



ISSN: 0028-8233



The intrauterine effects of a maternal winter diet of either kale or fodder beet on measures of calf stature and bone morphology at birth

Michaela J. Gibson, Chris W. Rogers, Penny J. Back, Keren E. Dittmer, Alvaro Wehrle-Martinez, Dawn E. Dalley & Roshean R. Woods

To cite this article: Michaela J. Gibson, Chris W. Rogers, Penny J. Back, Keren E. Dittmer, Alvaro Wehrle-Martinez, Dawn E. Dalley & Roshean R. Woods (09 Oct 2024): The intrauterine effects of a maternal winter diet of either kale or fodder beet on measures of calf stature and bone morphology at birth, New Zealand Journal of Agricultural Research, DOI: [10.1080/00288233.2024.2409749](https://doi.org/10.1080/00288233.2024.2409749)

To link to this article: <https://doi.org/10.1080/00288233.2024.2409749>



© 2024 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 09 Oct 2024.



Submit your article to this journal [↗](#)



Article views: 64



View related articles [↗](#)



View Crossmark data [↗](#)

The intrauterine effects of a maternal winter diet of either kale or fodder beet on measures of calf stature and bone morphology at birth

Michaela J. Gibson ^a, Chris W. Rogers^{a,b}, Penny J. Back^{a,b}, Keren E. Dittmer^a, Alvaro Wehrle-Martinez^a, Dawn E. Dalley ^c and Roshean R. Woods^c

^aSchool of Veterinary Sciences, Massey University, Palmerston North, New Zealand; ^bSchool of Agriculture and Environment, Massey University, Palmerston North, New Zealand; ^cDairyNZ Ltd., Lincoln University, Lincoln, New Zealand

ABSTRACT

Fodder beet and kale are commonly used winter crops in New Zealand dairy and dairy support farm systems. Fodder beet (FB) is known to have lower crude protein, phosphorus and calcium content compared to kale. The intrauterine effects of feeding FB during late pregnancy on bone growth and development in calves requires attention. This study aimed to examine the intrauterine effects of a maternal diet of either kale or FB on measures of calf stature and bone morphology at birth. In winters 2019 and 2020, two herds of pregnant Friesian × Jersey cows were fed either kale (2019, 2020 $n = 20$) or FB (2019, 2020 $n = 20$) supplemented with pasture baleage. Calves born from these mobs were euthanised within ten days of birth and a forelimb and section of rib including the costochondral junction were harvested for peripheral quantitative computed tomography and histological analysis. Calves from the FB treatment had reduced bone density and strength compared to calves from the kale treatment ($p < .05$). The effect of a nutritional deficit on bone morphology at birth emphasises the importance of sufficient nutrition in pre-calving diets. Future research is required to examine the long-term impact of dam undernutrition on calf growth and production.

ARTICLE HISTORY

Received 12 July 2024
Accepted 23 September 2024

HANDLING EDITOR


Rene Corner-Thomas

KEYWORDS

Beta vulgaris L; *Brassica oleracea* L; intrauterine nutrition; dairy cattle; wintering; bone strength; bone density; bone histomorphometry; bone histology

Introduction

New Zealand dairy farms utilise pasture-based feed systems with the use of crops to either supplement pasture during lactation or as the main diet component during the non-lactation ('dry') period to meet feed demand when pasture growth is low. The majority of dairy herds in New Zealand use a spring calving system (85%) with few farms using an autumn (3%) or split calving system (autumn and spring, 12%) (Edwards et al. 2021). Therefore, drying-off for spring calving cows typically occurs prior to winter when pasture growth is low.

CONTACT Michaela J. Gibson  m.gibson@massey.ac.nz

© 2024 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

During the non-lactating winter period, cows are offered different combinations of pasture, crop and conserved forages e.g. silage, baleage and straw. The relative quantity of crop and conserved forage as a proportion of total feed offered differs geographically due to variation in climate. In the southern regions of New Zealand, crops can contribute a large proportion of the winter-feed offered due to lower seasonal pasture growth and quality associated with colder temperatures. Two common winter crops are kale (*Brassica oleracea* L.) and fodder beet (*Beta vulgaris* L.) (Edwards et al. 2017). Kale is a common winter crop due to its higher yield of metabolisable energy and crude protein compared to ryegrass (Rugoho et al. 2014).

Fodder beet (FB) has become an increasingly popular winter feed option in the past decade (DairyNZ Economics Group 2019). The popularity of FB is associated with its high metabolisable energy, consistent quality, high yield and lower cost per kg of dry matter produced. In addition, FB can be fed in situ or lifted from the ground to be fed in another paddock or on a feed pad (Waghorn et al. 2018a). Although the high metabolisable energy in FB makes it a valuable tool to put condition on cows, if fed alone, it is known to be insufficient in dietary protein, phosphorus and calcium (Jonker et al. 2022). As a result industry recommendations suggest that FB should be fed in conjunction with pasture or other supplements to ensure adequate nutrition is provided and reduce the risk of metabolic and health issues such as ruminal acidosis and hypocalcaemia in dairy cattle (Waghorn et al. 2018b; Jonker et al. 2022). Additionally, FB has also been suggested to have an intrauterine effect on lamb bone development, with offspring of sheep wintered on FB being born with limb deformities and shortened limbs due to insufficient phosphorus and protein (Dittmer et al. 2017; Hammond et al. 2022). In a case study of lambs born to ewes grazed on FB over winter, 6% were born with a skeletal deformities consistent with nutritional congenital rickets likely due to insufficient phosphorous (Dittmer et al. 2017). Given this data, it is possible that intrauterine and preweaning nutritional deficits may have a direct effect on bone growth and development. The timing of the nutritional deficit alters how a bone responds, depending on its location and stage of maturity. For example, distal bones in the cow, such as the metacarpus, have limited growth after birth and are mature by one year of age (Guilbert and Gregory 1952), whereas proximal bones such as the humerus are later maturing (Gibson et al. 2022). The majority of intrauterine growth in cows, particularly of the musculoskeletal system, occurs in the third trimester (Mao et al. 2008). Thus, if the majority of the diet during the third trimester consists of winter crops low in protein and minerals that are required for bone growth, such as FB (Woods et al. 2023), the risk of intrauterine growth retardation may be increased.

