Final report

Nitrogen recycling as determinant for feed efficiency of Bos indicus cattle

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Abstract

Performance of cattle in northern Australia depends on the ability to recycle nitrogen back to the rumen, instead of eliminating it in the urine. Nonetheless, bulls are routinely selected on high-protein diets assuming similar rankings on low-protein diets. The results from 89 Brahman steers corroborate the initial hypothesis that feed efficiency on low-protein diets is not correlated with feed efficiency on nutrient-abundant diets and that efficiency on low-protein diets depends on the ability of the animal to conserve nitrogen via nitrogen recycling to the rumen. This is not the case with high-protein diets. It was demonstrated that feed efficiency measured as feed conversion rate, gain to feed ratio, residual gain, and nitrogen use efficiency, could be estimated with acceptable accuracy from the ^{15}N:^{14}N isotopic ratio in tail hair. Specific rumen bacteria populations were associated with better feed-efficiency, but only with the low-protein diet. Furthermore, a trial with 24 steers demonstrated that the N-isotopic ratio in tail hair can predict the response of cattle to urea supplements, and a trial with 479 steers demonstrated good heritability of this trait (43%). In conclusion, the N-isotopic ratio in tail hair can be used for early detection of more efficient animals when targeting performance in harsh environments.

Executive summary

Background

Some cattle are more efficient than others when exposed to a harsh environment and low-protein diets. Because of long drought periods and the low-protein values (<4-6% CP) of typical native pastures where digestibility is below 51%, the performance of cattle in most northern Australia production systems will depend on salvage mechanisms maximising nutrient utilisation and conservation. Nonetheless, when cattle are evaluated for feed efficiency, the industry standard is to use nutrient-abundant diets, with the assumption that the selected animals will perform as efficiently in more challenging environments. If feed efficiency is to be used for selection of *Bos indicus* cattle grazing rangeland pastures, it is essential to determine the robustness of rankings evaluated with nutrient-poor and nutrient-rich diets, with special emphasis on nitrogen use efficiency. However, rumen efficiency and nitrogen recycling are not easily measured, and the development of more practical techniques is important for large-scale evaluation of nitrogen use efficiency. Classifying cattle according to the nitrogen use efficiency would be important, not only from a genetic improvement perspective but also as a diagnostic for overall management practices aiming to improve nitrogen utilization and mitigate environmental impact.

Objectives

1. To determine the necessity of using low-protein diets when evaluating feed efficiency of cattle for the northern Australian production systems.
   The objective was achieved. The results indicate that there was no agreement in efficiency ranking between both diets and that nitrogen recycling parameters were important in determining feed efficiency in the low (8.8% CP)- but not the high (13.5% CP)-protein diet.

2. Provide elucidation of physiological processes responsible for better rumen efficiency in low-protein diets.
   The objective was achieved. The results indicate that steers with more efficient rumen fermentation lose less N in urine and maintain lower plasma urea nitrogen, emphasising the importance of N-recycling in determining rumen and feed efficiency.

3. Evaluate the potential for the development of a practical tool to monitor the efficiency of nitrogen usage in grazing cattle, via analysis of plasma and tail hair.
   The objective was achieved. When fed a low-protein diet, steers losing less nitrogen in urine and with better rumen and overall feed efficiency also had distinct N-isotopic profile in tail hair, indicating that the ^{15}N:^{14}N ratio in tail hair can be used to predict the nitrogen use efficiency of cattle.

4. Identify rumen microbial populations and microbial activities associated with more efficient animals.
   The objective was achieved. Microbial populations associated with feed efficiency were identified, but only for the low-protein diet, further corroborating the hypothesis that different physiological mechanisms are responsible for feed efficiency in animals receiving different diets.

5. Review of the project and analysis of potential value.
   The objective was achieved. Interviews with producers, extension agents, and stakeholders in the red meat industry indicated great demand for the ability to identify cattle with better nitrogen use efficiency in rangeland grazing systems. However, two conditions were identified affecting

the commercial value of this technology: the trait needs to be heritable and the relationship with efficiency needs to hold for cattle receiving urea supplements.

6. Estimate the heritability of nitrogen isotope ratios in tail hair.
   The objective was achieved. The nitrogen isotope ratio was measured in 492 Brahman and Droughtmaster steers with complete pedigree information. Heritability was estimated at 43%.

7. Determine the relationship between nitrogen isotope profile in tail hair and the response of cattle to urea supplements.
   The objective was achieved. Steers with lower levels of ^{15}N in tail hair were more efficient on both diets, with, or without a urea supplement.

Methodology

- **Appropriate diets for feed efficiency**: To test the hypothesis that feed efficiency of cattle fed low-protein diets is not correlated with feed efficiency in nutrient abundant diets, measurements were made with two diets fed to 89 growing *Bos indicus* cattle. Steers were fed in individual pens for two periods of 70 days, including an adaptation of 10 days. Feed efficiency was calculated as Feed conversion ratio, Gain to Feed ratio, Residual feed intake, Residual gain, and Residual feed intake and gain.

- **Understanding feed efficiency in low-protein diets**: The second hypothesis of this project stated that more feed efficient steers would lose less N in urine and faeces when fed the low-protein and high-protein diets. At the end of the 70-day feeding period in individual pens, steers were moved into metabolism crates for a five-day collection period after two days of adaptation. Throughout the collection period in metabolism crates, each steer's total daily faecal and urine output was collected, nitrogen recycling and rumen efficiency were quantified, as well as nitrogen use efficiency.

- **Development of a practical tool to select more efficient cattle**: To evaluate whether the ^{15}N:^{14}N ratio in tail hair could be used to identify more efficient animals when ingesting a low-protein diet, tail hair samples from the 89 steers were collected, washed, dried, cut into 10 mm sections and combined for mass spectrometry analysis of the C and N isotopic enrichment. The use of tail hair isotopes to predict cattle response to protein supplements was further validated in a trial with 24 steers receiving a low-protein diet for 8 weeks followed by 8 weeks of urea supplementation. The ^{15}N:^{14}N ratio in tail hair was used to predict cattle performance on both diets.

- **Estimating the heritability of nitrogen isotope ratios in tail hair**: Tail hair was collected from 492 tropically-adapted steers (Brahman and Droughtmaster) participating in the BREEDPLAN genetic evaluation system. Segments of the tail hair representing hair growth during the dry season were cut, processed and analysed for N isotope ratios. Heritability was estimated in a univariate animal model in the WOMBAT software using 3 generations of available pedigree.

- **Microbiome analysis:** To characterise the rumen microbial populations and identify microbial species associated with more efficient steers, rumen fluid samples were collected from the 89 steers evaluated for feed efficiency in two different diets. The genomic DNA in the rumen fluid was extracted, amplified and sequenced at the V3-4 region of the bacterial 16S rRNA gene. Taxonomy was assigned at 97% similarity to the Green Genes database.

Results/key findings

There was no agreement in efficiency ranking when steers were consuming a low-protein compared to a high-protein diet, demonstrating the possible need to use appropriate diets when targeting performance in harsh environments. Because more-efficient steers lost less N in the urine and used the available nitrogen 41% more efficiently than the less-efficient steers, more-efficient steers had a distinct N-isotopic ratio in tail hair. This project demonstrated that the ^{15}N:^{14}N ratio in tail hair can be used to predict feed efficiency in low-protein diets and the performance of steers when receiving a urea supplement. In addition, this trait was demonstrated to have good heritability (43%) in tropically adapted breeds.

Benefits to industry

This project demonstrated the possibility of an innovative way to detect more nitrogen-efficient cattle, which could be further developed and commercialised. The ability to classify cattle for nitrogen efficiency may be able to select more efficient steers with faster growth rates in grazing systems, to select more efficient cows able to maintain body condition during the dry period translating into higher fertility and calf survival, and to predict the response of individual animals to dry-season supplements.

Future research and recommendations

The continuation of this line of research may allow for a more efficient selection of animals when targeting performance in harsh environments. Therefore, this technology could be commercialised. Breeders' performance in northern systems, with low fertility rates and high calf wastage, is a major factor affecting northern beef businesses' profitability and sustainability. Extending this line of research to quantify the impact of nitrogen use efficiency on cow fertility and calf mortality may allow for easier selection of more efficient cows, able to make better use of the scarce amount of protein available.

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1. Background

The ability of cattle to grow and reproduce when ingesting low-protein diets is crucial for productive beef cattle systems in the seasonally dry tropics and subtropics. Because of long drought periods and the low energy and protein values of typical native pastures, the performance of cattle in most northern Australia production systems will depend on salvage mechanisms maximising nutrient utilisation and conservation. There are large differences between individual animals in their efficiency of feed use. For example, some cows require up to 50 per cent less feed per kg of calf weaned than other cows of similar size from the same selection line (Griffith *et al.* 2004). As expected, differences in feed efficiency will result in differences in productivity and profitability of the herd. Simulations performed with the Northern Australia Beef Systems Analyser demonstrated that an increase of only three percentage points in the digestion of poor quality forages with improved rumen efficiency would result in large gains in productivity and financial performance, leading to an increase of 57% in annual net profit (Ash *et al.* 2015). Thus, we currently have a scenario in which there is large herd variability in feed efficiency and small gains in feed efficiency would translate to massive gains in productivity. However, selecting cattle for better feed efficiency in rangeland grazing systems is not a trivial task.

A major issue of the beef industry is that, generally, bulls are selected on nutrient-abundant diets and they and their progeny are expected to perform as efficiently in more challenging environments, such as the ones imposed by tropical pastures during the drier periods of the year. If feed efficiency is to be used for selection of *Bos indicus* cattle grazing rangeland pastures, it is essential to determine the robustness of rankings evaluated with low- and high-protein diets, with special emphasis on nitrogen use efficiency. The productivity of beef cattle fed low-protein diets relies on the ability to retain more nitrogen (Stewart and Smith 2005) by mechanisms involved in nitrogen recycling (Silva *et al.* 2019). Thus, nitrogen use efficiency should be considered when using feed efficiency parameters to select animals for the dry tropics. However, rumen efficiency and nitrogen recycling are not easily measured, as it involves infusing solutions via the jugular vein for several days, and frequent faecal and urine collections. Therefore, the development of more practical techniques is important for large-scale evaluation of nitrogen use efficiency. Classifying animals on the farm according to the nitrogen use efficiency would be important, not only from a genetic improvement perspective but also as a diagnostic for overall management practices aiming to improve nitrogen utilization and mitigate environmental impact.

There is strong evidence that the efficiency of nitrogen utilisation can be inferred based on the isotopic ratio of ^{15}N:^{14}N in the diet and the animal. The more efficient animal will lose less ^{15}N-depleted urea in the urine, changing the ^{15}N concentration in body proteins. Strong correlations between the efficiency of nitrogen usage and ^{15}N values in plasma protein have been reported previously (Cantalapiedra-Hijar *et al.* 2015; Guarnido-Lopez *et al.* 2021). Furthermore, cattle fed with high protein diets have higher ^{15}N values than cattle fed with low-protein diets, reflecting the lower nitrogen efficiency in high-protein diets (Sponheimer *et al.* 2003).

Tail hair grows at about 1 cm every fortnight, and in doing so, incorporates the ^{15}N signature of the available amino acids. By analysing the ^{15}N content in tail hair segments, it is possible to determine periods of greater and lesser nitrogen efficiency and to classify animals accordingly to their nitrogen usage efficiency. Classifying cattle according to the nitrogen use efficiency would be important, not only from a genetic improvement perspective but also as a diagnostic for overall management practices aiming to improve nitrogen utilization and mitigate environmental impact.

2. Objectives

The objectives for this project were:

1. To determine the necessity of using low-protein diets when evaluating feed efficiency of cattle for the northern Australian production systems.

The objective was achieved. The results indicate that there was no agreement in efficiency ranking between low and high protein diets and that nitrogen recycling parameters were important in determining feed efficiency in the low- but not the high-protein diet.

2. Provide elucidation of physiological processes responsible for better rumen efficiency in low-protein diets.

The objective was achieved. The results indicate that steers with more efficient rumen fermentation lose less N in urine and maintain lower plasma urea nitrogen, emphasising the importance of N-recycling in determining rumen and feed efficiency.

3. Evaluate the potential for the development of a practical tool to monitor the efficiency of nitrogen usage in grazing cattle, via analysis of plasma and tail hair.

The objective was achieved. When fed a low-protein diet, steers losing less nitrogen in urine and with better rumen and overall feed efficiency also had distinct N-isotopic profile in tail hair, indicating that the ^{15}N:^{14}N ratio in tail hair can be used to predict the nitrogen use efficiency of cattle.

4. Identify rumen microbial populations and microbial activities associated with more efficient animals.

The objective was achieved. Microbial populations associated with feed efficiency were identified, but only for the low-protein diet, further corroborating the hypothesis that different physiological mechanisms are responsible for feed efficiency in animals receiving different diets.

5. Undertake an in-depth review of the project providing clear recommendations on future work and an in-depth analysis of perceived potential value to the industry in terms of productive advantage and environmental benefit.

The objective was achieved. Interviews with producers, extension agents, and stakeholders in the red meat industry indicated great demand for the ability to identify cattle with better nitrogen use efficiency in grazing systems. However, two conditions were identified affecting the commercial value of this technology: the trait needs to be heritable and the relationship with efficiency needs to hold for cattle receiving urea supplements. Therefore, two new objectives were added to this project.

6. Estimate the heritability of nitrogen isotope ratios in tail hair.

The objective was achieved. The nitrogen isotope ratio was measured in 492 Brahman and Droughtmaster steers with complete pedigree information. Heritability was estimated at 43%.

7. Determine the relationship between nitrogen isotope profile in tail hair and the response of cattle to urea supplements.

The objective was achieved. Steers with lower levels of ^{15}N in tail hair were more efficient in both diets, with or without a urea supplement.

3. Methodology

The main purpose of this project was to provide crucial knowledge of feed efficiency that may be considered to accelerate the genomic improvement of the northern herd. Our overarching hypothesis was that the ability of the animal to recycle nitrogen back to the rumen, instead of eliminating it in the urine, is crucial to determine feed efficiency when fed protein-deficient diets. To address this hypothesis, a series of experiments were conducted which are described below. The experiments were conducted at the Queensland Animal Science Precinct (Gatton, QLD, Australia), all procedures were done following the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were reviewed and approved by the University of Queensland Animal Ethics Committee (QAAFI/003/18, Appendix 1).

3.1 Appropriate diets for feed efficiency

This study is based on the concept that appropriate and representative diets must be used when selecting cattle genetics for specific scenarios. To test the hypothesis that feed efficiency of cattle fed low-protein diets is not correlated with feed efficiency in nutrient-abundant diets, measurements were made with two diets fed to growing *Bos indicus* cattle (Table 1). The first diet was a low protein (LP) ration with medium to high energy content and was formulated with insufficient rumen degradable protein to maximise the impact of nitrogen recycling on feed efficiency. The second diet (high protein, HP) contained the nutrients to meet all requirements for high growth rate, simulating animals fed a feedlot diet providing for high daily liveweight gains (ADG).

3.1.1 Animals, experimental design and facilities

This experiment was carried out between May and December of 2018 (steers born in 2016), between January and June of 2019 (steers born in 2017), and between September 2019 and March 2020 (second lot of steers born in 2017) at the Queensland Animal Science Precinct (Gatton, QLD, Australia). A sub-group of 45 Brahman steers was selected based on similar birth dates and BW from a cohort of circa 500 Brahman steers born in 2016, managed together, and sired from 50 possible bulls. A similar procedure was used to select 90 Brahman steers born in 2017. These 135 steers were genotyped using the Illumina BovineSNP50 BeadChip (Neogen, Gatton). From these 135 steers, 90 steers that maximized the genetic variability were selected and treated with moxidectin pour-on (Cydectin®) on arrival. The total number of steers was selected to confidently detect correlations equal to or greater than $r = 0.35$, with 80% of power. The experimental animals were allocated to one of nine blocks (10 animals each) based on their BW and housed in individual pens with concrete flooring, free access to water, and shading.

3.1.2 Diets and feeding procedure

All steers received two diets in two consecutive periods of approximately 70 days each, with the first 10 days of each feeding period used for adaptation. The LP diet was fed during the initial 70 days followed by the HP diet. The diets were Rhodes grass hay (*Chloris gayana* cv Kunth) and pelleted concentrates (Table 1). The hay was chopped in a single auger vertical mixer (PFG Australia, Melbourne, VIC) for approximately 15 to 20 minutes resulting in particles of varying sizes (average 5.5 cm). Steers were fed *ad libitum* with daily adjustment of the amount offered, at 0700h targeting 5% of refusals. The LP diet was formulated to supply circa 70% of the calculated requirement of rumen degradable protein (RDP) [88 g RDP/kg digestible organic matter intake (DOMI)], according to NASEM (2016). McLennan (2015) calculated the RDP requirements for microbial crude protein production to be 130 g RDP/kg DOMI, which would indicate that the LP diet in the present study was supplying 68% of the estimated requirements. Therefore, the term low protein diet in this study

refers to the lower than required supply of rumen degradable protein, which would stimulate the rumen recycling mechanism. In contrast, the term high-protein diet refers to the abundant dietary supply of rumen degradable protein, minimizing the impact of the rumen recycling mechanism on animal performance.

Table 1. Ingredients and chemical composition of the experimental diets.
DM, dry matter; CP, crude protein; RDP, rumen degradable protein; NIDN, nitrogen insoluble in neutral detergent; NIDA, nitrogen insoluble in acid detergent; and from, neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash; Lignin (sa), lignin determined by solubilisation of cellulose with sulphuric acid; DOM, digestible organic matter; ME, metabolizable energy; MP, metabolizable protein.

Item	Diet	
	Low-Protein	High-Protein
Ingredient (% DM)		
Rhodes grass hay	50	40
Concentrate	50	60
Concentrate composition (% DM)		
Barley ground	39	52.5
Sorghum ground	39.5	--
Wheat ground	5	5
Millrun	10	10
Canola meal	--	13
Soybean meal	--	13
Vegetable oil	1	1
Molasses	2.5	2.5
Limestone	1.3	1.3
Sodium bicarbonate	1	1
Ammonium sulphate	0.5	0.5
Vitamin/Mineral premix	0.2	0.2
Diet chemical composition		
DM (%)	92.4	92.7
CP (% DM)	8.8	13.5
RDP (% CP)	65.7	77.4
NIDN (%CP)	6.8	5.2
NIDA (%CP)	2.3	1.5
aNDFom (% DM)	48.1	39.9
Lignin (sa) (% DM)	2.5	2.3
Ether extract (% DM)	2.2	2.0
Ash (% DM)	1.6	1.3
DOM (% DM)	65.3	70.8
RDP/DOM (g/kg)	88.5	147.6
ME[1] (MJ/kg DM)	10.0	10.9
ME[2] allowable gain (kg/d)	0.91	1.33
MP[2] allowable gain (kg/d)	0.60	1.40

[1]Estimated as 0.81 x 19 MJ/kg of DOM (CSIRO 2017).
[2]Estimated based on requirements described in NASEM (2016).

Samples of hay and concentrates were collected throughout the experiment and bulked weekly for chemical analyses. Individual pen refusals were weighed daily for determination of dry matter intake (DMI). The steers were weighed unfasted, before feeding on two consecutive days at the beginning

of the feeding period in individual yards (day 0), mid (day 30) and final days of treatment (day 60). In addition, intermediate weighing's on days 15 and 45 were performed. Average daily gain (ADG) was estimated from the linear regression of all BW measurements over time.

3.1.3 Feed efficiency measurements

Feed conversion ratio (FCR) was calculated as the DMI per unit ADG, and G:F ratio was calculated as the reverse (i.e., ADG per unit DMI). Residual feed intake (RFI), residual gain (RG) and residual feed intake and gain (RIG) were calculated as follows: Feed efficiency as residual feed intake (RFI) was determined as the difference between the actual DMI and the expected DMI (Archer *et al.* 1997). Expected DMI was calculated from the linear regression of DMI over BW and ADG. In contrast, residual gain (RG) was estimated using the difference between the actual ADG and the expected ADG (Crowley *et al.* 2010). Expected ADG was derived from the linear regression of ADG over BW and DMI. Lastly, RIG was calculated as the difference between the real and the expected DMI and ADG (Berry and Crowley 2012). Expected DMI and ADG were estimated from the linear regression of DMI and ADG over BW.

3.1.4 Diet laboratory analyses

All samples of hay, concentrates and refusals were dried at 60°C for 72 h in a forced-air oven and ground through a 2 mm screen (Retsch ZM 200; Haan, NW, Germany). Final dry matter was corrected after drying samples for 24 h at 105°C. Organic matter content was calculated after combusting samples at 550°C for 8 h (Modutemp; Perth, WA, Australia). Ash-free NDF (aNDFom) was determined with an Ankom 200 fibre analyser (Ankom Technology Corporation; Fairport, NY, USA) with alpha-amylase using an adaptation of the procedure of Van Soest *et al.* (1991). Lignin (sa) was calculated by washing ADF samples in sulphuric acid at 72% for 3 h in a Daisy Incubator (Ankom Technology; Macedon, NY, USA). The N content of feed offered was determined by the Dumas combustion method (Sweeney 1989), using a LECO CN928 Carbon / Nitrogen combustion analyser (LECO Corporation; St Joseph, MI, USA). The crude protein of feed samples was calculated using the conversion factor of N x 6.25. Nitrogen insoluble in neutral detergent (NDIN) and nitrogen insoluble in acid detergent (ADIN) were determined in the residues from aNDFom and ADFom, respectively, using the LECO analyser. Ether extract content was determined after extraction with petroleum spirit with the SER148 Solvent Extraction Unit (VELP Scientifica S.R.L.; Usmate, MI, Italy).

3.2 Understanding feed efficiency in low-protein diets

When cattle are fed low-protein diets, in which microbial crude protein (MCP)production is limited by available dietary RDP, their productivity relies on their ability to retain more N within their body instead of excreting it in faeces and urine (Stewart and Smith 2005). In contrast, the ability of cattle to recycle N back to the rumen may not be important when fed protein-abundant diets, in which MCP is not restricted by dietary RDP. The second hypothesis of this project stated that more feed-efficient steers would lose less N in urine and faeces when fed both the LP and HP diets. Increasing nitrogen use efficiency is particularly important in cattle due to their inherently lower N retention rates when compared with other livestock species, leading to the high environmental and economic costs of N use in beef production systems (Zhao 2019).

3.2.1 Measuring digestibility and rumen efficiency

At the end of the 70-day feeding period in individual pens, steers were moved into metabolism crates for a five-day collection period after two days of adaptation. While in the metabolism crates, feed offered was fixed at 90% of the individual average daily intake during the five last days of the

previous feeding period. Throughout the metabolism crate collection period, total daily faecal output of each steer was collected. Faeces were weighed and mixed thoroughly, and a 10% sub-sample was retained, bulked and stored at 4°C. At the end of the collection period, the bulked faecal samples for each steer were mixed, weighed and duplicate sub-samples dried at 60°C to constant weight for N determination. These storage and drying conditions have been shown to not affect the total N content of cattle faeces (Juko *et al.* 1961). Duplicate samples were combined to provide a representative sample for that period.

Total daily urine output of each steer was collected over the same five days. Urine was collected in metal trays placed under the metabolism crates equipped with automatic pumps (Bowline 500GPH Bilge Pump, BCF, Toowoomba, QLD, Australia) that directed urine to a plastic container with 5% H_2SO_4 added to maintain urine pH below 4. Total daily urine output was weighed, mixed thoroughly and pH recorded. The targeted urine pH was between 3 and 4, if the urine sample had a pH above 4.5 it was discarded or if the pH was between 4.0 – 4.5, adjusted with more 5% H_2SO_4. A 10% sub-sample of acidified urine was collected each day and bulked over the collection period for individual steers and stored at 4°C for measurements of total nitrogen, N-NH_3 and purine derivatives for estimation of MCP production.

Purine derivatives in the urine were analysed according to the methods of George *et al.* (2006) and Czauderna and Kowalczyk (1997) using a Prodigy 250 x 46 mm, 5 µm, ODS C18 reverse-phase column (Phenomenex, Torrence, CA, USA). In brief, acidified urine samples were thawed, a buffer and internal standard added, this was then filtered through a 0.20 µm cellulose nitrate filter followed by a 300 mg C18 filter and analysed for purine derivatives concentration using high-performance liquid chromatography with quantification at 215 nm (Shimadzu Prominence HPLC with Photo Diode Array Detector; Kyoto, Honshu, Japan). Microbial protein production was calculated using the equation of Chen and Gomes (1992), with the value for excretion of endogenous PD for *Bos indicus* cattle from Bowen *et al.* (2006). The efficiency of MCP synthesis (EMPS) was calculated as g of MCP/kg digestible OMI (DOMI).

3.2.2 Measuring nitrogen metabolism and nitrogen use efficiency

After 70 days on the experimental diets, blood was collected from the jugular vein into lithium heparin coated vacutainers (Becton Dickinson; Franklin Lakes, NJ, USA) at 0, 2, 4 and 6 h after feeding. The vacutainers were inverted six to eight times and held at 4°C, for 20 to 30 min before centrifugation at 4,000 x g for 10 min. Plasma samples were then separated and stored at -20°C for the subsequent analysis of the urea concentration. Plasma urea nitrogen (PUN) was determined using a Beckman Coulter AU480 auto-analyser (Beckman Coulter Diagnostic Systems Division; Melville, NYC, USA), as described in Marsh *et al.* (1965). The rumen NH_3-N concentration was estimated using direct distillation (UDK 139 semi-automatic distillation unit; Rowe Scientific Pty Ltd; Wacol, QLD, Australia) followed by titration (Titralab 840 automatic titration unit; Hach; Dandenong, VIC, Australia), as described in Preston (1995).

Nitrogen retention was calculated as the total N intake minus total N excretion in faeces and urine, using the chemical composition of bulked samples of feed offered, faeces and urine samples from the collection period in metabolism crates. Nitrogen use efficiency (NUE) was calculated as the proportion of retained N (g/d) over digested N (g/d) (Archibeque *et al.* 2001).

3.3 Development of a practical tool to select more efficient cattle

Measurements of feed efficiency usually must be conducted over extended periods to allow the collection of representative feed intake and liveweight gain performance data (Asher *et al.* 2018),

and measurements of nitrogen use efficiency require intensive sample collection and laboratory analyses. However, the ^{15}N to ^{14}N stable isotope enrichment ratio of ruminant plasma proteins has been used as an alternative method to estimate nitrogen use efficiency (Cantalapiedra-Hijar *et al.* 2015). The objective of the present study was to evaluate whether the N-isotope ratio in tail hair measured by mass spectrometry could be used to identify more efficient animals when ingesting a protein-limiting diet.

