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ISOLATION BOX TEST (IBT) AND DAIRY CALF PERSONALITY TRAITS RELATIONSHIP WITH PERFORMANCE THROUGH WEANING

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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at the University of Kentucky

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ABSTRACT OF THESIS

ISOLATION BOX TEST (IBT) AND DAIRY CALF PERSONALITY TRAITS RELATIONSHIP WITH PERFORMANCE THROUGH WEANING

Personality tests are utilized to characterize individual differences in personality traits across species. Currently, there is no objective personality test that can be implemented on-farm to measure personality in dairy calves. Measuring personality of youngstock in the dairy industry may allow producers to utilize information on individual differences for management decisions and limit the investment in unfit individuals. The isolation box test is a personality test that has potential to be adapted and utilized for dairy calves. The main objective of this thesis is to develop, validate, and utilize an isolation box test for use on-farm in dairy calves. More specifically, the objectives of this thesis are to determine if an isolation box test can determine individual differences in dairy calves, if outcomes from the isolation box test agree with outcomes of established standardized personality tests, and if outcomes from an isolation box test can predict performance outcomes through weaning in dairy calves. The measures produced from an isolation box test were associated with outcomes of standardized personality tests (novel person, novel object, and startle tests) and growth through weaning. The isolation box test has potential to be used as a personality test in dairy calves on-farm.

KEYWORDS: temperament, behavioral characterization, individual variation

Megan Marie Woodrum Setser

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CHAPTER 1. REVIEW OF LITERATURE

1.1 Introduction

Across species, the individual differences in coping style—stable individual behavioral, and physiological response to stressors (Koolhaas et al., 1999)—have been associated with levels of performance, disease vulnerability, welfare, and ability to adapt to situations (Koolhaas and Van Reenen, 2016). Coping styles are only one of the components that make up the personality of animals. The personality traits of individuals can be measured through observations of behaviors in a natural or experimental environment and have been measured in non-human animals in the wild, laboratory, and domestic production environments. To begin this literature review, I will define the terminology used to discuss personality and draw similarities and discrepancies between the different terminologies used across the research fields; then, I will describe the basic methodology generally used to measure behaviors and personality traits in animals. The main aim of this literature review is to explore the methodologies used to determine personality traits across taxa and living environments, the predominant personality traits found for each respective species, and the relationships between personality traits and performance or fitness in species. The final goal of this review is to identify tests that can be used or adapted for use on-farm for livestock species with a focus on dairy cattle, including the potential of an isolation box test to measure traits in dairy calves.

1.2 Animal Personality Terminology

Personality in the field of animal research differs from the colloquial understanding of personality that people have for themselves and from the typical use of the term in common life. There are specific scientific requirements and definitions that determine if a species can express personalities. The personality of an individual is made up of many personality traits (Kaiser and Müller, 2021). For an individual to have a certain personality trait, it must vary across members of a population, be consistent across time, and consistent across contexts within the individual (Stamps and Groothuis, 2010; Kaiser and Müller, 2021). Personality traits cannot be measured directly but are inferred from behavioral responses to environmental conditions. To illustrate this concept, two animals can be introduced to a new object: one may stand far from the item and never approach it, and the other animal may approach and spend time interacting with the object. If a week later, the same individuals are exposed to a new person and react in the same manner as they did with the novel object, then the first individual may be labeled as having the personality trait "shy", and the second individual may be described as "bold". Another way to define personality traits is as the stable differences in behavioral tendencies across a population (Wolf and Weissing, 2012). The accumulation of various personality traits for an individual, such as boldness, fearfulness, and aggressiveness, would make up their personality.

Across the research fields of behavior and personality in animals, there is lack of consensus of what terminology is most appropriate, creating similarities and discrepancies. There are a multitude of terms used in different fields of research to describe personality and personality traits in non-human animals. A common term used to describe personality traits, or a collection of related behaviors consistently expressed across context and time, is 'behavioral syndrome' (Sih et al., 2004). This term is often used in the field of ecology. 'Temperament' is most often used in animal science fields, and also refers to the stable individual behavioral differences (Réale et al., 2007; Stamps and Groothuis, 2010). These terms are used interchangeably in various fields of research, and it is important to understand the overlap and discrepancies between terms when interpreting research. 'Coping styles' is a term used to refer to the stable individual difference in the behavioral and physiological response to stressors (Koolhaas, 2008). Coping styles can be divided into two categories: either a 'proactive coping style', indicating animals who had low hypothalamic-pituitary-adrenal axis (HPA axis) reactivity and high sympathetic nervous system reactivity in response to a stressor; or 'reactive coping style', referring to animals who had high HPA axis reactivity and high parasympathetic reactivity to a stressor (Koolhaas et al., 1999). The behavioral reflection of these different coping styles to stressors is species dependent (Koolhaas et al., 1999). While 'coping styles' is slightly different than the other terminology, it is closely related and involves looking at traits closely related to handling stress rather than the broader range of traits that fall under 'personality traits', 'temperament traits', or 'behavioral syndromes.' For this review, the primary terms utilized will be personality trait and personality, however I will use specific terms when appropriate.

1.3 Genetic Contribution to Personality Traits

Personality expressed by animals, like other phenotypes, is a product of the interaction between the environment and the genetics of the individual. An early illustration of this point is the 1940 study that was able to test rats' abilities to solve a maze, and breed a high performance and low performance line of rats based on time to solve the maze (Tryon, 1940). However, these studies were conducted with rats that were all reared in an unenriched environment that was standard for laboratory rats at the time. When the two strains were reared in various conditions (barren, unenriched, and enriched) the performance outcomes changed: the low performance rats were able to perform at the same level as the high performance rats when reared in the enriched environment, and both lineages performed poorly in the barren environment, similar to the original low performance rats reared in an unenriched environment (Tryon, 1940). Thus, both environmental and genetic components of personality expression can be utilized to better understand indiviudal variation. The genetic component of behavior research can be explored with molecular analysis or through the heritability of traits.

Our understanding of the genetic contribution to behavior and personality of animals has increased drastically in the last few decades. Identifying the genotypes linked to personality can be done in two ways. The first way is through genome-wide mapping, which investigates all the genetic material of an individual to find associations between the genome and specific personalities phenotypes, this technique is becoming more readily available (Van Oers et al., 2005). However, genome-wide mapping requires sorting through massive amount of genetic information for each individual. The second way is quantitative trait loci (QTL) mapping, which involves use of genomic markers and knowledge of the pedigree of the individuals/population being investigated (Van Oers et al., 2005). QTL mapping is limited in natural populations as it is more difficult to identify pedigrees, but it has great potential for use in domestic species with controlled breeding. QTL mapping in cattle, swine, and sheep was used to identify conserved genomic areas that relate to behavioral traits such as milking speed, temperament, aggression, flight speed, and maternal ability (Alvarenga et al., 2021). Identifying the genomic areas related to particular traits may allow for researchers to better answer the questions of how traits evolved.

Another method of looking at the genetic contribution of personality traits is to look at the heritability of phenotype expression of traits (Van Oers and Mueller, 2010). Various personality traits have been found to be heritable in dairy cattle (Haskell et al., 2014), beef cattle (Haskell et al., 2014), sheep (Beausoleil et al., 2008; Wolf et al., 2008; Lennon et al., 2009), and swine (Turner et al., 2009). The heritability of traits, such as docility, allows for it to be incorporated into breeding programs with the potential to improve animal welfare and productivity (Norris et al., 2014). Traits are considered highly heritable when the heritability estimates exceeds 0.40, and is considered lowly heritable when the heritability estimate is less than 0.15 (Cassell, 2009). The heritability of production traits is typically moderate to high (Suzuki and Van Vleck, 1994; Rios Utrera and Van Vleck, 2004; Safari et al., 2005), while the heritability of personality traits is typically low to moderate (Lennon et al., 2009; Turner et al., 2009; Haskell et al., 2014). Nonetheless, personality traits are important to consider as they relate to how individuals respond to handling, physiological response to stress in animals, and welfare, which can all impact productivity and fitness of individuals (Koolhaas and Van Reenen, 2016); thus, it is worthwhile to make these traits a focus in breeding programs. It is important to remember that genetics are only one component of personality traits - the context in which behaviors are measured (such as the environment and test conditions) are just as important to consider when interpreting the expression of personality traits (Koolhaas et al., 1999). Ultimately, the expression of personality not only has a relationship to the genetic makeup of an individual but also has a relationship with metabolic and physiological processes, and thus needs consideration in theses contexts.

1.4 Personality and Stress Physiology

The physiological responses to stressors through the HPA axis and the autonomic nervous system (ANS) are often measured alongside personality tests to characterize personality traits, and the sensitivity of individuals to these systems can have an impact on other bodily functions. Stress can trigger the release of cortisol via the HPA axis, and the release of norepinephrine and epinephrine via the ANS (Burdick et al., 2011). The hormones released from these pathways can be measured through blood, saliva, fecal, or hair samples to investigate the concentration of hormones in response to stressors. Other methods to investigate the physiological response to stressors is through heart rate and body temperature measurements, which both increase in response to stress and have been related to behavioral responses to stress in cattle (Hopster and Blokhuis, 1994; Mohr et al., 2002; Stewart et al., 2007; Stockman et al., 2011), swine (Rutherford et al., 2012), and sheep (Wickham et al., 2012). Individuals vary in their strength of response to stressors, and thus the physiological response varies as well (Koolhaas et al., 1999; Koolhaas, 2008).

The strength of the stress response in individuals is important as it relates to a plethora of biological processes, consequently affecting animals' welfare levels. A heightened stress response and hyperactive HPA axis are detrimental to animal welfare. One model for welfare in animals has three primary components: biological functioning (physical wellbeing), natural state (ability to express natural behaviors), and affective state (emotional wellbeing) (Fraser et al., 1997; Weary and Robbins, 2019). In animals, sustained high concentration of cortisol can suppress the immune system (Koolhaas, 2008; Burdick et al., 2011) and alter digestive function (Loerch and Fluharty, 1999).

Thus, hyperactive stress and physiological response can impact the biological functioning (immunity and digestive function) and the affective state (increased anxiety levels and fear) of individuals. The relationship between personality traits combined with physiological responses with health and welfare makes personality essential to understand the differences in performance and welfare level of animals. To properly take the individual differences in personality into account in these contexts, it is imperative that there is methodology to reliably measure personality traits, and these methodologies must be tailored to the species of interest.

1.5 Basics of Measuring Personality

Quantifying personality allows for researchers to better understand why some individuals may perform differently or be better suited to a particular environment. To measure personality traits of individuals requires knowledge on methods to measure and interpret behaviors, the main personality traits seen in animals, and the requirements that must be met to be a valid personality test. Two methods of assessing personality traits are subjective ratings and behavioral coding. Subjective rating is performed by an observer who is familiar with an animal, where a score is assigned to rate an individual based on a particular trait or behavior (Gosling, 2001). For example, a milker could assign a milking temperament score to a dairy cow on a scale of 1-5, with 1 being very calm and 5 being very excitable. Subjective scoring increases the risk for unaccounted variation, such as bias or inter-observer variation. Behavioral coding involves recording behaviors of an individual using predefined behaviors in an ethogram (Gosling, 2001), and can be done for an animal in a natural state or in response to a controlled stimuli in an experimental setting (Carter et al., 2013). Behaviors recorded for behavioral coding can fall into one of three categories: 1) a ‘latency’—time lapsed before performing a behavior, 2) an ‘event’ that takes place in a moment and is often recorded as frequency or count of times occurred, and 3) a ‘state’, which is a behavior or behavioral pattern that occurs over a longer period and is often recorded as a duration (Martin and Bateson, 2007). Four common methods for observing behaviors include the following: 1) ‘ad libitum sampling’, which is an open ended form of observations where any behavior of relevance is recorded, 2) ‘focal sampling’, which records all behaviors expressed by an individual for a defined period of time, 3) ‘scan sampling’, which records the number of individuals in the group performing behaviors of interest at set intervals of time, and 4) ‘behavior sampling’, which records the occurrence of a particular behavior expressed in a group of individuals and is often used for rare behaviors (Martin and Bateson, 2007).

Behaviors observed for an individual using behavioral coding or subjective scoring are interpreted to define personality traits for an individual. One proposed model for describing animal personality outlines 5 main personality traits: 1) “bold-shy”, 2) “active”, 3) “exploratory-avoidant”, 4) “aggressiveness”, and 5) “sociability” (Réale et al., 2007). Another model to describe animal personality follows the five factor model from human psychology (‘The Big Five’) and adds dominance for 6 main personality traits: 1) “neuroticism”, 2) “agreeableness”, 3) “extraversion”, 4) “openness”, 5) “conscientiousness”, and 6) “dominance” (Gosling and John, 1999). Animal personality in primates typically relies most heavily on the human based model of personality traits. Traits of individuals act as dimensions, and each individual can vary where they fall on any of the traits. Although this is a non-exhaustive list for all of the possible personality traits, it encompasses the main traits that are most commonly measured in non-human animals utilizing current tests to identify personality traits, hereafter referred to as personality tests.

The validity of a personality test is the consistency and strength at which it can measure the desired trait (Burns, 2008). The personality trait being measured is dependent upon which test is administered and whether it is a free choice or forced test (Carter et al., 2013). A personality test can measure more than one personality trait, and different personality tests can measure the same personality trait (Réale et al., 2007; Carter et al., 2013). The validity of a test can be determined by how it correlated to personality tests that are established to measure the personality trait of interest, as their outcomes should be correlated (Campbell and Fiske, 1959). However, if the personality test measured multiple traits, then its results can become convoluted, making the relationship between traits unclear. To validate a personality test that may measure multiple personality traits, a factor analysis or principal component analysis can be utilized. These analyses allow researchers to view multiple traits and determine if they are consistent across tests, situations, and time (Goldberg, 1992; Budaev, 2010). Personality research methodology originated in humans and with time was adapted for use in non-human primates, laboratory animals and then to livestock animals, and now the specific details for this development will be discussed.

1.6 Personality Traits Found in Various Species

Personality tests in the research setting have been developed for almost a century. The first empirical study with an aim to label animal personality was conducted in 1938 on chimpanzees, and utilized subjective scoring of individuals and principals from human psychology (Crawford, 1938). The initial work in animal personality research was in the area of comparative psychology. With time, studies that relied on subjective scoring evolved to have less personification. Eventually, behavioral coding, factor analyses, and principal component analyses were taken from human personality research and incorporated for use in animal personality research (Whitham and Washburn, 2017). By the mid-twentieth century, research on personality had spread across fields and incorporated many other species, including great tits, rodents, and livestock species. The development of personality tests and the definitions of personality traits are sometimes shared across taxa. While traits are shared between species, personality tests often require specific methodology based on the species being investigated, as the size, ability to perform some tasks, and the behaviors expressed vary broadly by species. The remaining portion of this review will focus on personality test methodologies across species, measured personality traits, and the relationship of personality traits with performance in wild, laboratory, and livestock species. This review of personality research will focus on how the research in wild and laboratory species has contributed to research in livestock species, specifically in dairy cattle.

Personality Traits in Non-human Primates

Personality research on non-human primates was the first research performed in this area of study, specifically in chimpanzees. One method for rating personality traits for chimpanzees uses subjective rating on a 7-point scale on 43 areas for individuals, and it scores chimpanzees for the personality traits emotionality, openness, surgency, dependability, agreeableness, and dominance (King and Figueredo, 1997). Decades of scoring chimpanzees using the King and Figueredo (1997) personality traits showed that dominance in chimpanzees is a heritable trait (Weiss et al., 2000), and that highly “agreeable” male chimpanzees and highly “open” female chimpanzees had increased longevity (Altschul et al., 2018). Another method involves assigning scores for the personality traits “psychoticism,” “extraversion,” and “neuroticism” based on human psychology (Eysenck, 1963). Scores of chimpanzees using the Eysenck (1963) personality traits were assigned by handlers familiar with the individual by filling out a questionnaire that gave a score 1-7 for 12 different adjectives for each individual. Chimpanzees that expressed less “extraversion” and less “dominance” and chimpanzees that expressed higher levels of “neuroticism” were more likely to be successful in completing a puzzle, while chimpanzees that expressed low “extraversion” and high “dominance” were more likely to abandon the puzzle before it was completed (Padrell et al., 2020). The relationship between the Eysenck (1963) personality traits and performance with a cognitive test indicates that this methodology for classifying chimpanzees is valid and that it is essential to consider personality when interpreting results from cognition tests on chimpanzees (Padrell et al., 2020). The subjective scoring of chimpanzees by handlers showed the utility of subjective scoring for analyzing personality traits, heritability of traits, and the relationship between personality traits and performance measures despite the risk of anthropomorphism.

