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Subacute ruminal acidosis reduces sperm quality in beef bulls[1]

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ABSTRACT: Breeding bulls are commonly fed high-energy diets, which may induce subacute ruminal acidosis (SARA). In this experiment, 8 Santa Gertrudis bulls (age 20 ± 6 mo) were used to evaluate the extent and duration of effects of SARA on semen quality and the associated changes in circulating hormones and metabolites. The bulls were relocated and fed in yards with unrestricted access to hay and daily individual concentrate feeding for 125 d before SARA challenge. Semen was collected and assessed at 14-d intervals before the challenge to ensure acclimatization and the attainment of a stable spermiogram. The challenge treatments consisted of either a single oral dose of oligofructose (OFF; 6.5 g/kg BW) or an equivalent sham dose of water (Control). Locomotion, behavior, respiratory rate, and cardiovascular and gastrointestinal function were intensively monitored during the 24-h challenge period. Rumen fluid samples were retained for VFA, ammonia, and lactate analysis. After the challenge, semen was then collected every third day for a period of 7 wk and then once weekly until 12 wk, with associated blood collection for FSH, testosterone, inhibin, and cortisol assay. Percent normal sperm decreased in bulls dosed with OFF after the challenge period ($P < 0.05$) and continued to remain lower on completion of the study at 88 d after challenge. There was a corresponding increase in sperm defects commencing from 16 d after challenge. These included proximal cytoplasmic droplets ($P < 0.001$), distal reflex midpieces ($P = 0.01$), and vacuole and teratoid heads ($P < 0.001$). Changes in semen quality after challenge were associated with lower serum testosterone ($P < 0.001$) and FSH ($P < 0.05$). Serum cortisol in OFF bulls tended to be greater ($P = 0.07$) at 7 d after challenge. This study shows that SARA challenge causes a reduction in sperm quality sufficient to preclude bulls from sale as single sire breeding animals 3 mo after the event occurred.

Key words: bulls, fertility, oligofructose, sperm, subacute ruminal acidosis

INTRODUCTION

Breeding bulls in Australia are commonly fed high-energy diets before sale, generally in the form of grain. These diets previously have been shown to negatively affect sperm quality in bulls (Coulter and Kozub, 1984; Coulter et al., 1987; Mwansa and Makarechian, 1991; Swanepoel et al., 2008). This phenomenon has been associated with increased adipose tissue deposition in the scrotal neck, impairing thermoregulation via the pampiniform plexus (Kastelic et al., 1996). Elevation of testicular temperature decreases both the efficiency of sperm production and sperm viability (Vogler et al., 1993). However, anecdotal evidence from veterinary practitioners and semen morphology laboratories suggests that sperm production can be affected early in the sale preparation process, before fat deposition occurring (P. McAuliffe, personal communication). The rapid introduction of diets containing readily fermentable carbohydrates may precipitate ruminal acidosis

(Owens et al., 1998), suggesting that the effect on sperm quality may be associated with either subacute ruminal acidosis (**SARA**) or acute ruminal acidosis. During SARA, rumen lipopolysaccharides become translocated into the peripheral circulation, triggering a systemic immune response (Khafipour et al., 2009). Metabolic acidosis arising from SARA previously has also been associated with increased cortisol secretion (Enemark, 2008). In bulls, stress-induced elevation of cortisol has an inhibitory effect on the secretion of LH and testosterone (Welsh and Johnson, 1981; Buckham Sporer et al., 2008), which interferes with normal spermatogenesis (Barth and Bowman, 1994), We hypothesized that stress associated with SARA would elevate the concentration of circulating corticosteroids, reduce circulating concentrations of gonadotropins, and deleteriously affect normal sperm production in the testis and during transit through the epididymis, consequently reducing sperm quality for at least a full spermatogenic cycle of approximately 71 d.

MATERIALS AND METHODS

Animals and Management

All experimental procedures were reviewed and approved by the University of Queensland Animal Ethics Committee number SVS/557/09. Ten 2-yr-old Santa Gertrudis bulls with a mean initial live weight of 794.0 kg (SD 55.6) were selected for use in this study. The bulls were selected from a seed stock herd located near Jandowae (26.78° S, 151.10° E), in southern Queensland, Australia, and then transported 260 km to the experimental site at Goondiwindi (28.52° S, 150.33° E). Selection criteria for inclusion in the experiment were based on bulls having passed consecutive standardized bull breeding soundness evaluations (Entwistle and Fordyce, 2003; Fordyce et al., 2006), conducted 30 d apart, with greater than 85% morphologically normal spermatozoa at both evaluations. Bulls were administered vitamin A, D, and E (Vitamec ADE; Cattlekare, Dandenong, VIC, Australia; 5 mL intramuscular [**i.m.**]) on arrival at Goondiwindi. Throughout the experiment, the bulls were group housed in feedlot yards with unrestricted access to Bambatsi panic (*Panicum coloratum* var. *makarikariense* cv. Bambatsi) hay fed in racks. Bulls were individually stalled once daily at 0800 h and fed commercial bull pellets (Ridley AgriProducts, Toowoomba, QLD, Australia) at 0.5% BW (DM basis). Samples of hay and pellets offered were taken, bulked over the course of the experiment, and subsampled for wet chemistry analysis by a private feed analysis laboratory (Table 1; Symbio Laboratories, Eight Mile Plains, QLD, Australia).

Table 1. Composition[1] (g/kg) of bulked Bambatsi panic (*Panicum coloratum* var. *makarikariense* cv. Bambatsi) hay and commercial bull pellets throughout the experiment[2]

Nutrient, g/kg	Bambatsi panic hay	Pellet
DM	918	923
Moisture	82	77
Protein	66	165
Fat	19	28
Ash	99	88
ADF	386	86
NDF	666	230
Calcium	3.0	14.8
Phosphorous	0.9	6.6

[1]All tests results except moisture are reported on a DM basis. Moisture is reported on an as-fed basis.

[2]Source: Symbio Laboratories, Brisbane, QLD, Australia.

Experimental Design

Upon arrival at the experimental site, bulls were allocated to either of 2 treatment groups balanced on semen morphology and liveweight. The animals went through an acclimatization period (−125 d to −1 d), challenge on Day 0, and a postchallenge (1 d to 88 d) period. The purpose of the acclimatization period was to allow a complete cycle of spermatogenesis to occur and to ensure that each bull was producing a stable spermiogram before the challenge period. At the end of acclimatization, 2 bulls were excluded, one due to failure to attain a stable spermiogram attributed to chronic seminal vesiculitis and the other due to poor temperament. The remaining bulls were challenged (Day 0) with either a single oral dose of oligofructose (**OFF**; Orafti P95; Beneo, Tienen, Belgium) at 6.5 g/kg BW or an equivalent sham dose of water (Control). Oligofructose is highly soluble and the dose was dissolved in water (8.75 g/kg BW) before administration. Treatments were administered into the rumen using a stirrup pump attached to a plastic stomach tube inserted through a stainless steel probang placed in the mouth of the bull. In addition, 500 mL water was flushed through the tube into the rumen after dosing to ensure that each bull received the allocated dose in full. Bulls were excluded from accessing hay and water for the period between 12 h before challenge commencement until 16 h after challenge. Pellets were withheld on the day of challenge and reintroduced at 72 h after challenge. Priming doses, equating to 5% of the main dose, were administered twice daily for 3 d preceding the challenge as described by Thoefner et al. (2004).

