Final report

Effect of feed withdrawal on truck effluent, animal welfare, carcase characteristics and microbiological contamination of feedlot cattle

Project code: B.FLT.5009

Prepared by: Melissa George[1], Daniel Meehan[2], Peter McGilchrist[3], Paul Jensen[4], Des Bowler[5], Andrew Hale[5], Joseph McMeniman[6], Matthew George[1]

[1]Bovine Dynamics Pty Ltd
[2]Meehan Agribusiness Consulting
[3]University of New England
[4]University of Queensland
[5]Management for Technology
[6]Meat & Livestock Australia

Date published: 22 June 2022

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

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

Executive summary

Background

Effluent produced during the transport of feedlot cattle is a by-product of the beef industry. Effluent must be well-managed to minimize its effect on the environment. Possible management techniques include capture of effluent in truck tanks and trailers with disposal at approved discharge points. Withdrawing feed may also be a possible management strategy for truck effluent, however no research has occurred to determine the effects of this practice in Australian feedlot cattle. This project was undertaken to determine the effect of feed withdrawal on truck effluent, animal welfare, carcase characteristics and microbiological contamination of feedlot cattle. Potential uses for feedlot truck effluent will be determined.

This purpose of this project is to give feedlot producers, transport businesses, processers, regulatory authorities, and governments data to make informed decisions regarding best management of effluent produced by feedlot cattle during transportation from feedlot to abattoir.

Objectives

This project fulfilled its objectives to determine the effect of duration of feed withdrawal on carcass yield, quality, and safety and the volume and composition of effluent captured. These results were measured over four seasons.

Methodology

A single-blinded randomized complete block design was used to compare four durations of feed withdrawal including 0, 4, 8, and 12 hours on truck effluent volume and composition, carcass characteristics, and microbiological contamination of feedlot cattle. Specifically, this project was completed in 4 blocks per season (16 blocks total) and included a total of 64 pens including 4943 domestic heifers. Cattle were loaded at a feedlot near Roma, QLD and transported to Brisbane for a total transit length of 545 km. Effluent volume and composition were measured at Dalby and Brisbane.

Results/key findings

Duration of feed withdrawal did not affect the amount of effluent captured in tanks or trailers at Dalby or for the total journey ($P > 0.05$). Of total effluent captured in tanks and trailers most was produced between Roma and Dalby (64%). From Dalby to Brisbane, effluent captured linearly decreased ($P \leq 0.01$) as time of feed withdrawal increased. The differences were however small, and within the capacity of the effluent tank and trailers (3.5, 3.0, 2.7, 2.7 kg/hd for 0, 4, 8, and 12 h, respectively). As a percent of total transit shrink, 36% was attributed to effluent captured in tanks or trailers across experimental treatments, with the rest being attributed to loss from animal and truck sources as detailed below.

Loss from animals (cutaneous and respiratory evaporation) and truck (evaporation, convection, spillage) was impacted by duration of feed withdrawal ($P = 0.03$), however the magnitude of the difference was small over the 545 km haul (0 vs 12 h, 68.2 kg per truck). There was no statistical difference between 0 and 4 hours of feed withdrawal ($P > 0.05$).

Hot carcase weight was affected by feed withdrawal ($P = 0.045$). As duration of feed withdrawal increased, hot carcase weight linearly decreased ($P \leq 0.01$). Compared to 0 h of feed withdrawal, hot carcase weight decreased for 4, 8 and 12 h by 1.2, 1.1 and 1.8 kg, respectively. No other carcase characteristics were affected by duration of feed withdrawal including rib fat, marbling, meat colour, and ultimate pH. Additionally, there was no effect of duration of feed withdrawal on the decline of pH and temperature of the *Longissimus lumborum*. Liver glycogen was affected by feed withdrawal ($P = 0.049$). As time of feed withdrawal increased, liver glycogen linearly decreased ($P \leq 0.01$). Compared to 0 h liver glycogen (4.64%), levels decreased by 5.4, 8.2, and 13.6% for 4, 8 and 12 h, respectively, reflecting mobilisation of glycogen reserves to maintain blood glucose status in fasting animals.

Total microbiological counts and the proportion of carcasses with microbiological contamination were not affected by duration of feed withdrawal ($P > 0.05$).

A variety of uses for the feedlot effluent from this project were explored including municipal sewer disposal, incorporation into standalone or red meat processor biogas facilities, and direct land application. Direct land application of effluent contingent on environmental licensing, land availability and infrastructure development for irrigation was determined to be the most appropriate use of effluent from this study.

Benefits to industry

The results of this trial provide a clear baseline for the volume and composition of effluent captured at multiple stops during a journey from feedlot to abattoir under Australian conditions. These results provide valuable data for future planning of means to capture, treat, and use feedlot cattle effluent produced during transportation.

Managing feed allocation to time of dispatch is an important consideration for lot feeders. This project provides an objective dataset to enable discussion with abattoir customers and supply chain participants on impacts of feed withdrawal on carcase traits and effluent.

Future research and recommendations

Future development opportunities exist to use the results of this study to design methods to capture feedlot cattle effluent produced during transport and subsequently means to treat, prepare, and capture value from this resource. Whilst being conducted in domestic grain-fed heifers, future research could focus on alternative market categories of Australian feedlot cattle. It should be noted the results of this report are only relevant to feedlot production and not grazing systems.

Table of contents

1. Background

Truck effluent from cattle transport has been identified as a potential environmental, commercial and social risk for the Australian beef industry. This presents a specific challenge to industry as large numbers of cattle converge in areas of urbanisation, where concentrations of beef processing facilities are located.

Possible management includes; (a) capture of effluent in truck tanks and appropriate disposal at approved discharge points, (b) removal of cattle from all feed sources (fasting) for specific duration prior to feedlot exit and/or (c) combinations of the above. Regardless of solutions a, b or c, the impost remains of appropriate applications – specific to this investigation – feedlot cattle generated manure.

It is a common industry practice in the grazing industry to remove access to feed for several hours prior to transport of cattle. This period of time where access to feed is removed is called a 'curfew' and in grazing cattle typically ranges from 4 to 12+ hours. This practice of feed withdrawal is designed to reduce the amount of faeces cattle produce during transportation and thus the total volume of effluent that must be managed during transportation and at the abattoir (Walker and Banney, 2011). Diets of grazing cattle differ from feedlot cattle in terms of both dry matter and digestibility. Whilst the benefits of a 'curfew' may be evident in cattle grazing lush green pasture with a high moisture content (80 to 90%), the effects in feedlot diets (< 30% moisture) have not been researched in Australia.

Total weight loss during transport (transit shrink) can be segmented into effluent that is captured in tanks and trailers; and loss from animal evaporation (cutaneous and respiratory) and truck effluent loss (evaporation, air movement, and spillage).

The collective activities of transportation, handling and lairage of cattle during the pre-slaughter period can be cumulatively stressful and contribute to reduced intramuscular glycogen at slaughter (Giannetto et al., 2011, Romero et al., 2013). Further, cattle subjected to time off feed during lairage of 36 – 84 h versus 12 h produced carcases with a higher incidence of dark cutting (Kreikemeier et al., 1998). More recently, Australian industry investigations (George and George, 2018) identified as duration from feedlot dispatch to final cattle slaughter increased, rumen temperature declined with a concurrent rumen pH increase indicating rumen available substrate exhaustion and therefore rate of fermentation was declining. In this same study (George and George, 2018), the reduced substrate availability directly contributed to producing carcases with significantly lower longissimus glycogen, an increase in dark cutting beef incidence, lower dressing percentage and carcase yield.

Pre-harvest conditions including transportation, lairage, and diet can impact the gut microflora, level of shedding, and hide load of various microorganisms associated with food safety of beef carcasses and products. However, the effect of lairage duration on food safety remains an area of contest with some reports that reduced lairage duration and time off feed improve food safety by reducing the pH of the digestive tract and improving gut membrane integrity.

However, others reports suggest reduced lairage duration and shorter time off feed leads to increased incidence of gut rupture during evisceration and subsequent carcass contamination due to increased gut fill. Thus, a primary area of focus for the present study is to determine the effect of total time off feed lairage duration on food safety parameters.

Cattle are a known reservoir for several important foodborne pathogens including multiple pathotypes of enterohaemorrhagic *Escherichia coli* and *Salmonella* spp. (Grau and Brownlie, 1965; Fedorka-Cray et al., 1998; Haines et al., 2000; Kunze et al., 2008). More specifically, cattle are known to carry Shiga toxin producing *E. coli* O157:H7 and several other non-O157 O serogroups including O26, O45, O103, O111, O121, and O145 which are major food safety concerns for the beef industry (Arthur et al., 2017). Cattle exposed to longer duration of lairage are known to have increased rumen pH favouring microbiological growth (Grau et al., 1968), and reduced gut membrane integrity which may increase the numbers of microorganisms present in the gastrointestinal tract and increase the incidence shedding from gastrointestinal tracts during slaughter. Other work in this area found the prevalence of *Salmonella* spp. in the rumen was greater the longer the time period between cattle exiting a property and slaughter (Grau et al., 1968). However, the time periods tested ranged from 24h to 168h and these durations of transport and lairage are much longer than those typical of current feedlot industry practices. The incidence of salmonellosis in cattle increases with stress such as transportation and lairage stress (Grau and Smith, 1974).

Therefore, objectives of this study were to determine;

1. The effect of feedlot feed withdrawal on truck effluent volume and composition at defined distances between a feedlot and beef processing plant
2. The effect of duration of feedlot feed withdrawal on animal welfare, liver glycogen concentration, carcase quality characteristics, and, the prevalence and microbiological load of enterohaemorrhagic *E. coli* and *Salmonella* spp. on beef carcasses
3. Presence of any seasonal differences to the effects of feed withdrawal duration on objectives (1) and (2)

2. Objectives

2.1 Determine the effect of duration of feedlot feed withdrawal on truck effluent volume and composition at defined distances between the feedlot and processing plant

The effect of duration of feed withdrawal on the volume and composition of effluent was successfully measured at two defined points from the feedlot to the processing plant, demonstrating this objective was fulfilled.

2.2 Determine the effect of duration of feedlot feed withdrawal on animal welfare, carcass characteristics and microbiological contamination of feedlot cattle

The effect of duration of feed withdrawal on liver glycogen levels, chiller assessment pH, pH and temperature declines, meat colour, marbling, hot carcase weight, and hide-on and chilled carcass standard plate, coliform, and *E.coli* counts was measured, demonstrating this objective was fulfilled.

2.3 Determine seasonal differences in the effect of feed withdrawal on truck effluent volume, animal welfare, carcass characteristics, and microbiological contamination of feedlot cattle

These effects were measured across four seasons, demonstrating this objective was fulfilled.

3. Methodology

3.1 Animal welfare

This project was completed under the approval of the Queensland Government Department of Agriculture and Fisheries Animal Ethics Committee (Animal Ethics Committee Reference Number: SA 2020/09/754).

3.2 Experimental design

A single-blinded randomized complete block design was used to evaluate the effect of duration of feed withdrawal on truck effluent volume and composition, animal welfare, carcass characteristics, and microbiological contamination of feedlot cattle.

The study compared four durations of feed withdrawal including 0, 4, 8, and 12 hours.

Specifically, this project was completed with 4 blocks per each season (16 blocks total) and included a total of 64 pens of cattle for a total of 4943 heifers. The experimental unit was pen.

3.3 General

The cattle were fed at Australian Country Choice's Brindley Park Feedlot in Roma, Queensland, Australian and processed at Australian Country Choice's Cannon Hill Abattoir in Brisbane, Queensland, Australia.

The cattle were non-implanted domestic heifers of a single market category that were certified by a veterinarian to be not detectably pregnant at the time of feedlot induction. The cattle had an average induction weight of 372.8 kg that were fed for 63.5 days on feed on a steam-flaked barley ration. Cattle breed type was mixed with a maximum of 50% bos indicus content.

A home pen of 320 head of cattle were randomised to 4 treatment pens 14 days prior to exit. Importantly, no cattle were mixed with new pen mates at this time. The four trucking pens were uniform in design (1,050 square meters, stocking density 14 square meters per head, 3.0 square meters of shade per head, 3.0 square meter water trough shared between two pens, concrete feed bunk 20 meters in length with 250 mm per head). The weight of each individual animal was recorded on the date of randomisation. Scales were calibrated prior to the commencement of each season and checked weekly prior to the induction session. Scale increments were accurate to +/- 1 kg.

Of those 4 trucking pens, each was assigned to one of 4 treatments for feed withdrawal duration (0, 4, 8, 12 hrs feed withdrawal prior to feedlot exit). Feed was removed from the bunks at specific times to ensure the duration of feed withdrawal was accurate. Cattle with 0h feed withdrawal duration had feed in their bunk until the time of exit. Cattle withdrawn for 4h had feed removed at 4:00 AM the day of feedlot exit. Cattle withdrawn for 8h had feed removed at 12:00 AM (midnight) the day of feedlot exit. Cattle withdrawn for 12h had feed removed at 8:00 PM the day of feedlot exit. The weight of the feed remaining in the bunk at the time of withdrawal was measured to allow calculation of the quantity of feed consumed in 24h prior to feedlot exit. All cattle exited their trial pens at similar times (approximately 8:00 AM day of exit). Cattle departed the feedlot at similar times (approximately 9:00 AM day of exit). The trial pen at the feedlot, dispatch order, truck, truck driver, lairage pens, and kill order were scheduled to ensure that all parameters were equally represented across treatments.

Cattle were transported in B-Double configuration trucks that were fitted with effluent tanks to allow measurement of effluent volume and sampling for compositional analysis.

The empty and loaded truck weight was measured at the feedlot to provide an exit weight on a pen basis for the cattle. The trucks travelled from Brindley Park Feedlot to Dalby Regional Saleyards for a distance of 305 km. A truck weight was measured at arrival, effluent was dumped and sampled for compositional analysis. The dumped truck weight was then measured prior to departure. Feedlot and Dalby weighbridge scale breaks were 20 kg. Feedlot weighbridge was calibrated prior to the commencement of each season. The amount of fuel consumed during the journey was measured.

The trucks travelled from Dalby Regional Saleyards to Cannon Hill, Queensland for a distance of 240 km. Truck weight was measured at arrival, cattle were unloaded, trucks were weighed unloaded, effluent was dumped and sampled, trucks were cleaned, and a clean truck weight was measured. The amount of fuel consumed during the journey was measured. The Cannon Hill weighbridge scale break was 50 kg.

The cattle arrived to Australian Country Choice's Cannon Hill Abattoir at 5:30 p.m. The cattle were unloaded and housed overnight in shedded lairage pens with cement cross-hatched floors with access to ad libitum water. Cattle were processed the next morning with an average stun time of 8:14 AM. Hence, cattle with 0h feed withdrawal prior to exit did not have access to feed for 24 hours prior to stun. Cattle with 4h feed withdrawal prior to exit did not have access to feed for 28 hours prior to stun. Cattle with 8h feed withdrawal prior to exit did not have access to feed for 32 hours prior to stun. Cattle with 12h feed withdrawal prior to exit did not have access to feed for 36 hours prior to stun.

Cattle were NLIS scanned by abattoir staff and visual identification was recorded and paired with body number to ensure accuracy of data.

Split carcasses entered the chillers for 8 hrs of draw down to 2°C , followed by a hold pattern at 5 °C. Carcasses were chilled for 22 hours prior to grading.

3.4 Ration dry matter, consumption and quality measurements

Cattle were fed a steam-flaked barley total mixed ration twice a day with the first delivery at 9:00 AM and second delivery at 2:00 PM. The finisher ration was sampled daily and dry matter testing was performed in duplicate. Feed intake was measured on a daily basis. The mean feed intake on a dry matter basis per individual over the 14 days prior to exit was recorded per pen.

At the defined time of feed withdrawal, bunks were shovelled and the feed remaining was weighed. Duplicate dry matter measurements were performed on the feed remaining in the bunk. The mean feed intake on a dry matter basis per individual for the 24 hours prior to departure was recorded per pen.

Ration analyses including dry matter, crude protein, fat, neutral detergent fibre, ash, metabolizable energy, calcium, phosphorus, magnesium, and potassium were performed for each block (n=16). Samples were taken from the trial pens directly following feed delivery the day prior to feedlot exit.

3.5 Weather measurements

The feedlot and abattoir were fitted with weather stations that measured temperature, windspeed, relative humidity, and precipitation at hourly intervals. For the feedlot, the measurements recorded at 7:00 AM on the day of feedlot exit was used for the analysis. For the abattoir, the mean of the sixteen measurements recorded from 5:00 PM the day of arrival at the abattoir until 8:00 AM the day of slaughter was used for the analysis.

3.6 Effluent volume and composition measurements

Cattle transport trucks with a B-Double configuration were fitted with effluent tanks. Trailer A was fitted with a 200-litre tank and trailer B was fitted with a 400-litre tank. Both upper and lower decks were bunded to reduce effluent spillage. All truck trailers were clean and empty prior to feedlot arrival.

Trucks were weighed at the following times on calibrated weigh bridges.

Feedlot Arrival (Empty)

Feedlot Exit (Cattle Loaded)

Dalby Regional Saleyards (Arrival, Cattle Loaded, Effluent tanks full)

Dalby Regional Saleyards (Exit, Cattle Loaded, Effluent tanks emptied)

Cannon Hill Abattoir (Arrival, Cattle Loaded, Effluent tanks full)

Cannon Hill Abattoir (Cattle Unloaded, Effluent tanks full)

Cannon Hill Abattoir (Exit, Cattle Unloaded, Effluent tanks empty, Truck washed)

The following formulas were used to calculate the weight of the effluent dumped at Dalby Regional Saleyards and Cannon Hill Abattoir.

Effluent dumped at Dalby Regional Saleyards = Truck weight at Dalby Regional Saleyards Arrival with cattle loaded and full effluent tanks – Truck weight at Dalby Regional Saleyards exit with cattle loaded and dumped effluent tanks

Effluent dumped at Cannon Hill Abattoir = Truck weight at Cannon Hill Abattoir with cattle unloaded and full effluent tanks – Truck weight at Cannon Hill Abattoir with cattle unloaded and effluent tanks dumped and truck washed.

A sample of effluent was taken at Dalby Regional Saleyards and Cannon Hill Abattoir for compositional and biochemical analyses. The detailed report for the compositional and biochemical analyses are included as Appendix 1.

3.7 Cattle weight measurements

Formulas for the calculation of cattle weights at feedlot exit, Dalby Regional Saleyard, and the Cannon Hill abattoir are listed below.

Cattle weight at feedlot exit per head = (Truck weight at feedlot exit with cattle loaded – Truck weight at feedlot arrival without cattle)/Number of cattle loaded on truck

Cattle weight at Dalby Regional Saleyards per head = (Truck weight at exit from Dalby Regional Saleyards following dumping of effluent – Truck weight at feedlot entry without cattle) / Number of cattle on truck

Live cattle weight at Cannon Hill abattoir per head = (Truck weight at arrival to abattoir – Truck weight following unloading of cattle) / Number of cattle on truck

3.8 Carcase measurements

Weight of the carcase with the hide on following exsanguination was recorded to yield a bled weight. This value was multiplied by 1.02 to yield a live weight estimate.

Hot standard carcase weight was recorded after evisceration and trimming according to the Aus-meat standard carcase trim requirements. After chilling for approximately 22 hours, chiller assessment was conducted by qualified plant graders. Body number, dentition, Aus-meat meat colour, MSA marbling, AusMeat marbling, pH at chiller assessment at the M. longissimus dorsi quartering site, fat colour, subcutaneous rib fat at the M. longissimus dorsi (Aus-meat standard site), P8 fat depth, left side bruise, right side bruise, left hot standard carcase weight, right hot standard carcase weight, total hot standard carcase weight, and eye muscle area at the M. longissimus dorsi quartering site. PH meters were calibrated prior to each grading session and every two hours within a session by qualified plant graders. Temperature probes were calibrated. Dressing percentage was calculated as the hot carcase weight divided by the truck weight times 100.

3.9 Carcass pH and temperature decline measurements

A total of 9 carcases per pen of cattle were selected for measurement of pH and temperature declines. Every 9th carcass was tested. Dual temperature and pH meters (WP-80M, TPS Pty Ltd, Brisbane, Queensland, Australia) were calibrated for temperature the day prior to each data collection session and calibrated for pH hourly during pH decline measurements. Hourly pH and temperature measurements of the *Longissimus lumborum* between L2 and L5 were recorded at chiller entry (0h), 1h, 2h, 3h, and 4h following chiller entry.

