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Wolf presence in the ranch of origin: Impacts on temperament and physiological responses of beef cattle following a simulated wolf encounter[1]

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ABSTRACT: This experiment evaluated temperament, vaginal temperature, and plasma cortisol in beef cows from wolf-naïve and wolf-experienced origins that were subjected to a simulated wolf encounter. Multiparous, pregnant, nonlactating Angus-crossbreed cows from the Eastern Oregon Agricultural Research Center located near Burns, OR (CON; $n = 50$), and from a commercial operation near Council, ID (WLF; $n = 50$), were used. To date, grey wolves are not present around Burns, OR, and thus CON were naïve to wolves. Conversely, wolves are present around Council, ID, and WLF cows were selected from a herd that had experienced multiple confirmed wolf-predation episodes from 2008 to 2012. Following a 50-d commingling and adaptation period, CON and WLF cows were ranked by temperament, BW, and BCS and allocated to 5 groups (d 0; 10 CON and 10 WLF cows/group). Groups were individually subjected to the experimental procedures on d 2 ($n = 3$) and d 3 ($n = 2$). Before the simulated wolf encounter, cow temperament was assessed and blood samples and vaginal temperatures (using intravaginal data loggers) were collected (presimulation assessments). Cows were then sorted by origin, moved to 2 adjacent drylot pens (10 WLF and 10 CON cows/pen), and subjected to a simulated wolf encounter event for 20 min, which consisted of 1) cotton plugs saturated with wolf urine attached to the drylot fence, 2) continuous reproduction of wolf howls, and 3) 3 leashed dogs that were walked along the fence perimeter. Thereafter, WLF and CON cows were commingled and returned to the handling facility for postsimulation assessments, which were conducted immediately after exposure to wolf-urine-saturated cotton plugs, wolf howl reproduction, and 20-s exposure to the 3 dogs while being restrained in a squeeze chute. Chute score, temperament score, and plasma cortisol concentration increased ($P \leq 0.01$) from pre- to postsimulation assessment in WLF but did not change in CON cows ($P \geq 0.19$). Exit velocity decreased ($P = 0.01$) from pre- to postsimulation assessment in CON but did not change ($P = 0.79$) in WLF cows. In addition, WLF cows had a greater ($P = 0.03$) increase in temperature from pre- to postsimulation assessments compared with CON cows. In conclusion, the simulated wolf encounter increased excitability and fear-related physiological stress responses in cows that originated from a wolf-experienced herd but not in cows that originated from a wolf-naïve herd.

Key words: beef cattle, physiology, stress, temperament, wolves

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INTRODUCTION

The reintroduction of grey wolves into the Yellowstone National Park directly contributed to the dispersion of wolf packs into the northwestern United States, including agricultural lands in Idaho and Oregon (Larsen and Ripple, 2006). As a result, wolves started to inhabit and hunt in livestock grazing areas, which increased the incidence of cattle–wolf interactions and cattle predation by wolves in both states (Idaho Department of Fish and Game and Nez Perce Tribe, 2013; Oregon Department of Fish and Wildlife,

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2013a). The economic and productive implications of predators on livestock systems is often evaluated based on the number of animals injured or killed (Treves et al., 2002; Oakleaf et al., 2003; Breck and Meier, 2004); however, these parameters may not be the only negative impacts that wolf predation causes to beef cattle systems (Kluever et al., 2008; Laporte et al., 2010).

The mere presence of predators alters stress physiology and behavior parameters of the prey (Creel and Christianson, 2008), particularly if the preyed animal was already subjected to similar predation episodes (Boonstra, 2013). More specifically, fear of predation may alter cattle temperament and stimulate adrenal corticoid synthesis (Laporte et al., 2010; Boonstra, 2013), which have been shown to negatively impact health, productive, and reproductive parameters in beef cattle (Cooke et al., 2009, 2012; Francisco et al., 2012). Based on this rationale, we hypothesized that wolf presence near cattle herds stimulates behavioral and physiological stress responses, particularly in cattle from herds previously preyed upon by wolves. Hence, the objective of this experiment was to evaluate temperament, vaginal temperature, and plasma concentration of cortisol in wolf-naïve and wolf-experienced beef cows that were experimentally subjected to auditory, olfactory, and visual stimuli designed to simulate a wolf encounter.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University–Eastern Oregon Agricultural Research Center (**EOARC**; Burns, OR) from January to March 2013. Animals used were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee.