Measurement and Sampling

Measurements during the acclimatization period occurred at 14-d intervals. During the postchallenge period,

measurements were taken twice weekly until 53 d and then at weekly intervals until 88 d. On each day of measurement, the bulls were moved from the feedlot yards at 0700 h and restrained in a veterinary crush. Unfasted liveweight was recorded immediately. Blood was then collected by tail venipuncture into 10-mL plain Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) using 1.2 by 38 mm needles (Precision Glide; Becton, Dickinson and Company). Samples were allowed to clot at room temperature and then centrifuged ($3,000 \times g$ for 20 min) at 4°C to separate the serum, which was transferred into cryovials and stored at −20°C for analysis of FSH, testosterone, inhibin, and cortisol concentrations.

Bull breeding soundness examinations were performed according to the standardized bull breeding soundness evaluation procedure (Fordyce et al., 2006), including semen analysis and categorization of sperm abnormalities. After preliminary stimulation via rectal massage to the ampullae, semen was collected using a Lane ejaculator (Lane Pulsator IV; Lane Manufacturing Inc., Denver, CO) and standard electroejaculation technique (Entwistle and Fordyce, 2003; McAuliffe et al., 2010). If a satisfactory sample could not be collected initially, the bull was released and semen was collected 1 to 2 h later. Crush side assessments of semen traits were made immediately after semen collection using methodology and standards as described by Youngquist and Threlfall (2007). The proportion of progressively motile spermatozoa was estimated immediately after semen collection by placing a drop of semen with a 1-mL plastic transfer pipette onto a prewarmed slide with a cover slip and viewed under a phase contrast microscope (400x magnification). If sperm could not be viewed individually, samples were diluted in a standard manner with warm PBS (approximately 1 drop semen with 2 drops PBS). This was done by mixing semen and PBS on the slide and covering a subsample with a cover slip. Three fields of view were examined across the slide, and the view yielding the greatest percent motile sperm was assessed. Only sperm moving, through their own propulsion, 1 body length per second were considered forward progressive motile sperm for the purpose of assessment. A further 1 to 2 drops of semen were placed into a 1-mL vial of phosphate-buffered formal saline for sperm morphological assessment.

A series of intensive measurements were made during the challenge period (Day 0) to define the extent of SARA and provide objective thresholds for therapeutic intervention if required. Baseline measurements of locomotion, behavior, cardiovascular (heart rate, packed cell volume [**PCV**], and total plasma protein [**TPP**]), respiratory, and gastrointestinal (rumen pH, rumen motility, rectal temperature, and fecal consistency) status were taken immediately before dosing at 0800 h (Time 0) and then repeated at 2, 4, 6, 8, 10, 12, 14, 16, and 24 h after dosing.

Locomotion assessment involved walking individual bulls around the perimeter of a holding yard for a period of 2 min before all other measurements commenced. A locomotion score was recorded using a scale in accordance with Sprecher et al. (1997) as 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, and 5 = severely lame. Behavior was subjectively scored as 0 = normal, 1 = mildly depressed or agitated, 2 = restless and distressed, and 3 = severe distress.

Heart rate was measured by recording pulse rate for 0.5 min. A blood sample was collected (as previously described), filled in microhematocrit tubes, and centrifuged ($10,000 \times g$ for 5 min at ambient temperature). Packed cell volume (%) was determined from a sliding scale using a microhematocrit tube reader. The TPP (g/L) was determined by placing a few drops of the serum from the PCV microhematocrit tube onto a refractometer (Cambridge Instruments Inc., Buffalo, NY) and directly reading the result in grams per liter. Respiration rate was determined over 0.5 min by counting the number of flank movements.

Rumen fluid samples were collected using a custom-designed stomach pump and plastic hosing (10 mm diameter) fitted to a collection vessel. The hose was fitted with a tapered stainless steel tube (maximum 20 mm diameter; 100 mm length) at one end for immersion in the rumen through a stainless steel probang placed in the mouth of the bull. The stainless steel tube had been bored out to allow the influx of rumen fluid into the hose. The first 250 mL of rumen fluid collected was discarded to remove the potential of saliva contamination. The following 250 mL collected was retained if free of saliva. The process was repeated until an uncontaminated sample was obtained. The pH of retained rumen fluid was immediately measured with a portable pH meter (Cardy Twin pH Meter; Spectrum Technologies Inc., Plainfield, IL). Rumen fluid was strained to remove large particles of digesta and then immediately centrifuged ($3,000 \times g$ for 20 min) at 4°C. The supernatant was drawn off and stored in cryovials at −20°C for analysis of ammonia, VFA, and lactate concentrations. Rumen motility was assessed by auscultation with a stethoscope placed over the left paralumbar fossa for a period of 5 min and recorded as either 0 = normal, defined as 1 to 3 rumen contractions/min; 1 = decreased activity, defined as less than 1 rumen contraction/min; 2 = absence of rumen sounds; and 3 = rumen atony or bloat. Fecal consistency was evaluated using a 5-point visual observation scale (Danscher et al., 2009) as 1 = dry and firm, 2 = normal; 3 = pasty and soft; 4 = diarrhea, thin; and 5 = watery diarrhea.

At 12 h after dosing, one of the oligofructose-treated bulls was displaying signs of acute ruminal acidosis. The bull was immediately drenched with sodium bicarbonate (1 g/kg of BW in 10 L water) and magnesium

oxide (0.5 g/kg of BW in 5 L water). Additional treatment was provided as flunixin (1 mg/kg of BW, intravenously), thiamine (10 mg/kg i.m.), penicillin procaine G (12 mg/kg i.m.), and tripelennamine hydrochloride (1 kg/kg i.m.). The bull was housed in a monitoring pen and provided access initially to hay only and then to water and hay at 16 h. All other bulls were provided access to hay and water after 16 h from the commencement of the challenge period. The bull provided with therapeutic intervention was monitored but excluded from invasive sampling at 14 and 16 h but included at 24 h. An additional dose of flunixin and tripelennamine hydrochloride was administered at 24 h after challenge.

Laboratory Analysis

Serum cortisol was determined using a commercial RIA kit (Clinical Assays, GammaCoat, Cortisol 125I RIA Kit; DiaSorin, North Ryde, NSW, Australia). The intra- and interassay CV was 2.4 and 3.6%, respectively. The limit of detection of the cortisol assay was 3.5 nmol/L.

The concentrations of FSH were measured in a single RIA using a method previously described (Martin et al., 1994). The intra-assay CV were 4.2, 6.2, and 7.1% for Control sera, with means of 1.09, 2.66, and 4.32 ng/mL, respectively. The limit of detection for the FSH assay was 0.05 ng/mL.

Serum testosterone concentrations were determined using a total testosterone RIA kit (Diagnostic Products Corporation, Los Angeles, CA) with testosterone standards prepared with purified hormone (Sigma Chemical Co., St. Louis, MO) in charcoal stripped castrate bovine serum as described by Evans et al. (1995). The intra- and interassay CV were 3.9 and 5.1%, respectively. The limits of detection for the assay were 0.02 ng/mL.

Serum inhibin was assayed using a heterologous RIA directed toward the α subunit of the hormone, measuring both inhibin A and B as previously described (Robertson et al., 1989). Intra- and interassay CV were 6.5 and 7.3%, respectively. The limit of detection of the inhibin assay was 0.02 ng/mL.

Rumen ammonia concentrations were measured using the direct enzymatic method using Infinity Ammonia Liquid Stable Reagent (catalog number TR60101; Beckman Coulter Australia Pty. Ltd., Lane Cove West, NSW, Australia) on an Olympus AU400 Autoanalyser (AHL NTM-56; Olympus, Mount Waverly, VIC, Australia). The interassay CV was 9.5%. Rumen VFA concentrations were analyzed by an Agilent series gas chromatograph with P6890 injection, 30 mm by 0.53 mm by 1.0 μm capillary column (Agilent Technologies Inc., Wilmington, DE), and ChemStation software (Agilent Technologies Inc.) based on method-

ology from Supelco Inc. (1975). The interassay CV for acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproate were 10.2, 7.6, 6.3, 8.0, 6.7, 7.5, and 6.5%, respectively. The lactate concentration in rumen fluid were analyzed using the UV method with a Boehringer Mannheim kit (catalog number 11 112 821 035; R-Biopharm, Taren Point, NSW, Australia) after deproteinization with perchloric acid and analyzed on the Olympus AU400 Autoanalyser.