In rhesus macaques, tests that subjectively scored the attention, motor ability, and reflexes of individuals during infancy were able to characterize individuals in the categories of “orientation,” “state control” (emotionality), “motor maturity”, and “activeness” defined by the Primate Neonatal Neurobehavioral Assessment (Schneider et al., 2001).These researchers found that individuals’ scores for orientation could predict learning ability later in life (Schneider et al., 2001). Additionally, the individual scores assigned using the Primate Neonatal Neurobehavioral Assessment were determined to be heritable in rhesus macaques (Champoux et al., 1999). Another test in infant rhesus macaques profiled “anxiety” or “fearfulness” expression by subjecting individuals to a human intruder tests that measured cooing, freezing, and aggressive behaviors in response to isolation, a human not making eye contact, and a human making eye contact. The human intruder test was able to distinguish individual variation in the magnitude of the response to the test, where a higher magnitude of response was interpreted as a higher level of anxiety or fear during the test (Kalin and Shelton, 1998). The level of anxiety expressed in the human intruder test was found to be consistent through time (Kalin et al., 2001), and the amount of freezing expressed during the test was found to be heritable (Rogers et al., 2008). A measure of the “fearfulness” and “boldness” in juvenile rhesus macaques was conducted using a free-choice novel object test in the home pen of the animals. This test measured the latency for the juvenile to leave their mother, the durations of time off mother, and time >2 feet away from their mother (Spencer-Booth and Hinde, 1969). Spencer-Booth and Hinde (1969) determined that there was individual variation in the expression of “fearfulness” and “boldness” in response to a novel object, and that it related to natural behavioral expression as it was associated with the amount of time the individual typically spent away from the mother. Finally, research in rhesus macaques incorporated factor analysis in animal personality research, and it utilized this analysis to interpret behaviors measured of social interactions between juvenile conspecifics or in response to isolation. Three main factors were found (“affiliative,” “hostile,” and “fearful”) that explained variation between individuals and resembled human personality traits of “extraversion,” “psychoticism,” and “emotionality”

(Chamove et al., 1972). Personality research in rhesus macaques demonstrates the transition from subjective scoring of individuals to behavioral coding and more complex analyses to eliminate unintentional anthropomorphism.

Personality investigation recently has been performed in other non-human primates besides chimpanzees and rhesus macaques. In marmosets, the personality traits measured using novel food tests (exposure to star fruit or jack fruit), a foraging under risk test (presented a desirable food with a threat present), and a predator test (plastic snake) that were repeated over a 4 year period found the personality traits "boldness" and "exploration" to be consistent through time (Šlipogor et al., 2021). In bonobos, personality traits "activeness," "boldness", "openness", and "sociability" were measured using behaviors measured in the home pen, multiple novel food tests, puzzle feeder tests, and predator tests; these test determined all four traits to be heritable (Staes et al., 2016). The traits measured by Staes et al. (2016) were also found to be related to the prevalence of abnormal behaviors, with bonobos that were less "active" showed coprophagy and head shaking more often, and bonobos that were more "social" spent more time plucking hair and less time engaged in coprophagy (Laméris et al., 2021). Abnormal behaviors, such as coprophagy, typically indicate poor mental state or ability to handle stress and thus poor welfare in individuals (Hopper et al., 2016). This demonstrates the relationship between personality traits and performance measures is not species specific in non-human primates.

Across different species of non-human primates, individual differences for various personality traits were found, with many traits showing long-term consistency and heritability. Additionally, relationships between performance measures, such as longevity or cognitive ability, with personality traits were found. Non-human primate personality studies have been the foundation for the incorporation of factor analysis to interpret behavioral coding which are both widely utilized in livestock species personality research.

Personality Traits in the Great Tit

The great tit has been consistently researched since the 1940s (Perrins, 1965), and is considered a highly intelligent species that has been shown to display levels of self-control on par with corvids and apes when given a transparent cylinder test (Isaksson et al., 2018). Much of the research on the personality traits of the great tit has utilized an exploratory test with five artificial trees in a room. The exploratory test measures the latency for individuals to approach four out five of the trees, and classifies great tits based on slow or fast exploration (Verbeek et al., 1996; Drent et al., 2003; Bibi et al., 2019). In juvenile great tits, dominance was related to exploration, as great tits that were fast explorers were more likely to initiate fights with other birds, and were more likely win fights (Verbeek et al., 1996). These findings led to classification of dominant, fast explorers as "active" individuals, and the submissive, slow explorers as "passive" individuals (Verbeek et al., 1996). These findings were later interpreted as "proactive" and "reactive" traits, respectively, by Bibi et al. (2019). "Exploratory" and "dominance" traits in great tits were shown to be related to dominance in relation to social feeding, as "proactive" individuals were more likely to be dominant at feeding meaning they were able to access a competitive feeder sooner, monopolize the feed for longer, and showed more aggressive behaviors (Bibi et al., 2019).

The exploratory personality trait in great tits is known to be heritable over multiple generations (Drent et al., 2003). Lines of great tits were bred based on "exploratory" traits and were bred into fast explorer and slow explorer lines. Glucocorticoid levels were measured in the fast, slow, and natural lines of great tits, and it was found that fast explorers had lower baseline levels of excreted corticosterone metabolites than the slow and wild lines of great tits, and the fast explorer line had lower levels than the slow line after a handling test to illicit a stress response (Stöwe et al., 2010). These findings exemplify the relationship between individual variation in coping styles and physiological response to stress described by Koolhaas et al. (1999). Individual ability to handle stress caused by isolation from siblings was consistent through time for juvenile great tits, and this ability related to the "exploratory" personality trait. The fast exploration line of animals were more sensitive to isolation than the slow exploration line. This finding was repeated in the natural line of great tits, where faster explorers were also more sensitive than their slower counterparts (Fucikova et al., 2009). This stress handling test, showed individual variation and was related to established personality traits in great tits and was consistent through time; therefore, it met the requirements of a valid personality test for great tits (Fucikova et al., 2009).

In great tits, tests of neophobia—fear around eating a novel feed or in the presence of a novel object—were also related to exploratory behavior. Slow exploration birds were more fearful in the neophobia test (Bibi et al., 2019). A variation of the neophobia test in great tits examined the time it takes to resume feeding nestlings in the presence of a novel object. Individuals who were faster to resume feeding were interpreted as less fearful, and expressed more antipredator behaviors, therefore indicating that they were overall more “bold” (Vrublevska et al., 2015).

Personality traits in in great tits show a shift from human based terminology to describe personality traits to the Réale et al. (2007) terminology that is used predominately in the livestock species. The personality traits “exploratory,” “dominance,” and “boldness” were thoroughly explored in great tits. Additionally, the trait “exploratory” was shown to be heritable and related to physiological and behavioral signs related to handling stress. This research indicated that personality traits in great tits were related to coping styles, further cementing the overlap between these two classification systems—personality traits and coping styles—of animal personality. Research in the great tit reflects the importance of personality traits to fitness of individuals to their environment, and it shows the importance of investigating personality traits in livestock species. The research in primates and great tits typically simulated or utilized the animals’ natural environments; in the next section, I discuss animal personality research in rodents which involved testing in synthetic or experimental settings.

Personality Traits in Mice and Rats

Personality is often investigated in mice and rats in order to learn more about human personality and personality disorders, especially in association with medication development and administration. However, for the purpose of this review, the personality traits and coping styles as they relate to rats and mice themselves will be the focus.

In rats, exposure to novel environments—placing an individual in an open arena separate from their home environment—elicits a fear response that can decrease with repeated exposure, indicating that habituation decreases fear response (Blanchard et al., 1974). The level of “fearfulness” an individuals expresses shows individual variation, and is related to novelty seeking behavior; rats that were highly reactive to a novel environment also explored more arms of a maze and visited them for shorter periods of time (Dellu et al., 1993). Therefore, highly reactive rats habituated more quickly to novelty and would seek out more novelty. In a mix of male and female rats, three principal personality traits—“anxiety”, “activity”, and “sexual preference”—were determined using a holeboard (arena with holes in the floor to measure head dipping), an elevated plus maze (maze with closed and open arms that measures time spent in each type of arm), and a sexual orientation test (exposure to sexually mature male and female simultaneously). Sex had an effect on the principal trait that explained the majority of the variance seen in the personality tests, with “activity” explaining most of the variation in females, and “anxiety” and “sexual preference” explaining most the variance in males (Fernandes et al., 1999). The strain of rat being investigated impacts the performance of rats in personality tests and cognitive tests (van der Staay et al., 2009), and impacts the level of anxiety expressed, especially when developed for low or high reactivity (Salomé et al., 2002). When interpreting results from various studies of rats, it is important to note that profiles of personality traits are dependent on the rat strain and sex being investigated; however, the behaviors and personality traits expressed are consistent regardless of these stipulations.

The relationship between personality traits and physiological response to stress is also seen in rats. Rats had their personality traits “anxiety,” “exploration,” and “activity” profiled using an open field test (exposure to an open space), a novel object test (exposure to an unfamiliar item), a light/dark box test (measures time spent in light or dark side of an enclosed box and how many crossings take place between sides), and a circular corridor test (a cylinder in a circular arena makes round alleyway). Rats were then tested in a forced swim test, where behavioral and hormonal response was recorded. Rats that were more “anxious” in the personality tests expressed higher levels of corticosterone after the forced swim test (Castro et al., 2012). This is supported by the findings that a strain of rats bred for high avoidance struggled more and had a higher cortisol response to a forced swim test when compared to a heterogeneous line of rats and a strain of rats bred for low avoidance (Díaz-Morán et al., 2012). Rats that are more “avoidant” and “anxious” are therefore more susceptible to hyper-activity of the HPA-axis.

Mice and rats express exploratory behaviors similarly (Drai et al., 2001), and there is often overlap in the types of personality tests used. In mice, the personality traits “activity,” “anxiety,” and “novel seeking” were successfully measured using an open field test, a holeboard test, a T-maze (3 armed maze where one arm is blocked initially and then later opened; also referred to as a Y-maze), and an elevated plus maze (Ibáñez et al., 2007). The personality traits determined by Ibáñez et al. (2007) were able to predict individual response to a light-dark test, a circular corridor, and a novel arena that automatically measured activity (Ibáñez et al., 2009). The mouse “activity” trait was able to predict level of activity in all three of the later personality tests, the “anxiety” trait of mice could predict time spent in the light portion of the box in the light-dark test, and the level of "novel-seeking" trait expressed could predict activity in the circular corridor (Ibáñez et al., 2009). Thus, these personality traits are consistent across contexts in mice. The traits "exploration," "activity," and "boldness" were found to be consistent through time in juvenile and adult mice by repeatedly testing mice at 6, 7, 12, and 24 weeks of age in a Y-maze, open field test, and novel object test (Schuster et al., 2017).

In mice and rats, personality traits "exploration," "activity," "boldness," and "novel seeking" have been shown to be repeatable across time and context and related to HPA-axis reactivity. The research in rats and mice successfully measures multiple traits using a combination of tests, demonstrating how different personality traits can make up the overall personality of an individual. However, much of the rearing and research for these animals is in a laboratory setting, and thus research examining the expression of such traits in a natural setting is limited. Similarly, livestock species often do not have exposure to what historically would have been their natural environment, and as such, the tests developed for use in laboratory rodents have been successfully adapted for use in livestock species. The novel environment, novel object, and light-dark tests developed in rats and mice have been used for personality tests in swine, sheep, and cattle. Before going into detail on the specifics of personality research in different livestock species and the associations with performance measures, the methodology for common personality tests and the personality traits measured by them in livestock will be covered.

1.7 Important Personality Tests in Livestock Species

Below are the details of various objective personality tests commonly used for research in livestock species. The personality traits measured by these tests have large overlaps in their measurements of “fearfulness,” but the tests also cover “exploration,” “activity,” and “sociability” (Boissy and Bouissou, 1995; Blache and Ferguson, 2005; Forkman et al., 2007; Yuen et al., 2017). The isolation box test is a new addition to these tests since it has not been seen across many livestock species and because it is designed with on-farm use in mind. Therefore, it is discussed with the potential to be adapted across species in mind.

Novel Environment Test

A novel environment test, also referred to as an open field test, measures the response of an individual to a new open area. The open field test was originally developed for use in rats to measure “exploration” and “activity” traits (Hall and Ballachey, 1932). However, with more time and research, it was determined that the introduction to a novel environment elicited a fear response as well, which could be reduced with habituation (Blanchard et al., 1974). The personality traits measured by this test can change based on whether it is a free-choice or forced test (Carter et al., 2013). If the test is free-choice, meaning the animal is allowed to enter and explore an arena or space under their own volition, it will be measuring “exploratory” and “active” traits; however, if the test is forced, it will also be measuring “fearfulness” and “boldness” (de Passillé et al., 1995; Carter et al., 2013). In rats and mice, an open field test is typically $1m^3$ and the duration of the time within the arena can vary (Carter and Shieh, 2015). The behaviors measured in rats and mice during open field test include, but are not limited to, distance traveled and regions of arena visited and duration of time spent in different areas of the arena (Carter and Shieh, 2015). The open field test was first modified from laboratory use in rodents for use in dairy cattle in New Zealand in 1975 to measure temperament (Kilgour, 1975). When used in dairy cattle, novel environment test arenas can vary in size based on the research group and the resources available. In the literature, test arenas have been 13.5m^2 (Van Reenen et al., 2005), 25m^2 (Lecorps et al., 2018b), and 35m^2 (Neave et al., 2019) and can vary in duration from 3 to 30 minutes in length (de Passillé et al., 1995; Van Reenen et al., 2005; Van Reenen et al., 2013; Neave et al., 2018). Behaviors measured in cattle can include the number of quadrants crossed, time spent exploring, time spent active, and number of vocalizations (Van Reenen et al., 2005; Neave et al., 2018). Like in rats, these behaviors are used to measure the personality traits "fearfulness," "exploration," and "activity" in dairy cattle (de Passillé et al., 1995). However, the level at which a novel environment test measures "fearfulness" in cattle is questioned, as it can have low correlations with novel objects (Boissy and Bouissou, 1995) or no correlations with novel person tests (Kilgour, 1975). A multiple test analysis, such as using a novel environment test in conjunction with a novel object test, may allow for an improved interpretation of fearfulness-boldness trait (Forkman et al., 2007; Canario et al., 2013)

Novel Object Test

The novel object test—exposure to an unfamiliar object—was also first used in rats who were exposed to a piece of wood or a metal tray (Chitty and Shorten, 1946), and has been used in rats and mice to measure latency to approach the object and time spent interacting with the object (Larsen et al., 2010; Schuster et al., 2017). These behavioral measures are used to interpret "fearfulness" and "boldness" in individuals. Novel object tests are typically in a test arena that an individual has already been habituated to or within their home pen to remove the influence of the novel environment on behaviors expressed. One of the earliest instances of a novel object test utilized in cattle is by Boissy and Bouissou (1995), which compares the outcomes of the novel object test to many fear-eliciting personality tests. In cattle, the novel object test typically takes place in the same arena that a novel environment test, so the novel environment test acts as the habituation period to the test arena for the novel object test. The duration of a novel object test for dairy cattle can vary from 3 to 15 minutes, and the object utilized can be anything from a large black bucket, a ball attached to a tambourine, a Pilates ball, an umbrella, a bag, or a pyramid, to name a few (Boissy and Bouissou, 1995; Van Reenen et al., 2005; Forkman et al., 2007; Hedlund and Løvlie, 2015; Neave et al., 2018). The behaviors typically measured during a novel object test are latency to approach, time spent interacting, type of interaction with the object, distance from object, and vocalizations (Forkman et al., 2007). The purpose of a novel object test is to measure "fearfulness" and "boldness" in response to novelty, and when used in conjuncture with other test such as the novel environment test allows researchers to differentiate these traits from "exploration" elicited from a new or open environment (Forkman et al., 2007).

Novel Person Test

One final type of novelty test is the novel person test (also referred to as a human approach test, human test, or novel human test). In livestock species, it is especially important to measure response to a human because of the necessary handling involved. The use of an unfamiliar human serves to eliminate any effect that a history a handler has with an individual may have on the outcomes of the test, which is an effect seen across species (McCall et al., 1969; Hemsworth et al., 1981b; Boissy and Bouissou, 1995). The novel person test typically involves a stationary human and measures time to approach and interactions with person, but another version of the test involves a forced approach that has the person approach the animal and measures flight distance and speed in response to the person (Hemsworth et al., 1981a). The duration of a novel person test in cattle can vary based on the research group and is typically 10 to 15 minutes (Boissy and Bouissou, 1988; Van Reenen et al., 2013; Lecorps et al., 2018b; Neave et al., 2018), and it is utilized to measure "fearfulness" (Waiblinger et al., 2006).

