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Growth Biology

Growth performance, antibody response, and mammary gland development in New Zealand dairy replacement bovine heifers fed low or high amounts of unpasteurized whole milk

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Abstract

This study evaluated the influence of feeding low and high preweaning allowances of unpasteurized whole milk (MA) on intake, selected blood metabolites, antibody response, mammary gland growth, and growth of New Zealand (NZ) dairy heifers to 7 mo of age. At 10 ± 2 d of age (study day 0), group-housed (six·pen^{-1}) heifer calves (Holstein-Friesian × Jersey) were allocated to low (4 L whole milk·calf^{-1}·d^{-1}; n = 7 pens) or high (8 L whole milk·calf^{-1}·d^{-1}; n = 7 pens) MA for the next 63 d. Calves were gradually weaned between days 63 ± 2 and 73 ± 2. Calves in each pen had ad-libitum access to clean water, pelleted calf starter, and chopped grass hay from day 1 to 91 ± 2 d. At 92 ± 2 d, all calves were transferred to pasture, grazed in a mob, and their growth and selected blood metabolites were measured until day 209. All animals were weighed weekly during the indoor period (to day 91) and then at days 105, 112, 128, 162, 184, and 209. Skeletal growth measurements and blood samples to analyze selected metabolites were collected at the start of the experiment, weaning, and then postweaning on day 91, and day 201. Specific antibodies against *Leptospira* and *Clostridia* were quantified in weeks 7, 13, and 27. Mammary glands were scanned using ultrasonography at the start of the experiment, weaning, and day 201. Feeding high vs. low amounts of MA increased the preweaning growth in heifer calves (P = 0.02) without negatively affecting postweaning average daily gain (ADG) (P = 0.74). Compared with heifers fed with low MA, high MA fed heifers had a greater increase in antibodies against *Leptospira* and *Clostridia* by 13 wk of age (P = 0.0007 and P = 0.06, respectively). By 27 wk of age, the antibody response was the same in heifers offered low or high MA. There was no effect of MA on the total size of the mammary gland, measured by ultrasonography, at weaning and 7 mo of age. However, the greater MA was associated with more mammary parenchyma (P = 0.01) and less mammary fat pad (P = 0.03) in back glands at 7 mo of age compared with heifers fed lower MA. In conclusion, feeding a high vs. a low amount of unpasteurized whole milk increased the preweaning growth of New Zealand replacement heifers without negatively affecting their ADG during postweaning under grazing conditions. Feeding more (8 vs. 4 L·d^{-1}) unpasteurized whole milk positively affected antibody responses early in life and mammary gland composition by 7 mo of age in dairy heifers reared for pasture-based dairy systems.

Lay Summary

This study evaluated the effect of unpasteurized whole milk allowance on intake, antibody response, mammary gland growth, and growth performance of heifers until 7 mo of age. Feeding greater (8 L·d^{-1}) vs. lower (4 L·d^{-1}) milk allowance to heifer calves increased preweaning body weight without having any negative effect on postweaning growth under grazing. Heifers fed high milk allowance had significantly better antibody responses against *Leptospira* and *Clostridia* by 3 mo of age and had more mammary parenchyma (potential milk making tissue), and less mammary fat pad (supporting tissue) by 7 mo of age.

Key words: growth, heifer, immunity, mammary, milk allowance

Abbreviations: ADF, acid detergent fiber; BHB, β-hydroxybutyrate; BUN, blood urea nitrogen; CP, crude protein; DM, dry matter; DMI, dry matter intake; DOMD, digestibility of organic matter in DM; HRP, Horse radish peroxidase; IgG, Immunoglobulin; IVOMD, in vitro organic matter digestibility; kg, kilogram; ME, metabolizable energy; Mcal, megacalorie; NDF, neutral detergent fiber; NEFA, non-esterified fatty acid; OD, optical density; OM, organic matter; OMD, organic matter digestibility; PBS, phosphate buffered saline; TMR, total mixed rations

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Introduction

Around 1.2 million heifers are reared annually to replace and expand New Zealand's (NZ) dairy herd (DairyNZ, 2018). Data from NZ shows that many replacement heifers fail to attain their target Body Weight (BW) at breeding (Handcock et al., 2016). Many factors, including preweaning and postweaning nutrition, and feeding management, could influence the growth rate of heifers (Khan et al., 2016). Recommended calf feeding systems in NZ were originally designed to be the least cost (Muir et al., 2002), where a restricted amount of milk (unpasteurized whole milk, transition milk, or milk replacer at 4 L·d^{-1}; 10% of the initial body weight) was fed to calves using once or twice a day feeding approaches. However, research with Holstein-Friesian calves reared for indoor systems has shown several benefits of providing greater milk allowance on preweaning growth, welfare, and health of calves (Khan et al., 2011a), and on mammary gland development (Geiger et al., 2016), and future milk production of replacement heifers (Soberon and Van Amburgh, 2013).

In NZ, replacement heifers (mainly Holstein-Friesian × Jersey, called Kiwi-cross) have different genetics, growth potential, and breeding goals than Holstein-Friesian heifers reared for European and North American dairy systems (Horan et al., 2004). Further, calf and heifer feeding and management practices in NZ differ from North American and European systems because of the differences in herd calving patterns (i.e., seasonal calving vs. year-around calving). Replacement heifers in NZ are reared on pasture and their postweaning growth response could differ compared with heifers reared on total mixed ration (TMR) diets for the indoor system. Therefore, we reasoned that the recommendations resulting in the positive effects of feeding greater amounts of milk on BW gain and mammary gland growth (Soberon and Van Amburgh, 2013; Geiger et al., 2016) were shown for European and North American Holstein-Friesian heifer rearing needed to be tested for NZ dairy replacement rearing systems. The influence of preweaning nutrition on calf immunity has been well researched; however, studies where immune responses were measured in calves reared on different preweaning nutritional regimes have shown inconclusive results (Donovan et al., 1998; Foote et al., 2005; Ballou, 2012). Most of the studies compared different levels of milk replacers (Nonnecke et al., 2003; Foote et al., 2005; Hengst et al., 2012) instead of unpasteurized whole milk, a natural feed for the calf which can supply high-quality nutrients and numerous bioactive compounds (Gill et al., 2000; Shingfield et al., 2008), and consequently could influence the immune system in developing calves. In humans, measuring the immune response to annual influenza vaccines has been used as a proxy for immunocompetence in a large number of studies of nutrition, physical exercise, and ageing (Dugan et al., 2020; Weyh et al., 2020; Mathot et al., 2021). There is growing evidence that the same approach is valid in cattle (Smith et al., 2018; Engler et al., 2022). Here we investigate the response against a combined vaccine against Clostridium ssp. and Leptospira ssp. that is commonly used in New Zealand (Yupiana et al., 2021; O'Hara, 2020).

Based on the expected differences in preweaning growth response of replacement heifers destined for pasture, we hypothesized that feeding a greater vs. a lower amount of unpasteurized whole milk would supply high-quality nutrients to heifer calves which would increase antibody responses, and mammary gland growth by weaning in heifers. We further hypothesized that biological advantages including greater growth, mammary gland growth, and antibody response achieved by providing high milk allowance would be sustained during postweaning in heifers on pasture up to 7 mo of age. This study aimed to evaluate the influence of feeding low and high allowance of unpasteurized whole milk on nutrient intake, selected blood metabolites, antibody responses against common cattle pathogens (*Clostridia* and *Leptospira*), mammary gland growth measurements using ultrasonography, and growth in dairy replacement heifers in a pastoral system up to 7 mo of age.

Material and Methods

The animal ethics application was approved by the AgResearch Ruakura Animal Ethics Committee (RAEC # 13648), including all procedures, sampling, and measurements as described below.

Calves, treatments, feeding, and management of calves

Heifer calves born at AgResearch's dairy farm at Tokanui, Waikato, NZ during spring 2015 (August and September) were collected from the calving paddock (twice daily), ear tagged, and their navels were dipped in iodine solution. All heifer calves were weighed and fed colostrum (4 L·calf^{-1}) within 24 h of their birth. All calves were group-housed indoors in a large pen (12 × 4 m), fed 4 L of unpasteurized whole milk divided into two equal feedings for the first 10 ± 2 d of their age.

On day 10 of age (0 d of the study), the calves were weighed, checked for signs of diarrhea, omphalitis, and coughing before allocation to the experimental pens. A total of 84 healthy heifer calves were selected and randomly allocated to 14 pens (six calves·pen^{-1}) and were kept indoors for the next 91 ± 2 d of the study. Each indoor calf pen was 6 × 4 m in size which allowed 4 m^2 space per calf during the first 91 ± 2 d of the study. All pens were cleaned before the experiment and were bedded with wood chips. The bedding material was kept dry during the experiment by regularly adding additional wood chips as required. The bedding was completely changed 3 times during the indoor period.