Sperm morphology assessments were performed by an experienced Australian Cattle Veterinarian–accredited morphologist from a commercial laboratory, blinded to the treatments, using 1,000x magnification under differential interference contrast microscopy (Nikon Eclipse 80i; Nikon Instruments, Tokyo, Japan). Morphological assessment involved systematically counting 100 cells across a wet mount slide and recording the number of abnormalities as described by Fordyce et al. (2006). Sperm abnormality categories included midpiece abnormalities, proximal cytoplasmic droplets (**PD**), abnormal tails and loose heads, pyriform heads, vacuoles and teratoid sperm, knobbed acrosomes, and swollen acrosomes (Fordyce et al., 2006). This sperm cell count allowed percent normal sperm (**PNS**) to be calculated (Entwistle and Fordyce, 2003).

Statistical Analysis

Data from the intensive challenge period including rumen fermentation products and pH, cardiovascular status, blood (PCV and TPP), and rectal temperature were analyzed using repeated measures ANOVA to examine within- and between-subject effects on treatment, time, and their interaction. Pretreatment means (Time 0) were available for all variables measured during the intensive challenge period, so each was fitted as a covariate in the relevant analysis where significant at $P < 0.1$. Plots of residuals versus fitted values were examined to assess the assumption of homogeneity of variance, and with the exception of rumen ammonia, acetic and caproic acid, total VFA, and rectal temperature, the assumptions were reasonably satisfied. These exceptions were skewed to the right, and therefore, a log transformation was applied before analysis.

Hormone concentrations, sperm motility, and percentage normal and abnormal sperm morphological traits were analyzed by fitting linear mixed models using the REML approach. Fixed effects in the model included treatment group (Control versus OFF), time, and the interaction between treatment group and time. Random effects in the model were the individual animal and the interaction between individual animal and time. For the percentage of morphologically normal sperm, plots of residual versus fitted values showed a reduction

Table 2. Mean ruminal fermentation parameters of pH, ammonia (NH_3–N), and total and molar VFA concentrations for bulls challenged with either oligofructose (OFF) or water (Control) and sampled[1] over 24 h

	Treatment				P-value		
Item	Control	OFF	LSD	LSR[2]	Treatment	Time	Treatment × time
n	40	38					
Ruminal pH	7.19	6.27	0.38		<0.001	<0.001	<0.001
NH_3–N,[3] mg/L	14.7	59.4		2.05	<0.01	<0.001	<0.001
Total VFA,[3] mM	52.7	40.6		1.15	<0.01	<0.001	<0.001
Acetate[3]	38.5	23.8		1.20	<0.001	<0.001	<0.001
Propionate	7.03	7.81	1.80		0.33	<0.001	<0.001
Butyrate	5.61	8.93	1.69		<0.01	<0.001	<0.001
Isobutyrate	0.61	0.14	0.06		<0.001	<0.001	<0.001
Valerate	0.31	0.19	0.09		0.016	<0.001	<0.001
Isovalerate	0.91	1.50	0.52		0.033	<0.001	<0.001
Caproate[3]	0.11	0.07		1.56	0.041	<0.001	0.12

[1]Bulls sampled at 0 (before treatment), 2, 4, 6, 8, 10, 12, 14, 16, and 24 h.

[2]LSR = least significant ratio; used for comparison of means instead of LSD due to data transformation. The larger mean should be divided by the smaller mean and the resulting ratio compared with the LSR.

[3]Values are expressed as back-transformed ($\log_e$) means.

in variation as the percentage normal approached 100%. A log transformation was, therefore, applied [$\log_e$ (100 − % normal)]. For sperm morphological abnormalities, plots of residuals versus fitted values showed the variation increasing with the mean. A Poisson model was fitted for all morphologically abnormal traits of sperm before analysis to stabilize the variance. Hormone concentrations, with the exception of inhibin, also required a log transformation before analysis.

Data from bulls removed at the conclusion of the prechallenge period were excluded from statistical analysis. Treatment means adjusted for other factors, missing data, and covariates (where appropriate) were calculated for each variable, along with SE of differences and LSD for comparing pairs of means ($P < 0.05$). All procedures were conducted using the statistical package GenStat (GenStat for Windows, 10th edition, VSN International Ltd., Hemel Hempstead, UK) .

RESULTS

Clinical Observations

Control bulls showed no clinical signs of SARA or acute ruminal acidosis during the 24-h monitoring period after challenge commencement. In contrast, 3 out of 4 bulls dosed with OFF demonstrated clinical signs by 10 to 12 h after challenge. There was, however, marked individual variability in the extent of clinical signs. These signs included changes in fecal consistency, ranging from soft feces to profuse diarrhea; a reduction in rumen motility; depressed behavior; and elevated rectal temperature. The single worst affected bull had a rumen fluid pH nadir of 4.94 and peak rectal temperature

of 40.6°C at 12 h after challenge. This bull had developed moderate lameness by 14 h after challenge. The remaining OFF-treated bulls ($n = 3$), including a bull that showed no clinical signs during the intensive 24-h monitoring period, developed mild lameness by 48 h after dosing. Lameness in all bulls treated with OFF did not resolve until 7 d after the onset of the challenge.

Rumen Fermentation

Table 2 lists the main effects of the challenge on rumen fermentation parameters. Treatment with OFF reduced the mean rumen pH ($P < 0.001$). The mean rumen pH of OFF bulls fell immediately after dosing ($P < 0.001$), reaching a mean nadir of 5.7 ± 0.2 at 8 h (Fig. 1A) before increasing at 12 h and 24 h ($P < 0.001$) to reattain values similar to those measured before the challenge. Mean rumen ammonia concentrations of bulls dosed with OFF were greater ($P = 0.003$) than those of Control bulls during the challenge period. Rumen ammonia concentrations showed a treatment × time interaction ($P < 0.001$). Bulls treated with OFF demonstrated greater mean rumen ammonia concentrations than Control bulls at 4, 10, 12, 14, and 16 h after dosing ($P < 0.001$; Fig. 1B).

