BRIEF COMMUNICATION: Can the effects of intrauterine growth retardation on bone morphology be overcome by first calving? - A preliminary study.

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Abstract

Intrauterine growth retardation, reduced bone density, and an increased risk of fracture were observed in a cohort of calves born to dams fed a diet of baleage and either fodder beet or kale during winter. This study aimed to examine whether the intrauterine effects of the dam's diet on bone development can be overcome by the time the calf reaches its first lactation. Heifer calves from these cohorts were grown, mated, calved, and milked under standard farm practice. At 11 weeks post-calving, heifers were slaughtered, and the humerus was collected and scanned using peripheral quantitative computed tomography (pQCT). There were no differences in pQCT measures of bone size, density, and strength between heifers whose dams were wintered on kale or fodder beet before calving ($p>0.05$). Results indicate subsequent bone growth and development after birth may compensate for the intrauterine effects of late gestation dam diet.

Keywords: Fodder beet; Kale; Protein

Introduction

New Zealand dairy farms operate under a pasture-based system, with crops and supplements used to compensate for feed deficits during winter. In the southern regions of New Zealand, fodder beet (*Beta vulgaris*) is a commonly used crop due to its high metabolisable energy, consistent quality, high yield, and low cost per kilogram produced (Waghorn et al. 2018). As a sole diet, fodder beet is not balanced for dairy cows due to its low crude protein, calcium, and phosphorus content (Jonker et al. 2022).

In cows, the majority of intrauterine growth, particularly musculoskeletal growth occurs in the last trimester of pregnancy (Mao et al. 2008). With 85% of herds in New Zealand being spring-calving, the last trimester of pregnancy most commonly occurs in winter when pasture yield and quality are low (Edwards et al. 2021). If fodder beet is not supplemented correctly to provide adequate protein and minerals, intrauterine growth retardation of bone can occur (Dittmer et al. 2017; Hammond et al. 2022). An earlier study reported that calves born to dams fed (wintered on) fodder beet while pregnant had reduced bone density and strength, and an increased fracture risk compared to calves born to dams fed kale (Gibson et al. 2024).. The aim of this study was to examine whether the intrauterine effects of the dam's diet on bone development can be overcome by the time the calf reaches its first lactation.

Materials and methods

This study was conducted with approval from the Ruakura Animal Ethics Committee, Hamilton, New Zealand (RAEC #14453 and #15106 (lactation)).

This study followed a second cohort of calves from a previous study that examined the intrauterine effects of maternal diet (fodder beet vs. kale, supplemented with baleage) on calf stature and bone at birth(Gibson et al. 2024). Calves from the first cohort were euthanised within 10 days of birth and a forelimb and section of rib including the costochondral junction were harvested for peripheral quantitative computed tomography and histological analysis. There was no difference in calf birth weight, wither height and crown rump length between calves born to each dam diet. Calves born to dams on the fodder beet treatment had reduced bone density and strength compared to calves from the kale treatment. In addition, calves born to dams wintered on FB had smaller girth size than calves born to dams wintered on kale (Gibson et al. 2024).

Calves were born in August-September 2020 at the Southern Dairy Hub research farm (Branxholme, Southland), from dams fed a winter diet of either kale (kale treatment) or fodder beet (fodder beet [FB] treatment), with both groups also having access to pasture baleage. Heifers in the current study (kale = 18 and FB = 17) were reared, mated, calved, and milked under standard farm practice in one cohort. Liveweight was recorded 17 times from birth until approximately 19 months of age. 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.

As calves, these heifers were fed on milk (once daily after the first two weeks), pasture and meal until they reached weaning weight (100 kg), after which they continued to graze pasture and were supplemented with calf meal. At six months of age, heifers were moved to a grazier and reared under standard farm practice. Pasture silage was allocated during times of low pasture availability (i.e. winter).

Heifers returned to the Southern Dairy Hub at the beginning of May 2022 and were transitioned onto a diet consisting of 8.7 kg DM of kale and 3.3 kg DM of baleage. Two weeks prior to their expected calving date, heifers were transported to a commercial farm and were offered a grass and baleage diet with minerals (400 g/cow/day of Transition Cow Advanced [Agvance Nutrition, Howick, NZ] and 15-40 g/cow/day of SoluPhos® [260 g P/kg; Agvance Nutrition, Howick, NZ]). Ten days before the expected calving date, heifers were moved to a calving pad with self-feed silage, hay, and straw, and continued to receive the transition mineral mix and SoluPhos®.

