Final Report

Project code: W.LIV.0280

Prepared by: PD Mansell, DS Beggs, TF Jubb, MFS Pyman & AD Fisher

University of Melbourne

Date published: July 2012

PUBLISHED BY

Meat & Livestock Australia Limited

Locked Bag 991

NORTH SYDNEY NSW 2059

Premature Lactation in Exported Dairy Cattle

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

The occurrence of premature lactation is recognised as being a significant problem in dairy heifers exported from Australia by long-haul sea voyage, causing wastage of exported animals predominantly due to devaluation or rejection of consigned animals as unfit for purpose by importers.

The risk factors that lead to the occurrence of premature lactation are poorly understood. Although a substantial number of theories exist, the cause of the condition has not been elucidated and methods to control the incidence of the condition have not been established.

Following a critical review of the available literature and surveying of interested personnel with experience of the long-haul transportation of dairy cattle, exposure of exported dairy heifers to zearalenone in pelleted ship rations is considered to be a plausible explanation, at least in part, for the occurrence of premature lactation in such animals. It is also the factor most amenable to further investigation. There is sufficient evidence to suggest that zearalenone may commonly contaminate pelleted feedstuffs fed to dairy cattle before and/or during long-haul transport, and the link between exposure and premature lactation is also biologically plausible.

To further investigate this hypothesis we propose the following strategy: Determine whether zearalenone contamination of rations loaded onto ships used for long-haul export voyages from Australia does occur and, if so, to what extent; Evaluate and validate a simplified method for measuring zearalenone in feedstuffs that would be practical for on-board testing of livestock rations; Compile more reliable data to document the occurrence of premature lactation in exported heifers and the risk factors presumed to contribute to the syndrome; and Explore the potential for interactions between various risk factors using land-based feeding trials.

Executive summary

The exporting of dairy heifers for use as dairy animals in the country of destination is an increasingly significant component of the Australian livestock export trade.

The occurrence of premature lactation is recognised as being a significant cause of wastage in dairy heifers exported from Australia by long-haul sea voyage.

The wastage is predominantly due to devaluation or rejection of the heifers as unfit for purpose by importers. Some affected animals subsequently suffer from mastitis that can be a cause of economic loss to both exporters and importers. However, there is little quantitative data that describes the epidemiology of premature lactation in exported dairy heifers. Most information about the condition is anecdotal and poorly documented.

Premature lactation is most likely the result of hormonal aberrations in the exported cattle. Although there have been various theories proposed, it would appear that the most biologically plausible explanation is that animals are being exposed to extraneous oestrogenic factors. The mycotoxin zearalenone is of particular interest, being known to have oestrogenic effects and is commonly found in manufactured stock feeds such as pellets used in the live export process. A broad epidemiological study is unlikely to generate data of sufficient quality or quantity to change the current hypothesis that zearalenone is a necessary cause. More needs to be discovered about the presence and persistence of zearalenone in export pellets before such epidemiological studies or feeding trials are developed and is the basis of the following recommendations.

Recommendations

1) A study should be commenced that involves the collection of representative samples of pelleted rations for the majority of shipments leaving Portland over 12 months. These samples should then be analysed for the presence of zearalenone. Supervising personnel for each of these shipments should be contacted immediately after the conclusion of each voyage to ascertain whether premature lactation was observed in any of the exported animals, and to what extent. Simultaneously, evaluation of a commercially available test kit for the analysis of zearalenone in pellets should be done to establish the practicality and validity of conducting zearalenone assays of feed on-board long-haul voyages.
2) A systematic program needs to be established for the collection of basic epidemiological data relating to premature lactation from voyages on which dairy heifers are consigned. In addition to existing reporting obligations, all long-haul voyages from Australia on which dairy heifers are consigned should be described by a report at end of voyage that documents the proportion of animals which were observed to suffer udder enlargement, the leakage of milk or clinical mastitis.
3) A smaller number of representative voyages should be identified and resources put in place to ensure the collection of appropriate detailed epidemiological data specifically relating to premature lactation en route. As part of this component, the presence and amount of zearalenone in ships' pelleted rations should be periodically monitored to establish whether animals are indeed exposed to this toxin during long-haul voyages, under what conditions and to what extent.
4) A land-based study should be considered that assesses the occurrence and amount of zearalenone in rations fed to dairy heifers under pre-embarkation conditions, and quantifies any association between the occurrence of zearalenone in feedstuffs and the subsequent occurrence of udder development, premature lactation and mastitis in dairy heifers prior to loading or during the course of the voyage.

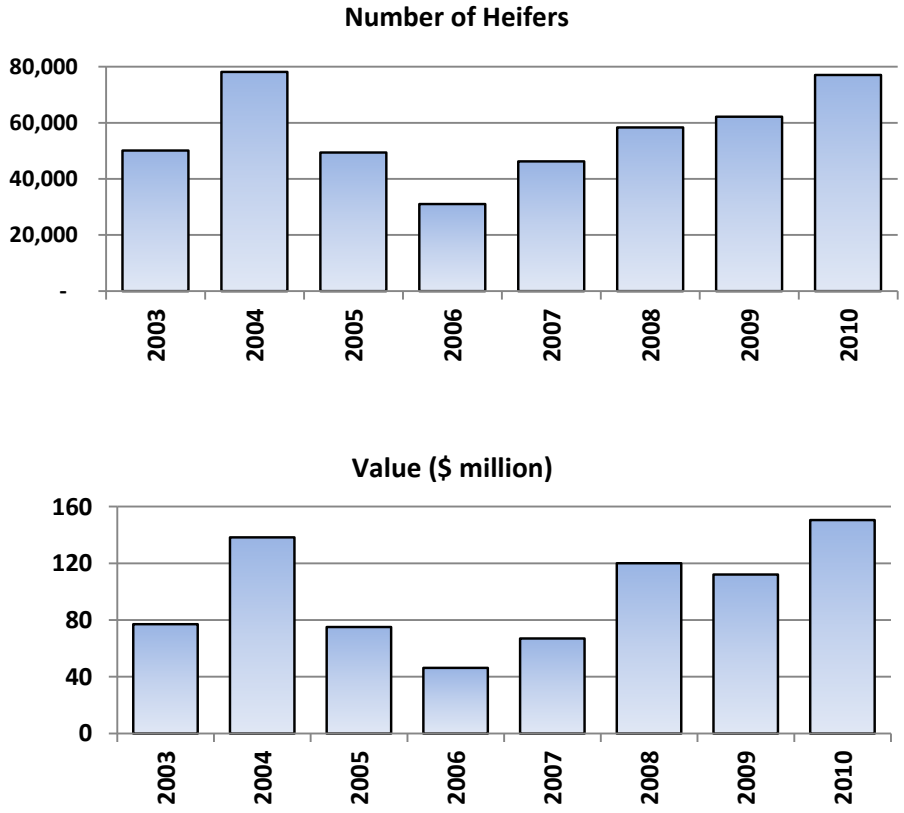
CONTENTS

1 Introduction

The exporting of dairy heifers for use as functional dairy animals in destination countries is now a substantial component of the Australian trade in exported livestock. Although the numbers of exported animals is small compared to the trade in cattle destined for slaughter, the value of the dairy animals is relatively high. This trade has become a significant source of income for Australian dairy producers, both by providing an additional market for the animals as well as by underpinning the prices received on the local market. The exportation of dairy heifers represents valuable commercial activity between Australia and a number of overseas trading partners. The economic benefit of the trade in dairy heifers has been well documented (CIE 2011).

Cattle have been predominantly recruited from dairy herds in south eastern Australia and preconditioned on land prior to being loaded onto dedicated livestock transport ships for sea voyage to Mexico, China, South East Asia, Pakistan, the Middle East, Russia and elsewhere. In 2010 approximately 77,000 dairy heifers worth about $150 million (at an average of about $2,000 per head) were exported from Australia, of which the majority (~85%) were from Victoria (see Figure 1).

Figure 1. Number and value of dairy heifers exported from Australia 2003 - 2010 *(Source: Australian Dairy Farmers Ltd)*



2 Problem of Premature Lactation in Exported Dairy Heifers

2.1 Definition of 'Premature Lactation'

2.1.1 Terminology

Premature lactation is the rapid development of the udder and the commencement of lactation not associated with the process of calving. Not all those animals that undergo udder enlargement during pregnancy subsequently produce milk within the mammary gland or leak milk from the teats. The term 'premature lactation' is used irrespective of the animal's pregnancy status, and irrespective of whether milk is observed to leak from the teats. A proportion of animals that do produce milk, especially those that subsequently leak milk, may also go on to develop mastitis in one or more quarters.

Premature lactation is sometimes also referred to as 'spontaneous lactation'. While these terms both refer to the same condition, the term 'premature lactation' is preferred and will be used throughout this report.

A variety of other terms have been used in the scientific literature to describe this or very similar conditions. These descriptions generally use words referring to the early or unexpected onset ('early', 'premature', 'spontaneous', 'precocious', 'induced' etc) and the nature of the udder change ('lactation', 'udder development', 'mammary enlargement' etc). The variety of descriptive terms is perhaps an indication that the condition is poorly understood and not well characterised.

2.1.2 Unrelated conditions

Premature lactation is not the same as 'udder oedema' or 'prepartum oedema' (sometimes colloquially known as 'flag') which is known to occur in dairy cattle in the last month of pregnancy. Udder oedema involves the oedematous swelling of the tissues adjacent to the udder or the udder itself but does not involve the lactational tissue of the gland. Udder oedema does not involve the initiation of lactation. Udder oedema could be easily confused with premature lactation by inexpert observers especially where close inspection or examination of animals is not feasible.

2.2 Consequences of Premature Lactation

There are potentially serious consequences if mammary development and premature lactation occur in dairy heifers as part of the live export process.

