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Zearalenone and Premature Lactation in Exported Dairy Cattle

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Abstract

Premature lactation has been a significant problem in dairy heifers exported from Australia by long-haul sea voyage. Exported heifers showing udder development and initiation of lactation *en route* represent a potential wastage of exported animals predominantly due to devaluation or rejection of consigned animals as unfit for purpose by importers in destination countries.

The risk factors that lead to the occurrence of premature lactation are poorly understood. A review of the available literature has been conducted previously, as well as an informal survey of personnel experienced in the long-haul transportation of dairy cattle. This indicated that exposure of exported dairy heifers to mycotoxins such as zearalenone in pelleted ship rations could be a plausible explanation for the occurrence of premature lactation in such animals.

To explore this hypothesis feed rations being loaded onto ships transporting dairy heifers for export were tested for the presence of a panel of mycotoxins, including zearalenone.

No significant contamination by a variety of mycotoxins was found in multiple feed samples collected from a number of sources.

Further work is needed to provide more substantial information about the incidence of premature lactation so that subsequent feed testing, or other investigations, can be more closely focussed on likely risk factors.

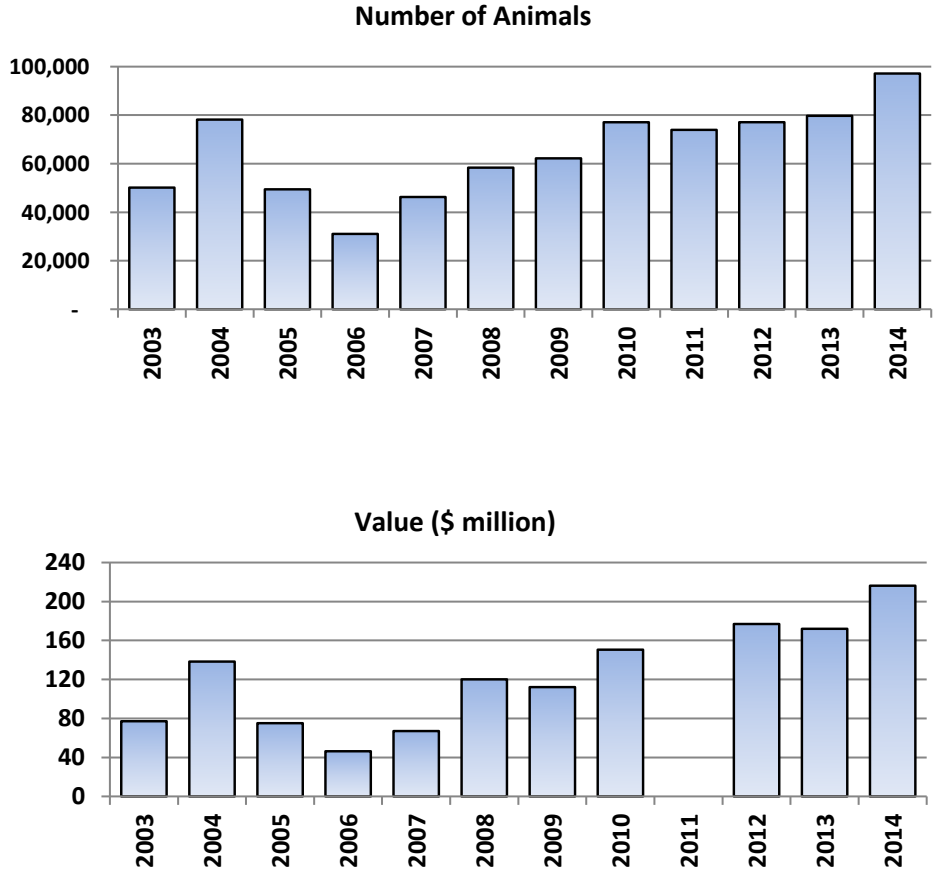
1 Introduction

The contents of this report are closely related to a report which has been previously published ("Premature Lactation in Exported Dairy Cattle"; W.LIV.0280). The earlier report provides a detailed explanation of the justification for the current work and other background detail. Some material from the earlier report is reproduced here for convenience. However the reader is strongly advised to read the two reports in conjunction.

