Final report

BeefLinks: "DietID" Feedbase mapping to raise productivity of cattle

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Abstract

The composition and nutritional characteristics of WA diverse and variable northern rangeland pastures are not well known, which in turn is a major driver of beef cattle productivity in WA rangelands. This project aimed to identify where and what cattle in WA rangelands graze and how this impacts their productivity. Grazing locations and spatial assessment of the cattle movement were estimated by georeferencing cattle using GPS-enabled devices. The grazing 'signatures' were obtained from plant DNA detected in animal faeces by DNA metabarcoding, and this information was then integrated with vegetation and plant nutritional profiles, to understand what makes up the grazing palate for beef cattle in WA rangelands. All of these inputs (grazing location, plant data and presence in faeces) were then linked to animal productivity parameters (i.e. live weight and live weight gain gain) towards developing better grazing management practices that improve production, deliver a more consistent supply of animals, and improve rangeland health.

The project was impacted by Covid-19 pandemic, restrictions on regional travel in WA, cyclone, flooding, pastoral access, and staffing issues. However, significant progress was made. In this project, a comprehensive approach was taken - from establishing a list of key plants species to be nutritionally mapped, cattle location, grazing patterns, diet selection to an overall effect on animal productivity has been have developed, applied and validated. A body of knowledge was generated of where and what beef cattle graze, and how it may affect the productivity data in the WA rangelands. It was found that there is a great spectrum of nutritive plant profiles (nutritive values, NV) in WA rangelands. It was also confirmed that faecal plant DNA (fDNA) metabarcoding can be used to obtain more precise information on what animals are actually grazing. It was detected where animals are grazing, and then from the land vegetation profiles and fDNA profiles, grazing plants and grazing palates identified. It was also revealed which attributes (vegetation profiles, NV, diet selection) may potentially improve animal production. This project provided an approach and methodology that can be now applied across different regions, landscapes, plant mosaics, as well as over longer period of time. There was also engagement with the key grower groups and producers in the region.

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Executive summary

1. Background

Cattle and grazing in rangelands

The Western Australian rangelands comprise of northern grasslands (Kimberley and Pilbara) and the southern rangelands, characterised by shrublands (Gascoyne, Murchison, Goldfields–Nullarbor). These are the main beef cattle production regions in the state, with land used for beef production estimated at 33M ha, and over 1 million animals at stocking rates of 1–3 cattle/km^2. Cattle in the pastoral industry are raised free-range, on native pastures and under natural conditions. The profitability of the northern WA beef industry is therefore driven by the nutritional attributes of the plants and what cattle choose to eat.

However, there is significant lack of information on the diet quality and the grazing profiles and palates. There are also significant gaps in understanding how to best (sustainably) utilize the feedbase when considering feed supply and type, rangeland mosaics and land types, any feed supplement requirement, and overall animal productivity and performance. There is evidence that cattle from WA rangelands entering the domestic and international supply chains are reflecting inadequate access to feed supply, poor in-paddock utilisation of feed and low feed conversation efficiencies. This poses a significant threat to the value of the supply chain. The costs of such production incur inefficiencies and costs manifested in pastoral businesses, at backgrounding or feedlotting, or during processing. A long-standing problem identified by cattle producers in the WA rangelands and in extensive pastoral systems is the lack of knowledge on nutritional status of grazing cattle and how best to influence it, particularly through grazing land management.

In the rangeland setting, the diet is not fixed over time and or to a specific grazing area, which poses a significant issue. As animals are in essence free ranging, their grazing palate changes daily. Animals in these production systems graze across diverse locations, characterised by different vegetation, topography and variable seasonal conditions and pasture quality. While animals are selective in what they graze, it is not known what they choose to eat, and if the plant selections made by individual animals always provides the optimal nutritional return. The nutritional characteristics of the plants are also prone to change, and little is known of these eco-geographical variations that may influence the NV of plants that cattle consume. Consequently, there are large differences in body condition and live weight (LW) and LW gain (LWG) in rangeland cattle of similar ages.

Understanding the factors that drive the efficient animal growth and utilisation of feed in the northern rangelands, is a key factor in the supply chain. The targets also include increased animal performance (feed to meat, fertility, time to turn off or greater efficiencies in feedlots), the ease of logistical management within the north-south supply chain, and reduced carbon footprint of production with concurrent enhanced market demand for products.

Another target is reducing the environmental impact of the beef industry, which include sustainable grazing, rangeland health and reduction of carbon (enteric methane) emissions. Identifying antimethanogenic plants in the grazing palate, as well as optimising animal production can help reduce enteric methane production and intensity from rangeland cattle. Further, cattle can affect rangeland health because certain plant species that are preferred by livestock may decrease through overgrazing. Knowing what animal graze and how to protect and better manage the vital natural resources will also help reduce environmental impact of livestock in the rangelands.

Methods to investigate rangeland grazing, diet selection and quality

Investigations around cattle grazing in rangelands are scarce and are limited to ad-hoc observations and anecdotal evidence of what is eaten by livestock. Current prediction of rangeland condition is based on the assessments made at monitoring sites during inspections. It is still relying on methodology from 1980's, where the assessment of rangeland cattle diet relies on visual rating of ground cover or remotely sensing and interpretation of the perceived grazing plants, what is palatable and what is not (DPIRD, 2019). The faecal near infra-red spectroscopy (F.NIRS) has a value predicting digestibility, crude protein, estimated non-grass component and predicted ADG of the diet of cattle grazing tropical pastures (Dixon & Coates, 2009). While a wide range of tropical grassland diets and animal house experiments were used to develop predictions of F.NIRS, these are not representative of WA rangeland plants.

Whilst these approaches provided useful information, the advances in research methodologies now allow more precise and accurate measurements of cattle grazing. With the development of new methodologies such as the global positioning system (GPS), animal tracking can be done with cattle fitted with the GPS-enabled devices (i.e. ear tags or collars) that can provide a real-time precise location of individual cattle – i.e. 'geo-referencing'(Gwatirisa et al., 2022). In parallel, advancement in molecular techniques have led to an emerging technology – faecal DNA (fDNA) metabarcoding that allows to reconstruct botanical composition of diets by detection of plant DNA in the faeces from free-roaming animals (Kartzinel et al., 2015; Valentini et al., 2009). Advantages of this technique is that it is non-invasive and allow large sample sizes, and determination of diet botanical composition at the species level is quicker given the automation of reading numerous plant fDNA sequences at a single time (Pompanon et al., 2012). In a controlled pen study, it has been shown that it can be used to predict composition of forage-type diets (Scasta et al., 2019).

The fDNA technology can therefore be applied for a more detailed and precise information on rangeland cattle diets. While GPS tracking enables detection of the specific location of the animal to match that with feed found at that location, the fDNA metabarcoding can be representative of what plant species they are consuming. Finally, monitoring and detecting individual cattle liveweight (LW) and liveweight gain (LWG) are important for estimation of nutritional requirements and health management.

The main research questions addressed in this project were – where and what do rangeland cattle eat; and can the grazing, and hence animal productivity and the effect of grazing on environment be improved in rangelands. The attempt was made to integrate these components – GPS positioning, grazing and plant nutritional profile, fDNA profiles and animal growth parameters in order to answer these questions.

Hypothesis

Diet in rangeland cattle is influenced by grazing location and individual preference. The nutritive values of plants will also vary according to location and land type. Animal productivity (measured by LW/LWG) will be related to the profile of plants that they graze, i.e. high performing cattle will readily access plants with high nutritive values compared to low-performing animals that will consume those of lower nutrition value.

2. Objectives

The overall objective of this project was to obtain quantitative data on NV, diet selection and the grazing patterns of the cattle in WA rangelands and to assess the link between diet selection and cattle growth rates.

Specific objectives were:

1. Quantify the diversity and variability of pasture species in the WA rangeland feedbase that supports beef production (literature search and database)
2. Examine animal grazing locations (via animal GPS positioning)
3. Examine NV of plant species that make up northern WA rangelands (via wet chemistry and NIRs spectral analysis)
4. Evaluate diet preference/grazing plant selection (via fDNA metabarcoding),
5. Investigate animal performance (LW/LWG from physical or WOW weighing)
6. Correlate animal diet selection with animal productivity

3. Methodology

3.1 Overview

The methodology comprised of synthesis of existing databases, followed by collection and nutritive analyses of targeted plants; geo-referencing of cattle grazing using using GPS devices (Ceres tags or GPS collars); identification of diet selection using fDNA metabarcoding; and productivity data using physical LW measurements.

There was one desktop study, followed by laboratory study and three distinct animal experiments. The desktop study collated information on plants likely to be consumed by rangeland cattle in north and central WA rangelands and developed a database. This information is integrated in the database titled 'CN30 - DietID DATABASE' (Appendix 1). The database evaluated following identifiers: Life form, Soil type/habitat, Interim Biogeographic Regionalisation for Australia (IBRA) Region, Location (station), Desirability in pasture, Grazing value, Palatability, Nutritive value, Medicinal/Toxic to livestock. Plants to progress were selected, collected and examined the NV.

Animal Experiment 1 was conducted in central WA rangelands and aimed to validate fDNA metabarcoding methodology for rangeland cattle by associating the plants present in the landscape with plants present in the faeces. Experiment 2 then examined diet selection in the northern WA rangelands using fDNA metabarcoding. Finally, Experiment 3 attempted to link geo-referencing and diet selection with animal productivity.

Apart from experimental studies, the project also identified and engaged supply chain stakeholders in Kimberley, Pilbara, Gascoyne and Southern pastoral regions. The engagements occurred either directly visiting producer farms, or attending/presenting meetings and conferences.

Methodologies used for distribution and nutritive profiles of grazing plants, animal movements and grazing distribution, diet profile/selection, and animal productivity are described below.

3.2 Database on WA rangelands plants

The approach for creating the database followed some of the principles set and applied in the FFI/MLA Project Enrich for native fodder shrubs (FFI, 2011), as well as those recently outlined for native plants in rehabilitation scenarios for the Pilbara region (Durmic et al., 2021). This was a stepwise process that involved a combination of discussions with leading producers in each region through the relevant regional grower group, pastoralists, researchers and other experts, and a targeted literature review of scientific peer review publications and databases.

Information was obtained from relevant literature published in scientific journals, industry and government reports, herbarium and survey data, online resources, catalogues, published books and encyclopaedias, extension publications, postgraduate theses, as well as any public plant databases that can be accessed. This was combined and further refined in consultations with leading producers in the different regions, other project members, as well as agriculture and livestock experts who have experience in the targeted regions. All information related to the species was compiled within the database, followed by a critical analysis of the data and reporting on the findings. The information was sought on the WA Rangeland plants relevant to their i) habitat, distribution and frequency in the landscapes; ii) biological and agronomical properties of the plants, iii) documented use in the livestock and grazing value; iv) plant nutritive profiles and rumen fermentability data (including effect on methane); v) any reported bioactivity, toxicity or anti-nutritive factors/effects, and any adverse or beneficial effects on animal health.

The information was filtered for grazing potential after a search in the FloraBase (https://florabase.dpaw.wa.gov.au/) where parameters 'south-west' and 'found in desert only' were removed. A database was then prepared in Excel format, to enable searching and sorting functions. Within the five themes listed above, separate 'attributes' were assigned, and then each attribute will be populated with a numerical or a descriptive value, or information transformed to common denominators such as to YES/NO (i.e. for desirable in pasture) or LOW/MODERATE/HIGH (i.e. for fodder value). When the information was found only at the genus level, or for another species in the genus, the attribute was assigned to all species in the same genus, but marked in brackets and italics. Once the information was compiled, the data was analysed with scoring and weightings against criteria to separate which species are more likely to provide livestock production benefits as well as forage diversity.

3.3 Nutritive values of rangeland plants

Distribution of grazing plants were done by identifying plants in the field, at specified times and locations. The information was recorded in the Fulcrum platform (https://www.fulcrumapp.com/) before collecting voucher specimen if required. Plant samples were collected in this manner: after the plant was selected, catalogued and identified, and voucher specimens (if plants were not identified botanically) were collected where required and identified by botanists at the WA Herbarium. Following this, a sample for analyses was collected. A handful of leaves and small branches was taken by hand, placed in a bag and chilled before being frozen at -20^{o}C. Three field replicates from each plant species were collected as separate, individual plants, 5 m – 100 m apart. The plant samples were then freeze-dried and ground before being submitted for analyses. Plant species were analysed in lab assays, by conventional wet chemistry and NIRS analysis to quantify *in vitro* dry matter digestibility (DMD) and protein (nitrogen), fibre and mineral concentration, as per published protocols (Van Soest et al., 1991).

3.4 Cattle movements and grazing distribution

The locations and grazing patterns were documented by tracking the cattle using 'smart' ear tags fitted with GPS, Ceres Tags (Figure 1a, https://www.cerestag.com/) or Vence CattleRider^{TM} (Figure 1b, https://vence.io/). Briefly, each animal is fitted with a tag or a collar that communicates with a portal (laptop, mobile) via satellite and sends in real-time the exact GPS location of individual cattle. Ceres Tag can fit in the palm of a hand and weighs 32 g. The technology works using low earth orbit satellite machine to machine (M2M) and Internet of Things (IoT) technology that enables monitoring of animals. Once tagged with the Ceres Tag, user receives continuous data points of their locations which

can be used to make observations about grazing patterns or movements of the cattle. In addition to this, cattle movement data was obtained in Experiment 3 using GPS cattle collars. This was done in conjunction with Beeflinks MLA P.PSH.1306 Virtual Fencing Technology Experiment 2, and the complete detailed there. Briefly, CattleRider™ collars (Vence, US) were used in conjunction to radio telemetry masts. Each collar was fitted with a unique number GPS sensor. Animals were contained for 27 days by virtual fence in a medium-sized paddock before sampling.