At 10 ± 2 d of calf age (i.e., day 0 of the study), the 14 pens of group-housed calves were allocated either to low (4 L of whole milk·calf^{-1}·d^{-1}; 7 pens) or high (8 L of whole milk·calf^{-1}·d^{-1}; 7 pens) unpasteurized whole milk allowance for the next 63 d. Treatments were balanced for initial age and body weight of calves.

Unpasteurized whole milk was collected daily from the AgResearch dairy farm at Tokanui and was warmed to 40 °C using a heater (Shoof International Ltd. Cambridge, NZ) before being fed to calves. All calves received their daily milk allowance in two meals of equal volume at 8:00 am (morning) and 4 pm (afternoon). In each pen, calves were fed milk through nipples attached to a group feeder (Stallion Milk Maid 10 Teat Milk Feeder, Stallion Limited, Palmerston North, NZ) designed for group-housed calves. There were 10 nipples attached to each milk feeder for 6 calves in a pen to provide enough feeding space and lower the competition at feeding. The milk feeders and nipples were washed and dried after each feeding.

The calves on both treatments were weaned gradually between days 63 ± 2 and 73 ± 2 of the study. The milk volume was reduced by approximately 20% after every 2 d and

Table 1. Chemical composition (% of dry matter, unless otherwise noted) of calf starter (n = 3), grass hay (n = 3) and pasture (n = 4) used in the study

	Calf starter	Grass hay	Pasture
Dry matter, % of fresh feed	87.3	86.6	16.8
Crude protein	18.7	9.5	19.7
Acid detergent fiber	6.6	39.2	28.7
Neutral detergent fiber	13.0	58.1	49.9
Ash	6.7	6.6	9.8
Organic matter	93.3	93.4	90.2
Metabolizable energy, Mcal·kg^{-1} DM	3.20	1.88	2.65
Soluble sugars	6.5	5.6	9.1
Starch	42.7	1.8	1.5
Crude fat	2.3	1.5	3.1
Non-structural carbohydrate	59.2	24.4	17.5
IVOMD[1]	90.1	53.1	77.1
Phosphorus	0.6		0.4
Potassium	0.8		3.2
Sulphur	0.2		0.3
Calcium	1.1		0.5
Magnesium	0.3		0.2
Sodium	0.5		0.1
Iron, mg·kg^{-1}	249		406
Manganese, mg·kg^{-1}	113		67
Zinc, mg·kg^{-1}	114		42
Copper, mg·kg^{-1}	26		8

[1]IVOMD, samples were incubated for 24 h to determine in vitro organic matter digestibility.

calves received no milk on day 74 of the study. Calves in each pen were given *ad libitum* access to clean drinking water, a pelleted calf starter (SealesWinslow Limited, Tauranga, NZ), and chopped grass hay *ad libitum* from day 1 to 91 ± 2 of the experiment (Table 1). In each pen, calf starter and hay were separately offered using plastic feeders (Stallion Limited, Palmerston North, NZ).

All calves remained indoors in their respective pens for 91 ± 2 d of the study to allow postweaning blood sampling, and measurements of feed intake, and body weight. Thereafter, all calves were transferred and transitioned to ryegrass-based pasture, grazed together as a single mob. Calf starter remained available (3 kg^{-1} calf^{-1} d, as fed basis) to the calves during the first 2 wk on pasture. The calf starter was removed gradually during the third week on pasture to put all heifers on 100% pasture (white clover and perennial ryegrass mixed pasture). A similar number of calves from both treatments were weaned and transitioned to pasture. The group size remained dynamic for the first 2 wk until all calves were weaned and transitioned to pasture. At 120 ± 2 days of the study, the calves were moved to the AgResearch Ruakura farm, Hamilton, NZ to graze white clover and perennial ryegrass-based pasture (Table 1) to allow monitoring of growth and sampling as detailed below. In both locations, a rising plate meter was used to determine the herbage mass per hectare and heifers were managed in a rotational grazing system. Throughout the grazing period, pasture availability was unrestricted, with heifers being in large paddocks with high herbage mass (3000–4000 kg Dry Matter (DM·ha^{-1}).

All calves were disbudded by a veterinarian within 3 wk of birth using a hot iron under general anesthesia. All calves received two vaccines at weeks 7 and 11 of the study (second vaccination): (1) Lepto-3-Way (Virbac, Hamilton, NZ), which is an inactivated leptospirosis vaccine containing the three serovars *Hardjo*, *Copenhageni*, and *Pomona*—the serovars most commonly identified with disease in livestock and humans in New Zealand. It also contains alaun as adjuvant and thiomersal as a preservative. (2) Covexin-10 (Merck Sharp & Dohme, Animal Health, Wellington, NZ), which contains 10 clostridial antigens, namely inactivated toxoid from *C. perfringens* type A, *C. perfringens* type B, *C. perfringens* type C, *C. perfringens* type D, *C. novyi, C. septicum, C. tetani, C. sordellii* and *C. haemolyticus*, and inactivated whole culture of *C. chauvoei*. It also contains thiomersal as preservative. Booster shots of both vaccines were given to the heifers in week 25 of the study as per normal farm practice. Calves were monitored daily for signs of diarrhea according to procedures described by McGuirk (McGuirk, 2008) and calves with diarrhea were treated with electrolytes and antibiotics on the recommendation of the herd veterinarian. Calves were treated every 4–6 wk with Reflex Pour-on (1 mL·20 kg^{-1} body weight, Alleva Animal Health, Auckland, NZ) to control internal and external parasites while on pasture.

Measurements, sampling, and analyses

Milk offered and refused in each pen was recorded daily to calculate pen milk intake for the first 73 d of the study. Samples of milk were collected weekly (n = 18) and analyzed at Milk TestNZ, Hamilton, NZ. Milk contained (mean ± SD) fat (4.9 ± 0.5%), protein (3.7 ± 0.1%), lactose (4.9 ± 0.1%), and total solids (14.3 ± 0.6%). Metabolizable energy in milk (5.95 Mcal·kg^{-1} DM) was calculated according to the NRC equation (National Research and Council 2001).

Calf starter and grass hay offered and refused were measured for each pen daily until 91 ± 2 d of study. Samples of each fresh feed (hay, calf starter) were collected fortnightly and pooled, and three samples for each feed were analyzed for DM and nutrient composition at Hill Laboratories, Hamilton, NZ. Calf starter was analyzed using wet chemistry procedures i.e., DM (Association of Official Agricultural Chemists (AOAC) 930.15), Crude Protein (CP) (AOAC 968.06), fat (AOAC 991.36), ash, and organic matter (OM); (AOAC 925.10; AOAC 942.05), neutral detergent fiber (NDF), acid detergent fiber (ADF), *and starch α-amylase (AOAC 996.11) (AOAC, 2012), using an Ankom auto analyzer*. Calf starter samples were subjected to in vitro organic matter digestibility (OMD) testing and digestibility of organic matter in DM (DOMD) was calculated from OMD using the Australian Fodder Industry Association Standard equation (AFIA, 2009). The metabolizable energy in solid feeds was calculated using the equation Metabolizable Energy (ME) = DOMD% × 0.16 (AFRC, 1993). Non-structural carbohydrates of starter feeds were calculated by difference where it = 100 – (CP + ash + crude fat + NDF). Minerals in starter feed were determined by inductively coupled plasma optical emission spectrometry after nitric acid/hydrogen peroxide digestion (Anderson, 1996). Grass hay and four pasture samples were collected and dried at 65 °C for 48 h. Dry matter content and nutritional composition were determined by near-infrared spectroscopy (Hill Laboratories, Hamilton, NZ). At each sampling, multiple pasture samples (> 25) were collected from each paddock just before the start of grazing. A pre-determined height of

5 cm was used to collect the pasture samples. The nutritional composition of the pasture and calf starter is given in Table 1.

All calves were weighed weekly during the indoor period (days 0–91), and then at days 105, 112, 128, 162, 184, and 209 of the study when they were on pasture. Calves were weighed individually using a digital weighing scale monitor (Gallagher TW1 Data Monitor, Hamilton, NZ) attached to a double load-bar scale (Technipharm, Rotorua, NZ). Body girth, hip height, and body barrel were measured at the start of the experiment (day 10 ± 2), at weaning (day 73 ± 3), and postweaning (day 91 ± 3 and d 201 ± 12). Hip heights were determined using a measurement stick, and body girth (smallest circumference behind the forelegs), and body barrel (maximum circumference around the belly) dimensions were measured using a measuring tape while the calf was standing on a flat surface and with its head in an upright position.