2.2.1 Decreased value (real and perceived) of animals at destination

Udder enlargement and the leaking of milk from teats are both visible characteristics of cattle that can be readily observed. In the absence of direct signs of pregnancy, both udder development and the production of milk can be taken by observers to be an indirect indication of the reproductive status of the animal. Animals showing signs of udder enlargement on-board or at the time of unloading may be suspected of being in a later stage pregnancy than they actually are. Non-pregnant animals with developed udders or which are producing milk may be assumed to be pregnant and close to term, or may be assumed to have calved recently, or may be suspected of having aborted. As such, importers may make inappropriate management decisions based on incorrect assumptions about the animal's pregnancy or lactational status.

Animals showing signs of lactation at the time of unloading at the country of destination may be rejected by importers as being unfit for purpose for a number of reasons:

- Importers who want to purchase non-pregnant cattle may incorrectly assume that cattle with enlarged or functional udders are actually pregnant and are in the process of 'bagging up' close to term.
- Importers intending to purchase pregnant cattle may have concerns that affected animals could have aborted or calved prior to or during transport. For cattle imported as pregnant animals, the expected calf may represent a significant component of the potential value of the animal. Any suspicion that such an animal is no longer pregnant decreases its perceived value.
- The initiation of lactation and the dripping of milk from the mammary gland represents a potential loss of colostrum (the highly concentrated 'first milk' produced by the mammary gland which is rich in fat, protein and, most importantly, immunoglobulins) that may jeopardise the survival of the calf subsequently born to that animal. The inappropriate release and loss of colostrum is recognised as being a significant management concern for the rearing of healthy, vigorous dairy calves (McNeil *et al* 2011).
- Exported dairy heifers that develop mastitis may be the subject of insurance claims as being unfit for their intended purpose as dairy animals in the country of destination.

Anecdotal reports indicate that in some export consignments, hundreds of high value animals may show signs of udder enlargement with or without the leakage of milk and sometimes almost entire consignments are affected. Some exporters have indicated that the costs associated with morbidity, mortality, insurance claims and dissatisfaction of customers have been very high although actual values are hard to document due to commercial sensitivities.

2.2.2 Mastitis

The proportion of affected animals that go on to develop mastitis is highly variable. Although mastitis in cattle may be relatively transient with complete resolution following self-cure, a significant proportion of animals that develop mastitis become chronically infected so that they either continue to produce abnormal milk that is unfit for human consumption, or they go on to suffer intermittent or recurrent clinical episodes of mastitis. Cows with existing mastitis infections in dairy herds represent a major source of contamination for other, non-infected cattle in the herd. As such, a significant component of the control of mastitis in dairy herds is the removal of chronically infected cattle from the herd. Hence, cows with mastitis have a shorter productive life as dairy animals which substantially decreases their attractiveness to buyers.

The teat orifice is by far the most common route of entry of bacterial pathogens into the mammary gland. Cattle with patent teat orifices are prone to infection by environmental pathogens especially when exposed to high levels of environmental contamination. In a pasture based production system this can occur when periparturient cattle are held on a calving pad or are exposed to poor hygienic conditions in holding yards, feed pads, laneways and similar environments. Mastitis due to environmental pathogens (classically *E. coli* and *S. uberis*) can have a variety of different clinical presentations including acute onset of severe clinical signs and even death of the animal if not managed appropriately.

3 Methodology

3.1 Literature Review

For this report, the authors accessed the peer reviewed literature by using the search engines available through the University of Melbourne's library system.

Search terms used (in various combinations) included: premature, spontaneous, precocious, induced, lactation, mammary, development, cattle, heifer, cow, dairy, zearalenone.

In addition, second-tier literature sources (online archives of professional associations, reference books, proceedings etc) that were available to the authors either through personal membership of associations or in their private literature collections were also consulted.

3.2 Targeted Surveys and Consultation with 'Experts'

Broad requests for information on the recognition of premature lactation in cattle from veterinarians in Australia, the United States of America and the United Kingdom were made through direct posting to mailing lists associated with the major professional groups of cattle veterinarians in these three countries (Figure 2). In Australia information was sought from the 1080 members of the Australian Cattle Veterinarians (ACV, a special interest group of the Australian Veterinary Association Ltd) through direct posting of a request on the ACV email mailing list. Similar approaches were made to the members of the American Association of Bovine Practitioners (6000 members, including approximately 200 from countries other than USA and Canada) and also the British Cattle Veterinary Association, through the British Veterinary Association.

Figure 2. Request for information from professional associations of cattle veterinarians

Dear Colleagues,

I am interested in hearing from cattle veterinarians who may have experience with or knowledge of premature lactation in heifers. (The condition is also known as spontaneous lactation.) The heifers may be non-pregnant or in early to mid pregnancy and undergo rapid mammary development and lactation. There have been occasional reports of this occurring in grazing or feedlot animals but more commonly it has been reported in exported dairy heifers on long haul voyages after a week or two at sea.

Premature lactation is not to be confused with udder oedema or prepartum oedema occurring in heifers in the last month of pregnancy.

Any information on where the condition may have been observed, the season, the type of cattle, their pregnancy status, diet, and outcomes for the animals, would be very helpful. And of course we are certainly interested in any ideas as to the cause.

Regards,

Subsequently, a more structured survey (see Appendices 1 and 2) was circulated to a more select group of individuals known by the authors to have direct experience with the exporting of dairy heifers from Australia. These people were either accredited shipboard veterinarians, onboard stockmen or supervisory personnel from exporting companies who had knowledge of the recruitment, loading, shipment and unloading of dairy heifers by long-haul sea voyage.

4 Current Knowledge about Lactation

4.1 Lactation in Normal Cattle

Lactation in cattle is the sum of three processes:

- Mammogenesis: *the physical development of the mammary gland and associated structures*
- Lactogenesis: *the process of differentiation of cells of the mammary alveoli within the gland, as a consequence of which the alveolar cells develop the capacity to secrete milk*
- Galactopoiesis: *the production of milk by the mammary glands*

The structural development of the mammary gland in cattle, like other species, is associated with the onset of puberty. The development of the hormonal mechanisms which result in the appearance of sexual function in heifers also leads to the growth of lactational tissues in the mammary gland. Oestrogen and progesterone both have an effect on the mammary gland, initiating the structural maturity and the functionality of the gland so that it is able to secrete milk at the end of pregnancy. The age at onset of puberty in heifers is variable, being dependent largely on breed and nutritional status. Oestrus occurs earlier in heifers that are on a higher level of nutrition. The physical growth of the mammary gland begins before the onset of actual secretory function within the gland. The physical appearance of the udder is a poor indicator of functionality of the gland. Lactation is determined by the amount and activity of glandular tissue within the udder rather than the size of the udder itself. Genetics and environment play a role in the development of the udder. Nutrition, both prior to and subsequent to puberty, affects the size and function of the udder. Poor nutrition limits the amount of glandular tissue that develops. Excessive nutrition can result in the formation of significant amounts of adipose tissue (fat) within the udder which may then limit the lactational capacity of the gland.

In normal cattle, immediately prior to calving, the mammary gland produces a specialised secretion called colostrum ('beastings') that contains a high concentration of fat, protein, sugars and immunoglobulins. This secretion is critical for the newborn calf as it provides a concentrated source of nutrition. It is also the predominant means by which passive immunity to disease is transferred from the cow to the calf. The production of colostrum is transient, lasting only for a few days in most animals, after which the mammary gland changes to the production of milk.

The onset of lactation is often associated with the dripping of milk or colostrum through the teat orifice of one or more of the quarters of the mammary gland. Release of colostrum or milk from the mammary gland results from an increased pressure within the gland as the fluid is secreted. Ejection of milk is also encouraged by muscular contraction of the alveolar tissues under the influence of the hormone oxytocin. The release of oxytocin is itself initiated by environmental stimuli. Physical massage of the

udder and teats, visual and auditory stimuli can all cause the sudden release of oxytocin from the hypothalamus. The teat orifice is the primary defensive structure that prevents the entry of pathogens from the environment into the gland itself. For most of the time in normal animals all four teat orifices are closed, occluding the teat canal and sealing the internal gland from the outside environment.

4.1.1 Mastitis

The presence of dripping milk indicates that the teat orifice is open and that entry of pathogens into the gland is possible. When the teat orifice is opened as part of the milk ejection process, the patent canal represents a potential route for bacteria to enter the gland and establish infection. The occurrence of an open teat orifice and environmental contamination of the adjacent teat skin greatly increases the risk of the quarter becoming infected with mastitis organisms. The dripping of milk when exposed to significant environmental contamination is recognised as being a major risk factor for the development of mastitis in commercial dairy cattle under common management systems (Brightling et al 2000).

The severity of bovine mastitis is highly variable ranging from subclinical infection (where there are no visible changes to either the teats, udder or milk and no clinical signs of illness in the cow) through to mild or moderate clinical mastitis (where there are visible changes observable in the milk and clinically detectable signs of inflammation in the udder) to severe mastitis (where the cow also shows signs of fever, depression and possibly dehydration consistent with substantial systemic illness) (Parkinson *et al* 2010). Even though there are no visible changes in the milk from subclinically infected quarters, milk from such glands is of lower quality and is harder to efficiently convert into processed dairy foods. Quite apart from the welfare aspects of clinical illness in cattle, the tissue damage associated with mastitis may lead to temporary or permanent loss of function of infected quarters. Very severe cases may lead to the death of the animal. Chronic, disabling infection may require salvage slaughter or euthanasia of the animal.

4.2 Induction of Lactation

4.2.1 Cattle

Premature lactation occurs in cattle that have not recently calved. The science of initiation of lactation in such cattle has been around for a long time as a 'treatment' for cattle that have difficulty conceiving. A review of the history and current state of knowledge regarding the factors we know can induce lactation is instructive in identifying those factors that might be present when premature lactation occurs on-board a ship.

