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Blood collection has negligible impact on scoring temperament in Angus-based weaned calves

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ABSTRACT

Temperament scoring typically occurs at the same time as vaccinations, germplasm collection, or both. Little is known about the impact of these procedures with scoring temperament. The objective was to determine the impact of blood collection (jugular venipuncture), representing a momentarily painful procedure, on different measures of temperament in weaning age calves. The hypothesis is blood collection will have little to no impact on temperament assessment. Calves of weaning age ($n = = 420$) were sired by Angus bulls and out of Angus-influenced commercial cows. Subjective methods included temperament score (1 to 5 scale, with 3 removed; 1 calm or docile and 5 wild or aggressive) and qualitative behavior assessment attributes of active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied, and relaxed (each scored on a 136 mm horizontal line with left equal to no expression and right equal to full expression). Objective scoring was captured by recording multiple weights on a four-platform standing scale with the standard deviation of weight over a specific time interval and its coefficient of variation as measures of temperament. During collection, calves were randomly assigned into before (scored before blood draw) and after (scored after blood draw) groups in sets of 5 as they came through the working facility. Traits were modeled using fixed effects of evaluation day ($n = = 2$), blood collection group (BCG; $n = = 2$), interaction of day and BCG ($n = = 4$), sex of calf ($n = = 2$), and evaluator ($n = = 4$ per method; subjective methods only) using an animal model based on pedigree and accounting for repeated measures using permanent environment variance method. The use of sequence of evaluation nested within day (SEQ) or age of calf (in days) were also assessed as covariates. Age at scoring was not impactful for modeling temperament traits and was not included in further analyses. Inclusion of the SEQ covariate was significant for 11 of 16 scoring methods (68.75%). Its inclusion resulted in lower estimates of additive genetic variation for 10 of 16 traits, suggesting it may be a temperament trait confounded with those analyzed and was excluded from the final model. The BCG main effect and its interaction with evaluation day were not significant for any temperament scoring method ($P > 0.05$). For this reason, it is reasonable to collect germplasm, give vaccines or both and score temperament in the same processing time frame to reduce handling needs.

1. Introduction

Cattle temperament, typically defined as the reaction of an animal to human handling (Burrow and Dillon, 1997), is known to have influences on many production aspects such as reproduction, immunity, and carcass characteristics (e.g., King et al., 2006; Burdick et al., 2011). Therefore, selecting based on temperament may be ideal to increase productivity and efficiency in the production system. Temperament is comprised of different behavioral characteristics such as shyness-boldness, exploration avoidance, activity, sociability, and aggressiveness (Réale et al., 2007), which makes it a challenging trait to appropriately record. Due to this, selection for temperament can be greatly influenced by the evaluation method used and the aspects of behavior that method is selecting on. Recommendations for scoring cattle temperament are typically during weaning age or as yearlings (Beef Improvement Federation, 2018), which also coincides with many other production

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needs such as vaccinations and germplasm collection. Vaccinations and germplasm collection (such as blood, tissue, or hair) represent momentarily stressful and painful situations that calves are exposed to. Published literature on how vaccination, germplasm collection, or a combination of those influenced temperament scoring is non-existent. Some literature suggests a relationship exists. For example, Core et al. (2009) observed lower correlations (10% or more difference) of temperament scores with eye white percentages in calves that were blood sampled and scored at the same time relative to those that were not. The authors suggested this change in behavioral reactions was due to adding blood sampling to the working environment, but this was not conclusively proven. To date, no other literature has been found that investigates this relationship. Therefore, the objective of this study was to determine the impact of blood collection on scoring temperament for different objective and subjective methods in weaning age calves. The hypothesis is that blood collection will have little to no impact on temperament assessment, regardless of scoring method used. As temperament is a complex trait to capture, literature is inconsistent with specific model parameters that need to be included (e.g., age at scoring and sequence at evaluation: König et al., 2006; Hoppe et al., 2010; Riley et al., 2014). Therefore, a secondary objective is to assess age at scoring (fixed effect or covariate) and sequence of evaluation (covariate) to determine appropriateness as model parameters for temperament traits. Results from this study are being applied to a longer-term project related to understanding evaluator influence on predictions of genomic merit in cattle temperament.

2. Materials and methods

2.1. Animals

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Dakota State University. Data used in this study were collected on October 6 and 7, 2014 at the North Dakota State University Central Grasslands Research Extension Center (CGREC) near Streeter, North Dakota. Calves scored ($n = = 420$) consisted of steers ($n = = 213$) and heifers ($n = = 207$) that were of weaning age (average age was 156.1 ± 15.8 d or 5.2 ± 0.5 mo). Calves were sired by Angus bulls and out of primarily Angus-influenced commercial cows (based on known pedigree). Pedigree was constructed based on known data that included dams and sires of calves, the dam's parentage (if known), and complete ancestry from the American Angus Association for registered bulls.

2.2. Collection. procedure

Calves scored for temperament in this project were also involved in other projects at the same time, therefore it was expected that calves encountered CGREC workers on a regular basis (1 to 2 days a week leading up to weaning). It was known that calves were taken through the chute facilities one to two times prior to temperament evaluation, but handling was minimal and in shorter duration than this data collection. Electrical shocks were not used to move cattle in previous work days and not allowed for this collection procedure. Individuals handling cattle through the facilities on evaluation days were instructed to maintain a normal flow and avoid undue noises or handling unless necessary.

