that digestive adaptation to the diet is achieved, therefore avoiding acidosis.

Regulations set out the minimum number of days in the feedlot before the voyage (Norris et al 1990). Sheep are to be in the feedlot for at least 3 clear days from November to April (Standard S3.8 bii) and 5 clear days from May to October (Standard S3.8 ai). It may take longer than this for the sheep to become adapted to the pelleted diet, generally around two weeks for all sheep to begin consuming feed (Juwarini et al 1981; Chapple et al 1987). The number of non-feeders generally decreases with time in the feedlot prior to shipping (Norris et al 1989), but it has been demonstrated that lot feeding adult wethers for a longer period (13 days) before an 18 day simulated voyage conferred no advantage on final body weight or numbers of non feeders, compared to shorter periods of lot feeding (3 or 8 days) (Norris et al 1992). In that work there were many more non-feeders at the end of 3 days of lot feeding, compared to at the end of 8 or 13 days, but on days 7, 14

and 18 of the simulated voyage, there was no difference in numbers of non-feeders between the groups (Norris et al 1992).

In some cases sheep can be held in feedlots for 2 to 3 weeks due to delayed departure of ships (Richards et al 1989).

Some feedlots consist of a small paddock where hay is fed initially and then gradually replaced by pellets, while others house sheep in sheds where pellets can sometimes be the only source of feed (Norris et al 1989).

Norris et al (1989b) found that over 80% of feedlot non-feeders began eating pellets within 5 days of being placed in shipping pens. This is despite there being less space per sheep in shipboard pens (Table A4.1.5) than during feedlotting (Standard S3.11c and d), and restricted feed trough space per head. It was suggested that short term acute stress of trucking to the ship may have somehow stimulated the appetite of these previously non-feeding sheep.

However, although many feedlot non-feeders begin eating once aboard ship, failure to eat pellets in the feedlot has been found to be a major risk factor for shipboard death (Norris et al 1989b). In that study it was found that sheep failing to eat late in the feedlot period had 6.9 times greater risk of death aboard ship due to inanition and 5.9 times greater risk of death due to salmonellosis than those who ate. It is believed that consistent feed intake is the key to preventing mortalities from both inanition and salmonellosis, and that several options for management of non-feeders at the completion of lot-feeding needed to be explored (Norris et al 1989b).

(i). Type of supplement

During live shipment, pellets are generally fed at 3% of body weight for younger sheep (up to and including 4 permanent incisor teeth) and 2% of body weight for those that are older (more than 4 permanent incisor teeth) (Appendix 4.2). Live shipping pellet specifications are as follows;

Moisture content	<12%
Ash	<13%
Crude protein	9 - 12%,
Urea	< 1.2%
Acid detergent fibre	18 – 35%
Metabolisable energy	>8 MJ/kg dry matter

Inappetence, or low feed intake of pellets has been attributed to inadequate preparation of sheep, physical environment, and the pelleted diets themselves (Round 1986). Norris et al (1989) raised concerns that failure to eat pelleted feed is a predisposing factor for death during live export, and work has been undertaken to improve or modify pelleted diets so as to reduce the numbers of sheep showing signs of inappetence during pre-embarkation adaptation periods. McDonald et al (1988b) demonstrated feed intake benefits and improved patterns of intake in feedlot sheep when either oaten or lucerne chaff was added to a pelleted feed. Over 70% of sheep fed pellets with either type of chaff visited the feed trough during the first 24 hours. When no chaff was fed, this level of feeding was achieved after 4 days. Over the 10 day experiment, intake of total feed per sheep putting its head in the trough increased from 1.10 kg/day for sheep fed pellets only to 1.27 kg/day for sheep fed pellets mixed with lucerne chaff ($P < 0.05$). These results were similar to those described by McDonald et al (1990) who also found that offering a mixture of hay and pellets, rather than pellets

alone increased the number of sheep eating in feedlots. It was suggested that when pellets only were fed, an unstable pattern of rumen fermentation may have caused a build up of lactic acid in the rumen resulting in the reduced feed intake. There was little fluctuation in daily feed intake when chaff was mixed with pellets (McDonald et al 1988b).