3.10 Sampling of carcasses

A subset of 10% of the carcases were sampled for further laboratory testing. Samples were taken for liver glycogen measurements, hide and chilled carcass microbiological samples, samples were collected from 10% of carcasses. Specifically, samples were taken from five carcasses per pen meaning that the 5th, 20th, 35th, 50th, and 65th carcasses processed from a pen were sampled.

3.11 Liver glycogen measurements

A minimum 3 g sample (approximately 2cm x 2cm x 2cm cube) was collected from the visceral surface of the liver near the bile duct. Samples were trimmed, placed into a plastic labelled tube with a screw-top lid, and placed on ice. Samples were frozen at -20°C until laboratory analysis. Laboratory analyses for muscle glycogen levels were conducted according to methods described by Coombes et al., 2014.

3.12 Microbiological measurements

Prior to hide removal, a 300 cm^2 area including 3 - 100 cm^2 sites including the flank, brisket, and butt were swabbed using a Whirl-Pack sponge. A 10x10 cm grid was used to ensure sample size accuracy. The swabs were tested for standard plate count, coliform counts, and *E. coli* counts.

Chilled carcass swabs were completed on a 300 cm^2 area including 3 - 100 cm^2 sites including the flank, brisket, and butt. One side of the sponge was used to sample the flank and brisket and the other side to sample the butt.

Testing was completed in accordance with the 'Microbiology manual for sampling and testing of export meat and meat products' (DAWR, Version 1.03, Dec 2018). Swabs were placed into individually labelled Whirl-Pack bags, stored at 1-5 degrees Celsius, and transported to the NATA accredited laboratory (Symbio Laboratories, Eight Mile Plains, Queensland, Australia) for analyses.

3.13 Statistical analyses

Data was analysed as a randomised complete block design with the experimental unit defined as the pen. The experiment was analysed using the PROC MEANS, PROC MIXED, PROC CONTRAST procedures of SAS (SAS Institute Inc., Cary, North Carolina, USA). Least square means were separated using the PDIFF procedure where significance was detected. For analyses, treatment and season were included in the model as fixed effects. Replicate was included in the model as a random effect. The season by treatment interaction was tested for all variables and was not significant and hence was removed from the model. Statistical significance of interactions and main effects were defined at $P \leq 0.05$ and a trend at $P \leq 0.10$ levels.

The GLIMMIX procedure of SAS was completed to calculate the proportion of carcasses and hides where microbiological contamination was present.

4. Results

4.1 Descriptive statistics

Simple descriptive statistics including average, standard deviation, minimum, and maximum values for 64 pens of research cattle are presented in Table 1. These results provide a general overview of the data set. The heifers (n=4,943) had an average induction weight of 372.9 ± 15.4 kg (mean ± standard deviation), were fed for 63.5 ± 0.6 days, had a draft weight of 458.8 ± 15.3 kg two weeks prior to feedlot exit, and weighed 480.6 ± 17.7 kg at feedlot exit. Cattle consumed on average 10.2 ± 0.5 kg of dry matter steam-flaked barley total mixed ration over the 14 days prior to feedlot exit.

B-double configuration trucks were loaded and departed the feedlot at 8:58 AM. Trucks travelled 305 km from the feedlot at Roma, Queensland to Dalby Regional Saleyards at Dalby, Queensland. The trucks arrived at Dalby, Queensland at 12:51 PM where the effluent was captured. Trucks departed Dalby, Queensland at 1:18 PM and travelled 224 km to arrive at the abattoir at Cannon Hill, Queensland at 4:25 PM. Cattle were unloaded, trucks re-weighed, washed, and re-weighed again at 7:07 PM. Cattle were processed the following morning at 8:14 AM with approximately 16 hours in lairage prior to slaughter. Carcases were chilled for approximately 22 hours prior to grading.

Cattle were 480.6 ± 17.7 kg at feedlot exit, 463.0 ± 15.9 kg at Dalby, 457.4 kg ± 15.8 kg at Cannon Hill, 442.6 ± 15.6 kg at the time of slaughter, dressed at 52.12 ± 0.52%, to yield a hot carcase weight of 250.5 ± 8.9 kg. Shrink was 23.1 ± 2.5 kg per head or 4.81% ± 0.40% on a percentage basis.

On average 407.5 ± 81.6 kg of effluent was dumped at Dalby, Queensland and 231.0 ± 81.8 kg was dumped at Cannon Hill for a total of 638.5 ± 114.8 kg of effluent captured across the journey. On average 1150.9 ± 241.7 kg of weight was considered 'loss' during the transportation and can be accounted to animal evaporation (cutaneous, respiratory) and truck losses (evaporation, wind movement, and spillage).

The mean temperature, windspeed, relative humidity, and rainfall for the feedlot and abattoir are included in Table 1.

Descriptive statistics for carcass quality grading and yield measurements are reported in Table 2. Carcases had an average MSA marbling score of 329.5 ± 35.0, Ausmeat meat colour of 2.3 ± 0.2, eye muscle area of 70.3 ± 1.8 cm^2 and ultimate pH of 5.56 ± 0.03. Mean liver glycogen percentage was 4.32% ± 0.66%. Standard plate counts, coliform counts, and *E. coli* counts are displayed in Table 2.

4.2 Effect of duration of feed withdrawal on cattle and carcase weights

There was no difference in entry weight ($p=0.46$), pre-transport treatment allocation (14-days prior to exit), draft weight ($p=0.55$), or days on feed ($p=0.84$) among the four treatments of feed withdrawal duration (Table 3).

At the time of feedlot exit, the duration of feed withdrawal had a significant effect on exit weight ($p \leq 0.01$), specifically as the period of feed withdrawal increased from 0 h to 12 h, there was a highly significant linear decrease in feedlot exit weight ($p \leq 0.01$), cattle weight at Dalby ($p<0.01$), cattle arrival weight at the beef processing facility ($p \leq 0.01$), dead weight ($p=0.01$) and hot carcase weight ($p \leq 0.01$). Cattle with access to full feed at the time of feedlot exit had hot carcass weights that were 1.8 kg heavier ($p \leq 0.05$) than cattle that were withheld from feed for 12 hours prior to feedlot exit. Dressing percentage was similar ($p=0.21$) between different durations of feed withdrawal.

These difference in cattle and carcass weights are consistent with the differences ($p<0.01$) in feed consumption in the 24 hours prior to exit. Specifically, cattle with 0 h duration of feed withdrawal consumed 4.7 kg more feed in the 24 hours prior to exit than cattle with 12 h duration of feed withdrawal demonstrating the cattle did consume feed overnight prior to feedlot exit.

Based on these weights, transport cattle shrink was significantly impacted ($p=0.01$) by duration of feed withdrawal and more specifically cattle with shorter duration of feed withdrawal had linearly higher ($p \leq 0.01$) shrink as compared to cattle with longer duration of feed withdrawal.

4.3 Effect of duration of feed withdrawal on effluent volume and composition

There was no effect ($p=0.98$) of duration of feed withdrawal on the weight of effluent captured at the Dalby Regional Saleyards (Table 3). Of total effluent captured in tanks and trailers most was produced between Roma and Dalby (64%). However, the weight of effluent captured from Dalby to Cannon Hill was significantly ($p<0.01$) by the duration of feed withdrawal with cattle with shorter duration of feed withdrawal linearly producing more ($p<0.01$) effluent during the second half of the transportation. The differences were however small, and within the capacity of the effluent tank and trailers (3.5, 3.0, 2.7, 2.7 kg/hd for 0, 4, 8, and 12 h, respectively). There was no difference ($p=0.11$) in the total weight of effluent captured across the entire transportation from Roma to Cannon Hill.

These results demonstrated that the weight of cattle shrink cannot be fully attributed to the effluent captured in the tanks. The majority of shrink is actually not captured as effluent and can be attributed to animal evaporation (cutaneous and respiration) and truck losses (evaporation, convection and spillage). This weight has been referred to as 'loss' in table 3. There was a significant ($p=0.03$) effect of duration of feed withdrawal on loss. Cattle with shorter duration of feed withdrawal linearly produced more ($p \leq 0.01$) 'loss' as compared to cattle with longer duration of feed withdrawal. The magnitude of the difference was however small over the 545 km haul (0 vs 12 h, 68.2 kg per truck). There was no statistical difference between 0 and 4 hours of feed withdrawal ($P > 0.05$).

A special report has been included as Appendix 1 titled 'The effect of duration of feed withdrawal on effluent composition'. This report includes a detailed compositional and biochemical analysis of feedlot effluent and an assessment of the value of truck effluent treatment. (Appendix 1).

4.4 Effect of duration of feed withdrawal on carcass quality, yield, and liver glycogen

There was no effect of duration of feed withdrawal on MSA marbling ($p=0.76$), meat colour ($p=0.78$), eye muscle area ($p=0.73$), rib fat thickness (=0.19), chiller assessment pH ($p=0.73$), loin temperature ($p=0.81$), or MSA index ($p=0.99$) (Table 3).

The percentage of liver glycogen was impacted ($p=0.049$) by duration of feed withdrawal. Carcasses that were exposed to a shorter duration of feed withdrawal had a linearly greater ($p<0.01$) percentage of liver glycogen as compared to cattle with longer duration of feed withdrawal. Specifically, cattle on full feed at the time of feedlot exit had 0.63% higher ($p \leq 0.05$) liver glycogen levels as compared to cattle with 12 hours feed withdrawal.

4.5 Effect of duration of feed withdrawal on microbiological parameters of hides and chilled-carcasses

There was no difference ($p>0.10$) in standard plate count, coliforms count, and *E. coli* count for hides or chilled carcasses between the four duration of feed withdrawal (Table 3). There was no difference ($p>0.10$) between durations of feed withdrawal in the proportion of hides with positive standard plate counts, positive coliform counts, and positive *E. coli* counts. There was no difference ($p>0.05$) between durations of feed withdrawal in the proportion of chilled carcasses with positive standard plate counts, positive coliform counts, or positive *E. coli* counts.

4.6 Effect of season on carcass characteristics, truck effluent, and microbiological parameters

The project was completed across four seasons to provide insights on the seasonal impact on effluent volumes. Importantly, there was no significant season by treatment interaction and hence the interaction was removed from the model. Although feedlot entry weights were similar ($p=0.22$) across seasons, draft weight, exit weight, dead weight, and hot carcass weight were significantly ($p<0.01$) impacted by season (Table 5). Cattle processed in the winter were lighter ($p<0.01$) than cattle processed in Spring, Summer, or Autumn. Cattle processed in the Spring had higher ($p<0.01$) shrinks than cattle processed in the Summer, Autumn, or Winter. There was no difference (p>0.10) in dry matter intake across seasons. While there was no difference (=0.44) in effluent captured at Dalby across seasons, a higher (p<0.01) weight of effluent was captured in the second portion of the transportation during Autumn and Winter as compared to Spring and Summer. Loss was significantly (p<0.01) impacted by season. Specifically, loss was higher (p<0.01) greatest in Spring followed by Summer, Autumn and Winter, respectively. Compared to winter, loss was 62.8% greater in spring perhaps reflecting greater evaporative losses during this period. Marbling, rib fat thickness, and loin temperature were significantly (p<0.01) impacted by season. Hide standard plate counts and *E. coli* counts were significantly (p<0.01) affected by season. Hide coliform counts tended (p=0.06) to be affected by season. Cattle processed in the Autumn had higher levels of microbes on hides as compared to other seasons.

4.7 Effect of duration of feed withdrawal on the decline of pH and temperature of *Longissimus lumborum*

All durations of feed withdrawal resulted in carcasses that dropped below pH 6.0 of the *Longissimus lumborum* within the temperature window of 35 to 15 degrees Celsius (Figure 1). All treatments followed similar patterns of pH and temperature decline.

Table 1. Descriptive statistics of pens of study heifers (n=64 pens, n=4943 heifers)

Variable	Mean	Stdev	Minimum	Maximum
Entry Weight, kg	372.9	15.4	332.0	394.6
Draft Weight, kg	458.8	15.3	429.8	485.8
Days on feed	63.5	0.6	62.1	64.1
Cattle weight at feedlot exit per hd, kg/hd†	480.6	17.7	446.6	513.6
Cattle weight at Dalby per hd, kg/hd	463.0	15.9	432.4	490.3
Cattle weight at abattoir per hd, kg/hd	457.4	15.8	427.1	484.4
Dead weight, kg*	442.6	15.6	412.4	466.6
Hot carcass weight, kg	250.5	8.9	233.4	263.9
Dressing percent, %	52.12	0.52	51.17	54.30
Transport cattle shrink, %	4.81	0.40	4.00	5.69
Transport cattle shrink per hd, kg/hd	23.1	2.5	18.3	29.2
Time of truck arrival to feedlot	7:47 AM	32:47	6:37 AM	9:03 AM
Time of truck exit from feedlot	8:58 AM	31:31	7:34 AM	10:13 AM
Time of truck arrival to Dalby Regional Saleyards	12:51 PM	32:45	11:21 AM	2:10 PM
Time of truck exit from Dalby Regional Saleyards	1:18 PM	33:57	11:56 AM	2:47 PM
Time of truck arrival to Cannon Hill	4:25 PM	36:46	3:06 PM	5:46 PM
Time of truck unloaded at Cannon Hill	5:28 PM	38:17	3:55 PM	6:50 PM
Time of truck washed at Cannon Hill	7:07 PM	48:13	5:05 PM	8:56 PM
Dry matter intake 24 hrs prior to exit, kg/hd	5.8	2.8	0.3	11.6
Daily dry matter intake 14 days prior to exit, kg/hd	10.2	0.5	8.8	11.4
Effluent captured Dalby, kg	407.5	81.6	240.0	580.0
Effluent captured Cannon Hill, kg	231.0	81.8	100.0	600.0
Total effluent captured, kg	638.5	114.8	380.0	940.0
Effluent captured Dalby per hd, kg/hd	5.3	1.0	3.2	7.5
Effluent captured Cannon Hill per hd, kg/hd	3.0	1.1	1.3	7.9
Total effluent captured per hd, kg/hd	8.3	1.5	4.9	12.1
Loss from feedlot to abattoir, kg	1150.9	241.7	790.0	1620.0
Loss from feedlot to abattoir per hd, kg	14.9	3.0	10.1	20.8
Feedlot temperature at time of exit, °C	14.8	7.6	-0.2	27.8
Feedlot windspeed at time of exit, km/h	12.0	8.1	1.5	27.4
Feedlot relative humidity at time of exit, %	71.0	6.9	53.1	84.9
Feedlot precipitation at time of exit, mm	0.00	0.00	0.00	0.00
Abattoir temperature during lairage, °C	18.8	3.0	13.0	22.5
Abattoir windspeed during lairage, km/h	6.6	2.2	3.8	11.2
Abattoir relative humidity during lairage, %	59.2	22.5	14.3	85.1
Abattoir precipitation during lairage, mm	0.14	0.29	0.00	0.99

*Dead weight was calculated as (dead bar bled weight x 1.02).

†Truck weights were considered outliers for 5 trucks resulting in a range of SEM being calculated.

Table 2. Descriptive statistics for carcass grading, liver glycogen, and food safety

Variable	Mean	Stdev	Minimum	Maximum
MSA marbling	329.5	35.0	234.5	382.6
AusMeat marbling	0.8	0.3	0.1	1.4
AusMeat meat colour‡	2.3	0.2	1.9	2.9
Fat colour	0.7	0.3	0.0	1.2
Eye muscle area, cm^2	70.3	1.8	66.5	74.6
Rib fat, mm	4.8	0.8	3.1	6.9
P8 fat, mm	10.0	1.4	7.7	12.8
Dentition	1.1	0.6	0.1	2.2
Chiller assessment pH	5.56	0.03	5.48	5.62
Loin temperature, ˚C	4.93	1.29	2.81	6.92
MSA index	60.50	0.48	59.40	61.42
Liver glycogen, %	4.32	0.66	2.88	6.24
Hide-on standard plate count, CFU/cm^2	2.4×10^4	1.7×10^4	6.8×10^3	1.0×10^5
Hide-on coliform count, CFU/cm2	4.6×10^2	3.9×10^2	5.1×10^1	2.3×10^3
Hide-on E. coli count, CFU/cm2	3.8×10^2	3.6×10^2	3.1×10^1	2.3×10^3
Chiller standard plate count, CFU/cm2	2.0×10^2	5.8×10^2	2.3×10^0	3.5×10^3
Chiller coliform count, CFU/cm2	2.2×10^0	7.9×10^0	0.0×10^0	4.3×10^0
Chiller E. coli count, CFU/cm2	5.2×10^1	7.3×10^0	0.0×10^0	4.3×10^0

‡Meat colour was scored as 1A=1.00, 1B=1.33, 1C=1.67, 2=2.00, 3=3.00, 4=4.00, 5=5.00, 6=6.00.

Table 3. Effect of feed withdrawal on carcase characteristics, truck effluent, and microbiological parameters

	Hours of Feed Withdrawal						
Variable	0h	4h	8h	12h	SE	*P-value*	*Linear P-value*
Entry Weight, kg	372.9	373.2	373.4	372.1	3.758	0.46	0.45
Draft Weight, kg	459.3	458.6	459.4	458.0	2.212	0.55	0.37
Days on feed	63.5	63.5	63.5	63.6	0.103	0.84	0.41
Cattle weight at feedlot exit per hd, kg/hd	483.6^{a}	482.2^{a}	480.6^{ab}	477.8^{b}	2.199-2.221*	<0.01	<0.01
Cattle weight at Dalby per hd, kg/hd	465.4^{a}	464.6^{a}	463.1^{a}	460.2^{b}	2.059-2.980*	<0.01	<0.01
Cattle weight at abattoir per hd, kg/hd	459.5^{a}	458.7^{a}	457.9^{a}	455.3^{b}	2.113-2.130*	0.01	<0.01
Dead weight, kg†	444.5	443.3	443.2	440.8	1.884	0.07	0.01
Hot carcase weight, kg	251.5^{a}	250.3^{ab}	250.4^{ab}	249.7^{b}	1.126	0.045	<0.01
Dressing percent, %	52.14	51.94	52.11	52.27	0.129	0.21	0.26
Transport cattle shrink, %	4.97^{a}	4.87^{ab}	4.70^{b}	4.69^{b}	0.075-0.078*	0.02	<0.01
Transport cattle shrink per hd, kg/hd	24.1^{a}	23.5^{ab}	22.6^{bc}	22.5^{c}	0.380-0.393*	0.01	<0.01
Dry matter consumed 24 hrs prior to exit, kg/hd	8.3^{a}	6.1^{b}	5.3^{b}	3.6^{c}	0.577	<0.01	<0.01
Daily dry matter intake 14 days prior to exit, kg/hd	10.2	10.3	10.3	10.1	0.133	0.19	0.51
Effluent captured Dalby, kg	409.7	404.5	409.8	413.8	21.98-22.44*	0.98	0.80
Effluent captured Cannon Hill, kg	269.1^{a}	229.5^{b}	211.5^{b}	206.2^{b}	14.89-15.28*	<0.01	<0.01
Total effluent captured, kg	679.1	634.0	621.3	620.0	28.40-28.92*	0.11	0.03
Effluent captured Dalby per hd, kg/hd	5.3	5.2	5.3	5.4	0.279-0.285*	0.98	0.78
Effluent captured Cannon Hill per hd, kg/hd	3.5^{a}	3.0^{b}	2.7^{b}	2.7^{b}	0.274-0.317*	<0.01	<0.01
Total effluent captured per hd, kg/hd	8.8	8.2	8.0	8.0	0.360-0.367	0.09	0.02
Loss from feedlot to abattoir, kg	1182.7^{a}	1181.8^{a}	1134.5^{ab}	1114.5^{b}	28.56-29.11*	0.03	<0.01
Loss from feedlot to abattoir, kg/hd	15.3^{a}	15.3^{a}	14.6^{ab}	14.4^{b}	0.367-0.373*	0.03	<0.01
MSA marbling	326.3	328.9	330.3	332.7	7.509	0.76	0.29
AusMeat marbling	0.81	0.82	0.84	0.85	0.069	0.90	0.48
AusMeat meat colour‡	2.31	2.35	2.30	2.33	0.059	0.78	0.96
Fat colour	0.73	0.73	0.74	0.77	0.065	0.84	0.45
Eye muscle area, cm^{2}	70.2	70.5	70.3	70.0	0.416	0.73	0.50
Rib fat, mm	4.7	4.7	4.8	5.0	0.135	0.19	0.04
P8 fat, mm	10.1	10.2	10.0	9.8	0.205	0.30	0.14
Dentition	1.1	1.1	1.1	1.1	0.094	0.42	0.10
Chiller assessment pH	5.56	5.56	5.57	5.57	0.009	0.73	0.27
Loin temperature, ˚C	4.98	4.93	4.87	4.95	0.109	0.81	0.68
MSA index	60.48	60.51	60.50	60.51	0.128	0.99	0.80
Liver glycogen, %	4.64^{a}	4.39^{ab}	4.26^{ab}	4.01^{b}	0.161	0.049	<0.01
Hide-on standard plate count, CFU/cm^{2}	2.6x10^{4}	2.8x10^{4}	2.3x10^{4}	2.1x10^{5}	3.8x10^{3}	0.20	0.06
Hide-on coliform count, CFU/cm2	4.8x10^{2}	5.0x10^{2}	4.0x10^{2}	4.4x10^{2}	9.3x10^{2}	0.81	0.56
Hide-on E. coli count, CFU/cm2	4.2x10^{2}	4.1x10^{2}	3.4x10^{2}	3.6x10^{2}	7.7x10^{1}	0.78	0.40
Chiller standard plate count, CFU/cm2	1.6x10^{2}	3.0x10^{2}	2.3x10^{2}	1.3x10^{2}	1.5x10^{2}	0.85	0.80
Chiller coliform count, CFU/cm2	2.5x10^{0}	1.3x10^{0}	3.8x10^{0}	1.3x10^{0}	2.0x10^{0}	0.78	0.90
Chiller E. coli count, CFU/cm2	1.9x10^{0}	1.2x10^{0}	3.8x10^{0}	1.3x10^{0}	1.8x10^{0}	0.71	0.92

†Dead weight was calculated as (dead bar bled weight x 1.02).

