

Effect of breed, plane of nutrition and age on growth, scrotal development, metabolite concentrations and on systemic gonadotropin and testosterone concentrations following a GnRH challenge in young dairy bulls

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Abstract

The onset of puberty in the bull is regulated by the timing of early GnRH pulsatility release from the hypothalamus, which has been demonstrated to be affected by plane of nutrition during calf-hood. The aim of this study was to determine the effect of plane of nutrition on growth rate, scrotal development, metabolite concentrations and exogenous gonadotrophin (GnRH) induced release of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (TT) in pre-pubertal bulls of two contrasting dairy breeds. Holstein-Friesian and Jersey bull calves were assigned to either a high or low plane of nutrition from 3 to 49 weeks of age. Intensive blood sampling was conducted at 16, 24 and 32 weeks of age, every 15 min from 30 min prior to intravenous administration of exogenous GnRH to 135 min after. Monthly blood samples were also collected and analyzed for insulin like growth factor 1 (IGF-

1), insulin, leptin, adiponectin and metabolite concentration. Insulin and IGF-1 were higher in bulls on a high plane of nutrition ($P < 0.001$) but were not affected by breed ($P > 0.05$). Leptin was not affected by plane of nutrition or breed ($P > 0.05$). Adiponectin tended to be higher in bulls on a high plane of nutrition ($P = 0.05$), but was not affected by breed ($P > 0.05$). Bulls on a high plane of nutrition had a greater concentration of LH in response to GnRH ($P < 0.05$) but there was no effect of breed ($P > 0.05$). FSH concentration was not influenced by breed or plane of nutrition but FSH concentrations did decrease with age ($P < 0.01$), while, LH was not affected by age ($P > 0.05$). Jersey bulls, particularly those on a high plane of nutrition, had higher TT production in the pre-pubertal period ($P < 0.001$). Using 28 cm as a proxy for age at puberty, bulls on a high plane of nutrition were predicted to reach puberty earlier than bulls on a low plane. In conclusion, the data clearly demonstrate that a high plane of nutrition positively affects several key nutritional and reproductive hormones which are critical to development of the hypothalamic-pituitary-testicular axis in dairy-bred bull calves.

Keywords: Calf-hood nutrition, puberty, insulin, IGF-1, hypothalamus, endocrinology.

1. Introduction

The advent of genomically-assisted selection has facilitated the early identification of elite sires within weeks of birth leading to an increased demand for their semen at a young age. This necessitates that bulls reach puberty as early as possible and produce an adequate volume of high quality semen to meet this demand. It is well established that a number of factors including breed and nutritional status may influence the timing of puberty in farm animals [1]. Management factors such as

nutrition can be used to hasten the onset of puberty [2] and sexual maturation in the bull [3], leading to an earlier availability of semen from these genetically elite sires which are in high demand. The timing of the early transient rise in anterior pituitary luteinizing hormone (LH) pulsatile activity is a critical factor in determining the age at which sexual maturity is reached [4]. This early rise has been reported to occur between weeks 10 and 18 of age in Angus and Angus x Charolais bull calves [5] and is thought to induce responsiveness of testicular Leydig cells to LH, leading to an increase in testosterone (TT) production [6]. This increase in TT is necessary for the differentiation of Sertoli cells and initiation of spermatogenesis [6]. The timing and magnitude of this early LH rise has been shown to be controlled by the metabolic status of the animal [7, 8].

The effect of early life plane of nutrition on sexual development in beef bulls has been demonstrated in two related studies [7, 8] in which bulls offered a low plane of nutrition from 10 weeks of age experienced a delay in the onset of puberty compared to those offered either a medium or high plane of nutrition. This delay was associated with lower TT concentrations during the peri-pubertal period. These studies demonstrate that testicular size in mature animals is positively influenced by increased systemic FSH, TT and particularly LH in early life. Other authors concluded that daily gains of greater than 1.2 kg/day during calf-hood should be targeted to ensure earlier attainment of puberty in bulls [9]. More recently, a higher plane of nutrition led to a greater scrotal circumference (SC) and thus hastened age at puberty in Holstein-Friesian bulls [10]. It has also been suggested that SC can be used as a predictor of sperm output potential in young dairy bulls [11]. The effect of pre-pubertal growth rate relative to estimated mature weight on the age at onset of puberty has been documented for Simmental, Angus and Hereford heifers [12, 13]. A

high plane of nutrition has also been used to achieve a heavier weight, at a younger age in heifers, leading to an associated decrease of approximately four months in age at puberty [14]. Earlier puberty work in beef bred bulls has also shown that early maturing breeds of bulls attain puberty at a younger age than late maturing breeds [4]. The effect of breed type (early v late maturing) on the age at onset of puberty has been well defined for heifers; however, there has been less work carried out for bulls.

Metabolic hormones such as leptin, adiponectin, insulin and insulin-like growth factor-1 (IGF-1) signal the nutritional status of the animal to the hypothalamus via receptors in the arcuate, neuro-peptide Y and pro-opiomelanocortin nuclei [15], which regulates the secretion of LH and FSH, mediated through the action of gonadotrophin releasing hormone (GnRH). These hormones are necessary for testicular and sexual development and in turn dictate the age at which puberty is attained [16-18]. Systemic concentrations of metabolic hormones such as IGF-1 have been shown to vary according to breed [19] and higher IGF-1 was consistent with greater LH pulsatility in Angus heifers, leading to more precocious puberty, when compared to other breeds. Additionally, IGF-1 concentrations were higher in Holstein-Friesian bulls offered a high plane of nutrition up to 31 weeks of age [10]. The influence of other metabolic hormones such as leptin on the functional development of the hypothalamic-pituitary-gonadal axis is not as clear [20, 21]. We hypothesized that increasing the plane of nutrition offered to bull calves of different breeds would positively influence metabolic hormones associated with increasing secretion of reproductive hormones. Due to a lower mature bodyweight we also hypothesized that Jersey bulls would have an earlier response to plane of nutrition than Holstein-Friesians.

The aim of this study was to determine the effect of age, breed and plane of nutrition on systemic growth rate, scrotal development, metabolite concentrations, as well as exogenous GnRH-induced release of LH, FSH and TT in pre-pubertal Holstein-Friesian and Jersey bulls.

2. Materials and Methods

All animal procedures performed in this study were conducted under experimental licence from the Irish Department of Health and Children (licence 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.

2.1. Animals and treatments

Holstein-Friesian (F) and Jersey (J) bull calves (n=34) with a mean (\pm S.D.) age of 21 (\pm 10.3) days and bodyweight of 47.4 (\pm 13.0) kg and 33.1 (\pm 5.13) kg, respectively, were sourced from commercial dairy farms and were blocked on breed, age, sire and bodyweight and assigned to either a high or low plane of nutrition. All calves were group housed indoors at Teagasc, Grange Beef Research Centre on sawdust-floored pens (balanced for treatment and breed) with a space allowance of 1.6 m²/calf. Calves were individually fed milk replacer and concentrate (Table 1) using an electronic feeding system (Förster-Technik, Vario, Engen, Germany). After five days acclimatization, at 26 (\pm 10.3) days of age, high F (n=9) and high J (n=8) received 1200 g (8 L at 150 g/L) and 800 g (6 L at 133.33 g/L) of milk replacer (23% crude protein, 18% lipid; Blossom Easymix; Volac, Co. Cavan, Ireland) daily, respectively, together with concentrate *ad libitum* (Lakeland Dairies, Monaghan, Ireland). Low F (n=11) were allocated 500 g (4 L at 125 g/L) of milk replacer plus a maximum of 1 kg

of concentrates daily while low J (n=6) were allocated 350 g (3.5 L at 100 g/L) of milk replacer plus a maximum of 1 kg of concentrates daily. Treatment diets were designed using National Research Council (2001) guidelines [22]. Bulls were weaned when consuming a minimum of 1 kg of concentrate for 3 consecutive days, at a mean age (\pm S.D.) of 83 (\pm 10.5) days. Following weaning, high F and high J were offered *ad libitum* concentrates, while low F received 1.7 kg and low J received 1.4 kg of concentrate daily. All bulls had daily access to approximately 0.5 kg of straw each from pre-weaning to turn-out. Bulls were turned out to grass at 16 weeks of age where high F and high J received grass and concentrate *ad libitum* while low F and low J both received grass *ad libitum* plus 0.5 kg of concentrates per day. All animals had constant access to fresh water. Bulls were weighed weekly until weaning and were weighed at 14 day intervals thereafter (Fig 1). Scrotal circumference measurements were taken at 14 day intervals using a scrotal measuring tape (Neogen®, Lexington, KY, USA.), beginning at 19 weeks of age (Fig 1). Both SC and weight measurements continued until bulls reached 49 weeks of age. A SC of 28 cm was used a proxy for age at puberty [23].

The health status of these animals from initiation of dietary treatments until 14 days post-weaning has been characterized in detail [24].

2.2. Blood sampling and analysis

Monthly bloods samples were collected via jugular venipuncture, beginning at approximately 4 weeks of age (Fig 1). On each occasion, blood was collected into either a 9 mL evacuated tube containing lithium heparin (Greiner Vacuette; Cruinn Diagnostics, Dublin, Ireland) for adiponectin, IGF-1, metabolite and leptin analysis or into a 6 mL K3 ethylenediaminetetraacetic acid (K3 EDTA; Vacuette, Cruinn

Diagnostics) for insulin analysis. Blood was centrifuged at 1750 g for 15 min; plasma was harvested and stored at -20 °C until analysis was performed.

Insulin-like growth factor-1 concentrations were determined using a radioimmunoassay (RIA) following acid-ethanol extraction using the method previously described by Beltman *et al.* [25]. The intra- and inter- assay coefficients of variation (CVs) were determined by replicating a low, normal and high reference sample, at the beginning, middle and end of each assay. Intra- and inter-assay CVs for IGF-1 were 12.7, 6.9, 5.1% and 7.5, 1.6, 4.1% for low, medium and high, respectively. The sensitivity of each assay was defined as the lowest concentration detectable. The sensitivity of the IGF-1 assay was 4 ng/mL. Insulin concentrations were determined using INS-IRMA (immunoradiometric assay) kits (DIAsource Immunoassays, Louvain-la-Neuve, Belgium), following the manufacturer's instructions. Intra- and inter-assay CVs for insulin were 5.5, 4.7, 4.1% and 13.9, 10.8, 11.2% for low, medium and high, respectively. The sensitivity of insulin assays was 1 ng/mL. Leptin concentrations were determined using enzyme immunoassay, previously described by Sauerwein *et al.* [26]. The sensitivity of the leptin assay was 0.6 ng/mL. Intra- and interassay CVs were 5% and 9%, respectively. Adiponectin concentrations were determined using enzyme immunoassay, previously described by Mielenz *et al.* [27]. The sensitivity of the adiponectin assay was 0.03 ug/mL. Intra- and interassay CVs were 5% and 10%, respectively.