Other studies have looked at manipulating pellet composition to try and increase feed intake and eliminate inappetence in pre-embarkation feedlots. In a study by McDonald et al (1994) “barley” pellets, formulated with 33% barley, 18% oats, 47% hay/straw and 2% minerals, were compared with pellets which had half the barley replace with lupins. Measurements were made of numbers of sheep visiting feed troughs, and daily feed intake, over an 8 day period. The inclusion of lupins in the pellet resulted in more sheep visiting feed troughs on days 3 to 6 and higher levels of feed intake on days 4 to 8 than feeding pellets without lupins. Similar results were obtained when virginiamycin was included in the “barley” pellet formulation (McDonald et al 1994). It was hypothesised in this study that the increased proportion of sheep eating with the inclusion of lupin grain or virginiamycin was due to the reduced risk of acidosis. However, as was also stated by these authors, adding virginiamycin to lupin pellets with chaff also increased the number of sheep visiting feed troughs. This would suggest that virginiamycin may have an effect on feeding behaviour other than through the control of acidosis.

The results obtained by McDonald et al (1994) differ somewhat to those of Bailey and Fortune (1992) who also studied the pellet composition during feedlotting and subsequent sea transport. Merino wethers were studied over a 21 day period which included an 8 day feedlot phase and 13 day journey by ship. Sheep were offered 2 diets during this period, a cereal straw and barley pellet, and lupin pellet of lupin hulls and kernel. Diet, in general, had little to do with the feed intake and weight change. During the feedlot phase, sheep given the barley pellet gained about 1 kg liveweight, while those fed the lupin based pellet lost 1 kg ($P < 0.001$). The source of sheep was considered to be the contributing factor to this difference rather than the feed. Some of the variation noted in the source of sheep may have been due to the amount of time they had been deprived of feed and water. The first 5 days of the shipping phase saw consumption of both diet treatments fall from about 1 kg per head per day (at end of feedlot phase) to about 0.5 kg per head per day. This period was associated with the animals adjusting to confinement, increased human activity and contact, and conditions of increasing temperature and humidity (Bailey and Fortune 1992). Of the sheep that died on board the shipping phase, most appeared to be the result of inappetence and starvation and again was not related to diet but the original farm source of sheep. The results obtained by Bailey and Fortune (1992) highlight the many mitigating factors involved in inappetent sheep during export.

(ii). Lactic acidosis

Lactic acidosis causes a reduction in feed intake and has been shown to contribute to losses in commercial feedlots prior to live export (Richards et al 1986). High levels of cereal grain in a pelleted diet can result in rapid fermentation, a drop in rumen pH, and the proliferation of bacteria which produce lactic acid; the accumulation of lactic acid further reduces the pH resulting in general acidosis (Rowe and Aitchison 1986). A drop in feed intake has been reported associated with a build up of lactic acid in the rumen on days 3-6 of the assembly period (McDonald et al 1988; Richards et al 1989). There have been several methods investigated for increasing the rate in which sheep begin to eat prior to live export and overcome the problem of acidosis (McDonald et al 1994; McDonald et al 1988; Norris et al 1986; Adams and Saunders 1992; Norris et al 1990).

(iii). Method of supplement delivery

Any form of supplement feeding whether it be pellet, whole grain, chaff, hay or block, requires the supplement to be delivery to the animal in a way that will be perhaps foreign to the animal. Depending on the industry, intended recipient, and the type of supplement, the method of delivery will vary and so too will the resulting incidence of inappetence. For example, feed intakes will vary markedly between adult sheep depending on the method used to deliver whole grain supplements, with greater variation seen when sheep are fed from a feeder compared to when supplement is trailed (Holst et al 1994). In their review of supplement delivery and intake, Bowman and Sowell (1997) summarised the percentage of non-feeders and the CV of individual consumption of block, dry, and liquid supplements. Over the range of animals, environments, and supplement formulations that were studied, the percentage of non-feeders averaged 14.3% for blocks, 15% for dry supplements, and 23.5% for liquid supplements. The CV of individual supplement consumption averaged 79% for block, 41% for dry and 60% for liquid supplements (Bowman and Sowell 1997).

The method of delivery of supplements for pre-embarkation feedlots and during shipping of sheep is limited to the use of dry pelleted feeds in feed troughs. Hence trough space per head and placement becomes important in limiting inappetence.