Figure 1a. Design of Ceres Tags, size and animal fitted with tag

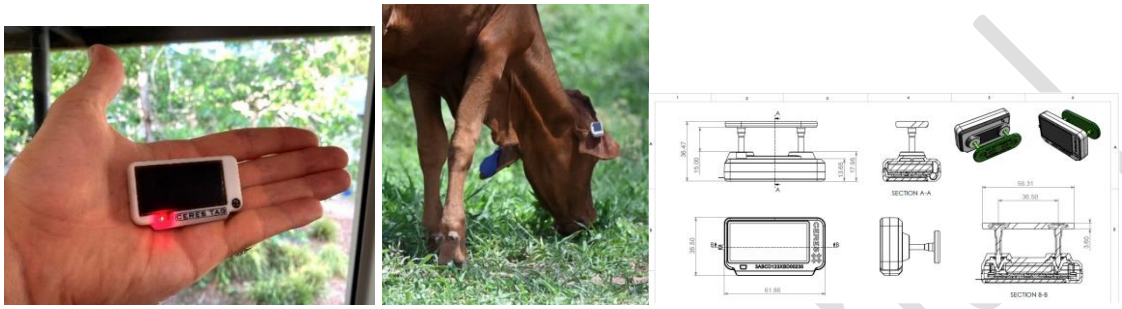


Figure 1b. Design of CattleRiderTM collars fitted on cattle

Mapipedia (https://mapipedia.com/) was used for geo-referencing of Ceres ear tag fitted cattle, while animals fitted with Vence CattleRider collars were tracked on HerdManager platform (https://aus.vence.io/#/main/management)

3.5 fDNA metabarcoding

Faecal matter collected off the ground (Experiment 1), or from the rectum of individual animal (Experiment 2 and 3) were subjected to fDNA metabarcoding to determine the plant composition of the diet, as per published methods for fDNA metabarcoding (Scasta et al., 2019). Briefly, up to 50 g of faecal material was collected off ground or per rectum in sterile DNA-se free vials and stored at -20^{o}C until analyses. The samples were analysed either in Argaly laboratory, France (https://www.argaly.com/home, Experiment 1), or eDNA Frontiers laboratory, Western Australia (Experiment 2 and 3, http://www.ednafrontiers.com/about/). The details of their methodologies are listed in Appendix 2.

3.6 Animal productivity data

Animal productivity parameters were based on measuring LW at the beginning and at the end of an extended grazing period and calculating LWG.

3.6 Data integration and analysis

Tools within Mapipedia platforms allowed creating and defining geo-references (i.e. paddocks, landscapes), and the addition of important features such as fences and water points. The movement of cattle based on the GPS data was analysed to produce desired outcomes such as 'heat maps' that show grazing location/movements of individual or groups of animals over time. The diet selection and grazing location was then correlated with animal productivity data for individual animals in the observed herd.

4. Results

4.1 Desktop and laboratory study - developing database and NV of WA rangeland plants

Introduction

Plant species with desirable nutritive profiles can be identified using chemical analyses of plant material for nutritive characteristics and an *in vitro* fermentation technique to examine fermentative properties of the plant, where microbial end-products such as total microbial gas and volatile fatty acid (VFA) production are used as an indirect indication of plant digestibility in the rumen. The same technique can also be used as an indication of the bioactivity of the plant, including likely anti-methanogenic potential. This approach was successfully applied to identify plants with nutritive and anti-methanogenic potential amongst Australian native shrubs (Durmic et al., 2010), alternative forages for dairy cattle in Victoria (Durmic et al., 2016) and beef production systems in northern Australia (Durmic et al., 2017).

The aim of this experiment was to identify plants likely to be consumed by rangeland and to examine their nutritive and fermentative profiles. Our hypothesis was that plant species in the WA Rangelands will vary in their chemical composition.

Materials and methods

Database was compiled based on approach described earlier. Over 300 documents (journals and other publications, databases and reports) were examined, and informal interviews with at least ten pastoralists conducted. Plants were collected from eight stations across the Pilbara, Gascoyne, Murchison and Goldfield regions of Northern Western Australia. The nutritive values were determined by NIRS predictions of diet quality as described earlier.

Results

1. Database

A comprehensive 'CN30-DietID DATABASE' (Appendix 1) was compiled. During the data exploration phase, it became evident that there is information about plants that are grazed by cattle in the region, and engagement with the key grower groups and producers within those regions has been invaluable. The database is now populated with a total of 246 plant species, with 133 having a reported fodder potential.

2. Nutritive values

A total of 10-15 key species from each general region/station were selected and collected (Table 1). Whilst they were collected in similar part of the year, there was diversity in land profiles, soil types

and other attributes. The analysis was done for a total of 108 plant samples, comprising of 92 species (some repeated across stations).

There was a range of nutritive value in plants collected. There were 22 species that had DMD higher than 50 g/100g, and the highest DMD was recorded in species *Calotropsis procera, Cucumis melo, Swainsona formosa, Ptilotus exaltatus, Crotalaria cunninghamii, Eremophila forrestii, E. maculata subsp. brevifola, Ptilotus polakii* and *Dodonaea lobulata,* and consequently these species also had the highest ME (i.e. 10-12 MJ/kg DM). Over half of the species tested had crude protein (CP) greater than 80 g/kg DM, and those with the highest CP content (i.e. greater than 200 g/kg DM) included *Swainsona Formosa, Maireana spp.* and *Enchylaena tomentosa.* There were some marked differences between same plant species collected at different locations (stations). For example, *Triodia pungens* from one station had better NV profile to that from the other station. Similarly, *Setaria dielsii* from one station had greater CP to that from the othet, but the greatest variability across nutritive value variables was seen in 'marker' species *Ptilotus obovatus.*

Key finding: The nutritive value for 92 WA rangeland plant species reported. Nearly half of these species may qualify as having good/advantageous nutritive value profile.

Table 1: The nutritive value of plant species collected and analysed in P.PSH.1245

Plant species	Common name	Station	NDF	ADF	DMD	OM	ASH	CP	ME MJ/kg DM
			g/100 g DM						
Acacia acuminata	Jam	Challa	53	37	34	92	8	14	4
Acacia aneura	Mulga	Weebo	53	21	59	92	8	13	9
Acacia bivenosa	Two-nerved wattle	Bullara	23	21	61	87	13	13	9
Acacia colei	Cole's Wattle	Hamersley	36	33	44	95	5	12	6
Acacia craspedocarpa	Hop Mulga	Weebo	59	23	60	89	11	15	9
Acacia linophylla	Bowgada	Menangina	60	31	48	94	6	8	7
Acacia pruinocarpa	Black Gidgee	Hamersley	36	26	40	92	8	12	5
Acacia pyrifolia DC. var. pyrifolia	Kanji bush	Hamersley	52	42	39	96	4	12	5
Acacia tetragonophylla	Kurara	Bullara	31	29	52	95	5	15	7
Acacia tetragonophylla	Kurara	Weebo	50	39	34	95	5	7	5
Acacia tetragonophylla	Kurara	Wyloo	37	34	48	95	5	14	7
Acacia victoriae	Bardie bush	Bullara	31	24	53	92	8	16	7
Acacia xiphophylla	Snakewood	Bullara	36	32	56	95	5	13	8
Acacia xiphophylla	Snakewood	Challa	41	32	46	96	4	11	8
Acacia Citrinoviridus	River Jam	Hamersley	36	28	45	96	4	13	6
Acacia colletioides	Wait-a-while	Challa	35	28	42	95	5	12	6
Aerva javanica	Kapok Bush	Yarrie	58	44	52	94	6	8	7
Allocasuarina huegeliana	Sheoak	Challa	56	29	42	94	7	8	6
Alysicarpus muelleri	-	Yarrie	76	46	40	98	2	3	5
Astrebla elemoides	Hoop Mitchel Grass	Hamersley	87	54	29	97	3	3	3
Atriplex spp.	Saltbush	Menangina	64	34	44	92	8	13	7
Bothriochloa ewartiana	Desert Bluegrass	Wyloo	70	43	49	96	4	7	7
Calotropsis procera	Giant Milkweed	Yarrie	36	23	79	80	20	15	12
Canthium latifolium	Wild Lemon	Menangina	47	26	51	95	5	10	9
Cassia spp.	Cassia	Weebo	57	21	56	90	10	12	9
Cenchrus setiga	Birdwood Grass	Wyloo	57	40	51	97	3	8	7
Cenchrus setiga	Birdwood Grass	Yarrie	80	51	36	99	1	2	5

Cenchrus ciliaris	Buffel Grass	Bullara	68	40	44	95	5	5	6
Chrysopogon fallax	Ribbon Grass	Wyloo	62	41	46	97	3	6	6
Chrysopogon fallax	Ribbon Grass	Yarrie	82	52	31	100	0	4	4
Cleome/Arivela viscosa	Asian Spiderflower	Hamersley	53	40	52	89	11	11	7
Clerodendrum tomentosum var. lanceolotum	Pilbara Lolly Tree	Hamersley	38	24	65	87	13	17	9
Corchorus incanus subsp. incanus	Jute	Yarrie	54	39	42	94	6	8	5
Crotalaria cunninghamii	Green Birdflower	Yarrie	29	28	68	92	8	13	10
Cucumis melo	Wild cucumber	Wyloo	39	25	71	88	12	17	10
Cymbopogon ambiguus	Lemon Scented Grass	Weebo	76	41	39	90	10	5	5
Dactyloctenium radulans	Button Grass	Wyloo	54	31	57	91	9	11	8
Dicanthium sericeum subsp. humilus	Annual Bluegrass	Wyloo	58	38	50	94	6	6	7
Dodonaea lobulata	Dead Hop Bush	Menangina	46	22	66	93	7	12	12
Enchylaena tomentosa	Ruby Saltbush	Hamersley	34	20	61	83	17	21	9
Eragrostis xerophila	Roebourne Plains Grass	Hamersley	68	41	47	97	3	7	6
Eragrotis eriopoda	Woolly butt	Challa	67	34	46	91	9	6	6
Eremophila cuneifolia	Compact Eremophila	Hamersley	42	26	64	92	8	16	9
Eremophila forrestii	Wilcox Bush	Menangina	43	25	67	91	9	15	11
Eremophila fraseri	Turpentine Bush	Weebo	47	26	58	94	6	14	9
Eremophila fraseri	Turpentine Bush	Hamersley	41	29	64	87	13	11	9
Eremophila fraseri	Turpentine Bush	Hamersley	41	28	62	93	7	12	9
Eremophila glabra	Tar Bush	Menangina	36	26	59	94	6	12	9
Eremophila longifolia	Berrigan	Challa	41	32	43	93	7	12	7
Eremophila maculata subsp. Brevifola	Fuscia Bush	Bullara	14	22	67	94	6	14	10
Eremophila oldfieldii	Pixie Bush	Menangina	45	24	58	94	6	12	9
Eremophilla spp.	Eremophila	Weebo	48	29	56	94	6	11	9
Eriochloa procera	Spring Grass	Wyloo	54	36	51	94	6	7	7
Evolvulus alsinoides var. decumbens	Tropical Speedwell	Hamersley	56	42	57	91	9	9	8
Exocarpos aphyllus	Naked Lady	Weebo	47	30	47	95	6	8	8
Gomphrena canescens	Bachelors Buttons	Yarrie	65	46	44	95	5	4	6
Hakea preissii	Needlebush	Challa	65	38	39	95	5	7	5
Halea lorea	Cork tree	Bullara	62	52	39	100	0	6	5

Indigofera linifolia	Narrow-leafed Indigo	Yarrie	38	29	54	93	7	10	8
Ipomoea muelleri	Poison Morning Glory	Hamersley	38	29	62	90	10	12	9
Maireana glomerifolia	Ball Leaf Bluebush	Weebo	69	41	35	92	8	8	5
Maireana planifolia	Bluebush	Hamersley	38	23	58	84	16	17	8
Maireana pyramidata	Sago Bush	Weebo	58	34	41	92	8	8	6
Maireana spp.	Blue bush	Challa	31	-7	53	80	20	22	10
Mirbelia platylobioides	Mirbelia	Yarrie	45	35	60	94	6	14	9
Panicum effusum	Native Panic Grass, hairy Panic Grass	Wyloo	62	39	49	94	6	10	7
Poaceae spp.	Grass	Weebo	81	53	26	92	8	3	3
Portulaca oleracea	Pigweed, Pigface	Hamersley	30	29	64	86	14	14	9
Ptilotus aervoides	Mat Mulla Mulla	Hamersley	61	40	46	94	6	11	6
Ptilotus auriculifolius	Ear-leaved Mulla Mulla	Hamersley	48	35	55	89	11	15	8
Ptilotus clementii	Tassel Top Mulla Mulla	Hamersley	58	42	48	90	10	12	7
Ptilotus divaricatus	Climbing Mulla Mulla	Challa	66	36	35	91	10	10	6
Ptilotus exaltatus	Pink Mulla Mulla	Hamersley	45	32	69	81	19	14	10
Ptilotus helipteroides	Hairy Mulla Mulla	Hamersley	63	41	46	89	11	10	6
Ptilotus obovatus	Cotton bush	Bullara	63	39	41	92	8	10	5
Ptilotus obovatus	Cotton bush	Challa	64	35	40	92	8	7	6
Ptilotus obovatus	Cotton Bush	Hamersley	59	45	51	90	10	9	7
Ptilotus obovatus	Cotton bush	Hamersley	57	40	43	94	6	7	6
Ptilotus polakii	Gascoyne Mulla Mulla	Hamersley	50	30	67	91	9	8	10
Ptilotus rotundifolius	Royal Mulla Mulla	Hamersley	55	42	41	93	7	11	5
Ptilotus schwartzii	Horse Mulla Mulla	Challa	61	29	46	92	8	12	7
Rhagodia eremaea	Thorny saltbush	Bullara	44	28	60	94	6	18	9
Rhagodia eremaea	Tall Saltbush	Weebo	71	41	34	92	8	7	6
Rhagodia spp.	Rhagodia	Challa	65	34	51	91	9	15	8
Rhynchosia minima	Mardie Clover	Yarrie	46	35	49	92	8	11	7
Scaevola spinescens	Currant Bush	Hamersley	38	28	64	92	8	15	9
Sclerolaena cornishiana	Cartwheel Burr	Hamersley	53	32	53	93	7	10	7
Senna artemisioides subsp. oligophylla	Bloodbush	Hamersley	29	24	54	95	5	13	8
Senna notabilis	Cockroach Bush	Hamersley	37	32	52	89	11	13	7