Concentrations of albumin, urea, total protein, β -hydroxybutyrate (BHB), glucose, non-esterified fatty acid (NEFA), triglycerides, and creatinine were determined as described by Lawrence *et al.* [28]. All metabolite concentrations were measured on an automatic analyzer (AU 400; Olympus, Tokyo, Japan). The inter assay CVs for low, medium and high standards were 1.65, 1.77 and 0.72%,

respectively. The sensitivities were 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, creatinine: 2.3 μ mol/L. Globulin concentration was calculated as the difference between total protein and albumin concentrations [29].

A GnRH intravenous challenge was carried out at 16, 24 and 32 weeks of age (Fig 1). At each challenge, a GnRH agonist (Buserelin Receptal®; Intervet Ireland Limited, Dublin, Ireland) was administered (0.05 mg/kg bodyweight, i.v.), immediately after the third blood sample was taken. Blood samples were collected at 15 min intervals for LH, FSH and at 30 min intervals for TT. Blood was allowed to clot at room temperature overnight and was then centrifuged at 780 g for 10 min. Serum was harvested and stored at -20°C until analysis. Sampling at 16 weeks of age was done based on the documented early gonadotropin rise normally occurring between 12 and 20 weeks of age [5]. Sampling was carried out at 24 weeks of age to profile the end of the first transient LH rise [5]. Bulls were sampled at 32 weeks as this time-point is associated with an increase in serum TT and a second rise in LH secretion associated with puberty in beef bulls [30].

All three hormones were analyzed in duplicate using RIA. Follicle stimulating hormone concentrations were determined using the method described by Crowe *et al.* [31]. The sensitivity of the assay was 0.05 ng/mL. Intra- and inter-assay CVs were 12.5, 9.4, 9.3% and 12.8, 6.9, 12.8% for low, medium and high, respectively. Luteinizing hormone concentrations were determined using the method described by Cooke *et al.* [32]. The sensitivity of the assay was 0.2 ng/ml. Intra- and inter-assay CVs were 8.7, 8.9, 8.7% and 8.4, 6.0, 8.4% for low, medium and high, respectively. Testosterone concentrations were determined using solid-phase RIA kits (Siemens, Los Angeles, CA, USA); following the manufacturer's instructions. The sensitivity of

the assay was 0.1 ng/mL. Intra- and inter-assay CVs were 5.0, 3.5, 2.4% and 16.2, 5.3, 4.9% for low, medium and high, respectively.

2.3. Statistical analysis

Area under the curve (AUC) was determined for FSH, LH and TT between 0 and 135 min relative to GnRH administration using Sigma Plot, version 11 (Systat Software, San Jose, CA). Basal serum concentration of each hormone (mean of two samples taken prior to GnRH injection) for each animal was included as a covariate in the statistical model. All data, including weight, SC, monthly blood samples and GnRH challenges were analyzed using the following procedures of Statistical Analysis Software (SAS version 9.3, Cary, NC, USA). Data were tested for normality (UNIVARIATE procedure) and, post-weaning bodyweight, metabolic hormone, metabolite, with the exception of albumin and glucose and reproductive hormone, with the exception of LH data were transformed to the power of lambda (TRANSREG procedure). Data were then subjected to repeated measures ANOVA (MIXED procedure). Age was included as the repeated term. Differences in individual least-square means were evaluated using the Tukey-Kramer adjustment. Diet, block, breed, age and their interactions were included in the model. The interaction term, if not statistically significant ($P > 0.05$), was subsequently excluded from the final model. The covariance matrix, namely simple, compound symmetry or toeplitz was determined for each variable by examining the Bayesian Information Criteria (**BIC**) (smaller is better). All results are presented as mean \pm s.e.m., unless stated otherwise.

3. Results

Bodyweight and scrotal circumference

There were no plane of nutrition by breed by age interactions among the main effects for either pre- or post-weaning bodyweight measurements ($P>0.05$). There was a plane of nutrition by age interaction for pre-weaning bodyweight ($P<0.001$; Fig 2). An effect of plane of nutrition on bodyweight was not evident until animals reached 4 weeks of age, after which bulls on the high plane of nutrition remained heavier than those on low plane of nutrition. There was also a breed by age interaction for pre-weaning bodyweight ($P<0.05$); F bulls were heavier than J at the start of the study (3 weeks of age); this difference did not reach statistical significance at either 4 or 5 weeks of age but was detectable again at 6 weeks of age after which F remained heavier than J until weaning. There was a plane of nutrition by age interaction for post-weaning bodyweight ($P<0.001$; Fig 3). Consistent with the pre-weaning period, bulls on a high plane of nutrition were heavier than those on a low plane; the magnitude of this difference increased after weaning and continued to increase until 49 weeks of age ($P<0.01$). There was a breed by age interaction for post-weaning bodyweight ($P<0.001$). There were small differences between breeds from 13 to 21 weeks of age; however, from 23 weeks of age, the bodyweight difference between F and J bulls increased until the end of the study at 49 weeks of age.

There was a plane of nutrition by breed by age interaction for SC ($P<0.01$; Fig 4). Holstein-Friesian bulls on a high plane of nutrition had a larger SC than those on a low plane of nutrition at 33, 37, 41 and 43 weeks of age. Scrotal circumference was also larger for F than J bulls on a low plane of nutrition from 31 to 49 weeks of age. Jersey bulls on a high plane of nutrition had a larger SC than either F or J bulls on a low plane of nutrition from 25 to 49 weeks of age, with the exception of low F from 45 to 49 weeks of age. Within plane of nutrition, there was no difference between either high F or J bulls ($P>0.05$). Low F had a larger SC than low J at 47 and 49 weeks of

age ($P < 0.05$). Predicted age at puberty, based on a threshold SC of 28 cm [33] and proportion of bulls predicted to be pubertal at 49 weeks of age from each diet group are presented in Table 2. Although not statistically significant, J bulls on a high plane of nutrition were predicted to reach a threshold SC of 28 cm 3 weeks younger than F bulls on a high plane of nutrition ($P > 0.05$) and 9 weeks younger than F bulls on a low plane of nutrition ($P < 0.01$). Holstein-Friesian bulls on a high plane of nutrition were predicted to reach a threshold SC of 28 cm 6.5 weeks younger than F bulls on a low plane of nutrition ($P < 0.05$). Based on SC measurements, only one J bull from the low plane of nutrition reached a threshold SC of 28 cm at 49 weeks of age; therefore, this group could not be directly compared to other groups.

Metabolic hormones and metabolites

There was a breed by age interaction for systemic concentrations of IGF-1 ($P < 0.01$; Fig 5); F bulls had higher IGF-1 than J bulls at the start of the study. However, serum IGF-1 concentrations converged at 8 weeks of age and were similar between the two breeds for the remainder of the study. There was also a plane of nutrition by age interaction for IGF-1 ($P < 0.001$); concentrations were higher in bulls fed the high plane of nutrition at 8 weeks of age, compared to those on the low plane of nutrition. Insulin-like growth factor-1 concentrations then converged until 24 weeks of age. From 24 to 28 weeks of age, bulls on the high plane of nutrition had higher IGF-1 concentrations than those on the low plane of nutrition. There was a plane of nutrition by age interaction for insulin concentration ($P < 0.001$; Fig 5). Bulls on a high plane of nutrition had higher insulin than those on low, at both 32 and 36 weeks of age. There was no effect of breed on insulin concentration ($P > 0.05$).

There was a breed by age interaction for adiponectin concentration ($P < 0.001$; Fig 6). Holstein-Friesian bulls had a higher concentration of adiponectin at 12 weeks of age in comparison to J bulls. Bulls on a high plane of nutrition tended to have higher adiponectin concentrations compared to bulls on a low plane of nutrition ($P = 0.05$). There was no effect of plane of nutrition or breed on systemic concentrations of leptin ($P > 0.05$; Fig 6). There was an effect of age ($P < 0.001$) on leptin with concentrations being higher in all bulls at 20 weeks than at 16 weeks of age ($P < 0.01$); no differences were detected at any other age ($P > 0.05$).

There was no plane of nutrition by breed by age interaction or breed effect for albumin concentration ($P > 0.05$; Fig 7). Bulls on a high plane of nutrition tended to have higher albumin concentrations compared to those on a low plane of nutrition ($P = 0.07$). There was a quadratic effect of age on albumin concentration ($P < 0.001$) which decreased between 4 and 12 weeks of age and then, following a large increase at 16 weeks of age, decreased to levels similar to those observed at the beginning of the experimental period. There was no plane of nutrition by breed by age interaction or plane of nutrition effect on creatinine concentration ($P > 0.05$; Fig 7). There was a breed by age interaction for creatinine ($P < 0.001$); J bulls had higher creatinine concentrations at all blood sampling time-points compared to F bulls. However, the magnitude of this difference between F and J began to decrease from 24 weeks of age with smaller differences particularly evident at 24 and 39 weeks of age ($P < 0.05$).

There was no plane of nutrition by breed by age interaction for globulin concentration ($P > 0.05$, Fig 7). There was a plane of nutrition by age interaction ($P < 0.05$); bulls on the high plane of nutrition tended to have lower globulin concentrations compared to those on the low plane of nutrition at 8 ($P = 0.06$) and 12

weeks ($P=0.09$) of age. Jersey bulls had a higher globulin concentration than F bulls ($P<0.001$). There was no plane of nutrition by breed by age interaction or effect of breed on glucose concentration ($P>0.05$; Fig 7). There was a plane of nutrition by age interaction ($P<0.01$), manifested as bulls on a high plane of nutrition having a higher glucose concentration compared to bulls on a low plane at 8 weeks of age.

There was no plane of nutrition by breed by age interaction for total protein concentration ($P>0.05$). There was a plane of nutrition by age interaction for total protein ($P<0.001$; Fig 7). Bulls on a high plane of nutrition had a lower total protein concentration than those on a low plane of nutrition at 8 weeks of age. Differences between planes of nutrition were not detected at any other age. Total protein concentrations were affected by breed ($P<0.01$) with J having a higher concentration compared to F bulls. There was no plane of nutrition by breed by age interaction for triglyceride concentration ($P>0.05$; Fig 7). There was a tendency for a plane of nutrition by breed interaction of triglyceride concentration ($P=0.08$). Jersey bulls on a low plane of nutrition had lower triglyceride concentrations than F bulls on a low plane of nutrition ($P<0.05$) and F bulls on a high plane of nutrition ($P<0.05$). Jersey bulls on a low plane of nutrition also tended to have lower concentrations than J bull on a high plane of nutrition ($P=0.09$). There was an effect of age on triglyceride concentration ($P<0.001$) as a result of a significant decrease in triglycerides between 12 and 16 weeks of age and also between 20 and 24 weeks of age.

