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# Enhanced early-life nutrition of Holstein bulls increases sperm production potential without decreasing postpubertal semen quality

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Enhanced early-life nutrition (~130% of required energy and protein) increased testes size and weight (~20–25%) and reduced age at puberty (~1 month) in beef and dairy bulls, compared with those fed 70% of dietary requirements. The objective was to determine effects of early-life (2–31 weeks) nutritional modulation on feed costs, predicted number of harvestable sperm and doses of semen, and semen quality. Calves (~1 week old) were randomly allocated into three groups that were fed 4, 6, or 8 L/day of milk (low [n = 8], medium [n = 9], and high groups [n = 9], respectively) from ages 2 to 8 weeks. Thereafter, they were weaned, transitioned onto barley silage-based diets, to receive ~70, 100, or 130% of recommended amounts of energy and protein (feed costs were ~CDN$280 more per bull to feed high versus low diets from 2 to 31 weeks). After 31 weeks, all bulls were fed a medium diet. Semen was collected, by electroejaculation, from 51 to 73 weeks, extended, chilled, and cryopreserved. Bulls fed high nutrition were numerically younger ($P = 0.45$) at sexual maturity (sperm with ≥30% progressive motility, ≥70% morphologically normal, and ≤20% abnormal heads), first acceptable post-chill sperm motility (>50%; $P = 0.66$) and first acceptable post-thaw motility (>25% progressive; $P = 0.25$) than bulls in the low-nutrition group. Semen from three bulls per group was used for *in vitro* fertilization (total of 1249 bovine oocytes); there were no significant differences among groups in fertilization percentage (mean ± SEM of 68.0 ± 8.7, 77.1 ± 3.5, and 68.7 ± 4.5% for low, medium, and high, respectively) or blastocyst yield (31.5 ± 5.6, 41.4 ± 4.9, and 33.7 ± 4.6%). On the basis of analysis of 2D gels of sperm proteins, 380 spots were identified on the fused master gel, but no spots were differentially expressed across groups. Overall, there were no significant differences in semen quality or sperm function among bulls fed three levels of nutrition from ages 2 to 31 weeks. However, bulls fed high-nutrition early in life had potential to produce more sperm that could be harvested and sold, which would increase profitability, thereby supporting enhanced early-life nutrition as a management tool to improve reproductive potential of dairy bulls.



## 1. Introduction

We recently reported [1] that enhanced early-life nutrition, both energy and protein, from ages 2 to 31 weeks resulted in bulls that were ~45 days younger at puberty (≥$50 \times 10^6$ sperm per ejaculate [2]) and they were also ~80 days younger when their scrotal circumference reached 28 cm, a proxy for puberty [3], compared with bulls that were underfed from 2 to 31 weeks. Furthermore, testis weight was increased by ~20 to 25% at age 74 weeks in well-fed bulls. In another recent study [4], increased early-life nutrition in bulls increased testes size. Larger testes are expected to increase the number of harvestable

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sperm, as efficiency of spermatogenesis, namely the number of sperm produced per gram of testicular parenchyma, is relatively constant among bulls ($10–14 \times 10^6$ sperm/g parenchyma [5–8]), and; therefore, daily sperm production is determined by paired testes weight [8].

It is well established that overfeeding bulls after puberty can reduce reproductive performance [9,10]; this is attributed to increased fat in the scrotal neck, which presumably interferes with testicular thermoregulation, thereby increasing testicular temperature and decreasing semen quality [10]. Although enhanced early-life nutrition increased testicular size and hastened puberty in our previous studies, its effects on postpubertal semen quality have not been well characterized. In one study [11], in bulls fed various diets from 11 to 31 weeks, sperm morphology did not differ significantly once bulls reached sexual maturity. In another study [12], dairy bulls were fed various levels of energy from early-life to age 4 years. Although the low-nutrition group at 80 weeks temporarily had lower average motility than the other groups, this difference disappeared as bulls matured.