3.3.1 Tissue sample collection

Immediately before relocating the steers to the metabolism crates, strands of tail hair were pulled from each steer, placed in paper bags, and stored at ambient temperature in a dry and dark place until further analysis. Five strands of the most recently grown 1 cm segment of tail hair, including the hair bulb, were combined for analysis of the N stable isotope ratios. Given that cattle tail hair grows circa 1 cm per fortnight (Schwertl *et al.* 2003), the analysed segment represents hair grown approximately during the last two weeks of the trial.

Blood was collected from the jugular vein at four time points (-1, 2, 4, and 6 hours after feeding) into a 10 mL lithium heparin coated vacutainer (Becton Dickinson; Franklin Lakes, USA) on the last two days of the feeding period. Plasma was obtained with centrifugation at 2,000 x g for 15 min and stored at -20 ^{o}C. The four plasma samples from each steer were pooled and 200 µl of sulfosalicylic acid (10% solution) added to 2 mL of plasma for protein precipitation. After centrifugation (4,500 x g for 20 min) and washing, protein precipitates were freeze-dried and approximately 2 to 3 mg used for nitrogen isotopic determination (Cantalapiedra-Hijar *et al.* 2015).

3.3.2 Tail hair processing and isotope analysis

The hair samples were first washed to remove contaminants. Hair strands of about 20 mm were soaked in deionized water in a 50 mL beaker, washed by ultra-sonication, the water discarded, and the beakers containing samples dried at 40 ^{o}C for 48 h. To remove fats and any other remaining contaminants the hair samples were soaked in a 2:1 mixture of methanol/chloroform before being re-washed with deionized water, soaked in deionized water for another 30 min, and rinsed again. Finally, the samples were dried at 40 ^{o}C for 48 h. Individual hairs were selected from each sample and cut into 10 mm long sections using a stencil. Five strands of the most recently grown 10 mm segment of tail hair were combined for analysis of the C and N isotopic enrichment.

Isotope ratio measurements were performed at the Stable Isotope Geochemistry Laboratory within the School of Earth and Environmental Sciences at the University of Queensland, using an IsoPrime100 isotope-ratio mass spectrometry (Isoprime Ltd, Cheadle, UK) with dual inlet and coupled with a vario Pyro cube (Elementar Australia Pty, Sydney). The stable isotope values are reported using the standard delta notation (δ per mil, ‰) calculated as follows: $\delta X\ (‰) = [(R_{sample} - R_{standard})/R_{standard}]$, where X is the element considered, and R is the ratio of the heavy to light stable isotope in the sample (e.g., $^{15}N/^{14}N$) and the standard ($R_{standard}$). The results are reported against the AIR international standard. The diet-animal fractionation ($\Delta^{15}N$) was calculated as the difference between the $\delta^{15}N$ of the animal tissue (both tail hair and plasma) and the $\delta^{15}N$ of the diet to provide comparative values for $\Delta^{15}N_{tail\ hair}$ and $\Delta^{15}N_{plasma}$.

3.4 Rumen microbiome

The main objective of this study was to evaluate the role of rumen microbiota in modulating feed efficiency in tropically adapted cattle receiving two diets with different protein contents.

3.4.1 Rumen fluid collection

At the end of each subperiod within experimental-block periods, rumen fluid was collected via oesophageal tubing at 0 h (before feeding) and 4 h after feeding on the same day. Rumen fluid was filtered through four layers of cheesecloth, and pH was measured immediately (Edge Benchtop HI2002, Hanna Instruments, Melbourne, Vic., Australia). Initial rumen fluid was discarded if saliva was visually observed. In addition, pH measurements were assessed to monitor whether samples were within optimum levels, ruling out saliva contamination. Subsamples were transferred into tubes for ammonia (NH_3-N) estimation (6 mL of rumen fluid + 2 mL 0.5 M H_2SO_4) and stored at −20°C. Further subsamples (1 mL) were immediately flash-frozen in liquid N and stored at −80°C for DNA extraction.

3.4.2 DNA extraction, sequencing and bioinformatics

The genomic DNA in rumen fluid samples was extracted after a bead-beating procedure and on-column purification (Popova *et al.* 2010). The quality and quantity of DNA were measured on a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, France). Approximately 15 µg of extracted DNA was submitted to Fluidigm amplification and MiSeq Illumina sequencing. The V3-4 region of the bacterial 16S rRNA gene was amplified via 515f (5'-GTGCCAGCMGCCGCGGTAA-3') and 806r (5'- GACTACHVGGGTWTCTAAT-3') primers. These sequences were processed using DADA2 (version 1.14) and R (version 3.6.0) software. The 16S rRNA bacterial gene sequences (forward and reversed) were trimmed to 240 and 160 bp and merged. Chimeras representing 2.5% of total sequences, were removed. Taxonomy was assigned at 97% similarity to the Green Genes database (V13.8).

3.5 Quantification of heritability of tail hair isotopes in cattle

Research has shown that nitrogen recycling plays an important role in differences in feed efficiency between individual animals, especially with low-protein rations (Silva *et al.* 2019). Recent studies demonstrated the potential of using the natural abundance of nitrogen isotopes ($\delta^{15}N$) in tail hair to evaluate urinary N excretion and urinary nitrogen: nitrogen intake in ruminants (Khanaki *et al.* 2021). Cattle with higher nitrogen use efficiency (NUE) are expected to have lower $\delta^{15}N$ in both plasma (Cantalapiedra-Hijar *et al.* 2015) and tail hair proteins (Silva *et al.* 2022), as less of the N intake is excreted to the urine. However, for $\delta^{15}N$ to be used by the cattle industry as an indicator of nitrogen use efficiency, its heritability needs to be determined. The objective of the present study was to use performance and pedigree records to identify the heritability of tail hair $\delta^{15}N$, and the correlation with performance traits.

Tail hair samples were collected from 492 Brahman and Droughtmaster steers participating in the Beef Information Nucleus (BIN) herd. All the steers were originally located at the Spyglass Research station and later transported to Taroom, QLD, where they were kept on Leucaena and oat stubble pasture. The steers experienced nutrient, especially protein, deficiencies during some stages of their life. For each steer, approximately 30-40 tail hairs were plucked making sure to get the hair bulbs. All hair samples were placed in labelled envelopes and stored in a cool, dry place. Using data from the Bureau of Meteorology, Australia, a time of the year representing the dry season was determined and the corresponding segment of hair was cut to represent the hair growth during this period. Tail hair samples were washed and processed as described in item 3.3.2. The history and pedigree information of the steers were supplied by Breedplan (Agricultural Business Research Institute, University of New England, Armidale, NSW).

3.6 Using tail hair analysis to predict response to urea supplements

Urea and molasses are commonly supplemented to cattle in northern Australia during the dry season to increase pasture intake, avoid liveweight loss and reduce cattle mortality (Gulbransen 1985). As urea is supplying readily available RDP, its use could minimize the importance of nitrogen recycling in determining feed efficiency. On the other hand, urea supplements are usually ingested just once a day, or less frequently in extensive grazing systems, and rumen urea is rapidly hydrolysed to ammonia in the rumen and absorbed into the blood circulation (Hall and Huntington 2008). The absorbed ammonia, after being converted back to urea in the liver, can be lost in the urine or recycled back to the rumen. Therefore, the effectivity of urea supplements to increase MCP production and cattle performance is likely also dependant on nitrogen recycling mechanisms. Our hypothesis was that cattle with better N-recycling ability, and lower $\Delta^{15}N_{tail\ hair}$, will have a better response to urea supplementation, as less N will be lost in urine and more microbial protein will be produced. Having the ability to screen individuals according to the potential to respond to urea supplementation would be of great value for the beef industry.

3.6.1 Location, animals, and diets

This trial was conducted between June and November of 2021 at the Queensland Animal Science Precinct (Gatton, QLD, Australia) and consisted of 24 Brahman steers with 238 ± 29 kg initial live weight (LW). Approval was granted by the University of Queensland Animal Ethics Committee (2021/AE000013, Appendix 1). Steers were blocked into two groups based on LW (high and low) and randomly assigned to individual pens (3x10m). The trial consisted of an adaptation period of 7 d before commencement of the trial, then two feeding periods: hay only and hay plus urea-molasses supplement, spaced by another 7 d adaptation period. During the hay only period, a low-protein hay was fed for 56 days (6.5% CP) to achieve maintenance requirements of steers. The hay was offered ad libitum, with daily adjustments to maintain refusals around 5%. After this period, the steers received the same low-protein hay and a urea-molasses supplement with 8% of urea (M8U). The M8U was offered at 10% of hay intake, on an as-fed basis and supplied approximately 40 g urea per day (Urea diet, 8.1% CP). The M8U supplement was chosen not only because of its widespread use in north Australia but also because molasses will supply fermentable energy, further stimulating the N-recycling mechanism (Obara *et al*. 1991).

Steers were weighed on two consecutive days, every fortnight, and average daily gain (ADG) calculated by regressing LW over time. The response to the urea supplement was calculated as ADG on the Urea diet – ADG on the Control diet.

3.6.2 Sample collection and analysis

Samples of hay and refusals were collected and pooled per week. An M8U sample was taken to represent the single container used in this study. Individual pen refusals were weighed daily to determine DMI. Hay as fed, hay residuals, and M8U were dried in a forced-air oven at 60^{o}C and ground to 2 mm (Retsch ZM 200; Haan, NW, Germany). Hay as fed samples were analysed for moisture, total N, NDF and ash. Hay residuals and M8U were analysed for moisture, total N and ash. Tail hair samples were collected at the end of the hay only period to determine the N-isotopic ratio without urea supplements, and then again at the end of the hay + urea-molasses supplement period. Tail hair samples were processed and analysed as described in section 3.3.2.

3.7 Statistical analysis

3.7.1 Understanding feed efficiency in low-protein diets

To evaluate the factors influencing feed efficiency measurements and to examine the consistency of animal rankings in both diets, the 85 steers (from the original 90 steers) for which data was available for both LP and HP diets were categorized into high, medium, and low FE (RFI, RG and RIG) groups based on being ± 0.50 SD from mean (Asher *et al.* 2018). One steer was removed from the trial due to aggressive behaviour, two steers were removed due to apparent ill health associated with low voluntary intake, and two steers were removed at the start of the trial due to injury (Appendix 3). Pearson correlation coefficients were determined using Proc CORR of SAS. Data were analysed as a completely randomized block design, using the MIXED procedure of SAS (SAS Institute Inc., 2019; version 9.4). The normality of residuals was evaluated by the Shapiro–Wilk test. Homogeneity of variances was assessed by the Levene test. The model included the fixed effect of RFI, RG or RIG groups and block as a random effect:

$Yij = \mu + \alpha i + Bj + eij$

where: Yij = dependent variable; μ = overall mean; αi = fixed effect of RFI, RG or RIG groups (i =low, medium or high); Bj = random effect of block j; eij = random residual variation. The chance-corrected agreement of the FE classifications between both diets was calculated with the Kappa analysis. Group means were compared using the LSMEANS option of the MIXED procedure and effects were considered significant at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

From the 89 steers evaluated for feed efficiency in the LP diet, 72 steers (81%) had robust data for the evaluation of nitrogen metabolism in metabolism crates. One steer was not evaluated because of aggressive behaviour, two steers were removed due to injury, four steers were removed because of failure in total urine collection, and data from 10 steers were removed from the analysis because of very low intake when inside the metabolism crates (less than 1.2 kg DM/100 kg BW).

3.7.2 Rumen microbiome

Differences in ruminal microbial abundance between high-and low-protein diets were determined using the DESeq2 package in R (Love *et al.* 2014). Alpha diversity indexes were analysed with the Phyloseq package (version 1.26.0) on R software, using the number of operational taxonomy units (OTUs) per sample (McMurdie and Holmes 2013). Bray–Curtis dissimilarities were estimated with vegan package 2.5-3 (Oksanen 2015) in R. Differences between low-and high-protein diets were calculated with permutational multivariate analysis of variance (PERMANOVA).

Ruminal microbial populations were associated with feed-efficiency parameters by using two alternative analyses. The abundance of individual bacterial genera was correlated with these parameters by using the PROC CORR procedure on SAS software. Further, principal-component analysis (PROC PRINCOMP) was performed for each diet, considering bacterial genera population to identify patterns between steers. Steers were grouped based on the four principal components, using PROC CLUSTER, explaining approximately 62% and 53.5% (R-square) of total bacterial variance composition across steers, for LP and HP respectively. Ward's hierarchical method was used for clustering analysis, and the MIXED procedure to calculate differences in feed-efficiency parameters among steer clusters based on ruminal bacteria populations.

Cluster means were compared using the LSMEANS option of the MIXED procedure, considering experimental period as a random effect, and cluster as a fixed effect. Differences were considered

significant at P ≤ 0.05 and tendencies were declared when P ≤ 0.10. Shapiro–Wilk test was performed to estimate normality of residuals, and homogeneity of variances was calculated using the Levene test.

3.8 Review of the project and analysis of potential value

The results from this project were constantly reviewed and presented to the industry to assess its potential value. The results have been communicated in different formats, such as publication of a thesis, manuscripts in scientific journals, presentations at national and international conferences, organization of workshops to discuss the results and plan future actions, and media communications (Appendix 4). In addition, we have hosted at Gatton the visit of several beef cattle producers, nutritionists, and cattle breeders interested in learning more about this project. The results were also presented at two field days in 2020, at McKinley and Redcliffe.

The potential for commercialization has also been discussed with different industry representatives, such as the Australian Brahman Breeders Association, Tropical Beef Technology Services, ST Genetics, and regional NABRC committees. The discuss on the applicability of a technique to rank cattle based on nitrogen use efficiency was focused on three basic questions:

1- Do you see value for your business in ranking cattle for nitrogen use efficiency?
2- How would you apply this knowledge to improve your business?
3- Which conditions should be met for you to purchase this information?

Based on the responses over the course of this project, we were able to adjust the objectives to address the main concerns from the industry representatives.

The impact of better nitrogen use efficiency was estimated based on a stable beef enterprise with 3,000 adult equivalents of carrying capacity. Total greenhouse gas emissions and emissions intensity were calculated based on the Australian's National Greenhouse Gas Inventory using the Greenhouse Accounting Framework calculator for beef (V1.8) and following the MLA Carbon accounting technical manual (Wiedemann and Dunn 2021).

4. Results

The results from the current project will be presented in sessions addressing the five initial objectives.

4.1 Appropriate diets for feed efficiency

If feed efficiency is to be used for selection of *Bos indicus* cattle grazing rangeland pastures, it is essential to determine the robustness of rankings evaluated with low- and high-protein diets. It has been reported that feed efficiency in different growth stages of animals fed similar diets has a moderate to high correlation (Hansen *et al.* 2017), but studies evaluating feed efficiency rankings in different diets are scarce. The objective of this study was to determine the necessity of using low-protein diets when evaluating feed efficiency of cattle for the northern Australian production systems.

4.1.1 Data description and individual variation

Feed intake (DMI) was highly variable within diets, averaging 2.00 ± 0.18 kg DM/100 kg BW (mean ± SD) when animals were fed a low-protein (LP) diet and 1.86 ± 0.21 kg DM/100 kg BW (mean ± SD) when animals were fed a high protein (HP) diet (Table 2). Average daily gain (ADG) was also highly

variable among the steers in both diets (Table 2). Under the LP diet, the mean ADG was 1.06 ± 0.26 kg/d (mean ± SD), while when receiving the HP diet, ADG averaged 1.16 ± 0.25 kg/d (mean ± SD). The difference in ADG between the two diets likely reflect the difference in energy content (10 vs. 10.9 MJ ME/kg).

Feed efficiency in this trial was calculated using four different criteria. When steers were fed the low-protein diet, the feed conversion ratio (FCR) averaged 7.3 while ranging from 4.4 to 16.4 kg DMI/kg ADG (Table 2); efficiency of gain (G:F) ranged from 0.06 to 0.23 kg ADG/kg DMI, RFI ranged from -1.25 to 1.62 kg, RG ranged from -0.48 to 0.52 kg, and RIG ranged from -5.87 to 4.68. The large variation in feed efficiency, even in animals of the same age, is not unexpected, as previous studies have also reported substantial variation (Archer *et al.* 2002; Herd *et al.* 2011; Manafiazar *et al.* 2015).

Table 2. Performance of steers fed with two diets differing in protein content.
BW, body weight; ADG, average daily gain; DMI, dry matter intake; FCR, feed conversion ratio; G:F, gain to feed ratio; RFI, residual feed intake; RG, residual gain; LP, low-protein diet with 90 g RDP/kg DOMI; HP, high-protein diet with 145 g RDP/kg DOMI; DOMI, digestible organic matter intake.

Traits	Diet	n	Mean	Min	Max	Std Dev
Initial BW (kg)	LP	89	341	264	452	45
	HP	85	406	302	515	53
Final BW (kg)	LP	89	403	314	510	45
	HP	85	469	371	605	53
ADG (kg/d)	LP	89	1.06	0.55	1.79	0.26
	HP	85	1.16	0.65	1.74	0.25
DMI (kg/100 kg BW)	LP	89	2.00	1.62	2.47	0.18
	HP	85	1.86	1.32	2.35	0.21
FCR	LP	89	7.3	4.4	16.3	2.0
	HP	85	7.4	4.4	12.0	1.7
G:F	LP	89	0.14	0.06	0.23	0.03
	HP	85	0.14	0.08	0.22	0.03
RFI	LP	89	0.0	-1.25	1.62	0.54
	HP	85	0.0	-1.55	1.45	0.64
RG	LP	89	0.0	-0.48	0.52	0.17
	HP	85	0.0	-0.35	0.41	0.16
RIG	LP	89	0.0	-5.87	4.68	1.75
	HP	85	0.0	-4.40	4.29	1.75

When receiving a high-protein diet, animals had similar mean FCR (7.4 kg DMI/kg ADG), ranging from 4.4 to 12.0 kg DMI/kg ADG (Table 2). Residual feed intake varied from -1.55 to 1.45 on the high-protein diet, RG varied from -0.35 to 0.41, and RIG varied from -4.40 to 4.29. It is not possible to make inferences about the effect of diet on RFI or RG, as these indexes are calculated for steers receiving the same diet (Fig. 1 and Fig. 2).

Figure 1. Variation in residual feed intake (RFI) of growing steers receiving either (a) a low-protein diet or (b) a high-protein diet. RFI is calculated as the difference between the observed and the predicted DM intake (solid regression line). Positive RFI indicates a less efficient animal.

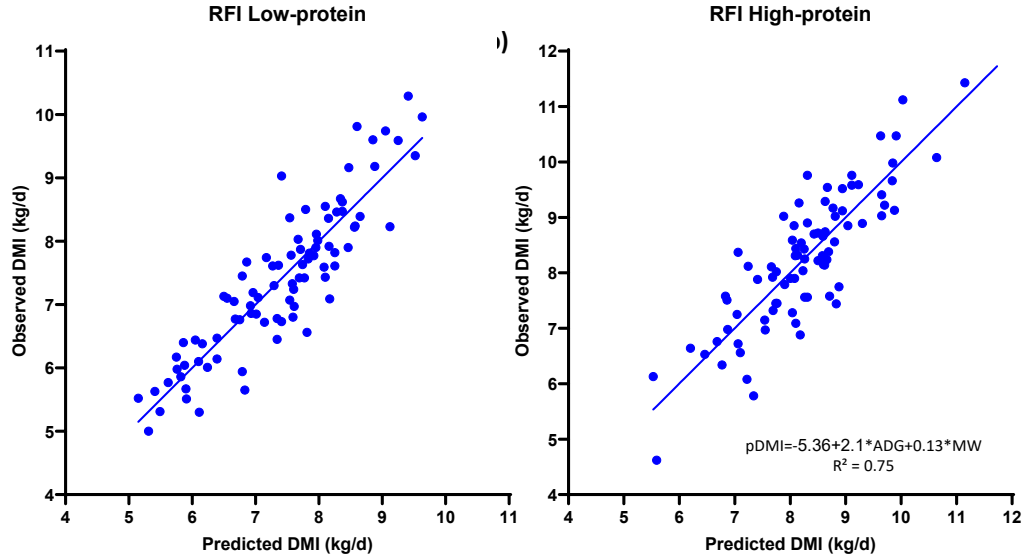
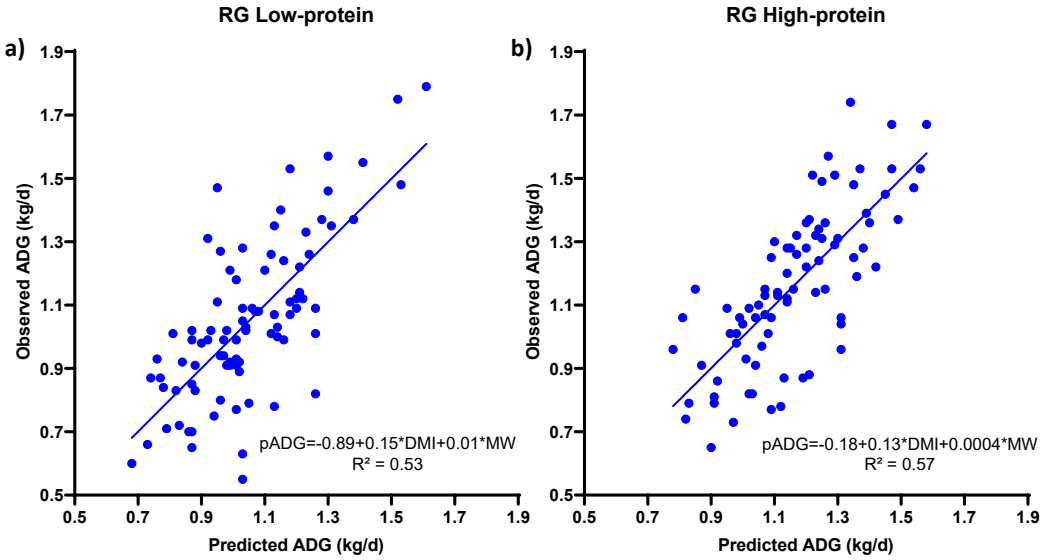


Figure 2. Variation in residual gain (RG) of growing steers receiving either (a) a low-protein diet or (b) a high-protein diet. RG is calculated as the difference between the observed and the predicted average daily gain (solid regression line). Positive RG indicates a more efficient animal.



Although all five measurements of feed efficiency were calculated using intake and body weight gain, it is relevant to note that these measurements were not all strongly correlated (Tables 3 and 4). RFI and RG had moderate (between 0.30 and 0.70) correlation (r = -0.465) in the LP diet and in the HP diet (r = -0.531).

Table 3. Pearson correlation coefficients comparing four different efficiency parameters in steers receiving a low-protein (LP) diet.

Item	FCR_LP	G_F_LP	RFI_LP	RG_LP	RIG_LP
FCR_LP	1				
G_F_LP	-0.946**	1			
RFI_LP	0.351**	-0.330**	1		
RG_LP	-0.701**	0.719**	-0.465**	1	
RIG_LP	-0.613**	-0.612**	-0.857**	0.854**	1

*P-value < 0.05; **P-value<0.01.

Table 4. Pearson correlation coefficients comparing four different efficiency parameters in steers receiving a high-protein (HP) diet.

Item	FCR_HP	G_F_HP	RFI_HP	RG_HP	RIG_HP
FCR_HP	1				
G_F_HP	-0.967**	1			
RFI_HP	0.335**	-0.369**	1		
RG_HP	-0.660**	0.635**	-0.531**	1	
RIG_HP	-0.568**	0.573**	-0.875**	0.875**	1

*P-value < 0.05; **P-value<0.01.

4.1.2 Ranking of animals in both diets

The initial objective of this trial was to evaluate the hypothesis that feed efficiency in a high-protein diet would be strongly correlated with feed efficiency in a low-protein diet. If this was the case, more efficient animals could be selected on normal, non-deficient diets and expected to perform with high efficiency when in more nutritionally challenging environments. There was a moderate correlation in intake between both diets (r = 0.43, P < 0.01), but low correlation for ADG (r = 0.27, P = 0.02, Table 5). Considering the five feed efficiency parameters, there were moderate correlations between the two diets for G:F ratio (r = 0.35, P < 0.01), RFI (r = 0.44, P < 0.01), and RIG (r = 0.33, P < 0.01). There was a low correlation for FCR (r = 0.23, P = 0.04) and no correlation for RG (r = 0.19, P = 0.09). Although significant, the moderate correlation coefficient means that RFI measured in the HP diet explained only 19% of the variation of RFI in the LP diet.

Table 5. Pearson correlation coefficients of efficiency parameters between steers receiving a low-protein versus a high-protein diet.
LP, Low-protein diet; HP, high-protein diet; ADG, average daily gain; DMI, dry matter intake; FCR, feed conversion ratio; G:F, gain to feed ratio; RFI, residual feed intake; RIG, residual feed intake and gain.

Item	ADG_LP	DMI_LP	FCR_LP	G:F_LP	RFI_LP	RG_LP	RIG_LP
ADG_HP	**0.267***	0.083	-0.206	0.300*	0.078	0.188	0.063
DMI_HP	0.264**	**0.429****	-0.227*	0.251*	0.343**	-0.077	-0.246*
FCR_HP	0.060	0.281*	**0.228***	-0.315**	0.103	-0.177	-0.164
G_F_HP	-0.065	-0.300*	-0.264*	**0.354****	-0.119	0.162	0.164
RFI_HP	-0.036	0.351**	0.171	-0.187	**0.439****	-0.198	-0.373**
RG_HP	0.048	-0.108	-0.056	0.131	-0.150	**0.188**	0.197
RIG_HP	0.048	-0.262	-0.130	0.182	-0.336**	0.221*	**0.326****

*P-value < 0.05, **P-value<0.01.

For further testing of the hypothesis, the 83 steers with performance data in both diets were categorized into low, medium or high groups according to their RFI and RG, based on ± 0.50 SD from

the mean (Asher *et al*. 2018). As shown in Table 6, there were 23 steers classified in the Low-RFI group, 34 in the Medium-RFI and 26 in the High-RFI group when evaluated with the LP diet; while there were 25 steers in the Low-RFI group, 32 in the Medium-RFI group and 26 in the High-RFI group when evaluated with the HP diet. Steers classified in the Low-RFI group had similar ADG but lower DMI than High-RFI steers in both diets.

Table 6. Performance of steers classified by residual feed intake in two diets differing in protein content.

LP, Low-protein diet; HP, high-protein diet; RFI, residual feed intake; BW, body weight; DMI, dry matter intake; ADG, average daily gain.

