# Beef production and biopreservative effects of dietary citrus and winery by-products

By

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# Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification. This dissertation includes one review article and two research articles published in peer-reviewed journals, and two articles were submitted for publication. The production of these articles was my primary responsibility and co-authored by my supervisors, as indicated below the relevant research chapters.

Date: March 2020

# Summary

The overall objective of the current study was to compare citrus pulp and grape pomace as dietary supplements and biopreservative for beef production and quality. Angus steers were fed 150 g/kg of dried citrus pulp (DCP) or grape pomace (DGP) as alternative fiber sources to wheat bran (control). Twenty-four steers (7 months old) were assigned to three dietary treatments (8 steers/ treatment) in a completely randomized design. Steer was the experimental unit. Steers were adapted for 21 d before 7 d of collecting feed, refusals, faecal and urine samples for determination of nutrient digestibility and utilisation. The digestibility trial was followed by a growth performance trial, which lasted 90d. During this period, dry matter intake (DMI), average daily gain (ADG) and feed efficiency data were collected. Post-feeding, steers were slaughtered and the left longissimus thoracis lumborum (LTL) from each carcass was sampled for physico-chemical meat quality attributes, shelf-life, major pro-oxidant fatty and volatile compound analyses, while the right LTL was sampled for the evaluation of eating quality attributes.

Overall, steers fed the DGP diet had the greatest intake of dry matter (DM), organic matter (OM), crude protein (CP), ash free neutral detergent fiber (aNDFom), ether extract and starch followed by steers fed the DCP and control diets ($P \leq 0.05$). Apparent digestibilities of DM, OM and aNDFom were greater ($P \leq 0.05$) for the DCP diet compared to the DGP and control diets. Feeding the DCP and DGP diets increased ($P \leq 0.05$) ruminal concentrations of total volatile fatty acids, acetate, isovalerate, acetate to propionate ratio, and reduced propionate concentrations compared to the control diet. The steers fed the control diet had the greatest urinary excretions of allantoin, uric acid and total purine derivatives followed by those fed the DCP and DGP diets ($P \leq 0.05$). The nitrogen (N) intake, faecal nitrogen, N retention and N efficiency utilization were DGP > DCP > control diets ($P \leq 0.05$). Feeding the DGP and DCP diets resulted in greater ($P \leq 0.05$) ADG and final weight compared to the control diet. Steers fed the DGP diet had greater ($P \leq 0.05$) DMI, warm and cold carcass weights than those fed the DCP and control diets. Shear force

and income over feed costs were greatest for the DGP diet followed by the DCP and control diets ($P \leq 0.05$). The DCP diet resulted in the greatest concentration of α-tocopherol in beef muscle tissues, followed by DGP and control diet ($P \leq 0.05$). The beef antioxidant activity was DGP > DCP > control ($P \leq 0.05$). During retail display DGP- or DCP-fed beef had greater L* ($P \leq 0.05$) and fewer ($P \leq 0.05$) coliforms than control diet fed beef. Beef TBARS and carbonyl contents were DGP < DCP < control ($P \leq 0.05$). Overall, antioxidant activity decreased ($P \leq 0.05$) while bacterial loads, TBARS and carbonyl contents increased ($P \leq 0.05$) during retail display regardless of the diet. Feeding the DGP or DCP diets increased ($P \leq 0.05$) the proportions of 18:2 n-6, 18:3 n-3 and total polyunsaturated fatty acid (FA) in LTL muscle. Beef from steers fed the control diet had greater ($P \leq 0.05$) concentrations of alcohol, ketones and aldehydes, and lower ($P \leq 0.05$) concentrations of sulphur containing compounds compared to the DCP- and DGP-fed beef ($P \leq 0.05$). Feeding the DGP and DCP diets produced about 10% less tender ($P \leq 0.05$) beef than the control diet. Overall, DGP improved nutrient intake, retention and efficiency of N utilization, growth performance, carcass attributes, beef shelf life, increase proportions of the main pro-oxidant fatty acids, and reduced aldehydes, ketones and alcohols associated with oxidation without compromising beef physicochemical and sensory quality. The current finding suggests that DGP may be a better fiber substitute and natural preservative in beef finishing diets than DCP

# Opsomming

Die algemene doel van die studie was om sitrus- en druiwepulp te vergelyk in terme van hulle potensiaal as voedingsaanvullings en biopreserveermiddels vir die produksie en kwaliteit van beesvleis. ‘n Porsie van 150 g / kg gedroogde sitruspulp (DCP) of druiwepulp (DGP) is aan Angus bulle gevoer as ‘n alternatiewe veselbron in die plek van koringsemels (kontrole). Vier-en-twintig bulle (7 maande oud) is lukraak ingedeel in drie dieetbehandeling groepe (8 bulle per behandeling) in 'n totaal ewekansige ontwerp, met ‘n bul as eksperimentele eenheid. Bulle is vir 21 dae aangepas voordat versameling van onbenutte voer, mis- en urienmonsters oor ’n tydperk van 7 dae versamel is vir die bepaling van die verteerbaarheid en benutting van voedingstowwe. Die verteerbaarheidstoets is gevolg deur 'n groeiprestasieproef wat 90 dae geduur het. Gedurende hierdie periode is droëmateriaalinname (DMI), gemiddelde daaglikse gewigstoename (ADG) en voerdoeltreffendheidsdata versamel. Na voeding is die bulle geslag en die linker *longissimus thoracis lumborum* (LTL) is van elke karkas versamel vir die ontleding van fisies-chemiese vleiskwaliteit eienskappe, rakleeftyd, hoof pro-oksidant vetsure en vlugtige verbindings, terwyl die regter LTL versamel is vir die bepaling van die eetgehalte eienskappe.

Oor die algemeen het die bulle op die DGP-dieet die grootste inname van droëmateriaal (DM), organiese materiaal (OM), ruproteïen (CP), mineraalvrye neutrale detergentvesel (aNDFom), eterekstrak en stysel gehad, in vergelyking met die bulle wat die DCP en kontrole diëte ingeneem het. Die oënskynlike verteerbaarheid van DM, OM en aNDFom was hoër vir die DCP-dieet in vergelyking met die DGP en kontrole diëte. Die voeding van die DCP- en DGP-diëte het ’n verhoging van die totale rumen vlugtige vetsure asetaat, iso-valeraat en asetaat tot propionaat-verhouding en die propionaatkonsentrasies verlaag, wanneer dit met die kontrole-dieet vergelyk word. Die bulle wat die kontrole dieet ontvang het, het die grootste urinêre uitskeiding van allantoïen, uriensuur en totale purienderivate gehad, gevolg deur die bulle wat die DCP- en DGP-diëte gevoer was. Die stikstof (N)-inname, fekale stikstof, N-retensie en N-

doeltreffendheidsbenutting was DGP> DCP> kontrole diëte. Die voeding van die DGP- en DCP-diëte het tot 'n beter ADG en finale gewig gelei in vergelyking met die kontrole-dieet. Bulle wat die DGP-dieet gevoer was, het ’n verhoogde DMI en swaarder warm- en koue karkasgewigte gehad as dié wat die DCP- en kontrole diëte ontvang het. Die skeursterkte en inkomste bo voerkoste was die hoogste vir die DGP-dieet, gevolg deur die DCP- en beheerdiëte.

Die DCP-dieet het gelei tot die hoogste konsentrasie van α-tokoferol in beesspierweefsel, gevolg deur die DGP- en kontrole dieet. Die antioksidant aktiwiteit van beesvleis was DGP> DCP> kontrole. Tydens kleinhandelvertoning het DGP- of DCP-gevoede beesvleis hoër L * waardes gehad en minder kolivorme as beesvleis van bulle wat die kontrole dieet ontvang het. Bees-TBARS en karbonielinhoud was DGP <DCP < kontrole. Oor die algemeen het die antioksidant aktiwiteit afgeneem terwyl bakterielading, TBARS en karbonielinhoud toegeneem het tydens kleinhandelvertoning, ongeag die dieet. Die voeding van die DGP- of DCP-diëte verhoog die verhoudings van 18: 2 n-6, 18: 3 n-3 en totale poli-onversadigde vetsure (FA) in die LTL-spier. Beesvleis van diere op die kontrole dieet het ’n hoër konsentrasie alkohol, ketone en aldehiede gehad en laer konsentrasie swaelbevattende verbindings, wanneer vergelyk met die monsters versamel van die DCP- en DGP-gevoerde bulle. ’n Metaalagtige vleis-aroma was meer prominent vir vleismonsters verkry van die DGP- en DCP-gevoerde bulle, in vergelyking met die kontrole dieet. Die voeding van die DGP- en DCP-diëte het ongeveer 10% minder sag beesvleis opgelewer as die kontrole-dieet. In die geheel het DGP voedingsinname verhoog, die retensie en doeltreffendheid van N-gebruik, groeiprestasie en karkaseienskappe verbeter. Die DCP – en DGP diëte het gelei tot ’n verlengde rakleeftyd, die verhoudings van die belangrikste pro-oksidant vetsure verhoog en laer vlakke van aldehiede, ketone en alkohole, wat met oksidasie verbind word, tot gevolg gehad. Fisio-chemiese en sensoriese kwaliteit is nie beïnvloed nie. Die bevindinge dui

daarop dat DGP 'n beter veselvervanger en natuurlike preserveermiddel in beesafronddiëte kan wees as DCP.

# Dedication

This research is specifically dedicated to God, my family and my wife.

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# Publications, under review articles & conference presentations

## Journal articles

1 **Tayengwa, T**., & Mapiye, C. (2018). Citrus and winery wastes: Promising dietary supplements for sustainable ruminant animal nutrition, health, production, and meat quality. *Sustainability (Switzerland)*, 10, 1–22. https://doi.org/10.3390/su10103718

2 **Tayengwa, T**., Chikwanha, O. C., Dugan, M. E. R., Mutsvangwa, T., & Mapiye, C. (2020). Influence of feeding fruit by-products as alternative dietary fibre sources to wheat bran on beef production and quality of Angus steers. *Meat Science, 161*. https://doi.org/10.1016/j.meatsci.2019.107969

3 **Tayengwa, T**., Chikwanha, O. C., Gouws, P., Dugan, M. E. R., Mutsvangwa, T., & Mapiye, C. (2020). Dietary citrus pulp and grape pomace as potential natural preservatives for extending beef shelf life. *Meat Science, 162*. https://doi.org/10.1016/j.meatsci.2019.108029

## Under review articles

1 **Tayengwa, T.,** Chikwanha, O.C., Raffrenato, E., Dugan, M. E. R., Mutsvangwa T., & Mapiye, C., 2020. Comparative effects of feeding citrus pulp and grape pomace on nutrient utilization in steers. *Animal, submitted*

## Popular articles

1 **Tayengwa, T.**, Chikwanha, O. C., Dugan, M. E. R., Mutsvangwa, T. & Mapiye, C. (2020). Fruit by-products provide a cheaper fiber feed for feedlot cattle . *Feed navigator.com. https://www.feednavigator.com/Article/2019/11/20/Fruit-by-products-provide-a-cheaper-fiber-feed-for-feedlot-cattle*

## Oral conference presentations

1 **Tayengwa, T.,** Chikwanha, O.C., Dugan, M.E.R., Mutsvangwa, T., & Mapiye, C. Growth performance, carcass and meat quality attributes of Angus steers fed dried grape pomace and citrus pulp. The 51st SASAS Congress, University of Free State Bloemfontein, 10-12 June 2019.

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prayers! Most importantly, I thank my loving and supportive wife Gloria Nyasha Chipangura for all the sacrifices, unconditional love and unending inspiration that you made on my behalf.

# Preface

This dissertation is presented as a compilation of seven chapters. Each chapter is introduced separately and is written according to the style of Meat Science. The whole literature reviewer (Chapter 2) was published in Sustainability. Chapter 3 is under review in Animal for publication, Chapter 4 and 5 were published in Meat Science. Chapter 6 has been submitted for publication Food Chemistry. As each chapter has been written as an individual entity, some repetition between chapters is unavoidable. The research chapters are prefaced by a summary of research performed, general introduction of the topic, culminating in a general discussion and conclusion of the project. The language, style and referencing are in accordance with specifications of Meat Science. The opinions expressed, and conclusions arrived at in this study are those of the author and are not necessarily to be attributed to the National Research Foundation, the Department of Animal Sciences, Stellenbosch University.

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|---|---|
| **Chapter 1** | General introduction, project objectives and hypotheses |
| **Chapter 2** | Citrus and winery wastes: Promising dietary supplements for sustainable ruminant animal nutrition, health, production, and meat quality |
| **Chapter 3** | Comparative effects of feeding citrus pulp and grape pomace on nutrient utilization in steers |
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# List of Abbreviations

| | |
|---|---|
| a* | Redness |
| ADG | Average daily gain |
| ADL | Acid detergent lignin |
| *a*NDFom | NDF assayed with a heat stable amylase and expressed exclusive of residual ash |
| C | Chroma |
| CFU | Coliform forming units |
| CP | Crude protein, being total N × 6.25 |
| CSIRO | Commonwealth Scientific and Industrial Research Organization |
| DAFF | Department of Agriculture, Forestry and Fisheries |
| DCP | Dried citrus pulp |
| DGP | Dried grape pomace |
| DM | Dry matter |
| DMI | Dry matter intake |
| DNPH | 2,4-dinitrophenylhydrazine |
| EE | Ether extract |
| FA | Fatty acids |
| FAO | Food and Agriculture Organization |
| FAOSTAT | Food and Agriculture Organization Corporate Statistical Database |
| FAMEs | Fat acid methyl esters |
| FCE | Food conversion efficiency |
| FRAP | Ferric reducing antioxidant power |
| g/ kg | grams per kilogram |
| GAE | Gallic acid equivalent |
| GP | Grape pomace |
| GC | Gas chromatograph |
| H | Hue angle |
| ICP-AES | Inductively coupled plasma-automatic emission spectrometry |
| IMF | Intramuscular fat |
| iNDF | internal neutral detergent fiber |
| ivNDF | in vitro neutral detergent fiber digestibility |
| IUPAC | International Union of Pure and Applied Chemistry |
| L* | Lightness |
| Lignin (sa.) | Lignin determined by solubilization of cellulose with sulfuric acid |
| LL | *Longissimus lumborum* |
| LSMEANS | Least square means |
| LT | *Longissimus thoracis* |
| LTL | *Longissimus thoracis et lumborum* |
| MDA | Malondialdehyde |
| ME | Metabolizable energy |
| mg/ kg | milligrams per kilogram |
| mg/ mL | milligrams per milliliter |

| | |
|---|---|
| MJ/ kg | megajoules per kilogram |
| mL | Milliliter |
| mM | Millimolar |
| MUFA | Monounsaturated fatty acids |
| N | Nitrogen |
| n-3 | Omega 3 |
| n-3: n-6 | omega 3 to omega 6 ratio |
| n-6 | Omega 6 |
| NDF | Neutral detergent fiber |
| NDS | Neutral detergent soluble |
| NFC | Non fibrous carbohydrates |
| NH3-N | Ammonia nitrogen |
| OM | Organic matter |
| PCA | Principal component analysis |
| PCC | Protein carbonyl content |
| PD | Purine derivatives |
| PUFA | Polyunsaturated fatty acids |
| RI | Retention index |
| RT | Retention time |
| SAS | Statistical Analysis Systems |
| SFA | Saturated fatty acids |
| SPME | Solid phase micro-extraction |
| SPME-GC-MS | Solid phase micro-extraction-gas chromatograph-mass spectrometry |
| TBARS | Thiobarbituric acid reactive substances |
| TPC | Total phenolic content |
| TVC | Total viable counts |
| VFA | Volatile fatty acids |
| UFA | Unsaturated fatty acid |
| WBSF | Warner-Bratzler shear force (Instrumental tenderness) |
| WCW | Warm carcass weight |
| WWF | Worldwide fund |
| µL | Microliter |

# Chapter 1 General introduction

## 1.1 Background

In South Africa, approximately 85% of beef in the formal market comes from feedlots (Department of Agriculture Forestry and Fisheries (DAFF), 2018, Kalaba et al., 2018). As in other water-scarce countries, feedlot beef production in South Africa is limited by shortage of feed ingredients due to prolonged droughts (DAFF, 2017; Kalaba et al., 2018; Pulina et al., 2017). This results in high prices of feed resources and reduced profitability since feed represents about 75% of the total variable costs of beef production (Arowolo & He, 2018; DAFF, 2017). In the last decade, for example, prices of wheat, maize and soybean meal have increased by 118, 160 and 108 %, respectively (DAFF, 2017; Wadhwa, Bakshi, & Makkar, 2015). This is compounded by climate change, which further limits fodder yield and quality in water-scarce areas (Pulina et al., 2017; Rust, 2019). Globally, the proportion of land allocated to fodder crops is small compared to the area allocated for food crops, and has been shrinking due to increased urbanization, industrialization and demand for food crops to feed the emergent human population (Pulina et al., 2017; Thornton, 2010; Wadhwa et al, 2013). In that regard, the feed supplies have remained short of normative requirements (DAFF, 2017; Gupta, Sing, Bhatt & Dey, 2014; Ramachandra, Taneja, Sampath, Anandan & Angadi, 2007), limiting realization of the actual livestock production potential worldwide (Dikshit & Birthal, 2010). To optimize feed shortages and profits for the feedlot industry it is, therefore, important to explore alternative and underutilized feed resources, especially non-conventional resources such as fruit by-products, which are produced in large quantities but not used for human food (Salami et al., 2019; Valenti, Luciano, Pauselli, Mattioli, Biondi, Priolo et al., 2018).

The bulk of South African feedlot cattle are fed grain-based diets, mostly comprised of cereal grains and their by-products as sources of energy, and oilcakes as sources of protein and unsaturated fatty acids (UFAs) (DAFF, 2018; Kalaba et al., 2018). This results in elevated levels of UFA in beef, which makes it susceptible to oxidation during storage (Cunha et al., 2018; Papuc et al., 2017). Oxidation of biomolecules (i.e., lipids, protein and haem pigments) causes deterioration of color and flavor, formation of rancid odors and toxic compounds, and promotes growth of undesirable microbes, which subsequently results in meat losses and wastage (Estévez & Luna, 2017; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). In South Africa for example, of the 31 million tons of food produced annually, 15% are lost in the form of meat and dairy products (WWF, 2017). It has been estimated that approximately 50% of meat losses or wastage occur during the postharvest phase, mainly as a result of oxidation and microbial spoilage (Papuc, Goran, Predescu, & Nicorescu, 2017; Simitzis et al., 2019). Antioxidants can be used to delay or inhibit oxidation in beef, but these are not all created equal, and some synthetics have toxic and carcinogenic effects (Ahmad, Gokulakrishnan, Giriprasad, & Yatoo, 2015; Hayes, Allen, Brunton, O'Grady, & Kerry, 2011). Increasing consumer preference for health beneficial natural foods has thus intensified the search for natural/alternative methods to retard oxidation in foods (Simitzis & Deligeorgis, 2018; Kumar et al., 2015).

Research on plant polyphenolic-based antioxidants as feed supplements or meat preservatives is currently being emphasized (Ahmad, Gokulakrishnan, Giriprasad, & Yatoo, 2015; Hayes, Allen, Brunton, O'Grady, & Kerry, 2011) as they are natural, perceived to be nutritious, safe and healthy. Specifically, plant polyphenol extracts are currently receiving attention as dietary sources of natural antioxidants and antimicrobials due to their potential for extending shelf life of meat and its by-products (Simitzis & Deligeorgis, 2018). It has been reported that feeding bioactive-rich plant by-products such

as bran, shells, skins, solid residues, husks, bagasse, seeds, peels and pomace from cereals, fruits vegetables, roots, tubers, spices, herbs, shrubs and trees could improve meat quality attributes including fatty acid composition, flavor and shelf-life. (Salami et al., 2019; Kumar et al., 2015; Tomovic et al., 2017).

In South Africa, citrus and grapes are major fruit crops, with an annual production of approximately 2.9 and 2.0 million metric tons, respectively (DAFF, 2018; USDA/FAS, 2019). This generates large volumes of waste with citrus pulp and grape pomace correspondingly contributing about 225 and 280 thousand metric tons annually (DAFF, 2018; USDA/FAS, 2019). This waste poses serious processing, storage and disposal challenges such as leachate generation and land fill gas (Khan, le Roes-Hill, Welz, Grandin, & Kudanga, 2015), thus presenting a huge cost to the fruit industry (Sharma, Mahato, Cho, & Lee, 2017; Khan, le Roes-Hill, Welz, Grandin, & Kudanga, 2015). Recent research shows the potential of citrus and grape byproducts as animal feed supplements (Chapter 4) or food preservatives (Luciano et al., 2017; Chikwanha et al., 2019; Pfukwa et al., 2019, Chapter 5). The usage of fresh citrus pulp and grape pomace as feed ingredients or natural preservatives in cattle finishing diets is, however, challenged by high moisture content, neutral detergent fiber and proanthocyanidins (Salami et al., 2019; Chikwanha et al., 2019; Wadhwa et al., 2013). Moisture content and proanthocyanidins can be reduced by drying, ensiling or co-feeding with other ingredients that have low content of phenolic compounds (Chikwanha et al., 2019; Mlambo & Mapiye, 2015; Wadhwa et al., 2015). Economic limitations on the transportation of fresh citrus pulp and grape pomace have also been found due to their bulkiness (Wadhwa et al., 2015). This can be minimized by pelleting, which reduces transport and storage costs (Arthington, Kunkle, & Martin, 2002; Bampidis & Robinson, 2006). In addition, pelleting can decrease feed waste through reducing ingredient selection, increase handling efficiency and decrease dustiness (Arthington et al.,

2002; Bampidis et al., 2006). This leads to shorter eating periods and improved palatability, which in turn, enhances animal performance, feed efficiency and profitability (Arthington et al., 2002; Bampidis et al., 2006).

Overall, DCP has relatively high metabolizable energy, starch, calcium, pectins, ascorbic acid, α-tocopherol, carotenoids and moderate linoleic acid (i.e. 15-45% of the total fatty acids) (Abeysinghe et al., 2007; Zou, Xi, Hu, Nie, & Zhou, 2016), whereas DGP has high crude protein, ether extract, fiber content (NDF and Lignin contents), minerals (phosphorus, zinc and copper), linoleic acid (i.e. 55-75%), bioactive compounds (total phenols, proanthocyanidins, flavonols and anthocyanidins) (Mattos, Tonon, Furtado, & Cabral, 2017; Teixeira et al., 2014), which have nutritional and biopreservative properties (Teixeira et al., 2014; Zou et al., 2016). It has been reported that inclusion of antioxidant-rich feed ingredients like DCP or DGP in ruminant finishing diets has the ability to confer protection against oxidation and/or reduce the extent of microbial spoilage during retail display of meat (Cunha et al., 2018; Papuc, Goran, Predescu, Nicorescu, & Stefan, 2017; Chikwanha et al., 2019; Guerra-Rivas et al., 2016, Inserra et al., 2014; Gravador et al., 2015; Mattos et al., 2017; Zou et al., 2016). The costs of DGP are comparatively lower than DCP, a common non-conventional substitute for wheat bran, the main conventional fiber ingredient in beef cattle finishing diets in South Africa (DAFF, 2018; Kalaba et al., 2018). However, there is limited information on DCP and DGP's feeding and biopreservative value for beef production and quality. In that regard, it was hypothesized that DGP could have superior economic, feeding and biopreservative value compared to DCP when included in a beef finishing diet.

## 1.2 Justification

There is a knowledge gap regarding utilization of citrus pulp and grape pomace as locally available and affordable sources of fiber and bioactive compounds in the feedlot and meat industries, especially in

developing countries. The present work will, therefore, provide data on nutrient utilization, growth performance, carcass and physicochemical attributes, shelf life, volatile and organoleptic profile of beef fed by-products from citrus and grapes grown in South Africa. Utilization of DCP and DGP as dietary supplements and natural biopreservatives could reduce feed shortages and prices thereby increasing profitability. In that regard, DGP and DCP are promising cheaper sources of fiber and bioactive compounds relative to wheat bran, thus offering affordable alternatives to farmers and new commercial opportunities to the animal feed and beef industries.

Use of these fruit by-products as natural biopreservatives in beef industry could improve healthfulness and eating quality of beef, and reduce beef losses and wastage associated with discoloration, rancidity and microbial spoilage. In that context, this will also reduce costs of feed additives, beef-borne illness associated with oxidative processes and microbial spoilage, and reduce the need for specialized processing and packaging often used in the beef industry to improve the appearance and maintain the quality of meat following long periods of storage. Furthermore, feeding citrus and grape by-products may subsequently improve storage of beef over extended periods and/or distribution over long distances, which could allow to export to distant markets. Utilization of DCP and DGP as dietary supplements in cattle finishing diets also has potential to reduce competition with conventional ingredients, which could be used as food sources for human consumption. And finally, the fruit processing industry may benefit from the utilization of DGP and DCP through reduction of economic- and ecological-costs related with its disposal. Overall, the current research will directly address sustainable development goals (SDGs) 2.1, 2.3 and 12.3, which seek to end hunger, improve food and nutrition security, and reduce food waste and losses, respectively.

## 1.3 Objectives

The broad objectives of the current study were to compare the beef production and biopreservative effects of dietary citrus and winery by-products under feedlot conditions. The specific objectives were to:

1. Compare the effects of feeding dietary DCP and DGP on nutrient utilization in Angus steers.
2. Determine the influence of feeding dietary DCP or DGP as alternative fiber sources to wheat bran on beef production and quality of Angus steers.
3. Assess the effects of feeding dietary DCP or DGP as potential natural preservatives for extending shelf life of beef from Angus steers; and
4. Assess the pro-oxidant fatty acid, volatile and organoleptic profiles of beef from steers fed diets containing DCP or DGP.

## 1.4 Hypotheses

The hypotheses tested included:

1. Incorporating DGP in Angus finishing diets improves nutrient utilization compared to DCP.
2. Supplementing DGP as alternative fiber source to wheat bran enhances beef production and quality of Angus Steers compared to DCP.
3. Feeding DGP extends the beef shelf life of Angus steers compared DCP.
4. Supplementing DGP relative to DCP improves the pro-oxidant fatty acid, volatiles and organoleptic profile of beef from Angus steers.

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# Chapter 2 Citrus and winery wastes: Promising dietary supplements for sustainable ruminant animal nutrition, health, production, and meat quality

ABSTRACT

Citrus and grapes are the most widely grown fruits globally, with one-third of total production used for juice and wine making. The juice and winemaking processes generate large quantities of solid organic wastes including citrus pulp and grape pomace. These fruit wastes pose serious economic, environmental, and social challenges, especially in low-to-middle-income countries due to financial, technological, and infrastructural limitations. They are, however, rich in valuable compounds which can be utilized in the ruminant livestock industry as novel, economical, and natural sources of cellulose, polyunsaturated fatty acids, and phytochemicals, which have nutritional, anthelmintic, antioxidant, and antimicrobial properties. Despite citrus and grape fruit wastes having such potential, they remain underexploited by the livestock industry in low-to-middle-income countries owing to lack of finance, skills, technology, and infrastructure. Inclusion of these fruit wastes in ruminant diets could combine the desirable effects of enhancing animal nutrition, health, welfare, production, and meat quality attributes with the prevention of challenges associated with their disposal into the environment. The current review explores the valorization potential of citrus and winery wastes as dietary supplements to sustainably enhance ruminant animal nutrition, health, welfare, production, and meat quality.

**Keywords**: fruit wastes; sustainability; ruminants; natural bioactive compounds; valorization

## 2.1 Introduction

Ruminants in low-to-middle-income countries far outperform all other domestic animals not only as a high-quality source for the human world food system, but also regarding other issues like being a source of energy/power, fertilizer, employment, income, capital accumulation, export earnings, and by-products and also having social and cultural significance (Pulina et al., 2017). In addition to supporting over 600 million smallholder farmers, livestock is an important risk aversion strategy for these resource-poor households in low-to-middle-income countries (Pulina et al., 2017). The global importance of ruminants and their products are increasing as consumer demand in the low-to-middle-income countries expands with population growth and rising incomes (Pulina et al., 2017; Thornton 2010; Wadhwa, Bakshi & Makkar, 2015). In that regard, it is estimated that at least 60 to 70% more ruminant livestock products should be produced to feed the population which is predicted to grow from 7.3 billion in 2015 to 9.5 billion in 2050, and most of this increases will be from low-to-middle-income countries (Pulina et al., 2017; Thornton 2010). Overall, there is a global need to sustainably increase ruminant production in dwindling arable areas and rangelands (Pulina et al., 2017). This must be achieved while minimizing the negative effects of livestock agriculture on the environment, which are associated with climate change, extreme weather events and threats from emerging diseases and parasites (Pulina et al., 2017; Thornton 2010).

Ruminant livestock production in low-to-middle-income countries, particularly in the tropics, suffers greater setbacks compared to in high-income countries, mostly owing to the available feed resources (Arowolo & He, 2018; Pulina et al., 2017; Thornton 2010). The growing demand for animal products in most low-to-middle-income countries imposes huge demand on the available feed resources (Arowolo & He, 2018; Pulina et al., 2017; Thornton 2010). In response to this demand, large amounts of feed

resources will be needed, compromising the sustainability of current feed production systems (Pulina et al., 2017; Wadhwa et al., 2015). On one hand, the availability of rangelands for ruminants has become limited, owing to the growing human population, urbanization, industrialization, and rising demand for utilization of agricultural land for food/biofuel production (Pulina et al., 2017; Thornton 2010). On the other hand, climate change is limiting the productivity and grazing capacity of rangelands, which negatively affects animal health, welfare, and production (Pulina et al., 2017). Besides these preharvest limitations, ruminant livestock production in low-to-middle-income countries is further challenged by severe postharvest losses caused by meat discoloration, rancidity, and microbial spoilage (Mlambo & Mapiye, 2015). To improve livestock nutrition, health, welfare, and meat shelf-life, the livestock industry often relies on extensive usage of synthetic chemicals including nutritional supplements, antiparasitics, antimicrobials, and antioxidants (Kumar, Yadav, Ahmad, & Narsaiah, 2015; Tomovic, Jakanovic, Sojic, Skaljac, & Ivic, 2017). Cases of chemical residues in meat, resistance to antiparasitic and antimicrobial drugs, and negative human health effects from some synthetic products are, however, increasing (Tomovic et al., 2017; Nordi et al., 2014). In addition, the affordability and accessibility of these compounds is a challenge among many smallholder farmers in low-to-middle-income countries due to their low socio-economic status (Pulina et al., 2017). Correspondingly, there is a paradigm shift towards the search for more “natural” and sustainable ways of managing animal nutrition, health, welfare, production, and meat healthfulness, safety, and shelf-life (Tomovic et al., 2017).

Low-to-middle-income countries are currently contributing about 51% to global total fruit production (Thornton, 2010). Of this global production, citrus and grapes are economically the most important fruit crops with approximately 100 and 80 million metric tons annual production, respectively (Thornton, 2010; USDA, 2018). As a result, large quantities of citrus pulp (14.4 million metric tons) and

grape pomace (9 million metric tons) are generated per annum with limited current economic value. Fruit wastes pose severe storage, processing, and disposal challenges including landfill gas and leachate generation, which are subject to stern regulations and end up as an expense rather than a profit for the processing industry (Wadhwa et al., 2015; Salami et al., 2019). The recent characterization of unutilized and discarded fractions of the fruit wastes indicates their potential candidature for processing and value addition (Muhlack, Potumarthi & Jeffery, 2018). In this regard, wastes from citrus and winery fruits are rich sources of natural cellulose, minerals, polyunsaturated fatty acids (PUFAs), and phytochemicals, which have nutritional, anthelmintic, antioxidant, and antimicrobial properties (Teixeira et al., 2014; Zou, Xi, Hu, Nie, & Zhou, 2016). These wastes, henceforth referred to as "by-products", can therefore be adopted in the citrus- and grape-producing low-to-middle-income countries as a strategy of economic advantage in ruminant diets as they have the potential to reduce feed shortages and costs and enhance animal nutrition, health, welfare, production, meat fatty acid profile, and shelf life. In addition, the utilization of citrus and winery by-products as dietary supplements can produce meat with human-health-promoting properties (Kumar et al., 2015; Tomovic et al., 2017; Salami et al., 2019) and help mitigate sustainability challenges that would arise from their disposal (Tomovic et al., 2017; Salami et al., 2019). The current review therefore explores the potential of utilizing citrus and winery by-products as dietary supplements for sustainable improvement of ruminant animal nutrition, health, welfare, production, and meat quality.

## 2.2 Nutrient and bioactive profiles of citrus and winery by-products

### 2.2.1 *Nutrient composition of citrus pulp and grape pomace*

Citrus pulp is the solid residue that remains after extraction of juice from fresh fruits. It contributes 50–70% of the fresh weight of the original fruit and is made up of the peel (60–65%), internal tissues

(30–35%), and seeds (0–10%) (Bampidis & Robinson, 2006). The genus citrus includes several important fruits, with the most important on a worldwide basis being sweet orange (C. sinensis, 67.8% of world citrus production), tangerine (C. reticulate, 17.9%), lemon (C. Limon, 6.3%), and grapefruit (C. paradise, 5.0%) (Bampidis & Robinson, 2006). Grape pomace comprises stalks (2%), seeds (47%), skin, and pulp (51%) on a dry matter basis (Beres et al., 2017). Globally, Sauvignon Blanc and Shiraz (Syrah) are among the most economically important cultivars of Vitis vinifera, which is the predominant species in genus Vitis.

