Evaluation of The Protect Pelleted Additive for High Grain Rations

A Research Report Prepared for ProAgni

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Contents

Executive summary

Growing Angus steers were offered a uniform quantity of barley-based rations at a feeding level of 2% of average LW, being either a control with Monensin (UNE-Monensin; n=5) or with a ProTect pelleted supplement (n=5) group. These inclusions were present in diets, from starter (45.2% barley) through to finisher rations (78.2% barley), using tempered barley with diets made up with mill run, whole cotton seed, wheat straw, oaten chaff, molasses and canola oil.

The Control UNE-Monensin supplement mix was prepared at the UNE feed mill with similar raw materials to those used in commercial manufacture of the ProTect pellet and contained canola meal (6%), wheat (34%), almonds hulls (40.0%), megamin (20%) and monensin (0.047%) and offered as mash. The ProTect pelleted supplement was received from ProAgni in pelleted form as 25 kg bags.

Either control supplement mash or protect pellet were offered at 550g/animal (5% as-fed in ration) each day in each phase of feeding period. Intraruminal pH boluses were placed in the rumen of all cattle at commencement of the study. Each feeding phase lasted for 7 days while the finisher phase was 16 days, during which methane emission were measured twice, DM and starch digestibility were monitored as was rumen pH and VFA concentration.

Findings may be summarised as below:

- In general, there was no difference in LW or total DM intake (DMI) of UNE-Monensin or ProTect pellet supplemented steers for the whole period of study, consisting of starter, intermediates and finisher phases.
- Pre and post-feeding ruminal pH values of cattle were safe and did not differ with diet.
- Protect pellet fed cattle tended to have a higher total volatile fatty acid (VFA) concentration compared to UNE-Monensin supplement fed cattle (P=0.06).
- Cattle on both diets exhibited very high proportions of propionate in ruminal VFA. Protect pellet supplement reduced the proportion of butyrate in the rumen volatile fatty acids
- There was no significant difference between UNE-supplement and ProTect pellet supplemented cattle in terms of liveweight, intake of dry matter (DM), or digestibility of starch or of total DM through the digestive tract.
- The data are completely consistent in showing ProTect is able to maintain a rumen fermentation that does not differ in any biologically important way from Monensin in respect to completeness of digestion or risk of ruminal acidosis.
- It could easily be published as a refereed paper to support use of ProTect pellet to obtain equivalent performance and nutrient digestibility compared to Monensin.
- The report is presented as a draft paper in which the discussion requires substantial development.

1. Introduction

The use of rumen modifying antibiotics including the feedlot industry standard ionophore 'Monensin' is increasingly being targeted in a global focus to reduce antibiotic use in agriculture and reduce risks to human health. In regard to this, ProAgni have developed a pelleted concentrate-based feed additive that may reduce the incidence of lactic acidosis in feedlot cattle, optimise feed efficiency and enhance performance. Based on their declaration, the ProTect pellet does not contain antibiotic which is more socially agreeable to use in cattle feed, so this experiment was conducted to quantify the effect of ProTect on the daily methane production and methane yield (g CH_4/kg dry matter intake) of cattle on a feedlot finisher diet. Furthermore, the study sought to prove if ProTect causes differences in rumen pH and in whole-tract DM and starch digestibility relative to that in Monensin supplemented cattle. In so doing the study served to identify possible advantages of ProTect in allowing safe adaptation (free of ruminal acidosis) to high grain diets without inclusion of in-feed antibiotics while also affecting the greenhouse gas cost of beef production.

2. Materials and Methods

2.1 Animals

Ten purebred Angus steers (18-19) months of age with mean live weight [LW] of 472.1 ± 4.81 kg) were individually housed in 1.8m × 3m respiratory chambers at the University of New England. The trial was approved by an Animal Ethics Committee (AEC; Authority No. AEC18-094) and was conducted in conformity with the *UNE Code of Practice for Experimental Animals,* the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2013,* the *NSW Animal Research Act 1985 and NSW Animal Research Regulation 2005*. All cattle were treated with anthelmintic and for infectious diseases (Bovilis® S and Ultravac® 5in1) prior to commencement and were allocated to the UNE-Monensin control (n=5) or ProTect (n=5) supplemented diets by stratified randomisation based on LW. Drinking water was available *ad-libitum* and pens were hosed out at least once per day and the automatic water systems in each pen checked and cleaned daily.

