Tropical beef cattle growth performance and meat quality in response to backgrounding on *Desmanthus* spp. pastures

By

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Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

College of Public Health, Medical and Veterinary Sciences

James Cook University, Townsville, Queensland, Australia

August 2022

Acknowledgements

I would like to thank the individuals who helped make my PhD journey incredible and successful. First, I express my sincere gratitude to my advisors, Professor Aduli Malau-Aduli, Mr Christopher Gardiner, Dr Robert Kinobe, Associate Professor Bunmi Malau-Aduli and Dr Edward Charmley for their guidance, knowledge, support and friendship both as a team and individually throughout my PhD journey. They created a very favourable working environment and gave me a platform to explore my research potential to the fullest. Words are not sufficient to fully express my appreciation. I will forever be grateful for the opportunity to learn from them.

I am thankful to Shedrach Pewan, Bénédicte Suybeng, John Otto, Jemma Starling, David Blignaut, Trevor Hall, Jenny Stanford, Wayne Flintham, Heitor Fleury, Melissa Mathews, Holly Reid, Steve Austin, Jess Simington, Stefania Maffei, Khalu Tomachy, Paulo Delbone and Ewerton Delbone for their assistance during the feeding trial and laboratory work. Appreciations go to the staff of CSIRO Oceans and Atmosphere Laboratory (Hobart, Australia) for fatty acids analysis, CSIRO Floreat (Perth, Western Australia) for diet nutrient composition analysis and CSIRO St Lucia (Brisbane, Queensland) for rumen ammonia nitrogen and volatile fatty acids analysis. Thanks to Oyelola Adegboye for his assistance with the experimental design and various statistical analyses. Special thanks to Bénédicte Suybeng and Shedrach Pewan for their friendship, emotional support and encouragement.

I thankfully acknowledge the Northern Australian Pastoral Company and Agrimix Pty Ltd for supplying experimental animals, and access to Cungelella cattle station, CSIRO Lansdown pasture research station, Wainui feedlot and Dingo Park feedlot for cattle management support.

Appreciation also goes to JBS Australia for their assistance with abattoir processing and grading of experimental steers.

I thank the Cooperative Research Centre Projects of the Australian Government's Department of Industry, Innovation and Science (Canberra, Australian Capital Territory) and the College of Public Health, Medical and Veterinary Sciences, James Cook University (Townsville, Queensland) for financial support throughout my PhD program.

I am very thankful to my parents Simon Mwangi and Mary Waitherero, siblings Hellen Mugure, Elijah Gachau, Esther Wambui, Anastacia Wangari, Cecilia Wanjiru and Jane Wangui, and nieces Shaleen Waitherero, Felister Waithera, Precious Waitherero, Maybelle Waitherero and Ruth Nyambura for the love, encouragement and support. Thanks to my mentors Prof Rina Meidan and Dr Mathew Gicheha for their continued encouragement and advice. Thanks to Umoja Kenya Townsville community and my friends Kipyegon Cheruiyot, Mugagga Kalyesubula, Wayne MacDonald, Ernest and Letizia Briet, Peter and Kylie Geary, Escain Kiwonde and Anthony Namodi for their encouragement and friendship.

Finally, I dedicate this thesis to the memory of my grandmother Hellen Mugure Gachau who peacefully left this world during my PhD journey.

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Declaration

I hereby declare that the research presented and reported in this thesis was carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 8th edition, 2013 and the James Cook University Animal Ethics Committee approved guidelines (Approval Number 2639) or the Commonwealth Scientific and Industrial Research Organisation Animal Ethics Committee approved guidelines (Approval Numbers 2019-38 and 21/05).

To the best of my knowledge, this thesis contains no material which has been accepted for the award of a degree by the University or any other tertiary institution and contains no material previously published or written by any other person, except where due reference is made in the text of the thesis.

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Financial support

Cooperative Research Centre Projects (CRC-P) [grant number CRC P-58599] from the Australian Government's Department of Industry, Innovation and Science

PhD scholarship funded by the College of Public Health, Medical and Veterinary Sciences, James Cook University

Data collection support

Professor Peter Nichols, Dr John Otto, Dr Shedrach Pewan, Dr David Blignaut, Dr Bénédicte Suybeng, Dr Stuart Denman, Dr Darryl Savage, Dr Glen Walker, Mr Wayne Flintham, Ms Melissa

Matthews, Ms Holly Reid, Ms Jessica Simington, Ms Jenny Stanford, Mr Steve Austin, Mr Heitor Fleury, Mr Trevor Hall, Ms Jemma Starling, Ms Elizabeth Hulm, Ms Wendy Smith, Ms Stefania Maffei, Mr Khalu Tomachy, Mr Paulo Delbone, Mr Jason Collins, Ms Donna Collins and Mr Ewerton Delbone.

Abstract

Dietary crude protein and dry matter digestibility are among the major factors limiting feed intake and weight gain of cattle grazing native and improved grass pastures in the subtropics of northern Australia during the dry season. Incorporating a suitable legume into grasses improves pasture quality and cattle weight gain, but only a limited number of legume species can establish and persist in the harsh semi-arid environment with vertosol soils. *Desmanthus* spp. (desmanthus) is a tropical and subtropical legume that is well adapted to such harsh environments. This thesis evaluated the effect of augmenting grass-based diets of tropical crossbred beef cattle with *Desmanthus* during backgrounding. The aims were to investigate changes in nutritive value of desmanthus with maturity and impacts on rumen fermentation, plasma metabolite profile, growth performance, carcass quality, meat fatty acid profile and associations with single nucleotide polymorphisms (SNP) in lipogenic genes as potential molecular markers for genetic improvement. The hypotheses tested were:

a) the nutritive value and *in situ* degradability of desmanthus changes with maturity and would differ between cultivars;

b) backgrounding steers on desmanthus-augmented grass pastures would elicit significant changes in plasma metabolites resulting in higher liveweight gains than in steers on grass only pastures;

c) tropical beef steers fed isonitrogenous diets supplemented with incremental levels of desmanthus would have similar growth rates, rumen fermentation and plasma metabolite profiles;

d) tropical beef steers backgrounded on grass only or desmanthus-augmented grass pastures with similar backgrounding growth performance would not differ in feedlot growth performance and carcass quality;

e) tropical steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce carcasses with similar characteristics and fatty acid composition; and

f) SNP in the fatty acid binding protein 4, stearoyl-CoA desaturase and fatty acid synthase genes are associated with chiller-assessed carcass traits and fatty acid composition within the loin eye muscle of tropical crossbred beef steers.

To test these hypotheses, six experiments were conducted using three desmanthus cultivars, namely *Desmanthus virgatus* cv. JCU2, *Desmanthus bicornutus* cv. JCU4 and *Desmanthus leptophyllus* cv. JCU7. First, the change in chemical composition and degradability of desmanthus with maturity stage at harvest were evaluated. This study was followed by the evaluation of weight gain, rumen volatile fatty acids, plasma metabolite profile, carcass quality and loin eye muscle fatty acid composition in response to desmanthus feeding. Finally, SNP in the lipogenic genes were identified using targeted Next-Generation Sequencing and their association with carcass traits and fatty acid profile were examined. The study findings demonstrate that:

1) All desmanthus cultivars were highly nutritious and degradable at early maturity, but declined in leaf to stem mass ratio, crude protein and degradability with advanced maturity associated with increase in the structural carbohydrates.
2) The crude protein of the leaf proportion remained above 16% for all cultivars even in the mature desmanthus forage.
3) Desmanthus at 11.5% of pasture botanical composition oversown in buffel grass under field conditions did not improve steers growth performance, plasma metabolite profile or carcass quality compared to the buffel grass-only pastures (negative control).

4) Under controlled experimental settings, increasing desmanthus levels in isonitrogenous diets reduced total rumen volatile fatty acid composition linearly.
5) Growth performance, molar volatile fatty acid proportions, plasma metabolites profile and loin eye muscle total SFA, MUFA or PUFA did not differ when steers were fed Rhodes grass diets supplemented with desmanthus only, desmanthus and lucerne or lucerne only (positive control) in isonitrogenous diets.
6) All steers gained weight at a rate above the 0.4 kg/day threshold required for cattle to attain the target slaughter weight for the prime beef market within 2.5 years of age. However, steers were lean after backgrounding but met the required minimum IMF for consumer-preferred palatability after feedlot finishing for 95 days. The recommended healthy n-6/n-3 PUFA ratio threshold of 4.0 was also maintained.
7) Potential SNP markers associated with carcass traits and fatty acid profile were identified.

Taken together, these findings indicate that desmanthus can be used for high growth performance to achieve prime market slaughter weight targets for beef cattle in the harsh environmental regions of northern Australia that lack a well-adapted legume. Furthermore, desmanthus can be used to augment diets of cattle grazing low quality grass pastures in northern Australia with no negative effect on meat quality. Feedlot finishing for a short period of time improved meat palatability. Finally, the identified SNP in lipogenic genes demonstrate that SNP can be used in marker-assisted selection to breed cattle with favourable meat fatty acid composition and carcass quality. Future studies are required to evaluate the effect of desmanthus on lipolysis and biohydrogenation of unsaturated fatty acids in the rumen, consumer-assessed sensory evaluation of meat eating quality, effects of SNP on fat regulatory gene expression and proteomics of tropical crossbred beef cattle.

List of Publications from Thesis

Published Peer-reviewed Journal Papers with 2021 Impact Factor (IF)

1. **Mwangi FW**, Suybeng B, Gardiner CP, Kinobe RT, Charmley E, Malau-Aduli BS, Malau-Aduli AEO. 2022. Effect of incremental proportions of *Desmanthus* spp. in isonitrogenous forage diets on growth performance, rumen fermentation and plasma metabolites of pen-fed growing Brahman, Charbray and Droughtmaster crossbred beef steers. ***PLoS ONE*** 17(1): e0260918 **(IF 3.752)** DOI: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0260918
2. **Mwangi FW**, Charmley E, Adegboye OA, Gardiner CP, Kinobe RT, Malau-Aduli BS, Malau-Aduli AEO 2022. Chemical composition and in situ degradability of *Desmanthus* spp. forage harvested at different maturity stages. ***Fermentation*** 8 (8): 377 **(IF 5.123)** DOI: https://doi.org/10.3390/fermentation8080377
3. **Mwangi FW**, Pewan SB, Otto JR, Adegboye OA, Charmley E, Gardiner CP, Malau-Aduli BS, Kinobe RT and Malau-Aduli AEO 2022. Towards sustainable sources of omega-3 long-chain polyunsaturated fatty acids in northern Australian tropical crossbred beef steers through single nucleotide polymorphisms in lipogenic genes for meat eating quality. ***Sustainability*** 14 (14): 8409 **(IF 3.889)** DOI: https://doi.org/10.3390/su14148409
4. **Mwangi FW**, Pewan SB, Otto JR, Adegboye OA, Charmley E, Gardiner CP, Malau-Aduli BS, Kinobe RT and Malau-Aduli AEO 2022. Single nucleotide polymorphisms in the fatty acid binding protein 4, fatty acid synthase and stearoyl-CoA desaturase genes influence carcass characteristics of tropical crossbred beef steers. ***Agriculture*** 12 (8): 1171 **(IF 3.408)** DOI: https://doi.org/10.3390/agriculture12081171

5. **Mwangi FW**, Blignaut DJC, Charmley E, Gardiner CP, Malau-Aduli BS, Kinobe RT a Malau-Aduli AEO 2021. Lipid metabolism, carcass characteristics and longissimus dorsi muscle fatty acid composition of tropical crossbred beef cattle in response to *Desmanthus* spp. forage backgrounding. ***Metabolites*** 11 (12): 804 **(IF 5.581)**
DOI: https://doi.org/10.3390/metabo11120804
6. **Mwangi FW**, Gardiner CP, Walker G, Hall TJ, Malau-Aduli BS, Kinobe RT, Malau-Aduli AEO 2021. Growth performance and plasma metabolites of grazing beef cattle backgrounded on buffel or buffel-desmanthus mixed pastures. ***Animals*** 11(8): 2355 **(IF 3.231)**
DOI: https://doi.org/10.3390/ani11082355
7. **Mwangi FW**, Charmley E, Gardiner CP, Malau-Aduli BS, Kinobe RT and Malau-Aduli AEO 2019. Diet and genetics influence beef cattle performance and meat quality characteristics. ***Foods*** 8(12): 648 **(IF 5.561)** DOI: https://doi.org/10.3390/foods8120648

Submitted Peer-reviewed Journal Manuscript Currently Under Review

1. **Mwangi FW**, Savage D, Gardiner CP, Charmley E, Malau-Aduli BS, Kinobe RT and Malau-Aduli AEO 2022. Feedlot growth performance and carcass traits of steers backgrounded on buffel grass or buffel-desmanthus mixed pastures. ***Frontiers in Veterinary Science*** **(IF 3.471)**

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List of abbreviations

ABS = Australian Bureau of Statistics

ADF = acid detergent fibre

ADG = average daily gain

ALA = alpha-linolenic acid

ARA = arachidonic acid

AT/MT = malonyl-CoA-/acetyl-CoA-acyl carrier protein-transacylase

BCS = body condition score

BGVD = Bovine genome variation database

BHB = beta-hydroxybutyrate

cDNA = complementary deoxyribonucleic acid

CLA = conjugated linoleic acid

CP = crude protein

CSIRO = Commonwealth Scientific and Industrial Research Organisation

CWE = carcass weight equivalents

DAF = Department of Agriculture and Fisheries

DHA = docosahexaenoic acid

DM = dry matter

DMD = dry matter digestibility

DMI = dry matter intake

DNA = deoxyribonucleic acid

DPA = docosapentaenoic acid

EMA = loin eye muscle area

EPA = eicosapentaenoic acid

FA = fatty acid

FABP4 = fatty acid binding protein 4

FAO = Food and Agriculture Organization

FASN = fatty acid synthase

FMP = fat melting point

GPS = global positioning system

HCW = hot standard carcass weight

He = heterozygosity

HWE = Hardy–Weinberg equilibrium

IMF = intramuscular fat

LA = linoleic acid

L/S = leaf to stem mass ratio

LW = liveweight

MAF = minor allele frequency

ME = metabolisable energy

MLA = Meat & Livestock Australia

MSA = Meat Standards Australia

MUFA = monounsaturated fatty acids

N = nitrogen

n-3 LC-PUFA = omega-3 long-chain polyunsaturated fatty acids

n-3 PUFA = omega-3 polyunsaturated fatty acids

n-6 PUFA = omega-6 polyunsaturated fatty acids

NCBI = National Center for Biotechnology Information

NDF = neutral detergent fibre

NEFA = non-esterified fatty acids

NH_3-N = ammonia nitrogen

NIRS = near infrared reflectance spectroscopy

OECD = Organisation for Economic Co-operation and Development

OM = organic matter

PCR = polymerase chain reaction

PCS = protein coding sequence

PIC = polymorphism information content

PPARγ = peroxisome proliferator-activated receptor gamma

PUFA = polyunsaturated fatty acids

QTL = quantitative trait loci

RDI = recommended dietary intake

RFI = residual feed intake

SCD = stearoyl-CoA desaturase

SEM = standard error of the mean

SFA = saturated fatty acids

SNP = single nucleotide polymorphisms

VFA = volatile fatty acids

Chapter 1: General Introduction

Australia is a major contributor to the global meat industry in terms of beef production and export. In 2020, Australia was ranked seventh in world beef production and second in beef exports after Brazil, with 1.4 and 2.5 Million tonnes of carcass weight equivalents (CWE), respectively (FAO, 2021). The beef cattle industry contributes significantly to the Australian economy, accounting for 24% ($14.6 billion) of the 2019-2020 total gross value of farm production (ABS, 2021). In addition, beef plays a significant role in nutrition. In 2020, Australian beef consumption was estimated to be among the top seven globally at 18.1 kg/capita (OECD, 2021). The Australian beef cattle population is currently 21 M head, occupies half of the Australian farms and accounts for 75% of the total agricultural landmass (ABS, 2021; ABS, 2022). Over half of the national beef cattle herd is in northern Australia, with 46% in Queensland and 16% in Western Australia and Northern Territory (MLA, 2019). Queensland alone accounted for 1.1% of the global beef herd in 2017 and 8% of world beef exports in 2016 (DAF, 2018).

In northern Australia's dry tropical environment, beef cattle rely mainly on extensive grazing on unimproved native pastures dominated by C_4 grasses with limited tree clearing and use of exotic pasture species. The C_4 grasses are grasses with a specialized photosynthetic pathway that minimizes photorespiration. This leads to increased photosynthetic performance in environments of high temperature and/or low carbon dioxide (Edwards and Smith, 2010; Hattersley, 1983; Hunt et al., 2014). The dry tropics are characterized by a distinct wet and dry season. Both seasons vary in length. For instance, the dry season varies from four to nine months of the year (Poppi et al., 2018). As a result, the quantity and nutritive value of pastures vary widely throughout the year. Pasture growth takes place in the wet season from November to April, resulting in increased green

herbage mass, crude protein (CP) content and dry matter digestibility (DMD). Towards the end of the wet/growing season and during the dry season, pasture senescence reduces green herbage mass, CP content, DMD and consequently cattle DM intake (DMI) (McCown, 1981; Allison, 1985). Thus, high cattle weight gains are observed during the wet season, which can exceed a kilogram per day (Poppi et al., 2018), but reduces in the dry season sometimes resulting in weight loss (Hill et al., 2009). This cycle continues until cattle reach market weight, which may take up to four years (McLennan, 2014; Poppi et al., 2018). Cattle gain approximately 100 – 130 kg/year grazing native pastures and 150 – 180 kg/year on introduced grasses (Winter et al., 1991). In extensive rangelands, high target slaughter weights to meet the ground meat trade in North America can be achieved with minimal inputs at 3.5 – 4.5 years of age and is profitable (O'Reagain et al., 2011; Poppi et al., 2018). However, prime markets require younger animals, hence the new marketing system of Meat Standards Australia (MSA) requires animals to be < 2.5 years of age and target weight of around 550 – 600 kg for their carcasses to meet the highest grade levels (Poppi et al., 2018).

Studies have reported that higher cattle growth rates to meet the MSA standards can be achieved through diet and genetic manipulation (Bryan et al., 2014; Poppi et al., 2018). Supplementing beef cattle grazing poor-quality tropical pastures with the limiting nutrients results in increased growth rate (Preston and Leng, 1987). Supplements optimize the availability of nutrients for rumen fermentative digestion and utilization of nutrients that are products of fermentation (Leng, 1990) and increase the mass of microbial protein flowing out of the rumen into the small intestine where it provides amino acids for the animal (Burrow, 2014). However, the cost of supplementation in extensive grazing systems is limiting (Poppi and McLennan, 2010; DAF, 2019). To reduce or eliminate the use of supplements during backgrounding (the period post weaning when cattle are

fed on pasture to enhance growth prior to finishing and slaughter; also referred to as the stocker phase), the backgrounding phase may be followed by a finishing period in the feedlot with nutrient-dense diets for a short period of about 70 – 100 days, or cattle can be fed on good quality pasture until slaughter (Poppi et al., 2018).

The potential of legume pastures to improve beef cattle production in the tropics is well recognized (McCown, 1981; Kanani et al., 2006; Hill et al., 2009). Incorporating highly digestible legumes into poor-quality grass-based pastures provides a renewable source of protein, improves animal energy intake, feed conversion, rumen function, and increases mineral and vitamin availabilities (Rochon et al., 2004; Kanani et al., 2006), thus improving cattle average daily gain and overall weight gain (Radrizzani and Nasca, 2014). Augmenting grass pastures with legumes of the genus *Stylosanthes* is reported to enhance cattle weight gains by 30 – 60 kg/head/annum compared to grass-only pastures (Coates et al., 1997). Similarly, leucaena is reported to increase cattle growth rate and stocking rate (Shelton and Dalzell, 2007; Beutel et al., 2018; Bowen et al., 2018). Legume forages retain higher green leaf mass for a longer period during the dry season compared to grass pastures (Hill et al., 2009). The leaf proportion of the plant is richer in membrane lipids compared to stems and seeds, thus higher alpha-linolenic acid (ALA), a fatty acid abundant in forages (Glasser et al., 2013). In addition, plant secondary metabolites such as tannins and saponins in legumes may modulate fatty acids lipolysis and biohydrogenation in the rumen (Kronberg et al., 2007; Alves et al., 2017). Moreso, ALA, the building block of the longer chain (≥ C20) omega 3 polyunsaturated fatty acids (n-3 PUFA) in the muscle through elongation and desaturation (Scollan et al., 2006; Scollan et al., 2014).

In northern Australia, pasture legumes have been in use for six decades (Mannetje, 1997), with more attention placed on the development of legumes adapted to the lighter textured soil (Coates

et al., 1997). Annual and perennial legume pastures such as *Leucaena leucocephala* (Leucaena), *Stylosanthes seabrana* (Caatinga stylo), *Clitoria ternatea* (Butterfly pea), *Macroptilium bracteatum* (Burgundy bean) and *Desmanthus* spp. (desmanthus) are suitable for the heavier textured cracking clay soils. They have been, or are being, developed/introduced to northern Australia where there is a dearth of suitable species (Schlink and Burt, 1993; MLA, 2001; Cook et al., 2005; Burnett et al., 2012). The few publications available indicate that these legumes can be used to improve cattle growth performance in tropical and subtropical northern Australia, although more data are required (Clem, 2004; Hill et al., 2009; Gardiner and Parker, 2012). In particular, desmanthus has shown more commercial potential for livestock production in northern Australia compared to other legumes adapted to the heavier textured cracking clay soils. This is because it grows in a wide range of soil textures and pH (Jones and Brandon, 1998; Cook et al., 2005; Gardiner et al., 2012). It is also drought tolerant (Schlink and Burt, 1993; Jones and Brandon, 1998) and withstands heavy grazing (Gardiner and Swan, 2008).

Cultivation of the pasture legume desmanthus in pastureland continues to grow in northern Australia, and over 50 000 ha of desmanthus have been established since 2012 (Gardiner et al., 2021). Desmanthus is a highly nutritious legume forage with CP ranging between 12% and 19% for whole leafy plants (Vandermeulen et al., 2018; Suybeng et al., 2021a) and at least 56% digestibility (Durmic et al., 2017). Augmenting low-quality grass pastures with desmanthus is reported to potentially reduce enteric methane emissions and improve rumen fermentation in cattle (Suybeng et al., 2020). Desmanthus is a tannin-containing legume (Vandermeulen et al., 2018; Suybeng et al., 2021b) that may modulate meat fatty acid composition by reducing fatty acids lipolysis and biohydrogenation in the rumen (Kronberg et al., 2007; Alves et al., 2017). Previous studies carried out to examine the effect of desmanthus on dry matter intake (DMI) and animal

liveweight gain in steers, sheep and goats reported positive outcomes (Gardiner and Parker, 2012; Marsetyo et al., 2017; Aoetpah et al., 2018). These studies suggest the possibility of improving beef cattle growth performance using desmanthus pastures. However, these previous studies were conducted indoors or in small paddocks (except one in 250 ha paddock), which do not represent practical commercial farm settings. In addition, literature gaps exist on the effect of desmanthus pasture backgrounding on carcass traits and meat quality of tropical beef cattle breeds, whose population has continued to grow over the years. For instance, the population of tropical beef cattle and their crosses increased from below 0.1% to 64% of Queensland's beef cattle herd between 1930 and 1982 (Bindon and Jones, 2001).

Tropical cattle breeds (*Bos indicus*) dominate the northern Australian beef herd due to their greater ability to grow and reproduce under harsh climatic conditions of high ambient temperatures, humidity, parasites, seasonally poor pasture quality and long walking distances while grazing compared to temperate breeds (*Bos taurus*) (Davis, 1993; Schatz et al., 2020b). These tropical breeds include purebred Brahman and breeds derived from creating stable crosses between Brahman and various *Bos taurus* breeds. The derived breeds include Droughtmaster, Braford, Brangus, Charbray and Santa Gertrudis (Davis, 1993). These cattle breeds have been used to serve the live export market to South East Asia, the primary market for northern Australian cattle (Schatz et al., 2020b). However, the growth rate and meat eating quality of *Bos indicus* cattle are comparatively lower (Bryan et al., 2014) than those of *Bos taurus* cattle (Johnson et al., 1990; Thrift and Thrift, 2002). The lower meat eating quality is attributable to less marbling related to the reduced intramuscular adipocyte volume (Cooke et al., 2020) and less tenderness due to reduced protein hydrolysis (Whipple et al., 1990), while the comparatively lower growth rate emanates from lower voluntary feed intake (Cooke et al., 2020). As a result, *Bos indicus* meat is

less preferred in the Australian domestic market (Schatz et al., 2020b) and fails to meet the requirements for prime export markets (Poppi et al., 2018). Broad consumer assessment of beef from cattle of various genetic, nutritional and environmental backgrounds in Australia demonstrated a measurable negative impact of *Bos indicus* content on meat sensory characteristics of tenderness, marbling and juiciness (Wolcott et al., 2009).

Overall sensory characteristics and healthy nutritional attributes are key factors of meat eating quality that strongly influence the willingness-to-pay decisions of beef consumers (Ardeshiri and Rose, 2018; Felderhoff et al., 2020; Garmyn, 2020). A rise in health concerns in the association of higher consumption of red meat and processed meat with increased risk of non-communicable chronic diseases such as cardiovascular disease, type 2 diabetes and gastrointestinal cancer has been reported (McAfee et al., 2010; Ekmekcioglu et al., 2018). Although controversy exists (Dehghan et al., 2017), this perception emanates from the high content of saturated fatty acids (SFA), low content of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) and variable trans-FA content (Bessa et al., 2015). The n-3 LC-PUFA confers anti-inflammatory effects (McAfee et al., 2010), improves brain and retinal development, maternal and offspring health, cognitive function and psychological status in humans (Mapiye et al., 2015). Current recommendations for various dietary fat fractions are 15 – 35%, <10%, <2.5 – 9%, <2 – 3% and <1% of total energy intake for total fat, SFA, n-6 PUFA, n-3 PUFA and trans fatty acids, respectively (WHO, 2002; FAO, 2008), and the ratios of PUFA:SFA at 0·45 and n-6:n-3 PUFA below 4 (Department of Health, London, 1994). Although seafood is the richest dietary source of n-3 LC-PUFA, its consumption in Western countries is insufficient (Byelashov et al., 2015). For instance, seafood contributes only 22 g of the Australian 152 g daily per capita meat consumption (Sui et al., 2016). As a result, there is increasing focus on studies aimed at elevating the levels of

beneficial n-3 LC-PUFA and reducing SFA in beef, especially in intramuscular fat, since it cannot be trimmed out (Scollan et al., 2006; Troy et al., 2016) while maintaining or improving meat eating quality (Woods and Fearon, 2009).

Although sex, age, pre-slaughter and post-slaughter handling are known to influence meat eating quality (Guerrero et al., 2013), genetic and dietary factors play a major role in beef quality outcomes (Coates et al., 1997; Johnston and Jeyaruban, 2014; Avilés et al., 2015). High concentrate diets lead to surplus energy consumption in cattle (Muir et al., 1998), increase carcass overall fatness and marbling (Duckett et al., 2013), which in turn, improves meat tenderness, juiciness and flavour (Pogorzelski et al., 2022). Pasture grazing or supplementing cattle with plant oil-seed meals, microalgae and fish oil increases n-3 LC-PUFA levels in meat (French et al., 2000; Scollan et al., 2001; Mapiye et al., 2013; Madeira et al., 2017). While dietary supplementation with fish oil increases n-3 LC-PUFA content in meat (Scollan et al., 2001), it has been shown to have a negative effect on meat flavour (Wood et al., 2003). The use of diet to manipulate beef fatty acid composition is complicated by rumen bacterial biohydrogenation of ingested unsaturated fats into more saturated fats (Harfoot, 1981; Menci et al., 2021). As a result, studies recommend the use of genetic selection of cattle to manipulate the fatty acid composition and meat quality characteristics (Bidner et al., 2002; Gao et al., 2007; Aboujaoude et al., 2018) or combining genetic selection and dietary manipulation (Chung et al., 2007).

The heritability of beef carcass and meat quality traits, including marbling and fatty acid composition, is reported to range from low to high (Pitchford et al., 2002; De Smet et al., 2004; Kelly et al., 2013; Savoia et al., 2019). Desirable meat quality traits and healthy fatty acid profile targets can be achieved by exploiting breed differences through crossbreeding or selection within breeds. For instance, there are numerous reports of breed differences in the fatty acid composition

of subcutaneous and intramuscular fat in cattle (Zembayashi et al., 1995; Malau-Aduli et al., 2000b; Park et al., 2018). Generally, *Bos taurus* cattle have higher marbling than their *Bos indicus* counterparts (Kelly et al., 2013; Park et al., 2018). However, the improvement of meat quality using traditional selection methods is difficult because its heritability varies with breed, and data collection is difficult and expensive because it is determined polygenically (Gao et al., 2007). Although several genes are reported to influence meat quality, their effects and interactions are not fully understood. Genes responsible for lipid synthesis and metabolism are reported to influence meat quality (Mannen, 2011). These include genes encoding the delta-5 (Δ5), Δ6 and Δ9 desaturases (Zietemann et al., 2010), stearoyl-CoA desaturases (*SCD*), fatty acid binding proteins (FABP) (Ordovas, 2007; Lee et al., 2010), fatty acid synthase (*FASN*) (Rempel et al., 2012) and growth hormone (Gao et al., 2007). These genes vary widely in polymorphism and expression between cattle breeds resulting in varying carcass fatness, fatty acid compositions (Laborde et al., 2011) and, subsequently, meat eating quality. Sequencing these genes can help to identify significant loci linked to meat quality traits that can be applied in cattle breeding programs using marker-assisted selection (Gao et al., 2007). Several genetic markers for meat quality traits like tenderness and marbling have been identified and commercialized (Johnston and Graser, 2010).

Genes responsible for lipid synthesis and metabolism, such as *SCD*, *FASN* and *FABP4,* are reported to influence carcass fat traits in Korean (Cho et al., 2008), Japanese (Dujková et al., 2015), American (Michal et al., 2006) and Australian cattle (Barendse et al., 2009). More studies are required to identify single nucleotide polymorphisms (SNP) in these genes and how the SNP may influence carcass traits and meat fatty acid profile, especially in the tropical breeds and their crosses raised in northern Australia. Given all the outlined factors affecting beef production and meat quality of cattle in northern Australia and the identified knowledge gaps in the published

literature, the series of studies reported in this thesis were designed to bridge these gaps. The primary focus was on the effect of desmanthus pasture backgrounding on livestock growth performance, carcass characteristics, meat quality, muscle fatty acid profiles and their associations with SNP in lipogenic genes in tropical beef cattle breeds. The studies were based on both extensive and intensive management systems of on-farm and group-feeding pen trials followed by feedlot finishing on high grain diets to answer the following research questions:

a) What is the nutritive value and *in situ* degradability of desmanthus and how does it change with maturity?
b) What are the growth performance, rumen fermentation and plasma metabolite characteristics of grazing beef cattle backgrounded on desmanthus-augmented grass pastures?
c) What are the growth performance patterns and plasma metabolite profiles of beef cattle fed incremental levels of desmanthus forage in isonitrogenous diets?
d) What are the loin eye muscle intramuscular fat contents, fat melting points and fatty acid profiles of beef cattle fed incremental levels of desmanthus forage in isonitrogenous diets?
e) Does desmanthus backgrounding influence feedlot growth performance and carcass traits?
f) What are the associations between SNP in *SCD*, *FASN* and *FABP4* genes with carcass traits, meat IMF and fatty acid composition of tropical crossbred beef cattle?

The main research objectives were: 1) To evaluate the nutritive value and rumen degradability of desmanthus forage; 2) To evaluate the impact of desmanthus pastures on tropical crossbred beef cattle backgrounding and feedlot growth performance, plasma metabolites, carcass traits and meat fatty acid composition; and 3) To investigate the associations between SNP in *SCD*, *FASN* and *FABP4* genes with carcass traits and fatty acid composition.

These objectives were tailored to 1) Provide beef cattle farmers with an alternative to achieve optimal finishing weights at an earlier age to meet the market specifications for prime carcass grade; 2) Identify and make early selection decisions about cattle with the potential to produce high quality meat without having to wait until slaughter; and 3) Provide beef consumers with meat products rich in the health beneficial omega-3 polyunsaturated fatty acids. Therefore, this thesis is structured into the following chapters:

Chapter 1: General introduction

Chapter 2: Literature review: Presents a comprehensive exploration of the published literature on the Australian beef industry with a specific focus on cattle genetic management and the impact of tropical pasture grazing, nutritional supplementation during feedlot finishing and fat metabolism-related genes on beef cattle performance and meat eating quality traits.

Chapter 3: Designed to address the first research objective; investigating changes in forage nutritive values and rumen degradability of three desmanthus cultivars with maturity and the first order interaction between maturity and cultivar. The tested hypothesis was that the nutritive value and *in situ* degradability of desmanthus would differ between cultivars and change with maturity.

Chapter 4: Addresses the second research objective and is divided into two sections: **Chapter 4.1** investigates the growth performance and blood plasma metabolite profiles of grazing beef cattle backgrounded on desmanthus-augmented grass pastures. The aim was to test the hypothesis that backgrounding steers on desmanthus-augmented grass pastures would elicit significant changes in plasma metabolites resulting in higher liveweight gains than in steers on grass-only pastures. **Chapter 4.2** investigates the growth performance, rumen fermentation and blood plasma metabolite profiles of tropical beef cattle fed incremental levels of desmanthus forage in

isonitrogenous diets. The aim was to test the hypothesis that tropical beef steers fed isonitrogenous diets supplemented with incremental levels of desmanthus would have similar growth rates, rumen fermentation and plasma metabolite concentrations.

Chapter 5: Investigates the feedlot performance and carcass traits of steers backgrounded on desmanthus-grass pastures. This chapter is divided into two: **Chapter 5.1** evaluates the feedlot growth performance and carcass traits of steers backgrounded on desmanthus pastures. The aim was to test the hypothesis that tropical beef steers backgrounded on grass only or desmanthus-augmented grass pastures with similar backgrounding growth performance would not differ in feedlot growth performance and carcass quality. **Chapter 5.2** investigates the loin eye muscle intramuscular fat content, fat melting point and fatty acid profile of beef cattle fed incremental levels of desmanthus forage in isonitrogenous diets. The aim was to test the hypothesis that tropical steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce carcasses with similar characteristics and fatty acid composition.

Chapter 6: Addresses the third research objective to test the hypothesis that SNP in the *FABP4*, *SCD* and *FASN* genes are associated with chiller-assessed carcass traits and fatty acid composition within the loin eye muscle of tropical crossbred beef steers. The chapter is divided into two sections: **Chapter 6.1** investigates the associations between SNP in *SCD*, *FASN* and *FABP4* genes with carcass traits. **Chapter 6.2** investigates the associations between SNP in *SCD*, *FASN* and *FABP4* genes with fatty acid composition within the loin eye muscle.

Chapter 7: Presents a general discussion and summary of the main findings from the series of studies, practical implications and recommendations for future research.

Appendices: Contains relevant but excluded supplementary materials from the thesis chapters, ethics approvals and copies of submitted and published peer-reviewed journal manuscripts emanating from this thesis.

Chapter 2: Literature Review

2.1. Beef Industry Overview

2.1.1. The role of beef consumption in human nutrition

Beef plays a significant role in global human nutrition. It is the third most consumed meat in the world after poultry and pork at 6.4, 14.0 and 12.2 kg per capita, respectively (OECD, 2021). Beef consumption continues to rise in line with growth in population and increase in household incomes. By 2027, it is estimated that beef consumption will be 8% and 21% higher in the developed and developing countries, respectively, compared to the 2015 to 2017 average (OECD and FAO, 2018). Beef is a nutrient-dense food that provides health-beneficial macro- and micro-nutrients for humans. A 100 g serving of beef provides more than the 25% recommended dietary intake (RDI) of protein, niacin, vitamin B_6, vitamin B_{12}, zinc and selenium, and more than 10% RDI of phosphorus, iron and riboflavin. Beef provides high quality protein that contains all the essential amino acids (Troy et al., 2016) and antioxidants such as carnosine and anserine (Williams, 2007; Asp et al., 2012).

2.1.2. The Australian beef industry

Cattle were introduced into Australia in 1788, and the intention was to assist with the country's development (Burrow, 2014). These cattle consisted of only European breeds until the introduction of Brahman cattle in the 1930s. However, the *Bos taurus* continued to dominate until the 1950s when more *Bos indicus* were introduced. A mix of *Bos taurus*, *Bos indicus* and their crosses led to a rapid increase in the cattle population that reached a peak of 29.8 M in 1976 (ABS, 2005). Currently, the Australian cattle population stands at 23.4 M head, comprising 21.1 M beef and 2.3

M dairy cattle (ABS, 2022). The red meat and livestock industry, comprising beef, sheep and goat industries, employed 195 800 people directly, and a further 249 000 people were employed in businesses servicing the red meat and livestock industry (MLA, 2021). Although Australia accounts for a small proportion of the global cattle herd and beef production at 1.6% and 3%, respectively, it contributes significantly to the beef export trade (MLA, 2021). In 2020, Australia exported an estimated 1.4 M CWE, which amounts to 66% of the total Australian bovine meat production (FAO, 2021).

Australian beef cattle production comprises cow-calf systems, backgrounding and finishing systems on pasture or feedlot over a wide array of agro-climatic zones ranging from the northern tropical to southern cool-temperate Mediterranean and alpine zones. A wide range of cattle genotypes, including temperate, tropically-adapted and crossbred genotypes (Greenwood et al., 2018; Smith et al., 2018), is a significant component of the Australian beef production system. Generally, the Southern Australian beef industry is characterized by a longer pasture growing season and more fertile soils that allow for intensive cattle grazing on smaller scale properties with cattle herds of less than 800 head and stocking densities of 0.2 to 2 adult equivalents per hectare (Campbell et al., 2014). On the other hand, beef production intensity varies widely across climates and topography in northern Australia, with cattle average herd sizes and stocking densities ranging from 1 500 to 12 000 head and 0.02 to 0.2 adult equivalents per hectare, respectively (Burrow, 2014). Northern Australia is home to a large proportion of the national beef cattle herd, with Queensland alone accounting for 46% and 47% (987,000 CWE) of the cattle herd and total beef and veal production in 2020, respectively (MLA, 2021).

2.2. Tropical Northern Australian Pastures and Beef Production

Beef production in northern Australia is heavily dependent on extensive tropical pasture grazing systems (Bezerra et al., 2018) of mainly native pastures dominated by C4 grasses (Hattersley, 1983). In the northern rangelands, pastures are mainly unimproved with limited use of exotic pasture species (Hunt et al., 2014). Black Speargrass (*Heteropogon contortus*) and *Aristida-Bothriochloa* grasslands dominate the more productive areas of eastern Queensland. In northern and western Queensland, Northern Territory and Western Australia Mitchell grass (*Astrebla* spp.), perennial tallgrass and shortgrass grass species, and spinifex (*Triodia spp*.) dominate (Tothill and Gillies, 1992; Hunt et al., 2014). *Stylosanthes* legumes are widely sown across northern Australia's light textured soils to improve pasture nutritive value (Cameron et al., 1996). On the contrary, there was a lack of suitable legume pasture for clay soils until recently (Pengelly and Conway, 2000). Clay soil typifies much of northern Australia pasture land (Robertson et al., 1997). For example, Vertosol (cracking clay) soils occupy 28% of Queensland's total area and are associated with grasslands, eucalypt woodland and brigalow (*Acacia harpophylla*)/gidgee (*Acacia cambagei*) forests (Soil Science Australia, 2015). The most predominant pastures in these clay soils are Mitchell grasses (*Asterbla* spp) and Flinders grasses (*Iseilema* spp), with few sown pastures such as buffel grass (*Cenchrus ciliaris*) in the Brigalow belt (Gleeson et al., 2012). With the exception of young leaves and seeds, native pastures are of relatively low nutritional value at the end of the summer growing season. During winter, growth is limited by temperature (Ivory and Whiteman, 2006), and most native pastures are susceptible to frost leading to a rapid decline in nutrient value (Wilson and Mannetje, 1978; McMeniman et al., 1986).

The pastures are highly seasonal, with growth occurring in the wet season (November to April) and ceases in 4 to 7 months of the year when conditions are too dry and/or too cold (McCown,

1981; Charmley et al., 2008). During the transition period from rainy to dry season, pastures decline in leaf to stem ratio caused by over 50% loss in leaf mass, CP content drops below 8% and the proportion of dead material increases, thus rendering the pastures less nutritionally beneficial and less palatable to cattle (Leng, 1990; Brandão et al., 2018). In addition, pastures deteriorate several years after tree clearing due to nitrogen run-down stress (Robertson et al., 1997). The resulting poor nutrition leads to poor reproductive performance, slow growth rate, loss of body condition, increased susceptibility to parasites and diseases, increased turn-off age (McCown, 1981; Kanani et al., 2006; Charmley et al., 2008) and increased enteric methane emissions (Johnson and Johnson, 1995).

Forage quality is determined by the nutrient concentration, intake, nutrient availability and partitioning of metabolized products within animals (Buxton et al., 1995). Low-quality forages contain less than 10% soluble sugars and starches, CP is below 8% and digestibility less than 55%. Utilization of these forages is limited by low intake due to physical fill limits and slow digestion as a result of high cell wall content and minimal nutrients available to support an efficient rumen microbial growth (Leng, 1990; Buxton et al., 1995). A summary of data from 11 studies depicted a linear relationship between forage CP content and liveweight gain in cattle. Forage CP below 5.6% resulted in weight loss of up to 6 g/kg metabolic body weight, but above 5.6% resulted in 5 – 27 g/kg metabolic body weight gain daily (Bowman et al., 1995).

2.3. Beef Cattle Responses to Under-Nutrition

Beef cattle use their evolutionary adaptation mechanisms, which are either short- (days), medium- (weeks) or long-term (months), to cope with periods of under-nutrition. Short-term adaptations are in response to diurnal feeding frequency or daily changes in feed intake; mid-term changes appear

within weeks of change; while long-term adaptations necessitate that the animals get into a new equilibrium involving different nutritional and physiological changes (Chilliard et al., 1998).

2.3.1. Decrease in liveweight

A decrease in liveweight (LW) after short-term underfeeding takes place due to gut-fill variation amounting to a 4 – 5 kg LW/kg decrease in DMI (Goodchild, 1985). For instance, digesta in the reticulo-rumen of a fed ruminant animal weighs up to 15% of body weight (Baldwin, 1984). Medium-term undernutrition leads to organ and tissue mass variation. Liver and digesta-free gastrointestinal track reduction of more than 50% was reported after three weeks of restricted dietary access to maintain body weight (Burrin et al., 1990). Mid- and long-term weight losses are due to decreases in the portal and hepatic blood flows as well as mobilization of fat, muscle and bone tissues in the reverse order of how they were deposited. The latest maturing tissues are more sensitive due to physiological priority (Baldwin, 1984; Chilliard et al., 1998).

2.3.2. Metabolic and body composition changes

2.3.2.1. Non-esterified fatty acids and ketone bodies

Storage triglycerides in the adipose tissue are hydrolysed to release fatty acids, which are oxidized directly to energy and broken down into ketone bodies (acetoacetate, hydroxy-butyrate and acetones) in the liver during periods of undernutrition (Baldwin, 1984). The liver also incorporates fatty acids into lipoproteins and triacylglycerols in the blood. The lipoproteins, triacylglycerols and ketone bodies act as energy sources in peripheral tissues (Figure 2.1) (Baldwin, 1984). Mobilized fatty acids from the adipose tissue results in elevation of blood non-esterified fatty acids (NEFA). The liver removes 10% of NEFA from the blood during each cycle pass and converts

half of all NEFA into ketone bodies. Therefore, an increase in NEFA results in an increase in blood ketone bodies (Johnson and Johnson, 1995). In addition, plasma NEFA concentration is positively correlated with total bilirubin concentration due to their transport concurrence (Couperus et al., 2021).

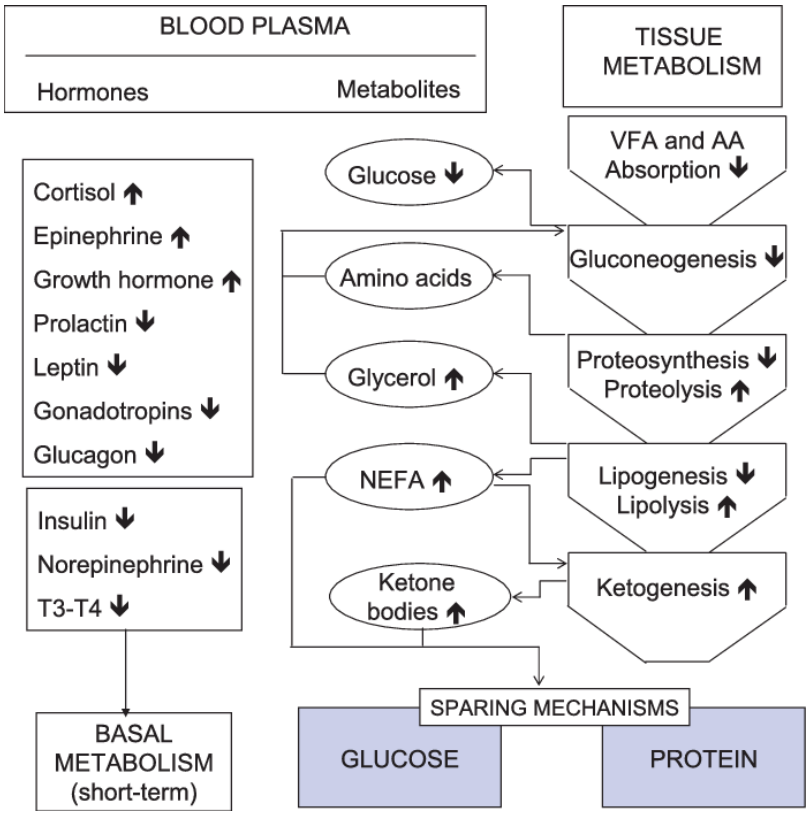


Figure 2.1. Metabolic and endocrine adaptations to undernutrition in the ruminant. NEFA: non-esterified fatty acids, VFA: volatile fatty acids, AA: Amino acids, T3-T4: thyroid hormones. Bold upward and downward pointing arrows indicate an increase or decrease in tissue metabolism, blood plasma hormone or metabolite levels, respectively (Chilliard et al., 1998).

2.3.2.2. Glucose

Propionate is the most abundant glucogenic acid among the ruminally released organic acids and it is the predominant substrate for gluconeogenesis in ruminants (Aschenbach et al., 2010). In

periods of undernutrition, gluconeogenesis from propionate decreases due to a decrease in propionate availability. This is partially compensated by gluconeogenesis from amino acid proteolysis, glycerol lipolysis and lactate recycling (Chilliard et al., 1998). Therefore, insufficient nutrient intake reduces plasma glucose levels (Ndlovu et al., 2007).

2.3.2.3. Creatinine

This metabolite is synthesized from irreversible dehydration of creatine phosphate and is positively correlated with animal muscle mass (Caldeira et al., 2007), although reports on the effect of diet nutritive quality on creatinine concentration remain controversial (Säkkinen et al., 2001; David et al., 2015). Therefore, a decline in plasma creatinine concentration is used as an indicator for a decline in muscle mass during periods of undernutrition in ruminants (Caldeira et al., 2007). Other plasma metabolites influenced by nutritional status include glycerol (Chilliard et al., 1998), urea (Säkkinen et al., 2001), albumin (Caldeira et al., 2007) and total proteins (Ndlovu et al., 2007).

The metabolic changes are controlled by teleophoretic hormones such as insulin, glucagon and norepinephrine and, together with decreased splanchnic tissue mass and variation in body composition, result in reduced energy expenditure. Mid-term experiments (several weeks) showed that portal-drained viscera, liver and skeletal muscles contributed to changes in energy expenditure of 17 – 61%, 14 – 44% and 5 – 7%, respectively (Ortigues and Doreau, 1995; Chilliard et al., 1998). These responses reduced growth rate and resultant beef quality since carcass fat content is a major factor that defines meat quality parameters like texture and taste (Smith et al., 1982; Yang et al., 1999).

2.4. Nutritional Supplementation to Improve Beef Cattle Performance on Low Quality Pastures

2.4.1. Feed supplements during grazing tropical pastures

Numerous studies indicate that high feed conversion efficiencies and medium to high production levels (Table 2.1) can be achieved by ruminants fed poor-quality tropical forages that are adequately supplemented with critical nutrients (Preston and Leng, 1987). Metabolisable energy utilization efficiency of forage can exceed that of grain-based diets when supplemented appropriately. The supplements optimize the availability of nutrients for rumen fermentative digestion and utilization of nutrients that are products of fermentation (Leng, 1990). Batista et al. (2016) observed that supplements with a high proportion of rumen degradable protein, favour nitrogen recycling and promote increased microbial protein synthesis in beef cattle. Supplementation of the drinking water of steers fed pangola grass (*Digitaria eriantha*) hay with Spirulina was found to increase ammonia-N concentration, propionate and branched-chain fatty acids in the rumen fluid. However, this study did not observe any positive effect of Spirulina supplementation on steer liveweight gains (Panjaitan et al., 2010). Non-protein N supplements are also supplied together with molasses to provide readily available energy for rumen microbes to synthesize microbial protein (Bowman et al., 1995).

Table 2.1. Impact of supplements on beef cattle performance.

Pasture	Cattle species	Supplement	Outcome	Reference
Urochloa decumbens	*Bos indicus*	Corn, Corn gluten, Soybean, Urea,	ADG up to 0.75 kg	Valente et al., 2013
Urochloa decumbens hay	*Bos indicus*	Pure casein, urea and ammonia	Increased NDF digestion	Batista et al., 2016
Urochloa decumbens hay	*Bos taurus* x *Bos indicus*	Urea, ammonium sulphate and albumin	Increased DMI and NDF digestion	Lazzarini et al., 2009
Urochloa brizantha	*Bos indicus*	Cottonseed meal, corn and urea	ADG of up to 0.3 kg	Fernandes et al., 2016
Urochloa brizantha	*Bos taurus* x *Bos indicus*	Soybean meal, urea and grain sorghum	ADG of up to 0.5 kg	Neves et al., 2018

ADG: average daily gain, NDF: neutral detergent fibre, DMI: dry matter intake

Supplements are reported to stimulate feed intake and liveweight gain to achieve up to one kg daily (Poppi and McLennan, 2010). Supplementing cattle with urea together with molasses or other readily available energy sources at 2.8% N increases forage intake and prevents liveweight loss (Bowman et al., 1995). However, the cost of supplementation during grazing in an extensive grazing system is a limiting factor. Therefore, it is mainly used for weaners and the breeding herd or whole herd survival (DAF, 2019; Poppi and McLennan, 2010).

2.4.2. Augmenting grass pastures with legumes

Incorporating a highly digestible forage into low digestible pastures supplies vitamin A, essential minerals, ammonia, peptides and amino acids. It also provides a readily fermentable fibre source that increases the fibrolytic bacteria numbers in the rumen, thus improving the total digestibility of the diet (Krebs et al.; Leng, 1990).

2.4.2.1. *Nutritional benefits of legumes*

Many studies have recognized the potential of legume pastures to improve beef cattle production in the tropics (McCown, 1981; Kanani et al., 2006; Hill et al., 2009). Legumes are rich in protein compared to tropical grasses due to the different biochemical pathways of carbon fixation during photosynthesis (Murphy and Colucci, 1999). The protein therefrom can avail a renewable protein source for cattle grazing low-quality grass pastures at a low cost (Osuji et al., 1993). Although the growth rate of cattle under grass-only or grass-legume pasture combination is similar early in the growing season, as the season progresses, legume-grass pasture-fed cattle gain more weight than grass-only fed cattle (Gillard, 1979). This is due to slower nitrogen decline in legumes compared to all-grass pastures that lead to higher nutrition value of mature or dry legume pastures (McCown, 1981). The introduction of legumes on grass-based pastures improves animal energy and protein intake, feed conversion and rumen function, as well as increases mineral and vitamin availabilities (Rochon et al., 2004; Kanani et al., 2006). Steers grazed on leucaena and *U. brizantha* pastures had higher weight gains compared to those on *U. brizantha*-only pastures (Radrizzani and Nasca, 2014). Furthermore, voluntary intake of legume forages is higher than that of grass pastures of similar digestibility due to lower resistance of legume forages to chewing, faster digestion rate and higher rate of particle degradation and clearance from the rumen (Steg et al., 1994; Lüscher et al., 2014).

2.4.2.2 *Rumen fermentation and protein degradation modulation*

Some pasture legumes are known to produce tannins that influence voluntary feed intake and digestive processes in the rumen (Jackson et al., 1996; Thi et al., 2005). Condensed tannins bind plant proteins during mastication to form a condensed tannin-protein complex. The tannin-protein

complex is highly stable and insoluble at pH 3.5 – 7.0 but soluble at pH < 3.0 and pH 8.5 (Barry and Manley, 1984). As a result, pasture tannin levels of 20 – 40 g/kg DM may bind to the dietary proteins, thereby shielding them from microbial degradation in the rumen (pH 5.7 – 7.1), but the complex solubilizes and releases the proteins in the abomasum (pH 2.5 – 3.5) and small intestine (pH 7.5 – 8.5) (Barry and Manley, 1984; Thi et al., 2005; Mangwe et al., 2020). This tannin-protein binding process increases the outflow of dietary protein to the duodenum, protein digestion and absorption in the small intestines (McSweeney et al., 2001; Puchala et al., 2005).

Moderate levels of tannins (< 50 g/kg DM) reduce protein degradation in the rumen without depressing rumen fibre digestion or voluntary feed intake (Thi et al., 2005; Piluzza et al., 2014). However, high levels of tannins (> 50 g/kg DM) are reported to reduce voluntary feed intake (Norton and Ahn, 1997), reduce rumen digestion of dietary structural carbohydrates (Barahona et al., 1997) and the readily fermentable carbohydrates (Barry and McNabb, 1999). High levels of tannins may cause toxicity due to the absorption of degraded products of hydrolysable tannins and the presence of a higher load of phenols in the bloodstream (Makkar, 2003). Barahona et al. (1997) reported that feeding sheep with *Desmodium ovalifolium* or *Flemingia macrophylla* with condensed tannin concentration > 80 g/kg DM reduced organic matter intake by 6 – 10%. It also reduced rumen organic matter digestibility by 7% compared to sheep fed the legumes and drenched with polyethylene glycol, a compound that binds and inactivates tannins. However, the reduction in rumen digestion of readily fermentable carbohydrates and hemicellulose is counteracted by increased post-ruminal digestion (Barry and McNabb, 1999). The reduction in feed intake at high tannin concentrations may be due to the astringency of tannins as a defensive mechanism of plants against consumption by herbivores (Thi et al., 2005).

The key product of microbial fermentation of carbohydrates in the rumen is volatile fatty acids (VFA), mainly acetate, butyrate and propionate, that are used as energy sources for the host animal. In addition, CO_2 and CH_4 (methane) are produced as by-products of fermentation and eliminated through eructation (Immig, 1996). Methane represents about 7.5% of gross energy intake in ruminant livestock fed low-quality diets (Johnson and Johnson, 1995; Johnson and Ward, 1996). The inclusion of tannin-containing legumes in the diet is reported to reduce enteric methane emission by directly inhibiting methanogenic activity or indirectly reducing hydrogen availability (Hook et al., 2010). This consequently improves feed utilisation and livestock weight gains (Barry and McNabb, 1999).

2.4.2.3 *Lipid degradation modulation*

Tannins may inhibit or slow down lipid biohydrogenation in the rumen (Toral et al., 2018). Dietary herbage lipid composition is made up of membrane lipids: glycolipids and phospholipids, while seed lipids are polar lipids, mainly triacylglycerides (Jenkins, 1993). After ingestion, dietary triglycerides and phospholipids are hydrolysed into glycerol, fatty acids and small amounts of mono- and diglycerides by microbial lipases in the rumen. Glycerol undergoes rapid fermentation to yield propionic acid as the major product (Garton et al., 1961), while unsaturated fatty acids are hydrogenated into saturated trans fatty acids by microbes (Baldwin, 1984; Jenkins, 1993). Biohydrogenation involves isomerization of cis-12 double bonds in unsaturated fatty acids to a trans-ll isomer, followed by hydrogenation of the cis-9 bond in linoleic acid by microbial reductase into saturated fatty acids (Figure 2.2) (Jenkins, 1993).

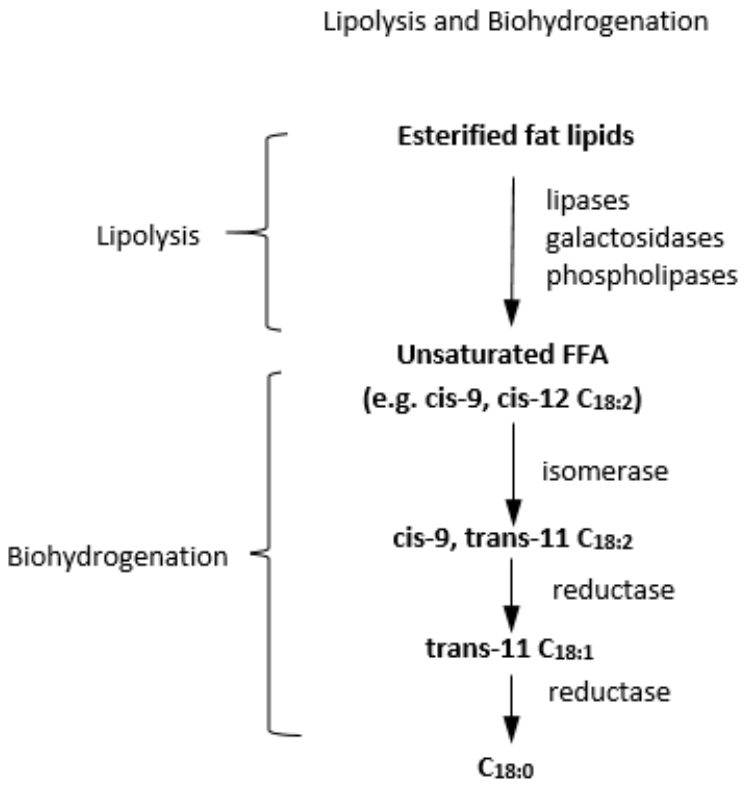


Figure 2.2. Key steps in lipolysis and biohydrogenation to convert esterified fatty acids to saturated fatty acids in the rumen. FFA: free fatty acids (Jenkins, 1993).

In an *in vitro* study, tannin extract from *Schinopsis lorentzii* reduced biohydrogenation of α–linolenic acid (ALA) from 43% to only 13% in flaxseed diet after 24 hours of incubation (Kronberg et al., 2007). Tannin-containing forage (Sainfoin) had no effect on ALA biohydrogenation, but diets containing tannin extracts (7.9% of dietary DM) reduced biohydrogenation by 20% *in vitro* (Khiaosa-Ard et al., 2009). Incubating hay and concentrate diets *in vitro* with tannins concentration of 1.0 mg/ml of cow buffered ruminal fluid increased vaccenic acid and reduced stearic acid concentration by 23% and 16%, respectively, compared to the control. Tannin was extracted from *Ceratonia siliqua*, *Acacia cyanophylla* and *Schinopsis lorentzii* (Vasta et al., 2009). Feeding lambs with *Cistus ladanifer* at 200 g/kg DM reduced complete rumen biohydrogenation by 36% in lambs but had no effect when fed at 50 g/kg (Alves et al., 2017). The inclusion of Sainfoin in a Timothy

grass silage diet of lambs increased the accumulation of ALA in rumen digesta (Campidonico et al., 2016). Protecting unsaturated fatty acids from biohydrogenation in the rumen increases the levels of unsaturated fatty acids absorbed through the intestines into the bloodstream. Plasma n-3 PUFA of steers infused sunflower oil into the duodenum was 2-fold compared to control (Scislowski et al., 2005). These studies were conducted in controlled environments and some used tannin extracts and/or included oil supplements in the feeds (Khiaosa-Ard et al., 2009; Alves et al., 2017).

2.4.2.4. *Parasite control*

Tannin-containing legumes in the diets impose higher parasite tolerance on different species of grazing animals (Niezen et al., 1995; Piluzza et al., 2014). Tannins exert their anti-parasitic effect by decreasing the viability of larvae, thus interfering with egg hatching and/or improved immunity as a result of improved protein nutrition from reduced rumen protein degradation (Min and Hart, 2003; Cresswell, 2007). *In vitro* and *in vivo* studies in small ruminants have reported significant effects of tannin extracts and tannin-containing legumes on faecal egg count, development of eggs to larvae and decreased larvae motility (Molan et al., 1999; Max et al., 2004). Naturally-infected lambs grazing chicory (*Cichorium intybus*) and birdsfoot trefoil (*Lotus corniculatus*) were reported to have fewer helminth parasites than lambs grazing ryegrass (*Lolium* spp.) or white clover (*Trifolium repens*). Birdsfoot trefoil grazing reduced faecal egg count significantly compared to all the other forages (Marley et al., 2003). Red deer calves infected with deer-origin gastrointestinal nematodes and lungworm (*Dictyocaulus* sp.) larvae for five weeks were allocated into either lucerne (0.1% condensed tannins; CT), birdsfoot trefoil (1.9% CT) or sulla (*Hedysarum coronarium L.*) (3.5% CT) and slaughtered after seven weeks. Abomasal nematode burdens had

significant negative linear relationships with dietary CT concentration, although no substantial differences were observed in faecal egg counts (Hoskin et al., 2000).

2.4.2.5. *Hedonic and eudaimonic well-being*

Dietary diversity can improve animal growth and production by providing the animals with the option to select and mix their diets to meet individual requirements and acquiring nutraceutical and prophylactic benefits linked with the ingestion of specific plant secondary metabolites at self-regulated safe quantities of intake (Provenza, 1996; Beck and Gregorini, 2020). The improved nutrition plane and health results to hedonic well-being relating to the balance between positive and negative emotions, and eudaimonic well-being relating to the ability of the animals to pursue their full potential (Harfeld, 2013; Beck and Gregorini, 2020).

Research exploring the effect of choice on livestock welfare reported that markers of physiological stress are reduced in ruminants offered a choice between foods that are diverse in nutrient composition compared to their counterparts fed a monotonous diet of all food options (Villalba et al., 2012; Catanese et al., 2013). In addition, negative energy balance is reported to be positively associated with oxidative stress in ruminants (Bernabucci et al., 2005; Singh et al., 2011). Therefore, improved nutrition from plant primary compounds and the improved antioxidant status from phenolic compounds obtained from legumes may reduce oxidative stress and consequently improve well-being in grazing cattle (Waghorn, 2008; Beck and Gregorini, 2020)

2.4.3. The use of legumes in northern Australia

For the past six decades, the northern Australian beef industry has used tropical pasture legumes (Mannetje, 1997). These legumes have been recognized as the best long-term alternative to

increase grass pasture productivity. However, adoption level remains low (MLA, 2017), accounting for less than 5 million hectares of planted area out of the total northern Australian land mass of over 450 million ha (comprised of the whole of Queensland, the Northern Territory and the part of Western Australia north of latitude 26°S) (Shelton et al., 2005). For a long time, most attention was directed at lighter textured soil pastures (Coates et al., 1997) and only little emphasis was placed on legumes adapted to dark clay soils, resulting in genotypes that were either not sufficiently productive or persistent (Cook et al., 2005; MLA, 1996; Schlink and Burt, 1993). However, attention has been directed to pastures of heavier textured soils of central and southern Queensland in recent years, leading to the development of more suitable annual and perennial legume species and varieties such as Caatinga stylo (*Stylosanthes seabrana*), butterfly pea (*Clitoria ternatea*), Burgundy bean (*Macroptilium bracteatum*) and desmanthus. In spite of this, only limited published literature on animal growth and performance from these legumes exists. Stylosanthes, butterfly pea and desmanthus-grass pastures were observed to improve weight gains compared to grass-only pastures numerically throughout the year (Hill et al., 2009). Steers grazed on butterfly pea-grass and Caatinga stylo-grass pastures had no difference in weight gain compared to grass-only pastures in the first year of establishment. However, 31 and 68 kg/ha difference, respectively, was observed over a five-year period (Clem, 2004).

Desmanthus has gained attention in recent years due to its palatability, high protein content, non-toxic characteristics, anti-methanogenesis demonstrated *in vitro* (Vandermeulen et al., 2018) and *in vivo* (Suybeng et al., 2020), and its ability to establish and persist well in clay soils (Schlink and Burt, 1993; Cook et al., 2005). Two species of desmanthus*, D. virgatus* and *D. leptophyllus,* were found in old trial sites after 25 years of establishment on black cracking clay soil surviving droughts, floods, frost and commercial grazing (Gardiner and Swan, 2008). Desmanthus is highly

nutritious with at least 14% CP in the whole plant and 22% in leaves (Gonzalez-V et al., 2005; Kanani et al., 2006). Desmanthus is, therefore, a good legume choice for the clay soils of northern Australia (Gardiner et al., 2004; Isbell, 2016). Since 2012, over 50 000 ha of desmanthus has been established in northern Australia (Gardiner et al., 2021) and necessitates studies to be carried out to determine its effect on beef cattle performance and meat quality. A short-term 90-day study reported up to 40kg higher liveweight in steers grazed on desmanthus-Buffel grass pasture than Buffel grass only (Gardiner and Parker, 2012). Supplementing Rhodes grass-fed goats with desmanthus increased dry matter intake, liveweight gain, loin eye muscle area and hot carcass weight significantly, compared to urea and cottonseed meal supplements (Aoetpah et al., 2018). Similarly, a 10-week study reported that desmanthus-Mulato grass diet increased liveweight gain in goats significantly compared to those on Mulato grass only (Marsetyo et al., 2017). In contrast, growing goats demonstrated poor acceptance of *D. bicornutus* compared to *Leucaena leucocephala* (Leucaena), *Medicago sativa* (lucerne) and *Lablab purpureus* (lablab), resulting in lower weight gains (Kanani et al., 2006).

2.5. Feedlot Finishing of Tropical Pasture-Backgrounded Cattle

Feedlot finishing is an important phase in the beef supply chain of pasture backgrounded beef cattle. In 2017, 50% of Queensland beef herds were finished in the feedlot with grain diets high in energy (DAF, 2018). Lot-feeding helps to finish cattle during periods of limited pasture availability. This allows beef products to meet the quality yardsticks of a wide range of markets, marketing of more even products, reduces farm-stocking pressure during the dry season and help to plan for the marketing season (MLA, 2019b; Pacheco et al., 2014). A comprehensive review by Drouillard and Kuhl (1999) reported that diet quality during backgrounding affects cattle

performance in the feedlot. Cattle grazed on poor pastures that restricted growth moderately led to complete compensatory growth during lot-feeding, while those backgrounded on poor pastures resulting to weight loss failed to achieve compensatory growth. Cattle grazed on endophyte-infected fescue were compared to those grazed on endophyte-infected fescue-clover mix and endophyte-free fescue. Cattle grazing on endophyte-infected fescue-clover mixture consistently performed best during grazing and finishing (Burton et al., 1994; Coffey et al., 2013). A meta-analysis of 20 dry-lot and 12 grazing studies showed that cattle fed high energy diets during backgrounding had lower final body weights than those grazing or fed on restricted energy. However, this study did not analyse the effect of dietary protein content (Johnson and DiCostanzo, 2017).

2.6. Meat Quality

The ultimate goal of the beef cattle industry is to provide consumers with beef that is safe and of high eating quality. The major determinant of meat quality is eating quality, influenced mainly by intramuscular fat content, fat melting point, tenderness, juiciness and flavour (Maltin et al., 2003). Carcass fat deposition and meat fatty acid composition play important roles in eating quality variation (Wood et al., 1999; Webb, 2006; Goszczynski et al., 2017; Testa et al., 2021). Consumption of high-quality beef fat is important to humans since it helps in the transport and absorption of fat-soluble vitamins and exerts a positive effect on immune response (Webb and O'Neill, 2008).