Citrus pulp contains up to 105 g/kg dry matter (DM) crude protein (CP), while grape pomace contains up to 123 g/kg DM CP (Table 2.1). The CP content of these fruit by-products is within the recommended metabolizable protein requirements for maintenance (60–80 g/kg DM) of ruminants (Salah, Sauvant, & Archimède, 2014). Furthermore, grape pomace has greater ether extract (EE) in g/kg DM compared with citrus pulp (Table 2.1). The EE levels of these fruit by-products are within the recommended range (<80 g/kg DM) for ruminants (Salah et al., 2014). In terms of neutral detergent fiber (NDF), citrus pulp has lower values than grape pomace (Table 2.1), but values for both fruit by-products lie within the recommended range of 170–330 g/kg DM for ruminants (Salah et al., 2014). In contrast, citrus pulp has a higher content of water-soluble sugars than grape pomace (Table 2.1). Gobindram et al. (2017) and Winkler et al. (2015) reported high metabolizable energy values for grape pomace compared to citrus pulp. However, both values were generally close to the recommended range of metabolizable energy (12.2 ± 2.0 MJ/kg average daily gain) requirements for growth of cattle (Salah, Sauvant, & Archimède, 2014). The lignin content in grape pomace is higher than that in citrus pulp (Table 2.1) and is above the 40 g/kg DM suggested as the threshold level at which DM intake and digestibility in ruminants could be depressed (Guerra-Rivas, Gallardo, Mantecón, del Álamo-Sanza, & Manso, 2017). The calcium, phosphorus, and magnesium content of citrus pulp and grape pomace are within the

recommended maintenance requirements for ruminants of 15.4 mg Ca/kg, 16 mg P/kg, and 12–16 mg Mg/kg body weight (Buchanan-Smith, 2016). The levels of the most limiting amino acids for ruminants, methionine and cysteine, in citrus pulp and grape pomace do not meet the required levels for maintenance (Buchanan-Smith, 2016). Use of citrus pulp and grape pomace in ruminant diets, therefore, requires supplementation of the diet with amino acids or use of amino-acid-rich ingredients to complement these fruit by-products. Overall, the observed variation in chemical composition reported in Table 2.1 can be explained by fruit origin, environmental factors, production techniques and cultivation conditions, dehydration methods, extraction procedures, and fruit cultivar. The nutritional composition variation of these fruit by-products implies that recommendations for inclusion level in ruminant diets can only be made after chemical characterization.

### 2.2.2 *Bioactive compounds in citrus and winery by-products*

#### *2.2.2.1 Citrus pulp*

Citrus pulp has relatively high proportions of PUFA, especially linoleic acid (15–45% of total fatty acids) (Assefa et al., 2017), but data are limited, and further research is recommended. Citrus fruits and their by-products are rich sources of bioactive compounds such as phenolics, flavonoids, and ascorbic acid. The total phenolic and flavonoid contents for citrus pulp range from 8.25 to 397 mg gallic acid equivalent (GAE)/g of extract (Ghasemi, Ghasemi, & Ali Ebrahimzadeh, 2009; Magwaza, Mditshwa, Tesfay, & Opara, 2017) and 0.3 to 31.1 mg quercetin acid equivalent/g of powder (Ghasemi et al., 2009), respectively. Ascorbic acid levels in citrus pulp range from 18.2 to 46.2 mg/100 mL (Zhang, Yang, & Zho, 2018). Overall, ascorbic acid and carotenoid contents of citrus fruits meet the recommended requirements for ruminants (Buchanan-smith et al., 2016; Zou et al., 2016).

**Table 2.1** Chemical composition of citrus and winery by-products

| Chemical Composition (g/kg) | Citrus Pulp [a] | | | Grape Pomace [b] | | |
|---|---|---|---|---|---|---|
| | Min | Max | Mean ± SD | Min | Max | Mean ± SD |
| Dry matter | 858 | 955 | 910 ± 5.0 | 351 | 955 | 918 ± 0.1 |
| Organic matter | 934 | 955 | 928 ± 3.1 | 866 | 938 | 910 ± 0.3 |
| Ash | 46.3 | 134 | 69.0 ± 1.40 | 33.0 | 134 | 60 ± 0.68 |
| Crude protein | 64.9 | 105 | 70.0 ± 1.50 | 54.0 | 123 | 115 ± 0.5 |
| Ether extract | 27.0 | 58.0 | 37.0 ± 2.00 | 52.0 | 71.0 | 68.0 ± 0.65 |
| Neutral detergent fiber | 155 | 387 | 242 ± 10.6 | 376 | 630 | 322 ± 2.5 |
| Neutral detergent solubles | 613 | 845 | 729 ± 10.2 | 373 | 744 | 678 ± 3.16 |
| Acid detergent fiber | 100 | 307 | 222 ± 10.4 | 317 | 550 | 326 ± 2.9 |
| Acid detergent lignin | 21.0 | 25.0 | 22.0 ± 4.90 | 161 | 446 | 197 ± 2.8 |
| Hemicellulose | 55.0 | 287 | 183 ± 4.2 | 59.0 | 313 | 208 ± 2.1 |
| Cellulose | 128 | 128 | 128 ± 3.1 | 540 | 540 | 540 ± 3.4 |
| Metabolizable energy (MJ /kg) | 105 | 128 | 119 ± 2.4 | 58.0 | 130 | 88.6 ± 5.31 |
| Mineral composition (g/kg) | | | | | | |
| Calcium | 4.90 | 22.4 | 17.0 ± 2.30 | 2.29 | 6.10 | 3.0 ± 0.05 |
| Phosphorus | 0.70 | 1.50 | 1.0 ± 0.20 | 0.60 | 3.10 | 2.6 ± 0.05 |
| Potassium | 6.60 | 11.6 | 9.3 ± 1.30 | 15.0 | 24.1 | 20.4 ± 0.41 |
| Magnesium | 1.00 | 2.10 | 1.3 ± 0.40 | 1.00 | 1.20 | 1.1 ± 0.03 |
| Sulphur | 0.80 | 1.20 | 0.8 ± 0.20 | 1.02 | 1.35 | 1.2 ± 0.02 |
| Sodium | 0.30 | 4.00 | 1.2 ± 1.30 | 0.10 | 0.90 | 0.7 ± 2.42 |
| Zinc (mg/kg) | 6.00 | 57.0 | 14 ± 14.0 | 7.30 | 14.6 | 11.3 ± 0.34 |
| Manganese (mg/kg) | 5.00 | 14.0 | 8.0 ± 3.00 | 14.2 | 21.0 | 16.8 ± 0.41 |
| Iron (mg/kg) | 46.0 | 170 | 80 ± 32.0 | 115 | 147 | 126 ± 2.9 |
| Copper (mg/kg) | 3.00 | 6.30 | 4.5 ± 1.00 | 7.30 | 13.4 | 9.8 ± 0.31 |

[a] Reported mean values were for *C. sinensis* and *C. reticulate blanco* cultivars in Egypt, Brazil, Morocco; USA, Turkey, Pakistan, and South Africa; [b] Reported mean values were for *Pinotage*, *Shiraz*, and *Blanc* cultivars in South Africa, Spain, Italy, Iran, and Chile; SD means standard deviation, Organic matter = 100 − Total Ash; Neutral Detergent Solubles = 100 − Neutral detergent fiber; Hemicellulose = Neutral detergent fiber − Acid detergent fiber. Sources: (Wadhwa et al, 2015; Nordi et al., 2014; Bampidis et al., 2006; Gobindram et al., 2017; Guerra-Rivas et al., 2017; Sharif et al., 2017; Chikwanha et al., 2018; Alnaimy, 2017).

#### *2.2.2.2 Grape pomace*

Grape pomace has elevated proportions of PUFA, particularly linoleic acid (55–75%) (Chikwanha, Raffrenato, Muchenje, et al., 2018), but data are again limited, and further studies are recommended. Grapes contain high levels of polyphenols, with as much as 70% retained in the pomace after the extraction of juice (Teixeira et al., 2014; Mattos, Tonon, Furtado, & Cabral, 2017). The main polyphenols

in grape pomace are phenolic acids, flavan-3-ols, flavonols, anthocyanins, and proanthocyanidins. Total phenolic and flavonoid contents among cultivars range from 55.5 to 153.8 mg GAE/g and 38.9 to 91.7 mg rutin equivalent/g, respectively (Teixeira et al., 2014, Mattos et al., 2017). Total tannin, monomeric anthocyanin, and proanthocyanidins contents in grape pomace extracts range from 54 to 152.2 mg GAE/g, 0.02 to 11.2 mg cyanidin-3-glucoside equivalent/g, and 21 to 51.7%, respectively (Teixeira et al., 2014, Mattos et al., 2017).

## 2.3 Influence of feeding citrus and winery by-products on ruminant nutrition, health and production

### 2.3.1 *Influence on dry matter intake*

Feeding high levels (>150 g/kg DM) of citrus pulp and grape pomace reduces dry matter intake (DMI) in ruminants (Chikwanha, Muchenje, Nolte, & Dugan, 2019; Guerra-Rivas et al., 2016; Lanza et al., 2015; Villarreal et al., 2006). The low DMI is attributed to the high fiber content and reduced palatability due to the sensation of astringency that proanthocyanidins confer on feed by binding with salivary proteins, giving an unpleasant feeling of dryness and harshness (Vasta et al., 2010; Jeronimo et al., 2010; Jeronimo et al., 2012). There is an increased DMI when the inclusion level of citrus and winery by-product is less than 150 g/kg DM in ruminant diets (Cribbs et al., 2015; Guerra-Rivas et al., 2016; Zhao et al., 2017). This increased intake for citrus and winery by-products might be due to their distinctive scent and taste and better palatability at lower inclusion levels (Wadhwa & Bakshi, 2013).

### 2.3.2 *Influence on rumen digestibility*

Rumen digestibility of DM, organic matter (OM), CP, and NDF decrease with increasing levels of citrus pulp and grape pomace above 150 g/kg DM in ruminant diets (Table 2.2). This might be connected

to the theory that polyphenols from these fruit by-products form complexes with natural polymers such as proteins and carbohydrates (Jayanegara & Palupi, 2010) and, therefore, may reduce their digestibility in ruminants. The binding property of bioactive compounds from citrus and winery by-products such as proanthocyanidins, eugenol, and limonene results from many free phenolic groups that form strong hydrogen bonds at numerous sites with proteins (Frutos, Hervás, Giráldez, & Mantecón, 2004; Mueller-Harvey et al., 2019). In addition, proanthocyanidins also form complexes with proteins through hydrophobic binding between the aromatic ring structure of tannins and hydrophobic regions of proteins (Frutos, Hervás, Giráldez, & Mantecón, 2004; Mueller-Harvey et al., 2019).

There is a decrease in CP digestibility with increasing levels of citrus and winery by-products in ruminant diets and the relationship is stronger than that of OM digestibility (Sharif et al., 2017, Javed et al., 2016). This suggests that polyphenols from citrus and winery by-products have a stronger interaction with proteins than do the other organic components in the diet, particularly fiber fractions.

**Table 2.2** Effects of dietary fruit by-products on the nutrient digestibility of ruminants

| Fruit By-Product | Inclusion (g/kg) | Animal | DM[a] (g/kg) | OM[b] (g/kg) | CP[c] (g/kg) | NDF[d] (g/kg) | ADF[e] (g/kg) | References |
|---|---|---|---|---|---|---|---|---|
| Citrus pulp | 25 | Lambs | 800 | 655 | 483 | 580 | - | Macedo et al., 2007 |
| | 50 | Lambs | 767 | 733 | 560 | 653 | - | Macedo et al., 2007 |
| | 75 | Lambs | 747 | 761 | 521 | 705 | - | Macedo et al., 2007 |
| Citrus pulp | 90 | Cows | 741 | - | 759 | 574 | - | Santos et al., 2014 |
| | 180 | Cows | 754 | - | 765 | 576 | - | Santos et al., 2014 |
| Citrus pulp | 1.25 | Steers | 530 | 540 | | 480 | - | Villarreal et al., 2006 |
| | 2.5 | Steers | 600 | 620 | | 510 | - | Villarreal et al., 2006 |
| Citrus pulp | 50 | Calves | 667 | - | 698 | 546 | 476 | Javed et al., 2016 |
| | 100 | Calves | 654 | - | 696 | 541 | 462 | Javed et al., 2016 |
| | 150 | Calves | 653 | - | 691 | 531 | 459 | Javed et al., 2016 |
| | 200 | Calves | 652 | - | 690 | 525 | 451 | Javed et al., 2016 |
| Citrus pulp | 60 | Lambs | 651 | 662 | 717 | 544 | - | Peixoto et al., 2015 |
| | 143 | Lambs | 658 | 669 | 725 | 554 | - | Peixoto et al., 2015 |
| | 218 | Lambs | 639 | 648 | 718 | 530 | - | Peixoto et al., 2015 |
| | 265 | Lambs | 658 | 666 | 731 | 554 | - | Peixoto et al., 2015 |
| Citrus pulp | 86.5 | Steers | 609 | - | - | 521 | 537 | Kim et al., 2007 |
| | 72.8 | Steers | 673 | - | - | 574 | 607 | Kim et al., 2007 |
| | 72.5 | Steers | 670 | - | - | 560 | 600 | Kim et al., 2007 |
| | 82.5 | Steers | 636 | - | - | 539 | 561 | Kim et al., 2007 |
| Citrus pulp | 100 | Lambs | 695 | 716 | 714 | 501 | 472 | Sharif et al., 2017 |
| | 200 | Lambs | 691 | 713 | 706 | 495 | 470 | Sharif et al., 2017 |
| Citrus pulp | 300 | Lambs | 681 | 705 | 703 | 488 | 465 | Sharif et al., 2017 |
| | 400 | Lambs | 678 | 704 | 692 | 471 | 461 | Sharif et al., 2017 |
| Grape pomace | 762 | Lambs | 453 | 510 | 345 | | 343 | Abarghuei et al., 2010 |
| | 300 | Wethers | 680 | 690 | 750 | 320 | 500 | Baumgärtel et al., 2007 |
| Grape pomace | 100 | Steers | 62.5 | 66.5 | 72.5 | 62.2 | 53.3 | Foiklang et al., 2016 |

[a]DM - Dry matter, [b]OM - Organic matter, [c]CP – Crude protein, [d]NDF- Neutral detergent fiber, [e]ADF – Acid detergent fiber

The greater negative effects of dietary polyphenols such as proanthocyanidins on CP digestibility related to fiber suggests that the effect of tannins on fiber digestion are a secondary effect (Frutos, Hervás, Giráldez, & Mantecón, 2004; Mueller-Harvey et al., 2019). Proteins have more possible binding sites with tannins than fiber. This is because fiber interacts with tannins through only hydrogen bonds, while protein may also form complexes with tannins through hydrophobic binding and covalent bonds (Frutos, Hervás, Giráldez, & Mantecón, 2004; Mueller-Harvey et al., 2019). It may be possible that proteolytic bacteria are more tannin sensitive than fibrolytic bacteria (Frutos, Hervás, Giráldez, & Mantecón, 2004; Mueller-Harvey et al., 2019). Only few studies have reported that dietary inclusion of 50–100 g/kg DM of citrus or winery by-products in ruminant diets enhances digestibility of DM, OM, CP, NDF, and ADF (Bahrami et al., 2010; Macedo et al., 2007). In general, utilization of citrus and winery by-products at inclusion levels of up to 150 g/kg DM in ruminant diets could be useful in finishing ruminants without negative effects on nutrient digestibility.

Bioactive compounds from citrus and winery by-products, such as polyphenols and essential oils, protect the dietary PUFA from biohydrogenation in the rumen, and/or suppress the growth and metabolism of rumen microbes responsible for biohydrogenation, particularly those involved in the last step, which is the conversion of *trans*-18:1 to stearic acid (Lanza et al., 2015; Gomez-Cortes, Guerra, et al., 2018). In this regard, selective inhibition of *Clostridium proteoclasticum* without influencing *Butyrivibrio fibriosolvens* results in more PUFA and their biohydrogenation products, such as vaccenic and rumenic acids, bypassing rumen biohydrogenation and being subsequently incorporated into animal tissues (Mapiye et al., 2015; Vasta, Makkar, Mele, & Priolo, 2009). Higher levels of citrus and winery by-products (≥200 g/kg DM) in the diet is more effective in modulating the fatty acid profile of ruminant meat (Correddu et al., 2013; Kafantaris et al., 2018; Lanza et al., 2015). Utilization of such high levels, however, has detrimental effects on DMI and nutrient digestibility.

### 2.3.3 *Influence on rumen fermentation*

Overall, rumen pH is not significantly affected by feeding citrus or winery by-products as sources of natural bioactive compounds (Table 2.3). Proanthocyanidins and essential oils lower the volume of ammonia nitrogen produced in the rumen, which improves assimilation of dietary amino acid in ruminants (Abarghuei, Rouzbehan, & Alipour, 2010; Francisco et al., 2018; Sparkes, Chaves, Fung, Van Ekris, & Bush, 2010; Tadayon, Rouzbehan, & Rezaei, 2017). The decline in ammonia nitrogen concentration is usually accompanied by a decrease in the production of isoacids because of the reduction in degradation of dietary proteins (Bodas et al., 2012). Furthermore, the effects of bioactive compounds on rumen ammonia concentrations are probably related to a reduction in the protozoal population, which plays a major role in ruminal feed protein degradation (Bodas, Prieto, García-González, Andrés, & Giráldez, 2012). Several studies have shown that ammonia nitrogen emanating from microbial protein degradation can be bound by polyphenols in a balanced chemical reaction regulated by the ammonia concentration to create a continuous supply of enough quantities of ammonia for microbial growth in the rumen (Patra & Saxena, 2011).

The inclusion of citrus or winery by-products in ruminant diets up to 150 kg DM improves the individual and total volatile fatty acid (VFA) profile (Table 2.3) by providing more lipogenic metabolizable nutrients (Foiklang, Wanapat, & Norrapoke, 2016; Piquer, Ródenas, Casado, Blas, & Pascual, 2009; Sparkes et al., 2010; Tadayon et al., 2017). This could be ascribed to a faster rate of feed intake and increased digestibility, since ruminal VFA production depends on the availability and utilization of substrates of rumen fermentation (Bodas et al., 2012). Therefore, addition of these by-products to concentrate diets supplies a greater amount of highly fermentable substrate to rumen microbes with a resulting increase in VFA concentration (Correddu et al., 2013). These fruit by-products

are rapidly and extensively degraded in the rumen due to their high concentration of pectins (Bampidis & Robinson, 2006), which have high rumen degradability However, reduced ruminal VFA concentrations have been reported in ruminants fed citrus and winery by-products (Berashati & Taghizadeh, 2009). This was attributed to low microbial activity and substrate degradation as a result of decreases in cellulolytic and total bacteria numbers in the rumen in response to proanthocyanidins in citrus and winery by-products (Correddu et al., 2013; Tadayon et al., 2017). The decline in VFA production may occur with changes in the quantities of main VFAs, such as increase in acetate and a decrease in propionate (Correddu et al., 2013; Tadayon et al., 2017).

**Table 2.3** The effects of citrus and winery by-products on rumen fermentation parameters

| Fruit By-Product | Inclusion (g/kg) | Animal | pH | VFA (mmol/L) | AA[1,2] | PA[1,3] | BA[1,4] | $NH_3N$ (mg/dl) | References |
|---|---|---|---|---|---|---|---|---|---|
| Citrus pulp | 477 | Steers | 6.40 | 131.0 | 0.64 | 0.14 | 0.15 | 135 | Taniguchi et al., 1999 |
| | 300 | Steers | 6.30 | 157.0 | 0.73 | 0.13 | 0.12 | 109 | Taniguchi et al., 1999 |
| Citrus pulp | 130 | Lambs | 6.53 | 75.4 | 0.64 | 0.18 | 0.14 | 5.69 | Piquer et al., 2009 |
| | 260 | Lambs | 6.57 | 72.8 | 0.64 | 0.20 | 0.12 | 6.09 | Piquer et al., 2009 |
| | 390 | Lambs | 6.63 | 71.5 | 0.66 | 0.18 | 0.11 | 4.42 | Piquer et al., 2009 |
| Citrus pulp | 300 | Ewes | 5.90 | 154 | 0.65 | 0.23 | 0.08 | 40.0 | Bodas et al., 2012 |
| | 100 | Steers | 6.37 | 154 | 0.59 | 0.21 | 0.11 | 124 | Kim et al., 2007 |
| Grape pomace | 50 | Steers | 6.67 | 116 | 0.63 | 0.26 | 0.11 | 10.2 | Foiklang et al., 2016 |
| | 76.2 | Lambs | 6.03 | - | - | - | - | 15.9 | Abarghuei et al., 2010 |
| Grape pomace | 20 | Buffaloes | 6.71 | 57.1 | 0.66 | 0.23 | 0.11 | 11.4 | Pretty et al., 2016 |
| | 40 | Buffaloes | 6.71 | 56.8 | 0.66 | 0.23 | 0.12 | 13.9 | Pretty et al., 2016 |
| | 60 | Buffaloes | 672 | 58.5 | 0.66 | 0.23 | 0.11 | 14.3 | Pretty et al., 2016 |
| Grape pomace | 150 | Lambs | 6.22 | 102 | - | - | - | 103 | Akbar et al., 2009 |
| | 300 | Lambs | 6.14 | 97.2 | - | - | - | 77.5 | Akbar et al., 2009 |
| | 450 | Lambs | 5.84 | 72.2 | - | - | - | 63.6 | Akbar et al., 2009 |

VFA means volatile fatty acid; [1] measured in mmol/L; AA[2] means acetic acid; PA[3] means propionic acid and BA[4] means butyric acid

### 2.3.4 *Effects on methane production*

Polyphenolic compounds from citrus and winery by-products have received attention for their capacity to decrease methane production when added to ruminant diets by suppressing the growth and activity of methanogens, such as *Methanobrevibacter* or *Methanomicrobium*, responsible for methanogenesis (Moate et al., 2014; Rochfort, Parker, & Dunshea, 2008). Dietary supplementation of essential oils from citrus and winery by-products such as eugenol and limonene in ruminant diets directly inhibits methanogenic *archaea* and/or indirectly reduce methane production by directly suppressing some microbial metabolic processes contributing to methanogenesis (Cobellis, Trabalza-Marinucci, & Yu, 2016). Essential oils may also cause changes in the archaeal community structure and/or in the activity of the methanogenesis pathway, consequently diminishing methanogen abundance and methane production (Author et al., 2008). They may also reduce methanogenesis by lowering the abundance of some protozoa that are symbiotically associated with *archaea* and can contribute up to 37% to rumen methane production. Essential oils reduce methane production in the rumen, with a reduction of up to 94% (Author et al., 2008; Patra & Saxena, 2011). This raises the opportunity for supplementing ruminant diets with feed ingredients rich in phytochemical–nutrient complexes as a mechanism to provide high feeding value while reducing methane production.

### 2.3.5 *Effects on nitrogen emissions*

Feeding citrus or winery by-products regularly results in a shift in nitrogen excretion from the urine to the feces (Correddu et al., 2013; Rochford et al., 2008). Urinary nitrogen is deemed more detrimental to the environment than fecal nitrogen, particularly concerning volatilization of ammonia nitrogen and leaching to groundwater reserves (Correddu et al., 2013). As a result, lowering the proportion of urinary nitrogen and raising the fecal nitrogen with the use of bioactive compounds from citrus and winery by-

products would allow for a decrease in ammonia nitrogen volatilization and would lessen environmental pollution. In that regard, further studies aimed at determining the effects of feeding citrus and winery by products on nitrogen emission and the associated mechanisms are important.

### 2.3.6 *Effects on nutritional disorders*

#### *2.3.6.1 Bloat*

The beneficial role of bioactive compounds from citrus and winery by-products on ruminant health and welfare is becoming a central issue in livestock production (Nordi et al., 2014; Correddu et al., 2013). Citrus or winery by-products could prevent bloat in ruminants because of bioactive compounds (Correddu et al., 2013, Tadayon et al., 2017). The reduction in gas production and prevention of bloat can be explained by the ability of proanthocyanidins to precipitate proteins during chewing and rumination and reduce protein solubility in the rumen (Correddu et al., 2013). This precipitation also decreases the incidence of bloat by lowering microbial activities, biofilm production, and ruminal gas production (Jayanegara & Palupi, 2010; Correddu et al., 2013). In addition, the interaction between polyphenols and protein can improve the flow of nitrogen leaving the rumen to the gut, enhancing protein utilization. The mechanism and ability of bioactive compounds from citrus and winery by-products such as polyphenols and essential oils, eugenol and limonene, to decrease gas production and prevent bloat is not clear (Jayanegara & Palupi, 2010; Correddu et al., 2013) and merits investigation.

#### *2.3.6.2 Ruminal parakeratosis and acidosis*

Inclusion of citrus and winery by-products below 200 g/kg DM in ruminant diets reduces the occurrence of parakeratosis and acidosis in ruminants (Bampidis & Robinson, 2006; Salami et al., 2016). However, when elevated levels of citrus pulp were fed, along with low levels of forage, rumen parakeratosis and acidosis occurred in lambs (Bampidis & Robinson, 2006). Dietary inclusion of

flavonoids from citrus and winery by-products such as quercetin in ruminant diets reduced the level of parakeratosis, which is an indicator of subacute ruminal acidosis (Benavides et al., 2013). Feeding either α-tocopherol from citrus pulp or carsonic acids in ruminant diets corrected the metabolic acidosis in growing lambs (Moran et al., 2013). Overall, there is dearth of information on how citrus and winery by-products influence rumen parakeratosis and acidosis, and further research is recommended.

### 2.3.7 *Effects of feeding citrus and winery by-products on animal health and welfare*

#### *2.3.7.1 Gastro-intestinal parasites*

Polyphenols and essential oils from citrus and winery by-products exhibit anthelmintic activity (Nordi et al., 2014; Santos et al., 2014; Azaizeh et al., 2013). An *in vivo* study showed a decline in the faecal egg counts (FEC) in goats and sheep offered elevated doses of essential oils rich in limonene and eugenol (Macedo et al., 2010). Furthermore, Squires et al. (2010) reported a decline of 97.4% in the FEC when inclusion levels of 95% of limonene and eugenol were administered at a dose of 600 mg/kg body weight. The decline in FEC can be attributed to the fact that limonene is toxic to bacteria and exhibits antioxidant effects in host animals. Limonene and eugenol tend to suppress cell growth and differentiation and, subsequently, embryogenesis of helminth eggs (Macedo et al., 2010). Intake of dehydrated citrus by-products by animals infected with *Haemonchus contortus* resulted in low hatching rates after 42 days of consumption, signifying a trend to shed less eggs to the environment (Nordi et al., 2014).

The direct effects of citrus and winery by-products rich in proanthocyanidins and essential oils on nematodes include inhibition of eggs and infective larvae and reduction of larvae mobility (Nordi et al., 2014; Bampidis & Robinson, 2006). Indirect effects include increasing protein availability, which strengthens the immune system, thus increasing the resistance to parasitic infections (Nordi et al., 2014;

Bampidis & Robinson, 2006). Furthermore, inclusion of grape pomace extract (30 and 60 g on kg fresh weight) in the diet of sheep with an intestinal parasitic infection reduced the development of the infection, indicating a direct anthelmintic effect of the proanthocyanidins (Nordi et al., 2014). It can be assumed that polyphenols and essential oils (limonene and eugenol) from citrus and winery by-products can control nematodes and are potential sources of natural anthelmintic agents in ruminants. Further research to clarify the efficacy and mechanism of the action and toxicity to the host parasites and the potential to develop formulations with isolated compounds is warranted.

*2.3.7.2 Effects on oxidative stress*

Inclusion of citrus or winery by-products in ruminant diets reduces the occurrence of oxidative stress (Havlin & Robinson, 2015; Kerasioti et al., 2017). Polyphenols in the form of monomers which are available and act as antioxidants by scavenging free radicals and disrupting oxidative reactions, protecting cells from oxidative damage and decreasing the danger of diseases associated with oxidative stress (Zhong & Dao-Wei, 2013). They also suppress molecular signaling pathways that are stimulated by oxidative stress (Hussain et al., 2016). Furthermore, polyphenols from citrus and winery by-products are involved in the suppression of reactive oxygen species (ROS) formation by either the inhibition of enzymes involved in their production, scavenging of ROS, or upregulation or protection of antioxidant defenses (Hussain et al., 2016). Polyphenolic antioxidants also reduce the catalytic activity of enzymes involved in ROS generation (Havlin & Robinson, 2015). The elucidation of the molecular mechanisms involved in the improvement of animal redox status after administration of feeds supplemented with citrus and winery by products would help in the development of low-cost interventions for pathological conditions associated with oxidative stress. There is limited information on the effects of feeding citrus and winery by-products on oxidative stress in ruminants; thus, it merits investigation.

*2.3.7.3 Effects on immune system*

Bioactive compounds such as flavonoids and proanthocyanidins from citrus and winery by-products respectively have received attention for their ability to enhance and modulate the immune system of ruminants. The main effects of flavonoids on immune responses may be derived from their different mechanisms of action such as protein binding, active site interference, or antioxidant effects (Provenza & VillaIba, 2010). Several flavonoids specifically affect the function of enzymes involved in generating inflammatory responses (Salem, Lopez, Robinson, 2012). Dietary flavonoids moderates the inflammatory response and have primarily inhibitory effects on T-lymphocytes (Correddu et al., 2013; Provenza & VillaIba, 2010). They modulate the cellular immune response *in vitro*, inhibiting the production of ROS by lymphocytes and granulocytes (Correddu et al., 2013; Provenza & VillaIba, 2010). Phenolic compounds from citrus and winery by-products reduce the proliferation of lymphocytes and production of immunoglobins and moderate the secretion of pro-inflammatory cytokines by myeloid cells (Provenza & VillaIba, 2010). Some flavonoids also alter immune response, which could be involved in immune surveillance of tumors. Quercetin suppresses antigenic stimulation of cytotoxic T-lymphocytes and inhibits natural-killer-cell-mediated cytolysis (Provenza & VillaIba, 2010). Proanthocyanidins can bind protein in the rumen, thereby making the protein unavailable for digestion and absorption until it reaches the more acidic abomasum. This high-quality protein bypass effect has the potential to enhance the immune response and increase resistance to internal parasites (Provenza & VillaIba, 2010). By-passing amino acids like arginine, glutamine, and cysteine can boost immune responses as these amino acids regulate activation of T- and B-lymphocytes, natural killer cells and macrophages, gene expression and lymphocyte proliferation, and the production of antibodies, cytokines, and other cytotoxic substances (Provenza & VillaIba, 2010). Further research is required to elucidate the

effects of feeding citrus and winery by-products and their availability on the immune system of ruminants.

### 2.3.8 *Effects on growth performance and carcass traits*

Recently, citrus or winery by-products have been widely used as an alternative feed and energy source in ruminants with no detrimental effects on animal growth (Wadhwa et al., 2015). Incorporation of 100 g/kg DM of citrus or winery by-products into ruminant diets enhanced average daily gain and feed conversion efficiency (Table 2.4). This can be attributed to polyphenols and essential oils in citrus and winery by-products which protect proteins from rumen degradation, thereby increasing intestinal protein absorption (Jeronimo et al., 2016). These bioactive compounds improve the efficiency of conversion of dietary protein to animal protein, which subsequently improves the growth performance (Jeronimo et al., 2016). Zhao et al (2018) and Ioannis et al. (2018) also reported that supplementation of ruminant diets with 100 g/kg DM of citrus or winery by-products increased body weight and average daily gain and reduced the feed-to-gain ratio (Table 2.4). At a high inclusion level (>150 g/kg DM), however, proanthocyanidins, eugenol, and limonene from these fruit by-products would impede feed intake due to their astringent nature. This, in turn, reduces the digestion of protein and other nutrients by overprotecting dietary protein, decrease ruminal microbial activity, and reduce endogenous digestive enzyme activities, thereby adversely influencing the growth performance of ruminants (Huang, Liu, Zhao, Hu, & Wang, 2017). It can be concluded that supplementing ruminant diets with up to 100 g/kg DM of citrus or winery by-products improves growth performance and reduces the feed-to-gain ratio of ruminants (Sharif et al., 2017; Chikwanha, Muchenje, Nolte, & Dugan, 2019).

At present, there is limited information on the effects of feeding citrus and winery by-products on carcass traits of ruminant animals. In one study, Macias et al. (2014) reported an increase in muscle pH

up to 45 min postmortem with dietary inclusion of ferulic acid from winery by-products in ruminant diets. Carcass traits were not affected by dietary inclusion of citrus or winery by-products in ruminant diets, except for dressing percentage, which was reduced with the addition of these fruit by-products in ruminant diets (Caparra, Foti, Scerra, Sinatra, & Scerra, 2007; Gómez-Cortés, Guerra-Rivas, et al., 2018; Moote, Church, Schwartzkopf-Genswein, & Van Hamme, 2014). Kafantaris et al. (2018) observed that an inclusion level of up to 100 g/kg DM of citrus or winery by-products in ruminant diets increased slaughter, warm, and cold carcass weights and *longissimus* muscle cross sectional area. This suggests that the inclusion of less than 100 g/kg DM of citrus or winery by-products in ruminant diets may have neutral to positive effects on ruminant livestock carcass attributes.