All animals were visually assessed a minimum of twice daily for any illness or health concerns (Plate 2.1). The 120 L plastic white tub used for feeding and were washed out daily. All daily activities including rations fed, refusals, and any indications of abnormality or cattle illness were recorded within a trial-specific book (feed book) kept on the premises.

Plate 2.1: Cattle housing arrangement in respiratory chambers inside the large animal facility of CART, UNE, and beef cattle used in the methane trial.

2.2 SmaxTec- Intraruminal pH bolus

After randomization and before offering test diets, a SmaxTec intraruminal pH bolus was inserted into the rumen of each animal by using a balling gun (Plate 2.2). The data emitted from the bolus is detected by an in-shed receiver and thereafter sent by satellite to the internet via the 3G telephone network, providing near- realtime monitoring which enable us to view the rumen pH value and recorded data every ten minutes.

Plate 2.2: Delivering of intraruminal pH bolus using balling gun by Mr Graeme Bremner, inside the large animal facility of CART, UNE.

2.2 Diets and Feeding

All main raw dietary ingredients were analysed before ration formulation (Table 1). The Angus steers allocated to the high grain diets (Table 2) were adapted to the finisher ration over a 21-day period consisting starter, T1 and T2 as per normal feedlot feeding practice.

Table 1: Nutrient composition of dietary ingredients and the rations as offered in the UNE-Monensin or protect pelleted supplement trial for starter to finisher phase feeding except no millrun on finisher. All data are expressed on a DM basis.

Component	Tempered barley	Cotton Seed	Mill run	Oaten chaff	Wheat straw
Dry matter	80.00	91.90	91.5	92.41	16.45
ME (MJ/kg DM)	13.57	13.00	10.0	5.00	12.23
Crude protein (%)	10.43	28.30	15	2.20	13.23
Starch (%)	62.62	1.39	21.45	2.15	11.64
NDF (%)	20.70	49.70	24.39	75.40	33.41

Offered high grain diet = 10.94 kg + 0.55 kg UNE-Monensin supplement or protect pelleted supplement as fed/day/steers. NDF = neutral detergent fibre and indicates the level of cell wall material in the sample.

Steers were brought from forage to the starter ration (45% tempered barley) for 7d, then the grain content increased to 56 and 67% in successive intermediate diets on days 8 and 15 of feeding, before the full finisher ration (78% tempered barley) was offered on day 22.

Table 2: Diet ingredients composition (g/100g) on an as fed basis of the starter, transition 1 (T1), Transition 2 (T2) and Finisher ration. Days in which each diet was fed is shown in parenthesis.

Ingredients (g/100g)	Starter (0-7 days)	T1 (d8-d14)	T2 (d15-d21)	Finisher (d22-d36)
Tempered barley*	45.2	56.4	67.4	78.2
Vegetable oil	0.0	0.9	1.8	2.8
Molasses	4.0	3.3tampered	2.6	2.0
Millrun	10.0	6.7	3.4	0.0
Whole cotton seed	9.7	8.7	7.7	6.7
Wheat straw	10.3	7.9	5.6	2.4
Oaten chaff	15.3	10.6	6.0	2.4
Monensin pellet or ProTect pellet	5.5	5.5	5.5	5.5
Total	100.0	100.0	100.0	100.0

*The moisture content of tampered barley was around 20 % and kept the similar moisture content all the way through by adding water depends on its instant moisture content prior after obtaining from mill before mixing diet.

The level of feeding of all steers in the trial was set at 9.45 kg DMI/head/day which was fed once daily in the morning in between 9.30am and 10.30am. The feed consumption occurred across a broader time scale, but the majority of animals consumed their ration within 90 minutes of it first being offered. There were a few minor instances of scouring or feed refusal, but all animals consumed 100% of the finisher ration offered for the last 16 days, which included the period of digestibility and methane studies.

2.2.1 Preparation Of UNE-Monensin Supplement

The supplement providing the Monensin was formulated and prepared to be as similar to the ProTect pelleted supplement as practical (Table 3). Almond hulls were obtained through ProAgni and other supplement ingredients were sourced locally. The almond hulls were ground using a roller mill to reduce the size and increase its mix-ability with other ingredient. While the intention had been to offer both the Monensin supplement and the ProTect supplement both as pellets, the Monensin supplement did not pellet well so the Monensin supplement was fed as a loose mash daily, mixed in with the main diet as were the ProTect pellets

Table 3: UNE-Monensin supplement (used as control) ingredient composition.