Animals and Diets

Multiparous, pregnant, nonlactating Angus-crossbred cows from EOARC (**CON**; *n* = 50) and from a commercial cow–calf operation (**WLF**; *n* = 50) near Council, ID, were used. Both locations used domestic herding dogs to move cattle across pastures or to the handling facility. The CON cows (age = 5.0 ± 0.1 yr; BW = 523 ± 6 kg; BCS = 4.80 ± 0.04; Wagner et al., 1988; approximately 6 mo in gestation) were randomly selected from the EOARC mature cowherd. The EOARC herd is reared and maintained near Burns and Riley, OR, and to date no known wolf packs exist or wolf-predation episodes have occurred in this region (Oregon Department of Fish and Wildlife, 2013a). Hence, CON cows were naïve to wolf presence and predation. The WLF cows (age = 4 ± 0.1 yr; BW = 513 ± 7 kg; BCS = 4.90 ± 0.06; Wagner et al., 1988; approximately 6 mo in gestation) were randomly selected from the commercial operation located near Council, ID. This region (i.e., McCall-Weiser Wolf Management Zone) supports active wolf packs (Idaho Department of Fish and Game and Nez Perce Tribe, 2013). Furthermore, the herd from which WLF cows were selected have experienced multiple confirmed wolf predation episodes from 2008 to 2012 when grazing summer pasture allotments (USDA-Animal and Plant Health Inspection Service, Idaho Wildlife Services, Boise, ID; confirmation documents are available on request to corresponding author) although none of the experimental WLF cows had been directly preyed upon or injured by wolves. Therefore, WLF cows were considered experienced with wolf presence and predation episodes.

The WLF cows were transported to the EOARC 50 d prior (d –50) to the beginning of the experiment (d 0). During this period (d –50 to d 0), CON and WLF cows were commingled and maintained in a single meadow foxtail (*Alopecurus pratensis* L.) dominated pasture (Wenick et al., 2008) harvested for hay the previous summer and had ad libitum access to meadow-grass hay (56% TDN, 65% NDF, 41% ADF, 1.07 Mcal/kg of NEm, and 8.2% CP), water, and a vitamin–mineral mix [Cattleman's Choice; Performix Nutrition Systems, Nampa, ID; containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 (IU)/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E]. Cows were also individually processed through the EOARC handling facility, but not restrained in the squeeze chute, once a week from d 50 to –2 to acclimate WLF cows to the EOARC personnel and facilities (Francisco et al., 2012).

On d 0, cows were ranked within previous wolf exposure status (CON and WLF) by temperament score (Cooke et al., 2012; by the same single technician), BW, and BCS and allocated to 5 groups of 20 cows each (10 CON and 10 WLF cows per group). Hence, groups were balanced for temperament score (2.58 ± 0.02), BW (518 ± 5 kg), and BCS (4.84 ± 0.05). Each group of 20 cows was maintained on individual meadow foxtail pastures (Wenick et al., 2008) harvested for hay the previous summer during the experimental period (d 0 to 3) and had ad libitum access to water and the previously described meadow-grass hay and vitamin–mineral mix.

Experimental Procedures – Simulated Wolf Encounter

Due to daylight limitations, 3 groups were randomly selected and received the experimental procedures on d 2 whereas the remaining 2 groups received the experimental procedures on d 3. Minimum, maximum, and average environmental temperatures on d 2 and 3 were, respectively, –8 and –2, 7, and 9, –1 and 4°C whereas average humidity was, respectively, 66 and 85% with no observed precipitation. While an individual group was being subjected to the simulated wolf encounter at the EAORC handling facilities, the other groups remained on their respective pastures. Groups were maintained on pastures that were ≥0.5 km distant from the handling facilities to prevent that cows perceived the simulated wolf encounter model while on pasture.