Setaria dielsii	Diel's Pigeon Grass	Hamersley	68	41	49	94	6	7	7
Setaria dielsii	Diel's Pigeon Grass	Wyloo	58	38	49	94	6	10	7
Solanum lasiophyllum	Flannel bush	Challa	70	35	52	92	8	9	7
Solanum lasiophyllum	Flannel bush	Hamersley	56	39	54	88	12	13	8
Sporobolus australasicus	Fairy Grass	Yarrie	85	54	35	97	3	2	4
Swainsona formosa	Sturt's Desert Pea	Hamersley	32	26	71	89	11	22	11
Tecticornia doleiformis	Samphire	Weebo	58	25	44	92	8	12	9
Themeda sp. Hamersley (leaves)	Kangaroo Grass	Hamersley	69	39	48	96	4	6	7
Themeda sp. Hamersley (seedhead)	Kangaroo Grass	Hamersley	78	46	41	98	2	3	5
Triodia pungens	Soft spinifex	Bullara	66	39	47	94	6	8	6
Triodia pungens	Soft spinifex	Yarrie	77	49	42	97	3	4	6
Triodia wiseana (seedhead)	Hard Spinifex	Hamersley	93	63	23	100	0	1	2
Urochloa gilesii ssp. occidentalis	Summer Grass	Hamersley	72	46	44	93	7	7	6
Urochloa occidentalis	Signal Grass	Wyloo	58	37	52	93	7	11	7
Vachellia farnesiana	False Mimosa	Hamersley	45	33	61	90	10	15	9

4.2 Experiment 1 - Validation of fDNA metabarcoding in rangeland cattle

Introduction

Rangeland cattle in Australia graze freely and roam on open land in search for pasture of good quality. To date, investigations around cattle grazing in rangelands are scarce and are limited to ad-hoc observations and anecdotal evidence of what is eaten by livestock. Very little investigation was done to determine which plants and what quantities are consumed, and what are nutritive and fermentative properties of plants consumed. The fDNA metabarcoding has been developed for environmental studies (Taberlet et al., 2018; Taberlet et al., 2012) and the methodology has been applied in dietary studies in wild ruminants (Craine et al., 2015; Kartzinel et al., 2015). In recent years, it has been extended to nutritional studies in cattle to quantify geographical patterns of cattle diets in North America (Craine et al., 2016), or where animals were fed known rations of forage plants (Scasta et al., 2019). However, research in free-roaming rangeland cattle in Australia is lacking and validation of the fDNA metabarcoding technique is necessary.

This experiment aimed to compare 'perceived grazing species' in central WA rangelands to what cattle may actually be grazing based on fDNA metabarcoding results.

Materials and methods

Experiment was conducted in Oct 2020 at three stations in WA Central Rangelands. Representative plants were identified within a 1 km radius of water points and based on abundance in the landscape and information from the station managers. Assumption was made that plants around watering point will be representative of plants that animals commonly graze. Plants were identified and catalogued. A total of 27 (9 per station) pooled faecal samples off ground were collected for fDNA analysis and sent to Argaly laboratory in France.

Results

The perceived key grazing species at three stations are listed in Table 2. Using this approach, 39 species were identified.

Table 2: List of perceived grazing species at three stations

Station	Plant label	Species	Latin name
Challa	C1P1	Wait-a-while	*Acacia colletioides*
Challa	C1P10	Unknown perennial grass	(*Poaceae spp.*) CHAL
Challa	C1P12	Woolly butt	*Eragrostis eriopoda*
Challa	C1P13	Snakewood	*Acacia xiphophylla*
Challa	C1P16	Kurara	*Acacia tetragonophylla*
Challa	C1P19	Rhagodia	*Rhagodia spp.*
Challa	C1P20	Prince of Wales Feather	*Ptilotus polystachyus*
Challa	C1P4	Sago Bush	*Maireana pyramidata*
Challa	C1P7	Jam	*Acacia acuminata*
Challa	C2P1	Mulga	*Acacia aneura*
Challa	C2P10	Fine leaf mulga	*Acacia assimilis*
Challa	C2P15	Horse mulla mulla	*Ptilotus schwartzii*
Challa	C2P18	Sheoak	*Allocasuarina huegeliana*
Challa	C2P2	Golden Bluebush	*Maireana georgei*
Challa	C2P8	Berrigan	*Eremophila longifolia*
Challa	C3P1	Flannel Bush	*Solanum lasiophyllum*
Challa	C3P14	Unknown perennial grass	(*Poaceae spp.*) CHAL
Challa	C3P16	Unknown perennial grass	(*Poaceae spp.*) CHAL
Challa	C3P4	Cotton Bush	*Ptilotus obovatus*
Challa	C3P8	Needlebush	*Hakea preissii*
Menangina	M1P13	Tar Bush Poverty	(*Eremophila glabra or E. alternifolia*)
Menangina	M1P15	Unknown shrub	tba
Menangina	M1P7	Wilcox Shrub	*Eremophila forrestii*
Menangina	M2P12	Pixie bush	*Eremophila oldfieldii*
Menangina	M2P4	Hop mulga	*Acacia craspedocarpa*
Menangina	M2P5	Bowgada (also known as Wanya)	*Acacia ramulosa var. linophylla*
Menangina	M2P8	Unknown Poverty Shrub	(*Eremophila glabra or E. alternifolia*)
Menangina	M3P1	Bead Hop Bush	*Dodonaea lobulata*
Menangina	M3P14	Wild Lemon	*Psydrax latifolia*
Menangina	M3P8	Unknown shrub	tba
Weebo	W1P12	Naked Lady	*Exocarpos aphyllus*
Weebo	W1P19	Samphire	*Tecticornia doliiformis*
Weebo	W1P22	Unknown Grass	(*Poaceae spp.*) WEB2
Weebo	W1P27	Unknown Grass 2	(*Poaceae spp.*) WEB1
Weebo	W1P4	Ball Leaf Bluebush	*Maireana glomerifolia*
Weebo	W1P7	Unknown tree	tba
Weebo	W2P5	Turpentine Bush	*Eremophila fraseri*
Weebo	W3P20	Silver cassia	*Senna artemisioides subsp. × artemisioides*
Weebo	W3P3	Thorny Saltbush, Tall Saltbush	*Rhagodia eremaea*

However, the fDNA analyses revealed a total of 76 plant species (Tables 3-5). At Challa, there were 64 plant species, at Weboo 51 and at Menangina 46; some of which overlapped between the stations, others (i.e. *Sweetia fruticosa*) that were unique to the station. However, upon more detailed analyses, many plants were found that are not (commonly) found in Australia, and only 38 of these are reported in WA Rangelands. There was significant individual variability between plant species (diversity and abundance of fDNA) between individual cattle faecal samples.

Table 3: Diversity and abundance (number of sequence reads) of plant species fDNA in 9 cattle dung specimens collected at Challa. In green – found in Australia

Scientific name	**Cattle id**								
	C1F1	**C1F2**	**C1F3**	**C2F1**	**C2F2**	**C2F3**	**C3F1**	**C3F2**	**C3F3**
Acacia sp.	187			2736	9564	4337	19634	19225	3703
Acacia acuminata			33121			2344		927	
Acacia aneura				9979		4781	3186	1975	1541
Acacia cuspidifolia	6592	31538	500			4932	7022		7849

Acacia tetragonophylla							1442		
Acacieae			2346			2344		927	
Amaranthaceae	505								
Aristida		185							
Asclepiadoideae		869							
Asteraceae		829							321
Atriplex			131			291	505		
Bassia eriophora									318
Brassiceae							682		
Caesalpinioideae	1152	8427	9813	3574	11114	5714	20554	24194	7207
Cardiochlamyeae						2429		3638	
Carex					765				
Caryophyllales	5746								
Chenopodiaceae					207		1452		
Chloridoideae									
Citrus	905	12002		4211			929		1738
Dodonaeaeae								1019	
Eleusininae									
Eremophila	4830		5821	3701		8169	12296	9145	2670
Eucalyptus longifolia			696					1019	
eudicotyledons	31857	4767	24531	27732	28242	29123	5729	13766	22930
Euphorbia		3468	2750	707				1389	1917
Euphorbia tirucalli								2691	
Euphorbiaceae		412	194						
Exocarpos sparteus								2691	
Fabaceae		425	33244						224
fabids		829	629	748	1437	1104		259	321
Faidherbia albida		187							
Heliantheae alliance			629	748	779	1104			
Indigofera tinctoria								1207	716
Lamiales			191				587	119	
lamiids	109			103		140	103		
Lessertia frutescens					658				
Macrosolen	275								
Macrosolen tricolor			2056			337	875		
Magnoliophyta		134	690	159	331	262	105	109	372
Maireana	36257	4901	22498	29847	30169	30498	6012	14132	24435
Malvaceae	22150	13117		6738	3185	26005	13952	22155	4472
Malvales		117		4800			1685		
Mesangiospermae	5439		1031	2211	831	2004	278	257	712
mimosoid clade	7557	34022	500	10110	1550	9955	11128	4348	9963
Nepetoideae								1349	
Nyctaginaceae							976		
Nyctanthes arbor-tristis							170		
PACMAD clade							2674		
Papilionoideae		1451							
Pentapetalae	23605	2999		7582	3185	25852	14708	5506	2734
Pentapetalae	6081	898	3927	3598		5507	10461	3924	3327
Poaceae								234	297
Poales								234	297
Proteaceae									
Ptilotus	6251						976		
Rhagodia eremaea			131			291	505		
rosids						153	682	16275	
Sapindaceae			696						
Solanaceae	2273								
Solanum lasiophyllum	2273								
Sweetia fruticosa			293						
Tecticornia lepidosperma					207		1452		

Table 4: Diversity and abundance (number of sequence reads) of plant species fDNA in 9 cattle dung specimens collected at Menangina. In green – found in Australia

Scientific name	Cattle id								
	M1F1	M1F2	M1F3	M2F1	M2F2	M2F3	M3F1	M3F2	M3F3
Acacia sp.	521		7566	13789	49515	22769	14506	14024	5473
Acacia acuminata						4267			5600
Acacia aneura				553	538	132			2800
Acacia cuspidifolia	1212		7564		2139	1422			
Acacieae						805			265
Asclepiadoideae							423		
Asteraceae	111		135						
Atriplex				521		278	788		
Bassia eriophora				123					
Caesalpinioideae	1058		8261	15421	57650	25505	16067	16334	5985
Citrus							3436	33456	8746
Dodonaeaeae						122	446		
Eremophila	8943		699	310	3396	2207	7980	18152	5396
Eucalyptus longifolia						122	446		
eudicotyledons	815		3873	6429	12434	9011	12526	22727	18888
Euphorbia									
Euphorbia tirucalli					1805	1319	4725	2236	15380
Exocarpos sparteus						922			
Fabaceae				295	449	3697			5335
fabids	12611		41684	5882	6689	3627			171
Frankenia	38528	27738	26206	27518		5329	2499		
Gentianales							3436	32736	8746
Hakea preissii					7426	1217			
Heliantheae alliance	11477		41275	5316	6689	3396			
Hypobathrum sp. SH-2010								126	
Lamiales	245				307	171	208	134	240
lamiids								583	
Lorantheae					155	367			
Magnoliophyta			107	119	699	431	172		361
Maireana	38221	27738	29465	33820	13732	15356	15664	24208	21811
Malvaceae					1106		1251		1456
Mesangiospermae					109				
Mesangiospermae	2155		513	627	2096	692	759	1481	2674
mimosoid clade	1212		7691	1578	9536	3795	1454	2310	2957
Nyctanthes arbor-tristis							108		
Pentapetalae	129		257	118	324		715		
Pentapetalae	7221		845	310	3441	2053	7664	18029	5156
Picea abies					196				
Proteaceae					5818	1217			
Quillaja saponaria									112
Rhagodia eremaea				521		278	788		
rosids	111		281		782		1251		1456

Santalum					1805	397	4832	2236	15682
Solanaceae			895						
Solanum lasiophyllum			895						
Spermatophyta					109				

Table 5: Diversity and abundance (number of sequence reads) of plant species fDNA in 9 cattle dung specimens collected at Weboo. In green – found in Australia

Scientific name	**Cattle id**								
	W1F1	**W1F2**	**W1F3**	**W2F1**	**W2F2**	**W2F3**	**W3F1**	**W3F2**	**W3F3**
Acacia sp.	19876	24235	30021	42705	35534	36818	24510	16517	64007
Acacia acuminata									
Acacia aneura		458	316	390	1573				
Acacia cuspidifolia				3763	352	4977		666	876
Asteraceae		464		178		118			
Atriplex				2065					316
Caesalpinioideae	22407	26648	33201	47478	39004	41997	26742	17975	74199
Caryophyllales					1085	187			
Chenopodiaceae	1548	910	1446	894				130	520
Chloridoideae		142							
Dodonaeaeae									
Eleusininae		10299							
Eremophila									216
Eucalyptus longifolia									
eudicotyledons	1117	4402	717	2400	9300	7294	6805	4577	7188
Euphorbia									
Euphorbia tirucalli	2766	710	633		775	1220	1789		3628
Euphorbiaceae									
Exocarpos sparteus	2766		633		775	1220	1789		3628
Fabaceae	495		684		142				153
fabids	19015	29061	12167	5137	994	20684	13830	13503	2209
Faidherbia albida									
Frankenia						234		339	
Gentianales									
Hakea preissii								211	
Heliantheae alliance	19015	29024	12167	4959	994	20566	13830	13503	2209
Hypobathrum sp. SH-2010									
Indigofera tinctoria									
Lamiales									
lamiids									
Lessertia frutescens									
Lorantheae									
Macrosolen									
Macrosolen tricolor									216

Magnoliophyta		150		158	452	345	331	239	465
Maireana	1117	4552	1642	2841	10486	8157	8144	5338	8211
Mesangiospermae		11244							
Mesangiospermae	356	495	308	120	389	768	794	497	405
mimosoid clade	1241	1803	1958	8514	5160	8680	1268	1373	10698
Musa				283					
PACMAD clade		803							
Pentapetalae	439	573	1471	292	438	992	1178	437	370
Proteaceae								211	
Ptilotus					1085	187			
Rhagodia eremaea									316
rosids		427							
Salicornia subg. Salicornia	8309	860	2814	580		403	947	2420	
Santalum		710							
Solanaceae	130								
Solanum lasiophyllum	130								
Tecticornia lepidosperma	9857	1770	4260	1474		403	947	2550	520

When comparing perceived grazing species to those found by fDNA metabrcoding, there was more species detected by later. Also, there was discrepancy between the species detected, with only two species were found as total match by both methodologies, and 8 were 'potential/partial match' (Table 6).