Startle Test

Startle tests, also referred to in literature as a surprise test, include the introduction of a sudden surprise to an individual. This test is used less often than novelty tests but remains a valuable tool to measure personality traits in livestock animals. Unlike novelty tests, the startle test is believed to solely measure "fearfulness" or "boldness" in individuals by exposing them to a "threat" (Yuen et al., 2017) rather than introducing individuals to a static novel object, person, or environment – which measures multiple personality traits (Forkman et al., 2007). Research that utilized startle tests in cattle and sheep have involved a puff of air in the face of an individual (Boissy and Bouissou, 1995; Schrader, 2002), dropping a ball into an arena (Vandenheede and Bouissou, 1993), or opening an umbrella (Lauber et al., 2006), but is not limited to these stimuli. Startle tests measure latency to reapproach the item that caused the startle or to resume eating feed next to the item that caused the startle, or time spent frozen after the startle. These measures allow for the interpretation of the personality trait "boldness" or "fearfulness" (Finkemeier et al., 2018). The use of the startle test with novelty tests can allow for differentiation of "fearfulness" and "exploratory" traits (Lauber et al., 2006; Yuen et al., 2017).

Isolation Box Test

The isolation box test was developed to classify sheep based on reactivity within a dark box (Murphy et al., 1994). The most recent version of the isolation box is a $1.5m^3$ enclosed box with an attached agitation meter that can measure movement and vocalizations over a set test period – typically 1 minute (Blache and Ferguson, 2005). The reactivity of sheep within the isolation box is an indicator of the trait "fearfulness," "activity," and stress in response to social isolation in individuals. The test classifies sheep as "more active" or "less active" (Blache and Ferguson, 2005; Murray et al., 2006; Beausoleil et al., 2008; Murray et al., 2009; Amdi et al., 2010). Currently the isolation box test has only been performed in sheep, but it has potential to be adapted for use in other species as we have seen with the development of novelty tests across species. Specifically, there is potential for the isolation box test to be adapted for use within dairy calves as an objective on-farm personality test. However, research is needed to validate the use of the isolation box test in dairy calves. To validate a personality test for use in a new species, it needs to be shown to be repeatable and to correlate with outcomes from personality tests that measure the same trait(s) (Carter et al., 2013). The utilization of these common standardized tests among other personality tests used in livestock animals will now be covered with an emphasis of the relationship of measured personality traits with performance and the ability of certain tests to be utilized on-farm in various livestock species.

Personality Traits in Swine

In livestock species, the relationship between personality traits and performance measures and how they tie back to profit or welfare level of animals are of particular importance. In young pigs, various tests have been utilized to measure “fearfulness” and “aggressive” personality traits. An older test to measure coping style is the backtest, which places an individual on their back and measures number of escape attempts. Individuals that have more attempts to escape are classified as “resistant” or as “high resistance,” and animals that have few to no attempts were classified as “non-resistant” or as “low resistance” (Hessing et al., 1993; Ruis et al., 2001; Bolhuis et al., 2003). Resistance level to the backtest was found to be heritable in swine (Iversen et al., 2017). Piglets that were subjected to a backtest and a mixing test to measure aggression showed that piglets that were “resistant” in the backtest were more aggressive during mixing (Hessing et al., 1993). In another study, half of the pigs scored as “low resistance” and half scored as “high resistance” pigs were raised in a barren environment, and the other half were raised in an enriched environment with bedding. The pigs were then subjected to an immune challenge test. The “low resistance” pigs had a higher immune response (higher antibody titers) than the “high resistance” pigs regardless of housing type. The “high resistance” pigs had no significant difference in immune response by housing type, but the “low resistance” pigs housed in an enriched environment had a lower immune response than “low resistance” pigs in a barren environment (Bolhuis et al., 2003). This indicates that there is relationship between immune response and both personality type and housing type for individuals. Finally, gilts were categorized by the backtest into “low resistance” and “high resistance” and then moved into social isolation housing at seven weeks of age. “High resistance” gilts adapted worse to isolation, with prolonged irregular body temperature for three weeks, while the “low resistance” gilts’ body temperature measures returned to normal after one day. Additionally, gilts that were “high resistance” had higher levels of noradrenaline levels three weeks into isolation (Ruis et al., 2001).

Sow group mixing often triggers aggressive behaviors between individuals (Verdon et al., 2015), and “aggression” and “dominance” of sows were shown to have stable individual differences (Horback and Parsons, 2016; Verdon et al., 2016). The level of “dominance” displayed by a sow can have an impact on their welfare level. A group of sows mixed at breeding had aggressive behaviors measured at feeding and plasma cortisol and skin lesions measured through gestation. Sows that were more “dominant” gained more weight and had fewer skin lesions, while “submissive” sows had the most skin lesions, and “subdominant” sows (intermediate between submissive and dominant) had the highest cortisol concentration two days after mixing (Verdon et al., 2016). The sows that are more “submissive” or “subdominant” would be the animals that would be the most affected due to lack of space or feed resources in sow group mixing, consequently diminishing their welfare level. The findings of Verdon et al. (2016) in relation to skin lesions were contradicted by another study that indicated that more “aggressive” or “dominant” sows were more likely to have skin lesions (Horback and Parsons, 2016). Sows that were more “dominant” and “aggressive” when groups were mixed also were able to access feed first (Horback and Parsons, 2016), which may relate to why “dominant” sows gained more weight in Verdon et al. (2016). The aggression of sows at mixing is a heritable trait (Løvendahl et al., 2005), and thus should be taken into consideration in breeding programs.

Sow mothering ability is important for the productivity of an operation. In more "fearful" sows (that were more avoidant of a person), a higher incidence of stillbirths was found (Horback and Parsons, 2016). At farms where there were fewer piglets produced per sow each year, there were more "fearful" sows in response to people than in higher producing farms, as indicated by quicker withdrawals from an approaching person and longer latencies to approach a stationary person (Hemsworth et al., 1981a). Young female pigs were subjected to an immobility test (holding pig on their back and measuring escape attempts), a novel object test, and a novel person test; these tests found consistency in "fearful" scores for individuals across 8 and 24 weeks (Janczak et al., 2003a). However, the magnitude of the response to the novel object and novel person decreased over time, leading to increased time spent exploring the novel person and object (Janczak et al., 2003a). This is likely due to the habituation of the young animals with time (Jensen, 1994). The "fearfulness" and "anxiety" traits of the young female pigs measured in response to the immobility test, novel object test, and novel person test (Janczak et al., 2003a) was related to maternal ability later in life (Janczak et al., 2003b). Gilts that expressed more fear in the presence of a person early in life later had longer farrowing durations, an increased number of piglets dying before three weeks of age, an increased number of stillborn piglets per litter, and an increased number of piglets dying with no milk in their stomach (Janczak et al., 2003b). Personality traits are related to mothering ability, and possible to measure in young individuals and has potential to be utilized to select for better mothering ability before a gilt is even pregnant the first time.

The backtest was developed specifically for use in swine and was shown to be related to the personality traits "fearfulness," "aggression," and "dominance" measured using other personality tests. The study of personality in swine shows the importance of understanding the individual to comprehend why certain individuals outperform others or adapt more easily to changes. This information can be used to better fit the care and housing needs of the diverse personality types among the swine or to create breeding program to produce animals that more fit for the existing environment. Therefore, personality traits are an important component to consider in management decisions regarding housing, feeding space, and breeding programs in swine. The backtest is an example of a personality test that was implemented on-farm as it is based around existent handling techniques used to castrate piglets and has the added benefit of being objective because the number of restraint attempts are utilized to categorize individuals. The adaption of the backtest for larger species, such as cattle, would be difficult because of their larger size even at a young age. However, in cattle, specifically in beef, a restraint test is utilized that typically involves the scoring of individuals in a chute but does not have a simple objective measuring system as we see in swine.

Personality Traits in Beef Cattle

In beef cattle production, the handling of cattle is less frequent and has led to the development of personality tests to be used during the limited handling encounters. One of these tests assigns a chute score from “calm” to “aggressive” typically on a scale of 1-5 (Grandin, 1993) or 1-6 (Tulloh, 1961; Turner et al., 2011) for cattle restrained in a chute. Exit/flight speed (time to cross a set distance), exit velocity (meters or feet per second), and exit scores (subjective classification of how fast or excitably an animal exits) are often used with chute scores as they can be determined from the release of the cattle from the chute (Vetters et al., 2013; Bates et al., 2014; Parham et al., 2019).

Subjective chute scores have been shown to be repeatable (Grandin, 1993; Voisinet et al., 1997), but have been shown to decrease with habituation to the chute (Curley et al., 2006; Hall et al., 2011). Exit scores subjectively assigned to cattle leaving a chute have been shown to be highly correlated with exit velocity (time to cover set distance divided by distance; Parham et al., 2019), and moderately correlated with flight speed (time to cover a set distance; Vetters et al., 2013). Breed and sex have been shown to affect chute scores and flight speed (Hoppe et al., 2010). The subjective exit scores assigned to cattle can vary by observer, or by day for a single observer (Vetters et al., 2013). When any subjective score is utilized to classify animals by personality, inter- and intra-observer variation should always be considered.

Recent studies have investigated an objective system of scoring cattle within a chute. Early use of an objective chute score categorized beef steers based on fluctuations in voltage to scale load cells in a chute for a 1 minute period (Sebastian et al., 2011). This developed into measuring objective chute score by placing cattle in a chute with a scale recorded the standard deviation of their weight at an interval of 5Hz for 10 seconds to produce a numeric measure of how active an individual was within a chute (Bruno et al., 2016; Bruno et al., 2018). The objective chute score was assigned at the same time a subjective chute score and exit velocity was given for each individual. The objective chute score was positively correlated with the subjective chute score (Bruno et al., 2016).

Personality tests in beef cattle are not limited to chute scores and flight speed but also expand to novel object and novel environment/open field tests for research purposes. One study involved 11 different personality tests on a group of steers and heifers including chute scores, flight speed, open field, novel object, and human approach tests;

the results of this study indicated that variation in behavioral responses from these tests was largely explained by two principle components, identified as "avoidance of humans" and "general level of agitation" (Kilgour et al., 2006). Expanding the personality tests administered to cattle allowed researchers to measure other aspects of beef personality, especially in regard to response to humans which was not measurable with just a chute score and flight speed. In another study, beef cattle were subjected to a social isolation test (essentially an open field test) and had flight speed measured; this study found that flight speed had a positive correlation with behaviors expressed in the social isolation test, including the number of subareas of the test arena entered (Müller and von Keyserlingk, 2006). This study indicated that there may be overlap in what is measured between an open field test and flight speed in beef cattle. While these more common personality tests performed in livestock species have been used with beef cattle, chute scores and flight speed/exit velocity measures remain more prevalent.

Chute scores and exit velocity (or exit scores) are related to physiological responses related to stress and immunity. In cattle that were more excitable when exiting, a consistently higher cortisol response was seen when compared to their calmer counterparts (Curley et al., 2006). Cattle with a slow exit velocity were found to have a higher antibody response to a vaccine than their fast counterparts (Bruno et al., 2018). This is in line with what is expected by Koolhaas, 2008, as more excitable or "fearful" animals are expected to release higher levels of cortisol (have hyperactivity of the HPA axis), and thus may have suppressed immune systems.

The use of accelerometers worn by beef cattle has allowed activity level to be compared to various personality trait measures. Cattle with low objective chute scores were found to be more active within their home pens, and cattle with fast exit velocities were also more active within their home pen (Bruno et al., 2018). Other research groups measured agonistic behaviors at feeding and home pen activity in addition to the normal chute score and flight speed measures. Cattle with a greater flight speed had higher activity in their home pen, and cattle deemed more "dominant" spent more time standing in the home pen (MacKay et al., 2013). Finally, cattle that were considered more "excitable" from chute scores had shorter feeding times, while cattle that were more "dominant" spent more time feeding than their more "submissive" counterparts (Llonch et al., 2018).

In beef cattle the personality traits associated with handling, often designated as "temperament", are related to performance, such as fertility or yield and quality of the carcass at slaughter. Cows deemed to have excitable temperaments from chute scores and exit velocity had lower pregnancy rates, calving rates, and tended to have lower kg of calf weaned per cow (Cooke et al., 2011; Cooke et al., 2012). Many studies using chute scores and exit velocity have shown that "calmer" cattle grow more than "excitable" cattle (Voisinet et al., 1997; Bates et al., 2014; Bruno et al., 2016; Bruno et al., 2018). A higher exit velocity was negatively associated with yield grade for cattle, and a higher chute score was negatively correlated with the amount of marbling present (Bates et al., 2014). In cattle assigned a catch score based on Grandin (1993) on difficultly to place in chute, cattle with a lower catch score ("calmer") had higher levels of marbling, and cattle with a slow first exit velocity had more tender ribeye than their more reactive counterparts (Hall et al., 2011). Therefore, results suggest that "calm" beef cattle grow more rapidly and have higher quality meat.

The personality tests developed in beef cattle revolve around procedures that are commonly used for routine handling, and the subjective scoring system for chute scores and exit scores makes the personality testing accessible for on-farm use. These subjective scores have successfully been able to categorize cattle for fearfulness during handling and to relate these measures to production traits. The objective measures for chute score and flight speed eliminate the bias or variation that can be caused by human error. While flight speed is an objective personality test that is easily used on-farm, the objective chute scores require more development to make practical for use outside the research environment. The objective chute score has only been explored in beef cattle and has the potential to be expanded. Finally, the utilization of precision technology in beef cattle and its connection to outcomes of personality tests shows the potential to expand this methodology across species, using this technology to measure individual variation in day-to-day behaviors that may relate to animal personality.

Personality Traits in Sheep

Like in the other species already explored, sheep show stable individual differences in various personality traits that are heritable and related to performance, and like in beef cattle, a common personality test in sheep involves measuring reactivity to restraint. This test is the isolation box test, which classifies sheep based on reactivity within a $1.5m^3$ enclosed box by using an agitation meter to measure movement and vocalizations over a 1 minute test period to then assign agitation scores (Blache and Ferguson, 2005). The reactivity of sheep within the isolation box is an indicator of fearfulness and activity in individuals and classifies sheep as “more active” or “less active” (Blache and Ferguson, 2005; Murray et al., 2006; Beausoleil et al., 2008; Murray et al., 2009; Amdi et al., 2010). The use of an isolation box test for sheep was shown to be highly repeatable, and could be successfully utilized to breed and select sheep based on temperament classification (Blache and Ferguson, 2005). The extremes from "more active" and "less active" sheep were selectively bred, and were able to successfully produce divergent flocks of sheep (Beausoleil et al., 2008). The isolation box test is one of the few personality tests that sheep producers can potentially use on-farm to consider temperament in their breeding programs.

The arena test, sometimes referred to as the conflict test, is a test that looks at the "sociability," "activity," and "fearfulness" of sheep by placing an individual in an arena with a person in between them and conspecifics visible through the wall (Boissy et al., 2005; Beausoleil et al., 2008; Wolf et al., 2008).The arena test can produce similar outcomes to the isolation box test, with sheep that were "more active" within an isolation box test also being classified as "active" from an arena test (Beausoleil et al., 2008). The traits "sociability" and "fearfulness" measured in the arena test were also shown to be heritable in sheep (Wolf et al., 2008). In the arena test, females were more "active" and avoidant ("fearful") of the humans than males (Boissy et al., 2005), which is a pattern seen in across many personality tests in sheep (Vandenheede and Bouissou, 1993).

Relationships between personality and productivity are also seen in sheep. The sociability and vigilance of lambs determined in a series of personality tests that exposed them to isolation, a novel environment, and humans, was associated with cortisol levels and pH of the meat at slaughter (Deiss et al., 2009), which has a direct impact on meat quality (Kim et al., 2014). Personality is also associated with growth, with "calm" lambs—determined from flight speed from a weight scale—having greater weights at the end of the fattening period compared to “aggressive” lambs (Pajor et al., 2008). Additionally, “more active” sheep in an isolation box had greater feed efficiency than “less active” sheep (Amdi et al., 2010). The social rank of ewes—determined from scan sampling measures of interactions with conspecifics in their home pen—was related to fertility and growth, with more “avoidant” ewes with a low social rank having lower fertility and lower body weights than their “affiliative,” “aggressive,” or “progressive” flock mates (Miranda-de la Lama et al., 2019). Finally, in regards to dairy production, dairy ewes that were “less active” in an isolation box produced more milk (Murray et al., 2006; Murray et al., 2009).

