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Personality of individually housed dairy-beef crossbred calves is related to performance and behavior

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The aim of this study was to evaluate differences in behavioral responses of individually housed dairy-beef crossbred calves to standardized personality tests (novel person, novel object, and startle test) and investigate associations of personality traits with performance and home pen behavior. Dairy-beef crossbred (Holstein x Angus) calves (n=29) were individually housed with *ad libitum* access to water and calf starter. Body weight was measured weekly and calf starter intake was recorded daily from day of arrival (8.5 ± 2.1 ; experimental day 1) for 76 days. Behavior within the home pen (eating, drinking, non-nutritive oral manipulation) and activity were recorded on experimental days 13, 32, 53, and 67 using a camera and a pedometer. The calves were subjected to standardized personality tests in their home pen at the end of the experimental period (80.7 ± 2.0 d of age), including a novel person test (stationary person in the corner of their home pen) and combined novel object/startle test (remote-controlled car in the pen, that suddenly moved when touched). A principal component analysis on the behaviors recorded from the tests (latency to approach person or object, time spent attentive and touching the person or object, and time spent inactive, playing and grooming) yielded 3 factors that explained 76.1% of the variance, and were interpreted as personality traits labeled “fearful”, “inactive”, and “bold”. These factors were examined in regression analyses for their associations with home pen behavior and performance. The factor “fearful” had negative associations with total average daily gain and average grain intake. In contrast, the factor “inactive” had positive associations with non-nutritive oral manipulation of buckets or walls. The factor “bold” had no significant association with any of the performance or home pen behavior measures. In conclusion, personality traits identified from standardized tests were related to performance and home pen behavior measures in individually housed, crossbred calves. These results complement work in group housed calves suggesting personality testing may be useful selective tools to identify high and low performing calves from an early age.

KEYWORDS

temperament, precision technology, behavioral characterization, individual differences, coping style, animal welfare

1 Introduction

Animal personality can be defined as individual differences in behavior that are consistent across contexts and time, and specific aspects of this behavioral repertoire are referred to as personality traits (Kaiser and Müller, 2021). These individual differences in behaviors may be assessed and interpreted from standardized tests, often by exposing animals to stressful situations (Finkemeier et al., 2018). In cattle, personality traits show long term consistency from early age to adulthood (Neave et al., 2020), and some traits are moderately heritable, such as handling traits of beef cattle and milking temperament of dairy cattle (Haskell et al., 2014). However, undesirable personality traits, such as fearfulness, could be inherited by future offspring in both beef and dairy cattle (Haskell et al., 2014; Dochtermann et al., 2015). This has implications for commercial practice as there are a number of studies showing personality is linked to individual performance, behavior, productivity, and welfare level (Haskell et al., 2014). Therefore, the measurement of personality traits across livestock production systems is vital to adapt management practices for improved production and animal welfare.

The environment that individuals are exposed to contributes to their expression of personality traits and behaviors (Dingemans et al., 2010). Calf management varies across farms and utilizes either individual, pair, or group housing. Housing directly impacts early social environments which have been associated with calf behavioral responses to novel stimuli. For instance, calves that were isolated were associated with increased scores that reflected fear (Jensen et al., 1997). This effect of the environment on expression of personality traits in animal have been seen in many species; rats that were individually housed (and thus socially isolated) showed abnormal responses to novel stimuli, including greater locomotor activity and more bouts of exploration (Sahakian et al., 1977). The expression of abnormal behaviors, such as excitable behaviors and fear of humans and/or confinement, can be detrimental to production in cattle as they have been linked to decreased growth rates, lower meat quality, and decreased milk production (Haskell et al., 2014). In Brahman cattle, poor temperament reduced DMI and ADG and had darker meat with smaller carcasses with less fat cover (Cafe et al., 2011). Hemsworth et al. (2002) reported that the avoidance distance in the human approach test is negatively associated with milk yield in cattle on farms. There is also evidence to suggest that abnormal responses such as stress and lack of sickness behaviours have a negative impact on immune function in cattle (Hulbert et al., 2011). Animals with personalities that are linked to chronic stress are at increased risk for long-term negative consequences (Koolhaas and Van Reenen, 2016).

The way animals interact with their environment, including the feeding environment, is also influenced by their personality traits. Calves that were more exploratory/active during novelty tests consumed larger quantities of solid feed and had higher average daily gains (Neave et al., 2018a). Thus, differences in personality traits may explain why some animals either fail or succeed in learning and adapting to their feeding environment (Neave et al., 2018b). Another aspect that may impact feeding behavior are novel diets that, when presented to ruminants, has been shown to decrease feed intake (Launchbaugh et al., 1997). This rejection of novel feeds, or food neophobia, has been measured in dairy cattle and was a contributing factor affecting feed intake (Cooke et al., 2006; Costa et al., 2020).

One method to measure personality traits is through the utilization of a standardized test that introduces a novel stimulus in

the environment and objectively measures individual behavioral response. This novel stimulus may be the environment, an object, or a person to measure personality traits such as fearfulness, exploration, and boldness in cattle (Forkman et al., 2007). The reactivity and responsiveness of calves during standardized tests can be attributed to a underlying personality trait such as fearfulness (Van Reenen et al., 2004). These tests may measure multiple traits which may make isolating a single trait such as fearfulness difficult. The introduction of an alternative standardized test to isolate the fearfulness trait, the startle test, may help differentiate between the expression of fearful and other personality traits such as exploration (Lauber et al., 2006). The use of a myriad of standardized tests allows for a more holistic view of personality traits in individuals. It is important to note that most of the personality research in calves are in animals that are group housed, yet individual housing is predominantly used in the dairy industry. The associations of personality traits with performance and behavioral indicators of calves in individual housing is therefore worthy of investigation.