Estimations on when longitudinal bone growth was restricted can be determined by evaluating sections of the bone. Long bones are divided into three main regions: the epiphysis, metaphysis and diaphysis. Longitudinal growth occurs in the metaphysis making it a dynamic site that is influenced by recent changes in growth. As the bone grows, the affected bone is pushed towards the diaphysis (middle of the bone) (Craig et al. 2016). Recent growth restrictions would be observed in the metaphysis whilst older growth restrictions would be observed in the diaphysis. Deficits in longitudinal bone growth are typically observed as a shorter bone length and stature (Hammond et al. 2022). Thus, if calves were exposed to insufficient nutrition in utero, they may be born smaller in size. Previous research has looked into how wintering cows on FB in late pregnancy impacts calf birth weight (Bryant and Pirat 2014; Moonsan et al. 2018), however,

there is limited information on how winter nutrition in dairy cattle alters intrauterine calf bone growth and development.

The aim of this study was to examine the intrauterine effects of a maternal diet of either kale or FB supplemented with pasture baleage on measures of calf stature and bone morphology at birth.

Materials and methods

Diet

All procedures had prior approval of the Ruakura Animal Ethics Committee, Hamilton, New Zealand (RAEC #14453).

The following outlines two trials that were run at the Southern Dairy Hub (Southland, New Zealand, 46°18 037.8''S, 168°18 046.1''E, 11 m asl) in 2019 and 2020 examining the effect of maternal winter diet on calf stature and bone development. Calves were born from dams offered a winter diet of either kale (kale treatment) or FB (FB treatment) with both groups also being offered pasture baleage. Samples collected in this experiment were opportunistically collected from a larger ongoing farm systems trial looking at the effect of feeding kale and FB pre-calving on cow production thus restricting the ability for variables in the 2019 calves to be completely replicated in the 2020 calves. Therefore, the 2019 born calves and 2020 born calves were treated and analysed as two separate cohorts.

Dams were transitioned onto their respective FB and kale crops at the beginning of their third trimester following industry good-practice management (Gibbs and Saldias 2014; Woods et al. 2023). The allocation and nutritional information for the kale and FB winter diets are presented in Table 1. Dams offered FB also received 50 g Dicalcium Phosphate (DCP, $\geq 18\%$ P, $\geq 21\%$ Ca, $\leq 0.18\%$ F, Farm Source) per cow per day dusted onto baleage and 70 g/cow/day of a loose lick (Magphos lick, 11% P, 20% Mg, 6.4% Na, Livestock Supplies). Utilisation of these has been assumed at 50% for DCP and 95% for loose lick. Cows were drafted off crops under standard farm practice of two weeks prior to their due date or due to changes in udder.

2019 born calves

At approximately 5–8 days old (median = 6 days, IQR = 5–7 days), 40 Friesian-Jersey cross bull calves from mixed age Friesian-Jersey cross dams (20 from dams offered kale with baleage, 20 from dams offered FB with baleage) from the Southern Dairy Hub were euthanized using captive bolt. Left and right forelimbs, costochondral junction and a section of ear (including the ear tag) were collected for labelling purposes. These were sent to Massey University, Palmerston North where the legs were disarticulated, and the humerus and metacarpus of each animal stored in separate labelled bags in a freezer until peripheral quantitative computed tomography (pQCT) scanning could occur. Bone slabs of the costochondral junction and humerus were placed in 10% neutral buffered formalin until processing for histology and histomorphometry.

2020 born calves

The 2020 cohort consisted of Friesian-Jersey cross heifer calves born from Friesian-Jersey cross heifer dams (10 from dams offered kale with baleage, 10 from dams offered FB with

Table 1. Kale and fodder beet dam diets in winter 2019 and 2020, data from the crop and baleage components of the diet.

Year	2019			2020	
	Dam diet	Recommended	Kale	Fodder beet	Kale
Time fully transitioned onto crops before calving (days)	–	90.8 ± 1.7	83.6 ± 1.7	70.6 ± 2.3	66.7 ± 1.8
Time on crop (days)	–	80.7 ± 1.0	75.0 ± 1.9	54.2 ± 1.4	52.2 ± 1.7
Time off crop before calving (days)	–	10.2 ± 1.0	8.3 ± 1.9	16.3 ± 1.3	14.9 ± 1.6
Cultivar	–	Regal	Jamon	Firefly	Feldherr
Crop allocation (kg DM/cow/day)	–	11.1	10.6	11.3	9.3
Estimated crop utilisation (%)	–	70	95	70	95
Baleage allocation (kg DM/cow/day)	–	3.5	3.4	3.2	3.4
Baleage utilisation (% assumed)	–	80	80	80	80
Total diet composition					
Metabolisable energy (MJ ME/cow) ^a	94.0 ^b	116	154	124	133
Crude protein (% DM)	12.0 ^c	14.6	11.6	16.4	11.2
Neutral detergent fibre (% DM)	35.0 ^c	35.0	21.5*	30.0*	25.3*
Soluble sugars and starch (% DM)	38.0	20.0	47.3*	20.0	40.5
	(maximum) ^c				
Phosphorus (% DM)	0.3–0.35 ^c	0.28	0.20 (0.29)*	0.31	0.25 (0.35)*
Potassium (% DM)	1.0 (maximum) ^c	2.01	1.70	2.56	2.29
Sulphur (% DM)	0.23 ^c	0.57	0.14*	0.59	0.14*
Calcium (% DM)	0.6–0.8 ^c	1.00	0.36 (0.41)*	1.19	0.42 (0.47)*
Magnesium (% DM)	0.22–0.28 ^c	0.18*	0.21 (0.32)*	0.18*	0.24 (0.36)*
Chloride (% DM)	0.25 ^c	1.55	1.03	1.33	1.12
Sodium (% DM)	0.2 ^c	0.76	0.63 (0.67)	0.33	0.66 (0.69)

Note: Total diet composition with and without mineral supplements included (50 g dicalcium phosphate/cow/day and 70 g loose lick/cow/day offered to dams on fodder beet diets). Numbers in brackets indicate diet composition value including the addition of mineral supplement.