Presimulation Assessments. The evaluated group was gathered in its respective pasture and walked to the handling facility, where cows were evaluated for temperament (chute score, exit velocity, and temperament score, by the same single technician; Cooke et al., 2012). Immediately after chute score evaluation, a blood sample was collected and a HOBO Water Temp Pro V2 data logger (Onset Company, Bourne, MA) was inserted intravaginally in each cow to record temperature at 30 s intervals. Each data logger was attached to a controlled internal drug-releasing device (Pfizer Animal Health, New York, NY) that did not contain hormones.

Simulated Wolf Encounter. Immediately after the presimulation assessment, cows within each group were sorted by previous wolf exposure as they exited the squeeze chute and subsequently moved to 2 adjacent drylot pens separated by a fence line (10 WLF and 10 CON cows in each pen). Pens were 17 by 17 m, located 0.1 km from the handling facility, and had no feed or water source. Furthermore, cows were not moved through these pens when walked from pasture to the handling facility for the presimulation assessment. After arrival in their respective pens, CON and WLF cows were immediately subjected to a simulated wolf encounter for 20 min. Specifically, wolf urine (Harmon Wolf Urine Scent; Cass Creek, Grawn, MI) was applied to 12 cotton plugs (Feminine care tampons; Rite Aid, Camp Hill, PA), and plugs were attached to the drylot fence line every 11 m (6 plugs/pen) before any experimental procedures on d 2 and 3. After cows were settled within each dry lot pen, wolf howls previously recorded from the wolf packs residing in Wallowa County, OR, were continuously reproduced using a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, CA) located 10 m from the dry lot pens; cows had no visual contact with the stereo system. Additionally, 3 dogs were conducted using a leash by 2 technicians outside the drylot perimeter fence during the entire 20-min simulation. The dogs were 2 adult German Shepherd females (BW = 34.5 ± 1.5 kg) to represent adult wolves and 1 adult Border Collie × Alaskan Malamute female (BW = 22 kg) to represent a young wolf. The maximum and minimum distances allowed between dogs and cows were 25 and 5 m, respectively. Dogs did not act aggressively or vocalize during the simulated wolf encounter.

Postsimulation Assessments. After 20 min of the simulated wolf encounter, WLF and CON cows were commingled and returned to the handling facility for removal of HOBO data loggers, temperament evaluation, and blood collection as in the presimulation assessments. However, cows were also subjected to the simulated wolf encounter during processing for postsimulation assessments. Three cotton plugs saturated with wolf urine were attached to the walls of the lead chute at 3-m intervals immediately before the squeeze chute, and 1 similar cotton plug was hung inside the squeeze chute (Silencer Chute; Moly Manufacturing, Lorraine, KS). Wolf howls were reproduced throughout the entire processing. Cows were also exposed for 20 s to the same 3 dogs while restrained in the squeeze chute. Dogs were handled via leash by 2 technicians in front of the squeeze chute, remained 5 m from the restrained cow, and did not act aggressively or vocalize during this process. After cows were exposed to all simulation components, blood was collected, HOBO data loggers were removed, and temperament was evaluated.

Immediately after the postsimulation assessments, the group was returned to its original pasture, cotton plugs were removed from the handling facility, wolf urine was reapplied to all cotton plugs (those attached to pens and handling facility), and the subsequent group was only evaluated after a 30-min interval to prevent residual wolf scent inside the handling facility during the presimulation assessment. Furthermore, the wolf howls were not reproduced and dogs were restrained in an enclosed barn during this 30-min interval to prevent unwarranted visual and auditory stimuli before the simulated wolf encounter.