On evaluation day, calves were moved through the working pens to the evaluation areas before being sorted for management purposes (Fig. 1). Four evaluators were randomly assigned 2 of 3 subjective scoring methods (6 total evaluators present) prior to evaluation. Scoring sheets for these subjective methods were provided to evaluators at least one week prior to evaluation days. This approach was intended to reduce bias and evaluator stress. Upon entering the working pens, calves first entered the silencer chute (Moly Manufacturing, Lorraine, KS), had their head caught, and were evaluated for docility score (Beef Improvement Federation, 2018), where each calf was evaluated for less than a minute. Docility score (DS) was a 1 to 6 scale, where 1 is calm and 6 is wild or aggressive. Following scoring, the body of the calf was squeezed and weaning weight was recorded. The calf was then moved to the four-platform standing scale (FPSS; Pacific Industrial Scale, British Columbia, Canada), where data was collected on each quadrant. The scale had a rubber mat placed on top (approximately 1.22 m wide by 2.44 m long) to improve traction and comfort for the animal. A worker controlled the computer and software connected to the FPSS. This worker was responsible for entering tag numbers as well as starting and stopping the software recording of weight. The calf remained on the FPSS for at least 45 s, where weight on the four platforms was recorded, on average, 15 times per second. The calf was not released from the FPSS until the outside testing area (subsequent in the evaluation process) was clear from the previous calf. Following the FPSS, the calf was released to a working pen (outside testing area; Fig. 1) for temperament score (TS) and qualitative behavior assessment (QBA) evaluation (Sant'Anna and Paranhos da Costa, 2013), where evaluators observed the calf's behavior and interaction with a human handler present from the observation area. Temperament score was a 1 to 5 scale with the intermediate value (3) removed, where 1 is calm and 5 is wild or aggressive following Sant'Anna and Paranhos da Costa (2013). This method is a similar scoring as the pen score described by Hammond et al. (1996), but the middle value is removed to encourage the evaluator to assess and choose the side of the scale the calf truly falls on rather than a middle value. Each assigned evaluator scored each calf for 12 QBA attributes including active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied, and relaxed (Sant'Anna and Paranhos da Costa, 2013). Each attribute was scored by placing a mark on a 136 mm line indicating the degree of expression, where far left indicated no expression (e.g., completely inactive) and far right indicated full expression (e.g., maximal activity). A worker was present in this outside testing area to calmly interact with the calf so that evaluators could assess the different aspects of these subjective scoring methods. Once all evaluators were finished scoring TS and QBA (less than 3 min per calf), the calf was sorted into a holding pen based on management needs. The working environment was controlled to reduce the influences of human interaction outside of the specific areas of handling for evaluation. Sequence of scoring was recorded on all calves within a day.

Calves entered the working facility in random groups for that day, where day 1 included calves from younger dams and day 2 included calves from older dams. Calves were assigned into one of two groups (BEFORE or AFTER) each day as they came through collection procedures. The first five calves each day were assigned into the BEFORE group, which meant calves were scored for all temperament methods before blood was collected at the silencer chute. This also meant calves in this group were sorted into pen A (Fig. 1) after being evaluated and remained in that pen until all calves had been processed for the day. These calves were then brought back into the facility for blood collection. Following the first five calves, the second five calves were assigned to the AFTER group, which meant calves were scored for DS, weighed, and bled before being scored for other temperament scoring methods. Calves in the AFTER group were sorted into pen B and not run through the facility again. This was repeated each day for every set of five calves until all calves had been worked. In all cases, blood was collected for each calf using jugular venipuncture into two 10 mL EDTA vacutainer tubes for DNA to be used in other objectives of a long-term aspect of this project. Due to handling procedures, DS was always scored prior to blood collection. As DS does not represent the same type of temperament evaluation method as the others (TS, QBA, and FPSS), it was not included in any analysis for this study.