In addition to a focus on group housed calves, available personality research has mostly focused on adult cattle, and previous work in pre-weaned animals has been performed in dairy calves. This lack of investigation on beef or dairy-beef crossbred calves in conjunction with the surge of interest in beef-on-dairy production creates a need for this investigation. Crossbreeding provides the opportunity to improve health and production efficiency of plants and animals through hybrid vigor (VanRaden and Sanders, 2003). There are clear benefits in reproduction, genetics, production, and increased value of surplus calves in the dairy industry (Berry, 2021). For example, dairy-beef crossbreeds improved calving ease in the dam and overall genetics of the herd (Eriksson et al., 2020) as well as higher yield and quality of the carcass (Bertrand et al., 1983). With the increased attraction to dairy-beef calves' production and potential monetary benefits to the producers, it is important to understand behavior and personality traits of crossbred calves, as these may have similar or different associations with performance and behavior than dairy or beef animals.

The main objective of this study was to evaluate a series of tests suitable for characterizing personality traits in individually housed dairy-beef crossbred calves. We aimed to 1) characterize behavioral responses of individually housed dairy-beef crossbred calves utilizing the novel person, novel object, and startle tests, and 2) evaluate the association between personality traits, calf performance and home pen behaviors, including feeding behavior and activity. We hypothesized that standardized behavioral tests adapted for use in the home pen of individually housed crossbred calves can reflect personality traits and will be associated with calf performance and home pen behavior.

2 Materials and methods

Dairy-beef (Holstein X Angus) crossbred bull calves (n = 31: 2 blocks) were sourced from a single commercial dairy producer in Indiana, USA and transported to the University of Kentucky Large Animal Unit in Lexington, Kentucky. Before arrival to the facility, calves were housed in individual hutches on the commercial dairy. Calves were 8.5 ± 2.1 days of age at arrival. These calves were

simultaneously enrolled in a 76-day study and were conducted under the approval of the University of Kentucky's Institutional Animal Care and Use Committee (IACUC #: 2019-3156). Block 1 calves (n=16) were 8.0 ± 2.0 d old (49.0 ± 5.6 kg) on arrival (Aug 10, 2020), and Block 2 calves (n= 15) were 9.0 ± 2.2 d of age (49.4 ± 9.0 kg) on arrival (January 26, 2021). Two calves were excluded from the study due to illness, so only 29 were utilized.

2.1 Calf management

Calves were housed individually indoors in an environmentally controlled room ($20.9 \pm 0.5^\circ\text{C}$ and $79.4 \pm 5.0\%$ relative humidity). Individual pens were 2.5 m in length and 2.6 m in width, totaling 6.5 m². All pens were fitted with rubber mats on the floor. Calves were socially isolated and could not see others between pens. Calves had *ad libitum* access to water and pelletized calf starter with 18% CP and 2.5% fat (Bagdad Feeds, Bagdad, Kentucky) in buckets. Calves were nipple-bottle fed 7 L/d of milk replacer at approximately 0830 and 1730h (Cow's Match Warm Front, Land O Lakes, Minnesota; 1 L, 150 g/L) that was divided into two equal meals until day 41. On day 42, milk replacer was reduced to 3.5 L/d between two equal meals. Calves were weaned on day 56 and continued to be managed post-weaning until completion of the experiment on day 76. Health checks were performed daily before morning feeding to assess for clinical signs of Bovine Respiratory Disease (BRD) and diarrhea following (Cantor et al., 2021). Four calves were diagnosed with a minor BRD case and received enrofloxacin subcutaneously (Baytril, Bayer, Leverkusen, Germany; 1 ml/15 kg) following the herd veterinarian protocol. Two calves were diagnosed with severe BRD and were treated with subcutaneous tulathromycin (Draxxin, Zoetis, Florham Park, NJ; 1.2 ml/45 kg) and intravenous flunixin meglumine (Banamine, Merck Animal Health, Madison, NJ; 0.5 ml/15 kg). Once weekly, calves were weighed to track growth and lungs were evaluated *via* ultrasonography to assess internal signs of pneumonia following (Ollivett and Buczinski, 2016). Calves indicating signs of illness were given treatment according to the protocol developed with the department veterinarian.

2.2 Home pen behavior and performance measures

Each calf was fitted with a pedometer (IceQube, IceRobotics Inc. Edinburgh, Scotland), validated for use in herd management and research (Trénel et al., 2009). This pedometer was a 3-axis accelerometer that uses algorithms to determine relative position to the ground and speed and direction of movement (Robert et al., 2009; Costa et al., 2021). Calf activity behaviors were measured daily for the duration of the experiment including standing time, motion index, step count, lying time, and lying bouts extracted from the pedometer. These activity measures were summarized from daily values to obtain an average per calf across the experimental period.

Individual body weight was measured and recorded weekly using a scale, then summarized to obtain an average daily gain (ADG) per

calf over the experimental period. Calf starter (grain) dry matter intake (DMI) was recorded daily by measuring grain orts and calculating disappearances daily intakes were summarized to obtain an average daily grain intake per calf over the experimental period.