There were no differences between the main effects of either plane of nutrition or breed on NEFA concentration ($P>0.05$; Fig 8). There was an effect of age on NEFA concentrations ($P<0.001$) which increased from 8 to 12 weeks, decreased from 12 to 20 weeks, increased again from 20 to 24 weeks and finally decreased from 24 to 28 weeks and thereafter remained unchanged to the end of the trial

period. There was a strong tendency for a plane of nutrition by breed by age interaction for BHB concentration ($P=0.06$; Fig 8). At 12 weeks of age, F bulls on a high plane of nutrition had a lower concentration of BHB than both F and J bulls on a low plane of nutrition. Concentrations of BHB increased from 4 to 20 weeks of age ($P<0.01$), after which BHB concentrations plateaued and were not different. No plane of nutrition by breed by age interaction was detected for urea concentration ($P>0.05$; Fig 8). There was a plane of nutrition by age interaction ($P<0.001$) manifested as bulls on a high plane of nutrition having a higher urea concentration at 24 weeks of age compared to those on a low plane of nutrition. There was an effect of breed on urea concentration ($P<0.05$) as F bulls had higher concentrations than J bulls.

Intravenous GnRH challenge

For statistical analysis, concentrations of each hormone were represented by AUC data; as such, results below are total AUC following GnRH administration. There were no plane of nutrition by breed by age interactions for concentrations of either FSH or LH ($P>0.05$; Fig 9, Fig 10). FSH concentrations were not affected by plane of nutrition or breed but were affected by age ($P<0.01$). Concentrations of FSH were highest at 16 weeks of age and decreased subsequently. Irrespective of breed, bulls on the high plane of nutrition had higher concentrations of LH compared with those on the low plane of nutrition ($P<0.05$). There was no effect of age on LH concentrations ($P>0.05$). There was a three-way interaction of plane of nutrition by breed by age for serum concentrations of TT ($P<0.001$; Fig 11). All bulls had low concentrations of TT at 16 weeks of age compared with either 24 or 32 weeks of age. Concentrations increased between 9 and 10-fold at 24 and 32 weeks of age, after a GnRH challenge, with J having consistently higher concentrations of TT than F ($P<0.01$), particularly those on a high plane of nutrition at 24 weeks of age.

4. Discussion

Increasing the plane of nutrition offered in the early calf-hood period has a positive effect on the metabolic status of bull calves. This, in turn, leads to an increase in concentrations of metabolic hormones such as IGF-1, which in turn stimulate GnRH secretion [34] and thus evoke a cascade of trans-tissue endocrinologically-mediated events, necessary for puberty to occur in the bull. The results of this study clearly demonstrate that both plane of nutrition and breed affect TT production in early calf-hood with higher systemic concentrations evident in calves offered a high compared to low plane of nutrition and J calves having higher concentrations than F calves.

Heavier bodyweight and a larger SC were observed in bulls fed a high compared with a low plane of nutrition, consistent with previous reports [7-9]. However, between weeks 41 and 49 the growth of J bulls on a low plane of nutrition plateaued. Grass was offered to all animals *ad libitum* and in addition the bulls on a low plane of nutrition were offered 0.5 kg of concentrate. The diet offered should have supported a growth rate in excess of 0.5 kg/day according to the national research council guidelines. Grazing groups were balanced for breed so the same diet was available to both breeds on a low plane of nutrition. Therefore, it is unclear why the growth rate of Jersey bulls was static while the Holstein-Friesians maintained an upward growth trajectory. The lack of a breed difference for SC was surprising considering the difference in bodyweight between the two breeds. However, when SC was expressed as a percentage of bodyweight, in order to account for differences in mature bodyweight, SC accounted for a greater percentage of bodyweight in J compared to F bulls (Table 2). It is also interesting that J bulls had a larger SC on a high plane of nutrition compared to that of F bulls while J bulls on a low plane of nutrition responded in the opposite manner, with a smaller SC than their F

contemporaries. This testicular growth retardation had a detrimental effect on the proportion of bulls which could be categorized as pubertal at 49 weeks of age, using the definition of achieving a SC of 28 cm [23].

In the present study, systemic concentrations of IGF-1 were not affected by plane of nutrition until 24 weeks of age, after which bulls on a high plane of nutrition had higher IGF-1 than bulls on low. Similar results have been shown in beef-bred bull calves fed either a control or restricted (75% of *ad libitum* control) diet, whereby, those on a control diet had higher IGF-1 concentrations at 26 weeks of age [8]. In contrast to these findings, nutritional effects on IGF-1 have been reported at 11 weeks of age in F bulls, where bulls on a high plane of nutrition had higher IGF-1 than those on low during 10 hours of intensive blood sampling [10]. The results of the two aforementioned studies have shown temporal associations between systemic concentrations of IGF-1 and LH. While no temporal relationships were observed between IGF-1 and LH in the current study, the overall effect of a higher plane of nutrition on LH is likely to have been mediated, to some extent, by increases in hepatic IGF-1 production. Indeed, receptors for IGF-1 have been detected in the pre-optic area of the hypothalamus, stimulating GnRH secretion [35] and also in anterior pituitary cell lines of rats; an increase in LH responsiveness to GnRH has been detected when pituitary cell lines are incubated in IGF-1 [36], highlighting the important association between IGF-1 and gonadotrophin secretion.

The effects of insulin on gonadotrophin secretion are inconsistent; in ewes intra-cerebral-ventricular infusion of exogenous insulin has resulted in both an increase [37] and decrease [38] in LH pulsatility. In heifers, a decrease in insulin, induced by fasting, has been associated with a reduction in LH pulsatility [39]. In the current study, a high plane of nutrition led to higher insulin concentration, though

concentrations were within the normal range low plane of nutrition did not display extremely suppressed levels of insulin. In this instance, insulin would be available to regulate blood glucose levels allowing maintenance of glucose homeostasis [40]. Animals in greater energy balance should have higher adiposity and therefore higher concentrations of leptin [41]. Nonetheless, leptin was unaffected by either plane of nutrition or breed in the current study and this lack of an effect of nutrition has previously been reported in young bulls that were offered different planes of nutrition [8, 10] perhaps due to the low levels of subcutaneous fat in these growing animals. Similar to insulin, leptin only plays an important, short-term role, in terms of GnRH secretion, during periods of fasting. In cows, short-term fasting appears to sensitize the hypothalamic-pituitary axis to exogenous leptin, which results in heightened secretion of both LH and insulin following treatment with exogenous leptin [42]. Since leptin receptors are not found on GnRH neurons, it is likely that kisspeptin acts as an intermediary between leptin and GnRH neurons; signaling metabolic status [43]. The effects of nutrition and breed on adiponectin concentrations in bulls have not been well characterized. In the current study, F bulls had higher adiponectin concentrations than J bulls at 12 weeks of age. In addition, bulls on a high plane of nutrition tended to have higher concentrations than those on low; both findings are in contrast to the previous reports. Similar to leptin, adiponectin is secreted by brown and white adipose tissue, but at higher concentrations than leptin [44] and in contrast to leptin, adiponectin is inversely related to body fat mass, in cattle [45]. Therefore, it would be expected that young dairy bulls with a higher potential for fat deposition (high vs low plane of nutrition) would have lower adiponectin concentrations. Increased adiponectin concentrations are associated with disturbances in GnRH pulsatility, in men [46] via down-regulation of *KISS1* gene; in *in vitro* hypothalamus

cell lines [47], indicating that adiponectin has an opposing effect to that of leptin on GnRH pulsatility.

Many of the metabolites analyzed in the current study are known to have an indirect role in reproduction, primarily as metabolic signals to the hypothalamus. Circulating leptin concentrations have been shown to be related to body adiposity [41] and triglyceride concentration [48] in cattle. This does not appear to be the case in the current study. Triglycerides are mobilized in response to negative energy balance-induced lipolysis [49]. If triglycerides are stored, there is a greater potential for adipose tissue to produce leptin; however, if triglycerides are mobilized, adipose tissue reserves may be reduced leading to a lower leptin concentration and thus reduce the stimulatory effect of GnRH secretion. The tendency towards lower triglycerides in the F bulls on a high plane of nutrition indicates a positive metabolic status, potentially facilitating greater leptin synthesis and secretion. The higher concentration of glucose in bulls on a high plane of nutrition is consistent with their greater feed intake and overall better metabolic status. The effects of glucose on the hypothalamus have been reviewed by Burdakov *et al.* [50] who concluded that glucose stimulated the pro-opiomelanocortin nuclei and inhibited the appetite-promoting neuro-peptide Y nuclei, processes which have been shown in sheep to be important for the secretion of GnRH. [51]. The higher urea concentration in bulls on the high plane of nutrition is likely as a result of higher protein intake. It has been shown that protein supplementation which leads to an increase in blood urea concentrations, has no apparent effect on steroid hormone concentrations or pregnancy rates in cows [52] or heifers [53]. In cows, an increase in blood urea concentrations has been shown to coincide with an increase in LH secretion [54], while others have reported that LH secretion is unaffected by changes in urea [55,

56]. It is more plausible that the increased LH secretion in calves on a high plane of nutrition, observed here, was more related to the increased systemic IGF-1 concentrations recorded.

There was no effect of breed or plane of nutrition on systemic concentrations of FSH, following GnRH administration, but FSH did decrease with age. At birth, FSH concentrations are typically high and begin to decline at 14 weeks of age and are at their lowest concentrations by around 25 weeks of age in the bull [34]. This period coincides with a period of rapid proliferation of immature Sertoli cells [33] suggesting that FSH concentration in the young bull calf plays a role in determining future semen production potential. This proliferation period may even be limited to the initial post-natal period; as administration of exogenous FSH to bull calves between 4 and 8 weeks of age increased the number of Sertoli cells per seminiferous tubule [57], observed following castration at 56 weeks of age. This would suggest that the early effects of nutrition on GnRH pulsatility may influence the proliferation of Sertoli cells in the young bull calf. It has been recently reported [10] that once FSH concentrations have declined they remain low, in agreement with the findings of this study. However, others [30] reported a second rise in FSH, beginning at 30 weeks of age and continuing to 45 weeks of age for Hereford and Hereford x Charolais bulls offered a common plane of nutrition. There was no evidence of such a rise at the 32-week sampling point of this study or it may have occurred after the final sampling point of this study. As FSH appears to be unaffected by breed or level of nutrition post-natally, it may be that concentrations reflect pre-natal in utero environment. For example, it has been shown, in cattle, that systemic FSH concentrations are increased when a low diet is offered, to the dam, pre-natally [58]. However, the

authors acknowledged that energy supplied, to dams, by the high diet may have been insufficient to cause an appreciable increase in FSH, in their offspring [58].