Beef fat is primarily categorized into three; subcutaneous, intermuscular and intramuscular fat (Dujková et al., 2015). Saturated fatty acids (SFA), whose levels are high in ruminant meat due to hydrogenation of dietary unsaturated fatty acids in the rumen, are associated with health risks such

as coronary heart disease (French et al., 2000; Scollan et al., 2006), although this association remains controversial (Dehghan et al., 2017; Astrup et al., 2019). Monounsaturated fatty acids (MUFA) are reported to be associated with a lower mortality rate (Guasch-Ferré et al., 2019), although other studies did not find any association (Dehghan et al., 2017). Increasing the level of n-3 polyunsaturated fatty acids (PUFA) in the human diet is important to overcome the imbalance resulting from high consumption of plant oils rich in linoleic acid (Scollan et al., 2001). Long-chain n-3 and n-6 PUFA improve growth, brain and retinal development, maternal and offspring health, cognitive function and psychological status in humans (Mapiye et al., 2015). Also, conjugated linoleic acid (CLA) and n-3 fatty acids confer anti-inflammatory effects (McAfee et al., 2010). Recommendations for various dietary fat fractions are 15 – 35%, <10%, <2.5 – 9%, <2 – 3% and <1% of total energy intake from total fat, SFA, n-6 PUFA, n-3 PUFA and trans fatty acids, respectively (FAO, 2008; WHO, 2002) and ratios of PUFA:SFA at 0·45 and n-6:n-3 PUFA below 4.0 (Department of Health London, 1994). As a result, there is increasing focus on studies aimed at elevating the levels of beneficial n-3 LC-PUFA and reducing saturated fatty acids in beef, especially in IMF, commonly referred to as marbling, since it cannot be trimmed out (Scollan et al., 2006; Troy et al., 2016).

Marbling is associated with carcass fatness. A positive correlation between total carcass fat content and subcutaneous fat thickness with marbling has been observed (Muir et al., 1998; Lorenzen et al., 2007). For instance, a genetic correlation of 0.91 between marbling score and muscle lipid content was reported (Hocquette et al., 2010). Increase in subcutaneous fat thickness from below 0.19 mm to over 1.40 mm transitioned marbling score from ‘devoid’ to ‘abundant’ (Jeremiah, 1996). An increase in carcass fat content and subcutaneous fat thickness from 187 to 217 g/kg and 6.6 to 8.3 mm, respectively, increased marbling score from 2.2 to 2.6 in steers (Steen et al., 2003),

while an increase in carcass fatness influenced fatty acid composition and PUFA:SFA ratio (De Smet et al., 2004). Marbling fat consists of more unsaturated fatty acids compared to other fats in beef, consequently a higher PUFA:SFA ratio. It also contains more oleic acid and less stearic acid (Insausti et al., 2004; Troy et al., 2016).

2.6.1. Effect of intramuscular fat on beef eating quality

2.6.1.1. Tenderness

Variation in tenderness is attributed to animal age, pre- and post-slaughter carcass handling, post-mortem pH decline, genetic make-up and carcass composition, mainly marbling (Wood et al., 1999; Hwang and Thompson, 2003; Špehar et al., 2008). Subcutaneous and intermuscular fats provide insulation for muscles to prevent cold shortening. Muscles cool down at a slower rate and rigour is attained at higher temperatures. Leaner lamb carcasses with lower marbling scores and less subcutaneous fat thickness were reported to be tougher than those with more fat (Smith et al., 1976; Sañudo et al., 1998). Similarly, Jeremiah (1996) reported a higher tenderness score for steers and heifers with higher subcutaneous fat thickness and marbling as scored by both trained and untrained panel of consumers. High marbling score as in Kobe beef that can exceed <200 mg/g fresh meat, cause dilution of fibrous proteins by soft fat, thus lowering the bulk density that may reduce resistance to shearing. Marbled fat cell expansion forces muscle bundles apart to result in opened up muscle structure (Wood, 1990; Juárez et al., 2012). Marbling fat concentration values between 3% and 7.5% of muscle are suggested to result in optimum tenderness and palatability (Smith et al., 1982; Smith, 2016).

2.6.1.2. Flavour

Animal nutrition status, diet, sex, breed and genetic make-up are factors that influence meat flavour (Arshad et al., 2018). Meaty flavour of cooked meat develops from a complex interaction of precursors from the fat and lean components of meat. Products of Maillard reactions between carbohydrates and proteins, such as pyrazines and thiazoles, and lipid degradation of aldehydes, alcohols and ketones, are the most important determinants of flavour (Mottram and Salter, 1989; Mottram, 1994). Hence, meat composition plays an important role in flavour, which could explain the increase in flavour intensity with age in meat animals (Wood et al., 1999). A trained panel reported higher flavour scores for beef from carcasses with higher subcutaneous fat thickness than those with minimal fat (Jeremiah, 1996). The fatty acid composition of fat also plays a significant role in meat flavour. Linolenic acid was found to be positively correlated with milky-oily and sour flavour in beef, while oleic acid was negatively correlated (Melton et al., 1982). Oleic acid is considered to be of major effect on the flavour of cooked beef (Lee et al., 2017). Fatty acid oxidative degradation to form alkyl radicals occurs faster in PUFA than MUFA, while linolenic acid derivatives, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are highly susceptible to oxidation giving rise to aldehydes (Wood, 1990).

Fats act as the storage site for skatoles and indoles, two compounds that play a significant role in meat flavour, but more so in sheep than cattle. They are produced in the rumen through microbial deamination and decarboxylation of tryptophan. When they exceed the liver's metabolism capacity, they are deposited in body fats, thus contributing to pastoral flavours in ruminant meat. At low levels, skatoles contribute to desirable odours and flavours, but at high levels, produce a nauseating faecal odour (Webb, 2006b; Webb and O'Neill, 2008). Finishing grass-fed cattle with concentrate diet for at least 54 days reduces pastoral flavour significantly (Dujková et al., 2015).

2.6.1.3. Juiciness

Meat juiciness is the initial impression of moisture released on the meat surface during chewing and the degree of induced salivation (Muir et al., 1998). Meat juiciness relies on water and fat contents, thus factors influencing water holding capacity and fat content of meat may influence juiciness (McMillin and Hoffman, 2009). Marbled fat provides lubrication between muscle fibres and increases the perception of juiciness by stimulating salivation while chewing (Juárez et al., 2012). In addition, fat prevents drying out of the meat during cooking (Wood, 1984). However, some studies did not find any positive correlation between beef subcutaneous fat and marbling with juiciness (Jeremiah, 1996).

2.6.2. Diet factors influencing beef intramuscular fat content and fatty acid composition

In beef, the lipid fraction generally contributes between 4 − 15% of carcass weight on fresh basis (Mapiye et al., 2013). Out of these, four-fifths are SFA, mainly composed of palmitic, stearic and oleic acid. The remaining fifth comprises 30 different fatty acids (Whetsell et al., 2003). In the intramuscular lipid fraction, values of 2 to 30% in the *Longissimus dorsi* (loin eye) muscle has been reported (Smith et al., 2009).

Lipid fraction and fatty acid composition are influenced by three major factors, namely, age of the animal, diet and breed. A study of fat content and fatty acid profile in three breeds of cattle reported an increase in IMF and saturated fatty acids percentage and a decrease in unsaturated fatty acids of loin eye muscle with age (Nürnberg et al., 1999). In contrast, Jersey and Limousin cattle showed decrease in total SFA and increase in MUFA (Malau-Aduli et al., 1997), while phospholipids showed a decrease in palmitic acid, stearic acid and oleic acid but an increase in PUFA with age (Malau-Aduli et al., 1998). Age had no effect on Japanese Black cattle fatty acid composition (Abe

et al., 2009). Beef cattle producers target early turn-off age of steers and heifers, usually below 28 months (Savage, 2005; Drouillard, 2018). Details on mechanisms in which age influences beef fatty acid composition are not given as this is beyond the intended scope of this review.

2.6.2.1. Basal diet composition

Composition of backgrounding and finishing diets influence beef fatty acid profile. Differences are reported between cattle fed pasture versus concentrate diets (French et al., 2000), pastures containing varying plant secondary metabolites (Priolo et al., 2005), and diets supplemented with oils (Dierking et al., 2010), vitamins and minerals (Kook et al., 2002; Dhiman et al., 2005).

Pastures versus concentrate diets: Beef from pasture raised cattle contains higher levels of n-3 PUFA and MUFA compared to concentrate-fed cattle (French et al., 2000; Nuernberg et al., 2005). Tume (2004) suggested that the effect is mainly attributable to individual ingredients in the diet and their combinations. Plants are the primary sources of n-3 PUFA due to their unique ability to synthesize ALA, which comprises at least half of the fatty acid content of forages. Alpha linoleic acid forms the building block of n-3 essential fatty acids, and its elongation and desaturation results in the synthesis of EPA, DHA and docosahexaenoic acid (DPA) (Scollan et al., 2006). Moreover, biohydrogenation of unsaturated fatty acids in the rumen is followed by microbial fatty acids synthesis from dietary long-chain fatty acids and *de novo* synthesis, amounting to 10 to 15% of bacterial dry mass that influences the fatty acid profile of absorbed lipids (Jenkins, 1993; Jacques et al., 2016). Feeding Angus crossbred steers on forages and pasture only increased muscle rumenic acid by four-fold compared to high-grain diet (Poulson et al., 2004). Continental crossbred steers fed on grass pasture had the highest intramuscular PUFA content and increasing dietary concentrate supplement led to a linear increase in SFA, increased n-6/n-3 PUFA ratio as well as a

decrease in PUFA/SFA ratio (French et al., 2000). Angus-cross steers backgrounded on pasture only were finished on corn-silage concentrate or pasture. Pasture-finished steers had 61%, 21% and 22% less total fat, oleic acid and total MUFA compared to the concentrate group. Individual (linolenic acid, EPA, DPA, and DHA) and total n-3 fatty acids concentrations and the ratio of n-6 to n-3 fatty acids were greater in forage than concentrate finished steers (Duckett et al., 2013). Grass-fed German Holstein and Simmental bulls had higher total PUFA and lower n-6/n-3 ratio than concentrate fed bulls, but total SFA was similar (Nuernberg et al., 2005). Similarly, grass-fed Hereford steers had higher PUFA, lower MUFA and n-6/n-3 ratio than concentrate-fed steers, while SFA was not affected (Realini et al., 2004). Fat deposition relies on consumption of surplus net energy (Park et al., 2018), hence grain feeding increases carcass total fat content due to high energy levels (Muir et al., 1998).

Time on feed also plays a significant role in beef fatty acid composition. Steers raised on a native range stocker operation were divided into eight groups, finished on a high concentrate diet and slaughtered serially at 28 days intervals from zero (control) to 196 days on a finishing diet. Carcass marbling score, subcutaneous fat thickness, loin eye muscle MUFA and total lipid percentage increased, while PUFA decreased with increase in days on concentrate diet. Differences in these parameters were observed after 112 days on the diet after which a plateau was reached (Duckett et al., 1993). These results should be interpreted with caution as age also affects carcass fat content and composition as well as marbling (Schönfeldt et al., 2010; Bednárová et al., 2013; Kelava Ugarković et al., 2013).

Pasture species: Some studies have reported the effect of pasture species on beef fatty acid composition, while some reported no difference. For instance, lucerne-finished steers had higher concentrations of linoleic acid and ALA than those finished on pearl millet and a combination of

Trifolium repens (white clover), *Poa pratensis* (blue grass), *Dactylis glomerata* (orchard grass) and *Festuca arundinacea* (tall fescue), but forage species did not affect total lipid content of the loin eye muscle in a 40-day study (Duckett et al., 2013). In a four-month study where steers grazed on tall fescue only or combined with *Trifolium pratense* (red clover) or lucerne, there was no effect of pasture on meat fatty acid content (Dierking et al., 2010). Similarly, rib eye rolls from steers finished on tall fescue and *Bromus commutatus* (meadow brome) or *Lotus corniculatus* (birdsfoot trefoil) for four months had similar marbling scores, n-6/n-3 ratios and total SFA, MUFA and PUFA, but EPA was higher in birdsfoot trefoil finished steers (Chail et al., 2016). The effect of pasture type on fatty acid composition was reported in a 90-day study where lambs with access to shrubs produced meat with higher percentage of ALA, n-3, n-6, total PUFA and lower MUFA and n-6/n-3 ratio than those on grass only, but total SFA was similar (Ramírez-Retamal et al., 2014). The effect of different forage species may be due to plant secondary metabolites. Cattle grazing botanically diverse pastures with different plant secondary metabolites had higher intramuscular n-3 and total PUFA compared to cattle grazing predominantly *Lolium* spp. (ryegrass) pastures with similar pasture fatty acid profile (Moloney et al., 2008). Red clover reduces ruminal biohydrogenation of PUFA, possibly due to the protective effects of the polyphenol oxidase enzyme (Lee et al., 2004). As discussed earlier, dietary tannin may inhibit or minimize rumen biohydrogenation of unsaturated fatty acids and increase the level of n-3 PUFA in the blood circulation. The loin eye muscle of lambs fed *Hedysarum coronarium L*. (sulla) containing 1.8% condensed tannins had 24% more ALA compared to lambs fed sulla and drenched with polyethylene glycol, a compound that binds and inactivates tannins (Priolo et al., 2005). Desmanthus contains up to 4.5% condensed tannins (Vandermeulen et al., 2018), hence grazing cattle on desmanthus pastures may increase n-3 PUFA in beef.

2.6.2.2. Oil supplements

Dietary supplementation with n-3 LC-PUFA-rich oils has been shown to increase PUFA in the meat of ruminants (Dierking et al., 2010) because at high concentrations, rumen microorganisms cannot hydrogenate these oils to any significant extent (Ashes et al., 1992) and oil supplements also enhance *de novo* fatty acids synthesis from their dietary precursors (Scollan et al., 2001). Steers supplemented with fish oil doubled the EPA and DHA contents in muscle phospholipids, while those supplemented with linseed increased the levels of ALA in muscle phospholipids from 9.5 to 19 mg/100 g and enhanced EPA synthesis from 10 to 15 mg/100 g in muscle with no effect on feed intake (Scollan et al., 2001). Lorenzen and colleagues (Lorenzen et al., 2007) reported over 80% increase in CLA in beef from soybean oil supplementation compared to the control during finishing of steers. Soybean oil supplement increased CLA in the adipose tissue of steers (Dhiman et al., 2005). Fish oil supplement increased n-3 LC-PUFA, including linolenic acid, EPA and DHA concentrations in the loin eye muscle of bulls and steers (Kook et al., 2002) and slightly increased the total fatty acids in supplemented steers compared to the control (Scollan et al., 2001). Diet-protected fish oil and free fish oil increased total muscle EPA and DHA from 13 to 19 mg/100 g and 3 to 12 mg/100 g, respectively (Richardson et al., 2004). Effect of oil on beef fatty acid composition is not unique to pure oil supplements. British x Continental crossbred steers were fed grass hay or red clover silage only or supplemented with either sunflower-seed or flaxseed concentrates to provide 5.4% oil in diet DM basis. Sunflower-seed or flaxseed supplements increased vaccenic, rumenic and n-6 fatty acids in the *Longissimus thoracis* muscle significantly. ALA was over two-fold in flaxseed compared to sunflower-seed supplemented steers (Mapiye et al., 2013).

2.6.2.3. Micronutrients

Vitamin A: Vitamin A or β-carotene deficiency results in elevated IMF content. Angus steers were fed low β-carotene and vitamin A cereal-based ration for 308 days with or without vitamin A supplementation before slaughter. Supplemented steers scored 19% less marbling and the loin eye muscle IMF content was 35% lower than the control (Kruk et al., 2008). Supplementing Japanese Black cattle with vitamin A after 15 months of age reduced marbling score significantly. A correlation of −0.38 was observed between marbling and serum vitamin A just before slaughter (Oka et al., 1998). Effect of vitamin A is proposed to be due to its derivative retinoic acid that restricts hyperplasia and/or by regulating the growth hormone gene resulting in a decrease in fat deposition (Hocquette et al., 2010). Trans-retinoic acid, a metabolite of retinol, subdues differentiation of preadipocytes by suppressing the expression of peroxisome proliferator-activated receptor gamma (PPARγ) gene (Hida et al., 1998; Smith et al., 2009).

Vitamin C: Domestic animals normally do not receive dietary vitamin C supplementation due to their ability to synthesize the vitamin in the liver (McDowell, 1989). However, plasma vitamin C levels in beef cattle drop below the normal 2.4 – 4.7 mg/L range during the late fattening period, showing that vitamin C plays an important role in adipogenesis (Kawachi, 2006). Supplemented Japanese Black cattle receiving high-concentrate diets with vitamin C during the late (Ohashi, 2000) or from middle fattening stage produced fatter carcasses with higher marbling scores than the control (Kawachi, 2006). Increased lipogenesis is as a result of the positive effect of vitamin C on adipocyte differentiation (Kawada et al., 1990).

Vitamin D and Calcium: 1α,25-dihydroxyvitamin D_3, the biologically active form of vitamin D, inhibits the differentiation of preadipocytes through direct suppression of PPARγ protein (Hida et

al., 1998). Since 1α,25-dihydroxyvitamin D_3 is critical for calcium homeostasis, low dietary intake of calcium leads to increased plasma 1α,25-dihydroxyvitamin D_3 that suppresses adipocyte differentiation and reduces marbling (Kawachi, 2006). Feedlot cattle with low plasma 1α,25-dihydroxyvitamin D_3 levels had higher marbling scores than those with higher levels (Montgomery et al., 2004). In contrast, high marbling scores were reported in Hanwoo steers finished on low calcium diets leading to high levels of plasma 1α,25-dihydroxyvitamin D_3 than in steers finished on high calcium diets (Lee et al., 2003). 1α,25-dihydroxyvitamin D_3 may exert two contrasting functions on adipogenesis; inhibit adipocyte differentiation and promote fat accumulation in adipocytes, depending on the animals' stage of growth (Kawachi, 2006).

2.6.3. Genetic factors influencing beef intramuscular fat content and fatty acid composition

2.6.3.1. Cattle breed

Generally, beef intramuscular fat content and fatty acid composition are of medium to high heritability, although low heritabilities have been reported in some studies (Table 2.2). For instance, heritability estimates for stearic acid ranging between 0.12 and 0.84 are reported (Malau-Aduli et al., 2000b; Pitchford et al., 2002; Nogi et al., 2011; Sakuma et al., 2017).

Table 2.2. Heritability estimates of intramuscular fat content and fatty acid composition in cattle

Cattle breed	Trait	Heritability	Reference
Japanese Black cattle	Marbling score	0.72	Inoue et al., 2017
	Oleic acid	0.63	
	MUFA	0.63	
Angus, Murray Grey, Hereford, Brahman, Belmont Red, Santa Gertrudis and Shorthorn	SFA	0.54	Kelly et al., 2013
	MUFA	0.53	
	PUFA	0.05	
Angus, Belgian Blue, Hereford, Jersey, Limousin, South Devon and Wagyu crossbred cattle	Marbling score	0.09	Malau-Aduli et al., 2000
	Stearic acid	0.12	
	SFA	0.22	
	MUFA	0.14	
Japanese Black cattle	Backfat thickness	0.34	Nogi et al., 2011
	Marbling score	0.51	
	Stearic acid	0.59	
	Oleic acid	0.78	
	SFA	0.66	
	MUFA	0.68	
	PUFA	0.47	
Jersey, Wagyu, Angus, Hereford, South Devon, Limousin and Belgian Blue	Backfat thickness	0.26	Pitchford et al., 2002
	IMF	0.18	
	Myristic acid	0.18	
	Palmitic acid	0.21	
	Palmitoleic acid	0.16	
	Stearic acid	0.14	
	Oleic acid	0.17	
Brahman	Backfat thickness	0.63	Riley et al., 2002
	Marbling score	0.44	
Japanese Black cattle	Marbling score	0.22	Sakuma et al., 2017
	Myristic acid	0.56	
	Palmitic acid	0.48	
	Palmitoleic acid	0.82	
	Stearic acid	0.84	
	Oleic acid	0.61	
	SFA	0.53	
	MUFA	0.57	
	PUFA	0.11	

IMF: intramuscular fat, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Studies have reported variation in beef fat deposition and fatty acid composition due to genetic differences between cattle. Beef breeds differ from milk breeds and such differences are well documented. Generally, meat type breeds are able to deposit more fat than milk breeds. Fatty acids synthesis was reported to be higher in beef cattle subcutaneous and perirenal adipose tissues than in same tissues from dairy cattle of similar age and weight (Vernon, 1981). Differences between Jersey and Limousin (Malau-Aduli et al., 1997; Malau-Aduli et al., 1998), Japanese Black and Holstein (Zembayashi et al., 1995) have been reported. German Holstein bulls had higher SFA and total PUFA compared to German Simmental bulls on similar diets, but breed had no effect on n-3 fatty acids (Dannenberger et al., 2005). Nuernberg et al. (2005) reported higher n-3, n-6, n-6/n-3 ratio and total PUFA in German Simmental beef bulls than in German Holstein bulls, but SFA levels were similar. The CLA isomer cis-9, trans-11 concentration was higher in the loin eye muscle of German Holstein than German Simmental bulls. In a 24-month study, Galloway, White-Blue Belgian and German Holstein bulls were fed on the same diet and slaughtered at different ages between zero and 24 months. Carcass subcutaneous fat and loin eye muscle intramuscular fat were highest in German Holstein and least in White-Blue Belgian. At birth, stearic acid, oleic acid and n-3 fatty acids were highest in the loin eye muscle of Galloway, while total unsaturated fatty acids, PUFA and n-6 fatty acids were highest in White-Blue Belgian. A similar profile was observed at 18 months of age except in n-3 fatty acids that were similar in Galloway and White-Blue Belgian, but lower in German Holstein (Nürnberg et al., 1999). Comparing the fatty acid profile of Japanese Black and Holstein steers subcutaneous neutral lipids showed lower myristic acid, palmitic acid, stearic acid and total SFA, but higher oleic acid, total MUFA and MUFA:SFA ratio in the Japanese Black than Holstein steers. However, intramuscular phospholipid fatty acid profile was not affected except for palmitic acid (Zembayashi et al., 1995). Simmental and Red

Angus steers at similar back fat finished level of 10 mm were compared for loin eye muscle fat profile. Total lipids, myristoleic acid, palmitoleic acid, vaccenic acid, along with n-6/n-3 ratio, were greater while EPA and total n-3 PUFA were lower in Simmental than Red Angus steers. Time on grain diet was a confounding effect in this study as the Angus spent 70 days less on the grain diet and were slaughtered 73 days younger than the Simental steers (Laborde et al., 2011).

Sires may influence the fatty acid content of their offspring. Japanese Black Wagyu cattle sired by different bulls were reported to have significantly different SFA and MUFA contents (Oka et al., 2002). These breed variances are probably due to differences in the activities of enzymes influencing gene expression and/or enzyme function (Scollan et al., 2006). The activity of Δ9-desaturase enzyme to convert palmitic to palmitoleic acid was observed to be greater in Simmental than Red Angus lipids (Laborde et al., 2011). These breed differences demonstrate the potential of improving beef intramuscular fat content and fatty acid composition through breed replacement, crossbreeding or selection of superior individuals within breeds (Kelly et al., 2013).

2.6.3.2. Genes that influence carcass fat content and fatty acid profile

Several genes are reported to be responsible for variation in fat content and fatty acid composition in beef. The genes encode for cocaine- and amphetamine-regulated transcript (Rempel et al., 2012), leptin (Shin and Chung, 2006), diacylglycerol O-acyltransferase, the growth hormone 1 (Barendse et al., 2006), sterol regulatory element-binding protein 1 (Bhuiyan et al., 2009), fatty acid synthase, stearoyl-CoA desaturase and fatty acid binding protein 4 (Zhang et al., 2008; Matsuhashi et al., 2011; Kaplanová et al., 2013; Dujková et al., 2015). This review focused on fatty acid synthase, stearoyl-CoA desaturase and fatty acid binding protein 4 genes.

Stearoyl-CoA desaturase (SCD): This gene encodes for Δ9 desaturase enzyme and introduces a single double bond in SFA to convert them to MUFA. For instance, the enzyme desaturates stearic acid to oleic acid and trans-vaccenic acid. High concentration of oleic acid in beef is associated with soft fat and overall palatability in Wagyu and Hanwoo cattle. As a result, high activity of Δ9 desaturase enzyme is associated with soft fat in beef (Westerling and Hedrick, 1979; Smith et al., 2009). The *SCD* catalytic activity is about double in bovine marbled muscle tissue than in the subcutaneous adipose tissue. This agrees with higher MUFA levels observed in the muscle than subcutaneous adipose tissue (Sturdivant et al., 1992; May et al., 1993; Archibeque et al., 2005). Expression and activity of the *SCD* gene is reported to increase after weaning (Lee et al., 2005) and preceding lipid filling in preadipocytes. Similarly, a gradual increase in *de novo* fatty acid biosynthesis is observed after weaning, indicating that *SCD* activity is required for lipogenic activity in the subcutaneous adipose tissue to develop (Smith et al., 2009). In another study, subcutaneous adipose tissue samples were collected from carcasses of pasture and feedlot cattle fed for 100, 200 and 300 days. Pasture-fed cattle adipose tissue had lower total SFA and higher total UFA than in feedlot cattle. Δ9 desaturase activity was much higher in pasture-fed than feedlot cattle (Yang et al., 1999).

The *SCD* gene expression varies between and within breeds. Full-length bovine *SCD* cDNA from 20 Japanese Black steers was compared. Two types of *SCD* genes with single nucleotide polymorphisms (SNP) in the open reading frame where valine (V) was replaced by alanine (A) were observed. The two *SCD* genes were genotyped and classified in 1003 Japanese Black carcasses into VV, VA and AA genotypes. Comparison of fatty acid composition from the carcasses showed that *SCD* type A gene was associated with higher percentage of MUFA with 0.8% effect and lower IMF melting point. They concluded that *SCD* is one of the causes of

genotype variation (Taniguchi et al., 2004b). In contrast, *SCD* (878C>T) SNP was observed to have no association with fatty acid profile in upper sirloin cuts of Aberdeen Angus and Blonde d'Aquitaine cattle (Dujková et al., 2015).

Fatty acid synthase (FASN): The *FASN* gene is abundantly expressed in the adipose tissue and encodes for fatty acid synthase, an enzyme that regulates the biosynthesis of long chain fatty acids. The enzyme plays a central role in *de novo* lipogenesis by catalysing all the reaction steps to convert acetyl-CoA and malonyl-CoA to palmitic acid. Association of *FASN* expression or polymorphisms with fat metabolism and obesity traits in cattle has been reported (Roy et al., 2005; Rempel et al., 2012). Analysing polymorphisms in thioesterase domain of *FASN* gene, which regulates the termination of fatty acid synthesis, in Hanwoo cattle showed a significant association between g.17924G>A SNP genotypes with palmitic and oleic acid concentrations. For instance, GG genotype had 3.2% and 2.8% higher oleic acid concentration than the AA and AG genotypes, respectively. However, they did not observe any significant association between g.17924A>G genotypes and other examined fatty acid such as myristic, stearic and linoleic acids (Bhuiyan et al., 2009). The GG genotype of g.17924A>G SNP was reported to result in higher unsaturated fatty acids and fairly lower amounts of SFA than the AG and AA genotypes in other studies (Morris et al., 2007; Zhang et al., 2008). Another study was carried out to determine exonic SNPs in the gene encoding *FASN* with fatty acid composition in Korean cattle. It was found that all the SNPs (g.12870T>C, g.13126T>C, g.15532C>A, g.16907T>C and g.17924 G>A), were associated with varying fatty acid compositions and marbling. Genotypes CC, TT, AA, TT, and GG were associated with higher MUFA and lower SFA (Oh et al., 2012a).

Some studies reported no relationship between *FASN* gene with fat thickness and marbling score. However, a significant relationship of the fat with DNA-protein kinase, known to play a role in transcriptional activation of *FASN*, was reported (Wong et al., 2009; Wong and Sul, 2009).

Fatty acid binding protein 4 (FABP4): The *FABP4* is a gene highly expressed in the adipose tissue and encodes for fatty acid binding protein 4 that belongs to a group of FABPs. These binding proteins play a significant role in absorption, transport and metabolism of fatty acids, and glucose homeostasis by interacting with peroxisome proliferator-activated receptors (Michal et al., 2006; Kulig et al., 2010). The 7516G>C SNP of *FABP4* was analyzed for association with IMF profile of upper sirloin cuts in Aberdeen Angus and Blonde d'Aquitaine cattle. The CC genotype in Angus cattle was 52% and 64% lower in Myristoleic acid, and 33% and 35% lower in linoleic acid than CG and GG, respectively. Blonde cattle CC genotype had higher arachidonic acid and EPA, but lower oleic acid and total SFA than the CG. The GG genotype was observed in only one bull (Dujková et al., 2015). Polymorphism in the g.7516G>C locus were analyzed for association with marbling score and subcutaneous fat depth in Wagyu x Limousin crosses. A positive relationship between CC genotype and lower marbling and fat depth was observed. The GC genotype had the highest scores while GG was in-between (Michal et al., 2006). The *FABP4* SNP were also reported to have an association with back fat thickness in Korean Native cattle (Cho et al., 2008).

2.7. Justification and Research Objectives of the Study

In northern Australia, the beef industry contributes immensely to the economy and it accounts for over half of Australia's beef exports. The industry relies heavily on native pastures that are highly seasonal and of low quality resulting in weight loss during the dry season and a high turn-off age of 3.5 to 4.5 years (Poppi et al., 2018). Although supplementing beef cattle with protein and energy diets improves weight gain, cost is limiting, hence supplementation is not an economical option in extensive grazing systems. However, nutrient-dense diets are used in feedlots to finish most northern Australian beef cattle following a backgrounding period on pasture. This produces a more even product that meets quality specifications for a wide range of markets. On the other hand, legumes are known to improve pasture and livestock production at a lower cost. However, most legumes do not survive or persist in clay soils prevalent in northern Australia. Desmanthus is one of the few pasture legumes identified to be persistent in clay soils in recent trials. Desmanthus is a highly palatable, high protein content, non-toxic tropical legume with potential to reduce enteric methane emissions, and the few available studies indicate that desmanthus can be used to improve pasture quality and subsequent beef cattle productivity in northern Australia.

However, only limited peer-reviewed published literature is available on the nutritive value of desmanthus and its effect on beef cattle growth and performance. These studies were either conducted indoors or in small sized paddocks (except one in 250 ha paddock) which may not be representative of the normal commercial farm settings. Therefore, there is the need to conduct more studies under commercial farm settings to determine the suitability of grass-desmanthus pastures in northern Australian beef cattle production system.

Tannin-containing pastures at 20 – 40 g tannins per kg DM are reported to increase polyunsaturated fatty acids in meat by reducing rumen biohydrogenation of unsaturated fatty acids (Carreño et al., 2015; Toral et al., 2018). There is the need to study the effect of desmanthus, a tannin-containing legume, on meat quality of cattle.

The majority of beef cattle in northern Australia are *Bos indicus* due to their ability to tolerate ticks, heat and poor quality pastures. However, meat quality from these cattle is low due to low marbling, tenderness and juiciness. These breeds are crossed with *Bos taurus* to improve growth rate and meat quality of several crosses and composite breeds such as Droughtmaster. It is irrefutable that genetic make-up plays a significant role in beef fat content and fatty acid profile.

SNP in several genes such as *SCD*, *FASN* and *FABP4* are reported to influence carcass fat traits in Korean and Japanese cattle as well as Australian temperate breeds such as Angus and Limousin. There is need to investigate the effect of these genes in the tropically adapted crossbred cattle of northern Australia. In addition, studies are required to determine finishing performance and carcass traits of the tropically adapted crossbred cattle of northern Australia backgrounded on newly introduced legume pastures, such as desmanthus, to enable industry players to exploit them for greater economic gains. Therefore, the research objectives required to address the identified gaps are: To evaluate the nutritive value and rumen degradability of desmanthus forage.

- To evaluate the impact of desmanthus pastures on tropical crossbred beef cattle backgrounding and feedlot growth performance, plasma metabolites, carcass traits and meat fatty acid composition.
- To investigate the associations between SNP in *SCD*, *FASN* and *FABP4* genes with carcass traits and fatty acid composition.

Chapter 3: Chemical Composition and *in situ* Degradability of *Desmanthus* spp. Forage Harvested at Different Maturity Stages

3.1. Introduction

Cattle production in the tropical and subtropical regions accounts for over 805 M head globally (Barendse, 2017) and over 15 M head are reared in the northern Australian states of Queensland, Western Australia and Northern Territory (MLA, 2020a). Cattle production in these environments is affected by wide seasonal variations in rainfall patterns and subsequently large variation in pasture quantity and nutritive value (Jones, 2003; Gusha et al., 2013). Seasonal fluctuations in pasture availability and nutritive value are major factors limiting livestock production on tropical grass pastures (Coates et al., 1997; Gusha et al., 2013). This is primarily due to declines in leaf to stem mass ratio (L/S), crude protein (CP) and soluble carbohydrates that reduce the nutritive value and palatability of pastures (Leng, 1990; Brandão et al., 2018). The poor pasture nutritive value leads to poor livestock reproductive performance, increased susceptibility to parasites and diseases, weight loss or slow growth rate and increased enteric methane emissions (McCown, 1981; Johnson and Johnson, 1995; Kanani et al., 2006; Charmley et al., 2008). The introduction of legumes into tropical grass pastures is reported to provide high quality forage to the grazing livestock for a longer period of time compared to grass only pastures (Hill et al., 2009). Augmenting low-quality grass pastures with forage legumes improves feed intake, rumen function and it increases mineral and vitamin availability (Rochon et al., 2004; Kanani et al., 2006). In addition, legumes increase soil nitrogen by symbiotic nitrogen fixation that improves growth of associated grasses, and some legume species contain secondary metabolites such as condensed

tannins that minimize protein degradation in the rumen (Lüscher et al., 2014; Schultze-Kraft et al., 2018).

Research on suitable pasture legumes for the heavier textured soils of semi-arid northern Australia where a scarcity of adapted and productive legume species exists, identified *Desmanthus* spp. (desmanthus) as a suitable candidate (Gardiner and Burt, 1995; Jones and Brandon, 1998; Pengelly and Conway, 2000; Hall and Walker, 2005; Gardiner et al., 2012). Desmanthus is a forage legume of the tribe Mimoseae of the subfamily Mimosoideae native to the Americas (Luckow, 1993), and varies from prostrate to erect, herbaceous to suffruticose and early to late maturing species (Cook et al., 2005; Suybeng et al., 2019). Desmanthus grows in a wide range of soil textures ranging from gravelly, sandy, loam to heavy clay soils of neutral to alkaline pH (Jones and Brandon, 1998; Cook et al., 2005; Gardiner et al., 2012); it is highly palatable, withstands heavy grazing (Gardiner and Swan, 2008), reduces enteric methane emissions (Vandermeulen et al., 2018; Suybeng et al., 2020) and is drought tolerant (Schlink and Burt, 1993; Jones and Brandon, 1998). Desmanthus is reported to survive with as little as 300 mm annual rainfall (Cook et al., 2005) and thrives with 500 to over 1000 mm average annual rainfall (Gardiner and Burt, 1995; Jones and Brandon, 1998). Studies have reported improved livestock growth performance (Rangel and Gardiner, 2009; Gardiner and Parker, 2012; Marsetyo et al., 2017), wool growth (Rangel and Gardiner, 2009) and carcass loin eye muscle area (Aoetpah et al., 2018) when grass-based diets are augmented with desmanthus. However, there is a dearth of peer reviewed literature evaluating the effect of cultivar and maturity stage at harvest on desmanthus nutritive value for ruminant livestock feeding. Therefore, this study aimed to fill this gap in the literature by evaluating the change in nutritive value and *in situ* digestibility of three desmanthus cultivars (JCU2; *D. virgatus*, JCU4; *D. bicornutus*, JCU7; *D. leptophyllus*) harvested at different maturity stages. The criteria for cultivar selection was based

on the ability of JCU2 to combine well with grass pastures in moderate fertility soils of the sub-humid environments, superior drought and grazing tolerance of JCU4 and superior leafiness and bulk of JCU7 (Cook et al., 2005). The study tested the hypothesis that nutritive value and *in situ* degradability of desmanthus differ between cultivars and with maturity stage at harvest.

3.2. Materials and Methods

This study was organized into two experiments; Experiment 1 evaluated the chemical composition and Experiment 2 investigated the degradability of desmanthus forage harvested at varying maturity stages.

3.2.1. Experiment 1 desmanthus establishment and management

Desmanthus was produced in Major Creek, Townsville, located in the sub humid tropical zone of North Queensland (19.39° S, 146.53° E) that receives 1136 mm mean annual rainfall with mean minimum and maximum temperatures of 19.9 °C and 29.0 °C, respectively (Australian Government Bureau of Meteorology, 2022). Soil analysis was carried out in a 12-ha plot of land before forage establishment to examine suitability of the plot for desmanthus production. The soils were dark greyish-brown 10YR/3/2 with a light clay texture and pH 6.7. Pure stands of three desmanthus cultivars, namely *D. virgatus* cv. JCU2, *D. bicornutus* cv. JCU4 and *D. leptophyllus* cv. JCU7 (Agrimix Pastures Pty Ltd, Ferny Hills DC, QLD, Australia), were planted on the 29th of November 2019, in 4 ha plot per cultivar at a sowing rate of 2 kg/ha. The plots were irrigated as follows; 15 – 22 mm/m^2 every two days for the first 10 days, every three to four days from day 11 to 30 and once a week onwards. Plots were not fertilized during desmanthus establishment but were top dressed with Natramin S (4.7% Ca, 0.07% P, 6.3% S, 2.8% K, 2.3% Mg, 23.3% Si, 5.1%

Fe, 3255 ppm C, 930 ppm Mn, 140 ppm Zn, 56 ppm Cu, 23 ppm Co, 18 ppm B and 6 ppm Mo; Ag Solutions, Gympie, QLD, Australia) at 400 kg/ha in February and urea at 100 kg/ha in March 2020. Weeds were controlled by spraying the plots with 3 L/ha glyphosate-based herbicide (Roundup; Monsanto, Kilda Road, Melbourne, Australia) mixed with 0.7 L/ha 2,4-dichlorophenoxyacetic acid (Titan amine; Titan Ag Pty Ltd, Princes Street NSW, Australia) four weeks pre-planting. Fluazifop-P (Fusilade Forte; Syngenta Australia Pty Ltd, Lyonpark Road, NSW, Australia) and clethodim (Select; Arysta Life Science Australia Pty Ltd, Hindmarsh Square, Adelaide, South Australia, Australia) were sprayed at 0.8 and 0.4 L/ha, respectively, three weeks after planting and twice more after forage was slashed back to prevent competition from grasses. All herbicides were mixed in 150 L/ha of water.

3.2.2. Samples harvesting and processing

Forage was slashed in February 2020 and sampling commenced after 11 days of regrowth. Samples were collected randomly by cutting at 5 cm above the ground and bulked to comprise eight samples per cultivar. Harvesting took place once a month after 11, 38, 72 and 103 days of regrowth (Table 3.1). Samples were transported in cooler boxes to the laboratory, separated into stem portion (stems and branches) and leaf portion (consisting of leaves, flowers and pods when present). Samples were dried in a forced-air oven at 55 °C for 48 hours to determine the dry matter (DM) content. Samples were then ground to pass through a 1 mm screen using a Cyclotec mill (Foss Tecator AB, Hoganas, Sweden) and analysed for CP, neutral detergent fibre (NDF) and acid detergent fibre (ADF) using the near infrared reflectance spectroscopy (NIRS) technique described by Norman et al. (2020). The L/S was calculated as dry leaf portion weight divided by dry stem portion weight in grams.

Table 3.1. Physiological maturity phase of desmanthus cultivars JCU2, JCU4 and JCU7 at harvest.

Harvest day	Cultivar		
	JCU2	JCU4	JCU7
	Experiment 1		
11	Vegetative	Vegetative	Vegetative
38	Pre-bloom	Pre-bloom	Vegetative
72	Developing seeds	Developing seeds	Vegetative
103	Mid-mature seeds	Mid-mature seeds	Full bloom
	Experiment 2		
78	Vegetative	Pre-bloom	Vegetative
122	Full bloom	Full bloom	Vegetative
168	Fully mature seeds	Fully mature seeds	Early-bloom

3.2.3. Experiment 2 desmanthus establishment and management

Desmanthus was established at Alligator Creek (19.21° S, 146.57° E) on 15 August 2020, after soil analysis to determine suitability of the site for desmanthus production. The soils were dark greyish-brown 10YR 3/2, heavy sandy loam texture with a pH of 6.3. The area receives 1136 mm mean annual rainfall with mean minimum and maximum temperatures of 19.9 °C and 29.0 °C, respectively (Australian Government Bureau of Meteorology, 2022). The plot was prepared with a tractor and rotary hoe to create a weed free seed bed. Single furrow per cultivar, approximately 40 m long and 4 m between rows, were made using a hand hoe and seeds sown by hand. The plots were rain fed but supplementary drip irrigation was used to occasionally water the plants to avoid water stress. The plot was not fertilized throughout the experiment. To mimic cattle grazing, approximately 300 g of the top 20 – 30 cm 'grab' samples of JCU2, JCU4 and JCU7 were collected

randomly 78, 122 and 168 days after establishment (Table 3.1) from three different locations in triplicates and stored at −20°C prior to processing.

3.2.4. Animal management

The animal use and management procedures were carried out according to the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013) and were reviewed and approved by the CSIRO Agriculture Animal Ethics Committee (Approval number 21/05). Three rumen fistulated Droughtmaster steers weighing 550 ± 48 kg were group housed in an open outdoor pen 18 m x 15 m. Steers were fed Rhodes grass (*Chloris gayana*) hay of 13.8% CP, 66.2% NDF and 32.9% ADF *ad libitum* and had free access to shade, water and mineral block (Trace Element Northern, Ollson's, Yennora, NSW, Australia) throughout the study period. A 14-day pre-experimental period was observed to gradually adapt the steers to the Rhodes grass hay diet. The adaptation period was followed by three weekly experimental periods. The study was organized in a randomized split-plot design with steers as the whole plots and cultivars as sub-plots to ensure that every steer received all the cultivars and maturity stages.

3.2.5. In situ incubations and chemical analysis

Samples were dried at 55 °C for 48 h in a forced-air oven to determine the DM content, and ground to pass through 2 mm screen with a Christy and Norris grinder (Christy Turner Ltd, Suffolk, England). Approximately 5 g of ground desmanthus forage were weighed into nylon bags measuring 9 cm x 14 cm and 45 µm pore size in duplicates. The nylon bags were enclosed in retaining sacs constructed of a mesh (3 mm x 5 mm) material that permits rumen fluid to percolate

completely. The sacs were inserted into the rumen and nylon bags added into the mesh sac using the sequential-in all-out method to ensure 0, 2, 4, 8, 12, 24, 48 and 72 h incubation periods. Rumen fluid pH was measured at every incubation time to ensure optimum rumen environment was maintained. All bags were removed from the rumen after the incubation period was completed and immediately submerged in ice cold water to stop farther microbial activity. The zero h bags were not incubated but were washed together with the incubated bags to determine the easily degradable component of the samples. Bags were washed with cold water using a domestic laundry machine with the short cycle and dried in a forced-air oven at 55°C for 72 h. Dry samples were weighed and duplicates combined and ground to pass through a 1 mm screen using the Cyclotec mill before chemical analysis. The NDF (without heat-stable α amylase) and ADF compositions were determined sequentially according to the methods of Van Soest et al. (1991) and Goering and Van Soest (1970), respectively using an ANKOM 220 Fibre Analyser (ANKOM Technology, Fairport, NY, USA). The nitrogen (N) component of 162 samples was determined by the Dumas combustion method (Sweeney and Rexroad, 1987) with a Leco CN628 N Analyser (Leco, St. Joseph, MI, USA). The N results were used to develop calibrations to predict N composition of the remaining 67 samples with the NIRS technique due to mechanical failure of the Leco CN628 N Analyser. The samples CP composition was calculated as total N × 6.25.

3.2.6. Data analysis

3.2.6.1. Calculations

The DM, CP, NDF and ADF degradability were calculated within steer and period. Duplicate values at each time were averaged and then fitted using the revised nonlinear regression equation

of Ørskov and McDonald (McDonald, 1981) (Equation 1) or the Ørskov and McDonald (1979) (Equation 2).

The following exponential equations were used based on preliminary model fitting (Figure 3.1);

$P = A + B(1-e^{-C(T-L)})$ for DM degradability……..…….(1)

$P = A + B(1-e^{-CT})$ for CP, NDF and ADF degradability……(2)

Where:

P is the proportion degraded at time T, A is the highly soluble fraction, B is the potentially degradable fraction, A+B is the total potentially degradable fraction, C is the rate of degradation of fraction B and L is the lag time.

The parameters (A, B, A+B, C, L) of degradation were estimated from the solution of the exponential equations (1) and (2) using the SAS procedure (PROC NLIN) from degradability data. Examples of the fitted segmented nonlinear (exponential) and exponential curve for DM and CP are displayed in Figure 3.1.

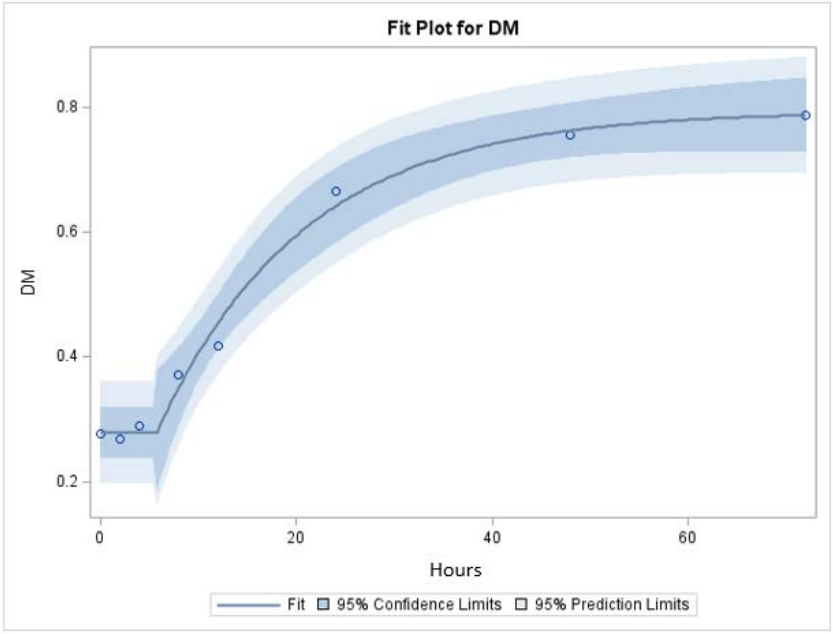
The effective degradable proportion (ED) was calculated as;

$ED = A + BC/(C+K)$……(3)

Where:

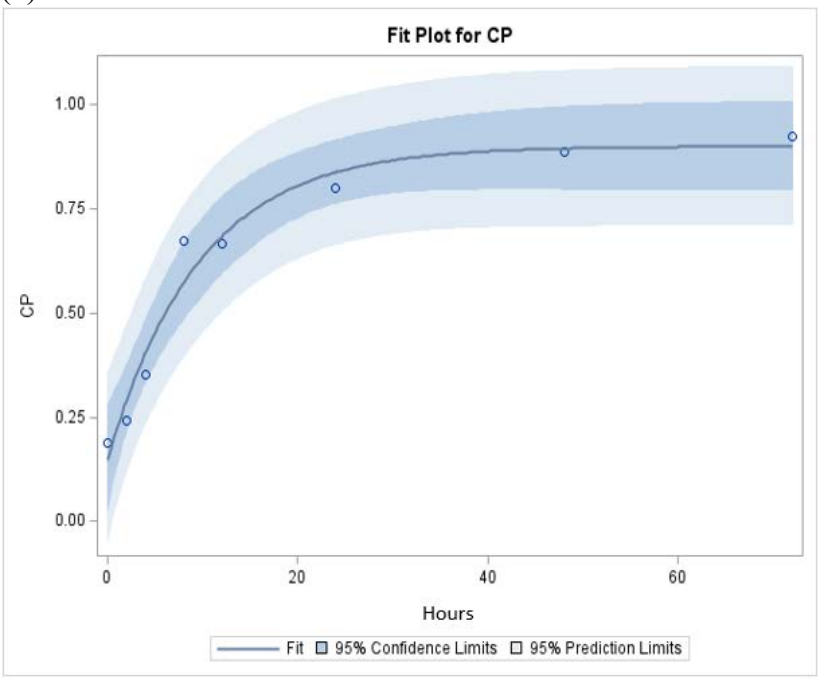
K is the particle outflow rate from the rumen (/h). The ED was estimated with slow and rapid outflow rates of 2%/h (ED2) and 6%/h (ED6), respectively.

(a)



A = 0.28 (0.02), B = 0.52 (0.03), C = 0.07 (0.01), L = 5.71 (1.12)

(b)



A = 0.15 (0.05), B = 0.75 (0.06), C = 0.10 (0.02)

Figure 3.1. Non-linear models for the estimation of degradation of (**a**) DM and (**b**) CP.

A is the intercept of the curve at 0h representing the highly soluble fraction; B is the asymptote representing the potentially degradable fraction; C is the fractional rate constant for degradation of B; L is the time lag.

3.2.6.2. Statistical analysis

All statistical data analyses were implemented in SAS version 9.4 and R version 4.0.1. Plant component nutritive values (leaf to stem ratio, DM, CP, NDF and ADF) and degradability variables (A, B, A+B, C, L, ED2 and ED6) were summarised as means and standard deviations. Analysis of variance (ANOVA) was used to determine if the nutritive values (4) and degradability (5) of desmanthus differ between cultivars and maturity stage at harvest. In addition, post hoc pairwise comparisons were carried out with Tukey's adjusted *P*-values.

The model for plant component nutritive values is of the form:

$$Y_{ij} = \mu + Cultivar_i + Maturity_j + (Cultivar \times Maturity)_{ij} + e_{ij} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where:

Y_{ij} is the nutritive value (leaf to stem ratio, DM, CP, NDF or ADF) for i^{th} cultivar at j^{th} maturity time;

μ is the overall mean;

$Cultivar_i$ is the fixed effect of i^{th} cultivar (JCU2, JCU4, JCU7);

$Maturity_j$ is the fixed effect of stage of maturity (11, 38,72, 103 days);

$(Cultivar \times Maturity)_{ij}\ is$ interaction between cultivar and stage of maturity (fixed effects);

$e_{ij} is$ random error.

e_{ij} i. i. d $\sim N(0, \sigma_e^2)$

The model for degradability variables is of the form:

$$Y_{ijk} = \mu + Cultivar_i + Maturity_j + (Cultivar \times Maturity)_{ij} + \alpha_k + e_{ijk} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Where:

Y_{ijk} is a degradability variable (A, B, A+B, C, L, ED2 and ED6) for i^{th} cultivar at j^{th} maturity time in steer k;

μ is the overall mean;

$Cultivar_i$ is the fixed effect of i^{th} cultivar (JCU2, JCU4, JCU7);

$Maturity_j$ is the fixed effect of stage of maturity (78, 122, 168 days);

$(Cultivar \times Maturity)_{ij}$ is interaction between cultivar and stage of maturity (fixed effects);

α_k is a random effect of the steer, and

e_{ij} is a residual error.

α_k i. i. d $\sim N(0, \sigma_\alpha^2)$

In addition, e_{ij} and α_k were assumed to be independent. Inferences were based on a 5% level of significance.

3.3. Results

3.3.1. Experiment 1: Leaf to stem mass ratio, dry matter and chemical composition

The effect of desmanthus cultivar, maturity stage at harvest and their interactions on L/S, DM, CP, NDF and ADF are presented in Table 3.2, Figure 3.2 and Figure 3.3.

Leaf to stem mass ratio. There was an interaction between cultivar and maturity on the L/S (P = 0.04; Figure 3.2), where the L/S of cultivar JCU7 declined more steeply than the other cultivars, JCU2 and JCU4. The L/S was higher in JCU7 for young desmanthus forage and higher in JCU4 for the mature forage, and L/S decreased linearly with maturity for all cultivars evaluated (P < 0.01).

Dry matter concentration. Significant interactions between cultivar and maturity were observed for DM composition of both leaf and stem portions ($P \leq 0.02$; Figure 3.3a and 3.3e). In both cases, there was a higher linear increase in DM composition of JCU4 with maturity compared to JCU2 and JCU7 which increased at a lower rate. Generally, the stem DM was highest in JCU4 and lowest in JCU2.

Crude protein composition. As shown in Table 3.2, an interaction between cultivar and maturity was observed for the leaf CP ($P < 0.01$) but not in stem CP ($P = 0.29$). There was a higher linear decline in leaf CP of JCU4 with maturity than JCU2 and JCU7 (Figure 3.3b). The stem CP declined with maturity for all cultivars ($P < 0.01$), but no significant difference between cultivar means was observed ($P = 0.63$).

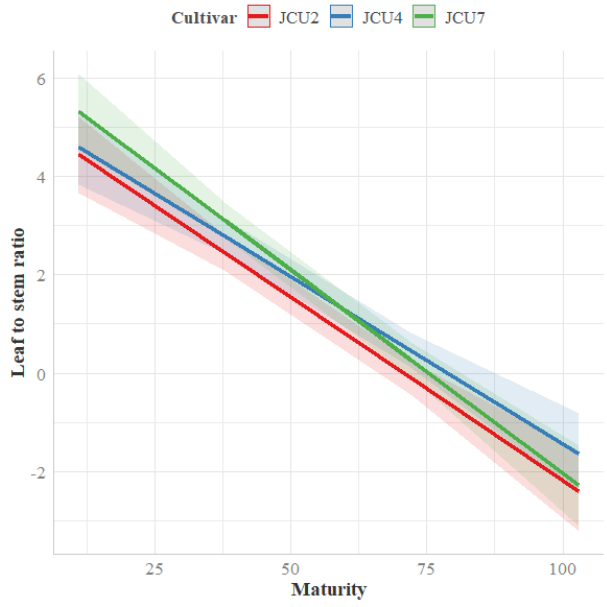


Figure 3.2. Effect plots for the interactions between cultivar and maturity on desmanthus leaf to stem mass ratio ($P = 0.04$).

Fibre composition. A significant interaction effect of cultivar and maturity on the stem NDF ($P < 0.01$) was observed but not in the leaf NDF ($P = 0.27$). The stem NDF increased substantially with maturity in JCU2 and JCU4 but not in JCU7 (Figure 3.3g). On the other hand, the leaf NDF declined with maturity ($P = 0.01$), and NDF was lower in JCU4 compared to JCU2 and JCU7 ($P < 0.01$). There were significant interactions between cultivar and maturity on the leaf and stem ADF ($P \leq 0.03$). The leaf ADF increased more steeply with maturity for JCU4 compared to JCU2 and JCU7 (Figure 3.3d), while the stem ADF increased with maturity for both JCU2 and JCU4 but not in JCU7 (Figure 3.3h).

Table 3.2. Mean leaf to stem mass ratio (L/S), dry matter (DM; %) and chemical composition (% DM) of desmanthus harvested at varying maturity stages

Variable[1]	Cultivar[2]	Maturity at harvest				Mean	SEM[3]	*P*-value		
		11	38	72	103			C	M	C*M
L/S	JCU2	2.2	0.60	0.74	0.89	1.1[a]	0.09	< 0.01	< 0.01	0.04
	JCU4	2.4	0.77	1.7	1.5	1.6[b]				
	JCU7	3.5	0.82	1.1	1.2	1.7[b]				
	Mean	2.7	0.73	1.2	1.2					
Leaf										
DM	JCU2	22.8	28.5	33.5	42.4	31.8	0.99	0.14	< 0.01	0.01
	JCU4	24.0	27.8	31.0	53.6	34.1				
	JCU7	25.9	26.3	31.9	44.2	32.1				
	Mean	24.2	27.5	32.1	46.7					
CP	JCU2	28.1	23.5	23.7	19.5	23.7	0.57	0.12	< 0.01	< 0.01
	JCU4	29.0	27.2	19.9	16.9	23.3				
	JCU7	24.3	23.4	23.0	18.7	22.4				
	Mean	27.1	24.7	22.2	18.4					
NDF	JCU2	47.1	49.6	51.5	38.2	46.6[b]	0.74	< 0.01	0.01	0.27
	JCU4	45.4	39.2	37.9	41.4	41.0[a]				
	JCU7	46.5	44.6	51.0	43.1	46.3[b]				
	Mean	46.3	44.5	46.8	40.9					
ADF	JCU2	14.6	24.5	27.1	25.1	22.8[a]	0.79	0.02	< 0.01	0.03
	JCU4	16.1	23.3	27.8	32.3	24.9[b]				
	JCU7	18.0	27.7	26.5	29.6	25.5[b]				
	Mean	16.2	25.2	27.1	29.0					
Stem										
DM	JCU2	19.5	31.2	41.0	42.2	33.5[a]	1.2	0.03	< 0.01	0.02
	JCU4	17.8	33.6	44.3	51.1	36.7[b]				
	JCU7	18.9	32.3	35.8	48.9	34.0[a]				
	Mean	18.7	32.4	40.4	47.4					
CP	JCU2	12.5	7.2	6.1	6.7	8.1	0.41	0.63	< 0.01	0.29
	JCU4	13.1	6.8	4.6	7.2	7.9				
	JCU7	11.6	6.2	8.4	7.9	8.5				
	Mean	12.4	6.7	6.4	7.3					
NDF	JCU2	54.2	65.6	68.8	67.1	63.9	0.92	0.88	0.01	< 0.01
	JCU4	53.9	67.3	72.8	64.2	64.6				
	JCU7	63.4	69.1	63.1	59.6	63.8				
	Mean	57.2	67.3	68.2	63.6					
ADF	JCU2	40.0	52.8	54.3	50.7	49.5	0.85	0.94	0.01	0.02
	JCU4	40.4	52.9	56.3	49.3	49.7				
	JCU7	46.7	55.5	50.3	44.8	49.3				
	Mean	42.4	53.7	53.6	48.3					

[1] DM; dry matter, CP; crude protein, NDF; neutral detergent fibre, ADF; acid detergent fibre

[2] JCU2; *D. virgatus*, JCU4; *D. bicornutus*, JCU7; *D. leptophyllus*

[3] SEM; standard error of the mean

C; cultivar effect, M; maturity effect, C*M; cultivar by maturity interaction: *P*-value based on ANOVA

[ab] Cultivar means with different superscripts were significantly different ($P < 0.05$)

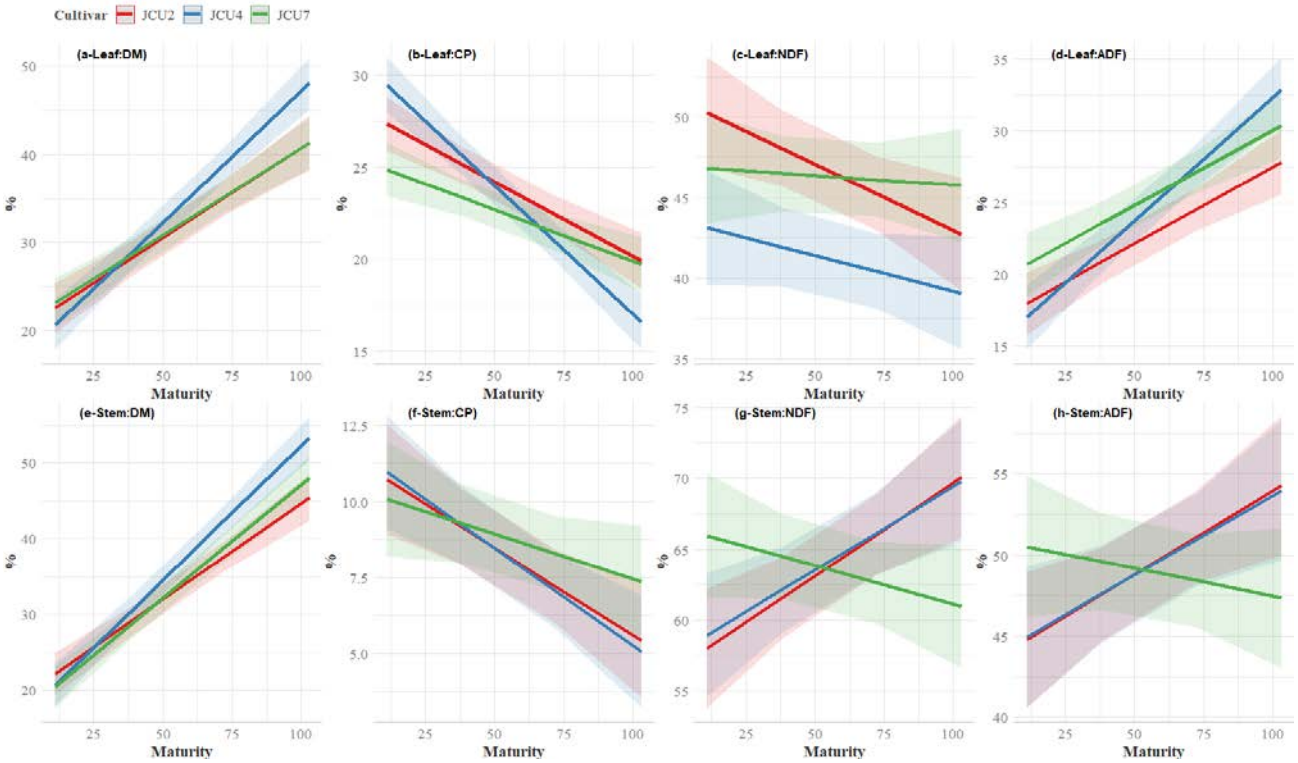


Figure 3.3. Effect plots for the interactions between cultivar and maturity at harvest on desmanthus leaf and stem portion dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) composition.

All interactions were significant except the leaf NDF ($P = 0.27$) and stem CP ($P = 0.29$).

3.3.2. Experiment 2: Forage dry matter and chemical composition

The forage DM, CP, NDF and ADF are presented in Table 3.3. These data were obtained from composite samples, thus not statistically analyzed. Generally, all the cultivars portrayed a numerical increase in NDF and ADF composition with increase in maturity. In contrast, the CP decreased with increase in maturity for JCU2 and JCU7, but JCU4 CP increased from day 78 to day 122 followed by a decline at day 168. The DM composition for JCU2 and JCU4 increased with maturity, while JCU7 DM was lowest on day 122 and highest on day 168. The mean DM,

CP, NDF and ADF were similar across cultivars with a maximum difference of 3.3% in ADF observed between JCU4 and JCU7.

Table 3.3. Dry matter (%) and chemical composition (%DM) of desmanthus forage harvested 78, 122 and 168 days after planting.

	JCU2				JCU4				JCU7			
Variable[1]	78	122	168	Mean	78	122	168	Mean	78	122	168	Mean
DM	26.1	29.4	33.2	29.6	27.4	26.6	34.3	29.4	28.3	28.1	30.3	28.9
CP	25.2	20.4	16.9	20.8	23.0	26.4	17.3	22.2	23.8	22.0	20.8	22.2
NDF	25.4	34.2	38.7	32.8	25.6	27.4	47.9	33.6	29.3	31.2	35.2	31.9
ADF	14.0	19.3	24.5	19.3	14.0	15.9	32.4	20.8	13.0	18.2	21.2	17.5

[1] DM; dry matter, CP; crude protein, NDF; neutral detergent fibre; ADF; acid detergent fibre
JCU2; *D. virgatus*, JCU4; *D. bicornutus*, JCU7; *D. leptophyllus*

3.3.3. Dry matter degradation

Table 3.4 presents the DM A, B, A+B, C, L, ED2 and ED6 of three desmanthus cultivars (JCU2, JCU4 and JCU7) harvested 78, 122 and 168 days after planting. There were no significant interactions between cultivar and maturity for all variables evaluated ($P \geq 0.17$). The lowest degradation of A was observed on day 168 for all cultivars ($P = 0.01$), whereas degradation of A was lower for JCU7 than JCU2 and JCU4 ($P < 0.01$). The B proportion of DM was influenced by cultivar with the highest degradation recorded for JCU7 followed by JCU2 and the least degradation was in JCU4 ($P < 0.01$). However, degradation of the A+B component and the ED2 were not significantly different between cultivars ($P \geq 0.25$), although degradation declined with maturity for all cultivars ($P < 0.01$; Figure 3.4c). The cultivar and maturity had significant effects on the lag time ($P \leq 0.04$). The lag time increased linearly with maturity (Figure 3.4e), whereas

JCU7 had the longest lag time. The ED6 was influenced by cultivar and maturity ($P < 0.01$). ED6 declined linearly with maturity for all cultivars (Figure 3.4g), JCU4 had higher ED6 than JCU7.

Table 3.4. Effect of cultivar and maturity stage at harvest of desmanthus on *in situ* dry matter (DM) degradation. Data presented in fraction unless otherwise stated.

Variable[1]	Cultivar[2]	Maturity at harvest					*P*-value[4]		
		78	122	168	Mean	SEM[3]	C	M	C*M
A	JCU2	0.30	0.27	0.26	0.28[b]	0.00	< 0.01	0.01	0.22
	JCU4	0.28	0.30	0.26	0.28[b]				
	JCU7	0.25	0.26	0.24	0.25[a]				
	Mean	0.28	0.28	0.25					
B	JCU2	0.53	0.48	0.42	0.48[b]	0.01	< 0.01	< 0.01	0.51
	JCU4	0.49	0.54	0.31	0.45[a]				
	JCU7	0.59	0.51	0.52	0.54[c]				
	Mean	0.54	0.51	0.42					
A + B	JCU2	0.84	0.75	0.68	0.76	0.01	0.30	< 0.01	0.66
	JCU4	0.78	0.84	0.58	0.73				
	JCU7	0.85	0.78	0.76	0.79				
	Mean	0.82	0.79	0.67					
C (/h)	JCU2	0.13	0.11	0.08	0.11[ab]	0.01	< 0.01	0.03	0.48
	JCU4	0.17	0.15	0.07	0.13[b]				
	JCU7	0.06	0.07	0.04	0.06[a]				
	Mean	0.12	0.11	0.06					
Lag (h)	JCU2	2.1	2.7	2.6	2.5[a]	0.31	< 0.01	0.04	0.17
	JCU4	3.0	3.1	3.0	3.0[a]				
	JCU7	4.0	5.7	6.1	5.3[b]				
	Mean	3.0	3.8	3.9					
ED2	JCU2	0.77	0.68	0.59	0.68	0.01	0.25	< 0.01	0.55
	JCU4	0.72	0.77	0.51	0.67				
	JCU7	0.71	0.66	0.59	0.65				
	Mean	0.73	0.70	0.57					
ED6	JCU2	0.67	0.58	0.49	0.58[ab]	0.02	< 0.01	< 0.01	0.53
	JCU4	0.65	0.67	0.44	0.59[b]				
	JCU7	0.56	0.54	0.46	0.52[a]				
	Mean	0.63	0.60	0.46					

[1] A; highly soluble fraction, B; potentially degradable fraction, A+B; total potentially degradable fraction, C; rate of degradation of fraction B, ED2; effective degradability with particle outflow rate of 2%/h, ED6; effective degradability with particle outflow rate of 6%/h
[2] JCU2; *D. virgatus*, JCU4; *D. bicornutus*, JCU7; *D. leptophyllus* [3] SEM, standard error of the mean
[4] C; cultivar effect, M; maturity effect, C*M; cultivar by maturity interaction: *P*-value based on ANOVA. [abc] Cultivar means with different uppercase superscripts per variable were significantly different ($P < 0.05$)

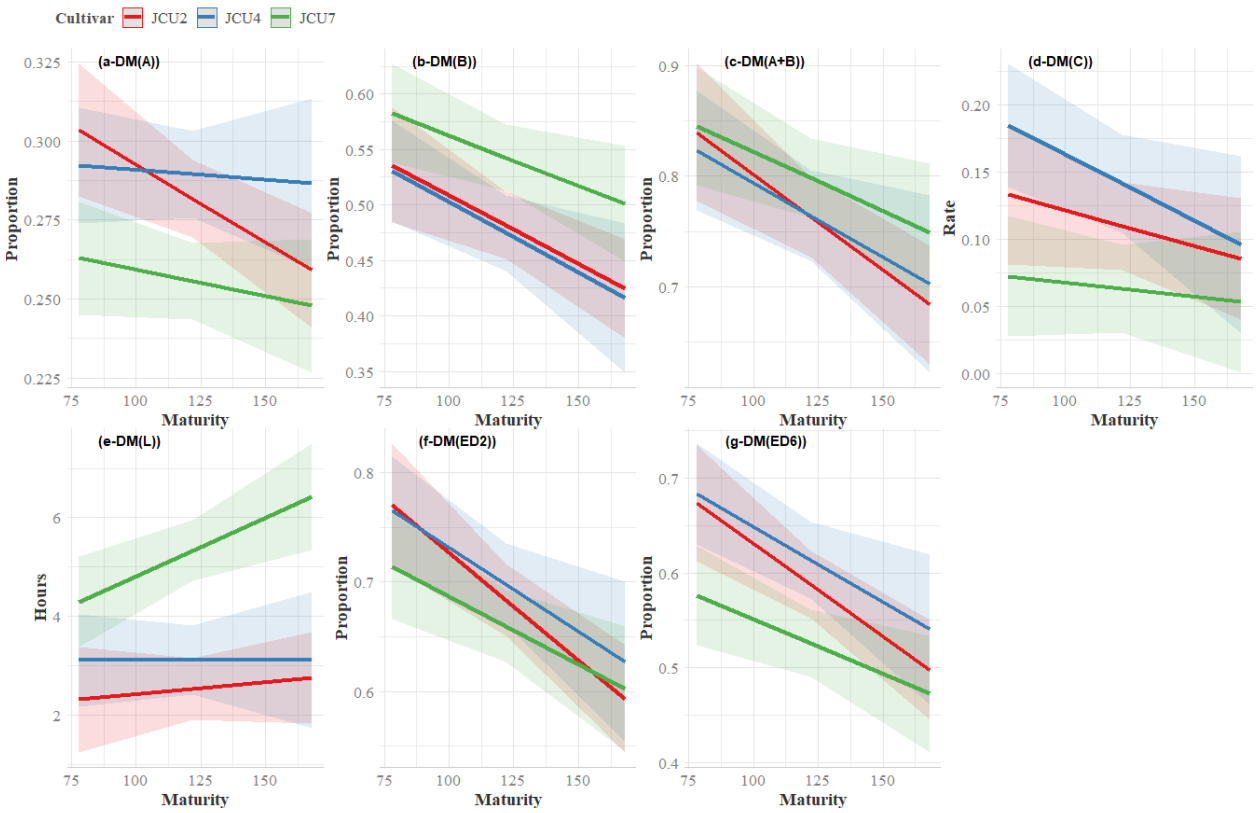


Figure 3.4. Effect plots for the change in *in situ* dry matter (DM) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. **(a)** A; highly soluble fraction, **(b)** B; potentially degradable fraction, **(c)** A+B; total potentially degradable fraction, **(d)** C; rate of degradation of fraction B, **(e)** L; lag time, **(f)** ED2; effective DM degradability with particle outflow rate of 2%/h and **(g)** ED6; effective DM degradability with particle outflow rate of 6%/h.