Feeding behaviors were recorded on days 13, 32 (prior to step down), 53 (prior to weaning), and 67 (one week post weaning) using a camera (Moultrie M40i, Moultrie Feeders Birmingham, AL) that recorded images of the water and grain buckets in 1-minute intervals. The use of time-lapse cameras has been validated for this purpose (Miller-Cushon and DeVries, 2011). The images obtained from the camera (n=164,457) were scored by a single observer for daily duration of feeding behaviors (defined as muzzle inside of the rim of the feed or water bucket; Miller-Cushon and DeVries, 2011), and daily duration of non-nutritive oral behaviors (defined as licking or biting buckets or walls, without the purpose of feed or water ingestion; Montoro et al., 2013). These two behaviors were summarized to obtain a daily average per calf across the 4 observation days. Other recorded behaviors were not accounted for, including when calves were out of view of the camera and behavior could not be determined. These behaviors were not of interest and thus were not included in the analysis.

2.3 Standardized personality tests

Calves underwent three standardized personality tests at d 75 of the experimental period within their home pen. Tests were performed in the following order for all calves: novel person then a combined novel object/startle test, adapted from (Woodrum Setser et al., 2022). All behaviors were continuously monitored with a camera (HERO9 Black, GoPro Technology, San Mateo, California) centrally mounted above each individual pen. During each test, a single observer sat out of sight outside the pen and recorded any audible vocalizations. All tests for an individual calf were performed within a single day, with no time between tests.

2.3.1 Novel person tests

Briefly, calves were temporarily removed from their home pen to allow for placement of a novel person inside their home pen. The novel person, who had never interacted with the calves before, was dressed in a neon yellow construction jacket and pants, and stood in the back corner of the calf's pen. The novel person had their hands inside their pockets, had the jacket's hood drawn up, and wore a medical face mask covering nose and mouth (i.e., eyes were visible). The person faced the front of the pen and was instructed to not interact with the calf. After a 10-minute test period, the calf was removed from its pen and held in the alley outside its home pen to allow the person to exit and to set up for the next test. Video observations and manual vocalizations were recorded for the duration of the novel person test. The behaviors measured were the latency to approach novel person, time spent touching novel person, exploring environment, inactive, walking, resting, attentive to person, grooming, and playing. Additionally, the frequency of bucking, withdrawals, and urination/defecation were recorded (see ethogram in Table 1).

2.3.2 Novel object/startle test

Immediately following the novel person test, the calf experienced a combined novel object and startle test. While the calf was held in the alley outside its home pen after the novel person test, a remote-controlled car (10-inch 20V Big Wheel Remote Control Monster Truck RC, Kid Galaxy, Manchester, New Hampshire) was placed up against the middle of the right wall panel inside the pen. The car remained motionless until the calf approached the car (defined as touching the car with its muzzle), or until 5 minutes lapsed without the calf approaching. Once approached, or after the 5-minute interval, the car sped across the pen toward the left wall panel, triggered using a remote control held by the researcher outside the pen that was out-of-sight of the calf. Once reaching the opposite wall, the car stopped movement and remained in this position for the remainder of the test. From the moment the car reached the opposite wall, calves were observed for an additional 5 minutes to determine whether the calf decided to re-approach the car after being startled.

Video observations and manual vocalizations were recorded for the duration of the novel object/startle test. The behaviors measured from video were latency to initially approach the object before the startle, latency to re-approach the object after the startle, and time spent touching the object, exploring environment, inactive, resting, walking, attentive to object, grooming, and playing. Additionally, the frequency of bucking, withdrawals, and urination/defecation were

recorded (see ethogram in Table 1). To ensure that test length was the same for all calves regardless of whether the calf approached the object initially, only latency to initially approach was recorded before the startle; all other behaviors were recorded after the startle, during the last 5 minutes of the test period. All behaviors that were recorded after the startle include latency to re-approach the object after the startle, and time spent touching the object, exploring environment, inactive, resting, walking, attentive to object, grooming, and playing. If a calf did not initially approach the object within 5 minutes, latency to initially approach was recorded as the maximum 5 minutes. If a calf did not re-approach the object after the startle (from the moment the car moved), latency to re-approach was recorded as the maximum 5 minutes. Duration and frequencies of all behaviors (except latency variables) were summed across the novel person test and novel object/startle test per calf. A single observer recorded all behaviors in the tests from video using a behavioral coding software (The Observer XT 14, Noldus Information Technology, Wageningen, The Netherlands) according to the ethogram (Table 1).

2.4 Statistical analysis

All statistical analyses were performed with SAS (version 9.4; SAS Inst. Inc., Cary, NC) with calf as the experimental unit. All variables

TABLE 1 Ethogram of behaviors recorded from the videos of the standardized personality tests (novel person, novel object, and startle tests) from individually housed Holstein x Angus crossbred calves ($n = 29$) tested in their home pen at 81.6 ± 2 d old.