Name of ingredients	Inclusions (g ingredient/100g)
Canola meal solvent	6.0
Wheat	34.0
Almond hulls	40.0
Megamin	20.0
Monensin stock	0.047

2.2.2. ProAgni ProTect Pelleted Supplement

We directly obtained the protect pellet supplement from ProAgni in 25kg bags and kept at room temperature. It has the following nutrient level (DM basis) as advised (Table 4).

Table 4: ProTect pellet supplement nutrient profile. *Source: ProAgni website: www.proagni.com.au*

Name of nutrients	Nutrient content (g/100g DM)
Crude protein	7.3
Crude fat	4.4
Magnesium	1.3
Sodium	2.8
Sulphur	1.4
Calcium	8.2
Phosphorus	1.2

2.2.3 Diet Analysis

Representative samples of the starter, T1, T2 and finisher (at three stages) were collected and sent away to determine dry matter and nutrient composition. Dry matter percentage for ration formulation purposes was estimated by oven drying samples at 80˚C for 72 hours or until a constant weight was achieved. Nutrient composition including dry matter (DM), metabolisable energy (ME), ash percentage, organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), starch and minerals were determined by the NSW DPI Feed Quality Service, Wagga Wagga Agricultural Institute, NSW, Australia and are shown in Table 5.

Table 5: Measured nutrient content of rations offered in the UNE-Monensin or ProTect pellet supplement trial for starter to finisher phase. All data are expressed on a DM basis.

Nutrients (g/100g DM)	Starter (0-7 days)	T1 (d8-d14)	T2 (d15-d21)	Finisher (d22-d36)
Dry matter (DM)	85.3	84.9	84.1	83.7
Neutral detergent fibre (NDF)	40	40	33.0	27.0
Acid detergent fibre (ADF)	20.0	22.0	16.0	12.7
Crude Protein	11.2	12.1	11.8	12.0
Dry matter digestibility (DMD)	70.4	69.8	74.6	80.9
Ash	4.4	4.2	3.5	2.9
Organic matter (OM)	95.6	95.8	96.5	97.1
Metabolizable energy (ME; MJ/kg)	11.8	12.1	12.7	13.8
Fat	4.7	6.2	6.0	7.2
Starch	19.0	28.0	35.0	46.3
Gross energy (GE; MJ/kg)	19.0	19.4	19.3	19.5

Offered high grain diet = 10.94 kg + 0.55 kg control supplement or protect pellet as fed/day/steers. NDF = neutral detergent fibre and indicates the level of cell wall material in the sample.

2.3 Sample And Data Collection

2.3.1 Feed Samples And Refusals

Representative random samples of starter, intermediate (T1 & T2) and finisher rations were taken daily and stored at -20˚C until analysis. Total ration refusals were collected individually from each pen when found and weighed daily with amounts recorded in the trial record book. However, there was very little feed refusal observed during the trial.

2.3.2 Estimation Of Dry Matter Digestibility (DMD)

All steers were fed 10 g/d of the indigestible marker chromium oxide (Cr_2O_3) from day 21 - 35 of the experiment. In the days leading up to the marker study, a spoonful of molasses was given into the empty feed tub before offering ration in every morning to get cattle used to Molasses as a carrier for the Cr_2O_3 From day 21 onwards, Cr_2O_3 powder (10g/head/d) was placed in the empty feed tub mixed into a spoonful of molasses (~50 mL) and offered to cattle before offering them the main ration (Plate 2.3). After the pens were hosed, fresh faecal samples from each animal were collected daily on days 30-37 in sterile 75ml specimen containers, either directly from the washed pen floor or by rectal sampling. The apparent digestibility (digestibility coefficient) of DM and of starch of the two dietary treatment group was then calculated using the formulae below:

DMD = 100* (1 –([Cr] in diet/[Cr in faeces])

Faecal output of DM (kg/d) = (Daily intake of Cr (g Cr)/Faecal Cr concentration (g Cr/kg DM)

Starch digestibility = 100x <u>(starch intake (g/d) – (faecal DM output(kg) x (faecal starch g/kg))</u>

Starch intake (g/d)