No significant interactions between cultivar and maturity were observed ($P \geq 0.17$).

3.3.4. Crude protein degradation

The degradation of CP and the rate of degradation are presented in Table 3.5 and Figure 3.5. The cultivar and maturity interaction was significant in the A and A+B ($P < 0.01$), but not in the B, C, ED2 and ED6 ($P \geq 0.11$). Degradation of the A component increased linearly with maturity for all cultivars ($P < 0.01$), but the increase was higher for JCU4. The overall degradation was highest in

JCU4 and lowest in JCU7 ($P = 0.01$). The A+B degradation was observed to decline with maturity for all cultivars ($P = 0.04$; Figure 3.5c). JCU7 had lower C, ED2 and ED6 than both JCU2 and JCU4 ($P < 0.01$), while the C and ED2 were observed to decline with maturity (Figure 3.5). The B component degradation was influenced by cultivar effects, with higher degradation observed for JCU7 than JCU4 ($P = 0.01$), but JCU2 did not differ from either JCU4 or JCU7.

Table 3.5. Effect of cultivar and maturity stage at harvest of desmanthus on *in situ* crude protein (CP) degradation. Data presented in fraction unless otherwise stated.

Variable[1]	Cultivar[2]	Maturity at harvest					*P*-value[4]		
		78	122	168	Mean	SEM[3]	C	M	C*M
A	JCU2	0.16	0.20	0.23	0.20[ab]	0.01	0.01	< 0.01	< 0.01
	JCU4	0.13	0.22	0.32	0.23[b]				
	JCU7	0.15	0.17	0.19	0.17[a]				
	Mean	0.15	0.20	0.25					
B	JCU2	0.72	0.63	0.57	0.64[ab]	0.01	0.01	< 0.01	0.11
	JCU4	0.66	0.70	0.44	0.60[a]				
	JCU7	0.72	0.71	0.65	0.70[b]				
	Mean	0.70	0.68	0.55					
A + B	JCU2	0.89	0.83	0.81	0.84	0.01	0.20	0.04	< 0.01
	JCU4	0.80	0.93	0.76	0.83				
	JCU7	0.88	0.89	0.85	0.87				
	Mean	0.86	0.88	0.81					
C (/h)	JCU2	0.09	0.08	0.08	0.08[b]	0.00	< 0.01	< 0.01	0.47
	JCU4	0.10	0.09	0.06	0.08[b]				
	JCU7	0.05	0.03	0.03	0.04[a]				
	Mean	0.08	0.07	0.06					
ED2	JCU2	0.76	0.71	0.70	0.72[b]	0.01	< 0.01	0.01	0.71
	JCU4	0.69	0.80	0.66	0.72[b]				
	JCU7	0.67	0.64	0.60	0.64[a]				
	Mean	0.71	0.72	0.65					
ED6	JCU2	0.60	0.57	0.57	0.58[b]	0.01	< 0.01	0.16	0.43
	JCU4	0.55	0.65	0.56	0.59[b]				
	JCU7	0.48	0.45	0.42	0.45[a]				
	Mean	0.55	0.56	0.52					

[1234] Fractions, abbreviations and effects are the same as in Table 3.4 *P*-value based on ANOVA
[ab] Cultivar means with different superscripts were significantly different ($P < 0.05$)

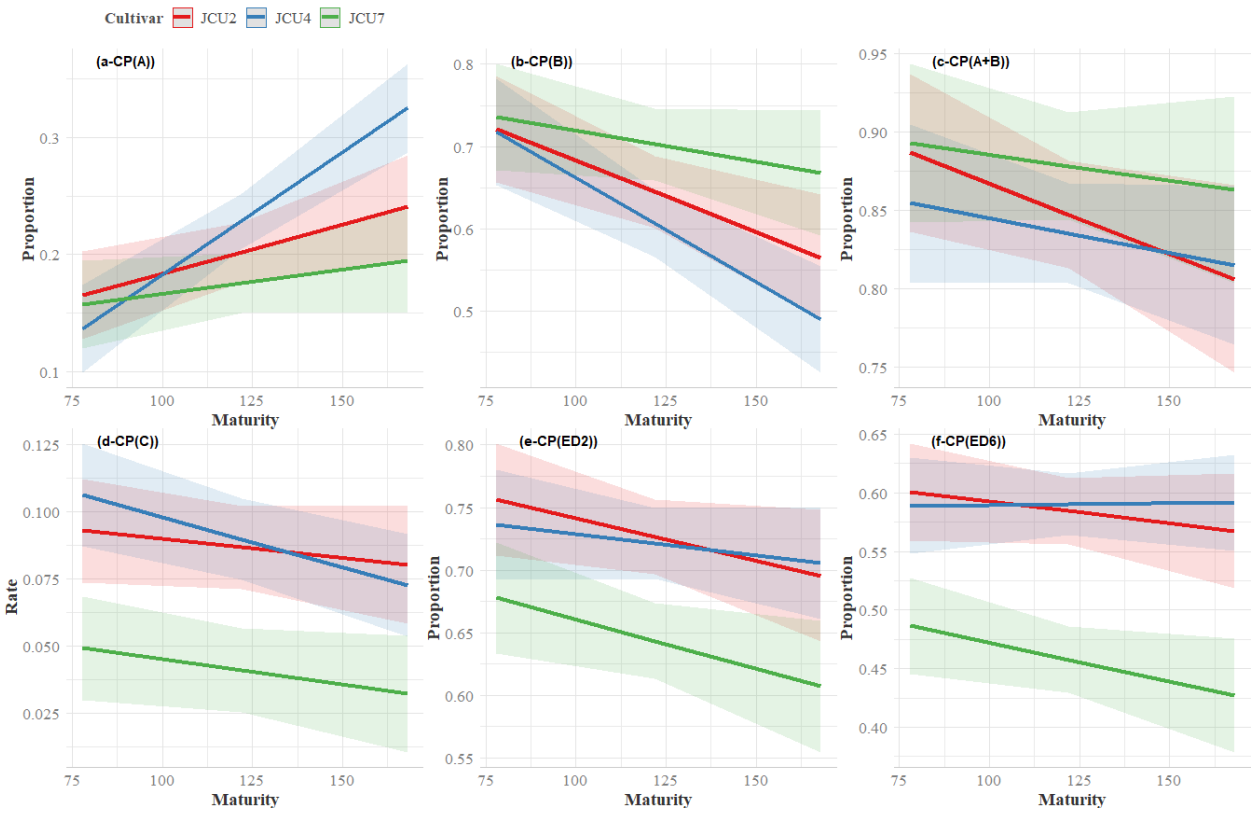


Figure 3.5. Effect plots for the change in *in situ* crude protein (CP) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. **(a)** A; highly soluble fraction, **(b)** B; potentially degradable fraction, **(c)** A+B; total potentially degradable fraction, **(d)** C; rate of degradation of fraction B, **(e)** ED2; effective CP degradability with particle outflow rate of 2%/h and **(f)** ED6; effective CP degradability with particle outflow rate of 6%/h.

Cultivar by maturity interactions were not significant except for the A and A+B proportions ($P < 0.01$)

3.3.5. Fibre degradation

Desmanthus potentially degradable fraction (B), rate of degradation of fraction B (C) and effective degradability (ED2 and ED6) of NDF and ADF in response to cultivar, maturity and their interactions are presented in Table 3.6 and Figures 3.6 and 3.7. There were no significant effects of the cultivar and maturity interactions on all the NDF and ADF variables evaluated ($P \geq 0.12$). Components B, C, ED2 and ED6 declined with maturity for NDF and ADF ($P < 0.01$). Significant

cultivar effects were observed for B and C of NDF ($P < 0.01$), and C in ADF component ($P = 0.02$). The degradation of the B component of the NDF was higher in JCU7 than in JCU2 and JCU4. However, JCU7 had lower C for both the NDF and ADF proportions.

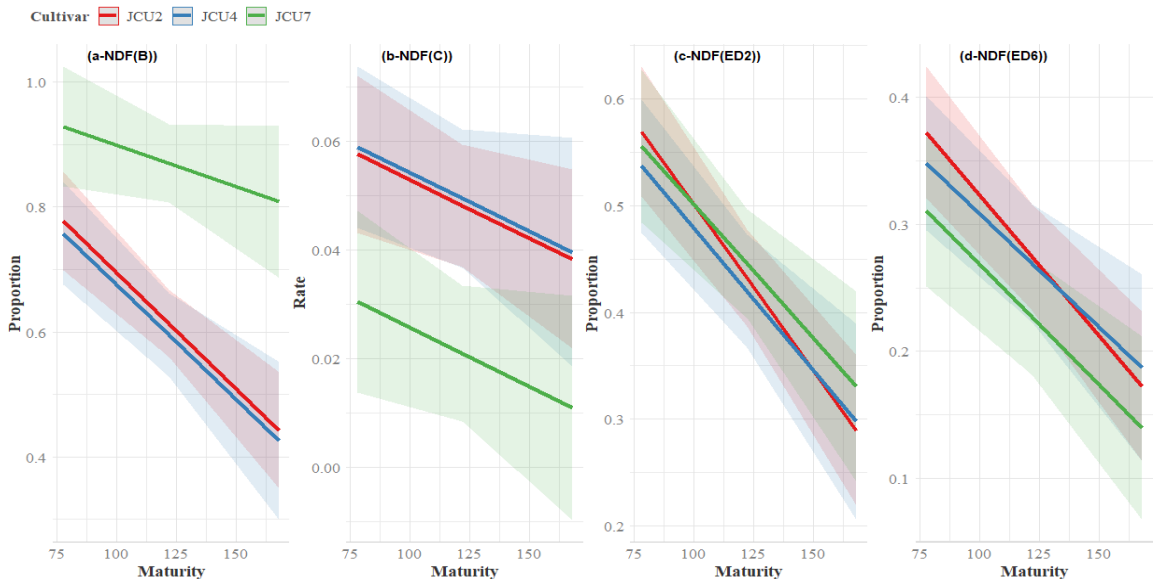


Figure 3.6. Effect plots for the change in *in situ* neutral detergent fibre (NDF) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. **(a)** B; potentially degradable fraction, **(b)** C; rate of degradation of fraction B, **(c)** ED2; effective NDF degradability with particle outflow rate of 2%/h and **(d)** ED6; effective NDF degradability with particle outflow rate of 6%/h.

Cultivar by maturity interactions were not significant ($P \geq 0.12$).

Table 3.6. Effect of cultivar and maturity stage at harvest of desmanthus on *in situ* neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradation. Data presented in fraction unless otherwise stated.

Variable[1]	Cultivar[2]	Maturity at harvest					*P*-value[4]		
		78	122	168	Mean	SEM[3]	C	M	C*M
NDF									
B	JCU2	0.77	0.62	0.43	0.61[a]	0.03	< 0.01	< 0.01	0.12
	JCU4	0.71	0.71	0.31	0.58[a]				
	JCU7	0.96	0.82	0.88	0.89[b]				
	Mean	0.81	0.71	0.54					
C (/h)	JCU2	0.05	0.04	0.03	0.04[b]	0.00	< 0.01	< 0.01	0.99
	JCU4	0.05	0.05	0.03	0.04[b]				
	JCU7	0.03	0.02	0.01	0.02[a]				
	Mean	0.04	0.04	0.02					
ED2	JCU2	0.56	0.43	0.27	0.42	0.02	0.70	< 0.01	0.75
	JCU4	0.50	0.51	0.19	0.40				
	JCU7	0.57	0.41	0.39	0.46				
	Mean	0.55	0.45	0.28					
ED6	JCU2	0.37	0.27	0.16	0.27	0.01	0.66	< 0.01	0.77
	JCU4	0.33	0.33	0.10	0.25				
	JCU7	0.32	0.20	0.18	0.23				
	Mean	0.34	0.27	0.15					
ADF									
B	JCU2	0.71	0.60	0.38	0.56	0.03	0.09	< 0.01	0.14
	JCU4	0.64	0.69	0.32	0.55				
	JCU7	0.77	0.65	0.71	0.71				
	Mean	0.71	0.64	0.47					
C (/h)	JCU2	0.04	0.02	0.02	0.03[b]	0.00	0.02	< 0.01	0.47
	JCU4	0.03	0.03	0.02	0.03[b]				
	JCU7	0.03	0.02	0.01	0.02[a]				
	Mean	0.03	0.02	0.02					
ED2	JCU2	0.49	0.31	0.21	0.34	0.02	0.77	< 0.01	0.29
	JCU4	0.39	0.42	0.19	0.33				
	JCU7	0.46	0.34	0.31	0.37				
	Mean	0.45	0.36	0.24					
ED6	JCU2	0.30	0.16	0.11	0.19	0.01	0.55	< 0.01	0.28
	JCU4	0.22	0.24	0.10	0.18				
	JCU7	0.26	0.17	0.15	0.19				
	Mean	0.26	0.19	0.12					

[1234] Abbreviations same as in Table 3.4; *P*-value based on ANOVA

[ab] Cultivar means with different superscripts were significantly different ($P < 0.05$)

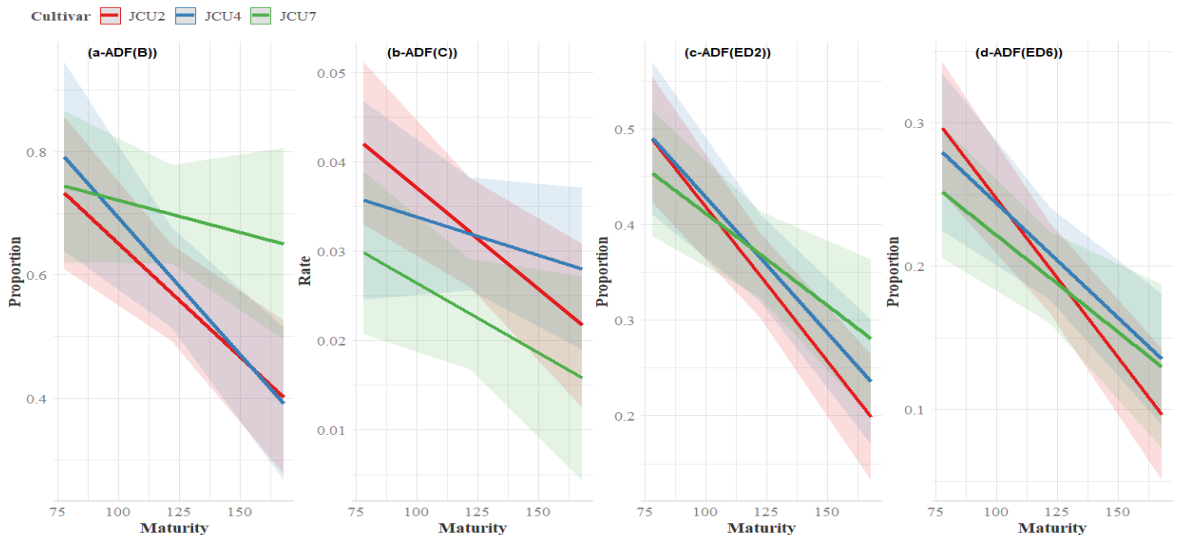


Figure 3.7. Effect plots for the change in *in situ* acid detergent fibre (ADF) degradation of *D. virgatus* (JCU2), *D. bicornutus* (JCU4) and *D. leptophyllus* (JCU7) with maturity. **(a)** B; potentially degradable fraction, **(b)** C; rate of degradation of fraction B, **(c)** ED2; effective ADF degradability with particle outflow rate of 2%/h and **(d)** ED6; effective ADF degradability with particle outflow rate of 6%/h. Cultivar by maturity interactions were not significant ($P < 0.14$).

3.4. Discussion

3.4.1. Leaf to stem mass ratio

The leaf proportion of forage contributes critically to the forage nutritive value because a decrease in leaf proportion is associated with increased forage NDF and ADF that are inversely correlated with digestibility (Lemàire and Bélanger, 2020). The L/S ranged between 0.7 and 3.5 in this study and this range was wider than the 0.9 − 1.3 range reported for unidentified species of desmanthus in Brazil (Calado et al., 2016). In this study, there was a decrease in the L/S with advancing plant maturity. These results concur with findings reported previously for *D. virgatus* and several other forage legumes such as *Medicago sativa*, *Trifolium repens* and *Desmodium intortum* (Wilman and Moghaddam, 1998; Suksombat and Buakeeree, 2006; Grev et al., 2020). The decline in L/S with

maturity is attributed to the decreasing leaf proportion due to senescence and increasing stem proportion as the plant matures (Buxton et al., 1985; Sheaffer et al., 2000). The finding that cultivar JCU7 had the highest L/S in this study was expected since JCU7 is described to be among the highest performing cultivars of desmanthus in leafiness (Cook et al., 2005). The slower decrease in L/S of JCU4 with maturity may be due to increase in leaf mass and production of pods that might have offset the stem proportion increase. Calado et al. (2016) reported increase in both leaves and stem yield with maturity of desmanthus harvested at three pod stages.

3.4.2. Chemical composition

The lower leaf NDF in JCU4 than JCU2 and JCU7 in this study agree with findings reported by Vandermeulen et al. (2018), but were in contrast with results of Durmic et al. (2017) who reported higher NDF in JCU4 than JCU2. The discrepancies may be due to differences in growth stage at harvest, plant part analysed or environmental factors (Suksombat and Buakeeree, 2006; Valderrama and Anrique, 2011; Gierus, 2013). This is because Durmic et al. (2017) analysed the leaves and young stems of up to 5cm long, propagated in an open environment, while Vandermeulen et al. (2018) analysed whole-plants grown in a semi-enclosed greenhouse and harvested from 10 cm above ground. Dietary digestible fibre is the main source of digestible energy in beef cattle fed forage diets and NDF provides physically effective fibre that stimulates rumination, salivation and reticulorumen motility which help elevate ruminal pH (National Academies of Sciences, Engineering and Medicine, 2016). Increase in dietary fibre is usually associated with reduced concentration of fermentable carbohydrates (Brandao and Faciola, 2019). Rumen microbial population growth is highly dependent on the fermentable carbohydrates. Insufficient supply of fermentable carbohydrates in relation to nitrogen reduces microbial protein

synthesis and increase nitrogen loss through urine (Hristov et al., 2005). Therefore, the increase in ADF and decrease in CP with maturity observed in this study may reduce rumen microbial protein synthesis. Generally, the ADF increased with maturity in both the leaf and stem proportions while NDF increased with maturity only in the stem proportion in this study. Structural carbohydrates are reported to increase with maturity for both the leaf and stem proportions in desmanthus (Suksombat and Buakeeree, 2006) and lucerne (Sheaffer et al., 2000), but the increase is less in leaves and higher in the stem because of lignin accumulation within cell walls during stem diameter expansion through cambial activity (Lemàire and Bélanger, 2020). The accumulation of structural carbohydrates in the lower stem internodes is accelerated as the plant stem height increases to provide plant support against mechanical constraint (Lemàire and Bélanger, 2020). Lignin content of the bottom two internodes in lucerne was reported to be 26.3% compared to 11.8% in the top four internodes (Vallet et al., 1998). The CP was higher in the leaf portion at ≥ 16.9% compared to the ≤ 7.9% in stem on day 103. In addition, the NDF was below 47% in the leaf and above 55% in the stem. Since grazing livestock select plant parts based on their nutritive need and availability (Buxton, 1996; Bailey and Brown, 2011), these findings indicate that animals can access high quality diet later in the pasture growing season from desmanthus leaves when the stems have declined in quality.

The observed similar CP composition between cultivars in this study agrees with the findings reported by Suybeng et al. (2021) for JCU2, JCU4 and JCU7, indicating that livestock grazing the different desmanthus cultivars separately or as a blend will obtain similar CP. The decrease in desmanthus CP with increase in maturity for both leaf and stem proportions observed in this study agree with findings reported by Suksombat and Buakeeree (2006) for *D. virgatus* harvested at 30-, 40- or 50-days regrowth, and may be due to increase in the fibre portion of the plant (Saylor et

al., 2021). Except for JCU4 stem portion on day 72, the forage CP was above the 5% to 5.6% threshold reported to prevent digestive disruption and body protein catabolism in cattle (Bowman et al., 1995; Durmic et al., 2017); this suggests that desmanthus can be used as a high-quality forage to prevent weight loss of cattle grazing low quality grass pastures. Since forage yield and quality are inversely correlated as maturity advances (Suksombat and Buakeeree, 2006; Ayyadurai et al., 2013), there is need to determine the optimum harvesting stage for desmanthus.

3.4.3. Dry matter degradability

The total potentially degradable DM (A+B) in this study (0.58 to 0.85) was within values reported for tropical legumes (Mupangwa et al., 2003; Gusha et al., 2013). The DM ED6 was lowest for JCU7 in this study though the total potentially degradable proportion did not differ between cultivars. In agreement with these findings, Vandermeulen et al. (2018) reported lower *in vitro* organic matter digestibility of JCU1 (*D. leptophyllus*) compared to JCU4 and JCU2 harvested in summer. The authors attributed the findings to higher polyphenolic secondary compounds in JCU1. Significantly higher total tannins were recorded for JCU1 compared to JCU2 and JCU4 (Vandermeulen et al., 2018), while total phenolics were reported to be higher in JCU7 compared to JCU2 (Suybeng et al., 2021b). The potentially degradable DM (B) proportion declined by 36.7%, 20.8% and 11.9% for JCU4, JCU2 and JCU7, respectively from day 78 to day 168 in this study resulting to overall highest B degradability in JCU7. These results are plausible since the forage NDF composition had increased by 87.1%, 52.3% and 20.1% for JCU4, JCU2 and JCU7, respectively. Increase in dietary NDF results in reduced concentration of the rapidly fermentable carbohydrates and consequently less energy for microbial growth and DM digestibility (Wilman and Moghaddam, 1998; Brandao and Faciola, 2019). As a result, diet NDF influences feed intake

and it has been reported to account for 15.3% variation in DM intake of Holstein cows in late gestation (Hayirli et al., 2002). However, the effective degradability with low particle outflow rate (ED2) from the rumen was not higher for JCU7 compared to other cultivars in this study due to low degradation rate and the long lag time. Hence, the findings of this study suggest that the three desmanthus cultivars should be utilized when they are still young (below 168 days for JCU2 and JCU4, and below 122 days for JCU7) to achieve high effective DM digestibility.

3.4.4. Fibre degradability

The rumen is the main site for fibre digestion since dietary fibre digestion is solely dependent on microbial fermentation as a function of the rate of degradation (C), proportion of the total potentially degradable fraction and the rate of feed passage from the rumen (Beauchemin et al., 2019). Dietary fibre is hydrolysed and fermented to produce fatty acids that are a major metabolic energy source for the animal and rumen microbial cells (Krause et al., 2003). Fibre escaping rumen digestion is only partially digested postruminaly in the hindgut and typically accounts for less than 10% of total tract NDF digestion in cattle (Huhtanen et al., 2006). Although the potentially degradable fraction (B) of NDF was higher in JCU7 compared to JCU2 and JCU4, there was no significant difference in the ED2 and ED6 fractions of NDF between cultivars. This may be due to the slow degradability rate and long lag time observed for JCU7 limiting the fermentation time of fibre in the rumen (Martineau et al., 2006). The decline in NDF and ADF effective degradability with maturity is conceivable due to the increase in lignification of plants with advanced maturity (Lemàire and Bélanger, 2020). Jung et al. (1997) reported −0.93 correlation between lignin composition and NDF digestibility of 36 forages.

3.4.5. Crude protein degradability

In the rumen, proteins are hydrolysed to peptides and amino acids which are incorporated into microbial cells or deaminated to form carbon dioxide, volatile fatty acids and ammonia (National Academies of Sciences, 2016). Greater ruminal ammonia nitrogen accumulation can compromise microbial fermentation and result in a reduced total volatile fatty acids concentration (Brandao and Faciola, 2019). The rumen degradable protein fraction provides nitrogen supply for rumen microbial activity and microbial protein synthesis, which is the main protein source for ruminants (Bowen et al., 2008; Keim et al., 2013). The rumen undegraded protein may be degraded post-rumen to provide a source of amino acids in the small intestines (Flachowsky and Lebzien, 2006). Higher rumen undegraded protein is associated with higher animal performance due to increased influx of essential amino acids into the small intestine and absorption into the bloodstream (Waghorn and Shelton, 1997). There was low ED2 and ED6 of CP for JCU7 that may have been caused by direct inhibition of microbial activity (Hook et al., 2010) and formation of tannin-protein complexes or higher ADF-bound protein that may protect diet protein from degradation in the rumen (Waghorn and Shelton, 1997; Makkar, 2003; Castro-Montoya and Dickhoefer, 2020). The low ED2 and ED6 of CP indicates that JCU7 may supply more rumen bypass protein to the grazing livestock compared to JCU2 and JCU4. Increase in the A fraction of CP with maturity observed in this study concurs with previous findings reported for temperate legume forages; *Medicago sativa, Lotus corniculatus, Trifolium ambiguum, Trifolium pratense* and *Trifolium repens* (Gierus, 2013). The author attributed the findings to the accumulation of assimilates characterized by increases in carbohydrates and reserve proteins after the high demands for self-replication or when growth declines. The increase in A fraction of CP with maturity may also be due to accumulation of CP with seed growth (Trevin and Gil, 2001).

The utilization of forages with CP and digestibility below 8% and 55%, respectively, is restricted by low DM intake caused by physical fill limits and slow digestion (Leng, 1990; Buxton et al., 1995). Thus, the high CP in the leaf portion in Experiment 1 and the 'grab' samples in Experiment 2 for all cultivars and maturity stages ranging from 16.9% − 29.0% and 16.9% − 26.4%, respectively, and the total potentially degradable DM ranging between 0.58 and 0.85, suggest that desmanthus can be utilized as a high-quality forage legume for grazing cattle in northern Australia. Since desmanthus is available in the market as a blend of several cultivars in Australia (e.g. Progardes ®), grazing ruminant livestock may benefit from both the highly rumen degradable CP of JCU2 and JCU4 for microbial protein synthesis, and the higher bypass protein from JCU7 for post rumen amino acids supply, more so from desmanthus forage grazed at a younger maturity stage. However, grazing is influenced by animal endogenous characteristics such as body condition, age and experience in addition to diet factors (Wyffels et al., 2020). Therefore, *in vivo* studies are required to evaluate the effect of desmanthus on rumen fermentation and growth performance of ruminants.

3.5. Conclusions

This study evaluated the leaf to stem mass ratio, chemical composition and *in situ* degradability of three desmanthus cultivars harvested at varying maturity stages. The leaf to stem mass ratio of desmanthus regrowth was highest on day 11 and the ratio declined more steeply in JCU7 compared to JCU2 and JCU4. The CP was similar between cultivars and higher in the leaf than stem portion, while fibre content was higher in the stem portion. The overall effective DM degradability at high particle outflow rate (ED6) was observed to be higher in JCU4 than JCU7. Therefore, the hypothesis that nutritive value and *in situ* degradability of desmanthus would differ between

cultivars and with maturity stage at harvest was accepted. These results indicate that differences exist between cultivars and higher livestock production may be achieved by utilizing the different cultivars in a blend and at early maturity stages. The high CP composition of the leaf portion in late maturity stages indicate the potential of desmanthus to produce a high-quality protein source later in the growing season when quality of grass pastures has declined. However, *in vivo* studies are required to evaluate the effect of desmanthus on rumen fermentation and growth performance of ruminants.

3.6. Summary

Desmanthus is a pasture legume that is well adapted to a wide range of soil textures and annual rainfall making it suitable for the vertosol soil sub-humid regions of northern Australia that lacks a suitable legume. However, the effect of cultivar and maturity stage at harvest on desmanthus nutritive value for ruminant livestock feeding is not widely studied. Therefore, this study evaluated the change in nutritive value and *in situ* degradability of desmanthus cultivars JCU2, JCU4 and JCU7 harvested at varying maturity stages to test the hypothesis that the nutritive value and *in situ* degradability of desmanthus differ between cultivars and with maturity stage at harvest. Experiment 1 was a completely randomized study where desmanthus was harvested at 11, 38, 72 and 103 days of regrowth, separated into the leaf and stem portion, dried and analysed for dry matter and chemical composition. In Experiment 2, 'grab' samples of desmanthus were harvested 78, 122 and 168 days after planting. Samples were dried and dry matter, crude protein and fibre degradation were determined using the *in situ* technique with three fistulated Droughtmaster steers in a randomized split-plot design with steers as the whole plots and cultivars as sub-plots. The results showed an interaction between cultivar and maturity on the leaf to stem mass ratio, leaf CP,

stem NDF and the leaf ADF ($P \leq 0.04$). The leaf to stem mass ratio declined more steeply with maturity in JCU7 compared to JCU2 and JCU4 ($P = 0.04$), while there was a higher decline in leaf CP of JCU4 than JCU2 and JCU7 ($P < 0.01$). The total potentially degradable fraction of DM and CP did not differ between cultivars ($P \geq 0.30$) but declined with maturity ($P \leq 0.04$). However, the effective DM degradability at high particle outflow rate (ED6) was higher in JCU4 than JCU7. Taken together, these results indicate that differences exist between cultivars and higher livestock production may be achieved by utilizing the different cultivars in a blend and at earlier maturity stages. Therefore, the hypothesis that nutritive value and *in situ* degradability of desmanthus differ between cultivars and with maturity stage at harvest was accepted. However, *in vivo* studies are required to evaluate the effect of desmanthus on rumen fermentation and growth performance of ruminants.

Chapter 4: Growth Performance, Rumen Fermentation and Plasma Metabolite Profile of Steers Backgrounded on Desmanthus-Augmented Diets

Chapter 4.1: Growth Performance and Plasma Metabolite Profile of Grazing Beef Cattle Backgrounded on Buffel or Buffel-Desmanthus Mixed Pastures

4.1.1. Introduction

Livestock production in the tropics plays a significant role in terms of animal numbers, total products output and employment globally (Oosting et al., 2014), but beef cattle production measured as annual live weight gain is low from tropical pastures compared to temperate pastures (Winter et al., 1991). In northern Australia's dry tropical environment, beef cattle rely mainly on extensive grazing of unimproved native pastures dominated by C4 grasses with limited use of exotic pasture species (Hattersley, 1983; Edwards and Smith, 2010; Hunt et al., 2014). The dry tropics are characterized by a distinct wet and dry season, both of which vary greatly in length (Poppi et al., 2018). As a result, the quantity and nutritive value of pastures vary widely throughout the year. Pasture growth takes place in the wet season of November to April, resulting in increased green herbage mass, crude protein (CP) content and dry matter digestibility (DMD). Towards the end of wet/growing season and during the dry season, pasture senescence reduces green herbage mass, CP content, dry matter digestibility and, consequently, cattle DMI (McCown, 1981; Allison, 1985). Thus, high cattle weight gains are observed during the wet season, which can exceed a kilogram per day (Poppi et al., 2018), but reduces in the dry season, sometimes resulting in weight loss (Hill et al., 2009).

The importance of tropical legume pastures to improve beef production has long been established (Castro-Montoya and Dickhoefer, 2020; Da Motta et al., 2020; Prudhomme et al., 2020). The integration of legumes into grass pastures increases protein and digestible energy intake resulting in improved cattle growth rate and reduced age at slaughter (Hill et al., 2009). In northern Australia, pasture legumes came to general use over five decades ago (Mannetje, 1997) and legumes of the genus *Stylosanthes* (Stylo) have a significant economic impact on light soils of tropical northern Australia (Clem and Hall, 1994; Coates et al., 1997), but there was no suitable legume pasture for the regions with cracking clay (vertosol) soils until recently (Pengelly and Conway, 2000). Vertosol soils play a significant role in northern Australian beef cattle production, particularly in the State of Queensland, which accounts for 46% of the Australian beef cattle herd (MLA, 2019a). Vertosol soils also occupy 28% of the total area (Soil Science Australia, 2015) and account for over 3.2 million ha of land (Weston et al., 1983) within the subcoastal north-eastern Australia between latitudes 16° S and 25° S (Clem and Hall, 1994).

Legumes of the genus *Desmanthus* spp. (referred to as desmanthus henceforth) can be utilised for pasture improvement. Hill et al. (2004) reported an increase in the use of legume-based pastures for livestock production in Australia due to financial pressure that has prompted the need for a more cost-effective protein source. As a result, over 35 000 ha of the three commercially available desmanthus species (*D. bicornutus*, *D. leptophyllus* and *D. virgatus*), have been established across many regions of Australia including Queensland, Northern Territory and northern New South Wales since 2012 (Gardiner et al., 2019). However, only limited literature exists on the effect of desmanthus pasture grazing on animal growth performance and none on plasma metabolites profile. A study on the effect of desmanthus on steer performance reported that steers grazing desmanthus/buffel grass pastures were 30 kg heavier than those grazing buffel-grass-only pastures

after 90 days (Gardiner and Parker, 2012). Goats fed *Brachiaria mulato* (Mulato) grass and supplemented with desmanthus at 27% DMI gained 17 g/day more than those fed Mulato grass only (Marsetyo et al., 2017). Supplementing sheep fed Mitchell grass (*Astrebla* spp.) basal diet with *D. leptophyllus*, *D. pubescens* or *D. virgatus* hay reduced weight loss from 5.83 kg/hd in control to between 1.33 and 2.33 kg/hd (Rangel and Gardiner, 2009). In contrast, growing goats fed *Sorghum bicolor* (Sudan grass) and supplemented with *D. bicornutus* at 40% DMI gained 16 g/day less weight compared to those supplemented with Leucaena, lucerne and lablab (Kanani et al., 2006). These studies were either indoor trials or conducted in small paddocks, which do not represent the extensive grazing systems of northern Australia. In addition, pasture legume levels of 27 – 40% used in these indoor studies may not be achieved.

Liveweight and body condition scores are traditional routine methods used to evaluate cattle nutritional status because they are quicker to perform and require less expertise, but they are associated with several limitations (Ndlovu et al., 2007). Bodyweight evaluates nutritional status by measuring growth as a function of cell enlargement, cell multiplication and incorporation of constituents from the environment, for example, in apatite deposition (Flier and Maratos-Flier, 2000). Change in body weight can result from tissue hydration, change in gut and bladder fill, pregnancy and parturition rather than change in body fat or protein content (National Research Council, 1996). Body condition score assesses the animal nutritional status over time as a function of the level of fatness on the animal (Nicholson and Sayers, 1987), but is less reliable due to the general subjective nature (Ndlovu et al., 2007). Plasma metabolites, on the other hand, provide an integrated index of nutrient supply adequacy in relation to nutrient utilisation (Chester-Jones et al., 1990) and provide an immediate indication of the animals present nutritional status (Pambu-Gollah et al., 2000). Animals grazing low-quality pastures during the dry season mobilize fatty acids from

the adipose tissue as a long-term response to the negative energy balance resulting in elevated NEFA and BHB (Abeni et al., 2004; Murillo-Ortiz et al., 2014). Supplementing animals fed low-quality grass diet with legumes improves their nutritional plane, thus minimizing catabolism to encourage anabolic processes. In addition to improving the nutritional plane of animals, legume supplementation improves their health status. Supplementing grass-fed sheep with *Moringa oleifera* was reported to increase blood glucose and immunoglobulin A levels (Prudhomme et al., 2020). In another study, calves supplemented with lucerne hay had lower plasma BHB compared to their unsupplemented counterparts (Movahedi et al., 2017). Although numerous reports on the effect of dietary legume supplementation on blood parameters in dairy cows exist (Soder et al., 2006; Pembleton et al., 2016), little information is reported on beef cattle (Ragni et al., 2018). Therefore, the primary aim of this study was to evaluate the growth performance and plasma metabolites of beef cattle backgrounded (the grazing period between weaning and finishing) on buffel grass pasture oversown with desmanthus during the dry season. The hypothesis tested was that backgrounding steers on low-level buffel grass-desmanthus mixed pastures would elicit significant changes in plasma glucose, bilirubin, creatinine, non-esterified fatty acids and β-hydroxybutyrate, resulting in higher liveweight gains than in steers on buffel-grass-only pastures.

4.1.2. Materials and Methods

All procedures in this study followed the James Cook University Animal Ethics Committee approved guidelines (Approval Number 2639) in accordance with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013).

4.1.2.1. Study Site

This dry season on-farm study from July 9th to December 3rd 2019 was carried out at Cungelella, a commercial beef pastoral property in central Queensland (24°41′ S, 147°10′ E), Australia. The mean annual rainfall of the farm is 598 mm with mean minimum and maximum temperatures of 12.7 °C and 29.1 °C, respectively (Collins et al., 2016; Australian Government Bureau of Meteorology, 2020). The soils are typically low in nitrogen and phosphorus, alkaline and contain moderate to high clay content (Collins et al., 2016). Two buffel grass-dominated paddocks were assigned as buffel grass (575 ha) and mixed buffel grass-desmanthus (520 ha) pastures. Desmanthus was sown in March 2018 in established buffel grass pastures. The paddock was sprayed with glyphosate-based herbicide (Roundup; Monsanto, Kilda Road, Melbourne, Australia) at the rate of 3 in 37 L (*v*/*v*) of water per ha and then desmanthus seed was aerial-sown at the rate of 3 – 5 kg/ha. Desmanthus (Progardes; Agrimix Pastures Pty Ltd., Ferny Hills DC, QLD, Australia) was a blend of *D. leptophyllus*, *D. virgatus* and *D. bicornutus* (cultivars JCU2, JCU4, JCU5 and JCU7), which range from early, medium to late maturing species (Gardiner et al., 2013; Gardiner, 2016). The pastures were not fertilised. After self-seeding re-establishment of buffel grass, both paddocks were grazed heavily in 2018 to control competition and for desmanthus to establish well (Miller and Stockwell, 1991). The paddocks were destocked in September 2019, before the start of the wet season that usually starts in November.

4.1.2.2. Animal Management

Four hundred 15–18-month-old weaned tropical composite steers of crossbred *Bos indicus* and *Bos taurus* genotypes, weighing 320 ± 21 kg as the initial average liveweight, were utilised in this set-stocked 147-day grazing trial. Prior to the experiment, the steers were grazing on buffel grass-

dominated pastures. Experimental steers were randomly assigned to either of the two pastures, buffel grass only (n = 200) or mixed buffel grass-desmanthus pastures (n = 200) at 2.87 and 2.60 ha/steer stocking rate, respectively, based on the farm manager's long knowledge of the paddocks' carrying capacity and remained constant throughout the trial period. Steers were not supplemented throughout backgrounding and were weighed on days 0, 49, 79 and 147 after the onset of grazing. Steers were brought from the paddocks at 09:00 hrs, left in the holding yards for one hour and weighed between 10:00 hrs and 14:00 hrs. Unfasted weights were recorded automatically (Gallagher 65 Scanlon Drive, Epping, Victoria 3076, Australia) and the average daily weight gain (ADG) was calculated by regression using the four weigh points. An a priori power analysis using G-Power was conducted to determine the appropriate sample size (Figure 4.1.1). A total sample size of 50 steers was required to achieve statistical power of 80% with a critical F-value of 4.0 for a large effect size and a significance level of 0.05. Therefore, twenty-five steers per paddock were randomly selected on day 0 for body condition scoring (BCS) using a five-point scoring system (1 – 5) (Ndlovu et al., 2007) and faecal samples taken in parallel with the weighing session. Blood samples were collected from these same 50 steers during days 0 and 147 weighing sessions.

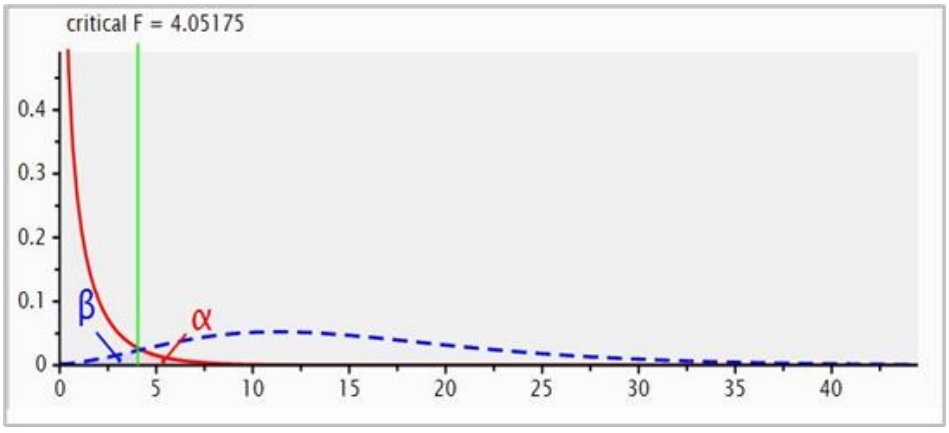


Figure 4.1.1. G-Power analysis for statistical power, critical F-value and sample size.

4.1.2.3. Pasture Sampling and Analysis

The Botanal technique (Tothill et al., 1992) was used, pre- and post-grazing, to estimate pasture yield, botanical composition, ground cover and woody cover (Tothill et al., 1992; McDonald et al., 1996). Since no substantial pasture growth was expected due to limiting moisture levels throughout the grazing period, grazing utilization was estimated as a percentage of the grazed stock as described by Stoddart (1935). Estimates were made in 0.50 × 0.50 m quadrats assigned on a 100 m × 100 m grid pattern on predetermined GPS points to ensure uniform sampling across the paddocks. The number of quadrats per paddock varied, with paddock size resulting to 595 and 507 quadrants for the buffel-grass-only and mixed buffel grass-desmanthus paddocks, respectively. Representative pasture samples were collected from both paddocks, four times over the course of the experiment; at the beginning, end and twice during grazing. Buffel grass and desmanthus were analysed as they were the dominant pastures, while *Acacia harpophylla* (brigalow) was the dominant woody cover, and steers were observed to browse on its leaves. Although they are palatable, *S. kali*, *U. mosambiencensis* and *Portulaca* spp. were not analysed because their contribution was minimal, below 5% of the pasture botanical composition. Buffel grass and desmanthus samples were harvested by cutting at 5 cm above the ground while brigalow samples consisted of leaves and soft branches approximately 10 cm long. Pasture samples were transported in cooler boxes and stored at −20 °C until being analysed in the laboratory. The samples were oven dried at 60 °C for 48 h, ground to pass through a 1 mm screen using a Cyclotec mill (Foss Tecator AB, Hoganas, Sweden) and analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), organic matter (OM), CP and DMD. Total nitrogen (N) was determined by the Dumas combustion method using a Leco CN628 N Analyser (Leco, St. Joseph, MI, USA) (Sweeney and Rexroad, 1987) and CP calculated using total N × 6.25. NDF (without heat-stable α amylase) and ADF

concentrations were determined sequentially using an ANKOM 200/220 Fibre Analyser (ANKOM Technology, Fairport, NY, USA) according to the methods of Van Soest et al. (1991) and Goering and Van Soest (1970), respectively, and hemicellulose was calculated as the difference between NDF and ADF. OM was determined by ashing the samples according to the methods of Faichney and White (1983). *In vitro* DMD was determined using a modified pepsin-cellulase technique (Clarke et al., 1982) and metabolisable energy (ME) was calculated as DMD × 0.172 − 1.707 (CSIRO, 2007).

4.1.2.4. Faecal Sampling and Analysis

To determine the nutritive value of the diet selected by the steers during grazing, faecal samples were collected from the rectum of 50 steers (25 from each paddock) and from random dung pats in each paddock close to the watering points on weigh days. Faecal samples were transported in a cooler box and stored at −20 °C awaiting laboratory analysis. The samples were dried and ground as previously described for the pasture samples. Faecal near infrared reflectance spectroscopy (FNIRS) (NIRSystems FOSS 6500) as described by Dixon and Coates (Coates and Dixon, 2007; Dixon and Coates, 2008) was used to determine CP, DMD, non-grass pasture proportion in the diet (comprising native and sown legumes, forbs and browse) and faecal N at the CSIRO Floreat laboratory (Floreat, WA, Australia). Spectral analyses, data manipulation and spectra calibrations were carried out using ISI (Infrasoft International) software NIRS 3 (Version 3.10, Port Matilda, PA, USA). The calibration equations used were developed for cattle grazing tropical and subtropical pastures (Coates, 2004; Coates and Dixon, 2008).

4.1.2.5. Plasma metabolites analysis

To assess the steers' nutritional and health status, blood samples were collected at the start and end of the grazing period from the sample 50 steers by caudal venipuncture into 10 mL heparin-containing BD Vacutainer tubes. Plasma was isolated using a portable horizontal bench-top centrifuge (StatSpin Express 4, Iris Sample Processing, Westwood, MA, USA) at 4000× *g* for 5 min at room temperature. Plasma samples were transferred into labelled 15 mL aliquot tubes and stored at −20 °C pending laboratory analysis. Plasma non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), total bilirubin, creatinine and glucose were analysed using the colorimetric, 3-hydroxybutyrate dehydrogenase, modified diazo, kinetic modified Jaffe and hexokinase methods of the AU480 chemistry analyser (Beckman Coulter, Inc. Brea, CA, USA), respectively, according to the manufacturer's procedures.

4.1.2.6. Statistical analysis

All data were analysed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). Growth performance and blood metabolites data were analysed using the General Linear Model procedure (PROC GLM) analysis of variance with the animal as the experimental unit. Backgrounding pasture, days since onset of grazing and their interactions were fitted as fixed effects, while liveweight (LW), NEFA, BHB, total bilirubin, creatinine and glucose were the dependent variables. The same model was used for the faecal parameters analysis with backgrounding pasture, month and their interactions fitted as fixed effects and faecal N, diet CP, DMD and diet non-grass as the dependent variables. Backgrounding pasture was the only fixed effect for the ADG analysis. Effects were declared significant at $P \leq 0.05$. Where significant, differences between means were tested by least significant difference (LSD) comparison test. Simple linear

regression using the PROC REG was used to determine the relationship between diet non-grass and CP or CP and DMD.

4.1.3. Results

4.1.3.1. Rainfall and pasture characteristics

Throughout the pasture establishment and grazing periods, the total annual rainfall was below average (598.2 mm/annum) at 421, 368 and 305 mm for the years 2017, 2018 and 2019, respectively (Table 4.1.1). The wet season preceding the grazing period commenced in October 2018 and ended in April 2019. The rest of the year was fairly dry, and the next wet season had not started by the time grazing period ended in December 2019.

Table 4.1.1. Monthly and total annual rainfall (mm) for the years 2017, 2018 and 2019.

	Month												
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual Total
2017	68	39	105	6	8	0	22	0	0	107	42	24	421
2018	19	118	36	6	0	24	4	10	4	68	41	38	368
2019	0	1	111	82	3	5	24	15	0	40	20	5	306

Table 4.1.2 presents DM yield, ground cover, woody cover and the five most dominant pastures species in the two paddocks. Native legumes and forbs such as *Rhynchosia minima*, *Sida* spp., *Convolvulus* spp., *Cleome viscosa* and *Abutilon andrewsianum* were below 0.2%. Buffel grass utilisation in the buffel grass and desmanthus paddocks was 36.5% and 48.7%, respectively, while desmanthus utilisation was 83.5%. Proximate analysis data of the pastures are presented in Table

4.1.3. CP was lowest in buffel grass and highest in desmanthus, while DMD and ME were higher in brigalow compared to buffel grass and desmanthus.

Table 4.1.2. Pasture characteristics of the buffel grass and desmanthus paddocks prior to commencing and at the end of the grazing period. Data presented in percentages unless otherwise stated.

	Buffel Grass Paddock		Desmanthus Paddock	
Variable	Pre-Grazing	End of Grazing	Pre-Grazing	End of Grazing
Ground cover	63.7	38.0	68.7	29.7
Woody Cover	0.7	0.7	0.5	0.7
Dry matter yield (kg/ha)				
Total yield	4066	1854	4509	1425
Cenchrus ciliaris	3532	1700	3372	1260
Desmanthus spp.			502	88.6
Salsola kali	8.0	4.2	131	3.9
Urochloa mosambicensis	158	57.2	112	28.1
Portulaca spp.	18.0	5.5	80.0	2.1
Botanical composition				
Cenchrus ciliaris	90.1	91.7	77.2	88.4
Desmanthus spp.	-	-	11.5	6.2
Salsola kali	0.2	0.3	3.0	0.3
Urochloa mosambicensis	4.0	3.1	2.6	2.0
Portulaca spp.	0.5	0.3	1.8	0.2

Table 4.1.3. Mean chemical composition and dry matter digestibility (± standard deviation) of buffel grass, desmanthus and brigalow leaves during the backgrounding period. Data are in %DM unless otherwise stated.

Variable	Buffel Grass	Desmanthus	Brigalow
DM (%)[1]	84.9 ± 3.1	68.3 ± 3.4	64.6 ± 1.6
Neutral detergent fibre	73.9 ± 1.0	62.8 ± 2.0	38.7 ± 0.5
Acid detergent fibre	43.4 ± 1.1	40.9 ± 1.6	25.5 ± 1.5
Dry matter digestibility	46.9 ± 1.1	48.4 ± 1.2	60.6 ± 1.1
Organic matter	93.1 ± 0.3	94.6 ± 0.5	91.8 ± 0.3
Ash	7.2 ± 0.2	5.4 ± 0.5	8.2 ± 0.3
Hemicellulose	30.5 ± 1.3	21.9 ± 2.0	13.2 ± 2.0
Crude Protein	4.4 ± 0.9	8.5 ± 1.4	7.5 ± 0.3
Metabolisable energy (Mj/kg DM)[2]	6.9 ± 0.1	6.8 ± 0.1	8.7 ± 0.2

[1]DM: dry matter

[2]Estimated from in vitro DMD as DMD × 0.172 − 1.707 (CSIRO, 2007); MJ: mega joules.

4.1.3.2. Diet selected during grazing

Diet CP and DMD were similar throughout the study for the steers on buffel grass, but varied significantly for the steers on desmanthus, with the lowest values recorded on day 49 (Table 4.1.4). Faecal N did not vary with backgrounding pasture but reduced significantly by the end of grazing ($P < 0.01$). There was no effect of pasture on the non-grass diet, but a decrease over time ($P < 0.01$) was observed, with the lowest values recorded on day 147. At the beginning of the study, there was no difference in the quality of diet selected by the two groups. The initial diet similarity is indicated by the similar CP, Faecal N, DMD and diet non-grass on day 0. Overall, DMD was higher for the buffel grass than the desmanthus steers (55.5% and 54.2%, respectively; $P < 0.01$).

Table 4.1.4. Effect of pasture backgrounding on dietary CP, DMD, diet non-grass and faecal N as estimated from faecal near infrared reflectance spectroscopy.

Variable	Paddock	Days since the Onset of Grazing					*P*-value		
		0	49	79	147	SEM	P	D	P*D
Diet CP (%)	Buffel grass	11.2^{ab}	11.7^{a}	10.9^{ab}	10.9^{ab}	0.995	< 0.01	< 0.01	< 0.01
	Desmanthus	11.2^{ab}	8.7^{d}	10.6^{bc}	9.9^{c}				
Faecal N (%)	Buffel grass	1.7^{a}	1.7^{a}	1.7^{a}	1.5^{b}	0.150	0.68	< 0.01	0.14
	Desmanthus	1.7^{a}	1.7^{a}	1.7^{a}	1.4^{b}				
DMD (%)	Buffel grass	53.9 ^{c}	55.1^{ab}	55.0^{ab}	55.9^{a}	1.24	< 0.01	< 0.01	< 0.01
	Desmanthus	54.1^{bc}	52.0^{d}	54.7^{bc}	53.8^{c}				
ME (MJ/Kg DM)[1]	Buffel grass	7.5^{c}	7.7^{ab}	7.7^{ab}	7.9^{a}	0.038	< 0.01	< 0.01	< 0.01
	Desmanthus	7.6^{bc}	7.2^{d}	7.7^{bc}	7.5^{bc}				
DNG (%)	Buffel grass	32.4^{a}	28.0^{bc}	26.2^{c}	19.9^{d}	4.91	0.65	< 0.01	0.08
	Desmanthus	31.8^{ab}	27.7^{c}	28.5^{abc}	17.3^{d}				

[abc]Means followed by different letters in the same row are significantly different between pastures and days at the $P < 0.05$.

[1]Estimated from in vitro DMD as DMD × 0.172 − 1.707 (CSIRO, 2007); MJ: mega joules, SEM: standard error of the mean, P: paddock; D: sampling day, P*D: paddock and days interaction, CP: crude protein, N: nitrogen, DMD: dry matter digestibility, DNG: diet non-grass.

A positive relationship was observed between the diet CP and non-grass (Figure 4.1.2; $P < 0.01$). CP increased with an increase in diet non-grass component, while DMD increased with an increase in diet CP (Figure 4.1.3; $P < 0.01$). However, diet non-grass accounted for only 16% variability in CP, while diet CP accounted for 34% variability in DMD (Figure 4.1.3).

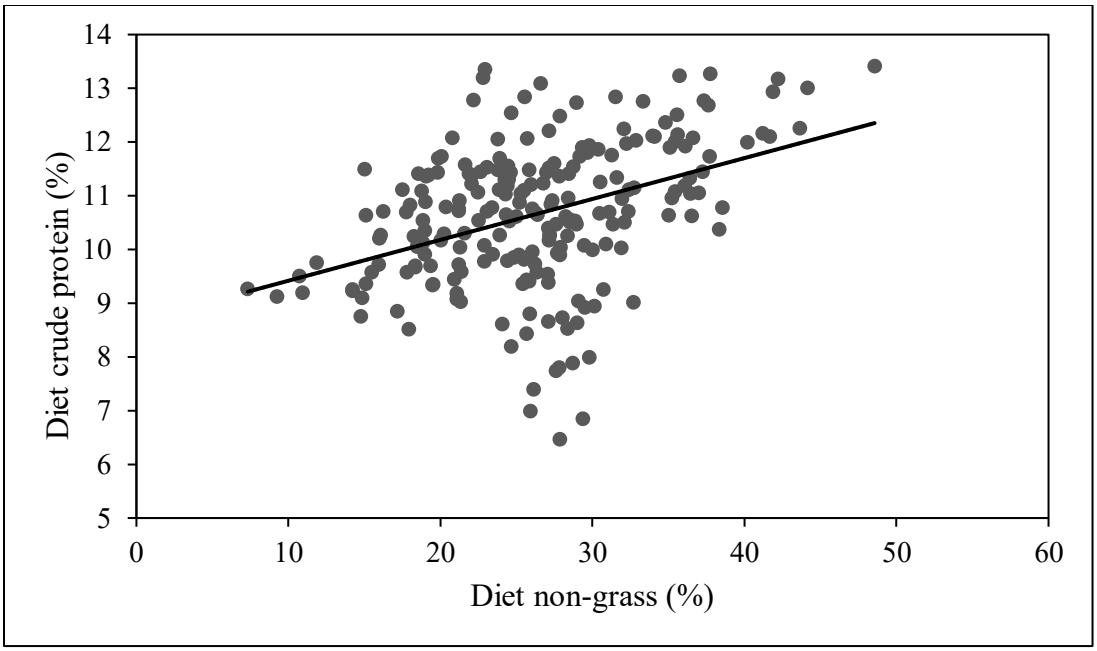


Figure 4.1.2. Relationship between diet non-grass and crude protein. Y = 8.66 + 0.076X; where Y = diet crude protein and X = diet non-grass, $R^2 = 0.16$, $P < 0.01$.

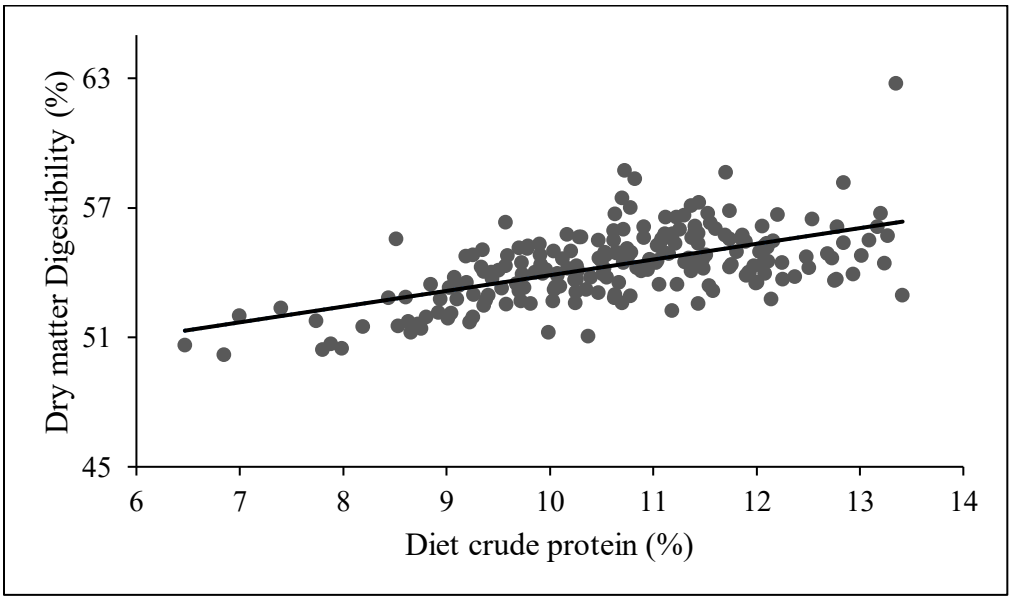


Figure 4.1.3. Relationship between diet crude protein and dry matter digestibility Y = 46.60 + 0.728X; where Y = %DMD and X = %CP, $R^2 = 0.34$, $P < 0.01$.

4.1.3.3. Plasma metabolites

Plasma metabolite data are presented in Table 4.1.5. No significant difference in plasma metabolites concentration was observed for steers backgrounded on desmanthus-buffel grass mixed compared to buffel-grass-only pastures, although NEFA tended to be higher for the buffel-grass steers ($P = 0.058$), whereas sampling period had a significant effect on all metabolites except NEFA. Total bilirubin ($P = 0.04$) and glucose ($P < 0.01$) decreased, while BHB ($P < 0.01$) and creatinine ($P < 0.01$) increased for both groups, although the BHB increase in the desmanthus group was not significant. An interaction between period and pasture ($P = 0.01$) was observed for the creatinine with a greater increase observed for the desmanthus than the buffel-grass steers.

Table 4.1.5. Effect of pasture backgrounding on plasma metabolites (least square means).

Metabolite	Pasture	Sampling Period		SEM	*P*-value		
		Day 0	Day 147		P	D	P*D
Total Bilirubin (µmol/l)	Buffel grass	2.9	2.2	1.21	0.41	0.04	0.09
	Desmanthus	2.8	2.3				
BHB (mmol/L)	Buffel grass	0.22	0.28	0.0603	0.35	< 0.01	0.68
	Desmanthus	0.21	0.25				
Creatinine (µmol/l)	Buffel grass	94.4	109.1	16.27	0.12	< 0.01	0.01
	Desmanthus	89.2	122.7				
NEFA (mmol/L)	Buffel grass	0.45	0.36	0.177	0.05	0.21	0.31
	Desmanthus	0.36	0.33				
Glucose (mmol/L)	Buffel grass	5.9	4.8	1.00	0.46	< 0.01	0.40
	Desmanthus	5.7	4.8				

BHB: β-hydroxybutyrate, NEFA: non esterified fatty acids, SEM: standard error of the mean, P: pasture, D: sampling day, P*D: pasture and sampling day interaction

4.1.3.4. Growth performance

Steer LW, BCS and ADG data are presented in Table 4.1.6. Backgrounding pastures did not affect steer performance. An increase in LW and BCS was observed throughout the study ($P < 0.01$). Steers final LW was 431 and 433 kg, and BCS was 4.1 and 3.9 for the buffel grass and desmanthus paddock steers, respectively.

Table 4.1.6. LW, ADG and BCS of steers backgrounded on buffel grass alone or with desmanthus.

Variable	Pasture	Days since the Onset of Grazing				SEM	*P*-value		
		0	49	79	147		P	Days	P*D
LW (kg)	Buffel grass	319^{d}	372^{c}	392^{b}	431^{a}	18.9	0.14	< 0.01	0.21
	Desmanthus	322^{d}	369^{c}	396^{b}	433^{a}				
BCS	Buffel grass	3.4^{c}	3.6^{bc}	3.5^{bc}	4.1^{a}	0.38	0.51	< 0.01	0.36
	Desmanthus	3.4^{c}	3.6^{bc}	3.5^{bc}	3.9^{ab}				
Overall ADG (kg/day)	Buffel grass	0.74				0.13	0.78		
	Desmanthus	0.75							

^{abcd} Means followed by different letters in the same row are significantly different between pastures and days at the $P < 0.05$. LW: liveweight, ADG: average daily gain, BCS: body condition score, SEM: standard error of the mean, P: pasture, P*D: pasture and days interaction.

4.1.4. Discussion

4.1.4.1. Pastures characteristics

The DM yield of the buffel grass pasture in this study (3.4 – 3.6 ton/ha) was lower than that average reported for the buffel grass pastures in the Brigalow region of Central Queensland (4.5 – 5.2 ton/ha) (Hacker and Waite, 2001). The low yield could be due to the below-average rainfall received during the study period (Cowie et al., 2007). Although desmanthus contributed a small proportion of initial pasture botanical composition (11.5%) in the study, pasture DM yield was 443

kg/ha higher in the desmanthus paddock compared to the buffel-grass-only paddock. This finding agrees with another study that reported an increase in pasture yield when legumes were oversown with grass pastures compared to grass-only pastures in the tropics (Gulwa et al., 2017). The presence of 11 – 33% legumes in temperate pastures was found to increase DM yield, but with a reduced yield benefit as the legume proportion increased to 67% or more (Bork et al., 2017). Legumes increase pasture productivity by contributing to increased light capture compared to pure grass stands (Khatiwada et al., 2020). Furthermore, nitrogen-fixing legumes promote grass growth by providing nitrogen for the companion grass if moisture is not limiting (Thomas, 1992; Thomas, 1995).

The CP of desmanthus in this study was lower than that reported for *D. leptophyllus*, *D. virgatus* and *D. bicornutus* grown in a semi-enclosed greenhouse in winter (11.2 – 18.9%) and spring (13.2 – 18.2%) seasons (Vandermeulen et al., 2018). Durmic et al. (2017) reported 12.2 – 21% CP in winter and 9.8 – 19.2% CP in spring. However, one cultivar- *D. virgatus* (Marc)- had a CP content of 6.2% in spring. The difference in CP between studies may be due to the plant part harvested. Durmic et al. (2017) analysed only the leaves and young stems, while Vandermeulen et al. (2018) analysed leafy whole-plants compared to the desmanthus whole-plants in this study that comprised of mature stems and few leaves. In this study, buffel grass CP was very low (4.4%). The low CP agrees with a review of studies carried out in Central Queensland that reported a decline in buffel grass CP to below 6% in winter (Poppi et al., 2018).

4.1.4.2. Diet selected during grazing

Dietary CP and DMD are the primary limiting factors of growth performance in cattle grazing low-quality pastures in the Australian subtropics during the dry season (Hennessy et al., 1983).

Limited CP levels result in below-optimal microbial growth required for structural carbohydrate digestion in the rumen, which in turn depresses feed intake (Hennessy et al., 1983; Leng, 1990). In this study, steers in both paddocks consumed diets with higher CP (8.8 – 11.6%), DMD (52.1 – 55.9%) and ME (7.3 – 7.9 MJ/Kg DM) compared to the CP (4.4 – 8.5%), DMD (46.9 – 48.4) and ME (6.8 – 6.9 MJ/Kg DM) obtained from the pasture proximate analysis. Although, the brigalow DMD and ME were higher at 60.6% and 8.7 MJ/Kg DM, respectively. Ruminants consume diets that differ from the average available biomass in plant species, plant parts and nutrient content (Gordon, 1995; Dove and Mayes, 2005) as a result of foraging behaviour influenced by short-term and long-term decisions, such as which plant to select, how long to search between bites and where to graze (Gordon, 1995). Hence, pasture samples do not adequately represent the diet consumed by grazing animals (Dove, 1998).

It was surprising to observe similar diet non-grass components in the consumed diet of steers in both paddocks. Steers on buffel grass might have consumed non-grass pastures from forbs, native legumes and woody shrubs. Bowen et al. (2018) reported 11% C3 forage biomass in cattle grazing C4 perennial-grasses-only pastures and attributed it to naturalised legumes and other dicots present in the pastures. The CP and DMD of selected pastures were lower than those selected by steers grazing the Leucaena-grass pasture (12.4% and 62%, respectively) (Dixon and Coates, 2008). However, CP was higher and DMD was similar to that reported for cattle grazing varying perennial grass pastures, forbs and shrubs that consumed a diet with 5.5 – 8.11% CP and 52.1 – 55.2% DMD (White et al., 2010). Although metabolisable protein is a better measure of protein requirement than CP (CSIRO, 2007), it was not possible to determine the metabolisable protein of the diet selected by steers in this study. Dixon and Coates (2005) reported that rumen degradable N is likely to be restrictive only when the DMD: CP ratio exceeds 8 to 10. In the current study, the

DMD: CP ratio ranged between 4.8 and 5.9 for both paddocks, indicating that rumen degradable N was not limiting (Dixon and Coates, 2005).

