Original Article

Measurement of tear production and intraocular pressure in conscious captive European fallow deer (*DAMA dama*)

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Abstract

Normal values for intraocular pressure (IOP) and tear production in conscious cervids have not been reported to date. Based on trends in zoological institutions to perform non-anaesthetized health exams, it is applicable to establish normal values in conscious animals, as anaesthesia and sedation can alter these parameters. The goal of this study was to estimate intraocular pressures using rebound tonometry and measure tear production values in a group of healthy, conscious, European fallow deer utilizing chute restraint. Evaluation of these values with regards to instrumentation and restraint variables will be assessed. Complete ophthalmic examinations, including estimation of IOP with rebound tonometry and measurement of tear production with Schirmer tear tests (STT) were performed on nine conscious European fallow deer (*Dama dama*) restrained in a chute. Correlations between IOP on the unspecified (P) and the equine (H) settings, as well as IOP and STT differences between left (OS) and right (OD) eyes were evaluated, in addition to assessment of correlations between right and left lateral recumbency on IOP and STT. Tear production measurements were 18.7 ± 5.1 mm min^{-1} with a 95% confidence interval (CI) range of 16.4–21.1 mm min^{-1}. Intraocular pressure measurements for the P setting were 16.1 ± 4.5 mmHg with a 95% CI range of 14.1–18.2 mmHg, and for the H setting were 21.5 ± 5.1 mmHg with a 95% CI range of 19.1–23.9 mmHg. No statistically significant difference ($P > 0.05$) was found between OS and OD in any test. Neither left nor right lateral recumbency was found to have a statistically significant effect on IOP or STT. This study represents the first assessment of ophthalmic parameters in conscious fallow deer with rebound tonometry and STT.

Keywords: Cervid, fallow deer, intraocular pressure, rebound tonometry, tear production, Schirmer tear test.

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Introduction

The European fallow deer (*Dama dama*) is a medium-sized cervid native to the Mediterranean region, although the current natural distribution extends through much of Europe and Anatolia (Schloeth 1990). Due to their aesthetic appeal and hardiness, fallow deer are commonly kept in captivity within zoological institutions and deer parks. Captive populations are farmed in multiple parts of the world for their meat, antler velvet and as trophy hunting game (Haigh & Smits 1990; Schloeth 1990).

Deer in captivity are susceptible to a variety of ophthalmic conditions via exposure to other captive and wild animals, congenital disease and infectious diseases of viral, bacterial and fungal origin. Of particular concern in a zoological setting are ophthalmologic diseases associated with advanced age such as glaucoma, cataracts and neoplasia (Inglis *et al.* 1983; Sakai *et al.* 2001). Ophthalmic disease can contribute to decreased production and economic losses in farming operations, expenditure of resources in a zoological setting and individual animal morbidity. Additionally, routine health assessment in captive cervids includes assessment of ophthalmologic parameters, in which an understanding of a normal range for certain values is necessary to determine what constitutes an abnormal versus normal state.

Therefore; the ability to recognize and assess ocular disease in cervids is important in managing captive populations.

Studies evaluating ophthalmic parameters in cervid species are scant in the literature, and current studies have utilized various methodologies for both cervid restraint and ophthalmic measurements. A study reported in Persian fallow deer (*Dama mesopotamica*) evaluated intraocular pressure (IOP) and tear production (STT) in a captive population. These parameters were measured in anaesthetized animals using applanation tonometry and STT (Ofri *et al.* 2001). Another study evaluated IOP via applanation tonometry, STT and conjunctival cytology, histology and culture in a conscious group of captive brown brocket deer (*Mazama gouazoubira*) utilizing in-hand physical restraint (Martins *et al.* 2007). A similar study documented IOP via applanation tonometry, STT, conjunctival parameters and orbital anatomy in a free-ranging group of sambar deer utilizing in-hand physical restraint (Oria *et al.* 2015). Modern trends in zoological veterinary programs include operant conditioning programs that allow examination of animals without the use of chemical sedation or anaesthesia. Given the current ability of many institutions to utilize a chute system for cervid restraint, it would be useful to determine normal ranges of intraocular pressure and tear production values in healthy, conscious, European fallow deer that are restrained in a chute system.