2.3. Data summary and statistical analysis

The FPSS is a novel objective approach to quantifying temperament

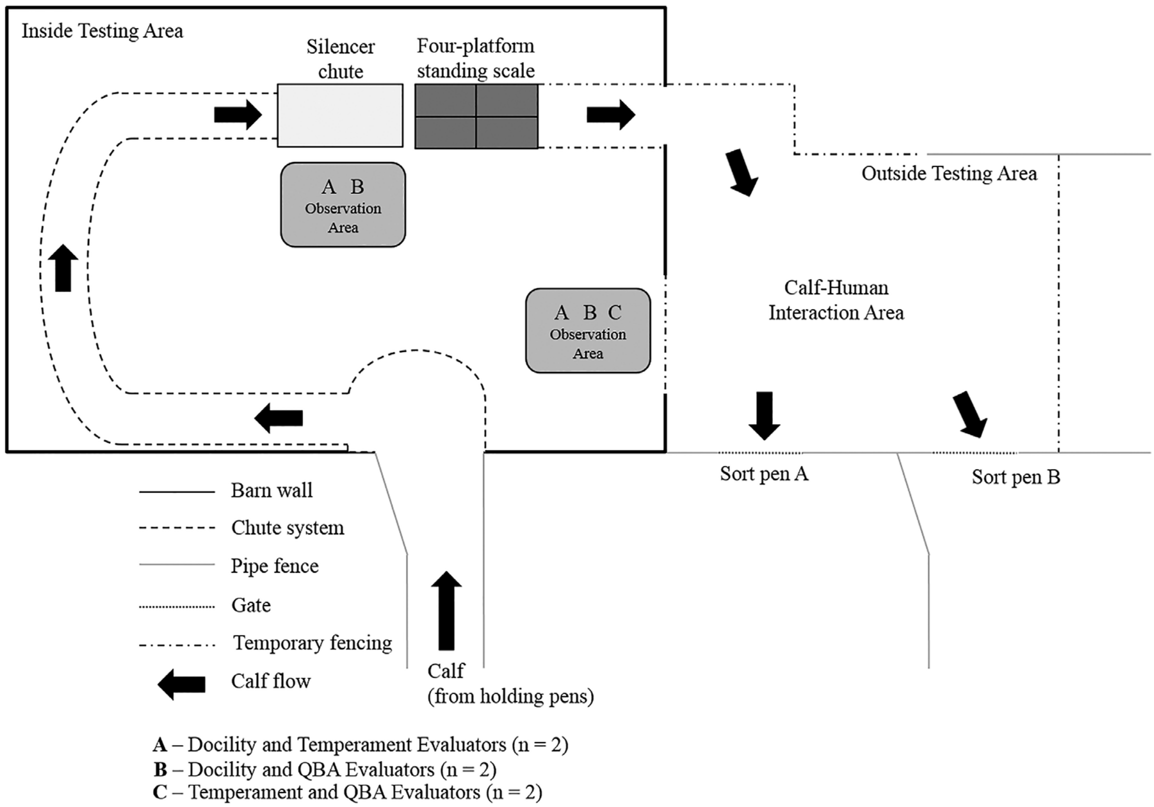

Fig. 1. Illustration of calf processing procedure for scoring of docility score (silencer chute), four-platform standing scale, temperament score (outside testing area) and qualitative behavior assessment (outside testing area) at the North Dakota State University Central Grasslands Research Extension Center. Blood was also collected in the silencer chute following docility scoring.

Table 1
Fixed effect *P*-values for temperament scoring methods when including or excluding age at scoring.[1].

Method[2]	μ	Day	BCG	Day x BCG	Sex	Age
Active	0.619,**	0.373, 0.205, 0.070	0.192, 0.183, 0.209	0.541, 0.540, 0.514	0.056, 0.055, 0.037	**0.025**, 0.261
Agitated	0.446,**	0.161, 0.098, 0.096	0.090, 0.099, 0.095	0.333, 0.346, 0.345	0.064, 0.051, 0.050	0.415, 0.987
Apathetic	***	0.016, 0.022, 0.059	0.792, 0.823, 0.776	0.922, 0.904, 0.855	0.160, 0.170, 0.144	0.096, 0.360
Attentive	***	***	0.155, 0.156, 0.151	0.265, 0.276, 0.292	0.005, 0.005, 0.004	0.224, 0.781
Calm	***	0.605, 0.501, 0.419	0.473, 0.466, 0.492	0.074, 0.076, 0.077	0.040, 0.039, 0.029	0.242, 0.748
Curious	***	***	0.452, 0.428, 0.467	0.389, 0.371, 0.340	0.368, 0.383, 0.333	**0.032**, 0.261
Distressed	***	***	0.594, 0.648, 0.578	0.167, 0.173, 0.163	0.005, 0.004, 0.007	0.441, 0.429
Fearful	0.196,**	0.008, 0.003, 0.005	0.338, 0.388, 0.343	0.622, 0.604, 0.615	0.011, 0.007, 0.010	0.924, 0.507
Happy	***	***	0.464, 0.463, 0.470	0.416, 0.398, 0.389	0.370, 0.364, 0.353	0.356, 0.951
Irritated	0.012,**	***	0.275, 0.288, 0.280	0.802, 0.800, 0.808	0.029, 0.024, 0.025	0.666, 0.903
Pos. Occ.	***	***	0.926, 0.892, 0.943	0.424, 0.422, 0.369	0.988, 0.965, 0.959	0.054, 0.197
Relaxed	***	0.054, 0.037, 0.021	0.237, 0.226, 0.252	0.123, 0.125, 0.128	0.187, 0.188, 0.145	0.188, 0.590
TI	0.400,**	***	0.797, 0.821, 0.817	0.306, 0.326, 0.304	0.157, 0.141, 0.136	0.537, 0.872
TS	0.755,**	0.075, 0.116, 0.183	0.166, 0.159, 0.179	0.167, 0.174, 0.169	0.085, 0.089, 0.060	0.070, 0.254
SSD	0.747,**	0.016, 0.017, 0.043	0.049, 0.036, 0.053	0.685, 0.662, 0.693	**, 0.002	0.070, 0.079
CVSSD	0.009,**	0.135, 0.073, 0.077	0.057, 0.047, 0.055	0.730, 0.684, 0.728	0.025, 0.019, 0.019	0.398, 0.249
N (out of 16)	10, 16, 16	10, 11, 10	1, 2, 0	0	7, 7, 8	2, 0

[1] Effect *P*-values are reported as 1) including age (d) as a fixed covariate, 2) including age as a fixed effect (n = 3), and 3) not including age. Evaluator was included in all models and was always significant (P < 0.001) except for Temperament Index (TI), regardless of how age was modeled. Values that are the same are only reported once. Values reported as *** indicate *P*-value < 0.001 for all three models or ** for two models consecutively. Model effects were used in ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), day of evaluation (Day; n = 2), blood collection group (BCG; n = 2), interaction of Day by BCG (n = 4), sex of calf (n = 2), evaluator (n = 4; only for subjective traits), with or without the age effect. This included fitting pedigree using calf as a random effect and repeated measures of subjective traits were accounted for using a permanent environmental effect.

[2] Subjective methods included qualitative behavior assessment (QBA) attributes of active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied (Pos. Occ.), and relaxed; Temperament Index (TI), the first principal component when using the 12 QBA attributes; and temperament score (TS). Objective methods included the standard deviation of the total weight recorded on 586 records from the four-platform standing scale (SSD) and the SSD divided by the average total weight on the same 586 records (CVSSD). The number of temperament scoring methods (N) that the effect is significant for are reported based on order specified previously.

Table 2
Genetic parameter estimates for temperament scoring methods when evaluating age at scoring in the statistical model.[1].