4.2.1.1 History

Protocols for the induction of lactation in non-lactating and non-pregnant dairy cows have been around for more than 60 years. Techniques have all used the ovarian hormones oestrogen and progesterone, alone or in combination, to stimulate the development of the mammary gland and to initiate lactation. Early attempts to induce lactation used 120-180 days of injections of oestrogen and progesterone. More recent attempts have added reserpine and/or bovine somatotropin (BST) and/or dexamethasone into the mix.

Smith and Schanbacher (1973) showed that lactation could be induced by treating cows for 7 days with 17β-oestradiol (1 mg/kg BW) and progesterone (0.25 mg/kg BW). Subsequent studies increased the treatment period from 7 to 11 or 12 days, although neither modification showed better rates of induction of lactation nor elimination of the milk production variation in cows artificially induced. Collier *et al* (1977) used reserpine injections on days 8, 10, 11 and 12 of the artificial induction lactation protocol with oestrogen and progesterone. Reserpine has been shown to stimulate a release of prolactin in cattle. Macrina (2011) assessed the use of BST in an attempt to increase milk production and reliability of response in pubertal heifers. This paper is interesting as it demonstrates that the protocols can induce lactation in heifers that have not had a calf. Most other work has been done in cows with demonstrated reproductive failure.

Jewell (2002) showed that synchronizing cattle with two injections of prostaglandin (such that they were between days 3-8 after oestrus when the program started) increased the response (although not the milk yield) of cattle to a lactation induction program. This was consistent with previous work by Erb *et al* (1976) that showed that cows with below average plasma progesterone at the commencement of a program were more likely to respond.

4.2.1.2 Hormonal Effects

Tucker (2000) reviewed the current understanding of different hormones role in mammogenesis and lactogenesis. Much of the following is a based on that article.

Oestrogen

In 1964, *in vitro* studies showed that oestrogen plus prolactin and growth hormone stimulated mammary growth. It was observed that growth of mammary tissue *in vitro* was especially strong if the medium containing oestrogen, prolactin, and growth hormone was supplemented with 5% serum. Subsequently, oestrogen was observed to induce secretion of growth factors from pituitary, kidney, and mammary tumour cells. Thus, it was postulated that growth factors secreted from extra-mammary tissues into serum may act via an endocrine mechanism to mediate the mammogenic effects of oestrogen. Oestrogen has been shown to induce the secretion of IGF-1 from the stromal tissue of the udder in cattle. IGF-1 and a host of other growth factors have been implicated in mammogenesis.

Oestrogen has been observed to be involved in initiating lactation in the periparturient period. With impending parturition, oestrogen is one of the first hormones to increase in concentration in the blood circulation. Oestrogen acts in at least two ways to initiate lactation: it causes release of prolactin from the anterior pituitary gland which in turn initiates lactation; and it increases the number of prolactin receptors in mammary cells.

Progesterone

It has been long known that exogenous progesterone acts synergistically with oestrogen to induce lobule-alveolar growth. This was supported by the observation that mammogenesis during pregnancy in cattle coincided with the increased secretion of both oestrogen and progesterone. Progesterone induces DNA synthesis at the end buds and along the walls of the mammary ducts, the sites where progesterone receptors are located. Furthermore, oestrogen increases the number of progesterone receptors. Thus the ovarian steroid hormones appear to regulate lobule-alveolar growth. It has not been definitively established whether oestrogen directly acts synergistically with progesterone or its receptor, or whether is it an oestrogen-induced growth factor that acts synergistically with progesterone to induce lobular-alveolar growth.

Whilst progesterone is essential in mammogenesis, it appears to be inhibitory to lactogenesis. Progesterone was observed to inhibit histological indicators of lactogenesis, and it has been shown that injections of progesterone during pregnancy prevent the normal initiation of lactose, α-lactalbumin, and casein synthesis (Kuhn 1969, Turkington and Hill 1969). Removal of progesterone via ovariectomy during pregnancy normally initiated lactation, but if ovariectomy was performed with concurrent removal of the adrenal or anterior pituitary, lactogenesis did not occur(Liu and Davis 1967). This reinforced the concept that positive (prolactin, adrenal glucocorticoids) as well as negative (progesterone) factors were involved in lactogenesis. A rapid decline in secretion of progesterone in the periparturient period in several species, including cattle, was observed to coincide with initiation of secretion of copious quantities of milk (Smith, Edgerton *et al* 1973). This finding provided additional correlative evidence of the pivotal role of progesterone in suppressing lactogenesis. Progesterone blocks lactogenesis in at least two ways: it suppresses the ability of prolactin to increase the number of prolactin receptors in the mammary glands; and blocks glucocorticoid receptors in mammary tissue, suppressing the lactogenic activity of the glucocorticoids.

Although progesterone inhibits the initiation of lactation, once lactation has become established, administration of progesterone appears to have no effect on milk yield - probably because the progesterone receptor was not present or at least not expressed in the mammary glands during this physiological state. Moreover, progesterone is observed to have a greater affinity for milk fat than for its own intracellular receptor, which would further minimise progesterone action at the mammary gland during lactation.

Prolactin

Prolactin is a much studied hormone. Care must be taken when interpreting laboratory results in experimental animals, as the effects of prolactin appear to be very species specific. Whilst in some species, prolactin is essential for mammogenesis, this does not appear to be the case in cattle, where prolactin levels do not change much during a normal gestation, when most mammogenesis occurs (Oxender *et al* 1972). However, prolactin was discovered to be critically important for the initiation of lactation in the periparturient period in several species, including cattle. Indeed in cattle, lactogenesis is the only function of prolactin that has been clearly established (Tucker 2000). (In monogastric species, prolactin is required for both the initiation and maintenance of lactation). In cattle, a surge in secretion of prolactin occurs several hours before parturition. Blockade of this surge with bromocriptine markedly reduces subsequent milk yield, and exogenous prolactin reversed this effect of bromocriptine. *In vitro* studies showed that prolactin in association with insulin and cortisol was required to induce secretion of milk proteins (Juergens *et al* 1965). Similar to the mechanisms described for mammogenesis, binding of prolactin to its receptor was subsequently found to initiate the lactogenic response. Following binding, a cascade of events is initiated that eventually turns on transcription of the genes that regulate secretion of milk proteins. However, some knowledge gaps remain. How progesterone interferes with the ability of prolactin to induce its own receptor during pregnancy, and where in the cascade cortisol interacts with prolactin are not understood.

Interestingly, increasing the light period from 8 to 16 hours per day in cattle increased concentrations of prolactin several fold and also increased milk yield by 6 to 10% (Peters *et al* 1978). However, there is no unequivocal evidence that prolactin is responsible for the galactopoietic responses to photoperiod. More recent studies suggested that long-day photoperiods stimulate secretion of IGF-I. However, as described later, there is doubt as to the galactopoietic activity of IGF-I. Therefore the

question of what mediates the galactopoietic responses to long-day photoperiods persists.

Growth Hormone

Whilst there are several studies showing that GH might increase the yield following induced lactation, it is thought that GH is not a major factor in the onset of lactation in cattle. It probably has effects on milk yield indirectly through IGF-1, or through simple changes in partitioning of energy and increased energy supply to the udder.

Glucocorticoids

The role of glucocorticoids in the regulation of milk synthesis has been reviewed (Tucker 2000). Cortisol is the predominant endogenous glucocorticoid in cattle, and the major function of cortisol at the mammary gland is to cause differentiation of the lobule-alveolar system. This glucocorticoid-induced differentiation is essential to allow prolactin to later induce synthesis of milk proteins. Injections of glucocorticoids into non-lactating cows with well-developed lobule-alveolar systems induced onset of lactation, although the quantity of milk subsequently produced was greater if prolactin secretion was also increased (Collier *et al* 1977). This constituted additional evidence for synergy among the hormones required for lactogenesis. As with many other hormones, glucocorticoid concentrations in blood were quantified during different physiological states of the lactation cycle. In general, glucocorticoid concentrations in blood remained low for the greater portion of gestation until just before parturition when they increased to a peak that coincided with delivery of the offspring. This peak occurred too late to account for the earliest stage of lactogenesis and may be associated with the stress of parturition. However, glucocorticoids in serum became bound to a protein, corticosteroid binding globulin (CBG), which inactivated the glucocorticoids. During the periparturient period, CBG decreased and free glucocorticoids markedly increased, which might explain the lactogenic activity of the glucocorticoids. Later, it was determined that glucocorticoids bind to specific receptors in mammary tissue, and regulate secretion of α-lactalbumin and β-casein.

Photoperiod effects on induced lactation

It is not the purpose of this review to extensively explore the physiological effect of photoperiod, however some brief comments seem worthwhile as photoperiod may have a significant effect on the effect of prolactin and IGF-1.

Cattle appear to be able to discriminate light at intensities as low as 5 lux. Light stimulates retinal photoreceptors that transmit an inhibitory signal to the pineal gland which prevents the secretion of melatonin. Dahl (2000) notes that long days (>15 h light) result in consistently higher circulating IGF-1 concentrations, and that exposure to long days in the third trimester of pregnancy results in a significantly increased periparturient prolactin surge in heifers.

Andrade (2008) found that in an exotic breed of sheep, lactation could be induced in spring but not in winter using an oestrogen/progesterone based program similar to the programs used in cattle. Akers (2006) commented “Greater milk yields for cows induced into lactation in the spring and summer (compared with winter) was also attributed to higher serum concentrations of prolactin” although the source of this is not referenced. This is consistent with the authors' personal experience in attempting to induce lactation in cattle.

4.2.2 Other species

In humans, the prolactin stimulant metaclopramide is used to pharmacologically induce lactation, for example in the intended mothers of surrogate pregnancies (Biervliet *et al* 2001). Metaclopramide is a dopamine antagonist, and is effective because dopamine inhibits prolactin release from the pituitary by stimulating the release of prolactin inhibitory factor from the hypothalamus (Albibi *et al* 1983).