Behavior	Definition
All tests	
Grooming	Duration of time calf spent licking or scratching own body with mouth or muzzle, or scratching own body with any limbs
Resting	Duration of time spent lying down, from the point the rear touches the ground until the point when the back legs are lifted to stand
Inactive	Duration of time spent with no movement, no interaction with the environment, and not attentive toward person or object
Walking	Duration of time calf moved around the pen, excluding running or trotting.
Environment exploration	Duration of time calf spent exploring the walls, ground, and other fixtures of the pen with tongue and muzzle
Locomotor Play	Duration of time spent running, jumping, or trotting around pen
Object/Person Play	Duration of time spent butting or mock butting the novel person or novel object
Bucking	Number of events where the calf lifts both hind legs off the ground and kicks backwards
Withdrawal	Number of events where the calf takes a sudden step or multiple steps backwards
Urination/Defecation	Duration of time spent with the tail lifted to expel urine or feces
Novel Person test	
Latency to approach	Time lapsed from the moment the calf enters the home pen, to the time the calf's muzzle makes contact with the novel person
Touching Person	Duration of time calf used muzzle to make contact with the novel person
Attentive to Person	Duration of time calf spent with head orientated towards person
Novel Object/Startle test	
Latency to initial approach	Time from the moment the calf enters the home pen, to the time the calf's muzzle comes in contact with the novel object
Latency to re-approach after startle	Time from the moment the car moves (start of startle) to the time the calf's muzzle touches the novel object
Touching Object	Duration of time calf used muzzle to make contact with the novel object
Attentive to Object	Duration of time calf spent with head orientated towards object

were inspected for normality utilizing the UNIVARIATE procedure and probability distribution plots in SAS. Grooming and inactive variables from the personality tests were not normally distributed and were transformed using \log_{10} . The variables withdrawal, resting, urinating, and defecating were removed from analysis since these rarely occurred and were not able to achieve a normal distribution with transformation.

A correlational multivariate analysis was used to identify common sets of behaviors across the different tests, followed by a subjective interpretation of the correlated sets of behaviors according to Costa et al. (2020). Labels were assigned to these sets of behaviors, guided by literature, which were interpreted as personality traits. A principal component analysis (PCA) with varimax rotation (PROC FACTOR) was used to reduce correlated measures from the novel person and novel object/startle tests, following analysis and reporting guidelines outlined by (Budaev, 2010). The PCA included 8 input variables: latency to initially approach novel object, latency to re-approach novel object after the startle, latency to approach novel person, time spent attentive to novel person or object, time spent grooming, time spent inactive, time spent touching novel person or object, and time spent locomotory and object playing. The variables walking and time spent exploring environment were removed from the PCA analysis as they lowered the Kaiser–Meyer–Olkin measure of sampling adequacy (requirement of 0.50 to conduct PCA). The correlation matrix was computed, and principal components were retained if eigenvalues were > 1 . Three principal components (factors) were retained that explained 76.1% of the variance in behavioral responses in the novel person and novel object/startle tests. Factor scores for each calf were extracted using the regression method; each calf received a score on a continuous scale for each factor (interpreted as a personality trait). These scores were then utilized to explore associations of factor scores (personality traits) with calf performance and home pen behaviors.

To explore associations between the factors scores (personality traits) and performance during rearing and home pen behaviors, linear regressions were performed (PROC MIXED). The factor scores yielded from the personality tests were the explanatory variables, and response variables were calf performance (ADG, daily grain intake), home pen feeding behaviors (eating time, drinking time, and non-nutritive oral behavior), and home pen activity (motion index, standing time, lying time, mean steps). Enrollment age, body weight at arrival, and block were included as fixed effects. Treatment with antibiotics (yes or no; $n = 6$ calves were treated) was also included as a fixed effect as a control for incidence of BRD throughout the study, but this variable was not significant. Fixed effects were removed from the model using stepwise backwards elimination if $P > 0.30$ starting with the least contributing effect. Findings were deemed significant if $P \leq 0.05$ and a tendency when $P \leq 0.10$.

3 Results

3.1 Personality traits from principal component analysis

The behavioral responses of calves during the novel person and novel object/startle test are reported in Table 2, and the variable loadings on each factor are reported in Table 3. Factor 1 explained 40.5% of the variance and yielded high positive loadings for latency to initially approach the novel object, latency to re-approach the novel object after startle, and time spent attentive to novel person or object. Calves with a higher score on this factor were labeled “fearful”. Factor 2 explained 18.8% of the variance and yielded high positive loadings for time spent grooming and time spent inactive. Calves that scored high on this factor were labeled as “inactive”. Factor 3 explained 16.9% of the

TABLE 2 Behavioral responses of Holstein x Angus crossbred calves ($n=29$) during the standardized personality tests (novel person and novel object/startle tests) when tested individually in their home pen at 81.6 ± 2 d old.

Variable	Mean	SD	Range
Latency to approach novel person	7.3	12.7	1.0-70.5
Latency to initially approach novel object	77.9	112.8	1.7-300.0
Latency to re-approach novel object	187.7	121.8	5.4-300.0
Attentive to novel person and novel object ¹	233.1	142.9	41.8-499.4
Touching novel person and novel object ¹	313.6	116.1	159.9-682.4
Grooming ¹	6.5	7.8	0-37.8
Inactive ¹	129.0	94.7	25.5-365.0
Play ^{1,2}	264.5	119.5	9.6-444.0
Exploring Environment ¹	37.1	30.9	0-136.5
Walking ¹	25.6	16.0	3.4-68.8
Bucking	0	0	0
Urinating/Defecating	0	0	0

Values are reported as mean, standard deviation, and range (minimum - maximum) (in seconds).
¹Total duration summed across the tests per calf, then averaged across calves.
²Total duration of locomotory play and object/person play.

TABLE 3 Coefficients (loadings) of each variable for the first 3 factors extracted from the principal component analysis of the behavioral responses of Holstein x Angus crossbred calves (n = 29) in the standardized personality tests.