**Table 2.4** Effects of citrus and winery by-products on the growth performance of ruminants

| Fruit By-Product | Inclusion (g/kg) | Animal | DMI (g/d) | ADG (g/d) | FCR | Reference |
|---|---|---|---|---|---|---|
| Citrus pulp | 1.25 | Steers | 90.3 | - | - | Villarreal et al., 2006 |
| | 2.5 | Steers | 87.3 | - | - | Villarreal et al., 2006 |
| Citrus pulp | 240 | Lambs | 790 | 178 | - | Lanza et al., 2015 |
| | 350 | Lambs | 756 | 179 | - | Lanza et al., 2015 |
| Citrus pulp | 50 | Calves | 800 | 517 | 0.12 | Javed et al., 2016 |
| | 100 | Calves | 814 | 528 | 0.12 | Javed et al., 2016 |
| | 150 | Calves | 829 | 533 | 0.11 | Javed et al., 2016 |
| | 200 | Calves | 300 | 539 | 0.11 | Javed et al., 2016 |
| Citrus pulp | 300 | Lambs | 858 | 188 | 4.59 | Caparra et al., 2007 |
| | 450 | Lambs | 880 | 165 | 5.37 | Caparra et al., 2007 |
| Citrus pulp | 300 | Lambs | 928.9 | 197 | 4.70 | Sparkes et al., 2010 |
| Citrus pulp | 150 | Lambs | 1230 | 289 | 4.31 | Francisco et al., 2018 |
| | 100 | Steers | 6130 | 1270 | 0.21 | Cribbs et al., 2015 |
| | 200 | Steers | 5960 | 1000 | 0.19 | Cribbs et al., 2015 |
| Citrus pulp | 100 | Lambs | 1350 | 67.5 | 0.05 | Sharif et al., 2017 |
| | 200 | Lambs | 1370 | 76.3 | 0.06 | Sharif et al., 2017 |
| | 300 | Lambs | 1390 | 71.7 | 0.05 | Sharif et al., 2017 |
| | 400 | Lambs | 1410 | 75.8 | 0.05 | Sharif et al., 2017 |
| Grape pomace | 50 | Lambs | 1.19 | 208 | 6.09 | Bahrami et al., 2010 |
| | 100 | Lambs | 1.22 | 237 | 5.55 | Bahrami et al., 2010 |
| | 150 | Lambs | 1.14 | 171 | 7.99 | Bahrami et al., 2010 |
| Grape pomace | 200 | Lambs | 1.04 | 140 | 8.08 | Bahrami et al., 2010 |
| Grape pomace | 50 | Lambs | 2512 | 283 | - | Guerra et al., 2016 |
| Grape pomace | 50 | Lambs | 2512 | 283 | - | Guerra et al., 2016 |
| | 100 | Lambs | 2495 | 258 | - | Guerra et al., 2016 |
| Grape pomace | 50 | Rams | 1379 | 1789 | 8.10 | Zhao et al., 2018 |
| | 100 | Rams | 1482 | 215 | 6.90 | Zhao et al., 2018 |
| Grape pomace | 100 | Lambs | 1142 | 120 | 0.10 | Calderon et al., 2018 |
| | 200 | Lambs | 1120 | 104 | 0.09 | Calderon et al., 2018 |
| Grape pomace | 300 | Lambs | 1240 | 107 | 0.08 | Calderon et al., 2018 |

DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio.

## 2.4 Effects of feeding citrus and winery by-products on meat quality

### 2.4.1 *Physico-chemical meat quality*

Dietary supplementation of citrus or winery by-products in ruminant diets have no effect on pH, drip loss, cooking loss, or proximate composition of ruminant meat (Francisco et al., 2018; Caparra et al., 2007). Moran et al. (2012) and Gomez-Cortes et al. (2018) found higher water holding capacity when natural polyphenols from winery by-products were included in ruminant finishing diets. This was attributed to the effects of bioactive compounds from winery by-products which have the ability to avoid loss of membrane integrity and protein cross-links by inhibiting and/or reducing the rate of oxidation in meat (Gómez-Cortés, Guerra-Rivas, et al., 2018; Lund, Heinonen, Baron, & Estévez, 2011). Dietary supplementation of citrus or winery by-products in ruminant diets increases meat redness (a*) in ruminants (Luciano et al., 2009; Resconi et al., 2018; Zhao et al., 2018). Overall, the mechanisms of how natural bioactive compounds in these fruit by-products affect meat redness are not clear but could be related to their ability to chelate iron in myoglobin, making it non-bioavailable for oxidation (Guerra-Rivas et al., 2016), and/or the resistance of myoglobin to oxidation (Kumar et al., 2015). Supplementation of 100 g/kg DM of citrus and winery by-products in ruminant diets improved the instrumental tenderness of ruminant meat by lowering shear force values (Zhao et al., 2018; Francisco et al., 2017). The reduction in shear force may be associated with the protection exerted by natural polyphenols from citrus and winery by-products against the oxidation of proteolytic enzymes during the ageing process (Zhao et al., 2018; Francisco et al., 2017).

### 2.4.2 *Effects on fatty acid composition*

Feeding citrus or winery by-products as dietary supplements were reported to increase the proportion of omega-3 and omega 6 PUFAs and their biohydrogenation products (Table 2.5). This could be ascribed to reduced rates of lipolysis by dietary polyphenols, which results in greater bypass of PUFAs and their biohydrogenation products reaching the small intestines for subsequent absorption and deposition in the muscle (Mapiye et al., 2015; Mlambo & Mapiye, 2015; Mueller-Harvey et al., 2019). The increase in percentages of PUFAs and their biohydrogenation products in ruminant meat varies with the level of citrus and winery by-products fed (Lanza et al., 2015; Gomez-Cortez et al., 2018). Overall, the proportion of PUFAs in ruminant meat has been reported to increase linearly with dietary levels of citrus or winery by-products up to 200 g/kg DMI, beyond which rumen function is impaired (Mapiye et al., 2015). Besides increasing the proportion of PUFAs, especially those purported to have human-health-promoting properties such as n-3 and n-6 PUFAs, as well as rumenic and vaccenic acids (Lanza et al., 2015; Resconi et al., 2018), Table 2.5 shows that the addition of citrus and winery by-products below <150 kg tends to lower monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) in ruminants. Contrary to the above findings, other researchers (Jeronimo et al., 2010; Correddu et al., 2013; Resconi et al., 2018) reported that dietary supplementation of citrus and winery by-products at levels lower than 150 g/kg DM in ruminant diets did not modify the fatty acid profile in longissimus muscle. Characterizing the individual and combined effects of specific phytochemicals in citrus or winery by-products or their combine effects on different biohydrogenation products could help to improve the proportions of human-health-promoting PUFAs in ruminant meat.

**Table 2.5** Effects of citrus and winery by-products on the meat fatty acid profile of ruminants

| Fruit By-Product | Inclusion Level (g/kg) | SFA | VA | RA | (g/100 g) Fatty Acid | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | MUFA | *n*-3 | *n*-6 | PUFA | Reference |
| Grape pomace | 100 | 40.45 | | | 37.5 | 2.17 | 13.7 | 16.4 | Kafantaris et al., 2018 |
| Citrus pulp | 250 | 36.5 | 1.39 | 1.08 | 30.9 | 5.57 | 20.3 | 26.9 | Lanza et al., 2015 |
| Citrus pulp | 350 | 35.9 | 1.56 | 1.02 | 32.5 | 5.48 | 20.4 | 26.9 | Lanza et al., 2015 |
| Grape pomace | 100 | 47.8 | - | - | 39.4 | 2.34 | 10.4 | 12.8 | Resconi et al., 2018 |
| Grape seed | 50 | 49.7 | - | - | 37.8 | 2.19 | 10.3 | 12.4 | Resconi et al., 2018 |
| Grape seed | 25 | 43.3 | 3.42 | - | 32.4 | 5.26 | 9.90 | 20.1 | Jeronimo et al., 2012 |
| Grape pomace | 50 | 67.8 | 4.76 | 0.78 | 17.1 | 1.01 | 5.09 | 8.94 | Correddu et al., 2013 |
| Grape pomace | 100 | 64.8 | 8.18 | 0.47 | 22.3 | 1.08 | 3.64 | 8.64 | Correddu et al., 2013 |
| Citrus pulp | 150 | 3.90 | 6.47 | 1.71 | 31.6 | 16.1 | 10.2 | 19.9 | Francisco et al., 2018 |
| Grape pomace | 50 | 36.6 | 2.10 | 1.00 | 34.9 | 5.3 | 6.61 | 28.4 | Gomez et al., 2018 |
| Grape pomace | 100 | 38.0 | 2.21 | 1.20 | 35.4 | 4.68 | 5.83 | 26.6 | Gomez et al., 2018 |

PUFA, polyunsaturated fatty acid; VA, vaccenic acid; RA, rumenic acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; *n*-6/*n*-3 is the ratio of omega 6 and omega 3 fatty acids.

### 2.4.3 *Effects on sensory quality of meat*

Regarding the use of citrus and winery by-products in ruminant diets, there are little if any studies that have investigated their effect on meat sensory attributes and volatile compounds. However, some bioactive compounds found in winery by-products, such as ferulic acid improves meat tenderness, juiciness, and flavor (Gonzalez-Rios et al., 2016). In contrast, Chaves et al. (2011) reported high off-flavor appreciation values in ovine meat supplemented with cinnamaldehyde and hesperidin, bioactive compounds present in citrus by-products. Citrus and winery by-products contain high levels of polyphenols which alter meat fatty acid composition (Kumar et al., 2015; Tomovic et al., 2017). The fatty acid composition of meat influences sensory quality attributes such as flavor, aroma, and juiciness (Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). In this regard, it is important to investigate the effects of citrus and/or winery by-products on ruminant meat sensory attributes and volatile compounds.

## 2.5 Effects of feeding citrus or winery by-products on retail meat shelf stability

### 2.5.1 *Myoglobin oxidation*

Feeding tanniferous-rich fruit by-products such as citrus or winery by-products delays or inhibits meat discoloration during storage, thereby extending its shelf life (Guerra-Rivas et al., 2016; Zhao et al., 2018; Inserra et al., 2014; Gravador et al., 2015). This could be attributed to the positive effects of dietary bioactive compounds on heme pigment concentration and metmyoglobin formation during storage. Changes in redness (a*) values over time describe meat color

deterioration from red to brown and reflect the myoglobin concentration and its redox state in meat (Luciano et al., 2009). The oxidation of myoglobin and the subsequent buildup of metmyoglobin at the meat surface are the key causes of meat color deterioration (Inserra et al., 2014). The actual mechanism by which these bioactive compounds influence meat color stability is not clear. However, it has been hypothesized that citrus or winery polyphenolic compounds may affect meat color shelf life by augmenting muscle overall antioxidant capacity and myoglobin resistance to oxidation (Kumar et al., 2015; Inserra et al., 2014).

### 2.5.2 *Lipid oxidation*

Dietary supplementation of tanniferous feeds in ruminant diets protects PUFAs in meat from oxidation during retail display (Kumar et al., 2014; Guerra-Rivas et al., 2016; Inserra et al., 2014; Zhao et al., 2018). Thus, dietary supplementation of citrus or winery by-products in ruminant diets may reduce oxidative deterioration (Inserra et al., 2014; Zhao et al., 2018) and the formation of rancid odors, off-flavors, and toxic compounds harmful to human health. A decline in the production of meat lipid oxidation products can also decrease meat discoloration and nutrient losses (Kumar et al., 2015). In addition, citrus or winery by-products may moderate the formation of lipid oxidation products that influence protein solubility, emulsification, water binding capacity, texture, and other rheological properties via the interactions between lipid and protein products (Inserra et al., 2014; Gravador et al., 2014). Overall, the mechanisms of the effect of natural bioactive compounds in citrus or winery by-products on lipid oxidation have not been fully explained. Nevertheless, phytochemicals are generally known to prevent lipid oxidation through numerous mechanisms, which result from their abilities to act as free radical scavengers, reducing

agents, metal chelators, and single oxygen quenchers (Kumar et al., 2014; Guerra-Rivas et al., 2016; Inserra et al., 2014; Zhao et al., 2018).

### 2.5.3 *Protein oxidation*

The rate and extent of protein oxidation in meat can be delayed, reduced, or prevented by feeding natural antioxidants in ruminant diets, including citrus or winery by-products (Gladine, Rock, Morand, Bauchart, & Durand, 2007; Gravador et al., 2014). Phytochemicals present in these by-products contribute to the antioxidative power protecting proteins from oxidation (Teixeira et al., 2014; Zou et al., 2016). Despite their being few studies devoted to the effects of dietary supplementation of citrus or winery by-products in ruminant diets on meat protein oxidation, there is some indication they may be efficient inhibitors of protein oxidation. These bioactive compounds reduce the destruction of amino acids to carbonyl compounds, which are known to affect protein functionality, solubility, and viscosity in meat (García-Lomillo & González-SanJosé, 2017; Gladine et al., 2007; Gravador et al., 2014). The reduction of carbonyl formation may positively improve some meat quality attributes, such as color, aroma, flavor, and tenderness, water holding capacity, nutritional value, juiciness, and biological functionality.

### 2.5.4 *Microbial growth*

Dietary citrus or winery by-products can retard the growth of many foodborne pathogens including *Escherichia coli*, *Salmonella Typhimurium*, *Enterobacteria*, *Pseudomonas*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Brochothrix thermosphacta* (Aziz et al., 2016; Guerra-Rivas et al., 2016; Correddu et al., 2013). Overall, the antimicrobial mechanisms of phenolic plant extracts manifest through attacking the phospholipid bilayer of the cell membrane,

disrupting enzyme systems and compromising the genetic material of bacteria (Wu, Zang, He, Pan, & Xu, 2013). The chelating ability of phenolic compounds can also deprive microbes of essential iron required for growth (Scalbert, 1991). Overall, there is little information on the effects of feeding citrus and winery by-products on the microbial shelf life of ruminant meat. Further studies to determine the effects of feeding fruit by-products to ruminants on meat microbiological quality are therefore warranted.

## 2.6 Potential utilization of citrus or winery by-products in low-to-middle-income countries and future perspectives

Valorization of citrus or winery by-products as supplementary feed ingredients for ruminant production holds great potential for smallholder farmers in low-to-middle-income countries producing these by-products. This is because the escalating demand for animal products, propelled by rising income, population, and urbanization in low-to-middle-income countries, imposes a huge demand on feed resources (Wadhwa et al., 2015). Sustainability of feed production systems is facing several challenges including land, soil, and water scarcity; food–feed–fiber–fuel competition; ongoing global warming and frequent and drastic climatic vagaries; and growing competition for arable land and nonrenewable resources such as fossil carbon sources, water, and phosphorus (Wadhwa et al., 2015). A key to sustainable ruminant production in low-to-middle-income countries is, therefore, the efficient utilization of locally available feed resources. These include reduction in wastage of organic content lost through the disposal of fruit by-products and broadening of the feed resource base through a quest for novel feed resources, especially fruit by-products which do not compete with human food.

Citrus or winery by-products have been shown to be rich sources of natural cellulose, minerals, vitamins, PUFAs, and phytochemicals, which have nutritional, anthelmintic, antioxidant, and antimicrobial properties (Teixeira et al., 2014; Zou et al., 2016). In that regard, these fruit by-products can be adopted in a plan of economic advantage in ruminant diets since they reduce feed costs and shortages, and improve animal nutrition, health, growth, carcass characteristics, physico-chemical meat quality attributes, and fatty acid composition (Figure 2.1), thereby generating income which can improve the living standards of smallholder farmers in low-to-middle-income countries. However, utilization of citrus and winery by-products can be limited by the presence of pesticides; thus, it is important to regularly monitor such contaminants before incorporating them into ruminant diets (Wadhwa et al., 2015). Owing to their seasonality, bulkiness, and high moisture content (600 g/kg), which expedites microbial spoilage, oxidation of organic macromolecules, and degradation of bioactive compounds (Sharma, Mahato, Cho, & Lee, 2017), these by-products also require preservation prior to utilization as animal feed. In that regard, simple and low-cost methods of practical handling such as dehydration and ensiling should be adopted to conserve these fruit by-products so that they can be fed to the livestock throughout the year or during periods of feed scarcity (Wadhwa et al., 2015). Given that ensiling losses are high and can have a negative impact on the farm environment, most studies recommend sun-drying because it is simple, inexpensive, and can be easily performed during the harvest season (Chikwanha, Raffrenato, Opara, et al., 2018).

For the sustainable use of citrus or winery by-products, it is crucial to seek low-technology-based agro-processing and value-adding opportunities for their utilization, especially in low-to-middle-income countries. This is because of limited financial resources, lack of storage

infrastructure and transport facilities, unstable or weak institutions, lack of information and skills, and inequitable empowerment and access to resources required for high-technology-based agro-processing and value addition. Collaboration of large companies producing citrus juice and wine in low-to-middle-income countries with smallholder farmers, which could see them establishing small feedlots to utilize citrus and/or winery by-products as feed ingredients in ruminant diets, is recommended. In that context, it could be important to create a proper fruit by-product supply chain system to connect fruit processors, ruminant livestock farmers, abattoirs and retailers, and ensure the availability of quality meat at a competitive price to consumers. The use of citrus and winery by-products can be a catalyst for organizations to realize that valorization of wastes not only lowers cost and increases processing efficiencies but also decreases the environmental pollution load. In addition, these fruit by-products may significantly contribute towards food and nutrition security by minimizing or preventing discoloration, rancidity, and microbial spoilage; maintain or enhancing nutritional and sensory quality; and extending the shelf life of animal-based foods (Figure 2.1). This may subsequently improve the distribution of animal sourced foods over long distances (Mlambo & Mapiye, 2015), which could help smallholder farmers to export to distant niche markets, thereby increasing their revenue. Supplementing citrus and winery by-products in ruminant diets may also reduce economic losses and food-borne illnesses associated with discoloration and with chemical and microbial spoilage. Moreover, the use of citrus and winery by-products could reduce costs for food additives, specialized processing, and packaging often used in the food industry to improve the appearance and maintain the quality of meat following long periods of storage and distribution (Mlambo et al., 2015).

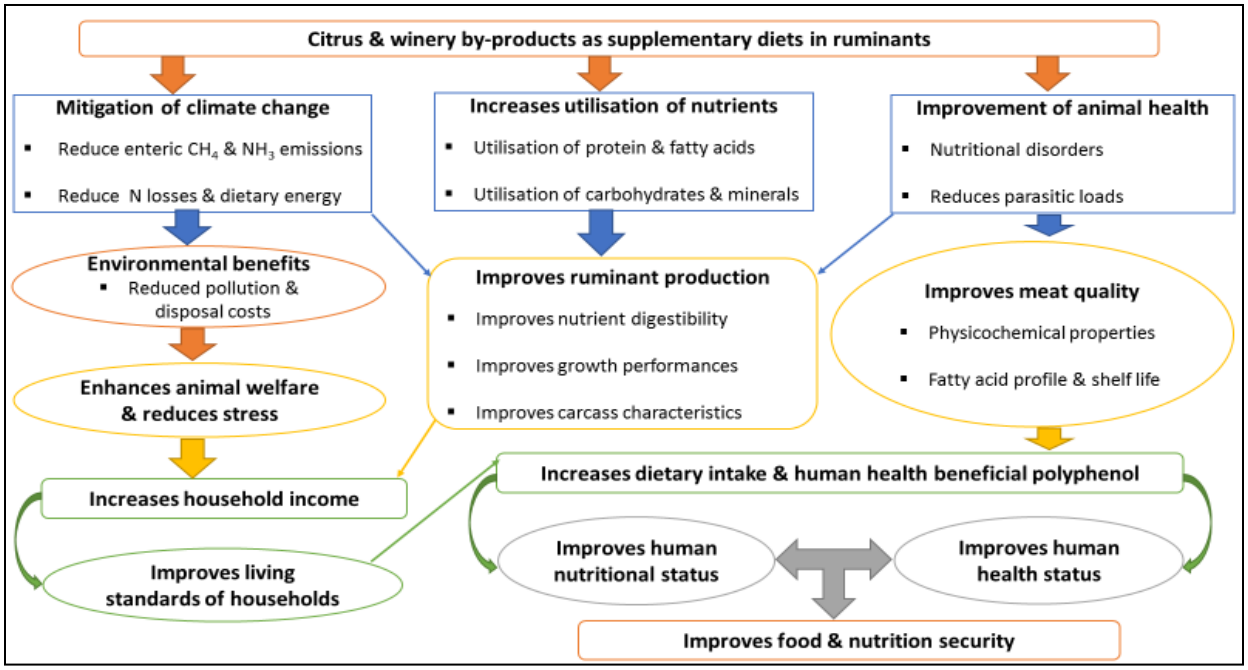


**Figure 2.1** A summary of the potential benefits of incorporating citrus and winery by-products into ruminant diets

Research to determine the optimum inclusion levels of these fruit by-products from specific varieties grown in different environments extracted and processed using distinct methods, among other variables, would be important. For full utilization, readily accessible lab(s) with adequate analytical capabilities would then need to be available for the complete quantification and classification of micronutrients and phytochemicals from citrus and winery by-products. Future research on the bioactivity, bioavailability, and toxicology of these phytochemicals and their mode(s) of action, stability, and interactions with other food ingredients should be conducted with cautious assessment of *in vitro* and *in vivo* studies. If all these components, including technical and economic feasibility challenges, product quality, and health safety, are in place, valorization of

citrus and/or winery by-products in low-to-middle-income countries producing these by-products could be completed.

## 2.7 Summary

Sustainable utilization of citrus and winery by-products as dietary supplements in ruminant production (up to 150 g/kg DM) holds the greatest potential in low-to-middle-income countries producing these by-products. These by-products have the potential to improve the DMI, nutrient digestibility, rumen fermentation parameters, health and welfare, growth performance, and carcass and meat quality attributes of ruminant animals. In that context, a sustainable food production system can be attained through the utilization of citrus and winery by-products to improve food, nutrition, and income security for resource-poor, vulnerable, and marginal populations who reside in low-to-middle-income countries. Continued location and product availability specific studies are, therefore, recommended to determine the effects of feeding citrus and/or winery by-products on meat fatty acid profiles, shelf life, microbiological quality, and volatile and flavor compounds in ruminants.

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# Chapter 3 Comparative effects of feeding citrus pulp and grape pomace on nutrient utilization in steers

ABSTRACT

Dried citrus pulp (DCP) or grape pomace (DGP) were compared as alternative fiber sources to wheat bran (control) in Angus steers diets. Fiber sources were included in diets at 150 g/kg, with eight steers (7-month old, 281 ± 15.7 kg) per diet. Twenty-four steers were assigned to three dietary treatments (8 steers/ treatment) in completely randomized design. Each steer was treated as an experimental unit. Steers were adapted to diets for 21 d before 7 d of sample collection. Overall, steers fed on DGP had the greatest intake of the nutrients assessed followed by DCP and control diets ($P \leq 0.05$). Apparent digestibilities of dry matter (DM), organic matter (OM) and ash free neutral detergent fiber (aNDFom) were greater ($P \leq 0.05$) for DCP diet compared to DGP and control diets. Feeding DCP and DGP diets increased ruminal concentrations of total volatile fatty acids, acetate and isovalerate, and acetate to propionate ratio, and reduced propionate concentrations compared to the control diet ($P \leq 0.05$). The steers fed the control diet had the greatest urinary excretions of allantoin, uric acid and total purine derivatives followed by those fed the DCP and DGP diets ($P \leq 0.05$). Nitrogen intake, fecal nitrogen (N), N retention and N efficiency utilization were in the order of DGP > DCP > control diets ($P \leq 0.05$). Overall, feeding DGP as alternative fiber source to wheat bran improved nutrient intake, retention and efficiency of N utilization but reduced apparent nutrient digestibility compared to DCP. Current finding suggests that DGP may be a better fiber substitute for wheat bran in beef diets than DCP.

**Keywords:** Nitrogen retention; Pectins; Proanthocyanidins, Purine derivatives, Volatile fatty acids.

## 3.1 Introduction

Non-conventional feed resources including fruit by-products are progressively becoming important substitutes for cereal grains (i.e., maize, wheat and barley) and their by-products (Bran and hominy chop) in livestock diets owing to climate change and high demand of cereals for human food (Hersom et al., 2010; Molina-Alcaide &Yáñez-Ruiz, 2008; Moote et al., 2014). Citrus (i.e., oranges, mandarins, lemons and grapefruits) and grapes are the world's major staple fruit crops, with about 60 to 80% of the yield being utilized for juice and winemaking (Beres et al., 2017; Mahato et al., 2018). In South Africa, the current production of citrus fruits and grapes per annum is approximately 2.9 and 2.0 million metric tons (MMT), respectively (DAFF, 2018; Kalaba et al., 2018; USDA, 2019). Processing of these fruits generates large quantities of waste with approximately 0.23 and 0.28 MMT of citrus pulp and grape pomace, respectively (DAFF, 2018; Kalaba et al., 2018; USDA, 2019). Disposal of these wastes is associated with environmental, economic and social challenges (Khan et al., 2015; Sharma et al., 2017). In vitro studies by Lashkari and Taghizadeh (2015) and Guerra-Rivas et al. (2016) with dried citrus pulp (DCP) and dried grape pomace (DGP) have shown potential for utilization by ruminants as fiber-rich feed ingredients based on their high degradability. This is particularly relevant due to intrinsic capability of ruminants to convert non-edible fibrous biomass into highly valuable food products owing to the consortium of rumen micro-organisms (Valenti et al., 2018; Van Soest, 1994). The

utilization of citrus and grape by-products from juice and wine industries presents an opportunity to decrease feed costs and cereal-grain dependence for the ruminant livestock industry.

Overall, utilization of DGP and DCP as feed ingredients in ruminant finishing diets is mainly limited by high neutral detergent fiber (NDF; 265-630 g/kg DM), proanthocyanidins (54.9-87.0 g/kg DM) and EE (50 -71 g/kg DM) contents (Chikwanha et al., 2018; Wadhwa and Bakshi, 2013). High contents of NDF (> 300 g/kg DM; Arelovich et al., 2008; Smith, 2008) and proanthocyanidins (> 40 g/kg; Waghorn et al., 1987; Waghorn et al., 2008) in ruminant feed decrease nutrient intake and apparent digestibility. A precautionary strategy could be to feed these fruit by-products at a dietary inclusion level of less than 200 g/kg DM (Vinyard et al., 2018; Cribbs et al., 2015; Bampidis & Robinson, 2006). Moreover, higher proanthocyanidins content can be reduced by drying (Chikwanha et al., 2018; Wadhwa et al., 2015) or co–feeding with other ingredients that have low content of phenolic compounds (Mlambo & Mapiye, 2015).

Currently, dried citrus pulp is one of the most common non-conventional substitutes for both rapid- (e.g., maize grain) and slow-fermenting (e.g., wheat bran) energy sources in ruminant finishing diets (López et al., 2014; Lashkari et al., 2017; Steyn et al., 2017; Sharif et al., 2018). Compared to dried grape pomace (a less established by-product), dried citrus pulp has lower EE, lignin and NDF content, and greater pectin content (250 g/kg DM) and neutral detergent soluble carbohydrates (NDSC) (Wadhwa et al., 2015; Bampidis & Robinson, 2006). However, dried grape pomace has greater crude protein (CP) content, palatability, and lower cost than dried citrus pulp (Wadhwa et al., 2015; Foiklang et al., 2016). To our knowledge there are few studies that have separately evaluated the nutrient intake, digestibility and utilization of dried citrus pulp (Villarreal

et al., 2006) and dried grape pomace (Vinyard, Myers and Chibisa, 2018) for beef production. Moreover, there are no studies that have compared nutrient intake, digestibility and utilization of these two ingredients in ruminant diets. The current study hypothesized that dried grape pomace may be a better substitute for wheat bran than dried citrus pulp in beef cattle finishing diets. The objectives of the present study were, therefore, to compare the effects of feeding 150 g/kg DM of either dried citrus pulp or dried grape pomace as alternate fibre sources to wheat bran on nutrient intake and digestibility, ruminal fermentation efficiency, microbial-N supply, N retention and efficiency of N utilization in steers.

## 3.2 Materials and methods

### 3.2.1 *Site of study*

The study was conducted at Mariendahl Experimental Farm (Stellenbosch University, South Africa; 33°50' 59"S; 18° 49' 31"E). The experiment was approved (ACU-2018-6738) by the Ethics Committee of the Stellenbosch University following guidelines of the South African National Standard (SANS 10386:2008) regarding the care and use of animals for experimental and scientific purposes.

### 3.2.2 *Preparation of citrus pulp and grape pomace diets*

Dried citrus pulp was purchased from Letaba Citrus Processors in Limpopo province, South Africa (23° 52' 47"S 30° 17' 48"E). Grape pomace (*Vitis vinifera L. cv*. Pinotage) was collected from Bellevue (33° 52' 48."S 18°45' 50"E), Spier (33° 58' 24.96" S 18° 46' 55.92"E) and Beyers Kloof (33° 53' 28"S 18° 49' 24"E) winery estates in the Western Cape Province (South Africa).

Post-pressing, fresh grape pomace (GP) was spread on plastic sheets and sun-dried for 7 days at Stellenbosch University's Welgewallen Experimental Farm (33° 56' 33"S 18° 51' 59"E, South Africa). The pomace was turned daily to aid drying and ensure that the moisture content was below 10% to avoid spoilage during storage. The DCP and DGP were milled through a 1-mm sieve (Wiley mill; Model 4, Thomas Scientific, Swedesboro, USA) for analyses of chemical composition and a 4-mm sieve for inclusion in diets. Samples for chemical analyses were stored at -20°C. Diets were formulated and prepared by a registered commercial feed manufacturer by replacing wheat bran in the basal diet (control) with either 150 g/kg of DCP or DGP meal (Table 3.1). Although DCP and DGP were used as fiber substitutes for wheat bran, their content of other nutrients also differed (Table 2). Diets were, therefore, balanced for crude protein (CP) and metabolizable energy (ME) for an estimated ADG of 0.2 g/g and 12 KJ/g DM, respectively (Salah et al., 2014). Diets were pelleted with steam conditioning at 80°C to a diameter of 6 mm.

### 3.2.3 *Steers, diets and experimental design*

Twenty-four, 7-month old Angus steers with an average body weight of 281 ± 15.4 kg were purchased from a commercial feedlot. The steers were tagged, drenched with Fenbendazole (MDS-Animal Health, Spartan, South Africa) and sprayed with Butox liquid (MDS-Animal Health, Spartan, South Africa) to control endo- and ecto-parasites, respectively. During the experimental period, steers were housed in individual pens (2 × 4 m) with sawdust bedding. Each pen was equipped with a feeder and a water trough. Steers were allocated to three dietary treatments (8 steers/ treatment) in a completely randomized design (CRD) to balance treatment groups for initial body weight with each steer treated as the experimental unit. All steers were then adapted to diets for 21 d followed by 7 d of sample collection for digestibility before growth trial which lasted for

90 d. A 21-day adaptation period was used; during this period steers were fed the pre-experimental diet (hay: experimental diet) replaced by gradually increasing amounts of the experimental diets. During adaptation, the level of hay in the diet was decreased from 75% (first 7 d) to 0% (last 7 d). After diet adaptation, steers were weighed after a 16-h fast from both food and water. This weight was used as the initial weight. Steers were fed once daily at 0700 h, with experimental diets being offered as pelleted total mixed rations. Diets were provided at 110% of the previous daily intake. Clean, fresh water was always available.

### 3.2.4 *Measurements and sample collection*

During the trial, all the feed offered, and refusals were weighed and recorded daily before feeding in the morning; and sub-samples from each steer were collected and kept frozen at -20 °C for the determination of nutrient intake. Urine and faecal samples were collected from each steer four hours post-feeding. Faeces were collected by grab sampling directly from the rectum. Spot urine sampling was done, using a chute by urethral palpation to induce urination (Da-Silva et al., 2016). Post sampling, approximately 500 ml of urine from each steer was acidified with 100 ml of 10% (v/v) sulfuric acid to minimize ammonia volatilization and to prevent bacterial degradation of purine derivatives. Sub-samples were taken, and 20 ml were diluted with 80 ml of distilled water to avoid precipitation of uric acid, then kept in freezer at -20 °C prior to determination of purine derivatives. For the determination of total N compounds, 100 ml of undiluted urine without 10% (v/v) sulphuric acid were stored at -20 °C. Sampling was conducted daily per steer, then daily urine samples were composited over the 7-d collection period prior to analyses. The feed, refusals and fecal samples were oven-dried at 60 °C for 48 h, then ground using a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 1-mm sieve and stored 4 °C pending analyses.

### 3.2.5 *Slaughter procedures and rumen liquor collection*

Post growth trial, the steers were transported to a commercial abattoir, located 83 km from the study site and then kept in lairage for 16 h before slaughter. Thirty minutes after slaughter, rumen contents were thoroughly mixed and rumen pH was measured using a portable pH meter (Crison PH25 pH meter, Lasec, South Africa) according to Chaves et al. (2011). The rumen contents were collected and then filtered through four layers of cheese cloth into 50-ml Greiner centrifuge tubes (Merck KGaA, Darmstadt, Germany). Tubes were kept on ice in a cooler box for transport to the laboratory. The rumen fluid was then centrifuged at 1000 × g for 10 min at 4 °C. A 0.1-ml sample of the supernatant was preserved with 5% phosphoric acid (v/v) for ammonia-N (NH3-N) analyses. For the determination of volatile fatty acids (VFA), 0.1 ml of 25% (w/v) metaphosphoric acid was added to 0.9 ml of supernatant. All the samples were kept at -20 °C pending further analyses.

## 3.3 Chemical analyses and calculations

### 3.3.1 *Nutrient composition and fiber analysis for DCP, DGP and experimental diets*

Feed, refusals and fecal samples were pooled to determine dry matter (DM, procedure; 934.01), ash (procedure; 942.05) and ether extract (EE, procedure; 920.39) according to AOAC (2002). The N content of feed, feces and urine was assayed using the Dumas technique (LECO® FP528, LECO Corporation, Miami, USA) (AOAC, 2002; procedure 968.06), and then N content was multiplied by 6.25 to determine the content of CP. The daily total volume of urine was predicted as follows: body weight (BW) × 29/creatinine concentration (mg/l; Valadares et al., 1999). The daily urinary excretion of nitrogenous compounds was determined by multiplying the

concentration of N in the sample by the estimated total volume of urine. The content of starch in the feed, refusals and faecal samples was assayed according to Hall (2009). Neutral detergent fiber (aNDFom) was assayed according to Mertens et al. (2002). The lignin (sa.) content was measured according to method of Raffrenato and Van Amburgh (2011).