Total rumen VFA, acetate, and valerate concentrations decreased over time in both treatment groups ($P < 0.001$). Treatment with OFF decreased the rumen fluid concentrations of mean total VFA ($P = 0.004$), acetate ($P < 0.001$), and valerate ($P = 0.02$) compared with Control bulls during the intensive challenge period. The treatment of bulls with OFF significantly lowered rumen concentrations of total VFA, acetate, and valerate between 10 and 16 h after challenge ($P < 0.001$; Fig. 1C, 1D, and 2B). The concentration of propionate
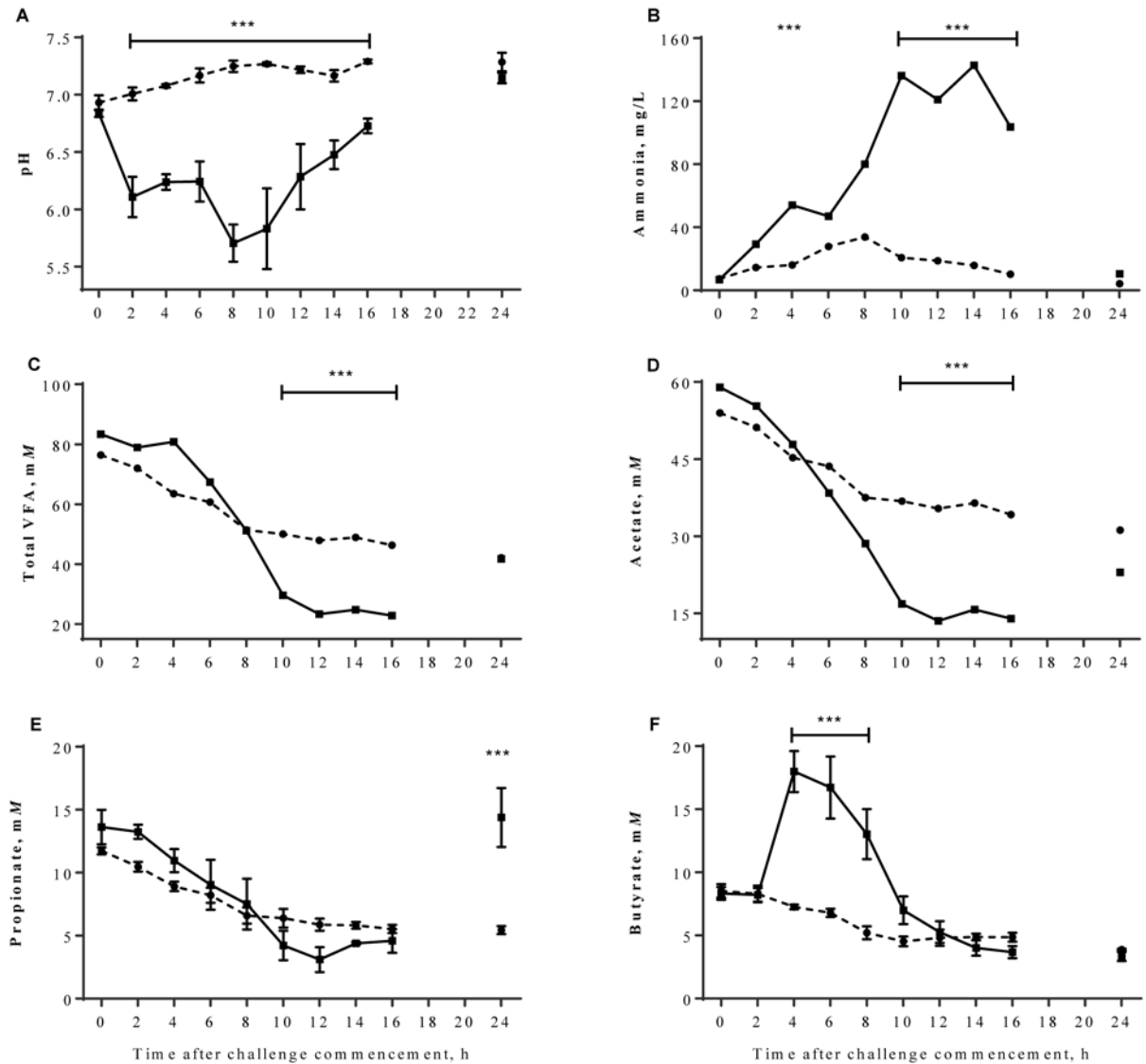
Figure 1. Mean pH and ammonia, total VFA, acetate, propionate, and butyrate concentrations in the rumen fluid of bulls challenged with either oligo-fructose (solid line; ■) or water (dashed line; ●) measured immediately before challenge (0 h) and then at 2, 4, 6, 8, 10, 12, 14, 16, and 24 h after the commencement of treatments. Values are mean (±SEM), with the exception of rumen ammonia, total VFA, and acetate, which are plotted as back-transformed means. Differences between treatments at time points are shown (***$P < 0.001$).

in rumen fluid declined over time in both treatment groups between dosing and 16 h after challenge ($P < 0.001$; Fig. 1E), but rumen propionate concentrations of OFF bulls were greater than those of Control bulls after 24 h ($P < 0.001$). Treatment with OFF increased mean butyrate concentrations of rumen fluid during the challenge period ($P = 0.003$). Concentrations of rumen butyrate in OFF bulls increased rapidly between 2 and 4 h to reach a peak before declining between 6 and 10 h, with only minor differences for the remainder of the challenge period (Fig. 1F). Rumen fluid butyrate concentrations in OFF bulls were greater than those in Control bulls at 4, 6, and 8 h after dosing ($P < 0.001$).

Bulls dosed with OFF had lower mean ruminal iso-butyrate concentrations than Control bulls at all measurements between 2 h and 24 h after challenge ($P < 0.001$).

There was no effect of time on rumen concentrations of isobutyrate in bulls receiving the Control treatment, with the exception of a reduction at 12 h ($P < 0.001$; Fig. 2A). In contrast, concentrations of isobutyrate in the rumen fluid of bulls receiving the OFF treatment linearly declined after dosing ($P < 0.001$) and had reached the limit of detection by 10 h after challenge. The effects of challenge on rumen isovalerate concentrations followed a pattern similar to butyrate. Bulls dosed with OFF had greater mean ruminal isovalerate concentrations ($P = 0.03$) across the challenge period. Treatment of bulls with OFF increased rumen isovalerate concentrations between 2 and 4 h. Concentrations then gradually declined, resulting in differences between treatments at both 4 and 6 h ($P < 0.001$; Fig. 2C). Concentrations of rumen caproate linearly declined between 2 and 24 h
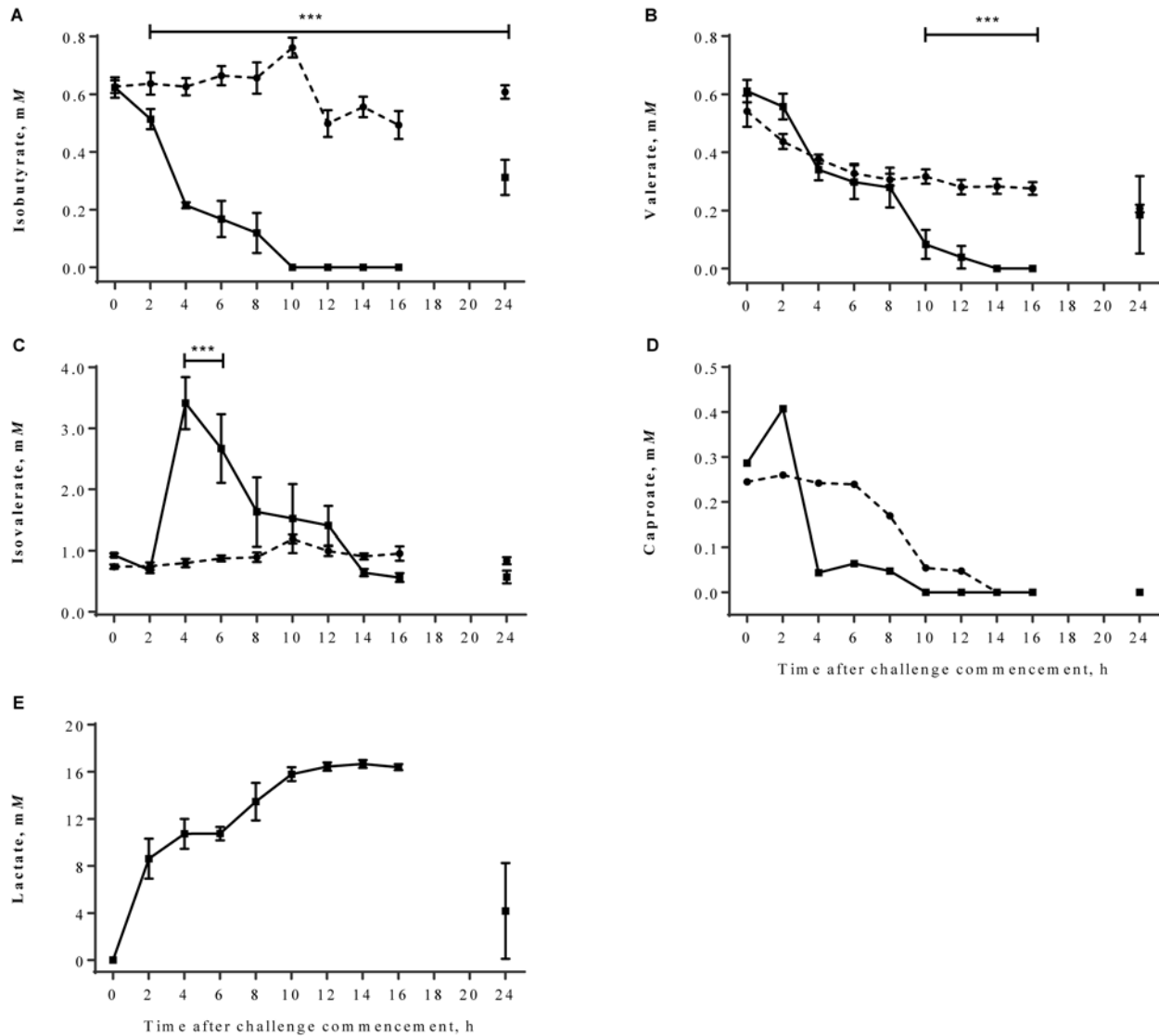
Figure 2. Mean isobutyrate, valerate, isovalerate, caproate, and lactate concentrations in the rumen fluid of bulls challenged with either oligofructose (solid line; ■) or water (dashed line; ●) measured immediately before challenge (0 h) and then at 2, 4, 6, 8, 10, 12, 14, 16, and 24 h after the commencement of treatments. Values are means (±SEM), with the exception of caproate, which is plotted as a back-transformed mean. Differences between treatments at time points are shown (***$P < 0.001$).

