REPRODUCTION

Effect of calfhod nutrition on metabolic hormones, gonadotropins, and estradiol concentrations and on reproductive organ development in beef heifer calves

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Abstract

This study examined the effect of plane of nutrition on the endocrinological regulation of the hypothalamic–pituitary–ovarian (HPO) axis in beef heifer calves during a critical sexual developmental window early in calf hood. Forty Holstein–Friesian × Angus heifers (mean age 19 d, SEM = 0.63) were assigned to a high (HI; ADG 1.2 kg) or moderate (MOD; ADG 0.50 kg) nutritional level from 3 to 21 wk of life. Intake was recorded using an electronic calf feeding system, BW was recorded weekly, and blood samples were collected on the week of age 5, 10, 15, and 20 for metabolite, reproductive, and metabolic hormone determination. At 19 wk of age, on sequential days, an 8-h window bleed was carried out for luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol analysis. To characterize anterior pituitary gland function, an intravenous GnRH challenge was conducted (19 wk of age). Blood was collected via a jugular catheter every 15 min for 135 min for the analysis of LH, FSH, and estradiol. Calves were subsequently euthanized at 21 wk of age; the anterior pituitary, metabolic organs, and reproductive tract were weighed, and ovarian surface follicular numbers and oocytes recovered were recorded. Mean ADG was 1.18 and 0.50 kg for HI and MOD, respectively, resulting in a 76.6-kg difference in BW ($P < 0.001$). Blood insulin, glucose, and IGF-1 concentrations were greater ($P < 0.001$) for HI compared with MOD. There was a diet × time interaction for leptin ($P < 0.01$); concentrations were greater in HI compared with MOD at 20 wk of age with no difference between treatments before this. Dietary treatment did not alter the concentrations of adiponectin or anti-müllerian hormone. There was a diet × time interaction for FSH, whereby MOD had greater concentrations than HI at 10, 15, and 20, but not at 5 wk of age. Over the duration of an 8-h window bleed (19 wk of age), serum concentrations of LH, LH pulse frequency, and LH pulse amplitude were unaffected by treatment, whereas FSH (0.23 vs. 0.43 ng/mL) and estradiol (0.53 vs. 0.38 ng/mL) concentrations were less than and greater, respectively, for HI than MOD ($P < 0.05$). Likewise, following a GnRH challenge, the area under the curve analysis revealed greater ($P < 0.01$) estradiol and lesser ($P < 0.01$) FSH concentrations in calves on the HI relative to MOD diet, whereas concentrations of LH were unaffected ($P = 0.26$) between treatments. Ovarian surface follicle numbers were greater ($P < 0.05$) in HI compared with MOD. Total reproductive tract, uterus, and ovarian tissue expressed relative to BW were greater ($P < 0.05$) for HI compared with MOD. In conclusion, enhanced nutrition in early calfhod advances the ontogeny development of the HPO axis.

Key words: early life nutrition, fertility, heifer, puberty, sexual maturity

Abbreviations

ADG	average daily gain
ADF	acid detergent fiber
AIC	Akaike information criterion
AMH	anti-mullerian hormone
AUC	area under the curve
BHB	beta-hydroxybutyrate
BW	bodyweight
CP	crude protein
DMI	dry matter intake
DM	dry matter
FSH	follicle-stimulating hormone
GIT	gastrointestinal tract
GnRH	Gonadotropin-releasing hormone
HPO	hypothalamic-pituitary-ovarian
IGF	insulin-like growth factor
LH	luteinizing hormone
MOD	moderate plane of nutrition
MR	milk replacer
NDF	neutral detergent fiber
NEFA	nonesterified fatty acids
PBS	phosphate-buffered saline
PEG	polyethylene glycol
RIA	radioimmunoassay

Introduction

Advancing the age at which puberty is attained in replacement beef heifers is fundamental to the financial and environmental sustainability of global beef production (Diskin and Kenny, 2014; Roberts et al., 2016; Kenny et al., 2017). The early onset of puberty is pivotal to an efficient seasonal calving beef production system, impacting submission rate, conception rate, age at first calving, and ultimately lifetime productivity and survivability of the breeding cow (Gasser, 2013; Perry, 2016; Roberts et al., 2016).

In cattle, the process of sexual maturation and puberty onset occurs in a gradual fashion, and this process is regulated by a complex network of biochemical processes and involves interaction amongst many key metabolic, neuroendocrine, and reproductive tissues ultimately culminating in the maturation of the hypothalamic-pituitary-ovarian (HPO) axis (Amstalden et al., 2014; Kenny et al., 2017; Cardoso et al., 2020). Intrinsic and extrinsic factors also influence the pubertal maturation process, the most notable of which are genetics and nutritional status, respectively (Perry, 2016; Cardoso et al., 2020).

Most of the research evaluating the impact of nutrition on age at puberty in suckled beef heifers has focused on nutritional changes after weaning (Roberts et al., 2009; Heslin et al., 2020), with limited opportunity to manipulate early-life nutritional status while suckling (without early weaning). However, many authors now agree that the potency of nutritional intervention affecting pubertal maturation is greater the earlier in life that it is applied, with improved nutritional status during critical developmental windows (Day and Anderson, 1998) early in juvenile development shown to have a much more potent impact on advancing the maturation of the reproductive axis and pubertal age, than nutritional interventions later in the rearing phase (Gasser et al., 2006a; Cardoso et al., 2014; Moriel et al., 2014). Indeed, Gasser et al. (2006a) showed with early-weaned suckled beef heifers offered diets that promote rapid rates of body weight gain between 3 and 7 mo of age, heifers reached puberty earlier and at lighter body weight than diet-restricted heifers with lower body weight gains. While the

suckled beef studies of Gasser et al. (2006a) and Moriel et al. (2014) were based on commencing differential feeding at 3 mo of age, a number of other studies (Shamay et al., 2005; Davis Rincker et al., 2011) using artificially reared calves have shown similar reductions in age at puberty in heifer calves offered a high plane of nutrition from a very young age (0 to 3 mo of age). Accordingly, a developmental window for nutritional imprinting of neuroendocrine functions that regulate reproductive maturation and age at the onset of puberty in heifers appears to exist quite early in juvenile development.