Method[2]	$\hat{\sigma}_P^2$	$\hat{\sigma}_a^2$	$\hat{\sigma}_{pe}^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$	$\hat{R}$
Active	1041.00 ± 36.08	15.66 ± 14.33	0.00 ± 0.00	1025.30 ± 37.35	0.02 ± 0.01	0.02 ± 0.01
	1043.20 ± 36.18	15.25 ± 14.71		1027.97 ± 37.56	0.02 ± 0.01	0.02 ± 0.01
	1043.30 ± 36.13	13.11 ± 14.42		1030.19 ± 37.63	0.01 ± 0.01	0.01 ± 0.01
Agitated	370.18 ± 15.88	49.15 ± 33.08	58.89 ± 27.78	262.15 ± 10.49	0.13 ± 0.09	0.29 ± 0.03
	370.05 ± 15.75	45.29 ± 32.08	62.61 ± 27.25		0.12 ± 0.08	
	368.94 ± 15.53	43.65 ± 30.72	63.14 ± 26.31		0.12 ± 0.08	
Apathetic	1612.59 ± 55.78	0.00 ± 0.00	0.00 ± 0.00	1612.59 ± 55.78	0.00 ± 0.00	0.00 ± 0.00
	1614.26 ± 55.86			1614.26 ± 55.86		
	1614.30 ± 55.84			1614.30 ± 55.84		
Attentive	643.81 ± 22.31	0.00 ± 0.00	13.09 ± 13.46	630.73 ± 25.18	0.00 ± 0.00	0.02 ± 0.02
	644.64 ± 22.35		13.94 ± 13.52	630.70 ± 25.18		
	644.02 ± 22.31		13.31 ± 13.46	630.70 ± 25.18		
Calm	852.39 ± 36.70	150.59 ± 86.87	33.47 ± 69.25	668.33 ± 26.66	0.18 ± 0.10	0.22 ± 0.03
	854.70 ± 37.07	154.78 ± 88.98	31.59 ± 70.80			
	852.82 ± 36.73	151.20 ± 87.59	33.30 ± 69.89			
Curious	874.86 ± 30.28	0.00 ± 0.00	0.00 ± 0.00	874.86 ± 30.27	0.00 ± 0.00	0.00 ± 0.00
	876.38 ± 30.34	0.00 ± 0.00		876.38 ± 30.34	0.00 ± 0.00	0.00 ± 0.00
	876.83 ± 30.34	1.49 ± 8.50		875.34 ± 31.27	0.002 ± 0.010	0.002 ± 0.010
Distressed	247.36 ± 10.03	21.40 ± 16.47	48.38 ± 14.94	177.59 ± 7.08	0.09 ± 0.07	0.28 ± 0.03
	246.95 ± 9.93	19.15 ± 15.48	50.21 ± 14.36		0.08 ± 0.06	
	247.58 ± 10.02	23.15 ± 16.23	46.84 ± 14.70		0.09 ± 0.06	
Fearful	238.96 ± 9.30	17.31 ± 14.24	37.97 ± 13.12	183.67 ± 7.33	0.07 ± 0.06	0.23 ± 0.03
	238.44 ± 9.22	14.84 ± 13.59	39.92 ± 12.81		0.06 ± 0.06	
	238.43 ± 9.18	15.64 ± 13.16	39.11 ± 12.47		0.07 ± 0.05	
Happy	1245.30 ± 43.07	0.00 ± 0.00	0.00 ± 0.00	1245.25 ± 43.07	0.00 ± 0.00	0.00 ± 0.00
	1246.60 ± 43.13			1246.56 ± 43.12		
	1245.20 ± 43.05			1245.15 ± 43.06		
Irritated	371.50 ± 16.62	61.10 ± 38.97	48.93 ± 31.73	261.48 ± 10.43	0.17 ± 0.10	0.30 ± 0.03
	372.21 ± 16.76	62.63 ± 39.87	48.10 ± 32.40		0.17 ± 0.10	
	370.90 ± 16.44	59.71 ± 38.02	49.71 ± 31.10		0.16 ± 0.01	
Pos. Occ.	705.15 ± 24.43	0.00 ± 0.00	0.00 ± 0.00	705.15 ± 24.43	0.00 ± 0.00	0.00 ± 0.00
	705.76 ± 24.45			705.76 ± 24.45		
	706.29 ± 24.46			706.29 ± 24.46		
Relaxed	879.16 ± 36.27	133.45 ± 78.77	41.03 ± 63.78	704.68 ± 28.10	0.15 ± 0.09	0.20 ± 0.030
	881.34 ± 36.60	137.47 ± 80.76	39.18 ± 65.22		0.16 ± 0.09	
	879.71 ± 36.31	133.27 ± 79.62	41.76 ± 64.55		0.15 ± 0.09	
TI	5.06 ± 0.18	0.03 ± 0.07	0.000 ± 0.000	5.03 ± 0.19	0.01 ± 0.01	0.01 ± 0.01
		0.03 ± 0.07		5.04 ± 0.19	0.01 ± 0.02	0.01 ± 0.02
		0.01 ± 0.07		5.05 ± 0.19	0.00 ± 0.01	0.00 ± 0.01
TS	0.78 ± 0.04	0.14 ± 0.11	0.28 ± 0.09	0.35 ± 0.01	0.18 ± 0.13	0.55 ± 0.03
		0.16 ± 0.12	0.27 ± 0.09		0.21 ± 0.14	
		0.17 ± 0.12	0.26 ± 0.09		0.21 ± 0.14	
SSD	583.42 ± 46.54	178.51 ± 109.86	–	404.91 ± 91.40	0.31 ± 0.18	–
	586.63 ± 47.67	199.07 ± 117.06		387.56 ± 95.93	0.34 ± 0.18	
	589.10 ± 47.31	190.88 ± 114.44		398.22 ± 94.59	0.32 ± 0.18	
CVSSD	0.0039 ± 0.0003	0.0011 ± 0.0007	–	0.0029 ± 0.0006	0.279 ± 0.167	–
				0.0028 ± 0.0006	0.294 ± 0.170	
				0.0029 ± 0.0006	0.280 ± 0.162	