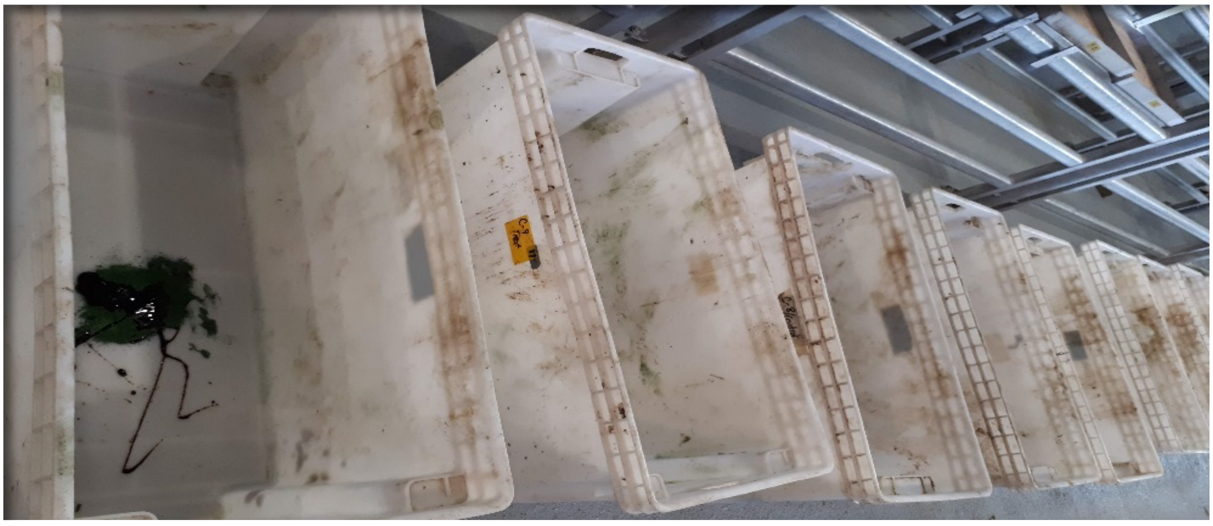

Plate 2.3: Green pigmented Chromium Oxide (Cr_2O_3) fed as an external marker for apparent digestibility analysis. Angus steers found it palatable when well mixed with molasses prior offered main ration.

2.4.4 Rumen Fluid Sampling

Each of the ten steers had rumen fluid taken by oesophageal intubation procedure on days 26 and 36, for pre-feeding sampling (Plate 2.5).

Plate 2.5: Collecting rumen fluid by using oesophagus method after respiratory chamber run

2.4.4.1 Rumen pH

In addition to the SmaXtec intraruminal pH bolus, rumen fluid samples were tested immediately post-retrieval as depicted in Plate 2.6 for pH using a twice calibrated Ecoscan pH 6 meter (Eutech Instruments, Pte Ltd. Singapore) and freshly obtained pH calibration standards.

2.4.4.2 Volatile Fatty Acid (VFA)

A 15-mL subsample of rumen fluid was placed into 25 mL McCartney bottles (acidified with 0.25mL of 18 M H_2SO_4) and frozen at -20˚C prior to VFA and NH_3-N analysis. Samples were subsequently thawed to room temperature and concentrations of VFA were ascertained by gas chromatography (GC) using a SMARTGAS Varian CP-3800 Gas Chromatograph (Varian Palo Alto, California USA) as described by Nolan et al. (2010).

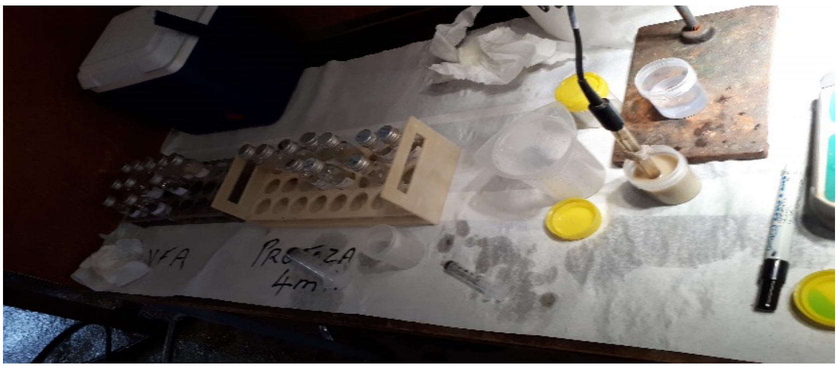

Plate 2.6: pH testing of rumen fluid immediately post sampling of animals by stomach tube procedure.