Non-grass pastures constituted between 17.4 – 32.4% of the diet consumed. This falls within the range reported for heifers grazing a mixture of Verano and Seca stylos with Sabi grass that selected 15 – 63% stylo (Coates et al., 1997). Among the factors that influence the diet composition of grazing animals are pasture species on offer, availability, palatability and nutritive value of the associated grass (Coates and Dixon, 2007). Leucaena in the diet was observed to decline steeply from 87% to 10% with reducing availability during the dry season (Dixon and Coates, 2008). In another study, where the entire cattle diet consisted of Mulga (*Acacia aneura*) during the dry season when Mulga was the only available forage, the Mulga proportion reduced to 30% during the wet season when moisture stimulated grass growth (Coates and Dixon, 2007). In grass-dominated pastures, cattle consumed 10% non-grass components during the pasture growing season, which increased to over 70% in the dry season (Coates and Dixon, 2007). Cattle grazing varying perennial grass pastures, forbs and shrubs consumed 19 – 49% non-grass components (White et al., 2010). In the Mitchell-grass-dominated pastures, the non-grass proportion in sheep and cattle diets was high during the wet season and reduced in the dry season. The authors attributed the trend to high palatability of the non-grass pasture species encouraging higher preference when available, but consumption dropped with a decrease in availability during the dry season (Orr et al., 1988; Coates and Dixon, 2007). In grass-dominated pastures consisting of just 2% forbs, cattle consumed up to 15% non-grass during the dry season, indicating high forb selection (Coates and Dixon, 2007). Forb and browse are often higher in N and metabolisable energy than grasses, especially when grasses are senesced (Holm and Eliot, 1980; Hall, 1981). These studies indicate that cattle can consume large amounts of palatable non-grass pastures when

not limited by availability. Desmanthus utilisation in the current study was very high (83.5%) suggesting that consumption was limited by availability. Therefore, a higher percentage of desmanthus legume in the pastures may be required for improved non-grass pastures and CP intake to be observed. Thomas (1992) suggested that 20 – 30% DM legume content is required for 10 – 40% pasture utilisation, and 35 – 45% DM legume at higher pasture utilisation levels of 50 – 70% for a productive and sustainable pasture.

4.1.4.3. Plasma metabolites

More accurate assessment of nutritional and health status in cattle can be achieved by including plasma metabolites analysis than from BCS and LW alone (Ndlovu et al., 2007). The glucose levels were similar to those reported for cattle grazing dormant pastures (Hersom et al., 2004; Murillo et al., 2015) and were within the normal range for beef cattle (2.5 to 5.5 mmol/L) (Mitruka and Rawnsley, 1977; Kaneko, 1997). The lack of difference in glucose concurred with results for cattle fed low-quality Sudan grass (*Sorghum* sp.) hay (CP 3.9%) supplemented with soybean alone or with pelleted Silver-grass (*Miscanthus* sp.) to achieve 9.6% CP levels (Asano et al., 2017). The decline in glucose from the start to the end of the grazing period is in agreement with results reported in other studies. For instance, rangeland-grazing beef cattle blood glucose decreased from summer, fall, winter to spring (Murillo-Ortiz et al., 2014). Similarly, a decline in blood glucose was reported for temperate-breed steers during the 'store' period (Beeby et al., 1988). The glucose decline over time can be explained by a decline in feed intake resulting from declining pasture availability (Ndlovu et al., 2007; Murillo-Ortiz et al., 2014).

Backgrounding pastures did not influence plasma NEFA concentration, indicating that steers were not mobilising body energy reserves in the current study (Abeni et al., 2004; Murillo-Ortiz et al.,

2014). NEFA levels are reported to increase with maturity of forage, which could indicate a negative energy balance (Abeni et al., 2004; Murillo-Ortiz et al., 2014). In this study, grazing started when the pastures had senesced; consequently, no difference in maturity over time was taking place. The increase in BHB levels over time for the buffel-grass steers was unexpected since there was no difference in the NEFA levels. However, this increase was marginal and the plasma BHB level was below a 1.2 mmol/L concentration reported as the threshold to indicate hyperketonaemia in cows (Benedet et al., 2019).

Creatinine is produced mainly in the skeletal muscles by the degradation of creatine and creatine phosphate to produce energy (Braun and Lefebvre, 2008) and it is commonly associated with renal disorders (Grünwaldt et al., 2005). Reduced creatinine levels are also indicative of prolonged tissue protein catabolism (Ndlovu et al., 2007). In this study, all the steers had creatinine levels within the normal range reported for cows (88.4 – 177 µmol/L) (Braun and Lefebvre, 2008) and bulls (98.7 ± 14.7 µmol/L) (Otto et al., 2000). Creatinine levels increased with time for both groups indicating that no catabolism was taking place but rather an increase in muscle mass (Agenas et al., 2006; Ndlovu et al., 2007).

Bilirubin levels were similar to the normal range reported for extensive range beef cattle (Otto et al., 2000; Grünwaldt et al., 2005) and the Angoni cattle on grass pastures (2.7 ± 1.4 µmol/L), although the quality of the pasture was not described (Otto et al., 2000). Issi et al. (2016) reported elevated total bilirubin levels in dairy cows diagnosed with subclinical and clinical ketosis. The authors associated the bilirubin increase with the existence of a functional disorder or liver damage. The similar levels of total bilirubin in the current study may indicate that the caloric intake of steers on both pastures was comparable. It is pertinent to state that going by the plasma metabolite

profiles, all the steers in this study were healthy; indicating that, with or without desmanthus inclusion in the diet of grazing steers, animal health status was not compromised.

4.1.4.4. Growth performance

The animal growth response to grass pastures oversown with legumes depends on legume yield and quality (Miller and Stockwell, 1991). Contrary to other studies that reported an increase in LW gain in cattle (Gardiner and Parker, 2012; Collins et al., 2016), sheep (Rangel and Gardiner, 2009; Ngo, 2017) and goats (Aoetpah et al., 2018) supplemented with desmanthus compared to their counterparts fed grass only diets, no difference was observed in this study. This could be due to the lack of increase in diet CP intake in the desmanthus paddock compared to the buffel grass paddock due to low desmanthus levels. An increase in weight gain for cattle supplemented with other tropical legumes has been reported (Cheffins, 1996; Radrizzani and Nasca, 2014; Bowen et al., 2018; Castro-Montoya and Dickhoefer, 2018). Zebu steers grazing low-quality standing hay supplemented with 0.8 kg DM Leucaena leaf meal improved daily weight gain from −0.3 to 0.26 kg (Rubanza et al., 2005). Pen et al. (2013) reported a 0.7 kg higher daily weight gain for cattle supplemented with *Stylosanthes guianensis* compared to cattle fed rice straw and *Brachiaria* spp. grass only. Similar to this study, Suybeng et al. (2020) reported no difference in LW gain between steers fed Rhodes grass only or supplemented with different levels of desmanthus. The authors attributed the results to low diet ME (6.1 – 8.2 MJ/Kg DM) and feed intake (1.2 – 1.6% per Kg LW) that resulted in low daily ME intake (22 – 39 MJ/Kg DM). In the current study, the selected diet contained at least 7.3 MJ/Kg DM ME, but feed intake could not be determined. Steers in both paddocks had similar weight gain and BCS, and no weight loss was recorded. The finding concurs with a review of eleven studies by Bowman et al. (1995) who reported that a pasture diet with CP

above 5.6% results in weight gain. Detmann et al. (2014) estimated that 10.8 g/kg CP is required to achieve the apparent equilibrium point where the N efficiency of utilisation is nil. A 5.6% CP level was achieved in both paddocks throughout the study while 10.8 g/kg CP failed to be achieved only on days 49 and 147 in the desmanthus paddock. This may indicate that dietary CP in this study was sufficient for rumen microbial growth (Hennessy et al., 1983; McCown and McLean, 1983; Hill et al., 2009). Regardless of the lower dietary CP on days 49 and 147 for the desmanthus steers, no effect on LW was observed. This could be due to the CP and DMD: CP ratio that persisted above 5.6 and 8, respectively (Bowman et al., 1995; Dixon and Coates, 2005), maintaining sufficient rumen function. Supplementing steers with 15%, 22% and 31% desmanthus was observed to improve rumen function as indicated by the increased total volatile fatty acids concentration in the rumen (Suybeng et al., 2020). Therefore, more studies are required to understand the effect of desmanthus on rumen function.

The ADG of steers grazing buffel grass-only pastures (0.74 kg/day) was within the 0.2 to >1.0 kg range reported for buffel grass pastures in the Brigalow region of Queensland (Poppi et al., 2018). However, this is higher than the 0.11 and 0.44 kg/day reported for buffel grass only and buffel grass-desmanthus pastures, respectively, in a similar environment (Gardiner and Parker, 2012), and −0.25 to 0.17 kg/day reported for steers grazing buffel grass-dominated pastures in the monsoonal climate region of Northern Territory during the dry season (Schatz et al., 2020a). The variance in ADG could be due to differences in stocking rate resulting in varying pasture availability. The stocking rate was 0.55 – 1.92 ha/steer compared to 2.57 and 3.02 ha/steer in the present study. Individual animal weight gain declines with an increase in stocking rate when not accompanied by increase in pasture biomass due to competition for forage (Hunt et al., 2014).

The main drivers of profitability in grazing systems are annual liveweight gain and the stocking rate (Poppi et al., 2018). Although the final liveweight for both groups was similar in the current study, the buffel grass-desmanthus mixed pastures paddock had a higher stocking rate compared to the buffel-grass-only paddock by 9.5%. Increasing the stocking rate increases the annual LW per ha (Dixon et al., 2020), promoting profitability (Poppi et al., 2018). In this study, liveweight gain per hectare was calculated to be 37.8 and 42.4 kg/ha for the buffel and desmanthus pastures, respectively. A strong correlation between the cattle stocking rate and the daily live weight gain ($R^2 \leq 0.93$) was reported for beef cattle grazing grass-dominated pastures with 5 – 8.1% CP (Ash et al., 1995). The authors associated the decline in LW as the stocking rate increased with reduced pasture availability.

Legumes offer the greatest weight gain advantage during the late wet and the dry seasons (Coates et al., 1997). This study took place during the dry season only; hence, the response of the steers to desmanthus pastures during the wet and transition seasons was not examined. Cattle grazing buffel grass and *Centrosema brasilianum* (Centro) were observed to select more Centro during the wet to dry transition season than during the dry season at 22.1 – 40% and 19.7 – 20.9%, respectively (Dixon et al., 2020). A similar trend was reported for *Chamaecrista rotundifolia* (Clements, 1996). However, low nutritive value and palatability of pasture in the seasonally dry subtropics of northern Australia are endemic in the dry season (Leng, 1990; Brandão et al., 2018), thus more controlled pen studies are required to determine the effect of varying levels of desmanthus on the rumen fermentation and growth performance of grazing cattle during the dry season. In addition, previous grazing nutrition is reported to influence the growth performance of cattle during the feedlot finishing phase and carcass traits (Drouillard and Kuhl, 1999). Further studies are required

to determine the feedlot growth performance and carcass quality of desmanthus backgrounded beef cattle.

4.1.5. Conclusions

This study evaluated the possibility of using desmanthus legume oversown in Buffel grass pastures to improve growth performance and plasma metabolites profile during the nutrient-limiting dry season in Northern Australia. The results showed no significant effect of desmanthus at low inclusion levels in backgrounding pastures on LW, weight gain and plasma metabolites. Therefore, the hypothesis that backgrounding steers on buffel grass-desmanthus mixed pastures would elicit significant changes in plasma glucose, bilirubin, creatinine, non-esterified fatty acids and β-hydroxybutyrate, resulting in higher liveweight gains than in steers on buffel grass only pastures was rejected. Though the lack of difference may be due to the high performance of the buffel grass pastures atypical for the dry season in this region, the main drivers of profitability in grazing systems are annual liveweight gain and stocking rate. The similar weight gain at higher stocking rate indicate that desmanthus may have the potential to improve profitability in the extensive grazing systems of northern Australia and other similar environments by improving pasture carrying capacity. Further research is required to investigate the effect of feedlotting and on-station pen feeding trial with the desmanthus legume to better understand its effect on growth, plasma metabolites, rumen volatile fatty acids, carcass traits and meat quality parameters of intramuscular fat content, fat melting point and muscle fatty acid composition in beef cattle. In addition, studies are required to evaluate the growth performance and plasma metabolites of cattle backgrounded on grass pastures oversown with higher levels of desmanthus.

4.1.6. Summary

Dietary CP and dry matter digestibility are among the major factors limiting feed intake and weight gain of cattle grazing native and improved pastures in the subtropics of Northern Australia during the dry season. Incorporating a suitable legume into grasses improves pasture quality and cattle weight gain, but only a limited number of legume pastures can establish and persist in cracking clay soils. This study aimed to evaluate the effect of desmanthus inclusion in buffel grass (*Cenchrus ciliaris*) pastures on the plasma metabolite profile and growth performance of grazing beef cattle during the dry season. The hypothesis tested was that backgrounding steers on buffel grass-desmanthus mixed pastures would elicit significant changes in plasma glucose, bilirubin, creatinine, non-esterified fatty acids and β-hydroxybutyrate, resulting in higher liveweight gains than in steers on buffel grass only pastures. Four hundred tropical composite steers were assigned to buffel grass only (n = 200) or buffel grass oversown with desmanthus (11.5% initial sward dry matter) pastures (n = 200) and grazed for 147 days during the dry season. Desmanthus accounted for 6.2% sward dry matter at the end of grazing period. Plasma metabolites results showed that β-hydroxybutyrate, creatinine, bilirubin, glucose and non-esterified fatty acids levels were within the expected normal range for all the steers, indicating that with or without desmanthus inclusion in the diet of grazing steers, animal nutrition and health status were not compromised. It was also evident that desmanthus inclusion in buffel grass pastures had no impact on the plasma metabolite profile, liveweight and daily weight gain of grazing steers. Therefore, the tested hypothesis of higher changes in plasma metabolite profile and higher liveweight gains due to backgrounding on low-level buffel grass-desmanthus mixed pastures does not hold.

Chapter 4.2: Effect of Incremental Proportions of Desmanthus in Isonitrogenous Forage Diets on Growth Performance, Rumen Fermentation and Plasma Metabolites of Pen-Fed Growing Brahman, Charbray and Droughtmaster Crossbred Beef Steers

4.2.1. Introduction

Australia is a major global beef producer. In 2019, Australia was the second-largest beef and veal exporter after Brazil and accounted for 14% of total global beef export. The northern region of Australia encompassing parts of three states including Queensland, Northern Territory and Western Australia accounted for 45%, 9% and 8% of the Australian national cattle herd, respectively (FAO, 2020; MLA, 2020a). Beef cattle are routinely backgrounded on extensive grazing systems and finished on pasture for the lean beef market, or energy-dense grain-fed prime beef markets (Poppi et al., 2018; MLA, 2020a). Northern Australian tropical beef cattle rely mainly on native grass with few sown grass and legume pastures. In these summer rainfall-dominant dry tropics and sub-tropics, cattle are able to selectively graze high quality pastures in the early wet season, but often lose body condition, experience slow growth and struggle to attain maintenance weight in the other seasons due to low diet CP and digestible energy, pasture senescence, frost and overall poor pasture quality (Leng, 1990; Ivory and Whiteman, 2006; Kanani et al., 2006; Brandão et al., 2018).

Augmenting grass pastures with legumes has been reported to improve diet CP and energy digestibility (Hill et al., 2009). Besides, legumes improve the yield and nutritive value of grass-based pastures since the nutritive value of the resultant diet is higher compared to grass-only diet

(Thomas, 1992; Thomas, 1995), with specifically profound effects in winter and spring (Miller and Stockwell, 1991; Coates et al., 1997). In northern Australian light-textured soils, tropical pasture legumes came into general use after 1960 (Coates et al., 1997; Mannetje, 1997; Hill et al., 2009). Benefits on the heavier textured soils (the Vertosols) are being evaluated after the development of suitable legumes (Pengelly and Conway, 2000). Among the new legume pastures developed is desmanthus, a legume native to the Americas. Desmanthus is reported to have the potential for use as a forage legume in extensive grazing systems and crop rotations (Hill et al., 2009). Two studies examining growth performance of livestock fed desmanthus-grass diet reported higher liveweight gain in cattle (Gardiner and Parker, 2012) and goats (Marsetyo et al., 2017) compared to *Cenchrus ciliaris* (buffel) and *Brachiaria mulato* (mulato) grass only diets. In contrast, growing goats fed *Sorghum bicolor* (sudan grass) and supplemented with *D. bicornutus* leaves gained less weight than those supplemented with *Leucaena leucocephala* (leucaena), *Medicago sativa* (lucerne) or *Lablab purpureus* (lablab) (Kanani et al., 2006). Beef cattle supplemented with incremental *D. leptophyllus* or *D. bicornutus* levels up to 31% dry matter (DM) had similar weight gain with their counterparts fed *Chloris gayana* (Rhodes grass) only diet, although desmanthus supplementation improved rumen fermentation (Suybeng et al., 2020). These studies indicate that there are discrepancies and inconsistencies in animal growth in response to supplementation with desmanthus. Therefore, more studies are required to determine the effect of desmanthus on beef cattle growth performance and change in rumen and plasma metabolites. Chapter 4.1 demonstrated that in an extensive grazing system typical of Central Queensland Brigalow region, backgrounding 400 beef cattle steers during the dry season for 147 days on buffel grass alone or buffel-desmanthus mixed pastures with desmanthus accounting for 11.5% pasture botanical composition, did not produce any significant differences in liveweight, daily weight gain

and plasma metabolites. The lack of difference was thought to be due to similar dietary CP levels for steers on both paddocks accessed through browsing on shrubs and forbs, necessitating the need for feeding steers isonitrogenous diets with varying levels of desmanthus inclusion in a controlled pen trial. Therefore, the aim of the current study was to evaluate the effect of increasing levels of desmanthus in isonitrogenous diets on beef cattle growth rate, rumen fermentation and plasma metabolites of tropical crossbred beef cattle. The hypothesis tested was that cattle fed isonitrogenous diets supplemented with incremental levels of desmanthus would have similar growth rates, rumen fermentation and plasma metabolites concentration.

4.2.2. Materials and Methods

This study was carried out at the CSIRO Lansdown Research Station, Queensland, Australia (19.59° S, 146.84° E) between March and July 2020. The station receives 861 mm mean total annual rainfall with mean annual min and max temperatures of 16.8 and 26.1°C, respectively (Jones, 2003; Australian Government Bureau of Meteorology, 2021a). All procedures in this study followed the CSIRO Animal Ethics Committee approved guidelines (approval number 2019-38) and the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013).

4.2.2.1. Animal management and diets

An *a priori* power analysis was conducted using G-Power to determine the appropriate sample size (Figure 4.2.1). A total sample size of 48 steers was required to achieve statistical power of 95% with a critical F-value of 4.06 for medium effect size and a significance level of 0.05. Therefore, forty-eight tropically adapted 24 − 28 months old Brahman, Charbray and

Droughtmaster crossbred steers weighing 332 ± 21 kg, were used for the study. The steers were fitted with insecticidal cattle ear tags containing a synergized formulation of zeta cypermethrin (Y.Tex Corporation, Cody, Wyoming, USA) and treated with an Ivermectin based pour-on parasite control (BAYMEC, Bayer Australia Ltd, Pymble NSW, Australia) at 1 ml/10 kg liveweight (LW) dosage at the beginning of the study for internal and external parasites control. Steers were group-housed in 12 open pens (4 steers per pen) with three pens per experimental diet in a completely randomized design. Steers were allocated to four groups based on their initial liveweight to ensure similar mean liveweight per pen. The pens were then randomly assigned to one of four diets. Each pen measured 60 m^2, fitted with 18 m^2 shade and 4 m by 1 m feed trough. Steers were allocated to one of the following four experimental diets: 0, 15%, 30% or 45% desmanthus on DM basis with Rhodes grass hay as the basal diet. Diet CP was adjusted by the inclusion of lucerne hay in the 0, 15% and 30% desmanthus diets, based on forage CP and not DM basis, to ensure all diets were isonitrogenous. The 0 desmanthus diet (Rhodes grass and lucerne) was used as a positive control. Lucerne was selected because it is the most widely grown perennial legume globally and has been extensively studied (Du et al., 2019). Desmanthus consisted of the three species, namely *D. virgatus*, *D. bicornutus* and *D. leptophyllus* fed in equal proportions. A mixture of these three species was used because *d*esmanthus is commonly marketed as a mixed product, e.g. Jaribu desmanthus comprising *D. virgatus cv Marc, D. leptophyllus* cv Bayamo and *D. pubescens cv Uman* in the 1990s (Cook et al., 1993) and Progardes® consisting of *D. bicornutus* cv JCU4, *D. leptophyllus* cv JCU7 and *D. virgatus* cv JCU2 and cv JCU5 to ensure that the best adapted cultivars eventually dominate while the less adapted cultivars take advantage of seasonal, land types and climate variation (Gardiner et al., 2013). Desmanthus establishment and management are described extensively in Chapter 3 and were not repeated herein. Each cultivar plot was divided

into three and harvesting was staggered between the plots to ensure similar maturity stage of the forage at harvest. Overgrown forage was slashed and used as mulch to encourage new regrowth. Steers were gradually adapted to the experimental diet within ten days (Cochran and Galyean, 1994). Throughout the study, steers had unlimited access to clean water and mineral block (Table 4.2.1; Trace Element Northern, Ollson's, Yennora, NSW, Australia).

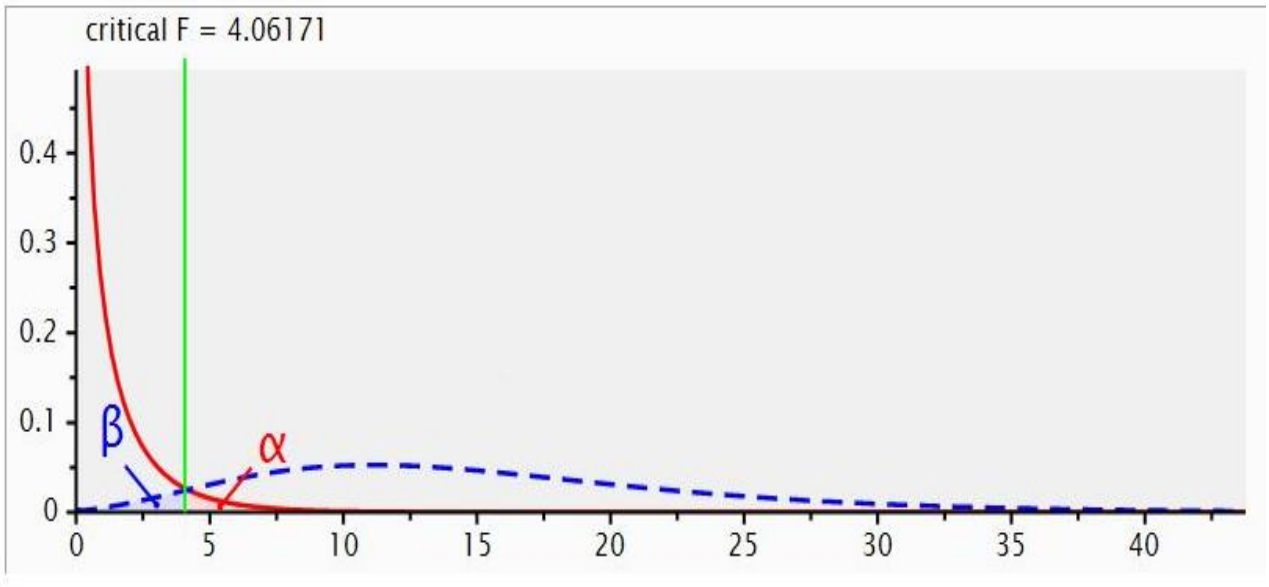


Figure 4.2.1. G-Power analysis for statistical power, critical F-value and sample size.

Table 4.2.1. Chemical composition of the mineral block.

Ingredient	Concentration
Molasses (%)	5
Sodium chloride (NaCl, %)	82
Macro ingredients (%)	
Calcium (Ca)	1
Phosphorus (P)	1
Sulphur (S)	0.8
Magnesium (Mg)	0.02
Micro ingredients (mg/kg)	
Copper (Cu)	1000
Cobalt (Co)	65
Ferrous Iron (Fe++)	1350
Iodine (I)	500
Selenium (Se)	26
Iron (Fe)	650
Zinc (Zn)	300

Desmanthus was harvested at the late bloom to full seed maturity stages and chopped with a flail type forage harvester (New Holland model 38 Crop-Chopper, Haryana, India) every morning, while Rhodes grass and lucerne hay were chopped with a tub grinder (Roto Grind model 760, Burrows Enterprises, LLC, Greeley, CO, USA) once a week. Experimental diets were mixed by hand and offered daily between 8:30 and 9:30 am after residuals were collected. Feed offered was adjusted to allow for 5-10% refusals. Refusals were weighed daily and a sample per pen stored at -20^{o}C, from which a weekly composite bulked sample per pen was obtained for DM and chemical analysis. Weekly samples of the desmanthus, lucerne and Rhodes grass were obtained throughout the study for DM and chemical analysis.

4.2.2.2. Feed intake, liveweight and body condition scores

Dry matter intake per pen was determined by the weight difference between feed offered and refusals collected 24 h after feeding. Steers were weighed at the start and end of the study, fortnightly for the first six weeks and monthly thereafter until the end of the study. The frequency of weighing changed after six weeks due to labour limitations resulting from COVID-19 pandemic related restrictions. All steers were weighed before feeding to reduce variation due to gut fill (Wishart et al., 2017; Cho et al., 2020). Unfasted LW were recorded automatically (Gallagher 65 Scanlon Drive, Epping, Victoria 3076, Australia) and the average daily gain calculated by regressing all fortnightly and monthly LW by time in days (Archer and Bergh, 2000). Body condition scores (BCS) were recorded monthly using the five-point (1 – 5) scoring system (Ndlovu et al., 2007).

4.2.2.3. Forage and refusals analysis

The forage offered and refusals DM, CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, DMD nutritive values were estimated using near infrared reflectance (NIR) spectroscopy at the CSIRO Floreat laboratory (Floreat, WA, Australia). The samples were dried in a forced-air oven at 60°C for 48 h and ground to pass through a 1 mm mesh with a Christy and Norris grinder (Christy Turner Ltd, Suffolk, England), the spectra collected using the Unity Spectrastar 2500X rotating top window system (Unity Scientific, Milford, MA, USA) and predictions were generated using the chemometric software package Ucal (Unity Scientific) as described by Norman et al. (2020).

4.2.2.4. Rumen fluid collection and analysis

Rumen fluid samples were collected in the morning prior to feeding at the start, middle and end of the experimental period (weeks 0, 10 and 20) from 24 steers to determine the effect of diet on rumen pH, ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA). About 200 ml of rumen fluid samples were collected from the ventral sac via oro-ruminal tubing using a reinforced plastic suction tube and a hand pump. The pH of each sample was taken immediately using a portable pH meter (Aqua-pH, TPS Pty Ltd, Brendale, QLD, Australia). Rumen fluid sub-samples of 8 ml were stabilized by adding 2ml of 25% metaphosphoric acid and stored at -80°C awaiting NH_3-N and VFA analysis. The rumen NH_3-N was analyzed using the colorimetric method of Chaney and Marbach (1962), while VFA were determined by gas chromatography (Shimadzu Corporation, Kyoto, Japan) as described by Gagen et al. (2014). Since rumen fluid with viscous appearance and pH of 7.5 or above indicate contamination with large volumes of saliva (Petrovski, 2017), samples with these characteristics were excluded in pH, total VFA and NH_3-N analysis.

4.2.2.5. Blood collection and plasma metabolites analysis

Blood samples were collected in the morning prior to feeding at the start, middle and end of the experimental period (weeks 0, 10 and 20) via jugular venipuncture into 10 ml sodium heparin blood Vacutainer tubes (BD, Sydney, Australia), centrifuged at 1425 x g for 20 min at 4 °C (Beckman Coulter, Inc. California, USA) to separate the plasma from the serum and stored at -80°C prior to analysis. All plasma metabolites were analyzed using the AU480 chemistry analyzer6 (Beckman Coulter, Inc. California, USA) according to the manufacturer's procedures. Plasma non-esterified fatty acids (NEFA) were analyzed using colorimetric method (Shimizu et al., 1980), beta-hydroxybutyrate (BHB) and glucose analyzed by the 3-hydroxybutyrate

dehydrogenase and hexokinase methods, respectively (Sacks et al., 2002), total bilirubin by the modified diazo method (McPherson and Pincus, 2016) and creatinine by the kinetic modified Jaffe method (Cholongitas et al., 2007).

4.2.2.6. Statistical analysis

Data were analysed using the SAS software version 9.4 (SAS Institute, Cary, North Carolina, USA), with an initial screening for data entry errors, outliers and data distribution done for all data sets. Mixed model (PROC MIXED) restricted maximum likelihood (REML) procedures in SAS fitted the fixed effect of diet and pen nested within diet as a random effect in the statistical model. Sampling week was analysed as a repeated measure and covariance structures were specified. Final LW and BCS were analysed by including the initial measurements as covariates. *P* values were deemed significant when below 0.05. When there was significant effect of diet, orthogonal polynomial contrasts were performed to test for linear, quadratic and cubic responses to increasing desmanthus proportion. The quadratic and cubic responses were dropped from the model because they were not significant for all variables tested. PROC CORR procedure fitted with Spearman's ρ test was used to calculate the residual correlations between diet, rumen and plasma metabolite parameters. Baseline rumen and plasma metabolite values were excluded in the correlation analysis because the quality of the pasture that cattle grazed before the study commenced was not analysed.

4.2.3. Results

4.2.3.1. Diet quality, intake and growth performance

The nutritive values of the forages are shown in Table 4.2.2 and the four experimental diets are presented in Table 4.2.3. Rhodes grass had the lowest CP and highest fibre contents compared to the legume forages. The lowest DMD and ME values were obtained in the Rhodes grass and JCU7 cultivar. The diets were formulated to be isonitrogenous hence the similar CP (P = 0.84). Diet NDF levels were similar (P = 0.40) but significant differences were observed in diets ADF, hemicellulose and ME levels ($P \leq 0.001$). There was a linear increase in ADF and decrease in hemicellulose and ME with increase in desmanthus proportion.

Table 4.2.2. Chemical composition (mean ± se) of the experimental diets.

Variable[1]	Rhodes grass	Lucerne	JCU2[2]	JCU4	JCU7
Dry matter (%)	87.9 ± 0.56	86.9 ± 0.62	31.6 ± 1.44	28.5 ± 1.25	34.3 ± 0.98
CP	9.0 ± 0.24	17.4 ± 0.70	13.7 ± 0.73	15.6 ± 0.61	11.9 ± 0.44
NDF	73.3 ± 0.40	47.6 ± 0.88	53.1 ± 1.24	50.4 ± 1.24	57.3 ± 0.75
ADF	42.6 ± 0.45	34.9 ± 0.86	39.6 ± 0.99	36.9 ± 0.82	41.7 ± 0.52
Hemicellulose	30.7 ± 0.26	12.7 ± 0.42	13.5 ± 0.84	13.5 ± 0.77	15.6 ± 0.56
DMD	51.8 ± 0.39	69.1 ± 1.34	55.1 ± 1.96	58.4 ± 1.58	50.6 ± 0.90
ME (MJ/kg DM)[3]	7.2 ± 0.067	10.1 ± 0.23	7.7 ± 0.33	8.3 ± 0.27	6.9 ± 0.15

[1]CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre; DMD: dry matter digestibility. Data are presented in percentage of dry matter (DM) unless otherwise stated.

[2]JCU2: *D. virgatus,* JCU4: *D. bicornutus,* JCU7: *D. leptophyllus*

[3]Estimated from *in vitro* dry matter digestibility as DMD × 0.172 − 1.707 (CSIRO, 2007).

Table 4.2.3. Effect of desmanthus proportion on the nutritive value, digestibility and dry matter digestibility to crude protein ratio over the feeding trial duration.

	% desmanthus in the diet					Linear
Variable[1]	0	15	30	45	SEM[2]	*P*-value
CP	11.6	11.6	11.5	11.4	0.11	0.84
NDF	65.2	64.9	64.6	64.4	0.15	0.40
ADF	40.1	40.4	40.8	41.1	0.07	< 0.01
Hemicellulose	25.1	24.4	23.8	23.3	0.14	< 0.01
ME (MJ/kg)[3]	8.1	7.9	7.6	7.4	0.03	< 0.01
DMD:CP ratio	8.7	8.5	8.5	8.5	0.09	0.91

[1]CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, DMD: dry matter digestibility; Values are presented in least square means. Data are presented in percentage of dry matter (DM) unless otherwise stated.

[2]SEM : standard error of the mean

[3]Estimated from individual forages *in vitro* DMD as DMD × 0.172 − 1.707 (CSIRO, 2007)

The DMI and nutrient intake data are presented in Table 4.2.4. Increasing the desmanthus levels in the diet decreased the DMI and subsequently CP, NDF, ADF, hemicellulose and ME intake ($P < 0.01$). The DMI decreased from 8.8 to 7.6 kg/day with increasing desmanthus proportion from 0 to 45% ($P < 0.01$).

Table 4.2.4. Effect of desmanthus proportion on nutrient intake.

Variable[1]	% desmanthus in the diet				SEM[2]	Linear *P*-value
	0	15	30	45		
DM	8.8	8.5	8.2	7.6	0.06	< 0.01
CP	1.0	1.0	0.95	0.89	0.01	< 0.01
NDF	5.7	5.4	5.2	4.8	0.04	< 0.01
ADF	3.5	3.3	3.2	3.0	0.02	< 0.01
Hemicellulose	2.1	2.0	1.9	1.8	0.02	< 0.01
MEI (MJ/day)	72.8	69.8	66.2	61.7	0.49	< 0.01

[1]Data are presented in kg/day unless otherwise stated. DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, MEI: metabolisable energy intake. Values are presented as least square means. [2]SEM : standard error of the mean

There were no significant differences in LW, BCS, ADG and feed to gain ratio observed between diets (Table 4.2.5). At the end of the study steers weighed between 419 to 434 kg ($P = 0.21$) with feed to gain ratio of 12.9 to 14.6 kg DMI per kg weight gained ($P = 0.31$).

Table 4.2.5. Effect of desmanthus proportion on the average liveweight, body condition score, daily gain and feed to gain ratio in supplemented steers.

Variable[1]	% desmanthus in the diet				SEM[2]	*P*-value
	0	15	30	45		
Overall liveweight (kg)	392.6	388.0	384.5	381.3	1.98	0.28
Final liveweight (kg)	434.0	439.5	426.7	419.3	4.49	0.21
Final Body condition score	3.3	3.4	3.3	3.2	0.05	0.33
Average daily gain (kg/day)	0.62	0.66	0.57	0.52	0.04	0.13
Feed:Gain ratio	14.2	12.9	14.6	14.5	0.35	0.31

[1]Values are presented as least square means.

[2]SEM : standard error of the mean

4.2.3.2. Rumen and plasma metabolite parameters

Rumen pH and metabolites data of steers fed increasing desmanthus level*s* in the diet are presented in Table 4.2.6. A linear decrease in total VFA with incremental levels of desmanthus in the diet was observed ($P = 0.02$). The rumen pH and all the other metabolites measured were similar for all the diets ($P \geq 0.07$). Proportion of desmanthus in the diet had no effect on plasma NEFA, BHB, glucose, creatinine and total bilirubin (Table 4.2.7; $P \geq 0.06$).

Table 4.2.6. Rumen volatile fatty acids (VFA), ammonia nitrogen (NH3-N) and pH of tropical beef cattle fed increasing levels of desmanthus.

	% desmanthus in the diet					
Variable[1]	0	15	30	45	SEM[2]	*P*-value
Total VFA (mg/100dL)	68.3	59.2	61.7	57.5	1.53	0.03
Acetate (molar %)	74.1	74.0	73.3	73.8	0.18	0.49
Propionate (molar %)	14.8	14.1	14.7	14.2	0.15	0.32
Acetate/Propionate ratio	5.0	5.2	5.0	5.2	0.06	0.37
Iso-butyrate (molar %)	1.4	1.5	1.3	1.4	0.05	0.63
n-butyrate (molar %)	6.6	7.1	7.2	7.3	0.09	0.08
Iso-valerate (molar %)	1.3	1.4	1.2	1.4	0.04	0.41
n-valerate (molar %)	0.60	0.65	0.66	0.66	0.00	0.08
n-caproate (molar %)	0.85	1.0	1.0	1.0	0.03	0.12
NH_3-N (mg/dL)	12.2	11.6	11.6	10.4	0.34	0.31
pH	6.9	7.1	7.1	7.1	6.58[3]	0.20

[1]Values are least square means.

[2]SEM : standard error of the mean

[3]SEM presented as H^+ concentration in nEq/L

Table 4.2.7. Plasma metabolites of tropical beef cattle fed increasing levels of desmanthus.

	% desmanthus in the diet					
Metabolite[1]	0	15	30	45	SEM[2]	*P*-value
NEFA (mmol/L)	0.52	0.40	0.44	0.50	0.02	0.92
BHB (mmol/L)	0.23	0.24	0.27	0.21	0.00	0.07
Glucose (mmol/L)	5.1	5.2	5.1	5.1	0.07	0.99
Creatinine (μmol/L)	108.3	106.8	109.1	113.7	1.41	0.41
Total bilirubin (μmol/L)	3.2	3.0	3.0	3.3	0.10	0.67

[1] Values are least square means. NEFA: non-esterified fatty acids, BHB: β-hydroxybutyrate.

[2] SEM : standard error of the mean

Medium to high residual correlations (0.41 – 0.83) were observed between diet parameters, rumen VFA and plasma metabolites (Table 4.2.8). Diet ADF was correlated to all rumen metabolites except NH_3-N and n-caproate. A negative correlation between diet ADF with total bilirubin and NEFA was observed. Diet CP correlated positively with rumen NH_3-N and propionate but negatively with acetate/propionate ratio, n-caproate and creatinine ($P < 0.05$).

Table 4.2.8. Residual correlation coefficients between diet, rumen and plasma metabolites.

	Diet parameters[2]				
Metabolites[1]	CP	NDF	ADF	Hem	DMD
Total VFA	0.20	0.65**	−0.68**	0.65**	0.51**
A/P ratio	−0.58**	0.41*	−0.56**	0.55**	−0.053
NH_3-N	0.63**	0.17	−0.02	0.06	0.46*
Acetate	−0.30	0.62**	−0.79***	0.72***	0.25
Propionate	0.64**	−0.3	0.45*	−0.45*	0.16
Iso-butyrate	0.26	−0.60**	0.64**	−0.66**	−0.21
n-butyrate	−0.17	−0.4	0.41*	−0.36	−0.38
Iso-valerate	0.03	−0.69**	0.72***	0.73***	−0.44*
n-valerate	−0.002	−0.73***	0.83***	−0.79***	−0.49*
n-caproate	−0.51*	−0.11	0.07	−0.04	−0.37
Total bilirubin	−0.25	0.58**	−0.62**	0.66**	0.17
Creatinine	−0.59**	0.09	−0.26	0.22	−0.24
Glucose	0.11	0.42*	−0.36	0.43*	0.30
BHB	−0.07	0.15	−0.16	0.19	0.06
NEFA	−0.37	0.39	−0.45*	0.51*	0.00

[1] VFA: volatile fatty acids, A/P ratio: acetate to propionate ratio, NH_3-N: ammonia nitrogen, NEFA: non-esterified fatty acids, BHB: β-hydroxybutyrate

[2] DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre, Hem: Hemicellulose, DMD: dry matter digestibility

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

4.2.4. Discussion

This study evaluated the effect of incremental levels of desmanthus in isonitrogenous diets on the feed intake, rumen fermentation, plasma metabolites and growth rate of tropical crossbred beef steers.

4.2.4.1. Diet quality, intake and growth performance

Rhodes grass and lucerne quality indicators were within the range reported in other studies (Kurihara et al., 1999; Popp et al., 2000; Gebregiorgis et al., 2012; Tomkins et al., 2015). Rhodes grass had lower CP and higher fibre content compared to lucerne and desmanthus. The observed difference agrees with previous studies that had reported grass to have lower quality than legume forages (Gebregiorgis et al., 2012; Dal Pizzol et al., 2017). The CP value of *D. virgatus* and *D. bicornutus* were within the values reported in previous studies carried out in northern Australia (Durmic et al., 2017; Vandermeulen et al., 2018; Suybeng et al., 2020). The CP content for *D. leptophyllus* was comparatively lower than the CP of *D. virgatus* and *D. bicornutus* (Durmic et al., 2017; Suybeng et al., 2020) but similar to that reported by Suybeng et al. (2020). Differences in values between studies may be due to soil fertility, climate, plant fraction and stage of maturity at harvest (Maasdorp et al., 1999). The quality differences between species may be due to differences in plant characteristics. For instance, desmanthus ranges widely from early to late maturing and herbaceous to suffruticose plant types (Gardiner et al., 2013; Vandermeulen et al., 2018). The fibre content was lower than that reported by Suybeng et al. (2020) but higher than that reported by Durmic et al. (2017). This difference may be due to the maturity stage and plant part collected. Unlike this study where the whole plant was used, Durmic et al. (2017) collected the leaves and

only 5cm long stems to mimic cattle grazing. Significant variation in plant part nutritive value has been widely reported (Baloyi et al., 2008; Grev et al., 2020).

Diets were formulated to ensure that they were isonitrogenous but increasing desmanthus proportion in the diet increased ADF content and reduced NDF and DMI. High indigestible fibre content reduces voluntary feed intake in ruminants due to low digestibility and high rumen fill effect (Pearson et al., 2006; Detmann et al., 2009). Dairy cows fed grass silage of varying maturity were observed to eat less as the ADF content increased (Rinne et al., 2002). Dietary fibre content has been widely used to predict digestibility. A negative correlation exists between indigestible fibre content and digestibility (Aufrere and Michalet-Doreau, 1988; Jančík et al., 2008). However, DMD is reported to restrict rumen fermentation and DMI when the DMD:CP ratio exceeds 8 to 10 (Dixon and Coates, 2005), but in this study, the ratio ranged between 8.5 and 8.7 indicating that DMD was not likely to limit rumen fermentation and feed intake.

Confined *Bos indicus* crossbred beef cattle weighing 400 kg fed an 8 MJ/kg DM diet require 40 MJ ME daily for maintenance and 69 MJ ME for 0.5 kg ADG (McLennan, 2015). In this study, the crossbred steers consumed 61 – 72 MJ ME daily; hence weight gain was expected. Steers in all experimental diets gained at least 0.5 kg/day. Typically, the annual ADG of cattle grazing northern Australian native grass pastures is 0.3 kg/day (Hill et al., 2009; Poppi et al., 2018). Poppi et al. (2018) reported that ADG ranging between 0.4 and 0.6 kg can be achieved using improved forage, and these gains are adequate to meet the target slaughter weight required for the prime beef market within 2.5 years of age. The observed significant drop in feed intake as the desmanthus level increased did not result in a significant difference in LW. The reduced feed intake may have improved digestibility by reducing the passage rate of digesta from the reticulorumen (Zanton and Heinrichs, 2009). Besides, desmanthus is a tannin-containing legume (Vandermeulen et al., 2018;

Suybeng et al., 2020). Condensed tannins can form complexes with proteins that protect CP from microbial degradation in the rumen, thus enhancing lower gastrointestinal tract digestion (Molina-Botero et al., 2019). Lack of significant difference between diets indicates that desmanthus alone or mixed with other high-quality legume forages can be used to supplement grass-based diets of beef cattle in northern Australia without any detrimental impact on productivity. Supplementation of grass-based grazing cattle with desmanthus was reported to improve growth rate (Gardiner and Parker, 2012). In contrast to findings of this study, incremental levels of up to 31% desmanthus resulted in only 0.2 kg ADG in confined steers even when 11.8% dietary CP was achieved (Suybeng et al., 2020). The authors attributed the low weight gain to low DMI. For a diet with ME of 7 MJ/kg DM, cattle weighing 300 kg (similar to those used in the study of Suybeng et al. (2020)) require 5.4 kg DMI/day (1.8% DM/LW), while cattle weighing 400 kg (similar to those used in this study) require 6.4 kg DMI/day (1.6% DM/LW) for maintenance (McLennan, 2015). Although DMI increased from 3.8 kg/day to 4.7 kg/day when steers were supplemented with 31% desmanthus, steers consumed 1.4% DM/LW in the study of Suybeng et al. (2020) compared to at least 2% in this study. The feed to gain ratio in this study (12 − 14) was similar to values reported for cattle fed high forage diets of similar CP. Continental crossbred steers fed a 70:30 forage to concentrate diet with 13% CP had feed to gain ratio of 10.4 – 16.9 (Kennedy et al., 2018), while *Bos indicus-Bos taurus* crossbred cattle fed 11% CP diet consisting of 5 – 24% concentrate supplement had 9.4 – 13.5 feed to gain ratio (Molina-Botero et al., 2019).

4.2.4.2. Rumen and plasma metabolites

Volatile fatty acids play a major role in ruminant nutrition (Dijkstra, 1994). They contribute 60 – 70% of metabolisable energy (Armentano, 1992; Seymour et al., 2005). The VFA levels vary with

animal feeding patterns and diet composition. Highly fermentable diets may raise rumen VFA levels to 200 mM, with the peak occurring 2 to 4 h after feeding. However, hay diets produce smaller fluctuations throughout the day and concentrations of less than 100 mM are common (Bergman, 1990). Previous studies reported increases in total rumen VFA with increases in diet digestibility (Park et al., 1994; Rinne et al., 2002). This agrees with findings of this study of a high negative correlation between ADF, a marker of diet digestibility, and total VFA. Total VFA concentrations decreased with an increase in desmanthus level in the diet. This could be due to several factors: a) The decrease in DMI associated with higher proportion of desmanthus in the diet that increased ADF and reduced feed intake (Armentano, 1992). Increase in ADF was associated with a decrease in total VFA concentration, in line with previous observations in a dual-flow continuous culture system where high levels of indigestible fibre resulted in low concentrations of rapidly fermentable carbohydrates and reduced VFA concentration (Brandao and Faciola, 2019). b) Condensed tannins may have formed complexes with proteins availing proteins for digestion in the lower gastrointestinal tract (Molina-Botero et al., 2019). This may increase microbial protein synthesis efficiency in the rumen (Gemeda and Hassen, 2018). On the other hand, lucerne is highly fermentable in the rumen (Ramírez-Restrepo and Barry, 2005), thus the high total VFA. Rumen NH_3-N levels of 10 mg/dl are required to maintain effective rumen microbial activity and maximize voluntary DMI in cattle fed low-quality tropical forage (Ortiz-Rubio et al., 2007; Sampaio et al., 2010). These levels (10.4 – 12.2 mg/dl) were attained in all the diets in this study.

The molar proportion of individual VFA is influenced by dietary composition and DMI (Brandao and Faciola, 2019). An increase in the proportion of dietary forage or fibre leads to an increase in acetate and decrease in propionate, butyrate, iso-butyrate, valerate and iso-valerate molar

concentrations (Rinne et al., 2002; Agle et al., 2010). Similar to this study, cattle grazing pasture of varying digestibility had similar rumen molar percentages of acetate, propionate, butyrate, iso-valerate and valerate (Hart et al., 2009). However, in contrast to this study, they reported an increase in iso-butyrate concentrations with increased diet DMD. However, the increase was numerically small and considered to be of no biological significance.

All plasma metabolites measured in this study were not affected by the dietary desmanthus levels. Cattle fed diets of similar CP as in this study had similar glucose and NEFA concentrations (Asano et al., 2017). Plasma NEFA originates from the mobilisation of stored fat. The similar NEFA concentration between diets may indicate that the steers had comparable energy balance (Rottman et al., 2015). In ruminants, negligible levels of glucose are absorbed across the portal-drained viscera from dietary sources. Propionate plays a major role as a precursor for glucose synthesis, contributing 46 – 73% to hepatic gluconeogenesis in cattle (Dijkstra, 1994). The similar molar proportion of propionate in the current study may have resulted to similar glucose levels between the diets. All plasma metabolites measured were within the normal range reported for beef cattle (Kaneko, 1997; Otto et al., 2000; Grünwaldt et al., 2005; Braun and Lefebvre, 2008). Similar levels of plasma metabolites indicate that an inclusion level of up to 45% desmanthus in the diet does not cause adverse effects on energy metabolism and health status of supplemented steers.

Overall, increasing the proportion of desmanthus in the diet did not influence LW, ADG, VFA molar proportion and plasma metabolites of steers fed isonitrogenous diets. These findings indicate that desmanthus can be used as a renewable protein source for beef cattle in the northern Australian beef cattle production system, particularly in the tropical semi-arid clay soil environments where lucerne is not adapted.

The northern Australian beef industry serves both the pasture-fed and grain-finished beef markets (MLA, 2020a). It is common practice for cattle backgrounded on pastures to be finished in the feedlot on energy-dense grain diets for short periods of about 100 days before slaughter (Greenwood et al., 2018). Further studies are required to determine the effect of desmanthus supplementation on feedlot performance and carcass quality of both pasture and feedlot finished cattle.

4.2.5. Conclusion

This study aimed to evaluate the weight gain, rumen fermentation and plasma metabolites of tropical crossbred steers in response to supplementation with incremental levels of desmanthus forage legume in isonitrogenous diets. The results showed similar weight gains, VFA molar proportion and plasma metabolites, but a decrease in total VFA with increase in dietary desmanthus levels. Hence, the hypothesis that cattle fed isonitrogenous diets supplemented with different desmanthus level*s* would have similar growth rates, rumen fermentation and plasma metabolites was accepted. The results indicate that desmanthus alone or in combination with other high-quality legume forages can be used to supplement grass-based diets of beef cattle in northern Australia. However, more studies are required to examine the effect of desmanthus supplementation on cattle feedlot performance and carcass quality.

4.2.6. Summary

Desmanthus, a tropically adapted pasture legume, is highly productive and has the potential to reduce methane emissions in beef cattle. However, liveweight gain response to desmanthus supplementation has been inconclusive in ruminants. This study aimed to evaluate weight gain,

rumen fermentation and plasma metabolites of Australian tropical beef cattle in response to supplementation with incremental levels of desmanthus forage legume in isonitrogenous diets. Forty-eight Brahman, Charbray and Droughtmaster crossbred beef steers were pen-housed and fed a basal diet of Rhodes grass (*Chloris gayana*) hay supplemented with 0, 15, 30 or 45% freshly chopped desmanthus forage on dry matter basis, for 140 days. Varying levels of lucerne (*Medicago sativa*) hay were added in the 0, 15 and 30% diets to ensure that all diets were isonitrogenous with the 45% desmanthus diet. Data were analysed using the Mixed Model procedures of SAS software. Results showed that the proportion of desmanthus in the diet had no significant effect on steer liveweight, rumen volatile fatty acids molar proportions and plasma metabolites ($P \geq 0.06$). Total bilirubin ranged between 3.0 and 3.6 μmol/L for all the diet treatments ($P = 0.67$). All plasma metabolites measured were within the expected normal range reported for beef cattle. Rumen ammonia nitrogen content was above the 10 mg/dl threshold required to maintain effective rumen microbial activity and maximize voluntary feed intake in cattle fed low-quality tropical forages. The average daily weight gains averaged 0.5 to 0.6 kg/day ($P = 0.13$) and were within the range required to meet the target slaughter weight for prime beef markets within 2.5 years of age. These results indicate that desmanthus alone or mixed with other high-quality legume forages can be used to supplement grass-based diets to improve tropical beef cattle production in northern Australia with no adverse effect on cattle health.

Chapter 5: Feedlot Growth Performance, Carcass Traits and Meat Fat Traits of Steers Backgrounded on Desmanthus-Augmented Diets

Chapter 5.1: Feedlot Growth Performance and Carcass Traits of Steers Backgrounded on Buffel Grass or Buffel-Desmanthus Mixed Pastures

5.1.1. Introduction

The collective effects of meat tenderness, juiciness and flavour are the most important sensory contributors to eating quality or overall eating satisfaction (Ferguson, 2004; Thompson, 2004) that exerts an influence on consumer satisfaction and decision to purchase (Felderhoff et al., 2020). Carcass marbling fat in the form of intramuscular fat (IMF) and subcutaneous fat are used by consumers as a visual cues for judging meat quality (Špehar et al., 2008; Frank et al., 2016). A survey by Testa et al. (2021) reported that up to 90% of Argentine consumers defined beef quality at purchase time on the basis of meat colour and marbling (Testa et al., 2021). Thompson (2004) reported a positive curvilinear relationship between IMF and beef flavour scores over a range of 0.3% to 15% IMF, which plateaued at higher IMF levels. Tenderness increases with an increase in marbling through a distortion of the connective tissue structure resulting in weakened tissue rigidity (Nishimura et al., 1999; Špehar et al., 2008). Also, high carcass fat content improves carcass water-holding capacity and consequently reduces drip loss (Santos-Silva and Portugal, 2001; Hwang and Joo, 2017) and insulates carcasses during chilling to prevent cold shortening (Savell et al., 2005).

Meat from tropical animals grazing forage has less intramuscular fat content and appears darker in colour than meat from grain-fed animals, thus, forage-backgrounded animals are usually

feedlot-finished on concentrate diets to increase IMF content with a markedly improved flavour, tenderness and juiciness (Scollan et al., 2006; Blanco et al., 2010; De Brito et al., 2017; Vega Britez et al., 2020). Backgrounding diet and weight gain influence subsequent finishing feed intake and growth performance, but results have been inconsistent (Drouillard and Kuhl, 1999; Dicker et al., 2001; Peripolli et al., 2018). Reuter and Beck (2014) reported that finishing ADG and DMI decreased as backgrounding ADG increased in cattle. Similarly, steers with low backgrounding weight gains were reported to have greater finishing ADG than those with higher backgrounding weight gains (Neel et al., 2007). Restricting the feed intake of steers during the backgrounding phase has been demonstrated to increase feed intake during the finishing period compared to steers with *ad libitum* access to feed (Loerch, 1990). In contrast, the DMI and ADG of beef steers during the finishing phase were not influenced by weight gain during the backgrounding period (Loken et al., 2009). These differences are dependent on the level of growth restriction during backgrounding that determines feedlot entry LW and the occurrence of compensatory weight gain during finishing (Drouillard and Kuhl, 1999; Peripolli et al., 2018). These findings highlight the need to better understand the effect of backgrounding beef cattle on desmanthus, a legume adapted to the cracking clay soil regions of northern Australia (Hill et al., 2009), on feedlot growth performance and feed intake.

Feeding ruminants on diverse forages is reported to influence carcass quality. For instance, hot carcass weight (HCW), marbling score and subcutaneous back fat thickness of Angus-cross steers grazing *Medicago sativa* (lucerne), *Cynodon dactylon* (bermudagrass), *Cichorium intybus* (chicory), *Vigna unguiculata* (cowpea), or *Pennisetum glaucum* (pearl millet) significantly varied (Schmidt et al., 2013). Augmenting *Chloris gayana* (Rhodes grass) hay diet with desmanthus was reported to improve *Longissimus dorsi* (loin eye muscle) area (EMA) and HCW compared to

cotton seed meal, urea or both in goats (Aoetpah et al., 2018). However, the difference may not persist when animals are finished in the feedlot before slaughter (Duckett et al., 1993; Kurve et al., 2016). While some studies have evaluated the impact of the finishing diets on carcass quality traits (Realini et al., 2004; Duckett et al., 2013), fewer studies have examined the effect of backgrounding on finishing growth performance and carcass quality (Dicker et al., 2001; Loken et al., 2009; Ferrinho et al., 2020). In addition, there is an existing knowledge gap on the feedlot growth performance of tropical beef cattle backgrounded on grass pastures augmented with desmanthus. Therefore, this study aimed to evaluate the feedlot growth performance and carcass quality of tropical beef steers backgrounded on buffel grass (*Cenchrus ciliaris*) only or buffel grass oversown with desmanthus (11.5% initial sward botanical composition) to similar feedlot entry weight. It was hypothesised that tropical beef steers backgrounded on buffel grass only or buffel grass oversown with desmanthus with similar backgrounding growth performance would have similar feedlot growth performance and carcass quality.

5.1.2. Materials and methods

This study was carried out according to the James Cook University Animal Ethics Committee approved guidelines (Approval Number 2639) and the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013).

5.1.2.1 Animals, diets and management

After backgrounding on buffel grass only or buffel grass-desmanthus mixed pastures for 147 days as described in Chapter 4.1, three hundred and twelve *Bos indicus* x *Bos taurus* tropical composite steers at 20 – 23 months of age and initial LW of 413 ± 24 kg were finished in a commercial feedlot

over a period of 110 days in South East Queensland. The feedlot receives annual mean rainfall, minimum and maximum temperatures of 584.4 mm, 12.0 °C and 27.0 °C, respectively (Australian Government Bureau of Meteorology, 2021b). Near-infrared reflectance spectroscopy of faecal samples indicated that the quality of forage consumed during backgrounding was 11.2 and 10.2% CP, 55.0 and 53.7% DMD, 7.8 and 7.5 MJ/Kg DM metabolisable energy and 26.7 and 26.4% diet non-grass forage for the buffel grass and buffel-desmanthus backgrounded steers, respectively. An *a priori* G-Power analysis to determine the appropriate experimental sample size was carried out as depicted in Figure 5.1.1 which showed that 50 steers were required to achieve an 80% statistical power with a critical t-value of 2.0 for a large effect size at a significance level of $P < 0.05$. Therefore, a representative cohort of 50 steers, comprising 25 steers from each backgrounding pasture, was housed in a pen fitted with GrowSafe systems (GrowSafe Systems Ltd., Airdrie, AB, Canada) to monitor individual feed intake, ADG and residual feed intake (RFI). The rest of the herd was group-housed in another pen and weighed at the start and end of the finishing period. Steers were housed at ≥ 11 m^2/head stocking density and had unlimited access to feed and clean water. The ingredient and nutrient compositions of the diets as well as feeding duration are shown in Table 5.1.1. The herd ADG was determined as the difference between the final and initial LW divided by the number of days between weighings. Three steers were omitted from the GrowSafe data analysis due to insufficient number of valid data points (less than 90 days) (Wang et al., 2006).

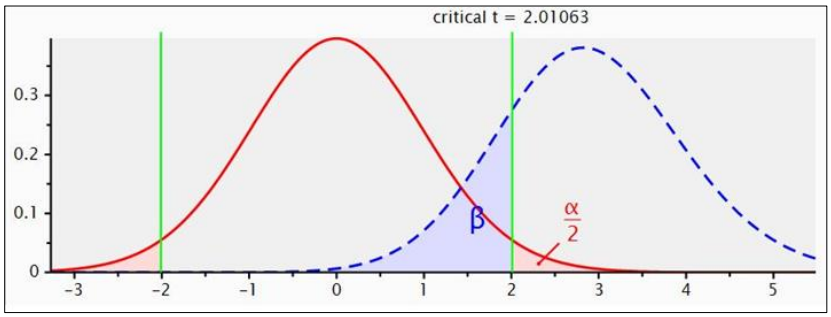


Figure 5.1.1. G-Power analysis for statistical power, critical t-value and sample size.

The management and transport procedures of steers followed the approved Meat Standards Australia (MSA) protocols (MLA, 2020b). Steers were slaughtered within 48 h of leaving the feedlot with a lairage period not exceeding 12 h. Carcasses were graded according to the AUS-MEAT and MSA grading standards (Meat Standards Australia, 2020). The recorded carcass traits included hot standard carcass weight (HCW), hump height, ossification, marbling, subcutaneous rib fat, rump fat at the P8 site, ultimate pH, loin temperature, EMA, fat colour, meat colour and grade code. Carcass assessment was carried out at the 12th rib 12 h after slaughter (Table 5.1.2). Dressing percentage was calculated as: Dressing percentage = (hot carcass weight / LW) x 100. The MSA Index was calculated as the sum of the predicted eating quality scores for 39 MSA cuts weighted by their relative proportion of total carcass weight (McGilchrist et al., 2019).

Table 5.1.1. Dietary ingredient and nutrient compositions of the feedlot finishing diets.

Variable	Diet 1	Diet 2
Ingredient, % as fed		
Days fed diet	1 – 10	11 – 110
Steam flaked Barley	0.0	25.5
Steam flaked Sorghum	0.0	12.5
Finisher supplement	0.0	4.5
Molasses	10.0	10.0
Whole cottonseed	0.0	5.0
Canola meal	0.0	7.5
Barley silage	30.0	12.0
Almond hulls	0.0	8.0
Cereal hay	60.0	15.0
Chemical composition		
Crude Protein (% DM)	8.6	14.5
Neutral Detergent Fibre (% DM)	50.7	29.3
Net Energy for Gain (MJ/Kg DM)	2.9	4.6
Net Energy for Maintenance (MJ/Kg DM)	5.3	7.2
Metabolisable Energy (MJ/Kg DM)	8.9	11.2
Ionophore (ppm)	0.0	19.7

Table 5.1.2. Chiller assessment of carcass traits

Variable	Score range
Hump height	Hump height is measured to determine the tropical breed content of a carcass
Ossification	Ossification is a measure of the physiological maturity of the carcass but can also increase with nutritional or health stress. It is assessed visually and is measured on a scale of 100 to 590, with higher scores indicating greater maturity and poorer eating quality.
Marbling	Assessment of marbling is at the loin eye muscle using the AUS-MEAT and MSA marbling reference standards, and it indicates the level of intramuscular fat content. Aus-marbling is scored 0 (devoid) to 9 (abundant) while MSA-marbling is scored 100 (devoid) to 1100+ (abundant)
Meat colour	Meat colour is assessed on the chilled carcass at the bloomed loin eye muscle against the AUS-MEAT colour reference standards from 1A (light) to 7 (dark)
Fat colour	Scored against the AUS-MEAT fat colour reference standards ranging from 0 (light) to 9 (dark)
Grade code	0 (all MSA specifications are met) or 1-9 (carcass does not meet all the MSA specifications)

5.1.2.2 Statistical analysis

Data were analysed using the SAS software version 9.4 (SAS Institute, Cary, North Carolina, USA). Preliminary data screening was carried out to check for data entry errors, outliers and data distribution. Data were analysed using the generalized linear mixed model in PROC GLIMMIX procedure with restricted maximum likelihood (REML) estimation technique. Backgrounding diet was fitted as the fixed effect, animal nested within backgrounding diet as a random effect and DMI, LW, ADG, RFI, feed to gain ratio and carcass variables as the dependent variables. *P* value was set at 0.05. Spearman's ρ correlation coefficients were computed using the PROC CORR

procedure to examine the correlation between feedlot performance and carcass variables. Thirteen steers were excluded from the analysis due to loss of electronic identification tags.

5.1.3. Results

5.1.3.1 Feedlot performance

Growth performance, DMI and feed efficiency data are presented in Table 5.1.3. The ADG of steers varied significantly (1.7 and 1.8 kg/day for the buffel and buffel-desmanthus groups, respectively), throughout the finishing period ($P < 0.01$). However, this difference did not reach statistical significance when the Growsafe data were analysed separately ($P = 0.48$). The initial LW, final LW, DMI, RFI and feed to gain ratios did not differ between the two groups ($P \geq 0.11$).

Table 5.1.3. Mean feedlot growth performance and feed intake

Variable	Buffel	Buffel-desmanthus	SEM[1]	*P*-value
Whole herd				
Initial liveweight (kg)	415.9	411.2	1.45	0.37
Final liveweight (kg)	610.8	613.6	2.43	0.50
Average daily gain (kg/day)	1.7	1.8	0.01	< 0.01
GrowSafe steers[50]				
Initial liveweight (kg)	406.2	394.5	3.69	0.11
Final liveweight (kg)	600.1	593.4	6.19	0.81
Average daily gain (kg/day)	1.7	1.8	0.03	0.48
Dry matter intake (kg/day)	9.6	9.7	0.15	0.64
Residual feed intake (kg DMI/day)	-0.1	0.1	0.08	0.50
Feed to gain ratio	5.4	5.3	0.07	0.68

[1]SEM, standard error of the mean

[50]Variable measured on the 50 steers with access to the GrowSafe system.

5.1.3.2. Carcass traits

All measured carcass traits were similar for steers backgrounded on either pasture type ($P \geq 0.31$; Table 5.1.4 and Figures 5.1.2 and 5.1.3). Carcass fat colour was light (score 0) for all carcasses except for 1% of the carcasses from both groups which were darker at score 2 ($P = 0.97$). The meat colour was light with majority of the carcasses (95% and 92% of the buffel and buffel-desmanthus steers, respectively) scoring between 1 and 3, and only 1% from both groups scored 5 (Figure 5.2.2; $P = 0.57$). All carcasses met the MSA grade code 0 except for 3% of the carcasses from steers on buffel grass only and 8% of the steers on buffel-desmanthus pastures, primarily due to high ultimate pH above 5.7 (score 4) and minimal subcutaneous rib fat below 3 mm (score 1) in 1% of the carcasses from steers backgrounded on buffel grass only (Figure 5.1.3; $P = 0.85$). All carcasses with grade scores of 4 had meat colour scores ranging between 3 and 5. The HCW were 343.8 kg and 345.6 kg for the buffel grass only and buffel-desmanthus backgrounded steers, respectively ($P = 0.58$).

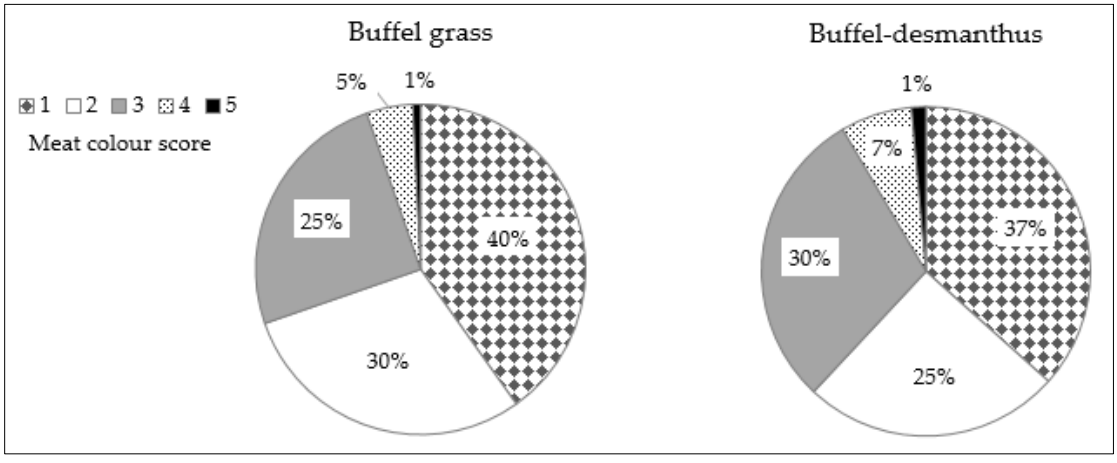


Figure 5.1.2. Effect of backgrounding pasture on steer meat colour score ($P = 0.57$)

Table 5.1.4. Mean carcass traits of feedlot finished steers after backgrounding on buffel or buffel-desmanthus pastures.