[1] Variance components and genetic parameter estimates are reported as 1) including age (d) as a fixed covariate, 2) including age as a fixed effect (n = 3), and 3) not including age. Estimates that were the same are only reported once per trait per parameter. Decimals are reported as relevant for that trait per parameter. Parameters included variances of phenotype ($\hat{\sigma}_P^2$), additive genetic ($\hat{\sigma}_a^2$), permanent environment ($\hat{\sigma}_{pe}^2$), and residual ($\hat{\sigma}_P^2$), as well as ratio of $\hat{\sigma}_a^2$ and $\hat{\sigma}_a^2 + \hat{\sigma}_{pe}^2$ to $\hat{\sigma}_P^2$ (heritability, $\hat{h}^2$, and repeatability, $\hat{R}$, respectively). Model effects were used in ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), day of evaluation (Day; n = 2), blood collection group (BCG; n = 2), interaction of Day by BCG (n = 4), sex of calf (n = 2), evaluator (n = 4; only for subjective traits), with or without the age effect. This included fitting pedigree using calf as a random effect and repeated measures of subjective traits were accounted for using a permanent environmental effect.

[2] Subjective methods included qualitative behavior assessment (QBA) attributes of active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied (Pos. Occ.), and relaxed; Temperament Index (TI), the first principal component when using the 12 QBA attributes; and temperament score (TS). Objective methods included the standard deviation of the total weight recorded on 586 records from the four-platform standing scale (SSD) and the SSD divided by the average total weight on the same 586 records (CVSSD). The number of temperament scoring methods (N) that the effect is significant for are reported based on order specified previously.

in cattle. Additional methods of utilizing the data it produced are still being investigated. The start point for these records were identified as described in Yu (2016) to ensure consistent and accurate starting points across calves, which entailed finding a weight record within a reasonable value to the weight captured on the silencer chute and balanced across the four platforms. The number of records used to calculate measures of temperament were determined by the minimum number of records available across all animals with valid scale data (n = = 586 records per calf for this study). Measures of temperament from the FPSS included the standard deviation of the total weight (SSD) over 586 records (for each calf) as well as the SSD divided by the average total weight over those same 586 records (i.e., coefficient of variation, CVSSD).

Following Sant'Anna and Paranhos da Costa (2013) and recommendations by Jolliffe (2014), the QBA attributes for each evaluator were analyzed in a principal component analysis using the

Table 3
Fixed effect *P*-values for temperament scoring methods when evaluating sequence effect in the model.[1].

Method[2]	Day	BCG	Day x BCG	Sex	Sequence (Day)
Active	0.069, 0.070	0.202, 0.209	0.520, 0.514	0.048, 0.037	0.110
Agitated	0.082, 0.096	0.068, 0.095	0.348, 0.345	0.106, 0.050	**
Apathetic	0.059	0.761, 0.776	0.857, 0.855	0.196, 0.144	0.161
Attentive	**	0.163, 0.151	0.280, 0.292	0.020, 0.004	**
Calm	0.419	0.493, 0.492	0.077	0.030, 0.029	0.964
Curious	**	0.512, 0.467	0.333, 0.340	0.190, 0.333	**
Distressed	**	0.519, 0.578	0.164, 0.163	0.015, 0.007	**
Fearful	0.004, 0.005	0.305, 0.343	0.570, 0.615	0.021, 0.010	**
Happy	**	0.511, 0.470	0.387, 0.389	0.196, 0.353	**
Irritated	**	0.218, 0.280	0.839, 0.808	0.060, 0.025	**
Pos. Occ.	**	0.955, 0.943	0.373, 0.369	0.486, 0.959	**
Relaxed	0.021	0.251, 0.252	0.129, 0.128	0.152, 0.145	0.773
TI	**	0.739, 0.817	0.310, 0.304	0.377, 0.136	**
TS	0.181, 0.183	0.171, 0.179	0.173, 0.169	0.079, 0.060	0.054
SSD	0.042, 0.043	0.050, 0.053	0.710, 0.693	**, 0.002	0.044
CVSSD	0.074, 0.077	0.050, 0.055	0.749, 0.728	0.011, 0.019	0.007
N (%)	10 (62.50)	0 (0.00)	0 (0.00)	7 (43.75) 8 (50.00)	11 (68.75)

[1] Effect *P*-values are reported as 1) including sequence nested within day effect (SEQ) and 2) excluding SEQ as a the fixed covariate. The mean (μ) and evaluator effects were included in all models and was always significant ($P < 0.001$). The only exception was for evaluator effect when modeling Temperament Index (TI), which was not significant ($P = 0.991$) regardless if SEQ was modeled. Values that are the same are only reported once. Values reported as ** indicate *P*-value < 0.001. Model effects were used in ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), day of evaluation (Day; $n = 2$), blood collection group (BCG; $n = 2$), interaction of Day by BCG ($n = 4$), sex of calf ($n = 2$), evaluator ($n = 4$; only for subjective traits). This included fitting pedigree using calf as a random effect and repeated measures of subjective traits were accounted for using a permanent environmental effect.

[2] Subjective methods included qualitative behavior assessment (QBA) attributes of active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied (Pos. Occ.), and relaxed; Temperament Index (TI), the first principal component when using the 12 QBA attributes; and temperament score (TS). Objective methods included the standard deviation of the total weight recorded on 586 records from the four-platform standing scale (SSD) and the SSD divided by the average total weight on the same 586 records (CVSSD). The number and percent of temperament scoring methods that the effect is significant for is reported in N (%) row.