^aCalculated as ME = DOMD × 0.14 + Crude Fat × 0.25 for fodder beet and ME = DOMD × 0.16 for kale; *Indicates lower than industry recommendations according to ^b (Nicol and Brooks 2007), ^c DairyNZ (2021)

baleage) and bull calves born from mixed age dams (10 from dams offered kale with baleage, 10 from dams offered FB with baleage). Calves were euthanised using captive bolt at approximately 3–10 days of age (median = 8 days, IQR = 6–9 days). Sample collection was performed using the same protocol outlined for the 2019 born calves.

Stature

Linear measurements (to the nearest cm) and liveweight (to the nearest kg) were taken within two days of birth. Linear measures of wither height, girth, wither-rump length (wither to tuber ischii) and leg length (from ground to point of olecranon) were obtained at the time of weighing. Humeral and metacarpal length were measured immediately prior to peripheral quantitative computed tomography (pQCT) analysis.

Peripheral quantitative computed tomography (pQCT)

Peripheral quantitative computed tomography scanning was carried out using the protocol previously reported by Gibson et al. (2021). Briefly, pQCT scanning was carried out using an XCT 2000 peripheral quantitative computed tomography machine (Stratec Medical, Pforzheim, Germany). For each bone, a 2 mm slice at the mid-diaphysis was obtained with a voxel size of 0.3 mm. In the metacarpus, bone length was defined as the distance between the lateral aspect of the lateral condyle of the fourth metacarpal and the proximal aspect of the lateral fourth metacarpal. The metaphysis and mid-diaphysis were defined as

25% and 50% of the total bone length starting at the lateral aspect of the lateral condyle of the fourth metacarpal. In the humerus, total bone length was defined as the distance between the proximal end of the humeral head at the lateral aspect, to the end of the trochlea at the distal end. The metaphysis and mid-diaphysis were defined as 25% and 50% of the total bone length, starting from the proximal end of the trochlea. Within the manufacturer's software, voxels $> 710 \text{ mg/cm}^3$ calcium hydroxyapatite (CaHA) were assigned as 'cortical' bone. Data derived from the scan included measures of total bone content, periosteal circumference, endosteal circumference and stress – strain index (SSI). The SSI is a derived measure of bone strength using pQCT data and avoids the requirement for destructive testing using techniques such as the three-point bending test. The SSI describes the ability of bone to withstand bending from lateral, dorso-palmar and torsional forces and is calculated by incorporating the index of material stiffness (bone mineral density) and bone geometry (cross-sectional moment of inertia) (Ferretti 1995). The pQCT variables will be referred to as bone parameters in the statistical models.

Bone histology

A rib section was taken from each calf for a total of 80 rib sections (40 from 2019 and 40 from 2020). Forty sections from calves with dams offered kale with baleage and 40 sections from calves with dams offered FB with baleage. Sections of ribs (costochondral junction) were cut using a band saw and fixed in 10% neutral buffered formalin solution. After 24 h ribs were decalcified in 10% hydrochloric acid (Decalcifier hydrochloric acid, Amber Scientific Ltd, Midvale, Australia), then trimmed to fit a histology cassette, dehydrated in graded alcohol and embedded in paraffin wax. Sections ($4 \mu\text{m}$) were cut using a microtome and stained with Haematoxylin and Eosin (H&E). After staining, bone sections were examined using a light microscope. Measures and photographs were obtained using Olympus cellSense imaging software (version 4.2). In each section the distance (length in micrometres (μm)) from the zone of hypertrophic chondrocytes to the deposition of bone matrix (zone of ossification) were measured at three different sites in the metaphysis and then the mean determined.

Bone histomorphometry

A section of the humeral metaphysis was taken from 20 calves born in 2019 cohort (10 from calves with dams on kale and 10 from calves with dams on FB) using a band saw and fixed in 10% neutral formalin solution for 24 h. Undecalcified humeral bone sections were then dehydrated in graded alcohol and placed in resin infiltration solution I (90% methyl methacrylate [MMA] and 10% dibutyl phthalate [BPO], Sigma-Aldrich, St. Louis, Missouri, United States) for 24 h, followed by resin solution II (90% MMA/10% BPO, Sigma-Aldrich) for 24 h. Samples were then placed in a resin embedding solution (95% MMA/5% BPO) for 24 h. Benzoyl peroxide (Luperox[®] A75, Sigma-Aldrich, St. Louis, Missouri, United States) was used as the polymerisation agent. Samples were cut at $6 \mu\text{m}$ using a super-microtome (Mikrotom 2050 supercut, Reichert Jung, Germany) and stained with Goldner's modified trichrome stain and Macneal's tetrachrome with Von Kossa. For sections stained with Goldner's trichrome stain an image of the entire section was taken at a magnification of $12.5\times$ to analyse bone area in

μm^2 and bone perimeter in μm . Also, at 400 \times magnification an image was taken each time osteoid was identified to measure mean osteoid area in μm^2 and mean osteoid perimeter in μm . This was performed on the entire section. For bone area standard BoneJ plugins (Domander et al. 2021), for ImageJ (version 1.53c, National Institute of Health, Bethesda, Maryland, USA) was used. Evaluation of bone perimeter, mean osteoid area and osteoid perimeter was measured using ImageJ software (version 1.53c, National Institute of Health, Bethesda, Maryland, USA). Finally, for sections stained with Macneal's, three random images at 200 \times magnification were taken. On each image resorption cavities were identified and counted manually. Results were compared between calves with dams on kale and calves with dams on FB.

Statistical analysis

Statistical analysis was conducted using the Statistical Analysis System software version 9.4 (SAS Institute Inc, Carey, NC, USA) with a level of significance set at $p < .05$. Due to the opportunistic nature of this research, variation in dietary factors, age of cow and calf sex between years could not be kept the same between the two years. Therefore, the calf cohorts (2019 vs 2020) were examined independently. All analyses were carried out using a general linear model with the model for calf birth weight, wither height, crown rump length and girth having the fixed effect of dam diet and sex (2020 cohort only). Models looking at the effect of diet on calf bone parameters included the covariate of birth weight and the fixed effect of sex (2020 cohort only) and diet. Birth weight was included as a covariate in the models to account for variation in calf size.

Results

Peripheral quantitative computed tomography

In 2019, calves in the kale treatment had a greater wither height, crown-rump length, metacarpus length and humerus length than calves in the FB treatment ($p < .05$, Table 2). In the 2020 born cohort, calves from the kale treatment had a greater girth size than calves from the FB treatment ($p = .019$).