More complex protocols are used to induce lactation in other farmed species. Early work in ewes by Head *et al* (1980) established the importance of both oestradiol-17-β and progesterone administration over 7 days, followed by hydrocortisone administration on days 18 and 20. Ewe milk yields were typically 25 to 50% of yields from postpartum animals. Greater milk production in ewes was achieved by a longer protocol developed by Mellor *et al* (1993), incorporating a 4-6 week period of daily subcutaneous (s.c.) injections of 100 mg oestradiol-17-β and 75 mg progesterone, followed by a 1-week period of 5 mg oestradiol-17-β daily, with or without 10 mg dexamethasone.

Probably due to the challenges of long-term daily injection regimens, Kann (1997) used a protocol of 7 daily injections of 0.5 mg/kg BW oestradiol-17-β and 1.25 mg/kg progesterone, followed by 1 mg/kg hydrocortisone on days 18, 19 and 20.

In dairy goats, Salama *et al* (2007) used a standardised protocol of daily s.c. injections of 0.5 mg/kg oestradiol-17-β and 1.25 mg/kg progesterone for 7 days, followed by 10 mg/day of intramuscular dexamethasone on days 18 and 20. All goats commenced lactation, with enhanced milk production occurring with the addition of 1 mg/day of the prolactin-stimulating agent reserpine on days 12, 14, 16, 18 and 20.

Finally, there is evidence of photoperiod and breed effects on the success of hormonal treatment to induce lactation in livestock species other than cattle. Ramirez Andrade *et al* (2008) used a standard protocol of 0.5 mg/kg oestradiol-17-β and 1.25 mg/kg progesterone for 7 days, followed by 50 mg/day of hydrocortisone on days 18, 19 and 20, in both Lacaune (a high yielding dairy breed) and Manchega ewes. Manchega ewes failed to establish lactation in winter, but were successfully induced to lactate in spring. Lacaune ewes were successfully induced to lactate in winter, and although milk production was approximately 50% of that caused by pregnancy and parturition, there were no differences in milk yield between nulliparous and multiparous induced ewes.

The evidence from other livestock species is that artificial induction of lactation can be achieved in nulliparous females of dairy breeds, with the administration of oestradiol and progesterone for a number of days, ideally combined with a subsequent corticoid administration.

4.2.3 Summary

Many studies over many years have looked at inducing lactation. Most protocols involve the administration of oestrogen and progesterone concurrently for 7 days or more. These protocols have been successful in pubertal heifers as well as adult cows suffering reproductive failure. Both mammogenesis and lactogenesis are needed to induce milk production, since most mammary development does not occur until during the first pregnancy. Other hormones are undoubtedly involved in both mammogenesis and lactogenesis, and protocols which add growth hormone, glucocorticoids, and reserpine (as a prolactin precursor) have been trialled in an attempt to more reliably induce lactation. Oestrogen and progesterone act synergistically to cause lobulo-alveolar development, and progesterone has the added effect of suppressing lactogenesis while this occurs. At the time of parturition, high cortisol levels aid in the induction of lactation because cortisol displaces progesterone from the mammary cell

receptors, thus reducing the progesterone-mediated block to prolactin receptor synthesis.

It seems that a combination of oestrogen, progesterone, prolactin and glucocorticoids work synergistically to cause the onset of lactation. The hormones IGF-1, insulin and a host of other growth factors are probably mediators but the mechanisms are not well understood.

Photoperiod is known to affect prolactin and IGF-1 concentrations.

5 Current Knowledge about Premature Lactation

5.1 Cattle

A previous project (Bovine Research Australasia 2003 [LIVE.217]) titled "*Investigating premature lactation in pregnant dairy females"* provides useful preliminary information. That report and other anecdotal reports indicate that premature lactation may occur in non-pregnant heifers and in pregnant cattle in early to mid-gestation and that it is primarily a phenomenon of dairy cattle exported by sea. Premature lactation in groups of animals is known to occur very rarely on land, although udder development without lactation has been anecdotally reported in Australia including in dairy heifers being prepared for export in quarantine facilities and fed the pelleted shipboard rations. The authors are aware of one anecdotal report of significant udder development in a group of dairy heifers on pasture in western Victoria.

5.1.1 Scientific literature

There are few reliable reports of premature lactation available in the peer-reviewed scientific literature. Those reports which have been published generally relate to cases of single animals in which ovarian dysfunction has been diagnosed or is suspected (Vestweber *et al* 1986, Whitacre *et al* 1988). In other clinical reports, the evidence upon which the tentative diagnosis of toxicosis was made is often scant (Bloomquist *et al* 1982).

5.1.2 Anecdotal reports and expert opinions

Reports of premature lactation in the 'second tier' scientific literature (proceedings, discussion groups etc) is very difficult to locate. Few of these sources of information are accessible using the common search engines or through library collections.

The American Association of Bovine Practitioners (AABP) maintains an archive of all discussions exchanged on their electronic discussion group by member veterinarians. A search of these archives produced a small number of reports relating to premature lactation, again primarily relating to individual or small groups of animals. These cases proposed a number of potential causes for the condition, including hormonal dysfunction due to ovarian tumours, initiation of lactation following physical stimulation of the mammary gland (by suckling by herd mates) or exposure to poor quality feed (such as heifers being fed conserved feedstuffs rejected by cows in the milking herd). The largest number of animals affected in one report was 17 of 68 Holstein heifers (10 - 14 months old) which developed premature development of the udder without any chance of having been mated. These animals had been fed a diet of rye grass and kikuyu which had been fertilised with chicken manure. Responses by AABP members to our call for information provided some opinions but little, if any, solid data. These

anecdotal reports also raised the potential for premature lactation to be the result of either the feeding of contaminated feedstuffs ("*fed mould spoiled crop corn refused by the lactating cow herd"* or *"feeding mouldy feed")* or the result of physical stimulation of the udder by suckling or other behaviour.

These suggestions were supported by the responses from members of the British Cattle Veterinary Association (BCVA) which raised the possibility of oestrogenic pastures, myotoxicosis and suckling behaviour as potential risk factors, although again no hard evidence was provided and no proposal of mechanisms beyond those described above.

The responses from both AABP and BCVA members highlighted that the transportation of dairy heifers on long-haul voyages is not done in either of their industries, and no-one in either the UK nor the USA industries claim to have experience of any similar management system that could be considered to be similar to the exporting of dairy heifers as it is done within the Australian industry.

Apart from one anecdotal report, Australian veterinarians did not report observing premature lactation in cattle on land.

The opinions of those with experience of the export of Australian dairy heifers provided some interesting insights into the condition. Those who did reply tended not to use the survey sheets provided to them but responded directly by phone or email. Premature lactation has not been observed in exported beef animals despite the fact that they undergo a similar process of pre-conditioning, loading and unloading, and are exposed to similar conditions whilst on-board during long-haul voyages. Suckling behaviour is rarely if ever observed amongst animals on-board, although it has been reported that the udders of cattle can be exposed to significant stimulation during periods when the decks are being washed down using pressure hoses.

> *"Dripping milk seems more evident after deck-washing, but I assumed mainly because this is when it is easiest to see milk on the floor."*

Premature lactation was observed by several of those who responded to our call for information, but the reported incidence was highly variable. Premature lactation was seen on some but not all voyages. Although few were able to provide robust numerical data on the incidence of premature lactation on voyages, the proportion of affected animals varied substantially between voyages, with some reporting only a few affected animals and some reporting more extensive 'outbreaks'.

> *"100% of pregnant Jerseys bagged up. About half moderately tight and half very tight and dripping milk. 60% of pregnant Friesians bagged up - half are mild to moderately tight and half very tight and dripping. 1 - 2% unjoined heifers on open decks are bagged up to minor or moderate degree." (Extract from AAV voyage diary)*

The observations of those who had experience of the condition were not entirely consistent. Some reported observing udder development and the dripping of milk in heifers at the time of loading, but most observed that udder development only became noticeable a week or more into the journey and only became a problem once the animals were subjected to more significant heat stress.

> *"During loading there were a few Jerseys noticed to be springing. The heifers had been in a feedlot for a month and fed hay and ship's pellets."*

> *"My impression is that the udder development gradually increases during the voyage, but dripping milk is not really observed until after the*

> *equatorial zone and continuous heat stress, and becomes a bit alarming in the days before discharge."*

The condition was seen in pregnant and non-pregnant cattle. A tendency was observed for mid-range body condition score animals or heavier, larger framed Holstein Friesian animals to be more likely to be affected. In contrast some respondents indicated that in their experience Jersey cattle were more susceptible. Premature lactation was not reported to be associated with observed abortion en route, but later gestation animals were considered to be most at risk.

No-one reported observing signs classically associated with the consumption of phytoestrogenic feeds (such as vaginal prolapses or excessive bulling) although the duration of exposure to extraneous oestrogen needed to cause these signs is probably substantially greater than the duration of oestrogen administration typically used in protocols to induce lactation pharmacologically.

Several respondents made reference to the potential for spoiled or contaminated feed to be a risk factor. Mould contamination of bagged feed which had been exposed to moist conditions had been observed. Others stated that, while they had seen no association with spoiled feed, there was a significant association with the feeding of high levels of pellets, ad lib pellets or the feeding of high protein pellets. If premature lactation was observed, it was reported that by reducing the amount of pellets fed and increasing the amount of roughage/chaff in the diet the condition could be managed and udder enlargement in affected animals usually resolved subsequently.

> *"Ad lib feeding of pellets is dangerous... no obvious link to spoilt feed... pretty much always relates to consumption of pellets... condition will affect between 10 - 90% of heifers..."*

Several respondents were of the opinion that any circumstances that lead to high levels of oestrogen and progesterone followed by 'stress' could be sufficient to initiate lactation in dairy cattle.

5.2 Other Species

A review of the literature showed that reports of premature lactation in species other than cattle are restricted to case reports and similar studies describing individual instances, rather than where multiple cases occur at once.