PRINCOMP procedure of SAS (SAS Institute, Inc., Cary, NC). The first principal component for each animal was outputted and used as the Temperament Index (TI). The TI was used as an additional temperament score to understand the impact of blood collection on temperament evaluation.

Statistical analyses were conducted in ASReml 4.2 (Gilmour et al., 2015) to allow for an animal model based on the current pedigree, appropriate distribution of the data, and model effects. Model effects included fixed effects of evaluation day ($n = = 2$), blood collection group ($n = = 2$), interaction of day and blood collection group ($n = = 4$), sex of calf ($n = = 2$), and evaluator ($n = = 4$) as well as random effects of calf with (additive genetic) and without (permanent environment) pedigree to account for repeated measures by calf. Repeated measures for each calf occurred due to 4 evaluators per method scoring the same calf. As each evaluator could not stand in the same location or at the exact same time for observing that calf on a given method, it is reasonable to assume that the environment changed based on evaluator perspective due to location. Modeling this way also allows repeatability across evaluators for a given calf to be assessed.

In all cases, the effect of sequence nested within evaluation day (SEQ) and age of calf (AGE) at scoring were investigated for fit in the model. The SEQ effect was always modeled as a fixed covariate, whereas AGE was considered as a fixed effect ($n = = 3$ levels of young, middle, or old based on calf ages) or fixed covariate (actual day of age at scoring). Variance components were estimated to observe the impact of these effects on modeling additive genetic, permanent environment, residual, and phenotype as well as heritability and repeatability. For this study, results from all models (inclusion and exclusion of SEQ and AGE effects) were generated to ensure comparison of blood collection group effect could be adequately made. Statistical significance was set at 0.05 for all models.

3. Results and discussion

3.1. Phenotypic summary

Summary statistics for each temperament scoring method by evaluation day, blood collection group, and their interaction are presented in Supplementary Table S1. Numerical differences existed between the means of blood collection groups that align with what may theoretically be expected (Suppl. Table S1). For example, calves had numerically higher scores for *active* in the AFTER group than the BEFORE group across evaluators, meaning that once the calves were bled they were more mobile (i.e., had higher amount of movement). Even so, standard deviations were relatively large and effect sizes were small, suggesting true differences would not exist once adjusted for environmental and genetic effects. This was further supported when investigating summary statistics of blood collection by evaluation day interaction.

3.2. Modeling age at scoring

Age of calf at scoring (AGE) can be considered a necessary model effect, especially if groups of calves differ markedly in age (Blanco et al., 2009; Burrow et al., 1988; Hoppe et al., 2010). In some cases, groups are similar in age at evaluation (i.e., the same cohort), but AGE is still fitted (Hoppe et al., 2010; Riley et al., 2014). It is unclear if this is necessary or dependent on the trait being evaluated. Calves in this study ranged from 118 d to 190 d for AGE, where models were assessed for inclusion of AGE as a fixed covariate, fixed effect, or exclusion. The fixed effect was created by dividing the age range into three groups of younger age (142 d or less, $n = = 78$), middle age (between 143 d and 167 d, $n = = 208$), and older age (168 d or greater, $n = = 134$) calves.

All models, including TS, assumed a normal distribution. A multinomial distribution was investigated for TS given its categorical nature, however, the Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) showed the multinomial distribution was a worse fit compared to fitting as a normal distribution (data not shown).

Table 4
Genetic parameter estimates for temperament scoring methods when evaluating sequence effect in the statistical model.[1].

Method[2]	$\hat{\sigma}_P^2$	$\hat{\sigma}_a^2$	$\hat{\sigma}_{pe}^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$	$\hat{R}$
Active	1042.20 ± 36.10	11.98 ± 14.20	0.00 ± 0.00	1030.18 ± 37.58	0.01 ± 0.01	0.01 ± 0.01
	1043.30 ± 36.13	13.11 ± 14.42		1030.19 ± 37.63		
Agitated	352.43 ± 14.43	37.63 ± 27.87	52.66 ± 24.00	262.13 ± 10.49	0.11 ± 0.08	0.26 ± 0.03
	368.94 ± 15.53	43.65 ± 30.72	63.14 ± 26.31	262.15 ± 10.49	0.12 ± 0.08	0.29 ± 0.03
Apathetic	1613.40 ± 55.82	0.00 ± 0.00	0.00 ± 0.00	1613.40 ± 55.82	0.00 ± 0.00	0.00 ± 0.00
	1614.30 ± 55.83			1614.30 ± 55.83		
Attentive	613.92 ± 21.26	0.00 ± 0.00	0.00 ± 0.00	613.92 ± 21.26	0.00 ± 0.00	0.00 ± 0.00
	644.02 ± 22.31		13.31 ± 13.46	630.70 ± 25.18		0.02 ± 0.02
Calm	853.54 ± 36.78	150.98 ± 87.64	34.24 ± 69.96	668.33 ± 26.66	0.18 ± 0.10	0.22 ± 0.03
	852.82 ± 36.73	151.20 ± 87.59	33.30 ± 69.89			
Curious	866.36 ± 29.98	0.00 ± 0.00	0.00 ± 0.00	866.36 ± 29.98	0.00 ± 0.00	0.00 ± 0.00
	876.83 ± 30.34	1.49 ± 8.50		875.34 ± 31.27	0.002 ± 0.010	0.002 ± 0.010
Distressed	237.53 ± 9.31	18.30 ± 13.63	41.65 ± 12.76	177.58 ± 7.08	0.08 ± 0.06	0.25 ± 0.03
	247.58 ± 10.02	23.15 ± 16.23	46.84 ± 14.70		0.09 ± 0.06	0.28 ± 0.03
Fearful	231.79 ± 8.76	12.51 ± 11.52	35.62 ± 11.27	183.67 ± 7.33	0.05 ± 0.05	0.21 ± 0.03
	238.43 ± 9.18	15.64 ± 13.16	39.11 ± 12.47		0.07 ± 0.05	0.23 ± 0.03
Happy	1228.2 ± 42.48	0.00 ± 0.00	0.00 ± 0.00	1228.24 ± 42.48	0.00 ± 0.00	0.00 ± 0.00
	1245.2 ± 43.05			1245.15 ± 43.05		
Irritated	354.93 ± 15.85	60.21 ± 39.21	33.27 ± 31.60	261.44 ± 10.43	0.17 ± 0.11	0.26 ± 0.03
	370.90 ± 16.44	59.71 ± 38.02	49.71 ± 31.10	261.48 ± 10.43	0.16 ± 0.10	0.30 ± 0.03
Pos. Occ.	674.47 ± 23.36	0.00 ± 0.00	0.00 ± 0.00	674.47 ± 23.36	0.00 ± 0.00	0.00 ± 0.00
	706.29 ± 24.46			706.29 ± 24.46		
Relaxed	880.29 ± 36.34	132.61 ± 79.61	43.00 ± 64.59	704.68 ± 28.10	0.15 ± 0.09	0.20 ± 0.03
	879.71 ± 36.31	133.27 ± 79.62	41.76 ± 64.55			
TI	4.79 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	4.79 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
	5.06 ± 0.18	0.008 ± 0.066		5.05 ± 0.19	0.002 ± 0.013	0.002 ± 0.013
TS	0.78 ± 0.04	0.16 ± 0.11	0.27 ± 0.09	0.35 ± 0.01	0.21 ± 0.14	0.55 ± 0.03
		0.17 ± 0.12	0.26 ± 0.09			
SSD	585.13 ± 47.09	191.60 ± 114.08	–	393.53 ± 94.15	0.33 ± 0.18	–
	589.10 ± 47.31	190.88 ± 114.44		398.22 ± 94.59	0.32 ± 0.18	
CVSSD	0.0039 ± 0.0031	0.0011 ± 0.0007	–	0.0028 ± 0.0006	0.285 ± 0.164	–
	0.0040 ± 0.0031			0.0029 ± 0.0006	0.280 ± 0.162	