Sheep are very gregarious animals; thus personality tests have focused on the magnitude of their reaction to isolation from their flock or interaction with their flock (Dodd et al., 2012). The “fearfulness” and “sociability” of individuals have been successfully measured repeatedly in sheep and shown to be related to important production traits (Dodd et al., 2012). Most notably, the use of the isolation box test in sheep is the first instance where a personality test was designed to be implemented in a commercial setting for use by producers (Blache and Ferguson, 2005). The isolation box has potential to be adapted to other species.

Across species—whether wild, laboratory, or livestock species—there are methodologies in place to measure personality tests in a research setting. A limited number of personality tests are suitable for use on-farm because they involve complex behavioral and statistical analysis. In swine, beef cattle, and sheep there are objective methods to classify personality traits on-farm through backtests, flight speed tests, and isolation box tests. In dairy cattle, as of today there are objective personality tests that can be used for research purposes, and subjective personality scores that can be used on-farm. The development of an objective personality test that could be implemented on-farm could be greatly beneficial to the dairy cattle industry in accurately identifying personality traits that could be used in management decisions.

Personality Traits in Dairy Cattle

Dairy cattle are one of the most extensively handled livestock species. Calves in the United States are almost always immediately separated from the dam and reared by producers, increasing the handling of the individual compared to the beef counterparts (Basarir and Gillespie, 2006) As adults, cows are milked a minimum of two times per day on top of any other handling performed for maintenance purposes. Therefore, it is especially important that personality traits of dairy cattle make individuals well suited to adapt to changes, to stay calm around handling, and to high production and performance results.

There are many different personality tests used on dairy cattle. The standardized tests used in beef cattle, such as subjective chute score (Grandin, 1993) and flight speed, were also explored in dairy cattle. Flight speed was consistent across time for dairy cattle, and dairy cattle with a greater flight speed were also more calm in response to an approaching person (Gibbons et al., 2011). This indicates agreement between flight speed and a human approach test that is used more commonly in dairy cattle studies. However, chute score was found to be not repeatable and thus not useful in dairy cows (Gibbons et al., 2011) Similar to how chute scores are designed around routine procedures in beef cattle, milking temperament scores are subjective scores assigned to dairy cows in the milking parlor. Subjective milking temperament scores are assigned on a scale, such as from "very nervous" (1) to "very quiet" (5), and can be assigned during udder preparation and milking (Szentléleki et al., 2015). Milking temperament is a system that can be easily utilized on-farm, and has been shown to be associated with milk yield and to be somewhat heritable (Marçal-Pedroza et al., 2020). However, the scales used to measure milking temperament vary widely and require training of observers for proper use, leading to the potential for human error and bias to impact scoring. An objective method to measure milking temperament is to count steps and kicks during udder preparation and during milking itself (Hedlund and Løvlie, 2015; Marçal-Pedroza et al., 2020). A drawback of milking temperament is that it can only be measured in adult cattle already in the milking herd, and thus cannot be used as a means of identifying replacement animals that should join the herd after years of investment into an individual.

Personality tests commonly used in dairy cattle research that can be used across age groups are the novel environment/open field tests, novel object tests, and novel person or human approach tests. These three tests combined measure "exploration," "activity," and "fearfulness" (de Passillé et al., 1995; Forkman et al., 2007). In dairy calves, personality traits determined through novel environment and novel objects tests were consistent from 3 weeks of age to 26 weeks of age (Van Reenen et al., 2005). Another group indicated that personality traits found in dairy calves through these tests were consistent from preweaning to post-weaning and were consistent from the onset of puberty to the first lactation (Neave et al., 2020). The upset between the post-weaning period and puberty is most likely due to individual plasticity and development during puberty. Additionally, personality traits determined in 7-month-old heifers were able to predict personality traits that related to milking performance during the first lactation (Van Reenen et al., 2013). Finally, the personality traits determined through novelty tests showed that calves that were highly "explorative" in novel environment, novel object, and novel person tests consumed starter sooner, had a higher starter and total DMI overall, and had a greater total ADG (Neave et al., 2018). The findings by Van Reenen et al. (2013) and Neave et al. (2018) support the notion that personality should be measured early in dairy cattle, where it would create the ability to tailor management and to select individuals that will have better performance as youngstock and in future production.

"Sociability" traits of dairy cattle can be measured as response to isolation, social disruption, or social interactions. Through these tests, dairy heifers show stable individual differences in response to social stress caused by moving individuals to a new group; tests also found that "avoidant" heifers in a new group had decreased lying and eating times (Nogues et al., 2020). In milking cows, feeding time (Johnston and DeVries, 2018) and lying time (Krawczel and Grant, 2009) are positively associated with milk production. Therefore, the animals who see an upset in feeding and lying behavior with social change would likely perform worse in the fresh period. Indeed, cows who spent more time facing their herd in a social isolation test, and thus were deemed more bothered by social stress, were found to produce less milk in their first lactation (Hedlund and Løvlie, 2015). Nogues et al. (2020) and Hedlund and Løvlie (2015) demonstrate how the differences in "sociability" can be measured and can be related to performance in dairy cattle.

The physiological response to stress associated with coping style is also seen in dairy cattle. In dairy calves, the calves classified as "fearful" by standardized tests, which exposed them to open field, novel object, and novel human tests, had higher vocalizations and higher ocular temperature after transportation than their “bold” peers (Lecorps et al., 2018a; Lecorps et al., 2018b). Calves that were more hesitant to approach a novel object had higher cortisol response to standardized personality tests than their peers that approached the novel object quickly and touched it more (Van Reenen et al., 2005). In dairy cows, the ability to adapt to milking showed consistent individual differences, and cows with high inhibition of milk injection also had greater increase in heart rate in the initial days being milked (Van Reenen et al., 2002). The variations in the ability to handle stress as a calf, heifer, and cow relate to the variations in coping style described by Koolhaas et al. (1999).

Personality traits and coping styles of dairy cattle have successfully been measured and related to performance measures throughout their lifetime. However, the objective measures of personality traits in cattle often involve multiple standardized personality tests, behavioral analyses, and statistical analyses that make them unpractical for use by producers. The subjective measures of dairy cattle, such as milking temperament, have potential for human error and bias, and do not allow producers or researchers to consider personality traits until they are in the milking herd—after years of investment into the animal. There is potential for other tests, such as the isolation box test used in sheep, or measures from precision technology to allow for objective and relatively quick measures of individual variation at an early age. More research is needed to develop these tests for use in dairy calves on-farm to allow for easier measurement of dairy cattle personality early in life.

1.8 Summary and conclusions

Animal personality research involves the measurement and interpretation of behaviors across a variety of situations. Many species, whether wild, laboratory, or livestock species, have had personality traits measured successfully, and have established relationships between personality and performance. The tests designed to measure personality traits can be subjective or objective but should be designed based on the expected behaviors for the species and personality trait being measured. Objective measures of personality through behavioral coding, automatic measures of activity, or feeding behavior eliminate bias or variation produced by humans. The accessibility of personality tests on-farm is limited, and no objective on-farm tests exist currently for the personality assessment of dairy calves.

The subjective scoring of animals on a simplified scale is most accessible to producers; however, it introduces issues with variation between observers or between a single observer over time. Examples of these subjective scoring systems include chute scores (Grandin, 1993), exit scores (Parham et al., 2019), and milking temperament scores (Szentléleki et al., 2015); all of these score based on the animal's reactivity to restraint/handling or speed to exit restraint. There has been research aimed at making objective measures for chute scores (Bruno et al., 2016; Bruno et al., 2018) and flight speed (Parham et al., 2019). Chute scores and flight speed are primarily used in beef cattle, but when explored in dairy cattle only flight speed was repeatable (Gibbons et al., 2011). However, these tests are not ideal for adaptation because habituation occurs from repeated exposure to a chute which reduces the reaction of individuals (Curley et al., 2006; Parham et al., 2019), restraint in a chute is not common practice in dairy calves, and milking temperament cannot be measured until individuals are in the milking herd. The backtest seen in swine is another objective personality test accessible for on-farm use for young animals (Hessing et al., 1993). However, adaptation to calves is impractical because of the greater size in cattle and could quickly become difficult or dangerous for handlers to restrain manually. The isolation box test has potential to be adapted for use in other species to profile personality traits individuals from a young age without use of complex standardized personality tests.

The relationship between individual variation and performance, the long-term consistency of production, and heritability of personality traits makes personality traits of individuals valuable information to producers. In dairy cattle, the novel environment, novel object, novel person, and startle tests can be used to objectively measure personality traits across all ages; however, these tests are labor intensive to conduct and analyze and thus is not practical for on farm use. The isolation box test was developed for use in sheep but has the potential to be implemented for use in dairy calves. Dairy calves remain the focus for the development of a more accessible personality test because it would provide information allowing for early management decisions to limit investment in poor performers and to select replacements that are more adaptable.

1.9 Thesis Objectives

The research described in this thesis aimed to investigate the development and utilization of an isolation box test for dairy calves on-farm. The specific aims of this research were to 1) develop an isolation box and methodology to measure behavioral responses of dairy calves subjected to an isolation box test, 2) determine if individual differences measured from the isolation box test correlate with behavioral responses and personality traits measured using traditional tests (novel object, novel person, and startle tests), and 3) examine the relationship between behavioral response measures in an isolation box and weaning performance measures.

CHAPTER 2. DEVELOPMENT AND UTILIZATION OF AN ISOLATION BOX TEST TO CHARACTERIZE PERSONALITY TRAITS OF DAIRY CALVES

2.1 Introduction

The ability to characterize animals based on personality has many practical applications in animal production such as management ease, genetic selection, and welfare implications for animals (Koolhaas and Van Reenen, 2016). To measure and utilize personality traits in a population there must be variance in traits across the population (Kaiser and Müller, 2021). In cattle, personality traits of "fear" (Forkman et al., 2007), "boldness" (Foris et al., 2018), "exploratory" behavior (Neave et al., 2020), "sociability" (Lecorps et al., 2018a) and coping style in response to stress (Van Reenen et al., 2005) are related to stable individual characteristics that can be reliably measured. The individual differences for these personality traits can impact how cows and calves handle the many transitions and stressors that they experience in the dairy industry and relate to overall performance. For example, in dairy cattle, a novel object test showed that cows that are calmer produce more milk and kick less within the milking parlor (Hedlund and Løvlie, 2015). Additionally, dairy calves that were categorized as more "explorative-active" during a novel environment test were shown to have a higher ADG and improved starter intake than other calves through weaning (Neave et al., 2018). Beef cattle with a slower exit velocity had higher ADG (Bruno et al., 2016), steers that are easier to catch in a chute had increased marbling, and steers with a slow first exit velocity had higher meat quality (Hall et al., 2011). Together these studies support the concept that cattle differ in how they handle novel or stressful situations and these differences relate to performance of these animals.

For a test to be sufficient to categorize animals based on personality traits (or temperament (Réale et al., 2007)), it must produce variation across a population, be consistent through time, and the behavioral responses measured should be consistent across situations (Kaiser and Müller, 2021). The most common tests used to measure personality traits in dairy calves are novelty tests which include a novel environment test, novel person test, and novel object test. Novelty tests have been shown to be consistent over time from the preweaned period through the first lactation in dairy cattle, with an exception at the onset of puberty (Neave et al., 2020). A startle (surprise) test has been shown to correlate with other personality tests that measure fear responses in heifers, such as novel environment and novel object tests (Boissy and Bouissou, 1995). In adult dairy cows, a puff of air used to startle the cow was shown to elicit reactions that were consistent through time (Schrader, 2002). The use of these standardized tests meets the requirements for characterizing personality traits in dairy cattle, but currently they are very intensive and not practical for on-farm use.

The isolation box test may be an alternative test that could quickly and efficiently test for personality differences in animals. Previously, the isolation box test was used to score sheep based on reactivity within a 1.5m^3 enclosed box by using a agitation meter to measure movement and counting vocalizations over a 1 minute test period to assign them agitation scores (Blache and Ferguson, 2005). The use of an isolation box test for sheep was shown to be highly repeatable and was successfully utilized to breed and select sheep based on personality trait classification (Blache and Ferguson, 2005). Additionally, the isolation box test was shown to be consistent across situations, with the sheep that were more active within an isolation box test also being classified as more "active" during a motivation conflict test with a person between the sheep and their flock mates (Beausoleil et al., 2008). Highly reactive sheep in an isolation box had greater feed efficiency than less reactive sheep (Amdi et al., 2010). Dairy ewes that were more calm in an isolation box produced more milk (Murray et al., 2006; Murray et al., 2009), but there is conflicting evidence whether calmer ewes also produce more milk protein (Sart et al., 2004; Murray et al., 2009). The isolation box test is a valuable tool for producers to determine personality traits and select animals based on these traits (Blache and Ferguson, 2005) and relates to important measures of productivity. There may be potential to adapt this test for use in dairy calves that would provide a quick and reliable test for personality traits.

Personaltiy tests that are already established for use in dairy calves have shown that personality traits were associated with feed intake and growth through weaning (Neave et al., 2018). These measures are of particular importance to producers as both feed intake (Heinrichs and Heinrichs, 2011) and weight gain (Chester-Jones et al., 2017) through weaning have been shown to be positively associated with milk production later in life. A new personality test for dairy calves, for instance the isolation box test, should also relate to these important production characteristics in early life to provide useful information about the individual. Thus, the relationship between the isolation box test and growth is worthy of investigation.

The main objective of this study was to develop an isolation box test to characterize personality traits of dairy calves on-farm. More specifically, we aimed to determine 1) if the isolation box test could identify individual differences in behavioral responses among dairy calves 2) if measures from the isolation box test were comparable to behavioral responses and personality traits derived from a novel person, novel object, and startle test, and 3) if personality trait(s) derived from the isolation box test are related to performance through weaning.

2.2 Materials and Methods

Holstein calves (n = 27) born at the University of Kentucky Coldstream dairy were enrolled into the experiment at 4 d of age between January and December 2020 under the approval of the University of Kentucky's Institutional Animal Care and Use Committee (IACUC #: 2019-3374).

Calves were removed from their dam within 6 hours and moved into a neonatal pen (3m × 3m) bedded with sawdust, within the calf barn, for 4 d. All calves had a birth weight recorded within 24h of discovery. Within this period, calves received either maternal colostrum or colostrum replacer, standard vaccines, had their navel dipped in an iodine solution, and received an ear tag. Also within this period, calves were assessed for passive immunoglobulin transfer with successful passive transfer defined as BRIX ≥ 8.4 % (Bielmann et al., 2010). At 4 d of age, calves were moved to a 4.6 m x 10.6 m group pen bedded with wood shavings for the experimental period with 6 ± 3 other calves. The group pens were emptied, disinfected, and re-bedded with sawdust every 2 weeks. Calves placed in the group pen had an automatic milk and solid feed feeder system (CF100, Forster-Technik, Engen, Germany) where calves were fed their first meal with human assistance and were assisted twice daily (0900 and 1700h) until independent consumption was observed. The automatic feeder system recorded daily milk replacer and starter consumption through weaning. Calves were allotted up to 14 L (140 g/L) milk replacer/d from the automated milk feeder (Cow's Match Cold Front; Land O' Lakes Animal Milk Products Co., Shoreview, MN) until 45 d of age, reduced to 50% allotment for 14 d, and then reduced to 20% allotment for an additional 7 d until complete weaning at 67d of age. Calves completed the study at 2 weeks post-weaning, at 81 d of age. Both the automated milk feeder and the calf starter feeder were calibrated weekly according to manufacturer instructions. All calves had *ad libitum* access to an automated waterer and chopped alfalfa hay in troughs.

Calf starter and chopped alfalfa hay were sampled weekly and immediately frozen at -20 °C. Later, the feed samples were weighed, dried in a forced air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) for 48 h at 55 °C, and weighed again to calculate % dry matter. Then, weekly samples were ground through a 1 mm sieve screen (Standard Model 3 Wiley mill, Arthur H. Thomas Co., Philadelphia, PA), composited into monthly samples, and sent to a laboratory (River Rock Lab, Watertown, WI) to determine nutrient composition (Table 2.1). Briefly, starch was analyzed using the acetate buffer only method as validated by Hall (2009). Crude protein was analyzed according to the Dumas method with a N analyzer (FP-528; LECO, St. Joseph, MI, USA; (Wiles et al., 1998). Crude fat, ash-free NDF, and ADF were calculated using Ankom Technology (Macedon, NY, USA). The ash-free NDF, and ADF were analyzed using the cell wall fractionation method as described in detail by (Surendra et al., 2018). Crude fat was analyzed using high temperature extraction (Ramos, 2005). The ME kcal/kg was calculated from chemical composition according to the equation TDN x 0.04409 x 0.82 (NRC, 2001).