Variable	Buffel grass	Buffel-desmanthus	SEM[1]	*P*-value
Hot carcass weight (kg)	343.8	345.6	1.39	0.58
Dressing percentage (%)	57.1	57.0	0.17	0.47
P8 (Rump) fat (mm)	15.8	16.0	0.37	0.87
Hump height (mm)	114.9	115.7	0.82	0.18
Loin eye muscle area (cm^2)	87.5	87.5	0.43	0.98
Ossification score	179.2	177.8	1.78	0.69
Aus-marbling score	1.1	1.0	0.03	0.54
Msa-marbling score	347.4	343.1	4.47	0.45
Subcutaneous rib fat (mm)	13.7	14.0	0.37	0.77
Ultimate pH	5.6	5.6	25.56[2]	0.31
Loin Temperature (°C)	5.8	5.7	0.07	0.91
MSA index	50.7	50.6	0.13	0.57

[1]SEM, standard error of the mean

[2]Expressed as H ions concentration in nEq/L

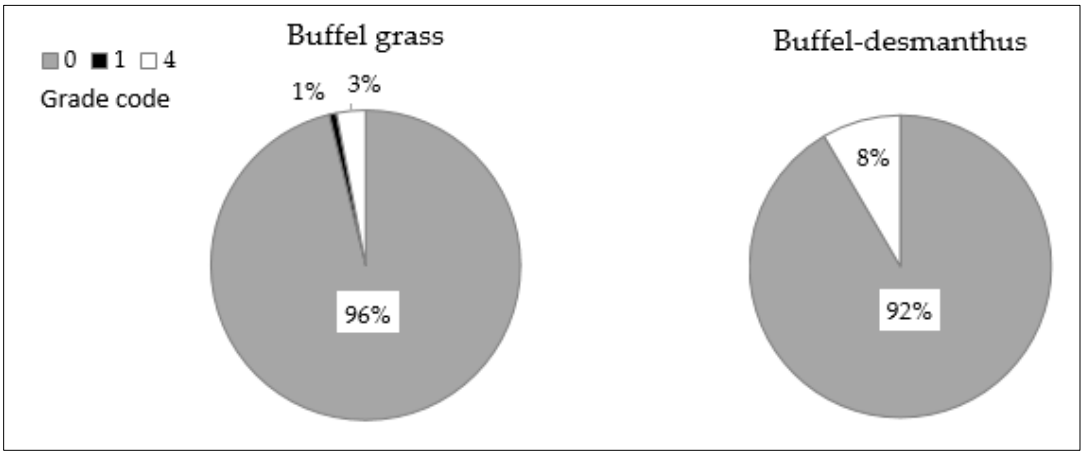


Figure 5.1.3. Effect of backgrounding pasture on steer carcass grade ($P = 0.85$)

5.1.3.3. Effect of feedlot growth performance on carcass traits

The effect of feedlot growth performance on carcass traits is presented in Table 5.1.5. Initial LW was negatively correlated with meat colour but positively correlated with loin temperature ($P < 0.05$). Final LW on the other hand, was positively correlated with hot carcass weight and EMA ($P < 0.01$), but negatively correlated with dressing percentage and meat colour score ($P < 0.05$). Both ADG and DMI were negatively correlated with EMA, while DMI had a negative correlation with meat colour and a positive correlation with loin temperature ($P < 0.05$). The RFI was positively correlated with loin temperature and grade code, while feed to gain ratio had a positive correlation with loin temperature only ($P < 0.05$). The P8 fat, hump height, ossification, marbling score, subcutaneous rib fat, ultimate pH and MSA index had no significant correlation with feedlot growth performance and feed efficiency parameters ($P > 0.05$).

Table 5.1.5. Correlation coefficients of feedlot performance parameters with carcass traits

	Initial LW[1]	Final LW	ADG	DMI	RFI	F:G
Hot carcass weight (kg)	−0.02	0.46**	0.11	0.02	−0.05	−0.2
Dressing percentage (%)	−0.02	−0.35*	0.02	0.05	0.08	0.00
P8 fat (mm)	0.14	0.06	0.11	0.09	−0.06	−0.08
Hump height (mm)	0.19	−0.02	0.01	0.13	−0.02	0.10
Loin eye muscle area (cm^2)	−0.15	0.39**	−0.29*	−0.33*	−0.14	−0.03
Ossification score	−0.03	0.01	0.19	0.07	−0.01	−0.18
Marbling score	0.04	−0.18	0.05	0.10	0.09	0.07
Meat colour score	−0.37*	−0.34*	−0.17	−0.34*	−0.08	−0.09
Subcutaneous rib fat (mm)	0.14	0.06	0.11	0.09	−0.06	−0.08
Ultimate pH	0.14	0.19	0.12	0.02	−0.19	−0.11
Loin Temperature (°C)	0.31*	0.18	−0.02	0.35*	0.31*	0.38**
Grade code	0.08	−0.27	0.00	0.19	0.29*	0.25
MSA index	−0.06	0.06	−0.10	0.03	0.19	0.14

[1]LW, liveweight; ADG, average daily gain; DMI, dry matter intake; RFI, residual feed intake; F:G, feed to gain ratio

* = $P < 0.05$, ** = $P < 0.01$

5.1.4. Discussion

5.1.4.1 Feedlot performance

The type of backgrounding pasture had no effect on feedlot DMI, final LW, and feed to gain ratio, although buffel-desmanthus backgrounded steers gained 100 g/day more than the steers backgrounded on buffel grass only. Studies that had demonstrated a significant effect of backgrounding weight gain on subsequent feed intake and feedlot growth performance (Peripolli et al., 2018) attributed the variation to compensatory gain and differences in feedlot entry LW. A review by Reuter and Beck (2014) reported that the ADG and DMI of finishing yearling cattle decreased as the backgrounding ADG increased. Steers with low backgrounding ADG of 0.23 kg/day were reported to have higher finishing ADG than steers with higher backgrounding ADG of 0.68 kg/day (Neel et al., 2007). Cattle fed restricted-intake diets during the backgrounding phase were reported to have higher feed intake and feed conversion ratio during the finishing period compared to those with *ad libitum* access to feed (Loerch, 1990). In contrast, Loken et al. (2009) reported similar finishing DMI, ADG and feed to gain ratio in beef steers fed to attain low (1.4 kg/day) or high (1.6 kg/day) weight gain during the backgrounding period. In the current study, the ADG during backgrounding was similar for steers on either pasture type (0.74 to 0.75 kg/day) (Chapter 4.1), hence the similar DMI, feed to gain ratio and final LW were expected.

The RFI did not differ significantly between the buffel-desmanthus and the buffel grass only backgrounded steers. A review by Kenny et al. (2018) reported that RFI of young growing beef cattle is influenced by diverse factors such as body composition, feeding behaviour and activity, intestinal cellularity and absorption, mitochondrial function and appetite regulation. These factors may be influenced by individual animal variability, with RFI reported to be moderately heritable

($h^2 \approx 0.33$) in dairy and beef cattle (Berry and Crowley, 2013). The lack of difference in this study may indicate that there were no major individual animal variabilities between animals in both groups. The RFI reported in the present study (–0.1 and 0.1 kg DMI/day) are within the values reported for Brahman (–0.1 ± 1.06) and tropical composite cattle (0.1 ± 1.17) (Barwick et al., 2009).

5.1.4.2 Carcass traits

All measured carcass traits were similar in both treatment groups. This may be due to similar growth performance during the backgrounding and finishing phases that resulted in similar final LW. A review by Reuter and Beck (2014) reported that cattle finishing body weight accounted for 27 – 70%, 9% and 22% of the variation in hot carcass weight, dressing percentage and loin eye area, respectively. *Bos indicus* breeds of cattle tend to produce less tender meat than the *Bos taurus* breeds due to lower proteolysis of myofibrillar proteins resulting from the high calcium-dependent protease inhibitor activity (Pringle et al., 1997). Genetics account for 30 to 50% of the variation in beef tenderness within breeds (Špehar et al., 2008), thus the need to determine the tropical breed content of a carcass. The similar hump height observed in this study indicates that tropical breed content did not vary between steers backgrounded on either pasture type. The similar ossification also indicates that carcasses from both groups did not differ in physiological maturity, a trait that significantly influences meat tenderness, flavour intensity, juiciness and acceptability scores (Špehar et al., 2008; Kopuzlu et al., 2018).

Carcass subcutaneous and IMF significantly influence meat eating quality. An increase in marbling is associated with increased tenderness due to reduced connective tissue rigidity (Nishimura et al., 1999; Špehar et al., 2008). Marbling is also positively correlated with juiciness, tenderness and

overall liking of beef (Hunt et al., 2014; Park et al., 2018; Wheeler et al., 1994). The Aus-marbling score in the present study was 1.1 and 1.0 for carcasses from steers on buffel grass only and buffel-desmanthus pastures, respectively. Bindon (2004) reported that the Aus-marbling score of 1 represents 3% intramuscular fat content in tropically adapted beef cattle breeds. This indicates that finishing steers backgrounded on buffel only or buffel-desmanthus steers in the feedlot resulted in intramuscular fat content within the levels required to meet the consumer-preferred overall beef palatability of 3 to 7% (Smith, 2016). Increased subcutaneous fat thickness is reported to improve tenderness due to reduced carcass chilling rate and increased enzyme activity that prevents cold shortening (Smith et al., 1976). Savell et al. (2005) recommended a minimum subcutaneous fat depth of 6.2 mm at the 12th rib to prevent cold shortening in cattle. In the current study, 13.7 – 14.0 mm fat depth was achieved in the carcasses of steers backgrounded on either pasture type.

Post-mortem glycogen is normally converted to lactate, resulting in muscle pH decline (Henckel et al., 2002). The muscle pH level in cattle usually declines from 7.0 at slaughter to approximately 5.4 – 5.6 within 18 to 40 h post-slaughter (Savell et al., 2005). The lack of difference in the average ultimate pH level in carcasses from steers backgrounded on either pasture type in the present study may indicate that there was no difference in muscle glycogen levels. All carcasses with a grade score of 4 (ultimate pH > 5.7) had darker meat colour with scores ranging between 3 and 5, while carcasses with ultimate pH of 5.7 and below had meat colour scores of 1 to 3. These results concur with the findings of Matarneh et al. (2017) that meat with ultimate pH of ≥ 6.0 appears darker in colour than pale meat of pH 5.4. Insufficient glycogen leads to premature termination of post-mortem metabolism resulting in ultimate pH > 6.0 and darker meat (Matarneh et al., 2017). Post-mortem pH decline coincides with muscle fibre diameter reduction, increase in extracellular space, myofibrillar shrinkage and drip formation. These muscle structural changes increase the intensity

of light penetrating or being transmitted into the muscle. The light is either absorbed by pigments or scattered by the structural components (Offer and Cousins, 1992; Hughes et al., 2014). Since the deeper transmitted light has higher absorption by muscle pigments and less light scattering (Swatland, 2004), the lower pH muscles appear lighter and the high pH muscles appear darker (Guerrero et al., 2013; Matarneh et al., 2017). In addition, myoglobin is the key pigment that influences meat colour, constituting 80% to 90% of total pigment in a well-bled muscle (Matarneh et al., 2017). The pH decline after slaughter gradually inhibits mitochondrial activity that results in increased oxymyoglobin levels that cause darker meat colouration (Zhang et al., 2018).

A rapid pH decline at higher temperatures contributes to protein denaturation and reduced solubility, resulting in reduced water-holding capacity and high drip losses. Denaturation of the myosin heads with falling pH at high temperatures also provides a shrinkage force resulting in heat shortening. On the contrary, a rapid temperature decline at high pH results in cold shortening (Hughes et al.; Thompson, 2002). Therefore, pH is commonly regarded as an indicator of fresh meat quality (Matarneh et al., 2017), with MSA developed pH/temperature window stipulating that pH should be above 6.0 at temperatures above 35 °C, and below 6.0 before the temperature falls below 12°C (Thompson, 2002). In the present study, the average pH 24 h post-mortem was 5.6 at loin temperatures of 5.8 and 5.7 for the carcasses from buffel grass only and buffel-desmanthus backgrounded steers, respectively. Although there were carcasses with ultimate pH levels above 5.7 which may indicate dark cutting (McKeith et al., 2016), the proportion (3% buffel and 8% buffel-desmanthus steers) was lower than that reported by Węglarz (2010) for cattle slaughtered at a similar weight (80%).

Since meat appearance influences customer decisions to purchase meat (Špehar et al., 2008), the minimum AUS-MEAT standard specifications for 100-day feedlot-finished cattle are 7 mm

subcutaneous rib fat depth, 1 – 3 meat colour score and 0 – 3 fat colour score (Meat Standards Australia, 2018). These specifications were met by the subcutaneous rib fat depth (13.7 – 14.0 mm) and fat colour (0 – 2), while only 6% and 8% of carcasses from the buffel grass only and buffel-desmanthus steers, respectively, failed to meet the 1 – 3 meat colour score in this study. The 4 – 8% MSA index non-compliance level observed in this study was within the level (4 – 9%) reported for cattle produced in Queensland and slaughtered between July 2019 and June 2020 (MLA, 2020c). These findings indicate that backgrounding steers on desmanthus did not have a negative impact on carcass quality. Therefore, desmanthus can be used to background beef cattle in northern Australia vertosol soil regions where there is a paucity of adapted pasture legumes (Pengelly and Conway, 2000), with no adverse effect on carcass quality.

5.1.4.3 Effect of feedlot growth performance on carcass traits

Measuring the response of individual animals may uncover valuable details compared with group means for traits with limited prediction ability such as feed efficiency and carcass quality (Reuter and Beck, 2014). In this study, RFI had no correlation with HCW and EMA. In contrast, highly feed efficient crossbred steers had higher HCW and EMA than less efficient steers (Russell et al., 2016). This may be due to the higher final LW of the highly efficient steers compared to this study where the final LW was similar between the two groups. The observed correlation between final LW with HCW, dressing percentage and EMA agrees with previous studies. Coyne et al. (2019) reported medium to strong correlations between final LW and carcass weight (0.25 – 0.92) in bulls and steers. Body weight was reported to account for 27 –70%, 9% and 22% variation in hot carcass weight, dressing percentage and EMA, respectively, in cattle (Reuter and Beck, 2014). Nogalski et al. (2014) also reported an increase in carcass dressing percentage with an increase in slaughter

weight in Polish Holstein Friesian and Limousin crossbred steers. The authors associated the difference with an increase in carcass fatness as slaughter weight increased. In this study, carcass fatness did not increase with final LW as indicated by the non-significant correlation coefficients. Contrary to the positive correlations in previous studies (Reuter and Beck, 2014; Coyne et al., 2019), the correlation between final LW and dressing percentage in this study was negative. Dressing percentage is a product of many factors that include LW, fatness, time off feed and water, sex and breed of the animal (Warmington and Kirton, 1990). Steers in this study were of the same composite breed, managed and transported together, therefore the time off feed and water, sex and breed did not influence HCW. The difference may be due to differences in gut fill during the final weighing as the steers were not fasted prior to weighing.

The negative correlation between final LW and meat colour score agrees with previous studies that reported a decline in meat lightness with an increase in slaughter weight (Teixeira et al., 2011; Majdoub-Mathlouthi et al., 2013). This may indicate a higher physical activity of steers with lower final LW compared to heavier steers. Liveweight is recognized as an important factor influencing social ranking in cattle where heavier individuals occupy higher dominance rank compared to the lighter individuals (Bouissou, 1972; Sołtysiak and Nogalski, 2010). Even in group housing with no restrictions on feed accessing, dominant animals displace the subordinates from the feeder in order to display their feeding patterns preference, while the subordinates may need to adjust their feeding behaviour to accommodate the more dominant individuals and avoid conflict (Llonch et al., 2018). The avoidance behaviour may greatly reduce eating and resting times, consequently increasing physical activity (Bouissou, 1980). Animals with higher physical activity tend to have darker meat due to an increase in muscle pigment as a result of high oxygen demand (Ekiz et al., 2019). Loin temperature was moderately correlated with initial LW (0.31), but the correlation with

final LW (0.18) was not significant. This observation is in tandem with large carcasses known to have a slower chilling rate compared to smaller carcasses (Ockerman and Basu, 2014). Since final LW was positively correlated with HCW, it is reasonable to assume that large bodied steers had large carcasses that reduced chilling rate and subsequently led to higher loin temperature 24 h post-mortem.

There is increased interest to produce meat with higher levels of the health beneficial long chain omega-3 polyunsaturated fatty acids and less saturated fatty acids in the intramuscular fat (Woollett et al., 1992; Krauss and Kris-Etherton, 2020). Meat fatty acid composition is influenced by many factors such as animal age, diet and genetic factors (De Smet et al., 2004; French et al., 2000; Hwang et al., 2018). In addition, several candidate genes are reported to influence carcass traits in cattle (Grigoletto et al., 2020). For instance, genetic polymorphisms in the fatty acid binding protein 4 (*FABP4*) are reported to influence marbling score and subcutaneous fat depth in Wagyu x Limousin F2 crosses (Michal et al., 2006) and marbling score in Hanwoo cattle (Lee et al., 2010). Therefore, there is the need for further studies investigating the fatty acid composition of meat from desmanthus backgrounded beef cattle and any associated interactions with backgrounding forage type and single nucleotide polymorphisms of fat metabolism related genes.

5.1.5. Conclusions

This study evaluated the feedlot growth performance and carcass quality of steers backgrounded on buffel grass only pastures or buffel grass oversown with desmanthus. The results showed no difference in final LW, DMI and carcass quality. Hence the hypothesis that tropical beef cattle steers backgrounded on buffel grass only pastures or buffel grass oversown with desmanthus with similar backgrounding growth performance would have similar feedlot growth performance and

carcass quality was accepted. The MSA index compliance level observed in this study was within the level reported for cattle produced in Queensland and slaughtered between July 2019 and June 2020. These findings indicate that backgrounding steers on desmanthus did not cause any adverse effect on carcass quality, thus desmanthus can be used to background beef cattle in northern Australia vertosol soil regions where there is a paucity of adapted pasture legumes.

5.1.6. Summary

Feedlot performance and carcass traits of tropical beef steers backgrounded on buffel grass (*Cenchrus ciliaris*) only or buffel grass oversown with desmanthus (11.5% initial sward botanical composition) were evaluated. It was hypothesised that tropical beef cattle steers backgrounded on buffel grass only or buffel grass oversown with desmanthus with similar backgrounding growth performance would not differ in feedlot growth performance and carcass quality. Three hundred and twelve *Bos indicus* x *Bos taurus* tropical composite steers, 20 – 23 months old and weighing 413 ± 24 kg, previously backgrounded on buffel grass only or buffel-desmanthus mixed pastures for 147 days were finished on a concentrate diet in the feedlot for 110 days before slaughter. Buffel-desmanthus backgrounded steers had slightly higher average daily gain (1.8 kg/day) than the buffel grass backgrounded steers that had 1.7 kg/day ($P < 0.01$). However, final liveweight and dry matter intake were not significantly different ($P \geq 0.50$). All the carcass traits measured were not significantly different ($P \geq 0.18$). Only 4% buffel grass and 8% buffel-desmanthus backgrounded steers fell short of the Meat Standards Australia (MSA) index, a level that is within the 4 – 9% reported for cattle produced in Queensland and slaughtered between July 2019 and June 2020. These findings indicate that desmanthus can be used to background beef cattle in northern Australia vertosol soil regions, where there is a paucity of adapted pasture legumes, with

no negative impact on feedlot performance and carcass quality. The hypothesis that tropical beef cattle steers backgrounded on buffel grass only pastures or buffel grass oversown with desmanthus with similar backgrounding growth performance would have similar feedlot growth performance and carcass quality was accepted.

Chapter 5.2: Carcass Traits and *Longissimus dorsi* Muscle Fatty Acid Composition of Tropical Crossbred Beef Cattle in Response to *Desmanthus* spp. Forage Backgrounding

5.2.1. Introduction

Beef is the third most consumed meat in the world at 6.3 kg per capita after poultry and pork at 15.1 kg and 11.8 kg per capita, respectively (OECD, 2021). World total meat production in 2020 from ovine, bovine, poultry, pig and other animals was estimated at 337.2 million tonnes carcass weight equivalent, 62% of which was produced in Brazil, Australia, the USA, the EU, China, India and Argentina (Food and Agriculture Organization of the United Nations, 2021). Therefore, meat is a significant global source of high quality protein, dietary lipids, minerals and B vitamins (Cabrera and Saadoun, 2014). As a result of the 2016 controversial epidemiological suggestion of red meat consumption being linked to increased risks of cancer, cardiovascular disease, obesity and diabetes by Troy et al. (2016), the recommendations by the American Heart Association (Arnett et al., 2019) and the World Health Organization (Bouvard et al., 2015) to reduce red meat consumption have been vigorously challenged by the Nutritional Recommendations Consortium (Johnston et al., 2019). Saturated fatty acids (SFA) have been reported to increase plasma low density lipoprotein cholesterol levels (Woollett et al., 1992), while other researchers reported an inverse relationship between SFA intake and the incidence of stroke (Krauss and Kris-Etherton, 2020). In some population-based studies (Van Vliet et al., 2021), the dietary intake of conjugated linoleic acid (CLA) has been inversely linked to the risk of breast cancer and colorectal cancer. In contrast, prospective cohort studies consistently support the role of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the primary prevention of atherosclerosis and cardiovascular

disease (Schulze et al., 2020) and a reduction in the omega-6/omega-3 (n-6/n-3) ratio known to be associated with a highly reduced risk of obesity (Simopoulos, 2016). In addition to health benefits, carcass fat influences meat quality and palatability by influencing meat tenderness, shelf-life, juiciness, flavour and market value (Wood et al., 2003).

Diet has a significant effect on meat quality (Hwang et al., 2018). Whereas numerous studies exist on increasing meat n-3 PUFA composition through dietary supplementation with fish oil, fish meal and vegetable oils (Girard et al., 2016; Flakemore et al., 2017a; Van Le et al., 2019), similar cattle supplementation studies in the extensive grazing systems of northern Australia are scanty, cost being a significant limiting factor (Neves et al., 2018). Forages contain a high proportion of total fatty acid as ALA, which is the building block of the longer chain (≥ C20) n-3 PUFA through elongation and desaturation (Scollan et al., 2006; Scollan et al., 2014). Plant secondary metabolites such as tannins and saponins may modify meat fatty acid composition by modulating rumen fatty acid lipolysis and biohydrogenation (Kronberg et al., 2007; Alves et al., 2017). For instance, the intramuscular fat (IMF) of lambs fed tannin-containing birdsfoot trefoil (*Lotus corniculatus*) and sainfoin (*Onobrychis viciifolia*) were shown to contain less SFA and more PUFA compared to those fed lucerne or red clover (Girard et al., 2016), and supplementing growing lambs with lucerne saponins at 0.05 – 0.2% DM for 90 days significantly increased muscle n-3 PUFA (Liu et al., 2021). These findings necessitate the need to study the effect of supplementing beef cattle with forage of the genus *Desmanthus* (desmanthus), a tannin-containing and vigorous tropical legume that is environmentally well-adapted to the harsh tropical and subtropical conditions of northern Australia (Schlink and Burt, 1993), on meat fatty acid composition. Desmanthus is a highly productive forage legume that thrives with as low as 500 mm annual rainfall, withstands high

grazing pressure and has been shown to reduce enteric methane emission in cattle fed a low-quality grass diet (Suybeng et al., 2019; Suybeng et al., 2020).

Backgrounding cattle prior to feedlot finishing with energy-dense diets is commonly practiced in order to meet the carcass specifications of premium beef markets. Approximately half of the beef cattle herd produced in northern Australia (DAF, 2018), the Mediterranean countries (Blanco et al., 2010) and 85% in the USA are finished in the feedlot (Kurve et al., 2016) for improved subcutaneous fat depth (Blanco et al., 2010) and IMF content that markedly increase flavour, tenderness and juiciness (Scollan et al., 2006). By the same token, finishing cattle on concentrate diets also has a tendency to increase meat SFA and reduce the health beneficial unsaturated fatty acid concentration (Yang et al., 1999; Woods and Fearon, 2009). A thorough search of the relevant literature indicated that no peer-reviewed literature exists on the intramuscular fatty acid composition of tropical crossbred beef cattle backgrounded on desmanthus forage. Therefore, this study aimed to fill this knowledge gap by evaluating the feedlot performance, carcass traits, IMF, fat melting point (FMP) and fatty acid composition of the loin eye muscle of tropical crossbred beef steers backgrounded on desmanthus forage with or without feedlot finishing. It was hypothesised that steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce similar carcass traits and fatty acid composition.

5.2.2. Materials and Methods

The backgrounding phase of this study took place at the CSIRO Lansdown Research Station, Queensland, Australia, between March and July 2020, while the finishing phase was carried out at a commercial feedlot 17 km from the research station from August to October 2020. The mean monthly minimum and maximum temperatures and rainfall were 15.5 °C, 24.9 °C and 26.0 mm,

respectively, while average minimum and maximum relative humidity were 55 and 65% during the experimental period. All procedures in this study were carried out according to the CSIRO Animal Ethics Committee approved guidelines (approval number 2019-38) and the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013).

5.2.2.1. Animals, diets and experimental design

Sample size determination, animal management and treatments are described in detail in Chapter 4.2. In brief, 48 Brahman, Charbray and Droughtmaster crossbred steers were backgrounded on Rhodes grass hay supplemented with incremental proportions of freshly cut desmanthus for 140 days in a completely randomised design. Desmanthus accounted for 0, 15%, 30% or 45% DM, and varying proportions of lucerne hay were added to the 0, 15%, 30% desmanthus diets to ensure that the diets were isonitrogenous to the 45% desmanthus diet (Table 5.2.1). Steers aged 28 – 33 months old weighed 332 ± 21 kg and 429 ± 31 kg at the start and end of the backgrounding phase, respectively, and they were group-housed in 12 outdoor pens with four steers in each pen and three pens per treatment. Each pen measured 60 m^2 and was fitted with 18 m^2 shade, water trough and 4 m by 1 m feed trough. The pen boundaries were portable metallic panels, and the floor was made of roadbase grade stone topped with crusher dust compacted and covered with soil. At the end of the backgrounding phase steers were separated into two groups based on liveweight. The two heaviest (453 ± 15 kg) steers per pen were slaughtered without finishing, whereas the other two steers (406 ± 25 kg) were transferred and fed at a commercial feedlot in accordance with the standard feedlot finishing rations. During the finishing phase, steers were housed in one outdoor pen allowing 11 m^2/head stocking density and were allowed unlimited access to clean water and

feed. The feedlot finishing phase lasted for 95 days following the 70 – 100 days finishing phase commonly practiced in Australia (Poppi et al., 2018). After finishing, steers were transported to a nearby commercial abattoir for slaughter and graded according to AUS-MEAT standards as described by the Agriculture and Resource Management Council of Australia and New Zealand (2001).

Table 5.2.1. Diet chemical composition, steers DMI and growth performance during backgrounding.

	Desmanthus Proportion in the Diet (%)			
Variable[1]	0	15	30	45
DM (%)	87.3	68.4	56.9	48.6
CP	11.6	11.6	11.5	11.4
NDF	65.2	64.9	64.6	64.4
ADF	40.1	40.4	40.8	41.1
Hemicellulose	25.1	24.4	23.8	23.3
ME (MJ/kg DM)[2]	8.1	7.9	7.6	7.4
DMI (kg/head)	8.8	8.5	8.2	7.6
Initial LW (kg)	332.0	330.2	332.7	332.8
Final LW (kg)	434.1	437.8	427.5	420.1
ADG (kg/day)	0.63	0.66	0.57	0.53

[1]CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ME, metabolisable energy; DMI. Dry matter intake; ADG, average daily gain; LW, liveweight. Data are presented in percentage of dry matter (DM) unless otherwise stated. [2]Estimated from in vitro dry matter digestibility as DMD × 0.172 − 1.707 (CSIRO, 2007).

Table 5.2.2. Fatty acid composition (% of total fatty acids) of the Rhodes grass, lucerne and desmanthus forages.

Variable[1]	Forage[2]					
	Rhodes Grass	Lucerne	JCU2	JCU4	JCU7	Desmanthus
C14:0	0.6	0.6	0.3	0.3	0.3	0.3
C15:0	0.9	1.6	0.4	0.3	0.5	0.4
C16:0	31.3	26.4	20.5	20.2	20.1	20.3
C16:1	2.8	3.9	3.2	2.7	2.7	2.9
C17:0	2.5	1.7	1.1	1.1	1.2	1.1
C17:1	0.3	0.2	0.2	0.1	0.2	0.2
C18:0	4.0	5.5	5.1	5.1	4.9	5.0
C18:1	3.3	4.0	5.3	5.4	6.4	5.7
C18:2n-6 (LA)	14.5	16.4	18.8	20.8	21.5	20.4
C18:3n-3 (ALA)	25.6	26.5	36.9	34.4	33.4	34.9
C18:3n-6	1.9	1.8	0.9	0.7	0.8	0.8
CLA	0.3	0.6	0.1	0.4	0.2	0.2
C19:1	0.0	0.2	0.4	0.2	0.1	0.2
C20:0	1.5	1.4	1.1	1.1	0.8	1.0
C20:1	0.6	0.7	0.4	0.6	0.4	0.5
C20:2n-6	0.0	0.1	0.1	0.1	0.1	0.1
C20:3	0.6	0.6	0.2	0.2	0.3	0.2
C20:4n-3	0.5	0.5	0.2	0.2	0.2	0.2
C20:5n-3 (EPA)	0.1	0.1	0.0	0.0	0.0	0.0
C21:0	0.4	0.5	0.4	0.4	0.4	0.4
C21:5n-3	0.2	0.3	0.1	0.1	0.1	0.1
C22:0	2.4	1.8	1.5	1.8	1.9	1.7
C22:1	0.3	0.9	0.1	0.4	0.2	0.2
C22:4n-6	0.0	0.1	0.1	0.2	0.1	0.1
C22:5n-3 (DPA)	0.9	0.2	0.0	0.0	0.0	0.0
C22:5n-6	0.0	0.2	0.0	0.1	0.0	0.0

C22:6n-3 (DHA)	0.5	0.3	0.2	0.3	0.1	0.2
C23:0	0.7	0.7	0.4	0.4	0.6	0.5
C24:0	2.6	1.8	1.9	2.0	2.6	2.2
C24:1	0.3	0.2	0.2	0.1	0.1	0.1
EPA + DHA	0.6	0.4	0.2	0.3	0.1	0.2
EPA + DPA + DHA	1.5	0.6	0.2	0.3	0.1	0.2
∑SFA	47.1	42.1	32.7	32.8	33.2	32.9
∑MUFA	7.7	10.1	9.7	9.6	10.1	9.8
∑PUFA	45.2	47.8	57.6	57.6	56.7	57.3
∑n-3 PUFA	27.9	27.9	37.4	35.1	33.8	35.4
∑n-6 PUFA	16.4	18.7	19.9	21.9	22.5	21.4
PUFA/SFA	1.0	1.1	1.8	1.8	1.7	1.8
n-6/n-3 PUFA	0.6	0.7	0.5	0.6	0.7	0.6

[1]IMF, intramuscular fat; FMP, fat melting point; CLA, conjugated linoleic acid; LA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; Total FA, sum of all fatty acids measured; ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids. ΣSFA = 14:0 + 15:0 + 16:0 +17:0 +18:0 + 20:0 + 21:0; ΣMUFA = 14:1 + 16:1n-13t + 16:1n-9 + 16:1n-7 + 16:1n-7t + 16:1n-5c + 17:1n-8 + a17:0 + 18:1n-7t + 18:1n-5 + 18:1n-7 + 18:1n-9 + 18:1a, + 18:1b + 18:1c + 19:1a + 19:1b + 20:1n-11 + 20:1n-9 + 20:1n-7 + 20:1n-5 + 22:1n-9 + 22:1n-11 + 24:1n-9; ΣPUFA = 18:4n-3 + 18:3n-6 + 18:2n-6 + 18:3n-3 + 20:2n-6 + 20:3 + 20:3n-6 + 20:4n-3 + 20:4n-6 +20:5n-3 + 22:4n-6 + 22:5n-3 + 22:5n-6 + 22:6n-3; Σn-3 PUFA = 18:3n-3 + 18:4n-3 + 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3; Σn-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6. [2] JCU2, *D. virgatus*; JCU4, *D. bicornutus*; JCU7, *D. leptophyllus*; desmanthus, average of the three desmanthus cultivars.

5.2.2.2. Loin eye muscle biopsy and carcass sampling

To determine the IMF, FMP and fatty acid composition, biopsy samples were collected from the loin eye muscle at the start and end of the backgrounding phase from the 12th – 13th rib interface based on the procedure described by Malau-Aduli et al. (1998). In summary, steers were restrained

in a crush, and the hair at and around the 12th – 13th rib interface was clipped. The clipped area was prepared aseptically and desensitised with local anaesthetic (Ilium Lignocaine 20 ®, Troy Animal Healthcare, Glendenning, NSW, Australia), and 3 g of muscle was taken. The incision was closed using absorbable monofilament suture material. Steers were prophylactically treated with Depocillin® (MSD Animal Health, East Bendigo, Victoria, Australia) both rounds and Metacam ® 20 mg/mL Solution for Injection (Boehringer Ingelheim Animal Health, Sydney, Australia Pty Ltd.) the second round of biopsies. Cetrigen ® (Virbac Australia Pty Ltd., Sydney, NSW, Australia) antibacterial wound aerosol and insect repellent was sprayed on and directly around the wound to prevent secondary infection and keep flies at bay. Steers were taken back to their respective pens and monitored twice daily until the wounds healed and no post-operative complications were recorded. Biopsy samples for the baseline analysis were taken from the left side of the animal and on the right side at the end of the backgrounding phase. Samples were placed on dry ice immediately after collection, transported to the laboratory and stored at −20 °C until analysis. For the carcasses, 10 g of the loin eye muscle were collected at the 12th and 13th ribs interface of the chilled carcasses 12 h after slaughter and stored at −20 °C until analysis.

5.2.2.3. Intramuscular fat, fat melting point and fatty acid composition analysis

The IMF content of biopsy and carcass samples was extracted and purified according to the modified method of Folch et al. (1957) as described by Flakemore et al. (2014). The procedure involved muscle sample homogenisation, overnight extraction using $CHCl_3$:MeOH (2:1 *v*/*v*) solvent mixture, phase separation with 5 mL of 10% KCl, manual removal of the upper inorganic layer and heat evaporation using porcelain crucibles to obtain the fat content. The IMF percentage was calculated as:

(Crucible with fat weight (g) − empty crucible weight (g))/sample weight (g) × 100

The FMP was analysed using the slip melting point method (American Oil Chemists' Society, 1998) as described by Pewan et al. (2020). Briefly, fat extracted for IMF content determination was melted in an oven at 100 °C for 1 – 2 min. The melted fat was transferred into capillary tubes and placed in a refrigerator at 4 °C for 10 min to allow the fat to solidify. Fat level was marked with a permanent pen and the capillary tube attached to the thermometer and suspended in a glass beaker with 80 mL deionised H_2O placed on a heating block. The heating block was gradually heated and the fat level closely monitored until fat melted and 'slipped' above the mark. The 'slip point' temperature was recorded as the FMP.

The fatty acid composition of the loin eye muscle samples was analysed using the gas chromatography–mass spectrometry procedure previously reported by Malau-Aduli et al. (2016). In summary, fatty acid analysis procedure included three steps. The first step was the lipid extraction: Total lipids of wet unground 1 g of muscle samples were extracted according to a modified Bligh and Dyer (1959) protocol. The protocol entailed a single-phase overnight extraction with CHCl3:MeOH:H_2O at 1:2:0.8 *v*/*v*, phase separation by addition of CHCl3:saline Milli-Q H_2O at 1:1 *v*/*v* and rotary evaporation of the chloroform phase at 40 °C to obtain total lipids. The second step was methylation: Total lipids aliquots were transmethylated in MeOH:$CHCl_3$:HCl at 10:1:1 *v*/*v* for 2 h at 80 °C. Milli-Q H_2O (1 mL) was added, fatty acid methyl esters extracted with hexane:chloroform at 4:1 *v*/*v* and flushed with nitrogen gas. The final step was the fatty acid quantification: The extracted fatty acid methyl esters were topped up to 1500 µL volume with an internal injection reference standard (19:0). The fatty acid methyl esters analysis was carried out using a 7890B gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent Technologies 7683 B Series autosampler, a split/splitless

injector, EquityTM-1 fused 15 m silica capillary column with 0.1 mm internal diameter and 0.1 µm film thickness (Supelco, Bellefonte, PA, USA) and a flame ionisation detector. The carrier gas was helium and initial oven temperature of 120 °C was increased to 270 °C at 10 °C /min rate and then to 310 °C at 5 °C/min. Fatty acid peaks were quantified using the Agilent Technologies ChemStation software (Palo Alto, CA, USA). The fatty acid identities were confirmed using a GC–mass spectrometric analysis with a Thermo Scientific 1310 GC attached to a TSQ triple quadropole (Thermo Fisher Scientific, Milan, Italy) PTV injector and Thermo Scientific Xcalibur software (Austin, TX, USA). The GC working conditions were as previously reported by Miller et al. (2006). Fatty acid percentages (%FA) and fatty acid contents (FA mg/100 g muscle) were calculated as (Flakemore et al., 2017b):

$$\%FA = (\text{individual fatty acid area}) \times (100)/(\text{sum total area of FA})$$

$$FA\ (mg/100\ g) = \text{Total lipid } (g/100\ g) \times 0.916 \times (\%FA)/100) \times 1000$$

where 0.916 was the lipid conversion factor based on the assumption that beef lipid contain ≈12% phospholipids and ≈1% cholesterol (Clayton, 2014).

5.2.2.4. Statistical Analysis

Data were analysed using the Statistical Analysis System software version 9.4 (SAS Institute, Cary, NC, USA). Initial data screening was carried out by computing summary statistics of means, standard deviations, minimum and maximum values to examine data for entry errors and outliers. Data were analysed by linear mixed model (PROC MIXED) procedure with the fixed effect of backgrounding diet (0, 15%, 30% and 45% desmanthus diets) and pen nested within diet as a random effect to determine the effect of backgrounding diet on IMF, FMP, fatty acid composition

and feedlot growth performance. The same model was used to examine the effect of backgrounding diet, feedlot finishing (feedlot-finished vs. unfinished) and their interactions on carcass traits and fatty acid composition. Baseline measurements of IMF, FMP and fatty acid composition were included as covariates in the model. When the effect of diet was significant ($P < 0.05$), orthogonal polynomial contrasts were performed to test for linear, quadratic and cubic responses to increasing desmanthus proportions. Significant interactions of backgrounding diet and feedlot finishing were separated using the Tukey–Kramer pairwise comparison test. The quadratic and cubic responses were eventually dropped from the model because they were not significant.

5.2.3. Results

5.2.3.1. Intramuscular fat, fat melting point and fatty acid composition

The backgrounding diet did not have a significant influence on the loin eye muscle IMF content, FMP or fatty acid composition ($P \geq 0.20$), with the exception of C22:0 (Docosanoic acid) ($P = 0.04$) and the n-6/n-3 PUFA ratio ($P = 0.01$), which increased linearly with an increase in the proportion of desmanthus in the diet (Table 5.2.3). The steers were lean, with IMF content ranging between 2.1% and 2.6% ($P = 0.50$) and FMP from 43.1 °C to 44.5 °C ($P = 0.94$). Although the desmanthus inclusion numerically increased total fatty acid content, this increase did not reach statistical significance ($P = 0.59$).

Table 5.2.3. Effect of desmanthus supplementation on intramuscular fat, fat melting point and fatty acid composition (least square mean) of the loin eye muscle of tropical crossbred beef cattle.

	Desmanthus proportion in the diet (%)					
Variable[1]	0	15	30	45	SEM[2]	*P*-value
IMF (%)	2.6	2.3	2.1	2.3	0.11	0.50
FMP (°C)	43.1	43.5	43.1	44.5	0.71	0.94
Fatty Acids (mg/100g)						
C13:0	0.2	0.1	0.3	0.1	0.03	0.51
C14:0	9.7	12.8	14.3	15.6	2.11	0.77
C14:1	1.4	2.1	2.1	2.8	0.40	0.69
C15:0	3.5	4.9	4.0	6.0	0.64	0.54
C16:0	156.9	209.9	203.5	215.7	21.83	0.67
C16:1	23.8	28.2	33.5	30.9	3.16	0.70
C17:0	9.4	11.0	12.4	11.7	1.06	0.78
C17:1	9.2	10.3	11.3	10.7	0.96	0.88
C18:0	102.7	135.2	137.1	139.6	11.40	0.60
C18:1 (Oleic acid)	190.0	250.3	288.6	258.8	28.48	0.58
CLA	3.1	4.1	5.4	4.9	0.49	0.48
C18:2n-6 (LA)	44.7	52.8	48.1	54.8	1.47	0.25
C18:3n-3 (ALA)	15.3	17.1	15.8	16.9	0.45	0.47
C18:3n-6	0.4	0.6	0.6	0.6	0.04	0.35
C18:4n-3	2.4	2.6	1.9	2.3	0.11	0.25
C19:1	1.4	1.6	2.1	1.9	0.21	0.64
C20:0	0.8	1.2	1.3	1.3	0.13	0.48
C20:1	1.6	2.2	2.3	2.5	0.22	0.38
C20:2n-6	0.7	1.1	0.9	0.9	0.06	0.30
C20:3	9.9	10.8	10.2	9.6	0.26	0.45
C20:4n-3	3.1	3.1	2.9	2.9	0.10	0.78
C20:4n-6 (ARA)	26.3	26.7	26.6	25.0	0.85	0.90
C20:5n-3 (EPA)	9.7	9.9	9.5	8.6	0.31	0.43

C21:0	0.2	0.3	0.3	0.3	0.01	0.98
C21:5n-3	0.2	0.3	0.3	0.2	0.02	0.56
C22:0	1.4	1.5	2.0	1.6	0.06	0.04[3]
C22:1	0.8	0.8	0.9	0.7	0.04	0.74
C22:4n-6	1.8	2.0	2.0	1.9	0.05	0.58
C22:5n-6	0.5	0.5	0.4	0.5	0.01	0.72
C22:5n-3 (DPA)	13.9	15.0	14.0	13.4	0.48	0.76
C22:6n-3 (DHA)	2.8	2.3	2.2	2.1	0.11	0.20
C23:0	1.6	1.6	1.8	1.6	0.04	0.60
C24:0	2.4	2.3	3.0	2.7	0.17	0.38
C24:1	1.1	1.0	1.2	1.1	0.04	0.60
EPA + DHA	12.5	12.3	11.8	10.7	0.40	0.41
EPA + DPA + DHA	26.4	27.2	25.8	24.1	0.83	0.62
Total FA	644.5	816.5	859.3	852.5	71.66	0.59
∑SFA	285.7	367.8	381.8	392.6	37.07	0.65
∑MUFA	229.7	296.1	342.1	310.7	33.35	0.60
∑PUFA	134.3	150.6	141.8	144.6	3.82	0.59
∑n-3 PUFA	47.4	50.3	49.2	46.2	1.28	0.70
∑n-6 PUFA	74.3	83.9	76.7	83.8	2.33	0.58
PUFA/SFA	0.6	0.5	0.4	0.5	0.03	0.59
n-6/n-3 PUFA	1.5	1.6	1.7	1.8	0.02	0.01[3]

[1] Abbreviations are as depicted in Table 5.2.2. [2] SEM, standard error of the mean. [3] Linear *P*-value.

The IMF, FMP and fatty acid composition data of forage backgrounded steers with or without feedlot finishing are presented in Table 5.2.4. No effect of the backgrounding diet was observed on the IMF, FMP and fatty acid composition ($P \geq 0.14$) except for the DHA levels that linearly declined with an increase in desmanthus proportion in the diet ($P = 0.01$). In contrast, the feedlot finishing of the steers increased the IMF, oleic acid, CLA, LA, ARA, ∑MUFA, ∑n-6 PUFA and n-6/n-3 PUFA levels and reduced ALA and ∑n-3 PUFA ($p \leq 0.04$). Feedlot finishing increased

DPA ($P = 0.04$) and ∑SFA ($P = 0.02$) except in steers fed 30% and 15% diets, respectively, while PUFA/SFA ratios decreased for the steers fed the 0 and 30% desmanthus diets ($P = 0.04$) but remained unchanged for the 15% and 45% desmanthus diets steers. No significant effect of feedlot finishing was observed on the EPA, DHA, EPA + DHA or EPA + DPA + DHA levels ($P \geq 0.16$). Interactions between diet and finishing were observed for the FMP, C14:1, C16:0 (palmitic acid), C16:1, C20:3, ARA, C21:5n-3 and C22:0 (Figure 5.2.1; $P \leq 0.04$). The FMP was lower for the feedlot-finished steers in all diets except the 15% desmanthus diet, and higher for the feedlot-finished than the unfinished steers at 43.6 °C and 42.0 °C, respectively. A reverse trend was observed for palmitic acid, where it was lower for the feedlot-finished than the unfinished steers at 236.5 mg/100 g and 280.6 mg/100 g muscle, respectively.

Table 5.2.4. Carcass intramuscular fat content, fat melting point and fatty acid composition of feedlot-finished and unfinished steers.

	Unfinished				Feedlot Finished					*P*-value[2]		
Variable[1]	0	15	30	45	0	15	30	45	SEM	D	F	D*F
IMF (%)	3.5	2.6	2.4	2.2	4.5	4.5	4.5	4.5	0.18	0.33	0.01	0.29
FMP (°C)	44.3	42.0	43.4	45.1	41.6	43.6	39.0	40.6	0.48	0.49	0.01	0.04
Fatty acids (mg/100g)												
C13:0	0.3	0.2	0.5	0.3	0.0	0.0	0.0	0.0	0.04	0.27	0.01	0.27
C14:0	3.9	18.9	8.9	18.5	16.6	13.4	36.3	17.5	2.63	0.42	0.07	0.06
C14:1	0.3	3.5	1.3	3.0	3.0	2.8	3.6	3.3	0.55	0.74	0.03	0.04
C15:0	2.2	5.2	4.3	3.9	3.2	3.6	7.7	3.8	0.55	0.30	0.46	0.25
C16:0	119.8	280.6	177.5	262.9	373.9	236.5	413.1	271.3	26.22	0.92	0.01	0.03
C16:1	20.5	38.8	27.6	39.2	58.1	30.7	72.9	36.9	4.34	0.62	0.02	0.04
C17:0	7.2	13.2	11.3	14.2	12.2	10.4	21.5	12.2	1.17	0.32	0.20	0.09
C17:1	7.2	11.9	9.9	12.7	20.4	11.4	23.4	13.9	1.35	0.59	0.01	0.09
C18:0	86.8	167.7	140.4	165.6	169.1	155.1	214.9	170.5	11.38	0.54	0.06	0.22
C18:1(Oleic acid)	151.4	331.0	244.5	316.9	581.6	356.4	649.0	407.1	39.88	0.77	0.01	0.08
CLA	2.5	4.4	5.5	5.5	6.4	5.5	8.9	6.4	0.60	0.35	0.04	0.68
C18:2n-6 (LA)	43.0	48.6	53.1	48.9	85.1	74.2	81.6	80.8	2.73	0.55	0.01	0.22
C18:3n-3 (ALA)	14.8	15.1	16.5	15.4	6.8	7.4	6.5	7.4	0.73	0.93	0.01	0.76
C18:3n-6	0.2	0.7	0.4	0.6	1.0	0.9	1.1	0.9	0.06	0.64	0.01	0.06
C18:4n-3	2.3	2.9	2.7	2.3	3.4	2.7	3.0	2.8	0.10	0.68	0.07	0.12
C19:1	1.0	2.0	1.8	2.4	2.3	1.3	2.8	1.6	0.22	0.61	0.64	0.15
C20:0	0.7	1.4	1.2	1.7	1.8	1.3	1.6	1.2	0.14	0.93	0.38	0.20
C20:1	1.2	2.4	2.3	3.4	4.3	3.2	3.9	3.5	0.30	0.87	0.01	0.19
C20:2n-6	0.6	1.2	1.0	1.1	1.5	1.0	1.4	1.1	0.08	0.94	0.08	0.05
C20:3	10.2	10.5	11.2	9.2	12.5	9.8	12.4	13.8	0.32	0.14	0.01	0.01
C20:4n-3	3.4	2.9	3.1	2.7	1.8	1.9	1.6	2.0	0.12	0.73	0.01	0.13
C20:4n-6 (ARA)	25.8	22.5	27.8	21.3	31.1	29.5	28.8	32.6	0.76	0.44	0.01	0.03
C20:5n-3 (EPA)	9.2	8.7	9.6	7.4	8.8	8.0	7.1	8.3	0.25	0.47	0.16	0.11
C21:0	0.2	0.3	0.3	0.3	0.1	0.1	0.2	0.1	0.02	0.93	0.01	0.48

C21:5n-3	0.3	0.3	0.3	0.2	0.4	0.3	0.3	0.3	0.01	0.17	0.06	0.01
C22:0	1.3	1.5	1.6	1.5	1.9	1.5	1.6	1.9	0.05	0.55	0.01	0.02
C22:1	0.7	0.8	0.9	0.7	0.9	0.9	0.7	0.7	0.04	0.59	0.90	0.11
C22:4n-6	1.8	2.0	2.2	2.0	3.6	3.5	3.4	3.7	0.01	0.89	0.01	0.37
C22:5n-6	0.5	0.5	0.5	0.4	0.7	0.6	0.5	0.6	0.13	0.50	0.01	0.17
C22:5n-3 (DPA)	13.6	13.7	15.4	12.8	16.6	15.9	14.2	14.9	0.37	0.60	0.04	0.20
C22:6n-3 (DHA)	2.5	2.0	2.2	1.8	2.7	2.1	2.2	2.0	0.09	0.01 [3]	0.38	0.97
C23:0	1.6	1.7	2.0	1.8	1.4	1.3	1.2	1.4	0.05	0.81	0.01	0.06
C24:0	1.6	1.7	1.9	1.8	2.0	1.6	1.7	1.8	0.05	0.68	0.89	0.24
C24:1	1.0	1.1	1.2	1.2	1.1	0.9	0.9	0.9	0.04	0.92	0.07	0.41
EPA + DHA	11.7	10.7	11.8	9.1	11.3	10.1	9.5	10.3	0.30	0.19	0.34	0.18
EPA + DPA + DHA	25.2	24.4	27.2	21.9	27.9	26.0	23.6	25.1	0.61	0.37	0.41	0.18
Total FA	523.7	1005	787.5	997.7	1513	1006.5	1635.5	1128.8	93.38	0.84	0.01	0.07
∑SFA	223.9	472.2	349.0	473.4	668.5	422.6	707.0	479.9	44.25	0.90	0.02	0.06
∑MUFA	184.7	391.5	289.6	381.7	675.7	407.2	766.0	468.1	46.70	0.75	0.01	0.07
∑PUFA	129.7	139.8	151.7	132.5	186.7	164.3	175.3	177.8	4.20	0.67	0.01	0.19
∑n-3 PUFA	45.9	45.7	49.9	42.7	40.3	38.2	34.9	37.7	1.14	0.77	0.01	0.27
∑n-6 PUFA	72.0	75.7	85.1	73.4	123.0	109.6	116.8	119.7	3.49	0.52	0.01	0.23
PUFA/SFA	0.6	0.4	0.5	0.4	0.4	0.4	0.3	0.4	0.03	0.59	0.04	0.06
n-6/n-3 PUFA	1.5	1.7	1.7	1.8	3.1	2.9	3.5	3.1	0.12	0.26	0.01	0.28

[1] Abbreviations are as depicted in Table 5.2.2. [2] D, diet; F, finishing, D*F, diet and finishing interactions. [3] Linear *P*-value.

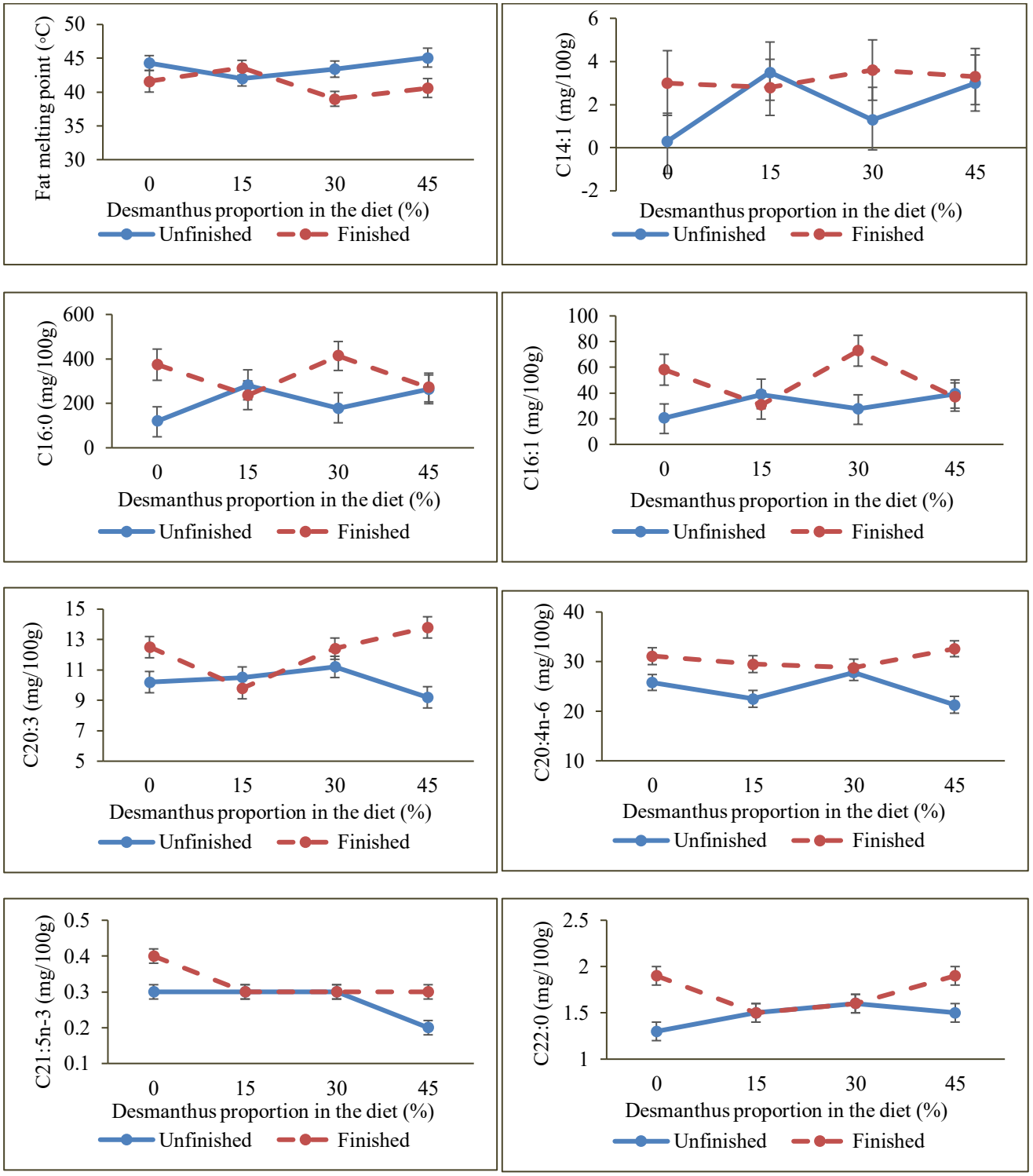


Figure 5.2.1. Effect of diet and feedlot finishing interactions on fat melting point (FMP), C14:1, C16:0, C16:1, C20:3, C20:4n-6, C21:5n-3 and C22:0 fatty acid composition ($P \leq 0.04$).

5.2.3.2. Feedlot growth performance and carcass traits

The initial LW, final LW and ADG of steers ranged between 330.2 – 332.8 kg, 420.1 – 437.8 kg and 0.53 – 0.62 kg/day, respectively, during the finishing phase (Figure 5.2.2). There was no significant difference in growth performance of steers backgrounded on either diet ($P \geq 0.36$).

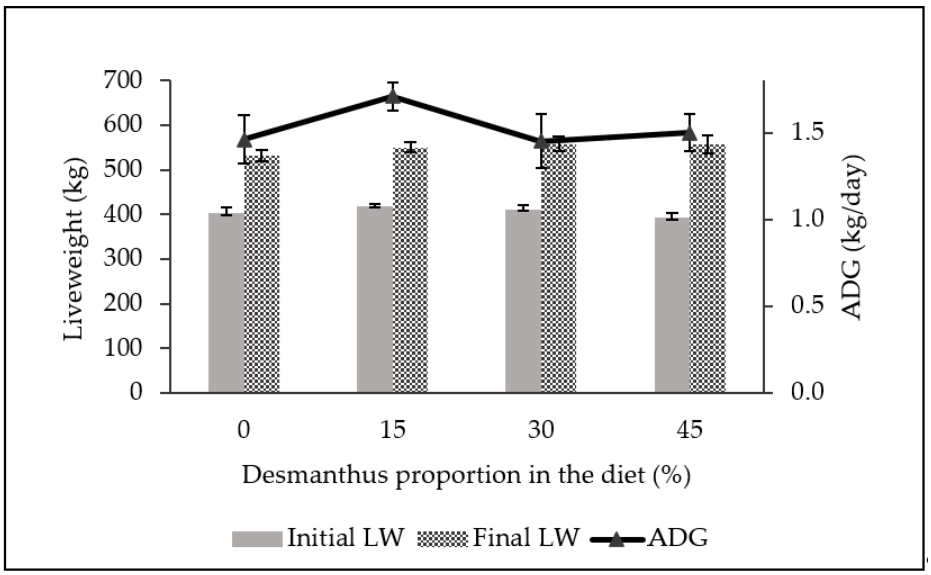


Figure 5.2.2. Mean (± SD) initial liveweight (initial LW; $P = 0.36$), final liveweight (final LW; $P = 0.63$) and average daily gain (ADG; $P = 0.41$) of steers during feedlot finishing after backgrounding on diets with incremental proportions of desmanthus.

The slaughter LW and carcass traits of backgrounded steers with or without feedlot finishing are presented in Table 5.2.5. Feedlot-finished steers had higher slaughter weight, subcutaneous fat depth at the P8 site, HCW and dressing percentage ($P \leq 0.04$), but no difference was observed between diets ($P \geq 0.28$).

Table 5.2.5. Effect of desmanthus proportion and feedlot finishing on slaughter weight and carcass traits.

	Unfinished				Finished					*P*-value[2]		
Variable[1]	0	15	30	45	0	15	30	45	SEM	D	F	D*F
Slaughter weight (kg)	463.8	457.7	443.5	447.5	532.8	550.5	558.0	557.3	8.11	0.96	0.01	0.21
HCW (kg)	225.3	219.0	216.7	220.8	267.2	275.0	274.2	274.9	4.42	0.98	0.01	0.63
Dressing percentage (%)	52.8	52.0	53.1	53.6	54.5	54.3	53.4	53.6	0.26	0.86	0.04	0.30
P8 fat (mm)	4.3	4.8	3.3	3.0	9.7	9.2	7.5	8.7	0.50	0.28	0.01	0.84

[1] HCW: hot carcass weight, P8 fat: Subcutaneous fat depth at the P8 (rump) site

[2] D: diet, F: finishing, D*F: diet and finishing interaction

5.2.4. Discussion

5.2.4.1. Intramuscular fat content, fat melting point and fatty acid composition

Steers backgrounded on diets augmented with incremental proportions of desmanthus or lucerne had similar IMF, FMP and fatty acid composition. These results agree with results reported in previous studies. For instance, Dierking et al. (2010) reported similar fatty acid composition in the loin eye muscle of steers finished on *Festuca arundinacea* (tall fescue) combined with either *Trifolium pretense* (red clover) or *Medicago sativa* (lucerne) pastures. Beef steers offered grass silage mixed with lupins and triticale silage or vetch and barley silage for 122 days had similar IMF content and fatty acid composition in the loin eye muscle (Kennedy et al., 2018). A study of steers finished on lucerne or mixed pasture consisting of *Poa pratensis* (bluegrass), *Dactylis glomerata* (orchardgrass), tall fescue and *Trifolium repens* (white clover) reported similar IMF and fatty acid composition, except for ALA, which was higher for the lucerne than the mixed pasture finished steers (Duckett et al., 2013). Steers fed *Lotus corniculatus* (birdsfoot trefoil) or *Bromus*

riparius (meadow brome) grass had similar IMF, SFA, MUFA, PUFA and PUFA/SFA ratio and n-6/n-3 ratio, although EPA was higher for those fed the birdsfoot trefoil (Chail et al., 2016). In this study, steers were lean with IMF of 2.1 – 2.3% after backgrounding. Muscle IMF accumulation depends on the balance between the uptake, synthesis and degradation of triacylglycerols, hence increasing the availability of net energy for fat synthesis during finishing results in higher IMF content (Scollan et al., 2006). Backgrounding diets had low ME content (7.4 – 8.1 MJ/kg DM), thus the low IMF content was expected. The increase in IMF, FMP and SFA after feedlot finishing steers agrees with previous studies (Scollan et al., 2006; Freitas et al., 2014; Hwang and Joo, 2017). The muscle IMF is positively correlated with carcass fatness, and an increase in carcass fatness is reported to improve meat tenderness through the insulation effect of the subcutaneous and intermuscular fat against the effect of refrigeration during carcass cooling and reduction in the resistance to shearing through the accumulation of IMF in the perimysial connective tissue (Wood et al., 1999). Muscle IMF is negatively correlated with drip loss and cooking loss, indicating that increasing IMF may improve meat water-holding capacity and consequently yield a higher juiciness score (Hwang and Joo, 2017). Therefore, a minimum of 3% IMF is required to meet consumer-preferred overall palatability (Smith, 2016). The 3% IMF was achieved in all the carcasses of feedlot-finished steers but only in the 0 desmanthus diet for the unfinished steers, indicating that finishing desmanthus-backgrounded steers is necessary to meet the consumer-preferred palatability score.

All the melting points were within the 30 – 50°C range reported for beef cattle IMF (Malau-Aduli et al., 2000b; Pitchford et al., 2002). Meat FMP is influenced by the melting points of its fatty acid components. For instance, stearic acid (C18:0) melts at 69.6 °C, whereas oleic acid (C18:1) melts at 13.4 °C; consequently, the concentration of stearic acid in beef fat has the greatest effect on

FMP (Turk and Smith, 2009). Stearic acid and palmitic acid are among the dominant fatty acids in beef fat, and both are reported to increase the fat hardness (Smith, 2016) and subsequently the FMP (Turk and Smith, 2009). In this study, there was no difference in stearic acid concentration between the steers backgrounded on the different diets, hence the similar FMP. However, the decrease in FMP after finishing may be due to an increase in carcass fatness, as indicated by increased IMF (Pitchford et al., 2002). Wood et al. (2003) reported that fat cattle have soft and oily fat due to increased oleic relative to stearic acid. The firmness of fat influences the economics of meat processing and the overall consumer acceptance (Turk and Smith, 2009), with softer fat preferred because it is easier to process during carcass boning and improves meat flavour (May et al., 1993). Besides, increased muscle oleic acid is associated with greater beef palatability, linked to the softness that provides a more fluid mouthfeel (Perry et al., 1998; Smith, 2016). Oleic acid is reported to lower blood low density lipoprotein and may increase the high-density lipoprotein making it a heart-healthy dietary fat (Smith, 2016). The similar oleic acid concentration and FMP in this study may indicate that the increasing proportion of desmanthus did not negatively impact carcass processing ease.

Plant metabolites such as the polyphenol oxidase present in red clover are reported to reduce the activity of plant lipases (Lourenço et al., 2008; Alfaia et al., 2009), while tannins may reduce lipid biohydrogenation in the rumen, although the ability of tannins to inhibit biohydrogenation remains controversial (Toral et al., 2018). A tannin-containing forage (Sainfoin; *Onobrachis* sp.) was reported to have no effect on ALA biohydrogenation. However, tannin extract-containing diets (7.9% of dietary DM) reduced biohydrogenation by 20% in vitro (Khiaosa-Ard et al., 2009), and the addition of sainfoin in a grass silage diet increased the accumulation of ALA in the rumen digesta of lambs (Campidonico et al., 2016). A recent study reported that supplementing growing

lambs with lucerne saponins at 0.05 – 0.2% DM for 90 days significantly increased muscle n-3 PUFA (Liu et al., 2021), although other studies reported that saponins had no effect on muscle n-3 PUFA, but it increased n-6 PUFA (Toral et al., 2018). Lucerne saponin levels of 0.8% to 2% are common (Tava and Avato, 2006). The lack of difference in the muscle SFA and unsaturated fatty acid concentration of steers backgrounded on the different diets in this study may be due to the presence of plant secondary metabolites in both legumes. Feedlot finishing steers reduced the ALA concentration by over 50% compared with the unfinished steers. This outcome was anticipated due to the high concentration of ALA in forage (Wood et al., 2003). Studies have reported that feeding ruminants with forage compared to concentrates with no added n-3 source results in higher concentrations of the health beneficial ALA, EPA, DPA and DHA in muscle lipids due to the high concentration of ALA (50 – 75% of the total FA) in forage (Lorenz et al., 2002; Dannenberger et al., 2005; Scollan et al., 2006). Alpha linoleic acid is the building block of the n-3 PUFA, and its elongation and desaturation result in the synthesis of EPA and DHA (Scollan et al., 2014). Finishing steers resulted in a decline in ALA, DPA and total n-3 PUFA, but EPA and DHA remained similar in this study. Concentrate feeding without n-3 supplementation reduces muscle n-3 PUFA levels due to the low ALA levels in concentrate diets (Lorenz et al., 2002; Steen et al., 2003). The linear decline in DHA concentration with an increase in the proportion of desmanthus in the diet was not expected since the dietary ALA concentration was higher in desmanthus (34.9%) than in lucerne (26.5%) and Rhodes grass (25.6%). The difference could have been due to the increased rumen biohydrogenation of ALA with an increase in the dietary desmanthus proportion or an effect of desmanthus on elongase and desaturase enzymes. Diet modifies meat fatty acid composition by influencing the expression of genes associated with fatty acid synthesis and metabolism (Joseph et al., 2010; Aboujaoude et al., 2018; Leal-Gutiérrez and Mateescu, 2019).

Therefore, more studies are required to examine the effect of desmanthus supplementation on the expression of lipogenic genes and examine if an interaction between lipogenic gene polymorphisms and the proportion of desmanthus in the diet exists.

The muscle fat level is inversely related to the PUFA/SFA ratio. As muscle fat increases, SFA and MUFA increase faster than PUFA, resulting in a decline in the relative proportion of PUFA, and consequently, in the PUFA/SFA ratio. Reviewing 24 studies, De Smet et al. (2004) reported a strong inverse relationship between IMF and the PUFA/SFA ratio in beef. The IMF increased from 1 to 4%, and the PUFA/SFA ratio decreased from 0.7 to 0.1 ($R^2 = 0.85$). The majority of muscle PUFA is found in the phospholipids, and only a small amount is present in the triacylglycerols. The PUFA/SFA ratio is mainly influenced by genetics and overall carcass fat level and much less by nutrition. The PUFA proportion of phospholipids is strictly controlled to maintain membrane properties, whereas the PUFA in triacylglycerols is strongly linked to the total fat content and is reported to vary from 0.2 to more than 5% in the muscle (De Smet et al., 2004; French et al., 2000). The PUFA/SFA ratios for the forage-fed and feedlot-finished steers (0.3 – 0.6) in this study were similar to those reported by Aldai et al. (2011) (0.4 – 0.7) and close or within the desired dietary ratio of 0.4 (McAfee et al., 2010).

Similar to findings of this study, German Holstein and Simmental bulls fed forage-based diets had higher n-3 PUFA and lower n-6/n-3 ratios than their concentrate-fed counterparts, although the SFA levels were similar (Nuernberg et al., 2005). On average, beef from pasture-fed steers contains higher concentrations of C20:3n-3, total n-3 PUFA and a lower n-6/n-3 ratio in the IMF relative to those finished in the feedlot (Freitas et al., 2014). Feeding steers with concentrates for two months before slaughter after forage feeding resulted in a decrease in n-3 PUFA and an increase in n-6 PUFA concentration in the muscle (Aldai et al., 2011). In another study, pasture-fed cattle

had lower SFA, MUFA and n-6/n-3 PUFA ratios, higher n-3 PUFA and similar PUFA levels compared to grain-fed cattle (Tansawat et al., 2013). French et al. (2000) also reported a lower n-6/n-3 PUFA in grass-fed than concentrate-fed cattle, and they associated the difference with the higher dietary n-3 PUFA levels in the grass compared to the concentrate diet. Dietary guidelines recommend that the n-6/n-3 PUFA ratio for human diets should be below 5.0 (Raes et al., 2004), and ratios below 4.0 are purported to have a potential to decrease the risk of coronary diseases and cancer, while ratios of 1.0 or 2.0 may contribute to the prevention of obesity (Simopoulos, 2016). The n-6/n-3 ratios in this study (1.5–3.5) were all below 5.0 and similar or close to those reported for British cattle (2.0 to 2.3) (Enser et al., 1998), German Simmental bulls and Holstein steers (1.3) (Nuernberg et al., 2002) and Alentejano cattle (1.8) (Alfaia et al., 2009). Findings of this study indicate that backgrounding beef cattle on desmanthus augmented forage and finishing them in the feedlot for a short period (95 days) produces meat with a healthy n-6/n-3 PUFA ratio.