after challenge commencement ($P < 0.001$; Fig. 2D). During the challenge period, mean rumen caproate concentrations were reduced in OFF bulls ($P = 0.04$).

Rumen fluid concentrations of lactate were below the limit of detection for Control bulls during the intensive sampling periods; consequently, we were unable to conduct statistical tests between treatments. However, ruminal lactate concentrations in OFF bulls increased immediately after challenge, reaching a plateau between 12 and 16 h ($P < 0.001$; Fig. 2E).

Rectal Temperature and Cardiovascular and Blood Variables

The main effects of the challenge on cardiovascular function and rectal temperature during the 24-h intensive monitoring period are listed in Table 3. Bulls treated with

OFF demonstrated greater mean rectal temperatures ($P < 0.001$) than Control bulls during the intensive challenge period. There were effects of time on rectal temperature ($P < 0.001$), which increased between dosing and 12 h before declining to 24 h (Fig. 3A). The mean heart rate of bulls challenged with OFF was greater than in Control bulls across the intensive measurement period ($P < 0.05$). There was an immediate increase in the mean heart rate of OFF treated bulls within 2 h of dosing ($P < 0.01$; Fig. 3B), maintaining a peak at 4 and 6 h, before declining at 8 h after challenge commencement. There were no effects of treatment on the mean respiration rate of bulls during the intensive challenge period. Although the respiration rates of Control bulls did not change (Fig. 3C), the respiration rate bulls treated with OFF demonstrated a gradual reduction, reaching a nadir of 23.3 ± 2.9 breaths/min after 14 h after challenge. Oligofructose-treated bulls had
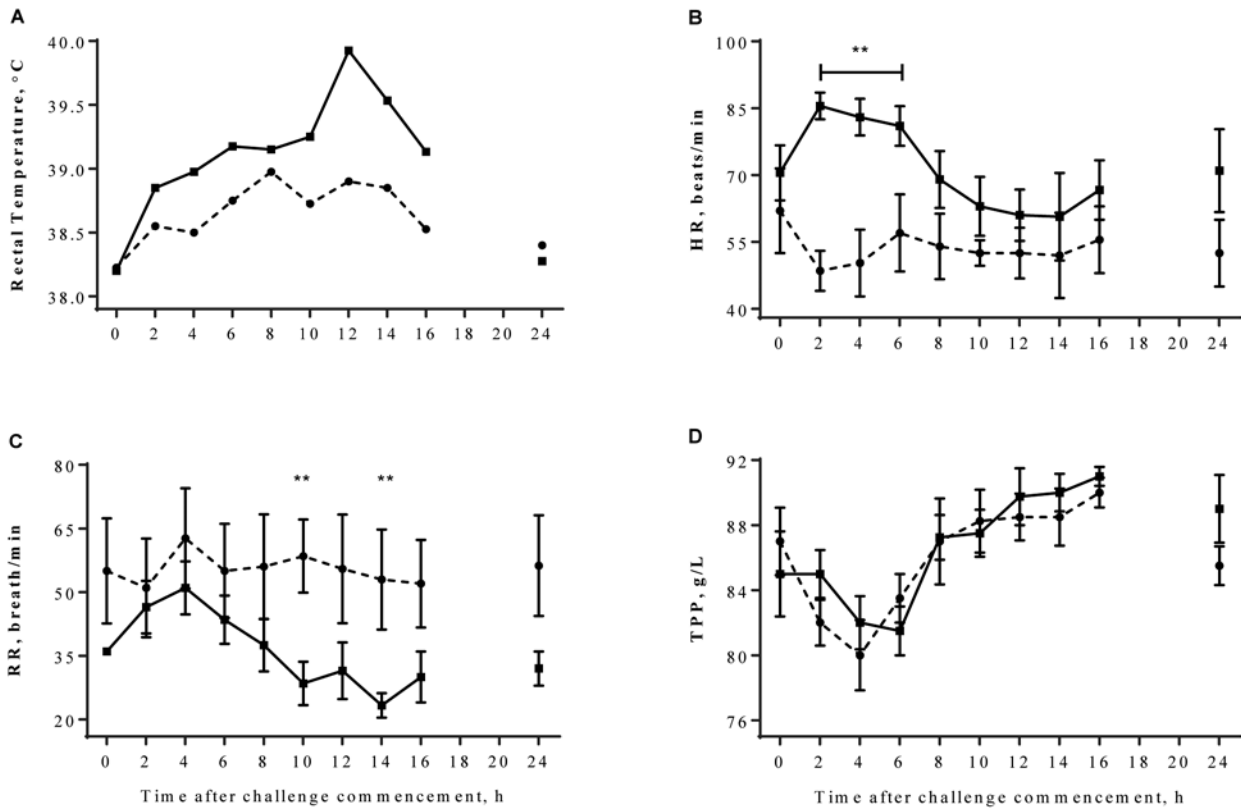
Figure 3. Mean rectal temperature, heart rate (HR), respiration rate (RR), and total plasma protein (TPP) concentrations of bulls challenged with either oligofructose (solid line; ■) or water (dashed line; ●) measured immediately before challenge (0 h) and then at 2, 4, 6, 8, 10, 12, 14, 16, and 24 h after the commencement of treatments. Values are means (±SEM), with the exception of rectal temperature, which is presented as a back-transformed mean. Differences between treatments at time points are shown (**$P < 0.01$).

lower respiration rates than Control bulls at both 10 and 14 h ($P < 0.01$). There was no effect of treatment on either PCV or TPP during the intensive challenge period. There were, however, effects of time on TPP ($P < 0.001$; Fig. 3D), with an initial decline between challenge commencement and 4 h, gradually rising between 8 and 16 h before returning to preinduction concentrations after 24 h.