Exported dairy animals represent a significant source of income for the Australian dairy industry. Although it is a small trade compared to the trade in cattle destined for slaughter, the value of the dairy animals is relatively high and the value of the trade as a whole is significant. The economic benefit of the trade in dairy heifers has been well documented (CIE 2011).

Dairy cattle for export have predominantly been sourced 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.

Figure 1. Number and value of dairy heifers exported from Australia 2003 - 2014 *(Source: Livecorp)*



2 Problem of Premature Lactation in Exported Dairy Heifers

2.1 Premature Lactation

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.

2.2 Consequences

The consequences of mammary development and premature lactation in dairy heifers during export can be serious. 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. Whether importers want pregnant or non-pregnant animals, the occurrence of premature lactation may create confusion regarding the animal’s pregnancy status and reduce the perceived value of affected animals. Animals which leak milk may also be at increased risk of mastitis, which detracts from the animal’s welfare and productivity.

2.3 Risk Factors

The initiation of lactation in mammals is a complex and poorly understood process. The cause of premature lactation in dairy cattle during long-haul sea voyages has not been established. A variety of theories have been proposed including:

- Stress associated with transport
- Feeding of high protein feeds
- Effects of day length on physiology
- Contamination of feeds with oestrogens of fungal/plant origin (eg zearalenone)

Figure 2. Factors required for induction of lactation, and potential influential risk factors (*from W.LIV.0280*)

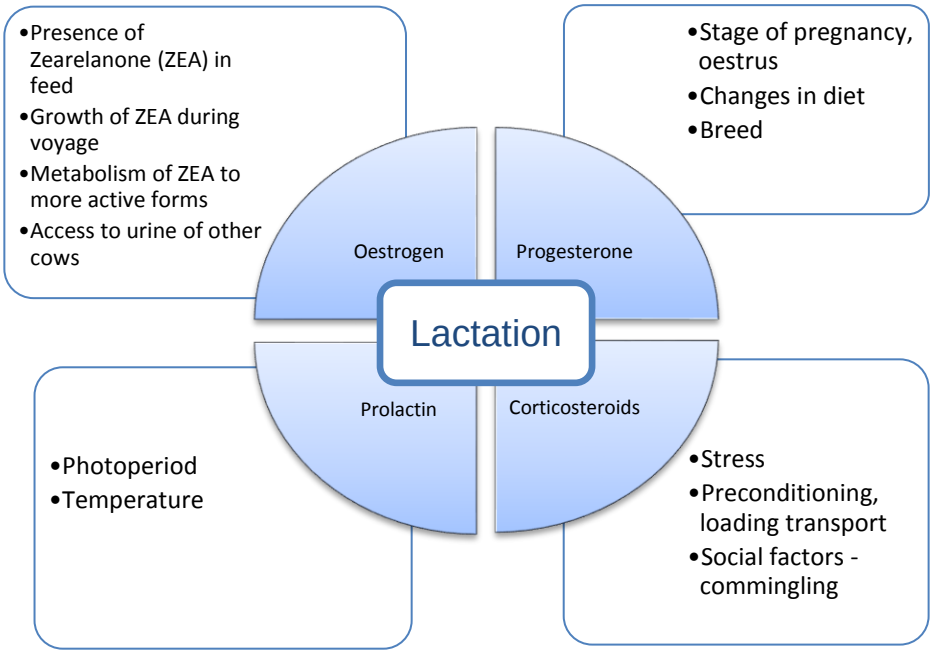


Table 1. Risk factors and potential mechanisms for premature lactation in exported dairy cattle (*from W.LIV.0280*)

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

As a result of the work reported previously (W.LIV.0280) contamination of feed with mycotoxins, especially zearalenone (ZEA), was considered to be a biologically plausible primary hypothesis.