Sample Analysis. Hay samples collected on d –50 were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF [method 973.18 modified for use in an Ankom 200 fiber analyzer (Ankom Technology Corp., Fairport, NY); AOAC, 2006], and NDF (Van Soest et al., 1991; method for use in an Ankom 200 fiber analyzer). Calculations of TDN used the equation proposed by Bath and Marble (1989) whereas NEm was calculated with the equation proposed by the NRC (1996).

Individual cow temperament was assessed by chute score and exit velocity as previously described by Cooke et al. (2012). Chute score was assessed by a single technician based on a 5-point scale where 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, and shaking of the chute, and 5 = violent and continuous struggling. Exit velocity was assessed by determining the speed of the cow exiting the squeeze chute by measuring rate of travel over a 1.9-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Furthermore, cows were divided in quintiles according to their exit velocity, within CON and WLF cows on d 0 and within group for pre- and postsimulation assessments, and assigned a score from 1 to 5 (exit score; 1 = cows within the slowest quintile and 5 = cows within the fastest quintile). Individual temperament scores were calculated by averaging cow chute score and exit score.

Temperature data from HOBO loggers were processed using the HOBOware Pro software (version 3.3.2; Onset Company). Only data obtained after the end of the presimulation assessments (when cows were gathered and moved to the dry lot pens) to the end of the simulated wolf encounter (when cows were commingled to return to the handling facility) were recorded and compiled into 5-min intervals. Hence, cows had 25 min of recorded vaginal temperature, with the initial 5 min collected before the simulated wolf encounter (presimulation assessment, when all cows were gathered but before moving to dry lot pens) and the remaining 20 min collected during the simulated wolf encounter (postsimulation assessments).

Blood samples were collected via jugular venipuncture into a commercial blood collection tube (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with 158 United States Pharmacopeia units of freeze-dried sodium heparin. After collection, blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min at 4°C) for plasma harvest, and stored at –80°C on the same day of collection. A bovine-specific commercial ELISA kit was used to determine plasma concentrations of cortisol (Endocrine Technologies Inc., Newark, CA) as previously used by our research group (Cooke and Bohnert, 2011). The intra- and interassay CV for the cortisol assay were, respectively, 6.2 and 6.7%.

Statistical Analysis. Pen within the evaluated group was considered the experimental unit. All data were analyzed using the MIXED procedure of SAS (version 9.3; SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects, with pen(previous wolf exposure), cow(pen), and group as random variables. The model statement contained the fixed effects of previous wolf exposure (CON and WLF), time (pre- and postsimulation assessments), wolf exposure × time interaction, and day of evaluation as independent variables (d 2 or 3 of the experimental period). The difference between post- and presimulation assessment values (final 5 min for vaginal temperature) was evaluated using a model containing the fixed effects of previous wolf exposure and day of evaluation as independent variable. Data were also analyzed using presimulation assessment as covariate. This model statement contained the fixed effects of previous wolf exposure, day of evaluation, and presimulation assessment values as independent variables in addition to time and the resultant interaction for vaginal temperature analysis. The specified term used in the repeated statement for variables with repeated measures was time, the subject was cow(pen), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least squares means or covariately adjusted least square means for the covariate analysis and separated using protected LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to previous wolf exposure status if no interactions were significant or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

The main hypothesis of this experiment was that the mere presence of wolf packs near cattle herds affects temperament and stimulates physiological stress responses known to impair cattle productivity and welfare (Cooke et al., 2009, 2012; Francisco et al., 2012), particularly in herds previously subjected to wolf predation (Creel and Christianson, 2008; Boonstra, 2013). To address this hypothesis, mature beef cows were subjected to an experimental model designed to simulate a wolf encounter, which was based on wolf-urine scent, prerecorded wolf howls, and 3 domestic canines physically similar to wolves. Accordingly, wolf scent and recorded howls have been successfully used to mimic wolf presence (Moen et al., 1978; Kluever et al., 2009), given that such stimuli can elicit similar behavioral or physiological responses by prey animals compared with the actual presence of the predator (Kats and Dill, 1998; Apfelbach et al., 2005). Likewise, Kluever et al. (2009) suggested that cattle may acquire a generalized fear response to domestic dogs, perhaps due to the physical and stalking predation characteristics shared among all canids (Nowak, 1999).