Between 4 and 6 h after feeding at all sampling time points, blood samples from the jugular vein were collected into 10 mL serum tubes, (Vacutainer, Becton & Dickinson, NJ, USA) from all calves at the start of the experiment (day 10 ± 2), at weaning (day 73 ± 2), and postweaning at day 91 ± 3, and day 201 ± 12 of the study. After a minimum clotting time of 30 min at ambient temperature, serum blood tubes were centrifuged at room temperature for 15 min at 2000 × *g*, serum harvested, and frozen at –20 °C until analysis for selected metabolites including β-hydroxybutyrate (BHB) (as a marker of rumen development), glucose, total protein, blood urea nitrogen (BUN), triglycerides, and non-esterified fatty acid (NEFA) concentrations. All blood metabolite tests were performed using the P800 module of the Roche Modular platform (New Zealand Veterinary Pathology, Hamilton, NZ).

Blood samples for antibody testing from all calves were collected by jugular venipuncture into serum tubes (Becton Dickinson, Franklin Lakes, NJ, USA) one day before vaccination, post-vaccination (10–14 d after second vaccination; week 13 of the study), and post-booster (10–14 d after booster vaccination; week 27 of the study). After a minimum clotting time of 30 min at ambient temperature, blood tubes were centrifuged for 15 min at 2000 × *g*, serum was harvested and stored at –80°C. Serum immunoglobulin (IgG) antibodies binding to antigens from *Clostridia* and *Leptospira* were determined via an in-house ELISA (Hopkirk Research Institute, Palmerston North, NZ). Two commercial veterinary vaccines were used as antigens: (1) Leptoshield 3 (Zoetis), which is an inactivated leptospirosis vaccine containing the three serovars *Hardjo, Copenhageni,* and *Pomona,* and thiomersal as preservative. (2) Ultravac 5in1 (Zoetis, Parsippany-Troy Hills, NJ, USA), which contains five clostridial, namely, inactivated toxoid from *C. perfringens* type D, *C. novyi* type B, *C. septicum,* and *C. tetani*, and inactivated whole culture of *C. chauvoei*. It also contains an aluminum salt as adjuvant and thiomersal as preservative. Using these antigens, vaccination-induced antibodies specific to *Clostridium perfringens* type A, *C. perfringens* type B, *C. perfringens* type C, *C. sordellii,* and *C. haemolyticus* remained undetected. Vaccines were diluted 1:10 in carbonate buffer, pH 9.6, 50 µL loaded into 96 well plates (Nunc MaxiSorp; Nunc, Roskilde, Denmark), and incubated overnight. The plates were then washed 3× with phosphate buffered saline (PBS) and blocked with PBS Tween 20 (PBST), containing casein (1% w·v^{-1}) and thimerosal (1:10,000 dilution; Sigma–Aldrich) for 30 min at room temperature. Serum samples were diluted 1:10 in PBST containing casein and thimerosal. 50 µL of diluted serum per well was added to the plates and incubated for 2 h at room temperature. The plates were washed 3× with PBS, conjugate (1:6,000 sheep anti-bovine Immunoglobulin G (IgG) Horse Radish Peroxidase (HRP), Bio-Rad, Hercules, CA, USA) was added (100 µL·well^{-1}), the plates were incubated for 1 h at room temperature, then washed five times in PBST. TMB substrate (3,3′,5,5′-tetramethylbenzidine, Sigma–Aldrich) was added and plates were incubated until the color had developed (≤ 30 min at room temperature). Reactions were stopped by adding 50 µL of 0.2M sulfuric acid and plates read at 450 nm using an ELISA plate reader (VERSAmax microplate reader; Molecular Devices Corporation, San Jose, CA, USA). Antibody levels were expressed as optical density (OD) readings at a serum dilution of 1:10.

Ultrasonography and image capture was performed by two people, on each mammary gland quarter of 60 heifer calves (5 of 7 pens·treatment^{-1}, 30 heifers·treatment^{-1}, resulting in 73%–80% statistical power), on three separate occasions: at the start of the study (day 10 ± 2; scan 1), at weaning (day 74 ± 3, scan 2), and once at postweaning (day 201 ± 12; scan 3) according to the method described in Molenaar et al. (2020) with some modifications. Calves in two pens did not receive ultrasonography to allow the future possibility of testing if there was an effect of scanning pre-weaning calf mammary glands on subsequent milk production. In the first image collection, due to the difficulty of obtaining images from standing small calves, the calves were restrained on their backs and ultrasound images of each quarter of the mammary glands were captured in a perpendicular and transverse plane to the calf's body using a SonoSite M-Turbo scanner with a 5 MHz 65mm sectorial probe (Fujifilm, Greenwood, SC, USA) using the minimum pressure required to capture images. The four quarters of the mammary gland were scanned with the transducer adjacent to the rearward aspect of the teats. At the second and third scans of the mammary gland, the mammary ultrasound protocol was repeated for each animal except that the heifers were restrained in a chute in a standing position, and mammary glands were scanned from the rear. Food grade vegetable oil was applied to the udder to ensure sufficient sonic contact between the probe and udder for high-quality ultrasonography. The probe was based directly beside the teat with the transducer in a parallel and perpendicular position relative to the body, angled at ±10° from the vertical depending on the side being examined, to obtain the maximum mammary image depth. The selected penetration depth of the ultrasound was adjusted as required to capture images. This allowed for the full dimension of the gland to be captured in the image. After capturing images, scoring was facilitated by collating animal numbers, image data, and live links to the images in Excel (Microsoft Office Professional Plus 2013). After size adjustment to match the image scan depth, captured images were manually scored by an operator blinded to the treatments. Images were displayed on a computer monitor, sized adjusted to match the scan depth, and a ruler was used to measure total mammary gland depth, combined cistern, and parenchymal depth, and fat pad depth in millimeters, directly off the screen. Fat pad tissue is hyperechoic and thus identified as the white contrast in the ultrasonic image, compared to parenchymal tissue (hypoechoic) which appeared as a darker shade on the image (Esselburn et al., 2015). Hence the mammary components were scored using conservative and liberal measures (Supplementary Figure S1) as described

by (Molenaar et al., 2020). Mammary gland measurements for forequarters and hindquarters were separately averaged for statistical analysis.

Statistical analyses

The pen was considered the experimental unit for all analyses; thus, individual animal responses were averaged by pen at each time point. R version 3.5.1 (R-Core-Team, 2019) was used for exploratory and basic statistical analysis. Data on mammary gland parameters were averaged for the front (forequarters) and back (hindquarters) mammary glands within an animal and analyzed separately. An ANOVA was performed using the base R "ANOVA" function where treatment was a fixed effect to analyze overall feed intake parameters. Daily nutrients and feed intake, body weight (for indoor and outdoor periods separately; indoor period = day 0–91 of the study, and outdoor period = day 92–209), average daily gain (preweaning, weaning, and postweaning), body measurements (body girth, hip height, and body barrel), blood metabolites, mammary gland parameters, and antibody test responses (pre-vaccination, post-vaccination, and post-booster) were analyzed as repeated measures using Restricted Estimate Maximum Likelihood analysis in lme4 R package version 1.1-17 (Bates et al., 2015). For the repeated measure analyses, time (sampling day of the study), treatment (high and low milk allowance), and their interaction (treatment × time) were used as fixed effects, and pen was used as the random effect. Body weight at the start of the trial and overall ADG were used as covariates for the analysis of mammary gland growth parameters. For blood metabolites, concentrations at the start of the study are used as a covariate. Subsequent statistics such as treatment means, and 95% confidence intervals or SEM were calculated using the predictmeans R package version 0.99 (Luo et al., 2014). The responses were fitted as a third-order polynomial over time. A total of 95% confidence intervals were calculated using the "covariate-means" function in the predictmeans R package. *P*-values for all model terms were evaluated using the lmerTest R package version 3.0-1 (Kuznetsova et al., 2017). The significant level was set as $P < 0.05$.

Results and Discussion

Overall, all calves remained healthy during the study with occasional diarrhea observed and they were treated according to the advice of a veterinarian. During the trial, no mortality, and no adverse behavior e.g. vocalization related to hunger or cross-sucking was observed in calves fed low or high milk allowance.

Feed and nutrient intake

Milk refusal was observed in high milk fed calves during the first week of the study. Thereafter, except for sick calves, calves on both treatments were consuming their milk allowance within 30 min. In contrast to our finding, where ad libitum milk allowance is offered from birth (Borderas et al., 2009; Frieten et al., 2017), calves start consuming 8 L·d^{-1} of milk by 10 days. In our study, 10 d old calves offered higher milk allowance took a week to achieve the full intake. Mean liquid milk intake (L·pen^{-1}·d^{-1}) during the experiment was 23.8 L and 44.8 L for high and low milk fed calves. In agreement with our result (Table 2), studies conducted with Holstein Friesian calves have demonstrated that calves can safely consume around 20% of their BW (Borderas et al., 2009; Frieten et al., 2017). Interaction between milk allowance × days on the study ($P < 0.01$) was significant for daily intake of hay, starter, and total solid (hay plus starter) feed DM intake (Figure 1). Calves receiving low milk allowance start consuming greater amounts of calf starter from week 4 of the study, and thereafter dry matter intake (DMI) of both calf starter, and total solid feed remained greater for calves that received low vs. high milk allowance during preweaning (Figure 1). Dry matter intake of calf starter and total solid feed was not influenced by milk allowance during postweaning (Figure 1). Overall, during the study, calves on high vs. low milk allowance had similar hay intake but a lower starter and solid feed intake (Table 2). Our result agrees with previous findings where greater milk allowance depressed the starter consumption (Jasper and Weary, 2002; Borderas et al., 2009). However, in contrast to previous findings (Jasper and Weary, 2002) the solid feed intake immediately after weaning was similar between low and high milk fed calves. Published literature indicates that housing, feeding, and weaning management influence the solid feed intake in calves (Costa et al., 2016; Khan et al., 2016). The calves in our study were reared in small groups with *ad libitum* availability of calf starter and hay from feeding buckets and were gradually weaned over 10 d. These factors may have contributed to promoting solid feed consumption during weaning(Khan et al., 2016) and thus reduced the negative influence of feeding high milk allowance on postweaning solid feed intake.