Table 6: Comparison between perceived grazing species and those identified by fDNA metabarcoding at three stations. Note – plants not found in Australia removed

Perceived grazing species	Plant fDNA detected
Acacia aneura	
Acacia sp.	
Acacia xiphophylla	*Acacia sp.*
Acacia acuminata	*Acacia sp.*
Acacia colletioides	*Acacia sp.*
Acacia tetragonophylla	*Acacia tetragonophylla*
Allocasuarina sp.	
Eragrostis eriopoda	*Eragrostis sp.*
Eremophila longifolia	
Hakea preissii	
Maireana georgei	
Maireana pyramidata	
Poaceae spp.	*Poaceae spp.*
Ptilotus obovatus	
Ptilotus sp.	
Ptilotus polystachyus	
Rhagodia spp.	
Solanum lasifolium	*Solanum lasiophyllum*

Agrostidinae

Acacia axillaris

Acacia clelandii

Acacia crassicarpa

Acacia dawsonii

Acacia floribunda

Acacia pubirhachis

Acacia sp.

Acacia victoriae

Andropogoneae

Aristida

Austrostipa

Aveninae

Cassia sp. AMT-2013

Cenchrinae

Cenchrus ciliaris

Cymbopogon caesius

Cymbopogon sp.

Elymus

Enneapogon asperatus

Enteropogon

Eragrostis desertorum

Eragrostis dielsii

Eragrostis kennedyae

Eragrostis lanicaulis

Eragrostis pergracilis

Eragrostis sp.

Eriachne

Eriachne helmsii

Eriachne pulchella

Hordeinae

Hordeum vulgare

Hyparrhenia anamesa

Leymus karelinii

Leymus sp.

Monachather paradoxus

PACMAD clade

Paspalidium geminatum

Pooideae

Setaria

Sorghastrum secundum

Sporobolus actinocladus

Stipeae

Thyridolepis
Thyridolepis multiculmis
Tripogonella
Triticeae

Discussion

It was found that cattle ate a greater diversity of species than the ones initially listed, and that species thought to be grazed were not found in the fDNA samples. Previous work using fDNA metabarcoding has shown notable surprises in diet botanical composition of free-roaming herbivores (Craine et al., 2015), and it is likely that cattle in WA Rangelands also have greater plant palate than originally thought. Degradation of fDNA in the sample off-ground, in the environment may limits the interpretation, particularly in hot regions like WA Rangelands. Despite these drawbacks, fDNA still demonstrated the potential to determine plant species grazed and their relative abundance in the diet. It is also possible that the plant identified as key grazing species do not fully represent the diet of the cattle, and that the most desirable plants to cattle would have been over-grazed around the water point and be in low numbers or non-existent at time of sampling. These plants would exist in higher densities with increasing distance from the water point as the cattle move towards the outer regions of their grazing range, so further studies are needed to expand search and identify such plants.

Key finding: The fDNA can detect greater range of grazing species in WA rangelands. Many additional species that have not been previously reported as grazed by cattle have been identified using this approach. Using off-ground faecal samples and foreign lab may limit the accuracy.

4.3 Experiment 2 – Diet selection in northern WA rangelands

Introduction

In Experiment 2, it was confirmed that fDNA metabarcoding may be a useful tool when identifying diet selection of WA Rangeland cattle. While we identified a range of grazing species is Central WA Rangelands, similar information is lacking for northern WA Rangelands. Further, we identified limitation when we used faecal samples off-ground and a laboratory outside of Australia.

The aim of this experiment was to obtain preliminary data in contrasting grazing environment (Central vs North); obtain individual cattle samples for DNA metabarcoding; validate local lab and regional and database for fDNA metabarcoding analysis; compare 'perceived grazing' to what cattle may actually be grazing based on DNA metabarcoding results; obtain some preliminary relationship between individual weights and the individual diet.

Materials and methods

The Experiment 2 was mirroring the work of Experiment 1 in the northern WA rangelands, but trying to overcome these limitations by collecting individual faecal samples obtained from rectum, and using local WA lab and regional plant database for fDNA metabarcoding. In addition, data of cattle movement were obtained using GPS-enabled cattle collars.

The DietID Experiment 3 was conducted in conjunction with the VFT Experiment 2 in P.PSH.1306. The experiment was conducted in May 2021 at Rio Tinto Hamersley station in WA North Rangelands, where cattle were grazing in a paddock for 1 month and GPS location tracked by GPS collars. A total of 10 individual faecal samples directly from cow rectum were collected, their start and finish weights and their GPS location.

The fDNA samples were analysed at the eDNA frontiers at Curtin University, https://research.curtin.edu.au/scieng/edna-frontiers/) and using regional plant sequence database (Plastids of the Pilbara, https://pilbseq.dbca.wa.gov.au/), in addition to GenBank.

Results

A total of 52 plant species were identified in the faecal sample of 10 cattle at Hamersley (Table 7). The diet composition in individual cattle varied considerably; for example in cattle #21, *Pluchea* and *Cullen* species seem to be predominant, while cow #79 seem to have more of *Vachellia* in its diet. There was again a discrepancy between what is perceived as main grazing plants to what animals were actually grazing based on fDNA metabarcoding results, and only two species i.e. *Vachellia farnesiana* and *Portulaca oleracea* were identified by both methodologies.

Table 7: Presence and distribution of plant species fDNA (number of sequence reads) in 10 cattle faecal samples collected at Hamersley station

Species	PilbSeq ID	Cattle id									
		1	33	79	47	65	21	57	69	83	85
Pluchea rubelliflora	33794										
Pluchea dentex	33793		-	449			16021			84	
Pluchea indica	NA										
Pluchea dioscoridis	NA										
Pterocaulon pycnostachyum	NA						2670				
Portulaca oleracea	NA		3959			676			1181	57	
Cullen graveolens	33646										
Cullen pogonocarpum	33624			90			64483	6285		69	
Cullen cinereum	22394										
Cullen australasicum	NA										
Cullen graveolens	33646										
Cullen pogonocarpum	33624										
Cullen cinereum	22394						1536				
Cullen australasicum	NA										
Otholobium sericeum	NA										
Rhynchosia minima	22367	138			27		6		814	24	
Rhynchosia malacophylla	NA										
Vachellia farnesiana	33637										
Vachellia tortilis	NA										
Vachellia farnesiana var. pinetorum	NA			5898	116		46				
Vachellia nilotica	NA										
Acacia pinetorum	NA										
Sida trichopoda	33661									167	13852
Sida trichopoda	33661	7									
Sida sp. (Shovelanna Hill)	33562			379	730	69	95		3827		14364
Sida sp. (Barlee Range)	33561										

Sida sp. (Articulation below)	33559										
Sida platycalyx	33558										
Sida sp. (dark green fruits)	33557										
Sida fibulifera	33556										
Sida sp. (spiciform panicles)	33555										
Sida sp. (verrucose glands)	33554										
Sida ectogama	33552										
Sida clementii	33551										
Sida sp. (Shovelanna Hill)	33562										
Sida sp. (Barlee Range)	33561										
Sida sp. (Articulation below)	33559										
Sida platycalyx	33558										
Sida sp. (dark green fruits)	33557										
Sida fibulifera	33556								13		107
Sida sp. (spiciform panicles)	33555										
Sida sp. (verrucose glands)	33554										
Sida ectogama	33552										
Sida clementii	33551										
Urochloa pubigera	22446										
Urochloa ramosa / Brachiaria ramosa	NA										
Megathyrsus maximus	NA										
Eriochloa villosa	NA				36						
Eriochloa procera	NA										
Sorghum bicolor subsp. drummondii	NA										

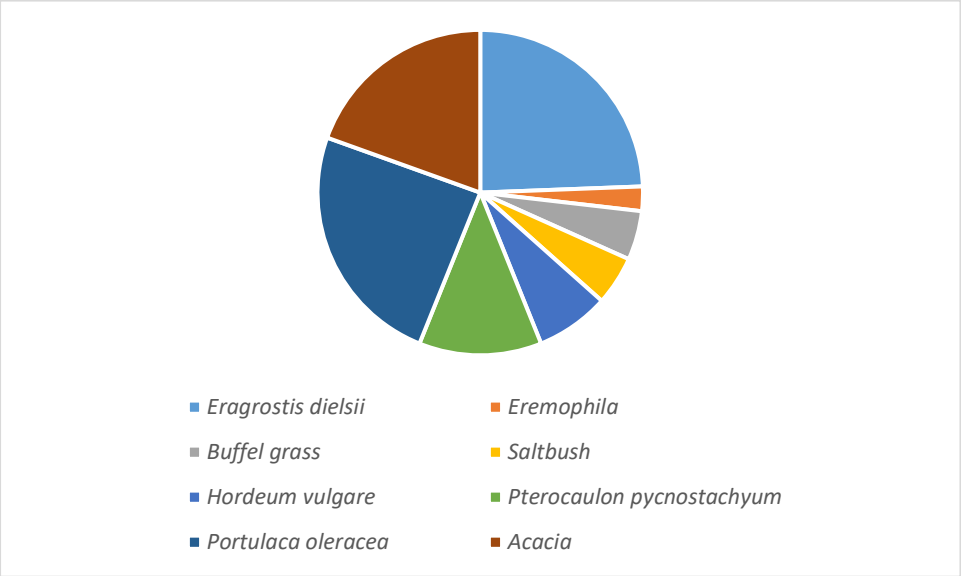
The presence of main species is summarized in the Table 8. It appears that *Eragrostis dielsii* and *Portulaca oleracea* were grazed by all 10 cattle, *Acacia* by 8 cattle, and other species by a smaller number of animals.

Table 8: Summary of key species grazed by individual cattle

Plant species	Cattle id										Present in diet of no of cattle
	1	2	3	4	5	6	7	8	9	10	
Eragrostis dielsii	■	■	■	■	■	■	■	■	■	■	10
Eremophila		■									1
Buffel grass				■	■						2
Saltbush			■					■			2
Hordeum vulgare					■	■	■				3
Pterocaulon pycnostachyum	■	■	■								5
Portulaca oleracea	■	■	■	■	■	■	■	■	■	■	10
Acacia			■	■	■	■	■	■	■	■	8

Based on this, it may be possible to visualise an 'average cattle diet' (Figure 2).

Figure 2. Illustration of an 'average cattle diet' based on fDNA metabarcoding results at Hamersley station Dunns paddock



Key findings: We found that cattle ate a greater diversity of species than the ones originally identified as key species for northern WA Rangelands. Using local lab provided more relevant and accurate results.

4.4 Experiment 3 – Cattle georeferencing, diet selection and productivity at Challa station

Introduction

In previous two animal experiments, it was revealed that fDNA metabarcoding can be used to reveal the diet selection in rangeland cattle. While these experiments validated the fDNA metabarcoding in WA rangeland cattle, they did not make an attempt to link these findings to grazing location or animal productivity. The objective of this study was to further obtain quantitative data on diet selection and the grazing patterns of the cattle at Challa station in the central WA Rangelands, and to assess any possible link between cattle movement, diet selection and cattle growth rates. Specific aims for Experiment 3 align directly with the overall project aims and include:

1. Profile vegetation and land systems
2. Examine cattle location and movements in the landscape over time
3. Identify diet preference and selection
4. Investigate animal performance over time
5. Examine possible links between animal location and diet with animal productivity

Materials and methods

1. *Profiling of vegetation and land systems*

Vegetation profiles were estimated based on the Windimurra Monitoring Sites reports for site 1-15 provided by the station owner. The land system map was constructed by Contour Environmental and Agricultural Consulting in 2023 (CEAC, 2023). This approach to mapping has been used in all of the regional rangeland surveys in Western Australia, where a land system is defined as 'an area with a recurring pattern of topography, soils and vegetation'. These recurring patterns were mapped using 1:50,000 scale aerial photography or other remotely sensed images and the land systems are then ground-truthed in the field to verify the mapping. Land systems then form the basis of condition assessments and carrying capacity calculations in the rangelands.

2. *Cattle movements*

Cattle location and movements were determined using geo-referencing via Ceres tags and Mapipedia. The tags were applied to 60 cattle when yarded during the May 2022 muster, and geolocations (latitude and longitude) of each animal were recorded approximately every four hours. The information was automatically downloaded onto Mapipedia platform for routine monitoring of stock movements. The positions of the animals were considered over a window of time as a movement trajectory in space. A density plot (heat map) of these positions for an individual animal, a group of animals, or the entire herd was estimated.

Diet preference and selection

This was assessed using land system approach and fDNA metabarcoding.

Firstly, the Mapipedia heat map of cattle locations were overlayed onto the land systems map to show land system preferences of each Ceres-tagged animal to determine the grazing behaviour over a 12-month period. Water points were added to determine the likely influence on cattle movements. Preferences for land systems were determined.

Faecal samples were taken from the 26 individual Ceres-tagged animals in April/May 2023 muster and subjected to fDNA metabarcoding at eDNA frontiers lab, as described earlier.

3. Animal performance

Growth performance of cattle was assessed using measuring LW at muster in 2022 and 2023, and calculating LWG. Cattle (n=60) were weighed at mustering in May 2022 and again in late April/early May 2023. Average daily liveweight change (LWG) was used to indicate animal performance over the period of 12 months. At the 2023 muster, 26 of the Ceres-tagged cattle were identified and sampled. The remaining animals were located on Mapipedia, but they could not be yarded during the muster. Animal performance was then classified according to daily weight change to high (> 0.400 kg/d), medium (0.250 – 0.400 kg/d), low (0.100 -0.250 kg/d) and very low (<0.100 kg/d).