Performance Measures

Health exams were performed daily at approximately 0830h from 4 d of age until 2 weeks post-weaning following Cantor et al, (2021). Briefly, calves had rectal temperature taken and were assigned a score for respiratory disease and diarrhea during the daily health exam. If illness was detected according to the health scores, calves were enrolled into a treatment protocol per standard farm procedures developed with the farm veterinarian and antibiotic treatments were recorded. Health information was utilized to control for disease in analyses regarding performance measures.

Weights were taken twice weekly from birth to 2 weeks post-weaning using an electronic scale (Brecknell PS1000, Avery Weigh-Tronix, LLC Brand, Fairmont, MN, USA). Average daily gain (ADG) was used as our main performance measure, which was calculated for the full milk allowance period (day of enrollment until day 45 of age; **Preweaning ADG)**, stepdown milk period (day 46 until day 66 of age; **Weaning ADG),** no milk allowance (day 67 until day 81 of age; **Post-weaning ADG**), and for the total experimental period from enrollment to 81 d of age (**Total ADG**).

Standardized Personality Test Procedures

Calves were subjected to three traditional personality tests (novel person, novel object, and startle tests) and the isolation box test at 24 ± 3 d of age. All behaviors from the novelty and startle tests were recorded via a camera mounted above the test arena. The test arena (4.88m wide, 4.88m long, 1.8m tall), located in the calf barn, was an enclosed space with solid plywood walls and bedded with wood shavings (Figure 2.1). During all tests, an observer sat outside the test arena and record vocalizations. Calves experienced all tests in a single day in the same order, with a 30-minute rest period in their home pen in between each test.

Novelty person and novel object tests: Before the start of the personality tests, calves were given a solo 30 min habituation period in the test arena where no person or object was present. Calves were first subjected to a novel person test, where the calf was walked from the home pen to the test arena for a 10-minute test period with an unfamiliar person dressed in a navy coveralls and wearing a navy baseball hat with their hands placed in their pockets and looking forward toward the entrance of the test arena. The novel person was positioned 1.22 m from the rear and centered between the left and right walls of the test arena (Figure 2.1). The novel person did not engage with the calf throughout the test period. At the end of the 10-minute period the calf was walked back to the home pen for the rest period between tests. Calves were next subjected to the 10-minute novel object test in the test arena; the novel object was a large 94 L black bucket placed in the same position as the novel person. At the end of the 10-minute period the calf was returned to the home pen for the rest period. The 10-minute length of the novel person and novel object tests was selected to allow as much time as possible for calves to choose to approach the human or object, but within practicalities of conducting several tests in a day on multiple calves.

Video observations and manual vocal observations were recorded throughout each of these test periods. The behaviors that were measured from the video were latency to approach novel person or novel object, time spent touching person or object, inactive, resting, attentive, playing, grooming, exploring the environment, and frequency of bucking, withdrawals and urination/defecation (see Table 2.2 for an ethogram).

Startle Test: The third test that calves were subjected to was the startle test where a red umbrella was placed through a hole in the right wall of the test arena (Figure 2.1). The umbrella remained closed until the calf approached the umbrella or if 5 minutes elapsed without the umbrella being approached. Approach was defined as the calf being within a head length of the umbrella. After the umbrella was opened, calves were observed for a further 5 min which allowed the calf the opportunity to re-approach the umbrella, or approach for the first time if they failed to do so before the umbrella was opened.

Video observations and manual vocal observations were recorded throughout the startle test period. The behaviors that were measured from the video were latency to approach closed umbrella and after startle, time spent touching umbrella, time spent inactive, resting, attentive, playing, grooming, exploring the environment, and frequency of bucks, withdrawals and urination/defecation (Table 2.2). Only latency to approach the umbrella was recorded before the umbrella was opened to ensure that the behavioral measures were for the same test period length across all calves. If a calf did not approach the umbrella before it was opened at 5 minutes, then latency to approach was recorded as 5 minutes. If calves did not reapproach the umbrella after it was opened, this latency was recorded as 5 minutes from the time the umbrella was opened.

Video recordings from the novelty and startle tests were watched by a single observer using an ethogram to record the respective behaviors described above. Observations were observed using a behavioral coding software (The Observer XT 14, Noldus Information Technology, Wageningen, The Netherlands).

Isolation Box Test Procedures:

After the startle test, calves were subjected to the final test in the series, an isolation box test. The isolation box was a metal frame enclosed in plywood sealed with an insulator foam to block out the light. The design and implementation of the isolation box test was based on the traditional box tests in sheep (Murphy et al., 1994) with amendments to make appropriate for use in calves and to operate within our facilities. The frame of the isolation box was supported off the ground by two legs in the front of the box and with wheels at the rear of the isolation box (Figure 2.2). The internal compartment of the box was 1m high, 1.4m long, and 0.6m wide. The front of the isolation box was on hinges to allow the calf to enter and exit. To record movements of the calf within the isolation box, five 3-axis and G force accelerometers (HOBO Pendant g Data Logger, ONSET, Bourne, MA, USA) were fixed to the outside of the box on the back, left, right, top-front and top-back (Figure 2.2).

At the time of testing, calves were moved from their home pen to the isolation box where their rear was faced toward the door of the isolation box. The isolation box was then shaken as a start signal across all 5 accelerometers fixed to the isolation box. Then the calf was pushed backwards into the isolation box and a five-minute timer was started with the close of the isolation box door. During the test, the observer sat and recorded any vocalizations that were emitted. Additionally, the observer recorded any incidence of urination or defecation through the isolation test period by checking the floor of the box at the end of the test. At the end of 5 min, the door to the isolation box was opened and the calf was permitted to exit and assisted by the observer if necessary. The isolation box was shaken a second time as a stop signal at the end of the test. The raw data from the accelerometers utilized for the isolation box test were plotted to check for irregularities (such as any shift of the axes) and to identify the start and stop signal that marked the test period. The start time of the isolation box observations was immediately after the start signal of the test. Only the 480 observations (240s) immediately following the start signal of the test period for each accelerometer were included for analysis; this was to ensure the number of observations were consistent across calves and to trim any movement caused by removing the calf from the isolation box.

Statistical Analysis:

All statistical analyses were performed with SAS (version 9.4; SAS Inst. Inc., Cary, NC) with the calf as the experimental unit. Additionally, R (version 4.1.0; https://www.R-project.org/) was utilized to create graphs and visualization (package: ggplot2). The data were scrutinized using the UNIVARIATE procedure and probability distribution plots in SAS.

We chose a correlational multivariate analysis approach to explore and extract common sets of behaviors across the different personality tests, following Costa et al. (2020). We then made a subjective interpretation of the meaning of these correlated sets of behaviors, guided by literature to assign labels to these sets of behaviors. Labels assigned to sets of behaviors were meant to assist in interpretation of personality traits for dairy calves. Before analysis, duration and frequencies of behaviors were summed across the novel person, novel object and startle tests, and the behavioral variables were transformed to achieve normality if required (either log10 or square root transformations that achieved an adequate normal distribution). The variable resting was removed from the analysis because it was not able to achieve normalcy with transformation. Frequencies of urination/defecation, bucking, and withdrawals were too few to be meaningfully included in the analysis. A principal component analysis (**PCA**) was used to reduce correlated measures of the traditional tests (novel person, novel object, and startle tests) which included 9 variables: time exploring environment, time inactive, time spent touching the novel person, novel object and umbrella, time spent playing, time spent attentive, and latency to approach the person, object, closed umbrella and open umbrella after the startle. The variables walking and inactive measured opposite traits and so only one was retained for the PCA; time spent inactive was retained as we predicted it would reflect freezing/inactivity behavior within the isolation box test. To achieve a sampling adequacy of 0.50, the variable time spent grooming was removed due to a low communality estimate (< 0.30). The analysis and reporting guidelines for the PCA followed (Budaev, 2010) where components were retained if they had an eigenvalue greater than 1. Three principal components were retained that explained 65.5% of the variance seen in the behavioral responses of calves in the novel person, novel object, and startle tests. Factor scores for each calf were extracted using the regression method and utilized for further analysis.

To characterize behavioral responses of calves during the isolation box test from the accelerometers attached to the isolation box, the mode for each axis for the 5 accelerometer positions on the isolation box was found for each calf to determine the baseline position for each axis. The area under the curve was calculated by subtracting the mode from each acceleration of each observation. These values were then squared to account for the negative values to only look at the magnitude of movements, and all axes from all accelerometer positions on the box were summed to create a total movement index (**TMI)** for each calf. Data for 3 calves were removed from the analysis due to malfunctions with the accelerometers.

Because the purpose of this study was to develop a new personality test, it was of interest to see how the isolation box TMI related to behaviors expressed in the traditional personality tests. To examine these relationships Pearson's correlations were performed between the TMI and behaviors that were retained in the PCA for the traditional tests. Correlation coefficients were categorized following Dancey and Reidy (2007) as 0.00 to 0.30 = weak; 0.30 to 0.60 = moderate; 0.60 to 1.0 = strong.

To determine whether factor scores from the traditional tests could be used to predict scores in the isolation box test, a linear regression was performed (PROC MIXED); scores on each factor from the PCA were the explanatory variables, and TMI from the isolation box test was the response variable. Birth weight, sex, age of calf on test day, and BRD score on test day were included as fixed effects, which were removed from the model using backwards elimination if P > 0.3. The F-value and P-value for each model for the explanatory factors in each model are reported.

To investigate the relationship between performance during rearing (ADG) and scores in the isolation box test and traditional tests, linear regressions were performed (PROC MIXED); the TMI or factor scores from the isolation box test were the explanatory variables, and Total ADG, Preweaning ADG, Weaning ADG, and Post-weaning ADG were the response variables. Birth weight, sex, age of calf on test day, and BRD score on test day were included as fixed effects. Additionally, prior treatment with antibiotics (yes or no) was included as a fixed effect in this model as a control for incidence of disease through weaning. Fixed effects were removed from the model using backwards elimination if P > 0.3. Findings were considered significant if $P \leq 0.05$ and a tendency when $P \leq 0.10$.

2.3 Results

Principal Component Analysis

The behavioral responses of calves across the novel person, novel object, and startle test, are presented in Table 2.3 and the loading of the behaviors for each factor are given in Table 2.4. Factor 1 explained 25.4% of total variance and had high positive loadings for time spent attentive, latency to approach novel person, latency to approach umbrella after startle, and a moderate negative loading for time spent touching the person, object or umbrella. Calves that loaded highly for factor 1 were labeled “fearful”. Factor 2 explained 21.0% of total variance and had high negative loadings for latency to approach the novel object, had a high positive loading for time spent playing with person, object, and umbrella, and a moderate positive loading for time spent touching the person, object, and umbrella. Calves that loaded highly for factor 2 were labeled “bold”. Factor 3 explained 15.6% of total variance and had high positive loadings for time interacting with the environment, and high negative loadings for time inactive. Calves that loaded highly for factor 3 were labeled “active”.

Comparing Isolation Box Test and Traditional Personality Tests

The distribution of the individual TMI scores ranged from 2.34 to 53.8 TMI and can be found in in Figure 2.3. The correlations between the TMI and the time individuals spent performing each of the behaviors scored in the 3 traditional personality tests can be found in Table 2.5. TMI had a relationship with specific behaviors expressed during the traditional personality tests; TMI had a moderate negative correlation with time spent exploring during the traditional tests ($r = -0.45$; $P = 0.03$), and a moderate positive correlation with the time spent inactive (r = 0.46; P = 0.03), indicating that a calf that had a high TMI (more active in responses to the isolation box test) spent less time exploring the environment during traditional standardized tests, and spent more time stationary and not attentive to the person, object or umbrella.

TMI was able to reflect personality traits interpreted from a PCA from the traditional personality tests as well. The distribution of the individual TMI scores from the isolation box test are plotted against each factor of the traditional personality tests PCA (Figure 2.4). TMI had a significant negative association with factor 3, "active", (P = *0.04;* Table 2.6), indicating that calves who were more active in the isolation box spent less exploring the test arena during the traditional standardized tests, and more time avoiding interaction with novelty. There were no significant associations between the TMI and factor scores 1, "fearful", or factor 2, "bold", found from the PCA.

Personality and Performance Measures

The Total ADG varied widely among individual calves (mean Total ADG of 0.89 ± 0.29 kg); variability for each of the preweaning, weaning and post-weaning periods can be found in Figure 2.5. The distribution of the individual Total ADG was plotted against the TMI scores from the isolation box test and each factor of the traditional personality tests PCA in Figure 2.6 and Figure 2.7 respectively. The relationships between the TMI from the isolation box test, and the factor scores for calves with the ADG in the four periods can be found in Table 2.7. There was a significant positive linear association between TMI and the Preweaned ADG and the Total ADG. Factor 1, "fearful", had a positive linear association with Preweaned ADG, and Post-weaning ADG. Factor 2, “bold”, had a positive linear association with the Weaning ADG and the Total ADG. Finally, Factor 3, “active”, had a negative linear association with Preweaned ADG, Weaning ADG, and the Total ADG.

2.4 Discussion

The study aimed to investigate the use of an isolation box test to characterize personality traits of dairy calves; the study also aimed to determine if behavioral measures from the isolation box test reflected personality traits measured with a novel person, novel object, and startle tests. There was individual variation in movement of calves within the isolation box, and calves with a higher total movement index (TMI) in the isolation box test were negatively associated with the calves classified as “active” in the novel person, novel object, and startle tests. Finally, calves with a higher TMI had a higher ADG during the full milk allowance period and in the total experimental period. Our results indicate that an isolation box test has potential for use in dairy calves and can measure the expression of a personality trait labeled as ‘active’. These findings show that measures of behaviors within an isolation box are related to performance through weaning, showing its utility in measuring personality in calves comparable to the traditional standardized personality tests and can show a relationship between personality traits and performance.

Isolation Box Test

In sheep, the isolation box tests have been utilized for decades to measure individual variation in response to isolation and restraint (Murphy, 1999; Beausoleil et al., 2008). The first requirement of a personality test is that it produces individual differences in behavioral response in a population (Kaiser and Müller, 2021). Regarding this criterion, we saw considerable variation in the TMI of calves in response to the isolation box test, indicating individual variation in response to confinement within a box. Individual variation in response to a form of restraint is seen in other tests in cattle, including for subjective chute scores of beef and dairy cattle (Hoppe et al., 2010; Gibbons et al., 2011) and objective chute scores utilizing the standard deviation of weight on a scale to measure beef cattle movement (Bruno et al., 2016). The variation in the response to the isolation box test is due to differences in how calves respond to involving isolation and restraint in a dark enclosed space. Although the personality trait measured with the isolation box tests must still be defined, it was negatively related to our "active" personality trait determined from PCA analysis of results from standard personality tests.

A second requirement for a personality test to be valid is that it agrees with other tests intended to measure the same personality traits, and therefore is measuring a personality trait consistenly across contexts (Carter et al., 2013; Kaiser and Müller, 2021). The comparison between traditional personality tests and the isolation box test may allow for interpretation of the personality trait being measured by this new personality test in dairy calves (Carter et al., 2013). The other personality tests utilized in this study include novel person, novel object, and startle tests that measure exploration, fearfulness, and activity in individuals (Forkman et al., 2007; Réale et al., 2007; Yuen et al., 2017). In sheep, there have been associations found between the isolation box test and other standardized personality tests, such as sheep that were more active within the isolation box test also were more active within an arena test and investigated a stationary person sooner when compared to their less active counterparts (Beausoleil et al., 2008). Therefore, we expected that calves that were more active in the isolation box test would be more active within the traditional standardized personality tests and would have shorter latencies to approach the novel person. In our study, we indeed saw an association between the TMI and factor 3, "active." However, contrary to our hypothesis and the findings of Beausoleil et al. (2008) with sheep, there was a negative association between TMI and "active" calves in the present study. This finding indicates that calves that had a higher TMI (were more active within the box) spent less time exploring the environment and more time inactive. This finding is supported by the positive correlation between TMI and time inactive and the negative correlation between TMI and time spent exploring the environment. This may indicate that calves that were highly reactive to the isolation box (i.e. showed greater movement when in a very confined space) were those that reacted to novelty and a startle by limiting their movement (i.e. more inactive).

