final report

FEEDLOTS

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Use of Enzymes for removing feedlot dags from the Live Animal

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Executive summary

A patent search has been conducted and there does not seem to be any Australian patent or published patent application that appears to be relevant to the removal of dags from cattle using enzymes. This allows further development of the process of enzyme removal of dags from cattle in Australia without patent infringement. However, it is possible that the inventors in the United Kingdom who have a UK patent could have either already filed application in Australian or could have filed an International application that designates Australia. To ensure that this project does not infringe an international patent legal advice recommends to repeat the patent search in 6 or 12 months time.

In general, the composition of dags from feedlots in Australia is composed of more lignocellulosic material than the faeces from UK feedlots. This means that the strategies to remove faeces from cattle using enzymes are likely to require a different mix of enzymes to that used in the UK. Laboratory results have demonstrated that decomposition of dags is achieved using concentrations of the enzymes laccase, cellulase and xylanase at around 10 units/mL glucose equivalent. Combinations of the enzymes laccase, xylanase and cellulase together with a dilute salt solution increased the efficiency of dag decomposition. The results demonstrated that of the three enzymes used, cellulase was the most effective enzyme at decomposing feedlot dags.

It was found that after 8-hrs of incubation with an enzyme mixture containing cellulase and laccase, dags became flakey and visible hairs were easily removed. These results indicate that dag decomposition is possible with enzyme solutions containing enzyme mixtures of cellulase and laccase in excess of 1 unit/mL and possible in the order of 10 units/mL.

Recommendation
Phase 1 results support further investigation into the removal of feedlot dags from cattle. The project team recommends that MLA and NRE consider moving to Phase 2 to further develop the process.

Introduction – Scope of the problem

The delivery and processing of dirty animals in abattoirs is an ongoing problem and an important issue for both the meat and leather industries. These concerns relate to the potential for faecal contamination of cattle carcasses at the point of presentation to the slaughter floor and increased processing time for hides, with an increased risk of bacterial damage to the hide. With dirty hides, it is also difficult to accurately determine the weight and therefore the value of the hide.

The Australian feedlot industry incurs significant costs each year in removing dags from live animals, paying processor charges for presenting dirty livestock for slaughter, and receiving lower meat values and poorer meat quality due to stress placed on stock during cleaning. In addition, there is a perceived increased risk due to food safety recalls. Faecal levels on cattle have been reported to be as high as 20 kg/animal with an average of around 3.7 kg/animal (Auer *et al.* 1999), with this being predominantly located around the rump and flanks. Removal of dung, especially hard-dried dung, can be difficult. Water washes, warm showers and rubbing animals with stiff brushes have proven to be ineffective and/or can damage and de-value the hide.

Dags are primarily composed of lignocellulosic materials (lignin, hemicellulose and cellulose), but this will vary with diet. Auer *et al.* (1999) showed that the dag is fixed to the hair fibre and not to the epidermis of the skin, accounting for the difficulty in removing dung, but providing the potential for non-invasive removal of dags. The bond between the dag and the hair fibre is a complex matrix of molecules arising from the dags chemical composition. Auer *et al.* (1999) concluded that some type of chemical solution would assist in breaking this bond. Auer *et al.* (1999) and Covington *et al.* (1999) have demonstrated that dung can be removed from animal hides under laboratory conditions. The work of these researchers has led to the lodging of a patent in the UK for the production and preparation of enzyme mixes for dung removal from animals.

The use of enzymes has the potential to achieve a faster and more complete removal of dags from cattle in Australia. It has been shown that dung can be removed from hides in the early stage of leather processing through the use of cellullase, xylanase and laccase enzyme mixtures. These enzymes attack the fundamental chemical composition of the dung, releasing it from entanglement and encrustment with the hairs on the hide (Covington *et al.* 1999). The chemical process has the potential to be readily adapted for the cleaning of cattle before slaughter to reduce the amount of faecal material entering the abattoir. For this process to be successful it must clean cattle but not lead to:

- residue in meat products;
- harm to the animal being cleaned;
- environmental impact; or
- harm to personnel applying the product.

This project set out to develop and test enzyme solutions comprised of naturally occurring substances that would be capable of breaking down the matrix bond

between the dag and the hair fibre without causing any undesirable outcomes (as specified above). The end result will be a technology that can be applied at the farm, in feedlots and/or immediately prior to slaughter at the abattoir.

Potential industry benefit

The processing of dirty animals represents a significant cost to the feedlot and the broader meat and leather industries. A treatment formulation of enzymes has the potential to provide a more cost effective, simple, natural, labour efficient method of removing dags from the live animal. The technology outlined in this project provides industry with a safe and effective means of reducing the number of unacceptably dirty stock being presented for slaughter.

Objectives

The following objectives were delivered within a short-term (6-month) study for MLA and NRE for delivery by the Rutherglen Research Institute, Department of Natural Resources and Environment. This was termed Phase 1 of the project.

Phase I - "Proof of Concept"

1. To determine the patent position and potential commercial limitations of using enzymes to remove dags in Australia.
2. To review literature on enzymes, their robustness in decomposing dags and their production to identify potential formulations for further consideration.
3. To identify the structure of dags on Australian cattle from different ration regimes that are representative of the Australian feedlot industry feeding practices.
4. To conduct "in vitro" experimentation to identify "best bet" formulations of enzymes for further testing.

Methodology

The methodology for Phase 1 of this project was based on patent review, literature review, *in vitro* laboratory based investigation and application to complete hides. Each of the milestones forms a "go/no-go" decision enabling the project to stop at any point if a major constraint to the technology is identified. This report details the findings from each component of Phase one of this project. Phase two is outlined for future investment by MLA and NRE.

This project has 3 distinct phases with the commencement and funding of each phase being dependent on the successful completion of the previous phase. This methodology was designed to minimise risk. A brief outline of the methodology is given below for Phase 1.

a) Patent review

Legal experts were engaged to undertake a further patent search and to provide advice on what can be done in Australia in accordance with any patent protection.

b) Literature review

A literature review was completed and considered dung composition and variation, attachment of dags to livestock, lignocellulose chemistry, enzymes and their culture, enzyme activity on dags and effectiveness, solution application and alternative approaches to removing dags from livestock.

c) Experiment 1 – Identify the structure of dags from Australian cattle

Dags were collected from animals fed on a range of common rations from two commercial feedlots and the Rutherglen Research Feedlot over nutritional experiments. These dags were analysed according to the method of Auer *et al.* (1999) in which dried dags are extracted with a range of solvents to determine the weight of cellulose, hemicellulose, lignin, ether soluble components, cold and hot water soluble components and protein. This analysis determined the type of enzyme mix likely to be most effective for dag removal.

d) Experiment 2 – Enzyme production

Some enzymes are commercially available from Sigma Chemical Co. eg. cellulase, hemicellulase, and laccase and these were used in Experiment 3. However, it has been shown that specific laccases derived from white rot fungi *Coriolus versicolor* are more effective than those derived from *Rhus vernificera* as with the Sigma product. Extraction of enzymes required the culturing of fungi on selected media followed by broth culture for 7 days. Enzyme extraction and assay of activity on culture broths was an important part of this process. The use of recombinant microbial DNA techniques for the synthesis of bulk amounts of enzyme was investigated at the completion of this experiment.

e) Experiment 3 – Enzyme treatment of dags

Individual enzymes and combinations of enzymes were used to treat pieces of dung from feedlot cattle. Dags were gently agitated in containers at different time intervals with enzyme solutions at room temperature to determine their effectiveness at dag removal. The most effective enzyme mixtures and concentrations were identified from this experiment for live animal applications, formulation development and stability in Phase 2 of the project.