Traits	Diet[A]	RFI Groups			SEM	*P*-value
		Low	Medium	High		H vs. L
n	LP	23	34	26	--	--
	HP	25	32	26	--	--
RFI	LP	-0.66	0.01	0.59	0.06	<0.01
	HP	-0.75	0.01	0.69	0.06	<0.01
BW (kg)	LP	375	378	381	27.7	0.42
	HP	444	445	444	31	0.99
DMI (kg/100 kg BW)	LP	1.82	1.99	2.15	0.05	<0.01
	HP	1.67	1.88	1.99	0.07	<0.01
ADG (kg/d)	LP	1.05	1.04	1.07	0.08	0.78
	HP	1.16	1.18	1.13	0.08	0.64

The chance-corrected agreement of the RFI, RG and RIG rankings between both diets was calculated with the Kappa analysis. (Landis and Koch 1977) described the relative strength of agreement associated with kappa statistics as follows: poor agreement, <0.00; slight agreement 0.00–0.20; fair agreement 0.21–0.40; moderate agreement, 0.40–0.60; substantial agreement, 0.61–0.80; almost perfect agreement, 0.81–1.00. There was slight agreement (Kappa = 0.14, *P* = 0.07) in the RFI classification of steers when evaluated in the two different diets (Table 7). Of the 25 steers classified as Low-RFI in the HP diet, only 12 were classified as Low-RFI in the LP diet while 6 were classified as Medium-RFI and 7 as High-RFI.

Table 7. Agreement in the classification of steers according to residual feed intake (RFI) in two diets differing in protein content.

Diet	RFI Groups	High Protein			Total	Kappa	*P*-value
		Low	Medium	High			
Low Protein	Low	12	7	4	23	0.14	0.07
	Medium	6	15	13	34		
	High	7	10	9	26		
	Total	25	32	26	83		

When the steers were classified according to their RG in the LP diet, there were 22 steers classified in the Low-RG group, 37 in the Medium-RG and 24 in the High-RG (Table 8). When evaluated with the HP diet, 25 steers were classified as Low-RG, 33 as Medium-RG and 25 as High-RG. Steers classified in the High-RG group had similar DMI but greater ADG than High-RG steers in both diets.

Table 8. Performance of steers classified by residual gain in two diets differing in protein content. LP, Low-protein diet; HP, high-protein diet; RG, residual gain; BW, body weight; DMI, dry matter intake; ADG, average daily gain.

Traits	Diet[A]	RG Groups			SEM	*P*-value
		Low	Medium	High		H vs. L
n	LP	22	37	24	--	--
	HP	25	33	25	--	--
RG	LP	-0.20	-0.01	0.21	0.02	<0.01
	HP	-0.19	0.01	0.18	0.01	<0.01
BW (kg)	LP	378	379	376	27	0.75
	HP	443	444	447	31	0.70
DMI (kg/100 kg BW)	LP	2.01	1.96	2.03	0.06	0.66
	HP	1.84	1.86	1.84	0.08	0.87
ADG (kg/d)	LP	0.86	1.03	1.28	0.07	<0.01
	HP	0.95	1.17	1.35	0.07	<0.01

There was no agreement (Kappa = -0.08, *P* = 0.28) in the RG classification of steers when evaluated in the two different diets. Of the 25 steers classified as High-RG in the HP diet, only 8 were classified as High-RG in the LP diet while 13 were classified as Medium-RG and 7 as Low-RG (Table 9).

Table 9. Agreement in the classification of steers according to residual gain (RG) in two diets differing in protein content.

Diet	RG Groups	High Protein			Total	Kappa	*P*-value
		Low	Medium	High			
Low Protein	Low	7	11	7	22	-0.08	0.28
	Medium	15	9	13	37		
	High	3	13	8	24		
	Total	25	33	25	83		

When the steers were classified according to their RIG in the LP diet, there were 22 steers classified in the Low-RIG group, 34 in the Medium-RIG and 27 in the High-RIG (Table 10). The same numbers were found when evaluated with the HP diet. Steers classified in the High-RIG group had lower DMI and greater ADG than Low-RIG steers in both diets.

Table 10. Performance of steers classified by residual feed intake and gain in two diets differing in protein content.
LP, Low-protein diet; HP, high-protein diet; RIG, residual feed intake and gain; BW, body weight; DMI, dry matter intake; ADG, average daily gain.

Traits	Diet[A]	RIG Groups			SEM	*P*-value H vs. L
		Low	Medium	High		
n	LP	22	34	27	--	--
	HP	22	34	27	--	--
RIG	LP	-2.04	-0.17	1.87	0.17	<0.01
	HP	-2.18	-0.07	1.85	0.16	<0.01
BW (kg)	LP	379	379	375	28	0.59
	HP	443	445	443	30	0.99
DMI (kg/100 kg BW)	LP	2.07	2.05	1.87	0.06	<0.01
	HP	1.94	1.89	1.74	0.07	<0.01
ADG (kg/d)	LP	0.91	1.11	1.11	0.08	<0.01
	HP	1.02	1.19	1.25	0.08	<0.01

There was a slight agreement (Kappa = 0.10, *P* = 0.20) in the RIG classification of steers when evaluated in the two different diets. Of the 275 steers classified as High-RIG in the HP diet, only 13 were classified as High-RIG in the LP diet while 8 were classified as Medium-RIG and 6 as Low-RIG (Table 11).

Table 11. Agreement in the classification of steers according to residual feed intake and gain (RIG) in two diets differing in protein content.

Diet	RIG Groups	High Protein			Total	Kappa	*P*-value
		Low	Medium	High			
Low Protein	Low	7	9	6	22	0.10	0.20
	Medium	12	14	8	34		
	High	3	11	13	27		
	Total	22	34	27	83		

In summary, this lack of agreement in steer's ranking indicates that, most likely, different physiological mechanisms are responsible for efficiency in both diets. The implication would be that it should not be expected that more feed-efficient animals selected in normal diets (high-protein) would also be the most efficient when facing a protein deficient diet (low-protein). The physiological mechanisms regulating feed efficiency will be discussed in the next session.

4.2 Understanding feed efficiency in low-protein diets

4.2.1 Rumen efficiency

In addition to feed efficiency, animals were classified according to rumen microbial efficiency. This measurement represents the efficiency of the rumen in converting available fermentable substrates into microbial protein and is called the efficiency of microbial protein synthesis (EMPS). The rumen efficiency of converting digestible energy (DOMI) into MCP was called EMPS1, and the rumen efficiency in converting digestible protein (DCPI) into MCP was called EMPS2.

Microbial protein production averaged 284 g/d, varying from 163 to 490 g/d. The rumen efficiency in using energy (EMPS1) averaged 72 g MCP/kg DOMI, varying from 43 to 120 g MCP/kg DOMI (Table 12) and the rumen efficiency in using protein (EMPS2) averaged 855 g MCP/kg DCPI, varying from 468 to 1620 g MCP/kg DCPI. These values illustrate the great variability in rumen efficiency even when cattle are receiving the same diet.

Table 12. Rumen fermentation parameters of Brahman steers receiving a low-protein diet.
MCP, microbial protein production; DOMI, digestible organic matter intake; DCPI, digestible crude protein intake; EMPS1, efficiency of microbial protein synthesis as MCP/DOMI; EMPS1, efficiency of microbial protein synthesis as MCP/DCPI.

Traits[A]	n	Mean	Min	Max	Std Dev
MCP (g/d)	72	284	163	490	69
MCP (g/100 kg BW)	72	71	39	112	18
DOMI (kg/d)	72	4.03	2.41	5.97	0.72
DCPI (kg/d)	72	0.35	0.20	0.55	0.07
EMPS1 (g MCP/kg DOMI)	72	71.9	42.7	120.5	18.3
EMPS2 (g MCP/kg DCPI)	72	855	468	1620	270

The total fermentation yield, as g MCP per kg BW, was moderately correlated with FCR and G:F ratio (r > 0.30, Table 13), as more efficient steers had greater MCP production. Rumen efficiency of using digestible energy (EMPS1) was not correlated with any feed efficiency measurements ($P > 0.10$). However, the rumen efficiency in using available protein was moderately correlated (r > 0.30) with FCR ($P = 0.01$) and G:F ratio ($P < 0.01$), and weakly ($r = -0.26$) correlated with RFI ($P = 0.03$). These results suggest that steers with better feed efficiency (lower FCR, higher G:F ratio and lower RFI) would also have better rumen efficiency (higher EMPS2). The investigation of the individual variation in the relative abundance of bacterial populations could help to identify factors modulating the variation in rumen efficiency.

Table 13. Pearson correlation coefficients between feed efficiency and rumen efficiency traits.
FCR, feed conversion ratio; G:F, gain to feed ratio; RFI, residual feed intake; RG, residual gain; RIG, residual feed intake and gain; MCP, microbial crude protein; EMPS1, efficiency of microbial protein synthesis as MCP/DOMI; EMPS1, efficiency of microbial protein synthesis as MCP/DCPI.

Item	FCR	G:F	RFI	RG	RIG
MCP (g/kg BW)	-0.329**	0.344**	0.007	0.038	0.020
EMPS1 (g MCP/kg DOMI)	-0.088	0.114	-0.194	-0.115	0.042
EMPS2(g MCP/kg DCPI)	-0.301*	0.320**	-0.262*	0.021	0.165

*P-value < 0.05, **P-value < 0.01.

4.2.2 Nitrogen metabolism

Parameters from nitrogen metabolism are presented in Table 14. Even though the animals were receiving the same diet, there was a large variation in measured parameters of nitrogen metabolism. As nitrogen can either be lost in urine or recycled back to the rumen, urinary N excretion can be used to assess nitrogen recycling. On average, animals were excreting 8 g of N/100 kg BW per day in the urine, ranging from 3.9 to 12.0 g of N/100 kg BW per day. On average, animals were retaining 22.9 g N/100 kg BW per day (positive balance); which represents an average nitrogen use efficiency of 40.1%. The retention of digested nitrogen, or nitrogen use efficiency (NUE) varied from 0.2 to 70 g/100 g of N digested. In other words, an animal with 0.2 NUE is losing almost all available N in urine and faeces, while an animal with 70 NUE is losing only 30% of the available N in urine and faeces.

Table 14. **Parameters of intake and nitrogen metabolism of steers fed a low-protein diet**.
N, nitrogen; NUE, Nitrogen use efficiency (g of digested N that was retained in body tissues per 100 g of N intake).

Traits	n	Mean	Min	Max	Std Dev
N-intake (g)	72	92.9	59.7	143.8	18.6
Faecal-N (g)	72	37.4	16.8	59.9	9.0
Urine-N (g)	72	32.5	16.6	58.1	9.1
Total N excretion (g)	72	70	44	103	15
N-intake (g/100kg BW)	72	22.9	17.4	31.4	3.3
Faecal-N (g/100 kg BW)	72	9.20	5.30	14.11	1.69
Urine-N (g/100 kg BW)	72	7.97	3.90	11.97	1.71
Total-N excretion (g/100 kg BW)	72	17.2	11.3	23.7	2.5
N-balance (g/100 kg BW)	72	22.9	0.1	48.0	11.6
NUE (g/100 g)	72	40.1	0.2	70.0	16.0

It is important to note that NUE was highly correlated with N excretion in urine (r = -0.79, P < 0.01), and not correlated with N excretion in faeces (r = 0.05, P = 0.66; Table 15). Therefore, nitrogen recycling is more important to explain individual differences in nitrogen use efficiency than differences in nitrogen intake or digestibility.

Table 15. Pearson correlation coefficients between nitrogen use efficiency and excretion.
N, nitrogen; NUE, nitrogen use efficiency; CP, crude protein.

Item	NUE	N balance
N balance	0.942**	1
CP Digestibility	0.330**	0.429**
N intake	0.391**	0.614**
Faecal N excretion	0.053	0.146
Urinary N excretion	-0.793**	-0.580**

*P-value < 0.05; **P-value<0.01.

There was also a large variation in the rumen ammonia and plasma urea concentrations throughout the day (Table 16). These differences can be explored to better characterize the process of nitrogen conservation and its association with feed efficiency parameters.

Table 16. Rumen ammonia (NH_3-N) and plasma urea in Brahman steers receiving a low-protein diet.

Traits[A]	n	Mean	Min	Max	Std Dev
Rumen NH_3-N (mg/L)					
0 h	72	41.7	3.0	161.7	23.4
4 h	72	32.6	1.5	115.8	21.0
Plasma Urea Nitrogen (nmol/L)					
0 h	72	2.9	1.0	6.2	1.1
2 h	72	3.4	1.2	6.2	1.0
4 h	72	3.0	0.8	6.0	1.2
6 h	72	2.5	1.0	5.3	1.1

[A]0 h, 2 h, 4 h and 6 h refer to time after the first feeding.

The initial hypothesis was that nitrogen recycling would be an important factor in determining feed efficiency in animals receiving a low-protein diet, and that it would not be as important for determining feed efficiency when the steers were fed a high-protein diet. Data in Table 17 indicates that when the steers were fed a low-protein diet, urinary N excretion was significantly correlated with feed efficiency parameters, such as FCR (r = 0.38, P < 0.01), G:F ratio (r = -0.33, P < 0.01), RFI (r = 0.24, P<0.05), and RIG (r = -0.26, P<0.05). Urinary N excretion was also correlated with the rumen efficiency parameters EMPS1 (r = -0.44, P < 0.01) and EMPS2 (r = -0.39, P < 0.01).

These results suggest that more feed-efficient animals lose less N in urine than less efficient animals, highlighting the importance of N-recycling in determining feed efficiency. In addition, more efficient steers (measured by G:F ratio, RFI, EMPS1 and EMPS2) had lower plasma urea-nitrogen (PUN) concentration. The lower PUN in the plasma could be reflecting a better ability to recycle N into the rumen, reducing the loss of N in urine.

Table 17. Pearson correlation coefficients between feed efficiency, rumen efficiency and nitrogen metabolism parameters of steers fed a low-protein diet.
FCR, Feed conversion ratio; G:F, gain to feed ratio; RFI, residual feed intake; RG, Residual gain; RIG, Residual feed intake and gain; EMPS1, efficiency of microbial protein synthesis (gMCP/kg DOM); EMPS2, efficiency of microbial protein synthesis (gMCP/kg DCP); NUE, nitrogen use efficiency; NH_3-N, ammonia nitrogen; PUN, plasma urea-nitrogen.

Item	FCR	G:F	RFI	RG	RIG	EMPS1	EMPS2
Faecal N excretion	0.165	0.011	0.236*	0.107	-0.071	0.006	0.042
Urinary N excretion	0.384**	-0.331**	0.265*	-0.182	-0.260*	-0.436**	-0.389**
N balance	-0.251	0.195	0.199	0.153	-0.023	-0.008	-0.159
NUE	-0.348**	0.277*	0.055	0.183	0.077	0.151	0.022
Rumen NH_3-N 0h	-0.175	0.181	0.158	0.159	-0.002	-0.048	-0.003
Rumen NH_3-N 4h	0.003	0.084	-0.140	0.182	0.188	-0.170	-0.148
PUN 0h	0.165	-0.286*	0.298*	-0.032	-0.189	-0.248*	-0.322**
PUN 2h	0.028	-0.083	0.234	0.017	-0.124	-0.338**	-0.376**
PUN 4h	0.058	-0.180	0.326**	0.036	-0.160	-0.393**	-0.439**
PUN 6h	0.024	-0.076	0.290*	0.064	-0.124	-0.442**	-0.449**

*P-value < 0.05; **P-value<0.01.

To further characterize the role of nitrogen metabolism on feed efficiency, the steers were divided into groups according to RFI, RG and RIG to detect differences in nitrogen metabolism (Tables 18 to 20). Following differences in DMI, more efficient steers calculated by RFI (low-RFI) had lower (P < 0.01) N intake in both diets than less efficient steers (high-RFI) (Table 18). When fed the LP diet, more efficient steers (low-RFI) had lower N excretion in faeces (P = 0.05) and a tendency for lower N excretion in urine (P = 0.10), resulting in 11% reduction in total N losses (P = 0.01). However, the reduction in N losses is caused by a reduction in total N intake, as there were no differences in N excretion between high-RFI and low-RFI steers in both diets when calculated as a proportion of N intake. In fact, because of a tendency for greater faecal N excretion, more efficient steers on the LP-diet had lower (P = 0.02) N balance than less efficient steers.

Table 18. Nitrogen metabolism of steers classified by residual feed intake in two diets differing in protein content.

RFI, residual feed intake; N, nitrogen; BW, body weight; NUE, Nitrogen use efficiency. LP, low-protein diet with 88 g RDP/kg DOMI; HP, high-protein diet with 148 g RDP/kg DOMI.

Traits	Diet[1]	RFI groups			SEM	*P*-value
		Low	Medium	High		H vs. L
N intake (g/100 kg BW)	LP	20.5	22.9	24.4	1.21	<0.01
	HP	32.4	33.7	36.2	2.43	<0.01
Faecal N (g/100 kg BW)	LP	8.50	9.18	9.52	0.52	0.05
	HP	9.47	9.23	10.23	0.33	0.11
Urine N (g/100 kg BW)	LP	7.37	8.19	8.31	0.68	0.10
	HP	18.9	18.9	20.1	1.08	0.29
Total N excretion (g/100 kg BW)	LP	15.8	17.4	17.8	1.02	0.01
	HP	28.3	27.9	30.2	1.02	0.19
Faecal N (g/100g N intake)	LP	41.5	39.9	38.9	1.09	0.09
	HP	29.3	27.2	28.5	1.49	0.42
Urine N (g/100g N intake)	LP	36.5	36.5	34.5	2.96	0.50
	HP	58.9	57.6	57.4	6.74	0.63
N excretion (g/100g N intake)	LP	77.9	76.5	73.4	3.35	0.18
	HP	88.2	84.9	86.0	8.09	0.50
N balance (g/100 kg BW)	LP	4.63	5.53	6.63	0.93	0.02
	HP	4.09	5.47	5.73	2.98	0.16
NUE[B] (g retained/100 g digested)	LP	37.2	39.1	42.8	5.25	0.29
	HP	15.9	21.1	22.2	9.94	0.16

There were no differences in N intake between low-RG and high-RG steers when fed the LP or HP diet ($P > 0.30$) (Table 19). When fed the LP diet, more efficient steers, high-RG, excreted less ($P = 0.03$) urinary N than low-RG steers, leading to lower ($P = 0.05$) total N excretion. When calculated as a proportion of N intake, more efficient steers excreted 16% less N in urine resulting in a 39% increase ($P < 0.01$) in NUE when fed the low-protein diet. In contrast, when fed the HP diet, there was no difference ($P = 0.85$) for urinary N excretion between high-RG and low-RG steers. Similarly, there was no difference for N balance ($P = 0.56$) or NUE ($P = 0.82$) between high-RG and low-RG steers fed the HP diet. There were no differences in the proportion of intake N excreted in faeces (reverse of N digestibility) between RG groups.

Table 19. Nitrogen metabolism of steers classified by residual gain in two diets differing in protein content.

RG, residual gain; N, nitrogen; BW, body weight; NUE, Nitrogen use efficiency; LP, low-protein diet with 88 g RDP/kg DOMI; HP, high-protein diet with 148 g RDP/kg DOMI.

Traits	Diet[1]	RG groups			SEM	*P*-value
		Low	Medium	High		H vs. L
N intake (g/100 kg BW)	LP	22.9	22.0	23.6	1.02	0.34
	HP	33.8	34.4	33.7	2.53	0.93
Faecal N (g/100 kg BW)	LP	9.60	8.66	9.42	0.50	0.70
	HP	9.53	9.59	9.71	0.36	0.72
Urine N (g/100 kg BW)	LP	8.85	8.04	7.67	0.57	0.03
	HP	19.2	18.9	19.8	1.06	0.63
Total N excretion (g/100 kg BW)	LP	18.5	16.7	17.1	0.79	0.05
	HP	28.4	28.3	29.5	1.01	0.43
Faecal N (g/100g N intake)	LP	41.5	39.4	40.1	1.27	0.40
	HP	28.0	27.8	29.2	1.51	0.28
Urine N (g/100g N intake)	LP	38.8	37.6	32.7	2.96	0.02
	HP	59.1	56.1	59.7	6.77	0.85
N excretion (g/100g N intake)	LP	80.3	77.0	72.8	3.49	<0.01
	HP	87.2	83.9	88.9	8.13	0.63
N balance (g/100 kg BW)	LP	4.45	5.28	6.51	0.95	<0.01
	HP	4.97	5.76	4.25	3.00	0.56
NUE[B] (g retained/100 g digested)	LP	32.3	37.8	44.8	5.46	<0.01
	HP	18.1	22.3	17.0	10.3	0.82

Driven by differences in DMI from RIG groups, high-RIG steers presented a tendency (*P* = 0.08) for lower N intake than low-RIG steers in the LP diet (*P* = 0.08) (Table 20). When calculated as a proportion of N intake, no differences were found in N losses of steers with different RIG in both diets. Also, no differences were found in the N balance (*P* > 0.40) or NUE (*P* > 0.30) in both diets.

When cattle are fed low-protein diets, their productivity relies on their ability to retain more N within their body instead of excreting it in faeces and urine (Stewart and Smith 2005), which may not be the case in protein-abundant diets. To test that, the second hypothesis of this experiment stated that more feed-efficient steers would lose less N in urine and faeces when fed the LP and HP diets. Utilizing cattle with higher NUE is particularly important due to its inherently lower N use rates when compared with other livestock species, hence increasing the environmental and economic costs of N use (Zhao 2019). Nitrogen use efficiency is strongly affected by the N intake (Huhtanen and Hristov 2009). Consequently, the association between feed efficiency and N metabolism should be assessed carefully, considering concomitant changes in intake (Cantalapiedra-Hijar *et al.* 2018), i.e., using RG. Classifying the steers based on RG resulted in groups with more similar DM and N intake than when classifying based on RFI.

In agreement with the initial hypothesis, the results from the present study demonstrated the importance of N preservation in determining feed efficiency when steers were fed a low-protein diet, as more efficient (high-RG) steers had lower N excretion in urine, lower total N excretion, greater N balance and greater NUE. It would be reasonable to argue that steers with greater genetic potential for muscle deposition would excrete less N, and that the potential for muscle growth would regulate N excretion, not the other way around. However, when fed the LP diet, muscle deposition is most likely limited by the available metabolizable protein rather than by the genetic

potential of the steers. In addition, the association between RG and N preservation was not found when the steers were fed the HP diet and the genetic potential of the steers could be expressed. High-RG steers presented similar N excretion, N balance and NUE in comparison to low-RG steers.

Table 20. Nitrogen metabolism of steers classified by residual feed intake and gain in two diets differing in protein content.
RIG, residual feed intake and gain; N, nitrogen; BW, body weight; NUE, Nitrogen use efficiency; LP, low-protein diet with 88 g RDP/kg DOMI; HP, high-protein diet with 148 g RDP/kg DOMI.

Traits	Diet[1]	RIG groups			SEM	*P*-value
		Low	Medium	High		H vs. L
N intake (g/100 kg BW)	LP	23.5	22.7	22.1	0.99	0.08
	HP	34.8	34.2	33.0	2.45	0.16
Faecal N (g/100 kg BW)	LP	9.49	8.89	9.11	0.49	0.43
	HP	9.76	9.66	9.34	0.36	0.42
Urine N (g/100 kg BW)	LP	8.47	8.55	7.43	0.57	0.05
	HP	19.0	19.7	18.8	1.05	0.90
Total N excretion (g/100 kg BW)	LP	18.0	17.4	16.5	0.78	0.05
	HP	28.4	29.3	28.2	1.13	0.90
Faecal N (g/100g N intake)	LP	40.1	39.1	41.5	1.17	0.39
	HP	27.9	28.5	28.3	1.55	0.72
Urine N (g/100g N intake)	LP	36.5	38.7	34.0	3.03	0.34
	HP	56.8	58.9	57.8	6.68	0.76
N excretion (g/100g N intake)	LP	76.6	77.8	75.5	3.35	0.62
	HP	84.9	87.4	86.2	8.08	0.71
N balance (g/100 kg BW)	LP	5.50	5.26	5.58	0.94	0.91
	HP	5.85	4.75	4.82	2.96	0.40
NUE[B] (g retained/100 g digested)	LP	37.7	36.4	41.3	5.46	0.43
	HP	24.0	21.7	19.1	5.66	0.32

In summary, the results of the current study suggest that different physiological mechanisms are responsible for variation in feed efficiency between animals eating diets differing in protein content. In addition, the results provide preliminary evidence that bulls targeted for improved performance in low-protein diets in dry tropical regions should not be selected on high-protein diets. Residual gain appears to be the most adequate measurement to study the effect of nitrogen use efficiency on feed efficiency, as it provides more homogeneous DM and N intakes across different feed efficiency groups. More efficient steers (high-RG) in low-protein diets lose less N in urine, resulting in higher NUE. This is not the case in protein abundant diets.

Encouragingly, there were about 10% of steers that had higher feed-efficiency in both diets and identifying these genetics would allow good efficiency both in grazing low-protein pastures or when fed a feedlot finishing diet.

4.3 Development of a practical tool to select more efficient cattle

Tail hair and plasma protein from the 89 steers fed the low-protein diet were processed as described and submitted for stable isotope analysis at the Stable Isotope Geochemistry Laboratory from The University of Queensland. Samples from two steers could not be analysed and were removed from the study. The diet-animal fractionation ($\Delta^{15}N$ and $\Delta^{13}C$) was calculated as the difference between

the $\delta^{15}N$ or $\delta^{13}C$ of the animal tail hair or plasma and the $\delta^{15}N$ or $\delta^{13}C$ of the diet. There was a large variation in the $\Delta^{15}N$ values across the 89 steers, especially when evaluated in tail hair (Table 21).

Table 21. Plasma and tail hair isotopic ratios for Brahman steers receiving a low-protein diet.
$\Delta^{15}N$, diet-animal fractionation of the ^{15}N and ^{14}N isotopes; $\Delta^{13}C$, diet-animal fractionation of the ^{13}C and ^{12}C isotopes.

Traits[A]	n	Mean	Min	Max	Std Dev
$\Delta^{15}N_{tailhair}$	87	4.47	2.66	7.88	1.01
$\Delta^{15}N_{plasma}$	87	5.67	4.67	7.41	0.54
$\Delta^{13}C_{tailhair}$	87	3.42	2.39	5.72	0.58
$\Delta^{13}C_{plasma}$	87	2.35	-0.58	3.36	0.81

There was a strong degree of correlation (r = 0.60) for the $\Delta^{13}C$ values evaluated either in plasma or in tail hair, and a moderate correlation (r = 0.48) for the $\Delta^{15}N$ values (Table 22).