Interaction between milk allowance × days in the study was significant ($P < 0.001$) for daily total DMI (Figure 2). Total DMI was greater for the first 6 wk in high vs. low milk fed calves. After 6 wk, calves on low allowance started eating comparable DMI to that of high milk fed calves (Figure 2). Calves with high milk allowance consumed more ($P < 0.001$) total feed DM overall during the whole study compared with calves offered low milk allowance (Table 2). The contribution of calf starter DM to daily total DM intake was affected ($P < 0.001$) by milk allowance × days in the study (Figure 2). After the first week of the study, the contribution of calf starter DM in daily total DM intake was greater in calves that

Table 2. Pen mean (± SEM) intake of milk (day 0–63), solid feeds (day 0–91) and nutrients (day 0–91) of group-housed heifer calves offered low (4 L·d^{-1}; $n = 7$) or high (8 L·d^{-1}; $n = 7$) milk allowance[1]

Intake, kg·pen^{-1}·day^{-1}	Low	High	*P*-value
As-fed milk intake	23.8 L	44.8 L	
Milk DM	3.4 ± 0.1	6.4 ± 0.1	0.001
Hay DM	2.1 ± 0.1	2.0 ± 0.1	0.210
Calf starter DM	7.2 ± 0.4	5.2 ± 0.4	0.009
Total solid feed DM[2]	9.2 ± 0.4	7.2 ± 0.4	0.004
Total DM[3]	11.5 ± 0.3	12.0 ± 0.3	0.001
Total crude protein	2.1 ± 0.1	2.4 ± 0.1	0.001
Total metabolizable energy, Mcal·kg^{-1} DM[4]	41.6 ± 1.1	49.9 ± 1.1	0.001
Total NDF[5]	2.1 ± 0.1	1.9 ± 0.1	0.001

[1]Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study, kept indoors until day 91 in pens (six heifer calves·pen^{-1}). [2]Solid feed dry matter (DM) = hay + calf starter; [3]total DM = milk DM + Solid feed DM. [4]Megacalorie Mcal·kg^{-1}. [5]Neutral Detergent Fiber (NDF)

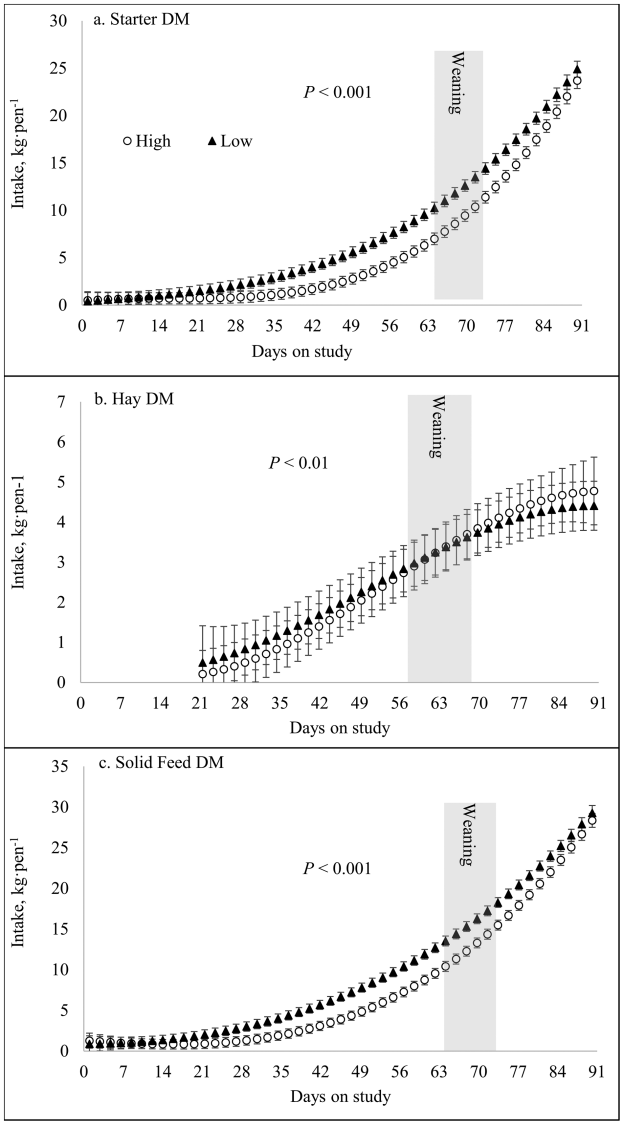

Figure 1. Pen mean (95% CI) daily dry matter (DM) intake of starter (a), hay (b), and total solid feed (starter plus hay; c) by group-housed calves offered low (4 L·d^{-1}; n = 7 pens) or high (8 L·d^{-1}; n = 7 pens) milk allowance. Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study, kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}). Effects of milk allowance, days on the study, and milk allowance × days on the study were significant ($P < 0.001$) for starter DM intake and total solid feed DM intake. Milk allowance was not significant ($P > 0.1$), days on the study ($P < 0.001$), and milk allowance × days on the study ($P < 0.01$) were significant for hay DM intake.

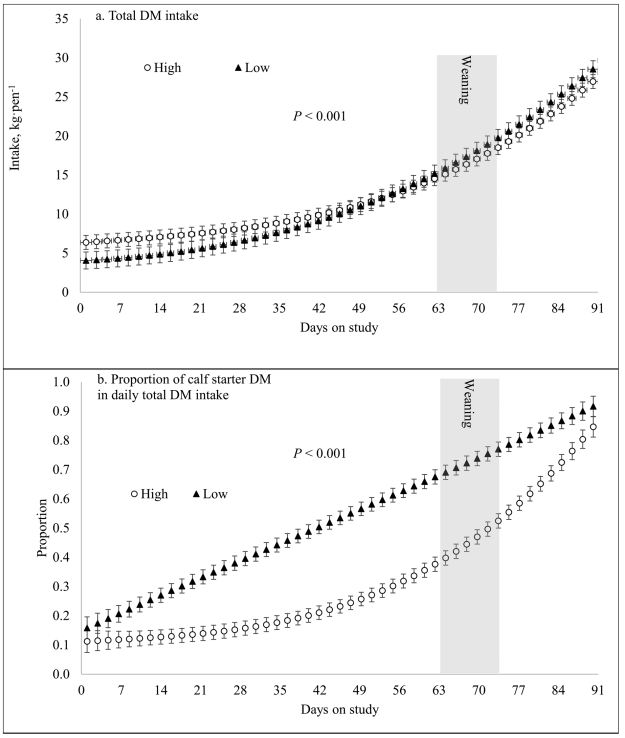

Figure 2. Pen mean (95% CI) daily intake of total dry matter (DM) (milk, calf starter, and hay; (a)), and proportion of calf starter DM in daily total DM intake (b) by group-housed calves offered low (4 L·d^{-1}; n = 7 pens) or high (8 L·d^{-1}; n = 7 pens) milk allowance. Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study and kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}). The effect of milk allowance was not significant ($P > 0.1$) for total DM intake. Effects of days on the study and milk allowance × days on the study were significant ($P < 0.001$) for total DM intake. The proportion of calf starter DM in daily total DM intake was influenced ($P < 0.001$) by milk allowance, days on the study, and milk allowance × days on the study.

received low vs. high milk allowance. Interaction between milk allowance × days in the study was significant ($P < 0.001$) for daily CP and ME intake (Figure 3). Compared with calves fed a low amount of milk, the calves on high milk allowance consumed more ME and CP before weaning, while ME and CP intakes were similar between high vs. low milk fed calves after weaning (Figure 3). Interaction between milk allowance × days in the study was significant ($P < 0.001$) for NDF intake (Figure 3). During pre-weaning, the calves on low milk allowance consumed more NDF than high milk fed calves which were driven by differences in solid feed intake. However, hay and therefore NDF intake was higher for high vs. low milk fed calves during post-weaning. Our findings agree with previous studies (Khan et al., 2011a; Curtis et al., 2018) which found that despite consuming a significantly greater amount of calf starter, calves reared on low milk allowance were unable to match total DM, CP, and ME consumption compared to high milk fed calves during the first 6 wk of age. Therefore, feeding calves high allowances of milk, at least during the first 6 wk of life, is desirable to reduce hunger, and to increase the supply of protein and energy to the calves for better preweaning growth (de Paula Vieira et al., 2008; Khan et al., 2011a; Khan et al., 2011b). However, efforts should also be focused on finding management strategies, and improving energy and protein density in starters for low milk fed calves to promote nutrient supply during the first 6 wk of age.