Variable	Factor 1	Factor 2	Factor 3
Grooming	0.05	0.83	-0.09
Inactive	0.11	0.82	-0.01
Latency to approach novel person	0.47	0.33	-0.43
Latency to initially approach novel object	0.86	0.12	0.01
Latency to re-approach novel object	0.88	0.05	-0.04
Attentive to novel person or object	0.95	0.09	-0.02
Touching novel person or object	-0.20	-0.27	0.85
Object/Person Play	-0.38	-0.44	-0.77
Eigenvalues	3.24	1.50	1.35
Variance Explained	40.5%	18.8%	16.9%
Interpretation	“Fearful”	“Inactive”	“Bold”

The eigenvalues are reported for each factor and variables with high loadings ($\geq \pm 0.63$) are in bold. The labels given to each factor are subjective interpretations of the correlated set of behaviors with high loadings; each factor is interpreted as a personality trait.

variance and yielded high positive loadings for time spent touching person or object and high negative loadings for time spent playing. Calves with a higher score on this factor were labeled as “bold”.

3.2 Associations between personality, performance and home pen behaviors

Calves spent (mean \pm SD) 38.1 \pm 10.6 min/d (range: 25.3 – 65.5 s/d) eating, 5.4 \pm 4.0 min/d (range: 0 – 13.5 min/d) drinking, and 25.4 \pm 11.4 min/d (range: 4.5 – 40.5 s/d) engaged in non-nutritive oral behavior while in their home pen. The relationships between calf performance, home pen behaviors, and the factor scores (personality traits) are outlined in Table 4. Factor 1 (“fearful”) had a negative association with total average daily gain and average grain DMI (Figures 1, 2). Factor 1 had no associations with any home pen behaviors. Factor 2 (“inactive”) had a positive association with average time spent licking (non-nutritive oral behavior; Figure 3). Factor 2 also tended to have negative associations with mean eating time, mean motion index, and mean steps. Factor 3 (“bold”) had no associations with any of the performance or home pen behavior measures.

4 Discussion

This is the first study to investigate personality traits in dairy-beef crossbred calves reared in social isolation, and to evaluate relationships between personality, performance, and home pen behaviors in these calves. Calves were reared in individual pens, with solid walls on each side of the pen to prevent physical and visual contact with other calves. The series of tests performed in this study were able to suitably characterize individual variation in performance and home pen behaviors. Calves that were more “fearful” consumed less grain and had reduced ADG, while calves that were more

“inactive” spent more time expressing non-nutritive oral behaviors (i.e., licking buckets or walls) and tended to spend less time eating and moving. These results have important implications for understanding individual variation in behaviors of calves during the rearing period, which could potentially signify animals with poorer performance and provide an on-farm selection strategy for rearing dairy-beef crossbred calves.

4.1 Personality traits of crossbred calves

Similar to previous study approaches in farm animals, personality traits were identified using a principal component analysis on the behaviors expressed during three standardized personality tests (Neave et al., 2018a; Costa et al., 2020; Whalin et al., 2022; Woodrum Setser et al., 2022). The pattern of factor loadings revealed how the novel person, novel object and startle tests can measure common or different aspects of calf personality. For instance, measures from the startle test (latency to initially approach and re-approach the novel object) loaded together with a measure from the novel person test (time spent attentive) on factor 1 (“Fearful”). The other factors, “Inactive” and “Bold”, were comprised of a combination of measures from all three tests (inactive, grooming, play, and touching the novel object and person). Notably, latency to approach the novel person did not load highly on any factor, indicating that a single measure from a single test was not sufficient to reflect a personality trait. The pattern of factor loadings from crossbred calves in this study supports previous work in dairy calves (Van Reenen et al., 2004; Neave et al., 2018a; Neave et al., 2019; Costa et al., 2020; Woodrum Setser et al., 2022) in the use of multiple standardized tests to identify personality traits in young cattle. It also supports a previous study (Woodrum Setser et al., 2022) in the use of a startle test to reveal individual variability in “fearfulness” in calves. However, research has yet to describe multiple personality traits in young beef calves.

TABLE 4 Relationships between factor scores (personality traits), performance and home pen behavior of individually housed Holstein x Angus crossbred calves (n = 29) during the 76-d experimental period.

Factor and variable	F-Value ¹	P-Value ²
Factor 1 (“Fearful”)		
Total ADG (kg/d)	26.35	<0.0001
Calf Stater DMI (kg/d)	25.47	<0.0001
Motion Index	0.29	0.59
Standing Time (h/d)	0.2	0.66
Lying Time (h/d)	0.44	0.51
Steps (steps/d)	0.31	0.58
Eating Time (min/d)	0.19	0.67
Drinking Time (min/d)	1.45	0.24
Licking Time (min/d)	0.11	0.74
Factor 2 (“Inactive”)		
Total ADG (kg/d)	0.98	0.33
Grain DMI (kg/d)	1.69	0.21
Motion Index	3.70	0.068
Standing Time (h/d)	0.01	0.94
Lying Time (h/d)	0.00	0.98
Steps (steps/d)	3.67	0.069
Eating Time (min/d)	2.90	0.104
Drinking Time (min/d)	0.00	0.96
Licking Time (min/d)	7.43	0.01
Factor 3 (“Bold”)		
Total ADG (kg/d)	0.06	0.81
Grain DMI (kg/d)	0.81	0.38
Motion Index	0.56	0.46
Standing Time (h/d)	0.00	0.97
Lying Time (h/d)	0.04	0.84
Steps (steps/d)	0.57	0.46
Eating Time (min/d)	0.08	0.78
Drinking Time (min/d)	0.94	0.34
Licking Time (min/d)	0.24	0.63
¹ Degrees of freedom (numerator, denominator) = 1,17		
² Significant P values (≤ 0.05) are bolded, and tendencies (≤ 0.10) are italicized		