(iv). Trough space and placement

Early studies looking at trough space requirements for sheep sought to minimise competitive aspects between sheep given grain supplements when grazing limited pastures (Arnold and Maller 1974). In that study, trough space per sheep was varied from 4 to 50 cm and aspects of competitiveness between sheep in mixed age and mixed breed flocks were reported. It was concluded that a minimum of 16 cm of trough space per sheep should be allowed when daily grain supplements are fed to grazing sheep (Arnold and Maller 1974). The rate of the disturbance of the sheep from the feed trough increased with decreased space per sheep. As the rate of disturbance increased a progressively greater proportion of sheep became non-feeders (Arnold and Maller 1974).

As reports of inappetence during live export of sheep became a source of concern, work was undertaken to assess the impact that trough space (Norris et al 1990) and trough placements (McDonald et al 1990) had on inappetence both on board ship and in pre-embarkation feedlots. Firstly McDonald et al (1990) examined at the behaviour of sheep in a pre-export feedlot situation when animals were fed from either troughs in the centre of a yard or troughs on the fence line. Paint soaked sponges located above troughs were used to mark and identify sheep which ate from troughs. When the feed trough was located in centre of the yard it took 2 days for 80% of the sheep to begin eating. This compared with 7 days when trough was on the fence line. The central location also resulted in a mean of 78 ± 2% of sheep being marked daily compared with 67 ± 2% for fence line ($P < 0.001$). Following this experiment, Norris et al (1990) simulated a 14 day sheep ship voyage applying various treatments to known inappetent sheep. Treatments included; normal quantities of feed (2% BW) and length of troughs (3 cm per sheep); extra trough length (8 cm per sheep); and extra feed (ad *libitum*). A control group of known feeders were included and were fed normal quantities of feed in standard length troughs. Like McDonald et al (1990), marker bars were used on days 1 to 3, 5 to 7 and 12 to 14 to identify sheep that fed. Results indicated that at least 85% of known non-feeders ate the pelleted feed during the simulated voyage, although more known feeders ate on days 1 to 3 and 5 to 7 compared to known non-feeders ($P < 0.01$). There was no significant

difference in feeding between non-feeders given extra trough length or extra feed compared to non-feeders given standard management at any stage of the simulated voyage (Norris et al 1990). It was concluded that sheep that die of inanition aboard ship are not inhibited from eating because of competition from other sheep or social dominance.

(v). Behavioural factors and prior feed recognition

One possible cause of inappetence is failure to recognise an unfamiliar substance as food. Familiarity of a food container may also be a component of this learning (Chapple et al 1987). Behavioural responses to novel feed (wheat) and feed troughs have been studied by Chapple et al (1987). Two groups of sheep with no previous experience of eating wheat or of feeding in feed troughs were individually penned and fed in troughs for 15 min/day over 20 days. One group was given wheat every day and the other hay for the first 12 days and then wheat for the next 8 days. The first group showed a gradual learning process whereby it took 14 days for all sheep to begin eating wheat. This reluctance or gradual learned behaviour is probably due to a combination failure to associate the trough with food and failure to associate wheat as a food source. The group of sheep offered hay first accepted it almost immediately suggesting that recognition of hay as a food overcomes any inhibitory effects of the trough on feeding behaviour. Sheep in this group also accepted wheat almost immediately when it was offered; however, the mean daily feed intake of wheat was low, suggesting that the animals must become familiar to wheat as a food. The end result of these behavioural observations was that there may be three phases involved in sheep learning to eat a “new” supplement. That is they must overcome fear of the trough and then of the supplement (neophobia), and then learn to prehend, chew and swallow the supplement (Chapple et al 1987).

Pre weaning experiences, particularly those related to maternal or other adult influences, are strong determinants of feeding behaviour, at least in the short term (Keogh and Lynch 1982) and possibly longer term (Green et al 1984; Lynch and Bell 1987), and may relate to the learning by sheep. In experiments feeding a wheat supplement, Lynch et al (1983) showed that lambs which first encountered wheat in the presence of their mothers ate significantly more wheat during post weaning tests than lambs that were exposed to wheat without their mothers being present. It was suggested that the transmission of feeding behaviour from ewe to lamb is a normal phenomenon. The mechanism behind this phenomenon remains unclear. Behavioural imitation, visual and olfactory stimulation, and cues via mother’s milk have been postulated (Lynch et al 1983). The mother’s experience of eating wheat also influences lamb intake post weaning. Lambs exposed to wheat with mothers that were familiar to wheat as a supplement ate significantly more than lambs exposed to wheat with naïve mothers (Lynch et al 1983). The interval between exposure and testing ranged from 1 to 10 weeks in these experiments and this interval did not seem to have any impact on the quantity of wheat eaten by groups of lambs.

