INTEGRATED TICK MANAGEMENT OF THE WINTER TICK, *Dermacentor albipictus* (ACARI: IXODIDAE), IN GRAZING CATTLE SYSTEMS

A Dissertation

by

SAMANTHA RAE HAYS

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Chair of Committee,	Pete D. Teel
Committee Members,	Jeffery K. Tomberlin
	Sonja L. Swiger
	Thomas B. Hairgrove
	David B. Anderson
Head of Department,	Phillip E. Kaufman

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ABSTRACT

The winter tick, *Dermacentor albipictus* Packard (Acari: Ixodidae), is a one-host tick species that parasitizes large ungulate hosts during fall and winter months when annual forage quality and quantity are low. Larvae remain inactive in clusters through the summer months to avoid unfavorable environmental conditions, a critical link in year-to-year infestations. *Dermacentor albipictus* is a biological vector of *Anaplasma marginale* Theiler, causal agent of bovine anaplasmosis, an important disease of Texas beef cattle. Tick control through Integrated Pest Management (IPM) strategies include habitat and wildlife management, grazing rotations, and cattle treatments with acaricides. Near infrared reflectance spectroscopy of bovine feces (fNIRS) may offer a non-invasive, economical, and efficient means of detecting tick infested animals. Investigations were conducted to 1) determine if artificial infestations of *D. albipictus* could be detected in cattle using fNIRS and if detection capability was sensitive to size of tick infestation and phase of on-host stage-specific tick development, 2) assess producer adoption of near infrared reflectance spectroscopy (NIRS) technology in grazing cattle systems, and 3) compare survivorship of *D. albipictus* larvae exposed to different saturation deficit environments in long-day and short-day photoperiods.

Cattle with *D. albipictus* infestations arising from as few as 1000 larvae were identified by fecal chemistry changes using fNIRS technology. In two separate trials, three animal pairs were infested with one of three treatment levels of *D. albipictus* larvae in a repeated measures experimental design. Significantly different fecal sample-cluster shifts occurred between two periods of pre-infestation, three stages of tick development, and a post-tick recovery period. Results from beef cattle producers surveyed at extension workshops over three years

characterized the enterprises of audience participants and found willingness for potential adoption of NIRS technology. Larval survivorship was highly associated with saturation deficit. As saturation deficit decreased, survivorship increased regardless of photoperiod treatment. Collectively, these results may guide future studies to determine the best IPM strategies for control of *D. albipictus* and the use of fNIRS to detect tick infestations in grazing cattle systems. Results may also guide extension program development to demonstrate best practices on new approaches and technologies that improve tick control.

DEDICATION

This work is dedicated to my heroes, my parents, and my PawPaw. Without them, I would not be who I am today.

Charles Keith Hays

Father, Hero, and Friend

You taught me to work hard for what I want in life and to love animals unconditionally. I will never forget the days of showing steers, hunting, fishing, and riding with you on the tractor. They are what shaped me into the person I grew up to be. I am proud to be your daughter.

Sheryl Raye Hays

Mother, Hero, and Friend

You taught me to be selfless, kind, loving, and a great person to any person I meet. I will never forget absolutely everything you have done for me throughout my life. You are my best friend, and I am proud to be your daughter.

Bobby Ray Childers

Grandfather (PawPaw), Hero, and Friend

Born: September 29, 1934

Died: October 30, 2016

You taught me to work hard and be a good person. I will never forget the sound of you tapping in your cowboy boots or the infamous Bobby Ray “finger point”. You always believed in me, and I know you would be proud that I finished my Ph.D. despite all the tough times.

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CHAPTER I

INTRODUCTION

Ticks (Subclass Acari: Order Ixodida) are highly specialized, obligate, hematophagous, ectoparasitic arthropods that feed on birds, mammals, and reptiles (Anderson 2002, Krantz and Walter 2009, Nicholson et al. 2019). There are approximately 900 tick species described (Barker and Murrell 2004) that are divided into three families: Argasidae, Ixodidae, and Nuttallielidae (Anderson 2002, Nava et al. 2009). Ixodidae is the largest and most economically important family comprised of approximately 700 species (Guglielmone et al. 2010, Sonenshine and Roe 2014). Ticks are believed to exceed all other arthropods in their ability to transmit a variety of infectious agents (Sonenshine and Roe 2014). Ticks may inject toxins that lead to host paralysis (Obenchain and Galun 1982, Oliver 1989). Infestations of ticks on livestock and wildlife can cause direct damage to animal hides and leave open wounds with the potential for myiasis and secondary microbial infections (Nicholson et al. 2019). Tick burdens on livestock can lead to loss of milk production, reduced weight gain, and decreases in overall production efficiency (Nicholson et al. 2019).

The winter tick, *Dermacentor albipictus* (Packard) (Acari: Ixodidae), is recognized as a one of approximately 12 Ixodid tick species that displays a one-host life cycle in which all three post-embryonic life stages blood feed on a single large host (Guglielmone et al. 2010, Sonenshine and Roe 2014). The majority of these one-host tick species produce multiple generations each year, including *Dermacentor nitens* Neumann (Acari: Ixodidae), *Rhipicephalus annulatus* Say (Acari: Ixodidae) and *R. microplus* Canestrini (Acari: Ixodidae) (Barbosa et al. 1995, Cruz et al. 2020, Labruna and Faccini 2020, Walker et al. 2003), which are all found in

Texas. *Dermacentor albipictus* has been described to complete one generation per year under natural conditions (Strickland et al. 1976). An ectoparasite of large ungulates, this species is widely distributed in North America (United States (US) and Canada) and can be found in the northern ranges of Mexico (Bishopp and Tremley 1945, Levin 2020). Under field conditions in Texas, adult female ticks drop off their host following a blood-meal and lay eggs on the ground in protected microhabitats during winter (December-February) or early spring (March) (Wright 1971). Larval ticks hatch in late winter and early spring (Wright 1969a). Larvae remain inactive in clusters through the summer (Howell 1939; Wilkinson et al. 1982) to avoid desiccating environmental conditions (Yoder et al. 2016). The effects of short-day photoperiod and cool fall temperatures activate larvae to quest (ascend vegetation) for hosts in late September and October (Wright 1969b).

The parasitic phase of *D. albipictus* has been previously described by Strickland et al. 1976 and Tolleson et al. 2007 stating that larvae will infest and attach onto a host to begin feeding from day 0-4, and then molt into nymphs from day 4-8. Nymphs will begin feeding from day 8-15 and then molt into adults from day 15-18. From here, adults will feed from day 18-35 where engorged females will drop-off from day 26-40. Adult males remain on the host or will move to another host to mate with other females. Winter tick feeding can cause irritation to an animal host resulting in nymphs and adults being groomed off. In most cases, *D. albipictus* larvae have been considered the only life stage that searches for a host, as compared to two-host and three-host ticks, which may help reduce the risk of failure in finding a viable host (Lysyk 2011). However, the capability of *D. albipictus* to survive being dislodged and acquiring a second host indicates this tick may complete its life history as either a one-host or two-host parasite (Barker et al. 1990, Cameron and Fulton 1926, Needham and Teel 1991).

Different tick life stages are inherently dependent upon the litter layer and vegetation to provide protection from adverse climatic conditions during growth and development, and for finding a viable host when they are seasonally active (Terry 2015). The length of time that ground-dwelling ticks reside in the litter layer and vegetation can vary and is dependent upon multiple factors of their immediate environment. *Dermacentor albipictus* larvae enter an inactive period during the summer season to avoid host-seeking during an unfavorable time of year when high temperatures and low humidity promote desiccation. Drew and Samuel (1985) outlined that under field conditions, the duration of inactivity *D. albipictus* larvae exhibit during the summer period can vary based upon geographical location: 2 weeks in Alberta (Drew 1984), 2-3 months in British Columbia (Wilkinson 1967), 3-6 months in Texas (Bishopp and Wood 1913), 4-5 months in Oklahoma (Patrick and Hair 1975), and 4-7 months in California (Howell 1939). Most recently, Terry (2015) stated that *D. albipictus* larvae remain inactive for three months in northeast Minnesota. These different durations of inactivity have various physiological explanations as *D. albipictus* larvae react to the environmental conditions associated with their geographical locations.

Dermacentor albipictus infestations can often occur in detrimentally high numbers (>50,000; Bergeron and Pekins, 2014, Drew and Samuel 1985, Mooring and Samuel 1998) on large ungulate hosts including livestock such as horses (Perissodactyla: Equidae; *Equus spp.*) and cattle (Artiodactyla: Bovidae; *Bos* spp.) and wildlife such as moose (Artiodactyla: Cervidae; *Alces alces*), elk (Artiodactyla: Cervidae; *Cervus canadensis*), and white-tailed deer (Artiodactyla: Cervidae; *Odocoileus virginianus*). Their seasonal activity is restricted to the fall, winter, and spring months, when tick infestations are easily unnoticed due to winter hair coats and occur when annual forage quality and quantity are low (Bishopp and Tremley 1945, Teel et

al. 1990). *Dermacentor albipictus* can affect wildlife by causing anemia, hair and weight loss, reduced weight gain, and even death (Glines and Samuel 1989). Irritation from *D. albipictus* feeding on moose can trigger them to groom uncontrollably, removing substantial portions of hair coat exposing bare skin causing moose to appear greyish-white exhibiting a ghost-like form which is known as "phantom moose disease" (Levin 2020). In Canada, cattle and horse deaths followed high infestations of winter ticks on animals left grazing scrublands during winter months of December and January (Cameron and Fulton 1926).

Dermacentor albipictus is recognized as either an experimental or known vector of several pathogens. It is an experimental vector of *Babesia caballi* Nuttal, a causal agent of equine piroplasmosis (Stiller et al. 1980, Scoles and Utei 2015, Levin 2020), and a known vector of *Klebsiella paralytica* Can, Wallace and Thomas, causal agent of moose disease (Bequaert 1945) and *Anaplasma marginale* Theiler, causal agent of bovine anaplasmosis (Stiller et al. 1980, 1981, 1983, Levin 2020). Bovine anaplasmosis is one of the most important diseases to beef cattle producers in Texas. *Dermacentor albipictus* is prevalent throughout Texas and is a known biological vector of *A. marginale*. Recent geographic (Hairgrove et al. 2014) and in-herd seroprevalence and PCR-prevalence (Hairgrove et al. 2015) of cattle exposure to *A. marginale* has been documented. Seroprevalence exceeded 25% in selected cow herds and exceeded 70% in selected bull herds (Hairgrove et al. 2015) west of Interstate Highway 35 in Texas. These findings associate the geography of the winter tick's distribution in this state where *A. marginale* is endemic.

Integrated pest management (IPM) strategies have been developed for ticks (Barnard et al. 1994, Williams 2010). These strategies rely on habitat management, grazing rotations, wildlife management, fencing, and cattle treatments with acaricides (Barnard et al. 1994,

Williams 2010). However, the on-animal application of acaricides has become the principle recommended tactic to control ticks. For tick management, producers are required to gather and physically inspect animals on a regular basis to determine tick presence and abundance and make informed decisions regarding management tactics (Barnard 1985, Williams 2010). Instead, producers treat for ticks when it is convenient to gather cattle to accomplish other management tasks such as sorting calves/cows, branding, and vaccinating. Treatments for ectoparasites applied at these times for prophylactic value are likely ineffective, but costly. Labor, time, animal stress, facilities wear, and expense are disincentives to IPM adoption. Surveys of producer practices show that cost cutting during difficult economic times is most often made in the management category of "animal health", especially ectoparasite management.

Hands-on inspection is the current recommended method to detect ticks on cattle. Tick inspection of cattle can be difficult for those who are untrained in conducting this task. Humans are only able to detect objects greater than or equal to 8 mm in size on cattle (Palmer et al. 1976), which is equivalent to the approximate size of most unfed adult ticks, or the size of engorged nymphs. Therefore, count data for attached tick larvae and nymphs are typically missing. The arduous task of inspecting for ticks on cattle is subject to many biophysical and human factors making probabilities of detection less than satisfactory (Teel et al. 2003). Prior research indicates near infrared reflectance spectroscopy of bovine feces (fNIRS) offers a non-invasive (not dependent on inspecting cattle), economical, and efficient means of detecting tick infested animals and monitoring effectiveness of tactics for tick suppression (Tolleson et al. 2015).

Near infrared reflectance spectroscopy (NIRS) is recognized as playing a key role in the diagnostic surveillance framework for agricultural and environmental management (Shepherd and Walsh 2007). Near infrared reflectance spectroscopy applications extend from soils, to

plants, to water, to crop and livestock product quality and processing, and to livestock health. These applications include biosecurity, bio-forensics, quality assessment and quality assurance. Global applications of NIRS for evaluating herbivore nutrition and physiology include parasite stress (Dixon and Coates 2009). Commercialization opportunities for NIRS applications are diverse. Near infrared reflectance spectroscopy analysis of bovine feces coupled with a computer-aided, decision support system (NUTBAL) has led to a diagnostic surveillance method for monitoring the nutritional status of grazing animals such as cattle, sheep (Artiodactyla: Bovidae; *Ovis aries*), goats (Artiodactyla: Bovidae; *Capra aegagrus hircus*), and white-tailed deer (Lyons and Stuth 1992, Cook 1999). The method of fNIRS coupled with NUTBAL was the basis for establishment of the Grazing Animal Nutrition Laboratory (GANLAB) by Texas A&M AgriLife Research (http://cnrit.tamu.edu/ganlab/) that offers a fee-based service for livestock owners for forage analysis. This service served approximately 4,200 clients and processed about 17,500 fecal samples in 2016. The value of this service is recognized and recommended by the United States Department of Agriculture (USDA), Natural Resources Conservation Service (NRCS) as part of its Conservation Stewardship Program having nutrition monitoring as a practice. The NRCS participation has driven an estimated 25 to 30% increase in GANLAB clients and samples each year. Research into the further development of NIRS applications is also part of the GANLAB mission (https://cnrit.tamu.edu/).