Semen Quality

Overall, PNS significantly decreased after treatment with OFF (Table 4; $P = 0.013$). There were interactions between treatment and time ($P = 0.046$), such that OFF bulls had lower PNS than Control bulls at 29, 74, 82, and 88 d after challenge (Fig. 4A). The reduction in PNS within OFF bulls was a consequence of a greater incidence of sperm defects after the challenge. Compared with Control bulls, the OFF bulls demonstrated an overall greater incidence of sperm midpiece abnormalities, predominately as distal reflex midpieces ($P = 0.011$), PD ($P < 0.001$), and vacuole and teratoid head defects ($P < 0.001$), during the postchallenge period (Table 4). There were also interactions between treatment group and time demonstrated for some sperm abnormalities. Oligofructose-treated bulls had greater midpiece abnormalities at 29 d ($P = 0.038$; Fig. 4B); PD at 16, 67, and

74 d ($P = 0.003$; Fig. 4C); vacuoles and teratoid heads at 67, 74, and 88 d ($P < 0.001$; Fig. 4D); and knobbed acrosomes at 82 d ($P = 0.049$; Fig. 4E). There were effects of time ($P < 0.001$) on sperm progressive motility during the postchallenge period (Fig. 4F). Progressive motility in OFF bulls (69.8 ± 1.8%) was not different from that in Control bulls (71.1 ± 1.4%) after challenge, nor were there interactions between treatment group and time.

Circulating Hormones

There were effects of time ($P < 0.001$) on serum concentrations of FSH, testosterone, inhibin, and cortisol in the 90-d postchallenge period. Bulls dosed with OFF had lower mean serum testosterone ($P < 0.001$) than Control bulls during the postchallenge period. There were also interactions between treatment and time for serum testosterone ($P = 0.002$), such that OFF bulls demonstrated lower concentrations than Control bulls at all measurements until 9 d and then at 19, 32, 60, and 74 d after treatment (Fig. 5A), There was no main effect of treatment on serum FSH; however, concentrations measured at 7, 23, and 32 d after challenge were lesser in OFF bulls than in Control bulls ($P = 0.03$; Fig. 5B). There was a trend toward an interaction between treatment and time for cortisol ($P = 0.07$), with OFF treated bulls tending to

Table 3. Mean rectal temperature, heart rate, respiration rate, packed cell volume, and total plasma protein of bulls challenged with either oligofructose (OFF) or water (Control) and sampled[1] over 24 h

Item	Treatment			LSR[2]	P-value		
	Control	OFF	LSD		Treatment	Time	Treatment × time
n	40	38					
Rectal temperature,[3] °C	38.6	39.2		1.01	<0.001	<0.001	0.24
Heart rate, beats/min	52.8	71.2	18.9		0.041	0.01	<0.01
Respiration rate, breaths/min	55	36.4	28.6		0.71	<0.001	<0.01
Packed cell volume, %	39.5	36.2	5.6		0.20	0.14	0.28
Total plasma protein, g/L	85.9	86.9	4.3		0.59	<0.001	0.30

[1]Bulls sampled at 0 (before treatment), 2, 4, 6, 8, 10, 12, 14, 16, and 24 h.

[2]LSR = least significant ratio; used for comparison of means instead of LSD due to data transformation. The larger mean should be divided by the smaller mean and the resulting ratio compared with the LSR.

[3]Values are expressed as back-transformed (log_e) means.

have greater concentrations at 5 d after challenge compared with Control bulls (Fig. 4C). There was no effect of treatment on circulating inhibin levels, nor were there interactions between treatment and time (Fig. 5D).

DISCUSSION

This is the first study to show that SARA can induce effects on sperm quality in bulls, with these effects persisting for at least 90 d. The reduction in PNS after SARA is an important finding because this semen quality trait is positively related to the calf output of beef bulls (Fitzpatrick et al., 2002; Holroyd et. al., 2002). Within the oligofructose-challenged bulls, PNS declined from 90 to 70% during the postinduction period. The attainment of 70% PNS is considered a threshold value of practical significance because bulls with the greatest calf output have PNS > 70% (Fitzpatrick et al., 2002). Should PNS fall below the 70% threshold, bull fertility is decreased (Barth, 2007), and such bulls are not recommended as suitable for single sire mating or for the collection of semen for freezing (Entwistle and Fordyce, 2003).

Effects on Spermatogenesis and Circulating Hormones

The observed sequence of sperm abnormalities over time, according to the position of the sperm in either the spermatogenic cycle stage or epididymal tract, was expected from a previous study (Barth and Bowman, 1994) where a similar sequence was observed after corticosteroid injection. Insult to the testicular environment has the greatest effects on sperm during spermiogenesis (Curtis and Amann, 1981) at the transition between round spermatid to specialized spermatozoon. This is because a cascade of linked developmental processes occur at this time including 1) formation of the acrosome from the Golgi apparatus, 2) compaction of the sperm chromatin, 3) organization of the mitochondria

to form the flagellum, and 4) initiation of cytoplasm resorption; abnormalities attendant to these structures will, therefore, be observed in the ejaculate. Sperm abnormalities such as acrosome abnormalities are the last to be observed after a single insult (82 d in this study) because this event occurs earliest in spermiogenesis (Barth and Oko, 1989) followed by vacuoles (67, 74, and 88 d) caused by aberrant compaction of chromatin, midpiece sheath abnormalities, and finally abnormalities occurring during passage through the epididymis such as distal reflex midpieces (29 d). This sequence of abnormalities was observed subsequent to SARA and the induced cortisol rise, except for an early rise in PD at 16 d. Distal reflex midpieces were elevated at 29 d, as this abnormality occurs as a result of perturbation in the epididymis (with a consequent reduction in total PNS) and, therefore, would be expected to be the first abnor-

Table 4. Mean percentage normal and abnormal sperm[1] collected from Santa Gertrudis bulls every third day for 53 d and then at 7 d intervals until 88 d after challenge with either oligofructose (OFF) or water (Control)

Item	Treatment		LSR[2]	P-value
	Control	OFF		
Percent normal sperm	88.0	83.3	0.81	0.013
Sperm abnormalities[3]				
MP	4.8	5.9	1.17	0.011
PD	2.1	2.6	1.13	<0.001
T+H	1.5	1.6	>100	0.85
PY	0.0	0.1		0.18
V+T	3.4	9.7	2.29	<0.001
KA	0.3	0.4	2.48	0.42
SA	0.1	0.2	2.14	0.61

[1]Values are expressed as back-transformed (log_e) means.

[2]LSR = least significant ratio; used for comparison of means instead of LSD due to data transformation. The larger mean should be divided by the smaller mean and the resulting ratio compared with the LSR.