Phase 1 Results

a) Patent review

Legal experts were engaged to undertake a further patent search and to provide advice on what can be done in Australia in accordance with any patent protection. A preliminary search was completed on both the Derwent World Patent Index (WPI) and INPADOC database by Davies Collison Cave (Patent and Trade Mark Attorneys) in May 2001. Limitations of this search include publication delay and the accuracy of the database. At this time the attorneys were not able to locate equivalents of the UK patent 232541 outside the United Kingdom. Discussions with one of the inventors (Christine Stella Evans) listed on patent 232541 revealed the need to undertake another more detailed review of patents relating to solutions, their manufacture and

live animal application. A summary of the patent search is given below and the full document from the attorneys is attached as Appendix A.

Summary of Patent search conducted by:
Mr Mark Roberts Patent Attorney FB Rice & Co

Six searches were constructed based on the information provided:

Search 1:
The intention of this search was to identify patent documents that may have been filed in any country and which could have Australian Equivalents, using the keywords (dung? or faec? or dag#) with (hide? or skin?) with remov? (enzym? or cellulas? or xylanas? or laccas?). A total of 13 documents were identified, but a brief review of the abstracts only identified UK Patent 2325241 as relevant. This document is the one that we were already aware of, from the inventors Anthony Dale Covington and Christine Stella Evens, entitled "Enzymic composition for the removing of dung from hides – containing cellulase, xylanase and laccase to break down the hair on the hide, for easy removal of the dung".

Search 2:
The intention of this search was to identify all recent live patents from the classification codes C14C 001 00 (Chemical treatment of hides, skins or leather eg. Tanning, impregnating, finishing; apparatus therefore; compositions for tanning) and C12S 007 00 (Processes using enzymes or micro-organisms to liberate, separate or purify a pre-existing compound or composition; processes using enzymes or micro-organisms to treat textiles or to clean solid surfaces of materials). A total of 10 documents were identified, but a review of abstracts and claims did not establish any patent as relevant.

The titles of the ten patents found are:
1. Tanning animal skin with wool or hair attached by pickling and tanning the skin whilst holding agent is present.
2. Appts. For treating hide with fluid – includes rollers or clamps for stretching hide during fluid application to open pores.
3. Type II endoglucosidase – used of releasing microorganism from surface to which it is bound at least in part by type II reactive linkage.
4. Prepn. of hides for tanning – using alkaline lipase in liming and/or baiting bath.
5. Use of silica sols for obtaining a hide which is called a stabilized pickled white or stabilized white.
6. Enzymatically-aided liming process.
7. A method for processing of hides or skins into leather, comprising enzymatic treatment of the hide or skin with a trypsin acting microbial protease.
8. Method for preserving animal hides.
9. Method and sytem for dehairing animals.
10. Improved hide treatment.

Search 3:
The purpose of this search was to identify all current patents with the inventor Anthony Dale Covington. Eleven records were identified, one of these was UK Patent 2325241, of which we were already aware. The other patents are entitled "Leather tanning process using aluminium (III) and Titanium (IV) complexes" filed in Italy, France, Canada, USA, South Africa and Germany, and the similar patent titled "Leather tanning process and agents" filed in New Zealand, and its equivalent titled "Tanning Leather using Aluminium and titanium complexes" filed in Australia. In

addition, the patents entitled "High Stability, organic tanning processes" and "Leather Tanning" both of which are filed in Great Britain.

A search of the inventor Christine Stella Evens did not identify any additional patents.

Search 4:
The purpose of this search was to identify equivalent patent or patent applications to UK Patent No. 2325241. No equivalent patents or patent applications were identified in existence outside the United Kingdom.

Search 5 and Search 6:
The purposes of these searches were to identify additional documents with the keywords and criteria already established in searches 1-4, of the databases PCTFULL and WPINDEX. Relevant additional documents were not identified.

Summary:
Using the above search criteria there does not seem to be any Australian patent or published patent application that appears relevant to removal of dung from hides. However, it is a clear possibility that unpublished patent applications could be in existence, especially in the United Kingdom. It is also possible that these inventors could have either already filed application in Australian or could have filed an International application that designates Australia. To determine whether such unpublished application is in existence, it is recommended that the search is repeated in 6 or 12 months time.

A full confidential patent search report is provided as an attachment. Note this document is not to be reproduced as it is subject to copyright protection. Additional copies of the report can be requested from Dr Bill Slattery at the Rutherglen research Institute if required.

Recommendation:
That in Phase two of the project (or in 6 months time) a further search be conducted in order to determine if any currently unpublished patent applications in Australia are pending.

Introduction:
The presence of dags (a combination of manure, soil, straw and hair) on feedlot cattle is a common problem in many parts of the world. It is particularly common during winter when the long coats of the cattle combined with muddy conditions favour the formation of dags (Wescombe 1994). Dirty cattle can carry up to 20 kg of dung on their hides (Dung Survey 1998, cited by Covington *et al.* 1999), but the average is around 3.7 kg of dung per animal (Raw Material Quality – Dung Survey 1996, cited by Covington *et al.* 1999). Dung contamination is a problem for the meat industry as it can result in penalty payments to the slaughter team, the need for extra staff to trim contaminated carcases, loss of meat production, and dirty hides being delivered to local and overseas hide processors (Wecombe 1994). In addition some downgrading of meat due to dark cutting (high pH) and ecchymosis has been attributed to aggressive pre-slaughter washing and handling (Wescombe 1994).

Dag contamination is a problem for the leather industry, as daggy hides require manual removal of dags prior to fleshing to prevent damage to the hide. As hides are offered for tender on a weight basis contamination with dags incurs a significant cost to the processor, in addition export dry-salted hides are discounted for any dirt they carry (Wescombe 1994).

A further concern to the meat industry is the effect of dags on the microbial contamination of carcases. Microbial contamination of raw meat is highly dependent on the conditions under which animals are slaughtered. Microorganisms can easily contaminate the surfaces of carcasses during skinning and evisceration. Roberts (1980) showed that even brief contact with faecal material could result in the contamination with over 10 million bacteria per sq cm. The subsequent shelf life of the contaminated product (Lambert *et al.* 1991) and the potential for human foodborne illness (Bean and Griffin 1990) can be affected by the extent of the initial microbiological contamination of red meat carcasses during the slaughter and dressing processes.

Ridell and Korkeala (1993) reported that the presence of a solid layer of dung on cattle hides prior to slaughter led to significantly increased microbial contamination of the dressed carcasses. This contamination was still evident even if the line speed of the processing was slowed and greater care was performed during the slaughtering procedures. The bacterial counts from carcasses originating from excessively dungy cattle were about 0.7 log units higher than from those containing either little or no dung (Ridell and Korkeala 1993). Ridell and Korkeala (1993) concluded that the higher microbial surface contamination of carcasses caused by the excessive dunginess of hide could not, in normal commercial scale slaughtering, be compensated for by greater care in work practices.

In contrast, Donkersgoéd *et al.* (1997) studied the association between dags attached to hides of beef cattle at slaughter and the subsequent bacterial contamination of carcasses. A total of 624 carcasses from 52 lots of cattle in southern Alberta were studied at a high-line-speed abattoir (HLSP) which processed 285 carcasses per hour and at a slow-line-speed abattoir (SLSP) which processed 135 carcasses per hour. Their study indicated that there was no consistent relationship between dag scores and bacterial contamination of carcasses. Changes in bacterial counts were generally less than 0.5 $\log_{10}/cm^2$ when associated with dag scores, surface wetness of hides, line speed, or removal of dags by shaving. Although there was no strong relationship between dags and subsequent bacterial

contamination of carcasses there was a relationship between dags and visual demerit of the carcasses according to industry specifications.
This lack of association between dags and subsequent bacterial contamination of carcasses has also been observed in Australia. A study which evaluated the effective of pre- and post-slaughter techniques used in Australia for removal of dags from cattle showed that there was no direct correlation between the dag loading of the live animal and the microbiological quality of the carcasse (Rowland *et al.* 1999).

The presence of dags on hides is a concern for processors because it increases the cost of processing by decreasing line speeds by up to 10 to 12% and increasing labour costs of through the employment of additional trimmers on the processing chain (Donkersgoed *et al.* 1997).