In both 2019 and 2020 calves born to dams wintered on kale had a greater cortical/subcortical bone area, content and density as well as SSI in the metaphysis of the metacarpus compared with calves from dams wintered on FB ($p < .05$, Table 3). In the mid-diaphysis of the metacarpus, there was no effect of treatment on bone parameters from calves born in 2019. In the 2020 cohort, calves in the kale treatment had greater total bone content and cortical/subcortical density and content than calves in the FB treatment.

Humeral bone length was greater in 2019 calves in the kale treatment than calves in the FB treatment ($p < .05$, Table 4). In 2019, there was a significant effect of dam diet on the metaphysis in the humerus such that calves in the kale treatment had greater cortical/subcortical bone area, content and density and SSI than calves in the FB treatment ($p < .05$, Table 4). Total bone content in the metaphysis and mid-diaphysis was greater in the kale treatment calves born in 2020 than FB treatment calves. In the mid-diaphysis, cortical/subcortical density and content was greater for 2020 born calves in the kale treatment compared to calves in the FB treatment.

Table 2. Measures of calves from dams offered a winter diet of baleage supplemented with either fodder beet or kale born in either 2019 or 2020.

	2019 born calves				2020 born calves			
	n	Kale	Fodder beet	p-value	Kale	Fodder beet	Diet	p-value
		20	20		20	20		
Birth weight (kg)	32.5 ± 0.8	31.5 ± 0.8	.397	30.0 ± 1.1	29.0 ± 1.1	.424	<.001	
Wither height (cm)	70.6 ± 0.6	67.9 ± 0.6	.002	68.0 ± 1.0	68.4 ± 1.0	.876	<.001	
Crown-rump length (cm)	57.8 ± 0.6	56.0 ± 0.6	.043	58.0 ± 0.9	57.5 ± 0.9	.667	<.001	
Girth (cm)	76.7 ± 0.9	75.5 ± 0.9	.308	75.2 ± 1.0	72.4 ± 1.0	.019	<.001	
Metacarpus length (mm)	157.5 ± 1.1	152.3 ± 1.1	.002	153.8 ± 1.4	153.3 ± 1.4	.785	.007	
Humerus length (mm)	150.2 ± 1.2	145.5 ± 1.2	.008	147.2 ± 1.6	144.8 ± 1.6	.225	<.001	

Table 3. Least squared means \pm standard error of bone parameters in the metaphysis and mid-diaphysis of the metacarpus adjusted for birth weight and calf sex (2020 only) between calves from dams offered baleage supplemented with either fodder beet or kale born in 2019 and 2020.

Bone parameter	2019 born calves				2020 born calves				
	Kale	Fodder beet	Treatment	p-value	Kale	Fodder beet	Treatment	p-value	
	Birth weight	Birth weight	Birth weight	Birth weight	Birth weight	Birth weight	Birth weight	Birth weight	
Bone length (mm)	157.1 \pm 0.9	152.6 \pm 0.9	.002	<.001	153.4 \pm 1.1	153.7 \pm 1.1	.830	<.001	.954
<i>Metaphysis</i>									
Periosteal circumference (mm)	81.9 \pm 0.1	83.4 \pm 1.0	.262	<.001	81.0 \pm 0.9	81.5 \pm 0.9	.679	<.001	<.001
Total bone area (mm ²)	535.3 \pm 12.8	556.6 \pm 12.8	.247	<.001	527.0 \pm 11.7	533.5 \pm 11.7	.696	<.001	<.001
Total bone content (mg/mm)	222.4 \pm 6.4	215.7 \pm 6.4	.464	<.001	218.0 \pm 5.1	206.2 \pm 5.1	.115	<.001	<.001
Cort/subcort bone area (mm ²)	113.0 \pm 3.2	98.2 \pm 3.2	.002	<.001	105.3 \pm 3.9	89.9 \pm 3.9	.009	.361	.107
Cort/subcort bone content (mg/mm)	88.0 \pm 3.3	73.2 \pm 3.3	.003	<.001	78.3 \pm 3.7	64.4 \pm 3.7	.011	.550	.398
Cort/subcort bone density (mg/mm ³)	773.8 \pm 10.6	736.5 \pm 10.6	.019	.024	740.4 \pm 9.6	710.3 \pm 9.6	.034	.588	.179
Stress strain index (mm ³)	524.7 \pm 23.9	437.4 \pm 23.9	.014	<.001	404.4 \pm 20.2	330.3 \pm 20.2	.014	.095	.879
<i>Mid-diaphysis</i>									
Periosteal circumference (mm)	60.3 \pm 0.6	60.2 \pm 0.6	.842	<.001	57.1 \pm 0.6	56.6 \pm 0.6	.511	<.001	.001
Total bone area (mm ²)	290.3 \pm 5.5	289.1 \pm 5.5	.874	<.001	260.9 \pm 5.3	256.7 \pm 5.3	.574	<.001	.001
Total bone content (mg/mm)	194.9 \pm 4.7	185.8 \pm 4.7	.186	<.001	177.1 \pm 4.0	164.9 \pm 4.0	.040	.016	.002
Cort/subcort bone area (mm ²)	175.7 \pm 4.0	168.4 \pm 4.0	.205	.001	157.4 \pm 3.8	148.3 \pm 3.8	.099	.018	.001
Cort/subcort bone content (mg/mm)	178.0 \pm 4.3	169.2 \pm 4.3	.156	.001	161.8 \pm 4.0	150.2 \pm 4.0	.047	.019	.008
Cort/subcort bone density (mg/mm ³)	1012.8 \pm 6.8	1004.2 \pm 6.8	.382	.243	1028.6 \pm 4.5	1014.7 \pm 4.5	.039	.750	<.001
Stress strain index (mm ³)	802.2 \pm 22.8	766.9 \pm 22.8	.285	<.001	682.2 \pm 20.0	647.8 \pm 20.0	.234	<.001	.009

Table 4. Least squared means ± standard error of bone parameters in the metaphysis and mid-diaphysis of the humerus adjusted for birth weight and calf sex (2020 only) between calves from dams offered baleage supplemented with either fodder beet or kale born in 2019 and 2020.