Nutritional control during early maturation in heifers exerts a substantial influence on the timing of puberty and leads to functional changes in the hypothalamic-pituitary pathways (Cardoso et al., 2020). Metabolic hormones (leptin, insulin-like growth factor-I, and insulin) and metabolites (glucose and fatty acids) are a major component of the complex neural signaling system involved in the conditioning and maturation of the HPO axis and serve as positive modulators of neuroendocrine signaling pathways that underlie the pubertal maturation process (Amstalden et al., 2014; Sartori et al., 2016; Cardoso et al., 2020). However, while knowledge of the biochemical interplays that trigger the pubertal process and sexual development has improved in recent times, much of the intricate mechanistic detail and underlying biology still elude us, especially during the key sexual developmental phases early in the heifer life. Therefore, the objectives of this study were to determine the effect of an enhanced plane of nutrition in early life (from 3 to 21 wk of age) on performance, feed efficiency, reproductive and metabolic organ growth, and the endocrinological regulation of the HPO axis and estradiol feedback, in beef heifer calves.

Materials and Methods

The work was conducted at the Teagasc, Animal & Grassland Research and Innovation Centre, Grange. All animal procedures performed were conducted under an experimental license from Health Products Regulatory Authority (HPRA) the Irish Department of Health and Children (license number B100/4516). Protocols were developed in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

Calves and management

Purebred Angus × purebred Holstein-Friesian heifer calves residing on high health status dairy farms were identified using the Irish Cattle Breeding Federation (ICBF) database [<https://www.icbf.com>]. From a well-characterized population, 40 heifer calves with a mean (±S.D.) age and body weight of 19 (±4) d and 51.2 (±7.8) kg, respectively, were sourced and transported to Teagasc Grange Research Centre. Upon arrival at the research center, calves were blocked by age, BW, and farm of origin and within block assigned to a high (HI) or moderate (MOD) plane of nutrition from 3 to 21 wk of life. The nutritional regimens were formulated based on (National Research Council, 2001) requirements to support a mean ADG of >1.2 and 0.50 kg for the HI and MOD nutritional treatments, respectively. Up to weaning, calves were individually offered milk and concentrate in pelleted form using an electronic feeding system (Vario, Foster-Technik, Engen, Germany) that recorded all feed-related events, including intake of both milk and concentrate, drinking speed, as well as the number of rewarded (when calves receive milk) and unrewarded (no milk dispensed) visits. Calves assigned to HI were offered a milk feeding plan as follows: 0 to 30 d (stage I): 10 liters of reconstituted milk daily; 30 to 35 d (stage II): 10

liters of milk daily gradually reduced to 6 liters; 35 to 42 d (stage III): 6 liters of milk daily; and 42 to 56 d (stage IV): 6 liters of milk daily gradually reduced to zero. Calves assigned to MOD were offered a milk feeding plan as follows: 0 to 50 d (stage I): 4 liters of milk daily and 50 to 56 d (stage II): 4 liters of milk daily gradually reduced to zero. Milk replacer (20% fat and 26% protein) was reconstituted to 15.0% solids. Calves assigned to HI were allowed to consume up to 2.5 liters of milk per visit and MOD were allowed to consume up to 1.5 liters of milk per visit, with meals a minimum of 2 h apart. The HI calves were offered concentrate ad libitum, whereas MOD received a stepped-up allowance, peaking at a maximum of 1 kg (fresh weight) of concentrate per day during the week of weaning. All calves were offered the respective preweaning diets for a minimum of 56 d and were weaned once they were consuming 1 kg (fresh weight) of concentrate for three consecutive days. Hay was provided as a source of roughage (250 g fresh weight/animal/day), and all calves had free access to clean fresh water. Post weaning, calves were penned in groups of 10 in accordance with the preweaning diet (two pens per diet). The HI calves were offered concentrate ad libitum, whereas MOD received 1 kg of concentrate daily. Both treatments were offered hay to appetite. Previously, we have shown that the HI and MOD rearing management protocols used have no adverse effects on calf immunity or health (Johnston et al., 2016). Additionally, calves were immunized against infectious bovine rhinotracheitis, bovine parainfluenza 3 virus, bovine respiratory syncytial virus, *Mannheimia haemolytica* serotypes A1 and A6, and *Salmonella dublin* and *Salmonella typhimurium* using Rispoval IBR-Marker live, Bovipast RSP, and Bovivac S vaccines.

Body weight and growth measurements

Calves were weighed using calibrated scales (Tru-Test XR3000, load bars XHD 10000, Auckland, New Zealand) at arrival and on a weekly basis throughout the duration of the experiment. Preweaning and postweaning ADG were determined by regressing animal BW over time using the REG procedure of SAS (SAS Inst. Inc., Cary, NC).

Feed sample collection and analysis

Samples of all feeds such as milk replacer (MR), concentrate, and hay offered were collected weekly and stored at -20°C . Feed samples were analyzed for CP, ADF, NDF, ash, ether, and gross energy as described by Kelly et al. (2010). Briefly, crude protein was determined using a Leco FP 528 nitrogen analyzer (Leco Instruments UK Ltd., Cheshire, UK). Acid detergent fiber and NDF were determined using the Ankom method (Ankom Technologies, Macedon, NY). Ash was determined after the ignition of a known weight of ground sample in a furnace (Carbolite Gero, Hope, UK) at 550°C for 4 h. The gross energy of milk powder, concentrate, and hay samples was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL). The chemical composition of MR, concentrate, and hay is presented in Table 1. The metabolizable energy value (MJ/kg) of the MR and concentrate was calculated using published values for the individual ingredients (National Research Council, 2001).