[1] Variance components and genetic parameter estimates are reported as 1) including sequence nested within day effect (SEQ) and 2) excluding SEQ as the fixed covariate. Estimates that were the same are only reported once per trait per parameter. Decimals are reported as relevant for that trait per parameter. Parameters included variances of phenotype ($\hat{\sigma}_P^2$), additive genetic ($\hat{\sigma}_a^2$), permanent environment ($\hat{\sigma}_{pe}^2$), and residual ($\hat{\sigma}_P^2$), as well as ratio of $\hat{\sigma}_a^2$ and $\hat{\sigma}_a^2 + \hat{\sigma}_{pe}^2$ to $\hat{\sigma}_P^2$ (heritability, $\hat{h}^2$, and repeatability, $\hat{R}$, respectively). Model effects were used in ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), day of evaluation (Day; $n = 2$), blood collection group (BCG; $n = 2$), interaction of Day by BCG ($n = 4$), sex of calf ($n = 2$), evaluator ($n = 4$; only for subjective traits), with or without the SEQ effect. This included fitting pedigree using calf as a random effect and repeated measures of subjective traits were accounted for using a permanent environmental effect.

[2] Subjective methods included qualitative behavior assessment (QBA) attributes of active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied (Pos. Occ.), and relaxed; Temperament Index (TI), the first principal component when using the 12 QBA attributes; and temperament score (TS). Objective methods included the standard deviation of the total weight recorded on 586 records from the four-platform standing scale (SSD) and the SSD divided by the average total weight on the same 586 records (CVSSD). The number of temperament scoring methods (N) that the effect is significant for are reported based on order specified previously.

Significance of model effects across traits are provided in Table 1 and random effect estimates are reported in Table 2. The only two models that AGE covariate was significant for included *active* and *curious* QBA (Table 1). In the case of *active*, inclusion of AGE covariate increased the additive genetic variance estimate compared to not including it, but only minimally (Table 2). The QBA *curious*, however, was opposite, where estimating additive genetic variance when including the AGE covariate was not possible compared to excluding the covariate (Table 2). Including AGE as a fixed effect showed that curiosity levels numerically decreased as calves got older (e.g., 52.98 ± 1.74 for younger calves, 51.18 ± 1.03 for middle age calves, and 49.30 ± 1.34 for older calves, relative to maximum of 136 mm level of expression). This indicates that AGE and curiosity levels may have genetic relationship and including it in the model is not appropriate. No other traits showed AGE to be significant (either fixed or as a covariate), where residual variance estimates were only decreased in 4 of the remaining 14 traits and any decreases were minimal (less than 0.11%; Table 2). Due to this, AGE was not included in subsequent comparisons for SEQ or the blood collection effect and suggests that calves of the same cohort are close enough in age to define it as the same phenotypic trait.

3.3. Modeling sequence of scoring

Anecdotal evidence suggests that including sequence effect would be detrimental to modeling temperament traits because it could remove genetic variation. Sequence of evaluation (or sometimes referred to as order of entry) could be considered an independent measure of temperament as more aggressive or otherwise independent animals may enter the chute system more readily than those with more gregarious attributes (e.g., König et al., 2006). There is a mix of literature that either include or exclude the covariate (e.g., Hoppe et al., 2010; Riley et al., 2010) due to significance, but do not investigate whether it is appropriate to use in the model. For this study, inclusion and exclusion of sequence nested within evaluation (SEQ) were assessed similar to AGE effect. Significance of model effects across traits are provided in Table 3 and estimates for random effect estimates are reported in Table 4.