Most papers are from the human medical literature, and describe cases where inappropriate lactation (termed ‘galactorrhea’) has occurred due to primary endocrine disorders, or where psychological factors and/or physical stimulation have initiated lactation. As an example of the first instance, a collection of 50 individual cases of galactorrhea was studied by Archer *et al* (1974) who found that 38% of cases were directly attributable to elevated serum prolactin concentrations associated with hypothalamic-pituitary problems and other endocrine dysfunction, as well as amenorrhea (acyclicity).

A case report by Mobbs and Babbage (1971) described the instance of an Australian woman, already a mother, who established lactation for feeding two successive adoptive babies. The lactation was thought to be induced by the psychological response of the adoptive mother to the babies causing endocrine changes, as well as the physical stimulation of having the babies sucking at the breast before lactation commenced. A more recent report (Szucs *et al* 2010) described the case of a woman achieving exclusive breastfeeding of adoptive twin babies, although this was facilitated by prolactin-enhancing drugs.

Animal case reports in the scientific literature are generally focused on instances of lactation caused by primary endocrine disorders. For example, Cortese *et al* (1997) describe a case of primary hypothyroidism in a female domestic dog that caused elevated prolactin concentrations and spontaneous lactation. Similarly, Buckrell and Johnson (1986) describe a case of acyclicity and spontaneous lactation in a bitch associated with a hypothyroid disorder. It is likely that dogs feature prominently in the non-human literature because they are more likely to undergo a veterinary examination and full diagnostic work up than other animal species.

Pseudopregnancy in bitches can stimulate mammary development, although full lactation is not generally described. If there is a mammary secretion, it is often described as serous in nature, although owners are advised to discourage bitches from licking or self-suckling in order to avoid further galactogenesis (Giesenberg 2004). Pseudopregnancy in the bitch is not uncommon, and is thought to be caused by a rapid fall in progesterone during metoestrus inducing a greater than normal rise in prolactin (Harvey *et al* 1999). The prolactin is thought to be responsible for the behavioural and mammary development manifestations of pseudopregnancy, and treatment, where necessary, is by the administration of prolactin inhibitors (Bastan *et al* 1998; Giesenberg 2004). Pseudopregnancy has also been described in rabbits (Szendro *et al* 2010) and goats (Wolff 2007). Although the aetiology in these species is not fully understood, mammary development and a degree of lactation can occur. The best described example of pseudopregnancy and lactation is in the dwarf mongoose (Creel *et al* 1991), where subordinate females undergo a pseudopregnancy and suckle the young of related females. This is thought to increase the fitness of the subordinate females by assisting some of their genes to continue in subsequent generations (Creel *et al* 1991).

Among animals there are reported instances of an adoptive mother undergoing spontaneous lactation, such as in a dolphin (Gaspar *et al* 2000), or due to endocrine imbalances associated with unusual tumours (e.g. uterine leiomyosarcoma in a sheep; Vemireddi *et al* 2007).

In summary, although the cases of premature lactation described for humans and non-bovine animals are useful for emphasising the hormonal changes necessary for mammary development and lactation in the absence of pregnancy, they do not provide evidence for potential causal mechanisms for multiple premature lactation cases in exported dairy cattle.

6 Zearalenone

6.1 Introduction

Zearalenone (ZEA), also known as F2-toxin, is a mycotoxin biosynthesised mainly by fungi belonging to the genus *Fusarium* species. It is a common contaminant of cereal feedstuffs worldwide including Australia and is thus a potential dietary source of oestrogens in the cereal-based pellet diet of cattle on-board a ship.

The situation on cattle ships where the diet of non-lactating dairy heifers is almost entirely cereal-based pellets is unique. (Pellets are rarely fed to non-lactating dairy cattle on land and then only as a small proportion of the total diet.) This situation rarely if ever occurs on land which may explain the extremely infrequent reports of premature lactation on land. The potential for high, prolonged intake of ZEA exists on ships and, as such, this oestrogenic mycotoxin is worthy of detailed review.

6.2 Background

Zearalenone exhibits a relatively low acute toxicity. However, ZEA binds to oestrogen receptors and may produce various oestrogen-disrupting effects, such as infertility, reduced serum testosterone concentrations and sperm counts, enlargement of ovaries and uterus, reduced incidence of pregnancy, and alterations in progesterone concentrations in animals, at relatively low levels. In mammals there are two stereoisometric metabolites (α and β) that are of most importance.

In 1996, six countries regulated ZEA, and it was regulated by 16 countries by 2003 (FAO 2004). The maximum allowable amount of ZEA in foods and feeds ranges from 50 to 1000 µg/kg in various countries (FAO 2004), and a total daily intake of 0.1 µg/kg was proposed as a margin of safety in humans.

6.3 Geographic Distribution

Fungi-producing ZEA contaminate corn and also colonise, to a lesser extent, barley, oats, wheat, sorghum, millet and rice. In addition, the toxin has been detected in cereal products such as flour, malt, soybeans and beer. Fungi of the genus *Fusarium* infect cereals in the field. Toxin production mainly takes place before harvesting, but may also occur post harvest if the crop is not handled and dried properly. This may be important in the context of export ships, for example ZEA levels could increase in feed at sea, or the feed may be able to be tested prior to voyage. Many of the toxigenic species of *Fusarium* are major pathogens of cereal plants, causing head blight in wheat and barley and ear rot in maize. Numerous experiments indicate that the high levels of ZEA reported to occur naturally in some samples of corn-based animal feeds result from improper storage rather than development in the field.

Zearalenone contamination of feed has been reported in just about every country in the world, including Australia. A comprehensive list is found in Zinedine (2007).

6.4 Metabolism of Zearalenone

Zearalenone is rapidly absorbed after oral administration, and metabolites are rapidly excreted in the bile. There are species differences reported in ZEA metabolism. In cattle, β-ZEA is the dominant hepatic metabolite, and excretion is almost solely via bile. Zinedine (2007) quotes a study where neither ZEA nor its metabolites were detected in muscle, kidney, liver, bladder, or dorsal fat of male cattle ingesting 0.1mg/kg feed per day. This has consequences for the investigation of premature lactation as urine/blood tests of animals on-board a ship are unlikely to be useful in determining the presence or absence of ZEA.

The reduced form of ZEA, zearalanol, has marked oestrogenic activity and a synthetic form of this (zeranol) was the active constituent of Ralgro®, marketed widely as an anabolic agent for both sheep and cattle until it was banned in 1989 by the EU.

6.5 Toxicity of Zearalenone

Zearalenone has a low acute toxicity (oral LD50 >2-20g/kg in rats, mice and guinea pigs). In oral toxicity studies of up to 90 days, Zinedine (2007) states that the effects seen in experimental as well as domestic animals appeared to be dependent on the interaction of ZEA or its metabolites with oestrogen receptors, and provides a large table of reported effects in a range of species. The only mention for cattle are

references to infertility, decreased production and sperm degeneration. Hyperoestrogenism is also mentioned by Placinta (1999).

6.6 Effect on the Endocrine System

Zearalenone and some of its metabolites have been shown to competitively bind to oestrogen receptors. The α-ZEA metabolite has 3 to 4 times higher oestrogenic activity than the ZEA itself. Whilst in most species, α-ZEA and β-ZEA are recognised metabolites of ZEA, being produced in both the liver and intestinal mucosa, Kiessling (1984) reported that the protozoa in rumen fluid "detoxified" 90-100% of ZEA to the more active α-ZEA. Potentially, different rumen conditions could result in more or less oestrogenic metabolites being formed.

In an immortalised pituitary cell line, ZEA bound to the oestrogen receptor with an affinity of 0.01 relative to oestradiol and induced prolactin secretion, leading Stahl (1998) to the conclusion that ZEA is active in the lactomorph cells of the pituitary.

Massart (2009) reports that exposure to ZEA is a likely trigger for precocious puberty in young girls.

6.7 Summary

Zearalenone, a mycotoxin produced by several bacterial species including the ubiquitous F*usarium spp*, is a common contaminant of cereal crops worldwide. It is thought that most ZEA contamination occurs on the plant, although ZEA levels may increase post harvest under improper storage conditions. Zearalenone has oestrogenic activity that may be enhanced in ruminants by the degradation to a more active metabolite (α-ZEA). Once absorbed, ZEA is rapidly metabolised and excreted. Cattle being fed typical levels of ZEA do not have measurable levels in any tissue that would easily be sampled on a ship. It is quite feasible that cattle being fed ZEA contaminated feed could develop oestrogen-related symptoms. Measuring ZEA in feed is probably the best way of assessing this as a risk factor for premature lactation. Zearalenone may well act as an exogenous source of oestrogenic activity in cattle on ships as a result of feed contamination. Factors such as the pH of the rumen may alter the metabolism of ZEA to more or less active forms. Assays of feed rather than samples taken from animals on-board a ship will be necessary to determine ZEA levels.

