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Original Article

Seminal plasma protein concentrations vary with feed efficiency and fertility-related measures in young beef bulls



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ABSTRACT

Fertility-associated proteins (FAP) found in seminal plasma indicate sexual maturity, which appears to be influenced by feed efficiency in cattle. This study characterized FAP via proteomics and verified associations of these proteins with feed efficiency, body composition and fertility-related measures in yearling beef bulls. Assessments including testicular ultrasonography, infrared thermography, seminal quality, seminal plasma proteomics, carcass composition, and reproductive organ biometry were obtained. From a population of 31 bulls, the seven most and least feed efficient (efficient, inefficient) bulls were used for categorical comparisons. Correlations between FAP, productive performance and fertility-related measures were determined. These traits were also correlated with orthogonal factors summarized from the FAP. Efficient bulls had increased epididymal sperm-binding protein-1 and decreased concentration of protein-C inhibitor compared to inefficient bulls. Correlations between FAP with age, body size, body composition, reproductive organ biometry, scrotal temperature, and seminiferous tubule maturity are reported. Acrosin and cathepsin D increased with development of the testes and osteopontin increased with greater numbers of mature seminiferous tubules. Phosphoglycerate kinase-2 was higher in animals with a higher scrotum temperature and a higher prevalence of sperm morphology defects. The principal factor indicated that FAP variability concentrations were positively correlated with age, reproductive organ biometry, body size and composition. Our results indicate that FAP

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changes with body size and sexual development, and demonstrates differences in the proteomics of bulls with diverging feed efficiency. This is related to the delay in the sexual maturity of efficient young bulls.

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1. Introduction

Reproductive success affects the profitability of the whole beef production chain, with bull fertility playing a key role in any production system [1]. Beef cattle breeding programs focus on improving feed efficiency using residual feed intake (RFI) [2]. This feed efficiency assessment measures the difference between observed and expected feed intake based on body weight, daily gain [3] and body composition [4]. While the improvement of feed efficiency is relevant to the beef industry, recent evidence suggests antagonistic relationships between fertility-related parameters and efficiency of feed utilization in young beef bulls. Feed efficient bulls appear to possess lower sperm quality [5,6], reduced scrotal circumference [7], abnormal scrotal thermoregulation, diminished testicular echogenicity and delayed development of the seminiferous tubules [8]. These features suggest delayed sexual maturity in bulls with increased feed efficiency.

Seminal plasma contains essential substances for sperm survival and viability, such as fertility-associated proteins (FAP). It is known that FAP have a variety of metabolic functions including energy metabolism at both organ and cellular levels [9]. Studies suggest that energy intake can influence fertility-related measures and seminal plasma composition [10,11]. More recently, seminal plasma was found to differ in composition with the plane of nutrition and body composition in mice [11]. Other seminal proteins including a precursor of glutathione peroxidase, spermadhesins and heat shock proteins are specifically over-expressed in poor-quality semen in humans [12], and may show altered expression with variation in thermoregulation in rams [13].

Evidence demonstrates delayed sexual maturity with increasing feed efficiency in young beef bulls [6–8] and with fertility-related measures [12,14] and body composition [14]. The FAP profile suggests that seminal proteins concentration may vary with feed efficiency. The objectives were: (a) to evaluate the relationships between seminal proteins with feed efficiency using proteomics and (b) to evaluate the concentration of FAP with body composition and fertility-related measures in young beef bulls.

2. Materials and methods

2.1. Bulls, experimental design and fertility-related measures

All procedures were approved by the University of Guelph Animal Care Committee following guidelines from the

Canadian Council on Animal Care [15]. A group of 31 *Bos taurus* bull calves (243 ± 33 d of age and 310 ± 54 kg of body weight), with an average breed composition of 60.5% Angus, 24.2% Simmental, 4.3% Limousin and 11% other European breeds were housed in an indoor pen at the Elora Beef Research Centre (University of Guelph, Canada) between December and March (winter, Northern Hemisphere). Bulls were allowed ad libitum consumption of a corn-based diet, similar to the diet described by Montanholi et al. [4]. Feed was provided through an automated feeding system (Insentec, B.B. Marknesse, The Netherlands) that allowed for individual feed intake assessment. Feed intake was assessed continuously for 112 days (d) and performance traits (body weight and ultrasound scans for body composition) were measured every 28 d. Residual feed intake was calculated through a regression of feed intake on body weight, gain and body composition [8]. A positive residual from this regression model indicates high-RFI (inefficiency), while a negative value indicates low-RFI (efficiency). Details on feed composition and productive performance evaluation are described by Montanholi et al. [4].

At the end of the productive performance test, bulls were subjected to a breeding soundness evaluation [16] and other assessments of sexual maturity and fertility-related measures as described by Fontoura et al. [8]. Briefly, the entire group of bulls was evaluated once, at an average age of 377 ± 33 d, for scrotal circumference (cm) and for semen quality (harvested by electroejaculation), with all the measures made in two consecutive days of sampling (16 bulls on day 1 and 15 bulls on day 2). Seminal quality parameters, namely semen concentration (millions of cells/mL) [17], normal (NMOR, %) and abnormal sperm morphology, including (head pathologies, PHEA; mid-piece pathologies, PMID; tail pathologies, PTAI; loose head pathologies, PLHE; proximal droplets, PPDP; and distal droplets, PDDP; %) were determined following the methodology described by Barth and Oko [18]. Sperm motility (%) was also determined, in samples extended using a Tris-EDTA and egg yolk extender, using an automated motility analysis software (Sperm Vision® CASA, Minitüb, Tiefenbach, Germany). Infrared thermography was used to assess average temperature and standard deviation (°C) at the top and bottom of the scrotum. Testis echogenicity was used to evaluate the testicular pixel intensity (pixels) and testis histomorphometry was used to measure the percentage (%) and size (mm²) of seminiferous tubules, which were classified as immature or mature.

2.2. Proteomic analysis of the seminal plasma

The semen allotted for proteomic evaluation was immediately centrifuged at $1000 \times g$ for 30 min. The supernatant was

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