Further studies have shown that prior feed recognition persists for longer durations (Green et al 1984; Lynch and Bell 1987; McDonald et al 1988a). For example, Lynch and Bell (1987) found marked behavioural differences between two groups of ewes fed grain supplements over 3 days. The group of ewes with experience of eating grain as lambs were all eating grain after 3 days, while less than 10% of control ewes (which had never seen grain) ate. These results were similar to those described by Green et al (1984). In that experiment lambs given access to wheat for 1 hour per day in the presence or absence of their mothers were tested again at 6, 12, 24 and 36 months of age to assess their acceptance of wheat as a food source. At all ages, lambs given access to wheat with their mothers ate considerably more wheat than those given access to wheat without their mothers

and controls, which had no exposure to wheat. In a similar experiment, Merino wethers with previous experience of supplements of pellets, oats or an oat/pellet mix, given either when they were lambs or when they were 18 months old, resulted in animals eating 35% more pellets over 5 days of lot feeding, than those which had never been fed supplements (McDonald et al 1988a). In this experiment there was no advantage in exposing the sheep to supplements as unweaned lambs versus at 18 months of age. In fact if anything results suggested that on-farm exposure to supplement feeds may be more effective when given immediately before export rather than earlier in life. Whether or not on-farm feeding improves feeding performance to the extent that deaths and live weight loss are reduced was not tested.

3.4.4 Pre-embarkation stressors

The management of sheep destined for live export changes markedly from their time on the farm to shipment. On farm selection and handling, road transport, handling and inoculations during assembly at the feedlot, altered diet and exposure to novel forms of feed and finally the shipping journey will occur in a period of about 1 month (Arnold and Chadwick 1978; Norris et al 1989; Bailey and Fortune 1992). These factors constitute many stressors to the sheep, with individual and cumulative effects, that may influence feed intake.

It has been suggested that there are two types of reactions to threats to homeostasis (stress). The first is a response to a situation that has already disturbed or will disturb the internal balance of the organism; including pain, severe thirst, and hunger. The other type of stress, which may be more relevant to inanition results from the perception of a threatening situation that may cause a disturbance in the homeostatic balance that the memory (limbic) system re-organizes and responds to by fear and anxiety (Nandi et al 2002). In overweight humans, studies have suggested that the hormonal stress axis becomes hypersensitive with increasing body fat (Björntorp 1996). The consequence is frequent over-stimulation of the stress axis, with elevated secretion of cortisol. Manifestations of this in humans include depression and loss of appetite, and effects on lipolysis, proteolysis, glycogenolysis, gastric function, and digestion. Stressors can also have effects on glucose allocation to the brain: with stress the allocation of glucose to the brain is increased while that to the body periphery is decreased (Peters et al 2004). The activated glucose allocation controlling ventromedial hypothalamus (VMH) is hierarchically superior to the appetite adjusting lateral hypothalamus (LH) and inhibits it, leading to a positive feedback cascade leading to persistent inappetence.

A. Transport and Fasting

Survey work conducted by Norris et al (1989b) indicted that total time off feed from mustering at the farm to arrival at the feedlot ranged from 1.0 to 42.9 hours. The effect of fasting duration on inappetence was investigated by McDonald et al (1990) who showed that fasting of sheep for 48 hours before lot feeding resulted in 18 ± 3% of the sheep not eating, which was significantly more than those marked as non-feeders when fasted for 24 hours or for less than 12 hours ($P<0.05$).

Results from Cole (2000) indicate that abnormal water and electrolyte shifts may be factors partially responsible for the decreased feed intake by ruminants subjected to feed and water deprivation stress. Appreciable water movements of plasma, extracellular, and ruminal water and electrolytes occur in ruminants during a meal. The quantity and/or timing of these shifts differ between animals that have been fed and animals that have been deprived of feed and water for several days (Cole 2000).

B. Mixing

The mixing of different lines of sheep from different sources is an unavoidable practise when sheep arrive at pre-embarkation feedlots and during the loading of ships (Arnold and Charlick 1978). Little scientific work has been undertaken to assess the relative risks that mixing alone has on social interactions of sheep and subsequent incidence of inappetence on sheep. Reports have been made citing mixing of sheep as a factor in inappetence (Arnold and Charlick 1978; Norris et al 1989b). However, Norris et al (1990) suggested that sheep that die from inanition aboard ship are not inhibited from eating because of competition from other sheep or social dominance.