The differences seen between our study and Beausoleil et al. (2008) may be due to differences in the behavioral measures and in the tests administered. Factor 3 in our study was interpreted as "active" based on Réale et al. (2007), which defines the personality trait activity as the general level of activity when not in a novel environment; factor 3 had high positive loading for environment exploration and high negative loading for time inactive. Therefore, a calf that loaded highly for factor 3 spent more time interacting with the ground and walls of the test arena and less time stationary. Beausoleil et al. (2008) interpreted activity level of sheep in the arena test as zones crossed, which was not recorded during our tests; our focus was on the interaction of the calf with the novel person, novel object, and umbrella.

A more likely reason for the study differences may be due to species differences because similar outcomes to ours have been seen in beef cattle. In beef cattle that were assigned objective chute scores (coefficient of variation of weight readings by a scale while steers were restrained in a chute), steers that had a higher objective chute score (more active) were actually less active within their home pen as measured by accelerometers (Bruno et al., 2016). This study indicated perhaps it is expected to see an inverse relationship between activity expressed to restraint and activity expressed in open environments in cattle.

The lack of significant relationship between TMI and the other two factors reveals just as much about what the isolation box test measures as the significant association with factor 3, “active.” Both factor 1, “fearful,” and factor 2, “bold,” have high loadings for behaviors that relate to how calves interact with novelty, specifically regarding calf latencies to approach and time spent interacting with the novel person, novel object, and umbrella from the startle tests. Unlike Beausoleil et al. (2008), we did not see a relationship between the latency to approach the human with the outcomes of the isolation box test. In cattle, the novel person, novel object, and startle tests are more notably considered fear tests (Boissy and Bouissou, 1995; Forkman et al., 2007). The relationships between the TMI and the 3 factors determined from the standardized personality tests indicate that the isolation box test is not measuring simply a fear response. These findings –that the isolation box test had individual variation within this population of dairy calves and was associated with outcomes from standardized personality tests– indicate that the isolation box test can used to characterize activity in dairy calves. Beyond testing the validity of the isolation box test as a personality test for dairy calves, we wanted to determine if the outcomes from it could be utilized to predict performance through weaning.

Our study was limited in its ability to test the consistency of the isolation box test through time. The three requirements in order to be considered a valid personality test are that the test must show individual variation, consistency across contexts, and consistency across time (Carter et al., 2013; Kaiser and Müller, 2021). The isolation box test was too restrictive to accurately measure activity in response to confinement within the isolation box test post-weaning. Future research should involve a new box design to be able to accommodate calves during their whole development period to be able to determine repeatability of outcomes from the isolation box test. However, the requirements that the isolation box test shows individual variation and consistency across contexts has been successfully met with this study.

Performance of Dairy Calves

Although the meal allowance and feed availability were consistent across all enrolled calves, there was large variation in the weight gains of calves. Variation in ADG among the study group is expected and has been found to be present when controlling for feed intake and meal plans (de Passillé et al., 2011; Neave et al., 2018). Many factors are associated with the ADG of dairy calves, including disease (Buczinski et al., 2021), passive transfer of immunity (Elsohaby et al., 2019), milk and starter availability (Rosenberger et al., 2017), and genetic potential (Coffey et al., 2006). An association of particular interest is the relationship between the personality and ADG in dairy calves: calves that were more “exploratory-active” within standardized personality tests had greater ADG during the period of milk reduction for weaning and overall testing period (Neave et al., 2018; Neave et al., 2019). We predicted that personality measured from the new isolation box test or the traditional standardized personality tests could be associated with the performance of the calves. Indeed, a positive association between ADG and TMI calculated from the isolation box test were found. No other studies have examined the relationship between outcomes from an isolation box test and growth in dairy calves, but similar studies exist in beef steers where an objective chute score was measured. Steers with a higher objective score had greater ADG (Bruno et al., 2018), indicating that more reactive steers during restraint in a chute had improved growth. However, these findings with the objective chute score are not typical of relationships seen between subjective chute scores and ADG (Voisinet et al., 1997; Turner et al. 2011; Bates et al., 2014). There was no relationship between the activity of sheep within a isolation box and ADG; however, sheep that were more active within the isolation box had a higher feed efficiency, growing at the same rate while consuming less feed (Amdi et al., 2010). The relationship between TMI and performance likely represents complex relationships between personality traits measured from standardized tests and real-life measures such as activity within the home pen or feeding behavior.

The relationship between the isolation box test behavioral measures and performance is likely due to the relationship between the personality trait “active” with stable individual differences in energy expenditure and feeding behaviors. In calves, personality traits have been associated with feed intake, growth, and feed efficiency (Neave et al., 2018; Neave et al., 2019). As the TMI in the isolation box was negatively associated with “activity” in the traditional personality tests, the relationship with performance measures becomes more complicated to interpret. However, in other studies, we have seen that calves that were more inactive in open field, novel object, and novel person tests had increased consumption of novel feed (Costa et al., 2020). Additionally, the calves that were more inactive in novel environment, novel person, and novel object tests had improved feed efficiency (Neave et al., 2019). Thus, the relationship between activity in the isolation box test and ADG may be due to reserved energy and willingness to investigate foods. Future work should investigate the factors that culminate in a higher performance of animals with expression of certain personality traits.

Associations between ADG and the factors derived from the PCA of the traditional personality tests were also found. Calves that loaded highly for factor 1 ("fearful") had greater ADG during the preweaning period. Calves that loaded highly for factor 2 ("bold") had greater total ADG and during the period of milk stepdown. Both factor 1 and factor 2 describe the interaction of the calf with novelty, and together appear to represent the shy-bold personality trait spectrum (Réale et al., 2007). Factor 1, "fearful," from our study loads many of the same (but inversed) behaviors as the factor "interactive" from Neave et al. (2018), indicating that our relationship between the expression of fearfulness/interactivity in the face of novelty agrees with the relationship found by Neave et al. (2018). Additionally, we found that calves that loaded highly for Factor 3 ("active") had a lower ADG overall, during full milk allowance and during milk stepdown. Neave et al. (2018) also found that calves that were more inactive had a greater ADG, though we found this relationship over more periods. This relationship may be seen because of less energy expenditure for calves. A social isolation test (similar to an open field test) in beef heifers disagrees with these findings as they found a negative correlation between time inactive and ADG, but this study did not employ a PCA approach (Müller and von Keyserlingk, 2006).

Activity in other studies is often measured by looking at the number of quadrants/zones crossed, typically in an open field or novel environment test (Müller and von Keyserlingk, 2006; Beausoleil et al., 2008; Neave et al., 2018). However, our study did not include a novel environment test and thus the comparisons between activity outcomes from our study with others is limited. The differences in behaviors recorded and personality tests administered may explain some of the discrepancies seen between our study and existing research. For instance, the behavior of exploring the environment in our study is loaded highly on factor 3 (“active”) which has a negative association with ADG, while in Neave et al (2018), it is loaded highly on a factor “exploratory/active” with positive associations with ADG. A key difference is that in Neave et al (2018) the ‘inactivity’ measure loaded on a different factor from the exploration measure (while these two measures loaded together on the same factor in our study). Importantly, our measure of exploration and inactivity was derived from behavioral responses during the novel object and novel human tests, while exploration in Neave et al (2018) was measured during the novel environment test only. This result suggests that relationship between ADG and exploration is related to the context in which it is measured. Regardless, both their study and ours demonstrates an association between personality and growth in dairy calves. These findings were able to be measured with the isolation box test in dairy calves.

The ability to measure a personality trait that is associated to performance in dairy calves with an isolation box test offers the potential to measure personality with a simplified test. With more development, this simplified test could be useful on-farm and for utilization by producers. Future research should focus on looking at the consistency of the isolation box test TMI scores through time, as we were unable to verify this during our study conditions due to the restrictive size of the box for older individuals. Finally, future research should look into computer learning models to accompany the isolation box test to assign TMI, allowing the findings to be more useful to producers without knowledge of statistical software.

2.5 Conclusion

An isolation box test, adapted from use in sheep, was able to measure variation in the behavioral response of dairy calves. The total movement index for calves calculated from the isolation box test was associated with activity personality trait scores derived from novel person, novel object, and startle tests. These results suggest that the isolation box test has potential to be utilized as a personality test in dairy calves. Additionally, outcomes from the isolation box tests were able to predict the growth of calves through weaning, indicating that the isolation box tests could be a useful tool for producers to characterize select their calves on the basis of personality.

Table 2.1. Feed Composition (Mean ± SD) of calf starter and hay offered to calves assessed for personality and performance through weaning and fed up to 14L/d milk replacer and calf starter by an automated feeder.

Variables % DM basis	Calf Starter[1]	Hay[2]
DM %	87.80 ± 2.34	96. 71 ± 0.28
CP %	22.29 ± 0.94	13.94 ± 2.81
Fat %	3.61 ± 0.70	1.41 ± 0.54
NDF %	12.39 ± 1.09	59.78 ± 13.55
ADF %	5.54 ± 0.61	39.62 ± 4.69
Starch %	36.63 ± 1.83	ND[3]
Ash %	7.65 ± 0.46	9.51 ± 1.23
Calcium %	1.12 ± 0.12	0.74 ± 0.12
Phosphorus %	0.54 ± 0.04	0.41 ± 0.06
Magnesium %	0.20 ± 0.01	0.22 ± 0.03
Potassium %	1.28 ± 0.06	2.56 ± 0.46
Sulfur %	0.26 ± 0.02	0.18 ± 0.04
ME (Mcal/kg)[4]	2.85 ± 0.01	2.20 ± 0.14

[1] Special Calf Starter and Grower, Baghdad Feeds, Baghdad, KY
[2] Late cut alfalfa farm hay (*Medicago sativa)* was provided to calves in a trough
[3] ND = not determined
[4] ME = TDN × 0.04409 × 0.82; calculated according to (NRC, 2001) calculations

Table 2.2 Ethogram of behaviors recorded from the video of the traditional personality tests (novel person, novel object and startle tests) from calves (n=27) tested individually at 24 ± 3 d of age.

Behavior	Definition
Grooming	Duration of calf licking or scratching themselves
Resting	Duration of lying time during test
Touching	Duration of calf using their muzzle to make contact, suckle, or lick the novel person, novel object, or umbrella
Exploring environment	Duration of time spent touching, licking, playing with the door, ground, and walls of the test arena
Attentive	Duration of time spent with the calf had and ears orientated toward the person, object or umbrella during the test
Locomotor Play	Duration of time spent running, trotting, galloping, jumping
Object/Person Play	Duration butting or mock butting the person, objects, or umbrella within the test arena
Inactive	Duration time spent stationary with no clear interaction with environment and not apparently being attentive toward person or objects
Bathroom	Duration of time spent lifting the tail to urinate or defecate
Latency to approach	Point when calf initially approaches the novel person, novel object, or closed umbrella in the startle test
Latency to approach after startle	Point in time that a calf approaches the umbrella after it is opened to produce a startling event
Bucking	Number of events where the calf lifts both hind legs off the ground and extended backwards
Withdrawal	Number of events when the calf takes a sudden movement backwards

Table 2.3 Time spent performing each behavior measured in the traditional personality tests (novel person, novel object and startle test). Values are mean, standard deviation and range, in seconds).

Variable	**Mean**	**SD**	**Range**
Exploring environment	244.8	101.7	78.6—436.1
Touching	222.9	116.4	0—437.0
Attentive	195.0	121.4	74.6—547.5
Play	64.8	78.5	0—285.7
Inactive	508.4	177.1	225.3—858.13
Latency to approach novel object	100.8	140.1	8.5—600
Latency to approach novel person	156.7	199.3	6.0—600
Latency to approach closed umbrella	141.3	113.6	9.4—300
Latency to approach after startle	153.4	126.5	4.8—300

Table 2.4 Coefficient loadings of each variable on the 3 factors extracted from the principal component analysis of behavioral responses in the traditional personality tests. The eigenvalue for each factor is reported, and the variables with high loadings (≥ ± 0.62 in bold) and moderate loadings (± 0.50 > ± 0.62, in italics) were utilized to interpret each factor. The labels generated for each factor are subjective interpretations of the correlated sets of behaviors and utilized to assist with the interpretation of the relationships with total movement index.

Variable	**Factor 1**	**Factor 2**	**Factor 3**
Exploring environment	0.17	0.04	**0.86**
Touching	*-0.50*	*0.53*	0.13
Attentive	**0.90**	-0.20	0.00
Play	-0.25	**0.87**	0.02
Inactive	0.20	-0.07	**-0.80**
Latency to approach novel object	0.11	**-0.75**	-0.04
Latency to approach novel person	**0.75**	-0.44	0.04
Latency to approach closed umbrella	-0.07	-0.49	-0.40
Latency to approach after startle	**0.88**	0.05	0.00
Variance Explained	25.4%	21.0%	15.6%
Interpretation (suggested label)	“Fearful”	“Bold”	“Active”

Table 2.5 Pearson's correlations between behavioral variables measured in each of the traditional personality tests (novel person, novel object and startle test) and total movement index measured in the isolation box test.

Variable	**r**	**P-value[1]**
Exploring environment	**-0.45**	**0.03**
Touching	-0.27	0.19
Attentive	0.02	0.92
Play	-0.02	0.92
Inactive	**0.46**	**0.03**
Latency to approach novel object	0.12	0.57
Latency to approach novel person	0.01	0.96
Latency to approach closed umbrella	-0.18	0.41
Latency to approach after startle	-0.07	0.73

[1]Significant P-values (≤ 0.05) are bolded, and tendencies (≤ 0.10) are italicized

Table 2.6 Relationship between total movement index (**TMI**) from the isolation box test and the factor scores extracted from the principal component analysis (containing behaviors from the standardized personality tests: novel person, novel object, and startle tests).

Factors from Novelty PCA (Explanatory Variables)	TMI		
	Effect Direction	F- Value[1]	P-Value[2]
Factor 1 (“Fearful”)	+	0.02	0.87
Factor 2 (“Bold”)	-	0.14	0.72
Factor 3 (“Active”)	-	**5.11**	**0.04**

[1]DF = 1, 20
[2]Significant P-values (≤ 0.05) are bolded, and tendencies (≤ 0.10) are italicized

Table 2.7 Relationship between ADG for each rearing period with total movement index (**TMI**) from the isolation box test and the factor scores extracted from the principal component analysis (containing behaviors from the standardized personality tests: novel person, novel object, and startle tests).

	Effect Direction	F-Value[1]	P-Value[2]
TMI			
Total ADG	**+**	**5.33**	**0.03**
Preweaned ADG	**+**	**7.38**	**0.01**
Weaning ADG	+	1.86	0.19
Post-weaning ADG	+	1.20	0.69
Factor 1 (“Fearful”)			
Total ADG	+	1.31	0.27
Preweaned ADG	**+**	**7.46**	**0.01**
Weaning ADG	+	0.13	0.72
Post-weaning ADG	+	*3.71*	*0.07*
Factor 2 (“Bold”)			
Total ADG	+	**5.16**	**0.03**
Preweaned ADG	+	1.09	0.31
Weaning ADG	**+**	**4.92**	**0.04**
Post-weaning ADG	+	1.00	0.33
Factor 3 (“Active”)			
Total ADG	**-**	**10.02**	**0.01**
Preweaned ADG	-	**5.02**	**0.04**
Weaning ADG	-	**10.00**	**0.01**
Post-weaning ADG	-	*3.32*	*0.08*

[1]DF = 1, 20

[2]Significant P-values (≤ 0.05) are bolded, and tendencies (≤ 0.10) are italicized

Figure 2.1 Diagram and dimensions of the test arena which is an enclosed space with solid plywood walls bedded with wood shaving located in the calf barn. The circle in the diagram represents the location of the novel person and novel object. The X in the diagram represents where the umbrella is located during the startle test. The doors to the test arena are seen open at the bottom of the diagram, with the entrance in the middle of the walls.

