finalreport

FEEDLOTS

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Assessment of an enzyme mixture for removal of dags from feedlot cattle

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Abstract

The presence of dags on cattle hides is a major problem to the feedlot and meat processing sectors due to animal discomfort during removal, costliness of cleaning and the potential to compromise food safety. Dags are formed from dung, dirt, chaff and urine; an earlier project (FLOT.214) demonstrated efficacious removal of dags from hides soaked in solutions of enzymes. We aimed to demonstrate proof of concept for the use of enzymes contained in a matrix applied to a small number of live feedlot animals using objective measures of dag removal. However, we were unable to demonstrate efficacy of soaking in enzyme solutions towards dry, hard dags even after extended soaking, or trialling a range of enzymes and concentrations. We identified matrices for delivering the enzymes to live cattle which were suitable for food animals, supported enzyme activity and trialled these against dags. The matrix with enzyme did not improve dag removal. We conclude that dag consistency and porosity are crucial factors in determining whether enzymes can improve dag removal, and prior to future commercial release of an enzyme solution for cattle treatment, objective testing should be conducted on a wide range of dag types and compositions.

Executive Summary

The presence of dags on cattle hides is a major problem to the feedlot and meat processing sectors due to concerns regarding the welfare and health of animals, increased costs associated with the cleaning and processing of daggy cattle, and the potential to compromise food safety through carcase contamination.

The composition of cattle dags is complex and variable but primarily composed of chaff, dung, soil and urine. Previous studies by Auer et al. (1999), and Tozan and Covington (2002) have shown that a mixture of enzymes such as cellulase, xylanase or hemicellulase and laccase have aided in the removal of dung from cattle hides prior to tanning. An earlier project supported by MLA 'FLOT.214 – Use of enzymes for removing feedlot dags' (Parker), studied the use of enzyme solutions to breakdown dags and demonstrated that decomposition was possible with similar enzyme solutions. The outcomes of that work were encouraging enough to warrant further investment to investigate aspects of the application of the enzymes to live animals.

We aimed to demonstrate proof of concept for the use of enzymes in a matrix to assist dag removal on a limited number of live feedlot cattle and report on the potential for improvement of the technology and the requirements for product development.

This project assessed in the laboratory suitable mixtures of commercially available enzymes that were likely to have a loosening effect on cattle dags when applied to live animals under conditions faced in feedlots. A delivery matrix in the form of a gel which would hydrate the dags and assist enzyme activity and hold the enzyme close to the dags rather than being lost in the run off, was also trialled. The gel selected for the final trial was selected to be suitable for use on a food animal, minimising irritation or other effects to the animal or feedlot handlers or interfering with enzyme activity. Animal ethics approval was granted for small trial of the enzyme-gel mixture on feedlot cattle at Rangers Valley Cattle Station NSW.

Prior to use of the enzyme mixture on live cattle, laboratory experiments were performed to repeat earlier analyses that had been conducted with daggy hides to obtain a benchmark, to improve on the formulation under laboratory conditions, and to evaluate the performance of the enzymes within a gel matrix. An objective measure of the force of removal of dags from animal hairs was developed and tested against dags soaked in enzyme solution versus buffer solution. Despite trialling a wide range of enzymes, concentrations and non-biological treatments (20 treatments in all), we were not able to show improvement with soaking in enzyme solution.

Variability in the force required to remove the dag from hair was high among the control and enzyme treatments, and this related to the type of dag, that is, whether it was a dag ball formed around a tight clump of hairs or a ball of dung ramified through a mat of hair. Despite the variability in force measurements, the enzyme soaked solutions were not significantly easier to remove. Parker (FLOT.214) found that enzyme treatment causes a virtual falling-apart of dags on hides after soaking. We did not find this in our analysis, after soaking in buffer or enzyme solution, all dag samples remained firm and intact.

We investigated the pH, viscosity and composition of a range of gelling substances, permitted food substances, which could be used as a matrix for enzyme application to cattle. Gelatine was

investigated further as it had the best combination of properties. Despite the addition of enzyme to gelatine causing a loss of viscosity, the gel-enzyme mixture coated the dags and remained for the treatment period. However, the gel-enzyme matrix did not improve the removal of dags from hair.

The average moisture content of the dags was increased by 6.6 and 7.7% respectively by exposing them to constant humidity for 7 and 14 d (final moisture content to around 20%). The increased moisture did not improve the ease of removal of hairs from the dags in either the control or enzyme treated group.

Although there are methodological differences between our study and Parker (FLOT.214) we can not say definitively what the cause of the different results are. It is most likely to be a consequence of different dag composition and properties used in the two studies especially dag hardness and integrity, and the lack porosity/water repellence of our dags. We have shown that enzyme solutions (particularly mixtures of cellulases and ligninases) will not improve removal of hairs from cattle dags of a composition like the ones tested in this project. The nature of the dag could have a large bearing on the success of enzyme treatments. Dry, hard, water repellent dags will be very difficult to treat under any circumstances.

Our other main objective was to report on the potential for improvement of the technology and the requirements for product development. We have shown that it is possible to formulate the enzymes into gels where their activities are maintained and the mixture fully coats the dag and remains hydrated during the exposure period.

Several companies have either developed enzyme formulations for dag removal from hides, or are developing enzyme formulations for live cattle dag application. It is recommended that these formulations be obtained and tested in the laboratory on a wide range of dags from Australian feedlots (as dags will differ in composition between seasons, diets and locations) before being released commercially.