Blood sampling

Blood samples were collected via jugular venipuncture, at 5, 10, 15, and 20 wk of age. For IGF-1, leptin, adiponectin, metabolites (albumin, urea, total protein, beta-hydroxybutyrate [BHB], glucose, nonesterified fatty acids [NEFA], triglycerides, and creatinine), and leptin, blood was collected into a 9-mL evacuated tube containing lithium heparin (Greiner Vacuette;

Table 1. Composition and chemical analysis ($\pm$ SD) of milk replacer, concentrate, and hay offered

	Milk replacer	Concentrate	Hay
Chemical composition, g/kg/DM			
DM, %	96.7 (± 0.15)	88.9 (± 0.66)	81.5 (± 1.11)
CP	266.3 (± 1.24)	167.9 (± 1.86)	107.5 (± 9.35)
NDF	5.1 (± 1.00)	204.3 (± 18.2)	583.5 (± 8.86)
ADF	12.0 (± 1.98)	103.1 (± 6.76)	349.9 (± 7.20)
Lipid ¹	203.0 (± 12.10)	30.8 (± 0.72)	17.5 (± 0.67)
Crude ash	65.7 (± 2.22)	68.8 (± 0.91)	74.6 (± 2.77)
Concentrate composition, %			
Rolled barley		26.5	
Soybean meal		25	
Maize		15	
Beet pulp		12.5	
Soy hulls		12.5	
Molasses		5	
Mineral and vitamins ²		2.51	
Vegetable oil		1	

¹Concentrate and hay using ether extraction, and MR using acid hydrolysis.

²Mineral and vitamin composition: vitamin A: 10 mIU/kg, vitamin D₃: 2 mIU/kg, vitamin E: 40 mg/kg, iodine: 8 mg/kg, cobalt: 40 mg/kg, copper: 88 mg/kg, manganese: 81 mg/kg, zinc: 139 mg/kg, and selenium: 11 mg/kg.

Cruinn Diagnostics, Dublin, Ireland). For insulin analysis, a 6-mL K3-ethylenediaminetetraacetic acid (Vacuette, Cruinn Diagnostics) tube was used. Blood was centrifuged at $1,750 \times g$ for 15 min, and plasma was collected and stored at -20°C before analysis. Blood samples were also collected into a 9-mL evacuated serum separator tube (Becton Dickinson, Dublin, Ireland) at the same time points for follicle-stimulating hormone (FSH) and anti-mullerian hormone (AMH) analysis. Blood was subsequently allowed to clot overnight and then centrifuged at $800 \times g$ for 10 min; serum was harvested and stored at -20°C for pending analysis.

Luteinizing hormone pulsatility and GnRH challenge

On the day prior to blood sampling, calves were fitted aseptically with indwelling jugular catheters, to facilitate intensive blood collection as previously described Kelly et al. (2017). The procedure was performed, using 12-gauge Anes spinal needles (Popper and Sons, Inc., New Hyde Park, NY) and polyvinyl tubing (approximately 1.47 mm i.d.; Ico Rally Corp., Palo Alto, CA; catalog No. SVL 105–18 CLR) attached to an 18-gauge needle at the blood collection end. Catheter patency was maintained by flushing with 3.5% sodium citrate after each blood collection.

At 19 wk of age, an 8-h window bleed (samples were taken via a jugular catheter at 15-min interval) were carried out for luteinizing hormone (LH), FSH, and estradiol analysis. The following day a GnRH challenge was used to quantitatively characterize the anterior pituitary gland function from HI and MOD treatment calves ($n = 16$ representative calves per dietary treatment). For the GnRH challenge, a GnRH agonist (Buserelin Receptal; Intervet Ireland Ltd., Dublin, Ireland) was administered at 0.05 mg/kg of BW, i.v. Relative to GnRH administration, blood samples were collected at -30 , -15 , 0 (GnRH administration time point), 15, 30, 45, 60, 75, 90, 105, 120, and 135 min, respectively, for gonadotropins (LH and FSH) and estradiol analysis.

Reproductive and metabolic organs

Calves were euthanized in an approved research abattoir at 21 wk of age (145 ± 3 d of age), and the reproductive tract was excised, washed with PBS, and trimmed of connective tissue. The weight of the entire reproductive tract as well as components such as ovaries, uterus, and cervix were recorded. Total ovarian surface follicle numbers were recorded. Following aspiration of surface follicles, follicular fluid was examined under magnification and the total numbers of oocytes recovered were reported. Additionally, the anterior pituitary, liver, heart, lungs, and gastrointestinal tract (GIT) were also excised and weighed.

Blood analysis and assay sensitivity

IGF-1

Concentrations of IGF-1 were determined using an RIA following acid-ethanol extraction (Beltman et al., 2010). The intra- and inter-assay coefficients of variation (CV) were determined by replicating a low, normal, and high reference sample at the beginning, middle, and end of each assay and were 12.5%, 6.6%, and 5.1%, and 13.7%, 8.4%, and 9.6% for low, medium, and high, respectively. The sensitivity of the assay, defined as the least concentration detectable, was 4 ng/mL.

Insulin

Concentrations of insulin were determined using insulin immunoradiometric assay (INS-IRMA) kits (DIAsource Immunoassays, Louvain-la-Neuve, Belgium) as previously described by Kelly et al. (2010). Intra- and inter-assay CV for insulin were 5.4%, 4.0%, and 4.3% and 4.9%, 6.5%, and 6.3% for low, medium, and high, respectively. The sensitivity of the assay, defined as the least concentration detectable, was 1 ng/mL.

Leptin and adiponectin

Enzyme immunoassays were used to determine the concentrations of leptin (Sauerwein and Häußler, 2016) and adiponectin (Mielenz et al., 2013). Intra- and inter-assay CV for leptin were 5% and 9%, respectively. Corresponding values for adiponectin were 5% and 10%. The sensitivity of the leptin and adiponectin assays, defined as the least concentration detectable, was 0.6 and 0.03 ng/mL, respectively.