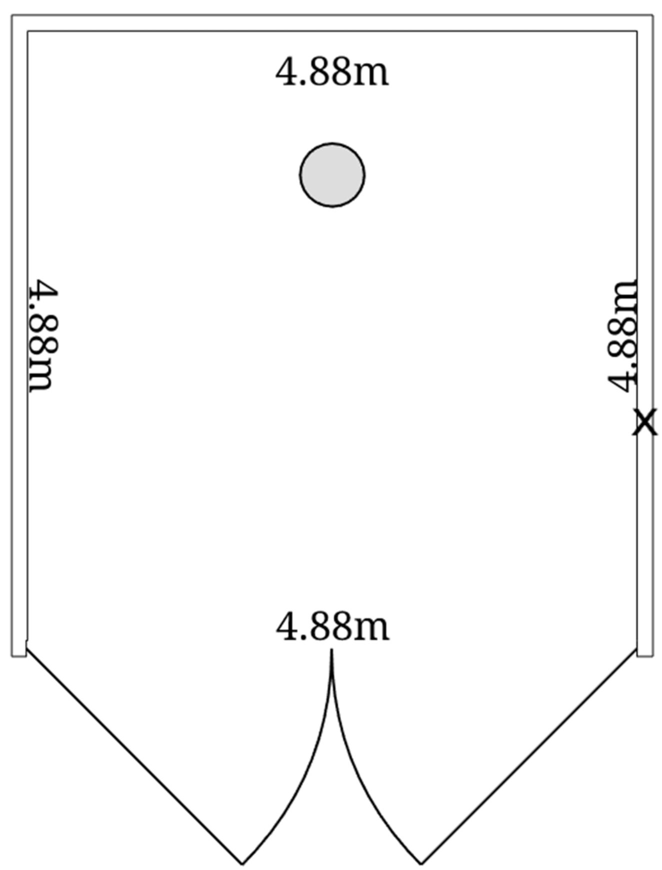


Figure 2.2 Diagram and the dimensions of the isolation box and the location of the accelerometers on the outside of the box. The positions on the outside of the isolation box for the accelerometers are 1) back, 2) left, 3) right, 4) top-front and 5) top-back.

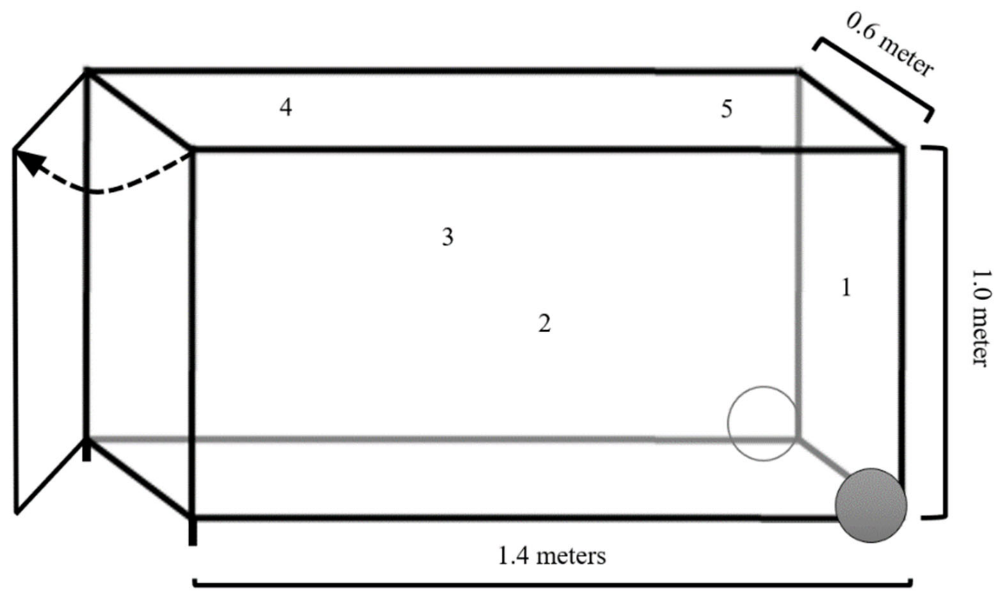


Figure 2.3 Boxplot showing the median (line) and interquartile range (box) of the the distribution of the Total Movement Index calculated for each calf from the isolation box test. Each color represents an individual calf.

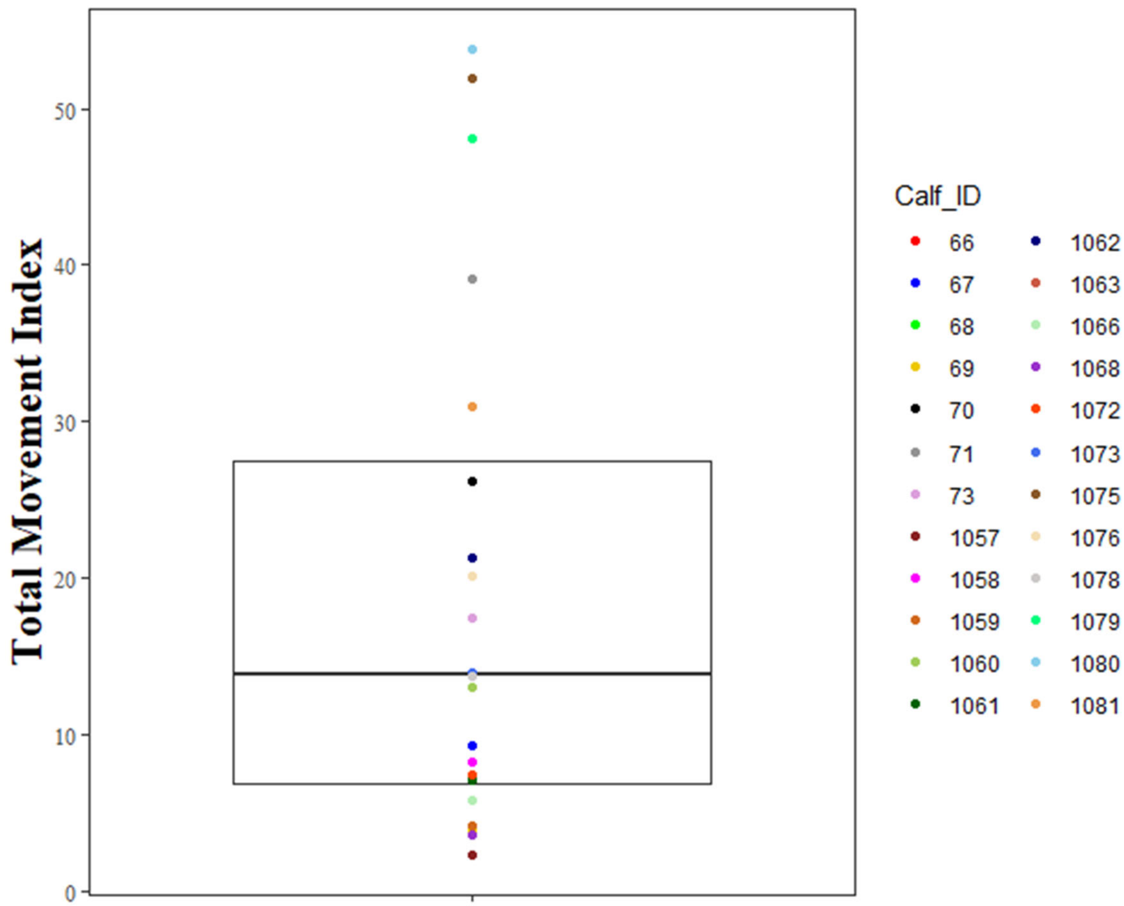
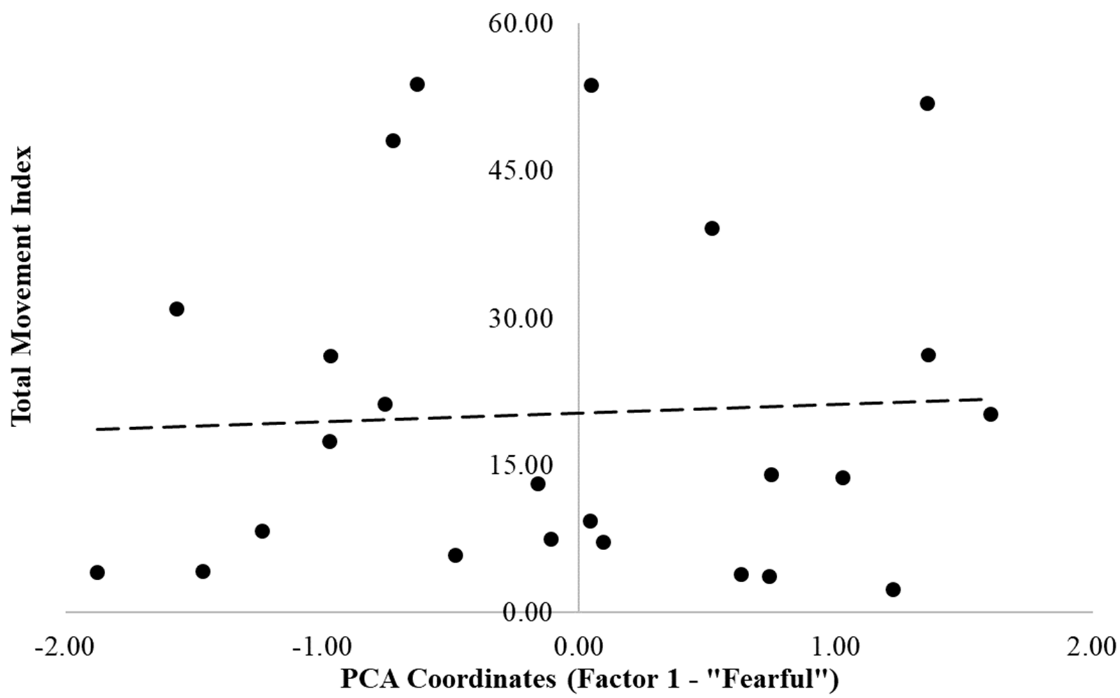
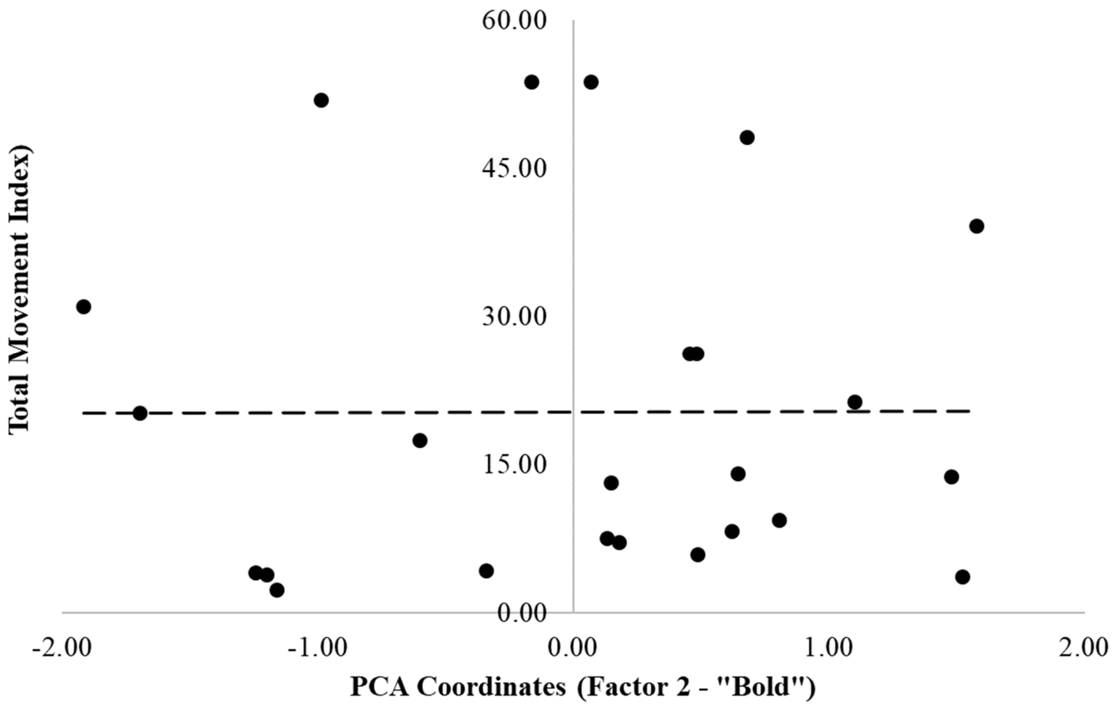


Figure 2.4 a-c Distribution of individual calf total movement index from the isolation box test plotted against each factor of the principal component analysis of the traditional personality tests: a) Factor 1 (“Fearful”), b) Factor 2 (“Bold”), and c) Factor 3 (“Active”). The linear regression trendline is presented for each plot (solid black line = significant regression, $P < 0.05$; dashed line = non-significant regression, $P > 0.05$)

a)

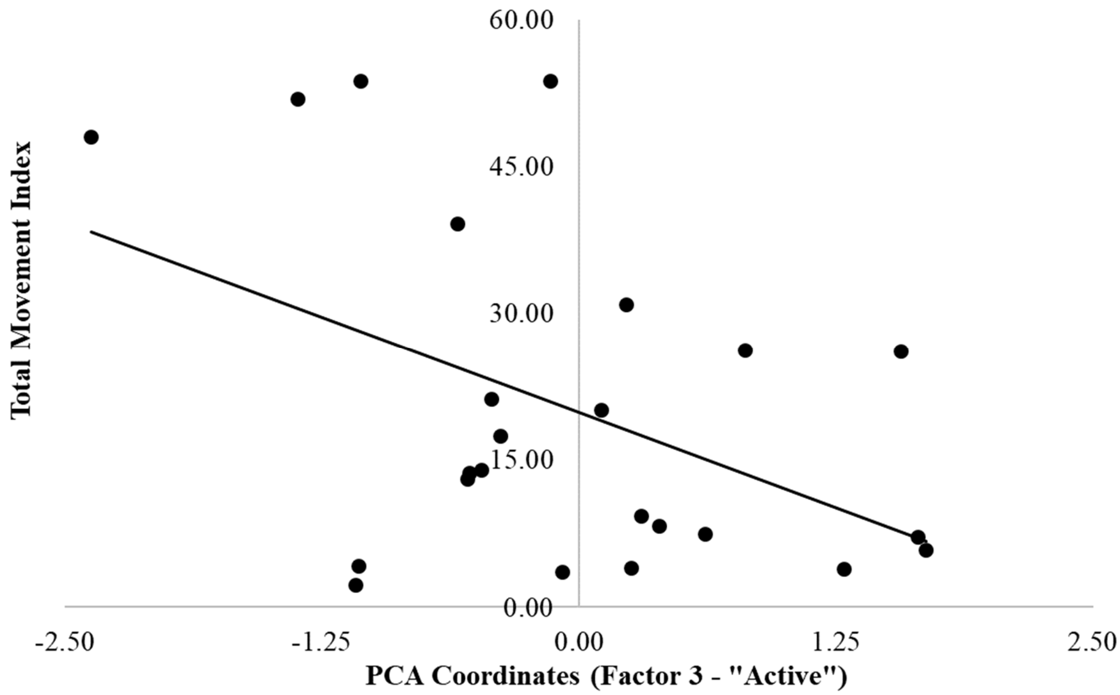


b)



60.00
45.00
30.00
15.00
0.00
-2.00
-1.00
0.00
1.00
2.00
Total Movement Index)
PCA Coordinates (Factor 2 - "Bold")

c)



60.00
45.00
30.00
15.00
0.00
Total Movement Index
-2.50
-1.25
0.00
1.25
2.50
PCA Coordinates (Factor 3 - "Active")

Figure 2.5 Boxplots showing the median (line) and interquartile range (box) of the average daily gain (ADG) for all calves for each rearing period: enrollment in the study to unenrollment (Total), the full milk allowance period (Preweaning ADG), the stepdown milk period (Weaning), and the no milk allowance period (Post-weaning). Each color represents an individual calf.