Bone parameter	2019 born calves				2020 born calves				
	Kale	Fodder beet	p-value		Kale	Fodder beet	p-value		
			Treatment	Birth weight			Treatment	Birth weight	
Bone length (mm)	149.9 ± 1.1	145.7 ± 1.1	.013	.015	146.8 ± 1.2	145.2 ± 1.2	.361	.002	.236
<i>Metaphysis</i>									
Periosteal circumference (mm)	113.5 ± 1.4	115.4 ± 1.4	.347	<.001	108.9 ± 1.6	108.0 ± 1.6	.693	<.001	<.001
Total bone area (mm ²)	1028.8 ± 25.8	1065.9 ± 25.8	.317	<.001	957.2 ± 29.4	941.4 ± 29.4	.707	.001	<.001
Total bone content (mg/mm)	390.9 ± 14.9	362.8 ± 14.9	.183	.001	355.7 ± 8.8	320.0 ± 8.8	.007	<.001	<.001
Cort/subcort bone area (mm ²)	149.8 ± 4.9	132.2 ± 4.9	.016	.002	140.0 ± 4.3	129.0 ± 4.3	.080	.098	.152
Cort/subcort bone content (mg/mm)	115.2 ± 4.1	96.3 ± 4.1	.002	.028	103.6 ± 4.2	95.7 ± 4.2	.188	.280	.380
Cort/subcort bone density (mg/mm ³)	768. ± 10.9	728.4 ± 10.9	.015	.136	739.9 ± 14.9	746.6 ± 14.9	.755	.706	<.001
Stress strain index (mm ³)	1082.3 ± 49.0	849.3 ± 49.0	.002	.010	899.5 ± 47.1	804.3 ± 47.1	.163	.037	.325
<i>Mid-diaphysis</i>									
Periosteal circumference (mm)	72.3 ± 0.7	72.8 ± 0.7	.598	<.001	69.8 ± 0.9	69.6 ± 0.9	.898	<.001	.012
Total bone area (mm ²)	417.0 ± 8.2	423.6 ± 8.2	.577	<.001	340.0 ± 10.4	389.3 ± 10.4	.966	<.001	.015
Total bone content (mg/mm)	286.9 ± 8.5	275.9 ± 8.5	.369	<.001	271.2 ± 6.3	252.9 ± 6.3	.049	<.001	<.001
Cort/subcort bone area (mm ²)	248.1 ± 6.6	237.6 ± 6.6	.269	.002	230.1 ± 5.3	216.8 ± 5.3	.084	<.001	<.001
Cort/subcort bone content (mg/mm)	261.6 ± 7.5	246.0 ± 7.5	.149	.002	245.9 ± 5.9	226.3 ± 5.9	.026	<.001	.016
Cort/subcort bone density (mg/mm ³)	1053.3 ± 6.8	1034.4 ± 6.8	.058	.556	1070.5 ± 6.0	1048.4 ± 6.0	0.014	.957	<.001
Stress strain index (mm ³)	1505.1 ± 43.4	1446.1 ± 43.4	.346	<.001	1386.9 ± 41.5	1307.0 ± 41.5	0.184	<.001	.0189

Table 5. Least squared means \pm standard error for distance to bone matrix deposition in the rib (μm) for calves born to dams fed either kale or fodder beet winter diets with baleage in 2019 and 2020.

	Kale	Fodder beet	<i>p</i> -value	
			Treatment	Birth weight
<i>n</i>	20	20		
Year				
2019	364.1 \pm 16.4	415.2 \pm 16.4	.035	.120
2020	308.9 \pm 12.4	313.5 \pm 13.0	.800	.398

Histology

In 2019, after adjustment for birth weight, calves in the kale treatment had a shorter distance between the zone of hypertrophic chondrocytes to deposition of bone matrix in the rib than calves from the FB treatment ($p = .035$, Table 5). There was no difference between treatments in mean distance (μm) from the zone of hypertrophic chondrocytes to deposition of bone matrix in the rib between calves born in 2020 ($p = .800$, Table 5).

Histomorphometry

Calves born in 2019 in the kale treatment had greater humeral bone area and bone perimeter and less resorption cavities than calves in the FB treatment as measured using histomorphometry (Table 6). There was no effect of treatment on osteoid area and osteoid perimeter in the humerus. Histomorphometry was not measured in the 2020 calves. In 2020 ribs from two calves from dams fed FB had healing microfractures in the primary spongiosa, which could be consistent with bone fragility. No microfractures were detected in the ribs of 2019 animals.

Discussion

Calves from the FB treatment had similar birth weight compared with calves from the kale treatment but demonstrated reduced linear growth as reflected in shorter wither height and crown rump length. The reduced linear growth was also reflected in reduced measures of bone size and strength in both the humerus and metacarpus. There was some variation between years in the magnitude of the differences between treatments, but not the direction of the difference, which may indicate a dose response

Table 6. Least squared means \pm standard error for bone area, bone perimeter and number of resorption cavities in the humerus of calves born to dams fed either kale or fodder beet winter diets in 2019.

	Kale	Fodder beet	<i>p</i> -value	
			Treatment	Birth weight
<i>n</i>	10	10		
Bone area (mm^2)	30.0 \pm 1.4	23.7 \pm 1.4	.005	.215
Bone perimeter (μm)	8146.8 \pm 211.0	7284.4 \pm 211.0	.011	.149
Number of resorption cavities	57.6 \pm 6.1	100.5 \pm 6.1	<.001	.582
Osteoid area (μm^2)	77.8 \pm 9.4	58.7 \pm 9.4	.172	.154
Osteoid perimeter (μm)	97.8 \pm 12.4	69.9 \pm 12.4	.134	.471

effect as the duration of the dam cohorts on the crops differed between years. This between year variation in the duration of time on crop reflects the practical difficulties with collection of data from on-farm system experiments.

The removal of the cows off crops in both years was based on predicted calving date and udder development with the aim to remove dams off the crop two weeks prior to calving. The source of calves differed between years with 2019 calves being born to mixed age cows and 2020 born calves being born to two-year-old heifers (heifer calves) and mixed age cows (bull calves). Heifers tend to be mated with the aim of calving earlier in the calving period than mixed age cows (DairyNZ 2023). As a result, the length of time on crops in 2019 and 2020 differed with dams of the 2020 born calves being on crops for approximately two weeks less than dams in 2019. This meant that calves in 2019 were exposed to a maternal nutrition restriction for longer.