FSH, LH, estradiol, and AMH

Concentrations of FSH, LH, and estradiol were analyzed using RIA. Serum concentrations of FSH were determined using the method of Crowe et al. (1997). The sensitivity of the assay was 0.05 ng/mL. Intra- and inter-assay CV were 3.8%, 4.8%, and 4.3%, and 14.2%, 15.7%, and 9.8% for low, medium, and high, respectively. Serum concentrations of LH were determined using the method of Cooke et al. (1997), modified so that the separation step (second antibody) used a polyethylene glycol (PEG) method. Briefly, after assay incubation with primary antibody, 100 μ L of 1% normal mouse serum in assay buffer was added. This was followed by 1 mL of goat-anti-mouse antibody (Equitech-Bio Inc., Kerrville, TX) diluted 1:100 in 5% PEG. Assay tubes were incubated for 1 h at room temperature and centrifuged for 20 min at $1,600 \times g$; then, the free fraction was separated by decanting the supernatant. The sensitivity of the assay was 0.05 ng/mL. Intra- and inter-assay CV were 9.1%, 15.2%, and 7.25%, and 2.5%, 4.6%, and 2.6% for low, medium, and high, respectively. Circulating concentrations of estradiol were analyzed using the methodology described by Perry and Perry (2008). Intra- and inter-assay CV were 3.9%, 8.2%, and 4.3%, and 6.1%, 4.7%, and 7.8% for low, medium, and high, respectively. Plasma concentrations (pg/mL) of AMH were analyzed using the Ansh Labs (Webster, TX) Bovine

AMH ELISA using a Dynex DSX Automated ELISA System (Dynex Technologies, Chantilly, VA) as described by Gobikrushanth et al. (2019). The assay has an analytical measurable range of 13.5 to 2,240 pg/mL. The AMH assay's least detection limit is 11 pg/mL, and intra- and inter-assay CV were <5%.

Plasma metabolites

Concentrations of albumin, urea, total protein, BHB, glucose, NEFA, triglycerides, and creatinine were determined using an automatic analyzer (AU 400; Olympus, Tokyo, Japan). The inter-assay CV for low, medium, and high was <10% for all metabolites. The sensitivity of the assay, defined as the least concentration detectable, was as follows: glucose: 0.02 mmol/L, urea 0.9: mmol/L, BHB: 0.1 mmol/L, NEFA: 0.072 mmol/L, triglycerides: 0.004 mmol/L, total protein: 0.8 g/L, albumin: 0.1 g/L, and creatinine: 2.3 μ mol/L. Globulin concentration was calculated as the difference between total protein and albumin concentrations.

Statistical analysis

Data were checked for normality and homogeneity of variance using histograms, Q-Q plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data that were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. Cluster was used to determine the average concentration of LH, LH pulse frequency, and LH pulse amplitude. The area under the curve (AUC) for FSH, LH, and estradiol between 0 and 135 min relative to GnRH administration was determined using Sigma Plot (version 11, Systat Software, San Jose, CA).

Variables with multiple observations such as BW, feed intake, hormone, and metabolite concentrations were analyzed using repeated-measures ANOVA (MIXED procedure), with terms for plane of nutrition (HI and MOD), time (age), and their interactions. Pen was included as a fixed effect, and the block was incorporated as a random effect in the statistical model. For the analysis of the GnRH challenge, basal serum LH, FSH, and estradiol concentration (mean of concentrations at 30 and 15 min before GnRH administration) for each animal was included as a covariate in the statistical model. Nonstatistically significant ($P > 0.10$) interactions were subsequently excluded from the final model. The type of variance-covariance structure used was chosen depending on the magnitude of the Akaike information criterion (AIC) for models run under compound symmetry, unstructured, autoregressive, heterogeneous first-order autoregressive, or Toeplitz variance-covariance structures. The model with the lowest AIC coefficient was selected. Differences between treatments were determined by *F*-tests using type III sums of squares. The PDIF command incorporating the Tukey test was applied to evaluate pairwise comparisons between treatment means. Mean values were considered to be statistically significantly different when $P < 0.05$ and considered a tendency toward statistical difference when $P \geq 0.05$ and < 0.10 .

Results

Early-life growth and feed intake

Calves on the HI diet were 105.8 kg at weaning and 189.6 kg at 21 wk of age. Corresponding body weight for their MOD diet contemporaries were 85.2 and 113.0 kg, resulting in a body weight differential between the treatment groups ($P < 0.001$)

of 20.6 kg at weaning and 76.6 kg at 21wk of age (Figure 1). Prewearing ADG was 0.91 kg and 0.56 kg for HI and MOD groups, respectively ($P < 0.001$). Corresponding postweaning ADG was 1.28 and 0.48 kg ($P < 0.001$). Overall, mean ADG ($P < 0.001$) between 3 and 21 wk of life was 1.18 and 0.50 kg for HI and MOD calves, respectively.

Mean MR and concentrate DMI daily were 1.01 and 0.51 kg and 0.48 and 0.61 kg for HI and MOD calves, respectively ($P < 0.001$) (Table 2 and Figure 1). Overall, daily DMI (MR and concentrate) during the preweaning phase was 1.51 and 1.09 kg for HI and MOD, respectively ($P < 0.001$). Cumulatively, calves on HI consumed 56.6 kg MR DM and 27.7 kg concentrate DM. Corresponding consumption for MOD was 26.9 kg MR DM and 34.3 kg concentrate DM. Daily energy intake was greater for HI than MOD calves ($P < 0.001$). Feed conversion ratio was higher for calves on MOD than HI ($P < 0.001$). The number of unrewarded feed visits for MR was greater ($P < 0.001$) for MOD than HI calves. Drinking speed did not differ between treatments.

Metabolic hormones and metabolites

Insulin ($P < 0.001$) and IGF-1 ($P < 0.001$) concentrations were consistently greater in HI compared with MOD calves (Figure 2). There was a diet $\times$ time interaction for leptin ($P < 0.01$), with concentrations greater in HI than MOD calves at 20 wk of age, but not prior to this. Adiponectin concentrations did differ between treatments ($P = 0.23$). There were no diet $\times$ time interactions detected for any of the metabolites (Table 3). Glucose ($P < 0.001$) concentrations were consistently greater in HI compared with MOD calves (Figure 2). Creatinine ($P < 0.001$) and BHB ($P < 0.01$) concentrations were greater in MOD compared with HI calves (Table 3). Globulin concentrations tended ($P = 0.08$) to be greater in calves on the MOD compared with the HI diet. Concentrations of albumin, total protein, urea, triglycerides, and NEFA were unaffected by treatment.