After calving, heifers were provided *ad libitum* grass and baleage was spread with an 80/20 calcium magnesium mix [containing calcium carbonate and magnesium oxide; Healthy Cow, AB LIME, Winton, NZ]. Heifers were milked once-a-day for eight days and offered an in-shed feed consisting of 0.33 kg/cow/day dried distillers grain, 0.33 kg/cow/day crushed grain, 0.33 kg/cow/day palm kernel expeller (PKE), and 165 g/cow/day of OptiPrill (Agvance Nutrition, Howick, NZ). After approximately eight days of once-a-day milking, the heifers were moved into the main milking mob and milked twice a day. They were offered approximately 23 kg DM/cow/day, consisting of grass with 1 kg/cow/day dried distillers grain, 1 kg/cow/day crushed grain, and 1 kg/cow/day PKE, plus 165 g/cow/day OptiPrill.

At approximately 11 weeks post-calving (26 months of age), heifers were slaughtered at a commercial abattoir and the left humerus was collected and sent to Massey University (Palmerston North, Manawatū) for subsequent analysis.

pQCT bone parameters

Peripheral quantitative computed tomography (pQCT; XCT 2000; Stratec Medical, Pforzheim, Germany) scanning was on the humerus (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 humerus, a 2 mm slice at the mid-diaphysis was obtained with a voxel size of 0.3 mm. 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 mid-diaphysis was defined as 50% of the total bone length, starting from the proximal end of the trochlea. Within the manufacturer's software, voxels >710 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.

Histology

Histology was undertaken using the same protocol outlined in Wehrle-Martinez et al. (2023). Humeri were longitudinally sectioned with a bandsaw, and then 3-4 mm thick slabs of the proximal humerus were obtained. The bone slabs were placed in 10% neutral buffered formalin solution until processing. During procesing, the bone slabs were demineralised using a hydrochloric acid-based product (Decalcifier hydrochloric acid, Amber Scientific Ltd, Midvale, Australia). Once sufficiently demineralised, four sections of bone from the proximal humeral metaphysis were trimmed and placed in tissue cassettes. The bone sections were then processed routinely, embedded in paraffin wax, and sectioned at 4 μm thickness, followed by staining with haematoxylin and eosin (Wehrle-Martinez et al. 2023).

The histology sections were examined microscopically, and images were taken with an Olympus cellSense imaging software (version 4.2). Physeal thickness was measured on section 1, at three random locations across the slide. Cortical thickness was measured on section 2, 10 mm below the bottom of the physis. Cortical resorption was assessed by counting the number of resorption canals in the cortex of section 4. A two-sample t-test was used to determine if there were any significant differences between the two groups of animals.

Statistical analysis

Statistical analysis was conducted using the Statistical Analysis System software version 9.4 (SAS Institute Inc, Carey, NC, USA). All analyses for pQCT bone parameters and histology measures are presented using means and standard error. Differences in bone measures between dam diets were examined using ANOVA with a significance level set at $p<0.05$. Stature measures were anlaysed using a general linear model with the fixed effect of dam diet and covariate of birth weight. Nine heifers were removed from the dataset due to either failing to get pregnant (FB=4, Kale=1) or the humerus was not collected at the abbatoir (FB=4). This left 26 heifers (FB=9, kale= 17) in the analysis.

Results

There was no significant difference in birth weight and average daily gain between treatments (Table 1, $p>0.10$). There was no effect of treament on calf stature measures (height, girth, length).

There was no significant difference in carcass weight, pQCT measures or histology between treatments (Table 1, $p>0.20$).

Discussion

Both the pQCT and histological analysis require the harvesting of the humerus from the animal of interest, as such this prevents serial sampling of the same individual during different stages of growth. In the subset of calves examined at birth, intrauterine growth retardation in the form of reduced bone mineral density and increased fracture risk was observed in the calves from dams fed fodder beet (Gibson et al. 2024). These differences were not present in the heifers from the same cohorts examined in early lactation in this study

Foetal programming in rats has indicating that offspring from dams fed a low protein diet had altered bone structure and density that persisted into later life (Lanham et al. 2008; Mehta et al. 2002). However, the magnitude of growth retardation from nutritional deficiencies is dependent on both the severity and exposure time during gestation (length of exposure and stage of gestation). The heifers in the current study were from the 2020-born cohort of the calf trial that was reported to have a shorter exposure to the nutritional challenge compared with the cohort from the previous year in the same calf trial. The differences in the calves (at birth) (Gibson et al. 2024), between dam diet treatments were less dramatic in 2020 than in 2019, which had a greater number of days of dam nutritional challenge (7.5 weeks vs 11 weeks respectively). Thus, the duration of nutritional challenge exposure may not have been sufficient to induce sustained impairment of humeral bone growth and development in the developing foetus. However, although the material bone properties were not different between the treatments, the tendency (p=0.06) for the humerus to be longer in heifers from the kale treatment indicating that dam diet may have impeded longitudinal growth at some stage.