Near infrared (NIR) light consists of wavelengths between ~800 and 2500 nm that exist just above the red portion of the visible spectrum. Organic molecules are complex, containing many different chemical bonds. Each of these bonds absorbs NIR light at a characteristic region of the spectrum. So, in the same way that humans perceive color by processing different amounts of visible light absorbed or reflected by a substance, NIRS detects the relative amounts of

different chemical bonds in that substance resulting in measurement of a composite spectrum. In the case of NIRS fecal profiling, scans are made in the 1100-2500 nm region of the NIR band. When dietary chemistry changes, the resulting products of digestion such as microbes and plant residue also change. The question then becomes whether the changes in dietary chemistry between non-infested and tick-infested periods can be associated with the stress imposed by tick blood-feeding under both artificial and field conditions.

Near infrared spectra of bovine feces can detect differences between tick infested and non-infested animals in controlled studies, as well as spectral changes in individual animals transitioning from pre-infestation, infestation, and recovery (Tolleson et al. 2000, 2004, 2010). These responses have been observed in cattle involving artificial infestations with 6 species of ticks: *Amblyomma americanum, A. maculatum, A. mixtum* (formerly *A. cajennense*), and *D. albipictus* (Tolleson et al. 2007), and *Rhipicephalus (Boophilus) annulatus* and *R. (B.) microplus* (Tolleson et al. 2013). In a study of cattle on a uniform diet and artificially infested with *A. americanum*, it was discovered that fecal chemistry was significantly different from pre-infestation levels for that period associated with tick attachment/early feeding and for a second period associated with peak blood-feeding by rapidly engorging females (Tolleson et al. 2002, 2004). These periods coincide with tick salivary secretions associated with feeding lesion development and the latter with maximum salivary fluid dosages during final rapid feeding to complete engorgement in female ticks (Brossard and Wikel 1997). Ticks modulate the immune system of cattle to obtain a blood meal over a period of days to weeks. Tick-associated immune system modulation is hypothesized to induce changes through the immune-endocrine-digestive system axis (Tolleson et al. 2010, Tolleson 2010). This may cause changes from baseline homeostasis resulting in distinct changes in fecal chemistry that are detectable by NIRS

(Tolleson et al. 2010, Tolleson 2010). Moreover, a field study of *Amblyomma*-infested cattle in north-central Oklahoma from February to April revealed NIRS fecal analyses differentiated tick-infested animals from animals protected by acaricide treatments. As acaricide protection waned and animals became re-infested, the differentiation between animal groups decreased until acaricide retreatment when separation of the groups, based upon fecal analysis, was again detected. These observations suggest potential value of fNIRS analysis as an IPM monitoring system for animal health (Tolleson et al. 2012).

Previous research has shown that feed intake of tick-infested cattle is depressed during periods of peak tick infestation, and that tick-induced stress responses will alter digestive physiological functions to impact changes in fecal chemistry profiles following acute tick infestation. However, the magnitude of changes in fecal chemistry profiles, and the time lag in these changes relative to peak periods of tick blood-feeding have not been clearly quantified. In earlier studies with three-host *Amblyomma* ticks, the period of peak stress was 9-14 days following a single cohort tick infestation, whereas a study with the one-host tick *D. albipictus* produced long-term chronic periods of tick infestation of approximately 20 days duration (Tolleson et al. 2007).

Future studies could further assess the feasibility of fNIRS technology and its potential to provide a non-invasive, economical, and efficient means of detecting tick infestations in grazing cattle systems without the need for physical inspection of individual animals. Controlling and reducing damages caused by arthropods in grazing cattle systems remains challenging due to the life-histories of the arthropods, the interactions of the landscape and grazing cattle system, and the operators' willingness to adopt new management techniques and technology for their system.

There are a variety of technologies available for adoption by cattle producers. This can include advanced breeding technologies (e.g., embryo transfer, artificial insemination, sexed semen), nutritional testing technologies (e.g., forage quality testing, NIRS for dietary diagnostic analysis and decision support system to be used as a nutritional monitoring system for grazing livestock), animal identification systems and record-keeping systems (e.g., GPS and RFID ear tags for animal identification and tracking, computerized record-keeping systems), implants to potentially increase weight gains, and veterinary services (e.g., bull breeding soundness exams, ultrasound) (Johnson et al. 2010, Pruitt et al. 2012, Selk et al. 2006, USDA 2009, Ward et al. 2008). Other technologies could include soil and water health associated with forage/grassland management and stewardship, grazing strategies, and marketing.

Extension services and the USDA have suggested the adoption of certain technologies, with careful consideration usually focused highly on the profitability and productivity of cattle operations. There are several factors that have been shown to affect technology adoption including: farm size, off-farm employment, risk assessment, and farm location (Dorfman 1996, Fernandez-Cornejo 2007, Fernandez-Cornejo et al. 2005, Ward et al. 2008). More factors that have been identified to influence technology adoption include human attributes such as education, years of experience, and age (Johnson et al. 2010, Ward et al. 2008).

Operator/farm size is considered a factor that fundamentally affects technology adoption and whether the operation can afford the initial cost of adopting the technology (Dorfman 1996, Johnson et al. 2010, Pruitt et al. 2012, Ward et al. 2008). For cow-calf operations, small-size operations can be categorized as managing 1-49 head of cattle with medium-size managing 50-199 head, and large-size managing greater than 200 head (USDA 2020). In relation to stocker/feeder cattle operations, Johnson et al. (2010) divided operation size into small (less than

100 head managed each year), medium (100-500 head managed each year), and large (greater than 500 head managed each year). Thus, depending on operation type, cattle herd size characterizations may vary. The probability and speed of adoption is also hypothesized to be positively related to the size of the operation (Gafsi and Roe 1979, Perrin and Winkelmann (1976), Wozniak 1987). Operation management goals are expected to affect technology adoption. A factor motivating technology adoption by large-size operations is the profitability of the technology (Johnson et al. 2010).

The importance cattle producers place on choosing technology is if it provides immediate economic benefits, reduces general labor, and whether their operation is producing sufficient income to avoid hiring off-farm employment. Through prior surveys, the primary disincentives of technology adoption by cattle producers include the overall cost and lack of labor, time, and facilities (Elliott et al. 2013, Pruitt et al. 2012, Ward et al. 2008). These deterrents suggest that the operation type (e.g., stocker/background operation, cow-calf operation) and management characteristics (e.g., on-farm and off-farm employment, facilities) influence the adoption of technology in cattle production systems. Further investigation into the different types of cattle operations and the operations management characteristics should permit extension service personnel to identify cattle producers that would profit from educational and/or incentive programs. Educational and/or incentive programs might encourage the adoption of new technology in grazing cattle systems. Technology adoption by cattle producers could greatly benefit the future of beef production and IPM tactics.

There were three main objectives in this dissertation. The first objective was to determine if artificial infestations of *D. albipictus* could be detected in cattle using fNIRS and whether its detection capability was sensitive to the size of tick infestation and to phase of on-host stage-

specific tick development (larval feeding, nymphal feeding, adult feeding). The second objective was to assess producer adoption of NIRS technology in grazing cattle systems. The third objective was to compare the survivorship of *D. albipictus* larvae in two photoperiod groups (long-day and short-day) exposed to varying saturation deficits.

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CHAPTER II

DETECTION OF WINTER TICK INFESTED CATTLE USING NEAR INFRARED REFLECTANCE SPECTROSCOPY ANALYSIS OF BOVINE FECES

Tick (Acari) parasitism in range cattle can occur year-round with direct production losses manifested through blood loss, irritation, poor rates of gain, weight loss, and loss of body condition (Barnard 1985, Teel et al. 1990, Williams 2010). Most tick species are seasonally active in the spring and summer months in the southern United States (US), while the one-host species *Dermacentor albipictus* Packard (Acari: Ixodidae), the winter tick, is active in fall (October through November) and winter (December through February). Winter tick infestations are easily unnoticed due to winter hair coats and occur when annual forage quality and quantity are low (Bishopp and Tremley 1945, Teel et al. 1990). *Dermacentor albipictus* infestations can often reach detrimentally high numbers (>50,000; Bergeron and Pekins 2014, Drew and Samuel 1985, Mooring and Samuel 1998) on large ungulate hosts including livestock such as horses (Perissodactyla: Equidae; *Equus spp.*) and cattle (Artiodactyla: Bovidae; *Bos* spp.) and wildlife such as moose (Artiodactyla: Cervidae; *Alces alces*), elk (Artiodactyla: Cervidae; *Cervus canadensis*), and white-tailed deer (Artiodactyla: Cervidae; *Odocoileus virginianus*). In Canada, cattle and horse deaths followed high infestations of winter ticks on animals grazing scrublands during the winter months of December and January (Cameron and Fulton 1926).

The winter tick is a known biological vector of *Anaplasma marginale* Theiler, causal agent of bovine anaplasmosis, which is one of the most important diseases of beef cattle in Texas, US. Recent geographic (Hairgrove et al. 2014) and in-herd seroprevalence and PCR-prevalence (Hairgrove et al. 2015) of cattle exposure to *A. marginale* have been documented.

Seroprevalence exceeded 25% in selected cow herds and exceeded 70% in selected bull herds (Hairgrove et al. 2015) west of Interstate Highway 35 in Texas, US. These findings associate the geography of the winter tick's distribution in this state where *A. marginale* is endemic. Interactions of cattle with ticks and rangeland landscapes supporting tick habitats and wildlife hosts pose substantial challenges to tick control and pathogen transmission.

Integrated pest management (IPM) strategies have been developed for ticks (Barnard et al. 1994, Williams 2010). These strategies rely on habitat management, grazing rotations, wildlife management, fencing, and cattle treatments with acaricides (Barnard et al. 1994, Williams 2010). However, the on-animal application of acaricides has become the principle recommended tactic to control ticks. Treatments for ectoparasites applied during routine management practices for other primary goals (e.g., vaccinations, branding, dehorning) for prophylactic value are likely ineffective and costly. For tick management, producers are required to gather and physically inspect animals on a regular basis to determine tick presence and abundance and make informed decisions regarding management tactics (Barnard 1985, Williams 2010). Labor, time, animal stress, facilities wear, and expense are disincentives to IPM adoption.

Hands-on inspection is the current recommended method to detect ticks on cattle. Tick inspection of cattle can be difficult for those who are untrained in conducting this task. Humans are only able to detect objects greater than or equal to 8 mm in size on cattle (Palmer et al. 1976), which is equivalent to the approximate size of most unfed adult ticks, or the size of engorged nymphs. Therefore, count data for attached tick larvae and nymphs are typically missing. The arduous task of inspecting for ticks on cattle is subject to many biophysical and human factors making probabilities of detection less than satisfactory (Teel et al. 2003). Prior research indicates that near infrared reflectance spectroscopy of bovine feces (fNIRS) offers a non-invasive (not

dependent on inspecting cattle), economical, and efficient means of detecting tick infested animals and monitoring effectiveness of tactics for tick suppression (Tolleson et al. 2015).

In a study of cattle on a uniform diet and artificially infested with *Amblyomma americanum* L. (Acari: Ixodidae), it was discovered that fecal chemistry was significantly different from pre-infestation levels for that period associated with tick attachment/early feeding and for a second period associated with peak blood-feeding by rapidly engorging females (Tolleson et al. 2002, 2004). These periods coincide with tick salivary secretions associated with feeding lesion development and the latter with maximum salivary fluid dosages during final rapid feeding to complete engorgement in female ticks (Brossard and Wikel 1997). Ticks modulate the immune system of cattle in order to obtain a blood meal over a period of days to weeks. Tick-associated immune system modulation is hypothesized to induce changes through the immune-endocrine-digestive system axis (Tolleson et al. 2010, Tolleson 2010). This may cause changes from baseline homeostasis resulting in distinct changes in fecal chemistry that are detectable by near infrared reflectance spectroscopy (NIRS) (Tolleson et al. 2010, Tolleson 2010). Moreover, a field study of *Amblyomma*-infested cattle in north-central Oklahoma, US from February to April revealed NIRS fecal analyses differentiated tick-infested animals from animals protected by acaricide treatments. As acaricide protection waned and animals became re-infested, the differentiation between animal groups decreased until acaricide retreatment when separation of the groups, based upon fecal analysis, was again detected. These observations suggest potential value of fNIRS analysis as an IPM monitoring system for animal health (Tolleson et al. 2012).

Previous research has shown that feed intake by tick-infested cattle is suppressed during periods of peak tick infestation in both natural seasonal peaks of tick activity and single artificial

tick infestations, and that tick-induced stress responses will alter digestive physiological functions to impact changes in fecal chemistry profiles following acute tick infestation (Teel et al. 2004, Tolleson et al. 2007, Tolleson et al. 2010, Tolleson et al. 2015). However, the magnitude of changes in fecal chemistry profiles and the time lag in these changes relative to peak periods of tick blood-feeding have not been clearly quantified. In earlier studies with three-host *Amblyomma* ticks, the period of peak stress was 9-14 d following a single cohort tick infestation, whereas a study with the one-host tick *D. albipictus* produced long-term chronic periods of tick infestation of approximately 20 d duration (Tolleson et al. 2007). Current and future studies to clarify temporal relationships of tick burden, host stress responses and changes in fecal chemistry profiles, are expected to further improve diagnostic capabilities of fNIRS.

Near infrared spectra of bovine feces can detect differences between tick infested and non-infested animals in controlled studies, as well as spectral changes in individual animals transitioning from pre-infestation, infestation, and recovery (Tolleson et al. 2000, 2004, 2007, 2010, 2013). These results have been discovered in six tick species: *A. americanum*, *A. maculatum* Koch (Acari: Ixodidae), *A. mixtum* Koch (Acari: Ixodidae; formerly *A. cajennense* Fabricius), and *D. albipictus* (Tolleson et al. 2007), and *Rhipicephalus annulatus* Say (Acari: Ixodidae) and *R. microplus* Canestrini (Acari: Ixodidae) (Tolleson et al. 2013). The objective of this study was to determine if artificial infestations of *D. albipictus* could be detected in cattle using fecal NIRS and whether its detection capability was sensitive to the size of tick infestation and to phase of on-host stage-specific tick development (larval feeding, nymphal feeding, adult feeding).