Figure 1. Growth trajectory of calves on aHI or MOD early-life nutritional diet (A). Prewearing milk and concentrate dry matter intake of calves on the HI or MOD planes of nutrition (B).

Table 2. Dry matter intake, energy intake, and feeding behavior in the preweaning period of calves offered a HI or MOD plane of nutrition¹

Trait	HI	MOD	SEM	P-value
Milk replacer intake, kg DM/d	1.01	0.48	0.04	<0.0001
Milk replacer intake, MJ/d	19.62	9.32	0.71	<0.0001
Cumulative milk replacer intake, kg DM	56.64	26.92	.	.
Concentrate consumption, kg DM/d	0.51	0.61	0.06	0.14
Concentrate consumption, MJ/d	5.47	6.78	0.62	0.14
Cumulative concentrate intake, kg DM	27.71	34.32	.	.
Total DMI, kg DM/d	1.51	1.09	0.05	<0.0001
Total ME intake, MJ/d	25.09	10.42	0.53	<0.0001
Cumulative DMI, kg DM	84.35	61.24	.	.
Cumulative ME intake, MJ	1,405.29	583.39	.	.
Feed conversion ratio (DMI/ADG)	1.64	1.93	0.12	<0.0001
Drinking speed, mL/min	883.30	872.90	7.21	0.20
Visits with feed	4.2	2.8	0.03	<0.0001
Visits without feed	7.3	10.8	0.1	<0.0001

¹HI, high plane of nutrition; MOD, moderate plane of nutrition.

Reproductive hormones and GnRH challenge

Plasma FSH and AMH hormonal profiles of calves on the HI or MOD early-life plane of nutrition are presented in Figure 3. A diet $\times$ time interaction was observed for FSH ($P < 0.05$) with MOD calves having greater concentrations than HI calves at 10, 15, and 20 wk of age, but no difference between treatments prior to this. Concentrations of AMH did not differ between treatment (HI = 852.3 pg/ml vs. MOD = 805.0 pg/ml; $P = 0.62$) at any sampling time.

The serum concentration of LH, FSH, and estradiol during an 8-h window bleed in heifer calves offered a high or moderate early-life plane of nutrition is presented in Figure 4. Over the duration of an 8-h window bleed (19 wk of age), serum concentrations of LH, LH pulse frequency, and amplitude were unaffected by treatment, whereas FSH (HI = 0.23 vs. MOD = 0.43 ng/mL) and estradiol (HI = 0.53 vs. MOD = 0.38 ng/mL) concentrations were less than and greater, respectively, for HI than MOD ($P < 0.05$).

The serum concentration of LH, FSH, and estradiol before and after GnRH challenge in heifer calves offered a HI or MOD plane of nutrition is presented in Figure 5. Following a GnRH challenge (19 wk of age), AUC analysis revealed greater estradiol (HI = 201.33 vs. MOD = 110.89; $P < 0.01$) and lesser FSH concentrations (HI = 154.86 vs. MOD = 219.18; $P < 0.01$) in calves on the HI relative to MOD diet, whereas concentrations of LH (HI = 1,351.88 vs. MOD = 1,222.08; $P = 0.26$) were unaffected between treatments. Post GnRH administration, FSH concentrations were greater ($P < 0.05$) for MOD than HI calves from 60 to 135 min, whereas estradiol concentrations were lesser ($P < 0.05$) for MOD than HI

Figure 2. Metabolic hormones and metabolites profiles in calfhoo from on a HI or MOD plane of nutrition.

Table 3. Characterization of blood metabolites of calves on a HI or MOD early-life plane of nutrition¹

Trait	Nutrition			Age, wk				P-value		
	HI	MOD	SEM	5	10	15	20	SEM	Nutrition	Age
Globulin	32.20	34.59	0.94	32.10	32.55	33.27	35.61	0.83	0.08	0.01
Albumin	33.80	33.12	0.26	32.75	33.47	34.24	33.43	0.28	0.25	0.01
Total protein	66.00	67.71	0.92	64.85	66.02	67.52	68.97	0.84	0.19	0.01
Creatinine	90.9	100.00	1.92	101.20	96.94	94.39	89.34	1.61	0.01	<0.0001
BHB	0.18	0.26	0.01	0.10	0.22	0.32	0.24	0.01	<0.0001	<0.0001
NEFA	0.10	0.13	0.01	0.11	0.11	0.13	0.10	0.01	0.11	0.51
Triglycerides	0.24	0.22	0.01	0.22	0.24	0.22	0.26	0.01	0.38	0.15

¹HI, high plane of nutrition; MOD, moderate plane of nutrition.

calves from 75 min to 135 min, but there were no differences between treatments prior to this.

Reproductive organ growth and ovarian surface follicles

Ovarian surface follicle number was greater ($P < 0.05$) in calves offered the HI compared with the MOD diet (HI = 67.1

vs. MOD = 38.3; Figure 6). The number of oocytes recovered following aspiration tended ($P = 0.07$) to be higher in HI than MOD calves. Calves offered the HI diet had greater ($P < 0.001$) total reproductive tract weight, as well as the cervix, uterus, and ovarian tissue compared with MOD calves on an absolute basis and when scaled for body weight (Table 4). There was evidence that the HI diet induced allometric growth of the total

reproductive tract, uterus, and ovarian tissue. The weight of metabolic organs such as the heart, lungs, liver, and GIT along with the anterior pituitary gland was greater for calves offered the HI compared with MOD diet ($P < 0.001$; Table 5). Liver weight

relative to body weight remained greater ($P < 0.001$) for calves on the HI compared with the MOD diet. The weight of the anterior pituitary, liver, and ovary was all positively related to one another ($P < 0.05$) on an absolute basis and when scaled for BW.