In theory the elimination of dirt, faeces and hair prior to removing the hide of a beef carcass should decrease the occurrence of pathogens on carcasses (Sofos 1999). However, the degree of contamination in the resulting meat, will also depend on plant design, manufacturing practices, sanitation and hygienic practices, as well as overall avoidance of environmental cross-contamination (Sofos 1999).

Dung composition:
The major components of the composition of dung are lignocellulosic materials (lignin, hemicellulose and cellulose) (Covington *et al.* 1999). Lignocellulose is generally considered to be the most abundant organic chemical on earth and is distributed widely throughout vascular plants where it forms the structural support system.

There are likely to be variations in the composition of dung due to differences in diet and the data in Table 1 are representative of feedlot conditions in the U.K. (Covington *et al.* 1999). From these data it can be seen that the major components of the dung are cellulose and hemicellulose (combined total of 58% by weight of the dung) and lignin (21%).

There is a paucity of Information on dung composition of cattle from Australian feedlots (S. Lott *pers. comm.*). Examination of the Australian literature showed that dung composition has been analysed regarding organic matter and mineral content but not lignocellulosic breakdown (Blair 2000; Lott 2000).

Table 1 Composition of dung (% based on dry weight basis).

Component	Content
Cellulose	30%
Hemicellulose	28%
Lignin	21%
Protein	6%
Ether soluble	10%
Cold water soluble	6%
Hot water soluble	4%

Source: Covington *et al.* 1999

Attachment of dags to livestock:
Examination of the dung attached to hides shows that the adhesion is between the dung and hair alone, there is no sticking of the dung to the epidermis (Covington *et al.* 1999). The dung, along with straw and soil, form a similar structure to concrete

when attached to the hair on the animal. Indeed, mixing wet cattle dung with hair is still used as a building material in many developing countries in the world (Covington *et al.* 1999). The process of adhesion involves the release of sugars that are leached out of the partly digested grains present in the dung (MLA fact sheet C0-003). These sugars are primarily polysaccharides, a polymeric form of carbohydrate, that forms a binder acting like a coat of paint covering each soil particle and each layer in the construction of the dag (MLA fact sheet C0-003). The dag is formed by the drying out process of each thin layer, this continual adhesion and hardening makes the dag very difficult to remove. The highest level of bacterial carcass contamination is produced by a dag that consists of a mud ball that dries to a powder after being coated with sugars. In this situation, when the dag is broken it explodes into a fine ball of dust (MLA fact sheet C0-003), releasing bacterial spores and cells that can spread over a large surface area.

The fact that the dung is attached to the hair and not the skin is an advantage and provides a means of removing the dags from live animals through the use of enzymes. Enzyme treatments are non-invasive and, when the correct enzymes are selected, they cannot target skin surface components, as they are specific to lignocellulosic components (Covington *et al.* 1999).

Lignocellulose chemistry:

Cellulose, a major polysaccharide of plants, serves a structural rather than a nutritional role. Cellulose is one of the most abundant organic compounds in the biosphere constituting 50% or more of all the carbon in vegetation. It is a linear polymer of β-1,4 linked D-glucose units (Bailey 1973). The β configuration allows cellulose to form very long straight chains. Each glucose residue is related to the next by a rotation of 180°, and the ring oxygen atom of one is hydrogen-bonded to the 3-OH group of the next (Stryer 1995), so that the basic repeating unit is cellobiose. Cellulose chains form numerous intra- and intermolecular hydrogen bonds, which account for the formation of rigid, insoluble microfibrils. The straight chain formed by β linkages is optimal for the construction of fibres having a high tensile strength.

Hemicellulose is the general term applied to a group of chemically heterogeneous carbohydrates that accompany cellulose in the leafy and woody structures of plants and in certain seeds (McDonald *et al.* 1981). They are closely associated with cellulose, cementing the microfibrils together, as well as covalently bound to lignin to give a lignocarbohydrate complex (Covington *et al.* 1999). The name hemicellulose is misleading as it implies that they are a precursor of cellulose which they are not. The hemicelluloses, like cellulose, are polymers of anhydro-sugar units linked by glycosidic bonds. Unlike cellulose molecules, however, hemicellulose molecules are much shorter, and are branched and substituted, and as a result they are usually non-crystalline. Hemicellulose can be classified broadly into two main types: (1) xylans; and (2) the gluco- and galactogluco-mannans. The xylans form the bulk of the hemicellulose fraction in annual or non-woody herbaceous plants (Bailey 1973). The basic structure of xylans is a main chain of β-1,4 linked D-xylose residue carrying short side chains.

The cellulose fibrils of plant cell walls are embedded in an amorphous matrix. The matrix of the young primary wall is composed mainly of cellulose and hemicelluloses, whereas the matrix of the mature secondary wall contains lignin as well as polysaccharide material (McDonald *et al.* 1981). Lignin is the one of the most abundant biopolymers on earth (Oxford Dictionary of Biochemistry and Molecular Biology 2000). Physical incrustation of plant fibres by lignin renders them

inaccessible to enzymes that would normally digest them (McDonald *et al.* 1981). Lignin is closely associated with the cellulose fibres and it is chemically bonded to hemicellulose. Chemically, lignin is an amorphous, three-dimensional aromatic polymer, formed at the sites of lignification in plants by enzyme mediated polymerisation of three substituted cinnamyl alcohols: p-coumaryl, coniferyl and sinapyl alcohols (Covington *et al.* 1999).

Enzymes:

Enzymes are proteins that act as catalysts to affect the velocity of a chemical reaction without appearing in the final products. Enzymes are produced by all living organisms and are responsible for many essential biochemical reactions in microorganisms, plants, animals and human beings. Enzymes are composed of amino acids and have the unique ability to facilitate biochemical reactions without undergoing change themselves.

Enzymes are categorised by the compounds they act upon; for example *proteases* break down proteins, *cellulases* break down cellulose, *lipases* split fats (lipids) into glycerol and fatty acids, and *amylases* break down starch into simple sugars. *Laccase* is a lignin-degrading polyphenol oxidase while *xylanase* breaks down xylan.

The resistance of cellulose to hydrolysis is caused both by the nature of the β-1,4 bonds and the crystalline state, however the β-1,4 linkage can be hydrolysed by cellulases (Covington *et al.* 1999). Cellulase is a multicomponent enzyme system generally considered to be composed of three main components: endoglucanase, exoglucanase and β-glucosidase. Fungi tend to produce extracellular cellulase, and those from *Tricoderma reesei, T. viride, T. koningii,* and *Fusarium solanii* are the best characterised (Covington *et al.* 1999). The endoglucanase attack cellulose in a random fashion, resulting in a rapid fall in the degree of polymerization, to produce oligosaccharide. They have little apparent capacity to hydrolyse crystalline cellulose. The cellobbiohydrolases (exogluconse) are considered to degrade cellulose by removing cellobiose from the non-reducing end of the cellulose chain. β-Glucosidases hydrolyse cellobiose and some soluble cello-oligosaccharides to glucose.

The enzymes that degrade xylans include β-1,4 xylanases and β-xylosidases. In general terms, the xylanases attack internal xylosidic linkages on the backbone and the β-xylosidases release xylosyl residues by endwise attack of xylooliogosaccharides.

The major enzymes found to affect lignin and lignin-model compounds are laccases (polyphenol oxidases), lignin-peroxidases and manganese-dependent peroxidases. White-rot basidiomycetes such as *Coriolus versicolor, Phanerochaete chrysoporium* and *Phebia radiata* have been found to secrete typical lignin degradation enzymes. These enzymes degrade lignin oxidatively, in a non-specific mechanism, by generation of cation radicals in substrate.