4. Correlating animal movement and diet with animal productivity

The 'heat map' of geolocation densities of all Ceres-tagged cattle was refined for cattle with growth performance data (n=26). These geolocations were overlayed onto the land systems map to determine the preferences of land systems for the various growth performance classes.

Results

1. Profiling of vegetation and land systems

There were 37 plant species detected across 15 monitoring sites, with most common and numerous being *Maireana pyramidata, Ptilotus obovatus, Soliva sessilis* and Triptra (Table 9).

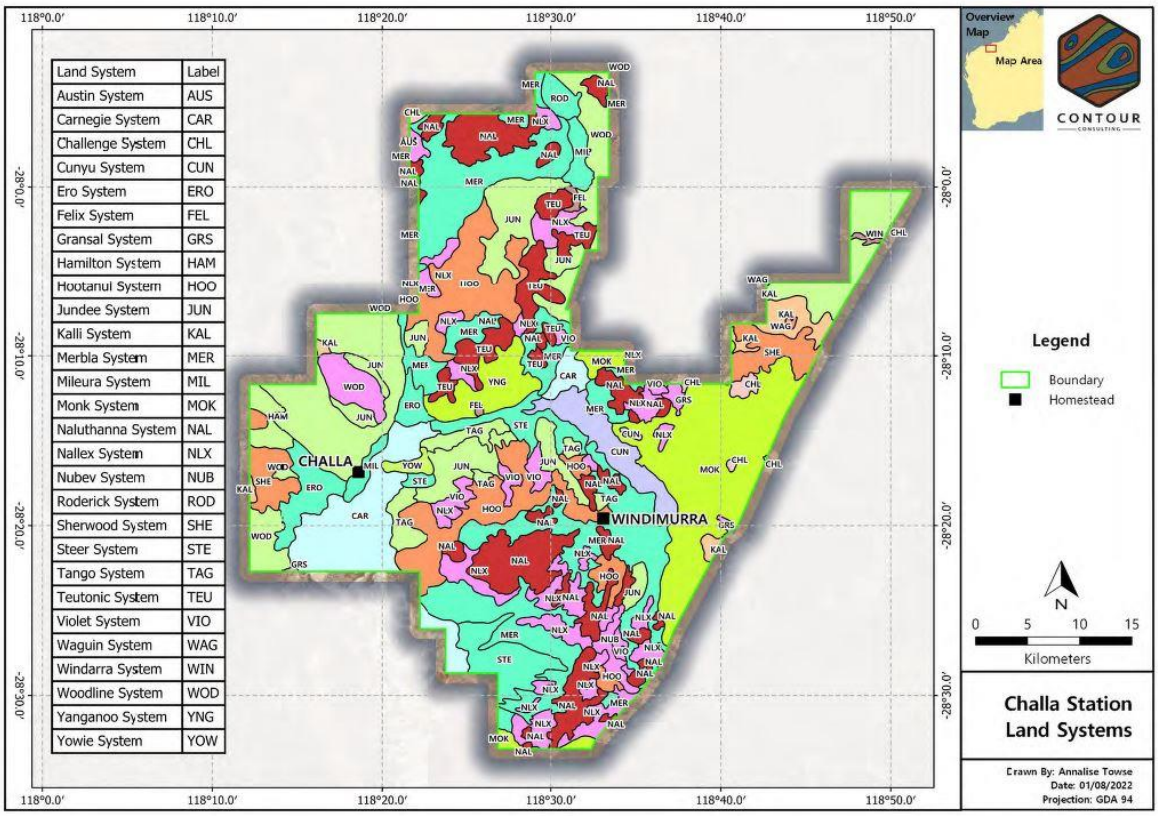
Table 9: Vegetation profiles (numbers of plants recorded) at Windimurra Monitoring Sites, Challa station Nov 2020

Latin name	Common name	Site number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acacia acuminata	Jam tree										1					1
Acacia sp.	Fine Leaf Mulga	1									5	2	5			
Acacia aneura	Mulga			2						2					2	6
Acacia craspedocarpa	Broad Leaf Mulga										1		1		3	
Acacia papyrocarpa	Western Myall		1					1								
Acacia ramulosa var. linophylla	Bowgata			3									1		2	
Acacia stellaticeps	Poverty Bush					1					2		2		1	
Acacia tetragonophylla	Kurara		1		2								2	1	1	2
Agonis flexuosa	Native Willow													1		
Asphodelus fistulosus	Wild Onion												1			
Atriplex bunburyana	Silver Saltbush					5										
Brachychiton populneus	Kurrajong															1
Cratystylis subspinescens	Sage				1											
Enchylaena tomentosa	Ruby Saltbush							1				1				1
Eremophila fraseri	Turpentine Bush			1							3					
Eremophila scoparia	Mulga broombush												1			
Eremophila sp.	Eremophila sp.															1
Frankenia sp.	Frankenia					3										
Maireana pyramidata	Sago Bush	13	23		5	8	4	17	12	9	4	20		18		
Maireana sedifolia	Blue Bush			1												
Ptilotus obovatus	Cotton Bush	1	1	4						10	22			1		25
Ptilotus sp.?	Gascoyne Mulla						7									
Ptilotus sp.?	Wire Mulla			3												
Rhagodia eremaea	Tall Saltbush				1						1	1		2		
Salsola australis	Tumbleweed									1						
Scaevola spinescens	Currant Bushes				2											
Senna artemisioides subsp. x artemisioides	Banana Leaf Cassia					1										
Senna artemisioides subsp. x coriacea	Desert Cassia								9							

Senna sp.	Green Cassia				1											
Senna sp.	White Cassia									2						
Sida calyxhymenia	Tall Sida		4								13	12				
Solanum lasiophyllum	Flannel Bush	4	1			2					1	1				2
Solanum orbiculatum	Wild Tomato										1					
Soliva sessilis	Bindi	5	3					13	100	9				3		
Tecticornia lepidosperma	Samphire							1								
unknown	Triptra		6	2	26	10	5	2		18	4			2	1	
unknown	Wild Lemon												1			

There was a total of 28 different land systems identified at Challa station (Figure 3)

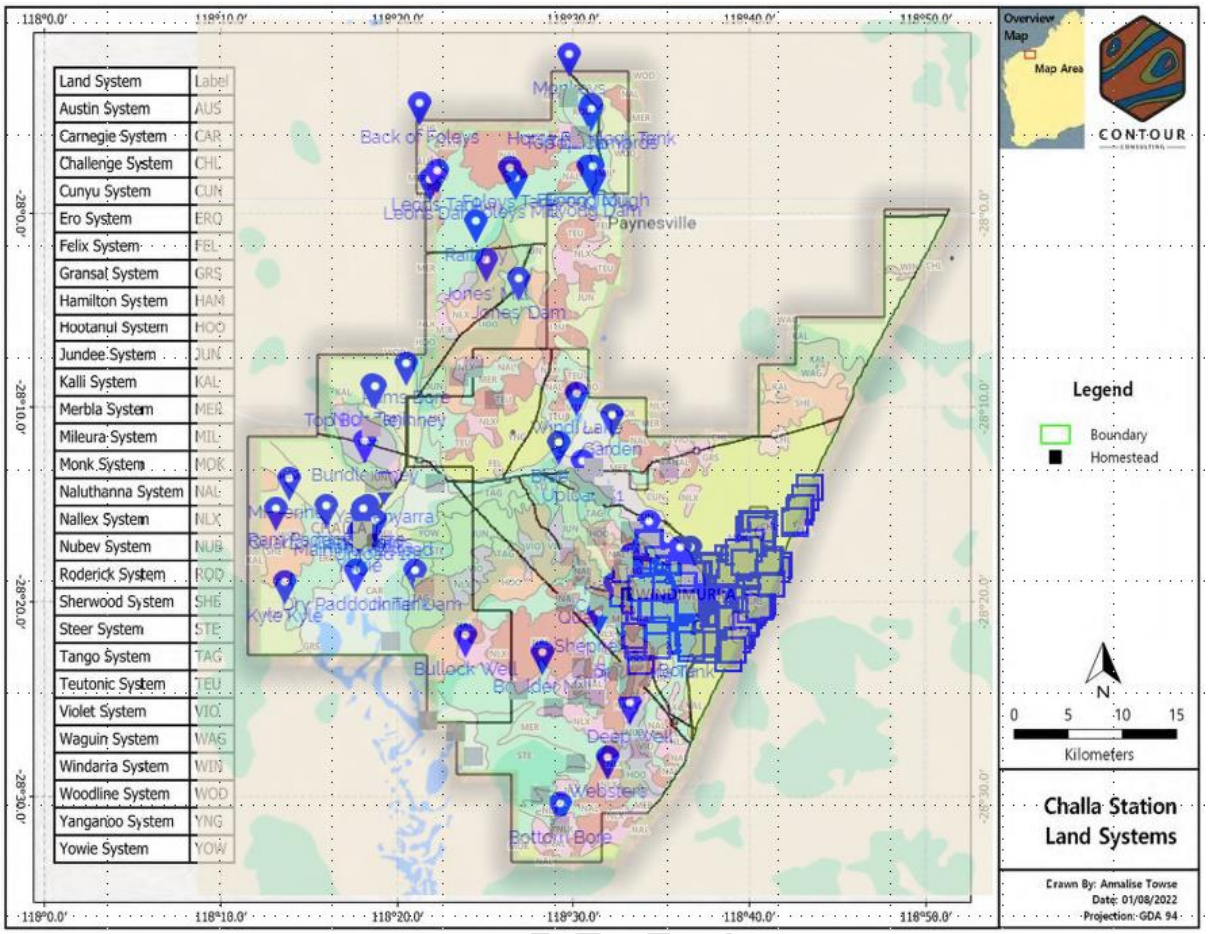
Figure 3. Challa station land systems



2. *Cattle location and movements*

Examining cattle geo-referencing data revealed that cattle had preferred areas in the landscape where the animals spend most of their time (Figure 4).

Figure 4. The preferred grazing area (blue squares) of a Ceres-tagged animal at Challa station over a 12-month period (May 2022 to April 2023) as affected by land systems and water points (blue markers).



3. *Diet preference/selection*

Diet preference

Only 7 land systems were found to be preferably grazed by cattle. The profiles of preferred grazing land systems are given in Table 10.

Table 10: Description of land systems preferred for grazing *- Cattle Unit equivalent to a 450kg steer or a dry cow.

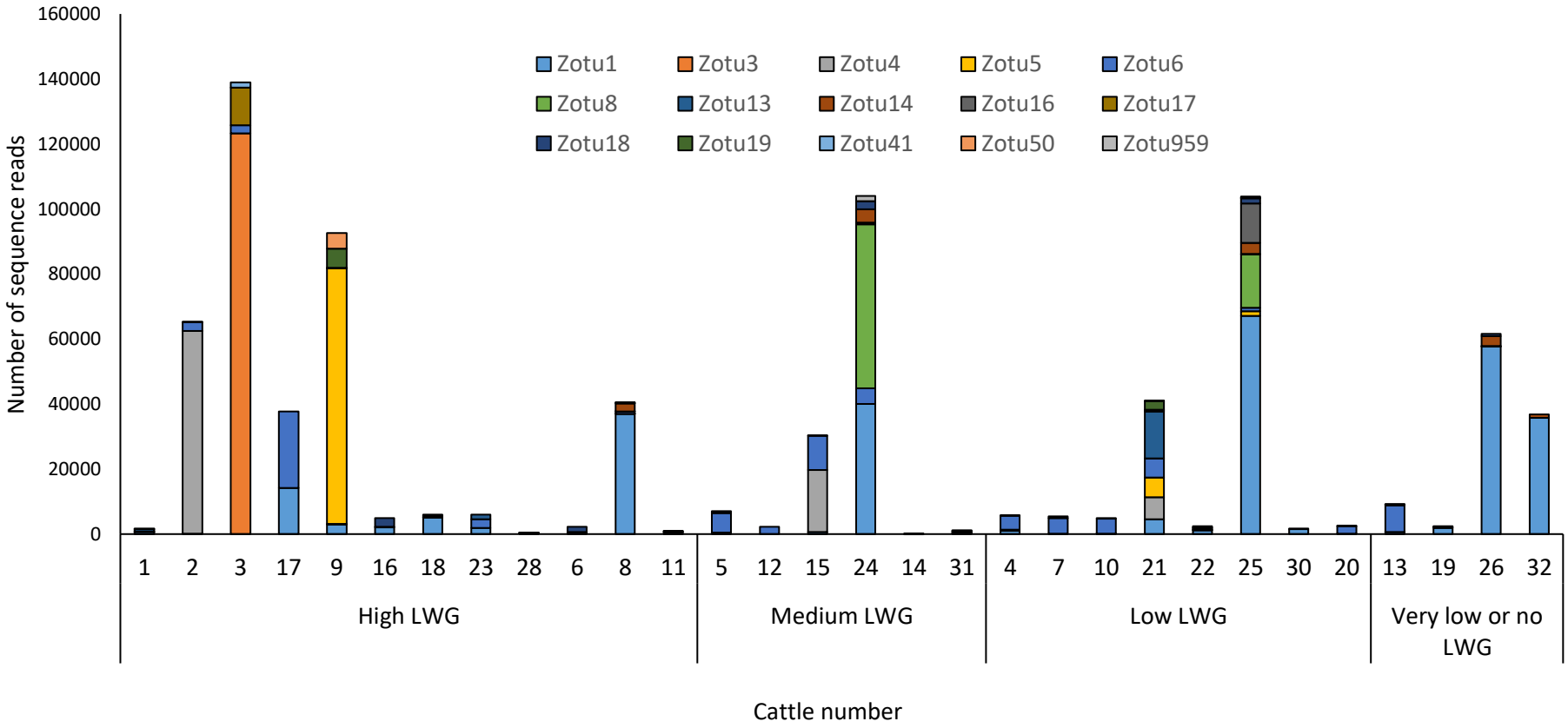
Land system	Description	Pastoral potential (ha/CU*)	% station
Merbla	Alluvial plains with clayey partly gilgaied soils, below gabbro hills supporting chenopod shrublands and tussock grasslands.	<35	16.1
Steer	Gravelly alluvial plains supporting chenopod shrublands.	42 - 63	3.8
Cunyu	Calcrete platforms, intervening drainage floors and channels and minor alluvial plains, supporting acacia shrublands, occasional casuarina woodlands and minor halophytic shrublands.	70 - 98	2.3

Naluthanna	Rough hills, tor fields and slopes of gabbro above lower stony plains with gilgaied drainage floors supporting mixed acacia shrublands with sparse halophytes.	70 - 98	6.4
Nubev	Gently undulating stony plains, minor limonitic low rises and drainage floors supporting mulga and halophytic shrublands.	70 - 98	1.5
Monk	Hardpan plains with occasional sandy banks supporting mulga tall shrublands and wanderrie grasses.	105 - 133	10.4
Nallex	Gently undulating stony plains supporting acacia tall shrublands and chenopod low shrublands.	140 - 203	8.9

Diet selection - fDNA metabarcoding

The fDNA revealed that as many as 85 organisational taxonomic units ('Zotu), with possible 707 different plant species were eaten by cattle (Appendix 3). The most common plant species found in the faeces are listed in Figure 5. The highest sequence read numbers were recorded with Zotu1 (clade containing *Eremophila*), Zotu5 and 13 (clades containing *Sida*), Zotu6 (i.e. clade containing *Maireana*), Zotu16 (clade containing *Indigofera*), and Zotu18 (clade containing *Acacia*). There was variation in individual cattle plant selection, but most cattle appeared to have consumed *Maireana* and *Eremophila species*.