The NDF digestibility (ivNDFd) was determined using the *in vitro* method with rumen fluid from two rumen-cannulated Holstein dairy cows offered a total mixed ration comprising of concentrate and lucerne hay (60/40%, w/w). Before the morning feeding (0700 h), rumen fluid was collected from each cow into pre-warmed insulated Thermos flasks. The rumen fluid was filtered through four layers cheesecloth and glass wool before incubation. Ground DCP and DGP samples (~0.5 g) were weighed in duplicate into 125-ml Erlenmeyer flasks and 40 ml of Van Soest buffer was added (Goering & Van Soest, 1970). Thereafter, the flasks were put in shaking water bath set at 39.5 °C. Carbon-dioxide was run through the flasks to maintain anaerobic conditions. Ten millilitres of rumen fluid were then added to each flask. The determination of ivNDFd was based on the difference between NDF content before and after incubation. The residual NDF was obtained by filtering the incubate after 24 and 48 h using a 50-ml sintered Gooch crucible porosity with Grade 2 Whatman glass microfiber filter (934-AH®, GE Healthcare, Pittsburgh, PA, USA). The final ivNDFd at 24 and 48 h was expressed as a percentage of the initial NDF content in the feed. The analyses were done in two runs and each sample analysis replicated twice.

The content of ME for DCP, DGP and experimental diets was computed following the method by CSIRO (2007). Non-fibrous carbohydrates were computed as 1000 g/kg - ([Ash + NDF + CP + EE] g/kg). Neutral detergent soluble (NDS) was computed by subtracting aNDFom (g/kg) from

1000g/kg. Pectin and sugar contents were calculated according to López et al. (2014) by subtracting the content of starch (g/kg DM) from NFC (g/kg) content. The mineral content of the citrus pulp, grape pomace and all the diets were first exposed to the microwave acid digestion and dissolution of the sample. Minerals were quantified by inductively coupled plasma-automatic emission spectrometry (ICP-AES) according to Sah and Miller (1992). All the analyses were performed in triplicate. The Folin-Ciocalteu colorimetric method as described by Makkar (2003) was used for the determination of total tannin content, and results were expressed as gram gallic acid equivalents per kg DM. Proanthocyanidins were determined following the procedure of Porter et al. (1986) and the results were expressed as g/kg DM leucocyanidin equivalent. Ascorbic acid content was determined colorimetrically (Mphahlele et al., 2016) and expressed as milligrams per kg DM. The α-tocopherol content was determined following the procedures outlined by AACC International (2000; method 86.06) and the results were expressed in mg/kg of α-tocopherol-equivalents.

### 3.3.2 *Nutrient intake and apparent digestibility*

Determination of daily nutrient intake per steer was computed as the difference between nutrients in feed offered and refusals. Apparent total tract nutrient digestibility of organic matter (OM), CP, EE, starch and aNDFom were determined indirectly using indigestible NDF (iNDF) as an internal marker. The 240-h Goering and Van Soest (1970) *in vitro* digestibility method was used to determine the residual *in vitro* neutral detergent fiber (ivNDF) in both feed and faecal samples for each steer (Schalla *et al*., 2012; Raffrenato *et al*., 2018). The faecal output, apparent and DM digestibility were calculated using the equations below:

$$Faecal\ DM\ output\ (g/kg) = \frac{iNDF\ intake\ (g/kg)}{faecal\ iNDF\ (g/kg)} \times 100$$

$$Apparent\ digestibility\ (g/kg) = \frac{nutrient\ intake\ (g/kg) - faecal\ output\ (g/kg)}{nutrient\ intake\ (g/kg)}$$

$$Dry\ matter\ \ digestiblity\ (g/kg) = 100 - \left(\frac{indigestible\ aNDFom\ in\ feed}{indigestible\ aNDFom\ in\ faeces}\right) \times 100$$

### 3.3.3 *Analyses of ruminal Ammonia–N concentration*

Ruminal $NH_3$-N was determined using the colorimetric technique of Broderick and Kang (1980). Briefly, duplicate samples of 0.5 ml rumen fluid (diluted × 20 in deionized water) were centrifuged at 2820 × g (Eppendorf MiniSpin®, Hamburg, Germany) for 5 min. Fifty microliters of supernatant were mixed with 2.5 ml of phenol reagent (0.05 g of sodium nitroferricyanide and 90% phenol [w/v] in 1-L of deionized water) and 2 ml hypochlorite reagent (5 g of sodium hydroxide and 5.25% sodium hypochlorite in 1-L of deionized water). The mixture was placed in a water bath at 95°C for 5 min and then transferred to an ice bath for 7 min. Absorbance at 630 nm was then measured using an ultra-violet-vis spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin, USA). Ammonia-N contents were calculated using ammonium sulphate standard curve.

### 3.3.4 *Analyses of ruminal volatile fatty acid concentration*

Ruminal fluid samples that were thawed overnight at 4°C, vortexed and then centrifuged at 12,000 × g for 10 min at 4°C. A 1-ml subsample of the supernatant was transferred into a micro-centrifuge tube and centrifuged at 16,000 × g for 10 min at 4°C, and volatile fatty acids were then quantified using a Thermo Scientific™ TRACE™ 1300, gas chromatograph (Thermo Electron S.P.A, Strada Rivoltana, Rodana, Milan, Italy) fitted with Thermo TriPlus RSH Autosampler (Switzerland) and a Phenomenex Zebron ZB-FFAP capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). A 1-µl volume of sample was injected into the GC using a 1:50 split ratio with a run time of 18 min. Crotonic acid was used as the internal standard. Thermo Scientific Xcalibur™ software was used for the final computation of VFA concentrations based on the retention times of the samples relative to the standard. The VFA were expressed as millimoles/l with the individual VFA later converted to mM/ 100 mM of the total VFA.

### 3.3.5 *Dietary fatty acid analyses*

Fatty acid methyl esters (FAME) of DCP, DGP and experimental diets were prepared in a 1-step extraction–trans-esterification procedure using chloroform (Sukhija and Palmquist, 1988) and 2% (vol/vol) sulfuric acid in methanol (Shingfield et al., 2003) and heptadecanoic acid (C17:0) as an internal standard. Fatty acid methyl esters were separated and quantified using a gas chromatograph (Agilent 7890A GC System; Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and a 60-m fused silica capillary column (0.25 mm i.d., 0.2- µm film thickness; CP-SIL 88, CP7489; Varian Ibérica S.A., Madrid, Spain) and hydrogen as the

carrier gas (207 kPa, 2.1 mL/min). Total FAME profile in a 2-µL sample volume at a split ratio of 1:50 was determined using the following temperature gradient program (Shingfield et al., 2003): the oven temperature was held at 70°C for 4 min, increased up to 110°C at a rate of 8°C/min, then increased up to 170°C at a rate of 5°C/min, held at 170°C for 10 min, and increased at 4°C/min to a final temperature of 240°C that was held for 14.5 min. The injector and detector temperatures were maintained at 255°C. FAME were identified by retention times relative to the Supelco™ 37 FAME mix, (Supelco, USA). The content of fatty acids was expressed as percentage of total FA. Analyses were conducted in quadruplicates and averaged.

### 3.3.6 *Analyses of purine derivatives and ruminal microbial N supply*

Post-thawing (25℃), the samples of urine were filtered through a Whatman No. 1 filter paper (Whatman Limited – GE Healthcare, Maidstone, UK). The daily total volume of urine was used to calculate the excretion of uric acid and allantoin according to Valadares *et al*. (1999). Total daily purine derivative (PD) excretion was then calculated as uric acid + allantoin (Chen and Gomes, 1992). The uric acid and allantoin contents of urine were measured by the method of Chen and Gomes (1992). Urine samples were analyzed in duplicate and if the difference exceeded 6%, analyses were repeated. Urinary allantoin and uric acid excretion rates (mmol/d) were computed by multiplying the molar concentrations by the urine volume. The endogenous PD excretion (mmol/d) was predicted using steer body weight at a rate of 0.385 mmol/BW0.75 per d (Chen and Gomes, 1995). Following the method of Valadares *et al*. (1999), total absorption of microbial purines was computed as: total purine derivative excretion – (0.385 × BW0.75)/0.85, where 0.85 is the absorptive efficiency of purines (Chen and Gomes, 1995). As reported by Chingala *et al*.

(2019), microbial-N was computed as: microbial N (g/d) = (purine absorption × 70)/ (0.134 × 0.83 × 1000), where 70 is the N content of purines (mg N/mmol), 0.134 is the mean ratio of purine-N: total-N measured for mixed rumen microbes, and 0.83 is the assumed digestibility of microbial purines (Chen and Gomes, 1995). The amount (g/day) of retained N was calculated as the difference between N intake and N excretion through faeces and urine. The N efficiency utilization was computed per animal as: g of retained N/g of ingested N.

### 3.3.7 *Statistical analyses*

All data were analyzed using GLIMMIX procedures of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA). Animal and diet were fitted in the statistical model as random and fixed factors, respectively. The least square means (LSMEANS) option of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA) was used to generate treatment means. Tukey's test was applied for LSMEANS separation. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

## 3.4 Results

### 3.4.1 *Dietary ingredients and chemical composition*

The ingredient composition of experimental diets is shown in Table 3.1. Overall, the substitutions of DCP and DGP in the diets were mainly for wheat bran, but additional substitutions were made to simulate industry conditions where diets were made iso-nitrogenous and iso-caloric resulting in equal estimated ADG. Specifically, addition of slightly higher quantities of maize to the DGP diet was mainly for balancing the energy content, whilst gluten was added in the control and DCP diets

to balance the N across treatments. Furthermore, due to higher level of calcium in DCP, more limestone was added to the control and DGP diets to balance its contents across the diets.

**Table 3.1** Proportions of feed ingredients in the experimental diets

| Ingredients, g/kg | Treatments | | |
|---|---|---|---|
| | Control diet | DCP diet | DGP diet |
| Dried citrus pulp (DCP) | - | 150 | - |
| Dried grape pulp (DGP) | - | - | 150 |
| White maize | 313.6 | 335.4 | 365.1 |
| Canola oil cake | 106.4 | 141.9 | 138.0 |
| Molasses | 80.0 | 80.0 | 80.0 |
| Lucerne | 100.0 | 100.0 | 100.0 |
| Wheat straw | 90.0 | 90.0 | 90.0 |
| Wheat bran (WB) | 231.7 | 50.0 | 51.4 |
| Gluten | 50.0 | 42.0 | - |
| Urea | 5.0 | 5.0 | 5.0 |
| Salt | 3.0 | 3.0 | 3.1 |
| Limestone | 19.7 | 2.2 | 16.9 |
| Fintech premix | 0.6 | 0.6 | 0.6 |

The chemical compositions and fatty acid profiles of DCP, DGP and experimental diets fed are presented in Table 3.2. Overall, the partial replacement of wheat bran with either DGP or DCP resulted in higher EE, aNDFom, lignin (sa.), proanthocyanidins and total tannins contents for the DGP diet, and starch, NFC, pectin and sugars, 24 and 48 h in vitro NDF digestibilities, ascorbic acid and α-tocopherol for the DCP diet. For all the diets, linoleic acid (18:2n-6) was the major fatty acid followed by oleic (18:1n-9), α-linoleic (18:3n-3) and palmitic (16:0) acids, respectively. The order of linoleic acid proportions was DGP > control > DCP diets. For oleic, α-linoleic and palmitic

acids, the DCP diet had greatest proportions followed by the control and DGP diets in that order. Overall, all the experimental diets as formulated had similar CP and ME.

**Table 3.2** Chemical composition, *in vitro* digestibility and fatty acid composition of dried citrus pulp (DCP), dried grape pomace (DGP), wheat bran (WB) and experimental diets

| Item | DCP | DGP | WB | Treatments | | | SEM[1] |
|---|---|---|---|---|---|---|---|
| | | | | Control diet | DCP diet | DGP diet | |
| Chemical composition (g/kg) | | | | | | | |
| Dry matter (DM) | 862.8 | 899.3 | 901.2 | 879.2 | 875.9 | 882.8 | 0.62 |
| Organic matter (OM) | 951 | 940.7 | 941.2 | 892.5 | 948.3 | 935.3 | 0.63 |
| Ash | 49.0 | 59.3 | 58.8 | 107.5 | 51.7 | 64.7 | 0.58 |
| Crude protein | 47.7 | 111.0 | 175.5 | 119.5 | 119.1 | 119.4 | 0.66 |
| Starch | 57.5 | 23.0 | 22.6 | 217.9 | 235.3 | 212.0 | 0.72 |
| Ether extract | 18.0 | 74.3 | 45.5 | 23.7 | 24.3 | 33.5 | 0.82 |
| Ash free neutral detergent fiber (aNDFom) | 145.8 | 317.6 | 385.4 | 164.4 | 172.7 | 183.5 | 1.41 |
| Neutral detergent soluble fiber (NDSF) | 854.2 | 682.4 | 614.6 | 835.6 | 827.3 | 816.5 | 1.28 |
| Acid detergent lignin (ADL, [sa]) | 6.2 | 205.9 | 41.4 | 21.7 | 19.2 | 48.0 | 0.71 |
| Metabolizable energy (MJ/ kg) | 11.1 | 8.2 | | 113.1 | 119.5 | 114.1 | 0.58 |
| Non-fibrous carbohydrates (NFC) | 739.5 | 437.8 | 342.7 | 584.5 | 632.2 | 598.9 | 0.58 |
| Pectin + sugar[2] | 683.0 | 414.8 | 320.1 | 367.0 | 396.9 | 386.9 | 0.47 |
| 24h *in vitro* neutral detergent digestibility (%) | 43.9 | 15.2 | 39.7 | 19.4 | 29.3 | 15.4 | 0.45 |
| 48h *in vitro* neutral detergent digestibility (%) | 62.1 | 23.0 | 44.4 | 40.0 | 53.2 | 22.1 | 0.58 |
| Calcium | 17.3 | 3.1 | 1.5 | 11.9 | 7.4 | 10.7 | 2.4 |
| Phosphorus | 1.1 | 2.7 | 10.9 | 4.4 | 4.1 | 3.9 | 0.3 |
| Potassium | 8.3 | 18.6 | 12.4 | 11.3 | 11.5 | 12.3 | 0.6 |
| Magnesium | 1.1 | 1.1 | 4.4 | 2.9 | 2.6 | 2.7 | 0.1 |
| Sodium | 0.1 | 0.1 | 0.6 | 1.8 | 1.5 | 1.7 | 0.2 |
| Iron | 1.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.02 |
| Aluminum | 0.2 | 0.2 | - | 0.2 | 0.2 | 0.2 | 0.02 |
| Copper, mg/kg | 3.1 | 19.1 | 12.2 | 14.3 | 18.6 | 13.9 | 2.6 |
| Zinc, mg/kg | 5.8 | 12.2 | 86.7 | 105.9 | 78.9 | 87.9 | 13.8 |
| Manganese, mg/kg | 19.8 | 11.4 | - | 70.1 | 52.6 | 62.9 | 8.7 |
| Total phenols g of gallic acid equivalent/kg DM | 51.4 | 177.3 | | | 32.5 | 89.6 | 4.3 |
| Total tannins, g of gallic acid equivalent/kg DM | 19.1 | 104.2 | - | - | 12.7 | 50.7 | 0.47 |
| Proanthocyanidins, % leucocyanidin equivalent | 8.1 | 33.3 | - | - | 5.7 | 24.1 | 0.47 |
| Ascorbic acid, mg/ kg | 427.0 | 174.0 | - | - | 316.1 | 114.2 | 0.46 |
| Alpha tocopherol, mg/kg | 74 | 47 | - | 9.8 | 16.8 | 12.6 | 0.12 |
| Fatty acid composition (% of total fatty acids) | | | | | | | |
| 14:0 | 0.6 | 0.6 | - | 0.1 | 0.2 | 0.1 | 0.01 |
| 16:0 | 16.7 | 16.0 | - | 8.2 | 8.5 | 7.3 | 0.07 |
| 18:0 | 2.3 | 1.0 | - | 0.4 | 0.6 | 0.6 | 0.04 |
| 18:1n-9 | 8.3 | 4.6 | - | 12.0 | 13.6 | 11.1 | 0.47 |
| 18:2n-6 | 56.2 | 58.9 | - | 66.6 | 63.5 | 69.3 | 0.17 |
| 18:3n-3 | 8.2 | 7.6 | - | 9.2 | 9.7 | 6.4 | 0.07 |

[1] SEM: Standard error of mean

[2] Pectin + sugars were calculated as NFC (g/kg DM) - starch (g/kg DM) according to López et al. (2014)

### 3.4.2 *Effects of feeding diets containing DCP or DGP on nutrient intake and digestibility*

Feeding the DGP diet resulted in greater ($P \leq 0.05$) intake of DM and EE compared to the DCP and control diets (Table 3.3). Steers fed the DGP diet had the greatest intakes of OM, CP, aNDFom and starch followed by those fed the DCP and control diets in that order ($P \leq 0.05$).

**Table 3.3** Effects of feeding diets containing dried citrus pulp (DCP), dried grape pomace (DGP), on nutrient intake and digestibility of Angus steers

| Item | Treatments | | | SEM | P-value |
|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | | |
| Nutrient intake (kg/d) | | | | | |
| Dry matter | 7.2[b] | 7.8[b] | 9.3[a] | 0.41 | 0.006 |
| Organic matter | 5.7[c] | 6.5[b] | 7.7[a] | 0.33 | 0.001 |
| Crude protein | 0.9[c] | 1.0[b] | 1.2[a] | 0.06 | 0.018 |
| aNDFom | 1.4[c] | 1.6[b] | 1.9[a] | 0.01 | 0.004 |
| Ether extract | 2.0[b] | 2.0[b] | 3.0[a] | 0.01 | <0.001 |
| Starch | 1.8[c] | 1.9[b] | 2.3[a] | 0.11 | 0.013 |
| Apparent digestibility (g/kg) | | | | | |
| Dry matter | 649[b] | 702[a] | 641[b] | 34.9 | 0.047 |
| Organic matter | 669[c] | 727[a] | 691[b] | 15.5 | 0.024 |
| Crude protein | 692 | 708 | 666 | 18.4 | 0.289 |
| aNDFom | 558[b] | 695[a] | 485[c] | 29.2 | 0.025 |
| Ether extract | 827 | 862 | 847 | 16.3 | 0.336 |
| Starch | 828 | 811 | 832 | 22.6 | 0.789 |

[a] Least square means with different superscripts in the same row are significantly different ($P \leq 0.05$)

Apparent digestibility of DM was greater ($P \leq 0.05$) for steers fed the DCP diet compared to those fed the DGP and control diets. Steers fed the DCP diet had the greatest apparent digestibility of OM followed by those fed the DGP and control diets, respectively ($P \leq 0.05$). The order of apparent digestibility of aNDFom was DCP > control > DGP diets. Diet had no influence ($P > 0.05$) on apparent digestibility of CP, EE and starch.

### 3.4.3 *Effects of feeding diets containing DCP or DGP on ruminal fermentation*

The effects of diets on ruminal fermentation parameters are shown in Table 3.4. Diet had no effect on ruminal pH ($P > 0.05$). Steers fed the DCP and DGP diets had lower ($P \leq 0.05$) ruminal concentrations of ammonia-N compared to those fed the control diet. Feeding the DCP- and DGP-containing diets increased ($P \leq 0.05$) the concentrations of total VFA, acetate, and isovalerate, and the ratio of acetate to propionate, and decreased concentrations of propionate compared to the control diet. Diet had no influence on rumen concentrations of butyrate, isobutyrate and valerate ($P > 0.05$).

**Table 3.4** Effects of feeding diets containing dried citrus pulp (DCP), dried grape pomace (DGP), on ruminal fermentation parameters of Angus steers

| Item | Treatments | | | SEM | P value |
|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | | |
| pH | 6.7 | 6.7 | 6.5 | 0.08 | 0.118 |
| Ammonia-N (mg/L) | 128.1[a] | 112.4[b] | 113.1[b] | 1.45 | 0.002 |
| Total VFA (mM) | 71.4[b] | 84.9[a] | 83.2[a] | 2.01 | 0.032 |
| VFA (mol/100 mol) | | | | | |
| Acetate | 60.4[b] | 67.1[a] | 66.1[a] | 0.84 | 0.001 |
| Propionate | 26.8[a] | 20.1[b] | 21.4[b] | 0.49 | 0.021 |
| Butyrate | 10.9 | 10.7 | 10.6 | 0.21 | 0.261 |
| Isobutyrate | 1.5 | 1.4 | 1.3 | 0.27 | 0.320 |
| Valerate | 0.1 | 0.1 | 0.1 | 0.03 | 0.910 |
| Isovalerate | 0.3[b] | 0.6[a] | 0.5[a] | 0.04 | 0.007 |
| Acetate: propionate ratio | 2.4[b] | 3.4[a] | 3.1[a] | 0.45 | 0.001 |

[a] Least square means with different superscripts in the same row are significantly different ($P \leq 0.05$)

### 3.4.4 *Effects of feeding diets containing DCP or DGP on purine derivatives and nitrogen balance*

Feeding diets containing DGP or DCP decreased ($P \leq 0.05$) total volume of urine, microbial-N supply and increased creatinine compared to the control diet (Table 3.5). Daily urinary excretions of uric acid, allantoin and total PD were in the order of control > DCP > DGP (Table 3.5). Steers fed the DGP diet had the greatest N intake, faecal N, N retention and N efficiency utilization followed by those fed the DCP and control diets in that order ($P \leq 0.05$).

**Table 3.5** Effects of feeding diets containing dried citrus pulp (DCP), dried grape pomace (DGP), on purine derivatives, microbial nitrogen supply and nitrogen balance of Angus steers

| Item | Treatments | | | SEM1 | P-value |
|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | | |
| Creatinine (mg/dL) | 9.7[b] | 14.2[a] | 13.1[a] | 0.81 | 0.001 |
| Total volume of urine (L/d) | 22.8[a] | 14.5[b] | 16.4[b] | 1.74 | 0.003 |
| Allantoin (mmol/d) | 67.7[a] | 41.7[b] | 37.7[c] | 91.47 | 0.030 |
| Uric acid (mmol/d) | 18.2[a] | 15.7[b] | 13.4[c] | 0.72 | 0.051 |
| Total purine derivatives excreted (mmol/d) | 85.9[a] | 56.4[b] | 51.1[c] | 13.19 | 0.041 |
| Microbial N supply (g N/d) | 62.1[a] | 41.1[b] | 40.8[b] | 11.58 | 0.010 |
| Nitrogen balance | | | | | |
| Intake N (g/d) | 146.2[c] | 163.7[b] | 181.9[a] | 9.00 | 0.019 |
| Faecal N (g/d) | 46.8[c] | 53.3[b] | 58.3[a] | 1.39 | 0.019 |
| Urinary N (g/d) | 61.7 | 62.7 | 64.7 | 5.04 | 0.211 |
| N retention (g/d) | 38.7[c] | 47.7[b] | 58.9[a] | 2.57 | 0.019 |
| Efficiency of N utilization (%) | 26.5[c] | 29.1[b] | 32.4[a] | 5.70 | 0.002 |

[a -c] Least square means with different superscripts in the same row are significantly different ($P \leq 0.05$)

## 3.5 Discussion

The greater contents of total tannins, proanthocyanidins, NDF and acid detergent lignin content observed in the DGP diet compared to DCP diet could be largely ascribed to the dietary inclusion of DGP as DGP had a greater content of fiber and phenolic compounds compared to DCP (Xu et al., 2016; Zou et al., 2016). The lower in vitro digestibility of the DGP diet compared to DCP diet could be due to the high total tannins, proanthocyanidins, and acid detergent lignin content reported for this diet. Polyphenols, particularly proanthocyanidins bind to nutrients and impede digestion in the rumen through substrate deprivation for ruminal microbes and inhibition of microbial enzymes (Frutos, Hervas, Giraldez & Mantecon, 2004). High acid detergent lignin content lowers digestion by forming a matrix over the NDF, thus preventing degradation of the cell wall by ruminal microbes (Abarghuei, Rouzbehan & Alipour, 2010).

The greater DM intake that was observed for steers fed the DGP diet compared to the DCP and control diets could be attributed to the pleasant odor and taste of DGP left after fermentation and pressing (Vinyard *et al*., 2018; Wadhwa & Bakshi, 2013), and its high relative palatability (Foiklang et al., 2016). The differences in nutrient intakes (i.e., OM, CP, aNDFom, EE and starch) are reflective of the observed differences in DM intake and/or chemical compositions of the experimental diets. Similar nutrient intakes were reported by Vinyard et al. (2018) who fed 150 g/kg DM DGP to cattle as a substitute to alfalfa silage in a total mixed ration.

The intermediate nutrient intakes observed for the steers fed the DCP could be attributed to intermediate DM intake due to the bitter taste, course texture and bulk density of the pellets of the DCP diet (Bampidis & Robinson, 2006; Zema et al., 2018). The aforementioned factors are a result

of the presence of essential oils, and the high acidogenicity and hydration capacity of DCP (Bampidis & Robinson, 2006; Zema et al., 2018). Hydration affects bulk density of DCP (303-324 kg/m$^3$) by initiating swelling capacity of the feed matrix due to absorption of water (Bampidis & Robinson, 2006; Brachet et al., 2015; Giger-Reverdin, 2000). This might have accounted for the lower nutrient intake for the DCP diet due to greater rumen fill (Santos et al., 2014). In addition, the intermediate intake of nutrients for the DCP diet could be related to the pungent odor. In particular, DCP is a heterogeneous raw material, rich in particular of active aromatic compounds (Bampidis & Robinson, 2006). The lowest nutrient intake observed for the control diet compared to fruit by-product supplemented diets could be linked to the high capacity (3.07kg/m$^3$) of wheat bran to absorb water and increase rumen fill (Brachet et al., 2015; Giger-Reverdin, 2000). Another explanation could be related to the high content of gluten (i.e. as needed to balance CP across diets) in the former diet. Addition of gluten has been previously found to decrease feed intake due to its large particle size which affects decreases passage rate, and subsequently increase rumen fill and reduce nutrient intake (Firkins et al., 1985, 1984; Staples et al., 1984).

The greater apparent DM, OM and aNDFom digestibility of DCP and control diets compared to DGP could be attributed to the addition of highly digestible gluten in these diets. It could also be partly explained by the intermediate DM intake of the former diets, which would result in lower rate of digesta passage through the gastrointestinal tract (GIT), thus potentially enhancing nutrient digestibility through elevated residence time. Consequently, the lower apparent DM, OM and aNDFom digestibility of the DGP diet may be attributed to greater DM intake observed for DGP diet compared to control and DCP diets, which may have resulted in greater rates of digesta

passage through the GIT, thus limiting DM, OM and aNDFom digestibility through reduced residence time.

The lower DM, OM and aNDFom digestibility observed for the DGP diet compared to DCP and control diets could also be attributed to its higher content of proanthocyanidins, ADL and EE (Abarghuei et al., 2010; Makkar, 2003; Palmquist, 1994; Pantoja et al., 1994). It has been reported that proanthocyanidins decrease OM and cell wall digestibility by either binding protein, cellulose, hemicellulose or pectins and/or directly inhibiting cellulolytic microorganisms thereby lowering microbial digestion (Abarghuei et al., 2010; McSweeney et al., 2001; Melaku, 2004). Proanthocyanidins may also diminish cell wall digestibility by binding bacterial enzymes and/or by forming indigestible complexes with cell wall carbohydrates (Barry et al., 1986). Several studies have reported that multiple phenolic hydroxyl groups in proanthocyanidins result in formation of complexes mainly proteins and, to a lesser extent, with polysaccharides, thus limiting their availability to animals (Abarghuei et al., 2010; Barry et al., 1986; Barry et al., 1999; Foiklang et al., 2016).

High lignin content lowers digestion by forming a matrix over the NDF, thus preventing degradation of the cell wall by microbes (Abarghuei et al., 2010). Recent studies suggest that the negative effect of lignin content in DGP on OM and aNDFom digestibility is more than the effect of proanthocyanidins (Abarghuei et al., 2010; Baumgärtel et al., 2007). Dietary fat (EE) content higher than 50 g/kg DM can lead to the formation of biofilm mainly around forage particles, negatively affecting ruminal microbes, thereby reducing digestion efficiency of aNDFom (Allen, 2000; Palmquist, 1988; Pantoja et al., 1994). In the currently study, EE may have had no or little

effects on aNDFom digestibility since its content for all the experimental diets were within the recommended range (<50 g/kg; Allen, 2000; Palmquist, 1994; Pantoja et al., 1994). The observed moderate aNDFom digestibility for the control diet compared to DGP diet could be explained by absence of proanthocyanidins, lower lignin and EE contents.

The elevated apparent digestibility of DM and OM observed for the DCP diet compared to control and DGP diets could be attributed to superior degradability of the NDSC in DCP. It has been reported that feeding DCP improves rumen conditions for cellulolysis (Bampidis & Robinson, 2006; Miron et al., 2002; Tadayon et al., 2017; Villarreal et al., 2006). Moreover, high pectin in DCP may have increased microbial adhesion to the surfaces of feed particles (Tadayon et al., 2017). Nam and Ray (2009) and Javed et al. (2016) reported that high nutrient digestibility might be attributed to total soluble solids and NDSC in DCP that are rapidly digested in the rumen. Likewise, improvement of the digestibility in these fractions seems to be linked with the low indigestible ADF and indigestible lignin content of citrus pulp cell wall (Lashkari & Taghizadeh, 2015). Overall, the high fiber contents in DCP, which are highly fermentable may lead to an increase in NDF digestion (Nam & Ray, 2009; Javed et al., 2016).

The lower ruminal concentration of $NH_3$-N produced feeding DCP compared to the control diet could be related to higher fermentation rates and microbial growth in the former diet (Lashkari et al., 2017; Lashkari & Taghizadeh, 2015). The greater and quicker supply of ruminal available carbohydrates (NDF, sugars and pectins) for microbial growth could have resulted in more efficient capture of $NH_3$-N (Javed et al., 2016; Piquer et al., 2009). The observed lower ruminal $NH_3$-N in steers fed DGP diet could be attributed to the lower gluten content of this diet and effects

of DGP polyphenols on protein and fiber digestion (Jayanegara & Palupi, 2010; Frutos et al., 2004). Similar to DGP, feeds containing 2.5% proanthocyanidins have been reported to decrease ruminal $NH_3$-N (Abarghuei et al., 2010; McSweeney et al., 2001). Overall, the effect of DGP proanthocyanidin content on ruminal protein metabolism has been attributed to its ability to bind plant protein, reduce activity of microbial enzymes and growth rate of proteolytic bacteria (Abarghuei et al., 2010; Besharati &Taghizadeh, 2009; Molan et al., 2001), and ultimately decreases rumen ammonia (Abarghuei et al., 2010; Foiklang et al., 2016).

High concentrations of ruminal $NH_3$-N observed for the steers fed the control compared to DCP and DGP diets could be linked to high rates of ruminal fermentation and rapid dietary CP degradation due to absence of tannins and proanthocyanidins. The $NH_3$-N concentrations in the rumen of all experimental steers were greater than 50 mg/L, which is the minimum level required by ruminal microbes to support optimum growth (Sinclair et al., 1993; Tadayon et al., 2017). These levels of $NH_3$-N are necessary to promote growth of fiber-degrading bacteria which use $NH_3$-N as N source, resulting in improved fiber digestion (Javed et al., 2016).

The high ruminal concentrations of total VFA, acetate and isovalerate observed in DCP and DGP diets compared to the control diet could be attributed to greater daily OM intake and digestibility observed for former diets, which might have increased OM fermentation in the rumen (Baluch-Gharaei et al., 2015; Tadayon et al., 2017). The high ratio of acetate and propionate for DCP and DGP compared to control diet, could be attributed to high proportions of acetate and propionate recorded in the current study. In that regard, it could be suggested that feeding the DCP and DGP diets may have resulted in the subsequent increase in the proportions of ruminal acetate

and a decrease in propionate (Sharif et al., 2018; Tadayon et al., 2017). The greater proportions of acetate, isovalerate and ratio of acetate to propionate in steers fed DCP and DGP diets agrees with previous studies (Piquer et al., 2009; Tadayon et al., 2017). Overall, the observed total VFA concentrations are within the recommended range of 70 –150 mM for ruminants (McDonald et al., 2011). Overall, it is recommended that esophageal tube method should be used to sample rumen fluid to come up with accurate values for ruminal fermentation parameters.

The decline of urinary excretion of allantoin, uric acid and total purine derivatives in steers fed DGP relative to the DCP and control diets could be attributed to proanthocyanidins and lignin contents in DGP diet. The findings that ruminal microbial N supply was lower for both DGP and DCP diets compared to control diet could suggest that the proanthocyanidins may have caused a decline in the microbial protein production by forming complexes mainly with proteins, thereby reducing their degradation in the rumen and subsequently reduced the excretion of purines in the urine (Abarghuei et al., 2010; Patra and Saxena, 2011). Overall, microbial N supply values observed in the present study were out of the recommended range of 278-419 g/d for both total and spot urine sampling methods (Valadares et al., 1999). In that regard, it is recommended to make use of metabolisable crates when conducting digestibility trial which will enable the researchers to measure the exact total urine and come up with the exact values of the total purine derivatives and ruminal microbial N supply that falls within the recommended range for beef cattle.