‡Meat colour was scored as 1A=1.00, 1B=1.33, 1C=1.67, 2=2.00, 3=3.00, 4=4.00, 5=5.00, 6=6.00.

*Truck weights were considered outliers for 5 trucks resulting in a range of SEM being calculated.

Table 4. Proportion of hides and chilled carcasses with microbiological contamination

	Hours of Feed Withdrawal				
Variable	0h	4h	8h	12h	*P-value*
Hide-on standard plate count, CFU/cm^2					
Positive count	127 (99.2%)	128 (100.0%)	128 (100.0%)	128 (100.0%)	1.00
No microbes detected	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Hide-on coliform count, CFU/cm2					
Positive count	123 (96.1%)	127 (99.2%)	124 (96.9%)	124 (96.9%)	0.52
No microbes detected	5 (3.9%)	1 (0.8%)	4 (3.1%)	4 (3.1%)	
Hide-on E. coli count, CFU/cm2					
Positive count	123 (96.1%)	127 (99.2%)	124 (96.9%)	124 (96.9%)	0.52
No microbes detected	5 (3.9%)	1 (0.8%)	4 (3.1%)	4 (3.1%)	
Chiller standard plate count, CFU/cm2					
Positive count	108 (84.4%)	102 (79.7%)	97 (76.4%)	92 (71.9%)	0.09
No microbes detected	20 (15.6%)	26 (20.3%)	30 (23.6%)	36 (28.1%)	
Chiller coliform count, CFU/cm2					
Positive count	11 (8.6%)	12 (9.4%)	3 (2.4%)	4 (3.1%)	0.051
No microbes detected	117 (91.4%)	116 (90.6%)	124 (97.6%)	124 (96.9%)	
Chiller E. coli count, CFU/cm2					
Positive count	9 (7.0%)	5 (3.9%)	3 (2.4%)	2 (1.6%)	0.15
No microbes detected	119 (93.0%)	123 (96.1%)	124 (97.6%)	126 (98.4%)	

Table 5. Effect of season on carcass characteristics, truck effluent, and microbiological parameters

	Season					
Variable	Spring	Summer	Autumn	Winter	SE	P-value
Entry Weight, kg	383.1	375.9	371.3	361.1	7.436	0.22
Draft Weight, kg	473.4^{a}	463.3^{ab}	460.9^{b}	437.8^{c}	4.215	<0.01
Days on feed	64.0^{a}	63.9^{a}	63.5^{a}	62.8^{b}	0.201	<0.01
Cattle weight at feedlot exit per hd, kg/hd	499.2^{a}	484.8^{b}	483.2^{b}	456.9^{c}	3.851-4.447*	<0.01
Cattle weight at Dalby per hd, kg/hd	479.5^{a}	465.8^{b}	466.1^{b}	441.9^{c}	3.605-4.163*	<0.01
Cattle weight at abattoir per hd, kg/hd	473.2^{a}	461.4^{b}	460.5^{b}	436.4^{c}	3.773-4.357*	<0.01
Dead weight, kg†	459.1^{a}	447.1^{b}	444.1^{b}	421.3^{c}	3.259-3.763*	<0.01
Hot carcase weight, kg	259.1^{a}	253.1^{ab}	252.1^{b}	237.7^{c}	2.129	<0.01
Dressing percent, %	51.90	52.23	52.30	52.03	0.175	0.35
Transport cattle shrink, %	5.21^{a}	4.82^{b}	4.70^{bc}	4.51^{c}	0.087-0.101*	<0.01
Transport cattle shrink per hd, kg/hd	26.0^{a}	23.4^{b}	22.7^{b}	20.6^{c}	0.423-0.489*	<0.01
Dry matter consumed 24 hrs prior to exit, kg/hd	5.3	5.5	6.3	6.1	0.701	0.70
Daily dry matter intake 14 days prior to exit, kg/hd	10.6	10.1	10.3	9.9	0.241	0.23
Effluent captured Dalby, kg	402.5	416.7	448.6	370.0	33.72-38.93*	0.44
Effluent captured Cannon Hill, kg	168.8^{a}	181.7^{a}	259.0^{b}	306.9^{b}	20.76-23.97*	<0.01
Total effluent captured, kg	571.3	598.3	707.9	676.9	45.32-52.33*	0.14
Effluent captured Dalby per hd, kg/hd	5.2	5.4	5.8	4.8	0.425-0.491*	0.45
Effluent captured Cannon Hill per hd, kg/hd	2.2^{a}	2.3^{a}	3.4^{b}	4.0^{b}	0.274-0.317*	<0.01
Total effluent captured per hd, kg/hd	7.3	7.7	9.2	8.9	0.575-0.664*	0.09
Loss from feedlot to abattoir, kg	1454.4^{a}	1220.8^{b}	1045.2^{c}	893.1^{d}	44.95-51.90*	<0.01
Loss per hd, kg	18.7^{a}	15.7^{b}	13.5^{c}	11.7^{d}	0.584-0.675*	<0.01
MSA marbling	360.0^{a}	332.1^{a}	298.1^{b}	328.9^{a}	13.094	0.02
AusMeat marbling	1.08^{a}	0.82^{ab}	0.58^{b}	0.84^{ab}	0.119	<0.05
AusMeat meat colour‡	2.27	2.19	2.36	2.46	0.01	0.25
Fat colour	0.93^{a}	0.74^{ab}	0.46^{b}	0.84^{a}	0.115	0.04
Eye muscle area, cm^2	71.3	69.9	70.8	69	0.614	0.051
Rib fat, mm	5.8^{a}	4.7^{b}	4.3^{b}	4.3^{b}	0.202	<0.01
P8 fat, mm	11.7^{a}	10.3^{b}	9.6^{b}	8.5^{c}	0.354	<0.01
Dentition	1.4^{a}	1.5^{a}	1.2^{a}	0.3^{b}	0.176	<0.01
Chiller assessment pH	5.56	5.55	5.58	5.57	0.016	0.65
Loin temperature, ˚C	6.50^{a}	5.18^{b}	4.96^{b}	3.08^{c}	0.172	<0.01
MSA index	60.62	60.37	60.35	60.67	0.225	0.65
Liver glycogen, %	4.39	4.23	4.21	4.47	0.179	0.68
Hide-on standard plate count, CFU/cm^2	$1.5x10^{4a}$	$1.8x10^{4a}$	$4.1x10^{4b}$	$2.4x10^{4a}$	$5.5x10^{3}$	<0.01
Hide-on coliform count, CFU/cm2	$3.8x10^{2}$	$3.0x10^{2}$	$7.8x10^{2}$	$3.6x10^{2}$	$1.4x10^{2}$	0.06
Hide-on E. coli count, CFU/cm2	$1.9x10^{2a}$	$2.6x10^{2a}$	$7.2x10^{2b}$	$3.5x10^{2a}$	$1.0x10^{2}$	<0.01
Chiller standard plate count, CFU/cm2	$3.2x10^{2}$	$3.9x10^{2}$	$9.2x10^{1}$	$1.3x10^{1}$	$1.5x10^{2}$	0.28

Chiller coliform count, CFU/cm2	$2.8x10^{0}$	$6.1x10^{0}$	$4.2x10^{-2}$	$1.4x10^{-2}$	$2.0x10^{0}$	0.12
Chiller E. coli count, CFU/cm2	$2.1x10^{0}$	$6.1x10^{0}$	$1.1x10^{-2}$	$1.2x10^{-2}$	$1.8x10^{0}$	0.08

†Dead weight was calculated as (dead bar bled weight x 1.02).

‡Meat colour was scored as 1A=1.00, 1B=1.33, 1C=1.67, 2=2.00, 3=3.00, 4=4.00, 5=5.00, 6=6.00.

*Truck weights were considered outliers for 5 trucks resulting in a range of SEM being calculated.

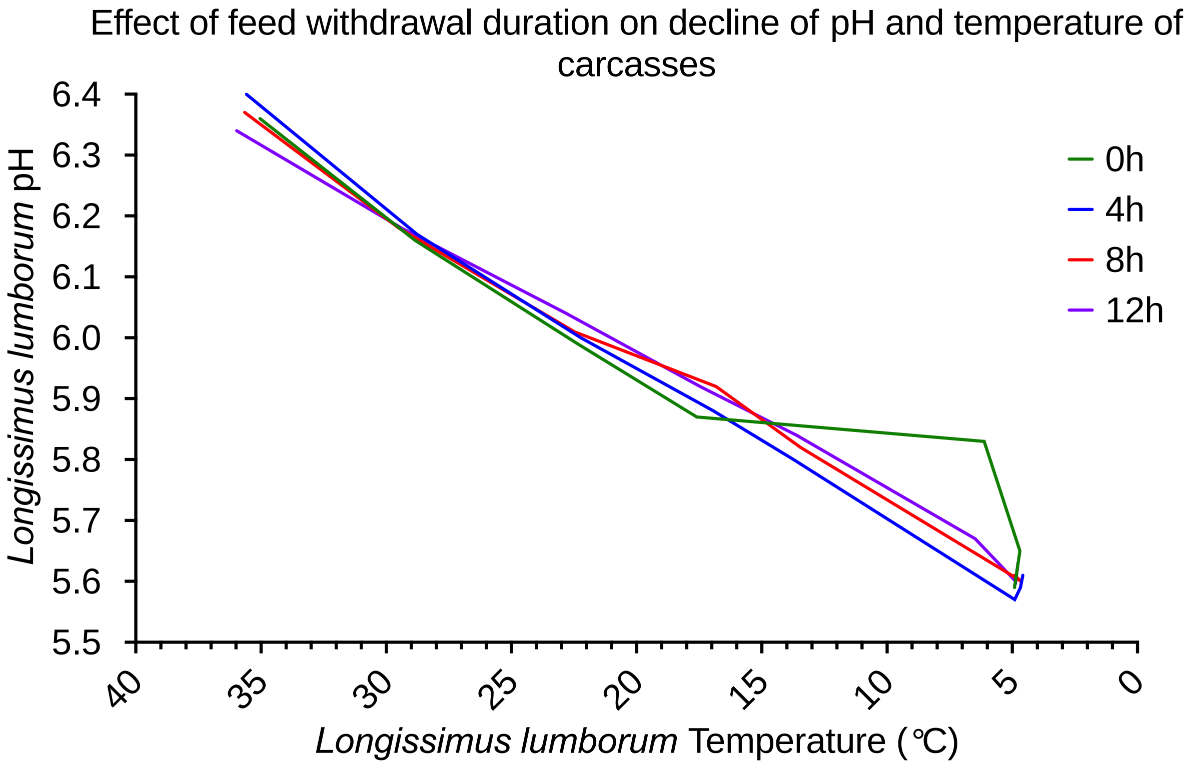

Figure 1. Effect of feed withdrawal duration on decline of pH and temperature of *Longissimus lumborum*

5. Conclusion

5.1 Key findings

- Reduced duration of feed withdrawal resulted in increased feed consumption in the 24 hours prior to feedlot exit and hence, increased cattle weight at feedlot exit. These cattle continued to be heavier throughout the entire journey from feedlot exit to abattoir arrival and abattoir stunning. These results demonstrate that cattle do consume feed overnight in the hours prior to feedlot exit.
- Reduced duration of feed withdrawal resulted in significantly heavier hot carcass weight. This increased hot carcass weight demonstrates the clear financial incentive for reduced duration of feed withdrawal even considering the increased feed costs.
- Cattle shrink along a journey includes effluent (36%) that is captured in truck tanks and loss (64%) that is not captured and includes animal evaporation (cutaneous and respiratory) and truck sources (evaporation, convection, spillage). As expected, reduced duration of feed withdrawal resulted in increased shrink and loss.
- Duration of feed withdrawal had no effect on the total amount of effluent captured during the first portion of the trip from feedlot to abattoir. However, during the second portion of the trip reduced duration of feed withdrawal resulted in a greater amount of effluent captured. The total amount of effluent captured demonstrates there is a source and potential opportunity for this by-product. Appendix 1 describes potential applications for truck effluent.
- Reduced duration of feed withdrawal resulted in higher liver glycogen levels, demonstrating that cattle use their liver glycogen reserves to maintain blood glucose homeostasis as the duration of time off feed increases.
- There was no effect of duration of feed withdrawal on carcass quality measurements including marbling, meat colour, and ultimate pH. Additionally, there was no effect of duration of feed withdrawal on the decline of pH and temperature of the *Longissimus lumborum.* These results clearly demonstrate that duration of feed withdrawal prior to feedlot exit has no effect on meat quality.
- There was no effect of duration of feed withdrawal on microbiological counts or proportion of positive counts for total plate counts, coliform counts or *E. coli* counts on hides or chilled carcasses.
- The results of this study are representative of a single market category: domestic, non-implanted heifers fed a feedlot diet. Results in cattle grazing pasture with different dry matter and energy may differ greatly and hence further research is required to make any inferences for grazing cattle.

5.2 Benefits to industry

- Managing feed allocation to time of dispatch is an important consideration for lot feeders. This project provides an objective dataset to enable discussion with abattoir customers and supply chain participants on impacts of feed withdrawal on carcase weight and truck effluent.
- Truck effluent is a valuable by-product of the feedlot and transport industries. Historically, effluent has been considered a risk to industry. However, this work highlights the potential for capture and application of this resource.
- The results of this trial provide a clear baseline for the volume of effluent captured at multiple stops during a journey from feedlot to abattoir under Australian conditions. These results provide valuable data for future planning of means to capture, treat, and use feedlot cattle effluent produced during transportation.
- While the results of this study demonstrate there are benefits to reduced duration of feed withdrawal, some of these benefits are potentially diminished due to the long duration of lairage applied in the present study. Specifically, the cattle in this study experienced 16 hours of lairage duration which may have compromised the value of reduced duration of feed withdrawal.

6. Future research and recommendations

- Future development opportunities exist to use the results of this study to design methods to capture feedlot cattle effluent produced during transport and subsequently means to treat, prepare, and capture value from this resource.
- Future research is required to determine the effect of duration of feed withdrawal on carcass yield, quality, and safety of grazing cattle. Feed is often withdrawn from grazing cattle greater than 12 hours prior to transport and hence there is immense opportunity for value capture in this area. In addition, other market categories of feedlot cattle may be researched.

7. Acknowledgements

The authors would sincerely thank Australian Country Choice for hosting this project, along with their livestock transporter, truck drivers and project management from Daniel Meehan of Meehan Agribusiness Solutions. The support from University of Queensland for effluent sampling and analysis is also recognised. This project was funded by Meat & Livestock Australia with the support of the Australian Lot Feeders’ Association as a research and development priority.

8. References

ARTHUR, T. M., N. KALCHAYANAND, G. E. AGGA, T. L. WHEELER, and M. KOOHMARAIE. 2017. Evaluation of Bacteriophage Application to Cattle in Lairage at Beef Processing Plants to Reduce Escherichia coli O157:H7 Prevalence on Hides and Carcasses. Foodborne Pathogens and Disease 14(1):17-22. doi: 10.1089/fpd.2016.2189.

COOMBES S.V., GARDNER, G.E., PETHICK, D.W., MCGILCHRIST, P., 2014. The impact of beef cattle temperament assessed using flight speed on muscle glycogen, muscle lactate and plasma lactate concentrations at slaughter. Meat Sci 98, 815-821.

FEDORKA-CRAY, P., D. DARGATZ, L. THOMAS, and J. GRAY. 1998. Survey of Salmonella serotypes in feedlot cattle. Journal of Food Protection 61(5):525-530. doi: 10.4315/0362-028X-61.5.525.

GEORGE, M.M and GEORGE, M.H., 2018. Effect of lairage timing and duration on rumen physiology and muscle glycogen. B.FLT.4002. Meat and Livestock Australia Limited, Locked Bag 1961, North Sydney, NSW 2059.

GRAU, F. H., and L. E. BROWNLIE. 1965. OCCURRENCE OF SALMONELLAS IN THE BOVINE RUMEN. Australian Veterinary Journal 41(10):321-323. doi: 10.1111/j.1751-0813.1965.tb01775.x

GRAU, F. H., L. E. BROWNLIE, and E. A. ROBERTS. 1968. Effect of some Preslaughter Treatments on the Salmonella Population in the Bovine Rumen and Faeces. Journal of Applied Bacteriology 31(1):157-163. doi: 10.1111/j.1365-2672.1968.tb00353.x

GRAU, F. H., and M. G. SMITH. 1974. Salmonella Contamination of Sheep and Mutton Carcasses Related to Pre-slaughter Holding Conditions. Journal of Applied Bacteriology 37(1):111-116. doi: 10.1111/j.1365-2672.1974.tb00421.x

GIANNETTO, C., FAZIO, F., CASELLA, S., MARAFIOTI, S., GIUDICE, E. & PICCIONE, G. 2011. Acute Phase Protein Response during Road Transportation and Lairage at a Slaughterhouse in Feedlot Beef Cattle. *Journal of Veterinary Medical Science,* 73**,** 1531-1534.

HAINES, H., J. BOBBITT, J. SIMONS, D. ROWLAND, and K. COATES. 2000. A review of process interventions aimed at reducing contamination of cattle carcasses, North Sydney, NSW.

KREIKEMEIER, K. K., UNRUH, J. A. & ECK, T. P. 1998. Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. *Journal of Animal Science,* 76**,** 388-395.

KUNZE, D. J., G. H. LONERGAN, T. M. PLATT, M. F. MILLER, T. E. BESSER, M. KOOHMARAIE, T. STEPHENS, and M. M. BRASHEARS. 2008. Salmonella enterica Burden in Harvest-Ready Cattle Populations from the Southern High Plains of the United States. Applied and Environmental Microbiology 74(2):345. doi: 10.1128/AEM.02076-07

ROMERO, M. H., URIBE-VELÁSQUEZ, L. F., SÁNCHEZ, J. A. & MIRANDA-DE LA LAMA, G. C. 2013. Risk factors influencing bruising and high muscle pH in Colombian cattle carcasses due to transport and pre-slaughter operations. *Meat Science,* 95**,** 256-263.

WALKER, K., and BANNEY, S. 2011. Maximising beef eating quality while meeting transport regulations. Meat and Livestock Australia Limited, Locked Bag 1961, North Sydney, NSW 2059.