[3]Sperm abnormalities include midpiece defects (MP), proximal cytoplasmic droplets (PD), abnormal tails and loose heads (T+H), pyriform heads (PY), vacuoles and teratoid heads (V+T), knobbed acrosomes (KA), and swollen acrosomes (SA).
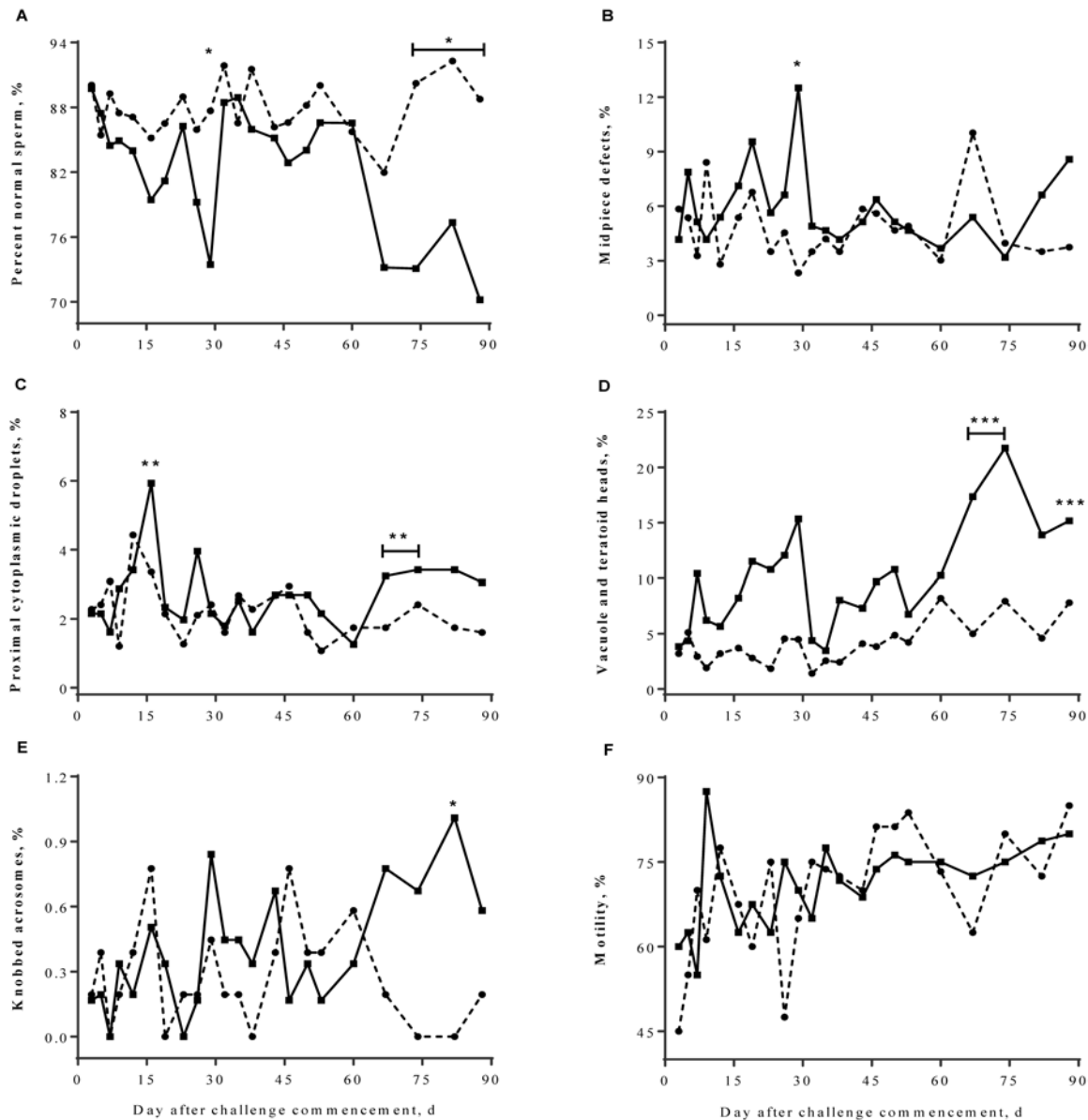
Figure 4. Mean sperm motility and back-transformed adjusted means for percentage normal and abnormal sperm (midpiece defects, proximal cytoplasmic droplets, vacuole and teratoid heads, and knobbed acrosomes) of bulls challenged with either oligofructose (solid line; ■) or water (dashed line; ●) at 6.5 g/kg BW collected every third day for 53 d and then at 7-d intervals until 88 d after challenge. Differences between treatments at time points are shown (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

mality observed after an insult. Such abnormalities have been previously associated with reduced testosterone in the epididymal fluid (Barth and Bowman, 1994).

There was a delay in initiation of the effect on PNS that was not anticipated. Cortisol in OFF peaked at 5 d after challenge, coinciding with the timing of observed lameness in treated bulls. The subsequent decline in FSH at 7 d corresponds with previously reported effects of glucocorticoids on gonadotropins (Chantaraprateep and Thibier, 1979). The episodic nature of FSH secretion in bulls (Perry et al., 1991), however, may mask a more direct relationship with cortisol than we were able to determine with once daily collection, with FSH concentrations also significantly lower at 23 and 32 d. Interestingly,

the apparent peak in cortisol and trough in FSH concentration were matched by the peak in midpiece abnormalities (notably distal midpiece reflexes) at exactly the interval expected if testosterone levels were reduced in the epididymal fluid during passage of the sperm at this juncture (Vogler et al., 1993). A standardized reduction in testosterone in the OFF treatment group was present throughout the postchallenge period. The immediate and sustained decrease in testosterone observed after the challenge similarly has been reported after induction of elevated circulating glucocorticoids at levels comparable to those reported here (Thibier and Rolland, 1976).

The accelerated display of vacuoles did not occur until 67 d, which is 62 d after the reported cortisol rise and
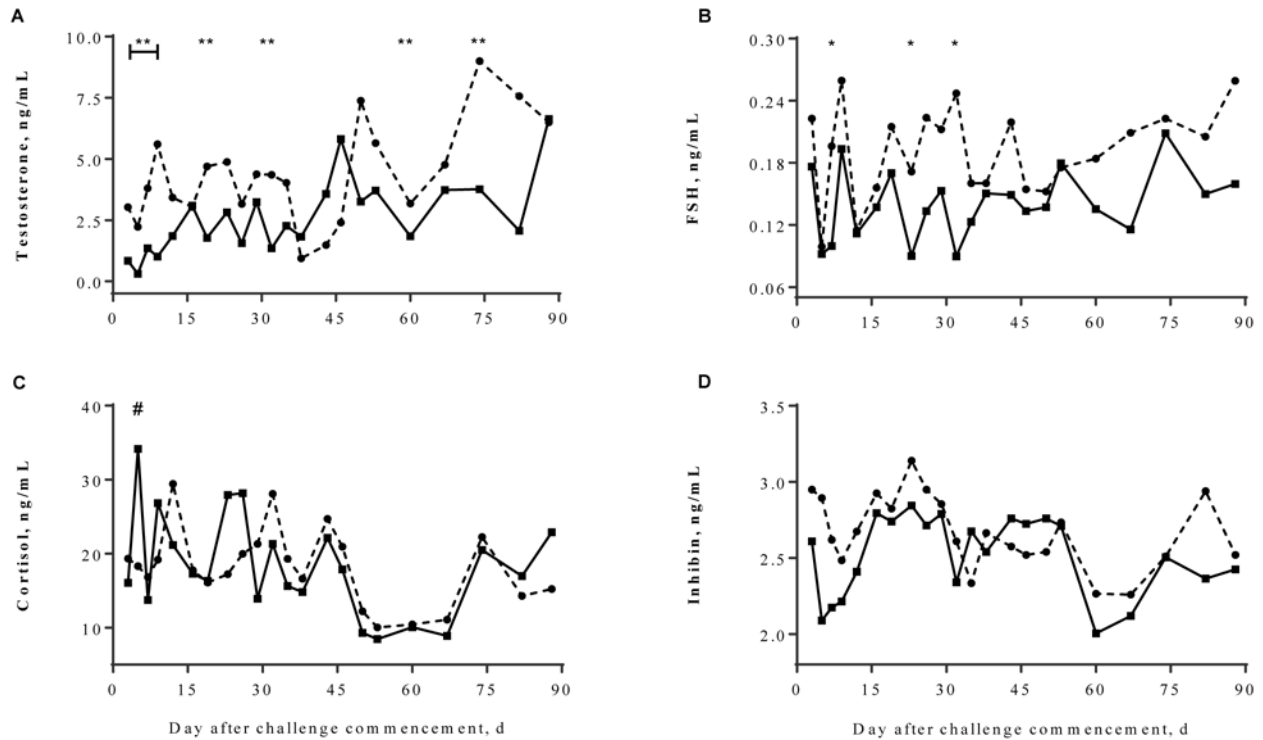
Figure 5. Mean serum testosterone, FSH, cortisol, and inhibin concentrations of bulls challenged with either oligofructose (solid line; ■) or water (dashed line; ●) at 6.5 g/kg BW from blood samples collected every third day for 53 d and then at 7-d intervals until 88 d after challenge. Values presented as back-transformed adjusted means with the exception of inhibin (average SED = 0.17). Differences between treatments at time points are shown (#$P < 0.1$; *$P < 0.05$; **$P < 0.01$).