Discussion

Considerable evidence indicates the potency of early-life nutritional intervention to affect the maturation of the reproductive axis (Kenny et al., 2017; Cardoso et al., 2020). Indeed, previous studies conducted across beef heifers (Gasser et al., 2006a; Moriel et al., 2014) and artificially reared heifer calves (Shamay et al., 2005; Davis Rincker et al., 2011) have consistently demonstrated the positive effect of early-life nutritional augmentation on advancing puberty in cattle. While knowledge on how nutritional status in early life may trigger sexual maturation and the pubertal process has improved over time (Perry, 2016; Cardoso et al., 2020), the intricate mechanistic biochemical regulatory processes involved are still poorly understood. The aim of this study, therefore, was to examine the sensitivity (endocrinological regulation) of the HPO axis and, in particular, estradiol feedback, in heifer calves offered an enhanced plane of nutrition during a critical sexual developmental window (Day and Anderson, 1998) early in calthood (3 to 21 wk of life).

Nutritive signals have a well-recognized role in the conditioning and maturation of the HPO axis in cattle, and the synthesis and secretion of GnRH and the gonadotropins FSH and LH from the hypothalamus and anterior pituitary, respectively (Perry, 2016; Kenny et al., 2017; Cardoso et al., 2020). Sexual maturation is initiated largely at the hypothalamic level, with metabolic signal thought to influence the secretion of GnRH by

Figure 3. FSH and AMH hormonal profiles of calves on a HI or MOD early-life plane of nutrition.

Figure 4. Serum concentration of LH, FSH, and estradiol during an 8-h window bleed in heifer calves (at 19 wk of age) offered a HI or MOD early-life plane of nutrition.

Figure 5. Serum concentration of LH, FSH, and estradiol before and after GnRH challenge at 19 wk of age in heifer calves offered a HI or MOD plane of nutrition.

changing the sensitivity of the reproductive neuroendocrine axis to estradiol (Perry, 2016; Cardoso et al., 2020). These metabolic signals can also modulate the secretion of gonadotropins, by acting either directly or indirectly at the level of the anterior pituitary gland (Zieba et al., 2005), and also the ovary to alter follicular growth dynamics and estrogen production (Amstalden et al., 2014). Nutritional stimulus appears most profound early in life during the heifer's development phase (2 to 6 mo of age) and feeding a high-energy diet during this critical developmental window hastens pubertal maturation, with dynamic changes occurring in the endocrine and steroidal feedback mechanisms within the reproductive axis (Gasser et al., 2006a, 2006b; Cardoso et al., 2014; Moriel et al., 2014), that expedites the maturation of the hypothalamus, pituitary gland, and reproductive tissues, with most components of the HPO axis fully competent by approximately 5 to 6 mo of age (Cardoso et al., 2020). Increased frequency of LH pulsatility signifies maturational changes in key hypothalamic regions of the brain and the anterior pituitary gland (Amstalden et al., 2014) and serves as the most accurate predictor of pubertal maturation in heifers (Day et al., 1984). Calves as young as 1 mo of age begin to

Figure 6. Effect of enhanced early-life plane of nutrition on ovarian surface follicle number and oocyte recovery.

secrete LH in pulses from the anterior pituitary gland under the influence of by pulsatile GnRH released from the hypothalamus (Cardoso et al., 2020). Enhanced nutrition during the heifer's developmental phase can accelerate the frequency of LH pulsatility, with Gasser et al. (2006d) conclusively reporting that in early-weaned heifers offered a high-energy diet (from 4 to 7 mo of age) an altered LH secretion and accelerated LH pulsatility were characteristically initiated from 6 mo of age onwards and extending up to the time of puberty onset. This acceleration in the onset of increased LH pulse frequency is a consequence of a gradual decline in the sensitivity of the hypothalamic/anterior pituitary axis to the negative feedback of estradiol (Kinder et al., 1995). Our data, generated during calthood, early in the heifer's developmental phase, tie in with these findings, as we failed to observe any effect of plane of nutrition on LH pulse frequency, LH pulse amplitude, or secretion of LH in response to exogenous GnRH as a consequence of strong steroidal negative feedback. However, in bull calves, an LH rise early in life is also considered essential to the subsequent timing of the puberty onset (Brito et al., 2007) with a greater response in LH pulsatility to nutritional intervention for bull compared with heifer calves. Indeed, data from our laboratory with young bull calves provide evidence that a high plane of nutrition from 2 until 39 wk of age

results in the greater secretion of LH in response to exogenous GnRH at 4, 6, and 8 mo of age (Byrne et al., 2018). The anterior pituitary gland also secretes FSH but in a nonpulsatile manner in response to hypothalamic GnRH release (Evans et al., 1994; Kenny et al., 2017). Although the research is limited in both sexes regarding the anterior pituitary gland's ability to secrete FSH during the juvenile developmental period, it appears that FSH concentrations in heifer calves generally rise within the first 3 mo of life, to initiate ovarian follicular development and the recruitment of a cohort of small- and medium-sized antral follicles (Rawlings et al., 2003). Thereafter, FSH concentrations typically decrease until the peri-pubertal period due to inadequate GnRH secretion (Rawlings et al., 2003; Perry, 2016). Our data reaffirm this scenario and further indicate that in calves on a high plane of nutrition, FSH concentrations decrease more quickly relative to their MOD diet contemporaries after 10 wk of age. We postulate that this decrease in FSH in calves on the HI diet is due to an earlier shift toward gonad-dependent suppression of GnRH, which develops progressively during the juvenile period and reflects an increase in responsiveness to negative feedback from estradiol (Day and Anderson, 1998). Alternatively, calves on the MOD diet were less sexually advanced, as the synthesis and secretion of gonadotropins appeared to be largely gonadal steroid-independent with the FSH secretion pattern characteristic of that typically observed