Contents

1 Background

The presence of dags on cattle hides is a major problem to the feedlot and meat processing sectors due to concerns regarding the welfare and health of animals, increased costs associated with the cleaning and processing of daggy cattle, and the potential to compromise food safety through carcase contamination. Currently, dags and their attached hairs are manually raked or mechanically removed from the animals after animals with dags are soaked with water. From the points of view of high water usage, manual handling by workers and the discomfort experienced by the animals the current dag removal practice is suboptimal. The use of enzymes to aid in the removal of dags is an approach that has been considered by the feedlot cattle and leather industry.

The composition of cattle dags is complex and variable but primarily composed of chaff, dung, soil and urine. Analysis of dung by Auer et al. (1999) showed it to be mainly composed of cellulose 30%, hemicellulose 28%, lignin 21%, protein 6% and ether soluble material (fats) 10%. Cellulose, the major polysaccharide of the cell walls of higher plants, is a crystalline linear polymer consisting of glucose subunits. Hemicellulose, associated with cellulose or bound to lignin, is composed of a mixture of 5-carbon and 6-carbon sugars such as xylans, glucans and mannans forming branched chains (Tozan and Covington, 2002). Lignin is an amorphous, 3-dimensional aromatic polymer which forms a protective matrix around the cellulose fibres and is covalently bonded to hemicellulose (Auer et al. 1999).

Previous studies by Auer et al. (1999), Parker (FLOT.214) and Tozan and Covington (2002) have shown that a mixture of enzymes such as cellulase, xylanase or hemicellulase and laccase have aided in the removal of dags from cattle hides prior to tanning. Cellulases and hemicellulases depolymerise the cellulose and hemicellulose, and laccase acts on lignin which are the major biological components of the cattle dags. An earlier project supported by MLA 'FLOT.214 – Use of enzymes for removing feedlot dags', studied the use of enzyme solutions to breakdown dags and demonstrated that decomposition was possible with enzyme solutions containing a mixture of cellulase, hemicellulase and laccase. The outcomes of the work are encouraging enough to warrant further investment to investigate aspects of the application of the enzymes to live animal.

A number of major industries already employ enzymes in their processes such as pharmaceutical/fine chemical transformations, animal feed, starch processing to ethanol, waste treatment, diagnostics, biotechnology, food (flavours, fats, oils, protein hydrolysis, and beverages), textiles and leather processing, paper bleaching and detergent production. The industrial enzyme market is dominated by hydrolases (including cellulases), proteases, and lipases. Many of the industrial enzymes in the food, detergent and pulp and paper industries are commodity products – produced in large amounts and competitive on price. For industrial applications, the expressed enzymes undergo minimal purification and formulation steps. Ideally, the enzyme is spray dried and packaged such that the enzyme is stable at room temperature for an extended period. Thus, the use of enzymes in industrial settings is growing and these processes are cost competitive.

2 Project Objectives

The aims of the project were to:

- Demonstrate proof of concept for the use of enzymes in a matrix to assist dag removal on a limited number of live feedlot cattle
- Report on the potential for improvement of the technology and the requirements for product development.

3 Methodology

3.1 Animal Ethics Approval

Prior laboratory research without use of animals has been conducted in this area. The project 'Use of enzymes for removing feedlot dags', used daggy hide pieces soaked in enzyme solutions to break down dags and demonstrated that decomposition was possible. However, that application method was better suited to the treatment of hides after slaughter of the animal. As it is the pre-slaughter removal of dags that is the pressing industry issue, we need an application method that will work on feedlot animals. Laboratory experiments can usefully show that the treatment may work but ultimately we need to test proof of concept in a pilot study on live animals in the feedlot. Several pilot studies may be required in the future to tailor the technique towards efficient feedlot operations.

We planned a pilot study for winter 2008 to demonstrate proof of concept for the use of a gel/enzyme mixture that can be applied safely to a sufficient number of dags on pre-slaughter feedlot cattle and test their relative ease of removal. We successfully applied to the CSIRO Livestock Industries FD McMaster Laboratory, Chiswick, NSW for approval to use vertebrate animals (2 daggy cattle) in an experiment **(**AEC approval No. 08/18.).

3.2 Enzyme selection and activity measurements

3.2.1 Selection and preparation of enzyme mixtures

Enzymes selected for use in the project closely matched the formulations used in the previous MLA project (FLOT.214) and their properties are given in Table 1.

The main combination of enzymes used was cellulase from *Aspergillus niger,* hemicellulase and laccase and the concentrations replicated those used in FLOT.214: cellulase (10 units ml^{-1}), hemicellulase (10 units ml^{-1}), and laccase (1 unit ml^{-1}). In addition, we trialled a number of enzyme treatments and concentrations for dag removal and other ingredients which included non-biological treatments such as Wool mix (Martha Gardener) and the non-ionic detergent Triton X-100 (t-octylphenoxypolyethanol). The ingredient concentrations are summarised in Table 2. All enzyme mixtures were prepared in 50mM acetate buffer, pH 5 unless stated. Control solutions were conducted using the buffer alone.

Table 1. Enzymes used in the study, source organism, Sigma-Aldrich catalogue number and stated activity.