Materials and Methods

Tick Colony and Maintenance

Dermacentor albipictus (F_2 generation in colony) used in this study were obtained from research colonies at the Tick Research Laboratory, Texas A&M AgriLife Research, College Station, Texas, USA. Ticks were maintained in sealed glass chambers under the following approximated environmental conditions: 25.0 ± 3.0°C, 80-85% relative humidity, and under a 14L:10D photoperiod. *Dermacentor albipictus* larvae were prepared for cattle infestation by placing them in a sealed glass chamber at 15°C, 80-85% relative humidity, and short-day photoperiod (8L:16D) for 10 d before beginning the study to activate them from quiescence/behavioral diapause (Wright 1969). The *D. albipictus* colony maintenance and rearing for this study was in accordance with the Institutional Animal Care and Use Committee (IACUC)-approved Animal Use Protocol (AUP) No. 2017-0345.

Animal Procedures

Bos taurus heifers (129 ± 1.5 kg) used in this study were approximately six months old, of uniform genetic background, had minimal to no prior tick exposure, and not previously treated for hematophagous ectoparasites. Each heifer received two vaccinations and an oral treatment prior to the pre-infestation acclimation period (Table 1). All heifers were pre-conditioned as a cohort. Pre-conditioning included daily haltering, halter training by a human walking them with a halter on and tying them up and brushing their hair coats to get them used to touch. Additionally, every other day they were walked through a sweep tub and squeeze chute to acclimate them to the facilities and being confined as tight places. The levels of human interaction all heifers received prior to being moved indoors to head stanchions helped ensure that the heifers were comfortable with humans and confinement and made the transition less

stressful on the heifers. Indoor facilities were specifically designed to maintain an ectoparasite free environment other than the ticks used in the study. Feed and water were provided *ad libitum* throughout the study. Heifers were maintained at the Tick Research Laboratory for the duration of the study in accordance with the Institutional Animal Care and Use Committee (IACUC)-approved Animal Use Protocol (AUP) No. 2017-0345.

Table 1. Pre-study animal health treatments for all six heifers administered weeks prior to the pre-infestation acclimation period.

Weeks	Vaccination	Company Information	Administration Type	Dosage
6	Liquamycin® LA-200	Zoetis, Parsippany, New Jersey, USA	Subcutaneous Injection	4.5 mL/45.4 kg body weight
4	Vision® 8 with Spur	Merck Animal Health, Omaha, Nebraska, USA	Subcutaneous Injection	Single 2 mL dose
4	Safe-Guard® Dewormer Paste (fenbendazole 10%)	Merck Animal Health, Summit, New Jersey, USA	Oral Treatment	5 g/100 kg body weight

Notes: Liquamycin® LA-200 was administered to all six heifers before they were shipped to the Tick Research Laboratory in College Station, Texas, USA. It was used to treat for pneumonia and shipping fever as these heifers were young and newly weaned when purchased. Vision® 8 with Spur was administered to all six heifers upon arrival to the Tick Research Laboratory in College Station, Texas, USA. It was used to prevent disease in the heifers, and it is considered standard for young beef cattle to receive this type of vaccination. Safe-Guard® Dewormer Paste (fenbendazole 10%) was given orally to all six heifers to possibly remove and control any internal parasites the heifers may have had prior to arriving at our laboratory or would pick up upon being moved to our laboratory.

Experimental Design

A total of six heifers were infested in each of the two trials separated by a resting period of 101 d (Figure 1). The experiment was conducted as a repeated measures experimental design with the replication source being fecal samples by day and the tick infestation levels as treatment (Table 2). In this study, the pre-infestation acclimation period served as the experimental control for each heifer. For Trial One, the six heifers were randomly assigned to a treatment group

resulting in three pairs. For Trial Two, a heifer that was assigned to a specific treatment group in Trial One was assigned to a different treatment group in the second trial based upon the tick infestation level they were exposed to in Trial One. Animals were chosen to be assigned in treatment groups this way for Trial Two due to each heifers' potential acquired immunity to the tick feeding from Trial One and ensure efficient tick infestations for each treatment group in the second trial. All heifers were weighed before and after each trial using an S3 Weigh Scale System (Tru-Test Datamars, Mineral Wells, Texas, USA).

Treatment groups consisted of three levels of tick infestation: low (1000 larvae), medium (4000 larvae), and high (8000 larvae). These levels of infestation were chosen to test the hypothesis as the subject of this study. Our observations of winter tick infestations on animals in the field can range from one to >100 ticks on a single host. The size of a single winter tick egg mass can range from 2700 to 4000 eggs (Bishopp and Wood 1913, Howell 1939) and subsequent larval clutches questing on vegetation range from 10 to 1000 ticks (Drew et al. 1985, Drummond et al. 1968). This supports the idea that minimally an animal could encounter questing larvae from a single batch of eggs where 1000 larvae would be a portion of a single egg mass. In line with our objective, there is a need to determine whether fNIR spectra can detect minimal levels of tick infestations.

Figure 1. Heifers used in both Trials One and Two.

Table 2. Both trials were conducted as a repeated measures experimental design with the replication source being fecal samples by day and the tick infestation levels as treatment. This table shows heifer numbers corresponding to each trial and experimental treatment group, and the source of replication for each trial.

Trial	Experimental Treatment Group	Heifer Number	Replication Source
Trial One (Fall 2018)	Low (1000 larvae)	3 5	1 fecal sample/day/heifer Total of 64 d 384 fecal collections total
	Medium (4000 larvae)	2 6	
	High (8000 larvae)	4 8	
Trial Two (Winter 2019)	Low (1000 larvae)	4 6	1 fecal sample/day/heifer Total of 57 d 348 fecal collections total
	Medium (4000 larvae)	3 8	
	High (8000 larvae)	2 5	

Experimental Controls and Timeline of Tick Infestations

For both trials, heifers were prepared for tick infestation through a 15-d pre-infestation acclimation period (Figure 2), which served as the experimental control period for each individual animal. The pre-infestation acclimation period was divided into two separate control periods. The first was an 8-d pre-infestation acclimation control period completed in an outdoor paddock behind the Tick Research Laboratory (pre-infestation control period outside; Days -15 to -8), where the heifers could graze 24 h per d on natural growing forages (primarily bermudagrass and rye grass), and each morning were provided 23 kg of coastal hay and 18 kg of creep feed. The second was a 7-d pre-infestation acclimation control period inside the laboratory (pre-infestation control period inside; Days -7 to -1), where each individual heifer was placed on a uniform diet ration that was fed twice a day consisting of 3 kg of creep feed and 0.11 kg of alfalfa cubes. Feed analyses for the creep feed and alfalfa cubes are described in A-1 and A-2 in Appendix A. On Day -7, heifers were moved inside to the animal use room (AUR) where they were randomly assigned into numbered elevated head stanchions to adjust them in the new environment and to the diet rations of creep feed and alfalfa cubes. Since each individual heifer was kept on a uniform diet ration during the pre-infestation control period inside the laboratory (Days -7 to -1) and during tick infestation (Trial One: Days 0 to 39; Trial Two: Days 0 to 33), the option to use each heifer's pre-infestation period as a control was viable (Tolleson et al. 2007). All environmental conditions were monitored for both trials inside the AUR where heifers were kept in elevated head stanchions. The environmental data for both trials are provided separately in Appendix A (Trial One: A-3; Trial Two: A-4). The photoperiod in the AUR for both trials was held at 14L:10D which is the standard light cycle used to rear ticks at the Tick Research Laboratory.

On Day 0, *D. albipictus* larvae were free released on each heifer down the midline of the back. Thereafter, all heifers were examined twice daily for tick attachment, engorgement status and molting. Daily logs (A-5 in Appendix A) for each heifer were implemented throughout the study to monitor feed and water consumption and to observe any behavioral and physical changes during tick infestation. Previous descriptions of the parasitic phase of the winter tick (Strickland et al. 1976, Tolleson et al. 2007) and our observations in rearing this species were the basis for establishing an anticipated timeline for tick feeding and development from an infestation initiated with a single cohort of larvae and relative host stress levels (Figure 3). Relative host stress was categorized generally by response to tick attachment, blood feeding and salivary modulation of feeding lesions in each developmental stage (Tolleson et al. 2010, Tolleson 2010). Days 0-4 were noted as larval feeding with molting to nymphs occurring from Days 4-8 (low stress period/early feeding). Nymphal feeding occurred from Days 8-15 with molting from Days 15-18 (low stress period/early feeding). Adult tick feeding occurred from Days 18-35 with drop-off of engorged adult females occurring from Days 26-39 (high stress period/peak feeding). Heifers were removed from elevated head stanchions on Day 39 in Trial One and Day 33 in Trial Two and put back into the paddock behind the Tick Research Laboratory. Immediately after heifers were moved back outside, they were treated with a synthetic pyrethroid to remove all remaining ticks and then allowed to recover until Day 48 in Trial One and Day 42 in Trial Two, marking the end of each trial. The post-tick recovery period is noted as Days 41 to 48 in Trial One and Days 35 to 42 in Trial Two. The post-tick recovery period is meant to serve as a time period to allow the heifers to recuperate from tick feeding and return to a state of homeostasis as they were in the pre-infestation acclimation controls periods outside and inside.

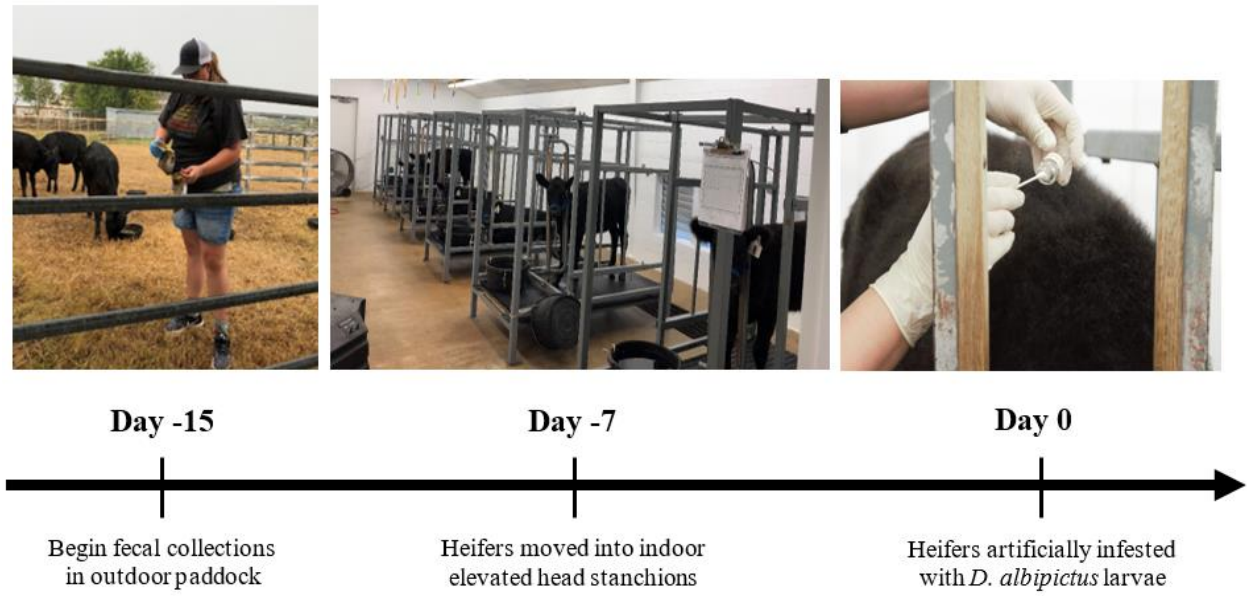

Figure 2. Eight-day outdoor pre-infestation acclimation control period (Days -15 to -8) followed by a 7-day indoor pre-infestation acclimation control period (Days -7 to -1). Daily fecal collections over the 15-day pre-infestation acclimation period served as the experimental control for each individual heifer (pre-infestation control period).

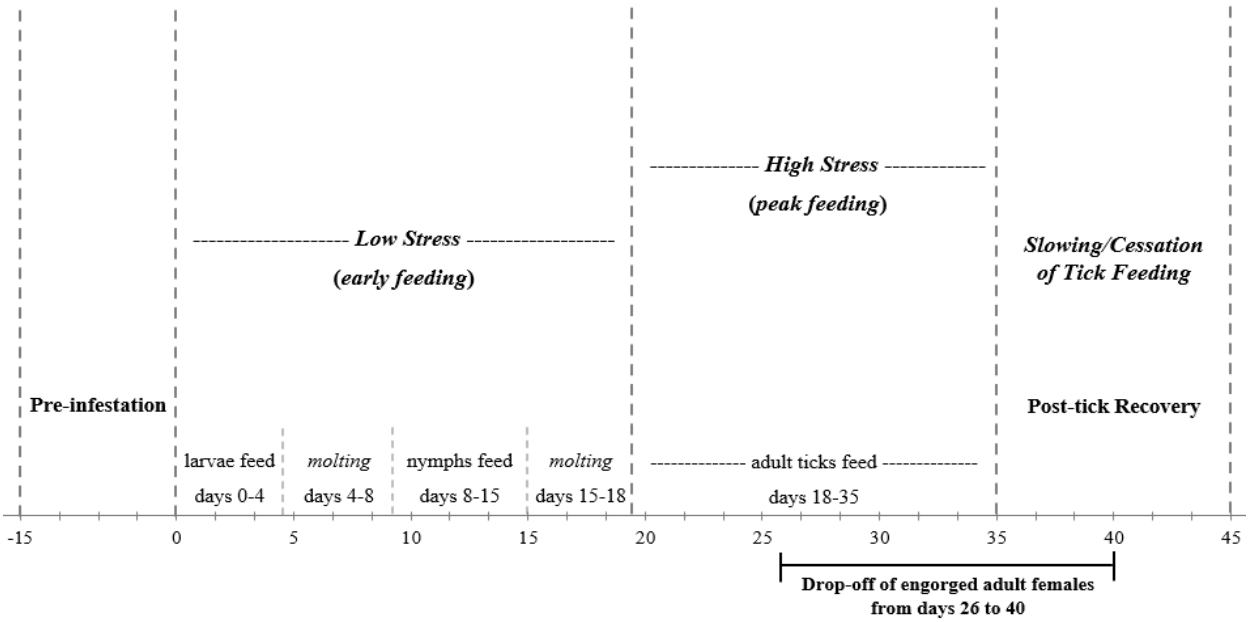

Figure 3. Anticipated sequence and duration of the *D. albipictus* feeding cycle by calendar day. Note: Day "0" represents the day that ticks are placed onto hosts to commence the infestation.