in heifer calves during the postnatal rearing phase (Evans et al., 1994; El-Sheikh Ali et al., 2017). An interlinked hormone to FSH is AMH with both hormones essential in ovarian folliculogenesis and known predictors of antral follicle count, ovarian reserve, and future fertility of female cattle (Ireland et al., 2008; Batista et al., 2016). El-Sheikh Ali et al. (2017) proposed that heifers exhibit a characteristic AMH plasma profile during postnatal life, such that high plasma AMH could be a possible biomarker for pubertal maturation. However, very few published studies have profiled plasma AMH in heifer calves during the early postnatal period (Rota et al., 2002; El-Sheikh Ali et al., 2017). In the current study, divergent postnatal nutrition did not alter circulating AMH concentrations. Indeed, previous work within our group by Mossa et al. (2013) showed a direct effect of maternal undernutrition during the first third of gestation in heifers on the ovarian follicle reserve, antral follicle count, and AMH concentrations of their female progeny during the prepubertal period, but systemic AMH was not related to or a predictor of age at the pubertal onset.

To quantitatively characterize the anterior pituitary gland function and responsiveness, a GnRH challenge was conducted, from this one could also monitor the sensitivity of the HPO axis and estradiol feedback pathways early in juvenile development. GnRH pulsatility has been documented to begin early in life at 2 wk of age onwards in the heifer (Evans et al., 1994). Once the hypothalamus acquires the capability to secrete GnRH, the anterior pituitary gland will respond to these stimuli but it is not instantaneous and the anterior pituitary gland takes time in order to mature and gain full responsiveness and the ability to secrete LH and FSH (Cardoso et al., 2020). Heifers as young as 1 mo of age have been shown to have adequate hypophyseal stores of LH and FSH and are capable of secreting gonadotropins in response to exogenous GnRH stimuli/challenge (Rawlings et al., 2003; Cardoso et al., 2020). In this study, following an exogenous GnRH challenge at 19 wk of age, heifer calves from HI dietary treatment had greater estradiol and lesser FSH concentrations compared with MOD-reared calves. Intuitively, in young calves during the sexual development phase, greater estradiol concentration coupled with strong (negative) hypothalamic

Table 4. Uterine and ovarian organ growth of calves on a HI or MOD early-life plane of nutrition

Trait	HI	MOD	DIFF ¹	SEM	P-value
Reproductive tract, % of BW	0.60	0.47	—	0.039	<0.0001
Ovaries, g	12.09	5.43	6.66	1.026	<0.0001
Ovaries, % of BW	0.065	0.048	—	0.006	<0.0001
Uterus, g	61.18	29.29	0.57	5.196	<0.0001
Uterus, % of BW	0.32	0.25	—	0.029	<0.0001
Cervix, g	33.39	16.24	17.15	2.181	<0.0001

¹DIFF, difference between HI and MOD.

Table 5. Anterior pituitary gland, GIT, and metabolic organ growth of calves on a HI or MOD early-life nutrition plane¹

Trait	HI	MOD	DIFF ²	SEM	P-value
Anterior pituitary gland					
Anterior pituitary gland, g	8.30	5.21	3.09	0.301	<0.0001
Anterior pituitary gland, % of BW	0.0044	0.0046	—	0.0002	0.52
GIT tract					
Entire upper GIT, kg	9.54	6.75	2.79	0.282	<0.0001
Entire GIT, % of BW	0.052	0.059	—	0.0020	0.12
Rumen and reticulum, kg	5.76	3.21	2.55	0.215	<0.0001
Rumen and reticulum, % of BW	0.03	0.03	—	0.0021	0.22
Omasum, kg	2.36	2.12	—	0.170	0.15
Abomasum, kg	1.53	1.24	—	0.290	0.50
Metabolic organs					
Heart, kg	0.84	0.54	0.3	0.039	<0.0001
Heart, % of BW	0.0044	0.0048	—	0.0002	0.38
Lungs, kg	2.25	1.27	0.98	0.059	<0.0001
Lungs, % of BW	0.011	0.011	—	0.0003	0.22
Spleen, kg	0.99	0.42	0.57	0.081	<0.0001
Spleen, % of BW	0.005	0.004	—	0.0005	0.18
Liver, kg	3.30	1.63	1.67	0.099	<0.0001
Liver, % of BW	0.018	0.014	—	0.0005	<0.0001

¹HI, high plane of nutrition; MOD, moderate plane of nutrition.

²DIFF, difference between HI and MOD.

sensitivity to estradiol results in the inhibition of GnRH neurons and reduced gonadotropin secretion, collectively signifying an advanced maturation of the HPO. Indeed, in young heifers, the restraint exerted by estradiol negative feedback is what prevents the coordinated function of the reproductive axis and thereby the continuance of estrous cycles or puberty (Perry, 2016).

Furthermore, follicular development in early life (first 2 or 3 mo of age) is a continuous process, with the number and size of gonadotrophin-responsive antral follicles increasing as the heifer matures (Evans et al., 1994). Greater estradiol output recorded in calves on the HI plane of nutrition was consistent with the observed greater ovarian surface follicle numbers and oocytes recovered following aspiration from this group at slaughter (21 wk of age), again demonstrating enhanced ovarian maturation and acceleration in the switch from positive to negative feedback of estradiol on GnRH pulsatility and gonadotrophin secretion. Interestingly, in an associated study, using tissue collected from these same animals (Kelly et al., 2020, unpublished), we observed that anterior pituitary gland expression of the FSH subunit beta and follistatin were downregulated at both a transcriptomic and proteomic levels in HI compared with MOD calves at 21 wk of age. We hypothesize that these differences in anterior pituitary gland functionality are most likely attributable to the rise in steroid secretion from ovarian follicles in the more sexually mature HI calves, which initiates the developing feedback mechanisms involving activin, inhibin, and follistatin that affect the pituitary's ability to produce and secrete FSH (Namwanje and Brown, 2016).