Enzyme	Source	Catalogue No.	Activity (units mg^{-1})
Cellulase	*Aspergillus niger*	C1184	1-2
Cellulase	*Trichoderma reesei*	C8546	6
Hemicellulase	*Aspergillus niger (crude)*	H2125	1-5
Xylananse	*Thermomyces lanuginosus* expressed in *A. oryzae*	X2753	≥2.5
Laccase	*Tramales veriscolor*	53739	22.4
Beta-glucosidase	Almonds	G4511	25.7
Lipase	*Candida rugosa*	L8525	39,800
Urease	Type IX from Jack Beans	U4002	70.4
Alpha amylase	Type X-A Fungal crude from *A. oryzae*	A0273	40

Table 2. Enzyme mixtures used in treatments and final concentrations.

Mixture concentration	Cellulase (mg ml^{-1})	Hemicellulase (mg ml^{-1})	Laccase (mg ml^{-1})	Additional enzymes/treatments (mg ml^{-1})
	A. niger			
x1	10	10	0.045	
x2	20	20	0.089	
x10	100	100	0.446	
x5 x10	50	100	0.446	
	T. reesei			
x1	1.6	10	0.045	
x2	3.2	20	0.089	
	A. niger			Lipase
x1	10	10	0.045	0.001
				Urease
x1	10	10	0.045	0.142
				Beta-glucosidase
x1	10	10	0.045	0.02
				Xylanase
x1	10		0.045	10
				Lipase (buffer pH 5 and 8)
				1
				Woolmix
				10% v/v
				Alpha amylase
				0.25
				Triton X-100
				0.1% v/v

3.2.2 Enzyme activity measurements

Cellulase, xylanase and hemicellulase activities were determined using the dinitrosalicylic colorimetric method (DNS) to detect reducing sugars (Miller 1959). The substrate used for cellulase was carboxymethylcellulose (CMC) and for hemicellulase, CMC and xylan. In brief, substrate (10 mg) was incubated in 50 mM sodium acetate buffer pH 5 with enzyme solution (5 mg) in a total volume of 4 ml for 1 h at 37°C. After incubation, 1.5 ml enzyme solution was combined with 1.5 ml of DNS and heated at 90°C for 10 min. The reaction was stopped immediately by addition of 0.5 ml of 40% potassium sodium tartrate solution. The absorbance of the solution was determined at 570nm using a Varian Cary 300 UV-Visible Spectrophotometer. The amount of reducing sugar produced was calculated by reference to a calibration curve using glucose as standard (for cellulase activity) or xylose (for hemicellulase activity). One unit of enzyme is defined as the amount of enzyme that will liberate 1 mole of reducing sugar from substrate per minute.

Laccase activity was determined by the method recommended by Sigma-Aldrich (1997). Enzyme solution (0.5 ml) was added to 2.2 ml of 100mM potassium phosphate buffer pH 6.5 at 30°C. The mixture was allowed to equilibrate to 30°C and 0.216 mM syringaldazine solution (0.3 ml) was then added with mixing. A 1 ml sample was taken and placed in a quartz cuvette and the increase in absorption at 530 nm was recorded for 5 min in a Varian Cary 300 Bio UV-Visible Spectrophotometer. 1 unit of enzyme activity produces a rate of change in absorbance of 0.001 per minute.

3.3 Objective assessment of dag removal from hairs

Dags were obtained through Armidale NSW DPI from feedlot operator Rangers Valley Cattle Station. Their appearance was of smooth balls of composite material, non-porous, with hairs fully coated in the dag and tuffs appearing at one end where they had been cut from the animals. Unlike a ball of dried dung, the dags were very hard, like concrete balls, and did not fracture or crumble. The dags were roughly bulb-shaped, their diameters varied widely and they ranged in weight from 3.5 to 35.0 g and were stored at 4°C until required. The moisture content was determined by drying pre-weighed dags at 100°C for 48 h and then re-weighing and calculating the loss due to water content.

Dags were weighed and each placed in a 100 ml glass beaker. Glass beakers were used to reduce loss of enzyme activity through absorption onto container surfaces. Enzyme solution (50 ml, Section 3.2.1) was placed in the beakers and the dags allowed to soak for 6 hours. All experiments were conducted under laboratory conditions of 22-23°C. The initial two experiments used only 25 ml of enzyme solution, and first experiment had soaking undertaken for 18 h. The dags were then washed twice in water before assessment.

Measurement of force required to remove the hair from the dags was determined using an Extech Model 475044 digital force gauge. The hair on the dag was placed in a clamp which was attached to a retort stand. The teeth of a large toothed, U shaped plastic comb were inserted between the dag and the clamp. The force meter, with a 120 mm extension rod and hook, was connected to a metal loop on the back of the comb (Figure 1). The comb was then pulled using the force meter and the force required to remove the hair from the dag recorded.

Figure 1. Dag removal equipment consisting of gripping comb, securing clamp and digital force meter with extension rod attached.

3.4 Gelling agents for application to dags

The gels selected were permitted food additives according to Food Standards Australia and New Zealand and were obtained commercially. Gelatine (McKenzie's), guar (Sigma-Aldrich Cat. No. G4129), gum arabic (Sigma-Aldrich Cat. No. G9752) and pectin (Sigma-Aldrich Cat. No. P8471, from apples). Guar dissolved in cold water, gum arabic required warm water and pectin and gelatine hot water to dissolve. The pH of the gels was measured using a pH meter (Jenco Model 6171). The viscosity of the gels at various concentrations was determined using a Rapid Viscosity Analyser (RVA) (Newport Scientific Model Super 3). Viscosity was measured using a 30 g gel sample at 25°C and 160 RPM. Viscosity is given in Rapid Visco Units (RVU) each of which equals 12 centipoise. The effect of cellulase, hemicellulase and laccase on the viscosity of gelatine was also determined. Enzymes were added to gelatine in similar ratio to that expected to be needed for dag removal (x2 Table 2) that is, 1 g each of cellulase and hemicellulase per 50 ml gelatine (prepared at 2.5% w/v) and 4.5 mg laccase per 50 ml gelatine solution.