Culture of enzymes:

Until recently, availability of enzymes, produced by microorganisms, have been limited to the quantities that could be produced in the host organism in which they were naturally derived (Enzyme Technical Association 2001). However, biotechnological techniques developed since 1973 has allowed the development of safe and efficient microbial hosts in which large quantities of many commercially useful enzymes can be produced. Enzymes that have not been readily available in adequate quantity can now be produced using this technology. Microbial cells, ie. a

bacterium, yeast or mould, are modified through natural selection or through classical breeding and selection techniques to produce desired enzymes. Since 1973 its been possible to transfer genetic material between cells from the same or different species to produce greater and more efficient quantities of enzymes. In addition, it has been easier to produce the desired enzyme with much less unwanted byproduct production (Anon. 2001).

Enzyme activity on dags

Covington *et al.* (1999) reported the results of a laboratory trial in which they used enzymes to remove dags from cattle hide. All the enzymes they used were obtained from Sigma Chemical Co., Poole, UK except for laccase, which was purified from liquid cultures of the white-rot fungus *Coriolus versicolor*. In the trial they treated 5 cm^2 pieces of dung clad hide with different enzymes, at pH 5.0 or 7.0 depending on their pH optima. The samples of hide pieces and enzymes were agitated gently on a rotary shaker at ambient temperature for up to 24 hours. Their results (Table 2) showed that treatment with cellulase and xylanase produced easy dung removal. Laccase showed the next effective enzyme treatment while treatment with protease and α-amylase had little effect. β-D-1,4-mannosidase and β-D-1,4-glucosidase also had little effect. Cellulase and xylanase treatments left the hairs intact and apparently clean and silky (Covington *et al.* 1999).

Table 2 Effects of enzymes on dung removal from pieces of dung-clad hides.

Enzyme	Units of activity of enzyme (U mL^{-1})	pH	Dag Removal[1] score
No enzyme	0	5	0
Cellulase	5	5	***
Xylanase	5	5	***
Laminarinase	5	5	*
β-D-galactosidase	5	5	*
Laccase	0.2	5	**
No enzyme	0	7	0
Protease	5	7	*
α-amylase	5	7	*
β-D-1,4-mannosidase	5	7	*
β-D-1,4-glucosidase	5	7	*

Source: Covington *et al.* 1999

[1]Dung removal was assessed by passing a spatula over the hair surface and giving a score using the following scale: *** easy removal; ** removed with moderate difficulty; * difficult and incomplete removal; and 0 no appreciable removal.

The reaction times for complete dung removal of dags using cellulase was 16 hours when either 2.5 or 5.0 units ml-1 were used whereas dung removal using xylanase took up to 24 h for complete removal (Covington *et al.*1999) (Table 3).

Table 3 Rate of dung removal from dung-clad hide pieces with cellulase and xylanase.

Enzymes	6h	12h	14h	16h	18h	20h	24h
Cellulase, (5 U/ml)	*	*	**	***	***	***	***
Cellulase, (2.5 U/ml)	*	*	**	***	***	***	***
Xylanse, (5 U/ml)	0	*	**	**	**	***	***
Xylanse, (2.5 U/ml)	0	*	*	**	**	**	***

Source: Covington *et al.* 1999

When they combined the two enzymes the time for removal of dung was reduced to 8 hours at ambient temperature (Table 4).

Table 4 Effect of a combination of enzymes on dung removal from pieces of dung-clad hides.

Enzyme(s) and Concentration	Score
0.016 units laccase	**
0.008 units laccase 5 units cellulase 5 units xylanase	****
0.008 units laccase 2.5 units cellulase 2.5 units xylanase	***

Source: Covington *et al.* 1999

The reaction time for cellulase, xylanase and laccase to remove dung from the hide pieces when they were used separately was between 18 and 24 hours. When used together the removal time was reduced to 6 hours and the dung was washed from the hide with no additional mechanical action (Covington *et al.* 1999).

Covington *et al.* (1999) state that the action of the enzymes was that cellulase hydrolyses the cellulose fibres, xylanse hydrolyses the xylan containing hemicelluloses, while breaking the covalent bonds linking lignin and hemicellulose, and laccase attacked the lignin component.

As Covington *et al.* (1999) were initially interested in the use of enzymes to remove dung from hides prior to leather production they needed to determine the activity of

the enzymes when salt was used. Hides are usually salted for preservation before delivery to the tanneries. They found that increasing the NaCl concentrations up to 3.0 M produced a doubling of the measure xylanase activity, while cellulase activity was enhanced by about 20% in the presence of 1.5 – 3.0 M NaCl.

Alternative approaches to removing dags from livestock:
The method most commonly employed to remove dags from cattle is washing. This can be done by either manual hosing and/or by soaking. Cattle subjected to a soaking period are sprayed with water over a longer period, enabling dags to become moistened and easier to remove (Wescombe 1994). Although manual hosing can be an effective method of removing excess dirt/mud and faeces prior to slaughter, it has limited success in cleaning secluded areas of the animal such as the underside, brisket and the inner-flanks. Wescombe (1994) concluded that pre-slaughter washing of cattle is capable of removing some dirt/mud and faeces from animals but that in the majority of cases, dags attached to cattle (particularly the long and fine haired *Bos taurus* breeds) are not successfully removed.

One technique used by an Australian feedlot is the Rockdale Robotic Dag Removal System (RRDRS). This system consists of a specially designed hydraulic crush that has robotically controlled arms with rotating drums on the ends of each arm. These drums are manipulated into position along side the animal while it is restrained in the crush. While the drum is rotating, the dags are pulled into the holes in the drum and are thus parted from the hair. An assessment of the RRDRS (Rowland et al. 1999) showed that it was not completely effective at dag removal, particularly from the legs and flanks of animals. The capital cost of the system is prohibitive for all but the largest of feedlots and the average cost incurred for treating stock is comparable with the knifing fee associated with slaughtering non-treated stock.

Another method of removing dags from cattle is the use of the Parke Rota Shear™. This is an air driven handpiece that utilises the technology of a rotating cutting blade; it can be used either pre-slaughter or post-slaughter. For pre-slaughter the cattle could be shorn on entry to the feedlot and/or just prior to slaughter. For post-slaughter shearing can be done just after the animal has been exsanguinated and elevated to the gambrels. In this instance the cutting lines (belly and inside of the rear legs) are shorn to facilitate cutting of the hide. An assessment (Rowland *et al.* 1999) of its effect on dags on hides showed that it was an effective treatment for the removal of dags and it increased the fleshing scores of hides and nullified the effect of hairballing. The only negative effect was uneven combing, but this would only affect the value if the hides were being tanned with the hair on (Rowland *et al.* 1999).

The Jarvis de-dagging tool (White 1997) is under development for use in abattoirs to remove dags from the carcase after sticking and prior to hide opening. It is designed to remove large contamination particles from along and around the hide opening cut lines from the point of brisket to the crutch. The de-dagging technology comprises a motorised head for breaking up dags and a system for removing the waste. At present this technology is waiting for commercial development for use in the meat industry.

Wescombe (1994) has also suggested that effective cleaning chemical solutions could be developed which would adhere to the hides of cattle for a desired period of time and would effectively reduce or prevent the formation of dags on cattle. Many chemical products have been tested for adherence to cattle hides over a period of several months. Commercial chemical products that have been tested include Hoescht Nuvalb, Wool Grease, Dow Corning Silicon, ICI Polyethylene Glycol (PEG), Mobil Vital Bunca 'A' and 'B', Johnson and Johnson Baby Oil, Johnson and Johnson

Cream, water based paint, Dupont MW133 and All Dull Colour (oil paint with and without kerosene). To date there is no commercial application of this technology.

In the United States they have a patented chemical process for dehairing carcasses to remove dags (Bowling and Clayton 1992). The dehairing process involves sequential spraying/rinsing at different pressures, temperatures and lengths of time with water and solutions of sodium sulfide and hydrogen peroxide. Comparisons of dehaired carcasses and conventional carcasses showed visible reduction of carcass contaminants as shown by a significant reduction in hair and other carcass defects and by lower amounts of trimmings generated from dehaired carcasses (Schnell *et al.* 1995).