Overall, the endocrinological changes we observed in the HPO axis in response to enhanced nutrition during early calfhood are indicative of a shift in the timing of the maturation of the reproductive axis consistent with hastened pubertal onset. In agreement, (Gasser et al. 2006a, 2006b, 2006c, 2006d) in their series of studies also demonstrated that increasing nutrient intake between 4 and 7 mo later in the sexual developmental window (Day and Anderson, 1998) successfully advanced the timing of puberty in beef heifers, with a comparable sequence of changes in the reproductive axis, follicular development, and estradiol negative feedback.

As regards morphological development, enhanced early-life nutrition promotes reproductive and metabolic organ development. The size (expressed on either an absolute or per unit of body weight basis) of the anterior pituitary gland, the liver, the ovary, and important interactive organs central to the activation of the reproductive axis were all positively related and responsive to nutritional stimulus in calf hood. In particular, both ovarian and uterine development were sensitive to prevailing nutritional status with ovarian and uterine weight positively related to early-life growth, whether expressed on either an absolute or per unit of body weight basis, signifying that HI dietary regimen induced allometric growth of the reproductive tract. During the first 5 mo of life, morphological development of the heifers reproductive tract (ovarian size, diameter of the uterus, vagina, and cervix) increases rapidly in size (Honaramooz et al., 2004), and reproductive tract scoring describing the relative development of the uterus as well as ovarian activity have long been cited as an accurate predictor of pubertal maturation and subsequent reproductive performance of young heifers (Holm et al., 2009).

Metabolic hormones and metabolites (nutritional "cues" or indicators) are major components of the complex neural signaling system involved in the regulation of sexual maturation in cattle (Amstalden et al., 2014; D'Occhio et al., 2019). Leptin, insulin, and IGF-1 respond to increasing nutritional status

and have direct or indirect influences (Williams et al., 2018) on the functioning of hypothalamic neurons and other cellular components, controlling the secretion of neuropeptides that regulate GnRH secretion and pubertal progression (Cardoso et al., 2020). Characteristically, maturation of the reproductive axis in heifers is associated with endocrinological modifications in both the reproductive axis and somatotrophic (insulin/IGF-1 pathways) growth axes (Diskin et al., 2003; Velazquez et al., 2008), with receptors for IGF-1 detected in the preoptic area of the hypothalamus, suggesting a stimulatory role in GnRH secretion and sexual development (Daftary and Gore, 2005). Furthermore, cell lines from the anterior pituitary gland incubated with IGF-1 showed an enhanced LH responsiveness to GnRH (Soldani et al., 1995). In early calfhood, IGF-1, insulin, and glucose are sensitive nutritional barometers, as the current study showed, with greater concentrations observed in calves on the high plane of nutrition at 5 wk of age onwards, reflective of the higher nutrient intake and growth of this cohort. The prevailing blood concentrations of IGF-1 have been consistently linked to the initiation of puberty in breeding heifers, with IGF-1 status in prepubertal calves cited as an excellent physiological predictor of sexual maturation and future reproductive success (Fortes et al., 2013; Rodríguez-Sánchez et al., 2015; Heslin et al., 2020). Leptin is well accepted as a robust indicator of increased adiposity and has been cited as an intermediary endocrinological signal of pubertal progression in prepubertal heifers (Zieba et al., 2005; Amstalden et al., 2014; Perry, 2016). Leptin transmits metabolic signals during juvenile development shown to modulate GnRH neuronal activity mediated via upstream neurons, including neuropeptide Y, kisspeptin, and proopiomelanocortin neurons (Cardosa et al., 2020). Nutritional manipulation during early calfhood has been shown to alter adipocyte hyperplasia and hypertrophy biochemical pathways (Tikofsky et al., 2001; Bascom et al., 2007). Adipose tissue produces the protein hormone leptin, and, in this study, circulating concentrations of leptin were positively influenced by the nutritional status of the heifers at 20 wk of age, but not prior to this. Similarly, in studies with artificially reared bull calves (Dance et al., 2015; Byrne et al., 2018), a response to prevailing nutritional status on the systemic concentration of leptin and body fat was not evident before 31 wk of age. Later in physiological development, Heslin et al. (2020) showed, in postweaning beef heifers (>8 mo of age), that greater body fatness and systemic concentrations of leptin were inversely associated with pubertal age.

Early-life nutritional programming has been shown to have a lasting influence on a range of key economically important traits in cattle production. Along with fertility gains such as the earlier onset of puberty and reduction in age at first calving, other productivity benefits such as improved health and robustness, enhanced mammary development, and greater milk production have been cited to arise from augmented nutritional management early in calfhood (Shamay et al., 2005; Drackley, 2008; Soberon and Van Amburgh, 2013). Early-life nutritional regimens for artificially reared calves have been classified into several categories but are commonly defined in commercial farm settings as either conventional/traditional or intensified (accelerated and enhanced). Conventional feeding systems typically support a growth rate of 0.2 to 0.6 kg/d during the preweaning period (2 to 3 mo of age), whereas calves reared on an intensive enhanced feeding programs are able to achieve in excess of 0.8 kg/d BW gain. In this study, calf growth rate was comparable with findings of other studies (Khan et al., 2007; Davis Rincker et al., 2011; Byrne et al., 2017a) examining intensified vs. conventional fed systems for artificially reared calves, with greater gain, structural growth,

and improved feed efficiencies, all evident in calves on the intensified fed regimen in early life. Additionally, the metabolite concentrations were very much within the normal biological range were reflective of the respective calf growth rate and an overall anabolic metabolic state.

In conclusion, enhanced nutrition during early calthood advances sexual maturity with direct effects on the HPO axis. Understanding the precise regulatory mechanisms involved in early sexual development will increase our ability to effectively manage replacement heifers for optimal reproductive performance. Improved nutritional regimens can then be formulated to consistently and cost-effectively ensure that a high proportion of replacement heifers reach puberty before the start of the breeding season, a central tenet of seasonal calving production systems. Ultimately, advancing the age at which puberty is attained in replacement beef heifers will not only shorten the generation interval and facilitate greater selection intensity, but also, most importantly, it will improve both the financial and environmental sustainability of beef production systems.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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