Dag removal using the enzyme gelatine solution was determined by placing the dags in a clamp attached to a retort stand. The dag was coated with a 2.5% w/v gelatine solution containing x2 concentration of cellulase, hemicellulase and laccase and allowed to stand for 6 h. The dags were then washed twice with water. The force required to remove the dag from the hair was determined by the method described in Section 3.3.

To determine whether the gels affected enzyme activity, enzymes were mixed into gels and their activity measured as described in Section 3.2.2 except that conversion of CMC to reducing sugars was determined using a colour meter rather than UV-Visible spectroscopy. Colour change in the

gelatine solutions was determined using a Minolta Croma Meter with a CR-310 (50 mm diameter) measuring head. Readings were taken using the Commission Internationale de l'Eclairage (CIE) L*a*b* system. L* measuring from black (0) to white (100), a* from green (-60) to red (+60) and b* from blue (60) to yellow (+60). Total change in colour is given by $\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ where ΔL*, Δa* and Δb* is the difference between the blank sample and the enzyme or calibration sample (Hunter and Harold, 1987). Colour of the solution was determined by placing the solution in a small petri dish which was then place on a white tile. Readings were then taken with the colour meter.

3.5 Increasing moisture content of dags

Next we tested whether there was any effect on removal of dags with increasing moisture content of the dag. A subsample of pre-weighed dags were equilibrated with glycerol/water solution in a sealed container which achieved an atmosphere of 70% relative humidity at 4°C and were held for 7 or 14 d. The samples were removed, weighed, divided into a control soaking and enzyme soaking groups and subjected to dag removal analysis as outlined in Section 3.3. One sample from each batch was left aside for measurement of total moisture content.

4 Results and Discussion

4.1 Effect of enzymes on dag removal

Weights of dags selected for each trial were matched between the control (buffer solution) and enzyme treatments in case the force required to remove dags was proportional to weight.

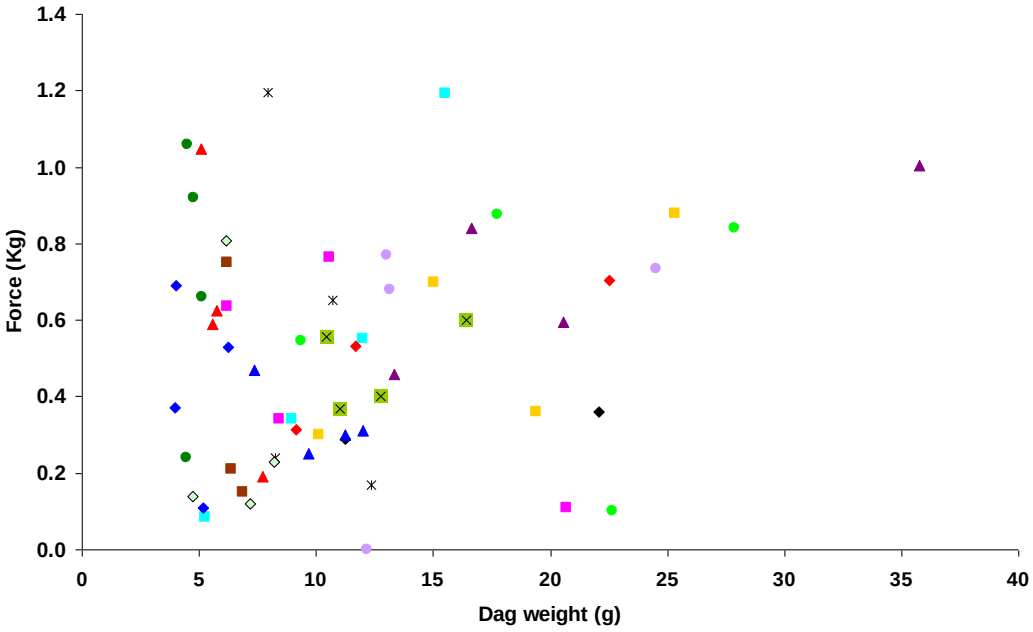

Figure 2. Force required to remove dags from cattle hair in control samples as a function of dag weight.

However, as is demonstrated in Figure 2, the force required to remove dags was not related to weight of dags (y=0.0359, r^2=-0.6486) and there is a large scatter in the measurements.

The amount of force required to remove the cattle hair from dags soaked in different concentrations of cellulase, hemicellulase and laccase compared with buffer controls is given in Figure 3. The results for the control and the treatments were variable as shown by the error bars per experiment. In general, enzyme treatments required similar force to controls to remove dags from hair although some experiments showed a trend to lower force required for enzyme treatments (e.g. 6 h 50 ml x1 and x2 experiments). High enzyme concentrations, x10, did not increase the action of the enzyme and resulting dag removal. In addition, there were examples among control and enzyme treatments where complete removal of the hair from the dags was not achieved. In these cases a force of over 1.4 kg resulted in the dags being pulled from the clamp.