It is also important to note that WLF and CON cows originated from different herds and regions of the Intermountain West and were reared in different management schemes. Hence, the impact of previous wolf exposure on the temperament and stress-related parameters evaluated herein cannot be completely distinguished from cow source. To address this concern, WLF and CON cows were commingled to receive the same management for 50 d before the beginning of the experiment and were processed weekly to familiarize all cows to personnel and handling facilities (Francisco et al., 2012). But more importantly, the temperament and physiological parameters evaluated herein are not being directly compared between CON and WLF cows. Instead, these parameters are being evaluated within each cow based on the changes between pre- and postsimulation values or using presimulation values as covariate for postsimulation assessments.

Both herds were also occasionally exposed to herding dogs and reared in areas with large populations of other canids such as coyotes and foxes (Idaho Department of Fish and Game, 2013; Oregon Department of Fish and Wildlife, 2013b). Therefore, differences in temperament and physiological responses between WLF and CON cows following the simulated wolf encounter should be mainly attributed to previous exposure to wolves and not to interactions with canids in general.

A previous wolf exposure × time interaction was detected ($P < 0.01$) for chute score and temperament score whereas a tendency ($P = 0.09$) for the same interaction was detected for exit velocity (Table 1). Chute score increased (time effect; $P = 0.01$) from pre- to postsimulation assessment in WLF cows but did not change in CON cows ($P = 0.72$), suggesting that the simulated wolf encounter increased fear-induced agitation during chute restraining only in WLF cows (Burrow, 1997). Accordingly, WLF cows had a greater ($P < 0.01$) positive change in chute score from pre- to postsimulation assessment as well as greater ($P < 0.01$) covariately adjusted chute score during the postsimulation assessment compared with CON cows (Table 1). Exit velocity decreased (time effect; $P = 0.01$) from pre- to postsimulation assessment in CON cows but did not change (time effect; $P = 0.79$) in WLF cows. Hence, CON had a greater ($P = 0.05$) negative change in exit velocity from pre- to postsimulation assessment but reduced ($P < 0.01$) covariately adjusted exit velocity during the postsimulation assessment compared with WLF cows (Table 1). Given that temperament score is based on chute score and exit velocity, this parameter also increased (time effect; $P = 0.01$) from pre- to postsimulation assessment in WLF cows but did not change in CON cows ($P = 0.75$), suggesting that the simulated wolf encounter increased excitability in WLF cows only. Accordingly, WLF cows had a greater ($P = 0.01$) positive change in temperament score from pre- to postsimulation assessment and greater ($P < 0.01$) covariately adjusted temperament score during the postsimulation assessment compared with CON cows (Table 1).

A previous wolf exposure × time interaction was detected ($P \leq 0.01$) for plasma cortisol (Table 2) as well as for vaginal temperature covariately adjusted to preassessment values (Fig. 1). Cortisol concentrations increased ($P < 0.01$) from pre- to postsimulation assessment in WLF cows but did not change ($P = 0.19$) for CON cows, indicating that the simulated wolf encounter induced a glucocorticoid stress response only in WLF cows (Sapolsky et al., 2000). Accordingly, WLF cows had a greater ($P < 0.01$) positive change in plasma cortisol from pre- to postsimulation assessments as well as greater ($P < 0.01$) covariately adjusted plasma cortisol concentrations during the postsimulation assessment compared with CON cows (Table 2).