Figure 5. Presence and distribution of plant species fDNA (number of sequence reads) and live weight gain (LWG) ranking in 30 cattle collected at Challa station in Apr/May 2023. Only sequence reads that were > 1000 shown. LWG ranking (g/d) - High>400; Medium 250 - 400; Low 100 - 249; Very low <100



Animal productivity

In Apr/May 2023 mustering, the LW was recorded in 49 animals. The LW was ranging from 121 to 376 (Figure 6a). Amongst 43 animals where LWG could be calculated (i.e. seen both in 2022 and 2023), there were 13 that lost weight since last weighing and 30 that gained weight (Figure 6b).

Figure 6a. LW of 49 cattle that still had Ceres tags by mustering Apr/May 2023

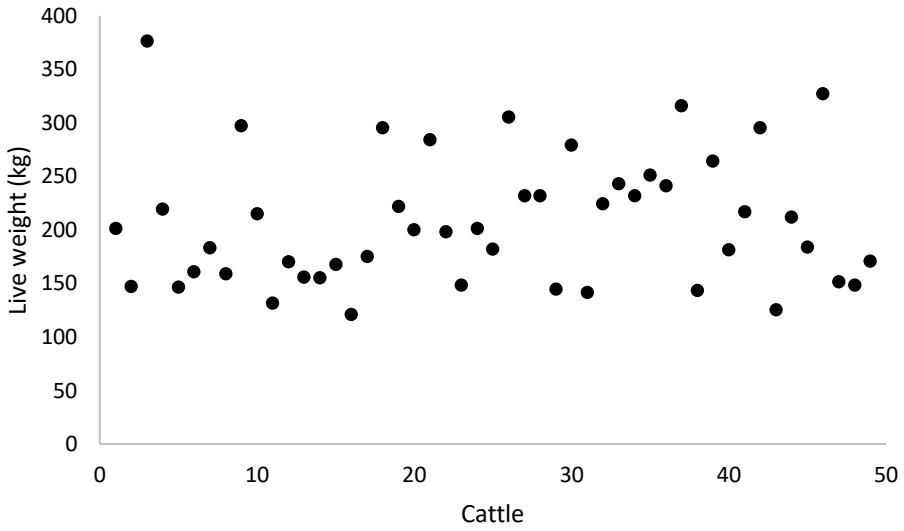
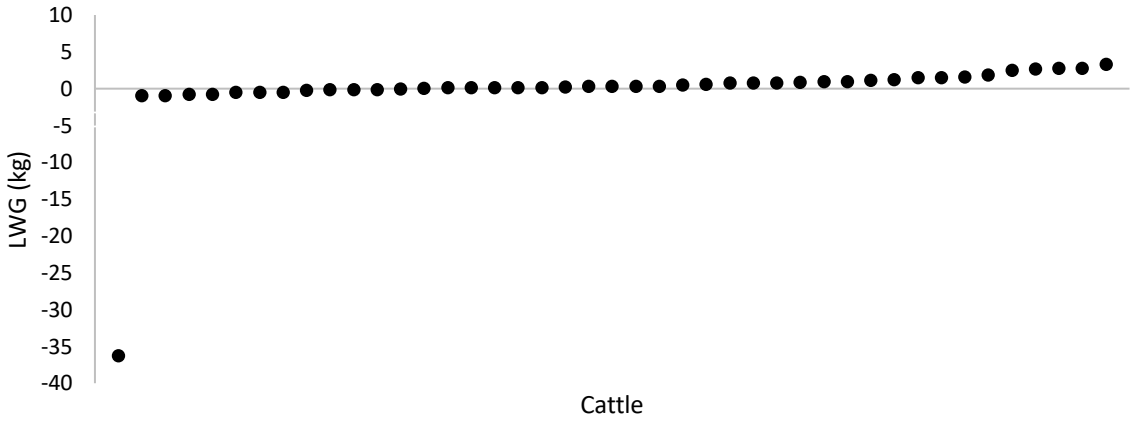


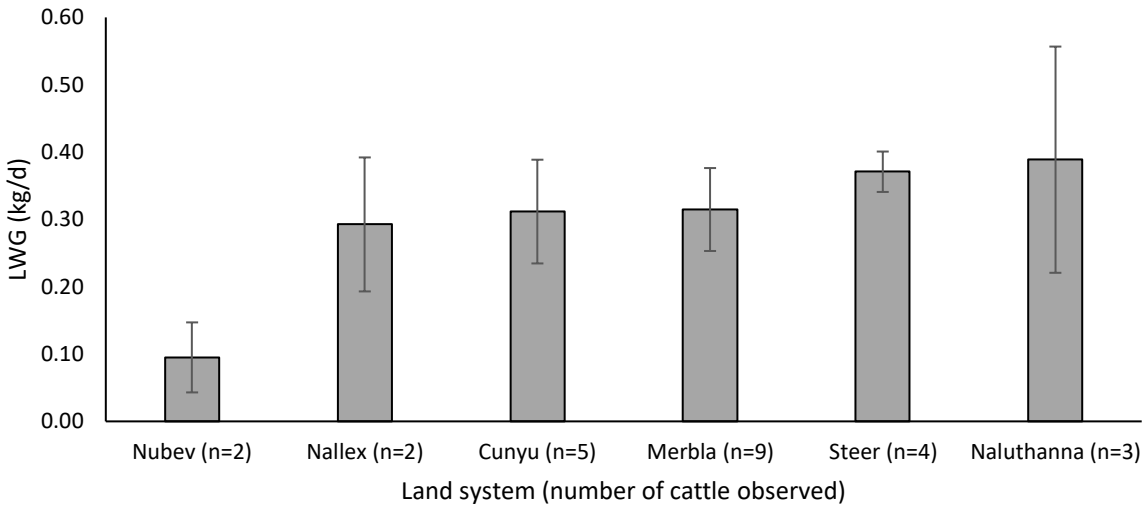
Figure 6b. The LWG in 43 cattle in period May 2022 – May 2023



Correlating animal location/movement with diet and animal productivity

The link between location and growth performance was assessed in 25 Ceres-tagged cattle. It was revealed that it varied in different land systems, ranging from 0.095 kg/d in Nubev to 0.389 kg/d in Naluthanna (Figure 7).

Figure 7. The LWG of individual cattle (n=25) at the preferred land systems from May 2022 to April 2023.



It appears that the growth performance was also affected by diet selection, as documented in Figure 5. Animals that had preference for species in Zotu 959, Zotu3, Zotu5 , and Zotu 41 seem to had better LWG to those that consumed Zotu1 or Zotu8 plant species.

Georeferencing of animals and alignment to vegetation characteristics.

One of the objectives of the research was to identify if georeferencing of animals could yield further information on the patterns of consumption of key vegetation characteristics. The federation of the georeferencing data series (collected using ceres ear tags) and the f DNA /vegetation data series was undertaken. Clear identification of animals with differing performance was observed with individual animals residing within different vegetation zones (Figures 8 and 9).

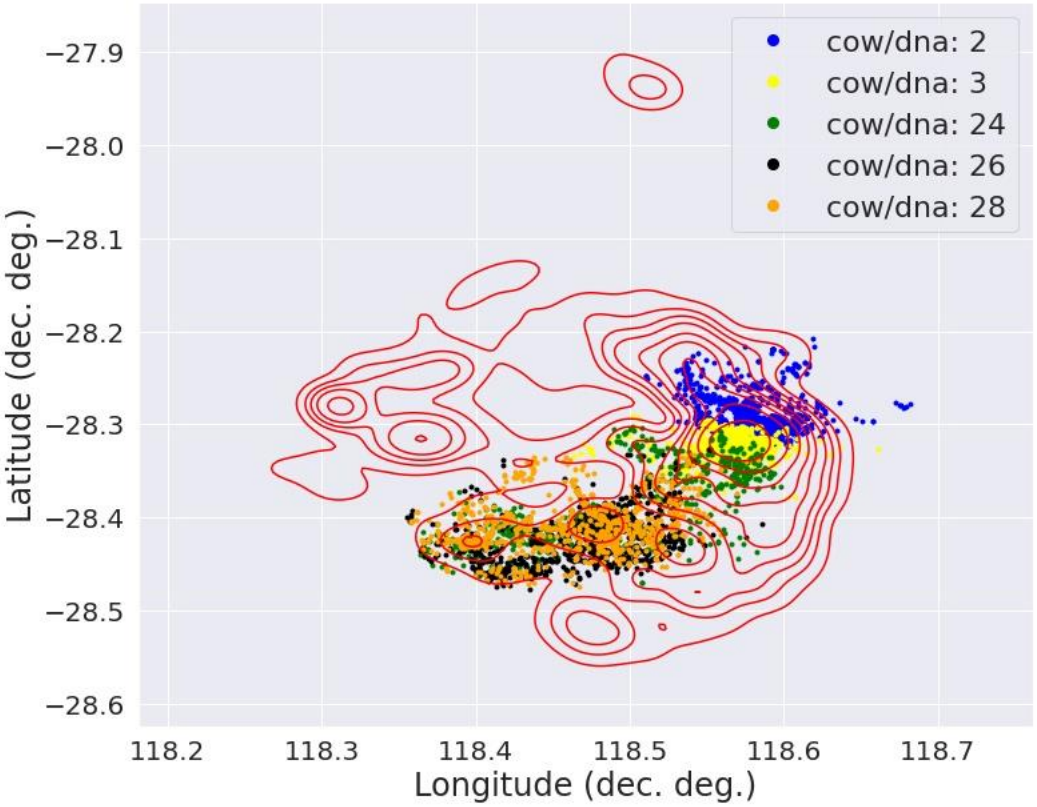


Figure 8 Location of cattle x fDNA federated data. Cattle #2 and #3 as high live weight gain and high abundance of Zotu3 (Abutilon clade) and Zotu4 (mixed species clade); Cattle #24 that had medium live weight gain and high abundance of Zotu1 (Eremophila clade) and Zotu8 (mixed species clade); and Cattle #26 and #32 that had very low live weight gain and high abundance of Zotu 1 only (Eremophila clade).

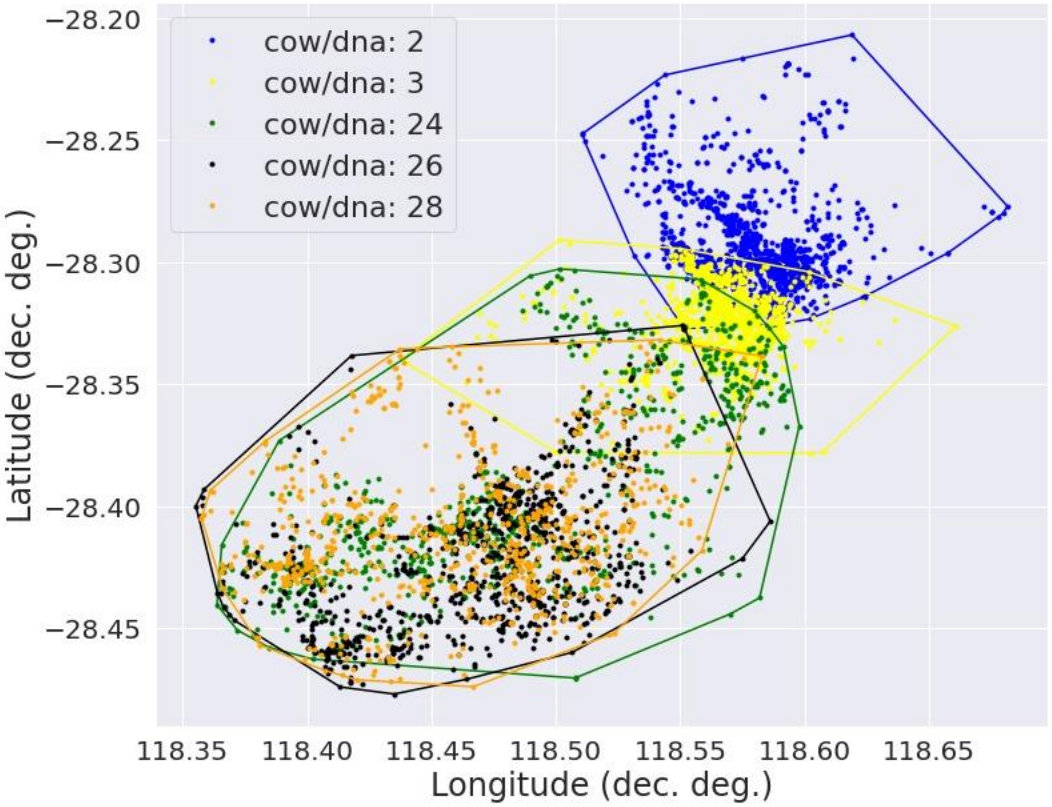


Figure 9 Kernel boundaries of cattle x fDNA federated data. Cattle #2 and #3 as high live weight gain and high abundance of Zotu3 (Abutilon clade) and Zotu4 (mixed species clade); Cattle #24 that had medium live weight gain and high abundance of Zotu1 (Eremophila clade) and Zotu8 (mixed species clade); and Cattle #26 and #32 that had very low live weight gain and high abundance of Zotu 1 only (Eremophila clade).