C. Stocking rate

Jenkins and Leymaster (1987) have used automated systems and electronic instrumentation to evaluate the effect of number of animals housed in a pen upon free choice feeding behaviour in male lambs. By limiting access to the feed stall to a single animal, data collected in this study include individual animal identification, time of initiation of feeding activity, duration of the feeding period, ambient temperature, and feed consumption at each feeding event. Lambs were housed in groups of 3, 7, 11 or 15 animals per pen given stocking densities of approximately 3.7, 1.7, 1.0 and 0.7 m^2 per head. As number of lambs within a pen increased, feed consumption per visit increased. Daily feed consumption per lamb was greatest for intermediate lamb numbers, and the number of visits and time spent feeding per lamb decreased as the number of lambs within a pen increased (Jenkins and Leymaster 1987). In sheep feedlots, the effect of stocking rate on feeding has also been investigated (McDonald 1986). Sheep stocked in yards at 0.27, 0.62, 1.42, 3.38 and 7.56 m^2 per head were classified as regular feeders if they were marked on every day of a 12 day assembly period. There was a significant association of regularity of feeding with stocking rate ($P < 0.001$), with 38, 59, 73, 75 and 75% regular feeders at each of the above stocking rates (McDonald 1986).

3.5 Effects of inappetence

Undernutrition of the sheep, due to partial or complete inappetence, results in mobilisation of body reserves, in response to energy deficits and the release of catabolic hormones. Important consequences of this are ketosis and hypoglycaemia.

In the normally fed ruminant with fermentation and production of the volatile fatty acids acetate, propionate and butyrate in the rumen, ketones are produced by conversion of butyrate to β – hydroxy butyric acid (BHB) and acetate to acetoacetate in the rumen epithelium (Heitmann et al 1987), and these can be used by peripheral tissues as an energy source. There is very little glucose absorption in the ruminant, and so gluconeogenesis from propionate and amino acids is required to produce glucose; the majority of this takes place in the liver.

As reviewed in Hietmann et al (1987), during fasting in ruminants circulating concentrations of ketones increase, due to mobilisation and metabolism of fats. Plasma insulin and pancreatic production of insulin decrease during fasting, while glucagon concentrations remain constant, such that there is a decreased ratio of insulin to glucagon, and therefore release of fatty acids from adipose tissue. The fatty acids are taken up by the liver, where oxidation occurs. Excessive mobilisation of fats leads to their accumulation in the liver, which may result in decreased hepatic function. The fats are oxidised to release energy, producing Acetyl-coenzyme A (Acetyl-CoA) and

NADH. The Acetyl-CoA can be further used in energy generation via the tricarboxylic acid (TCA) cycle, but this requires a supply of oxaloacetate (OAA) from the precursor propionate. If this is limiting, for instance in undernutrition, the Acetyl CoA is instead metabolised to acetoacetate (Radostits et al 2000). If there is greater production of ketones than can be used by peripheral tissues, accumulation in the blood can lead to acidosis and toxicity. Additionally, lack of OAA, either because there is insufficient propionate or because it is being used in metabolism of Acetyl CoA, means there is less gluconeogenesis, and tissues which use glucose for normal metabolism, such as the brain, may be depleted of energy, although there is evidence that the ovine brain can use ketones to some extent (Kammula 1976).

The usual clinical situations reported to cause hyperketonaemia and hypoglycaemia in sheep and cattle are associated with the heavy nutritional demands, particularly for glucose, in late pregnancy and early lactation, but similar metabolic consequences could be anticipated in starvation. Of particular interest in this regard is the finding of greater mortality in fatter sheep, which may be a result of fatty accumulation in the liver, and reduced hepatic function, similar to that which occurs in fatty liver syndrome in cattle. Thus, management and treatment of these diseases may provide useful strategies for persistently inappetent export sheep.

3.6 Anorexia in other animals

Various diseases and syndromes in other animals are also associated with anorexia and might give insights on mechanisms or treatments for inappetent sheep.