Although calves from the FB treatment born in 2019 had the same birth weight as the calves in the kale treatment, they had a shorter wither height and bone length for both metacarpus and humerus. As this effect was not observed in the 2020 born calves it is possible that the restriction in long bone growth was due to the longer period grazing FB in 2019. The result in the 2020 cohort was similar to observations by Moonsan et al. (2018) where dams were fed a diet with low or moderate crude protein for eight weeks prior to calving. Although the crude protein deficiency was greater in the study of Moonsan et al. (2018), than what was provided in the current study, they reported no effect of dam diet on birth weight and stature at birth. Thus, there may be a dose-dependent response to crude protein and mineral restriction in the dam, indicating that the negative effect may be more dependent on the duration of the exposure than the relative magnitude of the crude protein and mineral deficiency once below the 'minimum threshold'. This result suggests that the length of the dietary restriction plays an important role in determining the severity of intrauterine growth restriction on bone.

Differences in the effect of diet on stature between cohorts may reflect seasonal variation in crop growth and differences in the allocation of crops between years. Dams in the FB treatment in 2019 were fed 13.5% more kg DM/cow/d FB than FB dams in 2020. As a result, the metabolisable energy offered to the 2019 FB dams was greater than 2020 FB dams but the provision of crude protein and other important bone growth minerals such as phosphorus and calcium were lower. Therefore, the lower crude protein and minerals most likely became the limiting factor for bone growth and development. The absorption of phosphorus and calcium are heavily dependent on each other (Reinhardt et al. 1988). Additionally, some studies in humans have suggested that the impact of low protein on bone formation is dependent on having adequate calcium in the diet (Dawson-Hughes 2003; Vatanparast et al. 2007). Diets that are low in phosphorus have been shown to reduce the formation of bone matrix in growing rats and in pigs (Baylink et al. 1971; Sørensen et al. 2019).

Although there was only an effect of treatment on stature in the 2019 born calves, differences in bone morphology at a pQCT level were observed in both years in the metaphysis of the metacarpus. As bone in the metaphysis is associated with recent growth, this result was expected as the diet was fed to the dams in the winter just prior to calving. Although there was no difference in bone circumference between treatments, material properties of density and content were greater in calves in the kale treatment than in

the FB treatment. This indicates that the ability for bone to lay down matrix was reduced by the FB diet (i.e. protein deficiency). As a result of a lower bone content, calves from the FB diets had lower bone strength (SSI) and increased risk of fracture.

At the mid-diaphysis site in the metacarpus and humerus, calves from the FB treatment had lower measures of bone content than those from the kale treatment, though this difference between treatments was only significant in the 2020 calves. It is possible that the earlier removal of the 2020 dams off the crops provided an opportunity for some correction in dietary intake and this permitted some compensatory growth in the foetus. If this compensatory growth did occur, bone in the metaphysis would be pushed towards the mid-diaphysis as a result of longitudinal growth. However, due to bone turnover being a slow process relative to the timing of events in this study, it is possible that the magnitude in the differences between treatments in the two years is a result of variability in bone and low animal numbers.

Histological analysis of the ribs demonstrated that calves from the kale treatment laid down bone matrix on the scaffold of cartilage spicules earlier than calves from the FB treatment, which would have resulted in increased bone strength. Greater deposition in mineral content and bone strength was also observed in the morphometric analysis of the limb bones and the pQCT analysis at the metaphysis in both years for calves from the kale treatment. Although not statistically significant in 2020, changes were observed in the humeral growth plate in 2019 with calves from the FB treatment having a greater thickness of the growth plate compared with calves born to dams on kale. An increased thickness of the growth plate has also been reported in rats with intrauterine exposure of low maternal protein diet indicating an alteration in the programming of skeletal growth (Byers et al. 2000; Mehta et al. 2002a). This alteration in the programming of skeletal growth can have long-term implications on bone development (Mehta et al. 2002b; Lanham et al. 2008).

Bone histomorphometry analysis is used to study bone turnover, remodelling and structure (Eriksen et al. 1994; Vedi and Compston 2003). In this study, the humerus had less bone area and bone perimeter in calves from the FB treatment compared to the humerus from calves from the kale treatment. The lower percentage area occupied by mineralised bone observed in calves from the FB treatment indicates diminished bone formation rate in these calves that results in decreased bone length and strength. Interestingly, when active bone formation (osteoid area, osteoid perimeter) was determined as a percentage of overall bone area, there was no differences between groups. Due to greater bone area in calves born to dams on kale, there were a greater number of bone formation areas. This result suggests that the initial lower bone area in calves from the FB treatment may be perpetuated with age, as the overall number of active bone formation areas was also less in these animals.

Greater number of resorption areas in the humerus from calves from the FB treatment indicates increased osteoclastic activity and bone resorption. This results in reduced bone surface and hence reduced bone strength indicating an effect in the programming of the skeletal growth in calves born to dams on FB compared to kale. It is possible that the lower protein diet reduced oestrogen resulting in higher levels of follicle stimulation hormone and thus the formation and function of osteoclasts (Lanham et al. 2008). Alternatively, in humans low protein diets are associated with decreased calcium absorption from the intestine, resulting in increased parathyroid hormone secretion and thus

increased osteoclastic resorption (Kerstetter et al. 2000; Kerstetter et al. 2003). The greater bone removal could further compound the lack of bone formation surfaces available in calves born to dams on FB.

The sex effect on bone morphology observed in the 2020 born calves may reflect differences in the response of male and female calves to nutritional deficits. Studies performed in humans describe significant gender differences in bone growth and development (Schoenau et al. 2001). With only a sex effect examined in the 2020 born calves, further research is required to examine whether there are sex specific effects of intrauterine growth restrictions on calves, any probable long-term effects and whether animals can recover with time or not.

Studies on foetal/developmental programming in other species have shown that alterations in intrauterine growth can have long-term effects on organ structure and/or function and may result in, as appears to be the case in these calves, poor bone quality and potentially an increased risk of fracture in the future (Lanham et al. 2008; Reynolds et al. 2019). Research in rats has suggested that offspring from dams fed a low protein diet had altered bone structure, density and turnover that continued to be observed in later life (Mehta et al. 2002a; Lanham et al. 2008). This observation coupled with results in the current study suggest that the effect of maternal protein restriction in dairy calves may have a future effect on bone growth and development preventing peak bone mass from being attained. If peak bone mass is not achieved the risk of these offspring being affected by spontaneous humeral fractures as a result of osteoporosis may be increased (Wehrle-Martinez et al. 2022).