Fecal Sampling and Preparation for NIRS Analysis

Fecal samples weighing approximately 0.45 kg were obtained daily from individual heifers starting 15-days prior to tick exposure through completion of the post-tick recovery period (Trial One: Days -15 to 48, total of 64 d; Trial Two: Days -15 to 42, total of 58 d). Fresh, non-urine or feed contaminated feces was selected when possible. All samples were collected by hand off the elevated head stanchion in conjunction with morning feeding. Fecal samples were placed in 710 mL Whirl-Paks (Nasco Whirl-Pak®, Fort Atkinson, WI, USA) that were pre-labelled for each heifer's ear tag number, period during study, treatment group, and date. Fecal samples were then stored in an upright freezer (Frigidaire 0.58 cu. mt. Upright Freezer, Charlotte, North Carolina, USA) at -20°C until processed for NIRS.

Fecal samples were left to thaw for 24 h and then each sample was homogenized by moving the sample around in the Whirl-Pak. A subset of each homogenized sample was collected from the Whirl-Paks and placed into 15 x 9 cm paper boats where they were labeled with heifer number, collection date, and tick infestation period. The remainder of each fecal sample was returned to storage at -20°C. Labeled samples in the paper boats were then placed into a drying oven (Isotemp 737°F, Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) at 60°C for 72 h before being milled into 1mm particulates in a laboratory mill (UDY Cyclone model 3010-039, Fort Collins, Colorado, USA). The milled fecal samples were placed into labeled manila coin envelopes (Uline 3 3/8X6" Kraft Coin Envelope, model S-14720, Pleasant Prairie, Wisconsin, USA) and transported to the Texas A&M AgriLife Grazing Animal Nutrition Laboratory (GANLAB) in Temple, Texas, USA, where spectroscopic analysis was performed using a Foss® NIRS 6500 series scanning spectrometer with spinning cup attachment (Foss® Analytical, Hillerød, Denmark). Spectral data were sent to the Tick Research Laboratory

to perform stepwise cluster analyses using spectroscopy software GRAMS IQ™ v.9.3 (part of the Graphical Relational Array Management System, Thermo Fisher Scientific Inc, Waltham Massachusetts, USA).

Statistical Analyses

Stepwise Cluster Analyses

Spectral data were sent to the Tick Research Laboratory to perform stepwise cluster analyses using spectroscopy software GRAMS IQ. Stepwise cluster analyses were completed using raw spectral data from daily fecal samples for heifer pairs in each treatment level group (low, medium, and high) in Trial One and then in Trial Two from the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period. A discriminate analysis was conducted using a cross-validation calibration type with a standard normal variate de-trending process. Baseline effects were removed from the model by use of the 1st derivative of the spectra and using the Savitzky-Golay method. This method sorts the spectra by variation, looking at the entirety of the spectra, finding the most common variations, and assigning each variation as a primary "factor" or "variation". For the remainder of this chapter, we will refer to the "factor" or "variation" as factors. Each of the factors represents a unique fecal chemical profile (FCP) with successive factors representing unique sample chemistry. Once the first factor was assigned, the software examines the remaining spectra to find the next most common factor and assigns that as the next factor. The software repeats this process for the remaining spectra until all the possible factors are identified. Factors are listed from 1 to 25 with the most common variations listed first and less common factors listed last. Cluster analyses can be performed in GRAMS IQ™ with the three most dominant factors plotted as "x", "y" and "z" providing a basis for comparative

assessments. In the calculations performed by the GRAMS IQ™ software, eigenvalues are generated for each factor. These eigenvalues, a measure of the relative weight of a given factor, are a measure of the importance of the factors statistically. Using the total percent variance for each factor allows for a determination of the maximum number of factors that fit a model by calculating when the total reaches 100%, signifying that all variation has been accounted for by the listed factors.

Principal Component Analysis

A principal component analysis (PCA) was performed using SAS v.9.4 software system (SAS institute Inc., Cary, North Carolina, USA) to determine if any spectral cluster shifts that occurred in the stepwise cluster analyses were significantly different. The PCA was performed on spectral data from 384 observations in Trial One and 348 observations in Trial Two from the six heifers over the six Stages. Stages were designated as: Stage 1 = Pre-infestation Outside, Stage 2 = Pre-infestation Inside, Stage 3 = Larval Feeding, Stage 4 = Nymphal Feeding, Stage 5 = Adult Feeding, and Stage 6 = Post-tick Recovery. A multivariate analysis of variance (MANOVA) using SAS v.9.4 software system (SAS institute Inc., Cary, North Carolina, USA) was then performed on the three principal components using an alpha level of 0.05 in the following model: $(P_1, P_2, P_3) = Heifer(Treatment) + Treatment + Stage + Treatment * Stage + Residuals$. Terms in the model were defined as: 1) (P_1, P_2, P_3) are the first three principal components, 2) *Heifer*(*Treatment*) is the random nested effect of the individual Heifer for the three treatments, 3) *Treatment* is the effect of the three levels of infestation: Low, Medium, and High, 4) *Stage* is the effect of the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period, 5) *Treatment* $*$ *Stage* is the interaction effect of *Treatment* with *Stage*, and

6) *Residuals* is the random effect of all other factors that may impact the spectra. A MANOVA was performed on the three principal components to look at: 1) a difference in the means of the three principal components across the six Stages in the study, 2) a difference between the means of the three principal components across the three levels of tick infestation, and 3) an interaction between *Stage* and *Treatment* factors. The Wilk's Lambda test was used to detect any significant evidence of a difference ($P < 0.05$) for the results of the MANOVA performed on the three principal components. To compare the mean differences between the three levels of tick infestation by the six Stages in each Trial, a repeated measures model was conducted relating each of the three principal components separately. The three models took into account that the six heifers were measured multiple times over the course of each Trial. If there was significant interaction between *Stage* and *Treatment*, a comparison of treatment levels was made separately for each of the six Stages. A Bonferroni multiple comparison procedure was used to compare mean values of each principal component (P_1, P_2, P_3) for the three levels of tick infestation at each of the six Stages in each Trial. There was a total of 18 pairs of comparison (three pairs of Treatment means for each of the six Stages). Thus, the Bonferroni adjusted 0.05 critical level was 0.05/18 = 0.0028. A pair of Treatment means was declared to be significantly different if their *p*-value was less than 0.0028.

Results

Trial One (Fall 2018)

Heifer Body Weights

The beginning and end weights by heifer and treatment are provided in Table 3. All six heifers gained weight during Trial One. Weight gain ranged from 31.8 kg to 40.8 kg over the course of 64-days, which is equivalent to 1.1 kg to 1.41 kg per day. These are standard weight

gains for heifers in their growing weight class (Ringwall 2012) regardless of tick infestation. The lowest weight gains were in the high tick infestation level treatment group.

Table 3. Comparison of beginning and ending heifer weights following infestation with *D. albipictus* larvae at three infestation level treatment groups over the course of 64-days in Trial One.

Treatment Group Larval Infestation Level	Heifer Number	Beginning Body Weight (kg)	End Body Weight (kg)/ Weight Change (kg)
Low (1000 larvae)	3	122.5	156.5/34.0
	5	136.1	172.4/36.3
Medium (4000 larvae)	2	122.5	163.3/40.8
	6	138.4	170.1/31.8
High (8000 larvae)	4	127.0	161.0/34.0
	8	127.0	158.8/31.8

Engorged Female Drop

The result of each heifer's infestation was measured by determining the first date of engorged female tick drop, the daily and cumulative number of engorged females produced by each infestation, and the date of peak engorged female drop. Engorged *D. albipictus* females that dropped off the six heifers were collected three times daily. The approximate percentage of engorged female drop was calculated for each individual heifer based on a 50:50 sex ratio of females to males for *D. albipictus* (Barker et al. 1990) is summarized in Table 4. Graphical representations of daily engorged female drop for the six heifers are shown in Figure 4.

Table 4. Trial One engorged females drop data by heifer number for the three treatment groups. This table shows the number of engorged females, the percentage of engorged females collected from each heifer based on a 50:50 sex ratio of females to males, and the day(s) of peak female drop post-tick infestation.

Treatment Group	Heifer Number	Number of Engorged Females	Percent of Engorged Females Collected (50:50 sex ratio)	Peak Drop Day(s) Post-infestation
Low	3	166	33.2	32
	5	179	35.8	28
Medium	2	391	19.5	27
	6	597	29.9	29
High	4	1220	30.5	26
	8	698	17.5	33 and 34

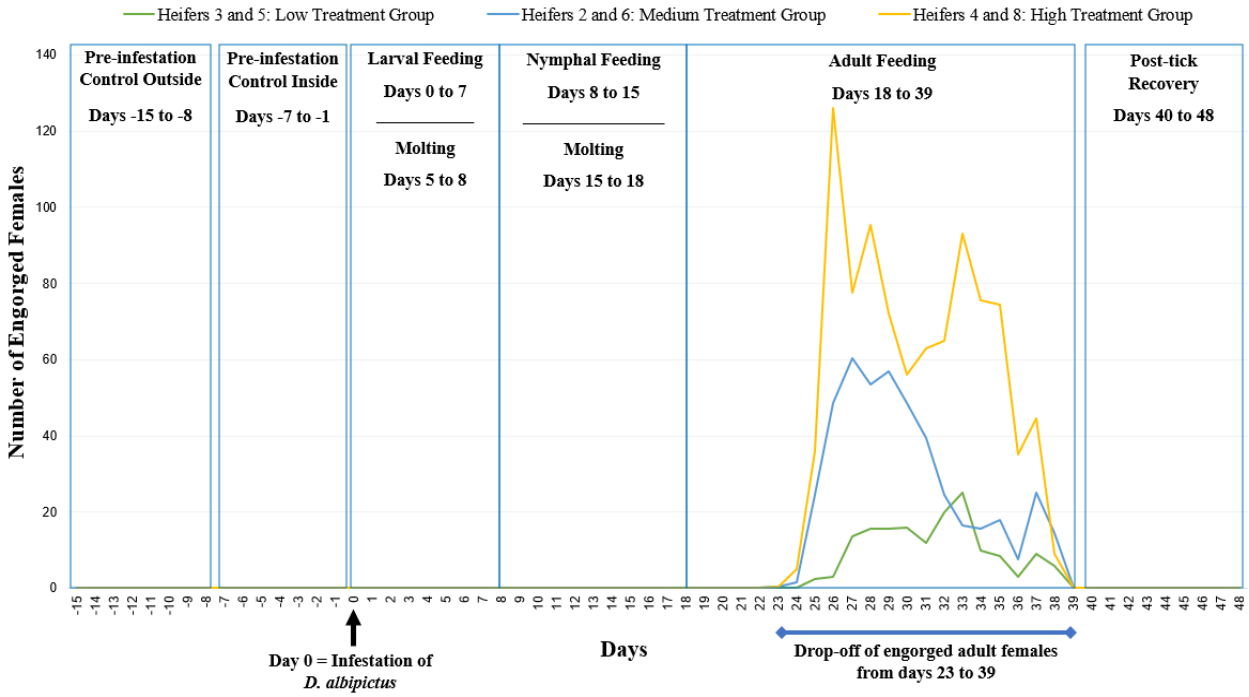

Figure 4. Number of engorged female *D. albipictus* collected per day from the start of female drop-off by heifer pairs in the three treatment groups in Trial One. Days are shown on the x-axis and the average number of engorged females collected from heifer pairs in the three treatment groups shown on the y-axis.

Stepwise Cluster Analyses for the Low Treatment Level Group

A discriminant analysis was conducted for raw spectral data from daily fecal samples for heifer numbers 3 and 5 in the low treatment level group from the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period (one fecal spectra per day per heifer). Spectral analysis of the entirety of the NIR spectra as well as part of the visible spectrum (400 nm to 2498 nm) identified non-contributing spectra. This resulted in a model that was not reliable, as the first three factors were only representative of 68.47% of the total spectral variation. The spectral range was then progressively limited through a series of analyses in order to maximize the contributing spectral range while mitigating the effects of non-contributing spectral range (noise). Repeated analyses were conducted using limited portions of the spectral range starting by examining the peaks and valleys, regions that exhibited high and low levels of reflectance, and whose first three factors explained the greatest amount of variation. The spectral region that yielded the most contribution to the first three factors was within the 576 nm to 1126 nm range. The analysis of spectra in the 576 nm – 1126 nm range produced 13 factors with the first three dominant spectral factors/variations representing 96.79% of the total variation among sample spectra for the low infestation treatment level (see Figures 5 – 7). This result permitted the sample spectra to be analyzed by cluster analyses with each of the three dominant "factors" or "variations" plotted as "x", "y" and "z".

The cluster analyses of the spectra from the pair of heifers in the low treatment level group resulted in a pattern of six clusters that depict shifts in fecal chemistry. Sample clusters were distinguishable for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick

recovery period in the cluster analyses (see Figures 8 through 14). The first was comprised of samples originating from Day -15 (15 d prior to infestation) through Day -6 (6 d prior to infestation; allowing for a 48-h rumen passage time) (Figure 8). The second cluster was comprised of samples within the period from Day -5 (5 d prior to infestation) through Day 1 (1 day post infestation; allowing for a 48-h rumen passage time) (Figure 9). Figure 10 is the daily fecal spectra for the two pre-infestation acclimation periods in one cluster analysis, showing the entire experimental control period for the low treatment group. The third cluster was comprised of samples from Day 2 (2 d post infestation) through Day 7 (7 d post infestation), which is the period of attachment, feeding and molting of larvae (Figure 11). The fourth cluster originated from samples from Day 8 (8 d post infestation) to Day 17 (17 d post infestation), corresponding to attachment, feeding, and molting of nymphs (Figure 12). The fifth cluster includes samples from Day 18 (18 d post infestation) to Day 41 (allowing for a 48-h rumen passage time), corresponding to the period of adult feeding and the period in which females complete their feeding and drop from the host (Figure 13). The sixth cluster was comprised of samples from Day 42 to Day 47, which consists of the period heifers were going through post-tick recovery (Figure 14). Spectral cluster shifts occurred representing time periods that are consistent with no tick feeding, low tick feeding, heavy feeding, and a period of post-tick recovery.