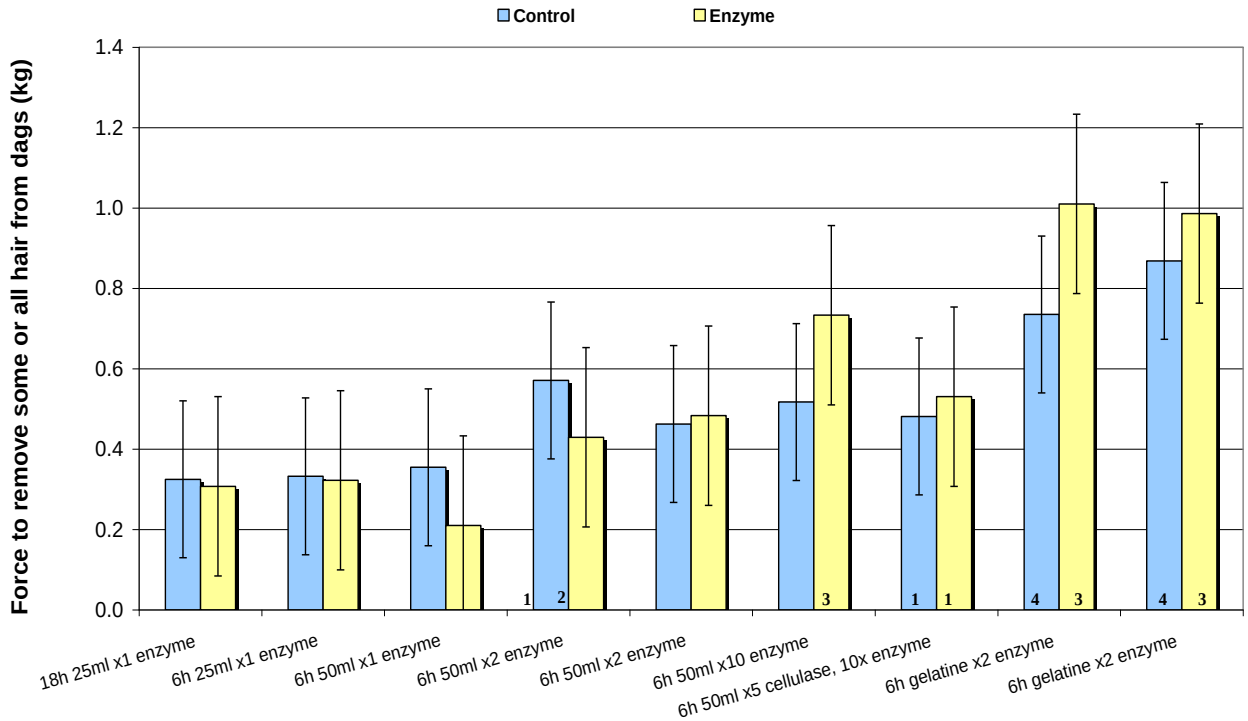

Figure 3. Force required to remove dags from cattle hair after soaking in buffer control (blue bars) or enzyme mixture (yellow bars) using cellulase, hemicellulase and laccase mixtures, with and without gelatine. Four dags were analysed per treatment or control and error bars represent standard deviations. The numbers in the columns represent dags where conplete hair removal was not achieved.

The effect of other enzyme and non-biological treatments on dag removal in comparison to control samples is given in Figure 4. Again the variability within experiments is high and the enzyme treatment would need to be very obviously effective for a clear difference to be measured. The lowest force for dag removal was obtained with the use of lipase in combination with cellulase and hemicellulase, and alpha amylase (Figure 4).