3.6.1 Anorexia of disease

Anorexia or decreased feed intake is a common part of the “sickness response” to a variety of diseases, most notably cancer, and also other chronic diseases such as chronic renal failure, heart failure, chronic obstructive pulmonary disease, as well as acute infectious or endotoxic disease. In some diseases this appears to result from the induction of cytokines, such as tumour necrosis factor (TNF) and interleukins (IL), with systemic or central administration of cytokines inducing the symptoms and signs of sickness behaviour, including anorexia (Wong and Pinkney 2004). Experimentally this has also been studied by the injection of lipopolysaccharide (LPS) which can induce endogenous release of such inflammatory mediators (Danzter 2001).

The cytokines IL-1β, IL6 and TNF-α can decrease food-motivated behaviour and food intake by acting directly in the CNS (Johnson 1998), through interactions with neurotransmitters such as NPY, α-MSH and CRF, and may also decrease food intake indirectly, through interaction with brain cells to trigger secondary signal such as prostaglandins. Peripheral cytokines also stimulate vagal afferent nerves, which transmit the stimulus to the central nervous system. It is also possible that there are links between cytokine release and stimulation of leptin production by adipocytes, which then will reduce food intake. Injection of LPS has been associated with increased leptin gene expression and secretion in laboratory animals (eg Finck et al 1998; Sachot et al 2004), leading to the hypothesis that leptin might be involved in regulating food intake in sick animals. However, other work has shown that this link between cytokines and leptin is not straightforward, and that other hormones may be involved. Intravenous injection of LPS, and of TNF, induced anorexia in Holstein cows, accompanied by increases in plasma cortisol and insulin, but with no effect on plasma leptin (Soliman et al 2002), while Kim et al (2007) reported an early response of insulin to LPS injection with a decrease in hypothalamic neuropeptide Y, followed by a later increase in plasma leptin.

Cytokines can also directly affect gastric motility and emptying as well as GIT peptides such as cholecystokinin (Plata-Salaman 2000; Schwartz 2002; Wong and Pinkney 2004)

In other diseases, there may be additional factors which depress food intake. Anorexia is common in uraemic patients with renal disease, due perhaps to cytokines, with high TNFα and low NPY serum concentrations (Aguilera et al 1998), and also due to the effects of poor renal function. Aguilera et al (2000) suggest that there is an excess of tryptophan in uraemia, and this results in hyperproduction of brain serotonin, which causes anorexia. There are also low concentrations of large neutral and BCAA in the cerebrospinal fluid, which allows greater tryptophan transport across the blood-brain barrier (Aguilera et al 2001)

3.6.2 Anorexia associated with stress

A reduction in appetite is commonly seen as a response to intensely acute or chronic stressors (Bernier 2006). Fish and rats have been studied most intensively to unravel the effects of stress on food intake. Bernier (2006) describes that the types of stressors that cause appetite suppression in fish can be divided into systemic, such as pathological (anorexia of disease) and environmental (eg hypoxia, increased water ammonia, changed salinity); physical (eg threats, chasing, handling, restraint); and processive, which includes social stressors such as social subordination, isolation, confinement.

In rats, “social defeat” such as happens when one rat is defeated by another aggressive rat (Meerlo et al 1997), is an acute psychological stressor that has been reported to cause changes in food intake that may last for several days. Immobilisation is also considered a strong psychological stressor, associated with a reduction in food intake (eg Valles et al 2000). The extent of this reduction in food intake appears to depend on both qualitative aspects of the stress (what type it is) and quantitative aspects including intensity and duration, with longer or more intense stressors resulting in some differences in duration and degree of anorexia. Valles et al (2000) reported that immobilisation of rats for periods of 20 mins to 6 hours depressed food intake for some days, and it is suggested that corticotrophin-releasing factor (CRF) may be involved in this stress-induced anorexia.

In fish, CRF is also considered to be the key mediator of the stress-associated reduction in food intake (Bernier 2006).

There are dose dependent and complex effects of cortisol on feed intake; for instance, cortisol may counteract the appetite suppressing effects of CRF and related peptides by negative feedback on CRF (Bernier 2006); however, chronic high doses of cortisol decrease feed intake.

Stress-induced anorexia could in part be due to serotonin (5HT) activation, as there is a general consensus that increased serotonin neurotransmission inhibits food intake (Leibowitz and Alexander 1998). However, supplying tryptophan (a serotonin precursor) in the food has context dependent effects on the anorexia and endocrine response, depending whether the animal is stressed or not; Höglund et al (2007) showed that pre-treatment of trout with dietary tryptophan attenuated stress-induced anorexia in the fish, perhaps because long term enhancement of central serotonin signalling could inhibit the HPA axis and therefore the stress response (such as is the case with long term administration of selective serotonin reuptake inhibitors; eg Jensen et al 1999; Jongsma et al 2005).