A key difference from other calf studies of personality is the application of behavior tests in the home pen rather than in a separate test arena. We also housed calves individually rather than in group housing, which is typical of current commercial practice for dairy and dairy-beef crossbreds (USDA, 2016). The factors from the PCA in this study were similar to other studies in group housed calves, but some key differences were also observed. The factor “fearful” in our study had high positive loadings for latency to approach and touch the object before and after the startle, and the time spent attentive looking toward the person and object. A previous study by Woodrum Setser

et al. (2022) in group-housed dairy calves used the same three personality tests as the current study, except these tests were performed in a test arena separate from the home pen; these authors reported a similar pattern of factor loadings to ours. Other studies that performed novel object and novel human tests in a test arena in group-housed calves (Lecorps et al., 2018; Neave et al., 2019) also showed a collective “Fearful” personality trait using similar relationships among behaviors from these tests. Together these studies suggest that “fearfulness” of calves is measurable across test situations (in home pen or test arena), across housing conditions

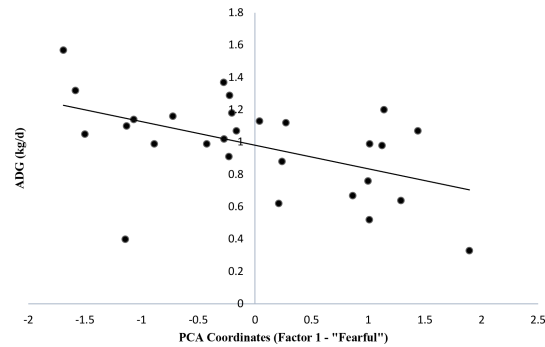


FIGURE 1

Average daily gain (ADG) of individually housed Holstein x Angus crossbred calves ($n = 29$) during the 76-d experimental period, plotted against Factor 1 ("Fearful") from the principal component analysis of the standardized personality tests. Each black dot represents a calf, and the linear regression trendline is presented ($P < 0.001$).

(individually or group-housed calves), and in both dairy and crossbred calves.

In this study, the factor "inactive" had high positive loadings for the time spent inactive and grooming during the standardized personality tests. This behavior "inactive", or its inverse "active", appears to consistently form a separate factor from other behaviors recorded across multiple personality tests, supporting that (in)activity forms an underlying personality trait in calves (Van Reenen et al., 2004; Van Reenen et al., 2005; Neave et al., 2018a). Notably, these previous studies in group housed calves also had a measure of exploring the environment that was well correlated with pen activity. In this study, time spent exploring the environment was removed from analysis as it lowered the MSA score of the overall PCA, suggesting it was not explaining sufficient variation in the behavior of our individually housed calves. This may be because our calves were tested in their home environment, which was not novel and was limited in size due to individual housing, so these conditions may not promote exploratory behaviors. This result may indicate that exploratory behavior may not be an informative measure of personality for individually housed calves tested in their home pen. It is also possible that there may be a breed effect causing this difference; more research on individually housed calves of various breeds may reveal the usefulness of this measure of exploration in describing personality of calves.

Finally, the "bold" factor in this study had high loadings of time spent touching the person or object which is consistent with Costa et al. (2020). However, our "bold" factor did not include the latency to approach the person, which was observed in group housed dairy calves exposed to the same tests (Woodrum Setser et al., 2022). The latency to approach the novel person is often interpreted as a measure of boldness or fearfulness in calves (Forkman et al., 2007), yet this measure could not explain sufficient variation in behavior in our calves. The average latency to approach the novel person was just 7 seconds, which could indicate that testing in the home environment (rather than a novel arena, as in most other studies; Forkman et al., 2007), or being individually housed, made calves more comfortable to approach a novel human. However, the novel person test is still useful to characterize personality, given other measures from the test contributed to the "Fearful" and "Bold" traits. Similar to Woodrum Setser et al. (2022), this study included a startle test that was expected to help distinguish measures related to fearfulness (i.e. a negative response to a real or perceived threat; Boissy, 1995) from measures related to boldness (i.e. the propensity to take risks, particularly when faced with novel situations; Toms et al., 2010). Indeed, the measures specific to the startle (latency to re-approach) and attentive behavior (reflecting vigilance following a threat; Welp et al., 2004) loaded together, providing support for the use of novelty and startle tests to characterize separate boldness and fearfulness traits in calves.

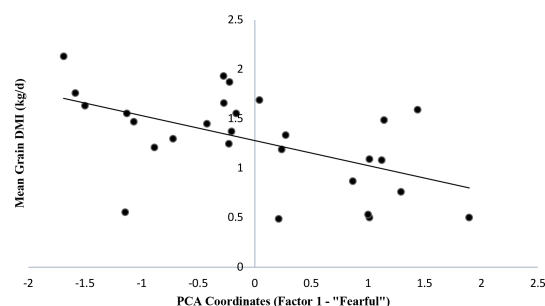


FIGURE 2

Average grain dry matter intake (DMI/d) of individually housed Holstein x Angus crossbred calves ($n = 29$) during the 76-d experimental period, plotted against Factor 1 ("Fearful") from the principal component analysis of the standardized personality tests. Each black dot represents a calf, and the linear regression trendline is presented ($P < 0.001$).