Growth performance

The body weight of calves was separately analyzed for both the indoor (day 0–91 of the study) and outdoor pasture grazing periods (day 91–209; Figure 4a). The effect of milk allowance was significant ($P < 0.001$) for the BW of calves during the indoor period. Interaction between milk allowance and days in the study was not significant for the BW of calves during the indoor period. The body weight of the calves at the start of the study was similar ($P = 0.71$), calves on high

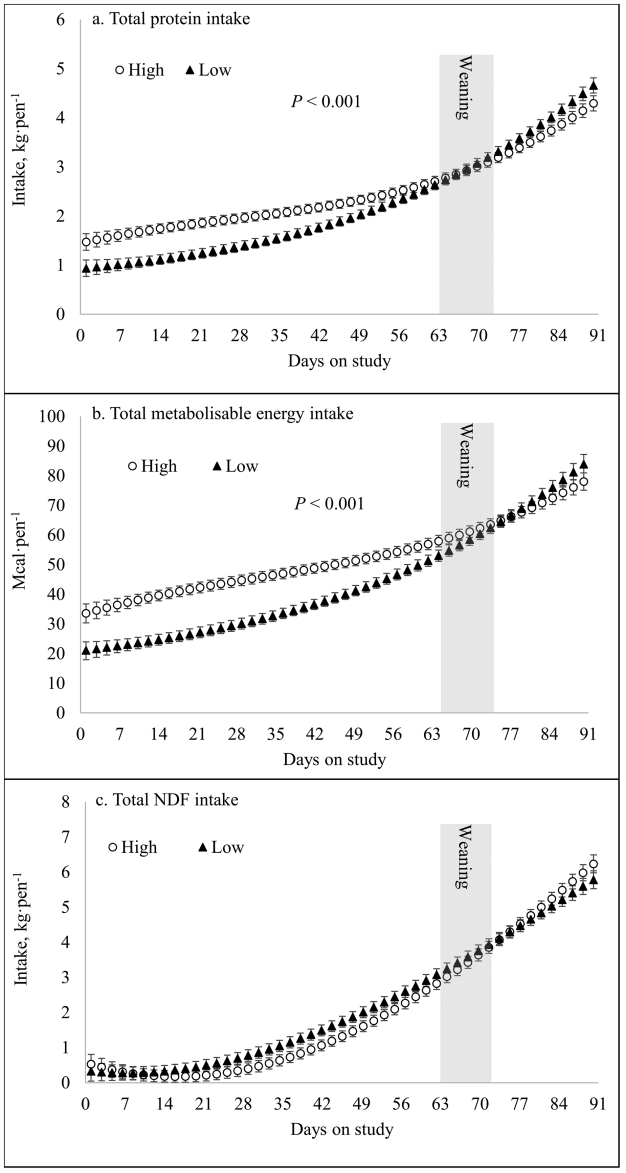

Figure 3. Pen mean (95% CI) daily total intake (milk, calf starter, and hay) of crude protein (a), metabolizable energy (b), and neutral detergent fiber (NDF) (c) by group-housed calves offered low (4 L·d^{-1}; n = 7 pens) or high (8 L·d^{-1} n = 7 pens) milk allowance. Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study and kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}). Effects of milk allowance, days on the study, and milk allowance × days on the study were significant (P < 0.001) for crude protein and metabolizable energy intake. The effect of milk allowance was not significant (P = 0.08) for NDF intake. Effects of days on the study and milk allowance × days on the study were significant (P < 0.001) for NDF intake.

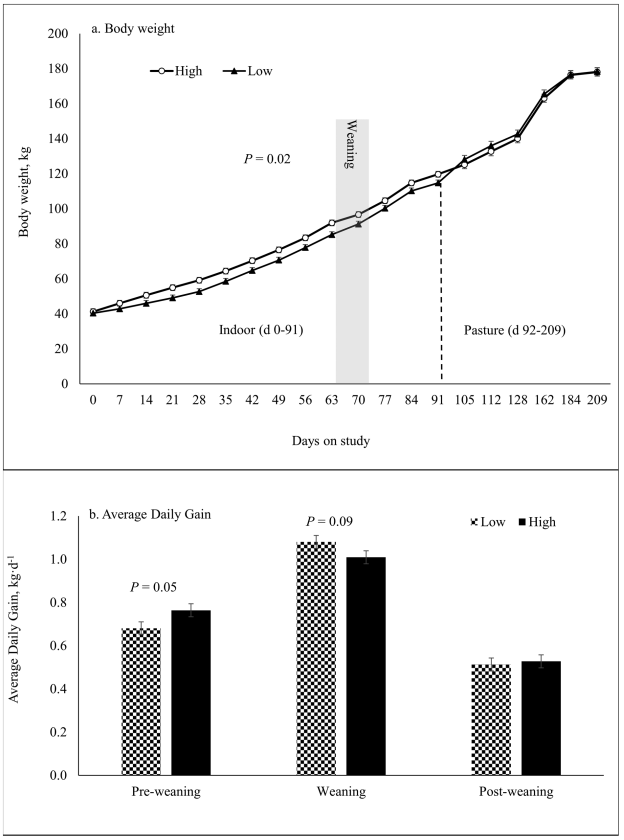

Figure 4. Pen mean (± SEM) body weight (a) and average daily gain (b) of group-housed calves offered low (4 L·d^{-1}; n = 7 pens) or high (8 L·d^{-1}) milk allowance (n = 7 pens). Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study, kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}) and then on pasture until the end (day 209) of the study. Bodyweight of calves was separately analyzed for indoor (d-0-91 of the study) and outdoor pasture periods (day 91–209). Effects of milk allowance and days in the study were significant (P = 0.02 and P < 0.001) respectively for the bodyweight of calves during the indoor period. Interaction between milk allowance and days in the study was not significant for the bodyweight of calves during the indoor period. Effects of milk allowance and interaction between milk allowance and days in the study were not significant (P = 0.09 and P > 0.1) respectively for the bodyweight of calves outdoor period. Days of study influenced the bodyweight of calves during the outdoor period. The average daily gain was not influenced by treatment however period and treatment × period interaction (preweaning, weaning, and post-weaning) were significant (P < 0.001).

milk allowance were heavier compared to calves fed less milk at the start of weaning (P = 0.02). Interaction between ADG and various periods (preweaning, weaning, and postweaning) was significant (P = 0.005; Figure 4b). The ADG was greater for high vs. low milk fed calves during the preweaning period (P = 0.05). During weaning, there was a trend toward greater ADG in low vs. high milk fed calves whereas ADG was similar between high and low milk fed calves during the postweaning period. Greater growth during the first 63 d in calves fed high vs. low milk allowance agrees with previous findings (Jasper and Weary, 2002; Borderas et al., 2009; Khan et al., 2011a) where greater milk allowance promoted preweaning growth. In our study, greater ADG and BW at 63 d could be attributed to greater DM, CP, and ME intake in calves fed high vs. low milk allowance. Interestingly, ADG during the weaning phase was around 1 kg·d^{-1} and was similar for calves that received low and high milk allowance. We suggest that rearing calves in small groups to promote peer learning (de Paula Vieira et al., 2008), weaning calves after 8 wk of age, gradual reduction of milk over 10 d (Khan et al., 2011b), and availability of a variety of feeds (Costa et al., 2016) have contributed to promoting solid feed intake in calves and therefore resulted in comparable ADG between calves fed high vs. low milk allowance during weaning. Effects of milk allowance and interaction between milk allowance and days in the study were

not significant (P = 0.08 and P > 0.1) for the BW of calves during the outdoor period (Figure 4a). The ADG between low and high milk fed calves was similar during postweaning on pasture (Figure 4b). This indicates that the provision of high vs. low milk allowance does not influence the postweaning growth performance of heifers under grazing conditions. However, postweaning ADG of heifers in our experiment was lower (0.52 ± 0.04, kg) than Holstein Friesian reared on TMR for indoor system (around 1 kg, (Rice et al., 2019)). In NZ, the majority of the dairy cows calve in spring (July–September). Replacement heifers are weaned onto pasture which coincides when pasture quality is declining in summer which limits the pasture intake and impedes the growth of the heifers (Burggraaf et al., 2020). Therefore, the lower ADG between this and other studies could be attributed to different genetics of the animals and postweaning feed quality. Body girth, body barrel, and hip height of group-housed heifer calves were not influenced by milk allowance at any measurement time (Table 3). Interaction between measurement time and milk allowance was also not significant for skeletal parameters. These results indicate that feeding dairy replacement calves 8 vs. 4 L of milk enhances preweaning growth without negatively affecting their postweaning growth on pasture until 7 mo of their age. Further, the provision of high-quality pasture or supplementary feeds should be explored to improve the postweaning growth of NZ replacement heifers.