Furthermore, there were identifiable differences in animal performance and residence location. Animals 2 and 3 within zotu 3 were gaining weight rapidly, and almost exclusively within the Abutilon vegetation characteristic zone compared to Animals 24, 26 and 32 who were georeferenced to areas with vegetation dominated by Eremophila (a low digestibility but potentially high antimethanogenic vegetation clade) or mixed Eremophila vegetation clades. Further information on these issues is presented in P.PSH.1262. These data are a preliminary analysis of an extensive and extremely complex data set. However, the analysis has demonstrated that the data collected and approaches to data federation are sufficiently granular to determine the environments where individual cattle are grazing, what those cattle may consume and how long those cattle reside within those specific vegetation environments.

Discussion

The current study demonstrated that it may be possible to use a combination of geo-referencing and molecular investigation tools to start understanding the diet selection in the WA rangeland cattle. It was obvious that cattle had some preferred grazing areas. In these areas, they appeared to have grazed a wide range of plants, but the most frequent and abundant in the faeces were likely to be *Maireana, Acacia, Indigofera, Eremophila* and *Sida*. The productivity (LW and LWG) was quite variable amongst individual cattle, with up to three-fold differences in these values between different land systems. It appears that the land system Naluthanna that was supporting mixed acacia shrublands and halophytes resulted in the highest LWG. The value of this finding is knowing of what animals are

eating (the most) in the landscape, as well as what may be supporting their productivity. Based on this study, we may only speculate that plants identified in the fDNA and in the landscape may be supporting animal production, as more studies are needed to confirm this observation.

The study also identified some obstacles and limitations to such approach. Firstly, there were some issues related with Ceres tags - while 60 cattle were initially fitted with tags, 11 tags either fell off, missing or were not functioning. Then, from these 49 cattle, only 43 had both LW data from 2022 and 2023, so that LWG could be assessed, and only 30 cattle were sampled for fDNA at mustering in 2023. Due to excessive flooding in the area, the cattle could not be accessed for the good part of 2022, so the faecal sampling and weighing occurred only once instead of four times. As the timing and limitation of access to the cattle throughout the year resulted in a single 'spot' sample taken for fDNA analyses, the diet selection captured in such sample may only be reflective of the diet selection near to mustering time, so further studies are needed with more frequent sampling throughout the year. Whilst the fDNA metabarcoding was conducted against a more-relevant WA rangeland library compared to a global library, it was initially developed for Pilbara species. Although there are many species that overlap between northern and central WA rangelands, this Pilbara database may still be missing key species present in the central WA rangelands.

The preliminary analysis of the federated georeferenced data, fDNA and vegetation characteristics provided the first insights into the relationship between animal performance and foraging theory within West Australian rangeland environments. Foraging theory in free ranging ruminants identifies that animal performance is closely linked to energy maximisation theory. Energy maximisation is defined as the animal of known genotype consuming a mix of species that optimise rate of digestion and hence yield of energy from the feed base. The key issue is that the animal must maintain intakes of feeds that result in synchronisation of nutrients for microbial processing as well as ensuring uptake of products of digestion. The limited data analysis demonstrated clear foraging environments for individuals with two animals geolocating in vegetation environments that seem to drive high levels of animal performance (northern location, abutilon zotu fDNA reference sites) whereas others maintain a georeferenced within rangeland vegetation zones that may be of lower nutrient density (reflected by lower animal performance). The reasons for these differences in georeferencing are poorly understood as all animals have an opportunity to access vegetation zones (free ranging) of differing nutrient profiles.

Furthermore, the data may provide opportunities to design strategic supplementation strategies. Strategic supplementation in rangeland environments should not be designed for the average animal within an average vegetation environment. If animals georeference to distinct locations within the rangeland environment, an understanding of animal performance and targets for the farming system are critical to designing efficient use of supplements. Supplementation is costly, and therefore, animals that maintain high levels of performance within a rangeland environment without supplements should not be the target to delivery of this technology. Arguably strategic supplementation should be used in environments where cattle are observed frequently (high rates of residence) but do not perform well (i.e. achieve the minimum benchmark for animal performance but no more, or do not achieve the benchmark performance). In the specific example presented in this report, the target for strategic supplementation to gain maximum benefit would be within zotu 3 and 4 that is where animal performance is suboptimal compared to animals within zotu 1 and 8 (above benchmark performance). By understanding the nutritive value of feeds within these different vegetation environments, supplements can be designed and tailored to the nutritional requirements benchmarked against animal groups within certain vegetation environments. This targeted approach would result in more animals achieving benchmark weights for transitioning to backgrounding and/or lot feeding. Inevitably, a producer cannot tailor supplementation strategy to all individuals but can achieve better use of supplements across broad groups of animals within known vegetation zones.

This project also has an important link to the work being conducted on antimethanogenic plants (P.PSH.1262) and the use of virtual fencing technologies (P.PSH.1306). By understanding the location and movement patterns of animals across the rangeland environments, this project identified vegetation clades that may have nutritional attributes that drive higher levels of animal performance. That information can be used by the pastoralist in the development of management plans for rangeland environments including the use of virtual fencing technologies and supplementation strategies. Virtual fencing technologies provide an opportunity to manage animals across extent rangeland areas. P.PSH.1306 has demonstrated, using large mobs of cattle, that animals can be maintained in specific areas of rangelands thereby maximising the use of the feed base. The work in our current project (P.PSH.1245) provides granular information on grazing preference and areas that are being actively grazed. Integration of virtual fencing would reduce the risk of landscape and biodiversity degradation through overstocking and over grazing of vegetation resources.

Key findings: Implementing Ceres tags and Mapipedia can be a useful tool for developing geo-referenced 'heat maps' of rangeland cattle and preferred land systems for grazing. There was a wide range in animal performances that can potentially be linked broadly to land systems and, through fDNA metabarcoding, to plant species diversity and the nutritional value that the diversity brings to the diet.

4.6 Engagement and communications activities

Awareness has been built through a number of meetings, forums, workshops and press releases, some of which are listed in Table 11 below.

The meeting in Broome in July 2022 included representatives from DPIRD, NABRC, KPCA and Pardoo Station and covered a number of current, new and prospective projects and programs of work that are focused on northern beef production. The main programs of work that were discussed were the recently announced extension to the DPIRD Northern Beef Development program (NBD; Trevor Price), the NABRC NB2 program (Lee Fitzpatrick), KPCA's innovation and adoption projects, Pardoo Station's MDC project (Kevin Bell) and BeefLinks (Vercoe). The purpose of the meeting was to inform each other of the work that was being undertaken and to use it to try and build partnerships across the programs and value add. There have been a number of follow up discussions with Trevor Price and Clinton Revell about the synergies between BeefLinks and the second phase of the NBD program where they intend to focus on genetics as a key opportunity. This is complementary to the feedbase focus in BeefLinks and the discussions we have already had with Dr David Johnston (AGBU) and the Repronomics project he leads. BeefLinks has also held a workshop through the Backgrounding project and released press articles in relation to the workshop, the backgrounding project and more broadly about BeefLinks that have all been designed to build awareness. Those specific activities have been reported in the Milestone report for that project.

We have been invited to participate in workshops and forums organized by grower groups to discuss and develop PDS and PGS projects. Some of these activities have been face to face but the majority have been via zoom. More specifically, both the Project Leader and the Project Manager have been invited to help discuss, develop and formulate PDS and PGS applications initiated by:

1. Joy Sherlock (Rangeland NRM). These meeting have included a large number of producers, a number of grower groups (GCG, WMG, MIG), consultants, DPIRD and BeefLinks. One of these

proposals progressed through to submission, but there were two more that the group decided needed more discussion and time to pull together properly because there were so many potential participants.

2. Gascoyne Catchment Group PPATT (P.PSH.2100) which is linked to PPSH.1233 Backgrounding project. The focus would be with pastoralists who are equally interested in the Diet-ID and nutritional mapping project.
3. MIG Executive Officer, Kathryn Fleahy. We have offered support to assist in developing a proposal around the most suitable feedbase for backgrounding properties that have an alliance with pastoralists and accept pastoral cattle for backgrounding. We think the offer of support will be accepted and that a proposal will be submitted in the next round.
4. Debbie Dowden. We are currently negotiating with Debbie Dowden, in collaboration with WALRC, a place on the program at the Southern Rangelands Pastoral Roundtable at Mt Magnet on October 2nd. The main project that I will present at that round table will be the Diet ID and nutritional mapping project and the link to CN2030.
5. Sim Mathwin, Rio Tinto. The project based around virtual herding with RioTinto is progressing and stems from a direct link to the DietID and nutritional mapping project.

Importantly, BeefLinks has been asked to help organize the annual Coral Bay meeting in partnership with West Midland Group and the Gascoyne Catchment Group. This meeting attracts pastoralists and backgrounders (amongst others from industry, State Departments, consultants, processors) from across the entire supply chain and is one of the pinnacles of meetings held for the northern beef industry each year. As part of the arrangement to co-coordinate the meeting, we have suggested that BeefLinks runs a specific workshops session designed to identify opportunities for PDS, PGS and other funding opportunities currently available at State levels. The timing of this meeting will be in November annually. It is one of the most important meetings on the calendar and is always well attended when it is held late in the year.

An industry workshop on nutritional requirements of cattle, delivered by Dr John Milton, was organised by BeefLinks via the West Midland Group as part of their BeefLinks project. The workshop was held at Joe and Jane de Pledge's Badgingarra property, 'Jandawanning' and was attended by 11 producers (there were others who attended from DPIRD and WMG). The de Pledge family is one of the most influential and progressive in the pastoral country and they own their own pastoral and backgrounding properties. It is our expectation that towards the end of the DIET ID project, the de Pledge family will be one of the producers who will be interested in hosting/participating in a PDS/PGS project. The workshop John gave was targeted at nutritional requirements of cattle in the backgrounding region but with a focus on the needs of cattle that had been transported from pastoral stations. John has a number of clients he advises in the pastoral region, is a keen supporter of the BeefLinks program and a key contributor to developing a specific WA module for the NutritionEDGE program. He is also well aware of the self-herding project and previous reports that have been finalised for MLA on a vision for the backgrounding region, the potential of virtual fencing and potential delivery mechanisms of anti-methanogenic supplements to the extensive production systems of the north. These interests will be extremely valuable to the backgrounding, DIET ID and CN2030 projects, and his network and trust amongst the pastoralist could prove invaluable.

The Project leader was involved in the organisation of Western Australian Livestock Research Council Winter Forum that was held as a face-to-face meeting in Perth on Friday, August 21 2020. The Forum program was intended for the special interests of the livestock research and extension community and associated practitioners. However, red meat producers were welcome to join the forum to get

an insight into current research and adoption priorities. Over 60 people attended the forum. The bulk of the participants came from the 3 universities (University of Western Australia, Murdoch University and Curtin University), CSIRO and DPIRD. There were a number of producers, consultants as well and the entire WALRC committee in attendance on the day. BeefLinks was included in the main program and feedback was positive. We were approached by two of the meat scientists at Murdoch University about the possibility of being part of the DietID project because of a new interest they have in the importance minerals play in improving meat quality.

Table 11: List of engagements with producers and supply chain stakeholders

Date	Location	Event	Title of activity	Industry group/Location
Nov-20	Coral Bay	GCG Annual Pastoral Forum	Courtney Martino and Phil Vercoe introduction of project and meeting with key stakeholders	Gascoyne Catchment
Mar-21	Karratha	KPCA Annual Innovation Conference 2021	Peter Hutton meeting pastoralists and other stakeholders	Kimberley Pilbara Cattlemen's Association
Aug-21	Rio Tinto Hamersley station	UWA-MLA Virtual fencing field day	Dean Revell Diet ID presentation	Pilbara/Gascoyne pastoralists
Mar-22	Online	West Midlands Group (WMG) online Seasonal updates Festival	Peter Hutton online presentation on rangeland plants for potential methane abatement	West Midlands Group stakeholders
Apr-22	Pilbara, Gascoyne	Engage with station and backgrounding properties: Bullara, Wyloo, Lyndon and Hamersley	Peter Hutton, Zoey Durmic and Lindsey Perry	Pastoralists interviewed included Tim and Edwina Shallcross (Bullara), Shanon and Clinton Thompson (Wyloo), and Sean Darcy (Lyndon station).
July-22	Broome	Meeting that was organised by DPIRD and MLA	Phil Vercoe as invited speaker presented the BeefLinks platform and projects. Zoey Durmic talked to pastoralist and stakeholders. Visit to DPIRD pivots.	Kimberley and Pilbara pastoralists, researchers, stakeholders
Sep-22	Bridgetown, WA	Bridgetown producer workshop	Peter Hutton presentation on diet identification and rangeland plants for methane reduction	Bridgetown producers
Nov-22	Tom Price	MLA BeefUp Forum	Phil Vercoe, Peter Hutton and Zoey Durmic meeting with pastoralists and other key stakeholders	Pilbara/Gascoyne pastoralists

Nov-22	Coral Bay	Gascoyne Catchment Group Annual Forum	Meeting pastoralists and other stakeholders: Peter Hutton, Erin O'Brien, Zoey Durmic and Phil Vercoe	Gascoyne Catchment
Mar-23	Broome	MLA MeatUp Forum	Zoey Durmic and Peter Hutton met pastoralists and other stakeholders	Pilbara/Gascoyne/Kiberely pastoralists

An internal (virtual) forum of MLA, UWA, Select Carbon and other invited agencies and/or companies will be held in October 2020 to socialise the project work and present key information on the technologies that are being deployed in Diet-ID, the potential use of data and information collected within the project and how those data will form the basis of adoption and practice change activities from 2021-2023.