Age had no effect on systemic concentrations of LH. This result was unexpected, as other studies have shown that LH is highest between 12 and 20 weeks, after which concentrations begin to decline [30, 33]. This rise could have occurred either before or after the 16 week sampling point employed in this study. However, as 16 weeks is in the center of the documented LH rise window it should have been the optimal time to detect a rise in LH. The higher secretion of LH in bulls offered a high plane of nutrition indicates that they had a greater store of LH within the anterior pituitary during the period of the brain's highest plasticity (the infantile period). This represents a positive effect of offering a higher plane of nutrition when trying to increase LH secretion, given that early increases in LH can hasten puberty and sexual maturity [8]. The relationship between plane of nutrition and IGF-1 in F bulls [10] and Angus and Angus x Charolais bulls [59] has been documented. Brito *et al.* (2007) found a moderate positive correlation between IGF-1 and leptin in beef-bred bulls. As reproductive hormones are regulated by the metabolic status of the animal, the higher energy intake of animals, on *ad libitum* intake is likely to lead to a stimulatory effect on the hypothalamus, leading to increased GnRH secretion and thus a higher pulsatile release of LH.

Serum TT concentrations were highest in bulls on the high plane of nutrition. Jersey bulls on this plane of nutrition produced more TT than F at 24 and 32 weeks of age and TT concentrations increased significantly with age for both breeds and nutritional groups. The level of TT detected at 24 weeks in the present study for J bulls is in agreement with other studies using dairy bulls [60]. Other studies also reported highest TT concentrations at 24 weeks when Angus or Angus x Charolais bull calves

were either offered either a high or a moderate plane of nutrition [61] and also when feeding maintenance diets to F bulls [6]. The overall increase in TT concentrations observed in these bulls coincides with a decrease in LH and FSH secretion at the same time-points. This observation is supported by the findings of others [5], where androgens were shown to have a negative feedback on gonadotrophin release in bulls. The treatment response of higher TT secretion at 24 and 32 weeks in these animals supports the argument for feeding bulls a higher plane of nutrition in the pre-pubertal period as TT plays a critical role in spermatogenesis [62]. While the importance of high concentrations of TT to spermatogenesis is not fully understood, low concentrations of TT are associated with a dramatic reduction in sperm production post puberty [63]. It is possible that the greater TT in J bulls is associated with SC; however; there was no effect of breed on SC. Furthermore, there is no evidence in the literature to suggest that J bulls have a greater population of Leydig cells than F bulls. There is evidence, however, that J bulls will reach puberty at a younger age than F bulls when both breeds are fed *ad libitum* diets [64]; therefore this increase in TT may be associated with earlier sexual development.

While measurement of age at onset of puberty, using traditional approaches [65] was beyond the scope of the current study the higher production of LH and TT observed, along with larger scrotal circumference suggests that bulls on a higher plane of nutrition would reach puberty and thus sexual maturation at a younger age, compared to their contemporaries offered a lower plane of nutrition based on the findings of [4, 10, 61]. For example, based on reaching a threshold SC of 28 cm, we estimate that bulls on a high plane of nutrition would be 7.5 weeks younger at puberty than those on the low plane of nutrition. This difference in age at puberty using a SC of 28 cm as a proxy has been reported in F bulls when offered a high or

low plane of nutrition for 31 weeks followed by a common, moderate plane of nutrition [10]. Ours is the first published study comparing F and J bulls of different planes of nutrition. Differences between breeds in estimated age at puberty were not found in the current study, mostly likely due to small sample size when the low J bulls were excluded. There was a numerical difference for age at puberty between F and J bulls (6 weeks) and this trend of J bulls being younger at puberty, than F has been reported previously and has been associated with the lower proportion of mature bodyweight that J bulls have to reach [64]. Notwithstanding this, within the confines of the current study, when maintained on a lower plane of nutrition F were estimated to be pubertal before J bulls.

In conclusion, this study has demonstrated that young dairy bulls on a high plane of nutrition have a better metabolic status as evidenced by higher systemic concentrations of IGF-1 and insulin and as a result have a greater potential for synthesis and secretion of LH than their contemporaries maintained on a lower plane of nutrition. This was consistent with higher TT production in the pre-pubertal period and coincided with increased testicular development and most likely earlier onset of puberty.

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Figure titles

Fig 1. Schedule of procedures carried out for the experiment. IGF-1: insulin-like growth factor 1; GnRH: gonadotrophin releasing hormone.

Fig 2. Effect of plane of nutrition and breed on pre-weaning bodyweight of pre-pubertal dairy bulls measured weekly; from the start of the trial at 3 weeks to weaning at 11 weeks of age. T= effect of plane of nutrition; B= effect of breed; A= effect of age; T*B*A= interaction effects; Error bars = \pm S.E.M. NS = non-significant

Fig 3. Effect of plane of nutrition and breed on post-weaning bodyweight of young dairy bulls measured bi-weekly from post-weaning to 49 weeks of age. T= effect of plane of nutrition; B= effect of breed; A= effect of age; T*B*A= interaction effects; Error bars = \pm S.E.M. NS = non-significant

Fig 4. Effect of plane of nutrition and breed on scrotal circumference of young dairy bulls measured bi-weekly from 19 to 49 weeks of age. T= effect of plane of nutrition; B= effect of breed; A: effect of age; T*B*A= interaction effects. Error bars = \pm S.E.M.

Fig 5. Mean insulin-like growth factor-1 (IGF-1; upper pane) and insulin (lower pane) concentrations in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition; B= effect of breed; A: effect of age; T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant.

Fig 6. Mean leptin (upper pane) and adiponectin (lower pane) concentrations in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition; B= effect of breed; A: effect of age; T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant

Fig 7. Mean plasma metabolite concentrations in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition, B= effect of breed, A= effect of age, T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant

Fig 8. Mean plasma non-esterified fatty acids (upper panel), beta-hydroxybutyrate (middle panel) and urea concentrations (lower panel) in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition, B= effect of breed, A= effect of age, T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant

Fig 9. Mean serum concentration of follicle stimulating hormone (FSH), before and after a GnRH challenge (marked by the arrow), in Holstein-Friesian and Jersey bull calves, at 16 (upper panel), 24 (middle panel), and 32 (lower panel) weeks of age; offered a high or low plane of nutrition. T= effect of plane of nutrition, B= effect of breed, A= effect of age, T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant

Fig 10. Mean serum concentration of luteinizing hormone (LH) before and after a GnRH challenge (marked by an arrow), in Holstein-Friesian and Jersey bulls, at 16 (upper panel), 24 (middle panel), and 32 (lower panel) weeks of age; offered a high or low plane of nutrition. T= effect of plane of nutrition; B= effect of breed; A= effect of age; T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant

Fig 11. Mean serum concentrations of testosterone (TT) before and after a GnRH challenge (marked by an arrow), in Holstein-Friesian and Jersey bull calves, at 16 (top), 24 (middle), and 32 (bottom) weeks of age; offered a high or low plane of

nutrition. T= effect of plane of nutrition, B: effect of breed, A= effect of age, T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant

Table 1. Ingredient and chemical composition of concentrate offered to calves.

Ingredient (%)	Concentrate
Barley	26.5
Soya Bean (dehulled)	25
Maize	15
Beet pulp	12.5
Soya hulls	12.5
Molasses	5
Minerals and Vitamins	2.5
Vegetable oil	1
DM	91.2
Energy (MJ ME/kg DM) ¹	11.06
Crude Protein (g/kg DM) ²	188
Crude Fiber (g/kg DM)	80
Ash (g/kg DM)	71
Crude oil (g/kg DM)	33

¹megajoules of metabolisable energy per kilogram of dry matter

²grams per kilogram of dry matter

Table 2. Effect of plane of nutrition and breed on estimated age at puberty in dairy bulls

	High J	High F	Low J	Low F	Diet	Breed
Scrotal circumference (% of bodyweight)	13 ± 0.15 ^{ac}	10 ± 0.10 ^b	14 ± 0.20 ^a	12 ± 0.10 ^c	***	***
Pubertal at 49 weeks of age	8/8 (100%)	9/9 (100%)	1/6 (17%)	8/11 (72%)	-	-
Age at puberty (weeks) ¹	34 ± 1.5 ^a	37 ± 2.5 ^a	-	43 ± 1.1 ^b	*	ns

^{a,b,c}= Different superscripts differ significantly within row. *= $P < 0.05$; ***= $P < 0.001$.

¹Based on attainment of scrotal circumference of 28 cm, measured bi-weekly from 19 weeks of age (Lunstra *et al.*, 1978).

Mean ± standard error of mean.

J= Jersey. F=Holstein-Friesian.

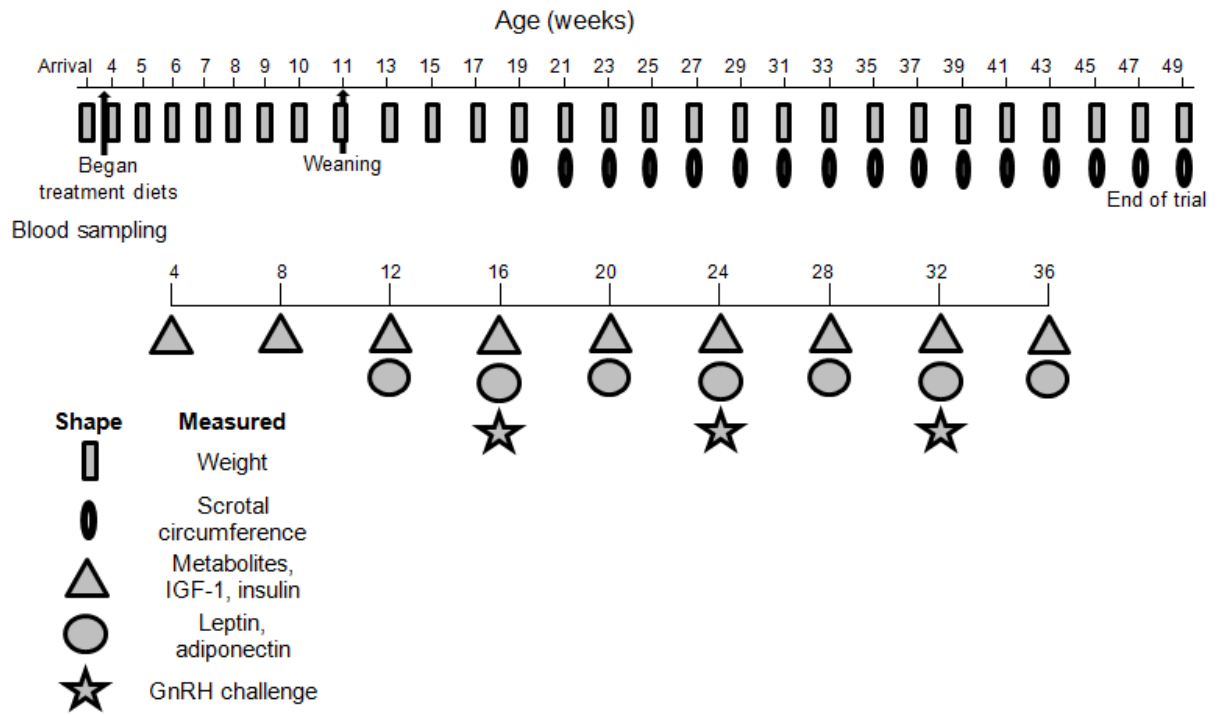


Figure 4.1. Schedule of procedures carried out for the experiment. IGF-1: insulin-like growth factor 1; GnRH: gonadotrophin releasing hormone.