Table 1. Temperament measurements of cows experienced with the presence of wolves (WLF; $n = 5$) or naïve to wolves (CON; $n = 5$) and subjected to a simulated wolf encounter[1,2]

Item	WLF	CON	SEM	*P*-value
Chute score, 1 to 5 scale				
Presimulation	2.27	1.85	0.11	0.01
Postsimulation	3.07	1.81	0.11	<0.01
SEM	0.11	0.11		
P-value[3]	<0.01	0.72		
Change[4]	0.78	–0.06	0.11	<0.01
Covariately adjusted[5]	2.91	1.92	0.11	<0.01
Exit velocity, m/s				
Presimulation	2.49	1.66	0.12	<0.01
Postsimulation	2.47	1.40	0.12	<0.01
SEM	0.12	0.12		
P-value[3]	0.79	0.01		
Change[4]	-0.02	–0.25	0.10	0.05
Covariately adjusted[5]	2.18	1.65	0.10	<0.01
Temperament score, 1 to 5 scale[6]				
Presimulation	2.97	2.08	0.12	<0.01
Postsimulation	3.37	2.05	0.12	<0.01
SEM	0.12	0.12		
P-value[3]	<0.01	0.75		
Change[4]	0.40	–0.04	0.10	0.01
Covariately adjusted[5]	3.06	2.34	0.09	<0.01

[1]Simulated wolf encounter consisted in olfactory (wolf urine; Harmon Wolf Urine Scent; Cass Creek, Grawn, MI), auditory [wolf howls reproduced on a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, CA)], and visual (3 adult female dogs conducted by leash, being 2 German Shepherd and 1 Border Collie × Alaskan Malamute).

[2]Measurements obtained before (presimulation) and immediately after (postsimulation assessment) the simulated wolf encounter. Cows were exposed to the simulated wolf encounter for 20 min in dry lot pens and when restrained in the squeeze chute immediately before the postsimulation assessment.

[3]Time comparison within WLF and CON cows.

[4]Calculated by subtracting presimulation values from postsimulation values.

[5]Postsimulation values covariately adjusted to presimulation values

[6]Calculated by averaging cow chute score (Cooke et al., 2012) and exit score. Exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score; 1 = slowest cows and 5 = fastest cows).

Vaginal temperature increased ($P < 0.01$) for WLF and CON cows during the simulated wolf encounter (Fig. 1). This outcome can be attributed to the handling and physical activity that cows endured during the experimental procedures (Mader et al., 2005) in addition to fear-related stress caused by the simulated wolf encounter because increased body temperature is a major component within the neuroendocrine stress response (Charmandari et al., 2005). However, WLF cows had a greater ($P = 0.03$) positive change in vaginal temperature from pre- to postsimulation assessments compared with CON cohorts (0.40 vs. 0.18°C, respectively; SEM = 0.06). Given that WLF and CON cows were handled similarly and walked the same distances during the ex-

Table 2. Plasma cortisol of cows experienced with the presence of wolves (WLF; $n = 5$) or naïve to wolves (CON) and subjected to a simulated wolf encounter[1,2]

Item	WLF	CON	SEM	*P*-value
Plasma cortisol, ng/mL				
Presimulation	17.9	13.1	1.5	0.04
Postsimulation	23.7	14.6	1.5	<0.01
SEM	1.5	1.5		
P-value[3]	<0.01	0.19		
Change[4]	5.8	1.5	0.8	<0.01
Covariately adjusted[5]	21.8	16.3	0.7	<0.01

[1]Simulated wolf encounter consisted in olfactory (wolf urine; Harmon Wolf Urine Scent; Cass Creek, Grawn, MI), auditory [wolf howls reproduced on a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, CA)], and visual (3 adult female dogs conducted by leash, being 2 German Shepherd and 1 Border Collie × Alaskan Malamute).

[2]Blood samples collected before (presimulation) and immediately after (postsimulation assessment) the simulated wolf encounter. Cows were exposed to the simulated wolf encounter for 20 min in dry lot pens and when restrained in the squeeze chute immediately before the postsimulation assessment.

[3]Time comparison within WLF and CON cows.

[4]Calculated by subtracting presimulation values from postsimulation values.

[5]Postsimulation values covariately adjusted to presimulation values.