9. Appendix

9.1 Effect of duration of feed withdrawal on effluent composition

Summary

Truck effluent is an emerging issue for the red meat industry. Truck effluent is comprised of manure, urine, bedding materials, and water collected within livestock transport vehicles. This project was designed to assess the composition of Truck effluent produced during the transport of livestock between farms, feedlots, and processing facilities. The project was designed to support a detailed sampling campaign over a 12-month period. The sampling campaign included samples from 64 trucks split into 3-month blocks. Curfew periods were varied throughout the trial to determine the impact of effluent production and composition. Samples were collected at a Dalby, representing a mid-point during transport, and at Cannon Hill representing the destination.

Waste volumes were approximately 5.3 kg/head at the Dalby mid-point with an additional 2.8 kg/head at the Cannon Hill destination. There was no practical difference in waste volumes at the Dalby mid-point with changes in curfew time. There was a more notable decrease in waste volumes at the Cannon Hill site with increasing curfew time. However, the mass of truck effluent waste ~8kg/head is small in comparison to waste volumes at a typical Australian Red Meat Processor and would have little practical impact on existing waste treatment processes.

Sewer disposal is not a viable option for Truck Effluent. The Truck Effluent significantly exceeds sewer acceptance criteria on several metrics (e.g., solids, N, P). Additionally, the cost of sewer disposal would be prohibitive at over $70-110/m^3 for the different samples, this provides substantial motivation for treatment using existing wastewater treatment infrastructure, either at i) onsite at a Red Meat Processor, ii) at a municipal wastewater treatment plant or iii) at an alternative industry site as a form of co-treatment.

The COD and solids concentrations of the Truck Effluent is 3-5x the typical concentration of combined wastewater at an Australian Red Meat Processing (RMP) facility. The Truck effluent is less suitable for stand-alone treatment in conventional lagoon processes due to this high concentration. However, considering the relatively low volumes of Truck Effluent there would be no technical barriers adding this material to existing RMP wastewater treatment processes, including treatment lagoons. High nitrogen concentration in the Truck effluent represents a risk of biological inhibition in conventional waste treatment and resource recovery processes, again this risk can be managed through dilution or co-treatment options.

While there are likely no strong technical barriers to treating Truck Effluent in existing RMP treatment processes (or treatment at municipal), the Truck Effluent is likely to result in a negative value proposition, i.e., the cost of managing the nutrients and/or residual solids from the Truck Effluent is likely to exceed the value of any biogas revenue. Analysis in this report indicated that the net cost of Truck Effluent ranged from ~$5/m^3 to ~$10/m^3; while the value proposition of treating truck effluent is negative, the treatment costs through an onsite treatment plant at an RMP are 80-90% lower than sewer disposal.

The value proposition could be improved through treatment at a remote or rural location where the nutrients are irrigated onto agricultural land at either low cost (or no cost) rather than removed through expensive biological treatment. In a scenario where the nutrients in truck effluent are applied to land through irrigation and valued at the same rate as chemical fertilizers, economic potential was up to $9.5/m^3, including an energy value of $3/m^3, a potential nitrogen value of $5/m^3 and >$1/m^3 each for phosphorous and potassium.

The land application of wastewaters is typically a licence condition and only viable within a relatively limited local area. Land availability and infrastructure development for irrigation would need to be considered as part of the treatment process development. This strategy may be effective for a portion of the waste (i.e., the mid transport), but would not be practical for waste at the destination (Cannon Hill).

Background

Truck effluent is an emerging issue for the red meat industry. Truck effluent is comprised of manure, urine, bedding materials, and water collected within livestock transport vehicles.

Manure and urine are waste streams produced by animals as part of their regular life cycle and this includes during animal transport operations. When the animals are transported to and from farms, feedlots, and meat processing plants, the effluent is collected on the transport vehicles, which may be fitted with purpose-built effluent holding tanks. Some amount of water is usually included either from rain or from washing trucks.

The composition of a truck effluent grab sample collected by the MLA Waste to Profits project is shown in Table 1 as an example. The composition in Table 1 shows a relatively high concentration of solids and a moderate to high concentration of nitrogen. Preliminary indications are that the waste may be suitable for energy recovery using anaerobic digestion, however, nitrogen/ammonia inhibition may be a challenge that limits the success of biogas technologies.

Table 1 Composition of truck effluent sample collected from MLA waste to profits project

Sample type	Truck Effluent
pH	7.15
TS (g/kg)	65.7 ± 0.3
VS (g/kg)	47.2 ± 0.2
Ash (g/kg)	18.5 ± 0.1
Total COD (g/kg)	58 ± 2
Soluble COD (g/kg)	16.4
Total VFAs (mg/kg)	6971
TKN (mg/kg)	3225
TAN (mg/kg)	1818
TP (mg/kg)	404
PO4-P (mg/kg)	33.3
VS/TS	0.72
TCOD/VS	1.2
sCOD/TCOD	0.29
TCOD/TKN	18
TCOD/TP	145

In general, the composition of truck effluent is expected to be mostly liquid with total suspended solids of 7-53 g/L reported in literature [1]. The composition and concentration of the truck effluent is expected to vary according to truck design and collection practices and/or animal handling practices. In terms of animal management, a curfew is applied where animals are taken off feed and water for a period prior to transport to manage stress and reduce excretion during transport. This is a widespread industry practice and is done in consideration of animal welfare. However, curfew periods are limited to minimise the effects on carcass weight and meat quality. The usual curfew duration is around four hours; however, the impact of varying curfew periods is one of the parameters being investigated through this project.

Despite curfews, significant amounts of excreta are expected during livestock transport and in many cases, effluent production exceeds the on-truck storage capacity. In many cases, effluent tanks must be drained at a mid-point during transport. A study by MLA indicates that 70% of the excreta is produced during the first 4 hours of travel [2], this is significant as the composition of truck effluent removed during mid-point drainage is likely to be different from truck effluent at the final destination.

This project was designed to assess the composition of Truck effluent produced during the transport of livestock between farms, feedlots, and processing facilities. The proposal was designed to support a detailed sampling campaign over a 12-month period. The sampling campaign consisted of samples from 64 trucks split into 3-month blocks. Curfew periods were varied throughout the trial to determine the impact of effluent production and composition. Samples were collected at a mid-point during transport and during at the destination.

The objective of the sample characterisation was:

1. Conduct standard chemical testing of truck effluent samples to identify concentrations of solids, organics, nutrients, and metals.
2. Conduct biochemical methane potential testing of select truck effluent samples to determine the potential to produce energy, fertilizers, and/or other value-add products.
3. Complete a desktop analysis of data collected during the project to determine waste treatment requirements and resource recovery/value-add opportunities related to truck effluent.
4. Complete a high-level desktop assessment of emerging/existing waste treatment technologies for truck effluent applications and/or co-treatment of truck effluent and animal processing wastes. Including existing waste infrastructure in operation at the ACC processing facility in Brisbane.

Composition Results

Composition of Truck Effluent – Dalby

Table presents the average composition of Truck Effluent samples collected from Dalby for various feed curfews ranging from 0 h to 12 h. In terms of waste treatment options and/or process design, there is no practical difference in the waste compositions with changing feed curfews.

Table 2 Composition of Truck Effluent Samples collected at Dalby with increasing curfew time

Time Off Feed		0h	4h	8h	12h	Average
TCOD	$g.L^{-1}$	44.5	44.8	42.2	40.8	43.1
SCOD	$g.L^{-1}$	13.96	14.03	13.99	13.41	13.8
TS	$g.L^{-1}$	41.58	41.74	38.87	37.06	39.8
VS	$g.L^{-1}$	29.88	30.07	27.94	26.81	28.7
Ethanol	$mg.L^{-1}$	40.34	34.94	30.00	24.81	32.5
Organic Acids	$mg.L^{-1}$	3722	3665	3305	3266	3490
TKN	$mg.L^{-1}$	3508	3917	4160	3801	3847
TAN	$mg.L^{-1}$	2359	2575	2788	2684	2601
TP	$mg.L^{-1}$	358	364	360	352	357
PO_4-P	$mg.L^{-1}$	16	17	22	28	20.8
Total Alkalinity	$mg.L^{-1}$	12255	12414	13010	12918	12649
Intermed. Alkalinity	$mg.L^{-1}$	4004	3754	3854	3714	3832
Partial Alkalinity	$mg.L^{-1}$	8251	8660	9157	9204	8818
pH		8.67	8.75	8.74	8.82	8.7
Conductivity	$mS.cm^{-1}$	19.38	20.09	19.78	19.67	19.7
SCOD/TCOD	ratio	0.31	0.31	0.33	0.33	0.32
TCOD/VS	ratio	1.49	1.49	1.51	1.52	1.50
VS/TS	ratio	0.72	0.72	0.72	0.72	0.72
TCOD/TKN	ratio	13	11	10	11	11
TCOD/TP	ratio	124	123	117	116	120
Alkalinity Ratio	ratio	0.49	0.43	0.42	0.40	0.43

Table 2. Effect of feed withdrawal on effluent elements at Dalby (n=64)

	Hours of Feed Withdrawal							
Variable	0h	4h	8h	12h	SE	P-value	Linear	Quadratic
Al (mg/kg)	73.33	80.18	75.28	72.89	7.672	0.39	0.68	0.17
As (mg/kg)	0.01	0.01	0.01	0.01	0.007	0.81	0.45	0.57
B (mg/kg)	0.73	0.86	0.84	0.79	0.116	0.31	0.50	0.09
Ba (mg/kg)	1.44	1.51	1.52	1.52	0.088	0.68	0.32	0.50
Ca (mg/kg)	1125.72	1163.64	1096.30	1031.32	60.666	0.16	0.07	0.22
Cd (mg/kg)	0.00	0.00	0.00	0.00	n/a	n/a	n/a	n/a
Co (mg/kg)	0.08	0.08	0.07	0.07	0.012	0.84	0.54	0.78
Cr (mg/kg)	0.95	1.08	1.00	0.94	0.089	0.21	0.66	0.07
Cu (mg/kg)	2.84	3.03	2.97	2.80	0.231	0.40	0.69	0.10
Fe (mg/kg)	212.50	236.59	216.62	223.24	20.167	0.66	0.85	0.55
K (mg/kg)	1962.18	2031.84	2011.81	1883.18	80.272	0.32	0.34	0.11
Mg (mg/kg)	310.28	312.17	294.34	271.21	14.960	0.02	<0.01	0.22
Mn (mg/kg)	9.56	10.09	9.88	10.02	0.423	0.73	0.47	0.59
Mo (mg/kg)	0.13	0.13	0.13	0.12	0.017	0.54	0.26	0.39
Na (mg/kg)	1074.41	913.83	783.48	736.24	39.889	<0.01	<0.01	0.11
Ni (mg/kg)	0.35	0.40	0.36	0.37	0.032	0.65	0.88	0.45
P (mg/kg)	413.38	423.12	424.62	415.66	21.255	0.92	0.89	0.50
Pb (mg/kg)	0.03	0.01	0.02	0.02	0.010	0.53	0.45	0.31
S (mg/kg)	383.56	375.70	383.00	361.98	13.350	0.46	0.24	0.54
Se (mg/kg)	0.12	0.11	0.13	0.14	0.015	0.57	0.24	0.48
Zn (mg/kg)	14.48	15.04	14.53	13.96	0.713	0.57	0.39	0.30

Composition of Truck Effluent – Cannon Hill

Table 3 presents the average composition of Truck Effluent samples collected from Cannon Hill for various feed curfews ranging from 0 h to 12 h. In terms of waste treatment options and/or process design, there is no practical difference in the waste compositions with changing feed curfews.

Table 3 Composition of Truck Effluent Samples collected at Cannon Hill with increasing time off feed

Time Off Feed		0h	4h	8h	12h	Average
TCOD	$g.L^{-1}$	28.2	27.3	26.6	25.5	26.9
SCOD	$g.L^{-1}$	7.51	7.38	7.26	6.66	7.2
TS	$g.L^{-1}$	27.47	24.61	25.33	23.78	25.3
VS	$g.L^{-1}$	20.43	18.28	19.11	17.85	18.9
Ethanol	$mg.L^{-1}$	19.13	12.69	9.06	9.25	12.5
Organic Acids	$mg.L^{-1}$	1910	1786	1712	1574	1746
TKN	$mg.L^{-1}$	1930	2350	2468	2351	2279
TAN	$mg.L^{-1}$	1508	1693	1815	1731	1687
TP	$mg.L^{-1}$	170	180	191	249	197
PO_4-P	$mg.L^{-1}$	9	11	14	16	12.6
Total Alkalinity	$mg.L^{-1}$	7217	7574	7897	7651	7584
Intermed. Alkalinity	$mg.L^{-1}$	2192	2087	2116	1998	2098
Partial Alkalinity	$mg.L^{-1}$	5025	5487	5781	5653	5486
pH		8.80	8.94	8.96	8.95	8.9
Conductivity	$mS.cm^{-1}$	12.57	12.75	13.06	13.90	13.1
SCOD/TCOD	ratio	0.27	0.27	0.27	0.26	0.27
TCOD/VS	ratio	1.38	1.49	1.39	1.43	1.42
VS/TS	ratio	0.74	0.74	0.75	0.75	0.75
TCOD/TKN	ratio	14.6	11.6	10.8	10.8	11.8
TCOD/TP	ratio	165.7	151.1	139.5	102.4	136.1
Alkalinity Ratio	ratio	0.44	0.38	0.37	0.35	0.38

Table 4. Effect of feed withdrawal on effluent elements at Cannon Hill (n=64)

	Hours of Feed Withdrawal							
Variable	0h	4h	8h	12h	SE	P-value	Linear	Quadratic
Al (mg/kg)	45.50	42.10	41.74	40.11	3.419-3.458*	0.21	<0.05	0.62
As (mg/kg)	0.00	0.00	0.01	0.00	0.00431-0.00445*	0.11	0.53	0.16
B (mg/kg)	0.59	0.56	0.56	0.54	0.0679-0.0681*	0.41	0.11	0.73
Ba (mg/kg)	0.85	0.81	0.83	0.85	0.0512-0.0519*	0.71	0.94	0.28
Ca (mg/kg)	600.06	563.00	547.67	507.43	28.38-28.94*	0.02	<0.01	0.94
Cd (mg/kg)	0.00	0.00	0.00	0.00	n/a	n/a	n/a	n/a
Co (mg/kg)	0.01	0.00	0.00	0.01	0.00532-0.00547*	0.18	0.33	0.05
Cr (mg/kg)	0.71	0.68	0.67	0.64	0.0461-0.0469*	0.45	0.11	0.93
Cu (mg/kg)	1.64	1.54	1.58	1.46	0.127-0.128*	0.22	0.07	0.81
Fe (mg/kg)	139.43	134.11	134.04	135.53	10.329-10.558*	0.95	0.74	0.66
K (mg/kg)	976.63	952.97	941.13	864.11	36.351-37.247*	0.05	0.01	0.36
Mg (mg/kg)	179.31	166.05	161.17	148.08	8.798-8.907*	<0.01	<0.01	0.99
Mn (mg/kg)	5.14	5.00	5.11	5.09	0.212-0.217*	0.94	0.95	0.71
Mo (mg/kg)	0.08	0.06	0.07	0.08	0.0122-0.0124*	0.25	0.84	0.05
Na (mg/kg)	592.72	529.64	498.79	451.27	19.669-20.173*	<0.01	<0.01	0.63
Ni (mg/kg)	0.20	0.19	0.18	0.19	0.0193-0.0199*	0.89	0.70	0.52
P (mg/kg)	225.90	224.04	235.09	224.57	15.097-15.236*	0.65	0.82	0.54
Pb (mg/kg)	0.01	0.01	0.01	0.02	0.00799-0.00824*	0.51	0.48	0.18
S (mg/kg)	257.86	254.09	264.52	251.48	8.964-9.169*	0.56	0.78	0.50
Se (mg/kg)	0.10	0.09	0.11	0.12	0.0145-0.0148*	0.52	0.21	0.45
Zn (mg/kg)	7.89	7.41	7.45	7.00	0.369-0.377*	0.16	0.04	0.95

*One sample was considered an outlier resulting in a range of SEM being calculated across the four treatments.

Notes on Truck Effluent Composition

A core aspect of this assessment is the potential for waste streams to be treated using anaerobic digestion or other common waste treatment technologies, which can be based on 5 key areas:

(a) **Solids Concentrations:** Solids concentration is linked to i) organic loading potential, where materials with higher solids can potentially achieve higher loading rates, ii) materials handling requirements, where materials with higher solids may be more difficult to handle, transport, and/or mix within the digester and iii) technology suitability, where co-substrates with very low solids are generally suited to different reactor technologies compared to co-substrates with very low solids. The fractionation between volatile solids and ash also provides an indication of inert material entering the digestion process. Ash will not contribute to AD and will either accumulate within the process or exit as residual solids. Therefore, co-substrates with very low ash content are preferred.

(b) **Chemical Oxygen Demand:** Chemical oxygen demand represents the energy potential of a substrate. Anaerobic co-digestion is generally applied to existing AD infrastructure, in this type of application reactor volumes are a fixed size. Adding co-substrates will generally increase the total volume of waste being treated in the reactor and this decreases retention time. Co-substrates with very high COD can be used to significantly increase organic loading to a reactor with only minor changes to volume loading and retention time. However, dosing of these substrates must be managed carefully to limit the risk of overload. The fractionation between soluble COD and particulate COD provides a qualitative indication of degradable fraction and speed of degradation. Co-substrates with high soluble fractions are more likely to have high degradability and rapid digestion kinetics.

(c) **Nitrogen Concentration:** Nitrogen is required in low amounts to support the growth of anaerobic digestion microorganisms. However, depending on the application, excess nitrogen can represent i) a significant cost associated with importing wastes for co-digestion (i.e., where nitrogen is mobilised and must be removed prior to discharge of the centrate) or ii) a value-add opportunity where nitrogen increases the fertilizer value of the centrate and/or solid digestate.

(d) **Phosphorous Concentration:** Phosphorus is required in low amounts to support the growth of anaerobic digestion microorganisms. However, depending on the application, excess phosphorous can represent i) a significant cost associated with importing wastes for co-digestion (i.e., where phosphorus is mobilised and must be removed prior to discharge of the centrate) or ii) a value-add opportunity where phosphorus increases the fertilizer value of the centrate and/or solid digestate.

(e) **Alkalinity:** Alkalinity is a form of dissolved inorganic carbon that has multiple functions within wastewater treatment processes. In anaerobic systems, an alkalinity concentration in the range from 2000 to 4000 mg/L as $CaCO_3$ is typically needed to offset the dissolved carbon dioxide produced within the process (CO_2 is a component of biogas). Higher concentrations may be required for waste streams that contain a large concentration of organic acids (Alkalinity ratios of 0.4 or lower desired). Alkalinity is also consumed in biological nutrient removal processes.

Considering the COD and solids concentrations, the Dalby Truck Effluent is 4-5x the typical concentration of combined wastewater at an Australian Red Meat Processing (RMP) facility (Appendix 2). The concentration of Cannon Hill Truck Effluent was less concentrated, but still 3-4x the concentration of typical combined RMP wastes. If looking at stand-alone treatment (i.e., dedicated treatment of Truck Effluent only), the concentration of Truck Effluent is very high for lagoon-based treatment processes, although this could be managed through a sufficiently large retention time within the lagoon.

The COD and solids concentrations of the Truck Effluent are similar to the concentrations of thickened Primary Sludge (PS) and Waste Activated Sludge (WAS) at many large municipal wastewater treatment plants. Mixed liquor reactors are more common for wastes at these solids' concentrations. However, if looking at treatment onsite at an existing RMP, the volume of truck effluent is relatively small with ~8 kg/animal of truck effluent compared to >1kL of wastewater per animal at a typical Australian RMP (often >2kL per animal). There would be no technical barriers to adding this material to existing RMP wastewater treatment processes, including treatment lagoons.

Alkalinity concentrations were high in all samples. Importantly, the alkalinity ratios were less than 0.5 in all samples and usually closer to 0.4. The alkalinity is likely to provide sufficient pH buffering for stable biological treatment.