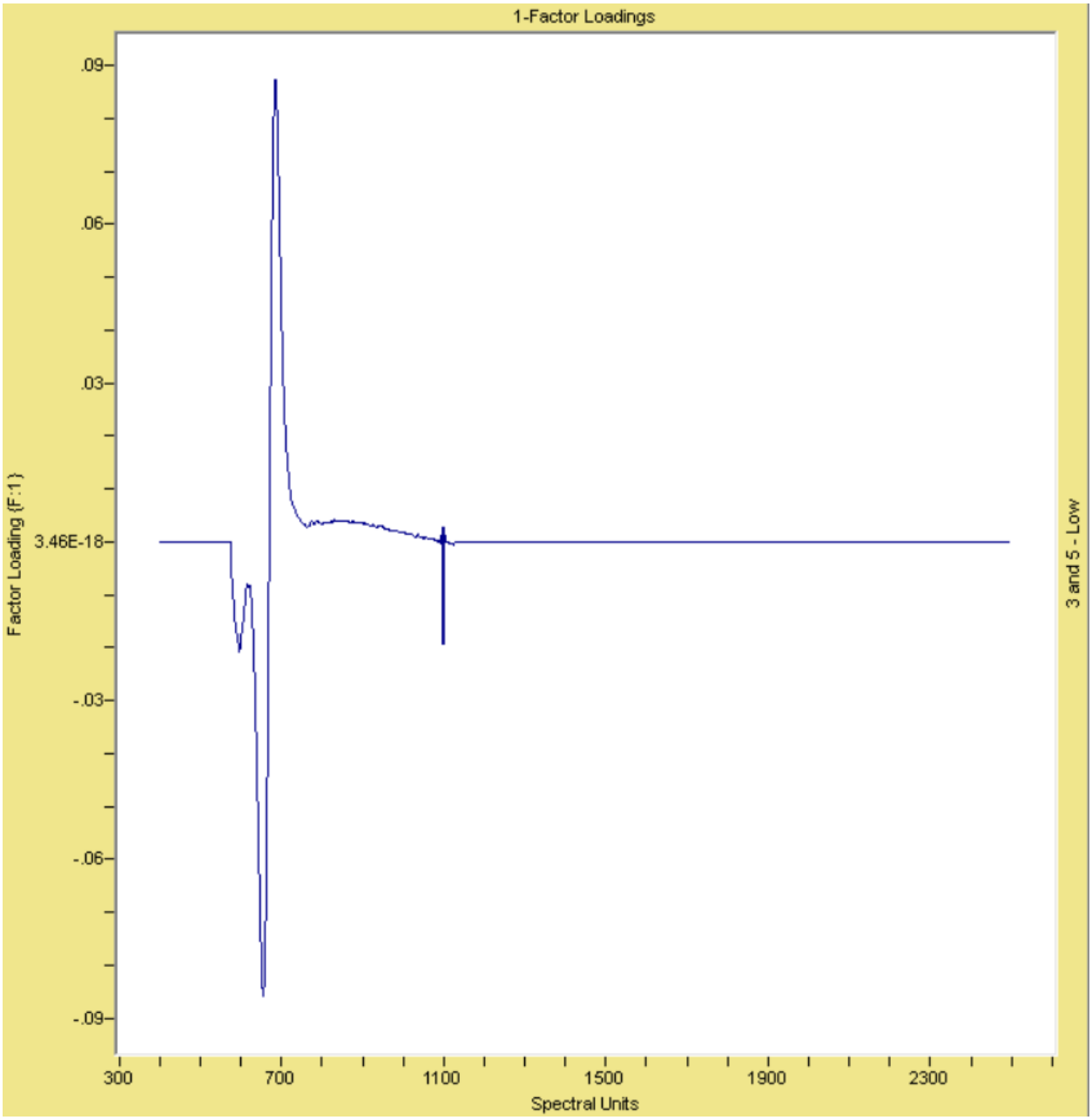

Figure 5. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the first most common spectral variation (factor) from the low infestation treatment level heifer numbers 3 and 5 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 86.55% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

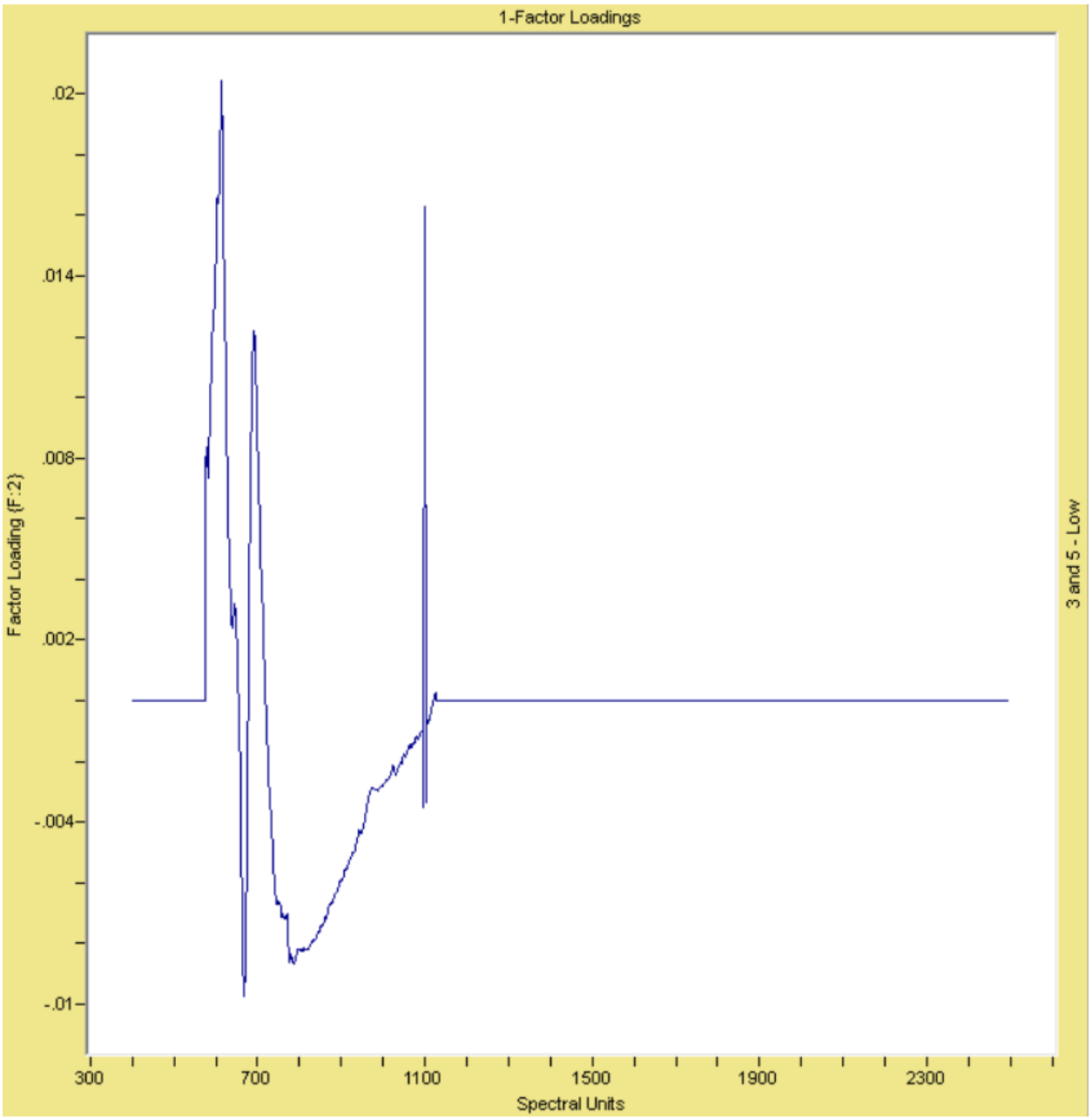

Figure 6. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the second most common spectral variation (factor) from the low infestation treatment level heifer numbers 3 and 5 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 7.39% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

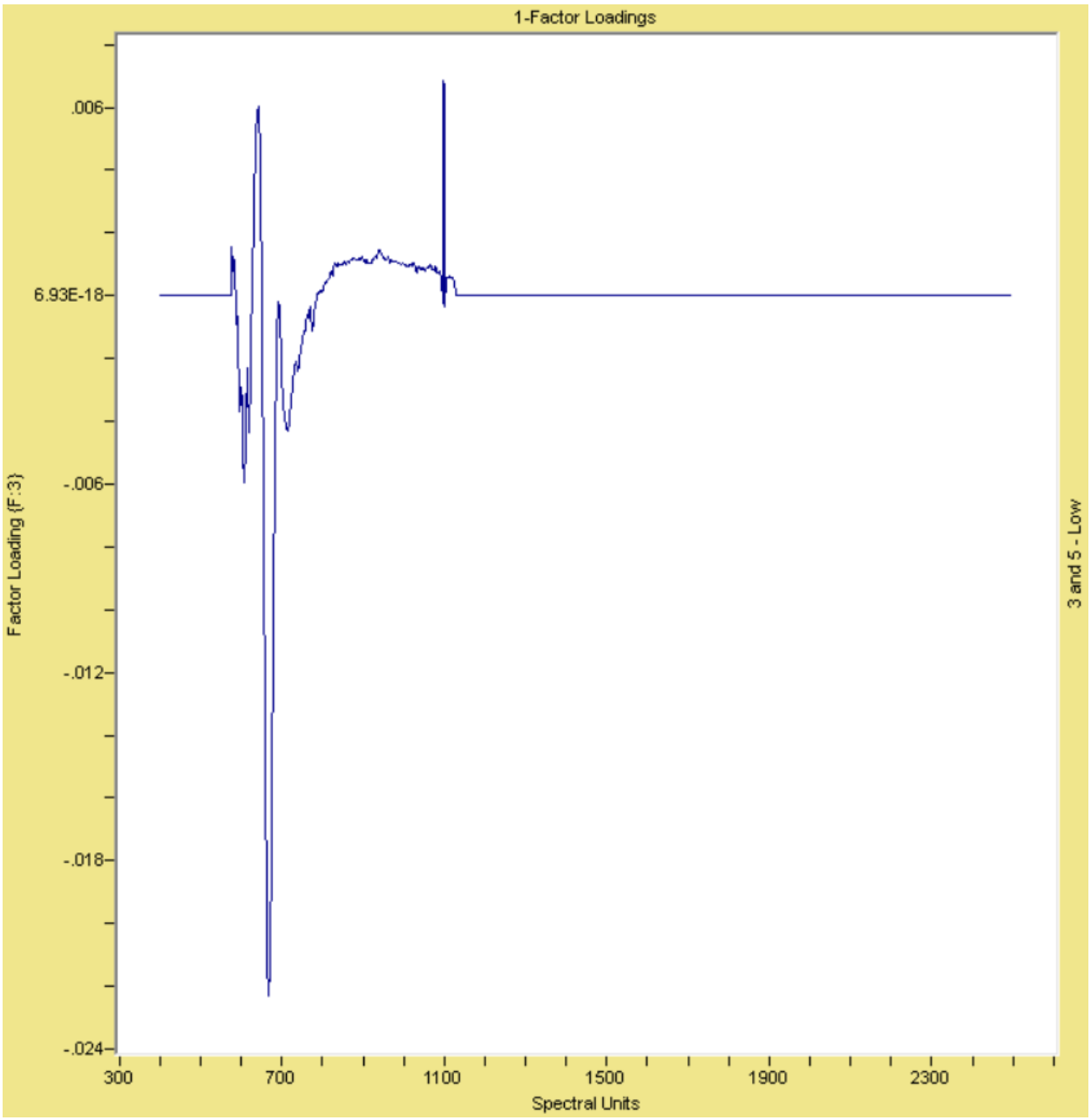

Figure 7. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the third most common spectral variation (factor) from the low infestation treatment level heifer numbers 3 and 5 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 2.85 % of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

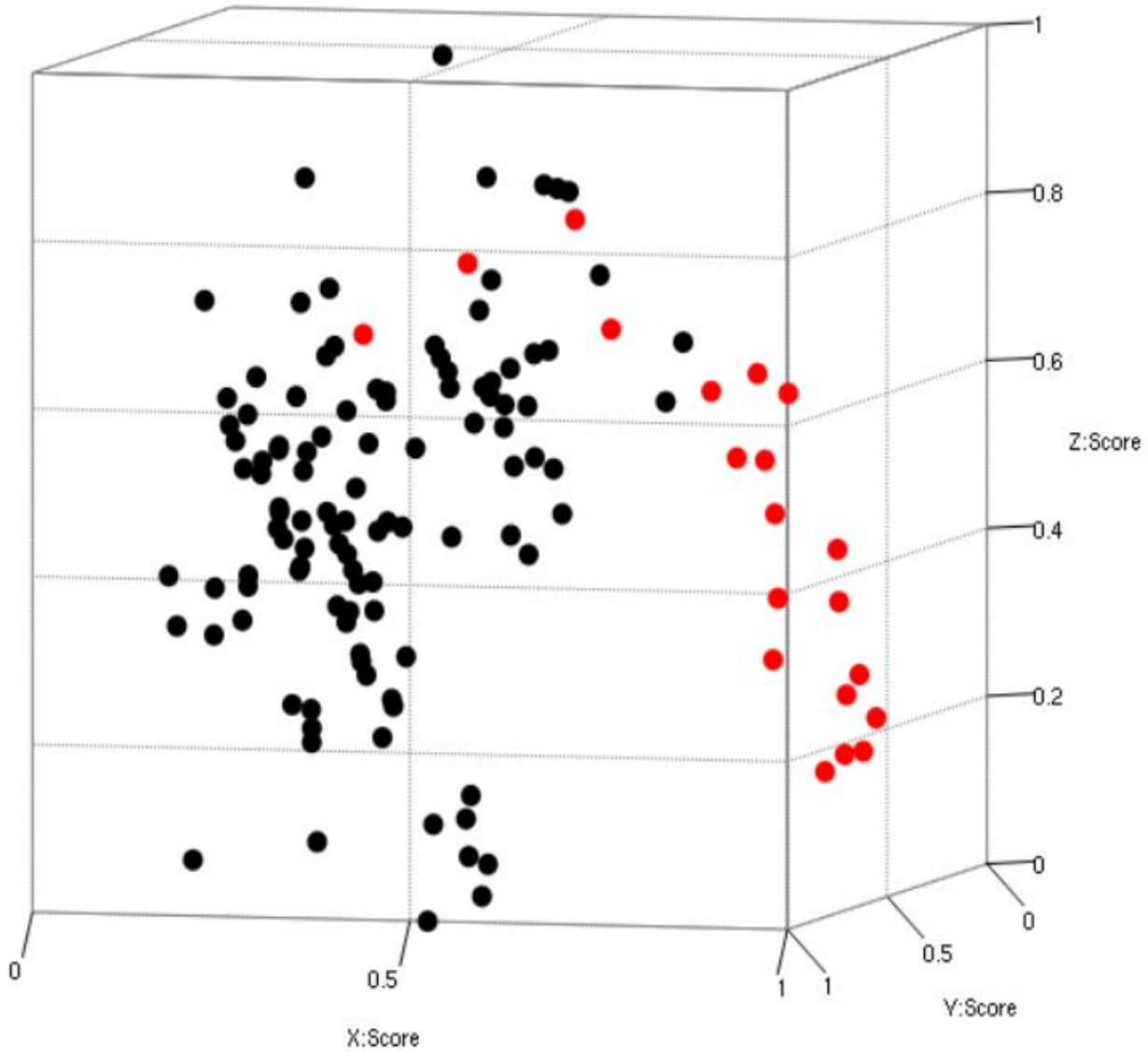

Figure 8. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day -6 during the Pre-infestation Outside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