Selected blood metabolites

Mean serum concentrations of selected metabolites are presented in Figure 5. Sampling time influenced (P < 0.001) all metabolites. Interaction between milk allowance and sampling time was significant (P < 0.05) for BUN, total protein, and triglycerides. Effects of milk allowance and interaction between milk allowance and sampling time were not significant for the rest of the metabolites. With the dietary shift from milk to solid feeds, the ability of calves to metabolize SCFA arising from rumen fermentation increases the circulating BHB (Baldwin et al., 2004). Circulating BHB can indicate the extent to which the rumen epithelium is developed and functioning (Quigley et al., 1991). Similar BHB concentration and similar ADG during postweaning in all calves indicate that calves that received high milk allowance have achieved a similar development of the rumen epithelium to calves fed low milk allowance immediately after weaning. Similar levels of serum glucose, BHB and NEFA indicate that preweaning milk allowance has no major effect on rumen development and energy metabolism in calves by weaning and during the postweaning period on pasture. Significantly higher concentrations of circulating triglycerides in low vs. high milk fed calves at weaning may be attributed to the higher starter and therefore fat consumption. Interestingly, serum total protein tended to be greater (P = 0.05) in high vs. low milk fed calves during postweaning sampling points. However, BUN concentrations at weaning and postweaning were greater (23.4%, P = 0.03 and 20%, P = 0.01, respectively) in low vs. high milk fed calves. In pre-weaned calves, BUN production is largely originating from milk AA (Quigley et al., 1991), however, fermentation and degradation of available plant protein in the rumen regulate the BUN concentrations in weaned calves (Khan et al., 2016; Batista et al., 2017). In our study, BUN was greater at weaning and postweaning sampling time points when calves consumed a similar amount of crude protein and energy (Figure 3). We are unable to establish a cause and effect of high BUN observed in low versus high milk fed calves. However, we postulate that these differences in total protein and BUN after calves were weaned and had similar nutrient intake could be attributed to the physiological changes at ruminal and cellular levels related to urea recycling and protein metabolism (Khan et al., 2016; Batista et al., 2017; Gerrits, 2019), which warrants further investigation.

Antibody responses

Anti-*Clostridia* and *Leptospira* IgG were detected in pre-vaccination serum and were similar in both high and low milk fed calves (Figure 6). Post-vaccination, calves on high and low milk allowance had greater concentrations of anti-*Clostridia*

Table 3. Pen mean (± SEM) body girth, body barrel, and hip height of group-housed heifer calves offered low (4 L·d^{-1}; n = 7) or high (8 L·d^{-1}; n = 7) milk allowance (treatment)[1]. The P-values are from a mixed-effect model with time (entire trial), treatment, and interaction as the main effects, and pen as the random effect

Parameters, cm·calf^{-1}	Time	Treatment		P-values		
		Low	High	Treatment	Time	Treatment × time
Body girth	Start of the study day 0	78.5 ± 1.1	78.6 ± 1.1	0.68	0.001	0.64
	End of weaning day 73	110.6 ± 1.1	111.8 ± 1.1			
	Postweaning (indoors, day 91)	116.7 ± 1.1	118.2 ± 1.1			
	Postweaning (pasture, day 201)	136.3 ± 1.2	135 ± 1.2			
Body barrel	Start of the study day 0	79.5 ± 1.4	79.6 ± 1.4	0.52	0.001	0.92
	End of weaning day 73	129.2 ± 1.4	127.3 ± 1.4			
	Postweaning (indoors, day 91)	133.3 ± 1.4	132.3 ± 1.4			
	Postweaning (pasture, day 201)	160.9 ± 1.6	160.4 ± 1.6			
Hip height	Start of the study day 0	77.5 ± 0.7	77.7 ± 0.7	0.56	0.001	0.45
	End of weaning day 73	92.2 ± 0.7	93.6 ± 0.7			
	Postweaning (indoors, day 91)	94.8 ± 0.7	95.7 ± 0.7			
	Postweaning (pasture, day 201)	112.1 ± 0.8	111.1 ± 0.8			

[1]Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64–73 of the study, kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}), and then on pasture until the end (day 209) of the study.

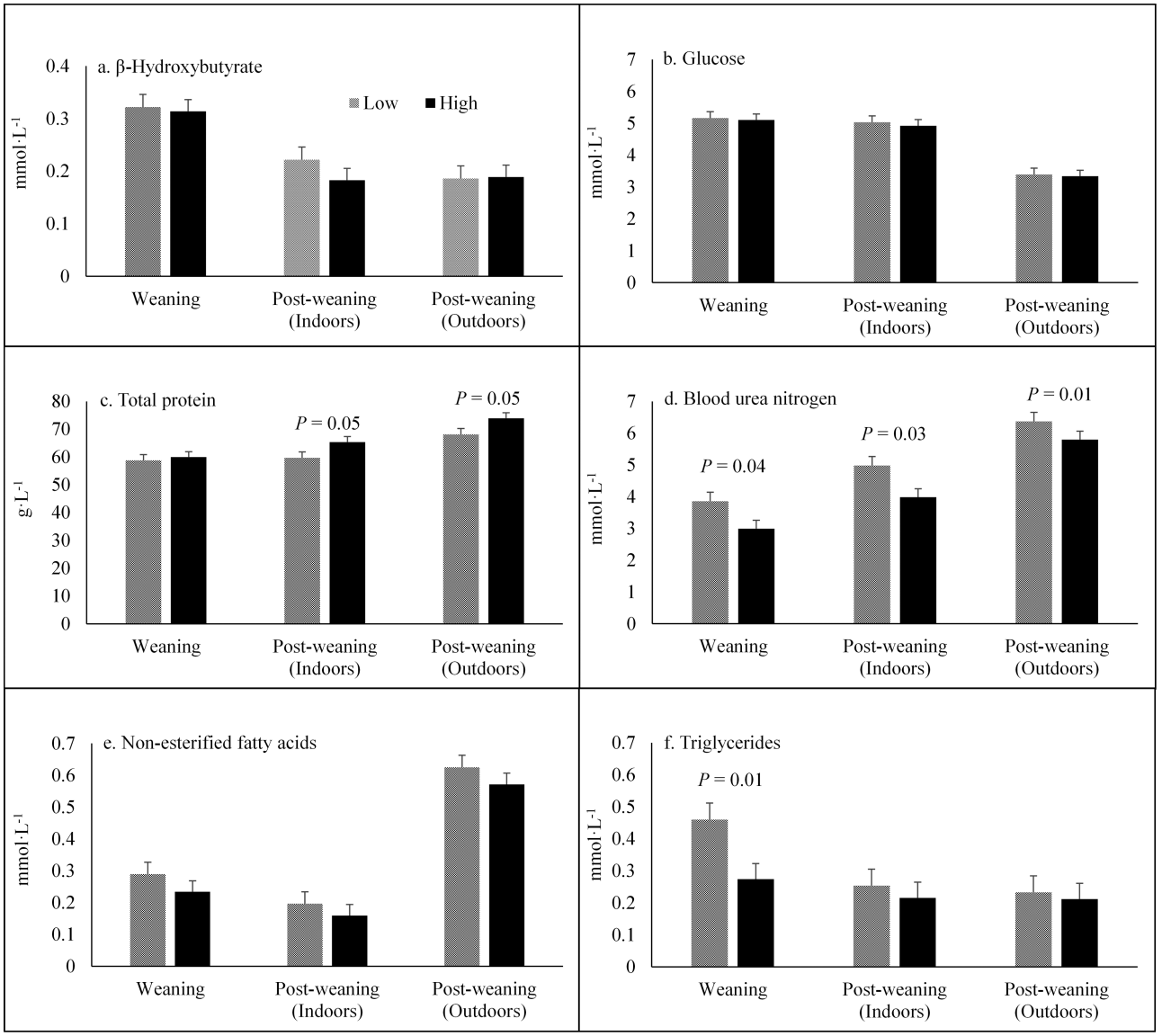

Figure 5. Means (± SEM) serum concentrations of β-hydroxybutyrate (a), glucose (b), total protein (c), blood urea nitrogen (d), non-esterified fatty acids (e), and triglycerides (f) in group-housed calves offered low (4 L·d^{-1}; n = 7 pens) or high (8 L·d^{-1}; n = 7 pens) milk allowance. Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study, kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}), and then on pasture until the end (day 209) of the study. Start, day 0 of study; weaning, 73 d of study; post-weaning (indoor), 91 d of study, post-weaning (pasture) 201 d of the study. Sampling time influenced (P < 0.001) all metabolites. Interaction between milk allowance and sampling time was only significant for total protein, blood urea nitrogen, and triglycerides (P < 0.05).