35 d after the last significant drop in FSH. This increase in vacuole abnormalities is further matched by a rise in proximal droplets, concomitantly producing a significant depression in PNS at the last 3 dates of measurement. This may imply that although SARA caused an initial single perturbation on spermatogenesis, there was a continued labile effect of the altered circulating metabolites on gonadotropin concentrations, which ultimately reduced sperm quality at least until the end of the trial.

Although the oligofructose challenge did not significantly affect sperm progressive motility, there was considerable variation observed within the treatment groups for this variable. Furthermore, it has been previously reported that motility does not have a significant impact on calf output within Santa Gertrudis bulls managed under Australian rangeland conditions (Fitzpatrick et al., 2002).

Acidosis Induction Model

The acidosis induction model chosen for this study was an oral drench of oligofructose, a highly soluble nonstructural carbohydrate. Under field conditions where bulls are fed high-grain diets, the substrate most likely responsible for the development of SARA or acute ruminal acidosis is starch. Although the composition of the bacterial community and rumen fermentation patterns are dependent on the substrate, oral dosing with oligofructose or a combination of starch and

fructose previously has been demonstrated as a suitable substrate to induce both SARA and acute ruminal acidosis in cattle (Thoefner et al., 2004; Danscher et al., 2009; Golder et al., 2012, 2014). In addition, it can be delivered as a compact, oral dose, which made it a logical challenge method for use in noncannulated, previously unhandled range bulls. The aim of this experiment was to initiate SARA without impacting animal welfare beyond a point whereby semen could not be collected immediately after the challenge period. Hence, the dose rate used in this study (6.5 g/kg BW) was substantially lower than in previous experiments using oligofructose as the sole substrate, where dosages of 13, 17, or 21 g/kg BW induced laminitis subsequent to acute ruminal and systemic acidosis (Thoefner et al., 2004; Danscher et al., 2009, 2010). Despite the comparatively lower dose rate in the current experiment, all bulls receiving oligofructose developed lameness, which persisted for 7 d, confirming the suitability of this substrate in both acidosis and laminitis challenge models.

Confirmation of Subacute Ruminal Acidosis Induction

Observed reductions in rumen motility and pH, coupled with diarrhea and lameness of the oligofructose-treated bulls, are consistent with diagnostic indicators of both SARA and acute ruminal acidosis

(Nocek, 1997; Krause and Oetzel, 2006; Vasconcelos and Galyean, 2008). The mean rumen pH nadir in this study was 5.7, which is close to the benchmark range of between 5.0 and 5.6 considered characteristic of SARA (Nagaraja and Titgemeyer, 2007). Under normal conditions, ruminal lactate concentrations do not exceed 5 mM, whereas concentrations in excess of 40 mM are indicative of acute ruminal acidosis (Owens et al., 1998). Although ruminal lactate concentrations during the current experiment increased within OFF bulls between 0 and 12 h after dosing, concentrations did not exceed 20 mM. Previous experiments have reported a similar pattern of increase and peak ruminal lactate concentrations for cattle experiencing SARA challenge (Brown et al., 2000; Krause and Oetzel, 2006). Systemic dehydration, which is characterized by increases in PCV and TPP, is commonly associated with acute ruminal acidosis (Brown et al., 2000; Thoefner et al., 2004; Danscher et al., 2009) as the rumen contents become hypertonic and fluid is drawn in from the extracellular compartment. In contrast, a range of studies have demonstrated that PCV remains unchanged under grain-induced SARA (Goad et al., 1998; Brown et al., 2000; Bevans et al., 2005; Schwaiger et al., 2013). In the current experiment using a soluble sugar as the challenge substrate, both PCV and TPP were not statistically different between treatments. The mean heart rate of OFF bulls was elevated during the challenge period, peaking at 80 to 85 beats/min between 2 and 6 h after dosing but below the threshold of 120 beats/min considered definitive of an acute ruminal acidosis diagnosis (Parkinson et al., 2010). Although rectal temperature is usually below normal under conditions of acute ruminal acidosis, elevated rectal temperatures corresponding with the range of values observed in OFF bulls during this experiment are associated with SARA (Radostits et al., 2006). We therefore conclude that the combination of clinical signs, rumen pH, and lactate concentrations suggests that bulls dosed with oligofructose in this study experienced SARA. Despite the clinical diagnosis of SARA, it should be recognized that responses to the challenge model occurred after a single SARA episode. This contrasts with some definitions of SARA, which describe the condition as repeated bouts of moderately depressed rumen pH that are between acute and chronic in duration (Krause and Oetzel, 2006). Additionally, although SARA is associated with reductions in feed intake and performance, this may occur in the absence of clinical signs (Owens et al., 1998). Therefore, caution should be exercised in extrapolating results from the single, relatively severe form of SARA induced in this experiment to milder or repeated bouts of SARA.

Effects on Rumen Fermentation

The observed rapid decline of rumen pH within OFF bulls between 0 and 8 h after challenge was expected based on previous experiments using this substrate (Thoefner et al., 2004; Danscher et al., 2009). The contrasting increase of rumen pH in Control bulls between 0 and 16 h after challenge may have been a consequence of the feed and water curfew, as previously shown (Cole and Hutcheson, 1981; Galyean et al., 1981). Lowering of rumen ammonia concentrations to a level below the accepted minimum threshold (50 mg/L) considered necessary for microbial protein synthesis (Satter and Slyter, 1974), as observed in the Control bulls, has also been demonstrated after a feed and water curfew (Galyean et al., 1981). The lack of fermentable carbohydrates due to the feed curfew is also the most likely explanation for the gradual reduction in rumen VFA concentrations in Control bulls until 16 h after challenge. Increasing rumen VFA concentrations, rather than lactate accumulation, is primarily responsible for the reduction in rumen pH during episodes of SARA in beef and dairy cattle (Osborne et al., 2004; Bevans et al., 2005; Krause and Oetzel, 2006). Despite a clinical diagnosis of SARA in the current experiment, the expected increase in total rumen fluid VFA production was not observed. A possible explanation is that the 2-h sampling frequency was insufficient to capture any increase in rumen VFA supply within OFF bulls experiencing SARA. Concentrations of rumen VFA in dairy heifers adapted to a moderate concentrate diet and challenged with starch (1.2% BW) and fructose (0.2% BW) have been reported to rise immediately after induction, peaking at 65 min and then remaining unchanged until at least 215 min (Golder et al., 2014). The addition of fructose to acidosis challenge models based on starch is known to increase rumen concentrations of butyrate and isovalerate but decrease isobutyrate and valerate concentrations (Golder et al., 2012). These findings are consistent with the results found in this study using oligofructose as the sole substrate.

Conclusion

We observed that after SARA challenge, there were associative negative effects on both circulating FSH and testosterone concentrations, thereby causing the disruption of spermatogenesis and sperm development within the epididymis. The reduction in PNS after the challenge was sufficient to preclude the use of these bulls from single sire mating or for the collection of semen for freezing for a period of at least 90 d. Although there was evidence of a transitory rise in corticosteroids shortly after the SARA challenge, further studies are necessary to confirm the physiological mechanisms involved. The study findings have significant economic and practical

implications for the bull breeding industry in Australia where current management practices may heighten the risk of a reduction in bull fertility, such that bulls are unable to be sold as viable breeding units or cause a reduction in herd fertility during the breeding season.

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