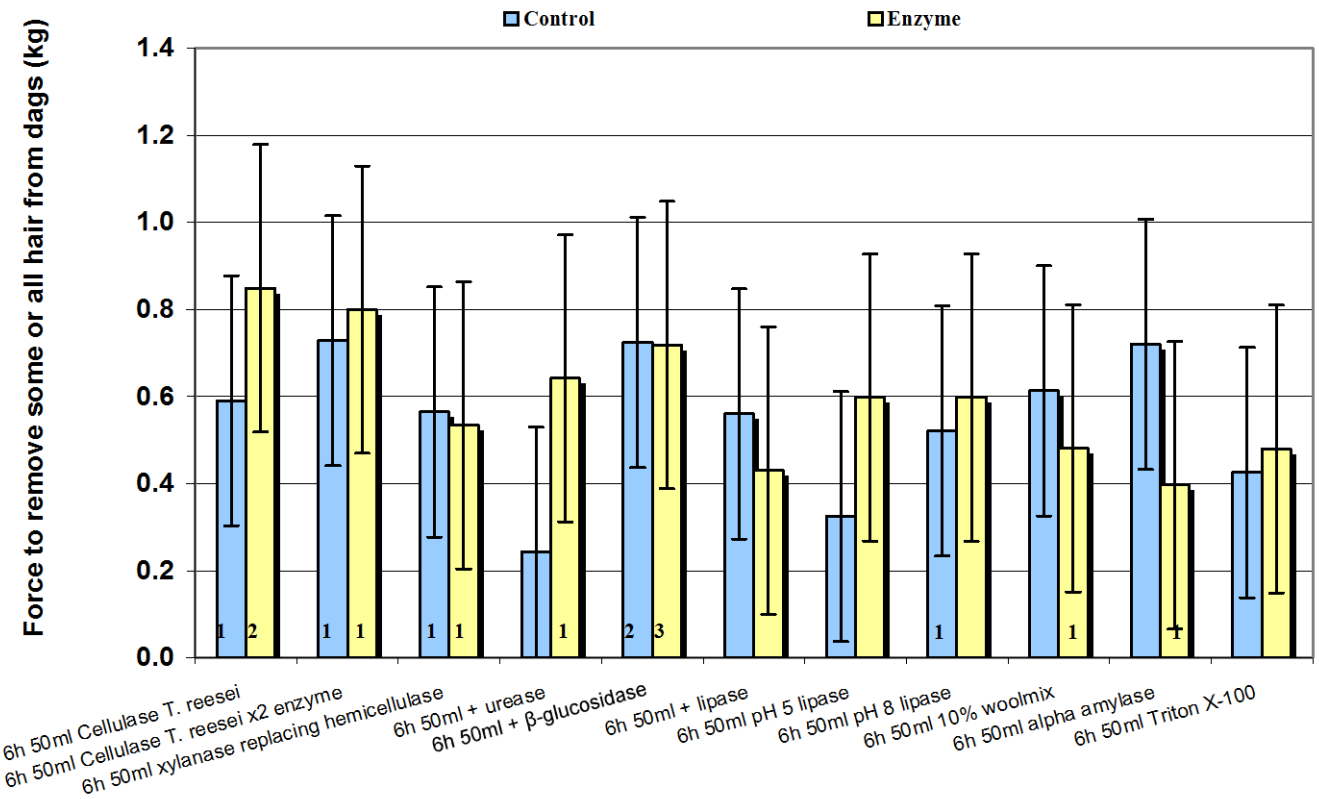

Figure 4. Removal of dags from cattle hair using a range of enzyme mixtures (yellow bars) compared with control dag removal (blue bars). Four dags were analysed per treatment and control and error bars represent standard deviations. Numbers in the columns represent dags where complete hair removal was not achieved.

The results obtained for removal of hair from the control dags (Figure 2) show how variable the results were between the dags used for the different experiments. The dags consisted of two main types: rounder, tightly formed dags that had formed around one clump of hair, and dags of a more irregular shape, attached in a more mattered fashion to a wider area of hair. Examples of these types are shown in Figure 5.

Removal of the dags from hair resulted in two type of response: complete hair removal (Figure 6) or partial hair removal (Figure 7). Where the result was partial hair removal, this is indicated by numbers within the relevant bars of each treatment group of Figures 3 & 4. When hair was not fully removed from the dags in our laboratory experiments, on animals this may mean that the dag would have other anchor points to remain attached.

Figure 5. Examples of different dag types examined. Dags in the top left and bottom right hand corners demonstrate the smooth rounded type formed around one clump of hair. Dags in the top right and bottom left hand corners demonstrate the irregular shaped dags formed around a mat of hair.

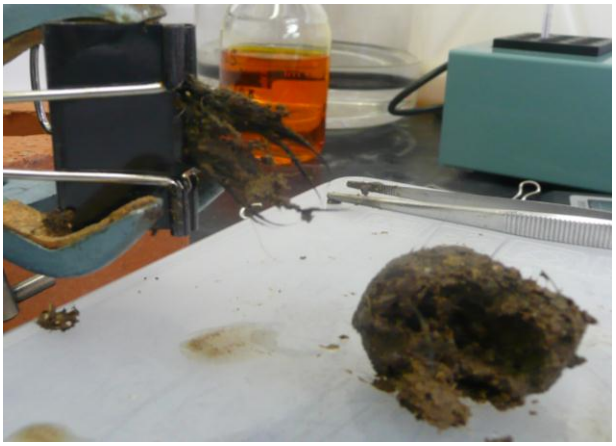

Figure 6. Full hair removal from a soaked dag.

Figure 7. Partial hair removal from a soaked dag.

Prior research conducted by Auer et al. (1999), Parker (FLOT.214) and Tozan and Covington (2002) found that enzyme treatment causes a virtual falling-apart of dung balls or dags on hides after soaking. We did not find this in our analysis, after soaking all dag samples remained intact as shown for a representative group in Figure 8.

Figure 8. Dags after soaking for 6 hours in solution. Beakers 1-4 (from left) enzyme solution and 5-8 control buffer solution.

4.2 Gel preparations and effect on dag removal

The consistency of the gels at different concentrations and the resulting viscosity and pH are given in Table 3. Gelatine was found to be the most suitable, forming a soft gel at concentration of 2.5% with a pH in a range that should work optimally with cellulase enzymes. Guar also exhibited similar properties to that of gelatine but was found to lose its gel consistency after a few days. Gum arabic required too high concentration to obtain any increase in viscosity and was not investigated further. Pectin formed a suitable gel but the pH of around 3.6 was quite acidic and possibly not suitable for enzyme performance.

The addition of enzymes to gelatine was found to cause a drop in viscosity as shown in Figure 9. Hemicellulase had the most dramatic effect reducing viscosity to zero. The enzymes caused the gelatine consistency to become uneven, lumpy and watery. This result was not expected as gelatine is composed of animal collagen (protein) which should not be affected by the activities of these enzymes.

The enzyme activities for cellulase and laccase in a gelatine formulation were compared with enzyme in buffer only. The effect of gelatine on enzyme activity was assessed with colour meter and a standard curve was generated in gelatine using glucose. Gelatine was found to have no effect on the activity of the cellulase or laccase.

The application of the enzyme to dags in a gelatine formulation gave the desired coverage and held the enzyme close to the dag (Figure 10) but was found not to improve hair removal. The force to remove some hair increased compared with the soaked dags, with complete hair removal not being obtained on nearly all of the dags tested in both the control and the enzyme treated samples, Figure 3. Penetration of the solution down the hair shaft and chaff pieces may have been much lower than that obtained when the dags were soaked in buffer or enzyme solution. Even though the gelatine became lumpy on addition of the enzymes it still provided a suitable covering of the dag.