The nitrogen concentrations of the Truck Effluent are relatively high, this will have implications on the cost of treatment, but also impacts the suitability and risk of common treatment technologies. TAN concentrations were >1,500 mg/L at Cannon Hill and >2,300 mg/L at Dalby. These concentrations are in the range where biological inhibition can occur in anaerobic treatment processes. Importantly, TAN represents only a portion of nitrogen in the waste streams, the TKN was up to 4,000 mg/L in the Dalby samples. This demonstrates that TAN concentration up to 4,000 mg/L could be reached during biological treatment (where organic nitrogen in TKN is converted to TAN). Anaerobic microorganisms can adapt to elevated nitrogen concentrations and therefore stand-alone anaerobic treatment of Truck Effluent is possible, however biological inhibition remains a risk for stand-alone treatment of Truck Effluent. This risk could be mitigated by diluting the wastes with water or through a co-treatment strategy.

Biochemical Analysis of Truck Effluent Samples

Assessment Methodology

Anerobic biodegradability of the truck effluent samples was determined using bench-scale Biomethane Potential (BMP) tests. Inoculum used in the tests was collected from a mesophilic anaerobic digester treating mixed sludge at a municipal wastewater treatment plant in Southeast Queensland. Truck effluent samples were collected at the mid-point site (Dalby) and the final site (Cannon Hill) with the truck wash in the 4th week of the February 2021 sampling campaign. The Samples were composited on site and stored in a cold room at 4˚C before composition analysis and BMP testing. The ratio of inoculum to substrate (ISR) used throughout all BMP tests in this report is 2.5 (VS basis). Triplicate blanks (inoculum only) were used to measure and correct for background methane produced from the inoculum; these corrections have been applied to all results presented. A summary of the experimental design used in the laboratory testing is shown in Table 2.

Table 2 Experimental Design used in the BMP testing of the samples

Substrate	Inoculum		Waste samples		ISR
	g wet	g VS	g wet	g VS	
Blank	160.0	3.52	-	-	-
Dalby Mid-point T1	128.8	2.84	31.2	1.14	2.5
Dalby Mid-point T2	126.3	2.78	33.7	1.11	2.5
Dalby Midpoint T3	124.8	2.75	35.2	1.10	2.5
Dalby Mid-point T4	130.3	2.87	29.7	1.15	2.5
CH Truck wash T1	99.0	2.18	61.0	0.87	2.5
CH Truck wash T2	99.7	2.20	60.3	0.88	2.5
CH Truck wash T3	108.2	2.38	51.8	0.86	2.5
CH Truck wash T4	102.3	2.25	57.7	0.90	2.5

Biochemical Methane Production

Cumulative methane production during the BMP tests is shown in Figure 1 and Figure 2 expressed on a substrate VS basis and substrate COD basis respectively. Methane potential from the samples ranged from 270 L CH_4.kgVS^{-1} to 310 L CH_4.kgVS^{-1}; this is towards the upper range of methane yields expected for manure samples collected from either i) beef feedlots or ii) cattle yards at beef processing facilities [3, 4]. In general, the methane potential appeared slightly higher for the Dalby mid-point samples.

Section 2.4 notes that nitrogen concentrations in the effluent samples were relatively high. Specifically, TKN was up to 4,000 mg/L in the Dalby samples. This demonstrates that TAN concentration up to 4,000 mg/L could be reached during biological treatment (where organic nitrogen in TKN is converted to TAN); and was identified as an inhibition risk. The BMP tests contain both inoculum and truck effluent samples; this set up condition essentially dilutes potentially inhibitory components in the truck effluent. However, we note that the BMP tests showed no signs of biological inhibition. This demonstrates that any potential inhibition risks from the truck effluent can be managed through dilution.

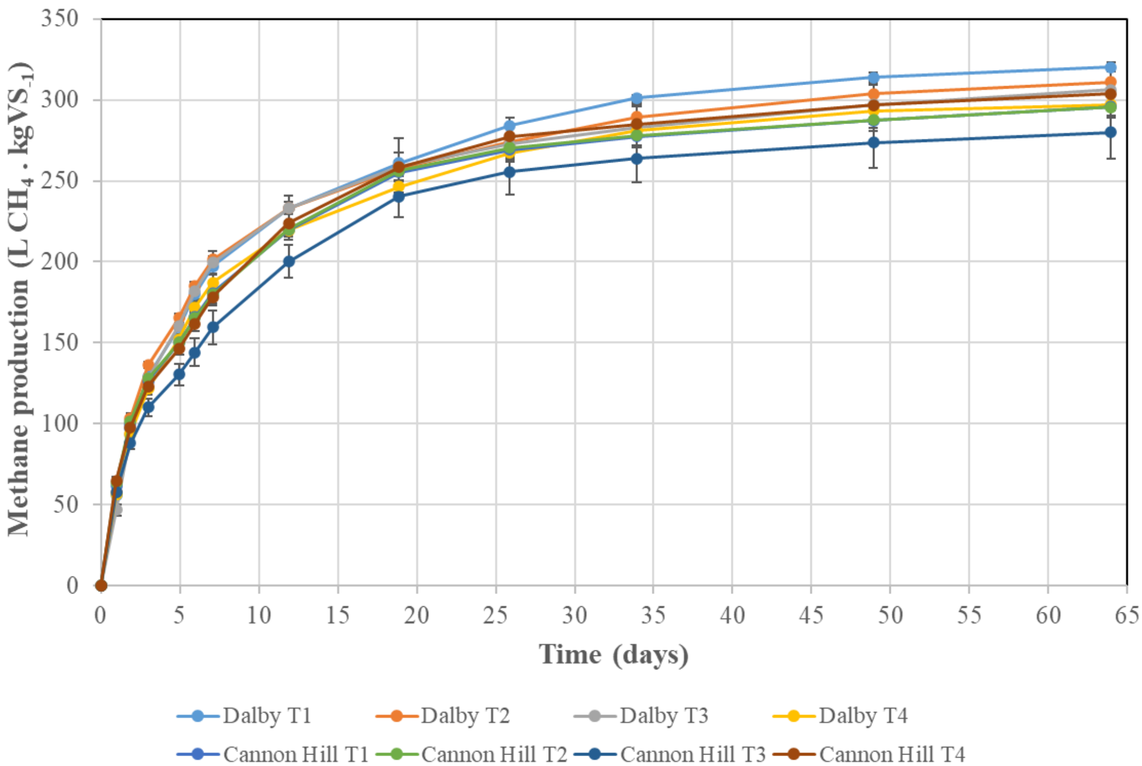

Figure 1 Methane production from BMP test digesting truck effluent samples at 37 ˚C using digested sludge (normalised to 0 ˚C and 1 atm and expressed as methane volume per mass of volatile solids)

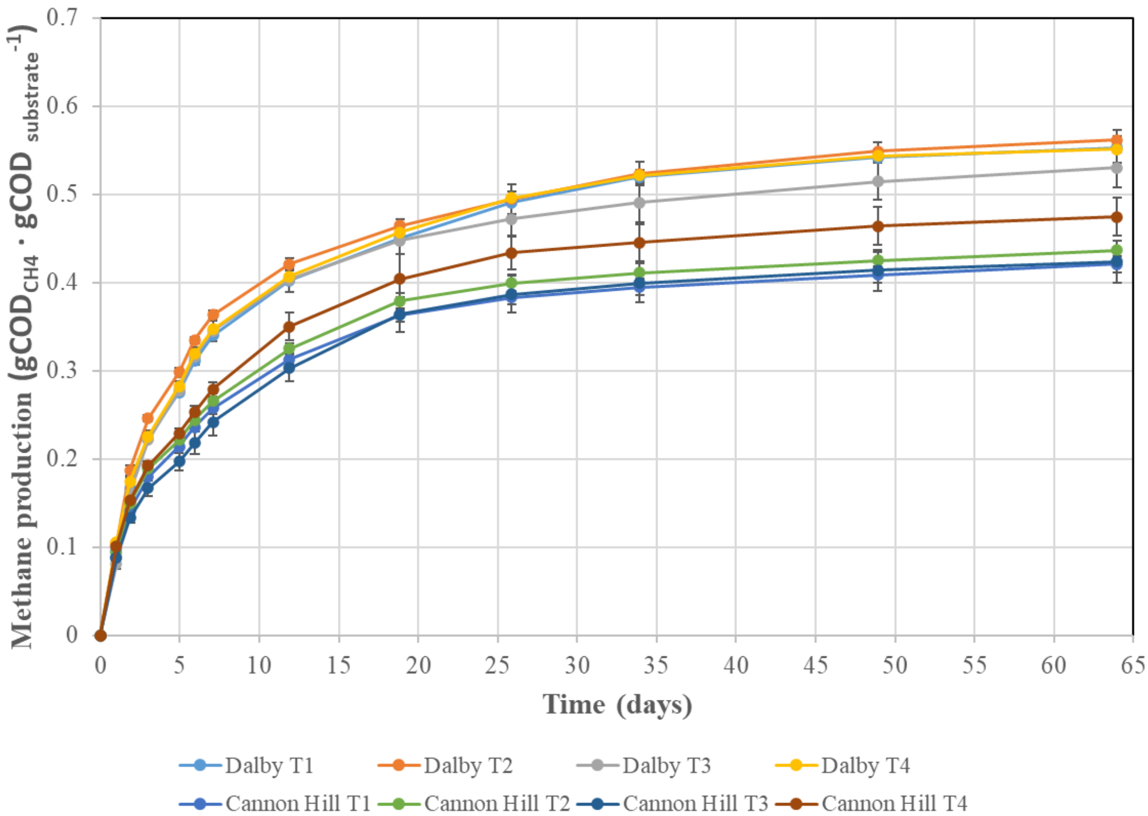

Figure 2 Methane production from BMP tests digesting truck effluent samples at 37 ˚C using digested sludge (normalised to 0 ˚C and 1 atm and expressed as a fraction of substrate COD added)

Anaerobic Degradability Analysis

Table 3 summarises the degradability parameters determined from model-based analysis of BMP test for each of the truck effluent samples in this report. Model based analysis showed a methane potential of the Dalby Mid-Point samples at 280 to 300 L·kgVS^{-1} corresponding to a degradable fraction of approximately 49-52%. The methane potential of the Cannon Hill Truck Wash samples was lower at 267 to 288 L·kgVS^{-1} corresponding to a degradable fraction of approximately 40-45%; this was a minor but consistent difference between the samples.

The apparent hydrolysis rate of the Dalby midpoint samples was estimated at 0.15-0.18 day^{-1}. The apparent hydrolysis rate of the Cannon Hill Truck Wash was marginally slower at 0.14-0.16 day^{-1} and does not represent a practical difference for anaerobic digester design. The results are consistent with manure from beef cattle and indicate a degradation time of approximately 25 days in a mixed liquor reactor.

Table 3 Kinetic parameters estimated from model analysis of BMP test degrading truck effluent samples

Substrate	Apparent Hydrolysis Rate (k_{hyd}): (d^{-1})	Degradable Fraction (f_d):	Methane Potential (B_0): ($L\ CH_4.kgVS^{-1}$)	Methane Potential: ($m^3.t^{-1}$ wet)
Dalby T1	0.15 ± 0.01	0.52 ± 0.02	300 ± 7	10.92 ± 0.03
Dalby T2	0.18 ± 0.01	0.52 ± 0.03	288 ± 7	9.49 ± 0.03
Dalby T3	0.17 ± 0.01	0.49 ± 0.02	286 ± 6	8.91 ± 0.03
Dalby T4	0.16 ± 0.01	0.52 ± 0.02	279 ± 7	10.78 ± 0.03
Cannon Hill T1	0.16 ± 0.01	0.40 ± 0.03	278 ± 7	3.98 ± 0.03
Cannon Hill T2	0.16 ± 0.01	0.41 ± 0.03	279 ± 7	4.06 ± 0.06
Cannon Hill T3	0.14 ± 0.01	0.40 ± 0.03	267 ± 7	4.92 ± 0.06
Cannon Hill T4	0.15 ± 0.01	0.45 ± 0.03	288 ± 8	4.50 ± 0.05

Assessment of Sewer Discharge

Cost Methodology

Sewer discharge has been assessed as a baseline option for management of Truck Effluent. Australia Country Choice is in the Brisbane Region of Queensland, therefore trade waste acceptance standards and trade waste charges applied by Urban Utilities (2021/22) have been used in the analysis.

Table 4 provides examples of Fees and Charges for Urban Utilities business customers in the Brisbane region. Trade waste costs will be estimated by determining the mass of contaminants per m^3 of waste and therefore the discharge cost per m^3 of that waste. BOD of samples was not directly measured during the sample campaign, during the analysis, the BOD load was estimated at 50% of the COD load.

Table 4 Examples of Trade waste Charges in the Brisbane Region of Queensland[1]

	Tariff	Units
Volume	$1.050	Per kL
Total suspended Solids (>500 mg/L)	$1.950	Per kg
Chemical Oxygen Demand (>1200 mg/L)	$1.160	Per kg
Total Kjeldahl Nitrogen (>60 mg/L)	$2.940	Per kg
Phosphorus (>14 mg/L)	$5.570	Per kg

1. Accessed: https://urbanutilities.com.au/business/business-services/trade-waste/trade-waste-charges-2021-2022

Table 5 shows the general trade waste acceptance standards for Urban Utilities. Urban Utilities notes that higher concentrations of these substances may be accepted to sewer, but that additional charges and/or permitting requirements may apply. Importantly, the Truck Effluent samples significantly exceed the general sewer acceptance criteria in a range of metrics, including COD, solids, N, and P.

Table 5 General Trade waste Acceptance Standards for Urban Utilities[1]

Substance	Values
Temperature	<38 °C
pH	6- 10.5
BOD	1000 mg/L (25 kg/d)
COD	2000 mg/L
Suspended Solids	500 mg/L
Total Dissolved Solids	5000 mg/L
Total Oil/Grease	200 mg/L
Gross Solids	Smaller than 13mm, settling velocity <3 m/h
Colour	Not detectable 1:100 dilution
Sulfate	2,000 mg/L
Sulfite	100 mg/L
Sulfide	1 mg/L
Ammonia	150 mg/L
TKN	150 mg/L (3.75 kg/d)
Phosphorus	20 mg/L (1.25 kg/d)
Boron	100 mg/L
Bromine	10 mg/L
Chlorine	10 mg/L

Cost to Sewer Discharge – Dalby Samples

Analysis of trade waste charges applicable to combined truck effluent at the Dalby collection point is shown in Figure 3. Trade waste liabilities ranged from $120/m^3 to $110/m^3, with an average value of approximately $117/m^3. The volume of waste at the Dalby site was approximately 400 kg/truck, corresponding to sewer costs of $44 to $48 per truck. Approximately 65% of this cost can be attributed to the solids content of the waste. Nutrients were a relatively minor contributor at approximately 10% of the cost.

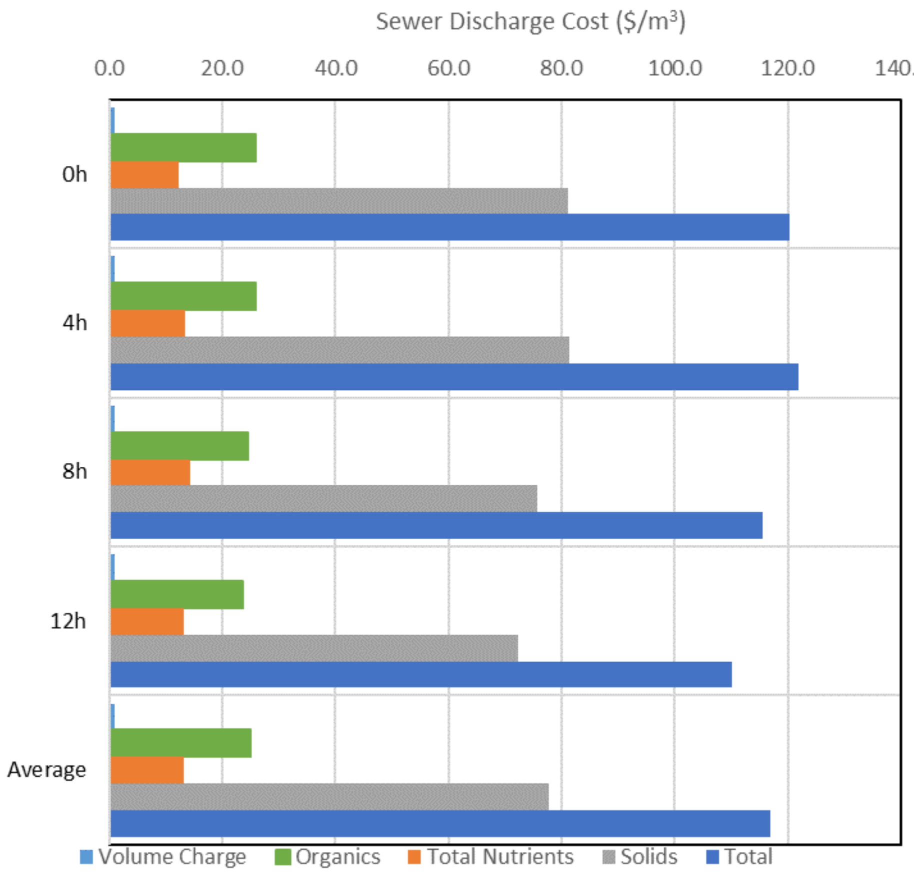

Figure 3 Trade waste costs for discharging Truck Effluent sample collected from Dalby into sewers in the Brisbane Region (Volume: $1.05/kL; BOD: $1.16/kg; Solids: $1.95/kg; TKN: $2.94/kg; TP: $5.57/kg).

Cost to Sewer Discharge – Cannon Hill Samples

Analysis of trade waste charges applicable to combined truck effluent at the Dalby collection point is shown in **Figure 4**. Trade waste liabilities for the Cannon Hill samples were lower and ranged from $77/m^3 (0h) to $70/m^3 (12h), with an average value of approximately $73.8/m^3. The volume of waste at the Cannon Hill site was also lower, ranging from approximately 270 kg/truck (0h) to 200 kg/truck (12h), this corresponded to lower estimated sewer costs at $21 (0h) to $15 (12h) per truck. Again, the solids content of the waste contributed approximately 65% of this cost and nutrients were a relatively minor contributor (~10% of the cost).

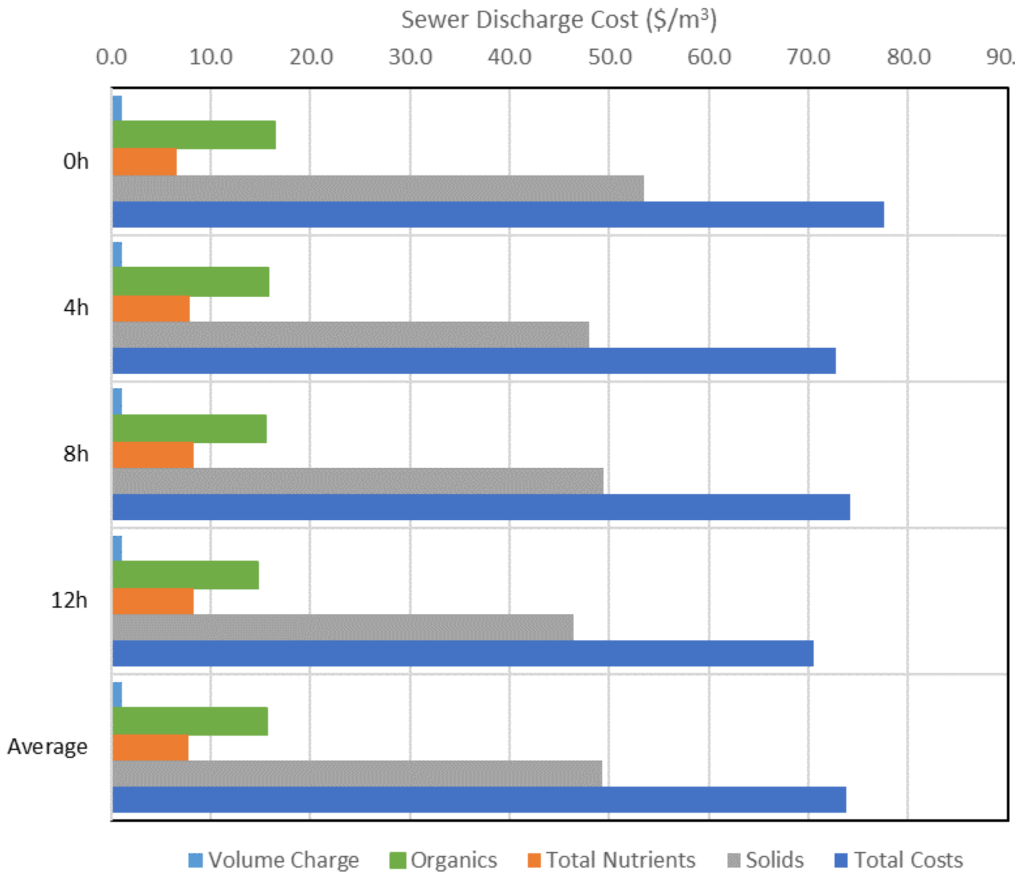

Figure 4 Trade waste costs for discharging Truck Effluent sample collected from Cannon Hill into sewers in the Brisbane Region (Volume: $1.05/kL; BOD: $1.16/kg; Solids: $1.95/kg; TKN: $2.94/kg; TP: $5.57/kg).