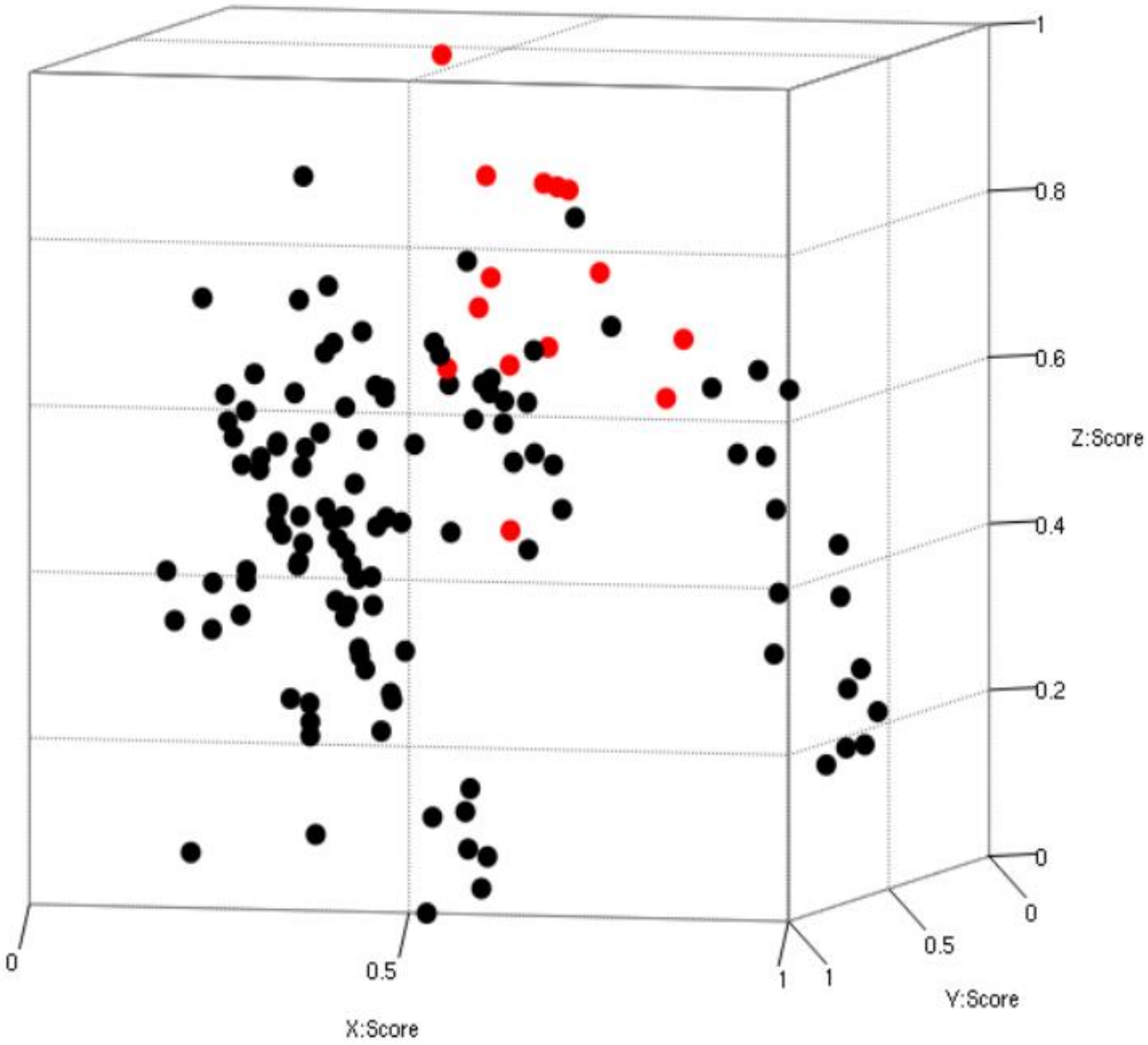

Figure 9. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Inside period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -5 to Day 1 during the Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

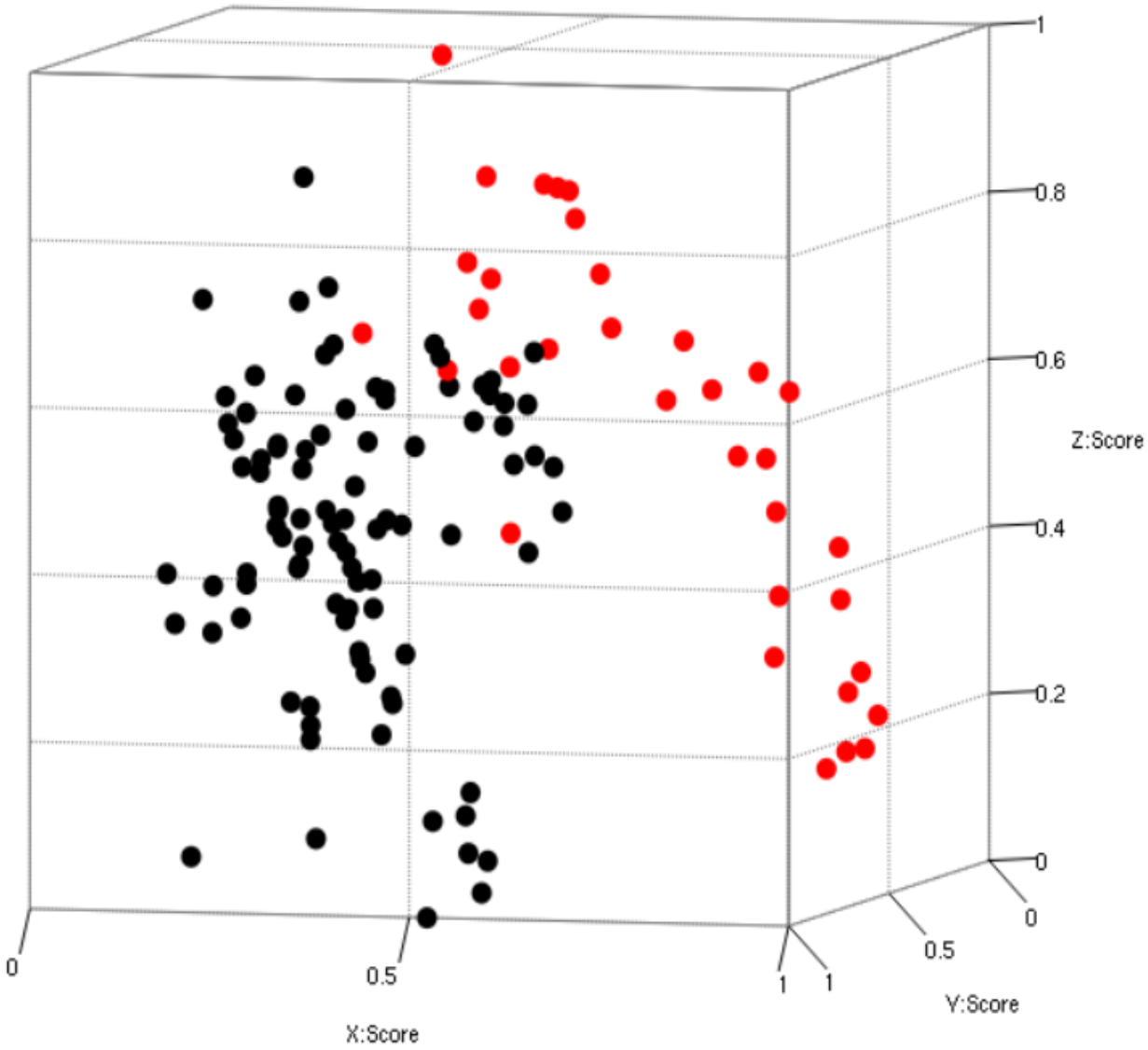

Figure 10. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside and Pre-infestation Inside period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day 1 during the Pre-infestation Outside and Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

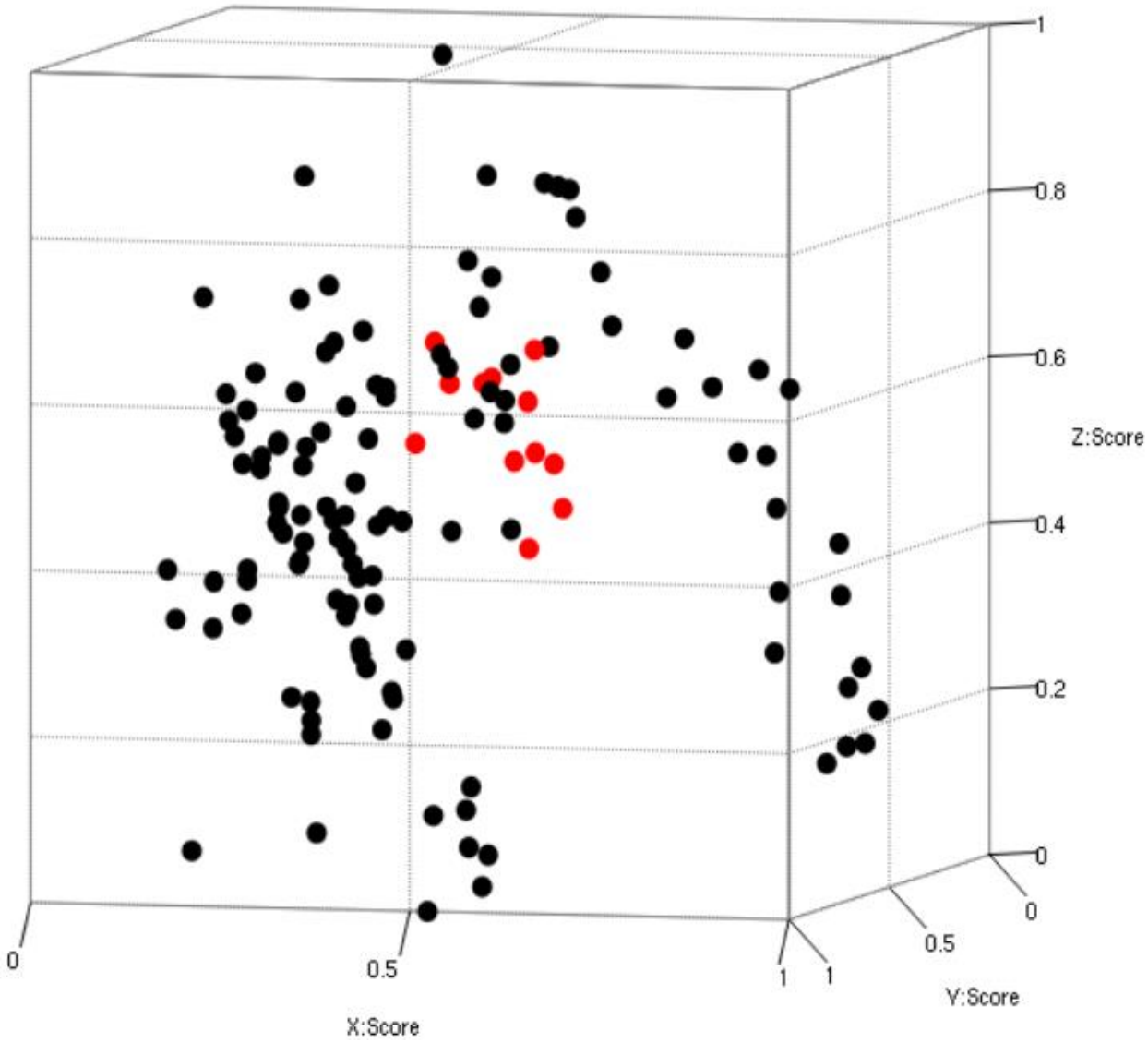

Figure 11. Three-dimensional cluster analysis of all daily fecal sample spectra for the Larval Feeding period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 2 to Day 7 during the Larval Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