Table 3. Properties of various gels evaluated for enzyme formulations.

Gel	Concentration (%, w/v)	Final viscosity	Consistency	pH
Gelatine	1.0	0	Fluid	6.80
	2.5	25.3	Soft set	6.26
	5.0	291.1	Forms lumps	5.33
	7.5	442.7	Forms stiff lumps	5.39
	10.0	Off scale	Forms hard stiff lumps	5.44
Guar	1.0	3.3	Fluid	5.92
	2.5	312.4	Set	5.94
	5.0	504.1	Stiff set	5.96
	7.5	Off scale	Very stiff	5.96
	10.0	Off scale	Very stiff	5.96
Gum arabic	1.0	0	Fluid	6.29
	2.5	0.1	Fluid	5.67
	5.0	0.2	Fluid	4.78
	7.5	0.2	Fluid	5.29
	10.0	0.2	Fluid	5.40
	50.0	27.0	Fluid	4.29
Pectin	1.0	0	Fluid	3.48
	2.5	52.1	Soft set	3.40
	5.0	367.8	Thicker set	3.64
	7.5	837.1	Sticky thick set	3.79
	10.0	Off scale	Very stiff forming lumps	3.67

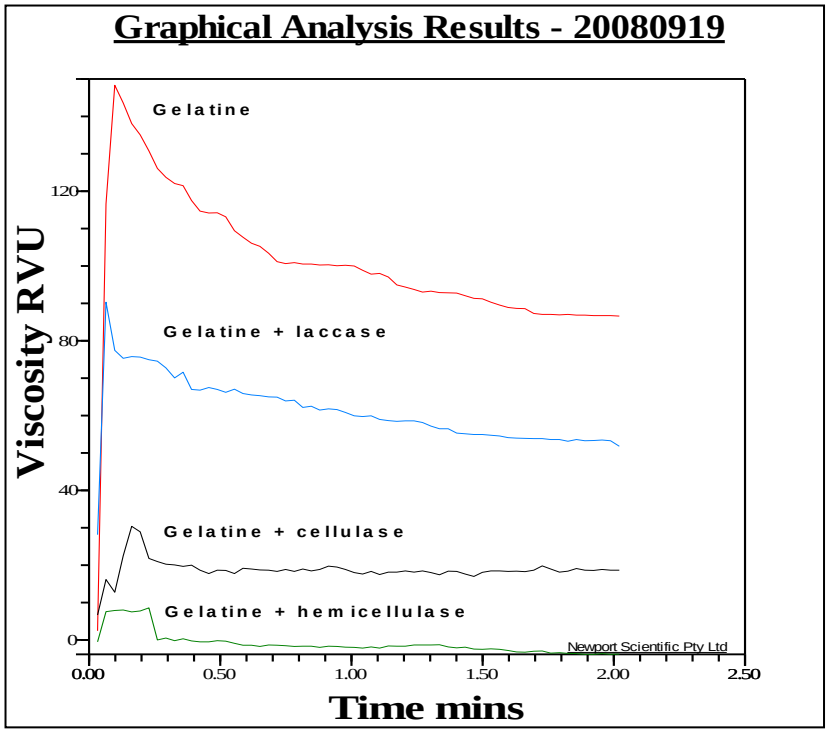

Table 4. The effect of cellulase, hemicellulase and laccase on gelatine viscosity.

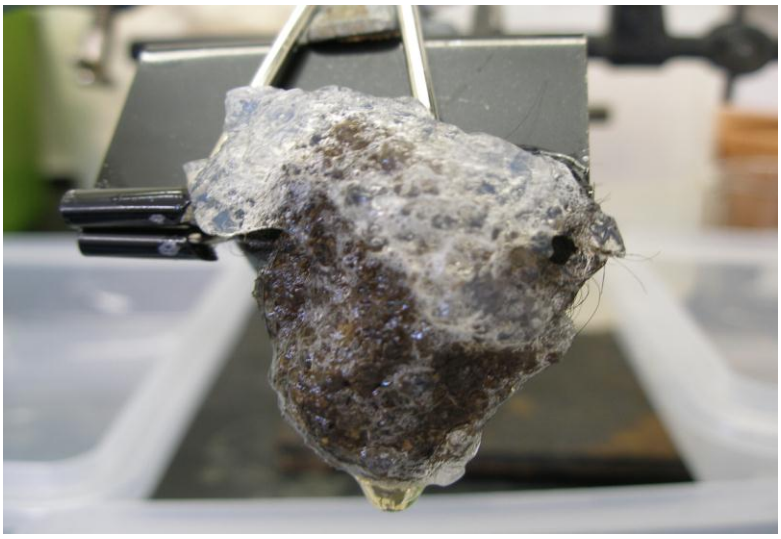

Figure 10. Complete coverage of the dag with enzyme mixture in gelatine formation.

4.3 Effect of moisture content of dags on enzyme-assisted removal

Dags were equilibrated at 70% RH for 7 or 14 days which raised their average moisture content by 6.6 and 7.7% respectively. As the dags were around 14% moisture content prior to humidifying, this brought the final moisture content to around 20%. The increased moisture did not improve the ease of removal of hairs from the dags in either the control or enzyme treated group as shown in Figure 11. The average force required to pull the dag from the hairs were higher overall in this experiment than previously which may be a function of the use of smaller dags in this experiment.