Table 22. Pearson correlation coefficients for carbon and nitrogen isotopes measured in tail hair and plasma.
$\Delta^{13}C$, diet-animal fractionation of the ^{13}C and ^{12}C isotopes; $\Delta^{15}N$, diet-animal fractionation of the ^{15}N and ^{14}N isotopes.

Item	$\Delta^{13}C_{tailhair}$	$\Delta^{15}N_{tailhair}$
$\Delta^{13}C_{tailhair}$	1	
$\Delta^{15}N_{tailhair}$	0.283*	1
$\Delta^{13}C_{plasma}$	0.596**	0.075
$\Delta^{15}N_{plasma}$	0.292**	0.479**

*P-value < 0.05; **P-value<0.01.

The correlation between C and N isotopic ratio and feed efficiency parameters can be observed in Table 23. Feed efficiency parameters were better correlated with the N isotopes than the C isotope ratio. In addition, N isotopic ratio measured in tail hair was better correlated with FCR and G:R ratio than N isotopic ratio measured in plasma proteins.

Table 23. Pearson correlation coefficients between feed efficiency, rumen efficiency and carbon and nitrogen isotopes measured in tail hair and plasma.
FCR, Feed conversion ratio; G:F, gain to feed ratio; RFI, residual feed intake; RG, Residual gain; RIG, Residual feed intake and gain; EMPS1, efficiency of microbial protein synthesis (gMCP/kg DOMI); EMPS2, efficiency of microbial protein synthesis (gMCP/kg DCPI); $\Delta^{13}C$, diet-animal fractionation of the ^{13}C and ^{12}C isotopes; $\Delta^{15}N$, diet-animal fractionation of the ^{15}N and ^{14}N isotopes.

Item	FCR	G:F	RFI	RG	EMPS1	EMPS2
$\Delta^{13}C_{tailhair}$	0.095	-0.069	-0.000	-0.091	-0.051	0.302**
$\Delta^{15}N_{tailhair}$	0.445**	-0.390**	-0.027	-0.306**	-0.154	0.309**
$\Delta^{13}C_{plasma}$	-0.233*	0.269*	0.090	0.107	0.006	0.258*
$\Delta^{15}N_{plasma}$	0.342**	-0.324**	-0.044	-0.295**	-0.141	0.156

*P-value < 0.05; **P-value<0.01.

To further evaluate the potential association between feed efficiency and isotopic ratios, the 87 steers were classified into three groups according to the diet-animal fractionation of the ^{15}N and ^{14}N isotopes ($\Delta^{15}N_{tailhair}$, Table 24).

Table 24. Feed efficiency and nitrogen metabolism parameters of steers classified by the diet-animal fractionation of the ^{15}N and ^{14}N isotopes ($\Delta^{15}N_{tailhair}$).
N, nitrogen; $\Delta^{15}N$, diet-animal fractionation of the ^{15}N and ^{14}N isotopes; DMI, dry matter intake; ADG, average daily gain; RFI, residual feed intake; RG, Residual gain; RIG, Residual feed intake and gain; FCR, Feed conversion ratio; G:F, gain to feed ratio; NUE, nitrogen use efficiency.

Item	$\Delta^{15}N_{tailhair}$ Groups			SEM	*P*-value
	Low	Medium	High		L vs H
n	30	35	22	--	--
$\Delta^{15}N_{tailhair}$ (‰)	3.81	4.40	5.46	0.13	<0.01
Performance					
BW (kg)	368	368	381	8.59	0.34
DMI (kg/d)	7.50	7.16	7.62	0.61	0.62
DMI (kg/100 kg BW)	2.04	1.95	1.98	0.06	0.19
ADG (kg/d)	1.10	0.99	1.01	0.08	0.18
RFI	0.08	-0.06	-0.06	0.09	0.32
RG	0.04	-0.01	-0.09	0.03	<0.01
RIG	0.09	0.09	-0.30	0.28	0.38
FCR	6.98	7.49	8.08	0.95	0.01
G:F ratio	0.148	0.139	0.136	0.016	0.06
Metabolism					
Urine N (g/100g intake)	31.9	36.3	37.4	1.74	0.03
Faeces N (g/100g intake)	38.3	38.1	41.8	1.23	0.05
N balance (g/d per 100 kg BW)	7.71	5.52	4.47	0.51	<0.01
NUE	48.0	40.7	35.4	2.98	<0.01

The classification of the steers according to the $\Delta^{15}N_{tailhair}$ in three groups demonstrated the significant association of $\Delta^{15}N_{tailhair}$ and feed efficiency parameters (Table 24). Steers with lower $\Delta^{15}N_{tailhair}$ had better feed efficiency, illustrated by higher RG ($P < 0.01$), lower FCR ($P = 0.01$), and a tendency for higher G:F ratio ($P = 0.06$) than steers with higher $\Delta^{15}N_{tailhair}$. Similar results have been reported previously for growing ruminants (Cantalapiedra-Hijar *et al.* 2018).

The association between $\Delta^{15}N_{tailhair}$ and feed efficiency is likely connected with N metabolism and the fact that more efficient steers in this low-protein diet are losing less N in the urine. Because cattle lose mostly ^{14}N in urine, more efficient steers would preserve more of the ^{14}N coming from the diet, therefore the tissue protein (tail hair) would have lower $\Delta^{15}N$ than less efficient steers (more similar to the isotopic profile of the diet). The results from the current study corroborate this hypothesis, as steers with lower $\Delta^{15}N_{tailhair}$ had a 15% reduction of N loses in urine and an 8.4% reduction of N losses in faeces, resulting in a 36% improvement in NUE.

In summary, the isotope analysis with 87 Brahman steers indicated that the classification of steers for feed efficiency, measured as feed conversion ratio, gain to feed ratio, residual gain, and nitrogen use efficiency, can be estimated from the tail hair with reasonable accuracy. These results represent an important step for the development of a tool for on-farm assessments of feed efficiency to guide cattle selection.

4.4 Rumen microbiome

The ruminal microbial composition and diversity differed between HP and LP diets ($P < 0.01$). As seen in Fig. 3, the Beta diversity index indicates that the distribution of operational taxonomy units

(OTUs) was different between diets ($P = 0.001$). Similarly, the Alpha diversity index indicated that steers fed the LP diet had greater bacterial richness (Chao1 and ACE; $P < 0.001$) than when these steers were fed the HP diet (Fig. 4). The same trend was observed in the Shannon index, which demonstrates the greater bacterial diversity regarding richness ($P < 0.001$) in the LP diet. There were no significant differences for the Simpson and Inverse Simpson indexes, indicating no alteration in evenness of microbial diversity (Fig. 4) between diets.

Figure 3. Beta diversity in rumen samples for steers fed low protein (LP - △) and high protein (HP - ▲) diets.

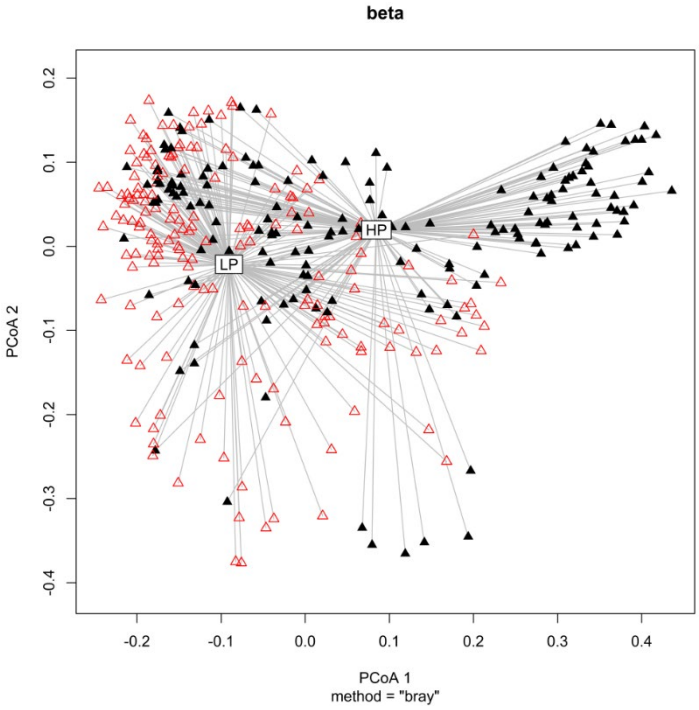
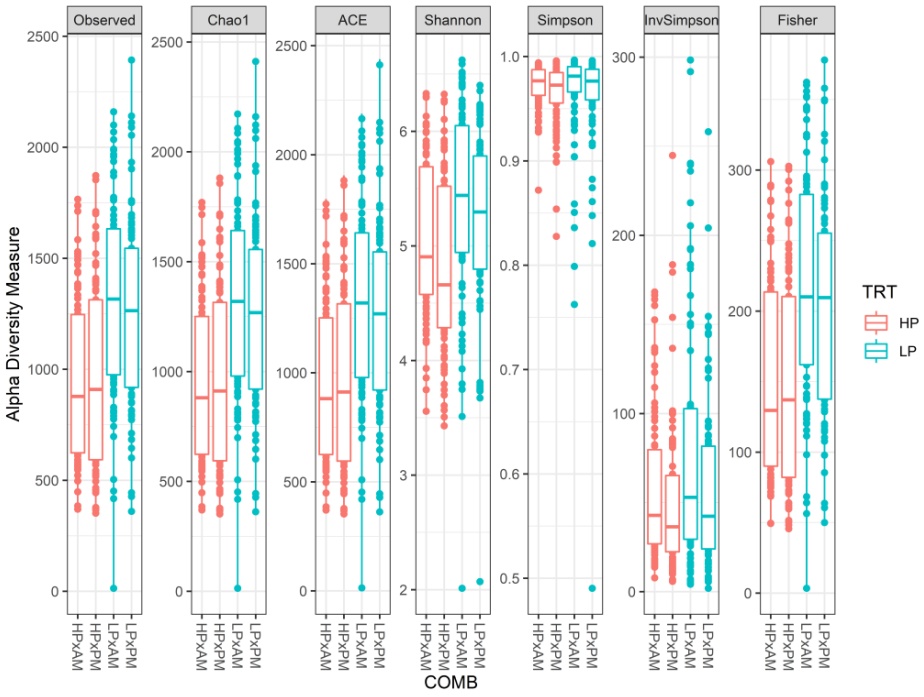
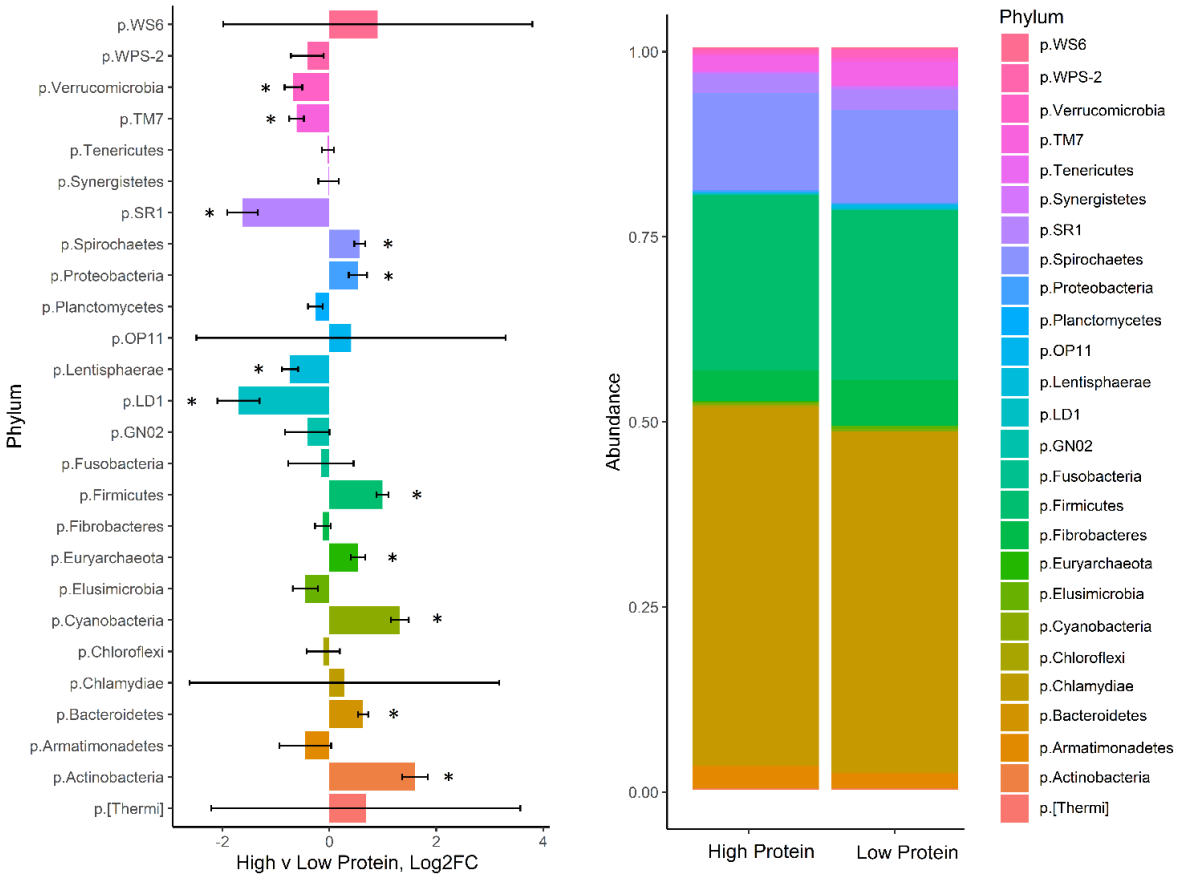


Figure 4. Between-sample diversity indexes for rumen samples from steers LP and HP diets.



The effect of diet on the rumen microbial population was evident. Six bacterial phyla, including the three most abundant phyla: Bacteroidetes, Firmicutes, Proteobacteria; and the archaeal phyla Euryarchaeota, were higher ($P < 0.001$) in the rumen of steers on the HP diet, compared to steers on the LP diet. Comparatively, Verrucomicrobia, SR1 and Lentisphaerae, were higher in the rumen of steers fed the LP diet (Fig. 5).

Figure 5. Relative abundance of rumen bacteria phylum and changes between low and high protein diets. *indicates a significant diet effect ($P < 0.01$).



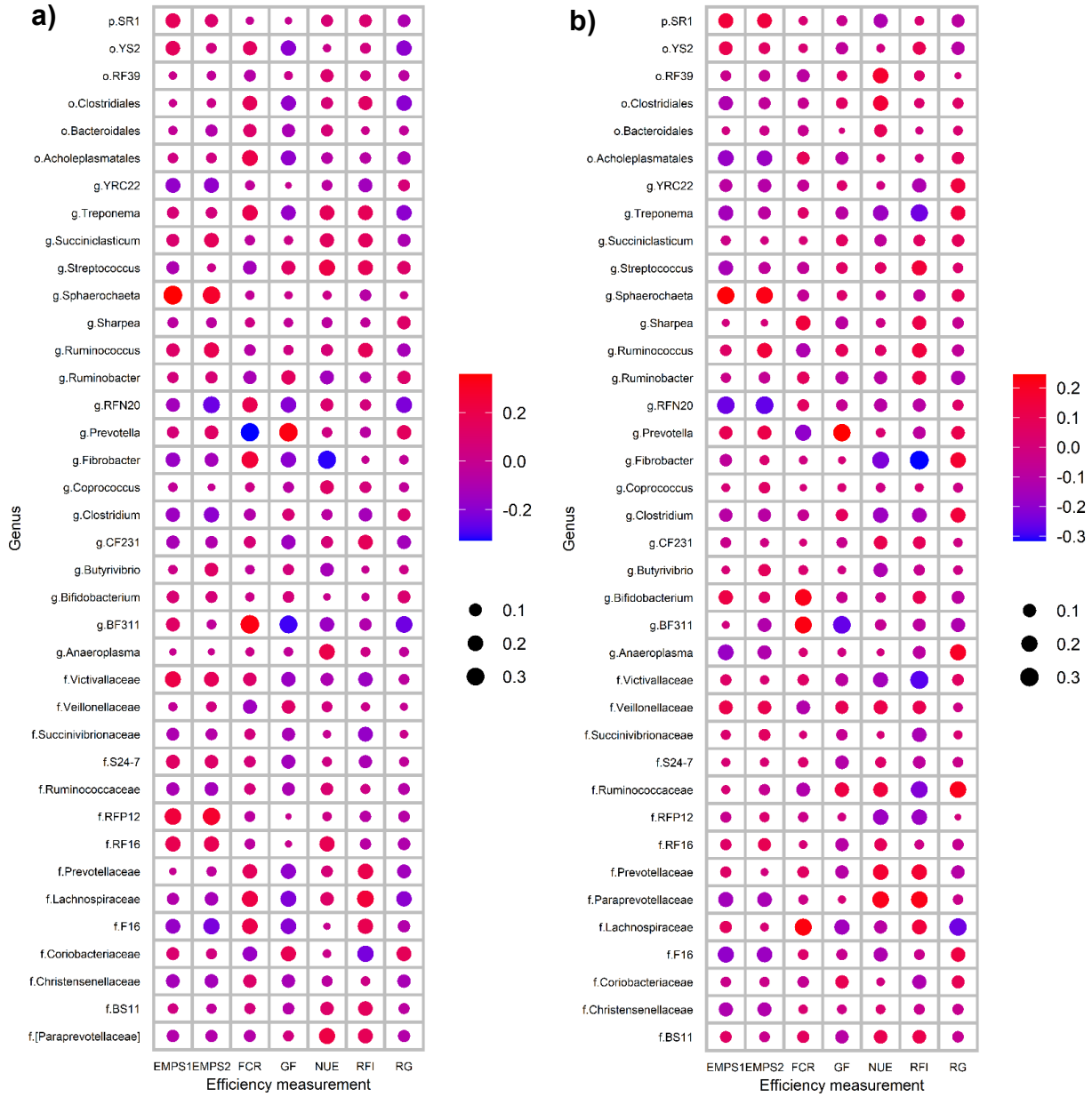
4.4.1 Rumen microbiome populations correlated with feed efficiency traits

Some of the most predominant genera of ruminal bacteria were correlated with feed efficiency parameters and these varied between the type of diet and sample collection time.

4.4.1.1 Associations in the low protein diet

As seen in the correlation heat map in Fig. 6a and Fig. 6b, there were about ten bacterial genera, such as *Treponema*, *Succiniclasticum* and *Streptococcus*, that had moderate and positive correlations with residual feed intake (RFI) in both sampling times. Nitrogen use efficiency (NUE) was correlated to eight bacterial genera, such as *Anaeroplasma*, *Streptococcus* and *Treponema*, having similar correlations despite the time of sampling. Interestingly, almost all the genera correlated with residual gain (RG) were moderate negative before feeding (Fig. 6a) and many of these correlations changed to positive after feeding (Fig. 6b).

Figure 6. Correlation between ruminal bacterial genera and rumen fermentation parameters a) before feeding and b) 4 hours after feeding in a low protein diet.

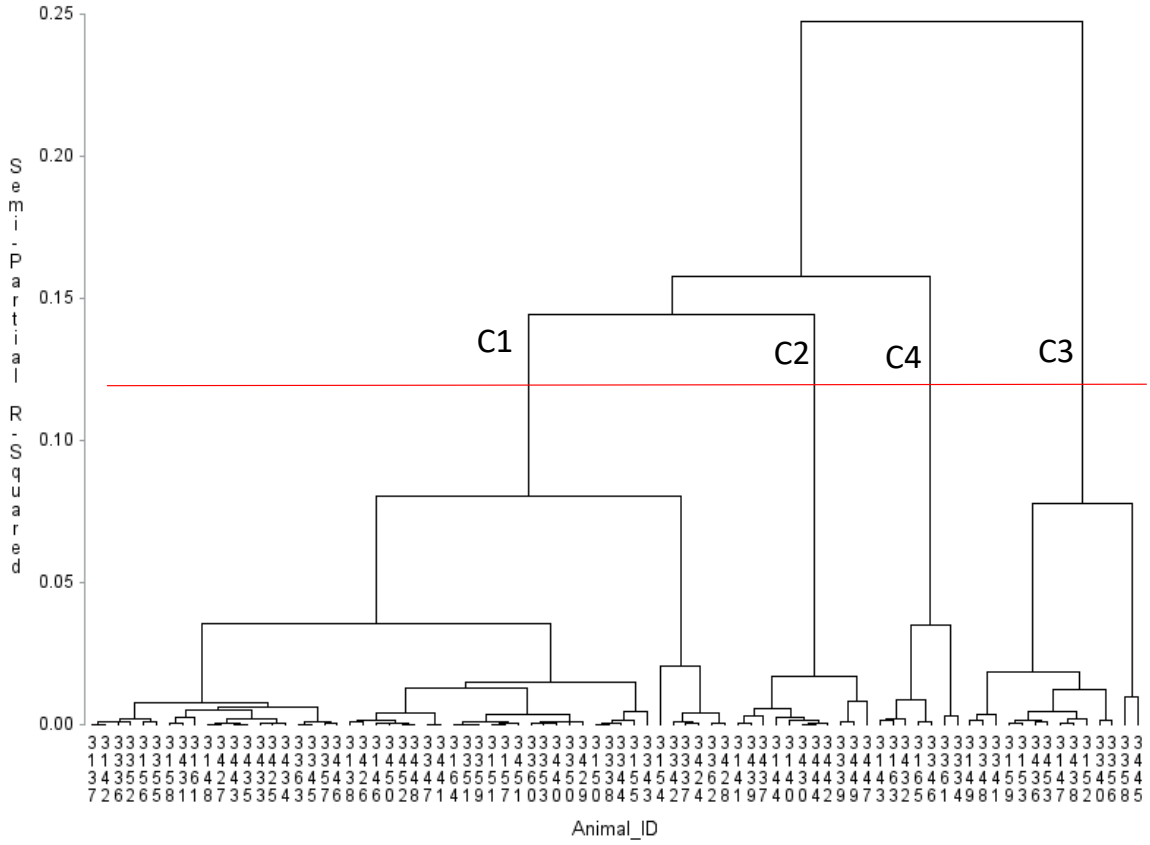


The finding that feed efficiency parameters are correlated with several microbial genera is of interest; however, a more comprehensive analysis is required to identify rumen profiles associated with better efficiency. The principal components analysis and clustering were used to identify groups of steers with similar rumen microbial profiles. For the LP diet, the first four principal components explained 69% (R-square) of the total variation in the population and were used for clustering. The Pseudo T analysis indicated the presence of four clusters of steers (Fig. 7) and grouping the steers into four clusters explained 62% (R-square) of the total variation.

The difference in the relative abundance of main bacterial genera responsible for the clustering of the steers is better visualized in Fig. 8. The main bacterial genera and families describing the four clusters in LP (Fig. 8) were *Prevotella*, *Ruminobacter*, *Bacteroidales*, *Ruminococcaceae*, *Fibrobacter*, *Succinivibrionaceae* and *Treponema*. Their population varied according to the cluster, e.g. in cluster 4, steers had a high relative abundance of *Prevotella* in both sample collection times, while the

steers in cluster 3 had a high abundance of *Ruminobacter* 4 h after feeding. Steers in cluster 2 had a high abundance of the unclassified genus belonging to the BS11 family in samples collected after feeding. Steers in cluster 1 had a low relative abundance of *Ruminobacter* in both collection times and a high abundance of a genus member of the *Succinivibrionaceae* family (Fig. 8).

Figure 7. Experimental steers, in a low protein diet, clustered (C1, C2, C3, and C4) by rumen bacteria genera using a principal component analysis.



The data presented in Fig. 7 and Fig. 8 illustrate the clear separation of the steers into four distinct groups according to the rumen microbial profile. In addition, it identifies the main bacterial genera responsible for the clustering of the steers into these four groups. So far, in this analysis, steers were grouped only based on the rumen microbial profile. What we are looking for is detecting if these four groups of steers that are different in rumen microbial profile are also different in feed efficiency parameters.

Comparing the feed efficiency parameters for the four clusters of steers (Table 25), it is clear that there were differences among the cluster of steers. The overall profile of the microbial population was not associated with the BW, total intake or ADG of the steers ($P > 0.30$). However, the overall profile of the microbial population was associated with RFI, RG, and rumen NH_3-N. Interestingly, the seven steers clustered together in Cluster 4 had lower rumen NH_3-N values before feeding than the other clusters and also had better feed efficiency, as measured by RFI and RG. Steers in Cluster 4 also had a high relative abundance of *Prevotella* across both collection times. In contrast, less feed efficient steers, i.e., Cluster 3, had a high relative abundance of *Ruminobacter* genus and the steers in Cluster 2 had a high abundance of an unclassified genus belonging to the BS11 family. *Prevotella* genus is most likely an important representative of feed efficiency for animals fed high forage because microbial species within this genus are responsible for a multitude of degradation activities

being able to utilise fibre components, like hemicellulose and pectin, or crude protein from various plant components (Koike *et al.* 2003). Furthermore, that is probably why this genus is actively participating in the degradation of plant cell wall components which in turn contributes to the growth of other fibrolytic bacteria, being linked to a higher synthesis of short-chain fatty acids, especially butyrate (Osborne and Dehority 1989).

Figure 8. Heat map of normalized relative abundance of rumen bacterial genera within four clusters in steers fed a low protein diet. Note: Squares in yellow means normalized abundance above 0.24.