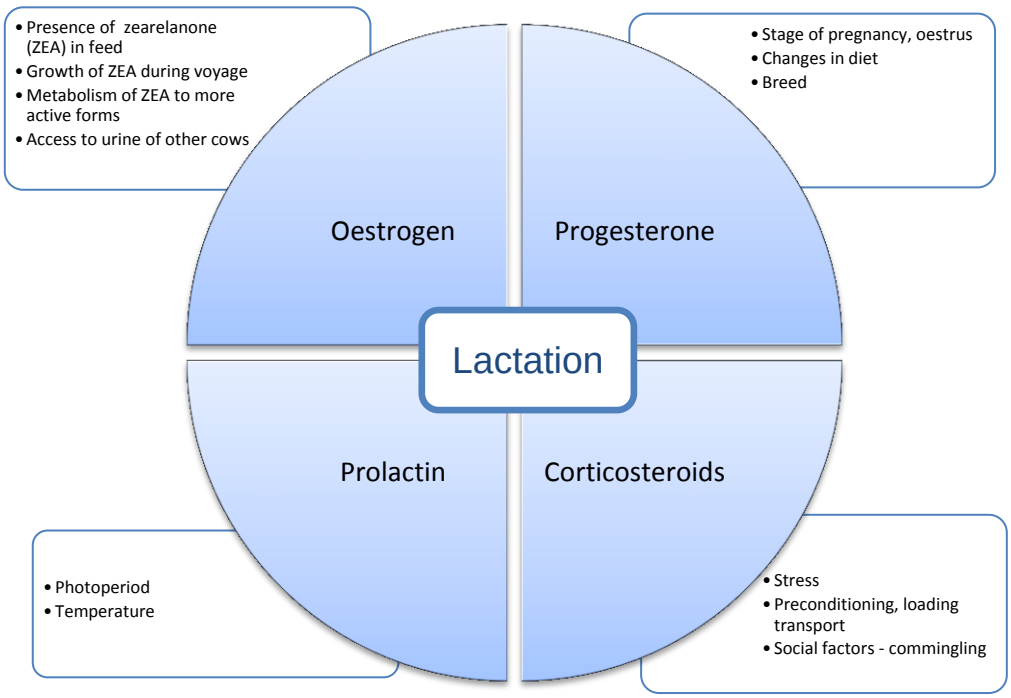
7 Proposed Risk Factors

The cause of premature lactation is not understood, although there are many speculative theories including stress, high protein feeds, day length effects on physiology and potential contamination of feeds with oestrogens of fungal or plant origin (eg zearalenone). Anecdotal information suggests that animals fed higher levels of pellets, of later stage of pregnancy and with more Jersey breed infusion are at greater risk. Beef breeds are so far not reported to be affected. It has apparently occurred in dairy cattle exported from other countries.

It is known from studies into both normal and artificially induced lactation that the components required for galactopoiesis are (at a minimum): oestrogens; progestagens; prolactin; and corticosteroids.

Thus factors that affect levels of these hormones may well be risk factors for premature lactation. A summary of these is shown in Figure 3.

Figure 3. Factors required for induction of lactation, and potential influential risk factors



At a practical level, the above diagram could be re-organised to show how a single risk factor might affect several hormones. This is described in Table 1.

Table 1. Risk factors and potential mechanisms for premature lactation in exported dairy cattle

Risk Factor	Potential mechanism of action
Presence of Zearalenone in feed	• ZEA is a fungal by-product with oestrogenic activity. Levels of ZEA intake might be influenced by level in feed at start of voyage, and growth of ZEA in feedstuffs during voyage
Diet	• If ZEA was present, changes in diet might affect rumen pH and in turn alter the metabolites produced, and thus increase the net oestrogenic activity in the diet • A sudden decrease in dietary intake might reduce portal circulation and metabolism of progesterone, resulting in higher progesterone levels • Changes in diet could cause stress and increased corticosteroid levels
Commingling and transport	• The stresses associated with commingling might cause increased corticosteroid levels • Exposure to the urine of other cows is a possible source of oestrogen/progesterone
Photoperiod and temperature	• Constant lighting conditions could simulate long day period conditions and affect prolactin and/or IGF levels • Light levels and temperature may affect the growth of the ZEA producing bacteria on board ship

8 Recommendations for Further Investigation

8.1 Basis to recommendations

This report has aimed to critically review the literature and to collate the accumulated expertise of people with experience of premature lactation in exported dairy heifers.

By considering the physiological basis of lactation, and critically reviewing established methods of artificially inducing lactation in cattle, we have arrived at a short list of factors which have potential to cause or at least contribute to the occurrence of this condition.

Although premature lactation is broadly recognised as being a problem of considerable economic and welfare significance for exported dairy heifers, the variable incidence of the condition between shipments, and even within groups of animals on the same voyage, has lead to there being a broad range of opinions about the possible causes among those professionals who have observed the condition.

This report has identified those factors which the authors consider are most worthy of further investigation. In making recommendations for the direction of further work, a number of questions were addressed.

8.1.1 Which explanations are plausible and most likely?

Of all the factors which may potentially increase the risk of premature lactation in exported dairy heifers, the focus of any further work should be on those factors which are considered to be scientifically plausible. Of those, emphasis should be given to those factors which could be considered to be most likely to be either sufficient or necessary to result in premature lactation in cattle on long-haul sea voyages.

> *A risk factor is a "sufficient cause" if the presence of that factor is enough on its own to cause the disease or outcome in question.*
> *A risk factor is a "necessary cause" if it must be present for the outcome to occur, but the presence of other risk factors may also be required to produce the outcome of interest.*

Hence, some suggested contributory factors extrapolated from other species and situations are fairly easily discounted. Although there have been a number of reports of premature lactation in cattle and other species due to ovarian pathology (e.g. tumours of ovarian or associated tissues), it is reasonable to assume that this would not explain the observations of premature lactation in the numbers of exported animals described. Similarly, although suckling behaviour between herd mates has been suggested by some as a risk factor associated with the initiation of lactation in some groups of animals under some conditions, this behaviour is rarely if ever observed in cattle on long-haul voyages and is unlikely to play a significant role under export conditions.

The initiation and stimulation of mammary development and lactation is primarily under hormonal control in normal animals. It is our belief that the most likely risk factors that could be explanatory are those that have a biological plausible mechanism for affecting the amounts of circulating oestrogen, progesterone, prolactin or other hormones in heifers either before or during shipment.

Factors which may alter the hormonal status of pregnant and non-pregnant cattle prior to or during shipment:

- Pregnancy status
- Stress
- Exposure to toxic components (e.g. zearalenone)
- Daylight variations
- Other factors

8.1.2 Which factors are amenable to more detailed investigation?

For those risk factors that are considered to be plausible and most likely, the allocation of additional resources should concentrate on those factors which are amenable to investigation under the practical constraints of resourcing and logistics associated with this industry.

The conduct of investigatory studies on-board a livestock export ship during a long-haul voyage can be problematic. Quite apart from the limited facilities available for close inspection or handling of animals, there is limited labour and other resources available.

At this stage we do not recommend the initiation of on-board studies which involve the collection of samples such as blood, urine or milk. These samples are likely to be difficult to collect, especially with the frequency and repetition that would be necessary to explore physiological changes in individual cattle over time. There is also limited ability to process samples on-board. Although we understand that it may be possible to process samples overseas or even to receive regulatory permission to return samples to Australia for processing under appropriate quarantine conditions, until a more clearly defined set of risk factors has been identified, the planning and resources that would be necessary for the conduct of such a study would be relatively expensive and of uncertain benefit. However, shipboard monitoring of cattle, recording of identified factors (e.g. temperature, deck, lighting, diet samples) of affected and non-affected animals will be essential in working out which combinations of factors put cattle most at risk.

Similarly, the conduct of intervention studies to explore the effect of factors such as hours of daylight are likely to be difficult to manage logistically and, at this stage, would not be considered to be a priority.

8.1.3 Which risk factors are amenable to control?

In considering which risk factors are worthy of further investigation as explanatory factors, emphasis should be given to those factors which could reasonably be amenable to manipulation, mitigation or control under the practical constraints of the industry.

It would seem sensible to focus limited research resources on the investigation of factors that could feasibly be avoided, controlled or mitigated within the constraints of the commercial exporting of dairy animals. If it is possible to identify one or more risk factors which are causally necessary to allow the occurrence of premature lactation then control basically becomes a matter of working out how one of those factors can be removed.

8.2 Recommendations

8.2.1 Zearalenone as the primary hypothesis

Considering the information presented above, exposure of exported dairy heifers to ZEA in pelleted ship rations is a plausible explanation, at least in part, for the occurrence of premature lactation in these animals. It is also the factor most amenable to further investigation. There is sufficient evidence to suggest that ZEA may commonly contaminate pelleted feedstuffs fed to dairy cattle before and/or during long-haul transport, and the link between exposure and premature lactation is also biologically plausible.

Consequently, to further investigate this hypothesis we propose the following strategy:

- Determine whether ZEA contamination of rations loaded onto ships used for long-haul export voyages from Australia does occur and, if so, to what extent;
- Evaluate and validate a simplified method for measuring ZEA in feedstuffs that would be practical for on-board testing of livestock rations;
- Compile more reliable data to document the occurrence of premature lactation in exported heifers and the risk factors presumed to contribute to the syndrome;
- Explore the potential for interactions between various risk factors using land-based feeding trials.

8.2.2 Determination of zearalenone in loaded pellets

As a first step to testing the ZEA hypothesis, it is appropriate to establish whether ZEA can be detected in rations loaded onto ships and to what extent exported heifers may actually be exposed to ZEA by consuming these rations. If ZEA can be detected in pellets being loaded onto ships there would be greater confidence in pursuing this line of investigation with subsequent studies. Assay of feedstuffs for ZEA is commercially available from a number of laboratories within Victoria.

It is recommended that representative samples of pelleted rations are collected as ships are loaded in Portland prior to each export shipment over the following 12 months. This will require the cooperation of exporting agents however preliminary contacts indicate that this will be forthcoming. Given the relatively large volume of pellets loaded, and the lack of knowledge of potential distribution of contamination (if any) within the pellets, it is recommended that multiple samples per shipment should be collected to ensure that a representative sample is collected for analysis. In light of early results, the number of samples to be collected per shipment may need to be modified. Samples would be submitted to a suitable commercial laboratory (potentially Agrifood Technology in Werribee, Victoria) for analysis of ZEA content.

The veterinarian or stockperson accompanying voyages would be briefed prior to sailing on how to detect premature lactation, asked to watch daily for its occurrence and record estimates of the proportion of different types of animals affected. A simple record keeping form would be developed and provided. At the conclusion of each export shipment the veterinarian or supervising stockperson would be contacted directly to ascertain whether premature lactation had been observed in any animals during the voyage and, if so, to what extent.