Fallow deer are generally considered among farmers to be relatively disease resistant and reports of ocular disease in fallow deer are limited (Inglis *et al.* 1983; Deegan 2002). These reports include *Listeria monocytogenes* induced keratitis and uveitis in a herd of fallow deer due to contaminated silage, and a single report of conjunctival melanoma in a captive 13-year-old fallow deer (Welchman *et al.* 1997; Sakai *et al.* 2001). Other ocular diseases reported in free-ranging cervids include *Moraxella ovis*, dermoid cyst and microphthalmia, *Yersinia pestis* endophthalmitis, and keratoconjunctivitis due to cervid herpesvirus-1, although no reports of these conditions exist in fallow deer (Inglis *et al.* 1983; Dubay *et al.* 2000; Edmunds *et al.* 2008; Gelmetti *et al.* 2010). The ability to assess ophthalmic parameters in captive fallow deer is of importance in maintaining healthy herds and assessing disease when it occurs. Knowledge of normal ophthalmic parameters specific to this species is imperative to recognize, evaluate and subsequently treat ophthalmic disease.

The goal of this study was to estimate intraocular pressures using rebound tonometry and measure tear production in a group of healthy, conscious, European fallow deer utilizing chute restraint. In comparison to previous published values in anaesthetized Persian fallow deer, IOP and STT were hypothesized to be higher in the conscious group. Chute position of right versus left lateral recumbency during examination was hypothesized to have no significant effect on IOP and STT values between left and right eyes. Establishment of reference value ranges specific to conscious animals can be of use to institutions in settings where chemical immobilization is not utilized for examination of this species.

Materials and methods

Study population

Approval for this research was obtained from the university animal care committee animal research ethics board at the University of Saskatchewan in compliance with the Canadian Council on Animal Care guidelines. Nine adult, healthy, captive fallow deer (eight females and one male) representing 18 eyes were utilized. The animals were housed in a fenced outdoor enclosure at the Saskatoon Forestry Farm Park and Zoo in Saskatoon, Saskatchewan, Canada on dirt and grass substrate with peripheral tree cover. An enclosed, roofed shelter bedded with straw was available at all times. Daily diet consisted of ad libitum mixed alfalfa and grass hay, and 2 kg of herbivore ration (Co-op feeds, Federated Co-operatives Ltd., Saskatoon S7K 3M9, Saskatchewan, Canada) offered in a raised feeder in the shelter. Two cups of oats or barley were offered every other day and water was available ad libitum.

As part of their routine examination, all animals received a physical examination, *Mycobacterium bovis* and serum brucellosis testing as required by the Canadian Food Inspection Agency (CFIA),

complete ophthalmic examination (including STT, IOP, fluorescein staining, slit lamp biomicroscopy and indirect ophthalmoscopy), hoof trim, deworming with ivermectin (Ivomec®, Merial Canada, Inc., Baie d'Urfé H9X 4B6, Québec, Canada; 0.2 mg kg^{-1} SC) and clostridial vaccination (Covexin® Plus, Merck Intervet Canada Corp., Kirkland, Québec, Canada; 2 mL/animal SC). Mid-cervical *M. bovis* purified protein derivative tuberculin testing (Bovine PPD tuberculin, CFIA Biologics Production Unit, Ottowa Laboratory Fallowfield, Ottowa K2H 8P9, Ontario, Canada; 0.1 mL/animal SC) was administered by a registered CFIA official. Blood for a complete blood count, plasma biochemistry panel and brucellosis titre was obtained from the jugular vein or lateral saphenous vein on all animals.

Data collection occurred on two separate days, 3 days apart with a group of five animals on the first day and four animals on the second day. Examination was performed in the morning between 0900 and 1100. Mean temperature during data collection was 1.8°C. For examination, animals were herded into the shelter and individually allowed into a chute with a lateral squeeze mechanism, which was then rotated 90° to position the deer in right or left lateral recumbency (see Figs. 1–3). Tuberculosis testing was performed first, followed by ophthalmic examination and data collection, physical examination, medication and vaccine administration, blood collection and hoof trim.