Within day, age at scoring had positive Pearson correlation coefficients with sequence (0.21 for day 1 and 0.04 for day 2). Although it was attempted to bring calves in randomly, calves of older age did enter the working facility later in the day more often than earlier in the day for the first day. This indicates, for day 1 at least, that AGE and SEQ may have similarities in their impacts on modeling each temperament

Table 5
Least squares means and standard errors for blood collection group of AFTER (scored after blood collection) and BEFORE (scored prior to blood collection) of different temperament scoring methods.

Method[1]	AFTER	BEFORE	Difference,% of scale[2]
Active	44.53 ± 1.35	42.51 ± 1.34	1.49
Agitated	22.62 ± 1.53	20.52 ± 1.52	1.54
Apathetic	59.86 ± 1.40	59.30 ± 1.37	0.41
Attentive	69.91 ± 0.91	71.75 ± 0.90	1.35
Calm	93.53 ± 2.56	94.73 ± 2.54	0.88
Curious	50.36 ± 1.06	51.42 ± 1.05	0.78
Distressed	15.07 ± 1.17	14.50 ± 1.16	0.42
Fearful	15.89 ± 1.03	14.98 ± 1.02	0.67
Happy	56.77 ± 1.23	58.01 ± 1.21	0.91
Irritated	22.41 ± 1.68	21.06 ± 1.67	0.99
Pos. Occ.	51.58 ± 0.93	51.67 ± 0.91	0.07
Relaxed	87.38 ± 2.46	89.39 ± 2.44	1.48
TI	0.01 ± 0.08	−0.01 ± 0.08	0.09
TS	1.86 ± 0.09	1.76 ± 0.09	1.67
SSD	48.33 ± 3.05	43.86 ± 3.03	3.93
CVSSD	0.12 ± 0.01	0.10 ± 0.01	6.64

[1] Subjective methods included qualitative behavior assessment (QBA) attributes of active, agitated, apathetic, attentive, calm, curious, distressed, fearful, happy, irritated, positively occupied (Pos. Occ.), and relaxed; Temperament Index (TI), the first principal component when using the 12 QBA attributes; and temperament score (TS). Objective methods included the standard deviation of the total weight recorded on 586 records from the four-platform standing scale (SSD) and the SSD divided by the average total weight on the same 586 records (CVSSD). Models for each trait were run in ASReml 4.2 (Gilmour et al., 2015) to fit an animal model with fixed effects of the mean (μ), day of evaluation (Day; $n = 2$), blood collection group (BCG; $n = 2$), interaction of Day by BCG ($n = 4$), sex of calf ($n = 2$), evaluator ($n = 4$; only for subjective traits). This included fitting pedigree using calf as a random effect and repeated measures of subjective traits were accounted for using a permanent environmental effect. [3]Difference was calculated using the absolute difference of least square means for a given temperament scoring method and dividing by the length of that method's scale. Length of scales included 136 mm (all QBA attributes), 21.18 (TI), 6 (TS), 113.65 lb (SSD), and 0.3013 (CVSSD). Expressed as a percentage.

trait. Unlike AGE, however, SEQ was significant for 68.75% of the models (11 out of 16; Table 3). Additive genetic variances increased for 10 of 16 temperament scoring methods (62.50%), where 7 of those (43.75% of all methods) had heritability estimates increase when the covariate was excluded from the model (Table 4). Changes in permanent environmental variance also occurred (Table 4), but the direction depended on the scoring method. In all cases, though, repeatability (i.e., the sum of additive genetic and permanent environmental variances divided by phenotypic variance) was increased in 7 of 16 methods (43.75%) when excluding SEQ, even for methods that a decrease in permanent environmental variance was observed. This supports the idea that sequence of scoring is a type of temperament trait (e.g., König et al., 2006) and should not be included in the statistical model for other temperament scoring methods.

3.4. Blood collection effect

The final model included fixed effects of evaluation day ($n = = 2$), blood collection group ($n = = 2$), interaction of day and blood collection group ($n = = 4$), sex of calf ($n = = 2$), and evaluator ($n = = 4$) as well as random effects of calf with (additive genetic) and without (permanent environment) pedigree to account for repeated measures by calf. The interaction of day by blood collection group was not significant across all traits ($0.077 \le P \le 0.855$; Table 3). Collecting blood was not impactful to temperament scoring, regardless of scoring method used (Table 3), which supports our hypothesis. Considering the scale of each trait, differences in blood collection groups' least square means differed minimally (no more than 6.64%; Table 5). Although Core et al. (2009) suggested blood collection could have caused issues in their study, our study suggests the impact would have been negligible. Due to this, the impact of blood collection to genetic parameter estimates would be negligible as well. Producers often vaccinate, collect germplasm such as blood, tissue, or hair, or both around weaning time, which may occur at the same time as temperament evaluation. All three of these instances indicate instances of momentary pain or discomfort that may stress the animal and cause changes in how it would be scored for temperament. Based on the results and how blood was collected in this study, it can be reasonably assumed that vaccinating, drawing blood, collecting tissue, or a combination of those would not impact temperament scoring thereby allowing them to be completed in the same timeframe to reduce handling needs and stress placed on the animal.

4. Conclusion

Across 14 subjective and 2 objective methods of scoring temperament, collecting blood or other single puncture procedures has negligible impact on scoring temperament in weaning age Angus-based calves. In finalizing model parameters to determine this outcome, it was found that age at scoring from calves of the same cohort or sequence during evaluation day model effects are not recommended for statistical models of temperament traits. Based on these results, it can be reasonably assumed that vaccinating, drawing blood, collecting tissue, or a combination of those would not impact temperament scoring. This demonstrates that handling needs and stress on the calf can be reduced by conducting these procedures during typical handling time periods needed by the producer or researcher.

Declaration of Competing Interest

The authors declare there are no conflicts of interest.

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Supplementary materials

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