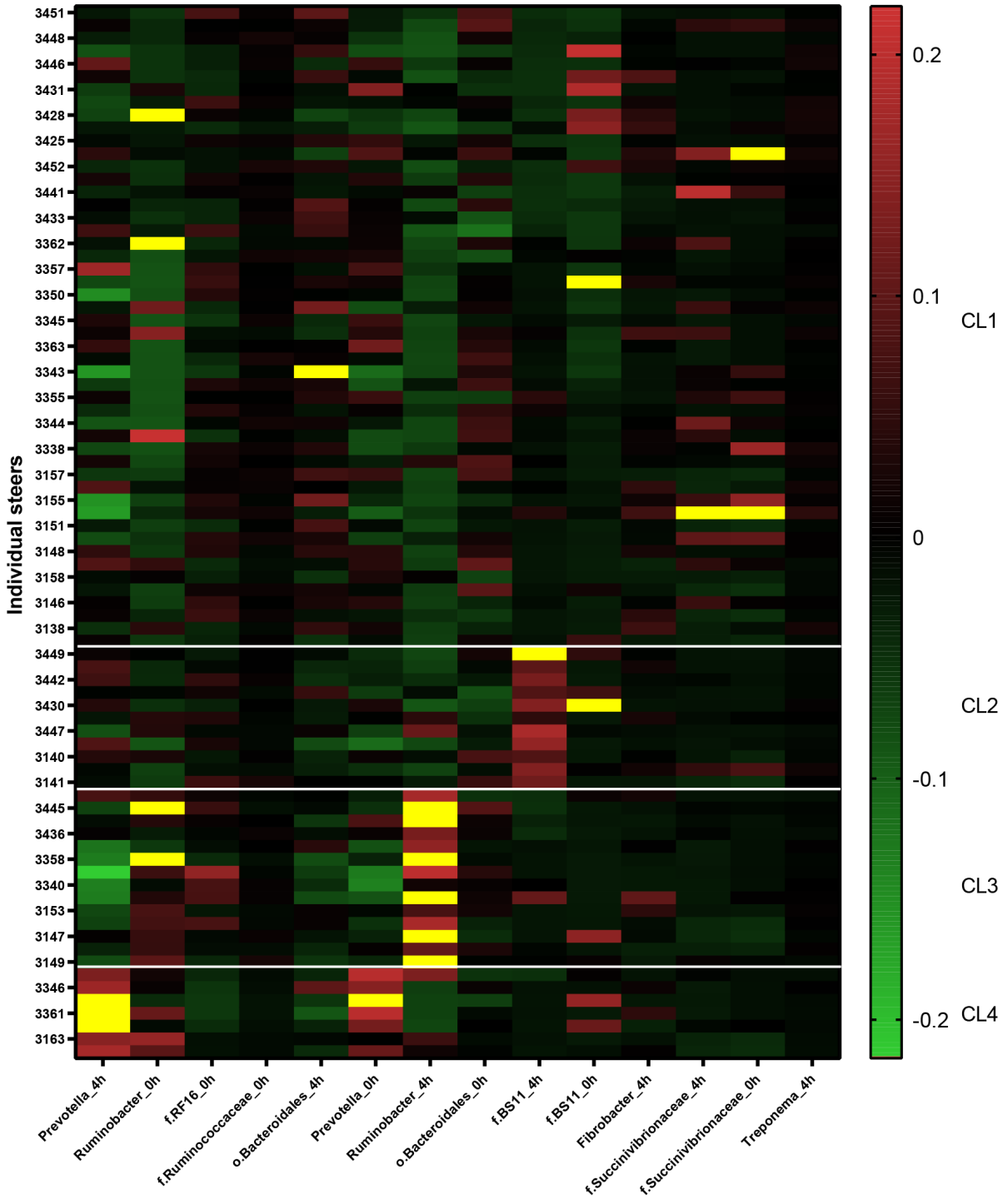


Table 25. Differences among clusters of steers classified according to the rumen bacterial profile on feed efficiency parameters when receiving a low protein diet.
BW, body weight; DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; G:F, gain:feed ratio; RFI, residual feed intake; RG, residual gain; NH_3-N, rumen ammonia nitrogen collected before feeding (0h) or after feeding (4h).

Efficiency Parameters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	SEM	*P*-value
BW (kg)	399	395	406	397	27	0.80
DMI (kg/d)	7.26	7.49	7.71	7.18	0.56	0.30
DMI (kg/100 kg BW)	1.96	2.05	2.02	1.93	0.07	0.32
ADG (kg/d)	1.06	1.00	1.04	1.07	0.10	0.90
FCR	7.12	7.70	7.88	7.07	0.92	0.16
G:F	0.147	0.135	0.137	0.152	0.017	0.28
RFI	-0.12^{ab}	0.19^{b}	0.15^{b}	-0.29^{a}	0.13	**0.06**
RG	0.04^{a}	-0.06^{ab}	-0.07^{b}	0.07^{a}	0.05	**0.09**
NH_3-N 0h (mg/L)	41.8^{a}	41.6^{a}	36.2^{a}	20.5^{b}	6.2	**0.01**
NH_3-N 4h (mg/L)	34.2	24.4	26.2	25.9	5.9	0.25

The analysis conducted here indicated that there was no significant difference ($P > 0.50$) between clusters for any of the rumen efficiency parameters evaluated (Table 26).

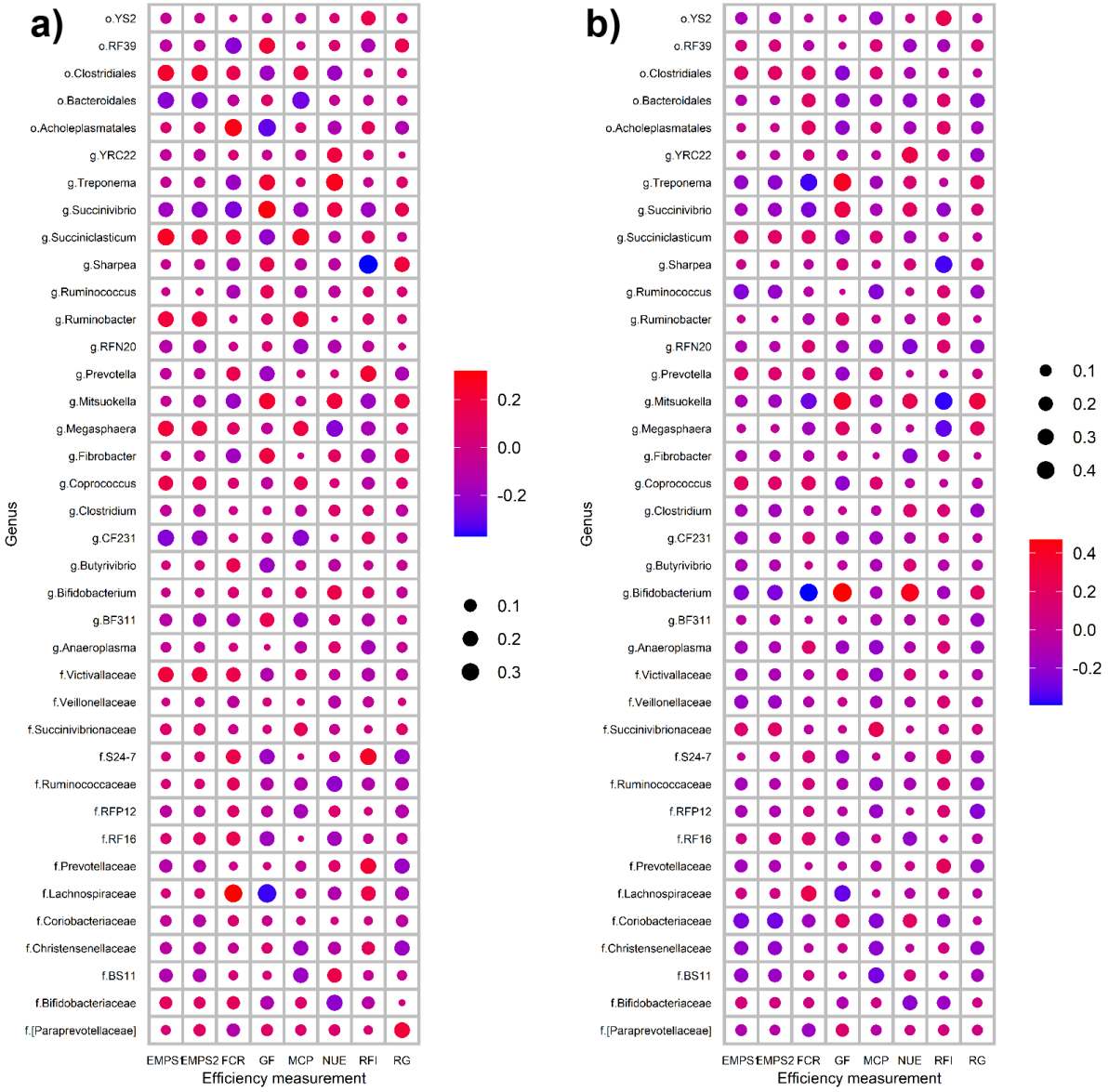
Table 26. Differences among clusters of steers classified according to the rumen bacterial profile on rumen efficiency parameters of animals fed a low protein diet.
MCP, microbial protein; BW, body weight; EMPS, efficiency of microbial protein synthesis; DOMI, digestible organic matter intake; NUE, nitrogen use efficiency.

Item	Cluster 1	Cluster 2	Cluster 3	Cluster 4	SEM	*P*-value
MCP (g/100 kg BW)	69.9	70.0	67.1	61.6	7.03	0.65
EMPS (g MCP/kg DOMI)	70.2	75.2	72.0	68.5	6.71	0.81
NUE (g retained N/g intake N)	41.2	46.6	39.4	37.5	5.79	0.63
Urine N/Intake N (g/d)	35.1	33.1	37.7	38.4	3.09	0.53

4.4.1.2 Associations in the high protein diet

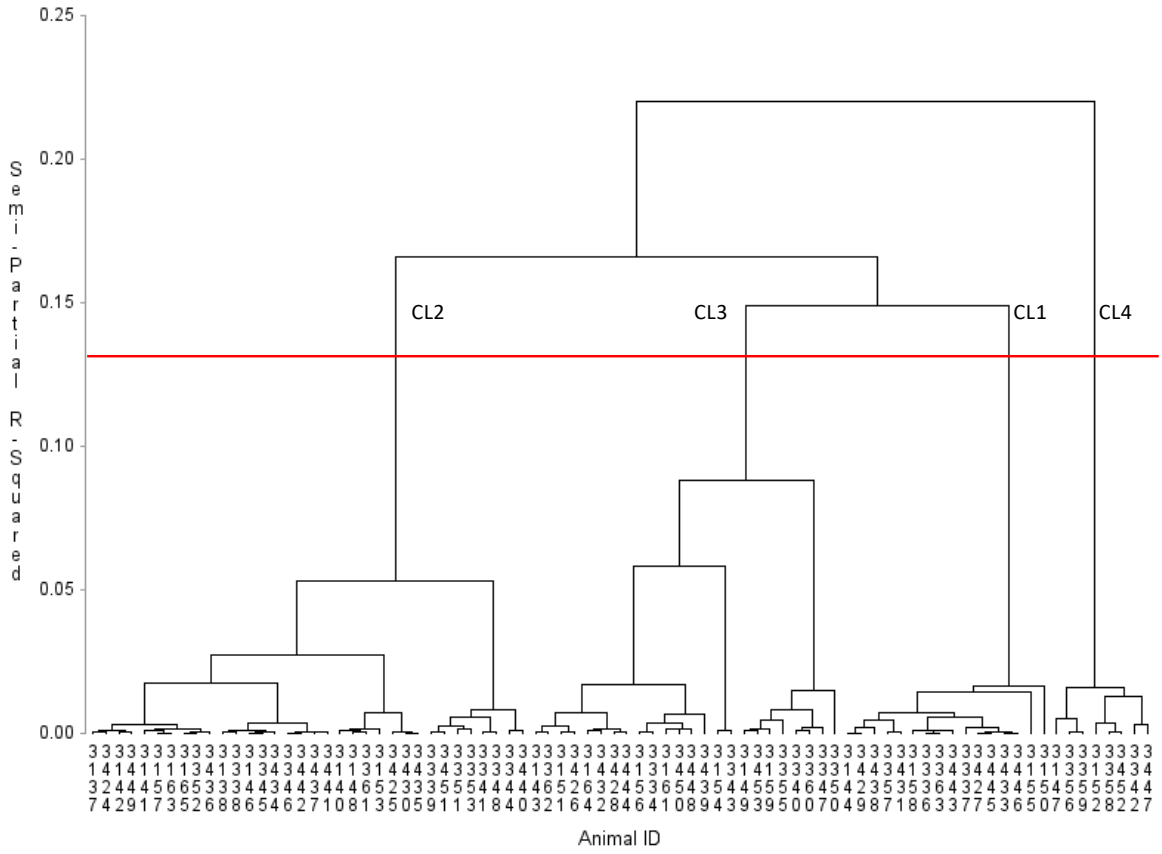
There were noticeably fewer interactions between bacterial genera and feed efficiency traits in animals fed the HP diet, but similarly to the LP diet, these correlations were mainly low to moderate (Fig. 9 a,b). In contrast, it is possible to observe that some of these, such as *Succinivibrio*, *Megasphaera*, unclassified genera belonging to *Victivallaceae* and *Lachnospiraceae* families, respectively, had more influence on feed efficiency traits when compared to animals in the LP diet. Interestingly, the strongest correlations occurred in different sampling times. Although, one could speculate that this is only happening because feed intake has major impact on rumen bacteria activities and growth.

Figure 9. Correlation between ruminal bacterial genera and rumen fermentation parameters a) before feeding (0 h) and b) after feeding (4 h) in a high protein diet.



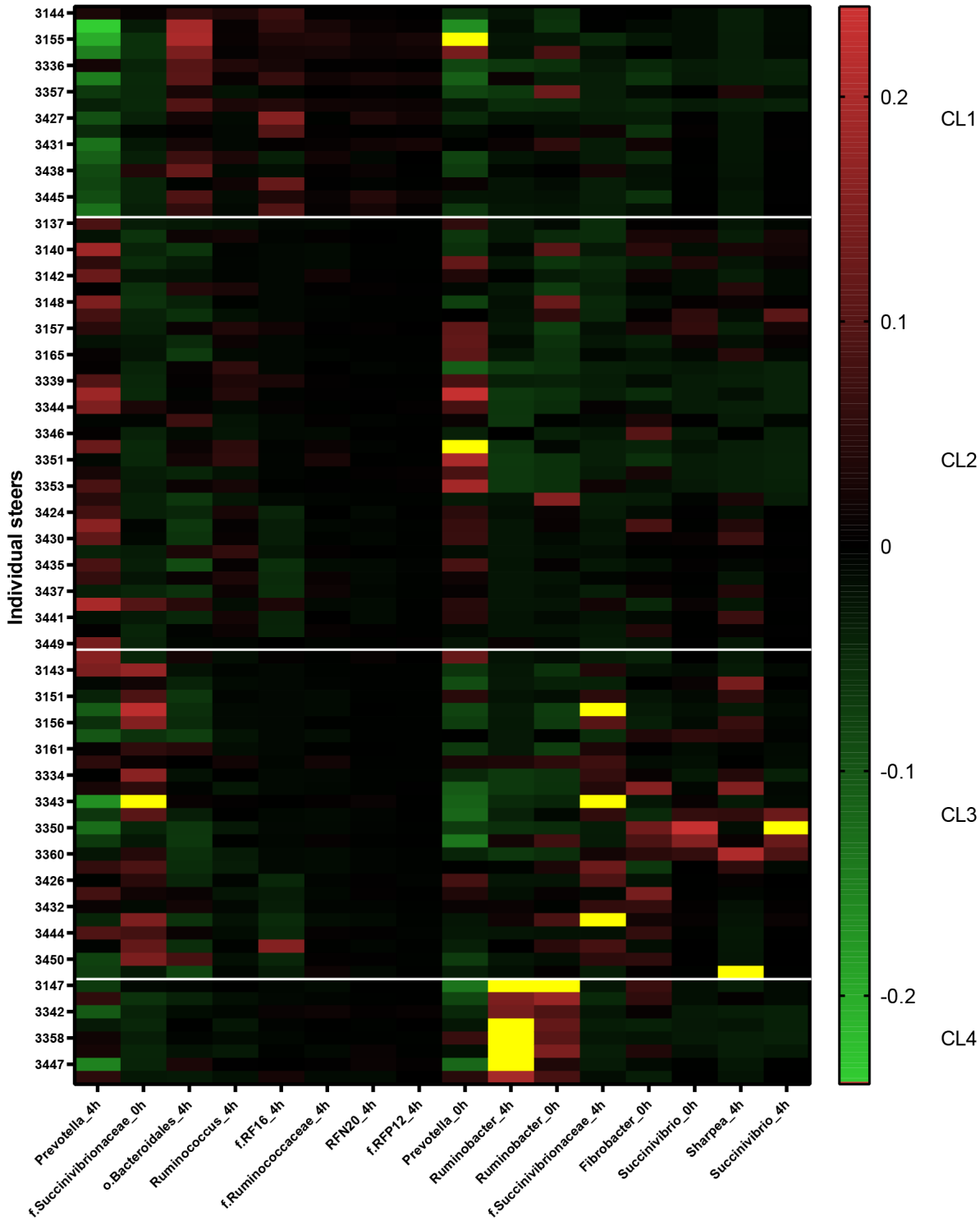
Principal components analysis identified four components representing 59.8% of the total variation (R-square). These four principal components were used to cluster the steers in the high-protein diet, and similarly to the low-protein diet, four clusters were identified (Fig. 10) with the Pseudo T-squared test and these clusters responded for 53.5% of the total variation (R-square).

Figure 10. Experimental steers, in a high protein diet, clustered (C1, C2, C3, and C4) by rumen bacteria genera using a principal component analysis.



The main bacterial genera responsible for the classification of steers into these four clusters were *Prevotella*, *Ruminococcus*, *Ruminobacter*, *Fibrobacter*, *Succinivibrio*, *Sharpea*, an unidentified genus from the *Succinivibrionacea* family, and two unidentified genera from the order *Bacteroidales*. Steers in Cluster 1 had a low relative abundance of *Prevotella* in rumen fluid in both collection times and a high abundance of unclassified genera of the *Bacteroidales* order 4h after feeding. In contrast, rumen fluid samples from animals in Cluster 2 were characterized by a high abundance of *Prevotella* genus in both collection times whilst experimental steers grouped in Cluster 3 had a high abundance of *Fibrobacter* before feeding, *Succinivibrio* at both collection times, and an unclassified genus member of the *Succinivibrionaceae* family 4h after feeding. Steers in Cluster 4 had a high abundance of *Ruminobacter* in rumen fluid from both sample collection times and low abundance of *Prevotella* at both collection times (Fig. 11).

Figure 11. Heat map of normalized relative abundance of rumen bacterial genera within four clusters in steers fed a high protein diet. Note: Squares in yellow means normalized abundance above 0.24.



Overall, there were no differences in feed efficiency traits between clusters (Table 27). Steers in Cluster 1 fed the HP diet had lower DMI in comparison to animals in clusters 2 and 4. Interestingly, these animals, i.e. Cluster 1, also had the highest NH_3-N concentration in the rumen fluid in comparison to experimental animals in the other clusters. The difference could be related to the

lower *Prevotella* abundance that, as previously mentioned, seems to play an important role in fibre degradation. That would, in turn, affect DMI as an indirect result of decreased fibrolytic bacterial growth. The rumen fluid samples from animals with lower DMI and high N-NH_3 concentration had a higher abundance of populations of *Bacteroidales* from an unknown genus. Although, *Bacteroidales* are known to participate in fibre degradation (Jin *et al.* 2018).

Table 27. Differences among clusters of steers classified according to the rumen bacterial profile on feed efficiency parameters when receiving a high protein diet.
BW, body weight; DMI, Dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; G:F, gain:feed; RFI, residual feed intake; RG, residual gain; NH_3-N, rumen ammonia nitrogen collected before feeding (0h) or after feeding (4h).

Efficiency parameters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	SEM	P-value
BW (kg)	439	444	444	441	30	0.972
DMI (kg/100 kg BW)	1.75^{b}	1.88^{a}	1.83^{ab}	1.93^{a}	0.08	**0.098**
ADG (kg/d)	1.03	1.17	1.17	1.20	0.09	0.253
FCR	7.51	7.48	7.17	7.59	0.87	0.705
G:F	0.137	0.140	0.147	0.143	0.02	0.486
RFI	0.02	0.04	-0.18	0.25	0.16	0.359
RG	-0.05	0.00	0.03	0.00	0.04	0.550
NH_3-N 0h (mg/L)	89^{a}	67^{b}	51^{c}	63^{bc}	7.3	**0.002**
NH_3-N 4h (mg/L)	74	75	66	60	12.3	0.581

As for the low-protein diet, there were no significant differences ($P > 0.50$) between clusters for rumen efficiency parameters (Table 28).

Table 28. Differences among clusters of steers classified according to the rumen bacterial profile on rumen efficiency parameters of animals fed a high protein diet.
MCP, microbial protein; BW, body weight; EMPS, efficiency of microbial protein synthesis; DOMI, digestible organic matter intake; NUE, nitrogen use efficiency.

Item	Cluster 1	Cluster 2	Cluster 3	Cluster 4	SEM	*P*-value
MCP (g/100 kg BW)	99	103	105	106	10.7	0.948
EMPS (g MCP/kg DOMI)	103	99	104	105	12.1	0.820
NUE (g retained N/g intake N)	18.7	24.6	20.9	17.7	8.4	0.526
Urine N/Intake N (g/d)	58	55	56	60	5.2	0.614

In summary, it was possible to identify specific rumen microbial profiles associated with better feed-efficiency, but only when fed the low-protein diet. It seems that bacterial populations within the genus *Prevotella* have a strong role in LP diets allowing other associated microbial communities to thrive. Likely, animals that are more efficient in challenging diets (low protein) can do it so because they can rely on specific microbial populations, which would not be relevant when there are no nutrients limiting feed efficiency traits

4.5 Heritability of tail hair isotopes in cattle

A total of 492 steers with complete pedigree information were used to estimate the heritability of the nitrogen-isotope ratio in tail hair. The data is described in Table 29.

Table 29. Descriptive data of the steers evaluated for heritability of nitrogen isotope ratio in tail hair.
ADG, average daily gain

Item	Brahman	Droughtmaster	SEM	P-value
2019 Cohort				
Number of steers	131	119		
Date of Birth	24/11/2018	19/11/2018	1.82	0.05
$\delta^{15}N$	9.65	9.62	0.07	0.75
Overall ADG	0.56	0.58	0.01	<0.01
ADG P1 (birth to Apr 2020)	0.46	0.49	0.01	<0.01
ADG P2 (Apr to Sep 2020)	0.65	0.66	0.01	0.63
ADG P3 (Sep 2020 to May 2021)	0.71	0.72	0.01	0.31
2020 Cohort				
Number of steers	137	105		
Date of Birth	26/11/2019	28/11/2019	2.41	0.52
$\delta^{15}N$	11.6	11.2	0.05	<0.01
Overall ADG	0.61	0.61	0.01	0.21
ADG P1 (birth to May 2020)	0.81	0.83	0.01	0.17
ADG P2 (May to Nov 2020)	0.39	0.41	0.01	0.01
ADG P3 (Nov 2020 to Jul 2021)	0.62	0.61	0.01	0.21

The steers were at the Spyglass research station during Period 1 and were moved to Taroom, Qld, during periods 2 and 3.

The heritability (h^2) of $\delta^{15}N_{tail\ hair}$ was estimated in a univariate animal model considering the fixed effect of contemporary group (year), breed, and ADG P1. The ADG P2 and ADG P3 parameters were not significant ($P > 0.20$) and were not included in the model.

The model used was:

$$\mathbf{y = Xb + Z_A a + e}$$

where **y** is a vector of observations, **X** is an incidence matrix relating observations to fixed effects (year, breed and ADG P1), $\mathbf{Z_A}$ is an incidence matrix relating observations to direct genetic effects, **a** is a vector of direct genetic effects and **e** is a vector of residuals.

Furthermore, var(**a**) = $\mathbf{A}\sigma^2_a$, var(**p**) =$\mathbf{I}\sigma^2_p$ and var(**e**) = $\mathbf{I}\sigma^2_e$ where **A** is the numerator relationship matrix, **I** is the identity matrix, σ^2_a is the direct additive genetic variance and σ^2_e is the residual error variance. The summary statistics for estimated genetic variance, residual variance, phenotypic variance, and heritability of $\delta^{15}N$ are shown in Table 30.

Table 30. Estimated genetic variance (σ^2_A), residual variance (σ^2_E), phenotypic variance (σ^2_P) and heritability (h^2) of $\delta^{15}N$ Brahman and Droughtmaster steers (standard errors in parentheses)

Item	σ^2_A	σ^2_E	σ^2_P	h^2
$\delta^{15}N_{tail\ hair}$	0.19 (0.07)	0.25 (0.06)	0.44 (0.03)	0.43 (0.14)

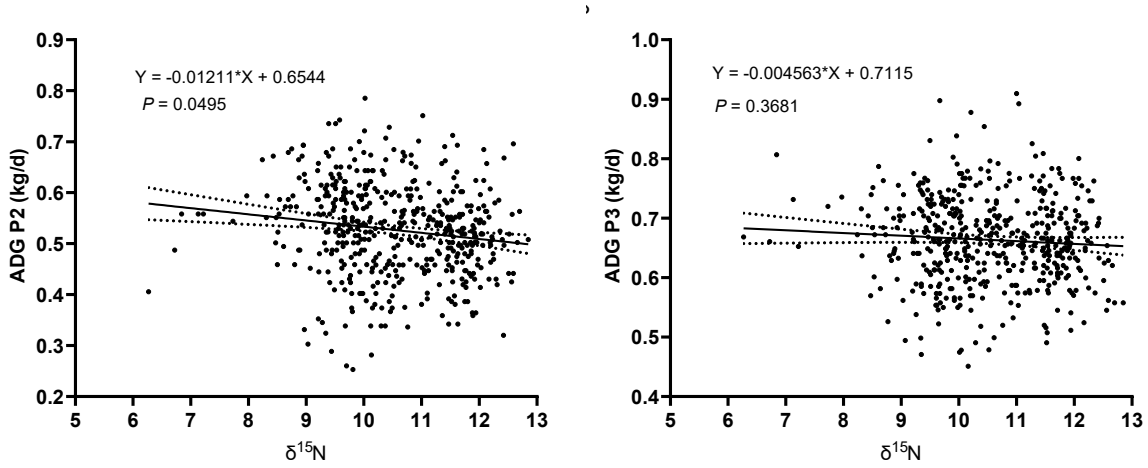
Direct heritability for $\delta^{15}N$ was estimated at 43% and significantly different from zero, with a standard deviation of 14%. Heritability of genetic traits varies from trait to trait. Whilst growth and carcase traits have moderate to high heritability (i.e., 20 to 60%), maternal traits have low heritability (10% or lower) in beef cattle. Therefore, it can be concluded that $\delta^{15}N$ is a moderately

heritable trait. This result opens an exciting possibility for the cattle industry for early detection of more nitrogen efficient cattle.

The present study was not designed to evaluate the correlation between $\delta^{15}N$ and cattle performance in low-protein diets. As discussed before, this correlation should exist only when nitrogen recycling is important in defining cattle performance. After weaning and transport to Taroom, the steers were raised with access to a Leucaena pasture and received protein supplementation during parts of the dry season, resulting in good growth during the dry season (ADG P2, Table 29). Therefore, the results presented below should be evaluated with caution.