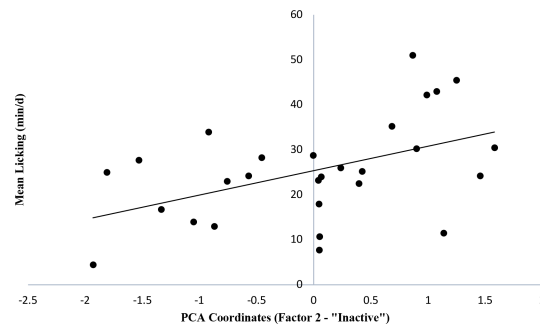


FIGURE 3

Average time spent licking (non-nutritive oral behavior in the home pen) of individually housed Holstein x Angus crossbred calves ($n = 29$) during the 76-d experimental period, plotted against Factor 2 ("Inactive") from the principal component analysis of the standardized personality tests. Each black dot represents a calf, and the linear regression trendline is presented ($P < 0.001$).

4.2 Personality and growth performance

Growth in both beef and dairy calves is indicative of performance as it relates to carcass weights in beef (Hennessy and Morris, 2003) and milk yield in dairy cows (Soberon et al., 2012). Thus, in the Holstein x Angus crossbred calves enrolled in this study, the key indicator of performance utilized was ADG, which was variable among individuals over the total study period. Average daily gain (ADG) was similar to group housed Holstein calves in Neave et al. (2019) and in individually housed Limousine x Holstein calves in Vestergaard et al. (2019). Despite differences in breeds and management practices, there are nevertheless similar ADG between these studies.

Personality could explain some of the differences in ADG observed between individuals. Calves that scored highly on the personality trait "fearful" had a negative association with overall ADG. The relationship between fearfulness and growth is seen across several studies in growing calves. For example, young beef calves that were highly reactive (while in the chute and high flight speed exiting the chute) had decreased weaning weight (Francisco et al., 2012; Torres-Vazquez and Spangler, 2016). In post-weaned beef cattle, calm individuals (less fearful, determined from subjective chute scores) have repeatedly been shown to have greater ADG (Voisinet et al., 1997) and feed efficiency (Reinhardt et al., 2009). In dairy calves, growth was also related to personality, where more "exploratory/active" calves in the novel environment test (Neave et al., 2018a), and more "bold" calves in novel person, object and startle tests were associated with higher average daily gains (Woodrum Setser et al., 2022). Research on personality of beef-dairy crossbred calves is limited, but there is similar evidence in crossbred cattle; individuals exhibiting less fearful behavior in a social separation test showed higher ADG (Müller and von Keyserlingk, 2006). Overall, despite the different housing systems, breeds, and personality tests across these studies, more fearful (or reactive) individuals appear to have poorer performance. This relationship may relate to calves being more reactive to changes in their environment such as daily handling that occurred and may be more cautious or vigilant toward their surroundings, leading to greater energy expenditure. These high arousal states from fearfulness may also affect feeding behavior and feed intake, consequently reducing growth.

4.3 Personality and feeding behaviors

There was also notable variation in feed intake and time spent eating per day among individual calves of this study; some of this variation could be explained by calves scoring highly on the "fearful" personality trait that were associated with reduced grain dry matter intake. Solid feed intake is a major driver of calf success during weaning, as grain intake in calves promotes rumen development and reduces negative behaviors and growth check at weaning (as reviewed by Khan et al., 2011). Few studies have examined the relationships between personality traits and solid feed intake in calves, but there appears to be support for such associations. One study that fed dairy calves using an automatic grain feeder found more "exploratory/active" calves (scored from a novel environment test) were positively associated with total grain intake and gain: feed ratio (Neave et al., 2018a), possibly because these calves were more likely to encounter or willing to sample alternate food sources while exploring their environment. The gain: feed ratio was not analyzed in our study but is worthy of investigation in future work, especially in crossbred calves. However, another study reported that "fearful" dairy calves (scored from novel human and object tests) were negatively associated with weaning age based on individual solid feed intake (i.e., more fearful calves consumed more solid feed intake and weaned earlier; Neave et al., 2019). The authors speculated these calves may be more reactive to the removal of milk and respond by increasing solid feed intake to complete weaning earlier. In contrast, Angus beef steers that were more reactive to restraint in a chute and had greater flight speeds upon release (interpreted as more fearful) were associated with lower dry matter intake at the feedlot (Cafe et al., 2011). These authors, among others (Petherick et al., 2002), have suggested this may be due to increased vigilant behavior and high arousal state leading to lowered feed intake; we suggest a similar mechanism may explain the reduced feed intake (and growth, as described above) in individually housed crossbred calves. Alternatively, a general reluctance of fearful calves to sample novel feeds (food neophobia; Costa et al., 2020) may drive this relationship. Overall, these studies provide evidence of a relationship between fearfulness and solid feed intake in both dairy and beef animals, although the mechanism behind this relationship remains to be explored.

Feeding behavior is also influenced by personality of the individual (reviewed by Neave et al., 2018b). We found no association between time spent eating grain and “fearfulness” personality trait, despite this trait having associations with reduced grain intake and ADG. However, we did observe a tendency for a negative association between time spent eating grain and “inactive” personality trait, yet this trait had no association with intake or ADG. These findings suggest that feeding rate (or bite size) may be a key influential behavior in calves of these personality traits. Eating time of solid feed has not been explored in young calves, but previous work in group housed dairy calves found stable and repeatable milk-feeding behavior patterns (Carslake et al., 2022). Individual differences in milk drinking speed (i.e., feeding rate) were related to “fearfulness” and visits to the milk feeder were related to “vocal/inactive” traits in calves using an automated milk feeder (Neave et al., 2018a; Neave et al., 2019). There may have been differences in milk feeding behaviors that were not measured in this study that may relate to personality of crossbred calves.