Increasing the proportion of desmanthus in the diet led to an increase in docosanoic acid concentration. These findings were contrary to those reported in a study where goats fed a basal diet of lucerne and augmented with *Cynodon dactylon* (Bermudagrass hay), *Lespedeza cuneate* (Sericea lespedeza) or *Pinus taeda L.* (pine bark*)* had similar docosanoic acid concentration in the loin eye muscle IMF (Lee and Min, 2021). Docosanoic acid is a long chain saturated fatty acid found in trace levels in beef (Zheng et al., 2018) from absorption of dietary sources (Cater and Denke, 2001) or produced directly from the biohydrogenation of DHA (Klein and Jenkins, 2011) or elongation of stearic acid (Sampath and Ntambi, 2005). Sheep fed grain pellets fortified with omega-3 oil had increased docosanoic acid concentration in the heart, liver and kidney, but not in the loin eye muscle (Pewan et al., 2021b). Since the docosanoic acid concentration was similar between lucerne and desmanthus forages in this present study, increasing the proportion of

desmanthus in the diet may have influenced docosanoic acid production through DHA biohydrogenation or stearic acid elongation. Although docosanoic acid is reported to increase serum cholesterol levels (Cater and Denke, 2001), its low proportion and absorption in the intestines, low bioavailability (Moreira et al., 2017) and low concentration in the muscle (Zheng et al., 2018) does not pose any detrimental impact on human health.

5.2.4.2. Feedlot growth performance and carcass traits

Steers backgrounded on the different diets had similar weight gains during the feedlot finishing phase. The similar performance could be explained by the similar plane of nutrition (11.4 – 11.6 CP and 7.4 – 8.1 ME/kg DM) and ADG (0.52 – 0.66 kg/day) of all steers during the backgrounding phase echoed by the similar plasma non-esterified fatty acids, glucose and β-hydroxybutyrate observed in Chapter 4.1. Steen et al. (2003) reported that heifers with low weight gains two months prior to the finishing phase had higher weight gains during finishing compared to steers who had higher weight gains prior. The authors associated the difference with compensatory growth in heifers, which was not the case in this study. The similar final LW between diets was expected due to similar initial weight and weight gain during finishing (Duckett et al., 2013).

It has been reported that the carcasses of cattle grazing on tall fescue mixed with sainfoin or lucerne pastures had similar weights and subcutaneous rib fat depths (Maughan et al., 2014) in agreement with this current study, where no significant differences were observed. Other studies have similarly demonstrated that cattle backgrounded on different grass pastures with similar LW at the end of backgrounding had similar LW, carcass weight, dressing percentage and fat cover after finishing in the feedlot for 180 days (Kurve et al., 2016). Carcass subcutaneous fat cover is essential to reduce the risk of cold shortening that creates myofibrillar toughening, resulting in

decreased meat tenderness (Turk and Smith, 2009). Thus, cattle are feedlot-finished on energy-dense diets to improve the subcutaneous fat depth (Blanco et al., 2010; Kurve et al., 2016). Backfat thickness of concentrate-finished cattle is reported to be higher than in their forage-finished counterparts in some studies (Steen et al., 2003; Blanco et al., 2010; Duckett et al., 2013), but not others (Blanco et al., 2012). This can be explained by the increased energy intake for fat synthesis when cattle are fed energy-dense diets during finishing compared to the backgrounding pasture diets (Scollan et al., 2006). In addition, the extensive fermentation of forage diets in the rumen promotes acetate production as the primary source of carbon that reduces lipogenesis, while concentrates increase glucose flow into the duodenum, thereby promoting lipogenesis (Blanco et al., 2010).

In this study, while there were no differences between diets in LW and carcass fatness, significant differences between feedlot-finished and unfinished steers were recorded. This tallies with the expected trends in dressing percentage between finished and unfinished steers and between diets. Blanco et al. (2012) reported that supplementing steers with barley improved the dressing percentage and HCW compared to steers fed on lucerne hay. Finishing Mertolenga steers for 100 days increased backfat thickness from 1.4 to 4.2 mm (Monteiro et al., 2014), but bulls finished to similar weights had similar HCW and dressing percentages (Blanco et al., 2010). Dressing percentage is influenced by breed, liveweight, carcass fatness and time off water (De Brito et al., 2016; Ladeira et al., 2018). Findings of this study indicate that backgrounding tropical crossbred beef cattle on desmanthus alone or mixed with other high-quality legumes may result in healthy meat, and palatability can be improved by feedlot finishing for a short period. These findings are essential for beef cattle producers in the environmentally harsh tropical and subtropical northern

Australian regions where desmanthus is one of the few legume forages that have adapted, established and persisted over several years.

5.2.5. Conclusions

Backgrounding steers on grass forage augmented with incremental proportions of desmanthus resulted in similar muscle IMF, FMP and fatty acid composition. Growth performance during finishing and ultimate carcass quality were comparably similar in all steers. Hence, the hypothesis that steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce similar carcass traits and fatty acid composition was accepted. Feedlot finishing increased carcass weight and fatness and maintained the n-6/n-3 ratio below 4.0. These findings indicate that backgrounding tropical beef cattle on desmanthus forage and finishing them in the feedlot for a short period (95 days) results in healthy and highly palatable meat. Further studies are required to examine the effect of backgrounding tropical beef cattle with incremental proportions of desmanthus forage on the expression of lipogenic genes associated with fat metabolism and meat quality.

5.2.6. Summary

The lipid metabolism, carcass traits and fatty acid composition of the loin eye muscle were evaluated in tropical crossbred steers backgrounded on desmanthus with or without feedlot finishing. It was hypothesized that steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce carcasses with similar characteristics and fatty acid composition. Forty-eight Brahman, Charbray and Droughtmaster crossbred beef steers were backgrounded for 140 days on *Chloris gayana* (Rhodes grass) hay augmented with 0, 15, 30

or 45 percent desmanthus on a dry matter basis. *Medicago sativa* (lucerne) hay was added to the 0, 15 and 30 percent desmanthus diets to ensure that they were isonitrogenous with the 45 percent desmanthus diet. After backgrounding, the two heaviest steers in each pen were slaughtered, and the rest were finished in the feedlot for 95 days before slaughter. Muscle biopsy samples were taken at the beginning and end of the backgrounding phase. Carcasses were sampled at slaughter for intramuscular fat (IMF) content, fat melting point (FMP) and fatty acid composition analyses. Increasing the proportion of desmanthus in the diet led to a linear increase in docosanoic acid ($P = 0.04$) and the omega-6/omega-3 polyunsaturated fatty acid ratio (n-6/n-3 PUFA; $P = 0.01$), while docosahexaenoic acid decreased linearly ($P = 0.01$). Feedlot finishing increased hot carcass weight, subcutaneous fat depth at the P8 site and dressing percentage ($P \leq 0.04$). The n-6/n-3 PUFA ratio was within the recommended < 5 for human diets. IMF was within the consumer-preferred ≥ 3% level for palatability. The hypothesis that steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce similar carcass traits and fatty acid composition was accepted. These findings indicate that a combination of tropical beef cattle backgrounding on desmanthus augmented forage and short-term feedlot finishing produces healthy and highly palatable meat.

Chapter 6: Association of Single Nucleotide Polymorphisms in Lipogenic Genes with Carcass Traits and *Longissimus dorsi* Muscle Fatty Acid Composition

Chapter 6.1: Single Nucleotide Polymorphisms in the Fatty Acid Binding Protein 4, Fatty Acid Synthase and Stearoyl-CoA Desaturase Genes Influence Carcass Traits of Tropical Crossbred Beef Steers

6.1.1. Introduction

Observable and measurable carcass traits are important in assigning meat quality values. The post-mortem measurements of subcutaneous fat depth and marbling are useful in carcass grading as indirect meat quality indicators (Goszczynski et al., 2017; Testa et al., 2021). Characteristics such as loin eye muscle area (EMA) and subcutaneous fat depth are indicative of the amount of saleable carcass, while marbling score is a vital indicator of meat eating quality (Raza et al., 2018). High beef quality standards are vital for consumer satisfaction influencing the decision to repurchase and maintain or increase the global market share of export-dependent beef industries (Scollan et al., 2006; Coleman et al., 2016). Tropical beef cattle breeds (*Bos indicus*) are predominant in northern Australia, (where over half of the Australian beef cattle herd is reared), due to their ability to adapt and survive in harsh environments characterized by poor feed quality, high ambient temperatures and high parasite load (Davis, 1993). However, tropical beef cattle have a low growth rate and produce comparatively tougher meat with lower fat content than the taurine breeds. Thus they are crossed with the taurine breeds to improve growth rate and meat quality without compromising their ability to survive in harsh environments (Greenwood et al., 2018). Therefore,

the measure of tropical breed content as determined by the hump height, is critical during carcass assessment (Polkinghorne et al., 2008).

Carcass traits are influenced by age, genetics and management, hence selective breeding to achieve long-term enhancement of economically important carcass traits is a relevant tool (Raza et al., 2018). However, it is difficult to attain efficient genetic gain using traditional breeding methods since meat quality measurements are obtained after animal slaughter, thus making it difficult to determine meat quality in a living animal (Tait et al., 2014; Ardicli et al., 2017). This creates a substantial genetic lag when progeny phenotypic carcass traits are used to select breeding sires (Raza et al., 2018). Advances in molecular genetics have led to the identification of candidate genes influencing meat quality traits (Li et al., 2013) and SNP loci in genes have been evaluated for their potential use as genetic markers for marker-assisted selection aimed at improving meat quality (Curi et al., 2006; Gill et al., 2009). The fatty acid binding protein 4 (*FABP4*), fatty acid synthase (*FASN*) and stearoyl-CoA desaturase (*SCD*) genes are among the candidate genes associated with carcass traits. The bovine *FABP4* gene encodes a cytoplasmic protein that binds long-chain fatty acids (FA) and other hydrophobic ligands (Michal et al., 2006). The protein is involved in the regulation of lipid hydrolysis and intracellular fatty acids trafficking (Michal et al., 2006), as a result, *FABP4* is considered a functional and positional candidate gene for fat content and distribution rate in the muscle (Barendse et al., 2009; Mannen, 2011). Associations of SNP in the *FABP4* gene with marbling score and hot carcass weight in Holstein bulls (Ardicli et al., 2017), carcass weight, marbling score and meat quality grade in Hanwoo cattle (Lee et al., 2010; Shin et al., 2012) and marbling score in Yanbian yellow cattle (Yin et al., 2020) have been reported. *FASN* encodes a multifunctional polypeptide of enzymes associated with fatty acid biosynthesis in the cytosol of animal cells (Li et al., 2004) and is considered as a candidate gene for marker-assisted

selection and breeding for intramuscular fat (IMF) for improved meat quality and carcass grades (Hillgartner et al., 1995). Several *FASN* SNP have been associated with backfat thickness, loin eye muscle area (EMA) and IMF content in Qinchuan cattle (Raza et al., 2018) and with hot carcass weight in Beefmaster, Brangus, Bonsmara, Romosinuano, Hereford and Angus beef cattle (Rempel et al., 2012). Polymorphisms of the *SCD* gene have been reported to be associated with marbling score, but not backfat thickness in Wagyu x Limousin cattle (Jiang et al., 2008). In Chinese Simmental cattle, SNP were associated with IMF, but not marbling score (Wu et al., 2012). In contrast, neither backfat thickness nor IMF in Spanish commercial beef cattle (Avilés et al., 2013) were associated with any SNP. Barendse et al. (2009) reported that *SCD* g.2502C>G SNP was associated with muscle IMF in Angus, but not Hereford, Shorthorn, Murray Grey, Belmont Red, Brahman and Santa Gertrudis cattle. These findings indicate that SNP-based genetic markers need to be tested and confirmed for the specific breeds of interest (Hocquette et al., 2012), thus the need for a targeted search for SNP in *FABP4*, *SCD* and *FASN* genes in northern Australian crossbred tropical beef cattle and determine their association with carcass traits. Therefore, this study aimed to detect SNP in the exons and introns of *FABP4*, *SCD* and *FASN* genes of northern Australian tropical crossbred beef cattle and investigate their associations with carcass traits. It was hypothesised that SNP in the *FABP4*, *SCD* and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle.

6.1.2. Materials and Methods

This study followed the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013) and was approved by the James Cook University Animal Ethics Committee (Approval Number 2639).

6.1.2.1. Animal management

Fifty tropical steers of crossbred *Bos indicus* and *Bos taurus* breeds were randomly selected and used for this study based on an *a priori* G-Power analysis to achieve 80% power with a 4.0 critical F-value for a large effect size and α of 0.05. Steers were backgrounded on buffel grass only or buffel grass and desmanthus pastures for 147 days as described in Chapter 4.1 and finished in a commercial feedlot for 110 days. The steers were managed and transported according to approved Meat Standards Australia (MSA) protocols (MLA, 2020b) and slaughtered within 48 h of leaving the feedlot with a lairage period not exceeding 12 h. Carcasses were graded according to the Aus-Meat and MSA grading standards (Meat Standards Australia, 2020). The traits of interest were hot standard carcass weight (HCW), hump height, marbling score, subcutaneous rib fat (back fat) thickness, rump fat thickness at the P8 site, EMA, meat colour and MSA index.

6.1.2.2. Blood collection and genomic DNA extraction

Blood was collected at the start of the backgrounding phase via caudal venipuncture into 4 ml EDTA-containing vacutainer tubes (BD, Sydney, Australia) and stored at -80°C prior to analysis. Blood samples were later thawed at room temperature and an aliquot of 2 mL used for genomic DNA extraction using NucleoSpin Blood Kit (Macherey-Nagel GmbH and Co. KG, Duren, Germany) according to the manufacturer's protocol. DNA yield and purity were quantified using a NanoDrop ND-1000 (Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) and diluted to 50 ng/µL with nuclease-free water.

6.1.2.3. Primer design

All primers were designed using the Geneious Prime software version 2020.2.5 (Biomatters Ltd, Auckland, New Zealand) and synthesised at Integrated DNA Technologies Pte Ltd (Queenstown, Singapore). *Bos taurus* (Hereford) sequences of *FASN*, *FABP4*, and *SCD* gene were obtained from the National Centre for Biotechnology Information database (GenBank) and used as references. The 15 kb *SCD* sequence (NC_ 037353.1) was split into three fragments and the 18 kb-long *FASN* (NC_ 037346.1) sequence was split into two overlapping fragments, while the 4 kb *FABP4* (NC_ 037341.1) gene was not fragmented before amplification (Table 6.1.1, Supplementary Figure S6.1.1).

Table 6.1.1. Primer sequences for target gene amplification

Gene[1]	Fragment	Primer	Sequence (5′ to 3′)	Annealing temp (°C)	Product size (bp)
SCD	1	Forward	GGAAGAAGACATCCGCCCTGAAAT	60	5092
		Reverse	AGGAAGCGAGATTGGCACTGTATG	60	
SCD	2	Forward	GGAAGAAGACATCCGCCCTGAAAT	60	10177
		Reverse	TGCCTCTGAGGGGATCTATTTGGT	60	
SCD	3	Forward	ATGAGCCACACTGTGAACAAACCT	60	2861
		Reverse	TTCTTTTTCTGGACAGGCAAGCCT	60	
FASN	1	Forward	TTGAGCTTCTGAGTATGATGGGAG	68	7302
		Reverse	ACCATCTATTATGCCTCCCTCAAC	68	
FASN	2	Forward	CTATAAGATCGGTGAGTCCTTGCA	68	8648
		Reverse	TAGTATTATTCACAGCTCCCTGGC	68	
FABP4	-	Forward	GCTAAGACTGCCTGTATGTTCCCC	60	3041
		Reverse	ACCTAGAGAAATAGACAATCGCCC	60	

[1]*SCD*, stearoyl-CoA desaturase; *FASN*, fatty acid synthase; *FABP4*, fatty acid binding protein 4

6.1.2.4. Target gene amplification

Gene amplification, library preparation, normalization, sequencing and data analysis procedures were carried out as described previously (Pewan et al., 2021a). In brief, three diverse long-range PCR approaches were tested to amplify the *SCD*, *FASN* and *FABP4* genes, and the optimum combinations were selected. Platinum SuperFi PCR Master Mix and Phusion Hot Start II High-Fidelity PCR Master Mix (Thermofisher Scientific, Scoresby, Australia) were used to amplify the *FABP4* and *SCD* genes, respectively, under similar PCR conditions. The amplification reactions were executed in a SimpliAmp Thermal Cycler (Thermofisher Scientific, Scoresby, Australia) in a total volume of 50 µL consisting of 25 µL of PCR master mix, 100 ng of DNA template and 0.5 µM of each primer in a 3-step procedure. The conditions were: single initial denaturation at 98 °C for 1 min, 35 cycles of denaturation, annealing and extension at 98 °C for 15 s, 60 °C for 15 s, and 72 °C for 9 min, respectively, followed by a final extension at 72 °C for 9 min and a 4 °C hold. The *FASN* gene fragments were amplified using PrimeSTAR GXL Master Mix (TaKaRa Bio Inc., Kusatsu, Shiga, Japan) in a 2-step protocol. The amplification reaction mix consisted of 1.25 units of polymerase, 10 µL of 5× buffer, 0.2 µM of each primer, 200 µM of dNTP mixture and 100 ng of DNA template in a total volume of 50 µL. The amplification reaction conditions included initial denaturation for 1 min at 98 °C and 30 cycles of denaturation and annealing combined with extension at 98 °C for 10 s and 68 °C for 10 min, respectively. The amplification products were visualised using gel electrophoresis in 0.8% agarose gel to evaluate success of the assay.

6.1.2.5. PCR products clean up

All PCR products were cleaned using the Sera-Mag SpeedBeads in a Zephyr NGS Workstation (PerkinElmer, Waltham, Massachusetts, USA) and quantified using the Promega dsDNA

Quantifluor System Kit (Ref: E2670, 00002484139) on the PerkinElmer Enspire Workstation. The six amplification products from the three genes were pooled at 0.4 nM, normalised to 2 ng/µL and then diluted to 0.2 ng/µL with 10 mM Tris-HCl at pH 8.0 for library preparation.

6.1.2.6. Library preparation and sequencing

Libraries were prepared with the Nextera XT DNA Library Prep kit (Illumina, CA, USA) according to the manufacturer's instructions. Libraries were purified using 0.6× Sera-Mag SpeedBeads (Merck KGaA, Darmstadt, Germany) and washed twice with 80% ethanol to get rid of unincorporated adapters and fragments shorter than 250 bp length. The DNA libraries were analysed for fragment size and concentration using Agilent High Sensitivity D5000 reagents and ScreenTape on the Tape Station 4200 (Agilent Technologies, Santa Clara, CA, USA) and further concentration determined using the QuantiFluor® dsDNA System (Promega, Madison, WI, USA). The fragment size and concentration data were used to normalise each library to 2 nM with 10 mM and pH 8.5 Tris-HCl and samples of the different steers were then pooled together. A 10 pM input of the pooled samples and 10% PhiX spike-in were used for sequencing on the Illumina MiSeq benchtop sequencer with a 500-cycle MiSeq Reagent Nano Kit v2 (Illumina, Inc, San Diego, CA, USA).

6.1.2.7. Data analysis

6.1.2.7.1. Sequence data analysis and calculations

Sequence data were downloaded from the Illumina Dashboard-BaseSpace Sequence Hub (https://basespace. illumina.com/dashboard, accessed on 27 July 2021) and analysed using the Geneious Prime software with NC_ 037353.1, NC_ 037346.1 and NC_ 037341.1 as the *SCD*,

FASN and *FABP4* reference sequences, respectively. The retrieved reads were trimmed and adapters removed using the BBDuk trimmer. The Phred quality score was set at 20 to increase the probability of calling true SNP to 99%. All short reads of 20 bp or less were discarded and low coverage regions were excluded during SNP calling. The allele and genotype frequencies were determined by direct counting, while the polymorphism information content (PIC) was determined using the GDIcall online calculator (http://www.msrcall.com/Gdicall.aspx) (accessed on 11 January 2022). Hardy–Weinberg equilibrium (HWE) and expected heterozygosity (He) were calculated as described previously (Nei and Roychoudhury, 1974). All monomorphic loci and SNP with minor allele frequency (MAF) below 0.01 were excluded.

6.1.2.7.2. Statistical analysis

Statistical data analyses were carried out using the R software version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Initial summary statistics were computed for means, standard deviations, distribution and range to identify any outliers. The Chi-Square test was used to determine if the SNP were in HWE. Distance-based hierarchical clustering of the SNP loci was used to examine the degree of linkage disequilibrium between each pair of loci (Lin et al., 2012) and the results presented as dendrograms and heat maps. Spearman's ρ correlation analysis was used to estimate the linear correlations between genomic variants and carcass traits. Linear models were fitted to investigate whether a gene SNP is associated with carcass traits using generalized least squares linear procedures to compute group means, while significant differences between least-square means were compared using the Tukey-adjusted multiple comparisons test. Differences were declared significant at $P < 0.05$.

6.1.3. Results

6.1.3.1. Genetic variants and population diversity

In a targeted evaluation of the associations between SNP loci in the *FABP4*, *SCD* and *FASN* genes with chiller-assessed carcass traits of tropical crossbred beef cattle, a total of 65 SNP loci were identified. These comprise 11, 27 and 27 SNP for the *FABP4*, *SCD* and *FASN* genes, respectively (Supplementary Table S6.1.1). Twenty-eight SNP were considered novel since they were not found in the Bovine Genome Variation Database (BGVD) (http://animal.nwsuaf.edu.cn/code/index.php/BosVar, accessed on 28 January 2022). Only seven SNP were predicted to be non-synonymous amino acid substitutions (Table 6.1.2). The SNP were in HWE except for g.50786977A>G and g.50790973C>A in the *FASN* gene (Supplementary Table S6.1.1; $P \leq 0.04$). The MAF, He and PIC for the SNP ranged between 0.11 – 0.47, 0.20 – 0.49 and 0.18 – 0.37, respectively, for all three genes. Correlation coefficients used to measure the degree of linkage disequilibrium between each pair of loci depicted in Supplementary Figure S6.1.2 – S6.1.4 showed the presence of local patterns forming four clusters for each gene.

Table 6.1.2. *FABP4*, *SCD* and *FASN* gene polymorphisms, protein coding sequence positions and non-synonymous amino acid substitutions

Gene[1]	SNP (dbSNP ID)[2]	PCS position[3]	Amino acid substitution
FABP4	g.44677959T>C (rs110757796)	220	Isoleucine to Valine
SCD	g.21272422C>T (rs41255693)	878	Alanine to Valine
FASN	g.50784533C>G (rs481622676)	2066	Alanine to Glycine
	g.50786496A>G	3145	Serine to Glycine
	g.50788575T>C (rs41919993)	4168	Tyrosine to Histidine
	g.50790973C>A (rs109149276)	5572	Leucine to Isoleucine
	g.50794099T>C	7277	Isoleucine to Threonine

[1] *FABP4*: fatty acid binding protein 4, *SCD*: stearoyl-CoA desaturase, *FASN*: fatty acid synthase

[2] SNP: single nucleotide polymorphism. Variant dbSNP ID are based on the Bovine Genome Variation Database (BGVD). SNP without dbSNP ID are not listed in BGVD.

[3] PCS: protein coding sequence

6.1.3.2. Correlations between SNP and carcass traits

The clustering patterns of the SNP loci are shown in the top panels of Figure 6.1.1 – 6.1.3 (A). The level of SNP heterozygosity and homozygosity varied widely between closely related individuals in the *SCD* gene compared to both *FABP4* and *FASN* genes. The correlation coefficients between the SNP and carcass traits are presented in the bottom panels of Figure 6.1.1 – 6.1.3(B). Most of the SNP loci in the *FABP4* gene were in linkage disequilibrium ($P < 0.05$). Correlations between SNP and carcass traits were observed in only three SNP. The g.44677205A>G (rs109388335), g.44677611G>C (rs41729172) and g.44679833A>G (rs133333024) SNP were all positively correlated with hump height ($P < 0.05$), while g.44677205A>G (rs109388335) was negatively correlated with EMA ($P < 0.05$). All the SNP identified in the *SCD* gene were in linkage disequilibrium ($P < 0.05$). Furthermore, g.21267896C>T (rs136334180) was not correlated with

g.21274479G>A (rs382184952) (Figure 6.1.2B). Polymorphisms on the g.21271392G>A (rs211294052) and g.21274479G>A (rs382184952) loci were positively correlated with HCW and EMA, respectively ($P < 0.05$). Moreover, g.21275851C>A (novel) was negatively correlated with P8 fat thickness, backfat thickness and fat class ($P < 0.01$), while g.21273692T>C (rs208058585), g.21276141C>T (rs41255697) and g.21276672A>G (rs41255698) were negatively correlated with carcass marbling score ($P < 0.05$). Most SNP detected in the *FASN* gene were in linkage disequilibrium (Figure 6.1.3B; $P < 0.05$). Positive correlations were observed in g.50784533C>G (rs481622676) and g.50792445C>T (novel) SNP with carcass P8 fat and backfat thickness, while g.50786221A>G (rs518879624) and g.50787362C>T (novel) were negatively correlated with carcass marbling ($P < 0.05$). Several carcass traits were significantly correlated with each other. For instance, HCW was positively correlated (≥ 30) with P8 fat, backfat thickness, EMA and MSA index, but negatively correlated with meat colour scores ($P < 0.05$). A highly positive correlation (≥ 80) between P8 fat with back fat and fat class was observed ($P < 0.001$). Hump height was negatively correlated with MSA index ($P < 0.001$), but not between hump height and other carcass traits.

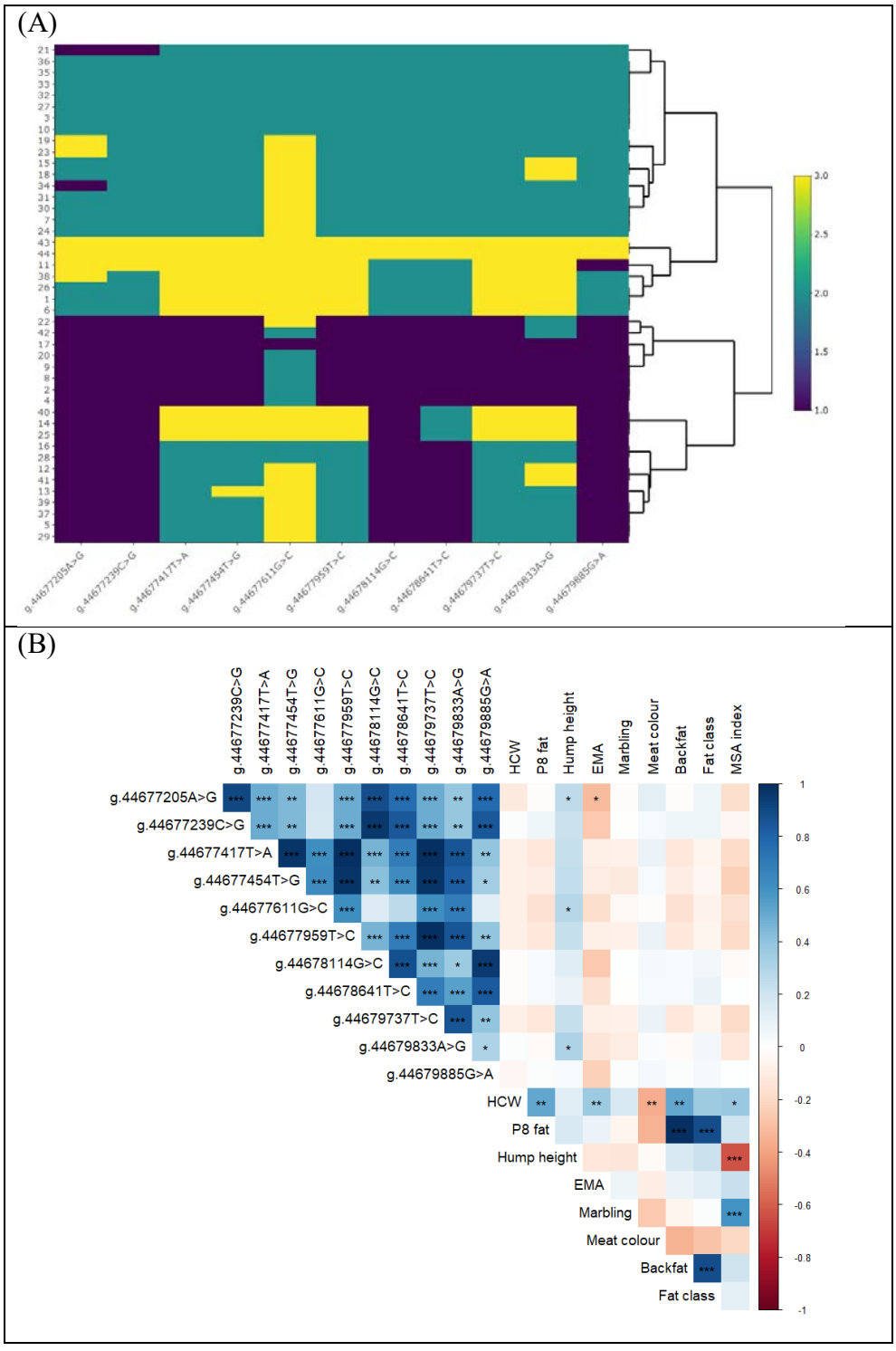


Figure 6.1.1. Single nucleotide polymorphisms on the *FABP4* gene. **(A)** Clustering map of genetic variants; homozygotes similar to the reference sequence (Hereford), heterozygotes and alternative homozygotes. **(B)** Correlations between detected SNP and carcass traits. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

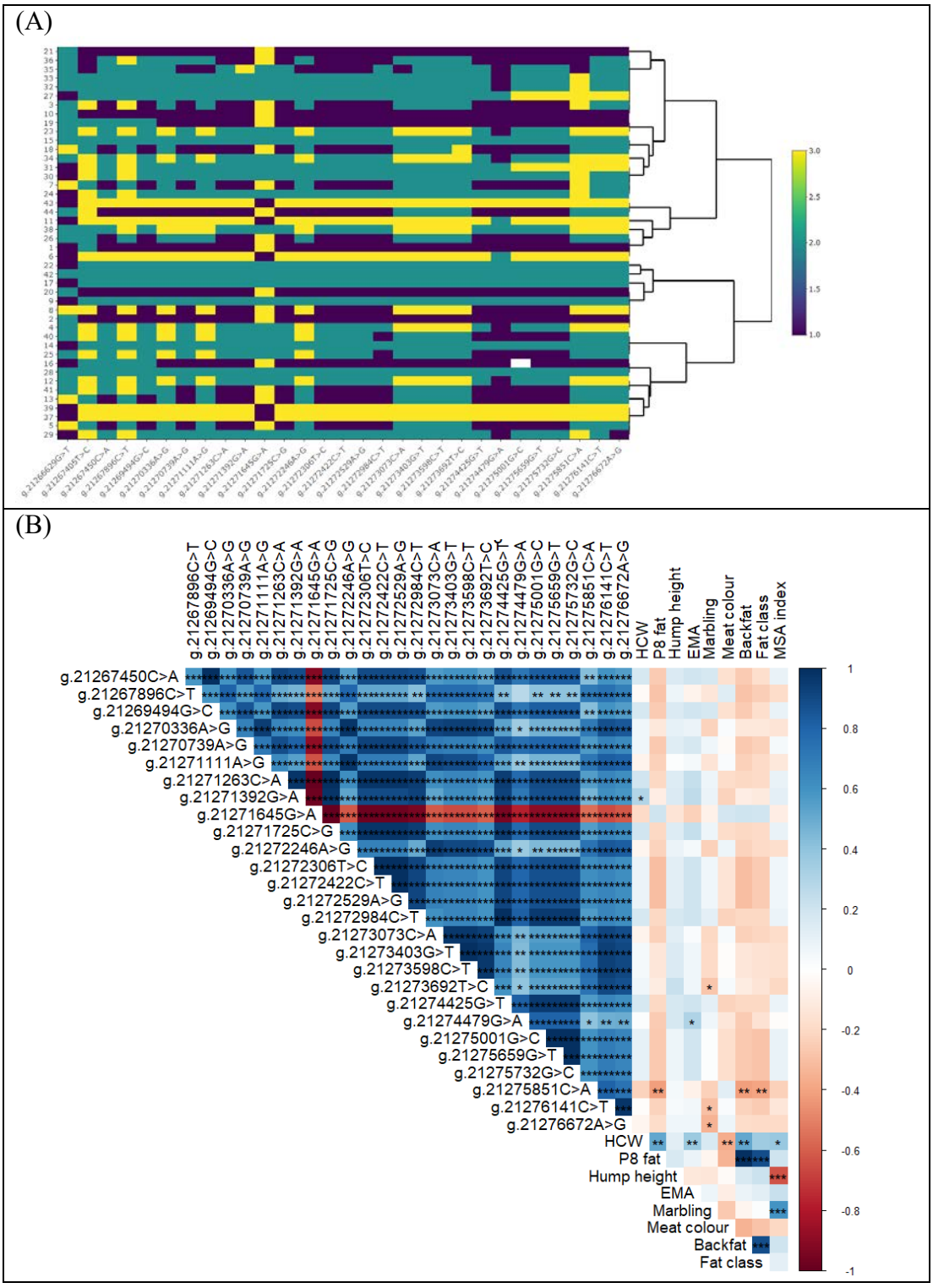


Figure 6.1.2. **(A)** Clustering map of genetic variants of the *SCD* SNP; homozygotes similar to the reference gene (Hereford), heterozygotes and the alternative homozygotes. **(B)** Correlations between SNP and carcass traits. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

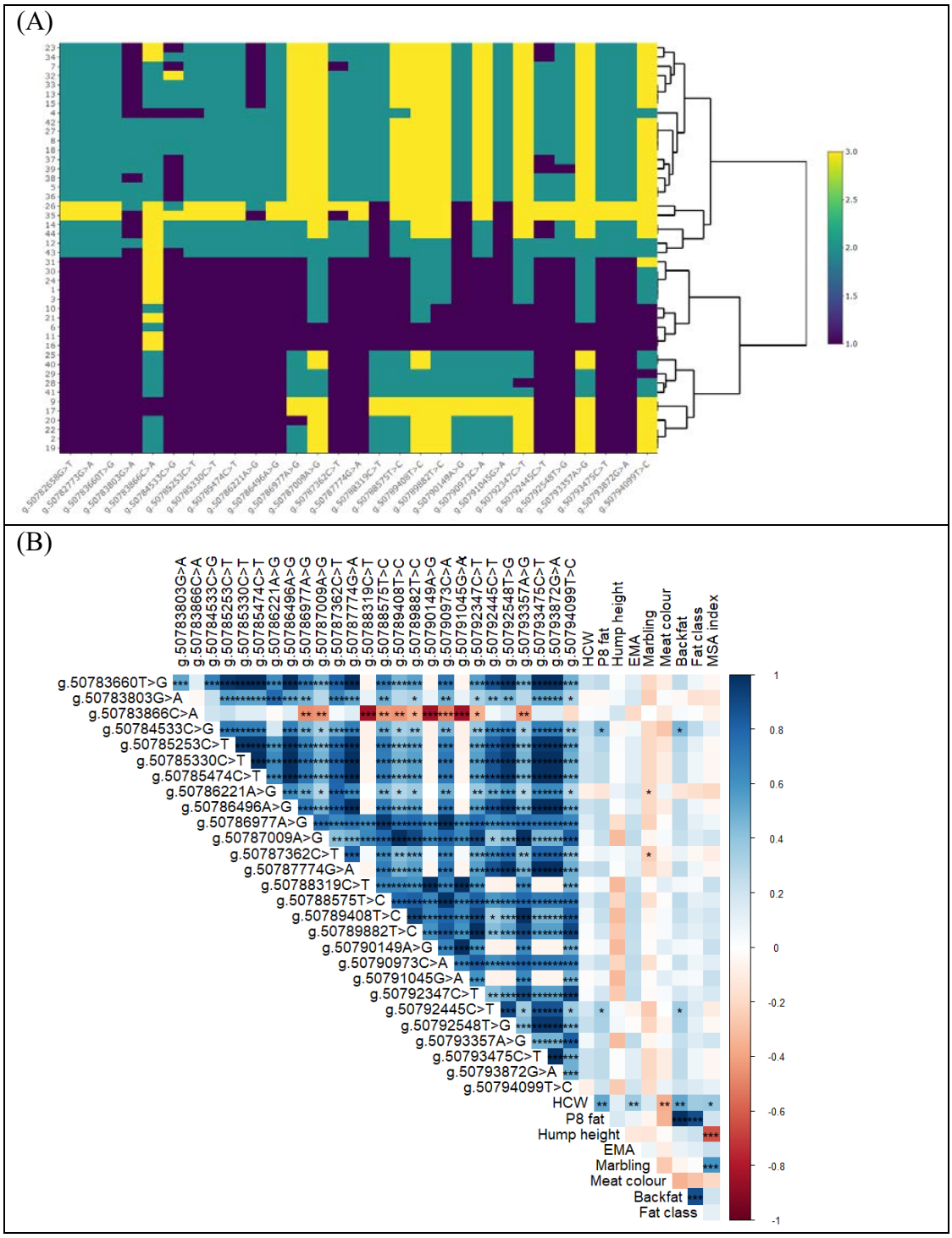


Figure 6.1.3. **(A)** Clustering map of genetic variants of the *FASN* SNP; homozygotes similar to the reference gene (Hereford), heterozygotes and the alternative homozygotes. **(B)** Correlations between genes SNP and carcass traits. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

6.1.3.3. Associations between SNP and carcass traits

Associations between *FABP4* g.44677205A>G (rs109388335), *SCD* g.21275851C>A (novel) and *FASN* g.50784533C>G (rs481622676) SNP with carcass traits are presented in Table 6.1.3. The *FABP4* g.44677205A>G SNP had a significant association with hump height ($P < 0.01$) and tended to be associated with EMA ($P = 0.05$). Multiple genotype comparisons at the g.44677205A>G locus showed that the GG genotype had higher hump height (125.8 ± 10.68 mm) compared to the AA (109.3 ± 13.39 mm) genotype ($P \leq 0.01$), but similar to the AG genotype ($P = 0.08$). The hump height difference between the AA and AG genotypes was insignificant ($P = 0.64$). EMA was significantly higher for the AA (88.4 ± 8.71 cm^2) than the GG (81.3 ± 3.88 cm^2) and AG (86.7 ± 4.93 cm2) genotypes (Figure 6.1.4; $P \leq 0.03$), but genotypic differences between AA and AG were not significant ($P = 0.81$). The AA genotype of *SCD* g.21275851C>A SNP had the lowest P8 fat, marbling, backfat and fat class, while the highest scores were observed for the CC genotype ($P < 0.02$). Multiple comparisons between *SCD* g.21275851C>A SNP variants and carcass traits indicated that P8 fat, marbling, backfat and fat class scores were significantly different between the CC and AA genotypes (Figure 6.1.5; $P < 0.03$). No significant associations were observed between the *FASN* g.50784533C>G variants and the carcass traits measured ($P \geq 0.107$).

Table 6.1.3. Least square means ± SD of carcass traits due to SNP in *FABP4* g.44677205A>G, *SCD* g.21275851C>A and *FASN* g.50784533C>G in northern Australian tropical crossbred beef cattle.

Variable[1]					*P*-value[2]
FABP4					
g.44677205A>G	Total (n = 44)	AA (n = 22)	AG (n = 16)	GG (n = 6)	
HCW (kg)	329.9 ± 26.15	331.1 ± 20.06	333.0 ± 32.81	317.6 ± 27.26	0.16
P8 fat (mm)	16.7 ± 5.80	17.0 ± 5.96	17.0 ± 6.41	14.6 ± 3.27	0.66
Hump height (mm)	112.5 ± 15.42	109.3 ± 13.39	111.8 ± 17.5	125.8 ± 10.68	< 0.01
EMA (cm^2)	86.8 ± 7.26	88.4 ± 8.71	86.7 ± 4.93	81.3 ± 3.88	0.05
Marbling (score)	361.5 ± 79.77	372.7 ± 83.05	353.1 ± 81.79	343.3 ± 67.13	0.86
Meat colour (score)	2.3 ± 1.11	2.1 ± 1.18	2.3 ± 0.96	2.5 ± 1.38	0.70
Back fat (mm)	14.7 ± 5.80	15.0 ± 5.96	15.0 ± 6.41	12.6 ± 3.27	0.74
Fat class (score)	3.8 ± 0.65	3.9 ± 0.75	3.8 ± 0.62	3.8 ± 0.41	0.98
MSA index	51.11 ± 2.32	51.3 ± 2.28	51.2 ± 2.6	49.6 ± 0.42	0.39
SCD					
g.21275851C>A	Total (n = 44)	CC (n = 9)	CA (n = 15)	AA (n = 20)	
HCW (kg)	329.9 ± 26.15	333.0 ± 38.21	333.2 ± 23.32	326.1 ± 22.44	0.75
P8 fat (mm)	16.7 ± 5.80	19.6 ± 6.73	18.6 ± 5.03	14.0 ± 4.88	0.01
Hump height (mm)	112.5 ± 15.42	105.5 ± 12.61	116.6 ± 15.99	112.5 ± 15.68	0.24
EMA (cm^2)	86.8 ± 7.26	85.8 ± 5.49	88.2 ± 8.60	86.2 ± 7.07	0.82
Marbling (score)	361.5 ± 79.77	421.1 ± 75.9	338.0 ± 83.85	352.5 ± 67.74	0.02
Meat colour (score)	2.3 ± 1.11	2.2 ± 1.20	2.3 ± 1.23	2.3 ± 1.03	0.98
Back fat (mm)	14.7 ± 5.80	17.5 ± 6.73	16.6 ± 5.05	12.0 ± 4.88	0.01
Fat class (score)	3.8 ± 0.66	4.2 ± 0.67	4.0 ± 0.59	3.6 ± 0.60	0.02
MSA index	51.1 ± 2.32	52.3 ± 2.18	50.9 ± 2.82	50.7 ± 1.90	0.19
FASN					
g.50784533C>G	Total (n = 44)	CC (n = 30)	CG (n = 12)	GG (n = 2)	
HCW (kg)	329.9 ± 26.15	327.2 ± 23.24	329.5 ± 24.73	377.5 ± 40.31	0.62
P8 fat (mm)	16.7 ± 5.80	15.5 ± 5.10	18.4 ± 5.84	25.0 ± 9.90	0.10
Hump height (mm)	112.5 ± 15.42	110.2 ± 14.97	110.2 ± 15.60	130.2 ± 14.14	0.92
EMA (cm^2)	86.8 ± 7.26	86.5 ± 7.90	87.4 ± 6.05	88.5 ± 6.36	0.69
Marbling (score)	361.5 ± 79.77	380.2 ± 91.95	350.2 ± 62.68	345.2 ± 49.50	0.23
Meat colour (score)	2.3 ± 1.11	2.5 ± 1.12	1.9 ± 1.15	2.0 ± 0.710	0.31
Back fat (mm)	14.7 ± 5.80	13.5 ± 5.10	16.4 ± 5.84	23.0 ± 9.90	0.10
Fat class (score)	3.8 ± 0.65	3.8 ± 0.65	3.9 ± 0.67	4.5 ± 0.71	0.38
MSA index	51.1 ± 2.32	50.9 ± 2.01	51.4 ± 3.24	51.8 ± 1.22	0.75

[1] *SCD*, Stearoyl-CoA Desaturase; *FASN*, Fatty Acid Synthase; *FABP4*, Fatty Acid Binding Protein 4; HCW, hot carcass weight; P8 fat; subcutaneous fat thickness at the rump site; EMA, loin eye muscle area; MSA index, Meat Standard Australia index.

[2] ANOVA *P*-value

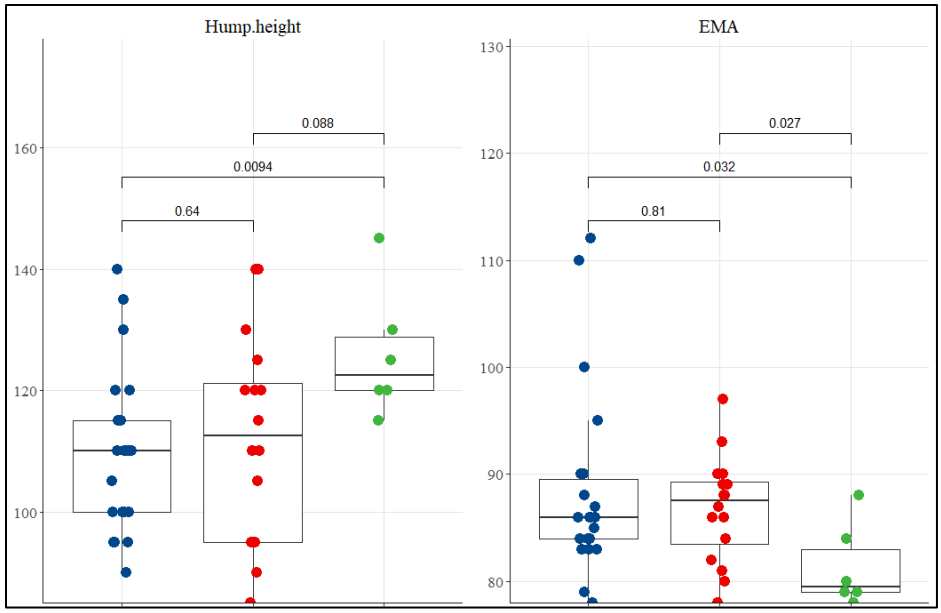


Figure 6.1.4. Multiple comparisons of hump height and loin eye muscle area (EMA) for *FABP4* g.44677205A>G genotypic variants AA (●), AG (●) and GG (●).

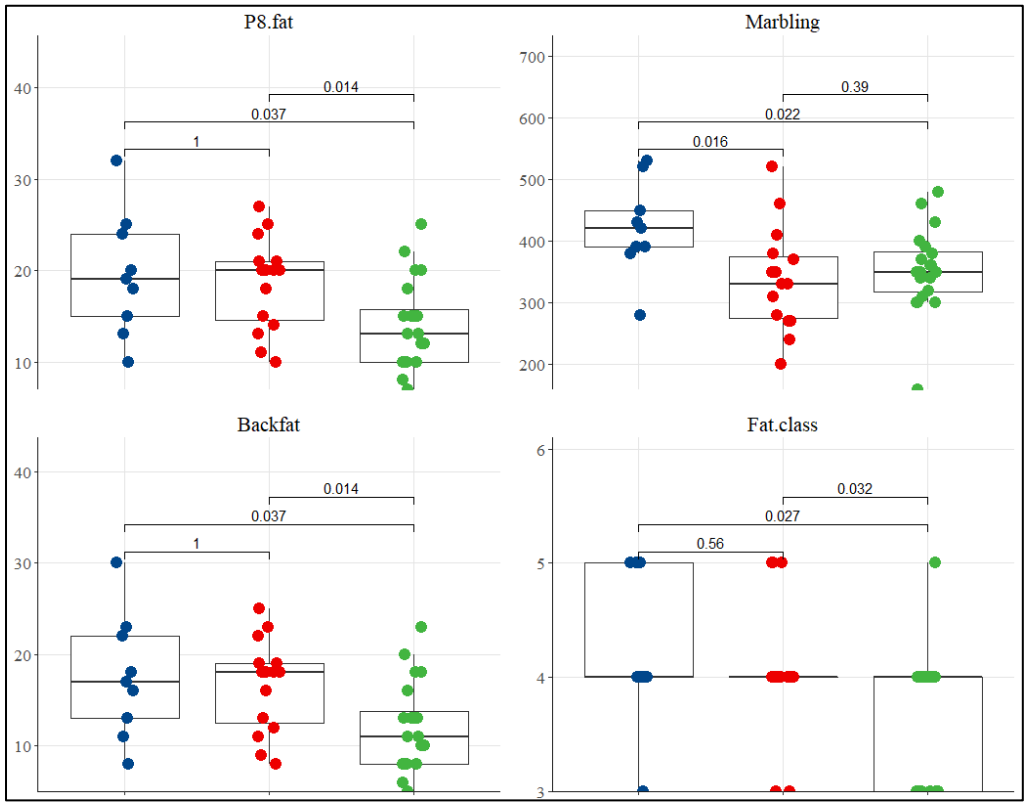


Figure 6.1.5. Multiple comparisons of P8 fat, marbling, backfat and fat class of *SCD* g.21275851C>A genotypic variants CC (●), CA (●) and AA (●).

6.1.4. Discussion

Chiller-assessed carcass quality measurements are obtained after slaughter, hence the data cannot be used to make management decisions such as culling the inferior and breeding the superior performing animals (Tait et al., 2014; Ardicli et al., 2017). Regions on chromosomes 1, 9, 14, 16, 19, 23, 26 and 29 had been previously identified to be associated with fatty acid composition and carcass traits in a cohort of *Bos indicus*, *Bos taurus* and tropical composite beef cattle using high-density data (Kelly et al., 2014). Other studies had reported that single allelic substitutions influence carcass traits and can be used to predict carcass quality in living animals (Jiang et al., 2008; Lee et al., 2010). In this study, a targeted Next-Generation Sequencing technique was used to identify SNP in the *FABP4*, *SCD* and *FASN* genes that may be used as molecular markers for carcass quality selection in northern Australian tropically adapted beef cattle. A marker PIC is one of the indicators of marker quality and it reflects the ability of a marker to detect polymorphisms between individuals in a population (Serrote et al., 2020). Markers with PIC values above 0.5 are deemed very informative, 0.25 – 0.50 are a bit informative and below 0.25 are minimally informative (Botstein et al., 1980). In this study, all the markers were informative with the exception of only one marker at the g.50783803G>A locus that had a PIC below 0.25.

6.1.4.1. Fatty acid binding protein 4 gene polymorphisms

The *FABP4* gene is a primary metabolic indicator of IMF deposition as it is located within the quantitative trait loci region that contributes to serum leptin; a protein involved in body fat regulation, and *FABP4* also encodes a protein involved in intracellular fatty acids trafficking (Jurie et al., 2007; Hoashi et al., 2008). Damon et al. (2006) reported that *FABP4* protein level was positively correlated with fat cell count and lipid content in porcine. Genetic variants of the *FABP4*

gene in cattle and their associations with meat and carcass traits have been reported (Michal et al., 2006; Barendse et al., 2009; Oh et al., 2012; Shin et al., 2012). The *FABP4* g.3691G>A SNP was associated with marbling score, while g.2834C>G was reported to be associated with HCW in Holstein bulls (Ardicli et al., 2017). The g.3473T>A SNP was reported to have a significant effect on carcass weight, while g.3631A>G significantly influenced marbling score in Hanwoo cattle (Lee et al., 2010). In another study, *FABP4* g.3691G>A SNP was reported to influence marbling score and meat quality grade in Hanwoo cattle (Shin et al., 2012). Five SNP (g.3496A>C, g.3745T>C, g.3533A>T, g.3767T>C, and g.3711G>C) were associated with marbling scores in Yanbian yellow cattle (Yin et al., 2020). However, these polymorphisms were not observed in this study. Furthermore, there were no correlations or associations between the identified *FABP4* SNP in the present study and carcass subcutaneous fat depth (P8 fat and backfat) or marbling score. These findings agree with a previous study (Hoashi et al., 2008) that reported no significant effect of g.44677959T>C (c.220) with subcutaneous fat nor marbling score in Japanese Black cattle. In contrast, Cho et al. (2008) reported a SNP association with backfat thickness in Korean cattle, while Goszczynski et al. (2017) reported an additive effect in cattle of varying breeds. Three SNP (g.44677205A>G, g.44677611G>C and g44679833A<G) were positively correlated with hump height in this study, with the homozygous GG genotype having the highest and AA the lowest measurements. Hump height is used to estimate the amount of tropical breed content of the carcass, with high values indicating higher tropical breed content and subsequently, lower meat eating quality (Pringle et al., 1997). Therefore, the correlations indicate that genetic variants of the three loci are influenced by breed in agreement with previous studies (Shin et al., 2012; Bartoň et al., 2016). Both EMA and subcutaneous fat depth indicate the amount of saleable meat from the carcass (Raza et al., 2018). The negative correlation between g.44677205A>G and EMA, and the

lack of correlation with subcutaneous fat depth, indicate that selecting for A allele may increase the amount of carcass saleable meat without increasing the subcutaneous fat that ends up being trimmed off (Kelly et al., 2013).

6.1.4.2. Stearoyl-CoA desaturase gene polymorphisms

The *SCD* gene is highly expressed in lipogenic tissues and encodes for an enzyme that desaturates SFA by introducing a *cis* double bond at the 9th and 10th carbon interface of the fatty acid (Bernard et al., 2013). The unsaturation of the fatty acid chain is a key determining factor of the melting temperature of triacylglycerols, thus influencing meat fat hardness (Kelly et al., 2013). Alleles associated with high *SCD* enzyme activity have been reported to be associated with increased marbling scores in Wagyu x Limousin cattle (Jiang et al., 2008). Similarly, three SNP were correlated with carcass marbling score in this study, where the C, T and G alleles of the g.21273692T>C, g.21276141C>T and g.21276672A>G loci, respectively, had favourably higher marbling scores. The missense mutation that causes substitution of valine (type V) to alanine (type A) at PCS position 878 (g.21272422C>T) had no effect on carcass traits measured in this study. These findings align with previous studies that reported no effect on backfat thickness in Spanish commercial beef and Chinese Simmental cattle (Wu et al., 2012; Avilés et al., 2013). In addition, the SNP was not associated with HCW, EMA, backfat thickness and marbling score of Japanese black cattle reported by Ohsaki et al. (2009). More studies are required to determine the mechanism behind the effect of *SCD* SNP reported in the Wagyu x Limousin cattle (Jiang et al., 2008) and lack of it in other cattle breeds (Ohsaki et al., 2009; Wu et al., 2012; Avilés et al., 2013). Accordingly, the study of Li et al. (2013) on the effect of the SNP on carcass traits of beef cattle populations indicated no significant effect on marbling score and meat colour soon after slaughter.

Genes responsible for fat deposition and metabolism are reported to influence meat colour since high fat levels accelerate lipid myoglobin oxidation and subsequently, meat discolouration, since marbling influences visually assessed meat colour (Fiems et al., 2000). The lack of effect of *SCD* SNP on meat colour observed in this study may be due to the short period between slaughter and carcass assessments.

6.1.4.3. Fatty acid synthase gene polymorphisms

The *FASN* gene encodes an essential homodimeric cytosolic enzyme critical for *de novo* lipogenesis by catalysing palmitic acid synthesis from acetyl-CoA and malonyl-CoA (Roy et al., 2005; Oh et al., 2018). Palmitic acid is a predominant fatty acid in beef and is used as a substrate for the synthesis of other fatty acids through elongation and desaturation (Sampath and Ntambi, 2005; Scollan et al., 2006). Fatty acid composition is reported to influence carcass traits (Hoehne et al., 2012; Yeon et al., 2013). The majority of association studies on *FASN* gene polymorphisms have focused on fatty acid composition (Abe et al., 2009; Matsuhashi et al., 2011; Li et al., 2012; Lee et al., 2014) with little emphasis on carcass traits (Oh et al., 2012; Raza et al., 2018). Polymorphism in the *FASN* thioesterase domain was found to have a significant effect on beef grade by influencing fat deposition in Korean cattle (Oh et al., 2018). Oh et al., (2012) examined the effect of five missense SNP in the *FASN* gene and reported an association of all the SNP with carcass backfat thickness and marbling score with no influence on carcass weight in Korean cattle. In another study, Raza et al. (2018) reported associations between g.13192T>C with backfat thickness and EMA, and g.13232C>T with IMF in Qinchuan cattle. Polymorphism in *FASN* influenced HCW, but no associations with subcutaneous fat thickness and marbling score were observed in crossbred beef cattle (Rempel et al., 2012), or with marbling score in the loin eye

muscle of purebred American Angus bulls (Zhang et al., 2008). Matsuhashi et al., (2011) reported no association between *FASN* g.16024A>G SNP with carcass weight and subcutaneous fat thickness of Japanese black cattle. In this study, polymorphisms in the g.50784533C>G and g.50792445C>T loci were correlated with subcutaneous fat depth, while g.50786221A>G and g.50787362C>T were correlated with marbling scores. The loci g.50787362C>T and g.50792445C>T are located in the intron, hence may influence on carcass fat content through regulation of alternative splicing or gene expression (Jo and Choi, 2015). Furthermore, the allelic mutations in g.50784533C>G locus in exon 13 resulted to amino acid substitution from alanine to glycine at PCS position 2066 that encodes for malonyl-CoA-/acetyl-CoA-acyl carrier protein-transacylase (AT/MT) domain (Oh et al., 2018). The AT/MT domain catalyzes the transfer of acetyl-CoA to the acyl carrier protein and transacylates the malonyl-CoA to the acyl carrier protein, which is the initial step in *de novo* fatty acids synthesis (Maier et al., 2006), thus the SNP may influence fatty acids synthesis. The differences between studies may be due to breed effect or in the SNP studied (Maharani et al., 2011; Li et al., 2021). Effects of genetic markers on phenotypic outcomes are often breed-specific, and may not be extrapolated to all cattle breeds (Shin et al., 2012; Avilés et al., 2013; Bartoň et al., 2016). Since marbling and MSA index scores were highly correlated, these findings indicate that SNP in the *FASN* gene may be used in marker-assisted selection for improved carcass grades and beef eating quality in northern Australian tropical crossbred cattle.

6.1.5. Conclusion

This study aimed to examine SNP present in the exons and introns of *FABP4*, *SCD* and *FASN* genes of northern Australian tropical crossbred beef cattle and examine their associations with the

chiller-assessed carcass traits. The results showed significant correlations between SNP in *FABP4* with hump height and loin eye muscle area, *SCD* SNP with carcass weight, loin eye muscle area and marbling while *FASN* SNP were correlated with subcutaneous fat thickness and marbling score. Therefore, the hypothesis that SNP in the *FABP4*, *SCD* and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle was accepted. These findings indicate the involvement of these genes in carcass traits of tropical crossbred beef cattle population of northern Australia and the potential to use SNP in carcass grade and meat quality improvement through marker-assisted selection.

6.1.6. Summary

This study explored the identification of single nucleotide polymorphisms (SNP) in fatty acid binding protein 4 (*FABP4*), stearoyl-CoA desaturase (*SCD*) and fatty acid synthase (*FASN*) genes that may influence the carcass traits of tropical crossbred beef cattle. The hypothesis tested was that SNP in the *FABP4*, *SCD* and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle. Fifty *Bos indicus* and *Bos taurus* crossbred steers were backgrounded on either buffel grass only or buffel grass and desmanthus mixed pastures for 147 days and finished in a commercial feedlot for 110 days. Steers were slaughtered within 48 h of leaving the feedlot within a lairage period not exceeding 12 h and carcasses graded 12 h after slaughter. Next-Generation Sequencing of the *FASN*, *FABP4* and *SCD* genes identified multiple SNP loci that were correlated and significantly associated with carcass traits. The *FABP4* g.44677205A>G locus was significantly associated with hump height and correlated with loin eye muscle area (EMA; $P < 0.05$). Polymorphism in the *SCD* gene g.21275851C>A locus was associated with subcutaneous fat depth and marbling score ($P < 0.05$).

The CC genotype had a higher subcutaneous fat depth and marbling score ($P < 0.05$) than the AA genotype. Significant correlations were observed between carcass marbling score and subcutaneous fat depth within the *FASN* SNP locus ($P < 0.05$). Therefore, the hypothesis that SNP in the *FABP4*, *SCD* and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle was accepted. These findings suggest that SNP in the *FABP4*, *SCD* and *FASN* genes may be used in carcass grade and meat quality improvement through marker-assisted selection of northern Australian crossbred beef cattle.

Chapter 6.2: Single Nucleotide Polymorphisms in Lipogenic Genes are Associated with *Longissimus dorsi* Muscle Fatty Acid Composition of Northern Australian Tropical Crossbred Beef Steers

6.2.1. Introduction

The fatty acid composition and intramuscular fat (IMF) content of beef resonate with cattle producers, processors and meat consumers due to their significant influences on shelf life (Hoa et al., 2022), eating quality (Pogorzelski et al., 2022) and human health (Patel et al., 2022). Studies suggest that dietary supplementation (Byrne et al., 2021; Correa et al., 2022), nutritional alteration (Monteiro et al., 2022), and selective breeding (Malau-Aduli et al., 2022) are management tools for manipulating meat fatty acid composition and beef quality. However, dietary manipulation of meat quality and fatty acid profile is challenging in ruminants due to rumen microbial lipolysis (Buccioni et al., 2012; Torres et al., 2021) and biohydrogenation of unsaturated to saturated fatty acids (Menci et al., 2021). Muscle fatty acid composition is less diet-dependent and more largely regulated by key lipogenic enzymes in fatty acid metabolism (Zhang et al., 2008; Ward et al., 2010).

Genetic selection and breeding of beef cattle provide a long-term, cumulative and permanent approach to improving meat fatty acid composition because of their moderate to high heritability (Malau-Aduli et al., 2000b; Malau-Aduli et al., 2000a; Malau-Aduli et al., 2000c; Malau-Aduli et al., 2000d; Inoue et al., 2011; Kelly et al., 2013). The identification of single nucleotide polymorphisms (SNP) in genes encoding key enzymes and proteins involved in fatty acids metabolism may improve the current fundamental understanding of underpinning genetic variants controlling muscle fatty acid composition. Several studies (Magee et al., 2010; Pannier et al., 2010;

Han et al., 2013; Li et al., 2013; Sugita et al., 2014; Chiaia et al., 2017; Gui et al., 2020; Pewan et al., 2021a; Fonseca et al., 2022) have shown that SNP can be used as genetic markers for improving IMF and muscle fatty acid composition in ruminant livestock. For instance, associations were reported between the growth hormone g.253 locus SNP with C14:0, C16:1 and C18:0 concentrations in Japanese Black cattle (Sugita et al., 2014), multiple autosomal SNP loci with C14:0, C16:0 and C18:0 concentrations in Nellore bulls (Chiaia et al., 2017), the diacylglycerol O-acyltransferase 1 gene SNP K232A and c.947 of μ-calpain gene with IMF in meat of five beef cattle breeds in Sweden (Li et al., 2013) and multiple SNP in the hormone-sensitive lipase gene with IMF of the Qinchuan and Nanyang cattle (Gui et al., 2020). Furthermore, the stearoyl-CoA desaturase (*SCD*) g.23881050T>C locus was significantly associated with IMF, C22:6n-3, and C22:5n-3, fatty acid binding protein 4 (*FABP4*) g.62829478A>T locus with IMF and fatty acid synthase (*FASN*) g.12323864A>G locus with C18:3n-3, C18:1n-9, C18:0 and C16:0 concentrations in Tattykeel Australian White lamb (Pewan et al., 2021a).

Three known candidate genes were selected for a targeted next-generation sequencing (NGS) of SNP in this study based on current knowledge of allelic substitutions encoding the *FASN*, *SCD*, and *FABP4* genes. The *FASN* is a complex homodimeric enzyme that regulates biosynthesis of long-chain fatty acids, and has been reported to be associated with fatty acid composition in Korean (Oh et al., 2012a), crossbred Jersey and Limousin (Morris et al., 2007), Japanese Black and Limousin crossbred (Mannen 2012; Zhang et al., 2008; Abe et al., 2009) and Angus (Zhang et al., 2008) cattle. Moreover, SNP in the gene encoding stearoyl-CoA desaturase (*SCD*), a rate-limiting enzyme that catalyses monounsaturated fatty acid (MUFA) synthesis, is reported to influence fat melting point (FMP), SFA, MUFA and polyunsaturated fatty acid (PUFA) composition in beef (Taniguchi et al., 2004; Jiang et al., 2008; Bartoň et al., 2010; Li et al., 2012). The fatty acid

binding protein 4 (*FABP4*) functions include fatty acid uptake, transport and metabolism (Bartoň et al., 2016), and the influence of *FABP4* genotypes on fatty acid composition is documented (Zalewska et al., 2021). For instance, the GG genotype of the c.388G>A, c.408G>C and c.456A>G SNP had higher MUFA composition in Korean cattle compared to the other genotypes (Oh et al., 2012b). However, SNP research on *Bos indicus* or *Bos indicus* x *Bos taurus* cattle maintained in harsh tropical environments of northern Australia is limited (Ferraz et al., 2020), hence the need for the present study in order to fill this knowledge gap.

Meat quality measurements are often attained after slaughter making it difficult to predict meat quality in living animals (Tait et al., 2014; Ardicli et al., 2017). Pewan et al. (2021) demonstrated that a combination of laboratory-based IMF, FMP, and fatty acid analyses of samples obtained through a minimally invasive biopsy sampling and Next-Generation Sequencing of polymorphisms in lipid metabolism genes is a suitable method to directly quantify the genetic worth of live animals for IMF and fatty acid composition. Therefore, this study aimed to identify targeted SNP in lipid metabolism related genes of tropically adapted crossbred beef cattle of northern Australia and determine associations with loin eye muscle fat characteristics.

6.2.2. Materials and Methods

All the study protocols followed the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, 2013) and were approved by the Commonwealth Scientific and Industrial Research Organisation Animal Ethics Committee (Approval Number 2019-38).

6.2.2.1. Animals, diets and experimental design

Sample size determination, animal management, diets composition and experimental design were previously described in detail in Chapters 4.2 and 5.2, and are not repeated herein. In summary, 48 Charbray, Brahman and Droughtmaster crossbred steers (28 – 33 months old steers with an initial average liveweight of 332 ± 21 kg were backgrounded on isonitrogenous diets of Rhodes grass hay augmented with either desmanthus, lucerne or both for 140 days in a completely randomised design. Steers were group-housed in 12 open outdoor pens and had unlimited access to clean water and mineral blocks with a five to ten per cent allowance for daily feed refusal. At the end of the study, steers were divided into two groups based on liveweight. The heavier steers (453 ± 15 kg) were transported to a commercial abattoir and slaughtered without feedlot finishing, while the lighter steers (406 ± 25 kg) were transferred to a commercial feedlot for finishing.

6.2.2.2. Loin eye muscle sampling and chemical analysis

A minimally invasive biopsy technique was used to collect loin eye muscle samples from the 12th −13th rib interface of the steers transported to the feedlot after backgrounding according to the protocol described earlier (Malau-Aduli et al., 1998). Samples from the steers slaughtered immediately after the backgrounding phase were collected from the 12th – 13th rib interface of the chilled carcasses 12 h after slaughter. The IMF of the biopsy and carcass samples was extracted as described by Flakemore et al. (2014), and FMP was determined using the slip-point method (Pewan et al., 2020). The fatty acid composition was evaluated using a gas chromatography-mass spectrometry procedure (Malau-Aduli et al., 2016).

6.2.2.3. Blood Sampling and genomic DNA extraction

Blood samples were collected into 10 mL EDTA-containing vacutainer tubes (BD, Sydney, Australia) via jugular venipuncture, transported in dry ice and stored at -80°C until needed for laboratory analysis. Blood samples were thawed at room temperature and genomic DNA was extracted from a 2 ml aliquot using the NucleoSpin Blood Kit (Macherey-Nagel GmbH and Co. KG, Duren, Germany) according to the manufacturer's instructions. DNA yield and purity were determined using NanoDrop ND-1000 (ThermoFisher Scientific Australia Pty Ltd, Victoria, Australia).

6.2.2.4. Primer design, amplification of target genes, clean-up of PCR products, library preparation, sequencing and data analysis

The procedures were carried out as described previously (Pewan et al., 2021a) with slight modifications and are described in Chapter 6.1. The target genes were amplified using the primer sequences presented in Table 6.1.1 and the gel image of the amplification products is presented in supplementary Figure S6.1.1. The Hereford cattle breed sequences NC_ 037353.1, NC_ 037346.1 and NC_ 037341.1 obtained from the GenBank database were used as the *SCD*, *FASN* and *FABP4* reference sequences, respectively.