2.4.4.3 Protozoa

A 4-mL subsample of rumen fluid was placed into 25 mL McCartney bottles containing 16 mL of formaldehyde-saline (4% formalin v/v) and stored at room temperature for the visual enumeration of ciliate protozoa. Protozoa were stained as described by Nguyen & Hegarty (2016) prior to enumeration on a Fuchs–Rosenthal optical counting chamber (0.0625 mm^2, 0.2 mm of depth) using a staining technique adapted from Dehority (1984). The protozoa were classified into large (>100 µm) and small (<100 µm) holotrich and entodiniomorph groupings.

2.4 Data Analysis

Effects of diet on DMI, LW, and digestibility of DM, GE and starch, together with VFA concentrations and proportions were all tested using a one way analysis (ANOVA) of variance in SPSS 22.

3 Results & Discussion

3.1 Topic 1: Rumen Fermentation Effects of ProTect

In this study all animals were adapted slowly to increasing grain contents up to 78% tempered barley in the finisher ration. In the first 7 days of feeding (starter ration) the average minimum pH across the 5 cattle in each treatment was 5.24 for control (Monensin) diet and 5.55 for ProTect indicating both presented an equal capacity to manage rumen acidity on introduction of grain. The average pH over this week of feeding was also near identical across treatments (6.58 Monensin, 6.57 ProTect).

Moving through to the finishing ration, pHs of both groups were high and not health threatening. (Fig 1; average pH of final week of finisher). The minimum pH reached in steers on UNE-Monensin and protect pellet supplements were 5.40 and 5.80 respectively and was stable for around 7 hours in both groups. There was almost no time on finisher when pH was under 5.5 (taken as the start of subacute ruminal acidosis) in either the UNE-Monensin supplement or ProTect pellet supplement groups, except in two animals for 30 minutes and one animal for 10 minutes respectively.

Fig: 1 Rumen pH as measured after sampling via the oesophagus on day 36

The intra-ruminal pH bolus data for the last five days on finisher ration is summarised in Table 6. In general, there was no difference observed between the groups in intra-ruminal pH data recorded via SmaxTec technology (https://www.smaxtec.com/en/).

Table 6: Rumen pH records apprehended by SmaxTec –Intraruminal pH bolus in Angus growing steers fed high grain diets with UNE–Monensin or ProTect pellet supplement over the last 5 days of finisher period.

Treatment	Time (h:m) pH under 5.5	Time (h:m) pH under 6.0	Minimum pH reached
UNE-Monensin	00.20	07.00	5.40
UNE-Monensin	None	03.30	5.79
UNE-Monensin	None	21.50	5.75
UNE-Monensin	00.10	06.00	5.45
UNE-Monensin	None	10.30	5.56
ProTect Pellet	None	01.10	5.97
ProTect Pellet	None	00.10	5.94
ProTect Pellet	None	07.25	5.80
ProTect Pellet	None	04.40	5.88
ProTect Pellet	00.10	23.02	5.44

High grain diets are typically associated with producing a high propionate percentage in the rumen and this is related to both the predominance of starch as the fermentation substrate and also the reduced pH of the rumen environment. In this study propionate percentage was high in both diets (29-38 moles/100 moles) but total VFA concentration was low, reflecting samples being taken pre-feeding at the low point of fermentation (Table 7). Despite being sampled pre-feeding, protect pelleted supplement steers tended to have a higher (P= 0.06) total VFA concentration in rumen fluid suggesting a greater rate of fermentation. ProTect animals also exhibited a lower butyrate molar percentage (P<0.01) and a lower acetate: propionate ratio than the UNE-Monensin group. In contrast, the tendency towards higher iso-butyrate (P=0.08) and iso-valerate (P=0.06) molar percentage was found in steers supplemented with ProTect pellet diets. These VFA result from amino acid catabolism suggesting that ProTect may enhance protein degradation in the rumen.

Table 7: Total volatile fatty acid (VFA) concentrations (mMoles/L) and VFA molar proportions in rumen contents of growing steers fed diet with UNE-Monensin supplement or ProTect pelleted supplement at day 26 or day 36 of feeding period.