Greater faecal N losses in steers fed DGP and DCP diets than those fed control diet could be due to formation of tannin-protein complexes in GIT, thereby, preventing N absorption (McSweeney et al., 2001). The enhanced N retention could be attributable to high concentration

of ruminal total VFA (Kim et al., 2007) observed in DGP and DCP diets, as this may have been used as source of energy by the animals to retain more N available from microbial protein and rumen undegradable protein in steers fed these diets. This increased N retention observed for DGP and DCP diets could be due to improved utilization of absorbed N. It has been suggested that proanthocyanidins might increase the efficiency of urea recycled to the rumen by reducing the rate of protein degradation and deamination in the rumen, and subsequently lowering ruminal $NH_3$-N (Waghorn et al., 1987, Foiklang et al., 2015). These findings contrast with the observations by Abarghuei et al. (2010) who reported that retained N was decreased in sheep fed DGP. The observed efficiency of N utilization in the present study falls within the recommended range of 10-40 percent in ruminants as reported by Calsamiglia et al. (2010).

## 3.6 Conclusions

Steers fed the DGP diet had greater intake of nutrients, N retention and efficiency of utilization, and lower apparent nutrient digestibility, microbial N supply and total PD compared to the DCP diet. Based on current findings, DGP could be a better alternative dietary fiber source compared to wheat bran and DCP. Follow up studies are thus warranted to compare the effects of feeding DGP or DCP as alternative dietary fiber sources to wheat bran on growth performance, carcass and beef quality attributes when included in beef cattle finishing diets.

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# Chapter 4 Influence of feeding fruit by-products as alternative dietary fiber sources to wheat bran on beef production and quality of Angus steers

ABSTRACT

The current study compared the growth performance, carcass and meat quality effects of feeding 150 g/ kg DM of dried citrus pulp (DCP) or grape pomace (DGP) as alternative dietary fiber sources to 7 months-old Angus steers for 90d. Twenty-four steers were assigned to three dietary treatments (8 steers/ diet) in completely randomized design. Each steer was treated as an experimental unit. Feeding the DGP and DCP diets resulted in greater ($P \leq 0.05$) average daily gain compared to the control diet. Steers fed on the DGP diet had greater ($P \leq 0.05$) dry matter intake, warm and cold carcass weights than those fed on the DCP and control diets. Shear force and income over feed costs were greatest for the DGP diet followed by the DCP and control diets ($P \leq 0.05$). Current findings suggest DGP is a better fiber source than DCP resulting in enhanced growth performance, carcass attributes and economic viability of feedlot steers.

**Keywords:** Animal performance; Beef quality; Carcass traits; Citrus pulp; Grape pomace; Profitability

## 4.1 Introduction

Beef feedlot production systems in South Africa account for more than 85% of the formal beef value chain (DAFF, 2018), and equates to those in some developed nations (Galyean, Ponce, & Schutz, 2011). Feedlot beef production in South Africa, as in other water-scarce countries, is not reaching its full potential because of the limited supply of feed ingredients, resulting in high prices of feed resources, and consequently, reduced profitability (Arowolo & He, 2018; DAFF, 2017a). To optimize feed shortages and profits for the feedlot industry it is, therefore, important to explore alternative and underutilized feed resources, especially non-conventional resources such as fruit by-products, which are produced in large quantities but not used for human food (Valenti, Luciano, Pauselli, Mattioli, Biondi, Priolo et al., 2018).

In South Africa, citrus and grapes are the major fruit crops, with an annual production of approximately 2.9 and 2.0 million metric tons, respectively (DAFF, 2018; USDA/FAS, 2019). This generates large volumes of waste with citrus pulp and grape pomace correspondingly contributing about 0.23 and 0.28 million metric tons per annum (DAFF, 2018; USDA/FAS, 2019). Recent characterization of dried citrus pulp (DCP) and dried grape pomace (DGP), however, shows they may have value as fiber-rich feed ingredients for ruminant animals (Muhlack, Potumarthi, & Jeffery, 2018). Citrus pulp is high in water-soluble sugars and pectin, whereas grape pomace is high in ether extract and linoleic acid (Teixeira, Baenas, & Viguera, 2014; Zou, Xi, Hu, & Zhou, 2016). The average metabolizable energy levels of DCP (Sharif, Ashraf, Mushtaq, Nawaz, Mustafa & Ahmad et al., 2018) and DGP (Besharati & Taghizadeh, 2010) are generally close to the recommended range of metabolizable energy (12.2 ± 2.0 MJ/kg average daily gain)

requirements for growth of cattle (Salah, Sauvant, & Archimède, 2014). The usage of fresh citrus pulp and grape pomace as feed ingredients in cattle finishing diets is, however, challenged by high water activity (Schmidt & Fontana, 2008), NDF (Wadhwa et al., 2013) and proanthocyanidins (Chikwanha et al., 2018) contents, which are above the recommended values of $<0.65a_w$ (Hemmingsen et al., 2008), 170–330 g/kg DM (NRC, 2007) and <40 g/kg (Waghorn, 2008) for ruminants. Water activity and proanthocyanidins can, however, be reduced by drying (Chikwanha et al., 2018).

Feeding DCP and DGP have either had neutral or positive effects on sheep growth, carcass and meat quality attributes compared to conventional fiber sources such as maize bran, soybean hulls, wheat bran and straw (Chikwanha, Muchenje, Dugan & Mapiye, 2019; Sharif et al., 2018; Zhao, Li, Ren & Zhang, 2018). There is, however, limited data on the effects of DCP and DGP on feedlot cattle performance (Hadjipanayiotou & Louca, 1976; Cribbs, Jennings, Burdick & Carroll et al., 2015), and no data available on their effects on carcass traits and beef quality. The costs of DCP and DGP are relatively low to wheat bran, a common fiber-rich ingredient used in beef cattle finishing diets in South Africa (DAFF, 2018; Dwyer, Hosseinian, & Rod, 2014). Overall, wheat bran has lower NDF (228-490 g/kg), proanthocyanidins (1.7-2.2 g/kg) and high crude protein (134-175 g/kg) compared to DCP and DGP (Bellaver et al., 2004; Kara, 2016; Stevenson et al, 2012). Metabolizable energy of wheat bran (8.6-11.2 MJ/kg) is slightly higher compared to that of DGP, but lower compared to DCP (Bellaver et al., 2004; Tahir, Khalique, Pasha, & Bhatti, 2002). Given results with sheep, it is hypothesized that DCP and DGP would have equivalent or better feeding value compared to wheat bran, and superior economic value due to differences in costs. The objectives of the present study were, therefore, to compare the effects of feeding 150 g/kg DM of

DCP or DGP as alternate fiber sources to wheat bran on growth, carcass attributes, beef quality and costs of production.

## 4.2 Materials and methods

### 4.2.1 *Study site*

The study site was as described in section 3.2.1.

### 4.2.2 *Preparation of citrus pulp and grape pomace diets*

The preparation of citrus pulp and grape pomace diets is as described in section 3.2.2.

### 4.2.3 *Steers, diets and experimental design*

The description of steers, diets and experimental design is as described in 3.2.3.

### 4.2.4 *Chemical analyses of ingredients and experimental diets*

The AOAC (2002) procedures were used to determine the contents of dry matter (DM, reference method 934.01), ash (reference method, 942.05) and ether extract (EE, reference method 920.30). The nitrogen content was assayed using the Dumas technique (reference 968.06; AOAC, 2002) with a macro-Nitrogen analyzer (LECO® FP528, LECO Corporation, Miami, USA) and multiplied by 6.25 to determine crude protein content. Starch content was determined using an assay kit (Total Megazyme kit KTSTA, Megazyme International Ireland Ltd., Wicklow, Ireland) (Hall, 2009). Neutral detergent fiber (aNDFom) was assayed using heat-stable α-amylase and sodium sulphite (Mertens, 2002). The content of acid detergent lignin (ADL) was measured following Goering & Van Soest (1970) method as adjusted by Raffrenato & Van Amburgh (2011).

All fiber components were expressed exclusive of residual ash. All the samples were replicated three times. The mineral content of the citrus pulp, grape pomace and all the diets were first exposed to the microwave acid digestion and dissolution of the sample. Minerals were quantified by inductively coupled plasma-automatic emission spectrometry (ICP-AES) according to Sah & Miller (1992). All the analyses were performed in triplicate.

The metabolizable energy (ME) content was estimated in MJ/ kg according to CSIRO (2007). Non-fibrous carbohydrate (NFC, g/kg of DM) was computed as 1000 - (g/kg NDF + g/kg DCP + g/kg lipid + g/kg ash). Neutral detergent soluble was calculated as 1000 - aNDFom g/kg. The Folin-Ciocalteu colorimetric method (Makkar, 2003) was used to measure total tannin content using a gallic acid standard curve (0.02-0.160 mg/ ml) and results were expressed as gram gallic acid equivalent per kg DM. Proanthocyanidins were determined according to Porter, Hrstich & Chan (1986) and expressed as grams per kilogram DM leucocyanidin equivalent. Ascorbic acid content was determined colorimetrically (Mphahlele, Fawole, Mokwena, & Opara, 2016), and ascorbic acid content was expressed as milligrams per 100g DM.

### 4.2.5 *Slaughtering and carcass characteristics*

Post-feeding, steers were transported to a Worcester abattoir (85° 36'.63" S, 98°19'.46" E, Worcester, South Africa), which is located 83 km from the study site. Steers were in lairage for 16 h without feed but had access to water. At the commercial abattoir, the animals were stunned using a non-penetrating captive-bolt and exsanguinated following Meat Safety Act (2000) regulations of South Africa. Hot carcass weights were obtained immediately post-dressing, by weighing all the carcasses. A calibration temperature of 35°C was used as a standard procedure for measuring pH

early post-mortem (Anders & Rosenvold, 2002). At 45 min and 24 h post-mortem, temperature and pH (Crison PH25 pH meter, Lasec, South Africa) were taken in triplicate between the 12th and 13th ribs of the right *Longissimus thoracis et lumborum* (LTL) muscle.

Carcass fatness was classed on a scale of 0–6 (0 = no visual fat cover,1 = very lean, 2 = lean, 3 = medium, 4 = fat, 5 = over-fat, and 6 = excessively over-fat) whereas conformation was categorized on scale of 1–5 (with 1 = a very flat carcass, 2 = a flat carcass, 3 = medium carcass, 4 = a round carcass, and 5 = very round carcass) according to the South African Meat Industry Company (2006). Carcasses were split, weighed and chilled at ± 3°C for 24 h. Warm and cold carcass weights were recorded 1 h and 24 h post-mortem, respectively. Dressing percentage was computed using the flowing formula: (Hot carcass weight / Body weight) × 100. Twenty-four hours post-mortem, the left LTL was removed for meat physicochemical analyses.

## 4.3 Sampling of meat and physicochemical attributes

Six cuts of (~2.5 cm thick) were excised from the left LTL and randomly allocated for the determination of meat colour and drip loss, proximate analyses, cooking loss and instrumental tenderness [Warner-Bratzler Shear force (WBSF)]. Physical and chemical analyses were conducted in duplicate, except for instrumental tenderness which was done using six repeated measurements.

### 4.3.1 *Colour and drip loss*

Meat surface was directly measured for colour measurements, 30 min after air exposure to allow blooming. Colour coordinates (lightness, L*; redness, a* and yellowness, b*) were measured

from three locations on the cut surface of individual steaks using the CIELAB colour meter (BYK-Gardner GmbH, Gerestried, Germany) with 11-mm diameter aperture and D65/10° for the illuminant/observer. Hue angle (H*) and chroma (C*) were computed as follows:

$H^{\circ} = tan^{-1}\left(\frac{b*}{a*}\right) \times 57.29$ (expressed in degrees); $C^{*} = \left(\sqrt{a^2 + b^2}\right)$

Drip loss was determined by suspending a standardized (50–60 g and approximately 30 × 60 × 25 mm) (Torres Filho, Cazedey, Fontes, Ramos, & Ramos, 2017) a sample of meat in an inflated plastic bag (n = 2) and placed in a 4°C refrigerator for 24 h (Honikel, 1998). Percent drip loss was calculated by dividing weight loss by initial weight and multiplying by 100.

### 4.3.2 *Proximate analyses of meat*

For proximate analyses, any visible subcutaneous fat was manually removed from the meat samples and homogenized using a knife mill (Knifetec™ 1095, Höganäs, Sweden). The homogenate was preserved through vacuum packaging and stored at -20°C pending chemical analyses. Determination of moisture (reference method 934.01) and ash (reference method 942.05) were performed following the procedures of the AOAC (2002). Total fat was measured by extracting with chloroform/methanol (2/1 v/v) as described by Lee, Trevino, & Chaiyawat (1996). Analyses of protein content was performed on the dried defatted meat (60 °C for 48 h) using DUMAS protocol (reference 968.06; AOAC, 2002). The analyses were performed in duplicate.

### 4.3.3 *Cooking loss and shear force*

Two, 2-cm steaks for cooking loss were weighed and placed in plastic bags and immersed in a water-bath at 80°C until the internal temperature reached 75°C, which was monitored by thermocouple (Hanna Instruments, Bellville, South Africa) placed in the geometric center of samples. After cooking, bags were cooled in a 4°C refrigerator. Thereafter cooled slices were slightly blotted with paper towels to remove excess water from the cooking process to obtain the final weight. Percentage cooking loss was calculated as [(weight of steak after thawing - weight of cooked steak)/weight of raw steak after thawing] × 100 (Honikel, 1998). Prior to determining instrumental tenderness, the cooked samples were chilled at 4°C for 24 h. Six cuboids (20×10×10 mm) were removed from each steak parallel to the fiber grain. Shear force was measured on each cuboid perpendicular to the fiber grain using an Instron 3345 (Universal) attached to a 1-mm thick, V-shaped-Warner Bratzler cutting blade (speed; 200 mm/ min; 500 N load cell). The instrumental tenderness values were recorded in Newtons as the average of all six cuboids/steak.

### 4.3.4 *Income over feed costs*

Income over feed costs (IOFC) were calculated according to Buza, Holden, White, & Ishler, (2014) using the following modified formulae below:

IOFC = Total income (TI) – Total feed costs (TFC) where:

TI = income generated after selling cold carcasses and;

TFC = feed costs per diet ×dry matter intake

### 4.3.5 *Statistical analyses*

All data were analyzed using GLIMMIX procedures of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA). Diet and animal were fitted in the model as fixed and random effects, respectively. Regarding DMI and ADG, initial weight was fitted in the model as a covariate, with day as a repeated measure. Treatments means were generated using the least square means (LSMEANS) option of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA). Tukey's test was applied for LSMEANS separation. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

## 4.4 Results

### 4.4.1 *Dietary ingredients and chemical composition*

The proportion of ingredients, chemical and mineral compositions and in vitro digestibilities of experimental diets, DCP and DGP fed are shown in Tables 3.1 and 3.2 (Chapter 3). Overall, the substitutions of DCP and DGP in the diets were mainly for wheat bran, but additional substitutions were made to simulate industry conditions where diets were made iso-nitrogenous and iso-caloric resulting in equal estimated ADG. Specifically, addition of slightly higher quantities of maize to the DGP diet was mainly for balancing the energy content, whilst gluten was added in the control and DCP diets to balance the N across treatments. Furthermore, due to higher level of calcium in DCP, more limestone was added to the control and DGP diets to balance its contents across the diets.

The replacement of wheat bran with either DCP or DGP resulted in an increase in EE and ADL contents for DGP, and starch, pectin and sugar content for DCP. For both DCP and DGP diets, the aNDFom and NFC contents increased whereas the ash content decreased as wheat bran was substituted with DCP or DGP. The DGP diet had greater contents of total tannins and proanthocyanidins than the DCP diet. Ascorbic acid was higher in the DCP diet than in the DGP diet. The DCP diet had the greatest ivNDFd at 24- and 48-h incubations, followed by the control and DGP diets. Most of the minerals were similar across the diets except for copper, zinc and manganese which were different across the diets. The DCP diet had the highest copper content and lowest zinc and manganese contents compared to the other diets.

### 4.4.2 *Growth performance and carcass traits of Angus steers*

Initial body weights were not different across diets ($P > 0.05$). Steers fed the DGP diet had greater DMI ($P \leq 0.05$) relative to steers fed the DCP and control diets (Table 4.1). Average daily gain and final body weights were greater ($P \leq 0.05$) for steers fed the DGP and DCP diets compared to those fed the control diet. The hot and cold carcasses of steers fed the DGP diet were heavier ($P \leq 0.05$) than those fed the DCP and control diets. Steers fed DGP and control diets had higher ($P \leq 0.05$) dressing percentages than steers fed the DCP diet (Table 4.1). Feed efficiency, carcass pH and temperature (i.e., 45 min and 24 h) were not affected by diet ($P > 0.05$). All carcasses of steers fed the DGP diet were classed as lean (fat class 2) with round conformation. For the DCP diet, 62.5% of the carcasses were very lean and round, 25% medium and round and 12.5%, very lean and round. Fifty-six percent of carcasses from steers fed the control diet were lean and round, whereas 22% were classed as very lean and round.

**Table 4.1** Effects of feeding diets containing dried grape pomace (DGP) and citrus pulp (DCP) on growth performance and carcass traits of Angus steers

| Items | Experimental diets | | | SEM[1] | P-value |
|---|---|---|---|---|---|
| | Control | DGP | DCP | | |
| Growth performance | | | | | |
| Initial body weight, kg | 281 | 282 | 280 | 14.6 | 0.740 |
| Final body weight, kg | 407[b] | 454[a] | 449[a] | 16.6 | 0.010 |
| Average daily gain, kg | 1.5[b] | 1.9[a] | 1.8[a] | 0.07 | 0.010 |
| Dry matter intake, kg/d | 8.5[b] | 10.7[a] | 9.1[b] | 0.37 | 0.001 |
| Feed efficiency | 0.2 | 0.2 | 0.2 | 0.01 | 0.430 |
| Carcass traits | | | | | |
| Hot carcass weight, kg | 222[c] | 249[a] | 235[b] | 9.37 | 0.003 |
| Cold carcass weight, kg | 215[b] | 242[a] | 228[b] | 9.07 | 0.004 |
| Dressing % | 55[a] | 55[a] | 52[b] | 0.01 | 0.032 |
| Carcass pH (45min) | 6.6 | 6.7 | 6.7 | 0.06 | 0.243 |
| Carcass pH (24h) | 5.6 | 5.5 | 5.5 | 0.09 | 0.652 |
| Carcass temperature (45min) | 36.1 | 36.0 | 36.0 | 0.19 | 0.921 |
| Carcass temperature (24h) | 10.4 | 10.1 | 10.0 | 0.52 | 0.883 |

[1]SEM: Standard error of means

[a-c] Least square means with different superscript within a row are significantly different ($P \leq 0.05$)

### 4.4.3 *Income over feed costs*

The IOFC for DCP and DGP diets are shown in Table 4.2. The feed cost/kg and TFC were lower for the DGP diet, moderate for the DCP diet and greater for the control diet ($P \leq 0.05$). Total income was greater for the DGP diet followed by the DCP and control diet, respectively ($P \leq 0.05$). The IOFC followed a similar trend to that of TI ($P \leq 0.05$).

**Table 4.2** Effects of feeding diets containing dried grape pomace (DGP) and citrus pulp (DCP) on income over feed cost (US$/ diet) of Angus steers

| Variable | Experimental diets | | | SEM[1] | P-value |
|---|---|---|---|---|---|
| | Control | DGP | DCP | | |
| Feed cost/kg | 0.18[a] | 0.16[c] | 0.17[b] | 0.01 | <0.001 |
| Total feed cost | 131.1[b] | 152.8[a] | 132.1[b] | 5.46 | 0.018 |
| Total income | 664.2[c] | 754.3[a] | 701.1[b] | 25.98 | 0.050 |
| IOFC[2] | 533.1[c] | 601.5[a] | 569.0[b] | 13.09 | 0.010 |

[1] SEM: Standard error of means

[2] IOFC means income overfeed cost

[a-c] Least square means with different superscript within a row are significantly different ($P \leq 0.05$)

### 4.4.4 *Physicochemical properties of Angus beef*

Diet had no significant influence on ($P > 0.05$) moisture, ash, intramuscular fat and crude protein contents of Angus beef (Table 4.3). Similarly, no effect of diet was observed on meat lightness (L*), redness (a*), yellowness (b*), hue and chroma ($P > 0.05$; Table 4.3). Cooking and drip loss were similar for all the diets ($P > 0.05$). Diet influenced the Warner Bratzler shear force, with DGP having the greater value followed by the DCP and control diets in that order ($P \leq 0.05$).

**Table 4.3** Effects of feeding diets containing dried grape pomace (DGP) and citrus pulp (DCP) on the physicochemical meat quality of Angus steers

| Items | Experimental diets | | | SEM[1] | P-value |
|---|---|---|---|---|---|
| | Control | DGP | DCP | | |
| Moisture, % | 75.0 | 74.6 | 74.7 | 0.27 | 0.570 |
| Ash, % | 1.1 | 1.1 | 1.1 | 0.04 | 0.470 |
| Intramuscular fat, % | 2.5 | 2.2 | 2.4 | 0.30 | 0.780 |
| Crude protein, % | 22.3 | 22.4 | 22.2 | 0.34 | 0.870 |
| L* | 39.5 | 37.5 | 38.6 | 0.72 | 0.170 |
| a* | 15.3 | 15.2 | 15.0 | 0.50 | 0.920 |
| b* | 10.6 | 10.6 | 10.7 | 0.53 | 0.980 |
| Hue angle (H*) | 35.0 | 35.0 | 35.4 | 1.14 | 0.930 |
| Chroma (C*) | 18.6 | 18.5 | 18.5 | 0.63 | 0.990 |
| Drip loss, % | 19 | 1.7 | 1.7 | 0.24 | 0.901 |
| Cooking loss, % | 40.1 | 39.2 | 38.7 | 0.72 | 0.410 |
| Instrumental tenderness,N | 63.0[c] | 82.1[a] | 73.2[b] | 5.16 | 0.050 |

[1] SEM: Standard error of means

[a-c] Least square means with different superscript within a row are significantly different ($P \leq 0.05$).

## 4.5 Discussion

Higher DMI observed for steers fed the DGP diet compared to the DCP and control diets may be linked to palatability. Such DMI was also evident in the studies of Vinyard & Chibisa (2018) who fed 150 g/kg DM DGP to cattle as an alfalfa silage substitute in a total mixed ration. Grape pomace has a pleasant odor and taste left after fermentation and pressing (Vinyard & Chibisa, 2018; Wadhwa et al., 2013). In addition, steers on the DGP diet may have consumed more to compensate for the energy deficit as reported by Hadjipanayiotou et al. (1976). The lower DMI observed for the steers fed the DCP compared to the DGP diet could be linked to the bitter taste, course texture and bulk density of the pellets of the DCP diet due to the presence of essential oil, high acidogenicity and hydration (Bampidis et al., 2006; Zema, Calabro, Folino, Tamburino, Zappia & Zimbone, 2018). Hydration affects bulk density of DCP (303-324kg/$m^3$) by initiating swelling of the feed matrix due to absorption of water (Bampidis et al., 2006). This might have

accounted for the decline of DMI of the DCP diet due to higher rumen fill (Santos, Lima, Schogor, Romero, De Marchi & Grande et al., 2014). Economic limitations on the transportation of fresh citrus pulp and grape pomace have also be found to be due to their bulkiness (Wadhwa et al., 2016). This can be minimized by pelleting, which reduces transport and storage costs (Arthington, Kunkle, & Martin, 2002). However, pelleting can reduce the volume of DCP and increases density by about 1.7 during immediate hydration (Bampidis et al., 2006), thereby giving DCP a high water-holding capacity of 4.3kg/kg of DM, which consequently lower its DMI.

The similar ADG and final weight observed when feeding the DGP and DCP diets despite the differences in DMI might be due to differences in digestibility between diets. The presence of easily digestible cell walls in DCP diet might have positively affected rumen microflora activity (Cribbs et al., 2015). It could also be due to a lower content of tannins, lignin and proanthocyanidins in the DCP diet and highly degradable NDF (Bampidis et al., 2006). Moreover, it has been proposed that the highly digestible fibrous fraction of DCP, like pectin, may increase the number of bacteria in ruminal fluid which enhances fibrous fraction of DCP degradation (Bampidis et al., 2006; Kim, Adesogan, & Arthington, 2007). The higher ADG and body weight observed for steers fed DGP and DCP diets compared to control may be attributable to the moderate amount of proanthocyanidins that were contained in the DGP and DCP diets. Moderate contents of proanthocyanidins (20-40g/kg DM) (Waghorn, John, Jones, & Shelton, 1987, Waghorn, Ulyatt, John & Fisher, 1987) can protect dietary proteins from ruminal degradation and inhibit the activities and growth of proteolytic bacteria (Jerónimo, Pinheiro, Lamy, Dentinho, Lopes, & Silva, 2016; Patra & Saxena, 2011; Rivera-Méndez et al., 2017). Reduced ruminal protein degradation can potentially enhance protein availability post-ruminally, thus increasing

amino acids absorption which, consequently, increases weight gain in growing steers as reported in literature (Javed, Sharif, Bhatti, Bilal, Ahmed & Ahmad et al., 2016; Kafantaris et al., 2018; Zhao et al., 2018).

The high carcass weights observed for steers fed the DGP diet correspond with observed DMI, ADG and final body weight. Similar findings were reported when feeding tannin-rich diets to ruminant animals (Jerez-Timaure & Huerta-Leidenz, 2009; Mapiye, Chimonyo, Dzama, Strydom, Muchenje & Marufu, 2009; Kafantaris, Kotsampasi, Christodoulou, Makri, Stagos & Gerasopoulos et al., 2018). High DMI and ADG increases muscle accretion, and subsequently results in high slaughter and heavier carcasses (Jerez-Timaure & Huerta-Leidenz, 2009; Mapiye et al., 2009; Kafantaris et al., 2018). Higher ADG and final body weights observed for the DCP diet compared to control diet are inconsistent with differences found in DMI, carcass weights and dressing percentage. The observed lower carcass weights and hot dressing percentage for steers fed DCP compared to the DGP diet could be linked to pectins in DCP and its hydration which interfere with ruminal fermentation resulting in greater rumen fill (Arthington et al., 2002; Bampidis et al., 2006; Santos et al., 2014). This is supported by the slightly faster rate and extent of ruminal starch degradation than pectin (Van Soest, 1994). The density of DCP after hydration in the rumen could also support to elucidate the observed effects on DCP intake. Indeed, hydration has been conveyed to stimulate swelling of citrus pulp (Arthington et al., 2002; Bampidis et al., 2006; Santos et al., 2014) and it may have accounted for the decline of DCP intake due to its possible greater rumen fill.

The lower feed cost/kg for DGP followed by DCP and control diet could be due to difference in nutrient composition of the ingredients used to formulate the diets. The high IOFC observed for steers fed the DGP compared to the DCP diet is mainly due to observed differences in TFC and TI. This may also be explained by a similar pattern for DMI, and consequently greater ADG, carcass and final body weights observed for the DGP diet. The findings that DGP and DCP did not affect LTL pH, drip loss and cooking loss are similar to previous findings (Chikwanha et al, 2019; Frances, Goeckner, Beckman, Murdoch, Doumit & Chibisa, 2018; Francisco et al., 2018). The present post slaughter LTL pH's were within the acceptable range (5.4-5.7) for beef carcasses (Węglarz, 2010; Hopkins, Ponnampalam, & Warner, 2014). The lack of colour differences between the DGP, DCP and control diets 24 h post-mortem are consistent with earlier findings (Caparra, Foti, Scerra, Sintra, & Scerra, 2007; Vasta, Mele, Serra, Scerra, Luciano & Lanza et al., 2009; Zhao et al., 2018). The lightness and redness values observed in the present study were above threshold values of $\geq 35$ and $\geq 14.5$, respectively, which consumers consider acceptable for beef colour (Cooper, Suman, Wiegand, Schumacher, & Lorenzen, 2018).

The observation that diet had no effect on the proximate composition of LTL muscle is in accordance with previous studies in ruminants (Caparra et al., 2007; Gómez-Cortés, Guerra, Gallardo, Lavin, Mantecon, Fuente & Manso, 2018; Zhao et al., 2018). The finding that steers fed diets containing DGP and DCP had greater instrumental tenderness values compared to the control diet suggest that somehow phenolic compounds may be interfering with early post-mortem proteolysis of meat by activating high levels of calpastatin, which inhibits and diminish the activity of μ-calpains thereby reducing the proteolysis required for tender meat (Caparra et al., 2007; Francisco et al., 2018; Kemp, Sensky, Bardsley, Buttery, & Parr, 2010).

## 4.6 Conclusions

Angus steers fed the DGP diet had superior growth performance, carcass attributes and profitability compared to those fed the DCP and control diets. These findings suggest that the DGP diet represent an economic advantage for beef producers as a fiber substitute for wheat bran in beef cattle finisher diets. Future studies are thus warranted to compare the effects of feeding DCP and DGP as fiber sources in cattle finishing diets on beef shelf life, fatty acid profiles, sensory quality and volatile and flavor compounds.

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# Chapter 5 Dietary citrus pulp and grape pomace as potential natural preservatives for extending beef shelf life

ABSTRACT

The shelf-life of beef was compared from 7-months old Angus steers (281 ± 15.4 kg initial body weight) fed 150 g/kg DM dried citrus pulp (DCP) or grape pomace (DGP) for 90 days. The antioxidant activity, bacterial load, and lipid and protein oxidation were evaluated on the *Longissimus lumborum* subjected to air-permeable packaging at days 1, 3, 5, 7 and 9 post-slaughter. Beef from steers fed DGP or DCP had higher L* values ($P \leq 0.05$) and fewer ($P \leq 0.05$) coliform counts than steers fed the control diet. Beef antioxidant activity was DGP > DCP > control ($P \leq 0.05$). Beef TBARS and carbonyl contents were DGP < DCP < control ($P \leq 0.05$). Overall, antioxidant activity decreased ($P \leq 0.05$), while bacterial loads, TBARS and carbonyl contents increased ($P \leq 0.05$) during retail display for all diets. Current findings indicate that DGP could be a better natural preservative than DCP when included in beef cattle finishing diets.

**Keywords**

Antioxidant, Bacterial growth, Beef, Oxidative stability, Phytochemicals

## 5.1 Introduction

In South Africa, approximately 85% of beef in the formal market comes from feedlots, which mainly rely on grain-based diets (DAFF, 2018). Overall, grain-fed beef tends to contain higher levels of unsaturated fatty acids, which are susceptible to oxidation during storage (Mapiye, Vahmani, Aalhus, Rolland, Baron, McAllister, et al.,2015; Scollan, Dannenberger, Nuernberg, Richardson, MacKintosh, Hocquette, et al., 2014). Oxidation of biomolecules causes deterioration of colour and flavor, formation of rancid odors and toxic compounds, and promotes growth of undesirable microbes, which subsequently results in meat losses and wastage (Estévez & Luna, 2017; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). Globally, 25-33% of food produced annually is wasted (FAO, 2019). This equates to about 1300 million tons of food, including 263 million tons of meat (FAO, 2019). In South Africa, of the 31 million tons of food produced annually, 15% of losses are meat and dairy products (WWF, 2017). It has been estimated that approximately 50% of meat losses or wastage occur during the postharvest phase mainly as a result of oxidation and microbial spoilage (Papuc, Goran, Predescu, & Nicorescu, 2017; Simitzis, Charismiadou, Goliomytis, Charalambous, Ntetska, Giamouri, et al., 2019).

To minimize losses, feed and food industries are currently using synthetically-manufactured antioxidants in feed (e.g. all-rac-alpha-tocopherol and ethoxyquin) (Salami, Guinguina, Agboola, Omede, Agbonlahor, & Tayyab 2016) and food (e.g. gallates, lactates and ascorbates) (Carballo, Caro, Andrés, Giráldez, & Mateo, 2018; Ribeiro, Santos, Silva, Pereira, Santos, da Silva Lannes, et al., 2019) to preserve meat. Nevertheless, new toxicological data on some of the synthetics has led to caution regarding their use, as emerging evidence has shown that such compounds have

potential toxic and carcinogenic effects (Ahmad, Gokulakrishnan, Giriprasad, & Yatoo, 2015; Hayes, Allen, Brunton, O'Grady, & Kerry, 2011). On the same, there is increasing consumer preference for natural products owing to their health benefits and this has intensified the search for alternative methods to retard oxidation in foods. This includes the use of natural antioxidants, including plant-based polyphenolic sources, which could be suitable natural preservatives for the meat industry (Guerra-Rivas, Vieira, Rubio, Martínez, Gallardo, Mantecón, et al., 2016; Salami, Luciano, O'Grady, Biondi, Newbold, Kerry, et al., 2019).