Indicative budget:	
Project management	5 days
Planning meeting with exporters	2 days
Development and testing of on-board scoring systems	1 day
Phone briefings/debriefings with stockperson or veterinarian accompanying the voyage	6 days
Data analysis and reporting	5 days
Laboratory testing: 60 samples @ $200/sample	$ 12,000
Total:	
Time of 19 days @ $1250/day	$ 23,750
Laboratory costs	$ 12,000
	= $ 35,750

8.2.3 Evaluation and validation of simplified zearalenone test methods

Should evidence be found to support the hypothesis that exported heifers may be exposed to ZEA by consumption of ship pellets, it would be beneficial to later studies if a simple, practical and accurate method was available to screen for the presence of ZEA in ship rations during voyages. For example, this could clarify whether exposure to ZEA may change throughout a long-haul voyage perhaps through degradation of feed held in a warm, moist environment. Although numerous simplified methods have been described, it is unclear whether any of these methods could be applied to on-board use. It is proposed that at the same time that the samples are being analysed as part of the above study (Section 8.2.1.1) a selection of the more promising 'kit' methods should be evaluated for accuracy and practicality for on-board use. The cost of this study would be heavily dependent on the cost of available kits. We have, as yet, been unable to establish which kits would be most worthy of evaluation. The completion of this component of the study is expected to add less than $10,000 to the cost of the study described above.

8.2.4 Structured data collection

It is unlikely that exposure to ZEA alone is sufficient to induce premature lactation. Other factors probably need to coexist. What these factors are is currently a gap in our knowledge of this condition. Further work to explore these factors and their relative importance is best done by epidemiological studies involving data collection on-board ship.

The authors consider it unlikely, at this stage, that a study to collect further epidemiological information would provide data of sufficient quality and quantity to remove the immediate need to determine if ZEA is implicated. Should suspicions be raised by finding ZEA in high levels in pellets associated with voyages where premature lactation occurs then further research projects would be indicated. If ZEA is not implicated then well managed observational studies combined with a high level of industry cooperation will be required to generate alternative hypotheses.

8.2.4.1 Broad standardised data collection

A major constraint to our current understanding of premature lactation in exported dairy heifers is the lack of well documented data detailing when the problem has arisen, at what rates, in which animals and under what conditions. In the production of this report it has become obvious that there is little data available to quantify or compare risk factors as they apply to shipments originating from Australia. It would be of great assistance in improving our understanding of this condition, and when formulating strategies aimed at reducing its impact, to be able to analyse consistent, comparable data from a number of voyages considered to be 'at risk' of having animals with premature lactation.

While accepting that livestock export ships are not set up for extensive data gathering, it should be possible to develop and implement a robust system to collect relevant data that describes the basic epidemiology of the condition. If possible it would be highly beneficial for shipboard staff to document the incidence of udder development and milk leakage in representative groups of animals on board using standardised descriptors. A draft of such a data collection system is provided (see Appendices). Data could be documented at journey's end as a retrospective summary but, ideally, would be collected regularly and more frequently throughout the voyage to provide more detail on chronological aspects. It would be preferable for this data collection to be done with the direct involvement of LiveCorp to ensure greater compliance. As is obvious from the discussion above, a major limitation to our understanding of this syndrome is the lack of standardised and reliably collected and compiled data from previous shipments.

Observational studies [October - June, Year 1 & 2]

Indicative budget	
Prepare and finalise animal ethics application (as appropriate)	4 days
Prepare and implement protocols for collection of observational data by AAVs and stockpersons accompanying all, or at least higher risk, consignments over this period.	15 days
Retrieve data collection forms and analyse data	5 days
Compile data and report	6 days
Total: 30 days @ $1250/day	**$ 37,500**

It would be preferable that such data would be gathered from all shipments of exported dairy heifers over a period of time or throughout the year. Apart from the additional workload imposed on the shipboard staff and some resources to compile and analyse the resultant data this is likely to be a relatively inexpensive and efficient use of limited resources. If necessary, instruction should be provided to those delegated to collect the data on-board prior to the loading of the shipment to ensure that they have a clear understanding of the issue, and are accurate in their detection and recording of relevant information.

8.2.4.2 Targeted Data Collection

A smaller number of representative voyages considered to be most 'at risk' should be identified and additional on-board resources allocated to ensure that data of sufficient

quality can be collected. The authors recommend that this should include the recruitment of a veterinarian to accompany at least two 'at risk' shipments, with the detection and recording of premature lactation as their primary task during the shipment. This will ensure that data collection on these shipments does not become secondary to other activities that may arise. It is unlikely that the collection of blood or urine samples during the voyage would be either practical or productive given the constraints of animal handling facilities on-board and the limited amount of analysis that can be conducted during the voyage.

It may also be feasible, depending on the outcome of the studies described above (Section 8.3.1.2), for basic assessment to be conducted on-board by suitably qualified personnel to ascertain the likelihood of fungal contamination developing in on-board feedstuffs during the voyage.

There is some risk in this targeted strategy, given the relatively unpredictable occurrence of premature lactation in exported cattle.

Observational studies [October - June, Year 2]

Indicative budget:	
Prepare and finalise animal ethics application (as appropriate)	4 days
A project team member to participate in two higher risk voyages to collect observational data, collect feed samples as required. Allow 30 days per voyage to allow for voyage ~21 days, monitoring at destination ~6 days and travel ~3 days.	60 days
Operating costs for travel and laboratory testing	$15,000
Retrieve data collection forms and analyse data (5 days)	5 days
Compile data and report	6 days
Total:	
15 days @ $1250/day	$ 18,750
60 days @ $1000/day	$ 60,000
Operating	$ 15,000
	= $ 93,750

8.2.5 Land-based feeding trials

While shipboard research is relatively difficult and expensive, some aspects of voyage conditions may be amenable to further investigation using land based studies at a much reduced cost and greater convenience. A number of reports have indicated that heifers have been seen to have developed udders or to be dripping milk prior to or at the time of loading. Zearalenone is a common feed contaminant and would seem to be a likely potential source of oestrogenic activity. As such it is worthy of further investigation. It is likely that other factors are required to occur in combination with exposure to ZEA to induce premature lactation.

Subsequent to the studies described above, and dependent on those results, we recommend that studies be further refined to explore aspects of udder development and milk leakage in heifers held under conditions similar to pre-embarkation preparation. In particular, it would be appropriate to conduct a well-controlled feeding trial that could explore the association between factors such as breed, body condition, stage of

pregnancy and the feeding of ship rations of known dietary composition (including ZEA contamination) with the incidence of udder enlargement and milk leakage. Such a controlled trial would also give the best opportunity to evaluate the potential for interaction between these factors.

9 References

Akers RM. (2006). "Major Advances Associated with Hormone and Growth Factor Regulation of Mammary Growth and Lactation in Dairy Cows." *Journal of Dairy Science* 89(4): 1222-1234

Andrade BR, Salama AAK *et al*. (2008). "Response to Lactation Induction Differs by Season of Year and Breed of Dairy Ewes." *Journal of Dairy Science* 91(6): 2299-2306

Barth AD. (1986) "Induced Parturition in Cattle" In: Morrow D.A. (ed.), Current Therapy in Theriogenology 2, Philadelphia, W.B. Saunders Co., 209-214

Biggs A. (2009) "Mastitis in Cattle" Crowood Press Ltd, UK [ISBN 978 1 84797 071 8]

Blowey R and Edmondson P. (2010) "Mastitis Control in Dairy Herds" CAB International, UK [ISBN 978 085236314 0]

Bloomquist C, Davidson JN and Pearson EG. (1982) "Zearalenone toxicosis in prepubertal dairy heifers" *Journal of the American Veterinary Medical Association* 180(2): 164-165

Bovine Research Australasia. (2003) "Investigating premature lactation in pregnant dairy females" Meat & Livestock Australia [LIVE.217]

Bramley AJ, Dodd FH, Mein GA and Bramley JA. (1992) "Machine milking and lactation" Insight Books, Berkshire England [ISBN 0 9519188 0 X]

Brightling P, Mein G, Hope AF, Malmo J and Ryan DP. (2000) "Countdown Downunder: Technotes for Mastitis Control", Dairy Research and Development Corporation, Melbourne [ISBN 0642 704 813]

CIE (2011) "The contribution of the Australian live export industry" The Centre for International Economics, Canberra.

Collier RJ, Bauman DE and Hays RL. (1975) “Milk production and reproductive performance of cows hormonally induced into lactation.” *Journal of Dairy Science* 58: 1524-1527

Collier RJ, Bauman DE and Hays RL. (1977) "Effect of reserpine on milk production and serum prolactin of cows hormonally induced into lactation" *Journal of Dairy Science* 60: 896-901

Coppock RW, Mostrom MS, Sparling CG, Jacobsen B and Ross SC. (1990) "Apparent zearalenone intoxication in a dairy herd from feeding spoiled acid-treated corn" *Veterinary and Human Toxicology* 32(3): 246-248

Delouis C, Djiane J, Kann G, Terqui M and Head HH. (1978) "Induced lactation in cows and heifers by short-term treatment with steroid hormones" *Annales de Biologie Animale Biochimie Biophysique* 18(3): 721-734

Dahl GE, Buchanan BA *et al*. (2000) "Photoperiodic Effects on Dairy Cattle: A Review." *Journal of Dairy Science* 83(4): 885-893

Erb RE. (1977) "Hormonal Control of Mammogenesis and Onset of Lactation in Cows–A Review" *Journal of Dairy Science* 60(2): 155-169

Frandson RD, Wilke WL and Fails AD (2003) "Anatomy and physiology of farm animals" Lippincott Williams & Wilkins, USA [ISBN 0 7817 3358 8]

Ha T. (2000) "Symposium: Hormonal Regulation of Milk Synthesis." *Journal of Dairy Science* 83: 874-884

Jewell T. (2002) “Artificial induction of lactation in nonbreeder dairy cows.” Master of Science (Dairy Science) - Faculty of the Virginia Polytechnic Institute, Blacksburg

Judd W. (2011) "Australian Dairy Farmers Submission to the Livestock Export Review" [www.livestockexportreview.gov.au/submission_received/australian_dairy_farmers]

Juergens WG, Stockdale FE *et al* (1965) Hormone-dependent differentiation of mammary gland *in vitro*" *Proceedings of the National Academy of Science* 54: 629-634