Ophthalmic examination

A complete ophthalmic examination was performed on each animal and included IOP estimation using a rebound tonometer (TonoVet®, Jorgensen Laboratories Inc.), tear production testing using STT (Color Bar, EagleVision Enteroptyx™, Memphis 38133, TN), slit lamp biomicroscopy (Kowa SL-17, Kowa Company, Ltd., Chuo-ku 103-8433, Tokyo, Japan), indirect fundoscopy with a 20 dioptre double aspheric lens (Volk Optical Inc., Mentor 44060, OH), retinal imaging (Optomed Smartscope Vet2, ARO Systems, Wodonga 3689, Victoria, Australia) and fluorescein staining (I-Glo JorVet, Jorgensen Laboratories, Inc., Loveland 80538, Colorado). The same

Fig. 1 Front view of the custom built cervid squeeze chute with tilt capability.

examiner (BSB) performed all ophthalmic examinations and intraocular pressure measurements to minimize variations in experimental technique. STT and fluorescein staining was performed by another examiner (REP) in all animals. All examinations were performed in the same order as follows: IOP measurement, STT measurement, slit lamp biomicroscopy, indirect fundoscopy, retinal imaging and fluorescein staining.

Intraocular pressure measurement

For IOP measurement, each animal was manipulated while in lateral recumbency to manoeuvre the head into a vertically oriented position. Each subject was allowed approximately 2 min in this position prior to taking IOP measurements. Care was taken to ensure that no compression of the jugular veins or cervical region occurred during measurement. A rebound tonometer with a single-use probe was held perpendicular to the surface of the cornea, approximately

Fig. 2 Back view of the custom built cervid squeeze chute with tilt capability.

Fig. 3 Side view of the custom built cervid squeeze chute with tilt capability.

4 mm from the central cornea. The setting of the device was set to P (undefined species) or H (equine), and measurements were obtained from both eyes on each animal using both settings. Six tonometry measurements were obtained for each the left (OS) and right (OD) eyes with a final mean displayed based on an average of four measurements, excluding the highest and lowest of the six total

measurements. Measurements were obtained in millimetres of mercury (mmHg) and only measurements with an acceptable standard deviation (≤2.5%) were included in the study. If an unacceptable standard deviation was acquired, the measurement was repeated until an adequate reading was obtained.

Tear production assessment

Tear production was measured for both eyes in each animal using a standard STT. Measurements were obtained from both eyes simultaneously with the animal's head manually oriented into a vertically oriented position. Measurements were obtained in millimetres over a one minute period.

Fluorescein eye stain

Fluorescein staining was performed using strips impregnated with 1 mg fluorescein sodium. One test strip was used per animal and each test strip was wetted with an ophthalmic irrigating solution (Eye Stream, Alcon Canada Inc., Mississauga L5N 8C7, Ontario, Canada) and touched to the dorsal bulbar conjunctiva. Excess stain was washed out of the eyes with irrigating solution, and the cornea was examined with a cobalt blue light on an ophthalmoscope head.

Statistical analysis

Statistics were derived utilizing Stata data analysis and statistical software (StataCorp LLC., College Station 77845, TX). Stem and leaf plots and Shapiro–Wilk tests were used to determine data normality for IOP, STT and differences between OS and OD for both IOP and STT. Wilcoxon signed-rank tests were used to compare data differences between eyes (OS vs. OD) and recumbency (left versus right lateral) for IOP and STT. Mean, standard deviation (expressed as ± SD) and 95% confidence interval (95% CI) were calculated based on a total of 18 eyes for all tests.

Results

All animals in the study population were determined to be healthy based on physical and ophthalmic

examination, and no precluding abnormalities were noted on CBC and plasma biochemistry results. Brucellosis and *M. bovis* testing were negative in all animals. No animals were excluded from the study, and nine fallow deer consisting of 18 eyes were included in the data population. On ophthalmic examination, one deer was noted to have an incipient anterior cortical cataract with overlying capsular pigmentation, and another sustained a 1 cm superficial dermal laceration to the inferior palpebrae of the right eyelid during chute restraint, which were determined to be non-significant factors in ophthalmic testing results. No fundic abnormalities were observed on indirect ophthalmoscopy. No uptake of fluorescein stain was noted in any eye.

There were no significant differences in IOP or STT between the left (OS) and right (OD) eyes ($P > 0.05$). Statistical analysis was used to compare STT values obtained in left and right eyes based on if the animal was in left or right lateral recumbency, and no statistical significance was found between mean OS and mean OD measurements regardless of the animal's body position in left lateral or right lateral recumbency during examination for both IOP and STT measurements (Table 1). The overall mean of 18 eyes for each IOP and STT had no statistical difference between animals in left lateral and right lateral recumbency ($P > 0.05$).