and anti-*Leptospira* IgG compared to pre-vaccination levels (P < 0.05). This increase in antigen-specific antibodies is the desired result after vaccination (Janeway et al., 2001) but it cannot be excluded that natural exposure to these pathogens contributed to the antibody response. At 13 wk of the study, the concentrations of antibodies against both *Clostridia* and *Leptospira* were significantly (P < 0.05) greater in calves fed greater vs. lower milk allowance, protecting at a time when maternal antibodies begin to wane (Windeyer and Gamsjäger, 2019). However, the concentrations of antibodies against both *Clostridia* and *Leptospira* were similar after booster vaccination (27 wk of the study). The increase in IgG concentrations from pre- to post-vaccination was more pronounced in calves fed greater milk allowance (*Leptospira* P = 0.0007, *Clostridia* P = 0.06), indicating that the greater milk allowance might have supported the primary antibody response. Although there was a further increase in antibody levels after the booster vaccine in both groups, there was no difference between treatments regarding the increase of IgG against *Leptospira* and *Clostridia* from pre-vaccination to post-booster, indicating that the antibody response in low milk fed calves had caught up to the antibody response level of the high milk fed calves. Our findings appear contradictory to previous studies (Nonnecke et al., 2003; Hengst et al., 2012) where the antibody response was largely unaffected by the amount and composition of milk replacer fed to calves. Our results demonstrate that feeding more unpasteurized whole milk has positively influenced the antibody response in calves around weaning and calves who received high milk allowance have acquired stronger immunity against *Clostridia* and *Leptospira* earlier than calves fed low milk allowance. Having a more effective immune response at an early age in high

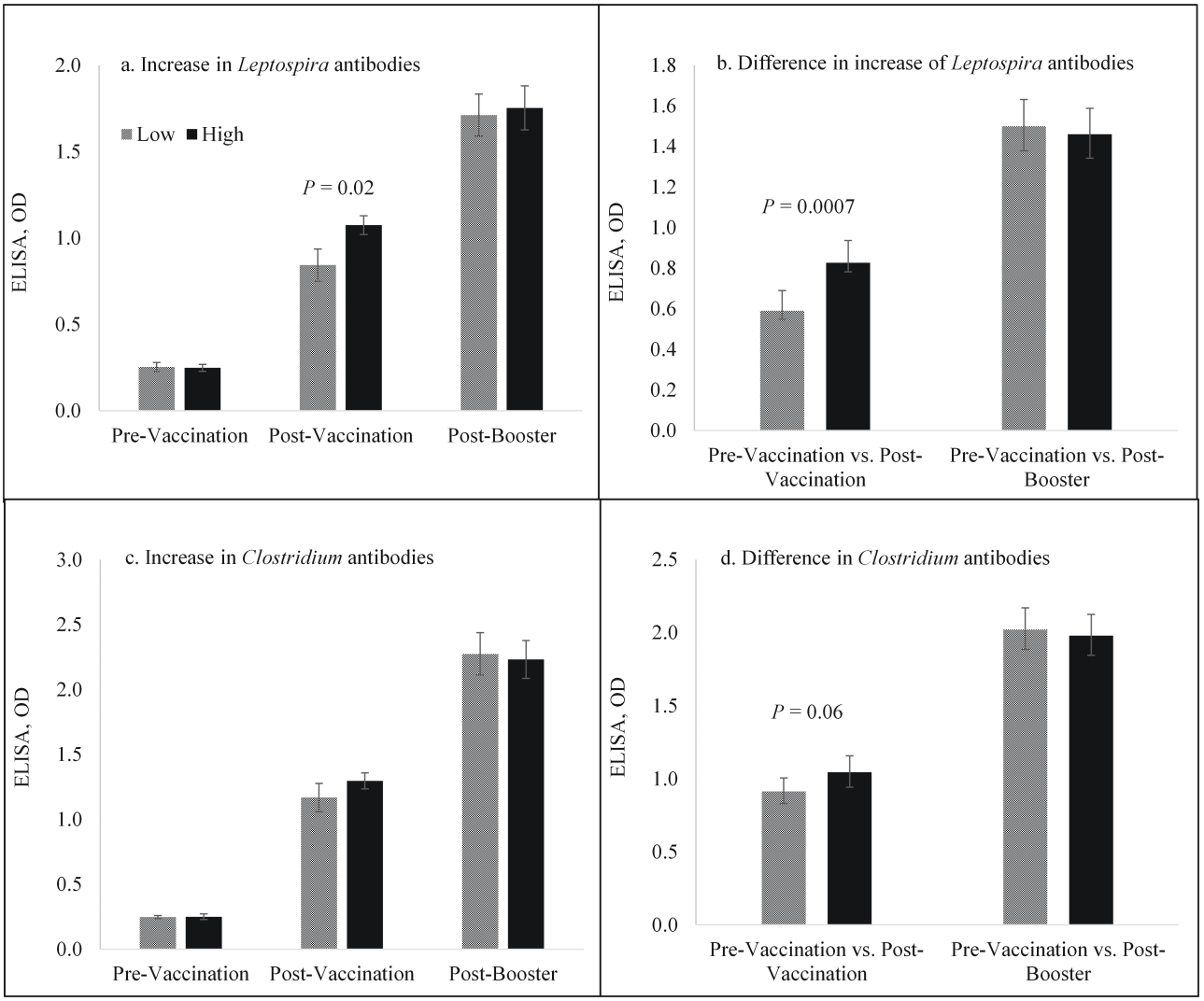

Figure 6. Antibody response by group-housed calves offered low (4 L·d^{-1}; n = 7 pens) or high (8 L·d^{-1}; n = 7 pens) milk allowance. Pen mean (95% CI) serum antibodies against *Leptospira* (a) and *Clostridium* (c) at pre-vaccination (week 6 of the study), post-vaccination (week 13 of the study) and post-booster (27 wk of the study). Pen mean (95% CI) difference in increase of serum antibodies against *Leptospira* (b) and *Clostridium* (d) from pre-vaccination to post-vaccination and post-booster, Unpasteurized whole milk was fed to all calves from day 0 (day 10 of calf age) to 73 of the study. All calves were weaned between day 64 and 73 of the study, kept indoors until day 91 of the study in pens (six heifer calves·pen^{-1}), and then on pasture until the end (day 209) of the study. Antibody levels were expressed as optical density (OD) readings at a serum dilution of 1:10. Effects of milk allowance (treatment), sampling time (time), and milk allowance × sampling time were significant (P < 0.001) for serum antibodies against *Leptospira*. Effects of milk allowance and sampling time were significant (P < 0.001), and milk allowance × sampling time was not significant (P = 0.09) for serum antibodies against *Clostridium*. Differences in serum antibodies against *Leptospira* and *Clostridium* between pre-vaccination and post-vaccination were affected (P > 0.0007 and P < 0.06, respectively) by milk allowance. Differences in serum antibodies against *Leptospira* and *Clostridium* between pre-vaccination and post-booster were not significant (P > 0.1) between high and low milk fed calves.

vs. low milk fed calves is an interesting finding which may contribute to reducing the risk of disease in calves during the high disease risk preweaning period (Cuttance et al., 2017; Urie et al., 2018). The underlying mechanism might be that compared with calves fed low milk allowance, the calves offered high milk allowance have consumed more energy and protein before and during the first vaccination. In previous studies, animals were fed different types and amounts of milk replacers (Foote et al., 2005; Hengst et al., 2012) instead of whole milk, and therefore the calves in those studies may have been partly deprived of nutrients and bio-actives from whole milk (Gill et al., 2000; Shingfield et al., 2008) that can influence the development of the immune system and its functionality in calves.