Conclusion:

From the literature it can be seen that to date there are no easy methods to de-dag cattle prior to slaughter. Dags can be a significant problem to the meat industry in the wetter periods of the year. Removal of dags prior to slaughter would alleviate the need for disposal of large amount of faecal (dag) material from the slaughter floor. Enzymes appear to offer an exciting alternative to mechanical treatments for removal of dags on cattle.

c) Experiment 1 – Identify the structure of dags from Australian cattle

Dags were collected from animals fed a range of common rations. Feedlot cattle dags were sourced from 3 locations; Rutherglen Research Feedlot, Rockdale Feedlot and Rangers Valley Feedlot.

All dags were analysed for the content of fibrous and fatty materials according to standard methods of extraction and are described briefly, as follows. Each dag sample was tested for its content of cellulose, hemicellulose, lignin, ether soluble components, cold and hot water soluble components and protein. Cellulose was determined by the method of Corbett (1963), hemicellulose by the method of Whistler and Feather (1965), lignin by the method of Adams (1965), ether soluble components, hot and cold water extracts from the Official Methods of Analysis of the Association of Official Analytical Chemists (1984) and protein by the method of Bremner and Mulvaney (1982).

These data were compared with similar analyses (Table 5) performed in the UK by Auer *et al.* (1999). These series of analyses were necessary in order to determine the type of enzyme mix likely to be most effective at dag removal.

In general, the composition of dags from feedlots in Australia are composed of more lignocellulosic or fibrous material than that of the faeces from UK feedlots (Table 5). This means that the strategies to remove faeces from cattle using enzymes is likely to require a different mix of enzymes to that suggested by Auer *et al.* (1999). In addition to these organic materials, the dag samples contained about 30% crushed stone and soil contaminants. These contaminants are typically derived from the feedlot pad surface and need to be taken into consideration when defining dag composition.

Table 5 The composition of feedlot dags from several feedlots in Australia compared with the data of Covington *et al.* (1999) from the UK.

Feedlot	Component (% dry weight basis)							
	Cellulose	Hemi - cellulose	Lignin	Protein	Ether soluble (Fats)	Cold water soluble (Gums)	Hot water soluble (Starch)	Total Ligno-cellulosic material
RRI Aug 2002	35	21	32	14	1	5	11	88
RRI Feb 2002	41	19	23	13	1	6	8	83
Rockdale	40	23	28	17	1	7	10	91
Rangers	32	17	29	15	1	9	10	78
UK (Covington *et al.*)	30	28	21	6	10	6	4	79

The composition of dags from cattle in Australian feedlots is considerably higher in protein and lignin and lower in hemicellulose than the composition of dags from cattle in UK feedlots. This difference is likely to be a reflection of the higher protein diets containing more fibrous material fed to feedlot cattle in Australia compared to those in the UK. In addition, this difference could also be related to a more varied content of bacterial microflora in the rumen of the animal and subsequently the content of enzymes available to digest plant cell walls and contents. The finding that there is considerably less ether soluble material or fats in the dags from Australian feedlots indicates that there is less fat in the diets of these cattle compared with UK feedlots or that microbes in the gut are more efficient in breaking these down.

The differences in dag composition between UK and Australian feedlot cattle are likely to mean that enzyme formulations required to breakdown the dag matrix bound or entrapped by the hair follicles will be different. For this reason we have focussed on the use of lignocellulosic enzymes in this project, as these materials constituted the major portion of the dag, similar to that found by Auer *et al.* (1999).

b) Experiment 2 – Enzyme production

Commercially available enzymes were purchased from the Sigma™ chemical company eg. cellulase, hemicellulase, and laccase for use in Experiment 3. However, it has been shown previously that specific laccases derived from white rot fungi *Coriolus versicolor* are more effective than those derived from *Rhus vernificera* as with the Sigma product. Extraction of enzymes required the culturing of fungi on selected media followed by broth culture for 7 days. The production of cellulosic enzymes by cultural methods provides a relatively cheap means of commercial production of enzyme formulations. Both the commercially available enzymes and the cultured enzymes were effective at dag decomposition, however the

commercially purchased enzymes were more effective than the cultured enzymes as shown in Figure 1.

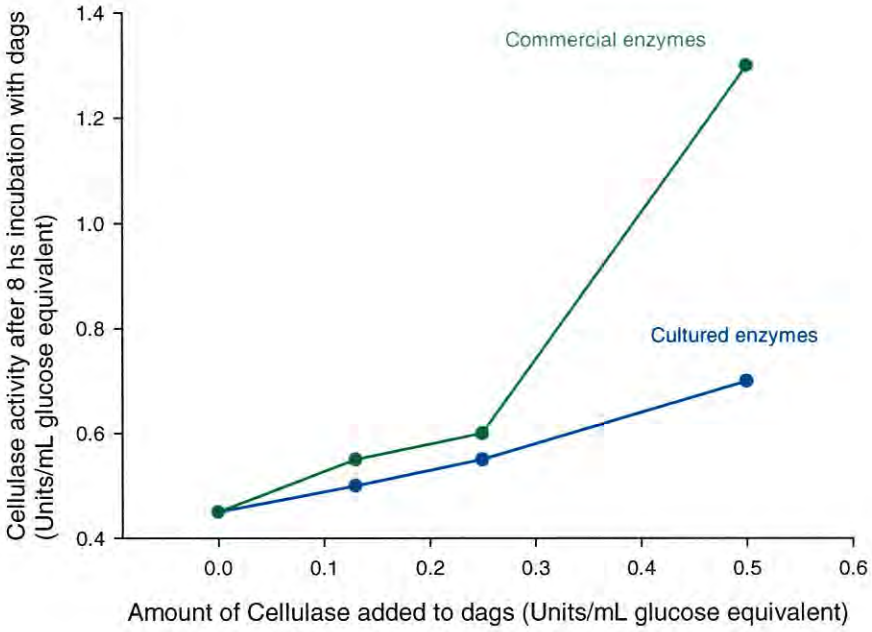

Figure 1 **The amount of cellulase activity after incubation of commercial and cultured cellulase with dags for 8 hrs at room temperature.**

These results demonstrate that cultured enzymes will be effective in the removal of dags from feedlot cattle. However, the activity and amount of enzymes cultured in this first study were not as effective as the commercially purchased enzymes. The commercial enzyme preparations are very costly and it is important to obtain enzymes cheaply if broadscale application of enzyme technology is to be adopted for the feedlot industry.

Further experimental studies are required that involve the production of enzymes from a range of similar microorganisms to that of the white rot fungi *Coriolus versicolor.* In addition, the conditions in which these enzymes are produced, such as appropriate growth medium, most suitable microbial cultures and growing conditions (eg. temp, ionic concentration of media, pH etc) need to be optimised so that maximum production of the cultured enzymes can be obtained. This will be essential in the next phase of the project, where cost to the industry in terms of application of enzyme formulation to animals will be extremely important. It is recommended that further experimentation with fungal cultures continue so that an optimum process may be identified before the next winter period and the formation of dags on feedlot cattle becomes an issue.

c) Experiment 3 – Enzyme treatment of dags

Individual enzymes and combinations of enzymes were used to treat pieces of dung from the hides of feedlot cattle. The dags were sourced from two commercial feedlots (Rockdale feedlot and Rangers Valley feedlot) and from two different nutrition experiments at the Rutherglen Research Institute R&D feedlot.

The treatment of dags after enzyme exposure for up to 18 hours at room temperature was through gentle agitation in a closed vessel. Enzyme activity was determined in each sample after incubation of the dag with specific enzyme mixtures. The enzyme activity was determined with the use of calibration curves and the dag decomposition was estimated by analysing the residue in solution for the presence of soluble high molecular weight compounds with catechol or like structures that absorb at a wavelength of 440 nm. This value was converted into a decomposition score by expressing it as an emission value. With a high score indicating a high degree of decomposition of the dag. This approach assumes that the catechols are a major intermediate in the decomposition of ring structures present in cellulose and lignin structures. Initial studies demonstrated that activities of 10 units /mL glucose equivalent of the enzymes laccase, cellulase and xylanase gave the lowest decomposition of dags (Figure 2).