Kiessling KH, Pettersson H *et al*. (1984) "Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria." *Applied and Environmental Microbiology* 47(5): 1070-1073

Liu TMY and Davis JW (1967) "Induction of lactation by ovariectomy of pregnant rats" *Endocrinology* 80: 1043-1050

Macrina AL, Tozer PR *et al*. (2011) "Induced lactation in pubertal heifers: Efficacy, response to bovine somatotropin, and profitability" *Journal of Dairy Science* 94(3): 1355-1364

Kuhn NJ (1969) "Progesterone withdrawal as the lactogenic trigger in the rat" *Journal of Endocrinology* 44: 39-54

Magliaro AL, Kensinger RS, Ford SA, O'Connor ML, Muller LD and Graboski R (2004) "Induced lactation in nonpregnant cows: profitability and response to bovine somatotropin" *Journal of Dairy Science* 87(10): 3290-3297

Massart F and Saggese G. (2010) "Oestrogenic mycotoxin exposures and precocious pubertal development" *International Journal of Andrology* 33(2): 369-376

McCarthy MR. (2002) "The Best Practice Management of Pregnant Dairy Cattle on Long Haul Voyages" Meat & Livestock Australia [LIVE.208]

McNeil J *et al*. (2011) "Rearing healthy calves: How to raise calves that thrive" Dairy Australia, Melbourne [ISBN 978-0-9871000-2-3]

Mellado ME, Antonio-Chirino E *et al*. (2011) "Effect of lactation number, year, and season of initiation of lactation on milk yield of cows hormonally induced into lactation and treated with recombinant bovine somatotropin." *Journal of Dairy Science* 94(9): 4524-4530

Oxender WD, Hafs HD and Edgerton LA (1972) "Serum growth hormone, LH and prolactin in the pregnant cow" *Journal of Animal Science* 35: 51-55

Parkinson TJ, Vermunt JJ and Malmo J. (2010) "Diseases of cattle in Australasia" VetLearn, Wellington New Zealand [ISBN 978 0 958 3634 4 7]

Peters RR, Chapin LT et al (1978) "Supplemental light stimulates growth and lactation in cattle" *Science* 199: 911-912

Placinta CM, D'Mello JPF *et al*. (1999) "A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins." *Animal Feed Science and Technology* 78(1-2): 21-37

Seeling K, Danicke S, Ueberschar KH, Lebzien P and Flachowsky G. (2005) "On the effects of Fusarium toxin-contaminated wheat and the feed intake level on the metabolism and carryover of zearalenone in dairy cows." *Food Additives and Contaminants* 22(9):847-855

Shin BS, Hong SH *et al*. (2009) "Physiologically Based Pharmacokinetics of Zearalenone." *Journal of Toxicology and Environmental Health Part A* 72(21-22): 1395-1405

Smith BL and Towers NL. (2002) "Mycotoxicoses of grazing animals in New Zealand." *New Zealand Veterinary Journal* 50 (3 supplement): 28-34

Smith KL and Schanbacher FL. (1973) “Hormone induced lactation in the bovine I. Lactational performance following injections of 17β-estradiol and progesterone.” *Journal of Dairy Science* 56: 738-743

Smith VG, Edgerton LA *et al*. (1973) "Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation" *Journal of Animal Science* 36: 391-396

Stahl S, Chun TY *et al*. (1998) "Phytoestrogens Act as Estrogen Agonists in an Estrogen-Responsive Pituitary Cell Line" *Toxicology and Applied Pharmacology* 152(1): 41-48

Sud SC, Tucker HA and Meites J. (1968) "Estrogen-Progesterone Requirements for Udder Development in Ovariectomized Heifers." *Journal of Dairy Science* 51(2): 210-214

Tanaka T, Teshima R, Ikebuchi H, Sawada J and Ichinoe M. (1995) "Sensitive enzyme-linked immunosorbent assay for the mycotoxin zearalenone in barley and Job's-tears" *Journal of Agricultural and Food Chemistry* 43: 946-950

Turkington RW and Hill RL (1969) "Lactose synthetase: progesterone inhibition of the induction of α-lactalbumin" *Science* 163: 1458-1460

Vestweber JG, Cole DE, Quadri SK and Schmidt MK. (1986) "Premature mammary development in a heifer with abdominal mesothelioma" *Journal of the American Veterinary Medical Association* 189: 804-805

Whitacre MD, Van Camp SD, Maclachlan NJ and Umstead JA. (1988) "Premature lactation in a heifer with sex cord-stromal tumor" *Journal of the American Veterinary Medical Association* 193(8): 946-948

Zinedine A, Soriano J et al. (2007) "Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin." *Food and Chemical Toxicology* 45(1): 1-18

10 Appendices

10.1 Survey sent to Australian 'live exporters'

10.2 End of Voyage Report (Premature Lactation) (DRAFT)

Premature Lactation in Exported Dairy Heifers

Concerns have been expressed about the wastage of animals due to premature lactation in some consignments of dairy heifers exported from Australia by ship.

Meat & Livestock Australia has asked the University of Melbourne Veterinary School to collect what information there is on this condition and use it to explore ways to reduce the frequency of this condition in exported dairy cattle.

As someone who is involved in the exporting of dairy cattle by ship, we would be grateful if you could complete the attached information sheet based on your own experience and opinions of this condition in exported dairy cattle.

- Premature lactation is the rapid development of the udder and the commencement of lactation without any link to calving. Not all those animals that undergo udder enlargement subsequently leak milk, but a proportion do proceed to drip milk and may also develop obvious signs of mastitis.

- Premature lactation is sometimes referred to as spontaneous lactation and these two terms refer to the same condition. Premature lactation is not the same as 'udder oedema' or 'prepartum oedema' which is known to occur in normal dairy heifers in the last month of pregnancy.

- Most reports of premature lactation in exported dairy heifers have been anecdotal and there is little reliable information available. Such information could be used to identify the factors that make heifers susceptible to this condition, and may allow the development of strategies to reduce the occurrence and severity of this problem on future shipments.

If you have comments that do not fit into the questions asked, please feel free to add these separately.

All information that you provide to us will be treated in confidence and no identifying information will be reported.

With thanks

Peter Mansell
Associate Professor

email: pmansell@unimelb.edu.au
fax: 03 9731 2366
phone: 03 9731 2222

Premature Lactation in Exported Dairy Heifers

Your Role and Experience

1. Your name (optional):_____
2. How many years have you been involved in the live export industry? _____Years
3. What is your primary role in the live export industry?_____
   _____
4. How many times have you accompanied voyages with shipments of dairy cattle? __
5. How many times have you had personal experience with premature lactation in exported dairy heifers as part of your on board or on shore role? _____

In your experience...

6. Which animals are more likely to show premature lactation during a shipment?

   [Please TICK if you think each factor is important. Provide details if possible.]

   - ☐ Of a particular BREED?_____
   - ☐ In particular BODY CONDITION?_____
   - ☐ PREGNANT or NON-PREGNANT?_____
   - ☐ STAGE OF GESTATION?_____
   - ☐ Detected to have ABORTED?_____
   - ☐ Other animal factors?.....

7. Which voyages are more likely to have animals show premature lactation?

   - ☐ En route to a particular country of destination (please specify)

     .....
   - ☐ Transported via a particular route during the voyage?

     .....
   - ☐ Transported during a particular time of year? (please specify month of loading when risk is highest) .....
   - ☐ Other (please specify)? .....

8. When are exported dairy heifers most likely to show signs of premature lactation?

   ☐ Prior to loading? .....

   ☐ During the voyage (How long after loading? _____)

   ☐ After unloading (How long after unloading? _____)

In your opinion...

9. What factors lead to premature lactation?

   ☐ Duration of voyage? .....

   ☐ Amount of feed provided prior to and during the voyage? .....

   ☐ Consumption of particular feedstuffs, for example...

      Type of Feed?.....

      Origin of Feed?.....

      Extent of spoilage apparent in Feed?.....

   ☐ Location of animals during the voyage, for example...

      Upper/Lower decks?.....

      Brightness/Variability of Lighting?.....

      Ventilation?.....

      Proximity to pregnant cows?.....

   ☐ Other? .....

In your experience...

10. What proportion of heifers that develop premature lactation subsequently develop mastitis?

11. Do heifers that show signs of premature lactation subsequently stop lactating? If so, under what conditions and how long does it take to return to 'normal'?

Please fax or email your responses as soon as possible to:
A'Prof Peter Mansell
University of Melbourne School of Veterinary Science
email: pmansell@unimelb.edu.au
fax: 03 9731 2366

End of Voyage Report on Occurrence of Premature Lactation

Meat & Livestock Australia has asked the University of Melbourne Veterinary School to collect information on the occurrence of premature lactation in consignments of dairy heifers exported from Australia by ship. We would be grateful if you could complete the tables below and return this form to:

Associate Professor Peter Mansell;

Faculty of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee Victoria 3030 Australia

email: pmansell@unimelb.edu.au; fax: 03 9731 2366; phone: 03 9731 2222

Premature lactation is the rapid development of the udder and the commencement of lactation without any link to calving. Not all those animals that undergo udder enlargement subsequently leak milk, but a proportion do proceed to drip milk and may also develop obvious signs of mastitis. Premature lactation is not the same as 'udder oedema' or 'prepartum oedema' which is known to occur in normal dairy heifers in the last month of pregnancy. Most reports of premature lactation in exported dairy heifers have been anecdotal and there is little reliable information available. Such information could be used to identify the factors that make heifers susceptible to this condition, and may allow the development of strategies to reduce the occurrence and severity of this problem on future shipments.

Ship's name	Exporter's name and contact details	Voyage dates	Ports of embarkation and discharge	Vet's /Stockperson's names and contact details

Line/consignee	No. of cattle	Location on ship	Breed	Age	Weight	Body condition	Stage of pregnancy	No. or % bagging up	No. or % lactating	No. with mastitis