Variable	Treatment				*SEM*	*P-Value*		
	UNE-monensin supplement (n = 5)		ProTect pelleted Supplement (n = 5)			*Treatment effect*	*Day effect*	*Treatment × day*
	d26	d36	d26	d36				
Total VFA (mM)	30.2^{ab}	22.0^{b}	36.7^{a}	30.6^{ab}	*2.06*	*0.06*	*0.07*	*0.77*
Acetate (mol %)	43.0	44.5	43.6	51.2	*1.60*	*0.25*	*0.15*	*0.33*
Propionate (mol %)	29.4^{b}	35.2^{a}	37.7^{a}	29.8^{b}	*1.70*	*0.66*	*0.76*	*0.05*
Iso-butyrate (mol %)	0.50	0.6	0.7	0.7	*0.04*	*0.19*	*0.46*	*0.38*
Butyrate (mol %)	19.6^{a}	13.6^{b}	10.5^{b}	11.6^{b}	*1.20*	*0.01*	*0.22*	*0.08*
Iso-valerate (mol %)	3.2	3.0	5.1	4.2	*0.41*	*0.07*	*0.48*	*0.69*
Valerate (mol %)	4.3^{a}	3.0^{ab}	2.5^{b}	2.6^{b}	*0.29*	*0.05*	*0.28*	*0.22*
Acetate:Propionate ratio	1.5	1.3	1.3	1.8	*0.10*	*0.51*	*0.34*	*0.08*

^{a,b} means with uncommon superscripts within a column are significantly different at P <0.05, SEM = pooled standard error of means

High grain diets are also associated with a low yield of methane (g CH_4/kg DM intake) and Monensin has been shown to further reduce this methane yield, though this effect is not sustained under prolonged feeding. In this study cattle supplemented with ProTect had a higher methane yield than Monensin cattle (P<0.05; Table 8) and this was consistent across the two measurement days. The methane yield of ProTect cattle (approximately 15 g CH_4/kg DM intake) was typical of that seen in grain fed ruminants. It is likely the difference between treatments reflects Monensin having a specific action reducing the availability of hydrogen, (a precursor for methane production) as an ionophore antibiotic, which is not present in the antibiotic-free ProTect.

Table 8: The CH4/kg DMI and CH4 g/d of growing steers fed diet with UNE-Monensin supplement or ProTect pelleted supplement at day 26 or day 36 of feeding period.

Variable	Treatment				SEM	*P*		
	UNE-monensin supplement (n = 5)		ProTect pelleted Supplement (n = 5)			*Treatment effect*	*Day effect*	*Treatment × day*
	d26	d36	d26	d36				
CH4/kg DMI	11.9^{ab}	9.3^{b}	15.6^{a}	14.1^{ab}	*0.10*	*0.03*	*0.29*	*0.76*
CH4 (g/d)	112.4^{ab}	88.2^{b}	147.0^{a}	133.7^{ab}	*9.38*	*0.03*	*0.29*	*0.76*

^{a,b,c} means bearing uncommon superscripts within a column are significantly different at P <0.05, SEM = pooled standard error of means.

3.2 Topic 2: Nutrient Intake, Digestion And Animal Growth

The feeding level used was selected during the period of diet adaption to ensure equal average DMI by cattle on UNE-Monensin or ProTect pelleted supplement diet (P>0.05). A fixed amount (11.49kg/day/animal including 0.55kg supplement as fed) of high grain feed was offered to each animal on either UNE-Monensin or protect pelleted group for the whole period of this study. The diet was typically fully consumed within 90 minutes of feeding because feed offered was below their potential voluntary feed intake. No feed refusal was observed during the whole period of study except a few refusals during the starter period.

There was no significant difference (P>0.05) in DMI (kg/d), faecal DM% or DMD% between treatments in steers on finisher phase feeding (Table 9). These results indicate that the both supplements performed equally in making high grain diets available to growing steers. This was also evident in the consistency of starch digestibility throughout the whole tract of UNE-Monensin and ProTect cattle, with no difference in starch digestibility through the tract or in total faecal starch output (= undigested starch)

Table 9: Nutrient digestibility of growing steers fed UNE-Monensin supplement or ProTect pelleted supplement on finisher period. *The measurements were made during an 8-day period of excreta collection*.