Rebound tonometry measurements were expressed in millimetres of mercury (mmHg) and were evaluated based on a 95% CI. Results were evaluated independently for the H setting and P setting for OS, OD and the mean of 18 eyes. Mean IOP of 18 eyes for the H setting was 21.5 ± 5.1 mmHg, and 95% CI range was 19.1–23.9 mmHg. Mean IOP of 18 eyes for the P setting was 16.1 ± 4.5 mmHg,

and 95% CI range was 14.1–18.2 mmHg (Table 2). Mean IOP values between OS and OD were not found to be significant for either setting ($P > 0.05$). The P setting (unspecified) and H (equine) setting on the tonometer were determined to have a statistically significant different range of IOP values between settings ($P = 0.002$). There were no significant differences in IOP or STT between the left (OS) and right (OD) eyes ($P > 0.05$). Evaluations of sex and age on IOP and STT were not performed due to an imbalanced sex ratio and unreliable age assessment of the population.

Schirmer tear tests were measured in millimetres per minute (mm/min) and results yielded a mean measurement of 18.7 ± 5.1 mm min^{-1}. Mean STT measurement range based on a 95% CI was 16.3–21.1 mm min^{-1} (Table 2).

Discussion

Tear production values and intraocular pressures in conscious European fallow deer in this study were higher than previously published values in anaesthetized Persian fallow deer (IOP 11.9 ± 3.3 mmHg, STT 10.5 ± 6.5 mm min^{-1}).(Ofri *et al.* 2001) Data collected in other cervids using manual restraint showed lower IOP and STT values in brown brocket deer (*Mazama gouazoubira*; IOP 15.3 ± 3.3 mmHg; STT 8.9 ± 1.8 mm min^{-1}) and lower IOPs and similar STT in sambar deer (*Rusa unicolor*; IOP 11.4 ± 2.8 mmHg; STT 18.8 ± 4.7 mm min^{-1}) compared to the conscious fallow deer in this study. (Martins *et al.* 2007; Oria *et al.* 2015) Data acquired in other artiodactyla species using conscious animals include bovine IOP (*Bos taurus*; 26.9 ± 6.7 mmHg), llama IOP (*Lama glama*; 16.96 ± 3.51 mmHg),

Table 1. IOP and STT values separated based on body position in left lateral ($n = 5$) or right lateral ($n = 4$) recumbency with *P*-values measured between left (OS) and right (OD) eyes in each position from nine captive conscious fallow deer restrained in a chute

Measurement parameter	Left lateral	*P*-value LL	Right lateral	*P*-value RL
Tear production (mm min^{-1})	20.2 ± 3.9	0.5	16.9 ± 5.8	0.71
Intraocular pressure H (mmHg)*, †	21.1 ± 5.4	0.49	22 ± 4.6	0.71
Intraocular pressure P (mmHg)*,†	14.9 ± 4.6	0.06	17.6 ± 3.8	0.36

*H indicates equine calibration setting; P indicates unspecified species calibration setting on tonometer. †A statistical significance exists between H and *P* IOP measurements ($P = 0.002$).

Table 2. Tear production and rebound tonometry results measured by Schirmer tear test and TonoVet® from nine captive conscious fallow deer (total eyes n = 18) restrained in a chute

Measurement parameter	Mean and SD	95% Confidence Interval
Tear production (mm/min)	18.7 ± 5.1	16.3–21.1
Intraocular pressure H (mmHg)*, †	21.5 ± 5.1	19.1–23.9
Intraocular pressure P (mmHg)*, †	16.1 ± 4.5	14.1–18.2

*H indicates equine calibration setting; P indicates unspecified species calibration setting on tonometer. †A statistical significance exists between H and P IOP measurements (P = 0.002).

alpaca IOP (*Vicugna pacos*; 16.14 ± 3.74 mmHg), camel IOP (*Camelus dromedarius*; OD = 27.1 ± 1.2 mmHg, OS = 28.2 ± 1.2 mmHg), barbary sheep (*Ammotragus lervia*; STT 27.22 ± 3.6 mm min^{-1}, IOP 19.47 ± 3.9 mmHg) and sanjabi sheep (*Ovis aries*; STT 18.52 ± 2.55 mm min^{-1}, IOP 9.37 ± 2.45 mmHg).(Gum *et al.* 1998; Nuhsbaum *et al.* 2000; Ghaffari *et al.* 2011; Marzok & El-Khodery 2015; Fornazari *et al.* 2016).