Value Proposition of Truck Effluent Treatment

Compatibility with Common Treatment Options

Results of the trade waste assessment demonstrated that the truck effluent exceeded sewer acceptance criteria on several metrics (e.g., solids, N, P). The trade waste cost assessment also showed a potential cost liability of over $100/m^3 for sewer disposal of truck effluent, this provides substantial motivation for treatment using existing wastewater treatment infrastructure, either at i) onsite at a Red Meat Processor, ii) at a municipal wastewater treatment plant or iii) at an alternative industry site as a form of co-treatment.

Common treatment methods for slaughterhouse wastewater vary widely, however, these wastewater treatment processes follow a consistent structure including primary treatment, secondary treatment, and tertiary treatment/polishing (summarised in **Figure 5**). Lagoon-based treatment processes are currently the most common form of technology at Australian RMP, in general lagoons are low intensity reactor designs and are applied to low or moderate strength wastes.

Mixed liquor reactors are more common for wastes at solids concentrations similar to the Truck Effluent. However, if looking at treatment onsite at an existing RMP, the volume of truck effluent is relatively small with ~8 kg/animal of truck effluent compared to >1kL of wastewater per animal at a typical Australian RMP (often >2kL per animal). There would be no technical barriers that prevent this material being added to existing RMP wastewater treatment processes, including treatment lagoons.

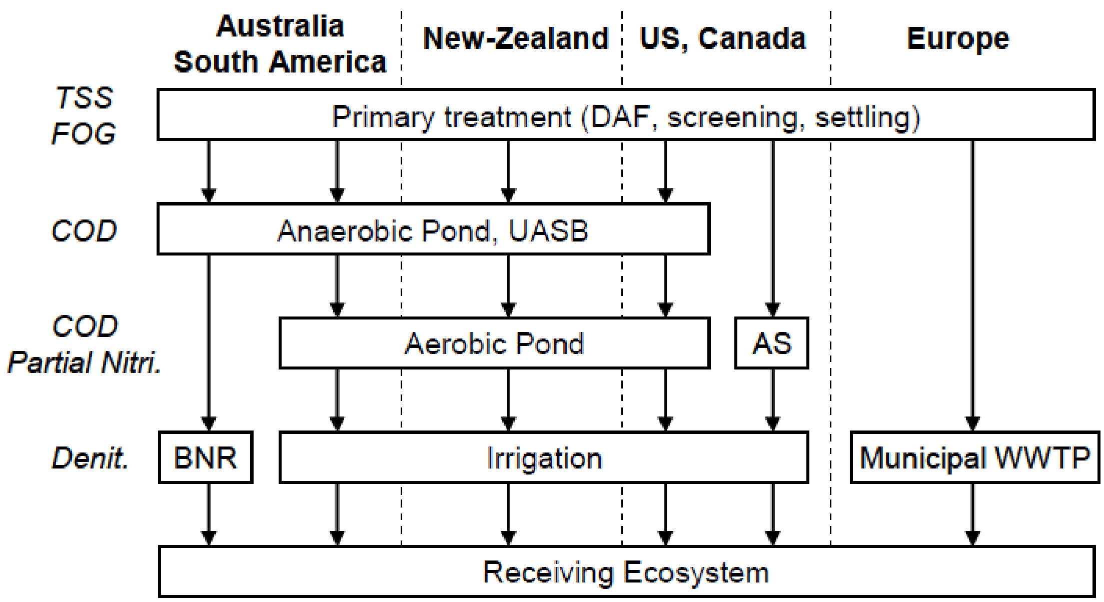

Figure 5: Principal wastewater treatment set-up of the meat industry [5]. Note: At some smaller Australian plants, primary treatment may be bypassed and/or raw effluent may be used for irrigation or land application.

Cost Methodology – Integrated Treatment

This section estimates the value proposition of truck effluent samples when added to an existing treatment process that incorporates anaerobic digestion followed by biological nutrient removal. The value proposition was developed to compare the overall value proposition of wastes using the difference between i) the potential biogas revenue from the waste and ii) the downstream treatment costs after AD to meet a typical environmental discharge license (costs to remove the nutrients and manage the solids); this results in a net value proposition of the waste.

The value proposition of each waste stream was assessed using 4 primary factors:

- Revenue from biogas energy, estimated at $10 per GJ and using a heating value of 55 MJ/kg for methane.
- Management of solids residues, estimated at $65 per T of wet solids (dewatered to 25% cake solids).
- Additional nitrogen mobilisation (to centrate) and removal cost, estimated at $2.9 per kg.

- Additional phosphorus mobilisation (to centrate) and removal cost, estimated at $5.7 per kg.

Notes:

1. This is not a complete economic analysis; it is a high-level assessment of economic potential considering major cost factors associated with wastewater treatment.
2. The removal costs of N and P are based on publicly available trade waste charges for Urban Utilities, these trade waste charges typically represent the cost of treatment operations to remove these compounds.
3. The solids disposal cost of $65/wet ton is based on beneficial land application managed through a third-party contractor in Queensland and may include transport in the range of 100 - 200 kms, this figure is intended for illustrative purposes.

Value Proposition of Truck Effluent – Dalby Samples

Figure 6 and Figure 7 show preliminary economic analyses for truck effluent samples collected from Dalby after feed curfews ranging from 0h to 12h. The first analysis (shown in Figure 6) assumes that all waste streams have a very high biological degradability, meaning that approximately 90% of the COD is converted to biogas, therefore representing the best-case scenario in terms of energy recovery. This has 2 additional consequences to the economics i) 90% of the nutrients are mobilised into the wastewater as ammonia and phosphate respectively and must then be treated/removed as part of the WWTP; and ii) 90% of the VS is converted to biogas resulting in lower residual solids. However, literature shows that cattle manure streams typically have a significantly lower degradable fraction and would therefore respond to treatment differently. The second economic analysis (shown in Figure 7) uses a more typical degradable fraction of 0.5 for each truck effluent stream.

Results in Figure 6 and Figure 7 demonstrate a negative economic potential for all truck effluent samples for the economic metrics used in the analysis. The negative economic potential ranged from \$9.40/m^3 (0h) to \$11.15/m^3 (8h) using a degradable fraction of 0.9; or \$9.90/m^3 (12h) to \$10.70/m^3 (8h) using a degradable fraction of 0.5. In this analysis, curfew time did not have a practical impact on the value proposition of the truck effluent and would not have a practical impact on the design or operation of a waste treatment plant.

The results shown in Figure 6 represent the upper limit of biogas production from the truck effluent wastes. In this scenario, biogas revenue from the truck effluent was potentially as high as \$5.50/m^3 for 0h off feed, reducing to \$5.05/m^3 for 12h off feed. However, the costs of managing nitrogen in the waste streams was \$11/m^3 to \$13/m^3; i.e., the cost of managing nutrients in the waste through conventional biological removal processes was more than double the predicted biogas revenue, this is a result of the consistently low COD/N ratio of the waste streams. While this analysis assumed near complete degradation of organic solids in the truck effluent, there was a significant concentration of inorganic solids in the waste, and this resulted in solids management costs of \$3.3 to \$3.8/m^3.

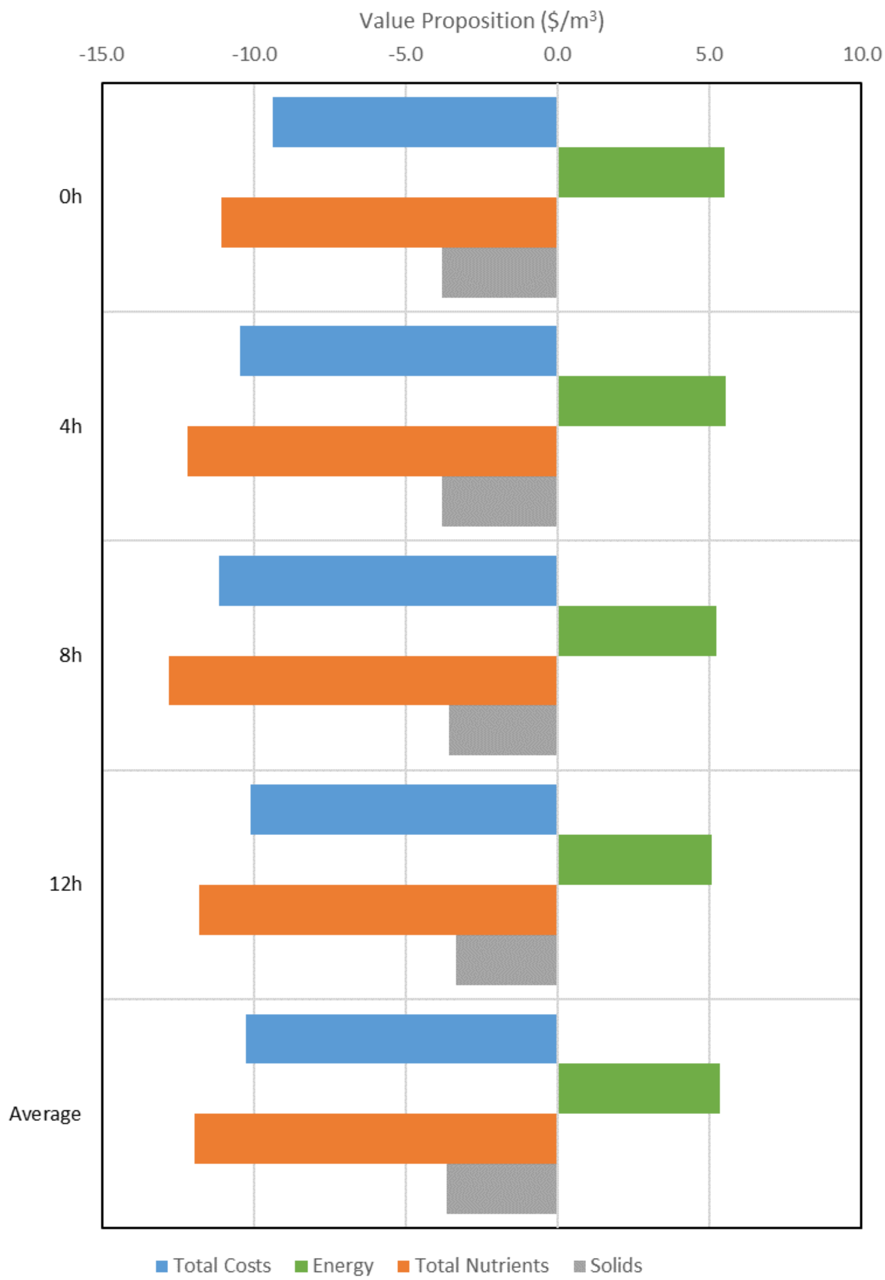

Figure 6 Value proposition of Truck Effluent collected from Dalby. During this assessment the biodegradable fraction was assigned at 90% to represent a best-case scenario for energy recovery, energy was valued at $10/GJ, nitrogen and phosphorus were assigned treatment costs of $2.9/kg and $5.57/kg. Solid's disposal costs were estimated at $65/wet ton and dewatered cake solids as 25% TS.

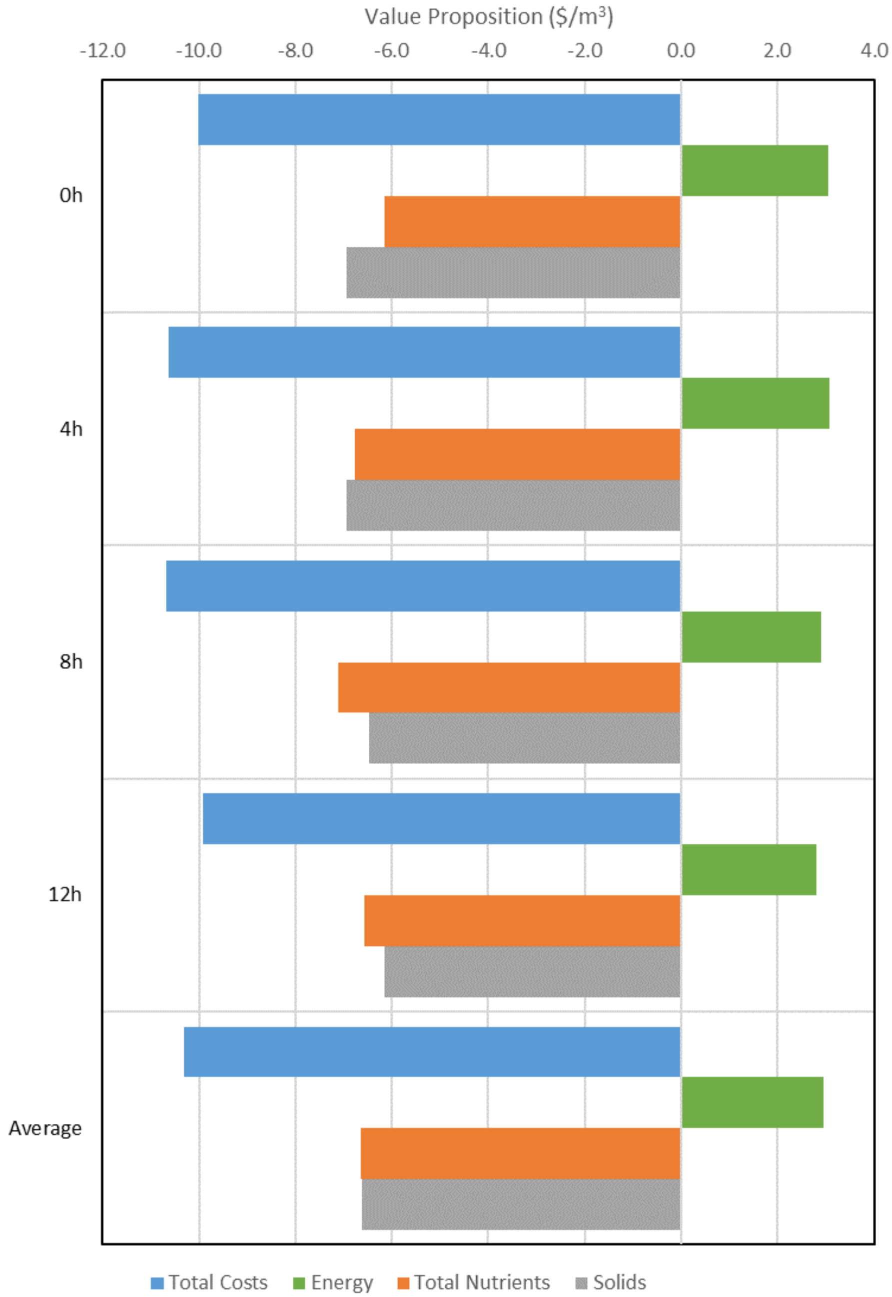

Figure 7 Value proposition of Truck Effluent collected from Dalby. During this assessment the biodegradable fraction was assigned at 50% to represent a more typical scenario for energy recovery from cattle manures, energy was valued at $10/GJ, nitrogen and phosphorus were assigned treatment costs of $2.9/kg and $5.57/kg. Solid's disposal costs were estimated at $65/wet ton and dewatered cake solids as 25% TS.

The results shown in Figure 7 a more typical biogas production from the truck effluent wastes. In this scenario, biogas revenue from the truck effluent was reduced to $3.06/m^3 for 0h off feed or $2.8/m^3 for 12h off feed. In this scenario, the nutrient mobilisation was lower (with nitrogen and phosphorus retained in the solids and not passed to the down-stream nutrient removal plant), However, the costs of managing the nutrients were still approximately double the value of the biogas. In this scenario, the cost of managing solid residues was the largest component at ~$6.5/m^3$, again double the value of the biogas.

While the value proposition of treating truck effluent is negative, the treatment costs through an onsite treatment plant at an RMP are 80-90% lower than sewer disposal.

Value Proposition of Truck Effluent – Cannon Hill Samples

Figure 8 and **Figure 9** show preliminary economic analyses for truck effluent samples collected from Cannon Hill after feed curfews ranging from 0h to 12h. The first analysis (shown in **Figure 8**) assumes that all waste streams have a very high biological degradability, meaning that approximately 90% of the COD is converted to biogas, therefore representing the best-case scenario in terms of energy recovery. This has 2 additional consequences to the economics i) 90% of the nutrients are mobilised into the wastewater as ammonia and phosphate respectively and must then be treated/removed as part of the WWTP; and ii) 90% of the VS is converted to biogas resulting in lower residual solids. However, literature shows that cattle manure streams typically have a significantly lower degradable fraction and would therefore respond to treatment differently. The second economic analysis (shown in **Figure 9**) uses a more typical degradable fraction of 0.5 for each truck effluent stream; this degradable fraction is consistent with the results of BMP testing presented in Section 3.

Results of the value proposition analysis for Cannon Hill samples was generally similar to that of the Dalby samples, although the Cannon Hill samples were consistently lower concentration and therefore the costs of treatment were also lower (on a volume basis).

As with the previous samples, Figure 8 and Figure 9 demonstrate a negative economic potential for all Cannon Hill truck effluent samples for the economic metrics used in the analysis. The negative economic potential ranged from $4.8/m^3$ (0h) to $6.3/m^3$ (12h) using a degradable fraction of 0.9; or $5.86/m^3$ (0h) to $6.43/m^3$ (8h) using a degradable fraction of 0.5. There was a general trend for the negative economic potential of the wastes to increase as the curfew increased (i.e., the cost of handling the wastes increased as the curfew time increased), however, the differences would not have a practical impact on the design or operation of a waste treatment plant.

The results shown in Figure 6 represent the upper limit of biogas production from the truck effluent wastes. In this scenario, biogas revenue from the truck effluent was potentially as high as $3.50/m^3$ for 0h off feed, reducing to $3.15/m^3$ for 12h off feed. Again, the costs of managing nitrogen in the waste streams was $6/m^3$ for 0h and $7.5/m^3$ for 12h off feed, i.e., the cost of managing nutrients in the waste through conventional biological removal processes was more than double the predicted biogas revenue, this is a result of the consistently low COD/N ratio of the waste streams. The costs of solids management in this scenario were still significant at ~$2.15/m^3$.

The trend is repeated in **Figure 9** for analysis estimating a typical biogas production from the truck effluent wastes. In this scenario, biogas revenue from the truck effluent was reduced to ~$1.8/m^3$. In this scenario, the costs of managing the nutrients (~$3.9/m^3$) and the costs of managing residual solids (~$4.1/m^3$) were each approximately double the value of the biogas. While the value proposition of treating truck effluent is negative, the treatment costs through an onsite treatment plant at an RMP are 80-90% lower than sewer disposal.