Mammary gland growth

Mammary gland depth and fat pad depth increased (P < 0.01) with the age of the heifer calves (Figure 7). Interaction between treatment and scan time was significant (P < 0.05) for hindquarter (back) parenchyma depth, fat pad depth, and parenchyma to fat ratio. Similar results were obtained using both scoring methods (liberal Figure 7, conservative Supplementary Figure S2). There was no significant difference in mammary gland total depth in calves receiving high vs. low milk allowance at weaning or by 7 mo of age in grazing heifers. There was also no significant effect of milk allowance on mammary gland component measures at weaning, but significant differences were observed at 7 mo of age in animals fed grass since weaning. A significant increase in

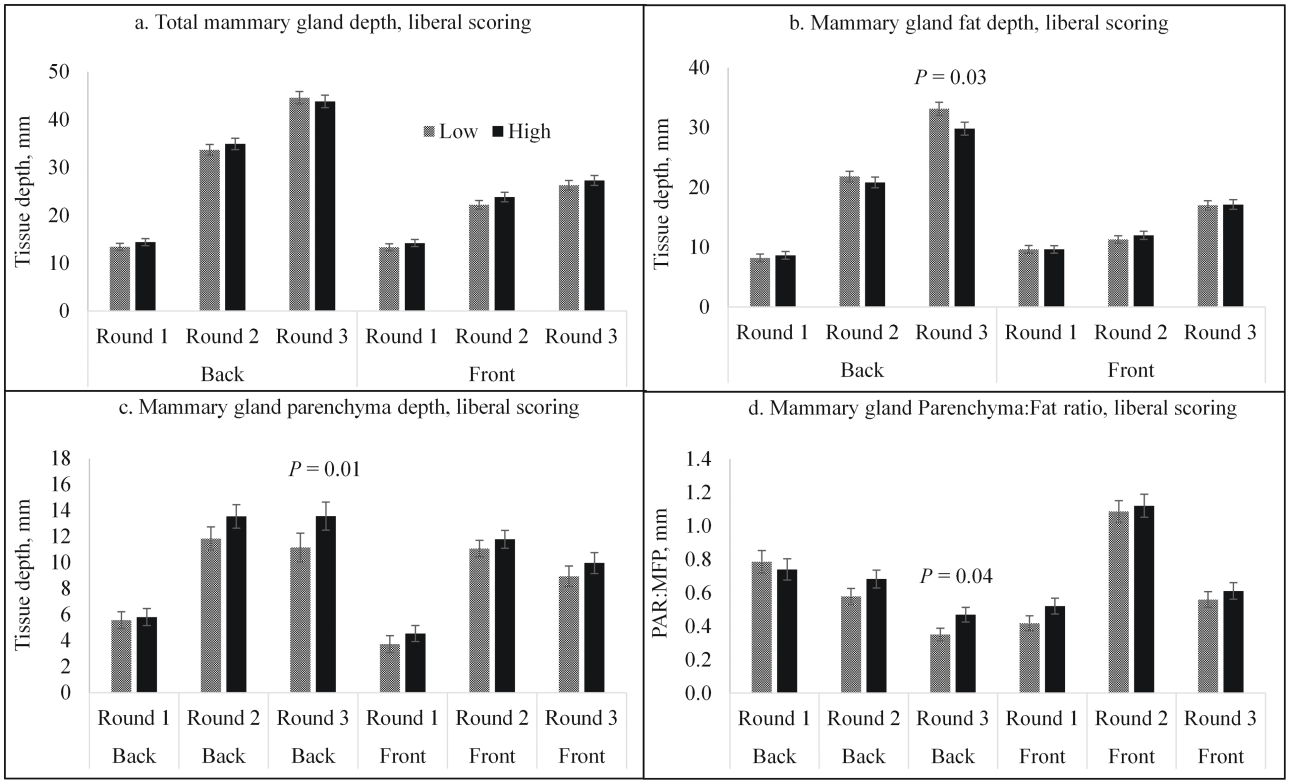

Figure 7. Mammary gland tissue measurements derived from liberal scoring of ultrasound images of group-housed calves offered low (4 L·d^{-1}) or high (8 L·d^{-1}) milk allowance. Ultrasonography was performed on each mammary gland quarter of 60 heifer calves (5 of 7 pens·treatment^{-1}, 30 heifers·treatment^{-1}, resulting in 73%–80% statistical power) on three separate occasions: at the start of the study (day 10 ± 2; scan 1) then once at weaning (day 74 ± 3, scan 2), and once at post-weaning (day 201 ± 12; scan 3). Numbers 1–3 on the *x*-axis indicate the scanning round. A total of four measurements are plotted. a. Total mammary gland depth (tissue depth (mm)); b. Mammary gland Fat Pad depth (MFP); c. Mammary gland PARenchyma depth (PAR); and d. Mammary gland PARenchyma to Fat Pad ratio (PAR:MFP). Mammary gland measurements for forequarters (front) and hindquarters (back) were separately averaged for statistical analysis. There was no significant difference in mammary gland total depth in calves receiving high vs. low milk allowance at weaning or by 7 mo of age in grazing heifers. There was also no significant effect of milk allowance on mammary gland component measures at weaning, but significant differences were observed at 7 mo of age in animals fed grass since weaning. A significant increase in hindquarters (back) PAR depth (P = 0.01) and a decrease in hindquarters (back) PAR:MFP depth (P = 0.03) were observed with a high vs. low milk allowance. These changes resulted in a greater (P = 0.04) hindquarters (back) PAR:MFP ratio with a high vs. low milk feeding level.

hindquarters (back) mammary gland parenchyma depth (P = 0.01) and a decrease in hindquarters (back) fat pad depth (P = 0.03) were observed with a high vs. low milk allowance. These changes resulted in a greater (P = 0.04) hindquarters (back) parenchyma to fat pad ratio with a high vs. low milk feeding level. These results support the previous studies which have reported a positive effect of preweaning nutrition on mammary gland growth (Geiger et al., 2016) with respect to parenchymal development. In contrast, others have reported no effect of preweaning nutrition on parenchymal development or have shown positive effects on fat pad development (Meyer et al., 2006; Daniels et al., 2008), also shown at weaning in our previous study (Molenaar et al., 2020). The difference here, showing a positive, carryover effect on parenchymal growth in animals fed a high relative to low milk intake weaning, with subsequent postweaning grazing lends support to the hypothesis that increasing the fat pad size creates a larger reservoir for the parenchyma to grow into (Hoshino, 1964; Neville et al., 1998; Hovey and Aimo, 2010), thus improving the overall growth of the mammary gland. At this stage, it would be pure speculation to venture a causal link, but this could be due to a long-term effect of signaling by milk components, or more likely, the additive endocrine effects of a large fat pad volume on parenchymal growth. The differences between our and previous studies could be attributed to the differences in animal genetics (i.e., Kiwi-cross vs. Holstein Friesian) and growth rate achieved during the study (e.g., change in the preweaning ADG was 390 vs. 820 g for Soberon and Van Amburgh, 2017 compared with 720 vs. 820 in our study), postweaning nature of the feeds (TMR and pasture), and the measuring techniques used (i.e., dissection vs. scanning). Around the time of the study, some reports were noted that ultrasonography may have a direct effect on cells (Johns, 2002; Furusawa et al., 2014; Yang et al., 2017), hence some calf udders did not receive ultrasonography in anticipation of comparing their milk yields with calves that did receive ultrasonography. Unfortunately, subsequent milk yields were unavailable, and we were unable to check if there was an effect of ultrasonography on mammary glands pre-weaning on milk production.

It is important to note that clear images can be obtained using ultrasonography in young animals at ~1-week-old, however in older animals, the images are harder to interpret due to the diminution of the signal intensity through the image as mammary gland size increases. The SonoSite equipment manufacturer (Fujifilm, Greenwood, SC, USA, 2017 personal communication) advises that while lipid (e.g.,

mammary fat pad) is more sonically dense and should appear brighter in the image, it also rapidly attenuates the signal and, hence, reduces the brightness of the reflected signal. Therefore, demarcation lines such as the mammary fat pad/abdominal wall were not clear in the images taken from most of the older animals hence, measured by one person self-trained by reviewing thousands of images and making comparisons with dissections and postmortem performed where, using a consistent interpretation of the whole image, considering feature shapes and pixel intensities. In our study, we used both conservative and liberal approaches to score the images as described by Molenaar et al. (2020). While both conservative and liberal scoring methods (Supplementary Figure S2) gave similar patterns of changes in mammary parameters for the treatment effects, more significant differences occurred with liberal scores and hence were presented in the manuscript, and conservative scores are provided for the reader as supplementary (Supplementary Figure S2). Further, due to the attenuation of ultrasound signals in larger glands, it was anticipated that front glands, being smaller, would be more informative than rear glands. However, here, this was not the case as while the front glands followed the pattern of changes of rear glands, significant results were observed with back glands but not with fore glands. These limitations show that using ultrasound scanning to study the mammary gland growth of heifers requires great care in the interpretation of the images and therefore further research using image analysis tools and machine learning is required to refine the use of this non-invasive technique to study mammary gland growth.

Conclusions

Feeding the high vs. the low amount of unpasteurized whole milk has promoted the growth rate of calves during preweaning in group-housed Kiwi-cross heifer calves without negatively affecting their growth during postweaning on pasture. At an early age (13 wk of age), calves that had received a greater milk feeding allowance had significantly greater serum-levels of antibodies against *Leptospira* and *Clostridia*. In addition, the differences in parenchyma to fat pad ratio at 7 mo of age found in our study show that preweaning milk allowance can influence mammary gland growth during postweaning in NZ dairy replacement heifers with potential consequences on future lactation.

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Conflict of Interest Statement

The authors do not have any conflicts of interest to declare.

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