It has been reported that inclusion of antioxidant- and antimicrobial-rich feed ingredients in cattle finishing diets has the ability to confer protection against oxidation and/or reduce the extent of microbial spoilage during retail display of meat (Cunha, Monteiro, Lorenzo, Munekata, Muchenje, de Carvalho, et al., 2018; Papuc, Goran, Predescu, Nicorescu, & Stefan, 2017). Dietary administration of natural antioxidants is more effective at protecting tissues from oxidation than application on muscle foods (Gravador, Jongberg, Andersen, Luciano, Priolo, & Lund, 2014; Guerra-Rivas et al., 2016; Inserra, Priolo, Biondi, Lanza, Bognanno, Gravador, et al., 2014). The physiological effects of polyphenol-rich fruits such as citrus, grapes, pomegranates and apples are currently receiving attention as dietary sources of natural antioxidants with potential value for extending shelf-life of meat and its by-products (Simitzis & Deligeorgis, 2018). For example, dried citrus pulp (DCP) is high in ascorbic acid, α-tocopherol, carotenoids and pectins (Abeysinghe, Li, Sun, De, Zhang, Zhou, et al., 2007; Zou, Xi, Hu, Nie, & Zhou, 2016), whereas dried grape pomace (DGP) is high in proanthocyanidins, flavonols and anthocyanidins (Mattos, Tonon, Furtado, & Cabral, 2017; Teixeira, Baenas, Dominguez-Perles, Barros, Rosa, Moreno, et al., 2014), compounds with biopreservative properties (Teixeira et al., 2014; Zou et al., 2016). Polyphénols

in DCP and DGP exhibit antioxidant and antimicrobial properties, which have the potential to inhibit microbial spoilage in beef (Mattos et al., 2017, Zou et al., 2016). The antimicrobial mechanism of α-tocopherol and polyphenols in DCP and DGP may manifest through attacking the phospholipid bilayer of bacterial cell membranes due to their chelating ability, disrupting enzyme systems, which can deprive microbes of essential iron required for their growth and also compromise their genetic material (Wu, Zhang, He, Pan, & Xu, 2013).

Several studies have highlighted that inclusion of DCP up to 350 g/kg (Gravador et al., 2014; Inserra et al., 2014; Luciano, Monahan, Vasta, Biondi, Lanza, & Priolo, 2017) and 200 g/kg DGP (Chikwanha, Moelich, Gouws, Muchenje, Nolte, Dugan, et al., 2019; Guerra-Rivas et al., 2016) in finishing lamb increased the resistance of muscle lipids and proteins to oxidative deterioration. Overall, the increased resistance of biomolecules to oxidation reported in these studies were linked to the possible antioxidant effects of polyphenols present in these by-products (Bodas, Prieto, Jordán, López-Campos, Giráldez, Morán, et al., 2012; Gladine, Rock, Morand, Bauchart, & Durand, 2007). Overall, there are limited studies comparing the effects of feeding DCP and DGP on shelf-life of beef. It was hypothesized that utilization of these polyphenolic-rich fruit by-products as dietary supplements could reduce discoloration, rancidity and microbial spoilage of beef. The objectives of the present study were, therefore, to compare the effects of feeding dried citrus pulp and grape pomace as natural preservative for extending shelf-life of Angus beef.

## 5.2 Materials and methods

### 5.2.1 *Study site*

The study site was as described in section 3.2.1.

### 5.2.2 *Preparation of citrus pulp and grape pomace diets*

Dried citrus pulp and grape pomace was as described in section 3.2.1.

### 5.2.3 *Steers, diets and experimental design*

The steers, diet and experimental design was as described in section 3.2.3.

### 5.2.4 *Chemical and fiber analyses for DCP, DGP and experimental diets*

Feed samples were pooled to determine dry matter (DM, procedure, 934.01), ash (procedure; 942.05), ether extract (EE, procedure, 920.39), and nitrogen (N; method 968.06) content using the Dumas technique (LECO® FP528, LECO Corporation, Miami, USA) according to AOAC (2002) procedures. The nitrogen (N) content was multiplied by 6.25 to obtain the CP content. The content of starch in feed was determined according to Hall (2009). The neutral detergent fiber (aNDFom) was assayed according to Mertens, Allen, Carmany, Clegg, Drouches, Wolf et al. (2002). The lignin (sa) content was measured according to method of Raffrenato & Van Amburgh (2011). The contents of ME for DCP, DGP and experimental diets were computed following CSIRO (2007) equations. Non-fibrous carbohydrates were computed as 1000 g/kg- ([Ash + NDF + CP + EE] g/kg). Neutral detergent soluble (NDS) was computed by deducting aNDFom (g/kg) from 1000 g/kg. Pectin and sugar were calculated according to López, Estellés, Moya & Fernández (2014) by subtracting the content of starch (g/kg DM) from NFC (g/kg) content. The Folin-Ciocalteu colorimetric method as described by Makkar (2003) was used for the determination of total phenols and tannin contents, and results were expressed on a gram gallic acid equivalent per kg DM basis. Proanthocyanidins were determined following the procedure of Porter, Hrstich & Chan (1986), and the results are reported as g/kg DM leucocyanidin equivalent. Ascorbic acid content

was determined colorimetrically (Mphahlele, Fawole, Mokwena, & Opara, 2016), and expressed as milligrams per kg DM. The content of α-tocopherol was determined following the procedures outlined by AACC (2000; method 86.06) and results are expressed in mg/kg of α-tocopherol-equivalents.

### 5.2.5 *Dietary fatty acid analyses*

The analysis of fatty acid of the experimental ingredients and diets were the same as described in section 3.3.5 of chapter 3.

### 5.2.6 *Slaughtering and sampling*

The steers were transported to a commercial abattoir located 83 km from the experimental site, and then kept in lairage for 16 h before slaughter. At the abattoir, the steers were stunned using a non-penetrating captive-bolt and exsanguinated. The carcasses were weighed, halved and kept refrigerated at 4℃. After 24 h of refrigerated storage, the left *Longissimus lumborum* (LL) muscle was removed from each carcass. All subcutaneous fat was trimmed from each sample before homogenization using a knife mill (Knifetec™ 1095, Höganäs., Sweden) for the determination of antioxidant activity, lipid and protein oxidation analyses, and stored at -80 °C pending analyses. Five, 2-cm thick slices steaks from left LL were aseptically cut with sterile knives and randomly placed onto polystyrene punnets lined with sterile stomacher bags (Curved 400, Grade Products Ltd., Leicestershire, England) for each of the different storage times (d 1, 3, 5, 7 and 9). The polystyrene punnets were then overwrapped with low-density polyethylene film (moisture vapor transfer rate: 585 g/$m^2$/24h/1 atmosphere, with oxygen permeability of: 2500 $cm^3/m^2$/24h/1 atmosphere, carbon dioxide permeability: 18000 $cm^3/m^2$/24h/1 atmosphere; Freddy

Hirsch, Cape Town, South Africa). The overwrapped punnets were then placed randomly in a cabinet illuminated with white fluorescent light (Philips TL-D 58W/33-640, cool white, 4600 Lumen) for 9-day at 4 ± 0.4°C, simulating retail display conditions. All the overwrapped punnets were rotated daily to minimize light intensity differences and possible temperature variations. On each sampling day, approximately 20 g of beef were sampled aseptically before taking colour measurements and stored at -20°C pending bacterial load determinations.

## 5.3 Shelf life analysis of beef

### 5.3.1 *Analysis of muscle α-tocopherol*

The content of α-tocopherol was determined using a modification of procedure described by Liu, Scheller, & Schaefer (1996), scaled up for 1g of tissue. Beef samples were thawed overnight at 4 °C and homogenized in ethanolic KOH containing 2, 6-di-tert-butyl-p-cresol (BHT) and ascorbic acid as antioxidants. The α-tocopherol was extracted into hexane and analyzed by reverse phase HPLC using a 5 µm silica column (HPLC Technology, Techsphere, Fisher Scientific, Loughborough, UK) with a mobile phase of 4% 1,4-dioxane / 96% n-hexane (v/v) at 40 °C with fluorescence detection. An internal standard of rac-5,7-dimethyltocol (Universal Biological Ltd, Stroud, UK) was used for the quantification of α-tocopherol. Results were expressed as mg α-tocopherol /kg fresh beef.

### 5.3.2 *Ferric reducing antioxidant power analysis (FRAP)*

The antioxidant activity of beef was determined using the ferric reducing antioxidant power (FRAP) method described by Descalzo, Rossetti, Grigioni, Irurueta, Sancho, Carrete, et al. (2007),

with some modifications. Briefly, 1 g samples of meat were homogenized (T18 digital ULTRA TURRAX®, IKA®, Staufen im Breisgau, Germany) at 9000 rpm for 2 min in 5 ml potassium phosphate buffer (pH 7.2), after which the homogenate was centrifuged at 4024×g for 30 min at 20 °C. The FRAP reagent was prepared immediately before the assay by combining 300mM acetate buffer (pH 3.6), 10mM 2,4,6- tri[2-pyridyl]-s-triazine (TPTZ) and 20mM ferric chloride in a 10:1:1 ratio. A 20 µl aliquot of the sample supernatant was combined with 180 µl FRAP reagent in a microplate well (Greiner Cellstar 96 well flat bottom plate, Sigma-Aldrich, St Louis, USA), and the absorbance was read at 593 nm after a 3 s shaking period (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). All samples were extracted and assayed in duplicate. The FRAP activity was quantified by comparison to a ferrous sulphate standard curve (0.1–0.8 mM, $R2 > 0.99$) and results were reported as millimoles ferrous equivalent/kg wet beef (mmol $Fe^{2+}$ eq /kg wet beef).

### 5.3.3 *Meat color*

Colour coordinates (lightness, L*; redness, a* and yellowness, b*) of beef were measured on three different locations using the CIELAB colour meter (BYK-209 Gardner GmbH, Gerestried, Germany) with an 11-mm diameter aperture and D65/10° for the illuminant/observer. The hue angle (H*) values were computed as $\tan^{-1}$ (b*/a*) and the chroma (c*) was calculated as $\sqrt{(a^{*2} + b^{*2})}$. For each parameter, at each storage time, the value was computed as the average value of three determinations per replica.

### 5.3.4 *Lipid oxidation analysis (TBARS)*

The extent of lipid oxidation in left LTL was determined by measuring thiobarbituric acid reactive substances (TBARS) using a modification method described by Lynch & Frei (1993).

Briefly, 1 g of beef samples were homogenized (T18 digital ULTRA TURRAX®, IKA®, Staufen im Breisgau, Germany) with 10 ml 0.15M potassium chloride for 20s at 6400 rpm. A portion (500µl) of the homogenate was combined with 250 µl 1% (w/v) 2-thiobarbituric acid in 50mM sodium hydroxide, and 250 µl 2.8% (w/v) trichloroacetic acid, and vortexed and placed in a water bath at 95 °C. After 1 h the tubes were allowed to cool, and 2 ml 1-butanol was added to each tube. The tubes were vortexed and centrifuged for 30 min at 4 °C at 2383 ×g (Sigma 2–16 K, Wirsam scientific, Cape Town, SA). A 200 µl aliquot of the supernatant from each tube was transferred to a 96 well clear microplate (Greiner Cellstar 96 well flat bottom plate, Sigma-Aldrich, St Louis, USA), and the absorbance was read at 532 nm (Spectrostar Nano, BMG Labtech, Or- tenberg, Germany). All samples (i.e., weighing 1g each) were extracted, assayed in duplicate, and TBARS were quantified using a 1,1,3,3-tetramethoxypropane (TMP) standard curve (0–20 µM, $R2 > 0.99$). Results were expressed as mg malondialdehyde/kg beef (mg MDA/kg beef).

### 5.3.5 *Protein carbonyl content (PCC) assay*

The oxidation of protein, as measured by the total carbonyl content, was evaluated using the colorimetric procedure detailed in the Protein Carbonyl Assay Kit technical bulletin (Sigma-Aldrich, St Louis, MO, USA; Sigma-Aldrich, 2015). 2,4-dinitrophenylhydrazine reagent was used to estimate carbonyl content in beef samples as nmol carbonyls/ mg protein read at 375 nm and an adsorption coefficient of 22 $mM^{-1}.cm^{-1}$ (for protein hydrazones). The protein concentration was quantified using a QuantiPro™ BCA (bicinchoninic acid) Assay kit (Sigma-Aldrich, St Louis, MO, USA; Sigma-Aldrich, 2015) read at 562 nm. For analyses, beef samples were extracted in duplicate, followed by duplicate analyses for each extract.

### 5.3.6 *Microbiological analyses*

After thawing beef samples overnight (24h) at 4°C, bags containing beef samples were aseptically opened in a 131 Bio-11-A microbiology cabinet (model AV30/70, Telstar Madrid, Spain). Ten g were then weighed into stomacher bags (Sterilin, Stone, Staffordshire, UK) using sterile tweezers and homogenized with 90 ml buffered peptone water 0.1% w/w in a stomacher blender (Interscience International, 78860 St Nom, France). Five-fold serial dilutions of homogenates were prepared in duplicate. Bacterial counts were determined as follows: 3M™ Petrifilm™ Aerobic Count Plates (3M, South Africa Pty Ltd., Cape Town, South Africa) for total viable count (TVC) (35 ± 1 °C for 48h ± 2 h); 3M™ Petrifilm™ *E. coli*/ Coliform count plates (3M, South Africa Pty Ltd., Cape Town, South Africa) for *Escherichia coli (E. coli)* and total coliforms (35 ± 1 °C for 24h ± 2 h; AOAC, method; 998.08) and 3M™ Petrifilm™ LAB Count Plates (3M, South Africa Pty Ltd., Cape Town, South Africa) for lactic acid bacteria (LAB) counts (48h ± 3h at 28 -37 °C). All bacterial counts were expressed as logarithms of colony forming units per gram of fresh beef (log CFU/ g beef).

### 5.3.7 *Statistical analyses*

All data were analyzed using the GLIMMIX procedures for repeated measures (Statistical Analysis Software (SAS) Institute Inc. Cary, NC, USA). Individual animals were the experimental unit. The standard model used was as follows: $y_{ijk} = \mu + T_i + A_{ij} + D_k + TD_{ik} + \varepsilon_{ijk}$, where $y_{ijk} =$ is the response variable at k$^{th}$ day on the j$^{th}$ steer assigned to i$^{th}$ treatment, $\mu =$ overall means; $T_i =$ is the fixed effect of the i$^{th}$ treatment (Control, DCP and DGP), $A_{ji} =$ is the random effect of j$^{th}$ steer in i$^{th}$ treatment; $D_k =$ is the fixed effect of k$^{th}$ day; $TD_{ij} =$ is the interaction effect of the i$^{th}$

treatment with $k^{th}$ day and $\varepsilon_{ijk}$ = is the residual error at $k^{th}$ day on $j^{th}$ steer and $i^{th}$ treatment. The least square means option of SAS (SAS Institute Inc. Cary, NC, USA) was used to generate treatment means. Tukey's test was applied for LSMEANS separation. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

## 5.4 Results

### 5.4.1 *Effects of feeding DCP and DGP on alpha tocopherol in beef muscle*

Diet × day interactions were not significant ($P > 0.05$) for concentration of α-tocopherol in beef muscle. The concentration of α-tocopherol in beef muscle tissues was however, affected by diet and day ($P \leq 0.05$; Table 5.1). The DCP diet had highest concentration of α-tocopherol in beef muscle tissues, followed by DGP and control diet in that order ($P \leq 0.05$; Table 5.1). Generally, concentration of α-tocopherol decreased throughout the retail display period ($P \leq 0.05$).

### 5.4.2 *Effects of feeding DCP and DGP on antioxidant activity and color of beef*

Diet × day interactions were not significant for antioxidant activity. The antioxidant activity of beef was however, affected by diet and day ($P \leq 0.05$). Beef from steers fed DGP diet had the highest antioxidant activity followed by the DCP and control diets ($P \leq 0.05$, Table 5.1). Generally, antioxidant capacity decreased throughout the retail display period ($P \leq 0.05$; Table 5.1). No diet × day interactions were observed for colour ($P > 0.05$). Diet affected L* ($P \leq 0.05$; Table 5.1), with beef from steers fed the DGP and DCP diet having greater values than steers fed the control diet ($P \leq 0.05$). Results of the effects of retail display duration on colour measurements are shown in Table 5.1. Retail display duration influenced all colour descriptors ($P \leq 0.05$; Table

5.1). Overall, L*, a*, b*, H* and C* values increased ($P \leq 0.05$; Table 5.1) from day 1 to day 5, and thereafter declined to day 9 (Table 5.1).

### 5.4.3 *Effects of feeding DCP and DGP on lipid and protein oxidation of beef*

Diet × day interactions were not significant ($P > 0.05$) for lipid and protein oxidation. The TBARS were influenced by diet and day ($P \leq 0.05$). Beef TBARS were in the order of DGP <DCP < control diets ($P \leq 0.05$, Table 5.1). The TBARS values generally increased over retail display period ($P \leq 0.05$; Table 5.1). The level of carbonyls in beef samples was affected by diet and day ($P \leq 0.05$). Beef from the steers fed control diet had highest carbonyl compounds followed by DGP and DCP diets, respectively ($P \leq 0.05$, Table 5.1). The level of carbonyls increased ($P \leq 0.05$) throughout the retail display period with a marked increase from day 5 onwards (Table 5.1).

**Table 5.1** Effect of diet and retail display period on α-tocopherol, antioxidant activity, lipid and protein oxidation of beef kept under retail display conditions. Values presented are the least square means with their pooled standard error

| Item | Treatments | | | Retail display period | | | | | SEM | P value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | 1 | 3 | 5 | 7 | 9 | | T | P |
| Alpha tocopherol (Vitamin E)[1] | $1.1^{z}$ | $3.4^{x}$ | $2.7^{y}$ | $3.9^{a}$ | - | $3.0^{b}$ | - | $1.9^{c}$ | 0.12 | 0.032 | 0.001 |
| Antioxidant activity[2] | $132.9^{z}$ | $153.9^{y}$ | $166.1^{x}$ | $189.7^{a}$ | $173.8^{a}$ | $146.3^{b}$ | $133.6^{c}$ | $119.3^{d}$ | 4.51 | 0.001 | 0.001 |
| Lightness (L*) | $40.3^{y}$ | $41.6^{x}$ | $41.8^{x}$ | $39.6^{c}$ | $41.9^{b}$ | $43.3^{a}$ | $41.4^{b}$ | $40.9^{b}$ | 0.25 | 0.017 | 0.001 |
| Redness (a*) | 15.4 | 15.3 | 15.5 | $14.1^{c}$ | $15.5^{ab}$ | $16.1^{a}$ | $15.5^{ab}$ | $14.8^{c}$ | 0.28 | 0.949 | 0.001 |
| Yellowness (b*) | 13.7 | 13.9 | 13.4 | $10.6^{d}$ | $14.6^{a}$ | $14.8^{a}$ | $13.5^{b}$ | $12.4^{c}$ | 0.23 | 0.221 | 0.001 |
| Hue angle (H*) | 41.6 | 41.2 | 41.9 | $35.1^{d}$ | $43.3^{a}$ | $43.1^{a}$ | $41.4^{b}$ | $39.4^{c}$ | 0.61 | 0.613 | 0.001 |
| Chroma (C*) | 20.7 | 20.5 | 20.8 | $17.5^{c}$ | $21.4^{a}$ | $21.5^{a}$ | $20.4^{b}$ | $19.6^{b}$ | 0.31 | 0.726 | 0.001 |
| TBARS[3] | $2.1^{x}$ | $1.6^{y}$ | $1.3^{z}$ | $0.8^{d}$ | $1.0^{d}$ | $1.2^{c}$ | $1.5^{b}$ | $1.9^{a}$ | 0.10 | 0.001 | 0.001 |
| Carbonyls[4] | $2.8^{x}$ | $2.1^{y}$ | $1.8^{z}$ | $1.0^{d}$ | $1.3^{d}$ | $2.0^{c}$ | $2.8^{b}$ | $3.9^{a}$ | 0.11 | 0.001 | 0.001 |

DCP: dried citrus pulp; DGP: dried grape pomace, T: treatments; P: period and SEM: standard error of mean

$^{xyz}$ Within row, different superscripts indicate differences between dietary treatments ($P \leq 0.05$),

$^{abcd}$ Within row, different superscripts indicate differences between days of storage ($P \leq 0.05$)

[1] Expressed as μg α-tocopherol/g of beef (mg α-tocopherol/g of beef),

[2] Expressed as as mmol ferrous equivalent/kg wet beef (mmol $Fe^{2+}$ eq/kg wet beef),

[3] Expressed as mg malondialdehyde/kg beef (mg MDA/kg beef),

[4] Expressed as nmol carbonyl/mg protein

**Table 5.2** Effect of diet and retail display period on bacterial load of beef kept under retail display conditions. Values presented are the least square means with their pooled standard error

| Item | Treatments | | | Retail display period | | | | | SEM | P value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | 1 | 3 | 5 | 7 | 9 | | T | P |
| Coliform count (CC)[1] | $1.8^{x}$ | $0.9^{y}$ | $1.0^{y}$ | $1.0^{c}$ | $1.1^{b}$ | $1.2^{b}$ | $1.4^{b}$ | $1.7^{a}$ | 0.16 | 0.004 | 0.012 |
| Total viable count (TVC)[1] | 4.6 | 4.4 | 4.2 | $3.4^{c}$ | $3.6^{c}$ | $3.9^{c}$ | $4.7^{b}$ | $6.5^{a}$ | 0.18 | 0.171 | 0.001 |
| Lactic acid bacteria (LAB)[1] | 2.8 | 2.6 | 2.7 | $2.3^{c}$ | $2.5^{c}$ | $2.7^{c}$ | $2.9^{b}$ | $3.2^{a}$ | 0.17 | 0.671 | 0.017 |

DCP: dried citrus pulp; DGP: dried grape pomace, T: treatments; P: period and SEM: standard error of mean

$^{xyz}$ Within row, different superscripts indicate differences between dietary treatments ($P \leq 0.05$),

$^{abcd}$ Within row, different superscripts indicate differences between days of storage ($P \leq 0.05$)

[1] Expressed as logarithims of colony forming units per gram of fresh beef (log CFU/ g beef)

### 5.4.4 *Effects of feeding DCP and DGP diets on bacterial counts of beef*

None of the bacteria assayed showed diet × day interactions ($P > 0.05$). *Escherichia coli* was not detected on any of the beef samples throughout the shelf-life study. The TVC remained stable for all the dietary treatments for the first 5 days and then increased until d 9 ($P \leq 0.05$; Table 5.2). No dietary effects were observed for TVC ($P > 0.05$). In contrast, diet influenced coliforms, with the DCP and DGP diets having lower ($P \leq 0.05$, Table 5.2) loads than the control diet. Day also influenced coliforms with day 9 recording the highest loads followed by day 7 ($P \leq 0.05$) and no differences were found between d 1, 3 and 5 ($P > 0.05$; Table 5.2). With regards to lactic acid bacteria (LAB), no differences ($P > 0.05$) were detected between dietary treatments. Loads for the LAB remained stable for all dietary treatments until d 7, with a marked increase on d 9 ($P \leq 0.05$; Table 5.2).

## 5.5 Discussion

The present study is the first to compare the effects of dietary DCP and DGP on α-tocopherol, antioxidant activity and shelf-life of retail displayed beef subjected to air-permeable packaging. The greater concentration of α-tocopherol in beef muscle tissues from DCP diet followed by DGP and control diets could be explained in the light of the higher concentration of α-tocopherol in the former diets compared to control diet. The findings that concentration of α-tocopherol in beef muscle tissues decreased from day 1 to 9 is supported by the fact that antioxidant activity decreases with storage time due to degradation/ oxidation (Descalzo & Sancho, 2008).

Beef from steers fed DGP had greater antioxidant activity (based on the FRAP procedure) than DCP and this could be attributed to a higher content of proanthocyanidins contained in former diet and observed moderate α-tocopherol in beef muscle tissues from steers fed DGP diet. Proanthocyanidins antioxidant activity has been reported to be 20 times more potent than ascorbic acid and 50 times more than α-tocopherol (Shi, Yu, Pohorly, & Kakuda, 2003; Uchida, Edamatsu, Hiramatsu, Mori, Nonaka, Nishioka, et al., 1987). The ability of proanthocyanidins and α-tocopherol to increase antioxidant activity would imply their possible absorption through the gastrointestinal tract and deposition in muscle tissues (Luciano et al., 2009). However, the high molecular weight and polymeric nature of proanthocyanidins limits their absorption in the small intestine. Nevertheless, hydrolysis of these to tri-, di- or monomeric compounds would make their absorption possible (Guerra-Rivas et al., 2016). The greater antioxidant activity for DGP-fed beef compared to control-fed beef could also be supported by the contents of ascorbic acid, total polyphenols and proanthocyanidins in DGP diet.

The greater antioxidant activity when feeding the DCP diet compared to the control diet could be due to greater contents of α-tocopherol observed in DCP diet and muscles tissues. It has been reported that α-tocopherol is not degraded in the rumen (Leedle, Leedle, & Butine, 1993) and when it reaches the small intestine it is absorbed and deposited in cell membranes and lipid depots, where it exhibits its antioxidant activity (Liu, Scheller, & Schaefer, 1996). Moreover, DCP had higher contents of ascorbic acid and polyphenols compared to control diet, which could have contributed to observed higher antioxidant activity for later diet. On one hand, it is known that a combination of α-tocopherol and ascorbic acid can enhance antioxidant status by acting as hydrogen donors (Ahn, Grün, & Mustapha, 2007; Brewer, 2011; Yeum et al., 2009). Yeum et al. (2009) reported this synergistic effect between ascorbic acid and α-tocopherol. Ascorbic acid regenerates α-tocopherol after α-tocopherol donates hydrogen electron to an oxidizing lipid (Brewer et al., 2011). On the other hand, phenolic compounds in DCP such as glycosylated flavonoids, naringin, naringenin and hesperidin possess strong antioxidant activities and/or properties and have been suggested to increase the antioxidant capacity of animal muscle tissues (Gladine et al., 2007; Gravador et al., 2014; Luciano et al., 2017; Zou et al., 2016). The DCP may have exerted its antioxidant activity by inhibiting pro-oxidant enzymes (nitric oxide synthase; lipoxygenase, xanthine oxidase and cyclooxygenase) (Zou et al., 2016). For example, Nakao, Murata, Itoh, Hanamoto, Masuda, Moriyama, et al. (2011) and Zou et al. (2016) observed that hesperidin, naringin and coumarins can directly decrease cellular free radical production by inhibiting xanthine oxidase. Nevertheless, their bioavailability and mechanism of action when ingested in animal diets is yet to be elucidated. It is known that dietary citrus flavonoids are poorly absorbed in the gastrointestinal tract, but if absorbed, especially their monomeric units, can be actively

metabolized and enhance antioxidant defenses (Gladine et al., 2007; Gravador et al., 2014; Luciano, Roscini, Mattioli, Ruggeri, Gravador, Natalello, et al., et al., 2017). However, possible indirect effects have been proposed to explain their potential antioxidant activities in vivo, such as the interaction with other antioxidants in the digestive tract (Halliwell, Rafter, & Jenner, 2005; Luciano et al., 2017).

Overall, the observed findings in the present study are in accordance with other shelf-life studies (Chikwanha et al., 2019; Guerra-Rivas et al., 2016; Simitzis et al., 2019) based on dietary α-tocopherol- and polyphenolic- rich lamb diets. The finding that antioxidant capacity plateaued from day 1 to 5 and then decreased to 9 is supported by the fact that antioxidant activity decreases with storage time due to degradation/ oxidation of antioxidants (i.e., α-tocopherol and polyphenols), which tends to reduce concentrations of antioxidant compounds in muscle tissues (Descalzo & Sancho, 2008).

The lack of differences among diets for the colour descriptors (i.e., a*, b*, H* and C*) is consistent with earlier findings for meat from lambs fed diets containing DCP (Caparra, Foti, Scerra, Sinatra, & Scerra, 2007) and DGP (Chikwanha et al., 2019; Luciano et al., 2009; Zhao, Li, Zhang, Liu, Ren, Zhang, et al., 2018). The higher values of L* observed for beef fed the DGP and DCP diets compared to the control diet could be due to high concentration of α-tocopherol in beef muscle tissues, which is an iron chelating agent promoting a lower blood haemoglobin concentration and probably lower myoglobin concentration prior to slaughter (Guerra-Rivas et al., 2016). High content of proanthocyanidins and ascorbic acid contained in DGP and DCP diets could be other factors influencing high L* values.

Polyphenols have been associated with reduced microbial biosynthesis of vitamin B12, which is the precursor for the synthesis of haem pigments (Priolo, Vasta, & Priolo, 2007; Vasta et al., 2008). In this regard, dietary polyphenolics may have reduced biosynthesis of haem pigments caused resulting in a lighter meat compared to the control diet. Specifically, a reduction of haemoglobin and, myoglobin could account for the lighter colour of the muscle in DGP- and DCP-fed steers (Priolo et al., 2007; Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000; Vasta et al., 2008). The increase in L* values up to day 5 of storage can be explained by structural changes of beef, especially protein denaturation, which subsequently results in greater dispersion of light and consequently increased L* (Warris, 2010). The L* values observed in the present study were within the range of 35.3-46.3, which is considered acceptable by beef consumers (Cooper, Suman, Wiegand, Schumacher, & Lorenzen, 2018; Holman, Van de Ven, Mao, Coombs, & Hopkins, 2017).

On one hand, the observed increase in a* from day 1 to day 5 could be linked to conversion of deoxymyoglobin to oxymyoglobin, which imparts a cherry-red colour associated with fresh beef by consumers (Faustman & Suman, 2017). The current findings concur with Gonzalez-Rios et al. (2016) who fed ferulic acid (i.e., a phenolic phytochemical found in plant cell walls) as dietary supplement to steers under commercial feedlot feeding conditions on meat quality and shelf life. These findings indicated that the typical beef redness colour begins deteriorating on day 5 due to presence of a dietary phenolic phytochemical. It has been proposed that the antioxidant properties of phytochemicals can be mediated by scavenging of free radical species such as reactive oxygen species or suppressing formation of free radicals by inhibiting some enzymes or chelating trace metals involved in free radical production or up-regulating or protecting antioxidant defense. On

the other hand, the decline of a* values observed from day 5 to 9 could be explained by the oxidation of myoglobin and/or oxymyoglobin to metmyoglobin, which subsequently results in brown colour linked to stale or spoiled beef by consumers (Faustman & Suman, 2017). The a* values observed in the present study were above threshold value of ≥ 14.5, which is considered the acceptable threshold for consumer acceptability of fresh beef consumers consider as an acceptable colour (Cooper et al., 2018; Holman et al., 2017). The increase in b* values up to day 5 could be probably attributed to oxidative processes which leads to production of Schiff pigments like lipofuschin from protein and lipid complexes as reported by Chelh, Gatellier, & Santé-Lhoutellier (2007). Yellowness (b*) is generally not associated with consumer preferences for beef and is, therefore, unlikely to influence consumer acceptability.

The observation that beef lipids from steers fed the DGP diet were more resistant to oxidation (i.e., lower TBARS) compared to those from steers fed the DCP diet could be attributed to higher antioxidant activity observed for the former diet due to combination of moderate concentration of α-tocopherol in beef muscle tissues from steers fed the DGP diet and high proanthocyanidins in DGP diet. The effects of natural antioxidants, particularly dietary α-tocopherol and phenolic compounds have been attributed to their ability to diminish oxidative damage of muscle indirectly by enhancing the natural defenses of the cell and/or directly by scavenging the free radicals species, chelating metal ions, quenching singlet oxygen, acting as reducing agents or through activation of antioxidant enzymes and combating disorders generated by phytochemical reactive oxygen/or nitrogen species (Guerra-Rivas et al., 2016; Jerónimo, Alfaia, Alves, Dentinho, Prates, Vasta, et al., 2012). The current findings concur with Chikwanha et al. (2019), Gladine et al. (2007) and Guerra-Rivas et al. (2016) who fed DGP to lambs and reported positive impacts on lamb meat

oxidative stability. Improved lipid oxidative stability (i.e., lower TBARS) observed for beef from steers fed the DCP diet compared to beef from steers fed the control diet could be due to observed higher concentration of α-tocopherol in addition to ascorbic acid and polyphenols in the former diet. It is reported that α-tocopherol preserves the integrity of muscle cell membranes, inhibiting the passage of sarcoplasmic fluid though it, as well as acting as a radical-quenching antioxidant, consequently preventing the oxidation of membrane phospholipids during storage (Faustman et al., 1998; Nassu et al., 2011). Furthermore, ascorbic acid and α-tocopherol may have had synergistic effects as discussed earlier.

It is noteworthy that after 9 days of storage, TBARS values of beef from steers fed the control diet exceeded 2 mg MDA/kg of beef, which has been suggested as a threshold for sensory detection of rancid flavors (Campo, Nute, Hughes, Enser, Wood, & Richardson, 2006). Generally, if the levels of antioxidants in muscle tissues decrease, the oxidative stability deteriorates (Descalzo & Sancho, 2008). The observed increase in TBARS during retail display could be attributed to exposure to pro-oxidants in muscle over time (Nassu et al., 2011). The findings in the present study are in agreement with ruminant studies (Chikwanha et al., 2019; Guerra-Rivas et al., 2016; Nassu et al., 2011; Inserra et al., 2014; Luciano et al., 2017; Simitzis et al., 2019), which reported TBARS corresponded to high antioxidant activities of dietary α-tocopherol and polyphenolic compounds.

The finding that beef from steers fed DGP had a lower content of carbonyl groups compared to DCP could be attributed to high antioxidant activity due to high levels of α-tocopherol and polyphenol observed in former diet as reported earlier in this study. The mechanism of α-tocopherol and polyphenols in inhibiting/or delaying the processes of protein oxidation is the same

as discussed earlier for myoglobin and lipid oxidation. It seems that the lower content of carbonyl groups found in beef muscle from steers fed DCP compared to control could be due to the lower formation of lipid oxidation products since amino acid residues (i.e. lysine and arginine) studied using the 2,4-dinitrophenylhydrazine method are common targets for reactive oxygen substances generated via lipid oxidation (Gravador et al., 2014; Muíño, Apeleo, de la Fuente, Pérez-Santaescolástica, Rivas-Cañedo, Pérez, et al., 2014). The ability of DCP to delay protein oxidation as observed in the present study can be linked to observed α-tocopherol in beef muscle tissues and the bioavailability of glycosylated flavonoids, naringin, naringenin and hesperidin as explained earlier for colour and lipid oxidation. Overall, the observed increase in carbonyl compounds during retail display could be attributed to the exposure to pro-oxidant factors that accelerate oxidation of proteins by inducing oxidative stress through inhibition of antioxidant systems (Carocho, Morales, & Ferreira, 2018). To date, no threshold values for protein carbonyl content in beef have been set. The protein carbonyls quantified in the present study (i.e., 1.8-2.8 nmol/mg proteins) are comparable with the results found in lambs fed DGP and DCP in previous studies (1.34-6.52 nmol/mg proteins) (Gravador et al., 2014; Chikwanha et al., 2019).