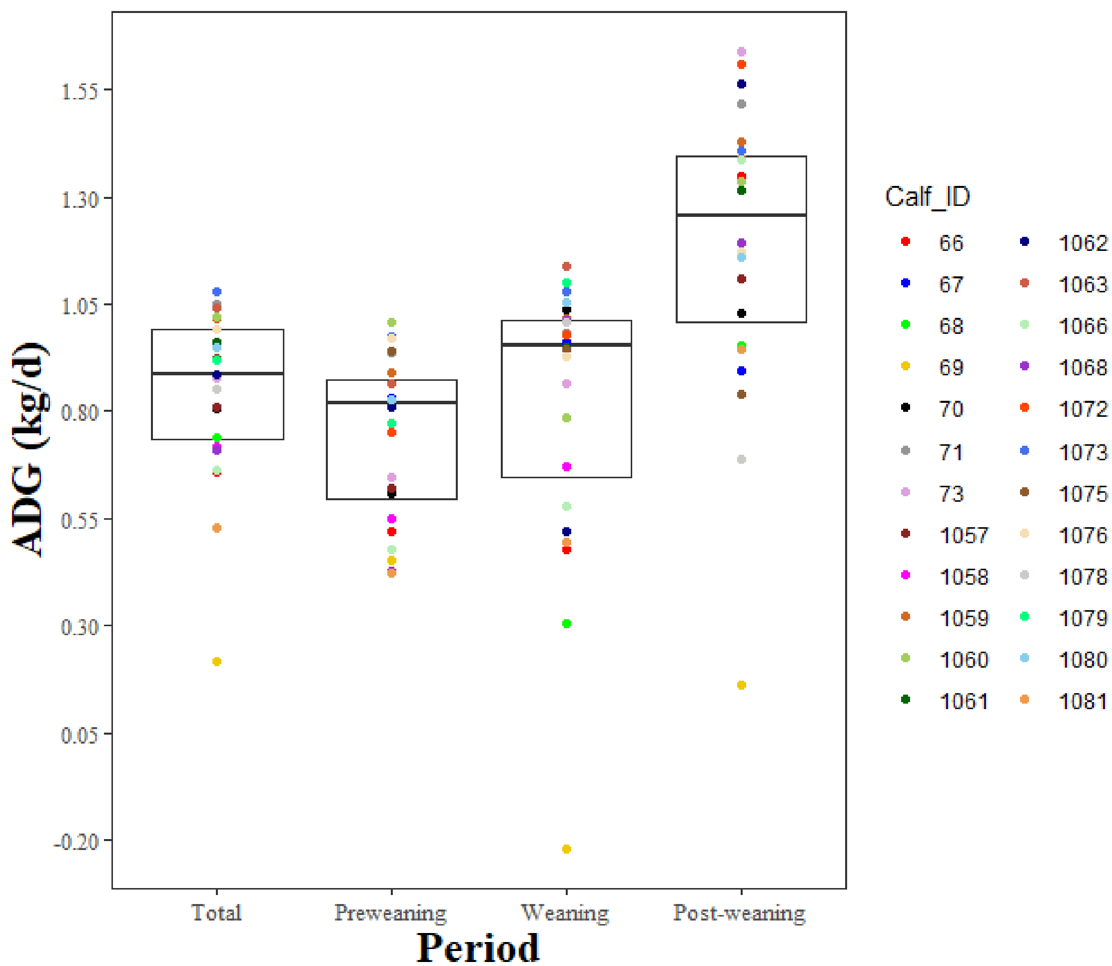


Figure 2.6 Distribution of individual calf average daily gain (ADG) from birth until 2-weeks after weaning (Total ADG) plotted against each the total movement index from the isolation box test. The linear regression trendline is presented for the plot (solid black line = significant regression, $P < 0.05$; dashed line = non-significant regression, $P > 0.05$)

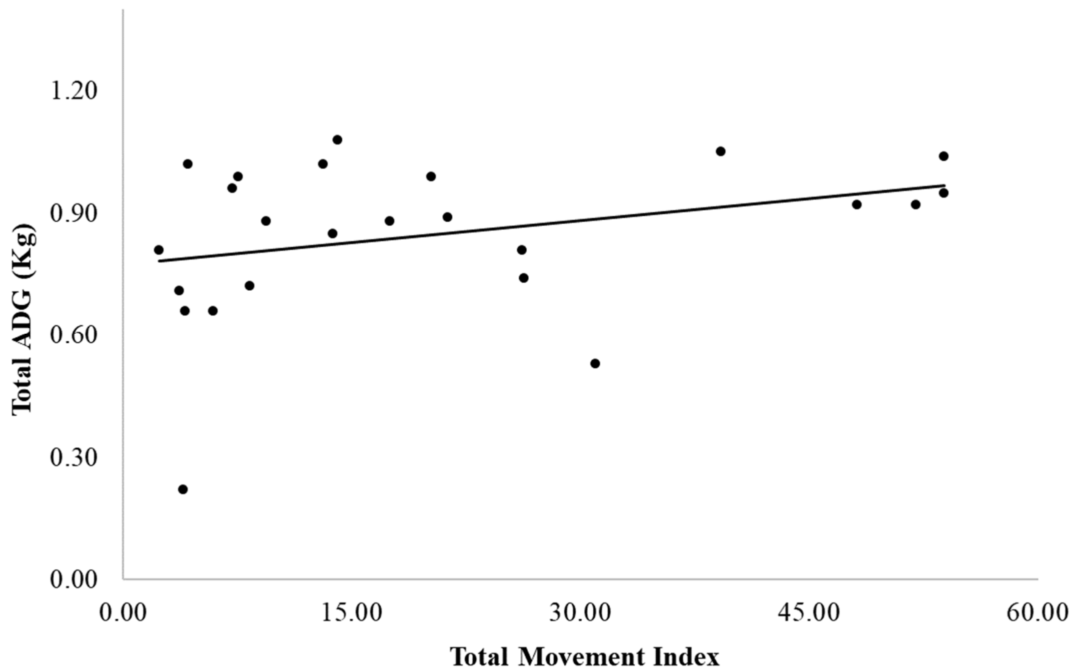
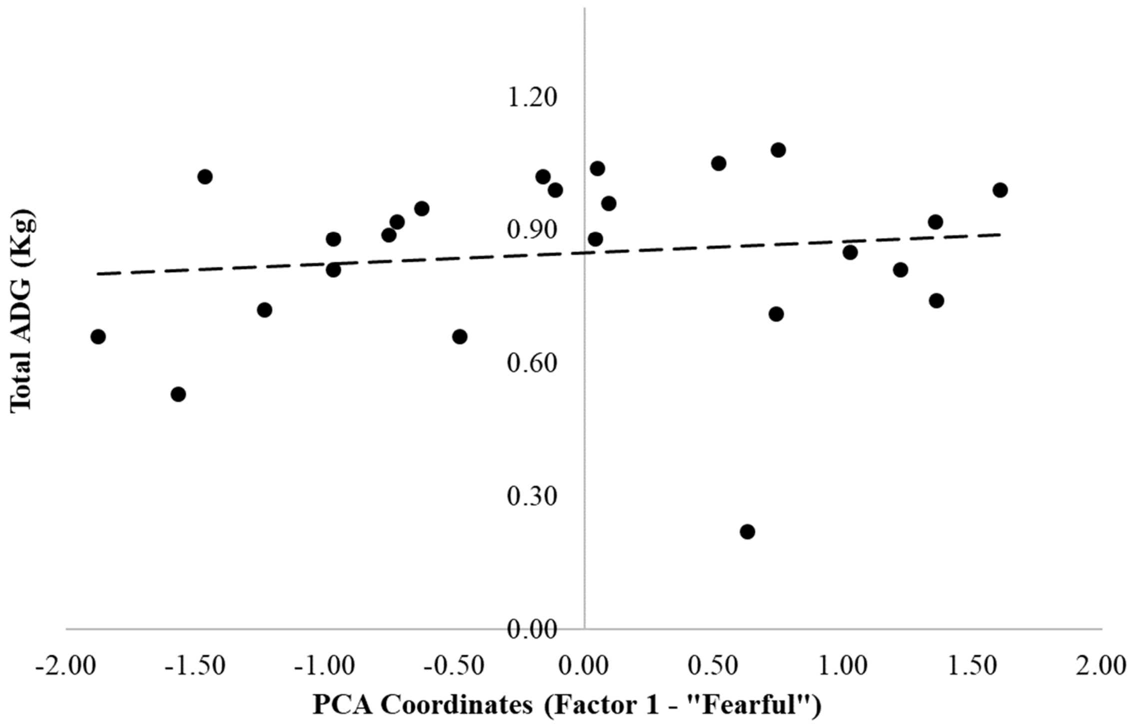
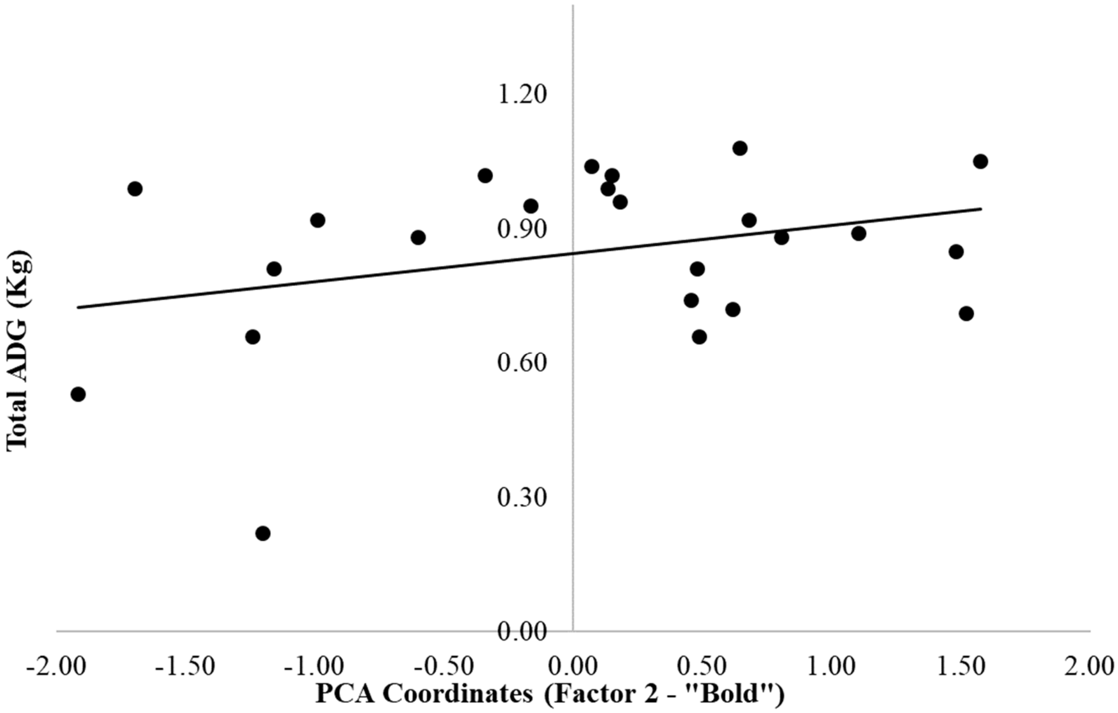


Figure 2.7 a-c Distribution of individual calf average daily gain (ADG) from birth until unenrollment 2 weeks after weaning (Total ADG) plotted against each factor from the principal component analysis of the traditional personality tests: a) Factor 1 (“Fearful”), b) Factor 2 (“Bold”), and c) Factor 3 (“Active”). The linear regression trendline is presented for each plot (solid black line = significant regression, $P < 0.05$; dashed line = non-significant regression, $P > 0.05$).

a)

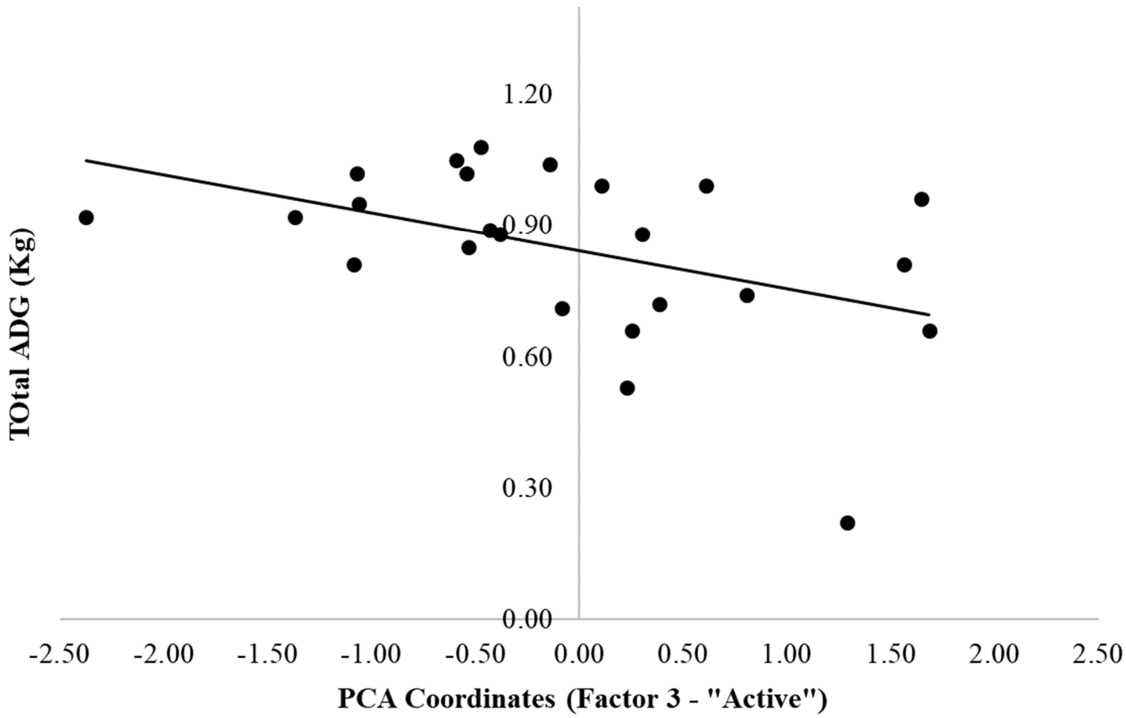


b)



1.20
0.90
0.60
0.30
0.00
Total ADG (Kg)
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00
PCA Coordinates (Factor 2 - "Bold")

c)


1.20
0.90
0.60
0.30
0.00
TOtal ADG (Kg)
-2.50
-2.00
-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50
2.00
2.50
PCA Coordinates (Factor 3 - "Active")

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VITA

EDUCATION

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PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Woodrum Setser, M. M, M. C. Cantor, J. H. C. Costa. August 2020. A comprehensive evaluation of microchips to measure temperature in dairy calves. *J. Dairy Sci.* 103.10 (2020): 9290-9300

ORAL PRESENTATIONS

Woodrum Setser, M.M, Neave, H. W., Costa, J. H. C. 2021. Isolation box test: development and investigation of its potential to measure personality traits in dairy calves. ISAE. Accepted.

Cantor, M.C., Neave, H.W., **Woodrum Setser, M.M**., and J.H.C. Costa. 2021. Predicting pre- and post-weaning performance of dairy calves: An investigation using precision technology data. For: American Dairy Science Association Annual Meeting. Accepted.

Woodrum Setser, M.M, Costa, J.H.C. 2020. An exploration of calf personality trats and their relationship to incidence of disease in the pre-weaned period, feeding behavior, and activity of pre-weaned calves. Youngstock Symposium. August 2020.

Cantor, M¸ **Woodrum Setser, M. M**, Costa, J.H.C. 2020. Predicting BRD: What following a cohort of calves daily tells us about feeding behaviors in an automated feeding system. Youngstock Symposium. August 2020.

Cantor, M, Costa, J.H.C, **Woodrum, M.M.** 2019. Are feeding behaviors associated with recovery for dairy calves treated for Bovine Respiratory Disease (BRD)? ISAE. August 2019.

Costa, J.H.C. Neave, H.W, **Woodrum, M.M**, Cantor, M.C. 2019. The use of precision technologies to monitor, selectively treat, and manage nutrition of dairy calves. ADSA. June 2019.

Woodrum, M.M, Costa, J.H.C, Cantor, M. 2019. Are Implantable microchips a reliable way to continuously measure body temperature in dairy calves? ADSA-SAD Original Research ORAL Competition. June 2019.

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POSTER PRESENTATIONS

Woodrum Setser, M.M, Cantor, M.C, Hayes, M, Mazon, G, Costa, J.H.C. 2021. Individual variation and reproducibility of infrared thermography of the eye and rectal temperature in dairy calves. ADSA. Accepted.

Cantor, M, **Woodrum Setser, M.M,** Renauld, D.L, Costa, J.H.C. 2020. Effect of colostrum replacer to ameliorate a disease bout in preweaned calves on an automatic feeder. ADSA. June 2020.

Woodrum, M.M, Cantor, M, Costa, J.H.C. 2019. Can we measure calves' temperatures with an implantable RFID microchip? University of Kentucky Undergraduate Research Showcase. April 2019.

Woodrum, M.M, Costa, J.H.C, Cantor, M. 2019. Using an implantable microchip for measuring body temperature in dairy calves. NCUR. April 2019.

Cantor, M.C., **Woodrum, M.M**., and Costa, J.H.C. Are feeding behaviors associated with recovery for dairy calves treated for Bovine Respiratory Disease (BRD)? 2019. In: International Society of Applied Ethology Conference (ISAE), Annual Meeting, 2019.