For the growth period after weaning, when the steers were in Taroom, the natural abundance of N isotopes ($\delta^{15}N$) was negatively correlated (P = 0.05, R^2=0.022) with average daily gain during the drier period (ADG P2), but not correlated (P = 0.37, R^2=0.005) with average daily gain during the wetter period (ADG P3), as can be seen in Fig. 12. Although significant, $\delta^{15}N$ in tail hair explained only 2.2% of the variation in ADG during the dry season.

Figure 12. Year-adjusted regression between natural abundance of nitrogen isotopes in tail hair ($\delta^{15}N$) and growth of steers during the dry (A) or during the wet season (B).



In summary, this is the first study to qualify the heritability of $\delta^{15}N$ in tail hair of cattle and the fact that this trait is moderately heritable further corroborate the potential use of nitrogen isotopes in tail hair to select cattle with better nitrogen use efficiency.

4.6 Using tail hair analysis to predict response to urea supplements

This trial aimed to test the use of tail hair isotopes as a predictor of nitrogen recycling efficiency and response to urea supplementation. Brahman steers were fed a hay only diet, followed by a second period of evaluation with urea and molasses supplementation. On average, the hay only diet supplied enough nutrients to maintain the steers (ADG = 63 g/d), with large variation in ADG among steers (min -125 g/d, max 181 g/d, Table 31).

Adding the urea supplement to the diet at an average of 39.4 g of urea per day, increased ADG to 266 g/d (min 68 g/d, max 446 g/d). The average response in ADG between the hay only and M8U supplement diets was 202 g ADG/d. As expected, there was a large variation in the growth response to the urea supplements. In one animal, urea supplementation increased ADG by only 4g/d, while in another animal growth was increased by 480 g/d. There was no variation among animals on the

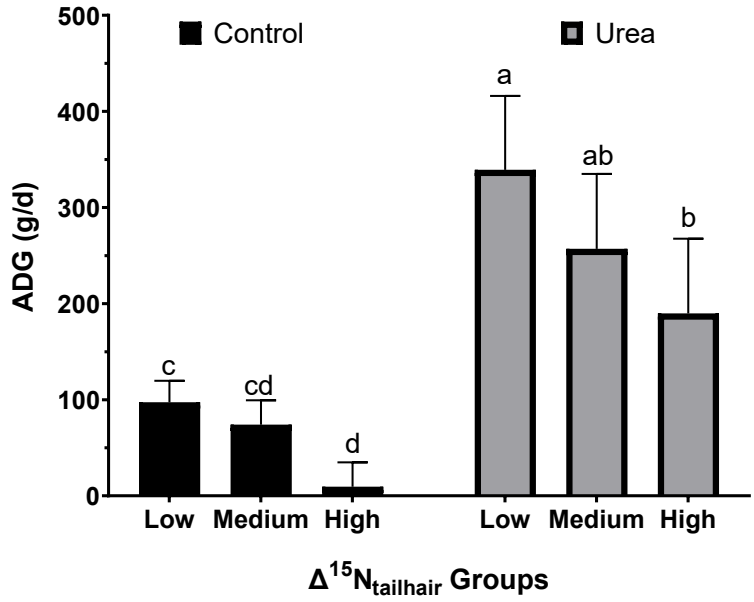
intake of M8U, as all animals were fed a fixed amount relative to their BW and consumed all the supplement provided.

Table 31. Growth performance of 23 Brahman steers receiving two experimental diets (with or without a urea supplement).
BW, body weight; ADG, average daily gain.

Traits	Diet	n	Mean	Min	Max	Std Dev
Initial BW (kg)	Control	23	238	172	302	30
	Urea	23	239	179	295	26
Final BW (kg)	Control	23	240	176	301	29
	Urea	23	254	187	306	29
ADG (g/d)	Control	23	63	-125	181	74
	Urea	23	266	68	446	114
DMI (kg/100 kg BW)	Control	23	1.62	1.23	1.88	0.16
	Urea	23	1.77	1.35	2.08	0.17
Response to supplement (g ADG/d)	Urea	23	202	4.18	480	120

Based on the distribution of $\Delta^{15}N_{tail\ hair}$, the 23 steers were classified into three groups: Low (n=9), Medium (n=7) or High (n=7). The growth rate of steers on both diets was associated with the N-isotopic profile in tail hair (Fig. 13). Steers with lower values of $\Delta^{15}N_{tail\ hair}$ had greater ADG on both diets than steers with higher $\Delta^{15}N_{tail\ hair}$ ($P < 0.01$). When receiving the urea supplement, steers with low $\Delta^{15}N_{tail\ hair}$ gained 339 g/d, while steers with high $\Delta^{15}N_{tail\ hair}$ gained only 190 g/d. The magnitude of response to the urea supplement ($ADG_{Urea} – ADG_{Control}$) was similar ($P = 0.19$) among the three groups (248, 178, and 179 g/d for the Low, Medium, and High $\Delta^{15}N_{tail\ hair}$ groups, respectively).

Figure 13. Effect of N-isotopic profile in the tail hair (Δ^{15}Ntail hair) on the performance of steers fed two diets, with or without urea supplementation.



These results demonstrated that the $\Delta^{15}N_{tail\ hair}$ analysis was successful in predicting the performance of steers in a protein-limiting diet (hay only) and in a protein-limiting diet with urea supplements (hay + urea-molasses supplement), reinforcing the applicability of this analysis to evaluate the N-

recycling ability of cattle. The 149 g/d difference in ADG between the Low and High $\Delta^{15}N_{tail\ hair}$ steers when receiving urea supplements represents 13.4 kg of BW difference during a 3-month supplementation period. In summary, even in cattle receiving a urea-molasses supplement, steers with better nitrogen use efficiency, as estimated by lower $\Delta^{15}N_{tail\ hair}$, had higher ADG than steers with higher $\Delta^{15}N_{tail\ hair}$.

4.7 Review of the project and analysis of potential value

Interviews with producers, extension agents, and stakeholders in the red meat industry indicated a huge demand for the ability to select more efficient animals in grazing systems. However, two conditions were identified as crucial for the commercialization of tail hair analysis as a predictor of nitrogen use efficiency:

1) The trait needs to be heritable, as the interest in classifying animals for efficiency is in the genetic improvement of the herd, not in annual management strategies.

2) Because cattle are commonly supplemented during the dry season when N levels in pasture are below 1%, the relationship between tail hair isotopes and feed efficiency in low-protein diets needs to hold when cattle are being supplemented with urea.

From this apparently simple but very important feedback, the project was modified to address these points. Both conditions proved to be true. Therefore, further interaction with the industry identified four distinct applications of the nitrogen isotopes technique:

1) Selection of more efficient breeders able to maintain body condition score during the dry period translating into higher fertility and calf and herd survival.

2) Selection of more efficient steers with faster growth rates during the year in grazing systems, especially during the dry to wet and wet to dry transition periods.

3) Selection of more efficient cattle with lower methane emissions.

4) Classification of weaners based on the potential growth rate when receiving a urea supplement during the dry season.

This project demonstrated that cattle with lower ^{15}N:^{14}N ratio in tail hair were 1.36-fold more efficient in using the available dietary nitrogen, resulting in better feed efficiency, as measured by residual gain, feed conversion ratio and G:F ratio (Table 24). For example, for the same level of intake, steers with lower ^{15}N:^{14}N ratio in tail hair would have 1.09-fold increase in growth rate. The impact of better nitrogen use efficiency in breeders was not evaluated in this project. However, a recent study from our group (Silva *et al.* 2022) demonstrated that more productive cows had lower ($P < 0.05$) $\delta^{15}N_{tail\ hair}$ during the dry season, indicating differences in N metabolism and possibly lower N losses.

To create an illustrative scenario, it is possible to model the impact of selecting breeders capable of using available nitrogen 1.36-fold more efficiently. For example, on a typical low-protein and low-energy diet, less efficient breeders would change from BCS 4 to BCS 3.5 in the last 6 months of gestation (34 kg of BW loss for a 480-kg cow, according to NASEM 2016). A more efficient cow would need to be only 1.06-fold more efficient in using the available nutrients from the pasture to be able to maintain BCS 4 during the same period. This relatively small change in feed efficiency of breeders (1.06-fold more efficient) would, nonetheless, result in significant increases in reproductive

performance. An increase in BCS 3.5 to BCS 4 at calving represents a potential increase from 62 to 72% of expected pregnancy rate in the herd (Schatz *et al*. 2011).

The impact of selecting steers with 1.09-fold better growth rate for the same intake would represent an additional 13 kg of annual liveweight gain in a region with an average of 145 kg of annual liveweight gain, such as the Mitchell grass pastures of North-Western Queensland. Taken together, the impact of better growth and reproductive efficiencies can be visualized in Table 32.

Table 32. Predicted impact of selecting cattle for better nitrogen use efficiency (NUE) based on the results from this project.

Items	Low NUE	High NUE	% change
Herd numbers			
Total adult equivalents	3 000	3 000	
Total number of breeders	2 143	2 066	-4
Body condition score before calving	3.5	4.0	+14
Pregnancy rate (%)	62	72	+16
Calf mortality (%)	10	10	
Weaning rate (%)	56	65	+16
Annual ADG steers (kg/d)	0.53	0.58	+9
Annual ADG heifers (kg/d)	0.49	0.54	+10
Livestock sales			
Male weaners sold[1]	600	671	+12
Heifers sold[2]	238	322	+35
Culled cows sold[3]	340	328	-4
Total sales (kg LW/year)	337 850	378 915	+12
GHG Emissions			
Total emissions (t CO_2eq/year)	6 709	6 776	+1
Emissions intensity (kg CO_2eq/kg LW)	19.8	17.8	-10

[1]Male weaners are sold at weaning.
[2]Excess heifers are sold at weaning.
[3]Empty cows are sold immediately after pregnancy check during the weaning muster.

The combined effect of better pregnancy rates and better growth efficiency resulted in an estimated 12% increase in saleable LW and an 10% reduction in emissions intensity. With the current cattle prices (450 ¢/kg LW), this would represent an increase of $184,792 in annual income for a stable 3,000 AE property. The numbers presented in Table 32 are a simple exercise to estimate the potential impact of better NUE on a breeding business and were created with the Ekonomou *et al* (2020) livestock emissions calculator. It is important to highlight that the hypothesis that cattle with lower ^{15}N:^{14}N ratio in tail hair, reflecting better NUE, would have better growth and reproductive performance in grazing systems has yet to be tested.

5. Conclusion

This project has demonstrated that different physiological mechanisms are responsible for variation in feed efficiency between animals eating diets differing in protein content. Therefore, the cattle industry should consider using appropriate diets, without excess protein, when evaluating bulls targeted for performance in harsh environments. More efficient steers in low-protein diets lose less nitrogen in urine, resulting in higher efficiency. This is not the case in protein abundant diets. These results have important application to the red meat industry, as it identifies nitrogen recycling and

the ability of individual animals to lose less nitrogen in the urine as a key trait to improve feed efficiency for the northern cattle herd.

As nitrogen efficiency is not easily measured, the application of the knowledge generated in this project requires the development of more practical techniques for large-scale evaluation. This project determined that both feed efficiency and nitrogen use efficiency in protein-deficient diets could be estimated from nitrogen isotopes in tail hair with reasonable accuracy.

In addition, this project demonstrated that the nitrogen isotope ratio in tail hair is a moderately heritable trait and can be used to predict the growth rate of cattle when receiving a urea supplement. This forensic tool being developed could allow quick diagnostic of nitrogen use efficiency of grazing cattle, enabling large-scale selection of more efficient animals.

6. Key findings

- There was a large variation in feed efficiency and nitrogen use efficiency in the population of Brahman cattle studied. The more efficient steers (29% of the studied population) had the same intake but 1.5-fold better growth rate (ADG) in a protein-deficient diet than the less efficient steers (data presented in Table 8).

- There was no agreement in efficiency ranking when steers were eating a low-protein compared to a high-protein diet, and nitrogen recycling parameters were important in determining feed efficiency in the low- but not the high-protein diet. Therefore, when targeting performance in harsh environments, it is important to use more appropriate diets during the performance trial.

- More efficient steers lose less N in urine and maintain lower plasma urea nitrogen, emphasising the importance of N-recycling in determining rumen and feed efficiency. More-efficient steers used the available nitrogen 41% more efficiently than the less-efficient steers. This trait is not important when the steers are fed with protein-abundant diets. These results indicate the possibility to select cattle for better nitrogen use efficiency, which would benefit the overall herd efficiency in northern systems and potentially reduce the need to overfeed nitrogen in feedlot diets.

- Microbial populations associated with feed efficiency were identified, but only for the low-protein diet, further corroborating the hypothesis that different physiological mechanisms are responsible for feed efficiency in diets differing in protein content.

- When fed a protein-deficient diet, steers losing less nitrogen in urine and with better rumen and overall feed efficiency also had a distinct nitrogen-isotopic profile in tail hair (lower $\Delta^{15}N$). This indicates the possibility to develop a practical tool to monitor feed efficiency of *Bos indicus* cattle fed protein-deficient diets.

- The response of steers to a urea and molasses supplement could also be predicted from the nitrogen isotopes on tail hair, reinforcing the applicability of this analysis to evaluate the N-recycling ability of cattle.

- The natural abundance of nitrogen isotopes in tail hair is moderately heritable (43%) in tropically adapted breeds, demonstrating the possibility of using this trait to select more efficient cattle.

7. Benefits to industry

This project demonstrated the possibility of an innovative way to detect more nitrogen-efficient cattle, which could be further developed and commercialised. The ability to classify cattle for nitrogen efficiency can be used to select more efficient steers with faster growth rates in grazing systems, to select more efficient cows able to maintain body condition during the dry period translating into higher fertility and calf survival, and to predict the response of individual animals to dry-season supplements.

8. Future research and recommendations

The continuation of this line of research will allow for a more efficient selection of animals when targeting performance in harsh environments. Therefore, this technology should be commercialised. Breeders' performance in northern systems, with low fertility rates and high calf wastage, is a major factor affecting northern beef businesses' profitability and sustainability. Extending this line of research to quantify the impact of nitrogen use efficiency on cow fertility and calf mortality can allow for easier selection of more efficient cows, able to make better use of the scarce amount of protein available.

A better understanding of the effects of lactation, gestation, and heat stress on cattle's nitrogen metabolism and on the nitrogen isotopic profile in tail hair would be necessary before the commercialization of this technique.

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10. Appendix

10.1 Appendix 1 – Animal Ethics Approval Certificates

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Office of Research Ethics
Director
Nicole Shively

Animal Ethics Approval Certificate

13-Apr-2018

Please check all details below and inform the Animal Ethics Unit within 10 working days if anything is incorrect.

Activity Details

Chief Investigator: Dr Luis Prada e Silva, Animal Science

Title: Nitrogen recycling as determinant for feed efficiency of Bos indicus cattle

AEC Approval Number: QAAFI/003/18

Previous AEC Number:

Approval Duration: 13-Apr-2018 to 13-Apr-2021

Funding Body:

Group: Production and Companion Animal

Other Staff/Students: Robert Dixon, Athol Klieve, Sheree Boisen, Diane Ouwerkerk, Rosalind Gilbert, Latino Coimbra, Lana Harriott, Brandon Fraser, Scott Cullen, Amy Hewitt, Stacey Groves, Maree Bowen, Karen Harper, Mary Fletcher, Sarah Meale, Veronica Costa Mota, Milou Dekkers

Location(s): Gatton - QASP

Summary

Subspecies	Strain	Class	Gender	Source	Approved	Remaining
Cattle		Adults	Male		90	90

Permits

Provisos

Approval Details

Description	Amount	Balance
Cattle (Male, Adults,)		
21 Mar 2018 Initial approval	90	90

Please note the animal numbers supplied on this certificate are the total allocated for the approval duration

Please use this Approval Number:

1. When ordering animals from Animal Breeding Houses
2. For labelling of all animal cages or holding areas. In addition please include on the label, Chief Investigator's name and contact phone number.
3. When you need to communicate with this office about the project.

It is a condition of this approval that all project animal details be made available to Animal House OIC. (UAEC Ruling 14/12/2001)

The Chief Investigator takes responsibility for ensuring all legislative, regulatory and compliance objectives are satisfied for this project.

This certificate supercedes all preceeding certificates for this project (i.e. those certificates dated before 13-Apr-2018)

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Page 1 of 1

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA
CREATE CHANGE

Research Ethics and Integrity

Animal Ethics Approval Certificate

Project Number: 2021/AE000013

Project Title: Urea supplementation to Brahman steers on a low protein diet

Version: 2.01

Chief Investigator (CI): Associate Professor Luis Felipe Prada e Silva

The University of Queensland

Other Participants:

Participant: Sheree Boison
Participant: Miss Alyssa Nicole Woodland
Participant: Mr Brodie Cole Dance
Alternative Investigator: Dr Brandon Fraser
Participant: Ms Chelsea Ruth Baker
Participant: Dr Danielle Schultz
Participant: Hunter Foxlee
Emergency contact: Ms Karen Elizabeth Eyre
Participant: Mr Latino Dos Santos Coimbra
Facility Manager: Ms Milou Helene Dekkers
Participant: Mr Mitchell John Harris
Participant: Mr Scott Andrew Cullen
Participant: Miss Stacey Groves
Student: Hung Ngo
Participant: Ms Samantha Ann Armour
Participant: Miss Sarah Kennedy

Funding Body (Ref#):

Approved Location(s): QASP-GATTON

Approval Duration: 31 Mar 2021 to 31 Mar 2024

Approving AEC: Production and Companion Animals AEC - PCA

Approval date: Monday, 30 August 2021

Summary

Animals approved for research

Subspecies	Strain	Class	Gender	Source	Approved	Remaining

10.2 Appendix 2 – Pellets composition

```
================================================       Riverina       (4684) ================================================
:                                                                                                                           :
:     Single-Mix          (Q )    NW OAKEY RIVERINA        {68} MAY 18             CUSTOMISED FORMULA        10:06 27/04/18  0002 :
: 1312.5/1.8                           (    9) Plant=0088      gbrown                                                      :
============================================================================================================================

            Time period: MAY 18                  Plant name:                          Optimization date: 26/04/18
            ***************************************************************************************************

            LP code: ZUQ6020RPU     Description: UQ TRIAL ENERGY PLT                              - STORED FORMULA -
            ***************************************************************************************************

 RM code       Description        Kgs     %   LP_min LP_max RMcost LOcost HIcost    Nutrient  Units   LP_min    Actual    LP_max
---------- -------------------- ------ ------ ------ ------ ------ ------ ------   ---------- ----- --------- --------- ---------
     10082 BARLE 10.0 HAMMER MI 780.0  39.0                                        [VOLUME]   %               100.0
     11602 SORGH 10.0 HAMMER MI 790.0  39.5                                        DRYMATTER  %              88.696395
     12242 WHEAT 12.0 HAMMER MI 100.0   5.0                                        PROTEIN    %              10.821825
     13540 MILLRUN 16.0         200.0  10.0                                        RDP        %               7.130325
     28100 RECYC VEG OIL - MIXE 20.0    1.0                                        UDP        %               3.654
     29100 MOLASSES 78 BRIX     50.0    2.5                                        PROT_DMA   %              12.200975
     30000 LIMESTONE            26.0    1.3                                        EQUIV_CP   %               0.6406
     32000 SODIUM BICARBONATE   20.0    1.0                                        EQ_CP_DMA  %               0.722239
     33500 AMMONIUM SULPHATE    10.0    0.5                                        UREA_EQUIV                 0.2228
     90030 RSF VITAMIN 4 PREMIX 2.0     0.1                                        C_FIBRE    %               4.680874
     90130 RSF MINERAL D PREMIX 2.0     0.1                                        NDF        %              16.486624
                                ------ ------               ------                ADF        %               6.175624
                          Total: 2000.0 100.0                                      STARCH     %              49.77
                                 ------ ------   Batch cost:                       NFC        %              55.0422
                                                                                  ME_RUM_MJ  MJ/KG            12.03445
                                                                                  ME_RUM_DM  MJ/KG            13.568139
                                                                                  NE_MAINT   MJ/KG             7.251972
                                                                                  NE_GAIN    MJ/KG             4.846607
                                                                                  NE_LACT    MJ/KG             6.847223
                                                                                  CALCIUM    %                 0.548671
                                                                                  PHOSPHORUS %                 0.34645
                                                                                  #CAL/PHO                     1.583695
                                                                                  SODIUM     %                 0.301025
                                                                                  POTASSIUM  %                 0.485861
                                                                                  MAGNESIUM  %                 0.237311
                                                                                  SULPHUR    MG/KG           2591.345
                                                                                  SALT       %                 0.0
                                                                                  COBALT     MG/KG             0.5
                                                                                  COPPER     MG/KG            12.0
                                                                                  FERROUS    MG/KG            50.0
                                                                                  IODINE     MG/KG             0.5
                                                                                  MANGANESE  MG/KG            40.0
                                                                                  SELENIUM   MG/KG             0.1
                                                                                  ZINC       MG/KG            40.0
                                                                                  FAT/EE     %                 3.338875
                                                                                  FLAVOPHOSP MG/KG             0.0
                                                                                  LASALOCID  MG/KG             0.0
                                                                                  MONENSIN   MG/KG             0.0
                                                                                  SALINOMYC  MG/KG             0.0
                                                                                  VIRGINIAMY MG/KG             0.0
                                                                                  HARDNESS   %/10              4.476
                                                                                  THROUGHPUT %/10              5.832
                                                                                  ABRASIVE   %/10              5.695

 &k2S
================================================       Riverina       (4684) ================================================
:                                                                                                                           :
:     Single-Mix          (Q )    NW OAKEY RIVERINA        {68} MAY 18             CUSTOMISED FORMULA        10:06 27/04/18  0001 :
: 1312.5/1.8                           (    9) Plant=0088      gbrown                                                      :
============================================================================================================================

            Time period: MAY 18                  Plant name:                          Optimization date: 26/04/18
            ***************************************************************************************************

            LP code: ZUQ6010RPU     Description: UQ TRIAL PROTEIN PLT                             - STORED FORMULA -
            ***************************************************************************************************

 RM code       Description        Kgs     %   LP_min LP_max RMcost LOcost HIcost    Nutrient  Units   LP_min    Actual    LP_max
---------- -------------------- ------ ------ ------ ------ ------ ------ ------   ---------- ----- --------- --------- ---------
     10082 BARLE 10.0 HAMMER MI 1050.0 52.5                                        [VOLUME]   %               100.0
     12242 WHEAT 12.0 HAMMER MI 100.0   5.0                                        DRYMATTER  %              89.613895
     13540 MILLRUN 16.0         200.0  10.0                                        PROTEIN    %              19.141825
     21530 CANOLA MEAL 38%      260.0  13.0                                        RDP        %              12.307425
     21840 SOYABEAN MEAL 46%    260.0  13.0                                        UDP        %               6.7969
     28100 RECYC VEG OIL - MIXE 20.0    1.0                                        PROT_DMA   %              21.360331
     29100 MOLASSES 78 BRIX     50.0    2.5                                        EQUIV_CP   %               0.6406
     30000 LIMESTONE            26.0    1.3                                        EQ_CP_DMA  %               0.714845
     32000 SODIUM BICARBONATE   20.0    1.0                                        UREA_EQUIV                 0.2228
     33500 AMMONIUM SULPHATE    10.0    0.5                                        C_FIBRE    %               6.999374
     90030 RSF VITAMIN 4 PREMIX 2.0     0.1                                        NDF        %              18.604624
     90130 RSF MINERAL D PREMIX 2.0     0.1                                        ADF        %               7.751124
                                ------ ------               ------                STARCH     %              32.43
                          Total: 2000.0 100.0                                      NFC        %              43.2447
                                 ------ ------   Batch cost:                       ME_RUM_MJ  MJ/KG            11.73445
                                                                                  ME_RUM_DM  MJ/KG            13.094454
                                                                                  NE_MAINT   MJ/KG             7.358472
                                                                                  NE_GAIN    MJ/KG             4.913607
                                                                                  NE_LACT    MJ/KG             6.926223
                                                                                  CALCIUM    %                 0.656621
                                                                                  PHOSPHORUS %                 0.489399
                                                                                  #CAL/PHO                     1.341686
                                                                                  SODIUM     %                 0.294325
                                                                                  POTASSIUM  %                 0.791361
                                                                                  MAGNESIUM  %                 0.314711
                                                                                  SULPHUR    MG/KG           3619.345
                                                                                  SALT       %                 0.0
                                                                                  COBALT     MG/KG             0.5
                                                                                  COPPER     MG/KG            12.0
                                                                                  FERROUS    MG/KG            50.0
                                                                                  IODINE     MG/KG             0.5
                                                                                  MANGANESE  MG/KG            40.0
                                                                                  SELENIUM   MG/KG             0.1
                                                                                  ZINC       MG/KG            40.0
                                                                                  FAT/EE     %                 2.879375
                                                                                  FLAVOPHOSP MG/KG             0.0
                                                                                  LASALOCID  MG/KG             0.0
                                                                                  MONENSIN   MG/KG             0.0
                                                                                  SALINOMYC  MG/KG             0.0
                                                                                  VIRGINIAMY MG/KG             0.0
                                                                                  HARDNESS   %/10              5.131
                                                                                  THROUGHPUT %/10              5.702
                                                                                  ABRASIVE   %/10              5.035
```

10.3 Appendix 3 – Adverse Events Report

08/16

The University of Queensland Animal Ethics

Adverse Event Report Form

Animal Ethics Unit, UQ Research and Innovation

Comment:	**Date Received:**	**AEC Group:**
	Current Ethics Approval No: Date/Signature Chairperson: / /	

Hard copy submission of this document is no longer required. Complete forms should be hand signed, scanned, and forwarded to the relevant committee via email. For further information, please see the Animal Ethics Website.

Please use this form when reporting an adverse event to the AEC as per *the Australian code of practice for the care and use of animals* (Current Edition):

2.4.18. *Investigators must take steps at all times to safeguard the wellbeing of animals by avoiding or minimising known or potential causes of harm, including pain and distress, to the animals. Steps include:*
- IX. *taking prompt action, including alleviating pain and distress and promptly notifying the AEC, in response to unexpected adverse events and emergencies, in accordance with institutional and AEC policies and procedures (see Clauses 2.1.5 [v] [d] and 3.1.24–3.1.25).*

2.4.34. *Investigators must provide the following to the AEC in accordance with AEC and institutional policies and procedures:*
- II. *prompt notification of any unexpected adverse events (see Clause 2.1.5 [v] [d])*

The AEC expects that, in the instance of an adverse event, that the AEC be notified immediately and any ongoing activities will cease until rectification/modification is made to the activity. If any clarification is required on adverse event reporting, please contact the Animal Ethics Unit Coordinator.