The content of α-tocopherol in the DCP and DGP diet compared to control diet was within the range recommended to meet physiological requirements of growing steers (10-40 mg/kg) (Hidiroglou, Cave, Atwal, et al., 1992; McDowell, Williams, Hidiroglou, Njeru, Hill, Ochoa, et al., 1996). Overall, observed α-tocopherol in beef muscle tissues from steers fed DCP and DGP diets compared to control fed beef muscle tissues falls within the recommended critical levels in muscle tissues (3-3.7 µg α-tocopherol/g) required to enhance oxidative shelf life (i.e., myoglobin, lipid and protein oxidation) in beef (Chan, Hakkarainen, Faustman, Schaefer, Scheller, & Liu,

1996; Faustman, Cassens, Schaefer, Buege, Williams, & Scheller, 1989; Liu, Lanari, & Schaefer, 1995).

Lower coliform counts observed in beef from steers fed the DCP and DGP diet compared to the control diet could be attributed to the chelating ability and antimicrobial potential related to the presence of α-tocopherol and phenolic compounds in citrus pulp and grape pomace, which can deprive microbes of essential iron required for growth (Mattos et al., 2017; Scalbert, 1991). In addition, the antimicrobial mechanisms of phenolics in DGP and DCP may manifest through attacking the phospholipid bilayer of bacterial cell membranes, disrupting enzyme systems and compromising the genetic material of bacteria (Wu, Zang, He, Pan, & Xu, 2013).

The result that diet had no effect on TCV, *E. coli* and LAB contrasts with previous studies (Rota, Herrera, Martínez, Sotomayor, & Jordán, 2008) providing evidence for the efficacy of α-tocopherol and polyphenols as antimicrobial agents, capable of altering bacterial cell membranes and microbial enzymatic metabolism with high antibiotic activity. In that regard several studies have shown that dietary supplementation with α-tocopherol (Buys, Nortjé, Jooste, & Von Holy, 2000; Smith, Morgan, Sofos, & Tatum, 1996; Luciano et al., 2011) and polyphenols (Ortuño, Serrano, Jordán, & Bañón, 2014; Papuc et al., 2017; Chikwanha et al., 2019) or quercetin (Andrés, Tejido, Bodas, Morán, Prieto, Blanco, et al., 2013; Cushine & lamb , 2005; Moon et al., 2011) reduced microbial populations responsible for meat spoilage during storage owing to the accumulation of these compounds or their metabolites in muscle tissues during the life of animals (Raccach, 1984).

The increase in TVC and LAB during retail display agree with previous findings, who reported that TVC and LAB remained stable for beef cattle fed dietary α-tocopherol and polyphenolics over the same retail display period. It is noteworthy that over 9 days, TVC, coliform and LAB did not surpass the 7 log CFU/g, a threshold value beyond which beef is deemed unsafe for human consumption (Kim, Song, & Jang, 2018; Kim & Yim, 2016).

## 5.6 Conclusions

Feeding DGP improved the shelf-life of beef during retail display through enhancement of antioxidant activity and reduction of coliforms, and lipid and protein oxidation compared to DCP and control diets. Based on current findings, DGP could be a better dietary natural preservative compared to DCP. Follow-up studies are thus, warranted to compare the effects of feeding DGP or DCP as dietary natural preservatives on fatty acid profile, volatile compounds and descriptive sensory quality when included in beef cattle finishing diets.

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# Chapter 6 Pro-oxidant fatty acid, volatile and organoleptic profiles of beef from steers fed on citrus pulp or grape pomace

ABSTRACT

The present study compared the effects of feeding 150 g/kg DM of dried citrus pulp (DCP) or grape pomace (DGP) to 7-months old Angus steers for 90 d on the major pro-oxidant fatty acids, volatile compounds and eating quality of beef. Twenty-four steers were assigned to three dietary treatments (8 steers/treatment) in a completely randomized design, with individual steer as the experimental unit. Feeding the DGP or DCP diets increased ($P \leq 0.05$) the proportions of 18:2 n-6, 18:3 n-3 and total polyunsaturated fatty acid (FA) in muscle. Beef from steers fed the control diet had greater ($P \leq 0.05$) concentrations of ketone and aldehydes, and lower ($P \leq 0.05$) concentrations of sulphur-containing compounds compared to the DCP- and DGP-fed beef ($P \leq 0.05$). Feeding the DGP and DCP diets produced less tender ($P \leq 0.05$) beef than the control diet. Current findings indicate that inclusion of either DGP or DCP in steer finishing diets can increase proportions of the main pro-oxidant fatty acids, reduce the concentrations of ketones and aldehydes, and negatively affect metallic aroma and tenderness of beef compared to the control diet containing wheat bran.

**Keywords** Antioxidant, Beef, Fatty acids, Phenolic compounds, Sensory attributes, Volatile compounds.

## 6.1 Introduction

Tenderness, flavor and juiciness are key determinants of ruminant meat palatability and acceptability by the consumers (Khan et al., 2015 Legako et al., 2015). These meat organoleptic attributes are primarily influenced by intramuscular fat content and composition of fatty acids (Wood et al., 2008). The effect of fatty acid on meat flavor is due to the production of volatile compounds during cooking and the involvement of these with Maillard reaction products to form other volatiles which contribute to flavor (Wood et al., 2003; Khan, Jo & Tariq, 2015; Kosowska et al., 2017). The pro-oxidant fatty acid profile of meat is particularly important in flavor development (Mezgebo et al., 2017; Piao et al., 2019). Tissue deposition of pro-oxidant fatty acids (i.e., 18:1 n-9, 18:2 n-6 and 18:3 n-3) is largely determined by their content and composition in the by diet and rumen microbial metabolism (Mapiye et al., 2012).

Dietary polyphenols can modulate fatty acid profiles in the rumen (Buccioni et al., 2017; Vasta et al., 2019), and contribute to the formation of volatile compounds and development of aroma compounds in beef (Ianni et al., 2019). These phenolic compounds have been reported to protect dietary polyunsaturated fatty acid (PUFA) from biohydrogenation in the rumen, and/or inhibit growth and metabolism of ruminal bacteria responsible for biohydrogenation, thereby, enhancing tissue deposition of PUFA and their biohydrogenation intermediates (Buccioni et al., 2017; Vasta et al., 2019; Vasta et al., 2009). In addition, feeding polyphenolic-rich diets (e.g., dried citrus pulp (DCP) or grape pomace (DGP) improves nutrient digestibility, growth performance, lamb meat quality and shelf life (Chikwanha, Moelich, et al., 2019; Chikwanha, Muchenje, Nolte, & Dugan, 2019; Francisco et al., 2017; Inserra et al., 2014) and beef (Ianni et al., 2019; Moote et al., 2014;

Chapter 3,4,5). However, the effects of feeding DGP or DCP on volatile compounds and eating quality of beef has not been investigated. Given DGP has greater total phenol and proanthocyanidin contents compared to DCP, it was hypothesized that inclusion of DGP in Angus steers diets could lead to increased levels of major pro-oxidant fatty acids in beef without adversely compromising eating quality. The objective of the present study was, therefore, to compare the effects of feeding DGP or DCP in finishing diets of Angus steers on major pro-oxidant fatty acids, volatile compounds and sensory quality of beef.

## 6.2 Materials and methods

### 6.2.1 *Site of study*

The study site was as described in section 3.2.1.

### 6.2.2 *Preparation of citrus pulp and grape pomace diets*

Dried citrus pulp and grape pomace were prepared the same as described in section 3.2.2.

### 6.2.3 *Steers, diets and experimental design*

The steers, diets and experimental design were the same as described in section 3.2.3.

### 6.2.4 *Chemical analyses of DCP, DGP and experimental diets*

DCP, DGP and compounded feed samples were pooled to determine dry matter (DM, procedure, 934.01), ash (procedure; 942.05) and ether extract (EE, procedure, 920.39), nitrogen (N; method 968.06) content using the Dumas technique (LECO® FP528, LECO Corporation, Miami, USA) according to AOAC (2002) procedures. The nitrogen (N) content for feed was assayed using the

Dumas technique (LECO® FP528, LECO Corporation, Miami, USA) (AOAC, 2002; method 968.06), and N content was multiplied by a factor of 6.25 to determine the content of CP. The content of starch in the fruit by-product and feed samples was determined according to Hall (2009). Neutral detergent fiber (aNDFom) was assayed according to Mertens, Allen, Carmany, Clegg, Drouches & Wolf et al. (2002) method. The lignin (sa.) content was measured according to method of Raffrenato and Van Amburgh (2011). The content of ME for DCP, DGP and experimental diets was computed using CSIRO (2007) equations. Non-fibrous carbohydrates were computed as 1000 g/kg- ([Ash + NDF + CP + EE] g/kg). Neutral detergent soluble (NDS) was computed by deducting aNDFom (g/kg) from 1000 g/kg. Pectin and sugar were calculated according to López et al. (2014) by subtracting the content of starch (g/kg DM) from NFC (g/kg) content. Minerals in DCP, DGP and compounded diets were measured after microwave/acid digested using inductive coupled plasma-automatic emission spectrometry (ICP-AES) according to Sah and Miller (1992). All analyses were performed in triplicate. The Folin-Ciocalteu colorimetric method as described by Makkar (2003) was used for the determination of total tannin content, and results were expressed as gram gallic acid equivalent per kg DM. Proanthocyanidins were determined following the procedure of Porter, Hrstich & Chan (1986), and the results were reported as g/kg DM leucocyanidin equivalent. Ascorbic acid content was determined colorimetrically (Mphahlele, Fawole, Mokwena, & Opara, 2016), and expressed as milligrams per kg DM. The content of α-tocopherol was determined following the procedures outlined by AACC International (2000; method 86.06) and results were expressed in mg/kg of α-tocopherol-equivalents.

### 6.2.5 *Fatty acid analysis of experimental ingredients and diets*

The analysis of fatty acid of the experimental ingredients and diets were the same as described in section 3.3.5 of chapter 3.

### 6.2.6 *Slaughtering and sampling*

The steers were transported to a commercial abattoir located 83 km from experimental site and kept in lairage with only water provided for 16 h before slaughter. At abattoir, the steers were stunned using a non-penetrating captive-bolt and exsanguinated following Meat Safety Act (MSA, 2000) regulations. After slaughter, the carcasses were weighed, halved and cooled at 4 ℃ for 24 h. The *longissimus thoracis et lumborum* (LTL) were excised from the right and left sides of the carcasses. For determination of fatty acids, 2.5cm thick steaks from each steer were removed from the 12th – 13th rib of the left LTL muscle, trimmed off visible fat and connective tissue, homogenized, vacuum-packed and stored at -80 ℃ pending analysis. Samples for the determination of beef volatile compounds were collected from left LTL muscle. Two grams of raw beef were weighed directly into solid-phase micro-extraction (SPME) headspace vials (20 ml, clear screw-thread vial, round bottom, USP 1, expansion 33), which were sealed using screw-thread magnetic caps with polytetrafluoroethylene/silicone septa, and were frozen at -80 °C until analysis. The right LTL were vacuum-packed and stored at -20 °C until sensory evaluation.

### 6.2.7 *Intramuscular pro-oxidant fatty acid analysis*

Lipids from raw beef (1 g) samples were extracted in 50 ml of 2:1 (v/v) chloroform: methanol (Folch, Lees, & Sloane-Stanley, 1957), which contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The samples were homogenized in the extraction solvent for 30 s at 7000 rpm

using a polytron mixer (IKA® T18 digital ULTRA TURRAX®), and then passed through extraction funnels fitted with glass microfiber filter papers (Whatman, GF/A, diameter 47 mm, Cat no. 1820-047, Sigma-Aldrich, Gauteng, South Africa). Heptadecanoic acid (17:0) was used as an internal standard (catalogue number H3500, Sigma-Aldrich, Gauteng, South Africa) to quantify the individual pro-oxidant fatty acids present in each sample, to each vial 0.5 ml of the 10mg 17:0/ml solution was added prior to lipid extraction. Methanol (19.4ml) was added as solvent to 0.6ml of sulfuric acid solution to make the transmethylating agent. A 250 µl sub-sample of the extracted lipids was subsequently transmethylated at 70 °C for 2 h using 2 ml of the transmethylating agent. After cooling to room temperature, the fatty acid methyl esters (FAMEs) were extracted after addition of 1 ml water using 2 ml hexane, and the FAME containing hexane layer was removed and dried under nitrogen in a 45 °C water bath. One hundred µl of hexane was then added to each dried FAME sample, of which 1 µl was injected on to the gas chromatograph using a 5:1 split ratio and helium as the carrier gas at a flow rate of 1ml/min. The FAMEs were analyzed using a Thermo TRACE 1300 series gas chromatograph (GC, Thermo Electron Corporation, Milan, Italy) equipped with a flame-ionization detector (FID) coupled to a CTC analytics PAL autosampler. Separation of FAME was done using a 60 m TR-FAME capillary column with an internal diameter of 0.25 mm and a 0.25 µm film (Cat. No. HY260M142P, Anatech, Cape Town, South Africa). The injector temperature was maintained at 250 °C. The oven temperature was programmed as follows: held at 50 °C for 2 min, and then ramped up to 180 °C at a rate of 25 °C/min for 5 min, followed by a ramping rate of 3 °C/min for 2 min until 260 °C. The FAME of each sample was identified by comparing the retention times to those of a standard FAME mixture (Supelco™ 37 Component FAME mix, Cat no. CRM47885, Supelco, USA), and

quantified by comparing the integrated areas to that of the internal standard. Results were expressed as percentage of total FAME

### 6.2.8 *Volatile compound analyses*

The volatile compound profile of the beef samples was determined after solid-phase micro-extraction, followed by gas chromatography–mass spectrometry (SPME-GC–MS). Sample vials were thawed and 100-µl internal standard solution (1000 µg/kg 3-octanol and anisole-d8 in methanol) was added. The vials were left to equilibrate for 10 min at 50 °C using a CombiPAL (CTC, Switzerland), before inserting a pre-conditioned fiber into the vial headspace above the sample. The fiber was pre-conditioned by heating in a GC injection port at 270 °C for 60 min and was coated with a 50/30 µm thick divinylbenzene/carboxen/poly-dimethylsiloxane layer (Supelco 57,298-U, Sigma). After a 20 min extraction period (with agitation), the fiber was retracted from the vial and inserted into the GC injection port. The SPME fiber was desorbed at 250 °C for 10 min, with the injection port operating in splitless mode. Separation of the volatile compounds was achieved using an Agilent 6890 N (Agilent Technologies, Palo Alto, CA, USA) GC with a polar Zebron 7HG-G009-11 ZB-FFAP capillary column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm) from Separations®, (South Africa). A single-ramp temperature program was used, with an initial temperature of 40 °C being held for 5 min, followed by an increase to 240 °C at 5 °C/ min and a final holding period of 2 min at 240 °C. The total run time per sample was 47 min. The carrier gas (helium) had a constant flow rate of 1.0 ml/min and the transfer line was maintained at 250 °C. The mass spectra for the separated compounds were obtained using an Agilent mass spectrometer detector (5975B, Palo Alto, California, USA), working with the electronic impact at

70 eV. The detector was operated in full scan mode (35–450m/z), and the ion source and quadrupole temperatures were maintained at 240 °C and 150 °C, respectively.

The chromatograms were processed using Xcalibur™ Software from Thermo Fisher Scientific (Massachusetts, USA). Concentrations reported for each peak are in µg/kg beef and were calculated using the area ratio and the concentration of the internal standard. Anisole-d8 was used for the quantification of all the chemical groups apart from the alcohols, for which 3-octanol was used. Compounds were tentatively identified by comparing their retention indices (RIs) and mass spectra of the house database (Wiley 6/NIST 11). Retention indices (RI) of the compounds were determined by running n-alkanes under the same chromatographic conditions and calculated according to Tao, Wu, Zhou, Gu, & Wu (2014) using the following formula:

$$\mathrm{RI} = [Rt(i) - Rt(n)/(Rt(n+1) - Rt(n) + n] \times 100$$

where: Rt(i) is the retention time (RT) of each targeted compound (i), Rt(n) and Rt(n+1) are the RTs of the n-alkanes eluting directly before and after the targeted compound (i) under the same chromatographic conditions. The n is the carbon number of the n-alkane eluting before the targeted compound (i). It must be emphasized that while the volatile compounds will henceforth be referred to by their IUPAC or common names, the identification of these compounds (apart from hexanal) is strictly tentative, as they have not been compared to pure standards for confirmation.

### 6.2.9 *Sensory analyses*

Prior to sensory evaluation analyses of LTL, samples were thawed for 24 h at 4 °C. The samples were placed in individual coded oven roasting bags (GLAD® Medium 250 mm x 400 mm) and positioned on a stainless-steel grid which was fitted on a stainless-steel oven roasting pan. A

thermocouple probe, attached to a handheld digital temperature monitor (Hanna Instruments, Bellville, South Africa), was inserted into the Centre of each meat sample (AMSA, 2015). The samples were roasted at 163 °C in two conventional electric Defy 835 ovens connected to a computerized electronic temperature system (Viljoen et al., 2001). When the samples reached an internal temperature of 70 °C (AMSA, 2015) they were immediately removed from the oven and left to cool for 15min remaining in their roasting bags. The samples were then removed from the roasting bags blotted dry and cut into $1cm^3$, perpendicular to the fiber direction. In order to minimize variation, only the centre cubes were used with the dryer outsides being trimmed off. The cubes were individually wrapped in aluminium foil and placed into glass ramekins with randomized three-digit codes. Each ramekin contained three wrapped meat cubes per treatment. Reheating of the samples took place in a preheated industrial forced convection oven (Hobart CSD 1012, France) at 70 °C for 8 min before the start of each session. Throughout the sensory analyses no salt (NaCl) or any form of seasoning was added to the samples. Descriptive sensory analysis was performed on one steak per animal per treatment. Equal numbers of samples per treatment were selected randomly for test sessions (n = 8) and rated for sensory attribute intensity.

Descriptive sensory analysis of the samples was performed by a trained panel with 11 members, all with previous experience in the sensory evaluation of beef. Prior to the testing phase, *Longissimus thoracis* (LT) was used to train the panel and the *longissimus lumborum* (LL) for the testing phase during eight sessions. Training was done according to the guidelines and recommendations of Belk et al. (2015). Reference standards were used during the training period to help the panel to define the attributes (Table 6.1). During the training period, nine aroma and flavor attributes and four textural attributes were decided upon and elucidated (Table 6.2). Each

panel member received four samples from each LT and reference sample during training. Panelists were allocated individual tasting booths fitted with computers with the Compusense five® software (Compusense, Guelph, Canada) program. Each animal was randomly assigned to a testing session and 8 testing sessions were performed (8 replications).

### 6.2.10 *Statistical analyses*

All data were analyzed using the GLIMMIX procedures (SAS Institute Inc. Cary, NC, USA). Individual animal was the experimental unit. The standard model was used as follows: $y_{ijk}= \mu +T_i+A_{ij}+\varepsilon_{ijk}$, where $y_{ijk}$ = is the response variable at kth day on the $j^{th}$ subject assigned to $i^{th}$ treatment, $\mu$ = overall means; $T_i$ = is the fixed effect of the $i^{th}$ treatment (Control, DCP and DGP), $A_{ji}$ = is the random effect of $j^{th}$ steer in $i^{th}$ treatment and $\varepsilon_{ijk}$ = is the residual error at kth day on $j^{th}$ steer and $i^{th}$ treatment. A similar model was used for sensory quality with diet as the fixed factor and session as random variable. The least square means (LSMEANS) option of SAS (SAS Institute Inc. Cary, NC, USA) was used to generate treatment means. Tukey's test was applied for LSMEANS separation. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Principle component analysis (PCA) was performed using XLstat software (Version 2019, Addinsoft, New York, USA), to visualize the relationships between the treatment groups and variables, based on a Pearson's correlation matrix.

**Table 6.1** Reference standards used for descriptive sensory analysis to interpret specific attributes

| Attribute | Standard reference |
|---|---|
| Beef meat | Roasted beef meat |
| Savoury 1 | Dissolve 5 ml of Bovril in 250 ml boiling water |
| Savoury 2 | Dissolve 5 ml of Marmite in 250 ml boiling water |
| Sweet-associated | Trimmings of roasted beef with fat |
| Livery | Pan fried beef liver = 7.5 (aroma and flavor) |
| Metallic | 0.10% potassium chloride solution = 1.5 (flavor) |
| Salty | 0.25% sodium chloride solution = 3.5 (flavor) |
| Rancid | Microwaved Wesson vegetable oil (3 min at high) = 7.0 (flavor) |
| Citrus pulp | 10 g of milled (1 mm) citrus pulp in 250 ml water |
| Grape pomace | 10 g of milled (1 mm) grape pomace in 250 ml water |

**Table 6.2** Definition and scale of final aroma, flavor and texture attributed used for descriptive sensory analysis

| Attributes | Description of the attributes |
|---|---|
| Aroma: 0 = None 100 = prominent | |
| Overall intensity | The intensity of the aromas on the first few sniffs |
| Beef | The aroma associated with a cooked beef steak |
| Savoury broth | Aroma associated with Bovril |
| Sweet associated | Aroma associated with the browning of a cooked meat surface (Maillard reaction) |
| Sour-associated | Aroma associated with sour substances |
| Metallic | The aroma of ferrous sulfate, associated with raw meat or blood like taste |
| Fatty | Aromatics associated with cooked animal fat |
| Liver-like | The aroma associated with pan fried beef ox liver |
| Rancid | Aromatics commonly associated with oxidised fat and oils; may include cardboard, painty and fishy |
| Flavor: 0 = None, 100 = prominent | |
| Beef | The flavor associated with a cooked beef steak |
| Savoury-broth | Flavor associated with Bovril |
| Sour associated | Flavor associated with sour substances |
| Metallic | The flavor of ferrous sulfate, associated with raw meat or blood like taste |
| Sweet-associated | Flavor associated with the browning of a cooked meat surface (Maillard reaction) |
| Salty | Flavor associated with sodium ions |
| Fatty | Flavor associated with cooked animal fat |
| Liver-like | The flavor associated with pan fried beef ox liver |
| Rancid | Flavor commonly associated with oxidised fat and oils; may include cardboard, painty and fishy |
| Texture | |
| Sustained juiciness: 0 = tough, 100 extremely juicy | The amount of moisture perceived during mastication (after 5-10 chews using the molar teeth) |
| Mealiness: 0 = none, 100 = abundant | The meat disintegrates into small gritty pieces in your mouth after 5 chews |
| Tenderness 0 = tough, 100 = extremely tender | The perceived tenderness during mastication (after 5-10 chews using molar teeth) |
| Residue: 0 = none, 100 = abundant | The amount of tissue left in your mouth after mastication (after 10 chews using the molar teeth) |

## 6.3 Results

### 6.3.1 *Fatty acid composition of experimental ingredients and diets*

For all the diets, linoleic acid (18:2n-6) was the major fatty acid followed by oleic (18:1n-9), α-linoleic (18:3n-3), respectively (Table 6.3). Palmitic and stearic acids proportions were similar across diets. The proportions of 18:1n-9 and 18:3n-3 were slightly lower in the DGP diet whereas proportions of 18:2n-6 were somewhat lower in the DCP diet (Table 6.3).

### 6.3.2 *Effects of feeding DCP and DGP on major pro-oxidant fatty acids in beef*

Intramuscular fat content was similar across diets ($P > 0.05$; Table 6.4). The proportion of 18:1n-9 was greater ($P \leq 0.05$) in control-fed beef compared to DCP- and DGP-fed beef ($P \leq 0.05$). The proportions of total PUFA, total n-6 PUFA, 18:2n-6, 20:4n-6, total n-3 PUFA and 18:3n-3 were affected by the diet ($P \leq 0.05$), with the DCP- and DGP-fed beef having greater ($P \leq 0.05$) proportions than control-fed beef.

### 6.3.3 *Effects of feeding DCP and DGP on volatile compounds in beef*

A total of 44 volatile compounds were tentatively identified and presented in Table 6.5. Dietary treatments influenced the profile of beef volatile compounds ($P \leq 0.05$; Table 6.5). Control-fed beef had greater ($P \leq 0.05$) concentrations of 2-propanol, ethyl amyl carbinol, isoamyl alcohol, 2-heptanol and 2-nonanol than DCP- and DGP-fed beef. Among the seven identified ketones, 2-butanone-3-hydroxy, 3-octanone, 2-heptanone and 2-nonanone were greater ($P \leq 0.05$) in control-fed beef than in DCP- and DGP-fed beef. Aldehydes were found at lower ($P \leq 0.05$) concentrations in DCP- and DGP-fed beef compared to control-fed beef. The DCP- and DGP-fed

beef had greater ($P \leq 0.05$) concentrations of sulphur compounds (dimethyl disulfide and 3,4-dihydrothienyl (3,4, b)-5-carboxythiophene) and heterocyclic compounds (1,3-nonadiene and cyclopropane, 1-heptyl-2-methyl) than in control-fed beef. None of the hydrocarbons and organic acids identified were influenced ($P > 0.05$) by diet.

**Table 6.3** Chemical composition, *in vitro* digestibility and fatty acid composition of dried citrus pulp (DCP), dried grape pomace (DGP), wheat bran (WB) and experimental diets

| Item | DCP | DGP | WB | Treatments | | | SEM[1] |
|---|---|---|---|---|---|---|---|
| | | | | Control diet | DCP diet | DGP diet | |
| Chemical composition (g/kg) | | | | | | | |
| Dry matter (DM) | 862.8 | 899.3 | 901.2 | 879.2 | 875.9 | 882.8 | 0.62 |
| Organic matter (OM) | 951 | 940.7 | 941.2 | 892.5 | 948.3 | 935.3 | 0.63 |
| Ash | 49.0 | 59.3 | 58.8 | 107.5 | 51.7 | 64.7 | 0.58 |
| Crude protein | 47.7 | 111.0 | 175.5 | 119.5 | 119.1 | 119.4 | 0.66 |
| Starch | 57.5 | 23.0 | 22.6 | 217.9 | 235.3 | 212.0 | 0.72 |
| Ether extract | 18.0 | 74.3 | 45.5 | 23.7 | 24.3 | 33.5 | 0.82 |
| Ash free neutral detergent fiber (aNDFom) | 145.8 | 317.6 | 385.4 | 164.4 | 172.7 | 183.5 | 1.41 |
| Neutral detergent soluble fiber (NDSF) | 854.2 | 682.4 | 614.6 | 835.6 | 827.3 | 816.5 | 1.28 |
| Acid detergent lignin (ADL, [sa]) | 6.2 | 205.9 | 41.4 | 21.7 | 19.2 | 48.0 | 0.71 |
| Metabolizable energy (MJ/ kg) | 11.1 | 8.2 | | 113.1 | 119.5 | 114.1 | 0.58 |
| Non-fibrous carbohydrates (NFC) | 739.5 | 437.8 | 342.7 | 584.5 | 632.2 | 598.9 | 0.58 |
| Pectin + sugar[2] | 683.0 | 414.8 | 320.1 | 367.0 | 396.9 | 386.9 | 0.47 |
| 24h *in vitro* neutral detergent digestibility (%) | 43.9 | 15.2 | 39.7 | 19.4 | 29.3 | 15.4 | 0.45 |
| 48h *in vitro* neutral detergent digestibility (%) | 62.1 | 23.0 | 44.4 | 40.0 | 53.2 | 22.1 | 0.58 |
| Calcium | 17.3 | 3.1 | 1.5 | 11.9 | 7.4 | 10.7 | 2.4 |
| Phosphorus | 1.1 | 2.7 | 10.9 | 4.4 | 4.1 | 3.9 | 0.3 |
| Potassium | 8.3 | 18.6 | 12.4 | 11.3 | 11.5 | 12.3 | 0.6 |
| Magnesium | 1.1 | 1.1 | 4.4 | 2.9 | 2.6 | 2.7 | 0.1 |
| Sodium | 0.1 | 0.1 | 0.6 | 1.8 | 1.5 | 1.7 | 0.2 |
| Iron | 1.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.02 |
| Aluminum | 0.2 | 0.2 | - | 0.2 | 0.2 | 0.2 | 0.02 |
| Copper, mg/kg | 3.1 | 19.1 | 12.2 | 14.3 | 18.6 | 13.9 | 2.6 |
| Zinc, mg/kg | 5.8 | 12.2 | 86.7 | 105.9 | 78.9 | 87.9 | 13.8 |
| Manganese, mg/kg | 19.8 | 11.4 | - | 70.1 | 52.6 | 62.9 | 8.7 |
| Total phenols g of gallic acid equivalent/kg DM | 51.4 | 177.3 | | | 32.5 | 89.6 | 4.3 |
| Total tannins, g of gallic acid equivalent/kg DM | 19.1 | 104.2 | - | - | 12.7 | 50.7 | 0.47 |
| Proanthocyanidins, % leucocyanidin equivalent | 8.1 | 33.3 | - | - | 5.7 | 24.1 | 0.47 |
| Ascorbic acid, mg/ kg | 427.0 | 174.0 | - | - | 316.1 | 114.2 | 0.46 |
| Alpha tocopherol, mg/kg | 74 | 47 | - | 9.8 | 16.8 | 12.6 | 0.12 |
| Fatty acid composition (% of total fatty acids) | | | | | | | |
| 14:0 | 0.6 | 0.6 | - | 0.1 | 0.2 | 0.1 | 0.01 |
| 16:0 | 16.7 | 16.0 | - | 8.2 | 8.5 | 7.3 | 0.07 |
| 18:0 | 2.3 | 1.0 | - | 0.4 | 0.6 | 0.6 | 0.04 |
| 18:1 n-9 | 8.3 | 4.6 | - | 12.0 | 13.6 | 11.1 | 0.47 |
| 18:2 n-6 | 56.2 | 58.9 | - | 66.6 | 63.5 | 69.3 | 0.17 |
| 18:3 n-3 | 8.2 | 7.6 | - | 9.2 | 9.7 | 6.4 | 0.07 |

[1] SEM: Standard error of mean

[2] Pectin + sugars were calculated as NFC (g/kg DM) - starch (g/kg DM) according to López et al. (2014)

**Table 6.4** Effects of feeding DCP and DGP on intramuscular pro-oxidant fatty acids of Angus steers (ether extract g/100g meat, fatty acids expressed as % of total fatty acids)

| Pro-oxidant Fatty acid | Treatments | | | SEM[1] | P-value |
|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | | |
| LTL ether extract | 2.5 | 2.4 | 2.2 | 0.30 | 0.780 |
| 18:1 n-9 | 20.7[a] | 18.3[b] | 17.9[b] | 0.96 | 0.025 |
| 18:3 n-3 | 3.1[b] | 5.4[a] | 5.2[a] | 0.32 | 0.002 |
| 18:2 n-6 | 4.8[b] | 7.0[a] | 7.2[a] | 0.39 | 0.001 |
| 20:4 n-6 | 3.2[b] | 5.0[a] | 4.9[a] | 0.33 | 0.003 |
| ∑ n-6 PUFA | 8.8[b] | 11.4[a] | 11.1[a] | 0.77 | 0.042 |
| ∑ n-3 PUFA | 3.5[b] | 5.5[a] | 5.3[a] | 0.30 | 0.005 |
| ∑ PUFA | 13.3[a] | 16.7[b] | 16.1[b] | 0.77 | 0.019 |

[a-c] Least squares means with different superscripts in the same row are significantly different ($P \leq 0.05$).