Zearalenone, also known as F2-toxin, is a mycotoxin biosynthesised mainly by fungi belonging to the genus *Fusarium* species. It is a relatively 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 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 was considered as being worthy of more detailed investigation. Investigation of this factor was also seen as being a reasonable first step given that testing of feedstuffs for contamination by mycotoxins is readily available and, if mycotoxins were found to be present, control of exposure to this factor may be possible through management.

3 Methodology

3.1 Feed Sampling

Samples of pelleted ship rations were sampled and submitted for analysis. Pellets were available from feedlot bunkers, bulk trucks and as bags. In expectation that any contamination with mycotoxins is unlikely to be evenly distributed, samples were collected from multiple trucks, and multiple samples were collected from some trucks throughout the transfer process.

Two shipments were involved, both loading dairy heifers at Portland Victoria. These were the 'Dareen' (loading July 2014) and the 'Bison Express' (loading December 2014).

Samples were submitted for testing within 48 hours of collection, during which time they were stored in individual plastic bags out of direct sunlight at ambient temperature and humidity.

3.2 Mycotoxin Testing

Samples were submitted to Agrifood Technology (Werribee, Victoria) for routine mycotoxin screening. The mycotoxins included in the screen were:

- Aflatoxin B1, B2, G1 & G2
- Deoxynivalenol
- Fumonisin B1 & B2
- HT2
- Nivalenol
- Ochratoxin A
- T2
- Zearalenone

Results were reported in parts per billion (ppb). The limits of detection for these mycotoxins ranged from 1 to 250 ppb.

4 Results

4.1 Mycotoxin Screening

Thirty six feed samples were submitted for mycotoxin screening.

Twenty one samples were collected as part of the first loading in July 2014, including 6 samples from a single truck and 6 samples from feed bunkers at the pre-loading feedlot.

Fifteen samples were collected as part of the second loading in December 2014. These samples included 6 samples from a single truck and 3 samples of bagged pellets. A sample of pellets from the ship's feed bunker was also available for testing.

The results of the mycotoxin assays are presented n Tables 4.1 and 4.2.

With the exception of 3 samples which contained detectable amounts of aflatoxin, no samples contained any of the tested mycotoxins above the test's limits of detection.

4.2 Incidence of Premature Lactation

The exporter reported that no premature lactation was observed in the first consignment of heifers loaded in July 2014. Information regarding the second consignment (December) was not available.

Table 4.1 Mycotoxin assay results for feed samples (July 2014)

		Detection Limit	Truck Samples															Feedlot Samples					
		(ppb)	A1	B1	C1	D1	E1	F1	F2	F3	F4	F5	F6	G1	H1	I1	J1	K1	K2	K3	K4	K5	K6
Aflatoxin	**B1**	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	9.6	-	-	-	-	-	-	-
	B2	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G1	<1.0	-	5.3	-	-	4.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deoxynivalenol		<250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fumonisin	**B1**	<200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B2	<200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HT2		<100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nivalenol		<250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ochratoxin A		<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T2		<100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zearalenone		<25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

An empty cell indicates a result below assay detection limit

Table 4.2 Mycotoxin assay results for feed samples (December 2014)

		Detection Limit	Ship Feed Bunker	Bagged Feed Samples			Truck Samples										
		(ppb)	1	1	2	3	A1	B1	C2	D3	E1	E2	E3	E4	E5	E6	F1
Aflatoxin	**B1**	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B2	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G1	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	G2	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deoxynivalenol		<250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fumonisin	**B1**	<200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B2	<200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HT2		<100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nivalenol		<250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ochratoxin A		<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T2		<100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zearalenone		<25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

An empty cell indicates a result below assay detection limit

5 Discussion

5.1 Possible Reasons for Failure to Detect Mycotoxins

There are a number of possible explanations for the failure to detect zearalenone and other mycotoxins in any of the samples tested.

As an initial step the sampling regime was relatively limited in scope and the occurrence of mycotoxins is likely to be unevenly distributed throughout ship rations. While the sampling strategy was designed to increase the chances of detecting mycotoxins, the reality of trying to detect a contaminant in tonnes of pellets by testing small numbers of samples is acknowledged.