Other animal models of anorexia have been developed that use separation, which results in severe weight loss, with increased 5HT and catecholamine levels in the hypothalamus (Siegfried et al 2003).

3.6.3 Melancholic depression

Cytokines may be involved in the impaired appetite in melancholic depression in humans (Andreasson et al 2007), in the same way that cytokines are involved in anorexia in the sickness response. It appears that human patients with depression show signs of immune activation, with increased circulating concentrations of inflammatory cytokines that could stimulate CRF release, which could in turn lead to anorexia.

3.6.4 Anorexia nervosa in humans

Anorexia nervosa is an eating disorder of humans of unknown aetiology, generally affecting females in adolescence, and very difficult to treat.

Södersten et al (2006a) suggested that for some people, reducing food intake (and increasing physical activity), as associated with the syndrome of anorexia nervosa, activate brain systems of reward so perpetuating the behaviour. Reduced food intake and increased physical activity activate CRF containing cells in the hypothalamus, which in turn activate dopamine and noradrenergic neurons, which are the neural basis for reward and attention (Zandian et al 2007). The continued anorexia leads to starvation, and then the resultant starvation causes the alterations in neurochemicals (rather than a psychiatric or physiological alteration that causes the reduction in food intake). Thus the endocrinological changes that are measured are most likely consequence not cause of the syndrome, with no evidence that reversal of the endocrine abnormalities of their starved condition affects the eating behaviour (Södersten et al 2006b).

There has been little success in managing or reversing the condition with pharmacological means; for instance selective serotonin re-uptake inhibitors (SSRI) have been used with varying success. As outlined above, serotonin (5HT) is an inhibitor of food intake, therefore increasing serotonin is unlikely to increase appetite and successfully treat anorexia nervosa (Zandian et al 2007).

3.6.5 Anorexia of aging

Aging in animals and humans is often associated with a decreased ability to maintain body weight and lean body mass in response to illness and disease, because of a loss of regulation of food intake and energy balance (reviewed by Wolden-Hansen 2006). The reasons for this are numerous, including a variety of social, psychological and physiological factors, sensory impairment, and the presence of disease, but also it may be due to loss of appetite. Older humans may have an apparent insensitivity to metabolic cues, failing to compensate for over-or under-feeding, which leads to inappropriate weight loss in response to illness or other stressors. Wolden-Hansen (2006) describes a rat model for age-related inappetence, dysregulation of energy balance, and weight loss, and cites references that suggest hyperleptinaemia, unrelated to disease states, contributes to the anorexia and wasting of the elderly animals. Aging male Brown Norway rats fail to appropriately increase feed intake after a 72 hour fast, and remain hyperinsulinaemic and hyperleptinaemic at the end of the fast, compared to younger animals. They also have a blunted neuropeptide Y gene expression in response to fasting, decreased AGRP and increased POMC and CART. Thus the aging animals have a reduced drive to eat and an increased drive to not eat – there are age-related decreases in

orexigenic signalling through AGRP/NPY neurones in the arcuate nucleus along with increases in anorexigenic signalling through POMC/CART neurons.

3.7 Potential methods of alleviating inappetence in export sheep

3.7.1 Feed additives

Hay is often fed in association with pellets during assembly and in pre-embarkation feedlots to sustain sheep which are slow to adapt to pellets, to encourage sheep to eat pellets, and to provide extra roughage to reduce the risk of acidosis (McDonald 1986). The effectiveness of such practises has been varied. Adding chaff hay to feed troughs in feedlots prior to export increases the proportion of sheep eating from the first day in the feedlot (McDonald et al 1988b). In direct contrast, McDonald et al (1994) concluded that the use of hay either in racks or sprinkled over the pellets was of little value and had no effect on feeding behaviour. In an attempt to improve feed intake and reduce inappetence, McDonald et al (1988b) examined the effects of spraying butyric acid, aniseed oil and molasses onto pelleted feeds. Over a 6 day period, molasses and aniseed reduced the number of sheep visiting the feed trough per day compared to sheep eating unsprayed pellets and pellets sprayed with butyric acid.