**Table 6.5** Effects of feeding DCP and DGP diets on profiling of volatile compounds of *Longissimus thoracis et lumborum* beef from Angus steers

| Volatile compounds | RT[1] | MSLM[2], % | RI[3] | Treatments | | | SEM[4] | P value |
|---|---|---|---|---|---|---|---|---|
| | | | | Control diet | DCP diet | DGP diet | | |
| Alcohols | | | | | | | | |
| Isoamyl alcohol | 14.9 | 90 | 1100.6 | 1.9 | 3.4 | 3.6 | 0.33 | 0.066 |
| Isoamyl acetate | 11.8 | 83 | 1000.6 | 1.0 | 0.7 | 0.9 | 0.16 | 0.312 |
| 2-Butanol | 9.0 | 78 | 900.6 | 1.2 | 1.0 | 1.2 | 0.07 | 0.125 |
| 1-Butanol, 3-methyl | 14.9 | 90 | 1100.7 | 12.3 | 17.4 | 6.6 | 4.14 | 0.203 |
| 2-Propanol | 6.3 | 90 | 800.5 | 5.8[a] | 2.6[b] | 2.6[b] | 1.60 | 0.033 |
| Ethyl amyl carbinol | 20.3 | 90 | 1300.5 | 4.9[a] | 2.1[b] | 1.9[b] | 0.32 | 0.047 |
| 2-Heptanol | 18.2 | 83 | 1200.6 | 0.3 | 0.3 | 0.2 | 0.10 | 0.096 |
| 2-Decanol | 23.6 | 83 | 1400.6 | 0.9 | 0.4 | 0.3 | 0.04 | 0.362 |
| 2,3-Butanediol | 24.2 | 90 | 1400.7 | 0.8 | 1.5 | 1.2 | 0.07 | 0.137 |
| 1-Hexanol, 2-ethyl | 22.8 | 78 | 1084.8 | 1.3 | 1.6 | 2.1 | 0.07 | 0.492 |
| 2-Nonanol | 23.6 | 83 | 1400.6 | 2.1 | 2.3 | 1.9 | 0.26 | 0.069 |
| Ketones | | | | | | | | |
| 2-Butanone, 3-hydroxy | 17.3 | 90 | 1289 | 5.9[a] | 2.6[b] | 2.5[b] | 1.81 | 0.047 |
| 3-Octanone | 16.1 | 95 | 1200.3 | 5.4[a] | 2.8[b] | 2.3[b] | 0.88 | 0.037 |
| 2-Hexanone, 5-methyl | 20.1 | 83 | 1300.5 | 0.2 | 0.3 | 0.3 | 0.08 | 0.312 |
| 2-Heptanone | 13.8 | 91 | 1100.4 | 3.5[a] | 1.2[b] | 0.8[b] | 0.46 | 0.046 |
| 2-Nonanone | 20.1 | 94 | 1300.5 | 3.4[a] | 1.9[b] | 1.7[b] | 1.07 | 0.016 |
| 2-Butanone, 3-hydroxy-3-methyl | 16.2 | 83 | 1200.3 | 2.1 | 1.2 | 1.7 | 0.73 | 0.534 |
| Acetoin (3 hydroxy-2-butanone) | 17.2 | 90 | 1200.5 | 2.4 | 5.3 | 10.9 | 0.97 | 0.271 |
| Hydrocarbon compounds | | | | | | | | |
| Benzene, methyl | 9.1 | 91 | 900.6 | 1.9 | 0.8 | 1.1 | 0.09 | 0.332 |
| Dodecane, 1,1-difluoro | 23 | 78 | 1401.0 | 04 | 0.2 | 0.2 | 0.03 | 0.267 |
| Dichloromethane | 6.1 | 94 | 800.5 | 0.2 | 0.1 | 1.5 | 0.01 | 0.722 |
| Methane, bromochloro | 9.4 | 97 | 900.7 | 1.5 | 1.8 | 2.5 | 0.35 | 0.662 |
| Cyclohexane, methylene | 13.4 | 87 | 1100.3 | 0.2 | 0.7 | 0.4 | 0.01 | 0.193 |
| Cyclooctane | 12.1 | 87 | 901.1 | 5.7 | 7.3 | 8.2 | 1.10 | 0.851 |
| 1-Undecene | 12.3 | 97 | 1100.6 | 3.2 | 7.1 | 8.4 | 1.13 | 0.342 |
| Trans nonene-3 | 12.1 | 90 | 1000.6 | 21.6 | 21.2 | 20.1 | 3.46 | 0.182 |
| 1-Propene, 1-(1-methoxy-1-methylethoxy) | 8.6 | 78 | 900.5 | 4.9 | 3.2 | 3.7 | 0.16 | 0.882 |
| Cycloheptene | 13.5 | 93 | 1100.3 | 0.2 | 0.7 | 0.5 | 0.02 | 0.623 |

Continued

| Volatile compounds | RT[1] | MSLM[2],% | RI[3] | Treatments | | | SEM[4] | P value |
|---|---|---|---|---|---|---|---|---|
| | | | | Control diet | DCP diet | DGP diet | | |
| Tetradecane | 20.2 | 78 | 1400.5 | 0.9 | 0.4 | 0.2 | 0.08 | 0.532 |
| Bicyclo [4.1.0] heptane | 13.7 | 87 | 1100.4 | 0.8 | 0.4 | 0.3 | 0.11 | 0.130 |
| Cyclopropane, 1-heptyl-2-methyl | 12.1 | 96 | 1000.6 | ND[4] | 8.3 | 5.25 | 1.34 | 0.782 |
| Bicyclo [7.1.0] decane | 13.5 | 90 | 1100.3 | 0.8 | 0.4 | 0.5 | 0.03 | 0.503 |
| Heterocyclic compounds | | | | | | | | |
| 1,3-Nonadiene | 9.7 | 83 | 900.5 | 0.1 | 0.3 | 0.3 | 0.02 | 0.085 |
| Cyclopropane, 1-heptyl-2-methyl | 12.2 | 90 | 1000.6 | 3.2$^{b}$ | 10.7$^{a}$ | 11.2$^{a}$ | 1.81 | 0.032 |
| Organic acids | | | | | | | | |
| Acetic acid | 21.8 | 90 | 1452 | 1.8 | 4.4 | 4.3 | 0.93 | 0.433 |
| Aldehydes | | | | | | | | |
| Nonanal | 20.1 | 72 | 1405 | 1.2$^{a}$ | 0.5$^{b}$ | 0.6$^{b}$ | 0.01 | 0.031 |
| Benzaldehyde | 23.6 | 72 | 1520 | 8.3$^{a}$ | 4.2$^{b}$ | 3.9$^{b}$ | 0.01 | 0.034 |
| Hexanal | 28.1 | 91 | 1071 | 6.2$^{a}$ | 2.1$^{b}$ | 1.9$^{b}$ | 0.21 | 0.009 |
| 2 methyl propanal | 21.7 | 88 | 819 | 4.8$^{a}$ | 1.9$^{b}$ | 1.7$^{b}$ | 0.93 | 0.042 |
| Sulphur containing compounds | | | | | | | | |
| Dimethyl disulfide | 10.2 | 98 | 1060 | 2.1$^{b}$ | 4.4$^{a}$ | 3.9$^{a}$ | 0.31 | 0.041 |
| Dimethyl trisulfide | 19.7 | 96 | 1364 | 0.2 | 0.8 | 0.5 | 0.03 | 0.230 |
| 3,4-dihydrothienyl (3,4,b)-5-carboxythiophene | 8.6 | 83 | 900.2 | 2.3$^{b}$ | 8.3$^{a}$ | 9.1$^{a}$ | 1.20 | 0.044 |

[a-b] Least squares means with different superscripts in the same row are significantly different ($P \leq 0.05$).

[1]RT means retention time

[2]MSLM means mass spectra library match

[3]RI means retention index

[4]SEM means standard error mean

### 6.3.4 *Effects of feeding DCP and DGP diets on sensory attributes of beef*

The effects of DCP and DGP inclusion on sensory attributes of beef are presented in Table 6.6. All the aroma, flavor and texture profile attributes were similar across the diets ($P > 0.05$), except for tenderness. The control-fed beef was 10% more tender ($P \leq 0.05$) than DGP- and DCP-fed beef ($P \leq 0.05$). No dietary effects were observed on sustained juiciness, residue or mealiness ($P > 0.05$).

**Table 6.6** Effects of feeding DCP and DGP diets on sensory attributes of *longissimus thoracis et lumborum* beef from Angus steers

| Attributes | Treatments | | | SEM[1] | P value |
|---|---|---|---|---|---|
| | Control diet | DCP diet | DGP diet | | |
| Aroma[2] | | | | | |
| Overall intensity | 76.4 | 76.1 | 76.2 | 0.78 | 0.278 |
| Beef | 69.7 | 70.3 | 70.1 | 0.76 | 0.393 |
| Savory broth | 25.6 | 26.1 | 26.3 | 0.69 | 0.779 |
| Metallic | 20.3 | 20.8 | 21.1 | 0.86 | 0.314 |
| Sweet associated | 26.2 | 27.1 | 27.3 | 0.76 | 0.562 |
| Sour-associated | 8.4 | 8.6 | 9.6 | 0.48 | 0.162 |
| Fatty | 11.9 | 11.9 | 11.9 | 0.61 | 0.996 |
| Flavor[2] | | | | | |
| Beef-like | 69.9 | 71.1 | 71.3 | 0.74 | 0.467 |
| Savory-broth | 27.3 | 26.6 | 27.2 | 0.85 | 0.796 |
| Sour associated | 16.1 | 14.9 | 15.2 | 0.71 | 0.433 |
| Metallic | 25.2 | 24.5 | 26.7 | 0.92 | 0.235 |
| Salty | 11.4 | 10.8 | 11.5 | 0.37 | 0.383 |
| Sweet-associated | 24.8 | 25.3 | 25.4 | 0.63 | 0.753 |
| Fatty | 11.9 | 12.6 | 12.3 | 0.66 | 0.765 |
| Texture[2] | | | | | |
| Sustained juiciness | 56.7 | 55.7 | 59.4 | 1.26 | 0.295 |
| Residue | 24.9 | 24.5 | 25.3 | 1.61 | 0.597 |
| Mealiness | 6.4 | 6.1 | 6.7 | 0.86 | 0.886 |
| Tenderness | 63.1[a] | 56.8[b] | 57.3[b] | 1.22 | 0.001 |

[a-b] Least squares means with different superscripts in the same row are significantly different ($P \leq 0.05$).

[1] SEM means standard error mean

[2] Means determined by a 100 point unstructured line scale (0 = low intensity, 100 = high intensity)

### 6.3.5 *Relationships between LTL pro-oxidant fatty acid, volatile compounds and sensory quality of beef*

A PCA was applied to visually explore the relationships among pro-oxidant fatty acids, volatiles and sensory profiles of steers fed DCP or DGP finisher diets (Figure 6.1). The first and second principal components accounted for 59.5 and 20.2% of the total variation, respectively (Figure 6.1). The control diet was clustered together with 18:1n-9, ketones, aldehydes, alcohols, sour-associated flavor, overall intensity aroma and tenderness at the center of the right quadrant. The DGP-fed beef was closely clustered with 18:2n-6, sulphur compound (3.4 dihydrothienyl (3, 4, b)-5-carboxythiophene, sustained juiciness, flavor (sweet associated, beef and metallic) and aroma (metallic, sweet associated, fatty and savory broth) attributes in the upper left quadrant. The DCP was closely clustered with PUFA, 20:4n-6, 18:3n-3, sulphur compound (dimethyl disulfide), beef aroma, fatty aroma, cyclohexane, methylene and 2 nonanol in the lower left quadrant.

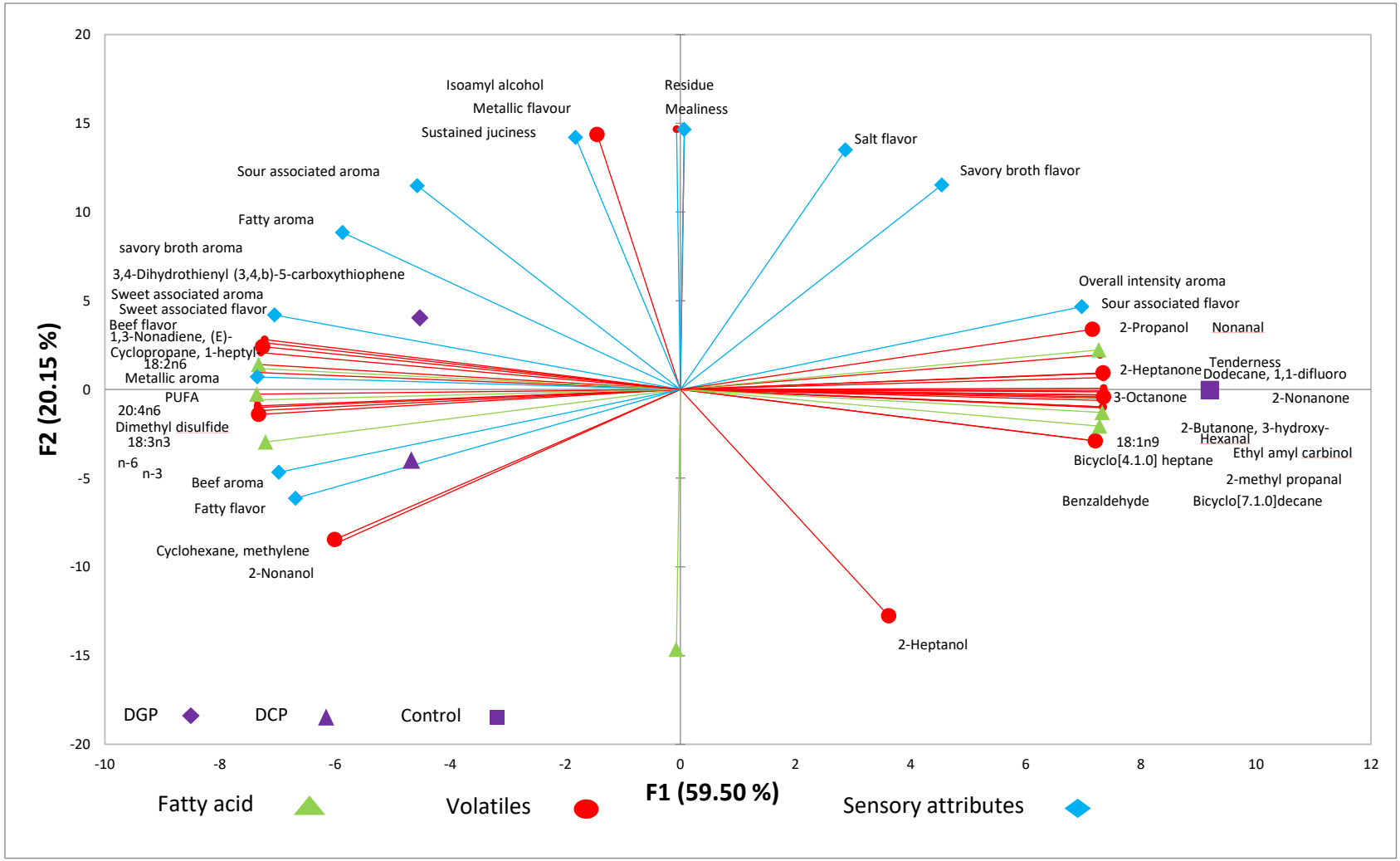


**Figure 6.1** Principal component analysis of variable plot of major pro-oxidant fatty acid, volatile and sensory profile of DCP-and DGP-fed beef

## 6.4 Discussion

To our knowledge, the present study was the first to compare the effects of feeding DCP and DGP on the major pro-oxidant fatty acids, volatile compounds and sensory quality profiles of beef from Angus steers. Increased total and individual PUFA in DCP- and DGP-fed beef could be attributed to high proportions of these fatty acids and contents of proanthocyanidins, in the diet. It has been postulated that polyphenols reduce ruminal lipolysis and protect PUFA from biohydrogenation in the rumen making them inaccessible to rumen microbes or their enzymes or interact by interfering with the microbial cell walls, for example, substrate deprivation and altering their membrane permeability systems, hence, slow or retard their growth and metabolism (Hajji et al., 2016; Luo et al., 2019; Mueller-Harvey et al., 2019). The higher proportions of 20:4 n-6 in DCP- and DGP-fed beef compared to control-fed beef could also be due to increase de novo fatty acid synthesis from 18:2 n-6 (Zhou & Nilsson, 2001). In addition, increased proanthocyanidins, α-tocopherol and ascorbic acid may have inhibited both the release of this 20:4 n-6 from phospholipids, and its utilization in the production of leuko-trienes (Jenkins & Atwal, 1995). The lower 18:1 n-9 observed for the DGP- and DCP-fed beef compared to the control-fed beef could be attributed to the absence of polyphenols known to inhibit biohydrogenation of monounsaturated fatty acids to saturated fatty acids (Vasta et al., 2019; Mueller-Harvey et al., 2019).

The lower alcohol concentration observed for the DCP- and DGP-fed beef could be attributed to the contents of proanthocyanidins, α tocopherol and ascorbic acid in grape pomace and citrus pulp, which may have reduced the rate of lipid and amino acid degradation, and consequently, their end product (i.e., aldehydes) (Ianni et al., 2019; Vasta et al., 2010; Chapter 5). This could also explain the observed decrease in the observed hexanal content in DCP- and DGP-fed beef, which concurred with the study of Descalzo & Sancho (2008). The greater concentrations of alcohols in control-fed beef volatiles compared to volatiles from DCP- and DGP-fed beef is in line

with the reported findings in the literature (Descalzo et al., 2007; Mezgebo et al., 2017; Morán, Giráldez, Panseri, et al., 2013). They have demonstrated that the formation of alcohols occurs through reduction of aldehydes, the main products lipid and amino acid oxidation, and their presence influences meat flavor. This effect is attributed to their relatively high threshold values, thus, exert additive effects on woody, oily, fatty and alcoholic flavor attributes (Elmore et al., 2005; Luo et al., 2019; Resconi et al., 2013). In accordance with other studies in beef (Vasta et al., 2007; Insausti et al., 2002; Saraiva et al., 2015), the alcohols identified in the present study are typical volatile markers of raw and unprocessed beef.

The lower levels of ketones and aldehydes observed in volatiles from DCP- and DGP-fed beef compared to control-fed beef could be attributed to a high lipid and carbonyl oxidative stability in the former diets due to high contents of proanthocyanidins, α-tocopherol and ascorbic acid (Chapter 5). The above-mentioned polyphenols have been reported to reduce the levels of ketones, aldehydes and alcohols from lipid oxidation by slowing down the oxidative processes affecting the lipid component (Ianni et al., 2019; Vasta et al., 2019). The greater concentration of hexanal and nonanal in beef volatiles from steers fed control diet compared to DCP- and DGP-fed beef could be due to instability of 18:1 n-9 in the former diet. Hexanal and nonanal are derived from oxidation of 18:1 n-9 and 18:2 n-6 (Elmore et al., 2005; Mezgebo et al., 2017). High concentration of hexanal has been reported to produce “green, paint like, herbal, rancid taste” and “fatty” aroma attributes (Elmore et al., 2005). Greater concentration of benzaldehyde in control fed-beef compared to DCP- and DGP-fed beef could have been derived from the degradation of 18:2 n-6 and 18:3 n-3 (Gravador et al., 2014; Vasta et al., 2011). In line with current findings, Mohamed et al. (2012) reported that degradation of 18:2n-6 can form benzaldehyde which is associated with development of grassy or fishy flavor attributes and are often not perceived as desirable beef flavors. The greater concentration of 2-methyl-propanal in control-fed beef compared to DCP- or

DGP- fed beef is likely to be associated with the strecker degradation of amino acid precursors (valine) rather than lipid oxidation (Bravo-Lamas, Barron, Farmer, & Aldai, 2018; Frank, Kaczmarska, Paterson, Piyasiri, & Warner, 2017).

The contribution of the aldehydes to beef volatiles was surprisingly small (4%), as they usually form a major component of the beef profiles, being both indicators of oxidation and important odor components (Andrés, Huerga, et al., 2014; Morán, Giráldez, Panseri, et al., 2013; Utama, Lee, Park, Jang, & Lee, 2018). However, the combined occurrence of a large alcohol component and a limited aldehyde concentration may suggest that the reducing mechanisms in beef steers were extremely active, converting aldehydes produced through lipid oxidation into alcohols before they could accumulate (Morán, Giráldez, Bodas, et al., 2013; Soncin, Chiesa, Cantoni, & Biondi, 2007). The control-fed beef volatiles, however, had greater aldehydes than DCP- and DGP-fed beef as visually supported by similar PCA associations. It has been reported that aldehydes are important intermediates in the formation of other flavor compounds like heterocyclic compounds associated with typical flavors (thiazoles, pyridine, thiophenes, oxazoles and other heterocyclics; Luo et al., 2019; Resconi, Escudero, & Campo, 2013). Generally, aldehydes are very important compounds that could give significant aromas either pleasant or rancid due to their low threshold values. On one hand, lower concentrations of aldehydes have generally been reported to give fishy and grassy, nutty and pungent scents (Mohamed, Man, Mustafa, & Manap, 2012; Mottram, 1998; Resconi et al., 2013). On the other hand, it has been reported that greater concentration of aldehydes could produce undesirable flavors (Legako et al., 2015). The greater concentrations of ketones detected in control-fed beef volatiles compared to DCP- and DGP-fed beef suggests a greater degree of microbial growth in these samples, as ketones are generally products of the microbial degradation of aspartate or catabolism of glucose (Luo et al., 2019; Resconi et al., 2013) and have been reported to increase with fat content since they are one of the product of lipid

degradation (Legako et al., 2015). Overall, observed ketones in this present study have been reported to generally give fruity, nutty, green, earthy, citrus, cheese and limonene aroma flavor attributes (Elmore et al., 2005).

Heterocyclic and sulphur compounds were significantly affected by diet and it may be possible that dietary DCP and DGP influenced the absorption and deposition of these compounds in the meat or their later release from the meat matrix during storage (Schreurs, Lane, Tavendale, Barry, & McNabb, 2008). The higher concentrations of 1,3-nonadiene, dimethyl disulfide and 3,4-dihydrothienyl (3,4,b)-5-carboxythiophene in DCP- and DGP-fed beef volatiles compared to control-fed beef could have directly come from the citrus pulp and grape pomace or as a product of the ruminal microbial fermentation of lignin (Resconi et al., 2018; Resconi et al., 2010; Mohamed et al., 2012). Furthermore, this could be a result of thermal degradation of thiamine which is reported to produce several heterocyclic and sulphur compounds like thiol, such as 1,3-nonadiene and disulfide (Khan et al., 2015). This is in line with the findings of Mohamed et al. (2012) who reported that sulphur and heterocyclic compounds normally originate or form during fermentation and can be considered important as odor constituents due to their low sensory threshold values. Overall, beef from all diets had low concentration of heterocyclic and sulphur compounds. This concurs with the findings of Vasta and Priolo (2006) who reported that heterocyclic and sulphur compounds occur in meat at very low concentrations. However, they are very potent contributors to meat flavor because of their low thresholds of sensory detection (Mottram, 1998; Resconi et al., 2013). Generally, sulphur compounds arise from sulphur-containing amino acids, cysteine and methionine, produced by proteolytic enzymes found in psychrotrophic bacteria (usually present in slaughterhouses).

The lack of differences among diets for hydrocarbons and organic acids is consistent with earlier findings for meat from ruminants fed diets containing polyphenolic compounds (Descalzo

et al., 2005; Rivas-Cañedo et al., 2013; Vasta et al., 2010). Although the threshold value of acetic acid is quite high, it might have a significant contribution to the aroma due to its abundance in DCP and DGP (Mohamed et al., 2012). Volatile compounds play a key role in meat's sensory attributes since they represent the major pathways of flavor development and therefore may potentially be used as markers for flavor development (Luo et al., 2019). Overall, the lack of intramuscular fat in this study could explain the lack of differences in the volatile compounds due to the diets. The findings that DGP- and DCP-fed beef was less tender than control-fed beef was consistent with instrumental tenderness values reported in Chapter 4, and maybe related to a lack of aging, enhanced membrane oxidative stability, and reduced post-mortem proteolytic activity (Kemp et al., 2010). Further research on extended aging or other intervention to improve tenderness in DCP and DGP fed cattle may, therefore, be in order.

## 6.5 Conclusions

The DCP- and DGP-fed beef had greater proportions of individual and total PUFA, and volatiles which were characterized by reduced concentrations of alcohols, ketones and aldehydes compared to control-fed beef. Feeding DCP and DGP diets produced less tender beef compared to control diet. Based on current findings, finishing steers with supplemented DCP and DGP could be a feasible strategy to modify pro-oxidant fatty acids and improve the volatile profile by decreasing the formation of volatiles associated with lipid and amino acid oxidation without compromising eating quality. Nonetheless, additional research to comprehensively analyse the fatty acid profile of LTL and address tenderness issues would be warranted.

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# Chapter 7 General discussion, conclusions and recommendations

## 7.1 General discussion

South African feedlot beef production is experiencing limited supplies of feed ingredients, resulting in higher prices of feed resources, and consequently, reduced profitability. Furthermore, beef production is associated with high post-harvest losses or wastage due to oxidative processes and microbial spoilage (WWF, 2017). Synthetic antioxidants are currently being used in feed and beef industries and are highly effective, but some may have negative effects on consumer health. It is, therefore, important to identify natural alternatives to synthetic antioxidants. In response to these challenges, low-cost alternative feed ingredients are being sought including natural ingredients which also improve beef oxidative stability and eating quality to meet consumer demands for nutritious, safe and heathier beef products (Brewer, 2011).

In South Africa, citrus and grapes are major fruit crops and generate large volumes of by-products including citrus pulp and grape pomace (DAFF, 2018; USDA/FAS, 2019). These fruit by-products are rich sources of fiber, PUFA and phytochemicals, which have nutritional, antioxidant and antimicrobial properties that enhance beef production, quality and shelf life. Despite citrus and grape fruit by-products having such potential, they remain underexploited by the feed and meat industries. The main objective of the current study was to compare beef production and biopreservative effects of dietary citrus and winery by-products. The hypothesis tested was that inclusion of dried grape pomace (DGP) or dried citrus pulp (DCP) in cattle finishing diets would improve beef production and quality of Angus steers compared to a control diet.

Chapter 3 hypothesized that feeding 150 g/kg of either DCP or DGP to Angus steers as fiber alternative sources to wheat bran would improve nutrient intake and digestibility, ruminal fermentation efficiency, microbial-N supply, and N retention. Steers fed on DGP had the greatest

intake of DM, OM, CP, aNDFom, EE and starch followed by DCP and control diets and this difference in nutrient intake could be due to observed differences in DM intake and /or chemical composition of the experimental diets. Lower DM, OM and aNDFom digestibility observed for the DGP diet compared to DCP and control diets could also attribute to its higher content of proanthocyanidins, ADL and EE (Abarghuei et al., 2010; Makkar, 2003; Palmquist, 1994; Pantoja et al., 1994). The elevated apparent digestibility of DM and OM observed for the DCP diet compared to control and DGP diets could be due to superior degradability of the neutral detergent soluble carbohydrates in DCP (Bampidis & Robinson, 2006). Low concentrations of ruminal $NH_3$-N observed for the steers fed the DCP and DGP diets compared to control diet could be linked to low rates of ruminal fermentation and slow dietary CP degradation due to presence of proanthocyanidins. This lower could be attributable to enhanced utilization related to increased microbial protein synthesis for DCP and DGP diet. The lower urinary excretion of allantoin, uric acid, total purine derivatives and microbial N supply in steers fed DGP relative to the DCP and control diets could be attributed to proanthocyanidins and lignin contents in former diet. Overall, feeding DGP as alternative fiber source to wheat bran improved nutrient intake, retention and efficiency of N utilization but reduced apparent nutrient digestibility compared to DCP. These findings show that DGP could be a better fiber substitute for wheat bran in beef finishing diets than DCP.

Chapter 4 tested whether feeding 150 g/kg DM of either DCP or DGP as alternate fiber sources to wheat bran improves growth performance, carcass attributes, beef quality and costs of production. The higher ADG and body weight observed for steers fed DGP and DCP diets relative to control diet may be attributable to the moderate amount of proanthocyanidins that were contained in the DGP and DCP diets. The steers fed on DGP had heavier carcass weights compared to DCP and the control diet, which could be related to greater DMI and the observed relatively

lower rumen fill and higher dressing percentages for the former diet. The high income over feed cost observed for the steers fed on the DGP compared to the DCP diet is mainly due to the observed differences in DMI, feed costs and total income. The observation that diet had no effect on physicochemical attributes is in accordance with literature. Findings suggest that DGP is a better fiber feed ingredient compared to DCP.

The hypothesis tested in chapter 5 was that feeding 150 g/kg of either DCP or DGP as natural preservative would extend the shelf life of beef from Angus steers. Overall, DGP-fed beef was observed to have the greatest antioxidant activity, and lower lipid and protein oxidation status in retail display followed by DCP- and control- fed beef, respectively. This was expected as beef with relatively high higher muscle α-tocopherol and total dietary phenolic compounds (i.e. total phenols and proanthocyanidins) tend to have better protective capacity against free radicals responsible for oxidative processes (Guerra-Rivas et al., 2016; Chikwanha et al., 2019; Inserra et al., 2014; Sales and Koukolova, 2011). Moreover, α-tocopherol cannot be synthesized by the animals and must be supplied by the diet, its presence in muscle tissues reflects dietary availability (Sales and Koukolova, 2011). The observed lower bacterial load in both DCP- and DGP-fed beef compared to control-fed beef could also be attributable to the high concentration of α-tocopherols in muscle tissues, and greater contents of total phenols and proanthocyanidins in these diets, which have been reported to induce antimicrobial effect on microbial cell membranes and/or cells walls. Moreover, the antimicrobial mechanisms of α tocopherols and phenolics in DGP and DCP may manifest through attacking the phospholipid bilayer of bacterial cell membranes, disrupting enzyme systems and compromising the genetic material (Wu, Zang, He, Pan, & Xu, 2013). Feeding DGP improved the shelf life of beef during retail display through reduction of coliforms and lipid and protein oxidation compared to DCP and control diets. Based on current findings, DGP could be a better dietary natural preservative compared to DCP.

In chapter 6, it was hypothesized that feeding 150 g/kg of either DGP or DCP could lead to increased proportions of major pro-oxidant fatty acids and volatile compounds without adversely compromising eating quality of beef from Angus steers. The DCP- and DGP-fed beef had greater proportions of individual and total PUFA compared to control-fed beef and this could be attributable to high proportions of these fatty acids and contents of proanthocyanidins, in the diet. Beef volatiles from steers fed on the control diet had higher concentrations of ketones and aldehydes, and lower sulphur compounds compared to DCP- and DGP-fed beef. This could also be attributed to the content of α-tocopherol, polyphenols and different types or proportions of fatty acids in diets which have been reported to influence the generation of volatile compounds by either reducing or sometimes inhibiting thermal lipid degradation upon thermal processing (Kosowska et al., 2017; Mottram, 1998; Resconi et al., 2013). Based on current findings, finishing steers with supplemented DCP and DGP could be a feasible strategy to improve fatty acid profile and decrease the formation of volatiles associated with oxidation without compromising eating quality, but further studies will be required to deal with LTL fatty acid profile, tenderness and further assess if slight changes in sensory properties would be detected at the consumer level or their willingness to purchase.

Compared to feeding DCP, DGP improved nutrient intake, retention and efficiency of N utilization, growth performance, carcass attributes, beef shelf life, profile of fatty acids and reduced concentration of aldehydes, ketones and alcohols associated with oxidation without compromising beef physicochemical. In that regard, it can be concluded that DGP may be a better fiber substitute and natural preservative in beef finishing diets than DCP.

## 7.2 Conclusions

Overall, feeding DGP as alternative fiber source to wheat bran improved nutrient intake, retention and efficiency of N utilization but reduced apparent nutrient digestibility compared to DCP. Furthermore, Angus steers fed on the DGP diet had superior growth performance, carcass attributes and income over feed cost compared to those fed on the DCP and control diets without compromising their meat quality attributes except for instrumental and panel tenderness. Feeding DGP also enhanced the oxidative and microbial shelf life of beef during retail display compared to DCP and control diets. The DCP- and DGP-fed beef had a lower concentration of volatile compounds associated with oxidation compared to control-fed beef. Based on the current findings, it can be concluded that DGP compared to the DCP represent an economic advantage for beef producers as a better fiber substitute and dietary natural preservative for wheat bran in beef cattle finisher diets, and extended studies to further investigate/solve issues with fatty acid profile of LTL, tenderness and palatability are warranted.

## 7.3 Recommendations

Based on current findings, it is recommended that DGP can be utilized as alternative fiber source to wheat bran in cattle finishing diets. This is because feeding DGP produces superior beef yield and quality compared to DCP and wheat bran. The meat industry is recommended to prioritize beef from steers fed on DGP compared to DCP and wheat bran as it is having long shelf life. Moreover, consumers are likely to pay premiums for such beef as they perceive it to be safe and healthy.

Fresh winery by-products are bulky, seasonal and highly perishable due to their high moisture content which subsequently result in high transport costs. This can be minimized by drying and pelleting, which reduces transport and storage costs. Small-scale wineries are recommended to sun-dry grape pomace to increase its shelf-life, reduce storage volume and decreases transport

costs. The dried pomace should then be sold to the feedlot industry to recover costs and generate extra income. Sun-drying may, however, not be feasible for large-scale wineries as it is a slow, weather-dependent, and subject to physiological and hygienic related problems. In that regard, fruit processing industry is urged to consider investing into drying equipment that can process bulky fresh fruit by-products over a short period of time. Collaboration of the fruit processing, feed and meat industries to establish feedlots and niche markets could optimize fruit by-products as feed ingredient and natural preservatives in cattle finishing diets.

## 7.4 Further research

Based on the discussed points in this thesis, following topics can be of interest for future research:

1. During the storage of DGP, antioxidants might be oxidized therefore further research is recommended to determine its shelf life.
2. The present study substituted wheat bran with several other ingredients in addition to DGP and DCP to make the diets isoenergetic and isonitrogenous as it is the standard practice for most commercial feed companies. However, this may have confounded effects of DGP particularly in terms of DMI. Future studies should do one-to-one substitution of test ingredients to ascertain the effects of DGP on beef production and quality.
3. Further research is necessary to determine the optimum inclusion level of DGP in cattle finishing diets which can be used to achieve sustainable returns in terms of income over feed costs.
4. A comprehensive study should focus on the assessment of bioavailability and bioefficiency of polyphenolic compounds in DGP as this will enable researchers to understand the fate of these polyphenols within the animal and meat matrices.

5. Future studies should focus on investigation and elucidation of the effects of feeding DGP on proteomics of meat. This will give some insights on the underlying molecular mechanisms behind meat quality attributes (i.e., tenderness, color, and water-holding capacity).
6. A comprehensive fatty acid analysis of beef fed DGP diets should be done using a 100m high-polarity capillary column to measure the long chain n-3 fatty acids and PUFA-biohydrogenation products which are known to effect human health, and could provide value added potential in the future through improved food safety and security. This evaluation was not conducted in chapter 6 of the present study owing to lack of resources that required for detailed ruminant fatty acid analysis.
7. Further research should focus on extended aging or other intervention to improve tenderness in DGP fed cattle and assess if changes in sensory properties would be detected at the consumer level or their willingness to purchase.

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