Chemical immobilization is generally required for ophthalmic examination of free-ranging cervids, and those not accustomed to confinement within an enclosure, for both the safety of handlers and practicality purposes. Currently, large farming operations of captive cervids prefer to handle their animals, if necessary, in a chute or stanchion system. Many zoological institutions that house captive cervids have operant conditioning protocols in place to make free-contact animal handling feasible or can utilize a restraint system to examine animals without sedation or anaesthesia. Therefore, determination of normal ophthalmic parameters in conscious fallow deer restrained in a chute system is of value to have accurate conscious assessments.

Animals in a zoological setting have been conditioned to tolerate various veterinary examination and medical procedures while conscious and restrained manually or in a restraint device to avoid the use of chemical immobilization, which can potentially pose risks greater than the benefits for any given procedure. (Phillips *et al.* 1998; Grandin 2000; Savasano *et al.* 2003) Several studies have assessed

values typically associated with stress responses, including glucose, creatine kinase and cortisol, to determine if conscious restraint and operant conditioning results in lower stress markers compared to non-conditioned or anaesthetized animals. A study performed in antelope showed serum cortisol levels in conditioned individuals to be significantly lower than reported reference values in non-conditioned animals. (Grandin 2000) Bongo at a zoological institution were conditioned to utilize a crate without the use of chemical or manual restraint and showed lower cortisol, blood glucose and serum creatine kinase values compared to animals chemically sedated. (Phillips *et al.* 1998). The animals in this study were manually restrained in a chute to allow examination, venipuncture, tuberculin testing, hoof trimming and ophthalmic assessment. Chute restraint was adequate to allow all handling, and animals were able to be returned to a normal routine immediately after examination. No significant morbidity or mortality was associated with restraint or procedures in this study. Based on risk assessment, chemical immobilization was deemed to be unnecessary in this group for the procedures performed. Current practices in zoological medicine utilize operant conditioning and manual restraint in lieu of chemical sedation for many minimally invasive procedures; therefore, it is important to develop reference ranges for parameters that can be affected by chemical agents.

Previous studies in various species have documented alterations in IOP and STT between conscious and anaesthetized animals. In a study of IOP and STT in koalas (*Phascolarctus cinereus*) anaesthetized with alfaxalone compared to conscious koalas, STT was found to be significantly (P < 0.0001) lower in anaesthetized koalas, but no significant alteration in IOP, as measured with applanation tonometry (P = 0.32), was found (Grundon *et al.* 2011). Anaesthesia in dogs and cats has been found to significantly alter both IOP and STT, although reports vary. A study in dogs showed no clinically significant effect of ketamine and midazolam anaesthesia on IOP, however a separate study evaluating ketamine and ketamine-midazolam combinations showed a clinically significant increase in IOP for up

to 20 min after administration (Hofmeister *et al.* 2006; Ghaffari *et al.* 2010b). Schirmer tear test measurements in dogs and cats have been reported to be significantly lower in animals undergoing general anaesthesia with various protocols. (Herring *et al.* 2000; Sanchez *et al.* 2006; Ghaffari *et al.* 2010a). In comparison to IOP and STT measurements obtained in fallow deer anaesthetized with an ultrapotent opioid and phenothiazine derivative, IOP and STT measurements obtained in this study were higher. Exact mechanisms of decreased tear production and IOP due to these anaesthetic agents is unknown; however, effects contributing to this potentially include alterations in somatic autonomic function, decreases in stress levels secondary to drug effects, alterations in cardiac output, vasoconstrictive effects on ocular structures and direct effects on conjunctival cellular tear film production.(Di Pietro *et al.* 2016) Given the variability in IOP and STT measurements associated with certain anaesthetic protocols in multiple species, the establishment of normal parameters in conscious, chute-restrained fallow deer can be applied to other fallow deer under similar restraint conditions.