6.2.2.5. Calculations and statistical analysis

Data analyses and the plotting of figures were conducted using the R software v.4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). The GDIcall online calculator (http://www.msrcall.com/Gdicall.aspx, accessed on 14 January 2022) was used to calculate SNP polymorphism information content (PIC). Hardy-Weinberg equilibrium (HWE) and expected

heterozygosity (He) were calculated according to the methods described by Nei and Roychoudhury (1974). The HWE was tested for each identified SNP locus using the Chi-square test. Summary statistics including range, means and standard deviations were computed and checked for data entry errors and outliers. The degree of linkage disequilibrium between each pair of loci was examined using distance-based hierarchical clustering of SNP loci (Lin et al., 2012) and the results presented as dendrograms and heat maps. Linear correlations between genomic variants and muscle IMF, FMP and fatty acid composition were estimated using Spearman's ρ correlations. Generalised least square procedure was used to fit linear models to investigate SNP associations with the loin eye muscle IMF, FMP and fatty acid composition. Differences between means were compared using the Tukey-adjusted multiple comparisons test with a threshold for significance set at $P < 0.05$.

6.2.3. Results

6.2.3.1. Genetic diversity of the identified single nucleotide polymorphisms

In total, 88 SNP comprising 16, 42 and 30 SNP for *FABP4*, *SCD* and *FASN* genes respectively, were identified (Supplementary Table S6.2.1). Thirty-five of the 88 SNP were not found in the Bovine Genome Variation Database (BGVD) (http://animal.nwsuaf.edu.cn/code/index.php/BosVar, accessed on 28 January 2022), and were deemed novel. All SNP had 0.11 – 0.50 minor allele frequency, 0.20 – 0.50 He and 0.18 – 0.38 PIC. All the SNP were in HWE except *FASN* g.50784824G>A (rs209227647), g.50785253C>T (novel), g.50786977A>G (novel), g.50788575T>C (rs41919993) and g.50790973C>A (rs109149276) ($P \leq 0.04$). Many of the identified SNP were located in the introns. The distance-based hierarchical clustering of SNP loci indicated presence of linkage disequilibrium between SNP loci (Figure S6.2.1-S4). The FABP4

SNP loci formed three clusters but g.44677611G>C (rs41729172) was not in linkage disequilibrium with other *FABP4* loci (Supplementary Figure S6.2.1). A similar trend was observed for the *SCD* gene SNP loci (Supplementary Figure S6.2.2) but not for the *FASN* gene. All the *FASN* SNP loci were in linkage disequilibrium with at least one other locus (Supplementary Figure S6.2.3). Only nine SNP were non-synonymous amino acid substitutions (Table 6.2.1).

Table 6.2.1. Single nucleotide polymorphisms of the *FABP4*, *SCD* and *FASN* genes, protein coding sequence positions and non-synonymous amino acid substitutions.

Gene[1]	SNP (Variant ID)[2]	PCS position[4]	Amino acid substitution
FABP4	g.44677959T>C (rs110757796)	220	Isoleucine to Valine
SCD	g.21272422C>T (rs41255693)	878	Alanine to Valine
FASN	g.50782773G>A (rs715140536)	1243	Alanine to Threonine
	g.50784533C>G (rs481622676)	2066	Alanine to Glycine
	g.50784824G>A (rs209227647)	2252	Arginine to Histidine
	g.50786496A>G[3]	3145	Serine to Glycine
	g.50788575T>C (rs41919993)	4168	Tyrosine to Histidine
	g.50789448C>T (rs516607144)	4693	Leucine to Phenylalanine
	g.50790973C>A (rs109149276)	5572	Leucine to Isoleucine

[1]*FABP4*: Fatty acid binding protein 4, *SCD*: Stearoyl-CoA desaturase, *FASN*: Fatty acid synthase
[2]SNP: Single nucleotide polymorphism. Variant dbSNP ID are based on the Bovine Genome Variation Database (BGVD).
[3]SNP not listed in BGVD
[4]PCS: Protein coding sequence

6.2.3.2. Correlations between single nucleotide polymorphisms, intramuscular fat, fat melting point and fatty acid composition

The clustering patterns of the SNP loci among steers with regard to the *FABP4*, *SCD* and *FASN* genes are presented in Figure 6.2.1(A), 6.2.2(A) and 6.2.3(A), respectively. There was less

variability in heterozygosity and homozygosity of closely related individuals for the *FASN* gene SNP compared to the *FABP4* and *SCD* genes. Most SNP were in linkage disequilibrium but a few SNP depicted no linkage Figure 6.2.1(B), 6.2.2(B) and 6.2.3(B). Four *FABP4* gene SNP – g.44677205A>G (rs109388335), g.44677239C>G (rs110383592), g.44678114G>C (novel) and g.44678641T>C (rs110490217) – were positively correlated with linoleic acid (C18:2n6). One SNP (g.44680048A>G; rs468994137) on the other hand, was negatively correlated with linoleic acid concentration, while g.44677587G>C (rs723716479) was positively correlated with conjugated linoleic acid (CLA) (Figure 6.2.1(B); $P < 0.05$). Fourteen *SCD* SNP were negatively correlated, while g.21266629G>T (novel) and g.21271645G>A (rs380628677) were positively correlated with eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) (Figure 6.2.1(B); $P < 0.05$). No correlation was observed between *FABP4* and *SCD* SNP with IMF, FMP, SFA or MUFA. In contrast, *FASN* g.50784242C>T (rs800844468) SNP was positively correlated with IMF, palmitic acid (16:0), oleic acid (18:1), SFA and MUFA (Figure 6.2.3(B); $P < 0.05$). The CLA concentration was negatively correlated with g.50787886A>G (novel), g.50788691T>C (rs526036338) and g.50788956C>T (novel) while g.50792445C>T (novel) was negatively correlated with EPA and the sum of EPA and DHA ($P < 0.05$). Several fatty acids were positively correlated ($P < 0.05$) and no significant negative correlations between quantified fatty acids were observed. Highly positive correlations (≥ 0.7) between palmitic acid, palmitoleic acid, stearic acid and oleic acid with the CLA, SFA and MUFA levels were observed ($P < 0.01$).

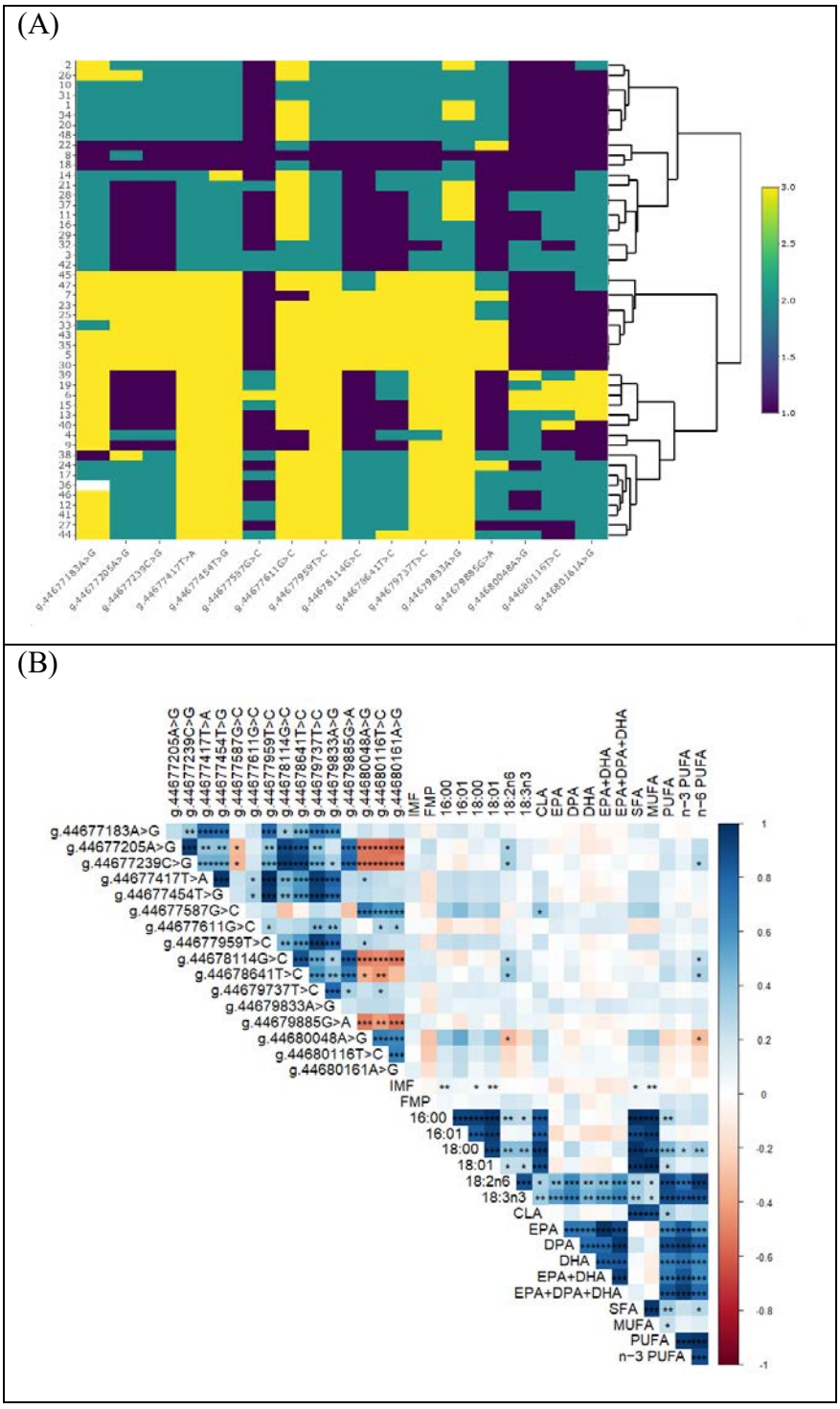


Figure 6.2.1. Single nucleotide polymorphisms in the *FABP4* gene. (A) Clustering map of genetic variants; homozygotes similar to the reference sequence genotype (Hereford), heterozygotes and alternative allele homozygotes. (B) Correlations between SNP and IMF, FMP and fatty acids. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

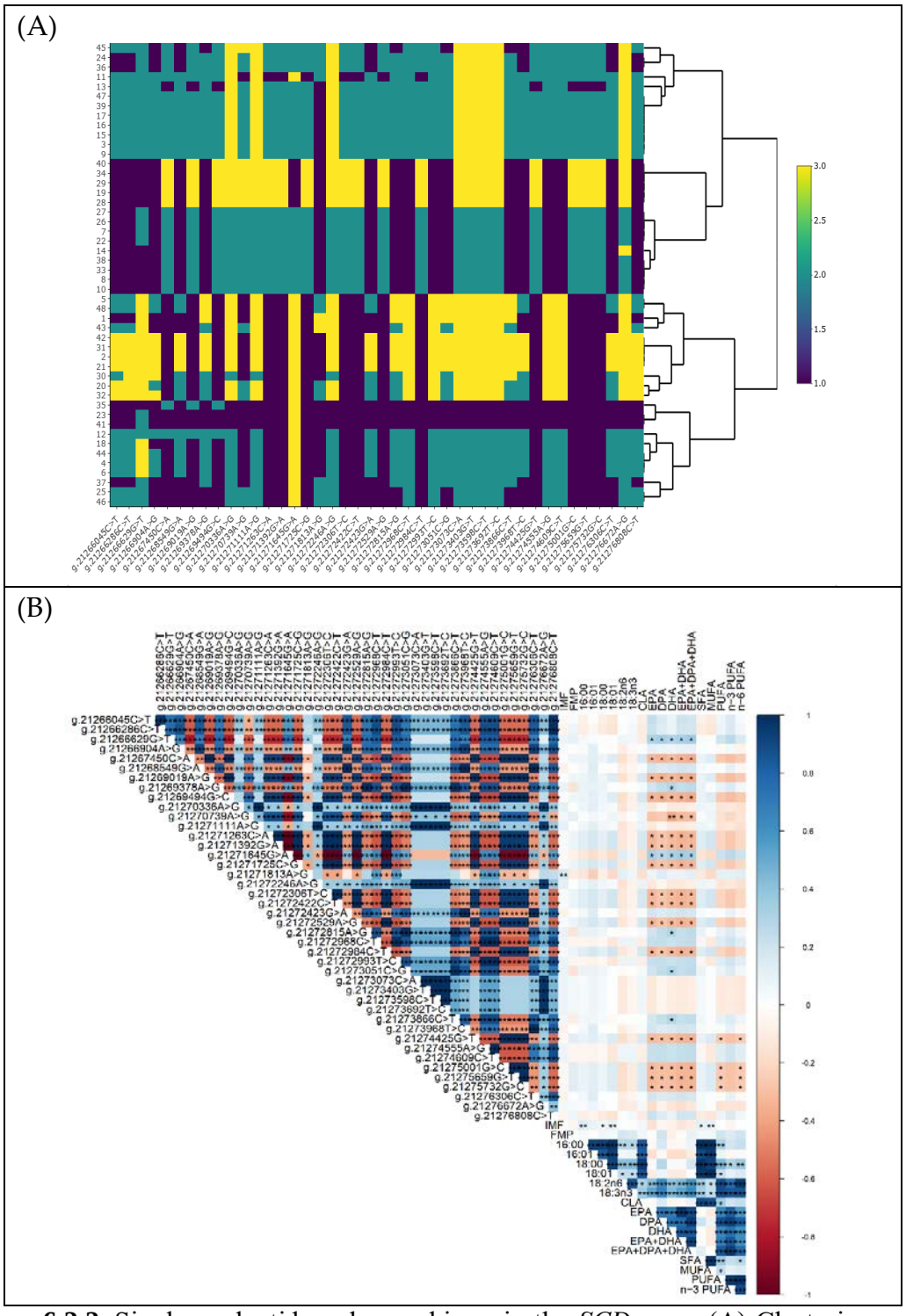


Figure 6.2.2. Single nucleotide polymorphisms in the *SCD* gene. (A) Clustering map of genetic variants; homozygotes similar to the reference sequence genotype (Hereford), heterozygotes and alternative allele homozygotes. (B) Correlations between SNP and IMF, FMP and fatty acids. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

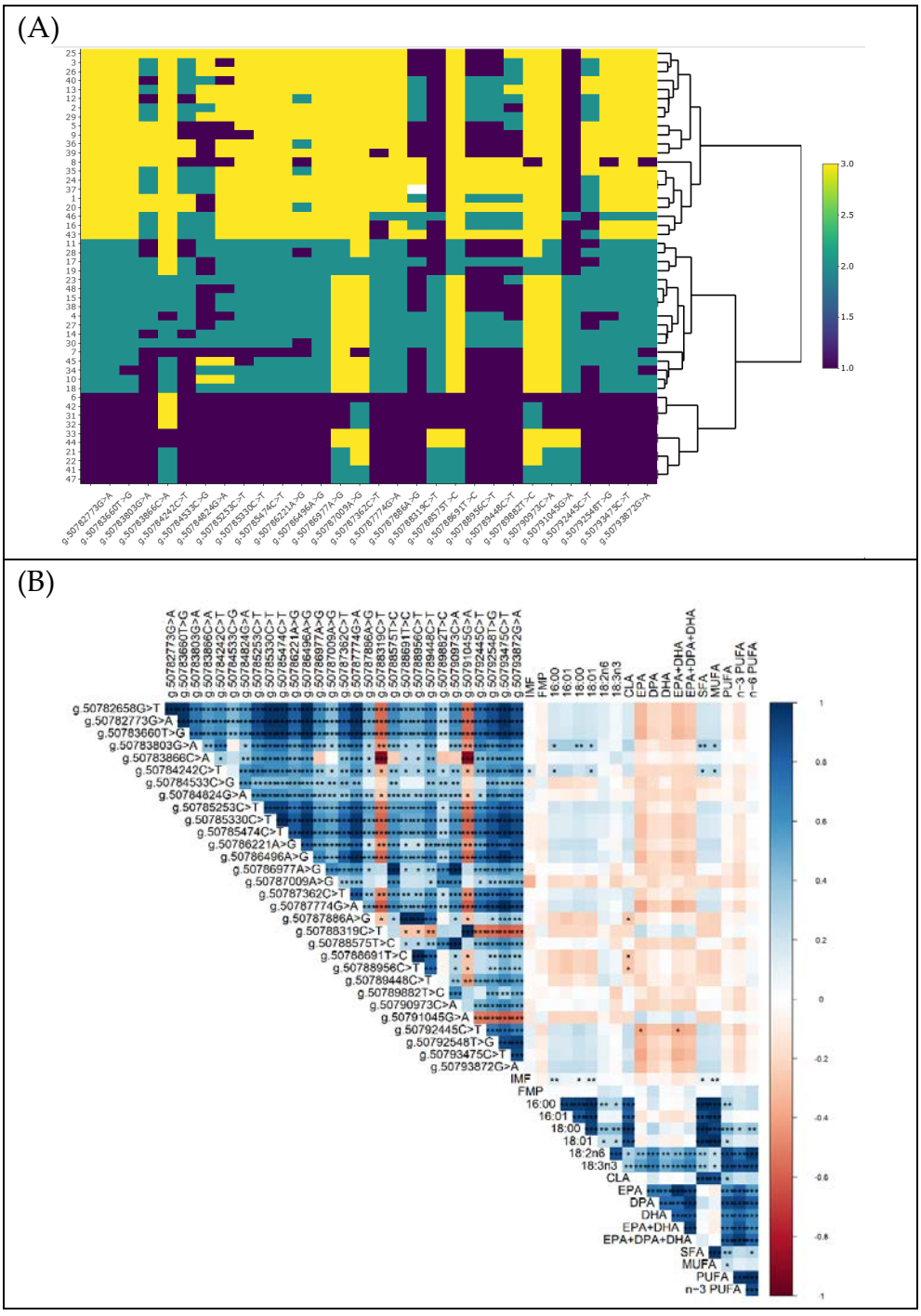


Figure 6.2.3. Single nucleotide polymorphisms on the *FASN* gene. **(A)** Clustering map of genetic variants; homozygotes similar to the reference sequence genotype (Hereford), heterozygotes and alternative allele homozygotes. **(B)** Correlations between SNP and IMF, FMP and fatty acids. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

6.2.3.3. Associations between single nucleotide polymorphisms, intramuscular fat, fat melting point and fatty acid composition

Associations between *FABP4* g.44677239C>G (rs110383592), *SCD* g.21266629G>T (novel) and *FASN* g.50783803G>A (novel) are presented in Table 6.2.2. No significant associations were observed between the *FASN* g.50783803G>A with IMF, FMP or any fatty acid examined. However, *FABP4* g.44677239C>G was significantly associated with linoleic acid (P = 0.03). Linoleic acid was lower for the CC than the GG genotypes at 45.8 ± 10.88 mg/100g and 54.5 ± 7.3 mg/100g, respectively (P = 0.02), but CG was not significantly different from the homozygotes (Figure 6.2.4). Significant associations between the *SCD* g.21266629G>T SNP with DPA, DHA, EPA+DHA and EPA+DPA+DHA were observed ($P \leq 0.02$). Multiple comparisons in Figure 6.2.5 indicate that EPA, DPA and DHA were significantly higher for the TT compared to the GG genotypes ($P \leq 0.03$). The DHA level was lower (P = 0.02) while EPA and DPA tended to be lower for the TT compared to the GT variants ($P \leq 0.08$). No significant difference was observed for EPA, DPA and DHA in GT compared to GG variants ($P \geq 0.47$). The IMF and FMP levels were not associated with either *FABP4* g.44677239C>G, *SCD* g.21266629G>T or *FASN* g.50783803G>A ($P \geq 0.38$).

Table 6.2.2. Least Square Means ± SD of loin eye muscle IMF (%), FMP (°C) and fatty acid concentrations (mg/100g fresh muscle) by genotype at the *FABP4* g.44677239C>G, *SCD* g.21266629G>T and *FASN* g.50783803G>A SNP loci.

Gene/SNP [1]					*P*-value[2]
***FABP4* g.44677239C>G**	Total (n = 48)	CC (n = 19)	CG (n = 19)	GG (n = 10)	
IMF	2.3 ± 0.75	2.1 ± 0.62	2.5 ± 0.9	2.2 ± 0.67	0.38
FMP	43.9 ± 4.79	42.7 ± 4.58	44.6 ± 4.83	44.9 ± 5.13	0.53
16:0 (Palmitic acid)	209.1 ± 149.68	194.3 ± 113.24	238.7 ± 185.99	179.5 ± 133.59	0.72
16:1 (Palmitoleic acid)	34.6 ± 34.72	40.5 ± 47.35	34.5 ± 25.6	23.3 ± 16.87	0.52
18:0 (Stearic acid)	128.6 ± 78.22	119.2 ± 66.31	139.4 ± 83.68	125.1 ± 92.35	0.61
18:1 (Oleic acid)	263.1 ± 195.31	244.6 ± 143.89	302.4 ± 245.96	221.4 ± 170.23	0.74
18:2n6 (Linoleic acid)	50.1 ± 10.2	45.8 ± 10.88 [a]	52.0 ± 9.62 [ab]	54.5 ± 7.3 [b]	0.03
18:3n3 (α-linolenic acid)	16.3 ± 3.14	15.7 ± 3.69	16.4 ± 2.85	16.9 ± 2.73	0.73
CLA	4.3 ± 3.33	4.2 ± 3.11	4.4 ± 3.49	4.1 ± 3.77	0.79
EPA	9.4 ± 2.18	9.2 ± 2.13	9.8 ± 2.31	9.1 ± 2.12	0.58
DPA	14.0 ± 3.37	13.0 ± 3.61	15.0 ± 2.64	13.9 ± 3.92	0.12
DHA	2.3 ± 0.8	2.3 ± 0.93	2.5 ± 0.69	2.2 ± 0.79	0.28
EPA+DHA	11.8 ± 2.77	11.5 ± 2.81	12.3 ± 2.76	11.4 ± 2.86	0.33
EPA+DPA+DHA	25.8 ± 5.79	24.6 ± 5.88	27.4 ± 5.23	25.3 ± 6.53	0.14
SFA	376.9 ± 254.18	351.5 ± 201.61	420.0 ± 298.96	340.8 ± 260.83	0.75
MUFA	313.4 ± 228.69	294.7 ± 173.9	358.2 ± 285.22	261.9 ± 199.01	0.69
PUFA	142.8 ± 26.51	134.2 ± 28.71	149.6 ± 23.23	146.3 ± 25.98	0.41
n-3 PUFA	48.2 ± 8.92	47.0 ± 10.06	49.9 ± 7.84	47.5 ± 8.96	0.55
n-6 PUFA	79.6 ± 16.18	73.0 ± 18.67	83.6 ± 13.12	84.5 ± 13.17	0.11
***SCD* g.21266629G>T**	Total (n = 48)	GG (n = 11)	GT (n = 22)	TT (n = 15)	
IMF	2.3 ± 0.75	2.2 ± 0.55	2.2 ± 0.62	2.5 ± 1.03	0.64
FMP	43.9 ± 4.79	42.9 ± 3.64	44.1 ± 6.17	44.3 ± 3.26	0.78
16:0 (Palmitic acid)	209.1 ± 149.68	182.2 ± 111.02	201.0 ± 116.16	240.2 ± 209.36	0.86
16:1 (Palmitoleic acid)	34.6 ± 34.72	27.4 ± 16.93	38.4 ± 44.8	34.2 ± 27.7	0.91
18:0 (Stearic acid)	128.6 ± 78.22	125.6 ± 76.49	122.6 ± 60.52	139.2 ± 102.52	0.82
18:1 (Oleic acid)	263.1 ± 195.31	241.6 ± 164.92	255.0 ± 152.87	290.1 ± 266.99	0.82
18:2n6 (Linoleic acid)	50.1 ± 10.2	50.0 ± 5.35	48.0 ± 10.93	53.2 ± 11.52	0.66
18:3n3 (α-linolenic acid)	16.3 ± 3.14	16.5 ± 2.66	15.5 ± 3.24	17.1 ± 3.28	0.49
CLA	4.3 ± 3.33	4.7 ± 4.46	4.3 ± 2.71	4.0 ± 3.4	0.57
EPA	9.4 ± 2.18	8.6 ± 1.61 [a]	9.2 ± 2.43 [ab]	10.3 ± 1.93 [b]	0.08

DPA	14.0 ± 3.37	12.9 ± 1.78 [a]	13.4 ± 3.75 [ab]	15.7 ± 3.2 [b]	0.03
DHA	2.3 ± 0.8	2.1 ± 0.51 [a]	2.2 ± 0.88 [a]	2.8 ± 0.74 [b]	0.02
EPA+DHA	11.8 ± 2.77	10.8 ± 1.89 [a]	11.4 ± 3.07 [ab]	13.1 ± 2.48 [b]	0.03
EPA+DPA+DHA	25.8 ± 5.79	23.7 ± 3.21 [a]	24.8 ± 6.31 [ab]	28.9 ± 5.51 [b]	0.02
SFA	376.9 ± 254.18	345.6 ± 210.17	360.6 ± 197.48	422.7 ± 348.72	0.89
MUFA	313.4 ± 228.69	287.2 ± 191.76	303.9 ± 181.66	345.7 ± 310.88	0.85
PUFA	142.8 ± 26.51	138.0 ± 15.98	137.8 ± 29.03	153.7 ± 26.98	0.31
n-3 PUFA	48.2 ± 8.92	45.5 ± 5.37	47.2 ± 10.49	51.8 ± 7.77	0.12
n-6 PUFA	79.6 ± 16.18	78.5 ± 7.52	75.9 ± 19.07	85.9 ± 15.07	0.40
***FASN* g.50783803G>A**	Total (n = 48)	GG (n = 20)	GA (n = 20)	AA (n = 8)	
IMF	2.3 ± 0.75	2.2 ± 0.71	2.3 ± 0.60	2.5 ± 1.14	0.49
FMP	43.9 ± 4.79	44.8 ± 3.56	43.1 ± 6.01	43.8 ± 3.62	0.40
16:0 (Palmitic acid)	209.1 ± 149.68	161.3 ± 84.44	211.4 ± 131.91	323.2 ± 248.45	0.24
16:1 (Palmitoleic acid)	34.6 ± 34.72	26.0 ± 13.60	41.8 ± 46.84	45.3 ± 31.24	0.17
18:0 (Stearic acid)	128.6 ± 78.22	103.2 ± 38.01	129.8 ± 76.27	189.3 ± 123.57	0.28
18:1 (Oleic acid)	263.1 ± 195.31	196.9 ± 114.57	279.5 ± 185.13	389.2 ± 309.04	0.16
18:2n6 (Linoleic acid)	50.1 ± 10.20	50.5 ± 10.29	48.9 ± 9.88	52.1 ± 11.72	0.95
18:3n3 (α-linolenic acid)	16.3 ± 3.14	16.3 ± 3.00	16.1 ± 3.34	16.8 ± 3.32	0.87
CLA	4.3 ± 3.33	3.4 ± 1.70	4.8 ± 4.01	5.6 ± 4.49	0.35
EPA	9.4 ± 2.18	9.9 ± 2.25	9.0 ± 2.05	9.3 ± 2.40	0.52
DPA	14.0 ± 3.37	14.7 ± 2.78	13.1 ± 3.45	14.5 ± 4.40	0.53
DHA	2.3 ± 0.80	2.4 ± 0.78	2.3 ± 0.8	2.1 ± 0.91	0.48
EPA+DHA	11.8 ± 2.77	12.3 ± 2.89	11.4 ± 2.5	11.4 ± 3.25	0.44
EPA+DPA+DHA	25.8 ± 5.79	27.1 ± 5.48	24.6 ± 5.34	26.0 ± 7.57	0.48
SFA	376.9 ± 254.18	294.0 ± 135.9	381.0 ± 229.59	574.6 ± 417.76	0.28
MUFA	313.4 ± 228.69	235.3 ± 132.97	332.1 ± 217.83	464.1 ± 359.61	0.20
PUFA	142.8 ± 26.51	143.9 ± 22.70	139.2 ± 25.97	148.9 ± 37.51	0.90
n-3 PUFA	48.2 ± 8.92	48.9 ± 8.18	47.4 ± 9.19	48.9 ± 10.91	0.82
n-6 PUFA	79.6 ± 16.18	81.5 ± 14.45	76.8 ± 16.70	79.6 ± 19.88	0.89

[1]IMF, intramuscular fat; FMP, fat melting point; CLA, conjugated linoleic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 and 21:0; MUFA is the sum of 14:1,16:1n-13t, 16:1n-9, 16:1n-7, 16:1n-7t, 16:1n-5c, 17:1n-8, 18:1n-7t,18:1n-5, 18:1n-7, 18:1n-9, 18:1a, 18:1b, 18:1c, 19:1a, 19:1b, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11 and 24:1n-9; PUFA is the sum of 18:4n-3, 18:3n-6, 18:2n-6, 18:3n-3, 20:2n-6, 20:3, 20:3n-6, 20:4n-3, 20:4n-6, 20:5n-3,

22:4n-6, 22:5n-3, 22:5n-6 and 22:6n-3; n-3 PUFA is the sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 PUFA is the sum of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6.

[ab] Means followed by different lowercase superscripts differ significantly

[2]ANOVA *P*-value

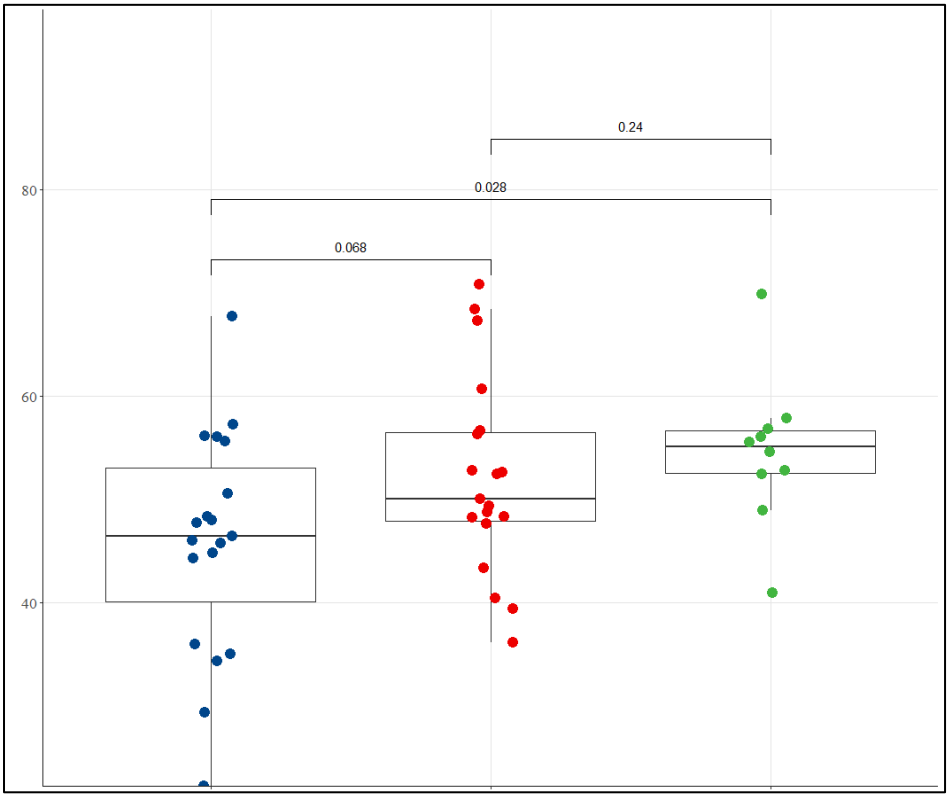


Figure 6.2.4. Multiple comparisons of loin eye muscle linoleic acid content between genotype variants at the *FABP4* g.44677239C>G SNP locus CC (●), CG (●) and GG (●).

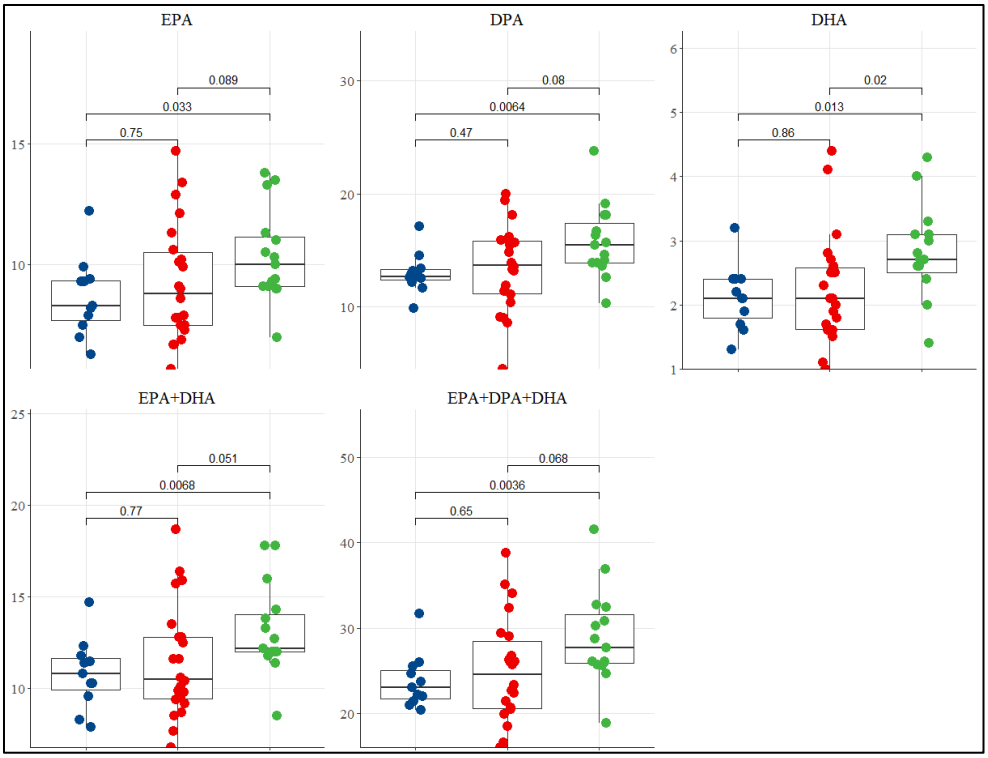


Figure 6.2.5. Multiple comparisons of loin eye muscle EPA, DPA, DHA, EPA+DHA and EPA + DPA + DHA content between genotype variants at the *SCD* g.21266629G>T SNP locus GG (●), GT (●) and TT (●).

6.2.4. Discussion

Meat fatty acid composition influences meat shelf life, eating quality and consumers' health (Colussi et al., 2017; Lee et al., 2017; Pećina and Ivanković, 2021). Although many studies have reported that diet modulation influences meat fatty acid composition, it is more difficult in ruminants compared to monogastric livestock due to microbial lipolysis (Buccioni et al., 2012; Torres et al., 2021) and biohydrogenation of unsaturated to saturated fatty acids in the rumen (Menci et al., 2021). As a result, meat fatty acids are more saturated in ruminant than in monogastric animals (Castillo-González et al., 2014; Alves et al., 2017). On the other hand, studies have reported that fatty acid composition is heritable (Malau-Aduli et al., 2000b; Pitchford et al., 2002; Nogi et al., 2011; Sakuma et al., 2017). A recent study by Sakuma et al. (2017) reported

medium to high heritability estimates of 0.48 to 0.85 for six out of the eight fatty acids analysed. Therefore, there is an increased research interest in breeding, selecting and producing farm animals with desirable fatty acid composition (Maharani et al., 2011).

Selection and breeding provide a long-term alternative to improving marbling level (Nguyen et al., 2021), and meat fatty acid composition (Das et al., 2022; Maiorano et al., 2022). Several SNP in genes encoding key enzymes and proteins involved in fatty acid metabolism have been reported as potential genetic markers for the improvement of IMF and fatty acid composition in different cattle breeds (Mannen, 2012; Oh et al., 2012a; Bartoň et al., 2016). This study examined SNP in the *FABP4*, *SCD* and *FASN* genes of northern Australian tropical crossbred beef cattle and identified SNP with significant influences on fatty acid composition of the loin eye muscle.

6.2.4.1. Fatty acid binding protein 4 gene polymorphisms

The *FABP4* gene is an important protein for long-chain fatty acid transport in mammals, and its polymorphism is associated with growth, fat deposition and carcass traits in cattle (Cho et al., 2008; Yan et al., 2018; Yin et al., 2020). Substitution of the G to C allele of the g.44677587 (rs723716479) loci was positively correlated with CLA, previously inversely linked with the risk of colorectal and breast cancer in some population-based studies (Van Vliet et al., 2021). The observed trend where the homozygous GG variant had the highest linoleic acid levels (almost 10 mg/100g higher than homozygous CC in the g.44677239C>G loci) may indicate higher inflammatory eicosanoids synthesis. Linoleic acid is a building block in the synthesis of arachidonic acid, the precursor for prostaglandins and other inflammatory eicosanoids (Jandacek, 2017). In contrast to findings of this study, variation in the g.44677959T>C (c.220) influenced palmitoleic acid in Japanese Black cattle (Hoashi et al., 2008). This discrepancy may be due to

epistatic interaction of the g.44677959T>C locus with polymorphisms at another locus in line with the observations of Xu et al. (2021) on the effect of polymorphisms on *FABP4* protein structure. They reported that the wild type protein with isoleucine in amino acid 74 had 58.33% sheet and 29.55% loop interactions that changed to 59.09% sheet and 28.79% loop when isoleucine was substituted with valine. This discrepancy may also be due breed differences since the Japanese Black cattle are reported to be genetically predisposed to producing carcass lipids with higher concentration of MUFA, including palmitoleic acid, compared to other cattle breeds such as Japanese Brown, Holstein or Charolais steers, likely due to the activity of the delta 9 desaturase on palmitic acid (Zembayashi et al., 1995; Gotoh et al., 2014).

6.2.4.2. Stearoyl-CoA desaturase gene polymorphisms

For most diets, approximately 70% to 95% and 85% to 100% n-6 PUFA and n-3 PUFA, respectively, are hydrogenated in the rumen (Van Tran et al., 2017). As a result, fatty acids are absorbed almost entirely as SFA and biohydrogenation intermediates comprising conjugated di- or trienoic fatty acids and trans-11 fatty acids, notably trans-vaccenic acid, due to chemical reduction of unsaturated fatty acids in the rumen by microorganisms in ruminants (Taniguchi et al., 2004a; Van Tran et al., 2017). Therefore, the composition of fatty acid stored in the fat depots mirror the action of *SCD* on fatty acid substrates (Kim and Ntambi, 1999). The enzyme *SCD* catalyses the desaturation of SFA and MUFA by inserting a cis-double bond in the delta (Δ) 9 position of SFA substrates, with a higher preference for palmitic acid and stearic acid substrates transformed into palmitoleic acid and oleic acid, respectively (Kim and Ntambi, 1999; Ohsaki et al., 2009; Maharani et al., 2011). Nucleotide substitution of C with T identified in the fifth exon of bovine *SCD* gene at the 878 CDS causes the replacement of the amino acid alanine with valine

(Taniguchi et al., 2004b). The replacement caused significantly higher MUFA and lower FMP in *M. trapezius* of CC compared to TT genotype cattle (Taniguchi et al., 2004b). Similarly, Flekvieh bulls with the CC genotype had lower SFA and higher MUFA compared to the TT, but CC and the CT genotypes were similar (Bartoň et al., 2010). The TT genotype of Chinese Simmental cattle were reported to have lower IMF compared to the CC genotype, but no difference was found between the heterozygous (CT) and either of the homozygous genotypes (Wu et al., 2012). Additionally, the SNP had a significant association with stearic acid, oleic acid, SFA and MUFA in Japanese black cattle with higher MUFA and lower SFA reported in animals with the CC variant (Ohsaki et al., 2009). In contrast, the SNP (g.21272422C>T) did not have significant effect on palmitic acid, stearic acid, palmitoleic acid or oleic acid in the present study. However, findings of this study concur with a previous study that reported no effect of the SNP with palmitic acid, stearic acid, palmitoleic acid or oleic acid in Canadian Angus and Charolais-based commercial crossbred beef steers (Li et al., 2012). Also, Dujková et al. (2015) found that the SNP did not influence fatty acid composition in Aberdeen Angus and Blonde d'Aquitaine cattle. Unsaturated fatty acids are synthesized through the activity of Δ5, Δ6 or Δ9 desaturases (Shingfield et al., 2013), hence the difference between studies may be due to the activity of other desaturases or other genes (Taniguchi et al., 2004b; Yokota et al., 2012). The *SCD* genotype was reported to explain only 4% of the MUFA composition in Japanese Black cattle (Taniguchi et al., 2004b), and 5% in MUFA and 4% oleic acid variation, respectively, in Wagyu x Limousin cattle (Jiang et al., 2008). The significant correlations between EPA, DPA and DHA with at least 16 *SCD* SNP observed in this study corroborate the findings of a previous study in sheep that recorded significant correlations between two *SCD* SNP and n-3 long-chain PUFA (Pewan et al., 2021a). The three n-3 long-chain PUFA are synthesized from alpha-linolenic acid through the activity of Δ6 desaturase

and Δ5 desaturase among other enzymes, but not Δ9 desaturase since alpha-linolenic acid already has a double bond between C9 and C10 (Calder and Yaqoob, 2009; Cherfaoui et al., 2012). Therefore, the correlation may be due to linkage disequilibrium between the *SCD* SNP and other loci responsible for the synthesis of n-3 long-chain PUFA. Nonetheless, the significant correlations of *SCD* SNP with the EPA, DPA and DHA with no influence on the SFA and MUFA observed in this study suggests that the SNP can be used as markers to select cattle for improved health beneficial n-3 long-chain PUFA with no negative influence on meat eating quality denoted by the lack of correlation with oleic acid; the most abundant fatty acid in beef that is reported to improve fat softness and meat palatability (Smith, 2016).

Seafood sources including fish, crustaceans and molluscs are recognized as the best dietary sources of long-chain n-3 oils (C. Wang et al., 2006). However, sustainability of seafood as a source of n-3 LC-PUFA is threatened by the global decline in wild-harvest fish stocks (Nichols et al., 2010), high cost of seafood (Kennedy et al., 2012) and low availability of seafood in many geographical locations (Walker et al., 2015). On the other hand, beef contributes significantly to meat intake as it is the third most consumed meat in the world at 6.3 kg per capita (Organisation for Economic Co-operation and Development, 2021). Therefore, the significant correlations of SCD SNP with the EPA, DPA and DHA suggests that marker assisted selection can be used to provide a sustainable source of dietary n-3 LC-PUFA in communities where beef constitutes a significant proportion of the diet.

6.2.4.3. Fatty acid synthase gene polymorphisms

The *FASN* gene is located in the BTA19 region where quantitative trait loci affecting milk fat content, meat fatty acid composition and related traits had been previously identified (Abe et al.,

2009; Zhu et al., 2017). The enzyme *FASN* catalyses the *de novo* synthesis of palmitic acid, a substrate for palmitoleic acid synthesis through desaturation, and stearic acid through elongation (Sampath and Ntambi, 2005; Scollan et al., 2006; Clarke and Nakamura, 2013). Genome-wide association studies with varying breeds of cattle have reported significant effect of *FASN* SNP on intramuscular composition of SFA, MUFA and linoleic acid (Uemoto et al., 2011; Li et al., 2016; Zhu et al., 2017; Bhuiyan et al., 2018; Dawood et al., 2021). Previous studies had reported that *FASN* polymorphism significantly influenced the intramuscular composition of oleic acid, SFA and MUFA in Fleckvieh bulls (Bartoň et al., 2016), and palmitic acid, palmitoleic acid, oleic acid, SFA and MUFA of the intramuscular adipose tissue in Japanese Black cattle (Abe et al., 2009; Matsuhashi et al., 2011; Yokota et al., 2012). Zhang et al. (2008) reported an additive effect of the g.17924A>G SNP on fatty acid composition, where the G allele was associated with higher MUFA and lower SFA compared to the A allele in purebred Angus bulls. The SNP also influenced palmitoleic acid and oleic acid composition in commercial crossbred beef steers (Li et al., 2012), and palmitic acid, palmitoleic acid, oleic acid, total MUFA, SFA and marbling score in Korean cattle (Bhuiyan et al., 2009; Yeon et al., 2013; Lee et al., 2014). In addition, *FASN* polymorphisms influenced SFA, MUFA and PUFA in Chinese Holstein cattle (Li et al., 2016). Oh et al. (2012) reported associations of five *FASN* exonic SNP with intramuscular fatty acid composition in Korean cattle. These findings align with this current study where *FASN* g.50784242C>T was positively correlated with IMF, palmitic acid, oleic acid, SFA and MUFA, while g.50783803G>A was correlated with palmitic acid, stearic acid, oleic acid, SFA and MUFA. Majority of the previous studies suggested that polymorphisms influenced the tissue fatty acid composition through amino acids substitutions on the b-ketoacyl reductase domain and the thioesterase domain by changing the spatial structure of the substrate-binding site (Abe et al., 2009; Bhuiyan et al.,

2009; Yeon et al., 2013). However, the g.50784242C>T was a synonymous mutation, while g.50783803G>A was in the intron, thus they did not influence the production of missense codons, but may have exerted their effect by changing the splicing regulatory sequences (Oh et al., 2012). The effect of g.50784242C>T on palmitic acid, oleic acid, SFA and MUFA may be due to the differences in IMF content. A review by De Smet et al. (De Smet et al., 2004) reported a linear increase in SFA and MUFA expressed in mg/100g muscle (r = 0.98) with IMF content.

Tissue CLA is primarily derived from endogenous synthesis from *trans-11* C18:1 (vaccenic acid) by the *SCD* activity (Mosley et al., 2006), and to a lesser extent, as an intermediate of microbial fatty acid biohydrogenation in the rumen (Guo, 2009; Dervishi et al., 2010). In this study, SNP of the g.50787886A>G, g.50788691T>C and g.50788956C>T loci were found to be correlated with high CLA levels. Although these polymorphisms were either synonymous or located in the intron, they may have influenced the function of *FASN* in palmitic acid synthesis, the substrate for *trans-11* C18:1 and subsequently CLA synthesis. These findings suggest that these loci may be used to select cattle with high CLA composition, a fatty acid associated with lower risk for atherosclerosis, diabetes and cancer (Dervishi et al., 2010; Van Vliet et al., 2021). Put together, these findings indicate that polymorphisms on the *FASN* gene can be used to select individuals for improved IMF and fatty acid composition of northern Australian tropical crossbred beef cattle.

6.2.5. Conclusions

This study aimed to investigate the targeted identification of SNP in the *FABP4*, *SCD* and *FASN* genes and their associations with fatty acid composition in the loin eye muscle of northern Australian tropical crossbred beef cattle. Single nucleotide polymorphisms on the *FABP4* gene significantly influenced linoleic acid, *SCD* was associated with long-chain n3 PUFA and *FASN*

impacted IMF, SFA, MUFA, CLA and EPA compositions. These findings not only provide insights into the genetic role of SNP in fat deposition and lipid metabolism in tropical crossbred cattle of northern Australia, but also their potential use in marker-assisted selection and breeding for improved meat eating quality. The tested hypothesis of significant associations between SNP loci encoding for the fatty acid binding protein 4, stearoyl-CoA desaturase and fatty acid synthase genes and human health beneficial omega-3 long-chain polyunsaturated fatty acids within the loin eye muscle of northern Australian crossbred beef cattle is therefore acceptable. This is the first study that demonstrates the presence of single nucleotide polymorphisms in lipogenic genes in northern Australian crossbred beef cattle which, constitute over 50% of Australian beef production and exports.

6.2.6. Summary

This study aimed to identify single nucleotide polymorphisms (SNP) in lipogenic genes of northern Australian tropically adapted crossbred beef cattle and to evaluate associations with healthy lipid traits of the longissimus dorsi (loin eye) muscle. The hypothesis tested was that there are significant associations between SNP loci encoding for the fatty acid binding protein 4 (*FABP4*), stearoyl-CoA desaturase (*SCD*) and fatty acid synthase (*FASN*) genes and human health beneficial omega-3 long-chain polyunsaturated fatty acids within the loin eye muscle of northern Australian crossbred beef cattle. Brahman, Charbray and Droughtmaster crossbred steers were backgrounded on Rhodes grass hay augmented with desmanthus, lucerne, or both, for 140 days and the loin eye muscle sampled for intramuscular fat (IMF), fat melting point (FMP) and fatty acid composition. Polymorphisms in *FABP4*, *SCD* and *FASN* genes with significant effects on lipid traits were identified using next-generation sequencing. The GG genotype at the *FABP4* g.44677239C>G

locus was associated with the highest proportion of linoleic acid than the CC and CG genotypes ($P < 0.05$). Multiple comparisons of genotypes at the *SCD* g.21266629G>T locus indicated that the TT genotype had significantly higher eicosapentaenoic, docosapentaenoic and docosahexaenoic acids than GG genotypes ($P < 0.05$). Significant correlations ($P < 0.05$) between *FASN* SNP and IMF, saturated and monounsaturated fatty acids were observed. These results provide insights into the contribution of lipogenic genes to intramuscular fat deposition and SNP marker-assisted selection for improvement of meat eating quality in northern Australian tropical crossbred beef cattle, hence an acceptance of the tested hypothesis.

Chapter 7: General Discussion, Conclusion and Recommendations for Future Research

The series of studies presented in this thesis evaluated the nutritive value of desmanthus and the effect of backgrounding tropical crossbred beef steers on grass-based diets augmented with desmanthus on feed intake, weight gain, rumen VFA, plasma metabolites, carcass traits and meat fatty acid composition. SNP in lipogenic genes and their associations with carcass traits and fatty acid composition were also evaluated. The hypotheses tested were:

a) The nutritive value and *in situ* degradability of desmanthus changes with maturity and would differ between cultivars; b) Backgrounding steers on desmanthus-augmented grass pastures would elicit significant changes in plasma metabolites resulting in higher liveweight gains than in steers on grass only pastures; c) Tropical beef steers fed isonitrogenous diets supplemented with incremental levels of desmanthus would have similar growth rates, rumen fermentation and plasma metabolite profiles; d) Tropical beef steers backgrounded on grass only or desmanthus-augmented grass pastures with similar backgrounding growth performance would not differ in feedlot growth performance and carcass quality; e) Tropical steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus would produce carcasses with similar characteristics and fatty acid composition; and f) SNP in the fatty acid binding protein 4, stearoyl-CoA desaturase and fatty acid synthase genes are associated with chiller-assessed carcass traits and fatty acid composition within the loin eye muscle of tropical crossbred beef steers.

In **Chapter 3**, variations in nutritive value attributable to the maturity stage at harvest of three desmanthus cultivars were identified. There was a decrease in the L/S with advancing plant maturity of all cultivars, in agreement with findings reported previously for *D. virgatus* and lucerne

(Suksombat and Buakeeree, 2006; Grev et al., 2020). The decline in L/S with maturity is ascribed to the decreasing leaf proportion caused by senescence and increasing stem proportion as the plant matures (Buxton et al., 1985; Sheaffer et al., 2000). The desmanthus CP was higher in the leaf (16.9 – 29.0%) than stem portion (4.6 – 13.1%), and it decreased with maturity for both leaf and stem proportions in this study. The decline in CP with maturity confirms findings reported by Suksombat and Buakeeree (2006) for *D. virgatus* harvested at 30 to 50 days old regrowth and could be due to an increase in the fibre proportion of the plant (Saylor et al., 2021). The high CP composition at late maturity stages of desmanthus leaf portion suggests that cattle can access high quality diet later in the growing season when the nutritive value of grass pastures has declined. Dietary NDF provides physically effective fibre that stimulates rumination, salivation and reticulorumen motility (National Academies of Sciences, Engineering and Medicine, 2016). By the same token, an increase in dietary fibre is usually associated with reduced concentration of fermentable carbohydrates (Brandao and Faciola, 2019). Therefore, the increase in ADF and decrease in CP with maturity observed in this study may reduce diet digestibility. The total potentially degradable DM (A+B) in this study (0.58 to 0.85) is within values reported for tropical legumes (Mupangwa et al., 2003; Gusha et al., 2013). Although the total potentially degradable proportion of DM did not differ between cultivars, the DM ED6 was lowest for JCU7 in this study. In addition, the ED2 and ED6 of CP was lowest in JCU7. In agreement with these findings, Vandermeulen et al. (2018) reported lower *in vitro* organic matter digestibility of JCU1 (*D. leptophyllus*) compared to JCU4 (*D. bicornutus*) and JCU2 (*D. virgatus*). The lower degradability was attributed to higher polyphenolic secondary compounds in JCU1 that may have caused precipitation of microbes and enzymes or formed tannin-protein complexes with proteins, a constituent of DM, making them unavailable for degradation (Waghorn and Shelton, 1997;

Makkar, 2003; Castro-Montoya and Dickhoefer, 2020). Suybeng et al. (2021b) also reported higher total phenolic composition in JCU7 compared to JCU2. The low effective CP degradability of JCU7 indicates that it may supply more rumen bypass protein to the grazing livestock availing more amino acids in the small intestines (Flachowsky and Lebzien, 2006), while the high rumen degradable CP of JCU2 and JCU4 might provide nitrogen supply for improved rumen microbial activity and microbial protein synthesis (Bowen et al., 2008; Keim et al., 2013). Taken together, these results provide an insight into the high potential of desmanthus to improve diet quality for cattle grazing low-quality grass pastures in sub-humid northern Australia with heavy clay soil.

Previous studies examining the digestibility of desmanthus for livestock production involved in vitro evaluation (Durmic et al., 2017; Vandermeulen et al., 2018), which does not account for all the comprehensive factors that influence feed digestibility in the rumen of a live animal (Kitessa et al., 1999). This is the first study to evaluate the degradability of desmanthus for utilization in the northern Australian beef cattle production using the *in situ* technique.

Grazing steers on grass pastures oversown with desmanthus (**Chapter 4.1**) did not elicit increased LW gain compared to steers grazing grass-only pastures, contrary to other studies that reported an increase in LW gain in cattle (Gardiner and Parker, 2012; Collins et al., 2016) and sheep (Rangel and Gardiner, 2009; Ngo, 2017) supplemented with desmanthus compared to their counterparts fed grass only diets. This could be due to the failure of desmanthus to increase the dietary CP, which is a major limiting macronutrient in livestock grazing low-quality tropical pastures (Bowman et al., 1995), possibly from the low desmanthus levels observed in this trial (11.5% pasture botanical composition) and the high contribution of browse that resulted in the similar diet CP for the buffel-grass only (negative control) and buffel grass-desmanthus grazing steers. To test this assumption, steers were fed Rhodes grass diets augmented with either lucerne (positive

control), desmanthus or both in isonitrogenous diets in **Chapter 4.2**. The results showed no difference in weight gain of steers between diets. Dietary CP is a major limiting macronutrient in livestock grazing tropical pastures during the dry season. Bowman et al. (1995) reported that a pasture diet with CP above 5.6% is required to prevent weight loss, while Detmann et al. (2014) estimated that 10.8 g/kg CP is essential to achieve the apparent equilibrium point where the N efficiency of utilisation is nil. The 5.6% CP level was achieved in all diets in both studies while 10.8 g/kg CP failed to be achieved in only a short period for steers grazing in the desmanthus paddock. These findings indicate that dietary CP was sufficient for rumen microbial growth. Increasing desmanthus proportion resulted in a linear decrease in DMI and total rumen VFA concentration of steers fed isonitrogenous diets (**Chapter 4.2**). These findings may be due to increase in diet ADF with increase in desmanthus proportion (Armentano, 1992). High dietary indigestible fibre composition is reported to reduce voluntary feed intake in ruminants due to low digestibility and high rumen fill effect (Pearson et al., 2006; Detmann et al., 2009). Although a negative correlation exists between indigestible fibre content and digestibility (Jančík et al., 2008), DMD is reported to restrict rumen fermentation and feed intake when the DMD:CP ratio exceeds 8 to 10 (Dixon and Coates, 2005). In this study, the ratio ranged between 8.5 and 8.7, indicating that DMD was unlikely to restrict rumen fermentation and feed intake. Desmanthus is a tannin-containing forage legume (Suybeng et al., 2021b). The lack of significant decrease in weight gain with increase in desmanthus proportion regardless of the decrease in feed intake and total rumen VFA concentration suggests that condensed tannins may have formed complexes with dietary proteins in the rumen making them available for digestion in the lower gastrointestinal tract (Molina-Botero et al., 2019). In addition, the 10 mg/dl rumen NH_3-N levels required to maintain

effective rumen microbial activity and maximize voluntary DMI (Ortiz-Rubio et al., 2007; Sampaio et al., 2010) were attained in all the diets in this study.

Typically, the annual ADG of cattle grazing northern Australian native grass pastures is 0.3 kg/day and 0.5 kg/day on introduced pastures (Hill et al., 2009; Poppi et al., 2018). These averages are obtained from high weight gain during the wet season, which may exceed one kg/day followed by weight loss during the dry season. Steers in all experimental diets (**Chapters 4.1** and **4.2**) gained at least 0.5 kg/day, which is above the minimum ADG of 0.4 kg/day required to meet the target slaughter weight required for the prime beef market within 2.5 years of age (Poppi et al., 2018). The similar levels of plasma metabolites of steers fed diets with or without desmanthus indicate that desmanthus can be added into diets with inclusion levels of up to 45% with no adverse effects on energy metabolism and health status of supplemented steers.

According to Thomas (1992), 35 – 45% legume biomass is required for 50 – 70% pasture utilisation for a productive and sustainable pasture. Therefore, the 11.5% in this study (**Chapter 4.1**) may be a limitation that prevented improvement in growth performance from being observed. Thus studies evaluating growth performance of cattle grazing desmanthus-augmented grass pastures with higher desmanthus proportions are required. In addition, this study was conducted in Central Queensland, which may not be replicated in other regions such as the Northern Territory, Western Australia and South Queensland, and did not encompass the year-to-year variation in rainfall and environmental temperatures. This necessitates the need to conduct longitudinal studies encompassing several years and in the other regions of northern Australia.

On the other hand, the size of beef cattle properties in northern Australia vary widely based on climatic conditions with an average of about 22 221 ha (Campbell et al., 2014) and are minimally

subdivided resulting in large paddock sizes. Paddocks as big as 12 000 ha in size are common (Hunt et al., 2007). Previous studies examining the effect of supplementing grass pastures with desmanthus on livestock performance were either indoor trials or grazing trials in small paddocks (≤ 250 ha) (Gardiner and Parker, 2012; Marsetyo et al., 2017; Aoetpah et al., 2018). Therefore, this is the only study that has reported findings from paddocks measuring over 500 ha which may represent practical pasture establishment challenges and the grazing behaviour of cattle in the commercial farm settings in semi-arid northern Australia (Hunt et al., 2007).

The backgrounding diet and weight gain may influence subsequent feed intake and growth performance during finishing (Drouillard and Kuhl, 1999; Dicker et al., 2001; Peripolli et al., 2018). Although for buffel-desmanthus backgrounded steers (**Chapter 5.1**), weight gain was 100 g/day more than the steers backgrounded on buffel grass only during feedlot finishing, this did not produce a significant difference in final LW between the two groups. On the other hand, steers backgrounded on isonitrogenous diets of Rhodes grass augmented with lucerne, desmanthus or both (**Chapter 5.2**) did not differ in feedlot finishing growth performance. The lack of difference in the present study may be due to similar backgrounding weight gain between treatment groups owing to the similar diet CP composition between the consumed diets, eliminating possible variation in compensatory gain or differences in feedlot entry LW (Peripolli et al., 2018). The carcass quality was not different for cattle backgrounded on the different diets (**Chapter 5.1** and **Chapter 5.2)**. This might have emanated from the insignificant variation in steers LW at slaughter. Reuter and Beck (2014) reported that cattle body weight at slaughter accounted for 27 – 70% and 22% of the difference in hot carcass weight and loin eye muscle area, respectively.

The fatty acid composition, FMP and IMF content of beef influences meat shelf life (Hoa et al., 2022), eating quality (Pogorzelski et al., 2022) and human health (Patel et al., 2022). Extensive

research has been conducted globally aimed to increase the concentration of the fatty acids purported to benefit human health and reduce fatty acids associated with detrimental human health outcomes (Scollan et al., 2014; Ladeira et al., 2018). Whereas numerous studies have reported increase in meat n-3 PUFA composition through dietary supplementation with fish oil, fish meal and vegetable oils (Girard et al., 2016; Flakemore et al., 2017a; Van Le et al., 2019), supplementation is limited in the extensive grazing systems of northern Australia due to cost (Neves et al., 2018). Increase in muscle unsaturated fatty acid composition in livestock fed diets with plant secondary metabolites such as tannins and saponins are reported and the increase is attributed to modulation of rumen fatty acid lipolysis and biohydrogenation of the dietary fatty acids in the rumen (Kronberg et al., 2007; Alves et al., 2017). Increasing desmanthus in the backgrounding diet did not influence the total SFA, MUFA or PUFA levels compared to the lucerne supplemented diets, although docosanoic acid levels and the n-6/n-3 PUFA ratios increased with increase in dietary desmanthus proportion as shown in **Chapter 5.2**. These results may be due to presence of plant secondary metabolites in both legumes (Suybeng et al., 2020; Liu et al., 2021). Steers were lean after backgrounding with IMF levels of ≤ 2.3%, but IMF increased to 4% after feedlot finishing for 95 days. Fat accumulation in the muscle is dependent on the balance between the uptake, synthesis and degradation of triacylglycerols, hence increasing the availability of net energy for fat synthesis during finishing increases fat accumulation compared to the low energy backgrounding forage diets (Scollan et al., 2006). Increase in muscle IMF enhances meat eating quality by improving meat tenderness through the insulation effect of the subcutaneous and intermuscular fat against the effect of refrigeration during carcass cooling (Wood et al., 1999). Furthermore, IMF reduces the muscle resistance to shearing through the accumulation of fat in the perimysial connective tissue (Wood et al., 1999), and improves meat

juiciness by reducing drip loss and cooking loss (Hwang and Joo, 2017). Therefore, a minimum of 3% IMF is required to meet consumer-preferred overall meat palatability (Smith, 2016). The essential 3% IMF level was achieved after feedlot finishing. In addition, feedlot finished steers had n-6/n-3 PUFA ratios below 4.0, a level purported to decrease the risk of coronary diseases and cancer (Simopoulos, 2016). Taken together, these results indicate that backgrounding steers on desmanthus-augmented diets followed by a short period of feedlot finishing can produce healthy meat of high eating quality. These findings present an important strength of this study because they are the only available findings on the feedlot performance, carcass traits and meat fatty acid composition of northern Australian cattle fed grass-based diets supplemented with desmanthus. Since the fatty acid composition in the muscle is highly dependent on the extent of fatty acids lipolysis and biohydrogenation in the rumen (Menci et al., 2021), studies on the effect of desmanthus on lipolysis and biohydrogenation of unsaturated fatty acids in the rumen are required. Furthermore, the ultimate goal of the beef cattle industry is to provide consumers with beef that is safe and of high eating quality. Thus, consumer-assessed sensory meat quality after backgrounding cattle on desmanthus-augmented pastures is required to determine consumer acceptance.

Carcass quality and meat fatty acid composition are influenced not only by diet but also by the genetics of the animal; thus, selective breeding to achieve long-term enhancement of economically important carcass traits is a relevant tool (Raza et al., 2018). Therefore, it was pertinent to evaluate SNP in the lipogenic genes and their association with chiller-assessed carcass traits and fatty acid composition of the IMF in the loin eye muscle. As shown in **Chapter 6.1**, no correlations or associations were observed between the identified *FABP4* SNP and carcass subcutaneous fat depth or marbling score. These findings concur with a study by Hoashi et al. (2008) that reported no significant effect of g.44677959T>C on subcutaneous fat or marbling score in Japanese Black

cattle. Conversely, Cho et al. (2008) reported a SNP association with backfat thickness in Korean cattle. These variations might be due to the difference in cattle breeds studied. Response in phenotypic outcomes due to effects of genetic markers are often breed-specific, and may not be extrapolated to all cattle breeds (Shin et al., 2012; Avilés et al., 2013; Bartoň et al., 2016). The AA genotype at the g.44677205A>G *FABP4* locus had significantly higher EMA than both the AG and GG genotypes with no effect on the subcutaneous fat depth. Loin eye muscle area and subcutaneous fat depth are used as indicators for the amount of saleable meat from a carcass (Raza et al., 2018). The effect of g.44677205A>G locus on EMA and the lack of it on subcutaneous fat depth indicate that selecting for AA genotype may increase the amount of carcass saleable meat without increasing the subcutaneous fat that ends up being trimmed off (Kelly et al., 2013). Three *SCD* SNP were correlated with carcass marbling score in this study, where the C, T and G alleles of the g.21273692T>C, g.21276141C>T and g.21276672A>G loci, respectively, had favourably higher marbling scores, in agreement with findings of Jiang et al. (2008) in the Wagyu x Limousin cattle. The results indicate that these SNP can be used to improve carcass palatability (Goszczynski et al., 2017; Testa et al., 2021).

Significant correlations between EPA, DPA and DHA with *SCD* SNP observed in this study (**Chapter 6.2**) are in agreement with the findings of a previous study in sheep that recorded significant correlations between two *SCD* SNP and n-3 long-chain PUFA (Pewan et al., 2021a). The synthesis of these three n-3 long-chain PUFA emanates largely from the activity of Δ6 desaturase and Δ5 desaturase on alpha-linolenic acid but not *SCD*, a Δ9 desaturase (Calder and Yaqoob, 2009; Cherfaoui et al., 2012). In fact, the Δ6 desaturase is considered to be the rate-limiting enzyme in n-3 long-chain PUFA synthesis pathway (Schulze et al., 2020). Therefore, the correlation may arise from linkage disequilibrium between the *SCD* loci and other loci in other

genes responsible for the synthesis of n-3 long-chain PUFA, necessitating further studies to evaluate the relationships between *SCD* SNP with other possible genes responsible for long-chain n-3 PUFA synthesis including fatty acyl elongases and fatty acid desaturases. Nonetheless, the correlations of *SCD* SNP with the EPA, DPA and DHA with no influence on the SFA and MUFA observed in this study suggest that the SNP may be used as markers to select cattle for improved health beneficial n-3 long-chain PUFA with no negative influence on meat eating quality.

The C allele on the *FASN* novel SNP at g.50792445C>T locus was associated with lower subcutaneous fat depth (**Chapter 6.1**) and higher meat EPA levels (**Chapter 6.2**), with no significant effect on IMF and marbling score. Eicosapentaenoic acid exhibits many beneficial effects on human health. It is required for normal functions and development of the brain and retina and it is reported to reduce oxidative stress, hyperlipidaemia and neurodegenerative diseases risk (Xiao et al., 2022). Thus, selecting for the C allele may increase the health benefits of the meat while maintaining the marbling-related palatability.

Polymorphisms in lipogenic genes are reported to influence carcass and meat fat composition through varying pathways such as change in enzyme activity (Jiang et al., 2008), regulation of alternative splicing or gene expression (Jo and Choi, 2015). Therefore, there is a need to evaluate the pathway in which the SNP observed in this study influenced fatty acid composition.

In summary, desmanthus is a productive and nutritious pasture legume that is well adapted to the vertosol soils of the dry regions of northern Australia where no suitable alternative legume species are adapted. The positive control study of pen-fed steers backgrounded on grass-based diet augmented with lucerne, desmanthus or both demonstrated that high desmanthus proportions (45% DM) can be used to obtain high growth rates during backgrounding on a forage-based diet.

However, desmanthus proportions at 11.5% of pasture botanical composition did not improve growth performance compared to grass-only pastures (Negative control) when browsing was not controlled. Taken together, these findings indicate that desmanthus can be used to improve growth performance of beef cattle in northern Australia that lacks a well-adapted legume where browsing is limited.

Generally, meat fatty acid composition, FMP and IMF did not differ in steers backgrounded on isonitrogenous grass diets augmented with desmanthus, lucerne or both, and although steers were lean after backgrounding, IMF increased to 4% after feedlot finishing for 95 days while the n-6/n-3 PUFA ratios remained below 4.0. These results denote that desmanthus can be used to augment diets of cattle grazing low-quality grass pastures in northern Australia with no negative effect on meat quality, and feedlot finishing for a short period of time is required to obtain the minimum IMF necessary for high meat palatability while maintaining healthy n-6/n-3 PUFA ratios.

Finally, meat fatty acids are more saturated in ruminants compared to monogastric animals and are difficult to modify using dietary alterations due to lipolysis and biohydrogenation in the rumen. In addition, chiller-assessed carcass quality measurements are obtained after slaughter, when it is too late to use the data for selection and breeding of the superior performing animals, and selective breeding based on progeny testing is limited by the substantial genetic lag. The SNP in the lipogenic genes identified in this thesis demonstrate that allelic substitutions can be used in marker-assisted selection to breed cattle with favourable meat fatty acid composition and carcass quality just by obtaining a blood sample from the target animals. Furthermore, the minimally invasive biopsy sampling can be used to directly quantify the exact phenotypic worth of live animals for IMF, FMP and fatty acid composition to inform management decisions.