These results indicate that at this enzyme activity the metabolic intermediate catechol structures had been degraded either by chemical oxidation or polymerisation reactions or microbial decomposition. The small increase at the decomposition score at the higher enzyme activity is likely to be due to the production of catechol from cellulose or lignin structures overtaking catechol decomposition rates. However, these results did not show a dramatic change in the ability to cut through dags after removal from the enzyme solutions (Table 6). These results demonstrated that of the three enzymes used cellulase was the most effective enzyme at decomposing feedlot dags.

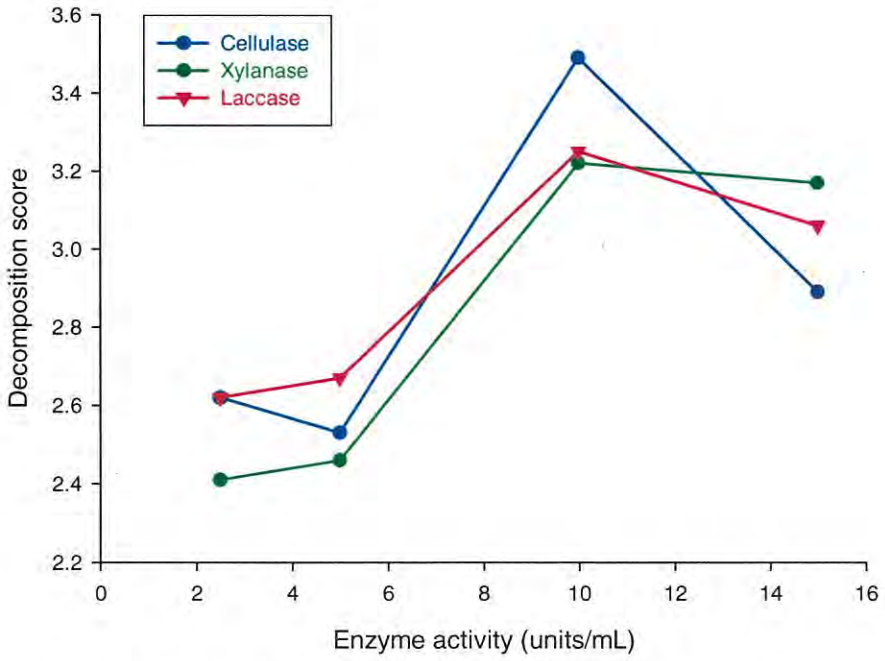

Figure 2 **The effect of enzyme concentration for the enzymes cellulase, laccase and xylanase on the decomposition of feedlot dags.**

Table 6 **Removal score for dags incubated with enzymes for different enzyme activities at room temperature after 21-hrs exposure.**

Enzyme	Enzyme activity (units/mL)	Dag removal score
Cellulase	1.1	**
	0.6	*
	0.3	*
	10.15	*
Laccase	15	*
	10	**
	5	**
	2.5	*
No enzyme control (Cellulase method)	0	**
No enzyme control (Xylanase method)	0	**

[1]Dung removal was assessed by passing a spatula over the hair surface and giving a score using the following scale: *** easy removal; ** removed with moderate difficulty; * difficult and incomplete removal; and 0 no appreciable removal.

It was expected that the dags incubated with enzymes at room temperature would have shown an increased ease of removal compared to the water control for the same time period of incubation. It was also expected that the increased exposure time to the enzyme would increase the dag removal score. However this was not found to be the case. It was also observed that the decomposition score of the incubation solution increased for each of the enzymes as the exposure time increased (Figure 3). This increase in decomposition is not unexpected due to the relatively higher amounts of cellulase and laccase relative to xylanase, where the former two enzymes are responsible for oxygenase reactions producing the intermediate catechol and like structures that absorb at 440 nm. Therefore it is likely that the removal of dags from the incubation solution and subsequent drying prior to cutting with a sharp spatula was an inappropriate method for estimating dag decomposition.

Data shown in Figure 3 indicate that as the incubation time increases the degree of decomposition also increases for all the enzymes. The decomposition score is seen to decrease with increasing enzyme rate for cellulase and xylanase, but was unchanged for laccase. It is clear from these results that at optimum level of enzyme somewhere around the 10 units / mL (glucose equivalent) is near optimal for dag decomposition.

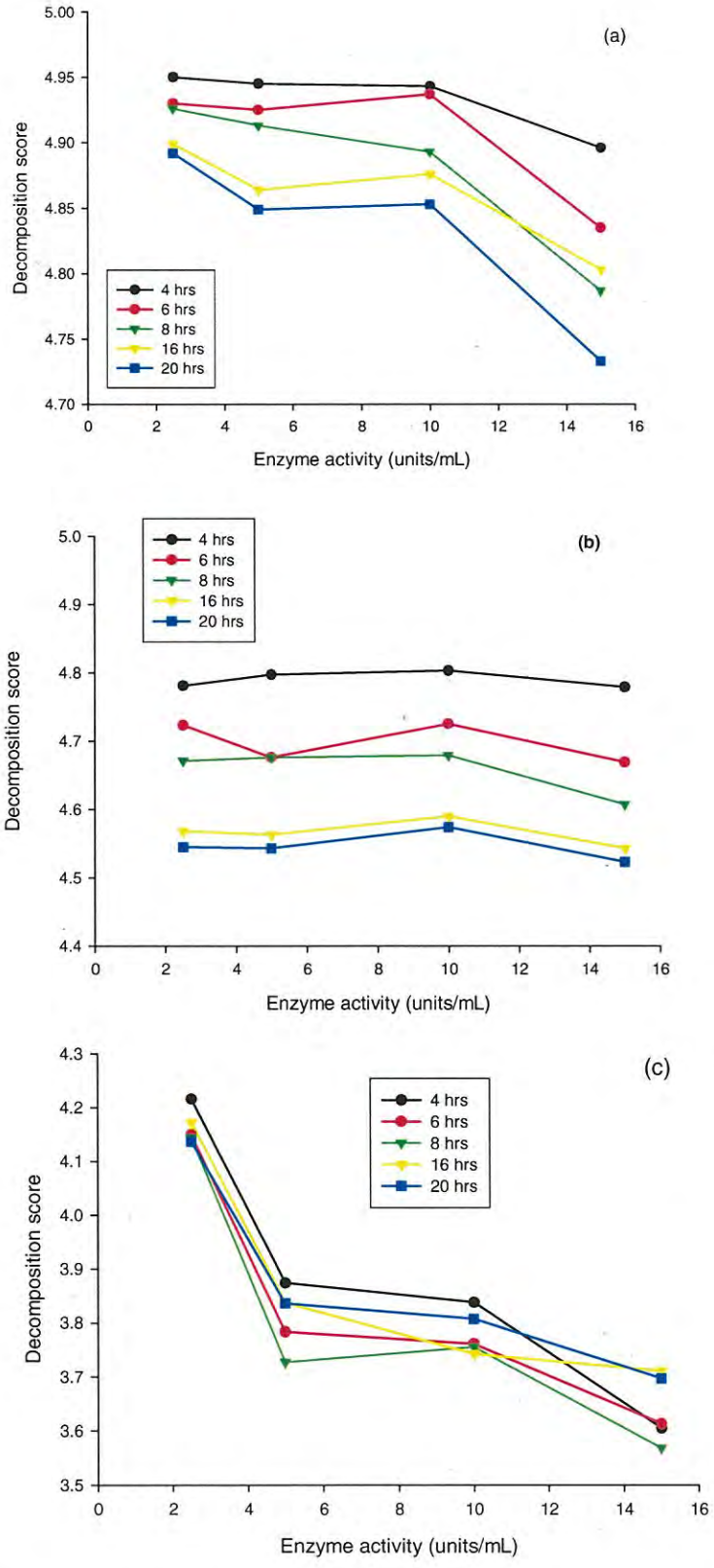

Figure 3 **The impact of incubation time and enzyme at 4 different concentrations on the decomposition of dags when exposed to (a) Cellulase, (b) Laccase and (c) Xylanase.**

Mixed enzyme preparations:

The preparation of solutions containing dags and mixed concentrations of the enzymes cellulase, laccase and hemicellulase were prepared to identify mix of enzymes required for decomposition of dags. All enzyme solutions were incubated with the same quantity of dag for up to 8 hrs at room temperature. All solutions were analysed for enzyme activity at the end of the incubation period to determine the longevity of the enzymatic process. It was found that the recovered enzyme activity for both cellulase and laccase increased with the increased rate of enzyme application (Figure 4). This demonstrates that in the test tube environment the enzymes are very active for periods of up to 8 hrs and are effective at decomposing plant fibres for at least this time period. These results indicate that it is most likely that enzymatic removal of dags from cattle is possible within the time frame of an overnight application of enzyme solution.