1. Project details

Project title:
QAAFI/003/18 Nitrogen recycling as determinant for feed efficiency of Bos indicus cattle

2. Investigator details

Chief Investigator:	*Dr Luis Prada e Silva*
Department/School:	*QAAFI – Centre for Animal Science*
Telephone:	*0421 833 376*
Email:	*l.pradaesilva@uq.edu.au*

REPORT (Questions 3-6 below)

3. Describe the event or incident:
Steer #3335 was limping on rear left leg and could not stand up on 03/04/2019. Reason for the injure was not clear, most likely occurred during routine procedure in the crush. Dr Brandon Fraser (UQ Vets) was present

and initially medicated the steer. After re-evaluation of the steer on the steer on the following morning, the steer was euthanized.

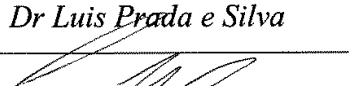
4. Describe what measures were undertaken at the time of the event to minimise impact on the animals:

The steer was immediately evaluated and medicated with anti-inflammatory drug on 03/04. As the animal was still with great difficult to stand up on the following morning the UQ Vets service was called on 04/04. After evaluating the animal under sedation including opioids, it was decided by Dr Victoria Churchill that because of the extent of the injury and the obvious signs of pain, the recommended procedure was to euthanize the animal, which was accepted by the chief investigator. The post mortem exam revealed a shattered femur and very low density bone. Further analysis on bone structure are pending. A likely underlying cause for this injury is lack of Phosphorous in the diet during the backgrounding phase in the property of origin, leading to weak bone structure and eventually to the fracture during the management.

5. Describe what measures have been undertaken, post event, to minimise a repeat of the incident or event: (Please see question 6 below if post event measures not yet undertaken.)

The current diet, although formulated to contain appropriate levels of Ca and P, is being analysed for mineral contents. The producer that owns the steers have being contacted and advice has been given to supplement all herd with Phosphorous. The management of animals during the experiment will continue to be done with patience and avoiding crowding of the animals in the crush. For future experiments, animals will be sourced from producers that are already supplementing with Phosphorous.

6. If this is a preliminary advice of an adverse event, and post-event measures have not yet been established, please describe the current situation regarding animal welfare:

Name of Chief Investigator: (please print or type)	*Dr Luis Prada e Silva*
Signature:	
Date:	24.4.2019

08/16

The University of Queensland Animal Ethics

Adverse Event Report Form

Animal Ethics Unit, UQ Research and Innovation

Comment:	**Date Received:**	**AEC Group:**
	Current Ethics Approval No: Date/Signature Chairperson: / /	

Hard copy submission of this document is no longer required. Complete forms should be hand signed, scanned, and forwarded to the relevant committee via email. For further information, please see the Animal Ethics Website.

Please use this form when reporting an adverse event to the AEC as per *the Australian code of practice for the care and use of animals* (Current Edition):

2.4.18. *Investigators must take steps at all times to safeguard the wellbeing of animals by avoiding or minimising known or potential causes of harm, including pain and distress, to the animals. Steps include:*
IX. *taking prompt action, including alleviating pain and distress and promptly notifying the AEC, in response to unexpected adverse events and emergencies, in accordance with institutional and AEC policies and procedures (see Clauses 2.1.5 [v] [d] and 3.1.24–3.1.25).*

2.4.34. *Investigators must provide the following to the AEC in accordance with AEC and institutional policies and procedures:*
II. *prompt notification of any unexpected adverse events (see Clause 2.1.5 [v] [d])*

The AEC expects that, in the instance of an adverse event, that the AEC be notified immediately and any ongoing activities will cease until rectification/modification is made to the activity. If any clarification is required on adverse event reporting, please contact the Animal Ethics Unit Coordinator.

1. Project details

Project title:
QAAFI/003/18 Nitrogen recycling as determinant for feed efficiency of Bos indicus cattle

2. Investigator details

Chief Investigator:	*Dr Luis Prada e Silva*
Department/School:	*QAAFI – Centre for Animal Science*
Telephone:	*0421 833 376*
Email:	*l.pradaesilva@uq.edu.au*

REPORT (Questions 3-6 below)

3. Describe the event or incident:
Steer #3349 laid down and could not stand up on 03/04/2019. Reason for the injure was not clear, most likely occurred during routine procedure in the crush. Dr Brandon Fraser (UQ Vets) was present and initially

medicated the steer. After re-evaluation of the steer on the steer on the following morning, the steer was euthanized.

4. Describe what measures were undertaken at the time of the event to minimise impact on the animals:

The steer was immediately evaluated and medicated with anti-inflammatory drug on 03/04. As the animal was still with great difficult to stand up on the following morning the UQ Vets service was called on 04/04. After Dr Victoria Churchill evaluated the steer, it was decided to treat the steer with oral fluids and electrolytes, painkillers, nursing care and deep bedding and revaluate in the next day. On 05/04 UQ Vets revaluated the steer (Dr Victoria Churchill) and because it was still with difficulty to stand-up and to urinate, the recommended procedure was to euthanize the animal, which was accepted by the chief investigator. The post mortem exam revealed a large haemorrhage area in the spinal cord. A probable underlying cause for this injury is lack of Phosphorous in the diet during the backgrounding phase in the property of origin, leading to weak bone structure and eventually to the damage in the spinal cord during management. Further analysis are pending.

5. Describe what measures have been undertaken, post event, to minimise a repeat of the incident or event: (Please see question 6 below if post event measures not yet undertaken.)

The current diet, although formulated to contain appropriate levels of Ca and P, is being analysed for mineral contents. The producer that owns the steers have being contacted and advice has been given to supplement all herd with Phosphorous. The management of animals during the experiment will continue to be done with patience and avoiding crowding of the animals in the crush. For future experiments, animals will be sourced from producers that are already supplementing with Phosphorous.

6. If this is a preliminary advice of an adverse event, and post-event measures have not yet been established, please describe the current situation regarding animal welfare:

Name of Chief Investigator: (please print or type)	*Dr Luis Prada e Silva*
Signature:	
Date:	24.4.2019

10.4 Appendix 4 – Scientific and Technical Communications

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

More efficient steers on good diets are not the most efficient ones on limiting diets

Peter Alexander Carmona Memmel

B. Sc. Agricultural Engineering

B. Sc. Animal Science

Supervisor: Dr. Luis Felipe Prada e Silva

Thesis submitted for the degree of Master of Animal Science at

The University of Queensland

School of Agriculture and Food Science

May 2019

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Comparison of Near Infra-Red Spectroscopy (NIRS) techniques to analyse tail hair as proxy to measure Nitrogen efficiency in cattle

Julia Harkin
Bachelor of Agricultural Science (Honours)

Candidate's ORCID

A thesis submitted for the degree of Bachelor of Agricultural Science at The University of Queensland in Year 2020
AGRC4614 Research Project

i

BACHELOR OF AGRICULTURAL SCIENCE

Animal Science

AGRC4616

Undergraduate Thesis

22 January 2021

The Use of Near Infrared Spectroscopy as a Prediction Technique of Feed Efficiency in Tropically Adapted Cattle using Tail Hair Samples

Jonathan Reid

School of Agriculture & Food Science

2021

CSIRO PUBLISHING
Animal Production Science
https://doi.org/10.1071/AN19234
Review

Nitrogen recycling and feed efficiency of cattle fed protein-restricted diets

L. F. P. Silva[A,D], *R. M. Dixon*[B] *and D. F. A. Costa*[C]

[A]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, 306 Carmody Road, St Lucia, Qld 4072, Australia.
[B]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, 25 Yeppoon Road, Parkhurst, Rockhampton, Qld 4701, Australia.
[C]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, Building 8150, Gatton, Qld 4343, Australia.
[D]Corresponding author. Email: l.pradaesilva@uq.edu.au

Abstract. The ability of cattle to grow and reproduce when ingesting low-protein diets is a crucial attribute for productive beef cattle systems in the seasonally dry tropics and subtropics. Nitrogen (N) recycling to the rumen is an important and known physiological mechanism allowing ruminants to efficiently grow in low-protein diets, but is usually disregarded in the nutritional models. This review discusses the role and magnitude of N recycling to provide additional N as microbial substrate in the rumen and in determining the efficiency of ruminants ingesting low-protein diets, to better understand the major factors regulating N recycling to the rumen. In addition to a review of the literature, study-adjusted regressions were used to evaluate various aspects of crude protein (CP) intake and availability, N recycling and excretion. There is large variation in N excretion and N-use efficiency among diets and among individuals, illustrating the opportunity for improvement in overall efficiency of cattle production. These data indicated that N recycling to the entire gastrointestinal tract supplies from half to twice as much N available for microbial growth as does the diet. Addition of rumen-degradable protein can increase rumen efficiency in using the available energy, as, conversely, the addition of fermentable energy can increase rumen efficiency in using the available CP. The present review has demonstrated that both are possible because of greater N recycling. Also, the importance of preserving the available N for determining individual variation in feed efficiency and the implications for selection are discussed. Nitrogen recycling can be controlled at both the epithelial wall of compartments of the gastrointestinal tract and at the liver, where ureagenesis occurs. Addition of fermentable energy can increase N recycling to the rumen and to post-ruminal tract by acting at both sites, and the mechanisms for this are discussed in the text. Although the effect of altering CP concentration in the diet has been substantially investigated, other factors potentially modulating N recycling, such as total fermentable energy, sources of protein and energy, hormonal modulation, and genetic variance, remain poorly understood. The selection of more efficient animals and development of diets with a lower environmental impact inescapably means further elucidation of the N-recycling mechanism.

Additional keywords: beef cattle, feed conversion efficiency, rumen function, urea.

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Feed efficiency and nitrogen use rankings of *Bos indicus* steers differ on low and high protein diets

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ARTICLE INFO

Keywords:
Brahman
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ABSTRACT

Nutrition represents the major operating cost of beef cattle production. Improvements on feed efficiency (FE) can lead to significant economic benefits and reduce the environmental footprint of red meat production. Usually, livestock selected for FE on high-protein diets are expected to perform as efficiently on low-protein diets. This experiment used 55 Brahman steers (346 ± 8 kg BW) to determine the agreement in FE rankings between a diet in which rumen degradable protein (RDP) was limiting and a protein abundant diet. It was suggested that the agreement would be low and FE in low-protein diets would be related to nitrogen (N) preservation mechanisms. A completely randomized block design was used. Each steer represented an experimental unit. Steers were fed in individual pens for two periods of 70 days, including an adaptation of 10 days, with diets supplying either 70 % or 100 % of their RDP requirements. Residual feed intake (RFI), residual gain (RG), and residual feed intake and gain (RIG) were determined based on average daily gain (ADG), dry matter intake (DMI), and body weight. Kappa analysis was used to determine the agreement in FE rankings between both diets. In the low-protein diet, ADG was 0.99 kg/d (0.38–1.53 kg/d), DMI averaged 1.9 kg/100 kg BW d^{-1} (1.6–2.3 kg/100 kg BW d^{-1}), RFI varied between −1.22 and 1.58, and RG from -0.62 to 0.53. In the high protein diet, ADG was 1.21 kg/d (0.64–1.74) and DMI averaged 1.8 kg/100 kg BW d^{-1} (1.0–2.3 kg/100 kg BW d^{-1}). RFI varied between −1.52 and 1.58 and RG from -0.36 to 0.41. Kappa analysis showed no agreement ($P > 0.10$) for RFI (−7 %), RG (2 %), nor RIG (−1 %) between diets. More efficient steers in the low-protein diets, measured as RG, excreted less ($P = 0.02$) N in urine as a proportion of BW and as a proportion of N intake, resulting in higher N use efficiency. This relationship was not present when steers were fed the high-protein diet ($P = 0.55$). These results suggest that different physiological mechanisms are responsible for FE regulation in both diets; thus, appropriate diets must be used when selecting animals for FE.

CSIRO PUBLISHING

SPECIAL ISSUE: AAAS 2022 | RESEARCH PAPER
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ANIMAL PRODUCTION SCIENCE

Rumen bacteria and feed efficiency of beef cattle fed diets with different protein content

M. C. Parra[A], D.F. Costa[A], S. J. Meale[B] and L. F. P. Silva[A,*]

For full list of author affiliations and declarations see end of paper

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Handling Editor:
Stephanie Muir

ABSTRACT

Context. Beef cattle feed efficiency is challenged in northern Australian production systems due to the limited dietary protein, leading to changes in rumen bacterial populations and fermentation outcomes. **Aims.** Two types of diets with different dietary protein contents were used to evaluate changes in rumen bacterial composition and diversity, aiming to correlate rumen bacterial populations with feed and rumen efficiency parameters. **Methods.** In total, 90 Brahman steers (341 ± 45 kg BW) were selected for this trial, but rumen fluid was collected from 85 Brahman steers, at 0 and 4 h after feeding, during a feed-efficiency trial. The steers were fed with a low-protein diet, including 70% rumen-degradable protein and 8.8% crude protein (CP) for 60 days, followed by a high-protein diet for the same period (13.5% CP). Liveweight and dry-matter intake measurements, as well as urine, faeces and rumen fluid samples, were collected to determine feed and rumen efficiency, and ruminal bacteria composition. Steers were clustered into groups using principal component analysis and Ward's hierarchical method, and differences in feed-efficiency parameters among clusters were compared. **Key results.** Rumen bacterial composition differed between diets ($P < 0.01$) and diversity changes were more related to bacterial richness ($P < 0.01$). In a low-protein diet, there were four distinct clusters of steers, on the basis of rumen bacteria, in which the most efficient steers, with a better residual feed intake ($P = 0.06$) and lower rumen ammonia concentration ($P < 0.01$) before feeding, had the highest relative abundance of *Prevotella* ($P < 0.01$). While in a high-protein diet, no differences were observed on feed or rumen fermentation parameters among steer clusters. **Conclusion.** In a low-protein diet, rumen bacterial shifting might contribute to upregulate nitrogen recycling, favouring feed efficiency. **Implications.** Identifying ruminal bacterial populations involved in nitrogen recycling upregulation might be useful to select the most efficient cattle fed low-protein diets.

Keywords: *Bos indicus*, feed efficiency, low protein diet, nitrogen recycling, *Prevotella*, rumen ammonia, rumen bacteria composition, rumen maturation, rumen microbiome.

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Animal Production Science
doi:10.1071/AN21508

Introduction

Maintaining a balanced energy:protein ratio in the diet is crucial for maximising rumen fermentation efficiency, supply of microbial protein, and growth efficiency of cattle (Poppi and McLennan 1995). Nevertheless, in the dry-tropic beef cattle-production systems dietary protein content is a limiting nutritional factor, challenging the rumen and feed efficiency and restraining growth rates of high genotypic-value animals. Ruminants have an extraordinary capacity to subsist consuming low-protein diets by altering host and rumen microbiology features. However, low dietary protein content tends to shift rumen bacterial populations, favouring the growth of non-ammonia-dependant bacteria (Marini and Van Amburgh 2003; Belanche *et al.* 2012). This microbial shift is accompanied by the upregulation of nitrogen (N) recycling from the liver into the rumen, with the main purpose of providing enough N for microbial growth with limited ruminal ammonia (NH_3-N) concentration (Reynolds and Kristensen 2008). These rumen bacterial changes, in addition to modifications of rumen cell-wall characteristics and urea transporters, facilitate N flow into the rumen. The hypothesis

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Using the natural abundance of nitrogen isotopes to identify cattle with greater efficiency in protein-limiting diets

L.F.P. Silva [a,*], R.S. Hegarty [b], S.J. Meale [c], D.A.F. Costa [a,1], M.T. Fletcher [a]

[a] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Saint Lucia, Australia
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[c] The University of Queensland, School of Agriculture and Food Sciences, Gatton, Australia

ARTICLE INFO

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Bos indicus
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Nitrogen use efficiency
Stable isotopes
Urine losses

ABSTRACT

The difficulty in selecting cattle for higher feed and nitrogen use efficiency (NUE) is an important factor contributing to poor growth and reproductive performance in dry-tropics rangelands. Therefore, the objectives were to examine the cattle variation in retaining nitrogen in a protein-deficient diet and the natural abundance of stable isotopes in body tissues as a practical alternative for the detection of more efficient cattle. In experiment 1, feed efficiency parameters were determined in 89 Brahman steers fed a protein-limiting diet for 70 days, followed by 7 days in metabolism crates for total collection of urine and faeces and calculation of nitrogen retention and NUE. The diet-animal fractionation of nitrogen isotopes ($\Delta^{15}N$) was quantified in tail hair and plasma proteins using isotope-ratio MS. There was a large variation in growth performance, feed efficiency and nitrogen losses among steers. Quantifying $\Delta^{15}N$ in tail hair ($\Delta^{15}N_{tail\ hair}$) resulted in stronger correlations with feed efficiency and nitrogen metabolism parameters than when quantified in plasma proteins. $\Delta^{15}N_{tail\ hair}$ was positively correlated with nitrogen losses in urine ($r = 0.31$, $P < 0.01$) and faeces ($r = 0.25$, $P = 0.04$), leading to a negative correlation with NUE ($r = -0.40$, $P < 0.01$). The group of steers with lower $\Delta^{15}N_{tail\ hair}$ had greater feed efficiency, lower nitrogen losses, and greater NUE. In experiment 2, for evaluation of isotope fraction as a predictor of reproductive performance, 630 Brahman-crossed cows were classified for reproductive performance for 2 years. From this group, 25 cows with poor reproductive performance and 25 cows with good reproductive performance were selected. Tail hair representing 7 months of growth were segmented and analysed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope enrichment. Reproductive performance was not associated with diet selection, as there was no difference in tail hair $\delta^{13}C$ between groups. However, more productive cows had lower ($P < 0.05$) tail hair $\delta^{15}N$ during the dry season, indicating differences in N metabolism and possibly lower N losses. In addition, cows with better reproductive performance and, therefore, greater nutrient demands, had similar body condition scores and a tendency ($P = 0.09$) for higher live weight at the end of the trial. In conclusion, the findings of the present study confirm that nitrogen isotope fractionation in tail hair can be used as a predictor of nitrogen losses, NUE, and reproductive performance of Brahman cattle on low-protein diets.

Implications

Some Brahman cattle are better able to cope with harsh environments than others, resulting in large differences in performance and the environmental impact of cattle production. We investigated a practical method to detect the more efficient animals in the herd when eating a low-protein diet. We found that more efficient steers and cows, with lower nitrogen losses in the urine, can be detected via tail hair analysis. These findings can assist in the development of a commercial method for the early detection of more efficient animals.

Introduction

Cattle grazing the tropical rangelands often must rely exclusively on low-quality grasses during the dry seasons when nitrogen (N) becomes the first limiting nutrient in their diet. Protein supplementation, when practised, is usually in the form of urea, a rapidly absorbed source of N. In such conditions, animals that utilise more

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1751-7311/

207 Measurements of feed efficiency in cattle fed protein-deficient diets

P. CarmonaA, D.F.A. CostaA, L. O. LimaA, K. J. HarperB, and L.F.P. SilvaA,C

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[B]The University of Queensland, SAFS, Gatton, Australia.

Introduction

Improvements in feed efficiency (FE) can lead to significant economic benefits and reduce the environmental footprint of any beef cattle production system. Animals selected for FE on high-protein (HP) diets are expected to perform as efficiently on low-protein (LP) diets. Thus, the objective of this experiment was to determine the agreement between FE rankings of beef cattle fed a HP and a LP diet.

Methods

In a completely randomized block design, thirty Brahman steers (398 ± 24 kg liveweight) were individually fed for two 70 day periods, with diets supplying either 70% (LP) or 100% (HP) of their rumen degradable protein requirements. Dry matter intake (DMI) and average daily gain (ADG) were measured, while feed conversion ratio (FCR), gain to feed ratio (G:F), residual feed intake (RFI) and residual gain (RG) were calculated. Kappa analysis and Pearson correlation coefficient were used to determine the agreement between the different FE measurements of both diets.

Results

There was no agreement or correlation between steers fed a LP and HP diet for any studied performance or FE traits (Table 1). RFI was strongly correlated with DMI (0.94, $P<0.01$) and RG was strongly correlated with ADG (0.91, $P=0.01$) for steers fed LP diets.

Table 1. Descriptive statistics and agreement coefficients of feed efficiency traits in different diets.

Items[A]	Diet LP					Diet HP				Kappa	*r*
	Mean	Min	Max	SD		Mean	Min	Max	SD	(P-value)	(P-value)
DMI (% LW)	1.94	1.62	2.3	0.15		1.69	1.04	2.01	0.2	-0.07 (0.55)	0.36 (0.06)
ADG (kg)	0.92	0.38	1.47	0.24		1.12	0.64	1.57	0.23	0.01 (0.98)	-0.05 (0.80)
FCR	9.46	5.6	23.57	3.17		7.65	5.71	10.53	1.35	-0.09 (0.44)	-0.10 (0.62)
G:F	0.113	0.042	0.179	0.025		0.134	0.095	0.175	0.022	-0.11 (0.40)	-0.13 (0.53)
RFI	0	-1.32	1.54	0.60		0	-1.47	1.52	0.73	-0.06 (0.68)	0.30 (0.13)
RG	0	-0.61	0.53	0.21		0	-0.34	0.31	0.18	-0.09 (0.44)	-0.07 (0.72)

Discussion

The present study confirms the large individual variation in FE in the Australian beef cattle herd. Negative kappa values indicate poor agreement in ranking of the animals on different diets. These results suggest that different physiological mechanisms are responsible for FE regulation in each diet; thus, appropriate diets targeting each scenario must be used when selecting animals for FE.

[C]*Corresponding author:* l.pradaesilva@uq.edu.au

208 Estimating diet nitrogen use efficiency from isotopes of tail hair in cattle

D.F.A. Costa[A], A.S.V. Palma[A], R.M. Dixon[A], M.T. Fletcher[A], and L.F.P. Silva[A,B]

[A]The University of Queensland, QAAFI, St Lucia, Qld 4072, Australia

Introduction

Cattle grazing tropical rangelands often have to depend on N-deficient pastures during prolonged dry seasons. Thus the ability of individual animals to best utilize the available diet N (i.e. N use efficiency; NUE) is likely directly related to performance. As tail hair grows amino acids are incorporated into hair proteins, and the relative abundance of the ^{15}N and ^{14}N isotopes ($d^{15}N_{tailhair}$) reflect a number of factors, including diet. Animals with higher NUE may have lower $d^{15}N_{tailhair}$, apparently because of lower urinary N excretion (Cantalapiedra-Hijar et al. 2015). Estimation of NUE from tail hair has obvious advantages for practical on-farm sampling. This study evaluated whether NUE was related to $d^{15}N_{tailhair}$ in *Bos indicus* cattle typical of northern Australia.

Methods

Twenty-eight Brahman steers (398 ± 7kg) fed a low-protein diet (70% of required RDP) for 70 days were classified as High (H), Medium (M) or Low (L) based on measured Residual Gain (RG) and Residual Feed Intake (RFI). NUE (g retained N/100 g digested N) was measured in metabolism crates. Tail hair was sampled on day 70, cut into 10 mm segments, and analysed for $d^{15}N_{tailhair}$ by mass spectroscopy.

Results

Fig. 1. shows that steers classified as more efficient by RG criteria (P=0.02) had lower $d^{15}N_{tailhair}$ (H vs L, P=0.02). However the steers were not classified by RFI criteria (P=0.80). Fig. 2. shows that there was a significant (P=0.02) relationship between NUE and the $d^{15}N_{tailhair}$ of H and L steers.

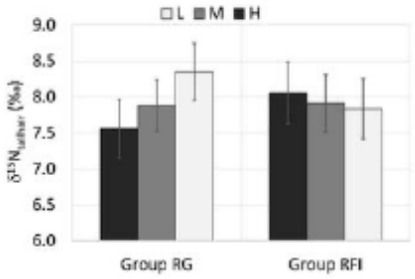

Fig. 1. $d^{15}N_{tailhair}$ of steers in groups classified according to residual gain or residual feed intake.

Fig. 2. $d^{15}N_{tailhair}$ correlated with nitrogen use efficiency of High (□) and Low (●) steers

Discussion

The results demonstrate that NUE could be estimated from $d^{15}N_{tailhair}$ with reasonable accuracy. These preliminary results indicate that it may be possible to estimate NUE and identify animals which are more efficient while requiring only simple non-invasive sampling on-farm.

References

Cantalapiedra-Hijar, G, Ortigues-Marty, I, Sepchat, B, Agabriel, J, Huneau, JF, Fouillet, H (2015) Diet-animal fractionation of nitrogen stable isotopes reflects the efficiency of nitrogen assimilation in ruminants. *British Journal of Nutrition* **113**, 1158-69.

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357 Rumen efficiency and nitrogen preservation of cattle

T. Breed[A], D.F.A. Costa[A], M. K. Bowen[B], and L.F.P. Silva[A,C]

[A]The University of Queensland, QAAFI, Centre for Animal Science, St Lucia, Qld 4072, Australia.
[B]Department of Agriculture and Fisheries, Rockhampton, PO Box 6014, Red Hill, Qld 4701, Australia.

Introduction

The rumen efficiency in converting available energy and nitrogen into microbial protein (MCP) is highly variable and fundamental to determine the performance of ruminants. In protein-limiting diets, better rumen efficiency can help to preserve the available nitrogen. The objective of this study was to investigate whether the rumen efficiency of cattle in using diet protein (EMPS1) was related to nitrogen excretion in urine.

Method

Twenty-eight Brahman steers were fed a protein restricted diet (LP) for 70 days providing 70% of the predicted required rumen degradable protein (RDP; 95 g RDP/kg DOMI), followed by 70 days on a high protein diet (HP; 145 g RDP/kg DOMI) providing 100% of the predicted RDP and were then classified into high (H), medium (M) or low (L) based on ±0.5 standard deviation from mean EMPS1. Rumen efficiency was determined after 7 days in metabolism crates and was defined as a) g of MCP per amount of crude protein intake (CPI; EMPS1) and b) g MCP per amount of digestible organic matter intake (DOMI; EMPS2). MCP was estimated by excretion of purine derivatives in urine.

Results

Steers classified as more efficient in transforming diet protein into microbial protein had similar DM intake and overall nitrogen use efficiency (NUE) on both diets (Table 1). Rumen efficient steers also had greater EMPS1 (by design), MCP, and EMPS2 on both diets. Steers with the lowest EMPS1 in the LP diet, but not in the HP diet, excreted a higher proportion of N intake in urine.