The findings of this thesis will aid beef cattle producers in northern Australia, research scientists and beef consumers to:

- Select a highly nutritious legume that is well adapted to the northern Australian sub-humid regions with vertosol soils.
- Utilise desmanthus forage at the optimum growth stage for cattle feeding.
- Ascertain that desmanthus does not cause negative effect on animal growth performance in extensive grazing settings and can be used to attain high weight gains of pen-fed cattle.
- Discover that meat from steers backgrounded on grass diets augmented with desmanthus and finished in the feedlot for a short period is healthy and of high palatability.
- Utilise the loin eye muscle biopsy as a suitable tool to determine the exact phenotypic worth of live animals for selection and breeding.
- Select superior producing cattle for breeding to improve carcass and meat quality using SNP in marker-assisted selection.

Strengths and limitations of this research

Strengths	Limitations
The grazing study was carried out in paddocks measuring 520 ha and 575 ha. This is the only study that has reported findings from large-sized paddocks of over 500 ha, which are representative of the typical commercial farm settings in semi-arid northern Australia. The utilisation of large paddock size is limited by uneven grazing due to heavy grazing around the water points and light grazing farther away, hence this study represents a more practical situation for replicating the findings in commercial farms.	In the grazing study, desmanthus accounted for 11.5% of botanical composition pre-grazing. This low level may have restricted the availability of desmanthus resulting to similar diet non-grass and CP for the steers grazing grass only or desmanthus-augmented pastures. Thus, grazing studies with higher desmanthus proportions are necessary to fully understand the effect of augmenting grass pastures with desmanthus in extensive grazing systems. Levels of 20 – 30% and 35 – 45% legume biomass composition are recommended for 10 – 40% and 50 – 70% pasture utilisation, respectively, for a productive and sustainable pasture.
This study evaluated the effect of cultivar and maturity stage at harvest on desmanthus *in situ* degradability for northern Australian beef cattle feeding. Previous studies evaluated desmanthus degradability *in vitro*, which does not account for all the comprehensive factors that influence feed digestibility in the rumen of a live animal.	The studies took place in central and north Queensland where climatic conditions and production systems may not be applicable to the other regions of northern Australia. Therefore, similar research in different regions, such as South Queensland, the Northern Territory and Western Australia, is required to confirm the findings of this study.
This is the only study that has evaluated the feedlot performance, carcass traits and meat fatty acid composition of northern Australian cattle in response to the inclusion of desmanthus in grass-based diets.	Each study evaluated performance in a single year. This did not accommodate the year-to-year variations in environmental temperatures and rainfall. Longitudinal studies encompassing several years are required to demonstrate the

	long-term effect of augmenting grass pastures with desmanthus.

Further research and experimental studies in the future are recommended to evaluate:

- Growth performance of cattle grazing desmanthus-augmented grass pastures with higher desmanthus proportions;
- The effect of desmanthus on lipolysis and biohydrogenation of unsaturated fatty acids in the rumen;
- The consumer-assessed sensory evaluation of meat eating quality after backgrounding cattle on desmanthus-augmented pastures;
- The relationships between *SCD* SNP with other possible genes responsible for long-chain n-3 PUFA synthesis including fatty acyl elongases and fatty acid desaturases;
- The effect of SNP in lipogenic genes on the expression and regulatory activities of fats and proteins.

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Appendices

Appendix 1. Supplementary Materials

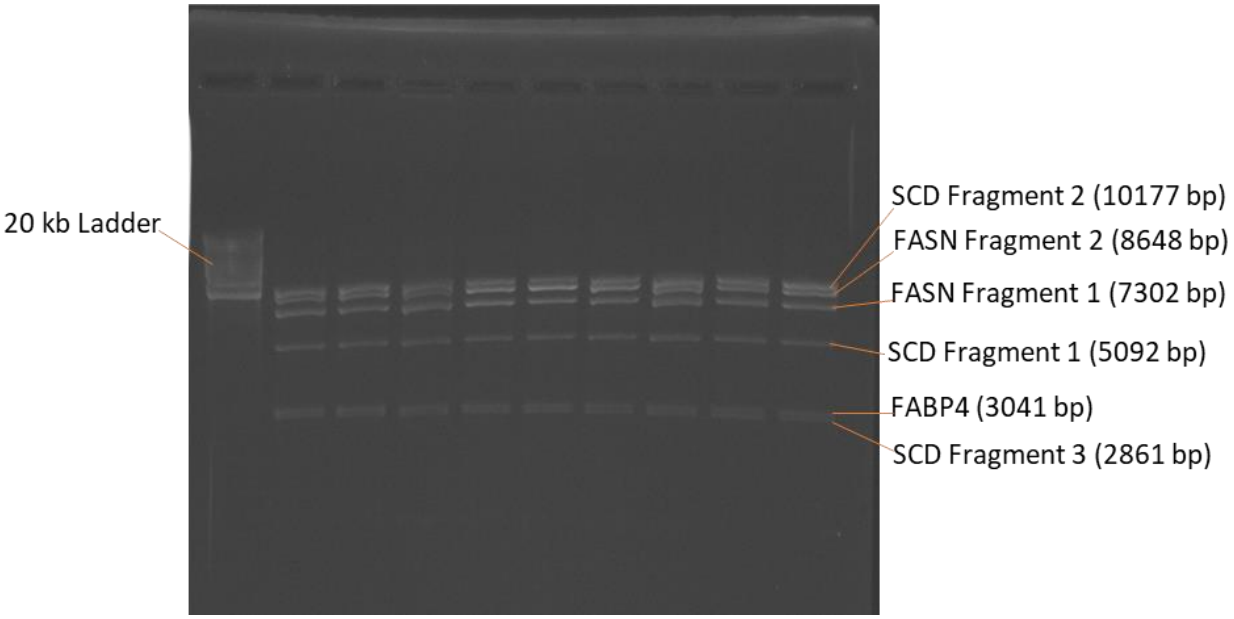

Figure S6.1.1. Gel image of the amplification products of the three target genes visualised in 0.8% agarose gel.

Table S6.1.1. SNP genetic variants identified in the *FABP4*, *SCD* and *FASN* genes

SNP[1]	dbSNP ID[2]	SNP location	Genetic diversity[3]				
			MAF	He	PIC	HWE χ^2	HWE *P-value*
FABP4							
g.44677205A>G	rs109388335	Intron 3	0.31	0.43	0.33	1.15	0.56
g.44677239C>G	rs110383592	Exon 3	0.29	0.41	0.32	0.37	0.83
g.44677417T>A	rs109014985	Intron 2	0.47	0.49	0.37	1.49	0.47
g.44677454T>G	rs134173517	Intron 2	0.46	0.49	0.37	0.88	0.64
g.44677611G>C	rs41729172	Intron 2	0.20	0.32	0.27	0.60	0.73
g.44677959T>C	rs110757796	Exon 2	0.47	0.49	0.37	1.49	0.47
g.44678114G>C	-	Intron 1	0.29	0.41	0.32	1.77	0.41
g.44678641T>C	rs110490217	Intron 1	0.33	0.44	0.34	3.59	0.16
g.44679737T>C	rs137643896	Intron 1	0.47	0.49	0.37	1.49	0.47
g.44679833A>G	rs133333024	Intron 1	0.40	0.48	0.36	0.72	0.69
g.44679885G>A	-	Intron 1	0.28	0.40	0.32	1.32	0.51
SCD							
g.21267450C>A	-	Intron 2	0.40	0.48	0.36	2.17	0.33
g.21267896C>T	rs136334180	Intron 2	0.34	0.44	0.34	0.35	0.83
g.21269494G>C	-	Intron 3	0.40	0.48	0.36	2.17	0.33
g.21270336A>G	rs41255689	Intron 3	0.44	0.49	0.37	0.15	0.92
g.21270739A>G	rs41255690	Intron 4	0.38	0.47	0.36	0.13	0.93
g.21271111A>G	rs132709487	Intron 4	0.46	0.49	0.37	0.07	0.96
g.21271263C>A	rs378548458	Intron 4	0.36	0.46	0.35	0.28	0.86
g.21271392G>A	rs211294052	Intron 4	0.37	0.46	0.35	0.01	0.99
g.21271645G>A	rs380628677	Intron 4	0.36	0.46	0.35	0.28	0.86
g.21271725C>G	rs378000803	Intron 4	0.36	0.46	0.35	0.28	0.86
g.21272246A>G	rs41255691	Exon 5	0.45	0.49	0.37	0.003	0.99
g.21272306T>C	-	Exon 5	0.35	0.45	0.35	0.09	0.95
g.21272422C>T	rs41255693	Exon 5	0.35	0.45	0.35	0.09	0.95
g.21272529A>G	rs383175036	Intron 5	0.35	0.45	0.35	0.09	0.95
g.21272984C>T	-	Intron 5	0.34	0.44	0.34	0.006	0.99
g.21273073C>A	rs211681261	Intron 5	0.46	0.49	0.37	0.88	0.64

g.21273403G>T	rs209591043	Intron 5	0.45	0.49	0.37	1.61	0.44
g.21273598C>T	-	Intron 5	0.45	0.49	0.37	1.61	0.44
g.21273692T>C	rs208058585	Intron 5	0.44	0.49	0.37	1.006	0.60
g.21274425G>T	rs384532210	Intron 5	0.34	0.44	0.34	0.006	0.99
g.21274479G>A	rs382184952	Intron 5	0.22	0.35	0.28	0.39	0.82
g.21275001G>C	rs211483324	Intron 5	0.37	0.46	0.35	0.46	0.79
g.21275659G>T	rs41255694	Exon 6	0.36	0.46	0.35	0.59	0.74
g.21275732G>C	-	Exon 6	0.36	0.46	0.35	0.59	0.74
g.21275851C>A	-	Exon 6	0.37	0.46	0.35	3.27	0.19
g.21276141C>T	rs41255697	Exon 6	0.43	0.49	0.37	0.54	0.76
g.21276672A>G	rs41255698	Exon 6	0.44	0.49	0.37	0.15	0.92
FASN							
g.50783660T>G	-	Intron 11	0.28	0.40	0.32	1.32	0.51
g.50783803G>A	-	Intron 11	0.11	0.20	0.18	0.72	0.69
g.50783866C>A	-	Intron 11	0.35	0.45	0.35	2.64	0.26
g.50784533C>G	rs481622676	Exon 13	0.18	0.29	0.25	0.30	0.85
g.50785253C>T	-	Intron 15	0.27	0.39	0.31	0.93	0.62
g.50785330C>T	-	Intron 15	0.28	0.40	0.32	1.32	0.51
g.50785474C>T	rs209214391	Exon 16	0.28	0.40	0.32	1.32	0.51
g.50786221A>G	rs518879624	Exon 19	0.17	0.28	0.24	1.85	0.39
g.50786496A>G	-	Exon 20	0.28	0.40	0.32	1.32	0.51
g.50786977A>G	-	Intron 21	0.38	0.47	0.36	7.94	0.01
g.50787009A>G	-	Intron 21	0.20	0.32	0.27	1.15	0.56
g.50787362C>T	-	Intron 21	0.25	0.37	0.30	1.98	0.37
g.50787774G>A	-	Intron 22	0.28	0.40	0.32	1.32	0.51
g.50788319C>T	-	Exon 23	0.34	0.44	0.34	4.36	0.11
g.50788575T>C	rs41919993	Exon 24	0.38	0.47	0.36	4.76	0.09
g.50789408T>C	rs41919992	Exon 27	0.20	0.32	0.27	1.15	0.56
g.50789882T>C	-	Intron 28	0.25	0.37	0.30	3.27	0.19
g.50790149A>G	-	Intron 29	0.34	0.44	0.34	4.36	0.11
g.50790973C>A	rs109149276	Exon 32	0.37	0.46	0.35	6.01	0.04
g.50791045G>A	-	Intron 32	0.34	0.44	0.34	4.36	0.11
g.50792347C>T	rs41919984	Exon 37	0.26	0.38	0.31	5.46	0.06
g.50792445C>T	-	Intron 37	0.21	0.33	0.28	0.002	0.99

g.50792548T>G	-	Intron 37	0.27	0.39	0.31	0.93	0.62
g.50793357A>G	rs41919985	Exon 39	0.20	0.32	0.27	1.15	0.56
g.50793475C>T	-	Intron 39	0.28	0.40	0.32	1.32	0.51
g.50793872G>A	-	Exon 41	0.28	0.40	0.32	1.32	0.51
g.50794099T>C	-	Exon 42	0.26	0.38	0.31	5.46	0.06

[1] SNP: single nucleotide polymorphism

[2] SNP without the dbSNP ID are not listed in the Bovine Genome Variation Database (BGVD)

[3]MAF: Minor allele frequency, (He): Expected heterozygosity, PIC: polymorphism information content, HWE: Hardy–Weinberg disequilibrium

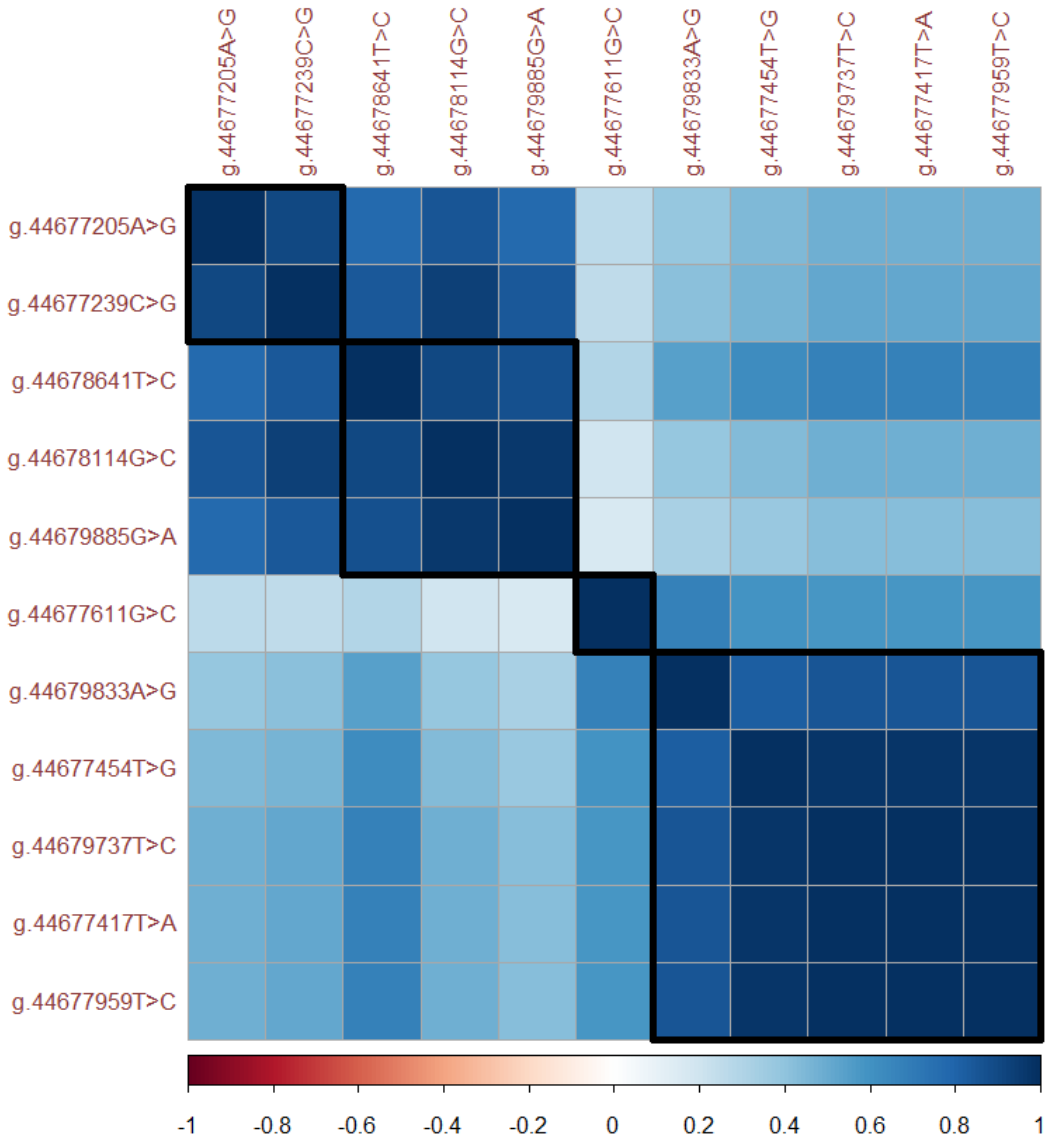

Figure S6.1.2. Correlation coefficients between all SNP in *FABP4* gene. The rectangle represents distance-based clustering of SNP loci.

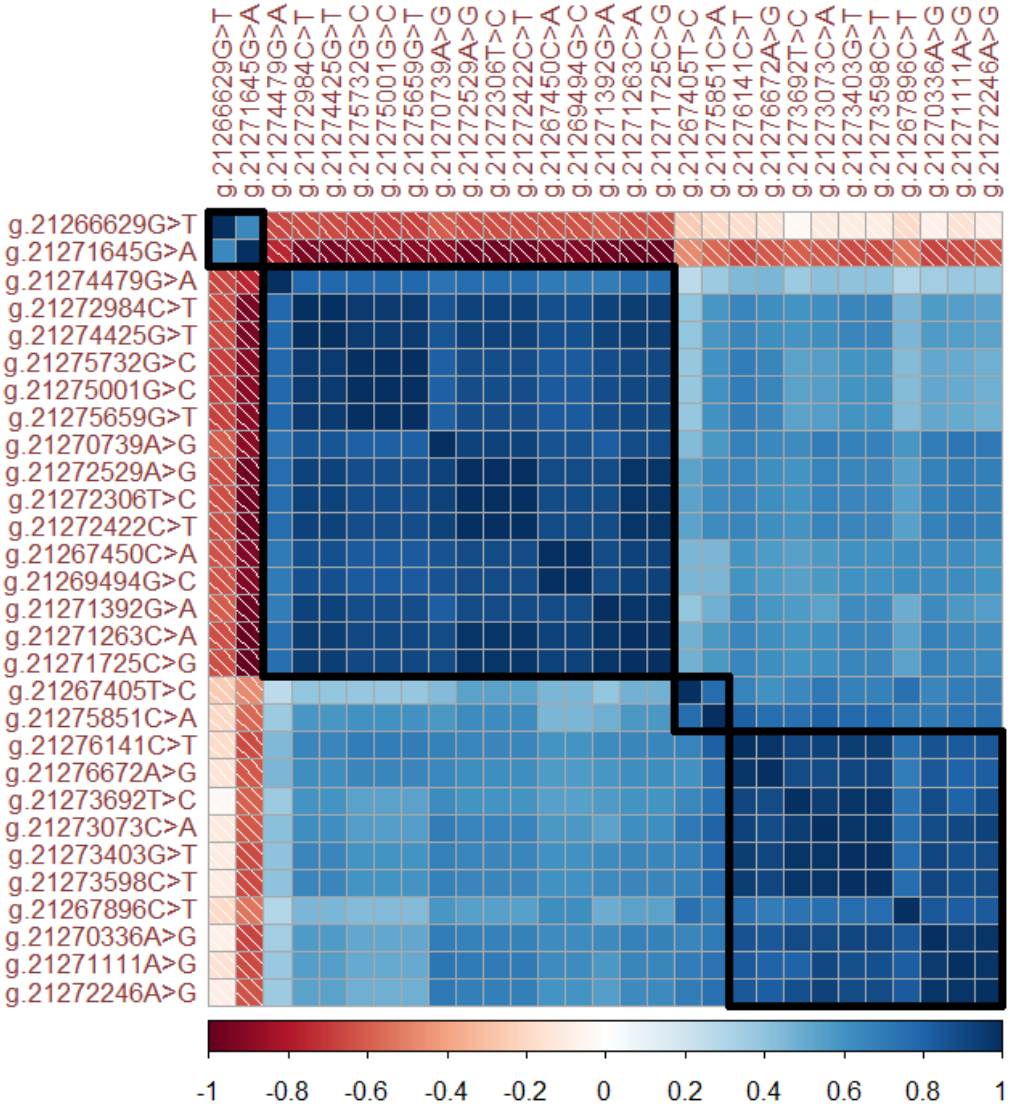

Figure S6.1.3. Correlation coefficients between all SNP in *SCD* gene. The rectangle represents distance-based clustering of SNP loci.

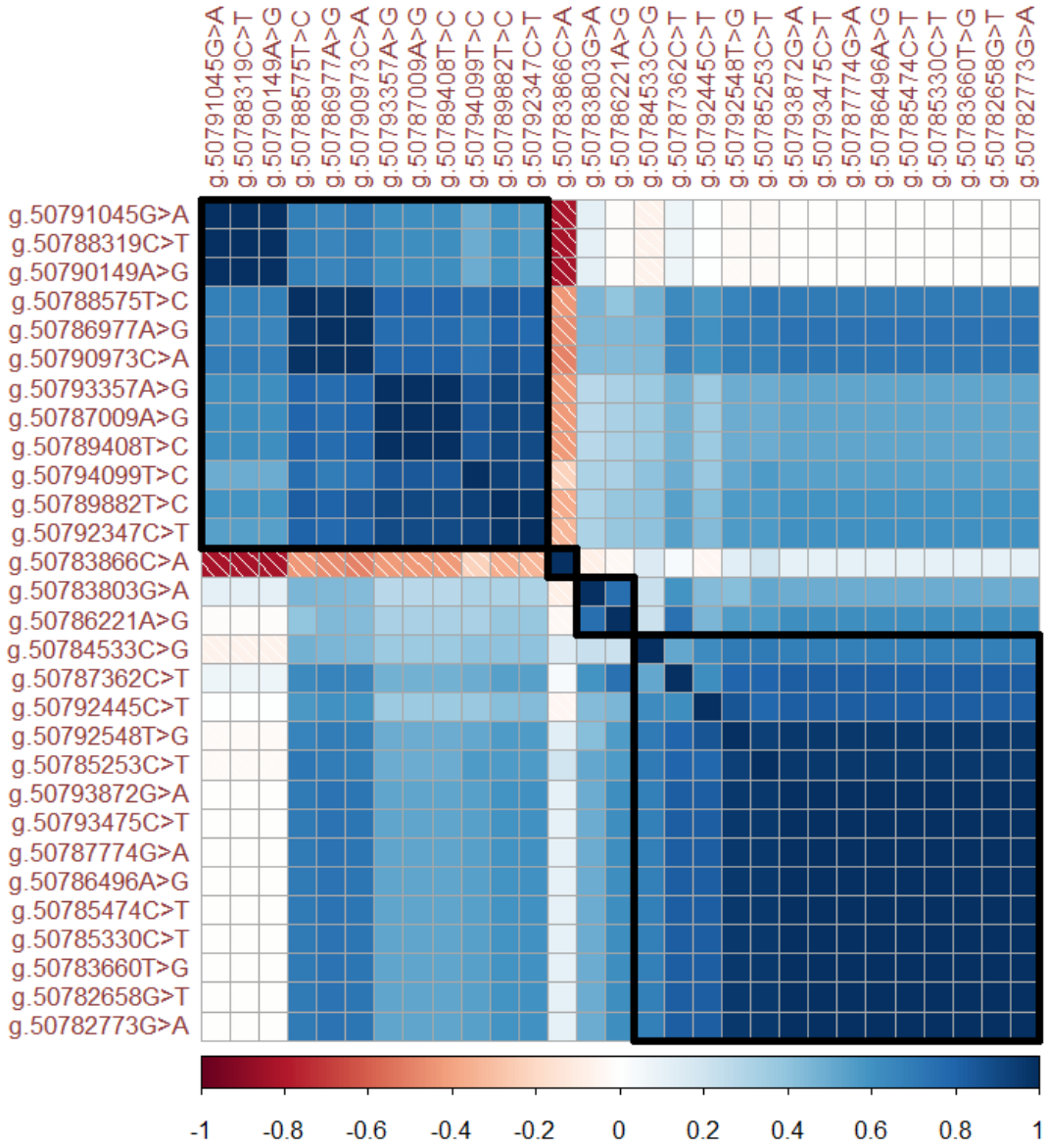

Figure S6.1.4. Correlation coefficients between all SNP in *FASN* gene. The rectangle represents distance-based clustering of SNP loci.

Table S6.2.1. Genetic variants detected in the *FABP4*, *SCD* and *FASN* genes of tropically adapted crossbred steers.

Gene/SNP [1]	dbSNP ID	Location	Genetic diversity [2]				
			MAF	He	PIC	HWE (χ^2)	HWE *P-value*
FABP4							
g.44677183A>G	rs109346428	Intron 3	0.29	0.41	0.33	0.01	0.99
g.44677205A>G	rs109388335	Intron 3	0.44	0.49	0.37	2.72	0.25
g.44677239C>G	rs110383592	Exon 3	0.41	0.48	0.37	1.55	0.46
g.44677417T>A	rs109014985	Intron 2	0.25	0.38	0.30	0.00	1.00
g.44677454T>G	rs134173517	Intron 2	0.24	0.36	0.30	0.04	0.98
g.44677587G>C	rs723716479	Intron 2	0.14	0.23	0.21	0.02	0.98
g.44677611G>C	rs41729172	Intron 2	0.16	0.26	0.23	9.59	< 0.01
g.44677959T>C	rs110757796	Exon 2	0.25	0.38	0.30	0.00	1.00
g.44678114G>C	-	Intron 1	0.36	0.46	0.36	1.02	0.60
g.44678641T>C	rs110490217	Intron 1	0.46	0.50	0.37	0.28	0.86
g.44679737T>C	rs137643896	Intron 1	0.27	0.40	0.32	0.12	0.94
g.44679833A>G	rs133333024	Intron 1	0.16	0.26	0.23	0.04	0.98
g.44679885G>A	-	Intron 1	0.36	0.46	0.36	1.02	0.60
g.44680048A>G	rs468994137	Intron 1	0.22	0.34	0.28	0.35	0.83
g.44680116T>C	-	Intron 1	0.25	0.38	0.30	0.59	0.74
g.44680161A>G	rs468130226	Intron 1	0.32	0.44	0.34	0.00	1.00
SCD							
g.21266045C>T	-	Intron 2	0.34	0.45	0.35	0.04	0.97
g.21266286C>T	-	Intron 2	0.35	0.46	0.35	0.38	0.82
g.21266629G>T	-	Intron 2	0.46	0.50	0.37	0.28	0.86
g.21266904A>G	-	Intron 2	0.29	0.41	0.33	1.79	0.40
g.21267450C>A	-	Intron 2	0.33	0.44	0.35	0.05	0.97
g.21268549G>A	rs208238577	Intron 2	0.28	0.40	0.32	0.02	0.99
g.21269019A>G	rs41255688	Intron 3	0.33	0.44	0.35	0.05	0.97

g.21269378A>G	rs210411417	Intron 3	0.36	0.46	0.36	0.15	0.92
g.21269494G>C	-	Intron 3	0.33	0.44	0.35	0.05	0.97
g.21270336A>G	rs41255689	Intron 3	0.25	0.38	0.30	0.00	1.00
g.21270739A>G	rs41255690	Intron 4	0.38	0.47	0.36	0.21	0.89
g.21271111A>G	rs132709487	Intron 4	0.25	0.38	0.30	0.00	1.00
g.21271263C>A	rs378548458	Intron 4	0.31	0.43	0.34	0.04	0.97
g.21271392G>A	rs211294052	Intron 4	0.31	0.43	0.34	0.04	0.97
g.21271645G>A	rs380628677	Intron 4	0.31	0.43	0.34	0.04	0.97
g.21271725C>G	rs378000803	Intron 4	0.31	0.43	0.34	0.04	0.97
g.21271813A>G	rs517923141	Intron 4	0.16	0.26	0.23	0.82	0.66
g.21272246A>G	rs41255691	Exon 5	0.25	0.38	0.30	0.00	1.00
g.21272306T>C	-	Exon 5	0.31	0.43	0.34	0.04	0.97
g.21272422C>T	rs41255693	Exon 5	0.31	0.43	0.34	0.04	0.97
g.21272423G>A	rs208932125	Exon 5	0.28	0.40	0.32	0.02	0.99
g.21272529A>G	rs383175036	Intron 5	0.31	0.43	0.34	0.04	0.97
g.21272815A>G	rs207511283	Intron 5	0.36	0.46	0.36	0.15	0.92
g.21272968C>T	rs209994060	Intron 5	0.44	0.49	0.37	1.13	0.56
g.21272984C>T	-	Intron 5	0.31	0.43	0.34	0.04	0.97
g.21272993T>C	-	Intron 5	0.44	0.49	0.37	1.13	0.56
g.21273051C>G	rs208495936	Intron 5	0.36	0.46	0.36	0.15	0.92
g.21273073C>A	rs211681261	Intron 5	0.24	0.36	0.30	0.04	0.98
g.21273403G>T	rs209591043	Intron 5	0.24	0.36	0.30	0.04	0.98
g.21273598C>T	-	Intron 5	0.24	0.36	0.30	0.04	0.98
g.21273692T>C	rs208058585	Intron 5	0.24	0.36	0.30	0.04	0.98
g.21273866C>T	-	Intron 5	0.38	0.47	0.36	0.59	0.74
g.21273968T>C	rs211184136	Intron 5	0.28	0.40	0.32	0.02	0.99
g.21274425G>T	rs384532210	Intron 5	0.31	0.43	0.34	0.04	0.97
g.21274555A>G	rs456785055	Intron 5	0.44	0.49	0.37	1.13	0.56
g.21274609C>T	rs209423801	Intron 5	0.44	0.49	0.37	1.13	0.56
g.21275001G>C	rs211483324	Intron 5	0.31	0.43	0.34	0.04	0.97
g.21275659G>T	rs41255694	Exon 6	0.31	0.43	0.34	0.04	0.97

g.21275732G>C	-	Exon 6	0.31	0.43	0.34	0.04	0.97
g.21276306C>T	rs521409231	Exon 6	0.28	0.40	0.32	0.02	0.99
g.21276672A>G	rs41255698	Exon 6	0.23	0.35	0.29	0.15	0.92
g.21276808C>T	-	Exon 6	0.35	0.46	0.35	0.38	0.82
FASN							
g.50782658G>T	rs519685521	Exon 9	0.39	0.47	0.36	3.06	0.21
g.50782773G>A	rs715140536	Exon 9	0.39	0.47	0.36	3.06	0.21
g.50783660T>G	-	Intron 11	0.40	0.48	0.36	4.41	0.11
g.50783803G>A	-	Intron 11	0.38	0.47	0.36	0.59	0.74
g.50783866C>A	-	Intron 11	0.24	0.36	0.30	0.97	0.61
g.50784242C>T	rs800844468	Exon 12	0.32	0.44	0.34	0.00	1.00
g.50784533C>G	rs481622676	Exon 13	0.32	0.44	0.34	3.91	0.14
g.50784824G>A	rs209227647	Exon 14	0.50	0.50	0.38	12.00	< 0.01
g.50785253C>T	-	Intron 15	0.43	0.49	0.37	6.27	0.04
g.50785330C>T	-	Intron 15	0.40	0.48	0.36	4.41	0.11
g.50785474C>T	rs209214391	Exon 16	0.40	0.48	0.36	4.41	0.11
g.50786221A>G	rs518879624	Exon 19	0.48	0.50	0.37	2.97	0.22
g.50786496A>G	-	Exon 20	0.39	0.47	0.36	3.06	0.21
g.50786977A>G	-	Intron 21	0.17	0.28	0.24	7.68	0.02
g.50787009A>G	-	Intron 21	0.11	0.20	0.18	3.80	0.15
g.50787362C>T	-	Intron 21	0.46	0.50	0.37	2.88	0.23
g.50787774G>A	-	Intron 22	0.40	0.48	0.36	2.24	0.32
g.50787886A>G	-	Intron 22	0.24	0.37	0.30	2.98	0.22
g.50788319C>T	-	Exon 23	0.23	0.35	0.29	0.18	0.91
g.50788575T>C	rs41919993	Exon 24	0.17	0.28	0.24	7.68	0.02
g.50788691T>C	rs526036338	Exon 24	0.26	0.39	0.31	4.23	0.12
g.50788956C>T	-	Intron 25	0.26	0.39	0.31	4.23	0.12
g.50789448C>T	rs516607144	Exon 27	0.31	0.43	0.34	4.95	0.08
g.50789882T>C	-	Intron 28	0.11	0.20	0.18	3.80	0.15
g.50790973C>A	rs109149276	Exon 32	0.17	0.28	0.24	7.68	0.02
g.50791045G>A	-	Intron 32	0.23	0.35	0.29	0.18	0.91

g.50792445C>T	-	Intron 37	0.40	0.48	0.36	0.80	0.67
g.50792548T>G	-	Intron 37	0.43	0.49	0.37	3.66	0.16
g.50793475C>T	-	Intron 39	0.40	0.48	0.36	2.24	0.32
g.50793872G>A	-	Exon 41	0.44	0.49	0.37	5.00	0.08

[1] SNP, single nucleotide polymorphism; *FABP4*, Fatty acid binding protein 4; *SCD*, stearoyl-CoA desaturase; *FASN*, fatty acid synthase.

[2] MAF: Minor allele frequency, (He): Expected heterozygosity, PIC: polymorphism information content, HWE: Hardy–Weinberg disequilibrium

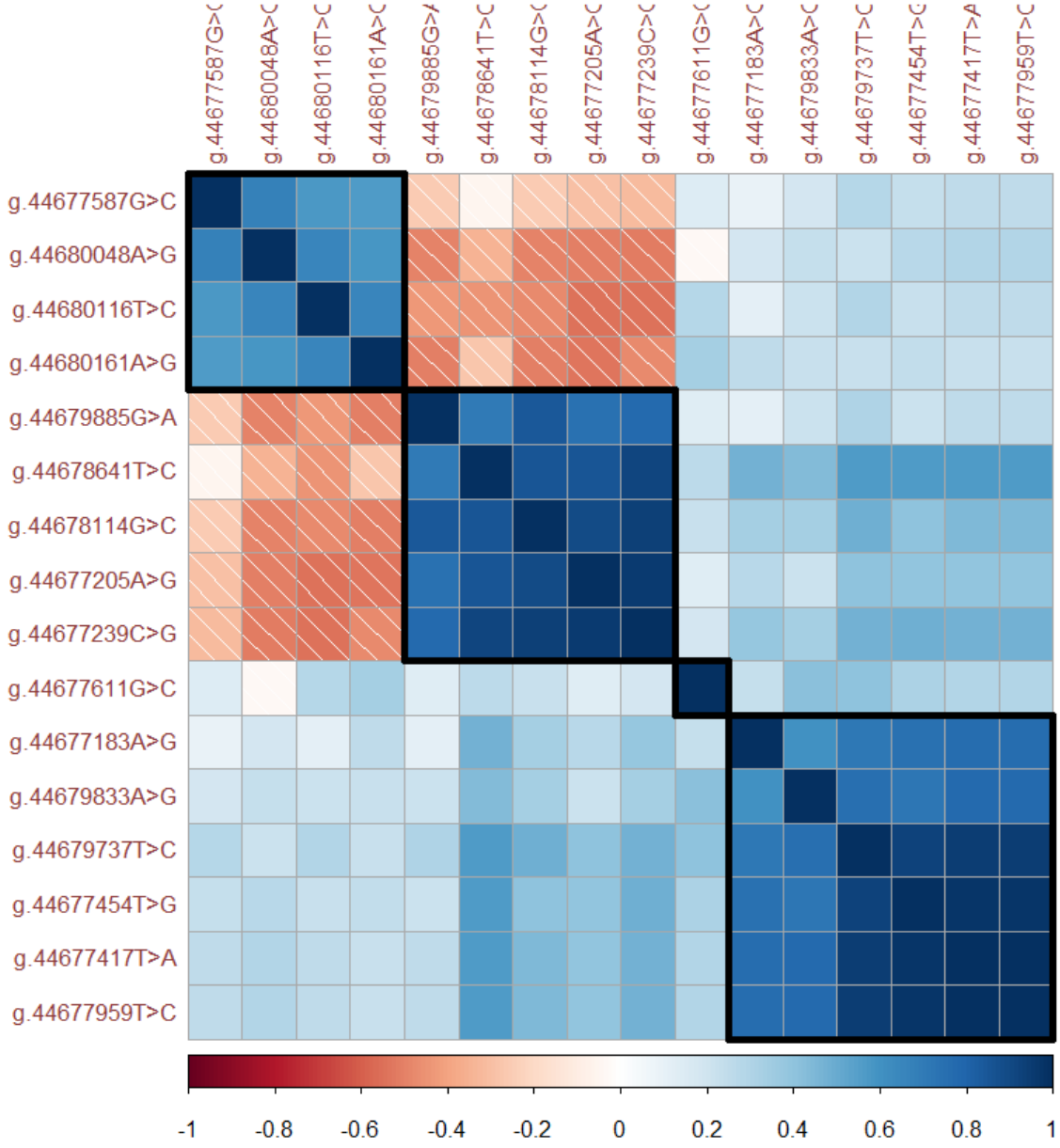

Figure S6.2.1. Correlation coefficients for all pairs of SNP loci of the *FABP4* gene. The rectangles represent distance-based clustering of SNP loci.

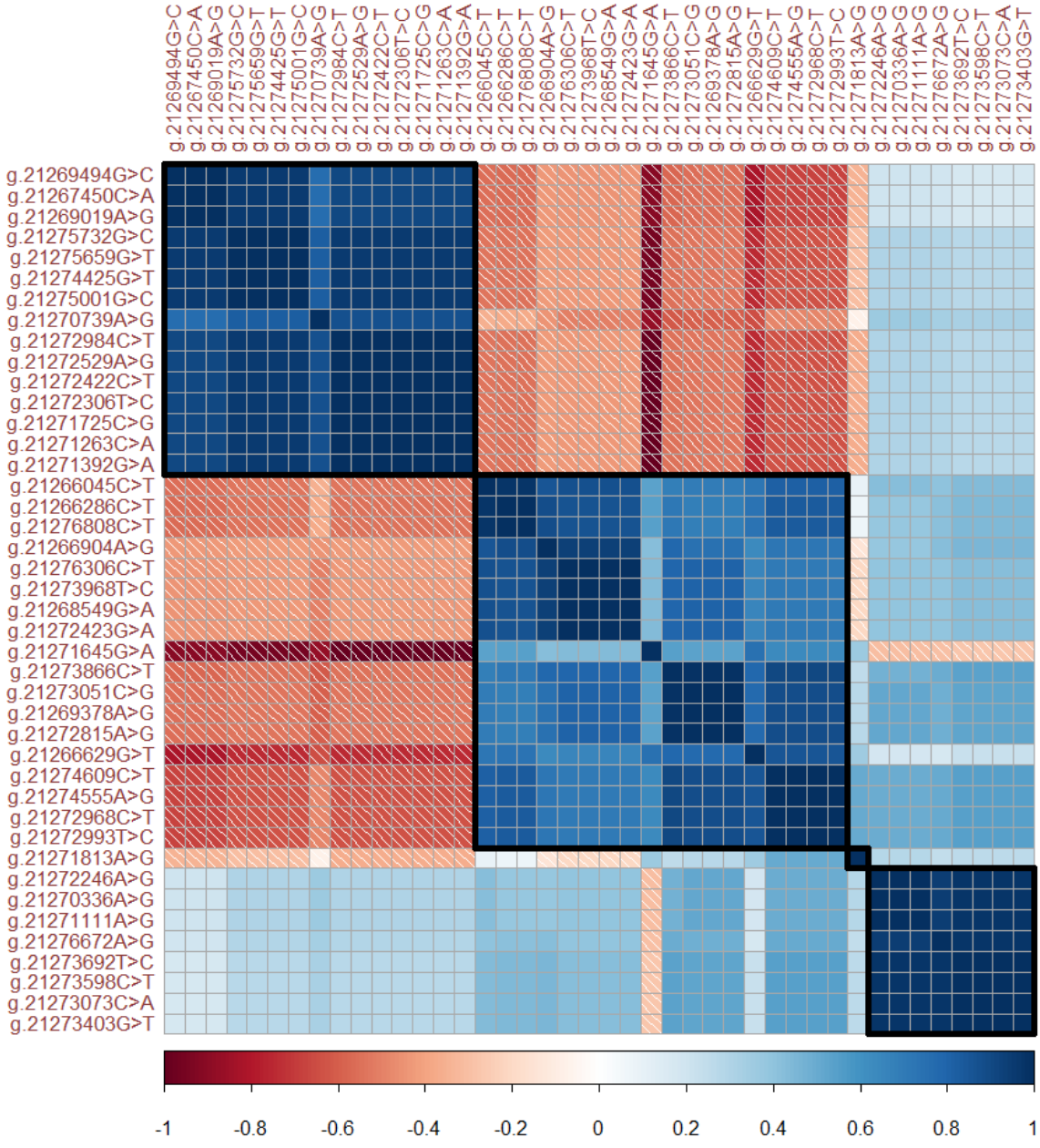

Figure S6.2.2. Correlation coefficients for all pairs of SNP loci of the *SCD* gene. The rectangles represent distance-based clustering of SNP loci.

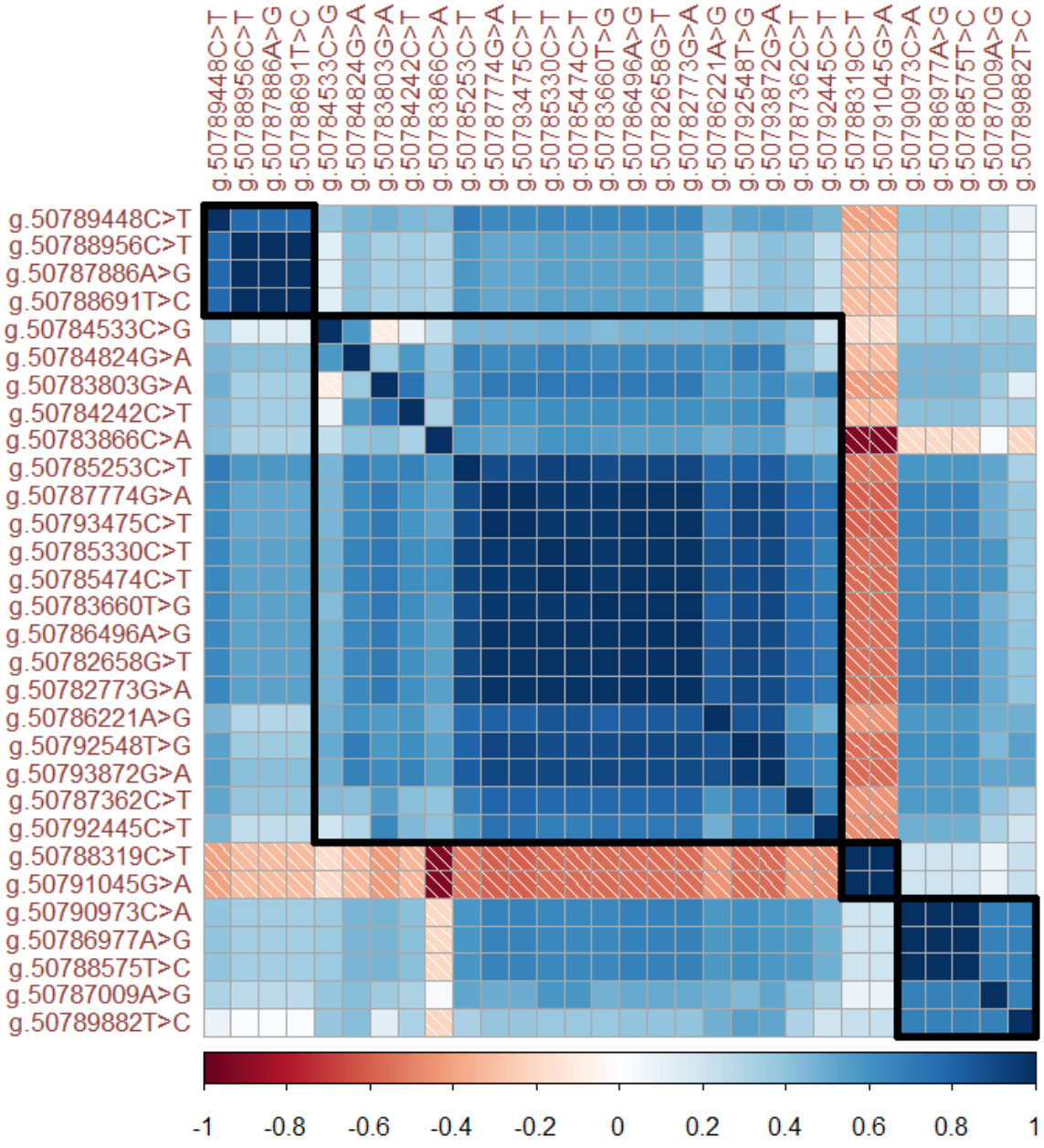

Figure S6.2.3. Correlation coefficients for all pairs of SNP loci of the *FASN* gene. The rectangles represent distance-based clustering of SNP loci.

Appendix 2: Animal Ethics Approvals

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CSIRO Queensland Animal Ethics Committee

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has been removed

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Appendix 3: Published Papers

foods

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Review

Diet and Genetics Influence Beef Cattle Performance and Meat Quality Characteristics

Felista W. Mwangi [1], Edward Charmley [2], Christopher P. Gardiner [1], Bunmi S. Malau-Aduli [3], Robert T. Kinobe [1] and Aduli E. O. Malau-Aduli [1,*]

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Received: 14 November 2019; Accepted: 27 November 2019; Published: 6 December 2019

check for updates

Abstract: A comprehensive review of the impact of tropical pasture grazing, nutritional supplementation during feedlot finishing and fat metabolism-related genes on beef cattle performance and meat-eating traits is presented. Grazing beef cattle on low quality tropical forages with less than 5.6% crude protein, 10% soluble starches and 55% digestibility experience liveweight loss. However, backgrounding beef cattle on high quality leguminous forages and feedlot finishing on high-energy diets increase meat flavour, tenderness and juiciness due to improved intramuscular fat deposition and enhanced mono- and polyunsaturated fatty acids. This paper also reviews the roles of stearoyl-CoA desaturase, fatty acid binding protein 4 and fatty acid synthase genes and correlations with meat traits. The review argues that backgrounding of beef cattle on *Desmanthus*, an environmentally well-adapted and vigorous tropical legume that can persistently survive under harsh tropical and subtropical conditions, has the potential to improve animal performance. It also identifies existing knowledge gaps and research opportunities in nutrition-genetics interactions aimed at a greater understanding of grazing nutrition, feedlot finishing performance, and carcass traits of northern Australian tropical beef cattle to enable red meat industry players to work on marbling, juiciness, tenderness and overall meat-eating characteristics.

Keywords: diet; genetics; meat quality characteristics; tropical beef cattle; stearoyl-CoA desaturase; fatty acid binding protein 4; fatty acid synthase; *Desmanthus* legumes; supplementation; growth performance

1. Introduction

Beef plays a significant role in global human nutrition. It is the third most consumed meat in the world after poultry and pork at 6.4, 14.0 and 12.2 kg per capita, respectively [1]. Beef consumption continues to rise in line with growth and increase in population and household incomes. By 2027, it is estimated that beef consumption will be 8% and 21% higher in the developed and developing countries, respectively, compared to the 2015–2017 average [2]. Beef is a nutrient-dense food that provides health-beneficial macro- and micro-nutrients for humans. A 100 g serving of beef provides more than the 25% recommended dietary intake (RDI) of protein, niacin, vitamin B_6, vitamin B_{12}, zinc and selenium, and more than 10% RDI of phosphorus, iron and riboflavin. Beef protein is of certain characteristics and contains all the essential amino acids [3] and provides antioxidants such as carnosine and anserine [4,5].

fermentation

MDPI

Article

Chemical Composition and In Situ Degradability of *Desmanthus* spp. Forage Harvested at Different Maturity Stages

Felista W. Mwangi [1], Edward Charmley [2], Oyelola A. Adegboye [3], Christopher P. Gardiner [1], Bunmi S. Malau-Aduli [4], Robert T. Kinobe [1] and Aduli E. O. Malau-Aduli [1,*]

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Abstract: This study evaluated the change in nutritive value and in situ degradability of *Desmanthus* spp. (desmanthus) cultivars JCU2; *D. virgatus*, JCU4; *D. bicornutus* and JCU7; *D. leptophyllus* harvested at varying maturity stages to test the hypothesis that the nutritive value and in situ degradability of desmanthus differ between cultivars and with maturity stage at harvest. In Experiment 1, desmanthus was harvested at 11, 38, 72 and 103 days of regrowth (maturity), separated into the leaf and stem portion, dried and analysed for dry matter (DM) and chemical composition. In Experiment 2, desmanthus was harvested 78, 122 and 168 days after planting (maturity). Samples were dried, and DM, crude protein (CP) and neutral detergent fibre (NDF) and acid detergent fibre (ADF) degradation were determined using the in situ technique with three fistulated Droughtmaster steers. The results showed an interaction between cultivar and maturity on the leaf to stem mass ratio, leaf CP, stem NDF and the leaf ADF ($p \leq 0.04$). The leaf-to-stem mass ratio declined more steeply with maturity in JCU7 compared to JCU2 and JCU4 ($p = 0.04$), while there was a higher decline in leaf CP of JCU4 than JCU2 and JCU7 ($p < 0.01$). The total potentially degradable fraction of DM and CP did not differ between cultivars ($p \geq 0.30$) but declined with maturity ($p \leq 0.04$). However, the effective DM degradability at a high particle outflow rate was higher in JCU4 than in JCU7. Taken together, these results indicate that differences exist between cultivars, and higher livestock production may be achieved by utilising the different cultivars in a blend and at earlier maturity stages. Therefore, the hypothesis that nutritive value and in situ degradability of desmanthus differ between cultivars and with maturity stage at harvest was accepted.

Keywords: legume pastures; tropical livestock; pasture quality; leaf to stem mass ratio

Citation: Mwangi, F.W.; Charmley, E.; Adegboye, O.A.; Gardiner, C.P.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E.O. Chemical Composition and In Situ Degradability of *Desmanthus* spp. Forage Harvested at Different Maturity Stages. *Fermentation* **2022**, *8*, 377. https://doi.org/10.3390/fermentation8080377

Academic Editor: Anusorn Cherdthong

Received: 6 July 2022
Accepted: 5 August 2022
Published: 9 August 2022

1. Introduction

Seasonal fluctuations in pasture availability and nutritive value are major factors limiting livestock production on tropical grass pastures [1]. This is primarily due to declines in leaf-to-stem mass ratio (L/S), crude protein (CP) and soluble carbohydrates that reduce the nutritive value and palatability of pastures [2]. Low nutritive value leads to poor growth and reproductive performance, increased susceptibility to parasites and diseases and increased enteric methane emissions [3–5]. The introduction of legumes into tropical grass pastures provides high-quality forage to grazing livestock for longer periods compared to grass-only pastures [6]. Augmenting low-quality grass pastures with

animals

MDPI

Article

Growth Performance and Plasma Metabolites of Grazing Beef Cattle Backgrounded on Buffel or Buffel-*Desmanthus* Mixed Pastures

Felista W. Mwangi [1], Christopher P. Gardiner [1], Glen Walker [1], Trevor J. Hall [2], Bunmi S. Malau-Aduli [3], Robert T. Kinobe [1] and Aduli E. O. Malau-Aduli [1,*]

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Simple Summary: Pasture quality and digestibility decline during the dry season resulting in weight loss or marginal weight gains of grazing cattle in the seasonally dry subtropics of northern Australia. Oversowing grass with legume pastures has shown potential to improve pasture quality and cattle weight gain. This study aimed to evaluate the change in steers' weight gain and plasma metabolites in response to grazing buffel grass pastures oversown with *Desmanthus* spp. (*Desmanthus*), a tropical legume adapted to cracking clay soils, compared to buffel-grass-only pastures. Results showed that *Desmanthus* at a low botanical composition had no effect on weight gain and plasma metabolites, although pasture yield and stocking rate were 443 kg/ha and 9.5% higher, respectively. Since the productivity of grazing systems depends on cattle annual weight gain and stocking rate, the practical implication of this study is that *Desmanthus* may improve the profitability of beef production in the dry tropics of northern Australia by improving pasture-carrying capacity with no adverse effect on cattle health status and growth performance.

Citation: Mwangi, F.W.; Gardiner, C.P.; Walker, G.; Hall, T.J.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E.O. Growth Performance and Plasma Metabolites of Grazing Beef Cattle Backgrounded on Buffel or Buffel-*Desmanthus* Mixed Pastures. *Animals* **2021**, *11*, 2355. https://doi.org/10.3390/ani11082355

Academic Editor: Igino Andrighetto

Received: 28 June 2021
Accepted: 6 August 2021
Published: 9 August 2021

Abstract: Dietary crude protein and dry matter digestibility are among the major factors limiting feed intake and weight gain of cattle grazing native and improved pastures in the subtropics of Northern Australia during the dry season. Incorporating a suitable legume into grasses improves pasture quality and cattle weight gain, but only a limited number of legume pastures can establish and persist in cracking clay soils. This study aimed to evaluate the effect of *Desmanthus* inclusion in buffel grass (*Cenchrus ciliaris*) pastures on the plasma metabolite profile and growth performance of grazing beef cattle during the dry season. We hypothesised that backgrounding steers on buffel grass-*Desmanthus* mixed pastures would elicit significant changes in plasma glucose, bilirubin, creatinine, non-esterified fatty acids and β-hydroxybutyrate, resulting in higher liveweight gains than in steers on buffel grass only pastures. Four hundred tropical composite steers were assigned to buffel grass only (n = 200) or buffel grass oversown with *Desmanthus* (11.5% initial sward dry matter) pastures (n = 200) and grazed for 147 days during the dry season. *Desmanthus* accounted for 6.2% sward dry matter at the end of grazing period. Plasma metabolites results showed that changes in β-hydroxybutyrate, creatinine, bilirubin, glucose and non-esterified fatty acids were within the expected normal range for all the steers, indicating that with or without *Desmanthus* inclusion in the diet of grazing steers, animal health status was not compromised. It was also evident that *Desmanthus* inclusion in buffel grass pastures had no impact on the plasma metabolite profile, liveweight and daily weight gain of grazing steers. Therefore, our tested hypothesis of higher changes in plasma metabolite profile and higher liveweight gains due to backgrounding on low-level buffel grass-*Desmanthus* mixed pastures does not hold.

PLOS ONE

RESEARCH ARTICLE

Effect of incremental proportions of *Desmanthus* spp. in isonitrogenous forage diets on growth performance, rumen fermentation and plasma metabolites of pen-fed growing Brahman, Charbray and Droughtmaster crossbred beef steers

Felista W. Mwangi[1], Benedicte Suybeng[1], Christopher P. Gardiner[1], Robert T. Kinobe[1], Edward Charmley[2], Bunmi S. Malau-Aduli[3], Aduli E. O. Malau-Aduli[1]*

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Citation: Mwangi FW, Suybeng B, Gardiner CP, Kinobe RT, Charmley E, Malau-Aduli BS, et al. (2022) Effect of incremental proportions of *Desmanthus* spp. in isonitrogenous forage diets on growth performance, rumen fermentation and plasma metabolites of pen-fed growing Brahman, Charbray and Droughtmaster crossbred beef steers. PLoS ONE 17(1): e0260918. https://doi.org/10.1371/journal.pone.0260918

Editor: Arda Yildirim, Tokat Gaziosmanpasa Universitesi, TURKEY

Received: June 28, 2021

Accepted: November 20, 2021

Published: January 4, 2022

Data Availability Statement: All research data are available from the James Cook University data at the following URL: Data citation: Mwangi, Felista (2021): Beef cattle growth performance in response to incremental levels of dietary desmanthus. James Cook University. https://doi.org/10.25903/5v97-fj22 DOI: 10.25903/5v97-fj22. https://research.jcu.edu.au/data.

Abstract

Desmanthus (*Desmanthus* spp.), a tropically adapted pasture legume, is highly productive and has the potential to reduce methane emissions in beef cattle. However, liveweight gain response to desmanthus supplementation has been inconclusive in ruminants. This study aimed to evaluate weight gain, rumen fermentation and plasma metabolites of Australian tropical beef cattle in response to supplementation with incremental levels of desmanthus forage legume in isonitrogenous diets. Forty-eight Brahman, Charbray and Droughtmaster crossbred beef steers were pen-housed and fed a basal diet of Rhodes grass (*Chloris gayana*) hay supplemented with 0, 15, 30 or 45% freshly chopped desmanthus forage on dry matter basis, for 140 days. Varying levels of lucerne (*Medicago sativa*) hay were added in the 0, 15 and 30% diets to ensure that all diets were isonitrogenous with the 45% desmanthus diet. Data were analyzed using the Mixed Model procedures of SAS software. Results showed that the proportion of desmanthus in the diet had no significant effect on steer liveweight, rumen volatile fatty acids molar proportions and plasma metabolites ($P \geq 0.067$). Total bilirubin ranged between 3.0 and 3.6 μmol/L for all the diet treatments ($P = 0.67$). All plasma metabolites measured were within the expected normal range reported for beef cattle. Rumen ammonia nitrogen content was above the 10 mg/dl threshold required to maintain effective rumen microbial activity and maximize voluntary feed intake in cattle fed low-quality tropical forages. The average daily weight gains averaged 0.5 to 0.6 kg/day ($P = 0.13$) and were within the range required to meet the target slaughter weight for prime beef markets within 2.5 years of age. These results indicate that desmanthus alone or mixed with

metabolites

MDPI

Article

Lipid Metabolism, Carcass Characteristics and *Longissimus dorsi* Muscle Fatty Acid Composition of Tropical Crossbred Beef Cattle in Response to *Desmanthus* spp. Forage Backgrounding

Felista W. Mwangi [1], David J. C. Blignaut [1], Edward Charmley [2], Christopher P. Gardiner [1], Bunmi S. Malau-Aduli [3], Robert T. Kinobe [1] and Aduli E. O. Malau-Aduli [1,*]

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Citation: Mwangi, F.W.; Blignaut, D.J.C.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E.O. Lipid Metabolism, Carcass Characteristics and *Longissimus dorsi* Muscle Fatty Acid Composition of Tropical Crossbred Beef Cattle in Response to *Desmanthus* spp. Forage Backgrounding. *Metabolites* **2021**, *11*, 804. https://doi.org/10.3390/metabo11120804

Academic Editor: Beate Fuchs

Received: 1 November 2021
Accepted: 26 November 2021
Published: 27 November 2021

Abstract: Lipid metabolism, carcass characteristics and fatty acid (FA) composition of the *Longissimus dorsi* (loin eye) muscle were evaluated in tropical crossbred steers backgrounded on *Desmanthus* spp. (desmanthus) with or without feedlot finishing. It was hypothesized that steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus will produce carcasses with similar characteristics and FA composition. Forty-eight Brahman, Charbray and Droughtmaster crossbred beef steers were backgrounded for 140 days on Rhodes grass (*Chloris gayana*) hay augmented with 0, 15, 30 or 45 percent desmanthus on dry matter basis. Lucerne (*Medicago sativa*) hay was added to the 0, 15 and 30 percent desmanthus diets to ensure that they were isonitrogenous with the 45 percent desmanthus diet. After backgrounding, the two heaviest steers in each pen were slaughtered and the rest were finished in the feedlot for 95 days before slaughter. Muscle biopsy samples were taken at the beginning and end of the backgrounding phase. Carcasses were sampled at slaughter for intramuscular fat (IMF) content, fat melting point (FMP) and FA composition analyses. Increasing the proportion of desmanthus in the diet led to a linear increase in docosanoic acid ($p = 0.04$) and omega-6/omega-3 polyunsaturated FA ratio (n-6/n-3 PUFA; $p = 0.01$), while docosahexaenoic acid decreased linearly ($p = 0.01$). Feedlot finishing increased hot carcass weight, subcutaneous fat depth at the P8 site and dressing percentage ($p \leq 0.04$). The n-6/n-3 PUFA ratio was within the recommended < 5 for human diets. IMF was within the consumer-preferred ≥3% level for palatability. The hypothesis that steers backgrounded on isonitrogenous diets augmented with incremental proportions of desmanthus will produce similar carcass characteristics and FA composition was accepted. These findings indicate that a combination of tropical beef cattle backgrounding on desmanthus augmented forage and short-term feedlot finishing produces healthy and highly palatable meat.

Keywords: carcass traits; meat quality; intramuscular fat; fat melting point; fatty acids; tropical beef cattle

1. Introduction

Beef is the third most consumed meat in the world at 14.4 kg per capita after poultry and pork at 33.0 and 22.9 kg per capita, respectively [1]. World total meat production in 2020 from ovine, bovine, poultry, pig and other animals was estimated at 337.2 million

agriculture

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Article

Single Nucleotide Polymorphisms in the Fatty Acid Binding Protein 4, Fatty Acid Synthase and Stearoyl-CoA Desaturase Genes Influence Carcass Characteristics of Tropical Crossbred Beef Steers

Felista W. Mwangi [1], Shedrach B. Pewan [1,2], John R. Otto [1], Oyelola A. Adegboye [3], Edward Charmley [4], Christopher P. Gardiner [1], Bunmi S. Malau-Aduli [5], Robert T. Kinobe [1] and Aduli E. O. Malau-Aduli [1,*]

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Citation: Mwangi, F.W.; Pewan, S.B.; Otto, J.R.; Adegboye, O.A.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E.O. Single Nucleotide Polymorphisms in the Fatty Acid Binding Protein 4, Fatty Acid Synthase and Stearoyl-CoA Desaturase Genes Influence Carcass Characteristics of Tropical Crossbred Beef Steers. *Agriculture* **2022**, *12*, 1171. https://doi.org/10.3390/agriculture12081171

Academic Editors: Ante Ivanković and Jelena Ramljak

Received: 13 June 2022
Accepted: 4 August 2022
Published: 6 August 2022

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Abstract: This study explored the identification of single nucleotide polymorphisms (SNP) in fatty acid binding protein 4 (*FABP4*), stearoyl-CoA desaturase (*SCD*), and fatty acid synthase (*FASN*) genes that may influence the carcass traits of tropical crossbred beef cattle. The hypothesis tested was that SNP in the *FABP4*, *SCD*, and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle. Fifty *Bos indicus* and *Bos taurus* crossbred steers were backgrounded on either buffel grass only, or buffel grass and desmanthus mixed pastures for 147 days and finished in a commercial feedlot for 110 days. Steers were slaughtered within 48 h of leaving the feedlot within a lairage period not exceeding 12 h and carcasses graded 12 h after slaughter. Next-generation sequencing of the *FASN*, *FABP4*, and *SCD* genes identified multiple SNP loci that were correlated and significantly associated with carcass traits. The *FABP4* g.44677205A>G locus was significantly associated with hump height and correlated with loin eye muscle area (EMA; $p < 0.05$). Polymorphism in the *SCD* gene g.21275851C>A locus was associated with subcutaneous fat depth and marbling score ($p < 0.05$). The CC genotype had a higher subcutaneous fat depth and marbling score ($p < 0.05$) than the AA genotype. Significant correlations were observed between carcass marbling score and subcutaneous fat depth within the *FASN* SNP locus ($p < 0.05$). Therefore, the hypothesis that SNP in the *FABP4*, *SCD*, and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle was accepted. These findings suggest that SNP in the *FABP4*, *SCD*, and *FASN* genes may be used in carcass grading and meat quality improvement through marker-assisted selection of northern Australian crossbred beef cattle.

Keywords: next-generation sequencing; backfat thickness; lipogenic genes; loin eye muscle area; marbling

1. Introduction

Observable and measurable carcass traits are important in assigning meat quality values. The postmortem measurements of subcutaneous fat depth and marbling are useful in carcass grading as indirect meat quality indicators [1,2]. Characteristics such as loin eye muscle area (EMA) and subcutaneous fat depth are indicative of the amount

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Article

Towards Sustainable Sources of Omega-3 Long-Chain Polyunsaturated Fatty Acids in Northern Australian Tropical Crossbred Beef Steers through Single Nucleotide Polymorphisms in Lipogenic Genes for Meat Eating Quality

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Citation: Mwangi, F.W.; Pewan, S.B.; Otto, J.R.; Adegboye, O.A.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Kinobe, R.T.; Malau-Aduli, A.E.O. Towards Sustainable Sources of Omega-3 Long-Chain Polyunsaturated Fatty Acids in Northern Australian Tropical Crossbred Beef Steers through Single Nucleotide Polymorphisms in Lipogenic Genes for Meat Eating Quality. *Sustainability* **2022**, *14*, 8409. https://doi.org/10.3390/su14148409

Academic Editors: Peter D. Nichols, Mike Packer and Kim Lee Chang

Received: 8 June 2022
Accepted: 5 July 2022
Published: 8 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: This study aimed to identify single nucleotide polymorphisms (SNP) in lipogenic genes of northern Australian tropically adapted crossbred beef cattle and to evaluate associations with healthy lipid traits of the *Longissimus dorsi* (loin eye) muscle. The hypothesis tested was that there are significant associations between SNP loci encoding for the fatty acid binding protein 4 (FABP4), stearoyl-CoA desaturase (SCD) and fatty acid synthase (FASN) genes and human health beneficial omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA) within the loin eye muscle of northern Australian crossbred beef cattle. Brahman, Charbray, and Droughtmaster crossbred steers were fed on Rhodes grass hay augmented with desmanthus, lucerne, or both, for 140 days and the loin eye muscle sampled for intramuscular fat (IMF), fat melting point (FMP), and fatty acid composition. Polymorphisms in FABP4, SCD, and FASN genes with significant effects on lipid traits were identified with next-generation sequencing. The GG genotype at the FABP4 g.44677239C>G locus was associated with higher proportion of linoleic acid than the CC and CG genotypes ($p < 0.05$). Multiple comparisons of genotypes at the SCD g.21266629G>T locus indicated that the TT genotype had significantly higher eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids than GG genotype ($p < 0.05$). Significant correlations ($p < 0.05$) between FASN SNP and IMF, saturated and monounsaturated fatty acids were observed. These results provide insights into the contribution of lipogenic genes to intramuscular fat deposition and SNP marker-assisted selection for improvement of meat-eating quality, with emphasis on alternate and sustainable sources of ω3 LC-PUFA, in northern Australian tropical crossbred beef cattle, hence an acceptance of the tested hypothesis.

Keywords: meat fatty acids; omega-3 fatty acids; intramuscular fat; next-generation sequencing; tropical beef cattle; marker-assisted selection

1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), sustainable diets are protective and respectful of biodiversity and ecosystems, nutritionally

Appendix 4: Submitted Manuscript Under peer Review

Original Research

Feedlot Growth Performance and Carcass Characteristics of Steers Backgrounded on Buffel Grass or Buffel-desmanthus Mixed Pastures

Felista Waithira Mwangi, Darryl Savage, Christopher Peter Gardiner, Edward Charmley, Bunmi Sherifat Malau-Aduli, Robert Tumwesigye Kinobe and Aduli Enoch Othniel Malau-Aduli

Frontiers in Veterinary Science
Animal Nutrition and Metabolism

Feedlot Growth Performance and Carcass Characteristics of Steers Backgrounded on Buffel Grass or Buffel-desmanthus Mixed Pastures

Felista Waithira Mwangi [1], Darryl Savage [2], Christopher Peter Gardiner [1], Edward Charmley [3], Bunmi Sherifat Malau-Aduli [4], Robert Tumwesigye Kinobe [1], and Aduli Enoch Othniel Malau-Aduli [1,*]

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Keywords: Tropical beef cattle, grazing, carcass traits, feedlot finishing, feed to gain ratio

Abstract

Feedlot performance and carcass characteristics of tropical beef steers backgrounded on buffel grass (*Cenchrus ciliaris*) only or buffel grass oversown with desmanthus (*Desmanthus* spp.; 11.5% initial sward dry matter) were evaluated. It was hypothesised that tropical beef cattle steers backgrounded on buffel grass only or buffel grass oversown with desmanthus with similar backgrounding growth performance will not differ in feedlot growth performance and carcass quality. Three hundred and