Various methods of intraocular pressure measurement are available, including indentation, applanation and rebound tonometry. Applanation tonometry has been used in the majority of artiodactyl IOP studies currently published and requires use of a topical ophthalmic anaesthetic (Gum *et al.* 1998; Nuhsbaum *et al.* 2000; Martins *et al.* 2007; Ghaffari *et al.* 2011; Marzok & El-Khodery 2015; Oria *et al.* 2015; Fornazari *et al.* 2016). Advantages of rebound tonometry include ease of use, decreased manipulation of the periocular structures, quick measurement acquisition and a lack of requirement for a topical corneal anaesthetic. Studies evaluating the reliability of rebound tonometry as compared to applanation tonometry in dogs and horses showed no clinically significant difference in techniques (Knollinger *et al.* 2005; Levia *et al.* 2006). Studies in various penguin species and Eurasian eagle owls (*Bubo bubo*) have shown a statistically significant increase in IOP measured with rebound tonometry as compared to applanation tonometry (Jeong *et al.* 2007; Bliss *et al.* 2015). A difference was found between IOP measured in this study as compared to applanation tonometry

performed in a different study of fallow deer; however, other factors including examiner technique, tonometer calibration, differences in animal group age, sex ratio, health, anaesthesia and restraint conditions could be contributing to this finding (Ofri *et al.* 2001). Rebound tonometry values were obtained on two different settings in this study, unspecified (P) and equine (H). Both estimates provided a statistically reliable reference range; however these ranges were considered statistically different when compared with each other. Therefore, if utilizing rebound tonometry to compare multiple measurements over time in the same animal, between members of a group of animals, or to other rebound tonometry measurements obtained in other groups or species of animals, it is recommended to utilize the same setting each time and compare to the same setting. Rebound tonometry in this study yielded a reliable IOP range without the need for a topical corneal anaesthetic or eyelid manipulation, as is necessary with applanation tonometry. Therefore, rebound tonometry can be considered for use in conscious cervids when corneal anaesthesia is not available.

Body position has been shown to alter IOP measurement in dogs placed in a dorsal recumbency position compared to standing and sitting, and head position in a downwards or upward position altered IOP in American flamingos (*Phoenicopterus ruber*) (Broadwater *et al.* 2008; Molter *et al.* 2014). Deer in this study were rotated into left or right lateral recumbency in a squeeze chute, and no significant alterations in IOP or STT were noted with respect to position. The deer heads in this study were manually held in a vertical plane and provided time to allow ocular pressure to stabilize, which could have accounted for the lack of effect of body position on results. This study was performed in a healthy captive population of fallow deer restrained in a chute. This setup may be a common setting in which cervid farms or zoological institutions may also restrain their cervids, requires less personnel training, and is generally considered a safer method of examination than full anaesthesia. Therefore the establishment of normal ranges for IOP and STT in conscious, chute-restrained deer is of clinical relevance. A limitation of this study included the positioning of deer within

the restraint chute in lateral recumbency, as other institutions may implement restraint chutes that maintain body position in a standing position which could influence IOP and STT measurements if compared to the values obtained in this study. Further investigation into normal values for IOP and STT measurements in conscious deer in standing chute restraint are warranted. Each animal in this study was undergoing full physical examination, blood sampling and tuberculosis testing; therefore a single measurement for IOP in each eye was obtained to reduce stress and time spent in the chute. Greater statistical reliability could be achieved by obtaining multiple measurements of IOP in each eye with averaging. To achieve this, the authors would recommend performing ophthalmic examination and obtaining IOP and STT measurements as a single restraint event not coupled with other procedures.

Conclusions

1. This study represents the first evaluation of intraocular pressure and tear production values in a conscious, chute-restrained group of healthy fallow deer via rebound tonometry and STT.
2. Body position in left or right lateral recumbency did not have a significant effect on either IOP or STT.
3. Values for both IOP and STT were found to be higher compared to values reported in an aneasthetized group of fallow deer in a separate study.
4. Application of these ophthalmic measurement methods in fallow deer can successfully be utilized in situations where ophthalmic examination is being performed in conscious animals.

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Conflict of Interest

No external funding was required for this study. The authors report no conflict of interest, relationship or interest influencing this study.

Contribution

All authors participated in the design of the study, collected the data, and analysed results. RP and MS interpreted the data and wrote the initial draft of the paper. BB assisted in the interpretation of the results. All authors collaborated in the preparation of the final paper and critically reviewed all sections of the text for important intellectual content.

Ethical statement

The authors verify that as per the journal's author guidelines, all ethical policies were adhered to in performing this study. The authors confirm that approval for this research was obtained from the university animal care committee animal research ethics board at the University of Saskatchewan in compliance with the Canadian Council on Animal Care guidelines.

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