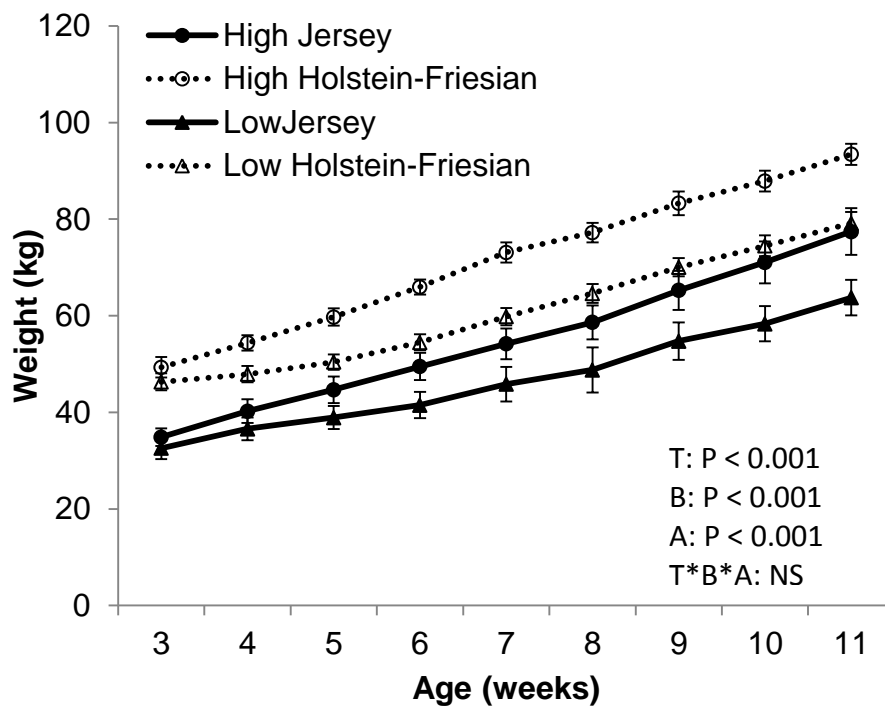


Figure 4.2. Effect of plane of nutrition and breed on pre-weaning bodyweight of pre-pubertal dairy bulls measured weekly; from the start of the trial at 3 weeks to weaning at 11 weeks of age. T= effect of plane of nutrition; B= effect of breed; A= effect of age; T*B*A= interaction effects; Error bars = \pm S.E.M. NS = non-significant

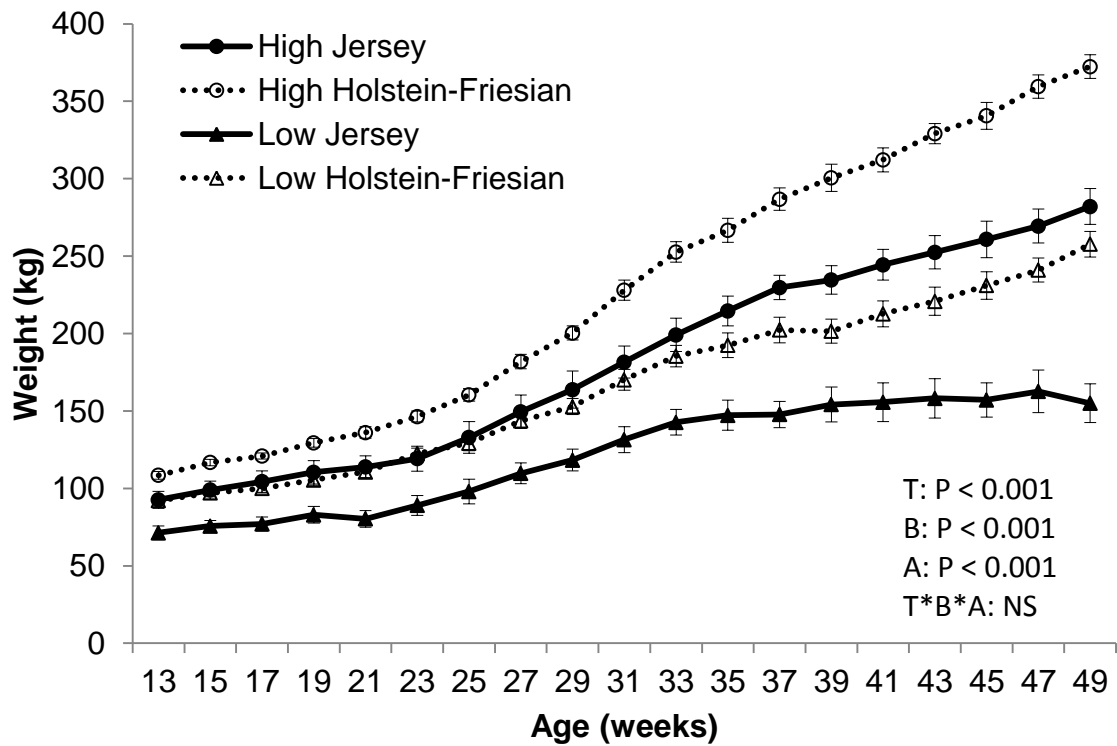


Figure 4.3. Effect of plane of nutrition and breed on post-weaning bodyweight of young dairy bulls measured bi-weekly from post-weaning to 49 weeks of age. T= effect of plane of nutrition; B= effect of breed; A= effect of age; T*B*A= interaction effects; Error bars = \pm S.E.M. NS = non-significant

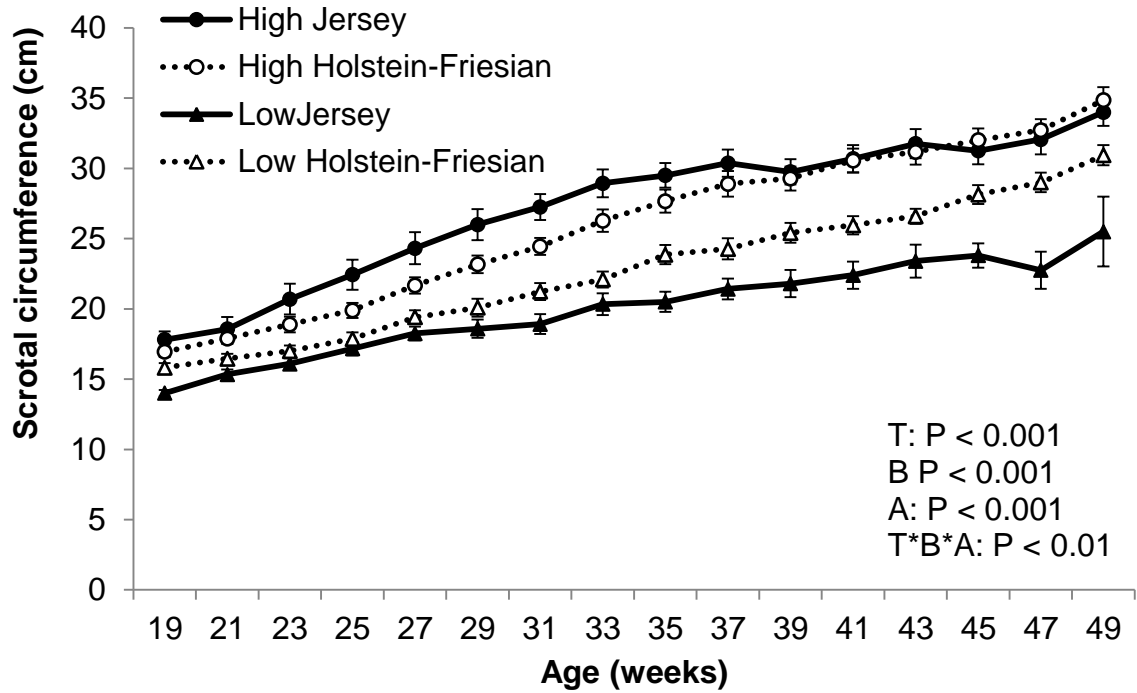


Figure 4.4. Effect of plane of nutrition and breed on scrotal circumference of young dairy bulls measured bi-weekly from 19 to 39 weeks of age. T= effect of plane of nutrition; B= effect of breed; A: effect of age; T*B*A= interaction effects. Error bars = \pm S.E.M.