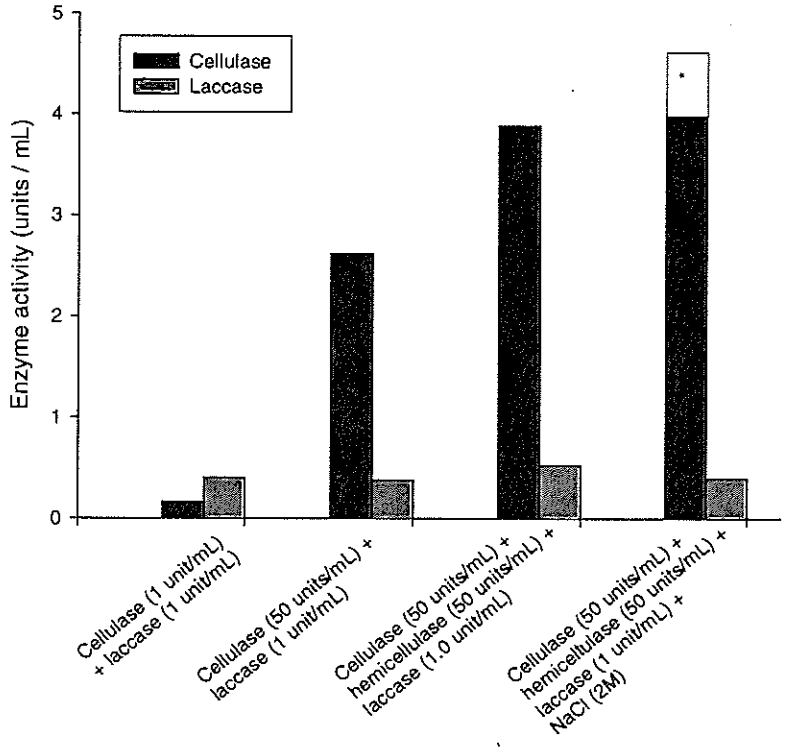

Figure 4 **Enzyme activities after 8-hrs incubation with dags for several enzyme mixtures.**

* = Response for this enzyme mixture will be greater than the shaded area (shown as the unshaded box) due to substrate exhaustion.

An estimation of the degree of decomposition of dags was made by measuring the amount of ring like structures, present in fibrous material, remaining after treatment of the dag with the enzyme mixtures. It was found that both the aromatic and catechol structures in the dag solution decreased with increasing enzyme activity (Figure 5). These results indicate that as the enzyme activity in mixed solutions increases so too does the rate of decomposition of dags.

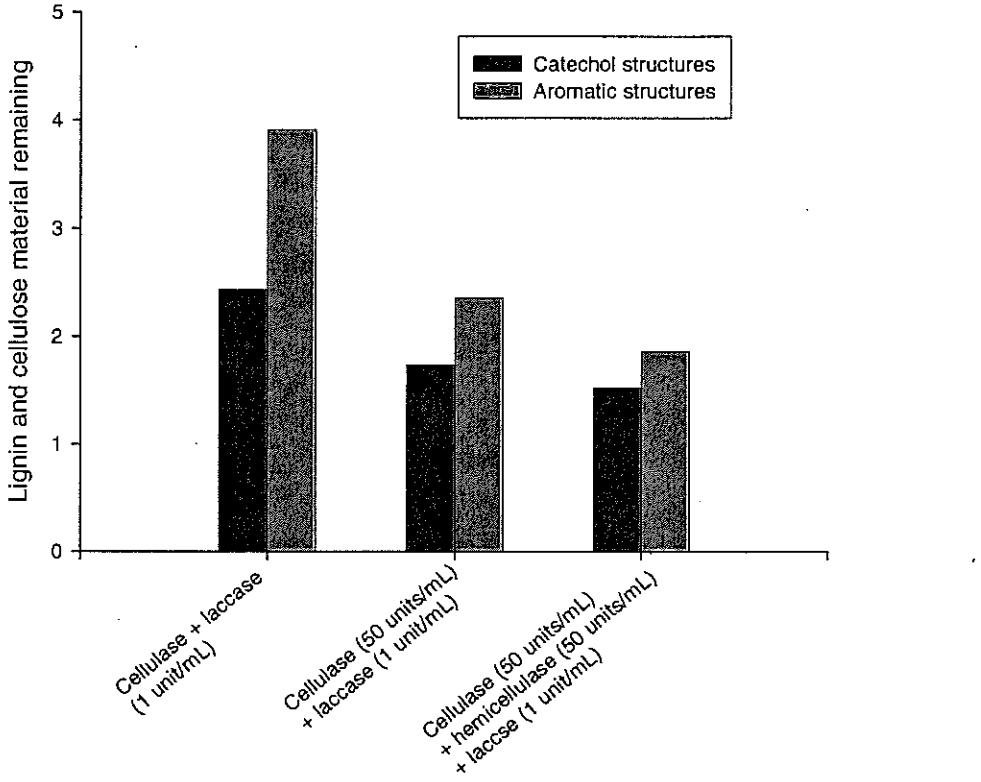

Figure 5 **Soluble lignin and cellulose materials remaining in solution after 6-hrs incubation with several enzyme solutions at room temperature.**

The high activities of enzymes used in this study have shown considerable decomposition of cellulose material and suggest that solutions of lower activity will be effective at dag decomposition. A visual examination of dags removed from the enzyme solutions showed that there was considerable decomposition with increasing enzyme activity and enzyme complexity (Table 7). It was found that after 8-hrs of incubation with an enzyme mixture containing cellulase, laccase, hemicellulase and a salt solution, dags became flakey and visible hairs were easily removed. Even at the lowest activity of laccase, but with a high activity of cellulase dags became flakey and hairs were loosely attached. At the very low activities dags remained hard and blocky and any visible hairs could not be removed. These results indicate that dag decomposition is possible with enzyme solutions containing enzyme mixtures of cellulase and laccase in excess of 1 unit/mL and possible in the order of 10 units/mL (as described earlier in figure 2).

Table 7 Visible scoring of dags treated with enzyme mixtures for a period of 8-hrs incubation at room temperature.

Dag treatment	Dag removal score	Visible appearance of dags
Cellulase (1 unit/mL) + Laccase (1 unit/mL)	*	Dags remain blocky Visible hairs firmly attached
Cellulase (50 units /mL) + Laccase (1 unit/mL)	**	Dags became flakey Visible hairs loosely attached
Cellulase (50 units/mL) + Hemicellulase (50 units/mL) + Laccase (1 unit/mL)	***	Dags became flakey Visible hairs loosely attached some easily removed
Cellulase (50 units/mL) + Hemicellulase (50 units/mL) + Laccase (1 unit/mL) + NaCl (2M)	***	Dags became flakey Visible hairs easily removed

Future recommendations and directions for further funding in the enzyme removal of dags from feedlot cattle project

Phase 2- "Pathway to commercialisation"

The most effective enzyme mixtures and concentrations identified in Phase 1 (solutions containing cellulase and laccase at activities of 10 units/mL) will be used for live animal applications, formulation development and stability in Phase 2 of the project. Promising treatments will then be applied to daggy cattle, water-washed after various time intervals, and measured for effectiveness of dag removal and hide damage.