Results in the current study emphasise that to maximise the benefits of using FB as a winter crop for pregnant dairy cattle, potential nutritional deficits in protein, phosphorus and calcium must be addressed to meet nutritional needs. When feeding FB, the inclusion of good quality pasture silage and/or pasture and mineral supplements in the diet is advised, particularly in late pregnancy to prevent intrauterine growth retardation in newborn calves.

Limitations

The opportunistic nature of this research restricted the ability to control factors such as time on crops between treatments, dam parity and the sex of the calves between years. In addition, the low number of calves prevented the examination of stature and bone morphology in older calves to determine whether the effect of intrauterine retardation would have been overcome if those calves were provided adequate nutrition after birth. Future research should aim to examine the long-term effects of maternal diet on dairy heifer growth and production.

Conclusions

Across the two years, despite variation in the measures showing differences, the overall trend indicates there was altered bone structure and development in calves from dams the offered FB diet compared to calves from dams offered the kale diet, resulting in predicted increased risk for fracture in calves from dams on FB. The variation in measures showing differences could indicate a year effect on the magnitude of the diet effect. This

may be related to either the timing of the feeding of the diets (duration and timing during foetal development), the magnitude of the exposure (quantity of feed), or the composition of the diet (for example protein, phosphorus content). Future research is required to expand this research on a larger scale and follow calves from birth to maturity to examine whether the effect of restricted crude protein or minerals on bone is overcome and the effect of further exposure to restricted protein on bone morphology later in the heifers' life. Results from the current study and future research will enable industry recommendations for pragmatic cost-effective winter-feeding solutions for dairy cows and replacements.

Acknowledgments

The authors would like to acknowledge Mathew Perrott, Petru Daniels and Evelyn Lupton for histology section preparation, Bryce Ilton and Kirsty Anderson for assistance with limbs transport. We would also like to thank the DairyNZ research technicians and the farm team at the Southern Dairy Hub for animal management and sample collection.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by DairyNZ [grant number CB1923]; Sustainable Farming Fund-Ministry of Primary Industries [grant number 405512]; PGG Wrightson Seeds.

Data availability statement

Data will be available from first author upon request.

ORCID

Michaela J. Gibson  <http://orcid.org/0000-0001-5970-4289>

Dawn E. Dalley  <http://orcid.org/0000-0003-3707-2051>

References

- Baylink D, Wergedal J, Stauffer M. 1971. Formation, mineralization, and resorption of bone in hypophosphatemic rats. *Journal of Clinical Investigation*. 50(12):2519–2530. doi:10.1172/JCI106752.
- Bryant R, Pirat M. 2014. Effect of feeding kale or fodder beet diets to dairy cows in late gestation on calf birth weight and skeletal development. 6th Australasian Dairy Science Symposium Proceedings, November 19–21, 2014, Hamilton, New Zealand. pp. 312–314.
- Byers S, Moore A, Byard R, Fazzalari N. 2000. Quantitative histomorphometric analysis of the human growth plate from birth to adolescence. *Bone*. 27(4):495–501. doi:10.1016/S8756-3282(00)00357-4.
- Craig L, Dittmer K, Thompson K. 2016. *Bones and joints. Pathology of domestic animals*. Amsterdam: Elsevier Health Sciences.
- DairyNZ. 2021. *Facts and figures: a quick reference guide for New Zealand dairy farmers*.

- DairyNZ. 2023. Calving pattern; [accessed 2023 Oct 24]. <https://www.dairynz.co.nz/animal/reproduction-and-mating/calving-pattern/>.
- DairyNZ Economics Group. 2019. Feed consumed by NZ dairy cows.
- Dawson-Hughes B. 2003. Interaction of dietary calcium and protein in bone health in humans. *The Journal of Nutrition*. 133(3):852S–854S. doi:10.1093/jn/133.3.852S.
- Dittmer K, Morley R, Smith R. 2017. Skeletal deformities associated with nutritional congenital rickets in newborn lambs. *New Zealand Veterinary Journal*. 65(1):51–55. doi:10.1080/00480169.2016.1241165.
- Domander R, Felder AA, Doube M. 2021. BoneJ2 – refactoring established research software. *Wellcome Open Research*. 6:37. doi:10.12688/wellcomeopenres.16619.1.
- Edwards J, Cuthbert S, Pinxterhuis J, McDermott A. 2021. The fate of calves born on New Zealand dairy farms and dairy farmer attitudes towards producing dairy-beef calves. *New Zealand Journal of Animal Science and Production*. 81:179–185.
- Edwards J, Mashlan K, Dalley D, Pinxterhuis J. 2017. A survey of dairy cow wintering practices in Canterbury, New Zealand. *Animal Production Science*. 57(7):1323–1329. doi:10.1071/AN16459.
- Eriksen EF, Axelrod DW, Melsen F. 1994. *Bone histomorphometry*, Raven Press.
- Ferretti J. 1995. Perspectives of pQCT technology associated to biomechanical studies in skeletal research employing rat models. *Bone*. 17(4):S353–S364. doi:10.1016/8756-3282(95)00313-3.
- Gibbs J, Saldias B. 2014. Fodder beet in the New Zealand dairy industry. *The South Island Dairy Event*: 1–8.
- Gibson M, Dittmer K, Hickson R, Back P, Wehrle-Martinez A, Rogers C. 2021. The mid-diaphysis is a poor predictor of humeral fracture risk indicating that predisposing factors are recent. *Ruminants*. 1(1):23–30. doi:10.3390/ruminants1010002.
- Gibson MJ, Adams BR, Back PJ, Hickson RE, Dittmer KE, Rogers CW. 2022. Live weight and bone growth from birth to 23 months of age in Holstein-Friesian, Jersey and Crossbred Heifers. *Dairy*. 3(2):333–344. doi:10.3390/dairy3020026.
- Guilbert H, Gregory P. 1952. Some features of growth and development of Hereford cattle. *Journal of Animal Science*. 11:3–16. doi:10.2527/jas1952.1113.
- Hammond KJ, Sandoval E, McKenzie CM, Lees S, Pacheco D, McCoard SA. 2022. The effect of a fodder beet versus rye-grass grazing regime during mid-to-late gestation twin-bearing ewes on dam and progeny performance and lamb survival. *New Zealand Journal of Agricultural Research*. 65(2–3):145–162. doi:10.1080/00288233.2021.1879175.
- Jonker A, Dalley D, Woods R, Dynes R, Pacheco D, Khan M, Edwards J, Hammond K. 2022. Modelling winter feeding practices with fodder beet in growing and dry dairy cattle. *New Zealand Journal of Animal Science and Production*. 82:39–44.
- Kerstetter JE, O'Brien KO, Insogna KL. 2003. Low protein intake: the impact on calcium and bone homeostasis in humans. *The Journal of nutrition*. 133(3):855S–861S. doi:10.1093/jn/133.3.855S.
- Kerstetter JE, Svastisalee CM, Caseria DM, Mitnick ME, Insogna KL. 2000. A threshold for low-protein-diet-induced elevations in parathyroid hormone. *The American Journal of Clinical Nutrition*. 72(1):168–173. doi:10.1093/ajcn/72.1.168.
- Lanham S, Roberts C, Perry M, Cooper C, Oreffo R. 2008. Intrauterine programming of bone. Part 2: alteration of skeletal structure. *Osteoporosis International*. 19:157–167. doi:10.1007/s00198-007-0448-3.
- Mao W, Albrecht E, Teuscher F, Yang Q, Zhao R, Wegner J. 2008. Growth- and breed-related changes of fetal development in cattle. *Asian-Australasian Journal of Animal Sciences*. 21(5):640–647. doi:10.5713/ajas.2008.70293.
- Mehta G, Roach H, Langley-Evans S, Taylor P, Reading I, Oreffo R, Aihie-Sayer A, Clarke N, Cooper C. 2002a. Intrauterine exposure to a maternal low protein diet reduces adult bone mass and alters growth plate morphology in rats. *Calcified Tissue International*. 71(6):493–498. doi:10.1007/s00223-001-2104-9.
- Mehta G, Roach H, Langley-Evans S, Taylor P, Reading I, Oreffo R, Aihie-Sayer A, Clarke N, Cooper C. 2002b. Intrauterine exposure to a maternal low protein diet reduces adult bone