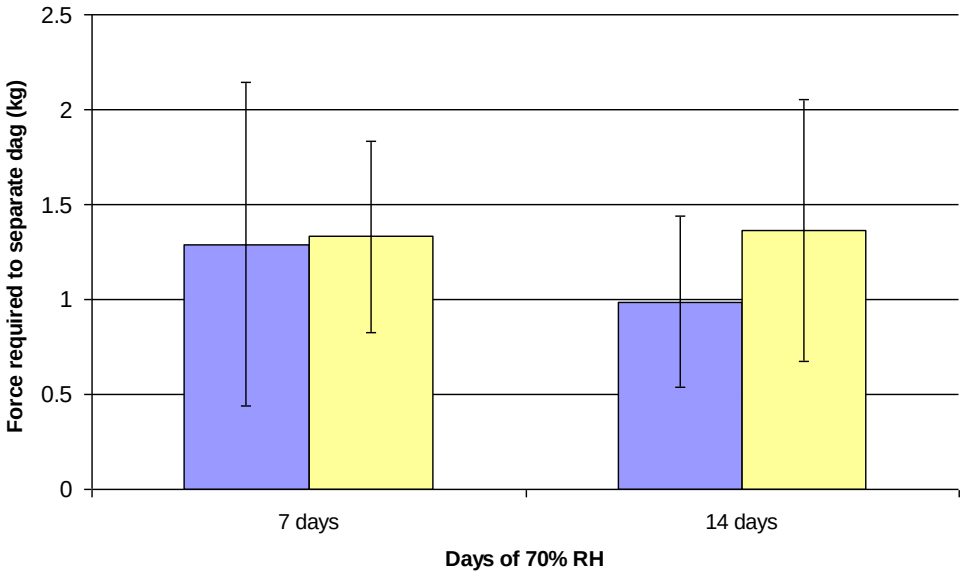

Figure 11. Force required to remove hair from dags with increased moisture content and soaked in buffer solution (blue bars) or enzyme solution x2 (see Table 2) for 6 h.

4.4 Comparison of enzyme-dag removal studies

Previous studies by Covington and Evans (1998), Auer et al (1999) have shown that solutions containing mixtures of cellulase with other enzymes aided in the removal of dung from hide pieces and Parker (FLOT.214) came to the same conclusion with daggy hides. There are several key differences between these studies and ours:

- Prior studies used hide pieces with dags attached and they used subjective methods of determining ease of dag removal by scoring the resistance of the dag to being pushed by a spatula. By contrast, we used dags with hairs attached that had been cut from cattle (and kept at 4°C) and we developed an objective method for measuring the force required to remove the dag from hair and noted full or partial hair removal.
- Additionally, the earlier studies soaked the dags for slightly longer periods e.g. 8 h although they noted this length of time was probably not necessary. Our most common soaking period was 6 h as we aimed to develop a method that could be applied to animals and removed as quickly as possible. Our initial studies conducted with 18 h soaking did not yield better results (Figure 3).
- In our study we have dag weights and moisture contents but these are unknown in the earlier studies.

Although there are methodological differences between our study and Parker (FLOT.214) we can not say definitively what the cause of the different results are. It is most likely to be a consequence of different dag composition and properties used in our study especially dag hardness and integrity, and the lack porosity/water repellence of our dags.

5 Success in Achieving Objectives

We did not achieve one of the main aims of the project which was to demonstrate proof of concept for the use of enzymes in a matrix to assist dag removal on a limited number of live feedlot cattle. Although we had animal ethics approval for a trial, the project did not progress to field testing of enzyme formulations because we were not able to demonstrate proof of concept under laboratory conditions. However, we have clearly shown that enzyme solutions (particularly mixtures of cellulases and ligninases) will not improve removal of hairs from cattle dags of a composition like the ones tested in this project. The nature of the dag could have a large bearing on the success of enzyme treatments. Dry, hard, water repellent dags will be very difficult to treat under any circumstances.

Our other main objective was to report on the potential for improvement of the technology and the requirements for product development. We have shown that it is possible to formulate the enzymes into gels where their activities are maintained and the mixture fully coats the dag and remains hydrated during the exposure period. However, the enzyme mixtures and other treatments we attempted in this project did not improve the removal of hair from dags.

6 Impact on Meat and Livestock Industry

The main impact on the meat and livestock industry is that for now, at least, the status quo is maintained and we have not able to demonstrate that enzymes were effective in removing dags from cattle hairs and hence offer an alternative treatment to the industry. Based on Parker (FLOT.214), some dags are likely to be susceptible to the enzyme treatment although the response may be variable. Therefore, dag removal remains a problem for the industry.

7 Conclusions and Recommendations

Variability in dag type with hairs, and the hardness of the dags used in this study resulted in the data obtained being inconclusive as to the beneficial effect of enzymes in dag removal, although, it has to be said, the result was not obviously effective in our study. Increasing the moisture content by ~7% did not improve the performance of enzymes for dag removal in our samples. The concept of applying enzyme mixtures in gels to dags was demonstrated although in this study it did not improve dag removal.

Several companies have either developed enzyme formulations for dag removal from hides, or are developing enzyme formulations for live cattle dag application. It is recommended that these formulations be obtained and tested in the laboratory on a wide range of dags from Australian feedlots (as dags will differ in composition between seasons, diets and locations) before being released commercially.

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