The conditions that lead to contamination of grain based pellets can be quite specific. Some mycotoxins are relatively stable in pellets while others can be more labile and may not be present in pellets that have been stored, or may only contaminate pellets which have been stored in particular conditions. Some anecdotal reports suggest that pellets held in ship feed bunkers can become grossly spoiled after exposure to moisture that forms on the walls of the feed bunkers. Such spoilage may indicate the growth of fungi or moulds on the pellets which may, in turn, result in the presence of mycotoxins on the pellets. No spoiled pellets were available for testing in the present study. The pellets from the ship's feed bunker which were available were in good condition. It may be that mycotoxins contamination occurs only after storage of pellets in ship feed bunkers, however there are significant logistical challenges to the testing of feedstuffs on ship.

Although reliable data is lacking, there is little evidence to suggest that the occurrence of premature lactation in exported dairy heifers is highly seasonal. The growth of fungi on cereal crops and the development of spoilage and contamination with mycotoxins in formulated pellets would be expected to be variable throughout the year. To complicate matters, some pellets may be formulated and almost immediately loaded onto ships, whereas other batches of pellets may be stockpiled prior to use. Pellets may also be 'recycled' and carried over between voyages, being exposed to a variety of poor storage conditions within the ship feed bunkers.

It must also be accepted that, however reasonable and biologically plausible as the original hypothesis appeared, it may be that mycotoxin (including ZEA) contamination of feedstuffs is not as common as hypothesised and that it has little or no association with the occurrence of premature lactation in exported dairy heifers.

Despite the limited scope of the sampling regime used in this study, in light of the complete absence of ZEA and almost all the other mycotoxins from all the samples collected it was considered difficult to justify continuation of feed sampling.

6 Recommendations for Further Investigation

6.1 Testing a Primary Hypothesis

This study was intended to test the primary hypothesis that ZEA contamination of feed could be a contributing factor for the development of premature lactation in heifers exported on long-haul voyages. Although a number of alternative hypotheses have been proposed, it was considered that mycotoxins were a reasonable starting point given the ease with which feed can be sampled and tested for the presence of mycotoxins contamination. Unfortunately the results have not supported our initial confidence that a biologically plausible mechanism had been proposed that could explain the occurrence of this condition.

Given the results of the current study we cannot recommend the expenditure of further resources pursuing the ZEA hypothesis until data is available to allow a more focussed investigation of risk factors.

6.2 Further Work Needed

6.2.1 Structured data collection

As indicated in our previous report, there is a significant gap in our knowledge of the epidemiology of premature lactation in exported dairy heifers. Although there are numerous anecdotal reports of premature lactation on multiple consignments, reliable data is lacking. Further work to provide this data will allow the investigation of possible risk factors and their relative importance. This is best done by epidemiological studies involving data collection on-board ship by personnel who are dedicated to the task and not distracted by other duties. Stockmen and on-board veterinarians have multiple responsibilities during a voyage and, despite their best intentions, have not proven to be a good source of data. Well managed observational studies combined with a high level of industry cooperation will be required to explore alternative hypotheses.

6.2.1.1 Broad standardised data collection

The recommendations from the initial report included…

> *"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… 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."*

A suggested data sheet to assist with the collection of relevant data was included. In order to encourage the collection of reliable data, consideration should be given to mandating the completion of such data sheets as part of the routine submission of voyage reports.

6.2.1.2 Targeted Data Collection

It would be preferable for data to be collected by personnel given the specific role of collecting it, as previously recommended…

> *"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."*

6.2.2 Land-based feeding trials

Research is relatively difficult and expensive to conduct on ship. It may be possible to test some alternative hypotheses using land based studies at a much reduced cost and greater convenience. Given the peculiarity of the ship ration fed to heifers, it would be interesting to ascertain whether there is any evidence of premature lactation in equivalent dairy heifers fed this ration for significant periods of time whilst held in similar confinement. Such an approach could allow investigation of a number of risk factors and any interaction between them simultaneously.

7 References

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