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# Effects of pipothiazine palmitate on handling stress and on the characteristics of semen collected by electroejaculation in bison (*Bison bison*) bulls

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ABSTRACT

Handling North American bison can pose risk to the handler and evoke stress in the animal. Moreover, this induced stress might affect qualities of semen collected by electroejaculation. The objective of this study was to investigate if a long acting neuroleptic tranquilizer (LAN) would reduce the stress of bison and thereby improve the quality of electroejaculated semen. Eight experimental replicates were conducted between May and November. In each replicate, the same six bison bulls were randomly assigned into LAN-treated ($n = 3$) and non-treated control ($n = 3$) groups. Pipothiazine palmitate (Piportil L4) was administered intramuscularly as a single dose of 100 mg in replicates 1–4 or 200 mg in replicates 5–8. Within each replicate, semen was collected by electroejaculation at 4, 6, 11 and 13 days post treatment. Behavioral parameters, sperm morphology and motility parameters were analyzed. A blood sample was collected before each electroejaculation and serum concentrations of testosterone, cortisol and corticosterone were determined. Treatment bulls with 100 mg of Piportil L4 reduced the restraint time and the struggling of bison bulls during handling compared to the control group ($P < 0.05$). Semen motility parameters and serum concentrations of testosterone, cortisol and corticosterone were not significantly affected when 100 mg of the LAN was administered ($P > 0.05$). However, giving 200 mg of Piportil L4 reduced the restraint time of bison bulls and the duration of semen collection ($P < 0.05$). Also, this treatment improved total and progressive sperm motilities when compared to the respective controls ($P < 0.05$). Interestingly, serum concentration of corticosterone, as an endocrine stress indicator, was decreased after administration of 200 mg of Pipothiazine palmitate, while testosterone concentrations were increased compared to those values in untreated control bulls (corticosterone: $0.10 \pm 0.01$ compared with $0.15 \pm 0.02$ ng/mL; testosterone: $9.11 \pm 1.68$ compared with $5.33 \pm 0.74$ ng/mL; $P < 0.05$). In conclusion, this study demonstrated that a treatment dose of 200 mg of Piportil L4 can decrease the behavioral and endocrine stress responses in bison bulls, which indirectly increasing testosterone concentrations and improving semen quality.

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## 1. Introduction

The rapid expansion of the bison industry in Canada is reflected in a 35% increase in the number of bison recorded between 2001 and 2006 (Firmage-O'Brien, 2008). Canadian bison producers have historically preferred natural breeding over the use of reproductive technology thereby limiting the potential for genetic gain. Improved breeding management practices and the application of reproductive technologies have been hindered in the bison industry by a lack of knowledge regarding reproductive pattern, infectious diseases, and the effects of stress induced by handling (Dorn, 1995; Lott and Green, 2002; Helbig et al., 2007b; Firmage-O'Brien, 2008). As wild animals, captive bison are subject to stressors that may negatively affect their wellbeing over the longer term (Omsjoe et al., 2009). Signs of extreme stress in bison include increased respiration, frothing at the mouth, aggression, and jumping or attempting to climb out of pens and alleyways. Individuals in a handling chute may sit down or refuse to move (Grandin, 1998). These unpredictable actions can lead to bison harming themselves or their handlers (Hawley and Peden, 1982; Grandin, 1998). Training bison to tolerate handling procedures to reduce some of the stress can be lengthy and is not always feasible in a large scale ranch situation (Grandin, 1998; Bornett-Gauci et al., 2006; Lessard et al., 2009).

Bison are seasonal breeders; their breeding season usually lasts from June to October with peak activity occurring in August (Lott, 1991). Semen is produced throughout the year, but greater sperm motility and fewer sperm defects were observed during the breeding season (Helbig et al., 2007b). Puberty in male bison is attained by approximately 16 months of age, when semen production reaches concentrations of ≥50 million of cells/mL with ≥30% progressive motility and 65% normal sperm morphology (Helbig et al., 2007a). Electroejaculation is the only method developed to date for safe collection of semen from bison (Helbig et al., 2007a,b; Lessard et al., 2009).