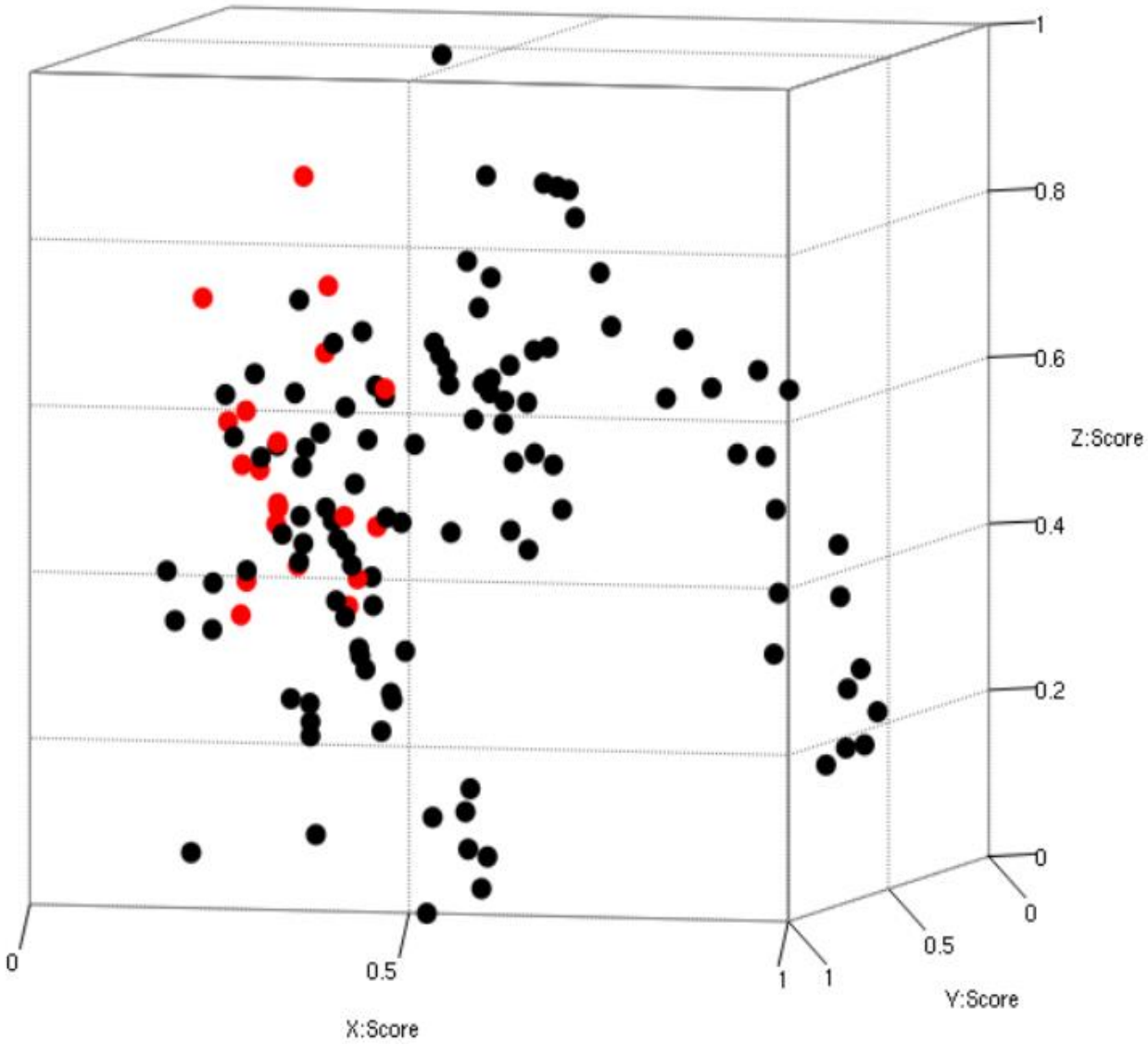

Figure 12. Three-dimensional cluster analysis of all daily fecal sample spectra for the Nymphal Feeding period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 8 to Day 17 during the Nymphal Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

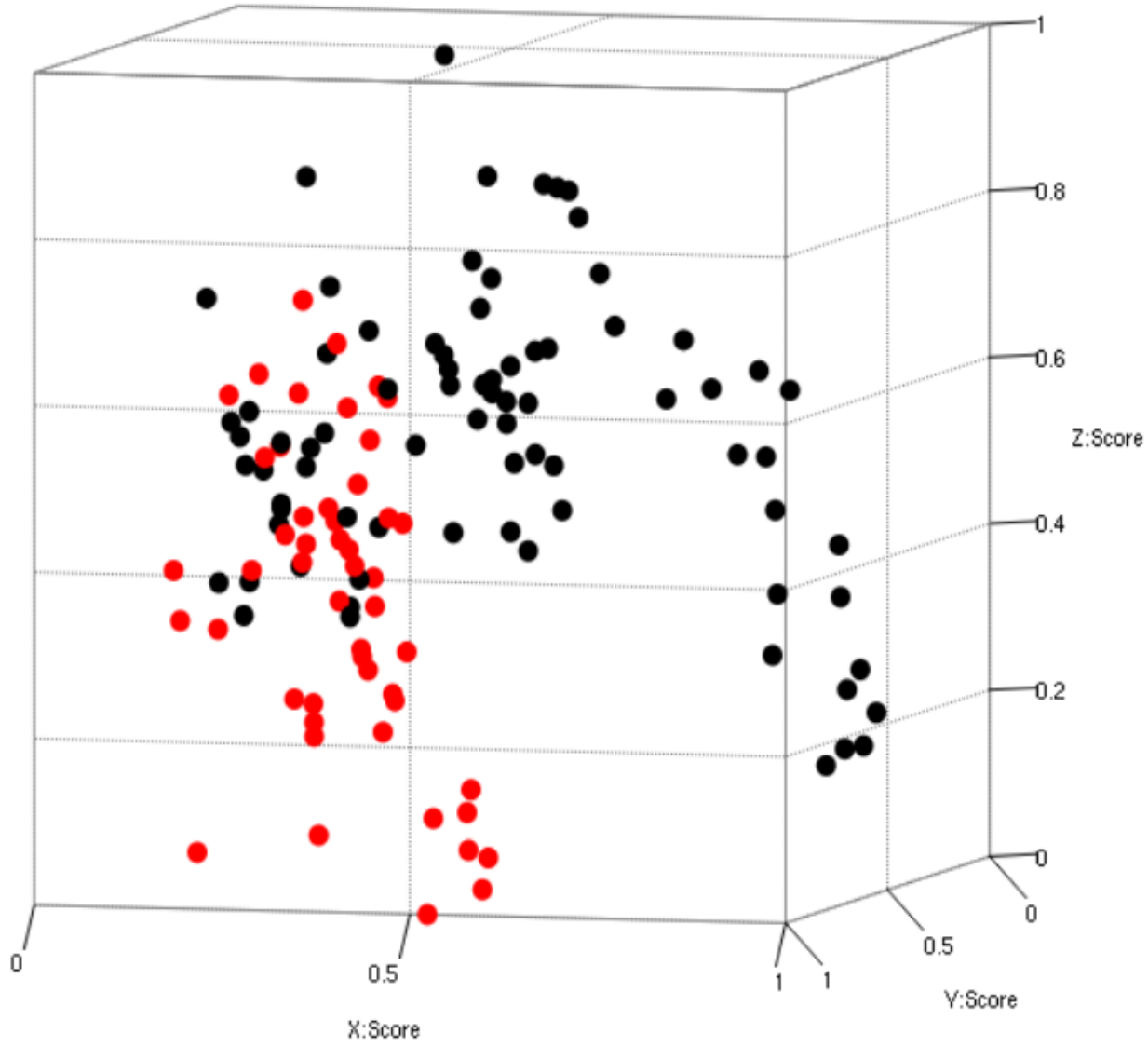

Figure 13. Three-dimensional cluster analysis of all daily fecal sample spectra for the Adult Feeding period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 18 to Day 41 during the Adult Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

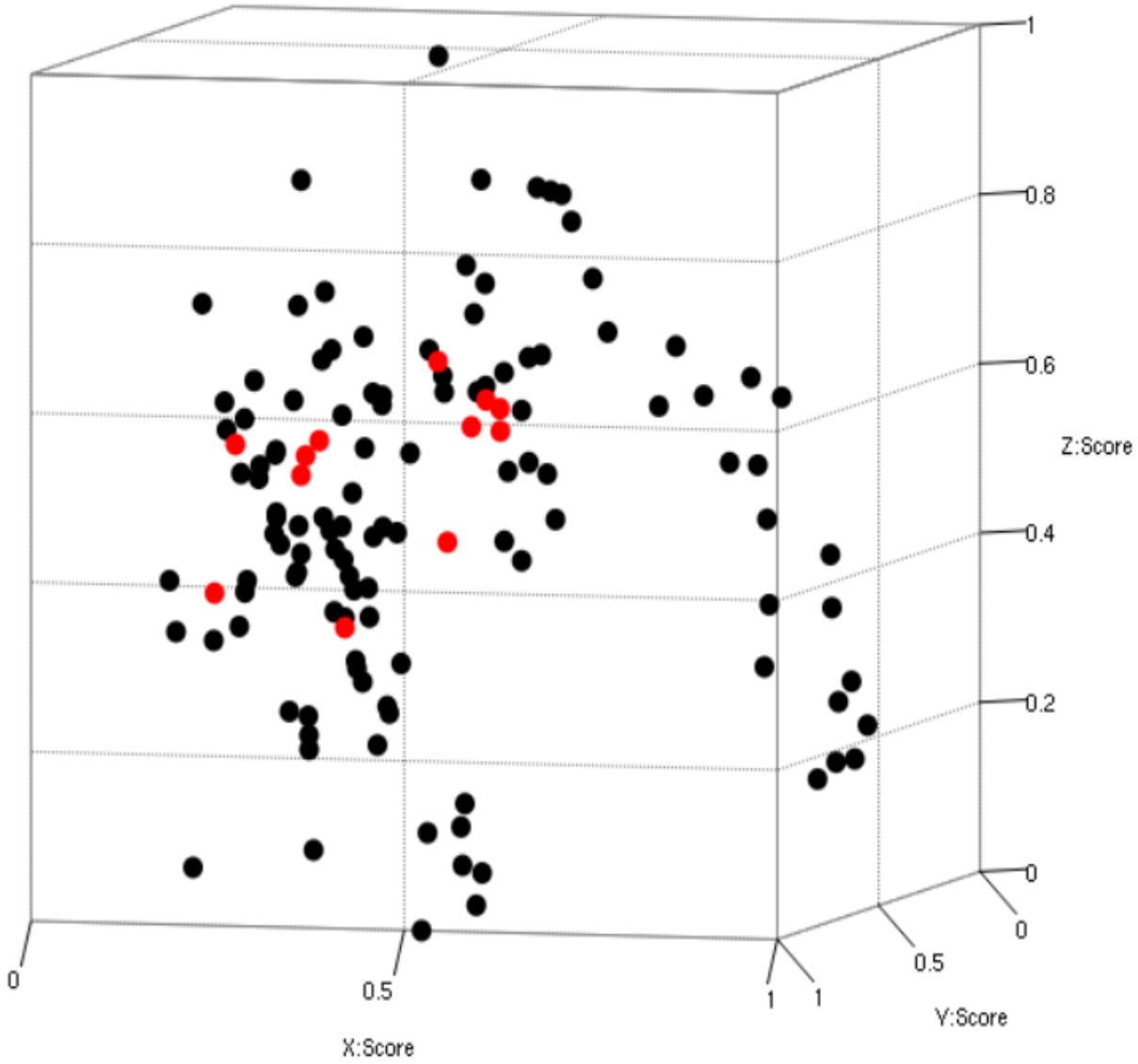

Figure 14. Three-dimensional cluster analysis of all daily fecal sample spectra for the Post-tick Recovery period for heifer numbers 3 and 5 in the low infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 42 to Day 47 during the Post-tick Recovery period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 96.79% of total spectral variation.

Stepwise Cluster Analyses for the Medium Treatment Level Group

A discriminant analysis was conducted for raw spectral data from daily fecal samples for heifer numbers 2 and 6 in the medium treatment level group from the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period (one fecal spectra per day per heifer). The analysis of spectra in the 576 nm – 1126 nm range produced 13 factors with the first three dominant spectral factors/variations representing 95.66% of the total variation among sample spectra for the medium infestation treatment level (see Figures 15 – 17).

The cluster analyses of the spectra from the pair of heifers in the medium treatment level group resulted in a pattern of six clusters that depict shifts in fecal chemistry. Sample clusters were distinguishable for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period in the cluster analyses (see Figures 18 through 24). The first was comprised of samples originating from Day -15 (15 d prior to infestation) through Day -6 (6 d prior to infestation; allowing for a 48-h rumen passage time) (Figure 18). The second cluster was comprised of samples within the period from Day -5 (5 d prior to infestation) through Day 1 (1 day post infestation; allowing for a 48-h rumen passage time) (Figure 19). Figure 20 is the daily fecal spectra for the two pre-infestation acclimation periods in one cluster analysis, showing the entire experimental control period for the medium treatment group. The third cluster was comprised of samples from Day 2 (2 d post infestation) through Day 7 (7 d post infestation), which is the period of attachment, feeding and molting of larvae (Figure 21). The fourth cluster originated from samples from Day 8 (8 d post infestation) to Day 17 (17 d post infestation), corresponding to attachment, feeding, and molting of nymphs (Figure 22). The fifth cluster

includes samples from Day 18 (18 d post infestation) to Day 41 (allowing for a 48-h rumen passage time), corresponding to the period of adult feeding and the period in which females complete their feeding and drop from the host (Figure 23). The sixth cluster was comprised of samples from Day 42 to Day 47, which consists of the period heifers were going through post-tick recovery (Figure 24). Spectral cluster shifts occurred representing time periods that are consistent with no tick feeding, low tick feeding, heavy feeding, and a period of post-tick recovery.