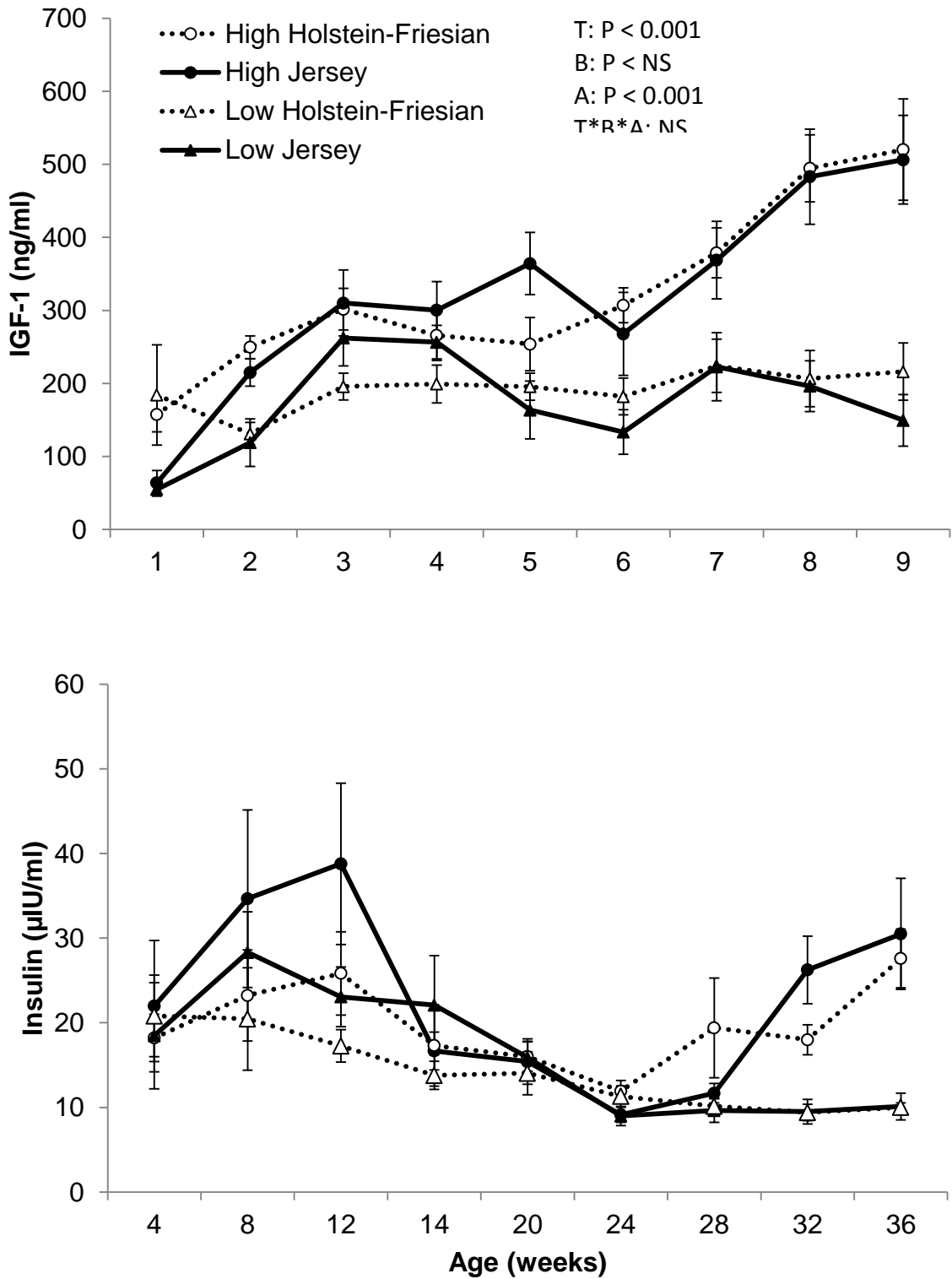


Figure 4.5. Mean insulin-like growth factor-1 (IGF-1; upper pane) and insulin (lower pane) concentrations in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition; B= effect of breed; A: effect of age; T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant.

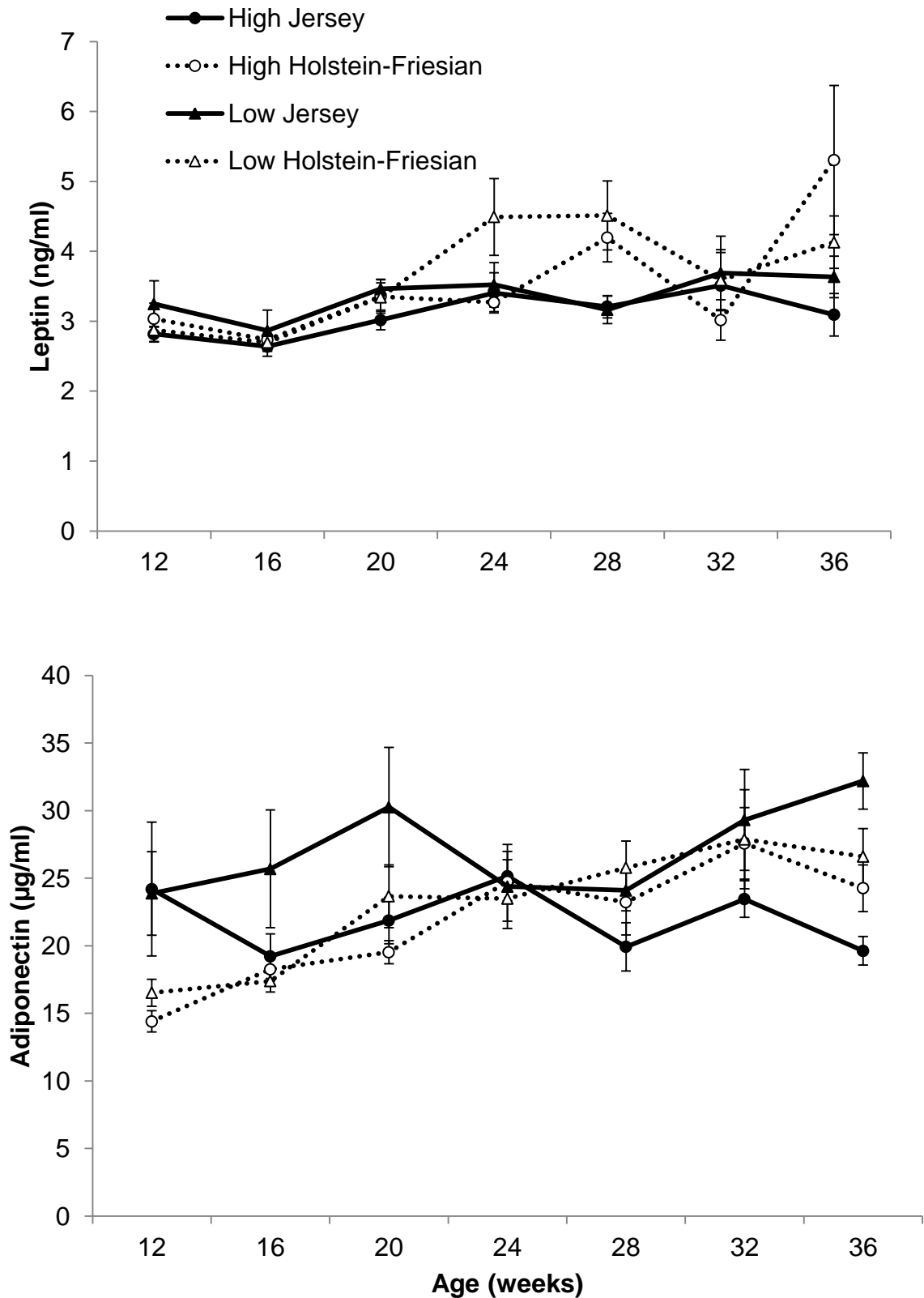


Figure 4.6. Mean leptin (upper pane) and adiponectin (lower pane) concentrations in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition; B= effect of breed; A: effect of age; T*B*A= interaction effects. Error bars = ±S.E.M. NS = non-significant