We measured other behaviors associated with the nutritional environment of calves, including water drinking duration and non-nutritive oral manipulation behaviors. Water drinking duration had no associations with any personality trait measured in this study; no previous work has explored variability in this behavior, water intake, or their possible relationships with personality, despite the importance of water for productivity and welfare in cattle (reviewed by Jensen and Vestergaard, 2021). A novel finding of this study was that non-nutritive oral behaviors in the home pen, specifically licking and sucking of feed or water buckets and fixtures, were expressed more in calves with higher scores for “inactive” personality. Non-nutritive oral behaviors are stimulated by ingestion of milk (De Passillé and Rushen, 1997), and these behaviors seem to be expressed more often in individual than group housed calves (Tapki, 2007), perhaps due to a lack of environmental complexity. These behaviors in our dairy-beef crossbred calves also occurred outside of milk feeding times, suggesting there may be underlying motivations to perform this behavior that are not related to milk ingestion. The reason why calves of the “inactive” personality performed non-nutritive oral behaviors more than other individuals may relate to how these calves respond to changes in their environment. When exposed to the novel and startling situations during the personality tests, these calves remained inactive (standing idle, performing no other behaviors) or performed grooming behaviors, which may indicate an incapacity to respond appropriately to environmental stimulation in their home pen. A lack of behavioral response to novel or unexpected stimulation may indicate a break-down in attentional processes arising from housing in impoverished environments (Wemelsfelder, 1991) and this lack of arousal can be an indicator of boredom (Burn, 2017). The fact that calves of the “inactive” personality tended to also show reduced activity in the home pen (measured using motion index and steps from accelerometers) may further support inactivity as a reflection of boredom in these calves (Hintze et al., 2020). Consequently, redirected behaviors toward immediately available stimuli in the housing environment may arise from a need to ‘do something’ (Wemelsfelder, 1993) and we suggest this may have manifested in more non-nutritive oral behaviors in calves of the “inactive” personality. Special management, such as providing environmental

stimulation, may be needed for these calves under individual housing conditions (da Silva et al., 2022). For instance, additions to the environment of individually housed calves can reduce the occurrence of non-nutritive oral behaviors, such as providing hay (Downey et al., 2022), stationary brushes (Horvath et al., 2020) or human contact (Doyle and Miller-Cushon, 2022). Activity patterns of calves in the home pen as a personality trait are rarely explored for links with other behaviors and performance, but the current study has revealed its potential importance and how this measure can be easily collected with technology. Alternatively, the isolation box test (brief restraint in an enclosed, dark box) identifies an “activity” personality trait, and has associations with both home pen activity and weaning performance in dairy calves (Woodrum Setser et al., 2022).

4.4 Study limitations

Behavioral responses to standardized tests should be consistent over time to be considered a personality trait (Carter et al., 2013). The novel person, novel object and startle tests used in this study have temporal consistency in dairy calves (Veissier et al., 1997; Van Reenen et al., 2004; Neave et al., 2020), but due to the terminal nature of the concurrent study we were unable to confirm temporal consistency of behavioral responses to these tests in our dairy-beef crossbred, individually housed calves. Future research should test the consistency of personality traits of dairy-beef calves through rearing, puberty, and at market weight, as these traits may change at key development periods (Neave et al., 2020) but nonetheless still predict later performance as mature animals. We acknowledge that the individual pens of this study may be larger than the individual pens in commercial dairies and that it may influence behavior. Research should be done utilizing the individual pens of commercial dairies to explore this. Finally, we acknowledge our measure of feeding, drinking and non-nutritive oral behaviors were limited to 4 days across preweaning, weaning and postweaning periods. Our study provides early evidence that variation in time spent engaged in feeding and non-nutritive oral behavior may be related to individual personality traits, which merits a more detailed investigation of how these behavioral patterns may predict calf performance at specific developmental periods.

5 Conclusion

Standardized personality tests conducted within the home pen were able to measure several personality traits in individually housed crossbred calves. These personality traits were able to explain some of the individual variation in ADG, grain DMI, time spent feeding and time spent engaged in non-nutritive oral behaviors in the home pen. These results indicate that personality testing can predict performance and home pen behaviors of individually housed crossbred calves, similar to findings in group housed dairy calves. Given individual housing remains prevalent in calf rearing systems, these tests could potentially be used on these farms to identify particularly “fearful” calves that are slow to

approach a novel object both before and after being startled. Testing could also be used to identify “inactive” individuals who spent more time engaging in non-nutritive oral manipulation of their environment. Both behavioral types may require targeted management to meet their behavioral needs and improve performance.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by University of Kentucky’s Institutional Animal Care and Use Committee (IACUC #: 2019-3156).

Author contributions

The authors report no conflict of interest. EM performed experimental design planning, data collection, and writing of the manuscript original draft. MW assisted with experimental design, data collection, statistical analysis, and manuscript editing. GM helped with data collection, and manuscript editing. HN assisted with experimental design, statistical analysis, and manuscript editing. JC assured the funds, performed experimental design planning, provided supervisory oversight of the project, manuscript editing and overall coordination of the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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