- mass and alters growth plate morphology in rats. *Calcified Tissue International*. 71:493–498. doi:[10.1007/s00223-001-2104-9](https://doi.org/10.1007/s00223-001-2104-9).
- Moonsan Y, Thomas C, Bryant RH, Edwards G. 2018. Brief communication: effect of maternal diet in late gestation on development of dairy calves.
- Nicol AM, Brooks I. 2007. The metabolisable energy requirements of grazing livestock. In: Rattray PV, Brooks I, Nicol AM, editors. *Pasture and supplements for grazing animals New Zealand society of animal production occasional publication no 14*. Hamilton: New Zealand Society of Animal Production; p. 151–172.
- Reinhardt T, Horst R, Goff J. 1988. Calcium, phosphorus, and magnesium homeostasis in ruminants. *Veterinary Clinics of North America: Food Animal Practice*. 4(2):331–350. doi:[10.1016/S0749-0720\(15\)31052-5](https://doi.org/10.1016/S0749-0720(15)31052-5).
- Reynolds LP, Borowicz PP, Caton JS, Crouse MS, Dahlen CR, Ward AK. 2019. Developmental programming of fetal growth and development. *Veterinary Clinics of North America: Food Animal Practice*. 35(2):229–247. doi:[10.1016/j.cvfa.2019.02.006](https://doi.org/10.1016/j.cvfa.2019.02.006).
- Rugoho I, Gibbs S, Edwards G. 2014. Dry matter intake and body condition score gain of dairy cows offered kale and grass. *New Zealand Journal of Agricultural Research*. 57(2):110–121. doi:[10.1080/00288233.2014.886598](https://doi.org/10.1080/00288233.2014.886598).
- Schoenau E, Neu C, Rauch F, Manz F. 2001. The development of bone strength at the proximal radius during childhood and adolescence. *The Journal of Clinical Endocrinology & Metabolism*. 86(2):613–618. doi:[10.1210/jcem.86.2.7186](https://doi.org/10.1210/jcem.86.2.7186).
- Sorensen KU, Shiguetomi Medina JM, Poulsen HD. 2019. Mineralisation of tubular bones is affected differently by low phosphorus supply in growing-finishing pigs. *Journal of the Science of Food and Agriculture*. 99(7):3628–3634. doi:[10.1002/jsfa.9583](https://doi.org/10.1002/jsfa.9583).
- Vatanparast H, Bailey DA, Baxter-Jones AD, Whiting SJ. 2007. The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. *The Journal of nutrition*. 137(12):2674–2679. doi:[10.1093/jn/137.12.2674](https://doi.org/10.1093/jn/137.12.2674).
- Vedi S, Compston J. 2003. Bone histomorphometry. In: Ralston SH, Helfrich MH, editors. *Bone research protocols*. Totowa, NJ: Humana Press; p. 283–298. doi:[10.1385/1-59259-366-6:283](https://doi.org/10.1385/1-59259-366-6:283).
- Waghorn G, Collier K, Bryant M, Dalley D. 2018b. Feeding fodder beet (*Beta vulgaris* L.) with either barley straw or pasture silage to non-lactating dairy cows. *New Zealand Veterinary Journal*. 66(4):178–185. doi:[10.1080/00480169.2018.1465484](https://doi.org/10.1080/00480169.2018.1465484).
- Waghorn G, Law N, Bryant M, Pacheco D, Dalley D. 2018a. Digestion and nitrogen excretion by Holstein–Friesian cows in late lactation offered ryegrass-based pasture supplemented with fodder beet. *Animal Production Science*. 59(7):1261–1270. doi:[10.1071/AN18018](https://doi.org/10.1071/AN18018).
- Wehrle-Martinez A, Lawrence K, Back PJ, Rogers CW, Gibson M, Dittmer KE. 2022. Osteoporosis is the cause of spontaneous humeral fracture in dairy cows from New Zealand. *Veterinary Pathology*. 60(1):88–100.
- Woods R, Dalley D, Edwards J. 2023. Effects of feeding fodder beet or kale in winter to dams and their heifer offspring on the heifer growth and production. *Animal Production Science*. 64(1). doi:[10.1071/AN22474](https://doi.org/10.1071/AN22474).