The results of the current study should be considered preliminary as there are limitations. Researchers were unable to enter the abattoir due to restricted access, thus preventing the identification of heifer carcasses. Although heifers were grouped into treatments during processing, individual heifer ID could not be linked to each carcass. As a result, humeral bone measurements could not be correlated with the liveweight and carcass weight of individual heifers to adjust for size differences. Given measures of the humerus are highly correlated with liveweight, adjusting for animal size would remove some variation in measurements (Gibson et al. 2019). If this study were to be repeated, it would be essential to ensure the identification of the humerus with heifer ID.

Table 1. *Means ± standard error of stature measures, bone parameters in the mid-diaphysis and histology of the humerus from heifers born to dams fed either kale (n=13) or fodder beet (n=13) at 26 months of age.*

	Fodder beet	**Kale**	**p-value**	
Weight measures			Birth weight	Treatment
n	9	17		
Birth weight (kg)	25.2±1.3	26.9±0.9	-	0.28
Average daily gain to 19 months (kg/day)	0.6 ± 0.01	0.6 ±0.01		0.25
Carcass weight (kg)	134.9±4.6	141.2±3.4	-	0.28
Height (cm)	67.5±1.0	69.2±0.8	<0.01	0.21
Girth (cm)	71.0±1.0	70.9±0.7	<0.01	0.92
Length (cm)	54.5±1.3	56.9±0.9	0.54	0.15
pQCT measures				
n	9	17		
Humerus length (mm)	256.2±2.0	261.2±1.6		0.06
Periosteal circumference (mm)	139.1±1.7	141.3±1.4		0.33
Total bone area (mm^2)	1542.0±38.1	1591.2±31.1		0.33
Endosteal circumference (mm)	93.1±1.8	96.6±1.5		0.16
Cortical bone thickness (mm)	7.3±0.1	7.1±0.1		0.24
Cortical bone density (mg/mm^2)	1288.8±5.3	1286.2±4.3		0.36
Cortical bone content (mg/mm)	1093.9±24.2	1088.4±19.7		0.86
Stress-strain index (mm^3)	11774.6±366.2	11988.5±299.0		0.65
Histology				
n	9	17		
Thickness of physis (μm)	470.5±25.5	452.1±18.5		0.56
Cortical thickness (μm)*	828.3±107.0	839.4±70.8		0.93
Number of cortical osteoclastic resorption cavities	9.6±1.7	8.6±1.2		0.67

**Missing 3 measurements due cut bone section not being long enough to measure cortical thickness (FB=2, Kale =1)*

Conclusion

The lack of differences in bone measures observed at two years of age between heifers whose dams were fed either kale or fodder beet prior to calving indicates that a linear growth pattern is sufficient to overcome intrauterine growth limitations. However, this conclusion may only be applicable to the duration and intensity of the dam's nutritional challenges experienced in this trial. It is important to note that the calves were managed and slaughtered under commercial conditions, which imposed logistical constraints that should be considered when interpreting the magnitude of the effect observed in the results. Additional research is required to determine whether the lack of differences in bone morphology at two years of age truly reflects the ability of bone to overcome the intrauterine effects of dam diet.

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References

Dittmer K, Morley R, Smith R 2017. Skeletal deformities associated with nutritional congenital rickets in newborn lambs. New Zealand Veterinary Journal 65**:** 51-55.

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.

Ferretti J 1995. Perspectives of pQCT technology associated to biomechanical studies in skeletal research employing rat models. Bone 17**:** S353-S364.

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**:** 23-30.

Gibson MJ, Rogers CW, Back PJ, Dittmer KE, Wehrle-Martinez A, Dalley DE, Woods RR 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**:** 1-16.

Gibson MJ, Rogers CW, Dittmer KE, Hickson RE, Pettigrew EJ, Back PJ 2019. Can bone measures of the bovine metacarpus predict humeral bone structure? New Zealand Journal of Animal Science and Production 79**:** 8-12.

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**:** 145-162.

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.

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.

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**:** 640-647.

Mehta G, Roach H, Langley-Evans S, Taylor P, Reading I, Oreffo R, Aihie-Sayer A, Clarke N, Cooper C 2002. 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.

Waghorn G, Law N, Bryant M, Pacheco D, Dalley D 2018. Digestion and nitrogen excretion by Holstein–Friesian cows in late lactation offered ryegrass-based pasture supplemented with fodder beet. Animal Production Science 59**:** 1261-1270.

Wehrle-Martinez A, Lawrence K, Back P, Rogers C, Gibson M, Dittmer K 2023. Osteoporosis is the cause of spontaneous humeral fracture in dairy cows from New Zealand. Veterinary Pathology 60**:** 88-100.