With increasing implementation of genomic selection in choosing future artificial insemination (AI) sires, there is a desire to start collecting and marketing semen from genetically superior bulls earlier in their life. Therefore, bulls that become sexually mature and produce freezable semen younger are favored. Furthermore, the number of insemination doses that can be produced and sold affects profitability. We reported benefits of increased early-life nutrition, with high-nutrition bulls having significantly larger testes at an earlier age than low-nutrition calves [1]. However, for the industry to adopt increased early-life nutrition in bulls, cost-benefits and consequences of this management on semen quality should be determined. Therefore, the objective was to determine effects of early-life (2–31 weeks) nutritional modulation on feed costs, estimated sperm available for harvest, estimated number of insemination doses, and semen quality, including sperm morphology, motility, sperm proteins, fertility, and viability. We tested the hypothesis that varying levels of nutrition from 2 to 31 weeks do not significantly affect sperm motility, morphology, or fertility in a homologous *in vitro* fertilization system, but increases estimated number of insemination doses.

## 2. Materials and methods

### 2.1. Bulls and treatments

Twenty-six Holstein bull calves were used; these were the same bulls used in a previous report from our laboratory [1]. In brief, calves were randomly allocated into one of three groups at approximately age 1 week to receive a low, medium, or high diet, from ages 2 to 31 weeks (low [n = 8], medium [n = 9], and high n = 9]). Calves were randomly allocated into groups so that average body weights of the three groups were similar. Unfortunately, birth weight data were not available, as calves were born on various dairy farms and soon thereafter, brought to a calf-rearing operation, where they were maintained until weaning. Calves were fed high-quality, reconstituted milk replacer (4, 6, or 8 L/day in low, medium, and high groups, respectively), from ages 2 to 8 weeks, and then transitioned onto barley silage–based diets, as described [1]. Calves were fed differential diets until 31 weeks, and thereafter, all were maintained on the medium diet. This experiment was conducted in accordance with the guidelines of the Canadian Council on Animal Care and was reviewed and approved by the Lethbridge Research Centre Institutional Animal Care Committee.

### 2.2. Cost of differential feeding

Costs of the three diets were estimated based on current market values of feed ingredients. Calves were fed three levels of milk from ages 2 to 8 weeks and then transitioned on to a silage-based total mixed ration. We estimated milk consumption given that they were on either 4, 6, or 8 L/day (low, medium, and high, respectively). The current cost of milk replacer in western Canada (CDN$4.65/kg) was used to calculate the cost of the milk portion of the diets (Table 1). After 8 weeks, calves were transitioned on to a silage-based diet. Feed costs during transition (8–11 weeks) were assumed to be similar among groups and were excluded from current calculations.

Feed intakes per pen were recorded on a daily basis from ages 11 to 31 weeks. The sum of all feed intakes combined for all calves in a nutrition group were calculated and then divided by the number of calves in that group, yielding total feed consumed on a per bull basis from 11 to 31 weeks. Diet composition has been reported [1]. In brief, all diets contained 1.6% vitamin-mineral premix (as fed). The low-nutrition diet group (n = 8) received barley silage (plus premix, but no concentrate). The medium-nutrition diet group (n = 9) received barley silage plus 4.8% rolled barley, 4.8% rolled corn, 3.8% canola meal, and 3.8% soybean meal, whereas the high-nutrition diet group (n = 9) received barley silage plus 9.7% rolled barley, 9.7% rolled corn, 7.6% canola meal, and 7.6% soybean meal. Using market prices in western Canada (Supplementary Table 1, online version only), the cost of the three diets for the entire differential feeding period (11–31 weeks) per calf was determined (Table 1).

### 2.3. Sexual development and testicular characteristics

Body weight and testicular growth characteristics of these bulls were measured monthly and have been reported [1]. Once scrotal circumference reached 26 cm, semen collection via electroejaculation was attempted every

**Table 1**
Diet costs (CDN$) for Holstein bull calves fed low, medium, or high diets from ages 2 to 31 wk.

| | Diet | | |
|---|---|---|---|
| | Low | Medium | High |
| Total milk replacer (L/bull; 2–8 wk) | 168 | 252 | 336 |
| Cost of milk replacer/bull | $97.65 | $146.48 | $195.30 |
| Total feed/bull (kg, as fed; 11–31 wk) | 1248.2 | 1420.6 | 1533.1 |
| Cost of diet/bull (11–31 wk) | $156.21 | $245.20 | $337.59 |
| Total cost of feeding | $253.86 | $391.67 | $532.89 |