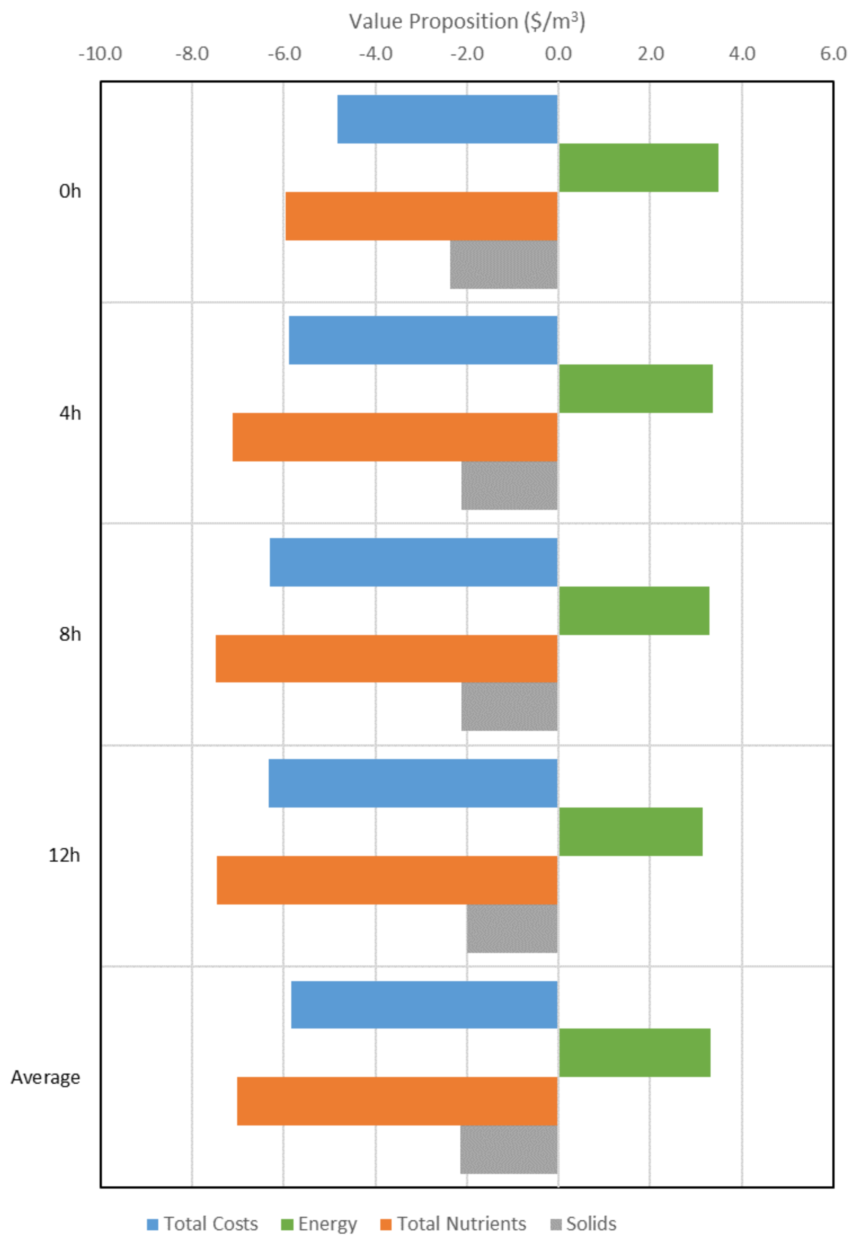

Figure 8 Value proposition of Truck Effluent collected from Cannon Hill. During this assessment the biodegradable fraction was assigned at 90% to represent a best-case scenario for energy recovery, energy was valued at $10/GJ, nitrogen and phosphorus were assigned treatment costs of $2.9/kg and $5.57/kg. Solid's disposal costs were estimated at $65/wet ton and dewatered cake solids as 25% TS.

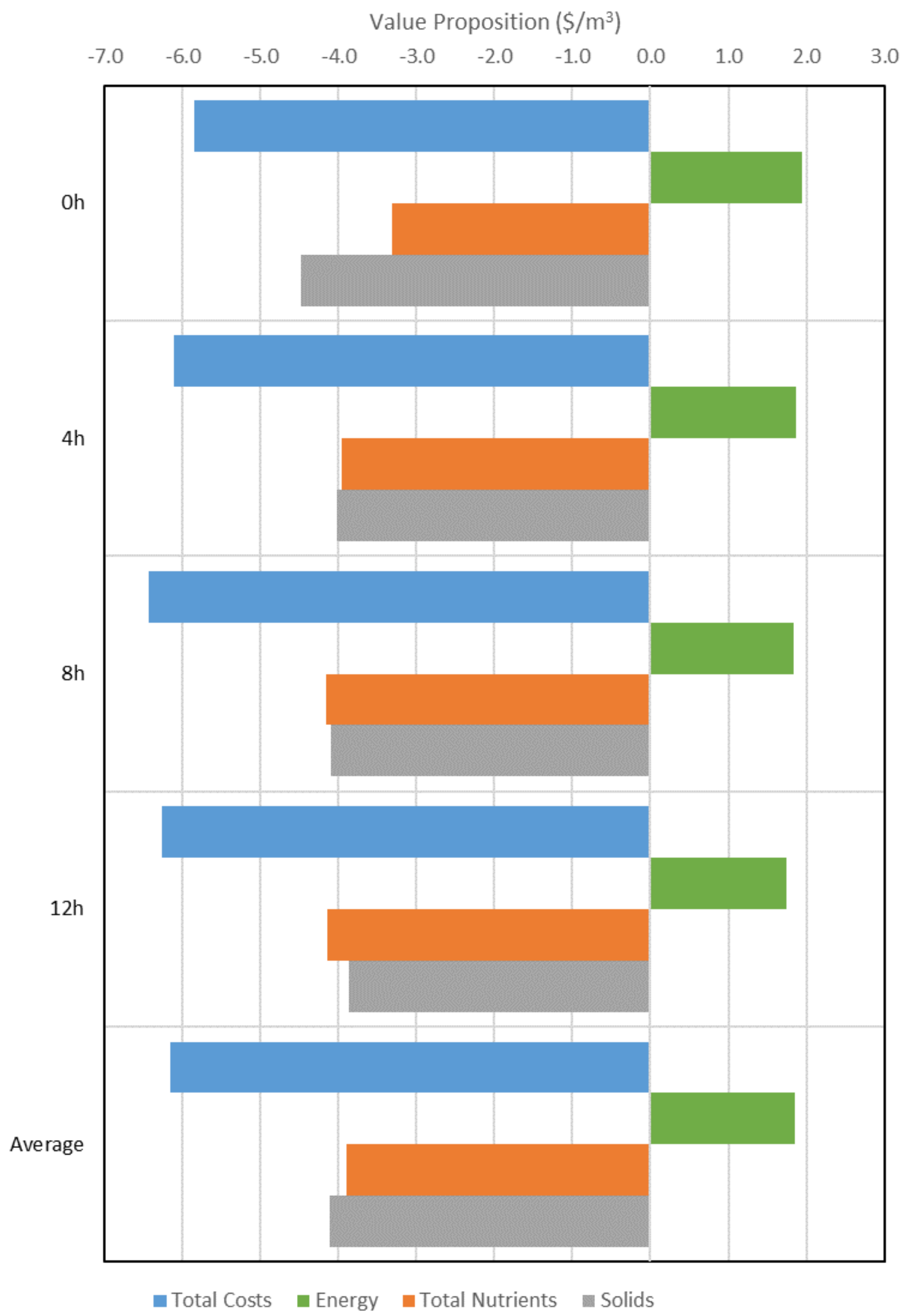

Figure 9 Value proposition of Truck Effluent collected from Cannon Hill. During this assessment the biodegradable fraction was assigned at 50% to represent a more typical scenario for energy recovery from cattle manures, energy was valued at $10/GJ, nitrogen and phosphorus were assigned treatment costs of $2.9/kg and $5.57/kg. Solid's disposal costs were estimated at $65/wet ton and dewatered cake solids as 25% TS.

Cost Methodology – Irrigation

This section estimates the value proposition of truck effluent samples using a dedicated treatment process that incorporates anaerobic digestion followed by land application of the liquid digestate.

Land application of wastewater/treated effluent is typically a licence condition and only viable within a relatively limited local area due to relatively high transport costs. Land availability and infrastructure development for irrigation would need to be considered as part of the treatment process development.

The value proposition for irrigation was developed to compare the overall value proposition of wastes considering i) the potential biogas revenue from the waste and ii) the potential value from irrigating the effluent and offsetting chemical fertilizer requirements. The value proposition of each waste stream was assessed using 5 primary factors:

- Revenue from biogas energy, estimated at $10 per GJ and using a heating value of 55 MJ/kg for methane.
- Nitrogen was valued at $1.3 per kg; based on a Urea price of $600/ton (June 2021).
- Phosphorus was valued at $3.7 per kg; based on a Triple Super Phosphate price of $750/ton (June 2021).
- Potassium was valued at $0.57 per kg; based on a potassium chloride price of $300/ton (June 2021).
- An irrigation cost of $0.10/kL was assumed. The SG of the effluent was assumed at 1 when estimating these costs.

Notes:

1. This is not a complete economic analysis; it is a high-level assessment of economic potential considering major cost factors associated with wastewater treatment.
2. This analysis assumes the anaerobic step operates as a mixed liquor reactor (CSTR or similar) and does not retain nutrients in the form of settled sludge.
3. N, P and K values are based on publicly available global fertilizer prices for June 2021.
4. Irrigation costs were based on high pressure overhead water cannons. This scenario was selected as it represented an upper range for irrigation costs of 375 kWh per 1 ML (corresponding to a total pumping head of 85m) and equating to $101.19/ML [6]. Irrigation costs for cattle manure effluent were previously reported at $5-50/ML, however this was based on 2002 dollars and 2002 energy costs (MLA FLOT.402 Final report).

Value Proposition of Truck Effluent Irrigation – Dalby Samples

Figure 11 and Figure 10 show preliminary economic analyses for truck effluent samples collected from Dalby after feed curfews ranging from 0h to 12h. Effluent fertilisers are less established and less optimised in comparison to chemical fertilizers, therefore it is not likely that effluent fertilizer would achieve the same market value as chemical fertilizers. To account for uncertainty in effluent fertilizer value, the first economic analysis (shown in Figure 10) assumes that the effluent nutrients are valued at 25% of the cost of chemical fertilizers (NOTE: there is no specific justification for selecting the value fraction of 25%, this was done to demonstrate a value range). The second analysis (shown in Figure 11) assumes that the nutrients present in the waste are valued at the same level as chemical fertilisers (Urea, STP and KCl for N, P and K respectively).

Using more conservative nutrient fertilizer values (Figure 10), the economic potential ranged from \$4.30/m^3 (12h) to \$4.60/m^3 (4h). Energy was the largest value component at approximately \$3/m^3. Phosphorous and Potassium were the lowest value components at approximately \$0.30/m^3 each. In this analysis, curfew time did not have a practical impact on the value proposition of the truck effluent and would not have a practical impact on the design or operation of a waste treatment plant or irrigation system.

In a scenario where the nutrients are valued at the same rate as chemical fertilizers (Figure 11), the economic potential is significantly higher at \$8.95/m^3 (0h) to \$9.54/m^3 (8h). Nitrogen was the largest value component at approximately \$5/m^3. The energy value remained at approximately \$3/m^3 in this scenario. Phosphorous and Potassium remained the lowest value components, although the value increased to \$1.3/m^3 each. In this analysis, curfew time did not have a practical impact on the value proposition of the truck effluent and would not have a practical impact on the design or operation of a waste treatment plant or irrigation system.

Anaerobic digestion followed by irrigation is a more attractive option for Dalby Truck Effluent due to the rural location and potential for local irrigation land.

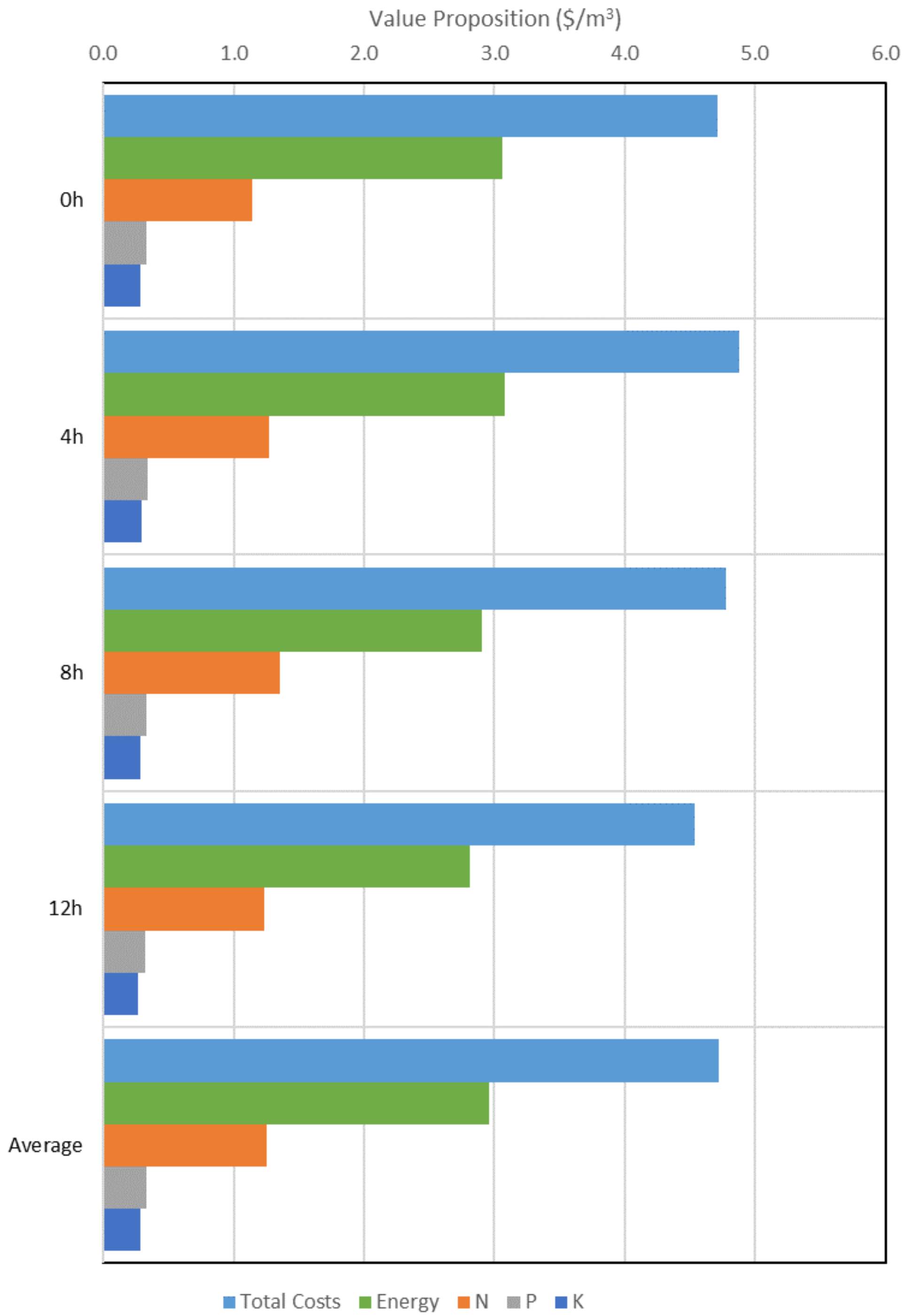

Figure 10 Value proposition of Truck Effluent collected from Dalby. During this assessment the biodegradable fraction was assigned at 50% to represent energy recovery from cattle manures, energy was valued at $10/GJ, nitrogen, phosphorus and potassium were valued at $0.325/kg, $0.925/kg, $0.14/kg based on 25% of the value of Urea, TSP and KCl.

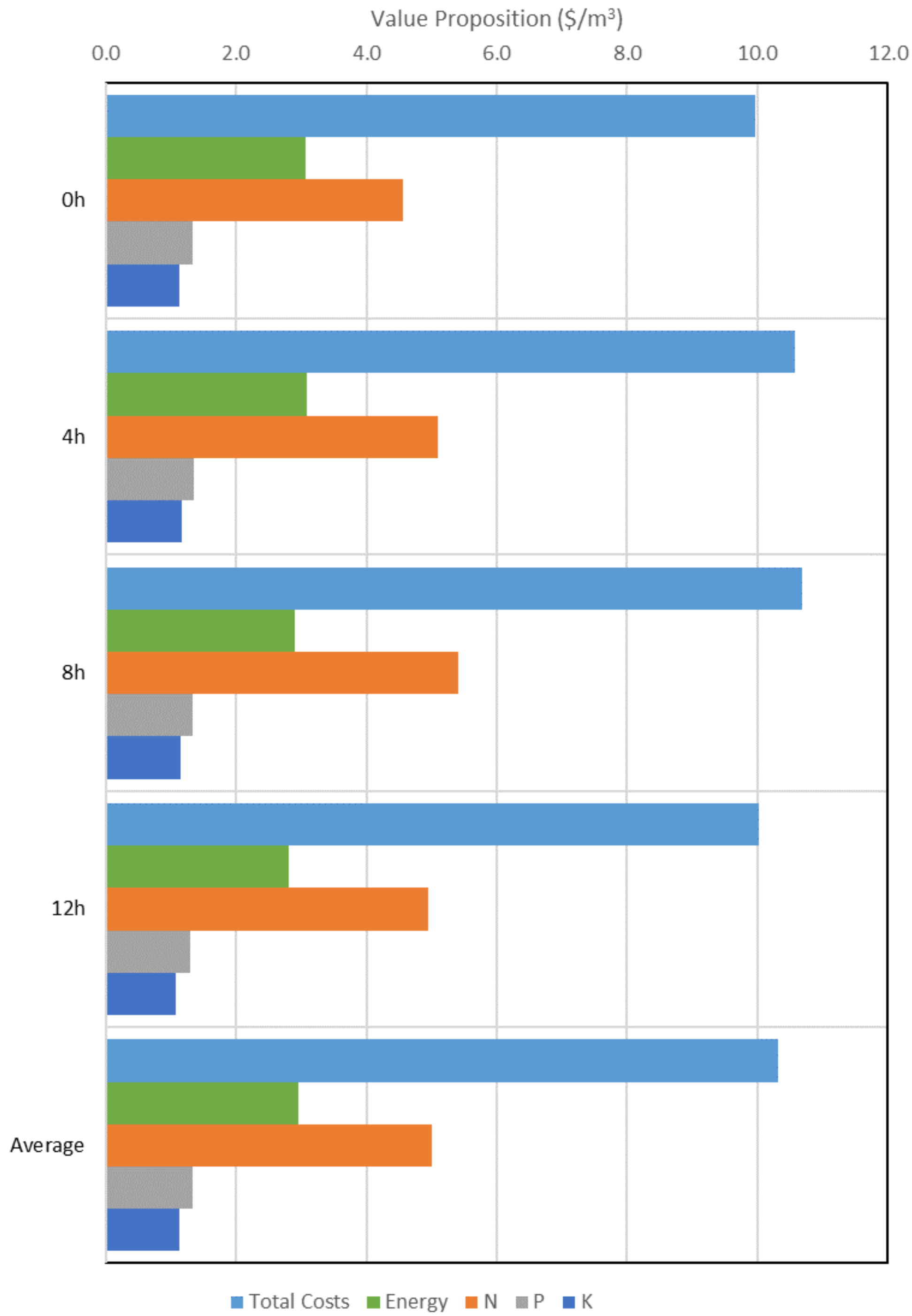

Figure 11 Value proposition of Truck Effluent collected from Dalby. During this assessment the biodegradable fraction was assigned at 50% to represent energy recovery from cattle manures, energy was valued at $10/GJ, nitrogen, phosphorus and potassium were valued at $1.3/kg, $3.7/kg, and $0.57/kg based on the value of Urea, TSP and KCl.

Value Proposition of Truck Effluent Irrigation – Cannon Hill Samples

Figure 12 and Figure 13 show preliminary economic analyses for truck effluent samples collected from Cannon Hill after feed curfews ranging from 0h to 12h. Figure 12 is based of effluent fertilizers achieving 25% the value of chemical fertilizers and Figure 13 is based on effluent fertilizers achieving 100% the value of chemical fertilizers.

Using more conservative nutrient fertilizer values (Figure 12), the economic potential ranged from \$2.77/m^3 (0h) to \$2.84/m^3 (8h). Energy was the largest value component at approximately \$1.9/m^3. Phosphorous and Potassium were the lowest value components at approximately \$0.20/m^3 each. In this analysis, curfew time did not have a practical impact on the value proposition of the truck effluent and would not have a practical impact on the design or operation of a waste treatment plant or irrigation system.

In a scenario where the nutrients are valued at the same rate as chemical fertilizers (Figure 13), the economic potential is significantly higher at \$5.54/m^3 (0h) to \$6.18/m^3 (8h). Nitrogen was the largest value component at approximately \$2.8 to 3/m^3. The energy value remained at approximately \$3/m^3 in this scenario. Phosphorous and Potassium remained the lowest value component, although the value increased to ~\$0.7/m^3 each. There was some variability between samples in this analysis, however curfew time did not have a practical impact on the value proposition of the truck effluent and would not have a practical impact on the design or operation of a waste treatment plant or irrigation system.