Table 1. Effect of rumen efficiency group [Low (L), Medium (M) or High (H)] on rumen efficiency parameters and N metabolism of Brahman steers

Items	Diet LP					Diet HP				
	L	M	H	SEM	LxH[A]	L	M	H	SEM	LxH[A]
EMPS1 (g MCP/kg CPI)	677	886	1309	40	<0.01	543	818	1555	59	<0.01
MCP (g MCP/100 kg BW)	50	66	85	3.6	<0.01	79	111	189	10.5	<0.01
EMPS2 (g MCP/kg DOMI)	60	71	103	3.4	<0.01	81	122	215	10.1	<0.01
DMI (kg DM/100 kg BW)	1.32	1.52	1.45	0.07	0.17	1.43	1.37	1.36	0.06	0.50
N excretion in urine (g N Urine/100 g N intake)	53.3	36.5	38.9	4.1	<0.01	69.9	65.8	61.6	7.2	0.31
NUE (g N retained/g N digested)	11.8	31.7	19.0	7.1	0.40	4.9	4.5	5.6	12.8	0.97

[A]Contrast between the L and H groups within diets.

Discussion

Rumen efficiency is an important mechanism governing overall feed efficiency of ruminants. The present study demonstrates that EMPS1 of cattle in the LP diet was connected with conservation of N, as more efficient animals excreted less N in urine. This was not the case for the HP diet, suggesting that different mechanisms regulate rumen efficiency in LP and HP diets.

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415 More efficient steers on good diets are not the most efficient ones on limiting diets. Peter Carmona[1], Luis Silva[2], Diogo Fleury Azevedo Costa[3], Lais Lima[4], [1]*The University of Queensland,* [2]*The University of Queensland,* [3]*The University of Queensland,* [4]*The University of Queensland*

Nutrition for a positive growth path represents the major cost of any beef cattle enterprise. Improvements on feed efficiency (FE) can lead to significant economic benefits and reduce the environmental footprint. Usually, animals selected for FE on high-protein (HP) diets are expected to perform as efficiently on low-protein (LP) diets. This experiment used 30 *Bos indicus* steers (398 ± 24 kg BW) to determine the agreement between FE rankings of beef cattle fed a LP or a HP diet. As hypothesis, it was suggested that the agreement would be high. A completely randomized block design was used, where each steer represented an experimental unit. Steers were fed in individual pens for two periods of 70 days, including an adaptation of 10 days, with diets supplying either 70% or 100% of their rumen degradable protein requirements. Average daily gain (ADG) and dry matter intake (DMI) were measured, while residual feed intake (RFI) and residual gain (RG) were calculated. Kappa analysis was

385 Feed efficiency of beef cattle in low-protein diets is driven by nitrogen use efficiency.

Diogo Fleury Azevedo Costa[1], Peter Carmona[2], Lais Lima[3], Brandon Fraser[4], Luis Silva[5], [1]*The University of Queensland*, [2]*The University of Queensland*, [3]*The University of Queensland*, [4]*The University of Queensland*, [5]*The University of Queensland*

Performance of cattle in rangeland systems is driven by the ability to efficiently use nutrients during periods of restricted availability. Thirty *Bos indicus* steers (398 ± 24 kg BW) were used to evaluate the relationship between feed efficiency (FE) and nitrogen use efficiency (NUE). The hypothesis was that FE would be related to NUE in protein restricted diets, but not in high-protein diets. Steers used in a completely randomized block design were classified by residual gain in low (LFE), medium (MFE) and highly feed efficient (HFE), after being fed for periods of 70 days with diets supplying either 70% (LP) or 100% (HP) of their rumen degradable protein requirements. After each 70-day period, animals were adapted to metabolism crates for two days, and NUE was measured for five days. About 10% of daily faecal and urine output of each animal was collected for N analysis. Results of N intake from LFE, MFE and HFE in the LP diet were 21.6, 21.2 and 22.2 g N/100 kg BW (P = 0.63), while the total N excretion was 20.3, 18.0 and 19.0 g N/100 kg BW (P = 0.45) for LFE, MFE and HFE, respectively. NUE values were 11.1, 26.9 and 28.0 g retained N/100 g of digested N (P = 0.04). In the HP diet, N intake was 31.6, 30.8 and 26.8 g/100 kg BW (P = 0.12) for LFE, MFE, and HFE, respectively. Total N excretion was 26.9, 28.8 and 27.5 g N/100 kg BW (P = 0.83), respectively. NUE in the HP diet was 21.0, 5.2 and -6.3 g retained N/100 g of digested N (P = 0.04). These results support the hypothesis that FE is dependent on NUE in protein restricted diets; whereas when evaluated in protein abundant diets, FE is not related to NUE.

Key Words: nitrogen efficiency, crude protein, Bos indicus

proceedings

MDPI

Abstract

Flight Zone as an Alternative Temperament Assessment to Predict Animal Efficiency †

Mariano Parra [1,*], Tia Breed [1], Alana Connolly [1], Emily Janz [1], Sarah Kennedy [1], Jonathan Reid [1], Andre Palma [2], Diogo Fleury Azevedo Costa [2] and Luis Felipe Prada e Silva Silva [2]

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[2] Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, The University of Queensland, Gatton 4343, Australia; a.soligovizeudepalma@uq.edu.au (A.P.); d.costa@uq.net.au (D.F.A.C.); l.pradasilva@uq.edu.au (L.F.P.S.S.)

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† Presented at the third International Tropical Agriculture Conference (TROPAG 2019), Brisbane, Australia, 11–13 November 2019.

Published: 8 April 2020

Abstract: Animal temperament evaluation can be included in the cattle selection program also because of an existing correlation with performance. However, there are different assessment methods such as flight speed (time and speed that an animal takes to leave the crush) and exit score (indicating in which pace it does). Flight zone (FZ) refers to the distance that an animal allows human proximity without signs of fear (e.g., moving away and/or aggression) and it was used in this study as an alternative approach to measure temperament without putting cattle through the crush. Apparently, there is no study correlating FZ with performance. Therefore, a pilot trial was conducted to evaluate the correlation between average daily gain (ADG), dry matter intake (DMI) and feed conversion ratio (FCR) of ten Brahman steers. Steers were classified into temperament groups (Docile <2 m; Moderate between 2 to 2.9 m; and Lively ≥3 m). Even though no significant differences were found for ADG ($P = 0.65$), DMI ($P = 0.36$), and FCR ($P = 0.46$), the docile group gained 133 grams/day more than lively counterparts, most likely because of the extra 50 grams consumed. Furthermore, lively steers required an extra 1 kg of feed per kg of gain in comparison to docile animals, 8.24 vs. 7.28 kg FCR, respectively. These results are promising and indicate that FZ could be an efficient way to measure temperament in cattle. Thus, in order to confirm these findings, a new experiment with a more representative number of steers (n = 30) will be conducted.

Keywords: cattle temperament; flight zone (FZ); average daily gain (ADG); dry matter intake (DMI); feed conversion ratio (FCR)

Author Contributions: This trial was design, directed and coordinated by L.F.P.S.S and D.F.A.C, also supporting with statistical analysis. All authors participated actively with data collection.

Funding: This research was funded by Meat and Livestock Australia (MLA).

Performance variation in Brahman steers supplemented with molasses and urea

B. C. Fraser[A,B], *K. Eyre*[A] *and L F. P. Silva*[A]

[A]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Qld 4343, Australia.
[B]Corresponding author. Email: B.fraser@uq.edu.au

Cattle in Northern Australia often graze low protein pastures. Supplementation of beef cattle with protein or non-protein nitrogen improves growth (Poppi and McLennan 2010). Plant-based protein supplementation can carry a significant cost to a producer, with urea being a more cost-effective option. Urea supplementation to cattle in Northern Australia is a common practice though more research is required to better understand the individual cattle variation in response to urea supplements. It was hypothesized that there is significant variation in the growth response to urea supplementation in Brahman steers.

This trial was conducted at the Queensland Animal Science Precinct in southeast Queensland and consisted of 24 Brahman steers with 238 ± 29 kg initial live weight (LW). Approval was granted by the University of Queensland Animal ethics committee. Steers were separated into two groups, based on initial LW, and randomly allocated to individual pens (3x10m) with ad libitum water, concrete floors with rubber mats on half and shade on half of the pen. After 7 days of adaptation to the facilities, steers received a low-quality Rhodes-grass hay (Control diet, 6.5% crude protein (CP)) for 56 days. Hay was offered ad libitum, with daily adjustments to maintain refusals around 5%. Steers were weighed on two consecutive days, every fortnight, and average daily gain (ADG) calculated by regressing LW over time. After this period, the steers received the same low-quality hay and a urea-molasses supplement with 8% of urea (M8U). The M8U was offered at approximately 10% of hay intake on an as fed basis, to supply 40 g urea per day (Urea diet, 8.1% CP). After 8 days of diet adaptation, the steers were weighed and diet intake and ADG measured for 56 days as described for the Control diet. The response to the urea supplement was calculated as ADG on the Urea diet – ADG on the Control diet. One steer had very low performance during the Control diet (-360 g/d) and was removed from the analysis.

This trial identified the large variation in performance of Brahman steers on a low-protein diet, with ADG ranging from –125 to 181 g/d with an average of 64 g/d. Thirty percent of the steers gained above 100 g/d, while 22% of the steers were losing weight (Fig. 1). The average response to the urea supplement was an increase of 202 g/d in ADG, varying from –11 g/d to 480 g/d. The increase in ADG with the urea supplement was less than 200 g/d for 43% of the steers, which can result in higher feeding cost per kg of extra gain. Considering that the steers consumed on average 500 g of the M8U supplement per day, at a cost of $500/tonne, the daily cost of supplement was $0.25/steer per day (not considering freight or on-farm costs associated with urea supplementation). Considering the current value of $5/kg LW for a growing steer and the average response to supplementation of 200 g/d, the benefit of urea supplementation was $1/steer per day and the average benefit-cost ratio was 4:1 (four dollars returned for each dollar invested). In the current experiment, the benefit-cost ratio would be below 2:1 to 30% of the steers (<100 g/d of response to supplement) and above 5:1 to 30% of the steers (>250 g/d or response to supplement).

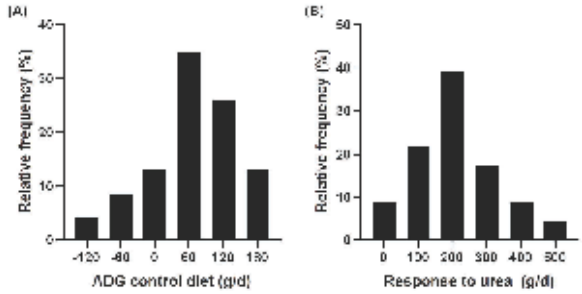

Fig. 1. Variation in growth performance of 23 Brahman steers on a low-protein diet (A) and response to a urea supplement (B).

In conclusion, this trial has shown significant variation between Brahman steers performance on the control diet and their response to a urea supplement, demonstrating the potential value from early identification of steers that are both able to perform well on low quality feeds and benefit from supplementation. Eliminating steers that will not benefit from supplementation would allow the industry to minimize expenses on this portion of the group of steers. Future studies are progressing to validate a practical tool for early identification of steers with low or high predicted response to a urea supplement.

Reference

Poppi D, McLennan S (2010) *Animal Production Science* **50**(6), 329–338.

We gratefully acknowledge Meat and Livestock Australia for funding this work.

lxxv

Australian Association of Ruminant Nutrition

AARN

July 23rd & 24th 2019

Rockhampton, QLD

DAY 1 JULY 23RD

CONFERENCE + DINNER

Headricks Lane, 189 East St, Rockhampton QLD 4700

TOPICS

Using nutrition to select more efficient cattle, improving calf health and survival

PROGRAM

Day 1 TUESDAY JULY 23rd Conference Headricks Lane, Rockhampton

8:30am	Conference registration, tea and coffee	
9.00am	Welcome	
9:05am	Using nutrition to select for more efficient cattle on protein limiting diets	**Dr Luis Prada e Silva** Senior Research Fellow , Uni of QLD
9.50am	Targeted cow nutrition to improve calf health and survival	**Dr Luis Prada e Silva** Senior Research Fellow , Uni of QLD
10:35am	**MORNING TEA**	
11:00am	Improving profit & resilience of beef businesses	**Dr Maree Bowen** Principal Research Scientist , DAF
11:45am	Pasture dieback + practical applications	**Stuart Buck** Agronomist, DAF
12.30pm	**LUNCH**	
1:30pm	Case study on mineral supplementation to on farm resources	**Jim Wade** Wade agricultural Consultants
2.15pm	Backgrounding and starter cattle + considerations In drought	**Dr Lachlan Strohfeldt** Veterinarian
3.15pm	Phosphorous research update	**Dr Rob Dixon** Senior Research Fellow, QAAFI, Uni of Qld
4:00pm	**Close**	
5:30pm	Pre dinner drinks	**Headricks Lane, Rockhampton**
6.30pm	Dinner	

Nitrogen recycling and feed efficiency in tropically adapted cattle

THURSDAY NOVEMBER 14th 2019

TROPAG
INTERNATIONAL TROPICAL AGRICULTURE CONFERENCE
11 - 13 NOVEMBER 2019 | BRISBANE

FREE REGISTRATION

Email: l.pradaesilva@uq.edu.au
Phone: 0421 833 376

This meeting is on the Thursday after the TropAg conference
tropagconference.org

SCHEDULE

09:00–9:30 Welcome
09:30–10:10 "The UQ N-recycling project: an overview" –Luis Prada e Silva, QAAFI
10:10–10:50 "Forensic predictions of feed efficiency" –Diogo Costa, QAAFI
10:50–11:20 – Morning Tea
11:20–12:00 "N-recycling in low protein diets" –Tryon Wickersham, Texas A&M
12:00–12:40 "The cattle selection program of ABBA" – Anastasia Fanning, ABBA
12:40–13:10 – Lunch

13:10 – 13:50 "Rumen efficiency in cattle grazing tropical pastures"–Maree Bowen, DAF
13:50 – 14:30 "Developing breeding objectives" – Catriona Millen- SBTS
14:30 – 14:40 - Break
14:40 – 15:20 "Key management and nutrition principles" - Mick Sullivan, DAF
15:20 – 16:00 "Modelling growth responses in low protein diets" – John Nolan, UNE
16:00 – 16:30 Final remarks

Located at Room S201, Hawken Engineering Building #50

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St Lucia QLD 4072, Australia

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The University of Queensland AUSTRALIA
QAAFI
The Queensland Alliance for Agriculture and Food Innovation (QAAFI) is a research institute of The University of Queensland (UQ), supported by the Queensland Department of Agriculture and Fisheries.
Queensland Government

beefup FORUM

mla MEAT & LIVESTOCK AUSTRALIA

Charters Towers

Friday 4 and Saturday 5 June 2021

Venue H M Clarke Saleyards & Equestrian Centre

Time Friday 2-4pm, Saturday 9-11am & 1-3pm

Friday session

Welcome
Barb Bishop - MLA BeefUp Forum Coordinator
Michael Lyons – NQBRC Chair

MLA
- **Update 2021** *Jason Strong Managing Director MLA*

Perspective on Successful Beef Production
- **Current Generation Perspective** *Russell Lethbridge Werrington Cattle Company*
- **NextGen Perspective** *Advancing Beef Leaders*

Friday night

Northern Beef Producers Expo Presentation Dinner – ticket purchase Presentation Dinner - Northern Beef Producers Expo (nbpe.com.au)

Saturday morning session

Welcome

Growing and producing protein
- **Grass, live weight & dollars** *Dr Geoffry Fordyce Centre for Animal Science, QAAFI, UQ*

Saturday morning session (continued)
- **Pasture options and management fundamentals** *Kendrick Cox Department of Agriculture and Fisheries Qld*
- **Feedbase options for extensive beef production** *Fran Lyons Basalt Creek*
- **NB2 project** *Lee Fitzpatrick Chair NB2 Management Committee*

Saturday afternoon session

Welcome

Agtech
- **Pain management** *Melissa Wooderson Centre for Animal Science, QAAFI, UQ*
- **Research update genomics** *Professor Ben Hayes, Centre for Animal Science, QAAFI, UQ*
  Tail hair testing for resilience on dry season feed *Associate Professor Luis Prada e Silva Centre for Animal Science, QAAFI, UQ*
- **Agtech in northern production** *Emily Corbett, John McLaughlin & Kelly Bethel*

Cost: Producers free; Non-Producers $50

RSVP: 31 May 2021

Contact: Barbara Bishop - 0408 999 009 - barbara@barbarabishop.com.au

CLICK HERE TO REGISTER

PRECISION BEEF

Improving breeding, feed efficiency, production and beef flavours

Wednesday 5 May 2021, 10am to 11:30am in the Paterson room, Rockhampton State High School

10:00 SEMINAR WELCOME
Prof Ben Hayes - Director, Centre for Animal Science, QAAFI at The University of Queensland
10:05 NEXT GEN PHD BEEF RESEARCH ON THE HORIZON
10:30 PRECISION BEEF RESEARCH
Prof Ben Hayes - Using genomics to more accurately select the most fertile bulls and heifers
A/Prof Luis Prada e Silva - Improving feed efficiency by measuring nitrogen in tail hair
Prof Mary Fletcher - Managing the impacts of Pimelea poisoning on cattle
A/Prof Heather Smyth - Flavour communicate your point of difference
11:00 INDUSTRY PANEL DISCUSSION
Russell Lethbridge, Werrington Cattle Company and MLA non-executive Director
Shannon Speight, Black Box Co, CEO
Bim Struss, Agforce, Regional Director Southern Inlands Qld
Hugh Killen, Australian Agricultural Company Ltd, CEO
11:30 SEMINAR CONCLUDES

MODERATOR MC

Jon Condon, Beef Central

As one of Australia's most experienced and respected agricultural journalists, Jon Condon has been part of the fabric of the nation's beef industry for his entire life. For 40 years he has specialised in reporting on the red meat and livestock industries, earning the trust and confidence of key stakeholders across the industry and developing a reputation for accurate, credible, informed and informative reporting.

RESEARCH PRESENTERS

Experts will share how research applied across the supply chain improves the precision of management, breeding, production and product quality.

Professor Ben Hayes

Prof Hayes is a world genomics expert and is the co-inventor of genomic prediction for traits in dairy and beef cattle. Ben has extensive research experience in genetic improvement of livestock, crop, pasture and aquaculture species, with a focus on integration of genomic information into breeding programs. He is also a member of the National Livestock Genetics Consortium Taskforce.

Professor Mary Fletcher

Prof Mary Fletcher is a natural product organic chemist and leads the Natural Toxin group within the Centre for QAAFI. Mary's current work focuses on the identification and analysis of natural toxins and other bioactives in a range of plants, fungi and agricultural products. Such toxins and bioactives can affect both human and animal health posing risks to livestock production, food safety and market access.

Associate Professor Luis Prada e Silva

A/Prof Luis Prada e Silva, is a leader in the area of ruminant nutrition. Luis brings perspective from the world's largest producer of beef, Brazil, where he had a previous appointment at the Universidade de Sao Paulo. Luis' has worked with different disciplines such as ruminant nutrition, ruminant physiology, rumen microbiology, ruminant reproduction, forage management, molecular biology, and economics of cattle production systems to improve cattle productivity.

Associate Professor Heather Smyth

A/Prof Smyth is a flavour chemist and sensory scientist who has been working with premium food and beverage products for the past twenty years. A/Prof Smyth has a special interest in describing and articulating food quality, understanding regional flavours of locally grown produce, and modelling food flavour and textural properties using instrumental measurements.

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

Queensland Alliance for Agriculture and Food Innovation

By Catherine Norwood

Identifying traits in cattle that allow them to thrive in challenging environments is the aim of new research to assist Australia's northern cattle producers.

Tail-hair samples routinely used for DNA testing in cattle are also providing valuable information about the ability of Brahman cattle to survive on the low-quality pastures typical in Australia's northern regions.

Dr Luis Prada e Silva, Senior Research Fellow, Centre for Animal Science, The Queensland Alliance for Agriculture and Food Innovation (QAAFI)

Being able to identify this adaptability trait is expected to help northern producers identify bulls that will produce offspring better suited to the local environment.

Livestock scientist with The University of Queensland, Dr Luis Prada e Silva (https://qaafi.uq.edu.au/profile/2311/luis-prada-e-silva), is investigating the ability of animals to adapt to pasture-based diets, which are often low in protein, as part of efforts to improve animal performance.

His project is a collaboration with the Department of Agriculture and Fisheries, The University of Queensland and Meat and Livestock Australia Donor Company.

A key trait that Dr Prada e Silva is focusing on is the ability of cattle to recycle nitrogen when challenged by low-protein diets.

Selecting nitrogen efficiency at the crushside

Shan Goodwin
@shangoodwinbeef

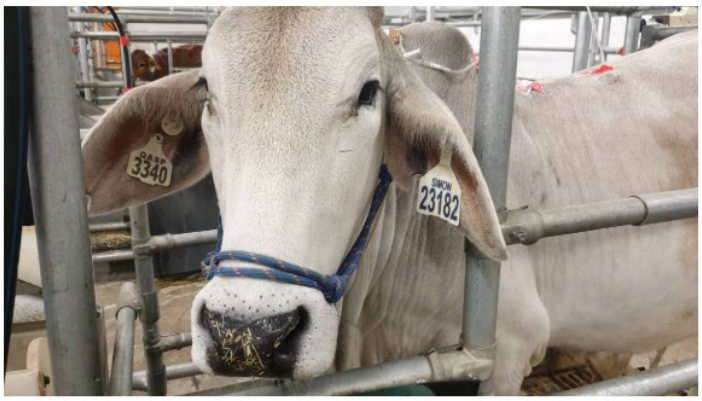
1 Feb 2020, 6:30 a.m. Beef

RESEARCH is showing enormous variance in the ability of northern cattle to preserve nitrogen where tropical rangeland diets are lacking in protein.

Animal production scientists from the University of Queensland have also come up with a forensic tool to make it easier to detect animals that are more nitrogen efficient at the crushside.

It's exciting work that has the potential to significantly boost productivity in northern herds during prolonged dry seasons.

Dr Luis Prada e Silva is leading the work looking into nitrogen recycling as a determinant for feed efficiency in Bos indicus cattle and while trials are still underway, answers have already become evident.



Researchers at Gatton have recorded the percentage of nitrogen lost in the

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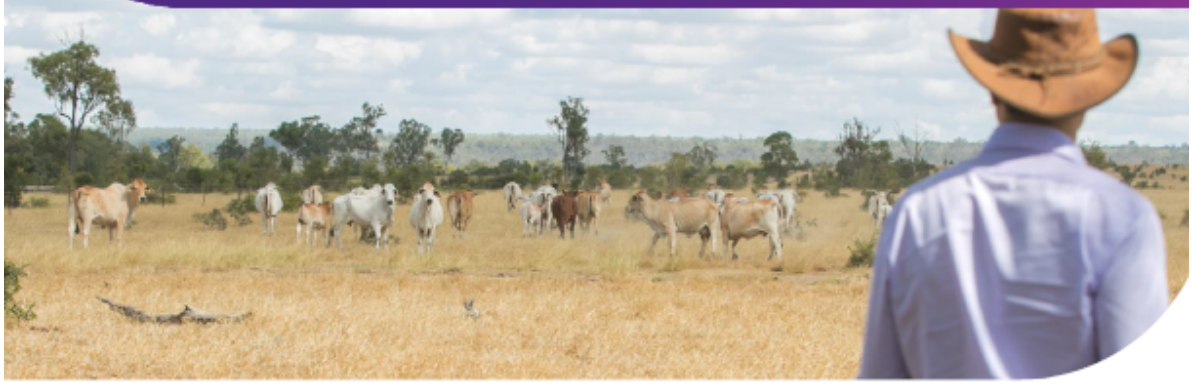


A practical tool to select for performance in harsh environments

Cattle performance on low protein diets can be estimated using ^{15}N stable isotopic ratio in tail hair.

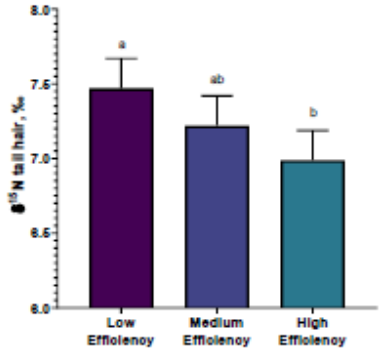
Cattle are amazing animals that can survive and perform in very harsh environments, especially the tropically-adapted breeds. When exposed to a low-protein diet, hot conditions, and water deprivation, adapted cattle will decrease urine losses and increase nitrogen recycling back to the rumen. On low protein diets, nitrogen recycling can provide 2-times more nitrogen to the rumen microbes than the diet.

There is huge variation of efficiency in a cattle cohort. A recent experiment at UQ with 90 Brahman steers demonstrated that Feed Conversion Ratio varied from 5 up to 12 kg of feed per kg of gain (140% variation!).

Also, more efficient cattle will lose less nitrogen in the urine, recycling the available nitrogen back to the rumen to produce more protein from the same poor-quality forage.

This discovery allowed us to develop a practical way to detect more efficient cattle. A more efficient animal will lose less nitrogen in the urine, changing the ^{15}N concentration in body proteins. Therefore, more efficient steers, the ones that were losing less nitrogen in the urine, had less ^{15}N on the tail hair.

An animal that is more efficient in harsh conditions will retain more N resulting in improved growth efficiency.



More efficient animals have less ^{15}N on the tail hair

Queensland Alliance for Agriculture and Food Innovation (QAAFI)

qaafi.uq.edu.au

CRICOS Provider Number 00025B

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Queensland Government

QAAFI is a research institute at The University of Queensland supported by the Queensland Government via the Queensland Department of Agriculture and Fisheries.

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QAAFI
Queensland Alliance for Agriculture and Food Innovation

Using ^{15}N isotopes in tail hair to select for performance

This research is co-funded with Meat and Livestock Australia and Queensland DAF.

A practical on-farm tool

The development of a practical diagnostic tool using ^{15}N stable isotopes in tail hair has a range of benefits

- An easy to collect on-farm diagnostic tool to estimate feed efficiency in cattle
- Practical way to identify efficient sires and cows to improve performance of the herd
- Upcoming research into the interaction between urea supplementation and N recycling opens the possibility of targeted supplementation to more or less efficient animals reducing supplementation costs and mitigating environmental impact
- Can be used in conjunction with existing genotypic testing
- This information can be used to improve growth and reproductive performance in the herd

Researcher Profile

Associate Professor Luis Prada e Silva

Principal Research Fellow
Centre for Animal Science, QAAFI

Assoc Prof Luis Prada e Silva is a leader in the area of ruminant nutrition. Luis brings perspective from the world's largest producer of beef, Brazil, where he had a previous appointment at the University of Sao Paulo. Luis' has worked with different disciplines such as ruminant nutrition, ruminant physiology, rumen microbiology, ruminant reproduction, forage management, molecular biology, and economics of cattle production systems to improve cattle productivity.

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Queensland Government

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