Long acting neuroleptics are a form of tranquilizer employed for translocation of wild animals, especially in Africa where large mammals are often moved between game reserves (Knox et al., 1990; Fick et al., 2007). Pipothiazine palmitate (Piportil L4), a long acting neuroleptic commonly used in wildlife, was originally used as an antipsychotic drug with long-acting sedative effects in non-agitated chronic schizophrenic patients (Lingjaerde, 1973). The effects of Piportil L4 can last from 3 to 6 weeks in humans (Lingjaerde, 1973), and thus offers the potential of minimizing the stress of handling wild animals and the number of times these animals need to be handled.

The objectives of the present study were to characterize the stress-reducing effect of Piportil L4 in bison undergoing semen collection, and to determine if this long acting tranquilizer has any effect on the quality and quantity of semen. We hypothesized that a single administration of pipothiazine will decrease bison sensitivity to the stress of handling and electroejaculation, resulting in greater semen quality.

## 2. Materials and methods

### 2.1. Handling of bison

Sexually mature (between 2 and 6 years old) bison bulls ($n = 6$), ranging from 496 to 630 kg, were used between May and November at the University of Saskatchewan Native Hoofstock Center (near Saskatoon, SK, Canada; 52° N latitude). The bison bulls had unlimited access to pasture/hay with fresh water and copper iodized salt licks. Bulls were housed separately from the females. Two out of six bison bulls used in this study were born at, and transferred from the Toronto Zoo while the rest were wild-caught. All bison bulls were occasionally handled for semen collection at the Native Hoofstock Center before the start of this study. Handling and semen collection procedures were done as described previously (Helbig et al., 2007b; Lessard et al., 2009) and were performed according to the guidelines of the Canadian Council as approved by the University of Saskatchewan Committee on Animal Care.

### 2.2. Experimental design

The present study included eight experimental replicates. In each replicate, the same bison bulls were randomly assigned into two groups ($n = 3$ per group) and given Piportil L4 (50 mg/mL, IM, Sanofi-Aventis Canada Inc., Laval, Quebec, Canada) or no treatment (control; Fig. 1). In Replicates 1–4, conducted between May to August, 100 mg of Piportil L4 was administered (per animal) while in replicates 5–8, conducted between August to November, the dose of Piportil L4 was increased to 200 mg (Fig. 1). There was a minimum of 7 days interval between the end of each replicate and beginning of the next replicate (Fig. 1). Within each replicate, semen was collected 4, 6, 11 and 13 days after drug administration (four semen samples/trial/bison; Fig. 1) by electroejaculation (Pulsator IV, Lane Manufacturing, Denver, CO, USA). A rectal probe (34 cm length and 7.6 cm diameter) having three electrodes was used to stimulate ejaculation by repeated impulses of $\sim 10.2_{VRMS}$ (root mean squared voltage), 2 or 3 s each. A similar pattern of stimulation was followed for all semen collections. In this pattern, stimulations started at power level 2 (from 1 to 9) and after five stimulations at each level it was increased to next power level until ejaculation completed. All collections completed at ≤power level 7. The duration from the beginning of stimulations to ejaculation was recorded and analyzed. The total volume of each ejaculate was recorded and the sample was stored in an incubator at 37 °C until it was transferred to the laboratory for semen analysis (<2 h). Each sample was analyzed for total motility, progressive motility, velocity curve line (VCL), velocity average path (VAP), and, velocity straight line (VSL) using a computer-assisted sperm analyzer (CASA; Sperm Vision 3.0, Minitube Canada, Ingersoll, ON, Canada). A fresh semen smear was prepared for each ejaculate and stained with nigrosin–eosin stain (Blom, 1950) to evaluate sperm morphology using a light microscope (1000× magnification).

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