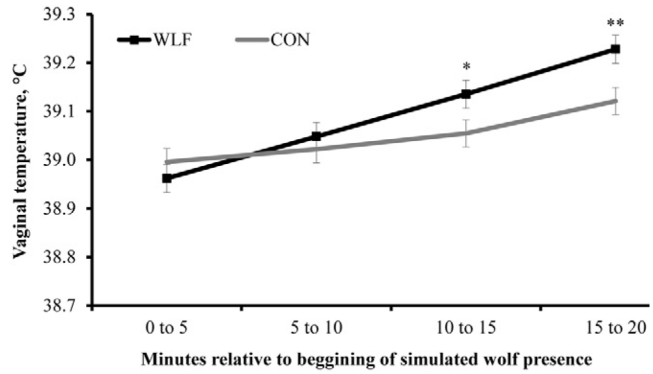

Figure 1. Vaginal temperature of cows experienced with the presence of wolves (WLF; $n = 5$) or naïve to wolves (CON; $n = 5$) and subjected to a simulated wolf encounter, which consisted in olfactory (wolf urine; Harmon Wolf Urine Scent; Cass Creek, Grawn, MI), auditory [wolf howls reproduced on a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, CA)], and visual stimuli (3 adult female dogs conducted by leash, being 2 German Shepherd and 1 Border Collie × Alaskan Malamute). Temperature was recorded by HOBO Water Temp Pro V2 data loggers (Onset Company, Bourne, MA) attached to a controlled internal drug-releasing device (Pfizer Animal Health, New York, NY) that did not contain hormones. Values recorded during the 5 min before simulated wolf encounter served as covariate ($P < 0.01$); hence, results reported are covariately adjusted least squares means. A previous wolf exposure × time interaction was detected ($P < 0.01$). Comparison within time: ** $P = 0.01$, * $P = 0.05$.

perimental procedures, this difference detected in vaginal temperature change can be attributed to a greater fear-related stress that WLF cows endured during the simulated wolf encounter.

Supporting our hypothesis, WLF cows became more excitable and had an increase in plasma cortisol and vaginal temperature following the simulated wolf encounter, suggesting that cows familiar with wolf presence and predation may endure fear-related behavioral and physiological stress responses (Charmandari et al., 2005) when in close proximity with wolves. Conversely, temperament and plasma cortisol concentrations in CON cows were not impacted by the simulated wolf encounter, and the marginal increase in vaginal temperature can be attributed to the handling and physical activity associated with the experimental procedures (Mader et al., 2005). Therefore, wolf presence may not be perceived as a stressor in cows still unfamiliar with predation and interaction with this predator. To our knowledge, no other research has evaluated temperament and physiological stress parameters in beef cows previously exposed or not to wolves and subjected to a simulated or actual wolf encounter. Hence, results described herein are novel and cannot be properly compared with the limited existing literature within this subject. Nevertheless, Boonstra (2013) described that fear of predation and its behavioral and physiological consequences are based on the anticipatory memory of the attack. Consequently, cows that have not yet been preyed upon by wolves may not experience a fear-related stress response when interacting with wolves for the first time due to the lack of adverse memories from previous predation episodes. In contrast, the behavioral and physiological stress responses detected herein in WLF cows are known to impair performance, reproductive, and health parameters in cattle (Cooke et al., 2009, 2012; Francisco et al., 2012). These results support the assumption that the impacts of wolf presence and predation on beef cattle systems are not limited to cattle death and injuries but may also extend to overall productivity and welfare of the herd (Lehmkuhler et al., 2007). Consequently, more research is warranted to directly evaluate the productive and economic consequences that wolves bring to beef cattle operations, including studies with authentic wolf packs, cattle from the same management and genetic background, and assessment of cattle performance, reproductive, and health parameters.

In conclusion, the simulated wolf encounter used herein increased excitability and fear-related physiological stress responses in cows previously exposed to wolves but not in cows unfamiliar with this predator. Therefore, the presence of wolf packs near cattle herds may negatively impact beef production systems via predatory activities and subsequent death and injury of animals, as well as by inducing stress responses that may impair cattle productivity and welfare when packs are in close proximity to previously preyed herds.

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