This phase of the project will require several components as listed below.

1. To optimise the preparation of enzymes and the development of a formulation for effective dag decomposition in both laboratory and field experiments.
2. To evaluate, under field conditions, efficacy, stability and practicality of the enzyme formulation derived from Phase 1.
3. To identify effects of the enzyme formulation on live animals (ie. mucous membranes, etc), humans applying the product, and environmental impact (ie. disposal, reuse).
3. To identify requirements for application of the product to live animals in a commercial context.
4. To investigate the commercial availability and cost structure of enzyme formulation preparation.

Phase 3- "Commercialisation"

A further phase of the project is foreshadowed that will ensure the successful route to market for this new product.

This phase is dependent on the successful outcome of Phase 2 and will include the following elements.

1. To identify practical application and washing technology and involve a commercial partner in the manufacturing and marketing of an application and disposal process.
2. To identify and involve a commercial partner in the manufacturing and marketing of a formulation to remove dags from live cattle.

It is expected that Phase 2 and 3 could be achieved within a 12 month period subject to the availability of feedlot dags and feedlot cattle containing daggy hides.

There are components of Phase 2 that could be achieved without the need for daggy feedlot cattle. Specifically parts 1, 3 and 4.

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milestone report

MLA project code:	**FLOT.214**
MLA project title:	**Enzyme removal of dags from cattle**
Project leader:	**Dr Tony Parker**
MLA project manager/coordinator:	**Des Reinhart**
Milestone number:	**4 - Addendum to contract FLOT.214**

Abstract

This experiment completes the FLOT.214 project by investigating the effects of enzyme solutions on dag/dung removal from cattle hides. The experiment reported was designed on the earlier project reports and draws on conclusions from the final report in its design.

Pieces of dung clad hide were allocated to one of two treatments:

1) an enzyme mixture of Cellulase (10u/mL), Hemicellulase (10u/mL) and Laccase (1u/mL) (n = 6)
2) A control solution of distilled water (n = 6).

The hides were incubated for eight hours in their respective treatment solutions before dags were removed mechanically. Dag removal scores were assessed following the treatment solution and mechanical dag removal. There was a difference in dags removal scores (P = 0.005), with greater removal of dung from the enzyme treated hides than the distilled water treated hides.

The results suggest that the enzyme treatment has potential to remove dags from cattle hides. However, further work is required to ensure that animal and operator safety is addressed.

Project objectives

The objective was to assess the effects of enzyme removal of dags/dung from cattle hides.

Success in achieving milestone

The final experiment has been completed and demonstrated promising results for the use of enzymes in removing dags from cattle hides.

Overall progress of the project

This milestone report brings the FLOT.214 project to a close.

Recommendations

It is clear from the final report and attached addendum that the use of enzymes to remove dags/dung from cattle shows promise. Further work is required to assess the occupational health and safety requirements for people administering the enzyme treatments to cattle. Additionally, the effects of the enzyme treatments on animals and meat safety also needs to be addressed.

Appendices

Experiment 4 – Addendum to contract FLOT.214
Enzymatic removal of dung from beef cattle hides

AJ Parker

Department of Primary Industries,
Primary Industries Research Victoria, Livestock Systems, Rutherglen, Victoria

Summary

This experiment completes the FLOT.214 project by investigating the effects of enzyme solutions on dag/dung removal from cattle hides. The experimental design was based on results from earlier experiments that suggested that enzyme solutions may have an effect in cleaning dags/dung from cattle.

Pieces of dung clad hide were allocated to one of two treatments:

1) an enzyme mixture of Cellulase (10u/mL), Hemicellulase (10u/mL) and Laccase (1u/mL) (n = 6)
2) A control solution of distilled water (n = 6).

The hides were incubated for eight hours in their respective treatment solutions before dags were removed mechanically. Dag removal scores were assessed following the treatment solution and mechanical dag removal. There was a difference in dag removal scores (P = 0.005), with greater removal of dung from the enzyme treated hides than the distilled water treated hides.

The results suggest that the enzyme treatment has potential to remove dags from cattle hides. However, further work is required to ensure that animal and operator safety is addressed.

Introduction

The presence of dags on cattle hides is a major problem to the meat and feedlot industries due to an increase in the cost of processing and concerns regarding the welfare and health of animals. Previous experiments have studied the use of enzyme solutions to break down dags and have demonstrated that decomposition was possible with enzyme solutions containing a mixture of cellulase (10u/mL), Hemicellulase (10u/mL) and Laccase (1u/mL). An incubation period of eight hours was required to achieve removal of hair from the dag structure (Slattery, Pers. Comm).

Materials and Methods

Pieces of dung clad hide, each $10cm^2$, were allocated at random to one of two treatments:

1) an enzyme mixture of Cellulase (10u/mL), Hemicellulase (10u/mL) and Laccase (1u/mL) (n = 6)
2) A control solution of distilled water (n = 6).

The hides were uniformly covered in dags/dung that were dry and hard. The dags were 2 - 3 cm in diameter and 3 – 4 cm in length. Flakes of dung were also dispersed throughout the hide (Figure 1). The enzyme solution recorded a pH of 6.42 and distilled water pH 6.85. Incubation of the hides was carried out at ambient laboratory temperature (24.5°C. Hides were placed into glass jars and filled with 250mL (w/v) of enzyme solution or distilled water dependent upon treatment allocation. Glass jars were capped and agitated every hour for eight hours.

After eight hours in solution, the hides were removed and lightly washed under tap water before assessment of dag removal score. Dag removal score was assessed by passing a spatula over the hair surface and giving a score based upon ease of removal (Table 1). Statistical analysis was performed using a one tailed students t-test.

Table 1. Dag removal score (based upon Covington et al. 1999)

Score	Description
4	Hide is clean of dags and no need to scrape
3	Easily removed
2	Removed with moderate difficulty
1	Difficult and incomplete removal
0	No appreciable removal

Results

The dag removal was greater (P = 0.005) for the hides that had been in the enzyme solution (3.6) than for those in the distilled water (1.5) treatment (Table 2). One hide sample from the water group was given a score of 4 contributing to an elevated value for the water group. However for all other samples in the water group, dags were still visible after the incubation period. There were no dags attached to the hides that had been subjected to the enzyme treatment for eight hours (Figure 1).

Table 2. The effect of an enzymic treatment or distilled water on removing dung from hides (mean ± S.D)

	Treatments	
	Distilled water	Enzyme solution
Dag removal score*	1.5 ± 1.37	3.6 ± 0.52

Enzyme solution = Cellulase (10u/mL), Hemicellulase (10u/mL) and Laccase (1u/mL) * P < 0.01

Figure 1. Effects of 8 hours of incubation in distilled water (left hide) or in an enzyme solution (right hide) on dung removal.

Discussion

This experiment demonstrates the effectiveness of using enzyme solutions in the removal of dung from hides. Previous experiments suggested that eight hours was required to remove dung from hides. However, it is the opinion of this investigator that this could be decreased with a low-pressure application of the enzyme solution onto hides or animals. When assessing the hides for dag removal score it was noted that when scraping the dung balls from the hide of the distilled water group considerable hair was pulled off in the

process. Conversely there was no need to scrape the hides from the enzyme treated group and little hair was lost.

Consideration of the safety of the enzymes used on live animals and humans needs to be addressed. While cellulose and hemicellulose are not a part of mammalian anatomy and therefore the enzymes that attack these compounds may not affect mammals, the material safety data sheets (MSDS) for the enzymes used warn against contact with human skin, hair or mucous membranes and against ingestion of the compounds. The MSDS for the enzymes further state that the effects on animals are unknown with no testing carried out by the manufacturer to ascertain any likely side effects.

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