5. Conclusion

5.1 Key findings

This project developed and validated a novel approach for feedbase mapping in the WA Rangelands. Firstly, the geo-referencing via Ceres tags and GPS collars is identified as a valuable tool to reveal where animals are grazing. In conjunction with other data such as land systems and profiles, revealed a preference for certain locations and vegetation profiles and allowed identification of preferred grazing areas. It was also identified that there is a wide choice of plants in the rangelands with favourable NV, which in turn can advance productivity of beef cattle. The fDNA metabarcoding had proven to be good tool for more accurate identification of what cattle actually graze. Some preliminary data on diet selection are obtained and potential grazing palate in the central and northern WA Rangelands revealed. The fDNA analysis also enabled discovery of many additional species that have not been reported earlier using classical approaches. Using a local laboratory and database proven to have the advantage over using a foreign laboratory and a global database, in the sense that it allowed more precise identification of plants that are present in Australia and the region. It appears that preliminary data on animal LWG can be linked to their grazing location and plants to identify what is driving the productivity.

In this project, we also identified some obstacles and limitations to such approach. For example, initial objective was to obtain real-time data on individual weights using Walk-Over-Weighing (WoW). This approach was reliant on cattle accessing water points. However, the unusual rain pattern generated excess water on the targeted property, which discouraged cattle to access water points, and hence not going through WoW scales. In future studies, other approaches may be considered such as offering supplementary feed or attractant to ensure that cattle are using WoW.

There was also limitation on how many cattle were able to be accessed to collect faecal samples from. This was reliant on weather conditions and consequently only limited number of samples were collected and analysed. The weather conditions and ability to access rangeland cattle therefore needs to be factored in when planning further trials. Another limitation to fDNA metabarcoding is that it is just a spot sample – i.e. it does not represent whole rang e of plants that animal may have grazed, as the plant DNA may be digested well before sample was taken. From DNA analyses perspective, there were also some inaccuracies when the foreign laboratory was used, as they mis-detected plant species not present in Australia. The local plant database, whilst having relevant plant species was also limited, as it only contains around 200 sequences of plant species, mostly from Pilbara.

5.2 Benefits to industry

Quantifying diet composition and selection of beef cattle in WA Rangelands can help in decision-making by pastoralists to achieve desired objectives through predicting outcomes of grazing strategies and identifying key plant species to guide management. Such information is critical because individual cattle consume different quantities of nutrients and animal nutritional needs fluctuate. Diet composition of animals in extensive and spatially heterogeneous environments is difficult to identify because it is hard to locate animals, plant composition varies spatial and temporal and finding, identifying and analysing plants is difficult, complex, and time consuming.

The information that is collected will be used to form guidelines for grazing management decisions and improved production. A better understanding of these factors will guide management decisions and potentially improve production. The potential production benefits from improved management decisions include improved performance of individual animals, a reduced 'tail' in the spread of liveweights, higher reproduction rates, and greater predictability in achieving target live weights.

6. Future research and recommendations

This project, (and other outputs from the WA BEEFLINKS) will form the basis for developing grazing management practices that improve production, deliver a more consistent supply of animals, and improve rangeland health. One of the main outcomes from this project is the development of guidelines for improving grazing management to better utilise the mosaic of feed sources available to increase productivity and sustainability of beef production. These guidelines will be a blueprint for all northern WA beef producers to enhance the supply chain at its origin and, in combination with the BeefLinks – Growing WA backgrounding through adoption project, improve the efficiency down the entire supply chain from north-south. The fDNA metabarcoding is a unique method still in development, but as technology advances and its use becomes more widespread, the procedure will become standardized and accurate. Ideally, a pilot study for each new application should be conducted to ensure that the sampling and analysis design is appropriate to detect the target.

Database quality and imprecise taxonomic resolution are issues yet to be overcome. Future work should focus on i) improving the reference library with more sequences of plant species relevant for the whole WA rangelands; ii) understanding limitations of the method, i.e. that multiple species may be attributed to the same Zotu; iii) greater verification of fDNA metabarcoding results using field-derived plant composition data, especially in rangeland settings where plant diversity can be intrinsically high.

While the current project was guided to collect and analyse plants based on the pre-existing information and knowledge, the later geo-referencing detection of preferred grazing area and looking at fDNA revealed what cattle may have grazed. Once these are defined, it is be possible to go back to these areas and look specifically for plant species identified with the later approach. To extend further on this, those specific plants can be collected and analyse for NV. This can then truly match land system x plant species x nutritive values x what animals are grazing x productivity.

These preliminary findings also need to be validated further over a range of land conditions and plant biodiversity profiles by collecting more plants that can be added to the local fDNA database and matching with fDNA grazing profiles and changes in LW. It may be useful to access faecal samples from other trials in free ranging or setup more controlled experiments where the diet profile is well known and defined. It is also possible to setup a highly controlled digestion experiments (i.e. *in vitro* in batch, Rusitec, dual fermentors, or *in vivo* in fistulated animals) to examine how enteric digestion may affect recovery of different plant species DNA .

Apart from the animal productivity, the current approach of geo-referencing coupled with fDNA metabarcoding may be a very useful tool in for estimating rangeland land and pasture condition, and help in managing land use. For example, it can monitor the grazing pressure on vulnerable plant communities. In rehabilitation efforts, by exposing land to some cattle and then follow-up with their fDNA metabarcoding analysis, it can potentially allow early detection of plants that are not visible in the landscape or low in biomass.

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Appendix

Appendix 1 – CN30-DietID DATABASE

Attached as Excel

Appendix 2 – Methodologies used for fDNA metabarcoding

Argaly laboratory

The plant DNA the faecal samples was extracted using the Phosphate buffer method (Taberlet et al., 2012). The DNA extracts were recovered in a total volume of 100 µL of elution buffer before applying the DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer protocols. The DNA extracts were recovered in a total volume of 100 µL of elution buffer. The DNA was carried out using one plant generalist primer pair(Sper03) and four primer pairs specific to several plant families of interest. For plant tissue samples, the selection of primer pairs for amplification was based on the plant family or genus (see table below). For the 'unidentified' plants (indicated in red), all primer pairs were used for amplification. Each amplification of faecal samples was repeated four times. Each tissue sample DNA was amplified a single time. Each sample was recognized by a specific combination of two 8-base long tags attached to the forward and reverse primer in 5'. This combination was used to assign sequences to PCR replicates or samples during the bioinformatic analysis. After amplification, amplicons were purified using the MinElute PCR purification kit (Qiagen GmbH) and pooled for sequencing. The pooled amplicons were sent to FASTERIS (Geneva, Switzerland) to proceed to library preparation according to the MetaFast protocol and sequencing (Taberlet et al., 2018). An Illumina MiSeq 2*150 was ran with one library per marker, except for the Cass01 and Acac01 markers that were pooled in the same library.

Table 12. Primers used for amplification of plant DNA in the Argaly lab analysis

Primer pair target and name	**Region**	**Reference**	**Selection of tissue samples**
Generalist (Sper03)	rbcl	Taberlet et al. unpublished,modified from Fahner *et al.* (2016)*	All
Poaceae specific (Poac01)	ITS1	Taberlet *et al.* 2018**	SEC029 / SEC044 / SEC045 / SEC049 / SEC054 / SEC057 / SEC060 / SEC061 / SEC063
Acacia specific (Acac01)	ITS1	Taberlet/Bonin unpublished.	SEC029 / SEC031 / SEC032 / SEC036 / SEC037 / SEC038 / SEC044 / SEC045 / SEC049 / SEC054 / SEC057 / SEC060 / SEC061 / SEC063
Cassia specific (Cass01)	ITS1	Taberlet/Bonin unpublished.	
Solanaceae specific (Sola01)	ITS1	Taberlet/Bonin unpublished	SEC029 / SEC043 / SEC044 / SEC045 / SEC049 / SEC054 / SEC057 / SEC060 / SEC061 / SEC063

Different controls were performed at each step of the protocol to monitor possible contaminations and to allow a better interpretation of the results: 1 extraction negative control for each type of sample, 1 PCR negative control and 1 PCR positive control and 2 tagging system controls for each PCR.

Success of amplification and purification were verified using electrophoresis (E-Gel Precast Agarose Electrophoresis System, Thermo Fisher Scientific).

Raw reads were analyzed using the OBITools software suite specially dedicated to the handling of metabarcoding data (Boyer et al., 2016). For each marker, paired-end reads were first assembled with the program illuminapairedend, keeping only sequences with an alignment score higher than 40. Aligned sequences were assigned to the corresponding sample using the program ngsfilter, by allowing two and zero mismatches on primers and tags, respectively. After sequence dereplication using obiuniq, bad-quality sequences (i.e. containing "N"), sequences whose length fell outside the expected size interval and sequences observed less than ten times in at least one PCR replicate were filtered out. The obtained sequences (MOTUs, or Molecular Operational Taxonomic Units) were taxonomically assigned using the program ecotag of the OBITools, which compares the sequence to a database of full-length reference metabarcodes. For the Sper03 marker, two databases were used independently. The first one ("EMBL reference database") was built from the EMBL public database (release 143) by running the ecoPCR program (Ficetola et al., 2010). More specifically, ecoPCR carried out an in silico PCR on the EMBL sequences using the Sper03 primers, with three mismatches allowed per primer. The obtained reference database was further curated by keeping only sequences assigned at the species, genus or family levels. The second database ("local reference database") gathered 34 metabarcode sequences (20 unique sequences) obtained by amplifying the DNA extracted from the plant tissues provided by Select Carbon using the Sper03 primers (see "Local reference databases" paragraph below). For the Sola01 and Acac01 markers, the EMBL and local sequences were combined to constitute a unique reference database. For Poac01 and Cass01, the taxonomic assignment was performed using only the EMBL reference database.

After taxonomic assignment, further data filtering was performed in R to remove spurious or contaminant sequences that can bias ecological conclusions drawn from DNA metabarcoding data (Calderón-Sanou et al., 2020). More specifically, we discarded from our datasets (1) MOTUs with a best identity with EMBL (and local when available) metabarcodes < 90%, as they are potential chimeras; (2) MOTUs that were more numerous in the negative control replicates than in true PCR replicates, as they are contaminants; and (3) PCR replicates with a low sequencing coverage (<1000 sequences), except for the marker Cass01 for which all PCR replicates were kept. After aggregating all PCR replicates per sample, sequences that were present less than 100 times per sample were set to zero to exclude low-level tag jumps contaminations. After this filtering, all conserved samples had a sequencing coverage > 1000 sequences.

Raw reads obtained after sequencing Acac01, Poac01, Sola01 or Sper03 amplicons from plant tissues were processed together with those from feces amplicons until the taxonomic assignment step (see above). Then, for each tissue sample, the most abundant sequence was kept as reference sequence for the corresponding plant taxon and marker if the following two criteria were met: the sequence showed more than 1000 reads in the sample, and it was 50% more frequent than the second most abundant sequence. For the nuclear markers (i.e. all except Sper03), there was a possibility of two equally abundant sequences in the same sample. When this situation occurred, both sequences were kept as reference sequences.

The sequences were read against published sequences in GeneBank (https://www.ncbi.nlm.nih.gov/genbank/)

eDNA Frontiers laboratory

Each faecal sample was sub-sample to 200-280mg, homogenised using the Tissue Lyser, and DNA extracted with a modified Qiagen PowerSoil Pro kit protocol (Qiagen, Germany) using an automated QIAcube extraction platform (Qiagen). All extractions were undertaken in a dedicated PCR-free laboratory, and extraction controls processed alongside samples. Extractions were eluted in a final volume of 100µL AE buffer.

To determine the required dilution for optimal amplification, PCR reactions were performed in duplicate on each extraction by adding DNA template directly to the PCR master mix (neat), then performing two serial dilutions (1in10, and 1in100). The PCRs were performed at a final volume of 25µL where each reaction comprised of: 1× PCR Gold Buffer (Applied Biosystems), 0.25mM dNTP mix (Astral Scientific, Australia), 2mM $MgCl_2$ (Applied Biosystems), 1U AmpliTaq Gold DNA polymerase (Applied Biosystems), 0.4mg/mL bovine serum albumin (Fisher Biotec), 0.4µM forward and reverse primers, 0.6µl of a 1:10,000 solution of SYBR Green dye (Life Technologies), and 2µL template DNA. PCRs were performed on StepOne Plus instruments (Applied Biosystems) with the following cycling conditions: 95°C for 5min, followed by 50 cycles of: 95°C for 30sec, 49°C for 30sec, 72°C for 45sec, then a melt-curve analysis of: 95°C for 15sec, 60°C for 1min, 95°C for 15sec, finishing with a final extension stage at 72°C for 10min.

After selection of the optimal dilution (neat, 1in10, or 1in100), PCRs were repeated in duplicate as described above but instead using unique, single use combinations of 8 bp multiplex identifier-tagged (MID-tag) primers as described in Koziol et al. (2019) and van der Heyde et al. (2020). Master mixes were prepared using a QIAgility instrument (Qiagen) in an ultra-clean lab facility, with negative and positive PCR controls included on every plate to ensure the validity of results. A sequencing library was created by combining samples into mini-pools based on the PCR amplification results from each sample. The mini-pools were then combined in roughly equimolar concentrations to form a library. The library was then size selected (200-600 bp cut-off) using a Pippin Prep instrument (Sage Sciences) with 2% dye-free cassettes, cleaned using a QIAquick PCR purification kit, quantified on a Qubit (Thermo Fisher), and diluted to 2nM. The library was sequenced on an Illumina MiSeq instrument using a 500-cycle kit with custom sequencing primers.

Raw metabarcoding sequence data was analysed using the eDNAFlow pipeline (Mousavi-Derazmahalleh *et al*., 2021) with custom filtering parameters applied (--minAlignLeng '12', --minLen '150', --minsize '2', perc_identity '95', --qcov '100', --minMatch_lulu '97').

Generated ZOTUs were queried against the nucleotide database NCBI (GenBank) and the PilbSeq database (https://pilbseq.dbca.wa.gov.au) and assigned to the species level where possible or dropped back to the lowest common ancestor if multiple possible taxonomic assignments were given.

Appendix 3 – Presence and distribution of plant species fDNA (number of sequence reads) in cattle sampled at Challa station in April/May 2023

Attached as Excel