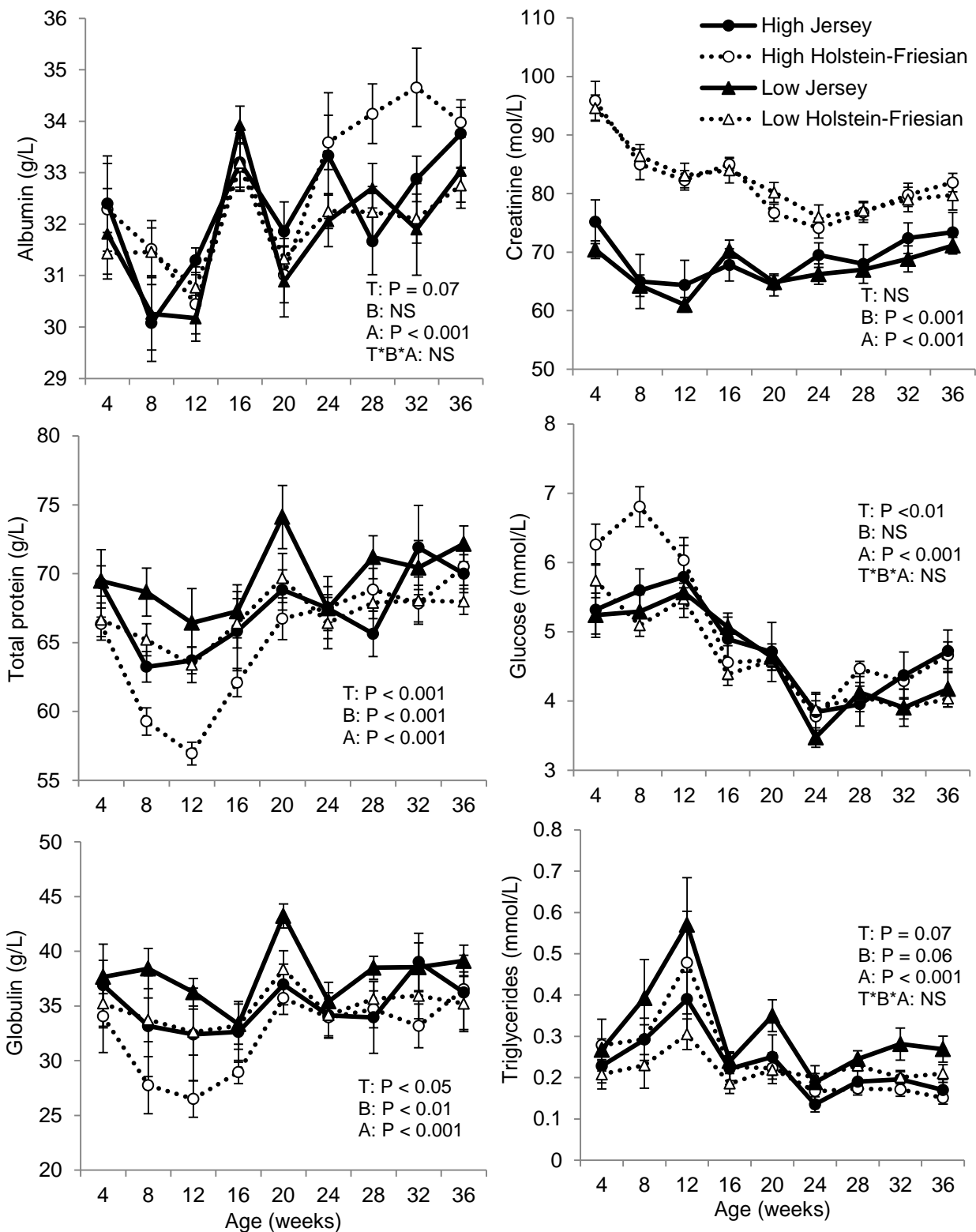
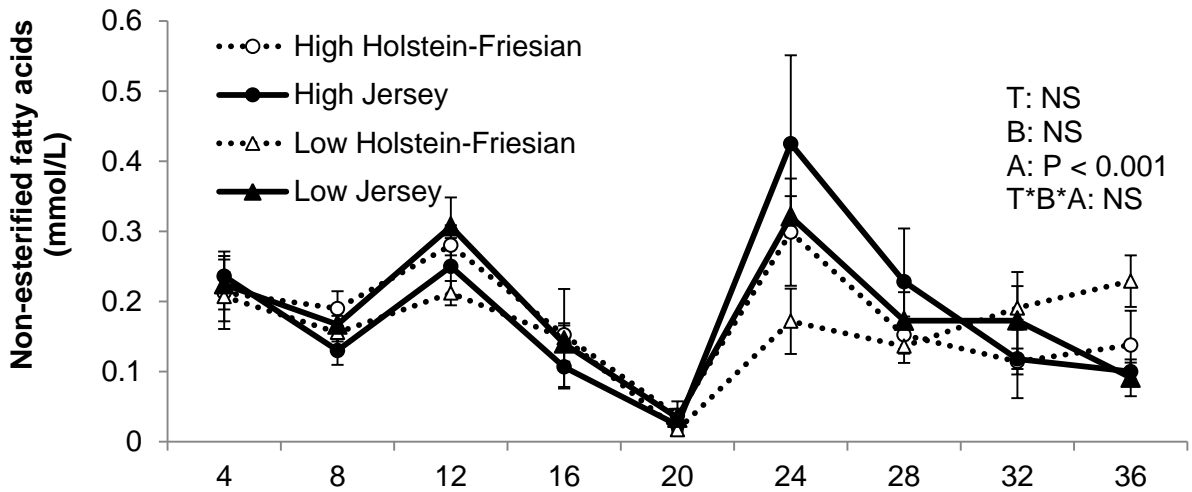
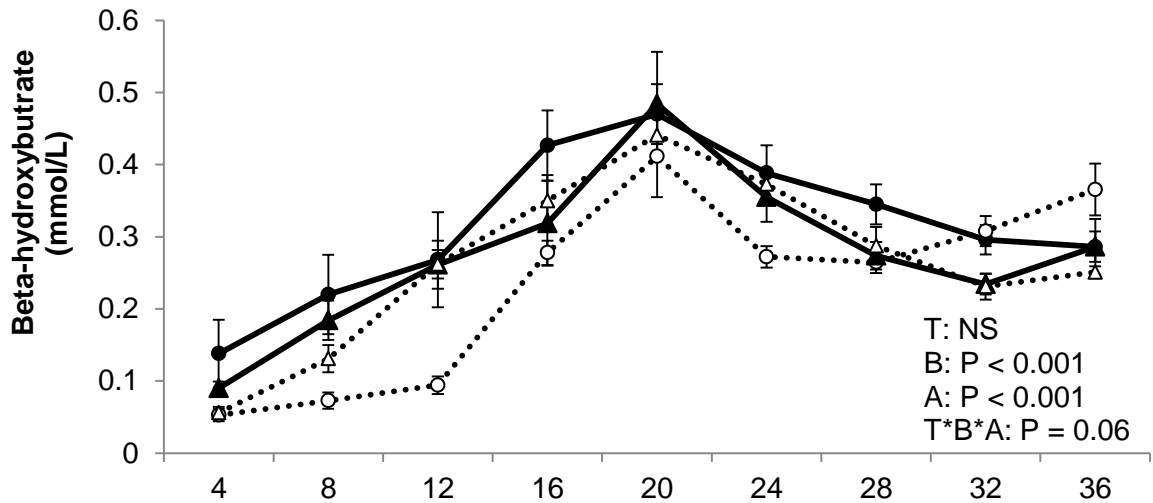


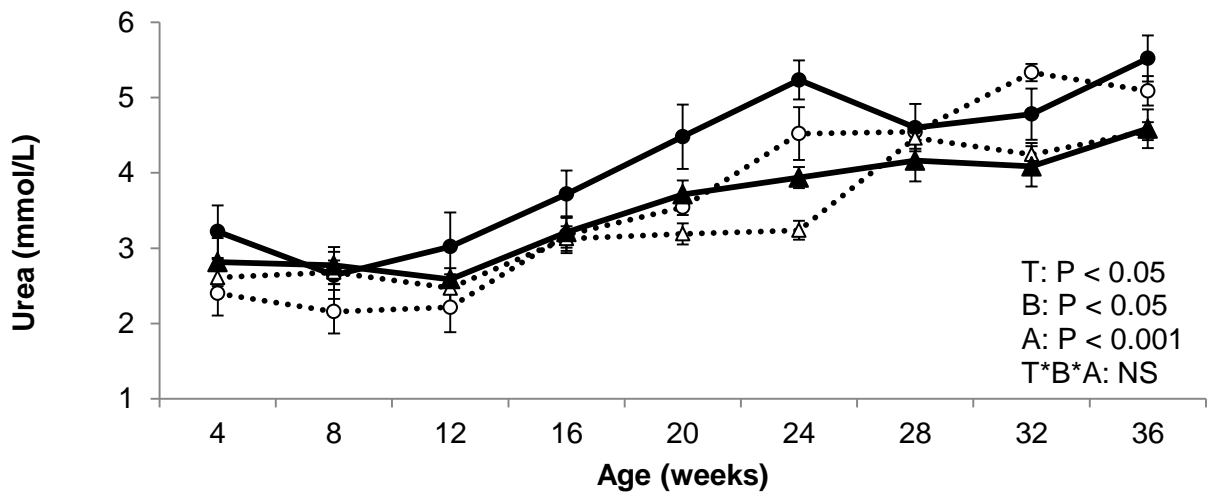
Figure 4.7. Mean plasma metabolite concentrations in Holstein-Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane of nutrition, B= effect of breed, A= effect of age, T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant



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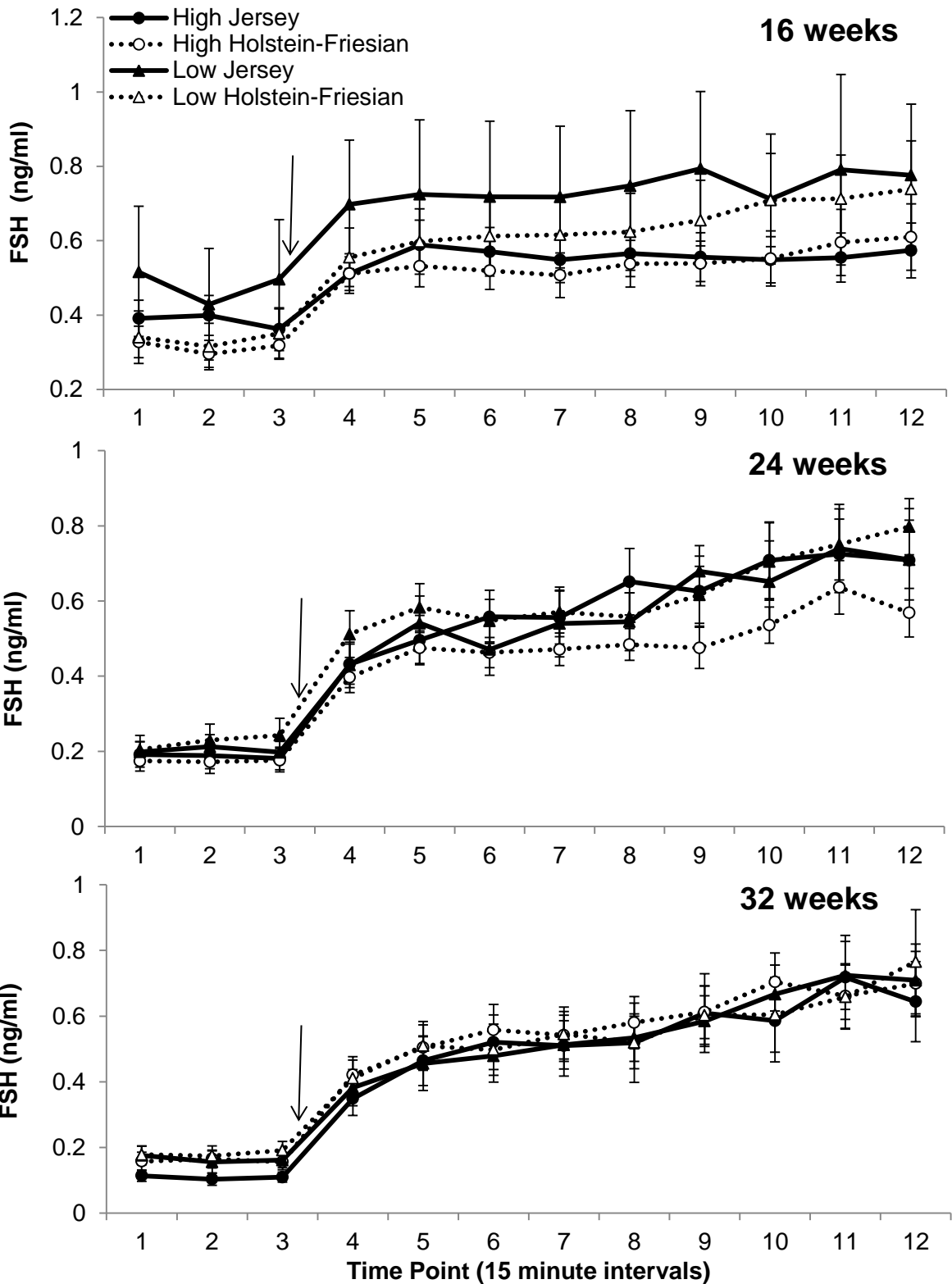


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4 **Figure 4.8.** Mean plasma non-esterified fatty acids (upper panel), beta-
 5 hydroxybutyrate (middle panel) and urea concentrations (lower panel) in Holstein-
 6 Friesian and Jersey bulls; offered a high or low plane of nutrition. T= effect of plane
 7 of nutrition, B= effect of breed, A= effect of age, T*B*A= interaction effects. Error
 8 bars = ±S.E.M. NS = non-significant