While the economics of anaerobic digestion followed by irrigation appear attractive for Cannon Hill Truck Effluent, the urban location makes identification of suitable agricultural land for irrigation more challenging. This is likely not a viable option.

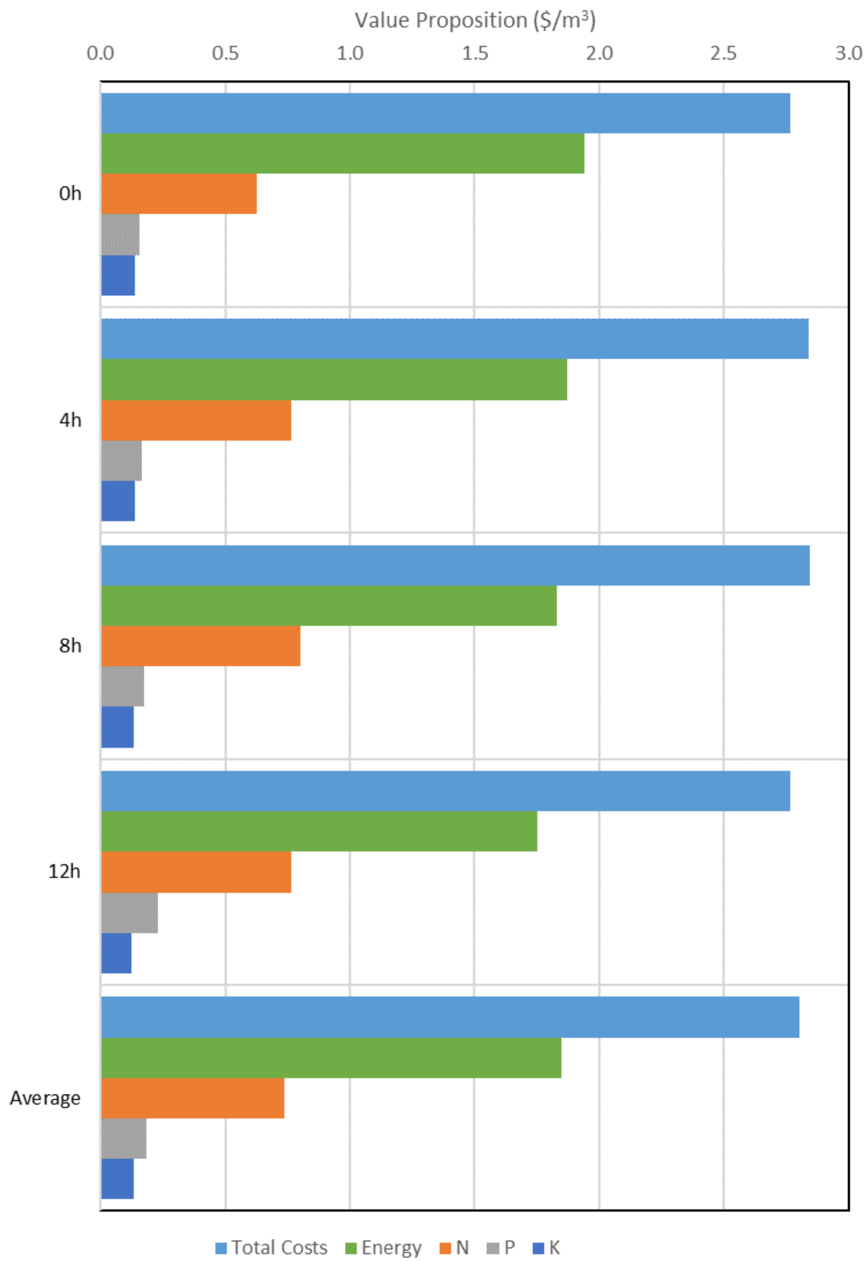

Figure 12 Value proposition of Truck Effluent collected from Cannon Hill. During this assessment the biodegradable fraction was assigned at 50% to represent energy recovery from cattle manures, energy was valued at $10/GJ, nitrogen, phosphorus and potassium were valued at $0.325/kg, $0.925/kg, $0.14/kg based on 25% of the value of Urea, TSP and KCl.

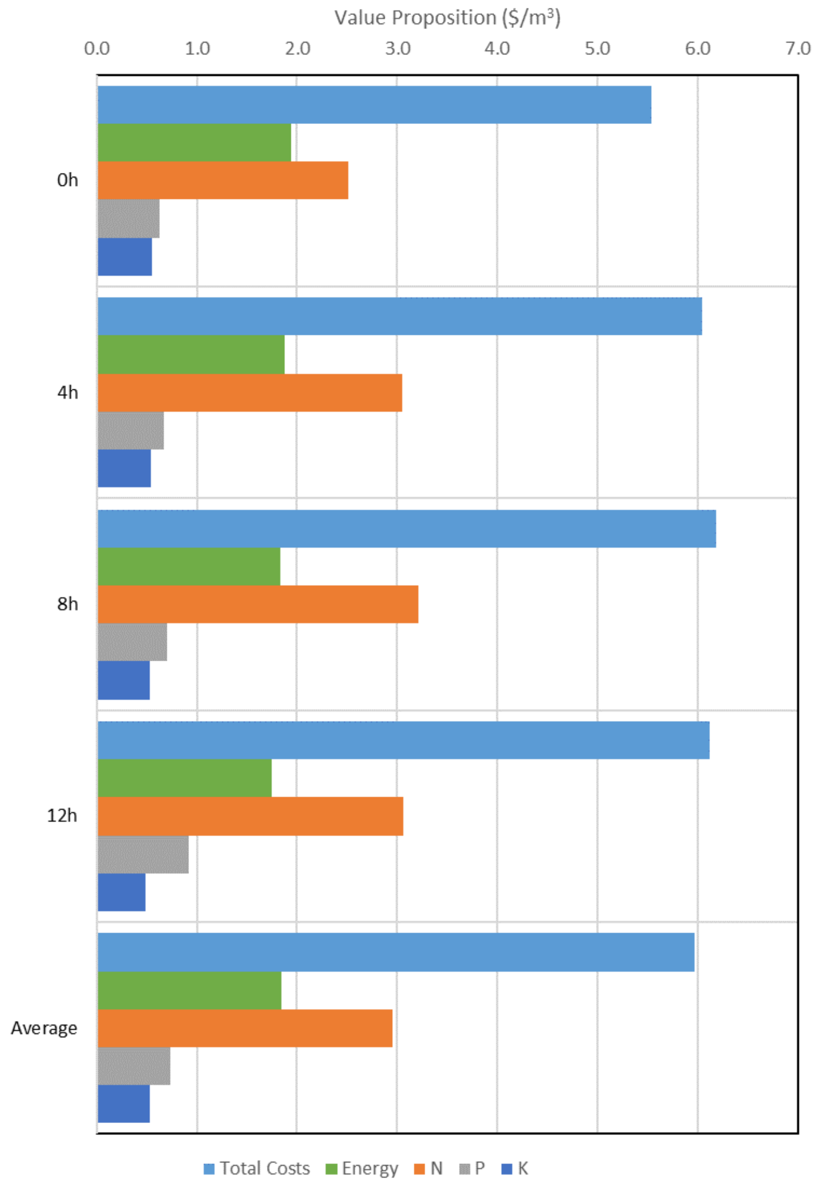

Figure 13 Value proposition of Truck Effluent collected from Cannon Hill. During this assessment the biodegradable fraction was assigned at 50% to represent energy recovery from cattle manures, energy was valued at $10/GJ, nitrogen, phosphorus and potassium were valued at $1.3/kg, $3.7/kg, and $0.57/kg based on the value of Urea, TSP and KCl.

Comparison of Truck Effluent Irrigation Value to Feedlot effluent

Table 6 shows the estimated nutrient value of truck effluent from this study compared to the nutrient value of cattle feedlot effluent reported in MLA project FLOT.402 (published in 2002). The value of truck effluent was estimated to be an order of magnitude higher than the feedlot lot effluent; this is partly due to the much higher nutrient concentrations in truck effluent (e.g., the concentration of N in truck effluent is more than an order of magnitude larger than concentrations in feedlot effluent).

The land application of wastewaters is a typically a licence condition based on the mass of nutrients applied per land area. Irrigation is typically only viable within a relatively limited local area due to the costs of pumping the effluent longer distances. The high concentrations of nutrients in truck effluent, and therefore higher value per volume would, increase the distance effluent could be transported while maintaining a positive value proposition.

The Dalby Mid-point effluent contained 700 – 100 mg/L Sodium; excess Sodium in soil (sodicity) is an issue in many areas and may impact the value of truck effluent used for irrigation. Sodium concentration was shown to decrease as feed curfew increased, this may be a strategy to manage the sodium content in the effluent. Alternatively, gypsum or lime can be used to add calcium to soils to balance the sodium content and maintain soil health.

Note: the gross value of truck effluent is estimated in **Table 6**, however the net worth of truck effluent is influenced by:

- Chemical composition after anaerobic treatment and therefore fertiliser value (the degradable fraction of the effluent is approximately 50%; up to half of the nutrients could be retained in a treatment pond in the form of settled sludge, therefore reducing the fertilizer value of the water effluent).
- The value of the water component.
- The costs of any clean water required to dilute the effluent for application (can be added after pumping to the land application area).
- The potential costs of soil additives such as gypsum or lime that may be required to offset the sodium content of effluent and maintain soil health.

The net-value of truck effluent achieved through irrigation is a promising area for future consideration.

Table 6 Nutrient value of Truck Effluent compared to Nutrient Value of Cattle Feedlot Effluent reported by MLA (FLOT.402, 2002)

Component	Average Dalby kg/ML	Average Cannon Hill kg/ML	FLOT.402 (2002) kg/ML
N	3850	2270	190
P	360	200	50
K	1970	930	1515
Cl	Not Tested	Not Tested	420
Assigned Nutrient Value	$1,800 - $7,400	$1,050 - $4,200	$30 - $200/ML#

Not adjusted for inflation

References

[1] A Practical Guide to Providing Facilities for Stock Effluent Disposal from Trucks, in, National Stock Effluent Working Group, New Zealand, 2013.
[2] E. McGahan, K. Baker, M. Burger, S. Wiedemann, R. Davis, C. Ouellet-Plamondon, P. Watts, Review of effluent spillage and animal welfare during livestock transport: a discussion paper, in, Meat and Livestock Australia, 2010.
[3] P.D. Jensen, T. Sullivan, C. Carney, D.J. Batstone, Analysis of the potential to recover energy and nutrient resources from cattle slaughterhouses in Australia by employing anaerobic digestion, Applied Energy, 136 (2014) 23-31.
[4] P. Gopalan, P.D. Jensen, D.J. Batstone, Biochemical methane potential of beef feedlot manure: Impact of manure age and storage, Journal of Environmental Quality, 42 (2013) 1205-1212.
[5] R.L.G. Lemaire, Development and fundamental investigations of innovative technologies for biological nutrient removal from abattoir wastewater, University of Queensland, 2007.
[6] J. Welsh, J. Powell, Oppurtunities for energy innovation in Australian irrigated sugarcane, in: S.R.A. report (Ed.), Ag Econ, Burren Junction, NSW, Australia, 2017.
[7] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, J.B. Van Lier, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays, in, 2009, pp. 927-934.
[8] APHA, Standard Methods for the Examination of Water and Wastewater, 22nd ed. , in, American Public Health Association, Washington, DC, 2012.
[9] L.E. Ripley, W.C. Boyle, J.C. Converse, Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes, Journal of the Water Pollution Control Federation, 58 (1986) 406-411.
[10] J.A.C. Cowan, F. MacTavish, C.J. Brouckaert, E.P. Jacobs, Membrane treatment strategies for red meat abattoir effluents, Water Science and Technology, 25 (1992) 137-148.
[11] M.R. Johns, Developments in wastewater treatment in the meat processing industry: A review, Bioresource Technology, 54 (1995) 203-216.
[12] G.S. Mittal, Characterization of the effluent wastewater from abattoirs for land application, Food Reviews International, 20 (2004) 229-256.
[13] W.P. Tritt, F. Schuchardt, Materials flow and possibilities of treating liquid and solid wastes from slaughterhouses in Germany. A review, Bioresource Technology, 41 (1992) 235-245.
[14] R. Borja, C.J. Banks, Z. Wang, Performance and kinetics of an upflow anaerobic sludge blanket (UASB) reactor treating slaughterhouse wastewater, Journal of Environmental Science and Health - Part A Environmental Science and Engineering, 29 (1994) 2063-2085.
[15] C.E.T. Caixeta, M.C. Cammarota, A.M.F. Xavier, Slaughterhouse wastewater treatment: Evaluation of a new three-phase separation system in a UASB reactor, Bioresource Technology, 81 (2002) 61-69.
[16] C.T. Li, W.K. Shieh, C.S. Wu, J.S. Huang, CHEMICAL/BIO-FLUIDIZED BED TREATMENT OF SLAUGHTERHOUSE WASTEWATER, Journal of Environmental Engineering, 112 (1986) 718-728.
[17] N.T. Manjunath, I. Mehrotra, R.P. Mathur, Treatment of wastewater from slaughterhouse by DAF-UASB system, Water Research, 34 (2000) 1930-1936.
[18] J. Martinez, L. Borzacconi, M. Mallo, M. Galisteo, M. Vinas, Treatment of slaughterhouse wastewater, in, 1995, pp. 99-104.
[19] L.A. Núñez, B. Martínez, Anaerobic treatment of slaughterhouse wastewater in an Expanded Granular Sludge Bed (EGSB) reactor, in, 1999, pp. 99-106.
[20] J.M. Russell, R.N. Cooper, S.B. Lindsey, Soil denitrification rates at wastewater irrigation sites receiving primary-treated and anaerobically treated meat-processing effluent, Bioresource Technology, 43 (1993) 41-46.
[21] G. Sachon, Waste water from slaughterhouses: Management and treatment, LES EAUX RESIDUAIRES DES ABATTOIRS DE BETAIL GESTION ET TRAITEMENT, 39 (1986) 27-45.
[22] S. Sayed, L. van Campen, G. Lettinga, Anaerobic treatment of slaughterhouse waste using a granular sludge UASB reactor, Biological Wastes, 21 (1987) 11-28.

[23] S. Sayed, W. De Zeeuw, The performance of a continuously operated flocculent sludge UASB reactor with slaughterhouse wastewater, Biological Wastes, 24 (1988) 199-212.
[24] T.W. Stebor, C.L. Berndt, S. Marman, R. Gabriel, Operating experience: anaerobic treatment at packerland packing, in: 44th Purdue Industrial Waste Conference, Chelsea, MI, 1990.
[25] N. Thayalakumaran, R. Bhamidimarri, P.O. Bicker, Characterisation of aerobic bio treatment of meat plant effluent, in, 2003, pp. 53-60.

Appendix 1: Composition Analysis

A summary of analytical methods to be used in the project is shown in Table 6. For analysis of soluble fractions, samples are filtered through a syringe filter (0.45 µm PES membrane) prior to analysis.

Table 6 Summary of analytical methods proposed for the project

Test	Method
BMP	The biochemical methane potential (BMP) of samples is assessed using methods developed in conjunction with the International Water Association (IWA) Anaerobic biodegradability, Activity and Inhibition Task Group [7], and summarised in the appendix.
TS, VS, Ash	Total solids (TS), Volatile Solids (VS) and ash content are measured according to Standard method 2540G of the American Public Health Association [8].
TSS, VSS	Total solids (TS), Volatile Solids (VS) and ash content are measured according to Standard method 2540G of the American Public Health Association [8].
pH	Benchtop pH electrode and meter (Thermo Fisher Scientific).
Chemical Oxygen demand (COD)	Estimates the organic content of a sample. Also an order of magnitude estimate of chemical energy present in the sample (i.e. the energy released by each gCOD converted to CO_2 and H_2O by being chemically oxidised). Chemical oxygen demand (COD) was measured using Merck Spectroquant® cell determinations and a SQ 118 Photometer (Merck, Germany). Total and soluble fractions are measured.
Conductivity	Benchtop conductivity probe and meter (Thermo Fisher Scientific).
Alkalinity	Measured by titrating a volume of sample with HCl to end points of pH 5.7 and pH 4.3. Partial alkalinity was determined using the pH 5.7 endpoint and represents alkalinity contributed by hydroxides, ammonia, carbonate and bicarbonate. Intermediate alkalinity was determined as the difference between alkalinity to pH 5.7 and alkalinity to pH 4.3 and represents the contribution by organic acids. The alkalinity ratio (α) is defined as the ratio of partial alkalinity to intermediate alkalinity; with ratios <0.3 representing a healthy process [9].
VFA and Alcohols	Volatile fatty acids (C2 to C6) and alcohols (ethanol, propanol, butanol) in samples are determined by gas chromatography using a flame ionisation detector (FID) and a polar capillary column (DB-FFAP).
Fats, Oil and Grease	Merck Photometric tests
NH_4-N* & PO_4-P*	Flow Injection Analysis (Lachat Instruments).
TKN & TP	Sample is first digested with sulfuric acid, potassium sulphate and copper sulphate catalyst in a block digester; then analysed via FIA (Lachat Instruments).
Metals & nutrients	ICP-OES (Agilent Technologies); targeting select isotopes of Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, & Zn.
Sulphur	Dried and pulverised sample is combusted in a high temperature furnace in the presence of strong oxidants / catalysts. The evolved S (as SO_2) is measured by infra-red detector
Temperature	Temperature measurements can be taken at time of collection by an infrared thermometer.

*samples were filtered through a syringe filter (0.45 um PES membrane) prior to analysis.

Appendix 2: Example Composition of Combined Wastewater at Red Meat Processing Facilities

The composition of combined wastewater at these Australian red meat processing facilities is shown in Table 7, while the compositions of slaughterhouse wastewater as reported in international studies are shown in Table 8. A comparison of Table 7 and Table 8 shows that wastewater from Australian slaughterhouses is concentrated by international standards, both in regards to organic contaminants (COD) and nutrient (N and P).

Table 7 Composition of combined wastewater at Australian slaughterhouses compared with literature values

	Volume m^3 day^{-1}	TCOD mg L^{-1}	sCOD mg L^{-1}	TS[b] mg L^{-1}	FOG mg L^{-1}	N mg L^{-1})	P mg L^{-1}
Literature Concentration[a]	-	2,000-10,000	-	500-2,000	100-600	100-600	10-100
Site A	2420	12,893	1,724	8,396	2,332	245	53
Site B	3150	9,587	1,970	4,300	783	232	50
Site C	2110	10,800	890	7,530	3,350	260	30
Site D	2150	12,460	2,220	7,400	1,200	438	56
Site E	1600	10,925	1,195	6,118	1,569	272	47
Site F	167	7,170	1,257	3,806	1,915	182	27

a. Based on [10-13]
b. Literature values are TSS (mg/L), study values are TS (mg/L)

Table 8: Characteristics of slaughterhouse wastewater after primary treatment/solids removal [5].

Reference	Country	TCOD mg L^{-1}	SCOD mg L^{-1}	FOG mg L^{-1}	TKN mg L^{-1}	NH_4-N mg L^{-1}	TP mg L^{-1}
Borja et al. [14]	Spain	5,100	-	-	310	95	30
Caixeta et al. [15]	Brazil	2,000-6,200	-	40-600	-	20-30	15-40
Li et al. [16]	China	628-1,437	-	97-452	44-126	25-105	10-16
Manjunath et al. [17]	India	1,100-7,250	-	125-400	90-150	-	8-15
Martinez et al. [18]	Spain	6,700	2,400	1,200	268	-	17
Nunez and Martinez [19]	Spain	1,440-4,200	720-2,100	45-280	-	-	
Russell et al. [20]	NZ	1,900	-	-	115	30	15
Sachon [21]	France	5,133	-	897	248	-	22
Sayed et al. [22]	Holland	1,500-2,200	-	-	120-180	-	12-20
Sayed et al. [23]	Holland	1,925-11,118	780-10,090	-	110-240	-	13-22
Stebor et al. [24]	US	4,200-8,500	1,100-1,600	100-200	114-148	65-87	20-30
Thayalakumaran et al. [25]	NZ	490-2,050	400-1,010	250-990	105-170	26-116	25-47