3.7.2 Electrolytes

There is no available literature to suggest that the use of electrolytes decreases the risk of inappetence in sheep feedlots. There has been published correspondence that sodium supplementation may prove beneficial in inappetent sheep on board livestock ships (Black 1990; Black 1997). Black (1990) suggests that during inanition, sheep become sodium deficient due to lack of intake, accompanied by a reduction in salivary recycling. In the absence of sodium in the GIT, ingested water remains in the GIT. The end result is inanition with excessive GIT fluid and signs of concurrent severe dehydration (Black 1990). On a voyage from New Zealand to Saudi Arabia treatment with seawater was initiated, due to post-mortem examinations of inappetent sheep revealing enteritis defined by excessive fluid in the GIT (Black 1997). In this report, sheep showing signs of inappetence were treated with 10 ml of seawater subcutaneously and 10 ml orally. The rationale was that the provision of sodium allowing water trapped in the GIT to be absorbed by sodium pump action and thus alleviating dehydration. The author reported that the great majority of animals treated with the seawater therapy recovered, but this was not a controlled study.

The only other study reporting the use of electrolytes on board live export ships was conducted using cattle, and reported that animals offered electrolytes had an increased weight gain compared to control animals (Beatty et al 2007). There was no indication in this experiment that cattle were affected by inappetence.

3.7.3 Management

As previously mentioned, pre-embarkation stressors on sheep have been thought to contribute to inappetence. However, there are no direct links that reducing time on trucks or in feedlot, decreasing stocking rate, or increasing trough space will have any direct impact in reducing the incidence of inappetence resulting in death. Norris et al (1990) maintained that the best option for management of inappetent sheep is to identify and modify on farm management factors that are responsible for producing the non feeders.

4 Conclusions and recommendations

4.1 Conclusions

Inappetence continues to be a concern in the live export of sheep, resulting in mortality both directly and indirectly due to its association with salmonellosis. Current research is identifying central pathways for appetite control along with the hormones, neurotransmitters, and feedback systems involved. A better understanding of appetite control, as well as information regarding voluntary food refusal in other species may provide avenues of new investigation, in particular in relation to anxiety and stress and the relationships with inanition. There are many factors which apparently affect feed intake and feeding of sheep, and efforts should continue to identify and correct any on-farm, pre-embarkation, and animal factors that predispose to inappetence. Additionally, those factors that influence metabolic consequences of short term fasting, such as fatness and season of the year, should be considered in management of exported animals.

4.2 Recommendations

A number of projects can be suggested to continue to address the problem of inappetence in sheep. It is recommended that a workshop be conducted with researchers and industry representatives to finalise details of such projects, which could then commence in 2009. Suggested projects and approaches are:

1. Identify and address on-farm factors that are related to a higher incidence of inappetent sheep from particular farms and at particular times of the year. This would involve retrospective analysis of mortality data from voyages, going back to farm of origin, and with a specific survey developed to collect information about management practices. Of importance in this work will be the accurate identification of cause of death of sheep, to distinguish those that die due to persistent inappetence from those that die due to acute or chronic salmonellosis, including those that may develop salmonellosis as a result of persistent inappetence. If specific factors can be identified as associated with inappetence, then management strategies can be developed to minimise these risks.

2. Investigate specific hypotheses about why some sheep become inappetent. For instance:

(i) Shy feeding sheep will become inappetent again, under stressful situations. While it appears that most sheep recommence eating when on the ship, it would be useful to identify such a group of repeatedly or persistently inappetent sheep, as such animals could be a useful research flock for further testing to be conducted to determine if there were differences in physiology, temperament, and/or behaviour compared to sheep that maintained their feed intake.

(ii) Sheep that have no background in eating supplementary feed are less likely to eat pelleted feed than other types of supplements eg whole grain, total mixed ration with grain and chaff, or hay. Testing this hypothesis will give information about the feeding preferences of sheep and then can be further developed to consider various backgrounding of sheep learning to eat supplements of different types, and whether that reduces the number of animals that do not eat in an intensive situation. It could also lead to recommendations about the type of food for sheep in intensive situations, particularly at introduction.

(iii) Calm sheep are less likely to become inappetent when exposed to a range of physical and psychological stressors, compared to nervous sheep. Lines of sheep can be selected on temperament, which indicates a genetic component to temperament. This may influence their response to a variety of management stressors, such that some animals may be genetically predisposed to a heightened stress response including inappetence in particular situations. The range of stressors that can be examined includes handling, transport, mixing and social stress, and periods of fasting, which are all commonly encountered factors in the live export process.

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