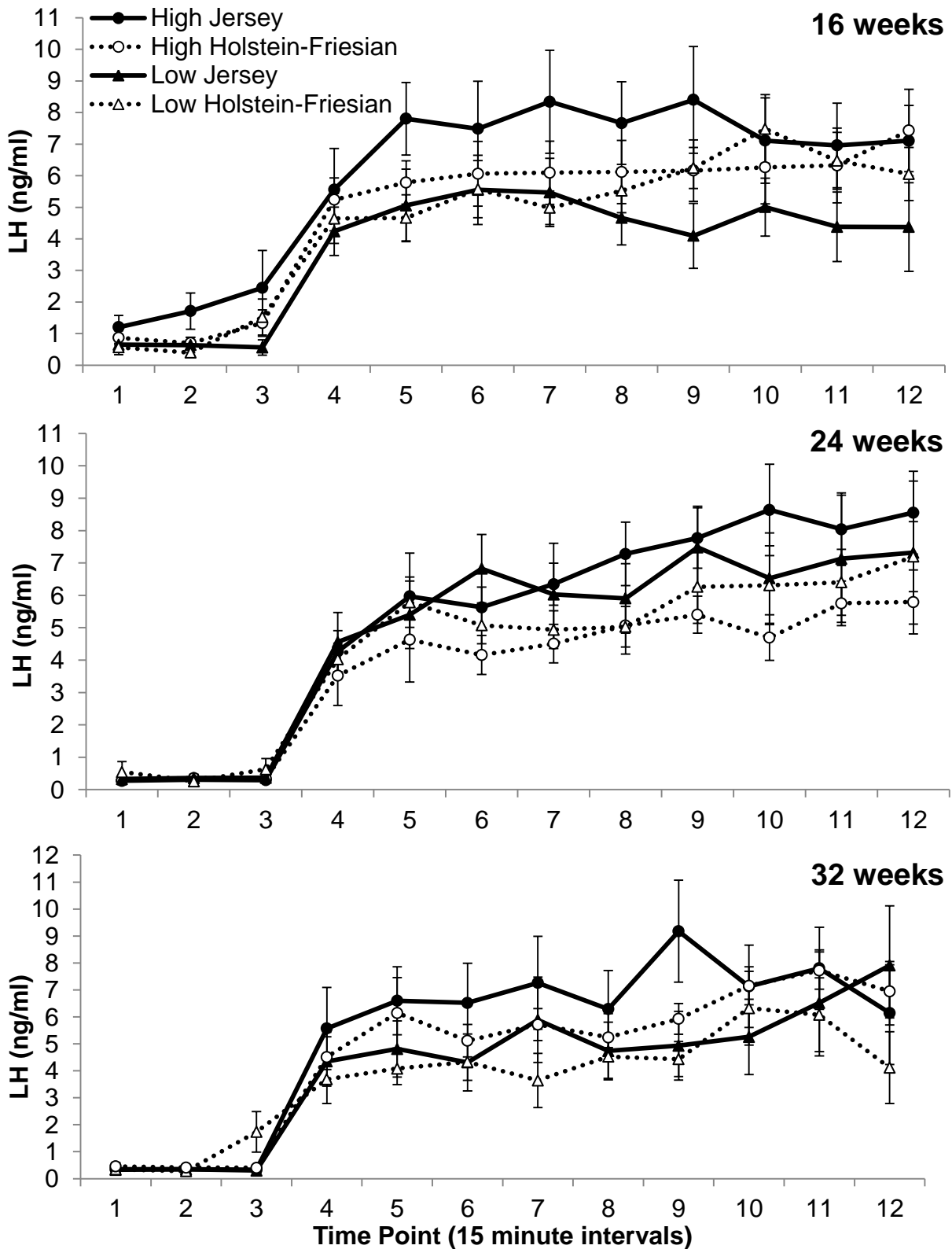


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12 **Figure 4.9.** Mean serum concentration of follicle stimulating hormone (FSH), before
 13 and after a GnRH challenge (marked by the arrow), in Holstein-Friesian and Jersey
 14 bull calves, at 16 (upper panel), 24 (middle panel), and 32 (lower panel) weeks of
 15 age; offered a high or low plane of nutrition. T= effect of plane of nutrition, B= effect
 16 of breed, A= effect of age, T*B*A= interaction effects. Error bars = \pm S.E.M. NS =
 17 non-significant

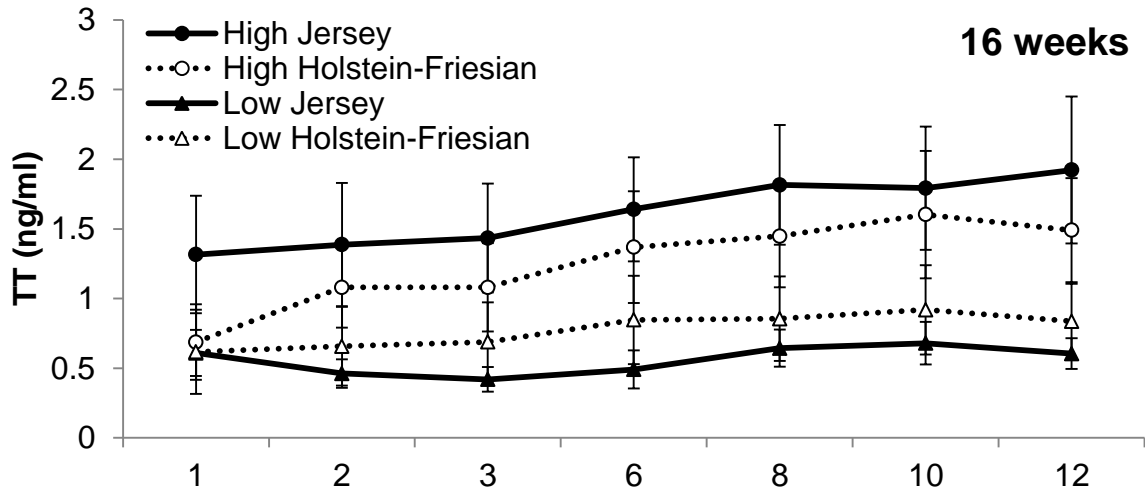


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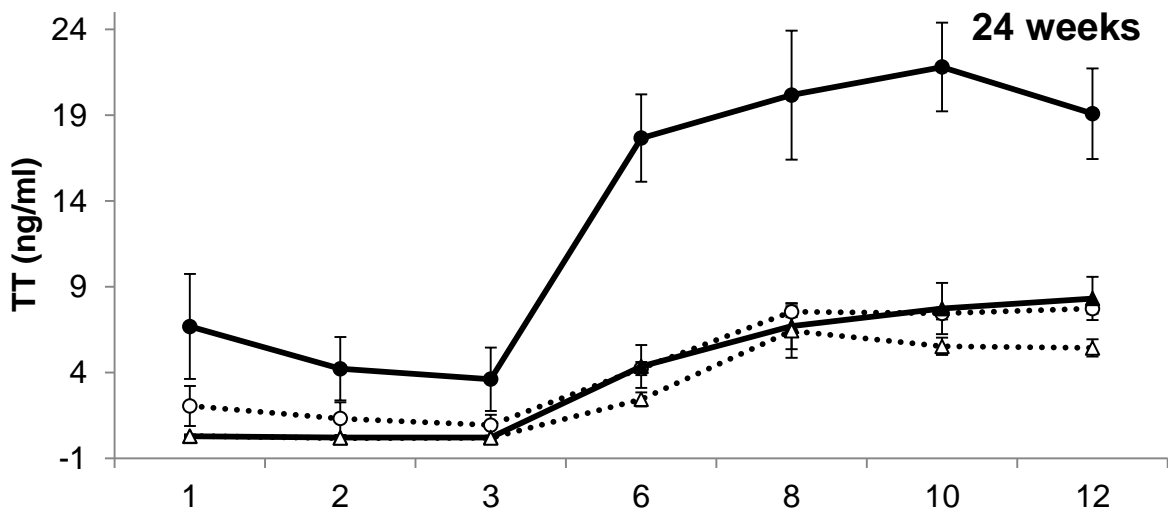
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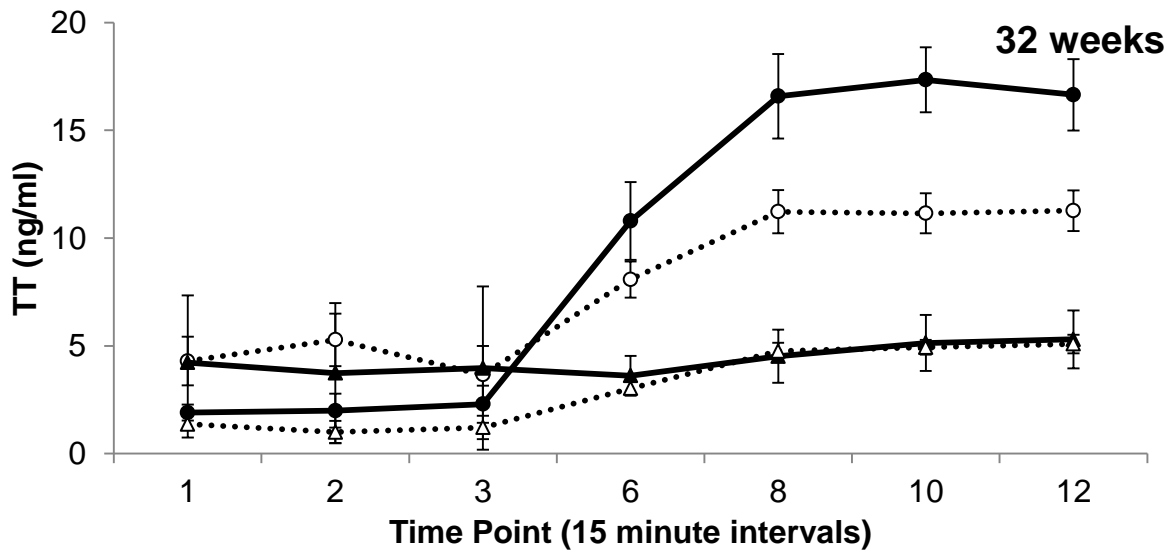
21 **Figure 4.10.** Mean serum concentration of luteinizing hormone (LH) before and after
 22 a GnRH challenge (marked by an arrow), in Holstein-Friesian and Jersey bulls, at 16
 23 (upper panel), 24 (middle panel), and 32 (lower panel) weeks of age; offered a high
 24 or low plane of nutrition. T= effect of plane of nutrition; B= effect of breed; A= effect
 25 of age; T*B*A= interaction effects. Error bars = \pm S.E.M. NS = non-significant



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29 **Figure 4.11.** Mean serum concentrations of testosterone (TT) before and after a
 30 GnRH challenge (marked by an arrow), in Holstein-Friesian and Jersey bull calves,
 31 at 16 (top), 24 (middle), and 32 (bottom) weeks of age; offered a high or low plane of
 32 nutrition. T= effect of plane of nutrition, B: effect of breed, A= effect of age, T*B*A=
 33 interaction effects. Error bars = \pm S.E.M. NS = non-significant