	Treatment			
	Control-UNE supplement n = 5	ProTect Pelleted Supplement n = 5	*SEM*	*p-Value*
DMI (kg/d)	9.24	9.24	*0.00*	*1.00*
Faecal DM (%) (last 10 days)	20.28	19.58	*0.31*	*0.255*
DM digestibility (%)	76.44	76.09	*0.49*	*0.727*
Starch digestibility (%)	97.38	96.90	*0.30*	*0.459*
Starch excreted (g/d)	114.34	134.66	*13.0*	*0.467*

The average LW results over time are summarised in Table 10. During the whole 5-weeks of feeding, no difference in LW was observed between the treatment groups either on starter, intermediate or finisher phase rations.

Table 10: Live weight (LW, kg/head) at different stages of feeding high grain ration supplemented with UNE-Monensin supplement or ProTect pellet supplement in growing steers.

	Treatment			
Live weight (kg/head)	UNE-Monensin supplement n = 5	ProTect Pelleted Supplement n = 5	*SEM*	*p-Value*
Initial	473.0	472.4	*4.8*	*0.954*
D7- Starter	485.2	490.0	*3.2*	*0.492*
D14 –T1	485.0	480.0	*3.4*	*0.491*
D21 –T2	492.6	492.4	*3.5*	*0.979*
D28- Finisher1	503.0	504.4	*3.9*	*0.868*
D36 – Finisher2	518.0	516.0	*4.9*	*0.851*

SEM = pooled standard error of means.

3.3 Discussion

Cereal grains are the principle energy substrate used in cattle production in Australia. Over 3.5Mt are fed annually and increasingly there is a desire to improve utilisation of cereal grains (ALFA Strategic Plan). In feedlots this has been achieved by increasing rumen degradation by physical damage to the grain (rolling/tempering/flaking) and changing the availability of the starch by using water to start hydrolysis or gelatinising (by heat and steam) to accelerate ruminal hydrolysis. In addition, it is standard practice to add an ionophore (most often Monensin) to improve rumen function, reduce the incidence of acidosis and improve feed use efficiency. While Monensin unquestionably reduces enteric emission in the short term, results for the long-term persistence of methane suppression arising from Monensin across low and high grain diets has not been shown (Guan et al., 2006). Subsequent studies have shown divergence in persistence of efficacy, so further investigations of the persistence of Protect C should be undertaken.

So for reasons of reduced dependence on antibiotic rumen modifiers and reducing enteric methane emissions, there is considerable interest in finding alternative means of moderating the fermentation patterns of ruminants without causing acidosis or compromising the completeness of the diet and of its key ingredient, starch.

The results presented confirm that

(1) ProTect is as effective as Monensin in maintaining a safe intraruminal pH during adaptation and finishing of feedlot cattle. By restricted feeding cattle (in difference to *ad-libitum* feeding), this trial created an artificially high risk of acidosis as restricted feeding encourages more severe and irregular feeding bouts so more dramatic pH fluctuations. The fact that the time spent at low pH was no worse in ProTect relative to Monensin identifies ProTect is a valid alternative to Monensin as a tool to manage ruminal acidosis.

(2) ProTect enabled the whole-tract digestibility of diet DM to be sustained. This is consistent with a stable rumen ecosystem and freedom from foregut or hindgut acidosis. This was also reflected in the consistently high starch digestibility of Protect C versus UNE-Monensin cattle and the lack of difference in faecal starch output between groups. Together this digestibility data indicate the ProTect cattle were as effective as Monensin cattle in digesting starch and, in combination with pH data, can be seen to do this with equally low risk of lactic acidosis

(3) Both diets supported a rapid starch-based fermentation with propionate proportions at the top of those normally observed. There were a number of minor differences in VFA proportions, including a slightly lower butyrate percentage in Protect C cattle. Further investigations and research is required to fully understand these anomalies.

(4) While the Protect C was directly compared to Monensin for methane mitigation, there was no 'control' group to certain baseline methane production. Repeating this research with a non-medicated control would be beneficial to capture the true impact of Protect C on methane mitigation.

(5) While it was clear that Protect C did not reduce methane production as effectively as monensin in the trial period (36 days) further understanding regarding Protect C impact on overall methane production over a longer time period is required. The lack of certainty of monensin's long term effect on emissions may mean the long-term mitigation achieved by Protect C may differ little from that of monensin fed cattle.

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