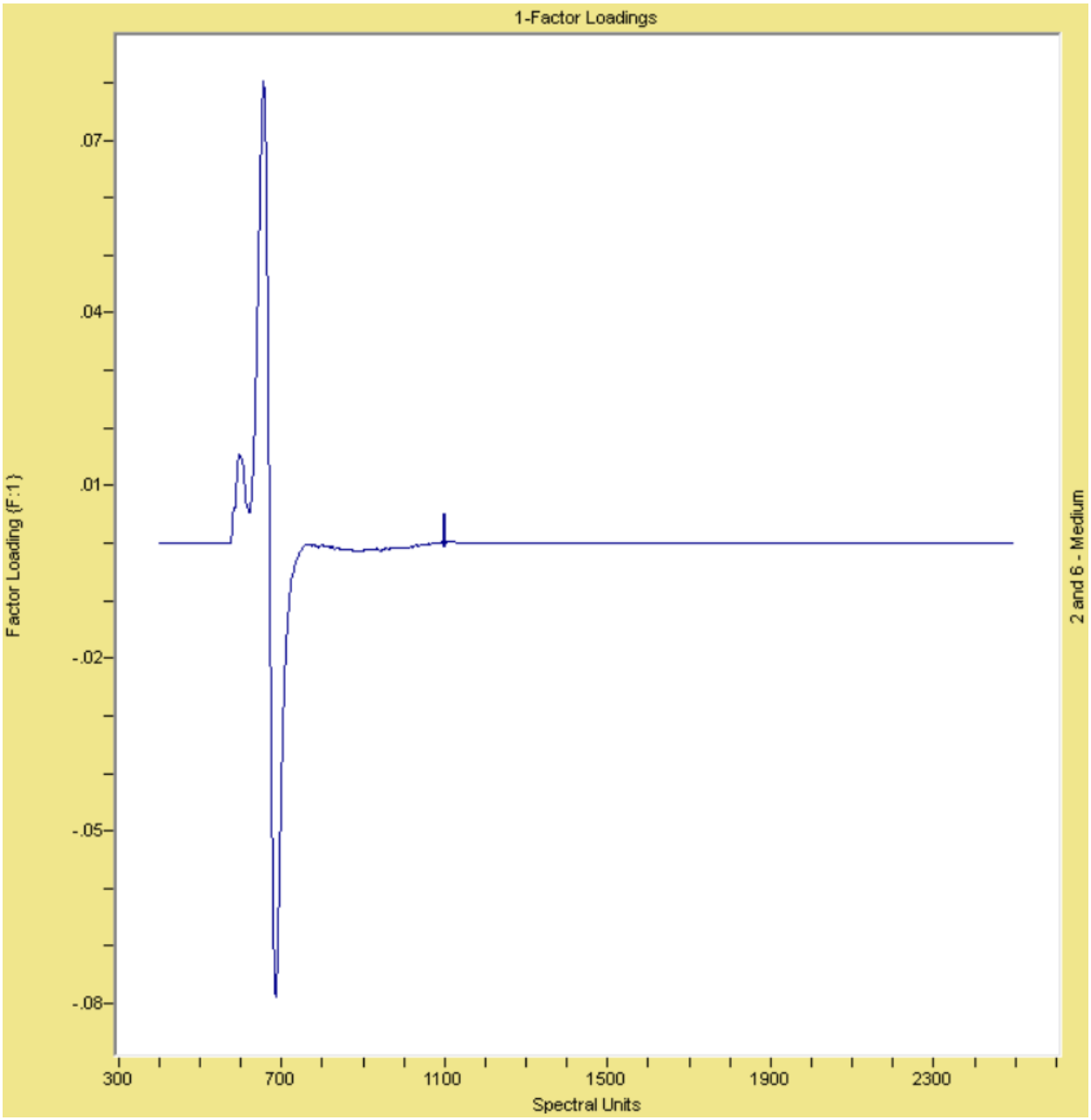

Figure 15. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the first most common spectral variation (factor) from the medium infestation treatment level heifer numbers 2 and 6 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 83.11% of the total variation within this medium infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

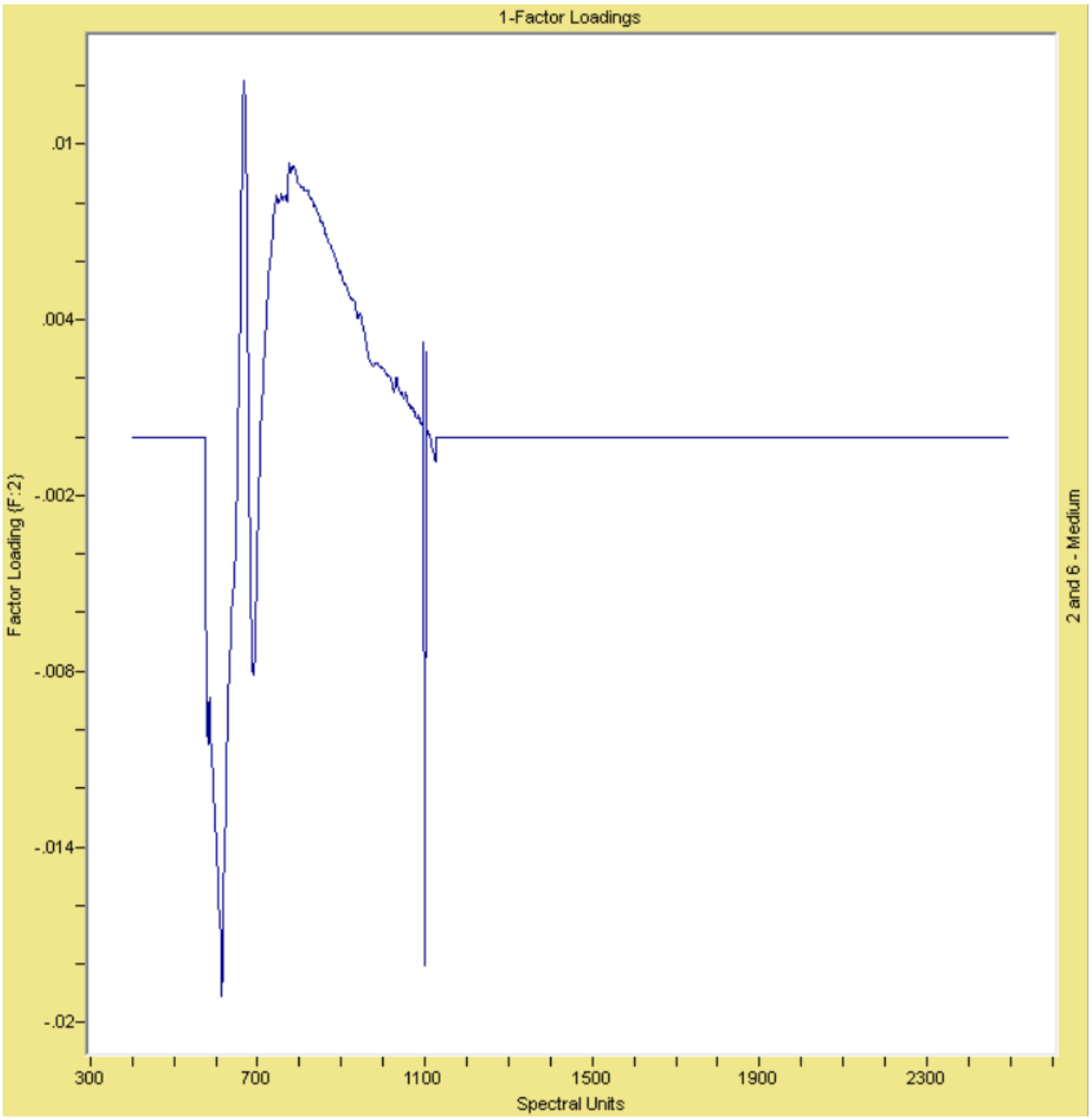

Figure 16. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the second most common spectral variation (factor) from the medium infestation treatment level heifer numbers 2 and 6 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 9.01% of the total variation within this medium infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

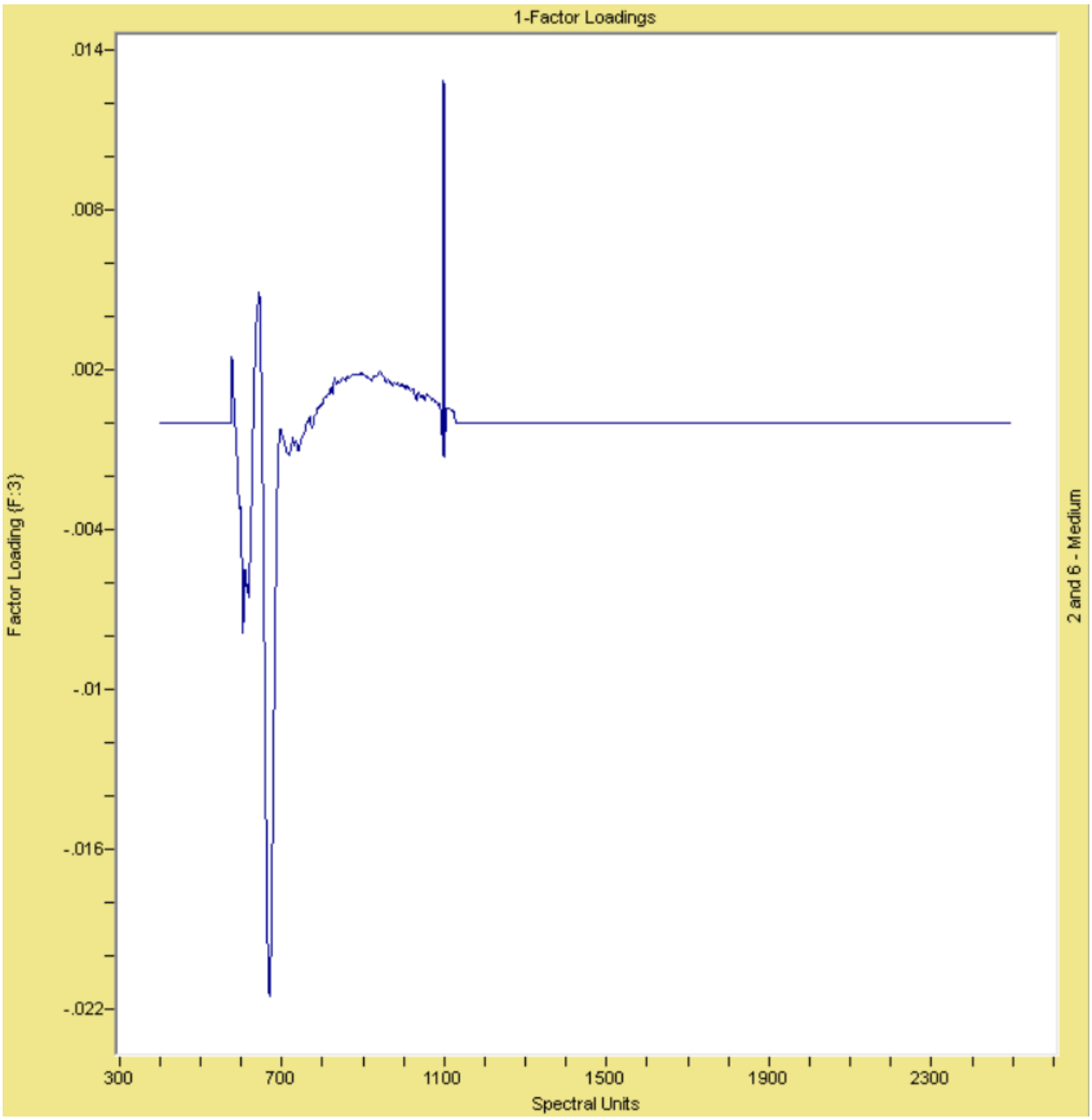

Figure 17. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the third most common spectral variation (factor) from the medium infestation treatment level heifer numbers 2 and 6 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 3.54% of the total variation within this medium infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

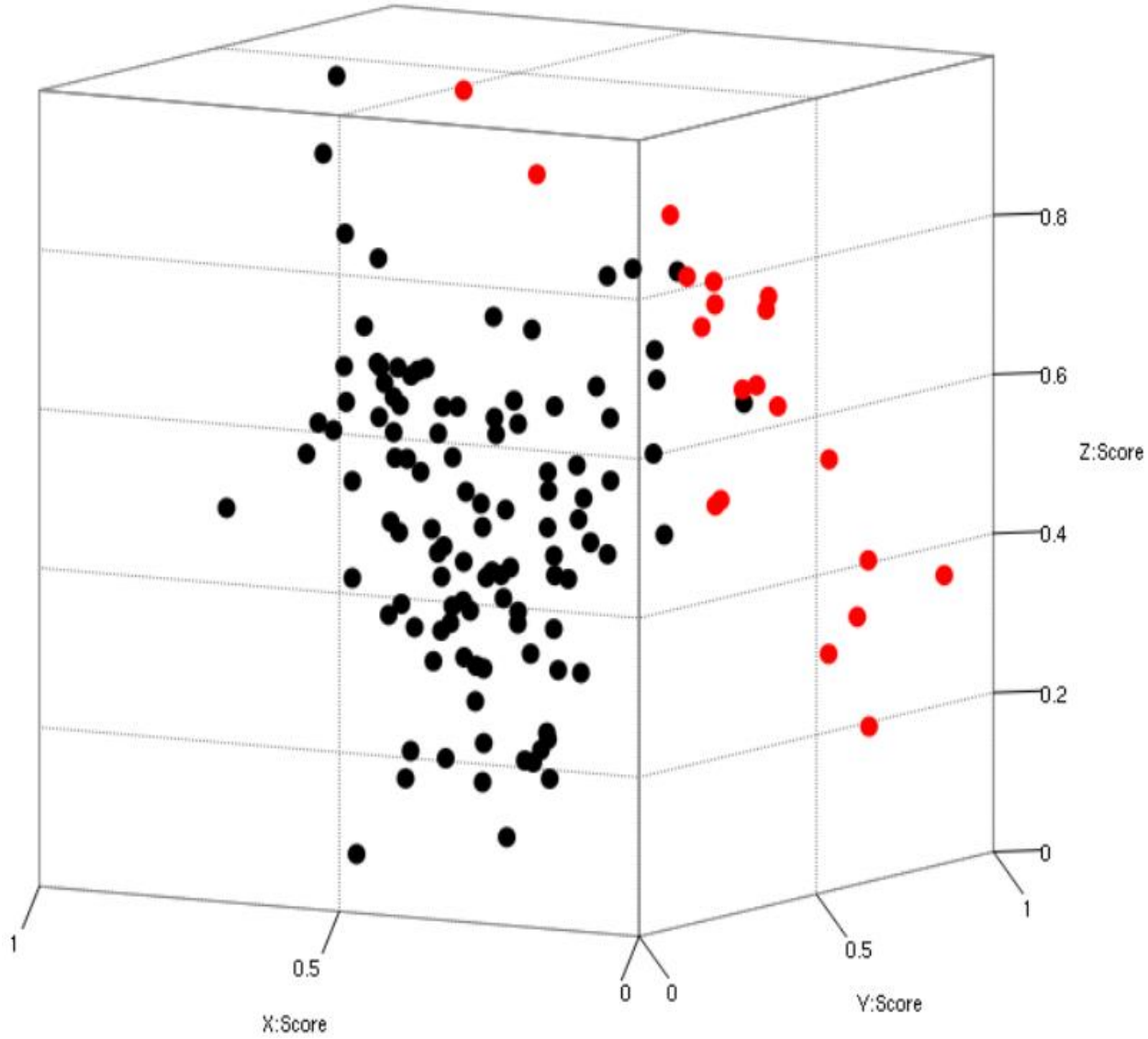

Figure 18. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day -6 during the Pre-infestation Outside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

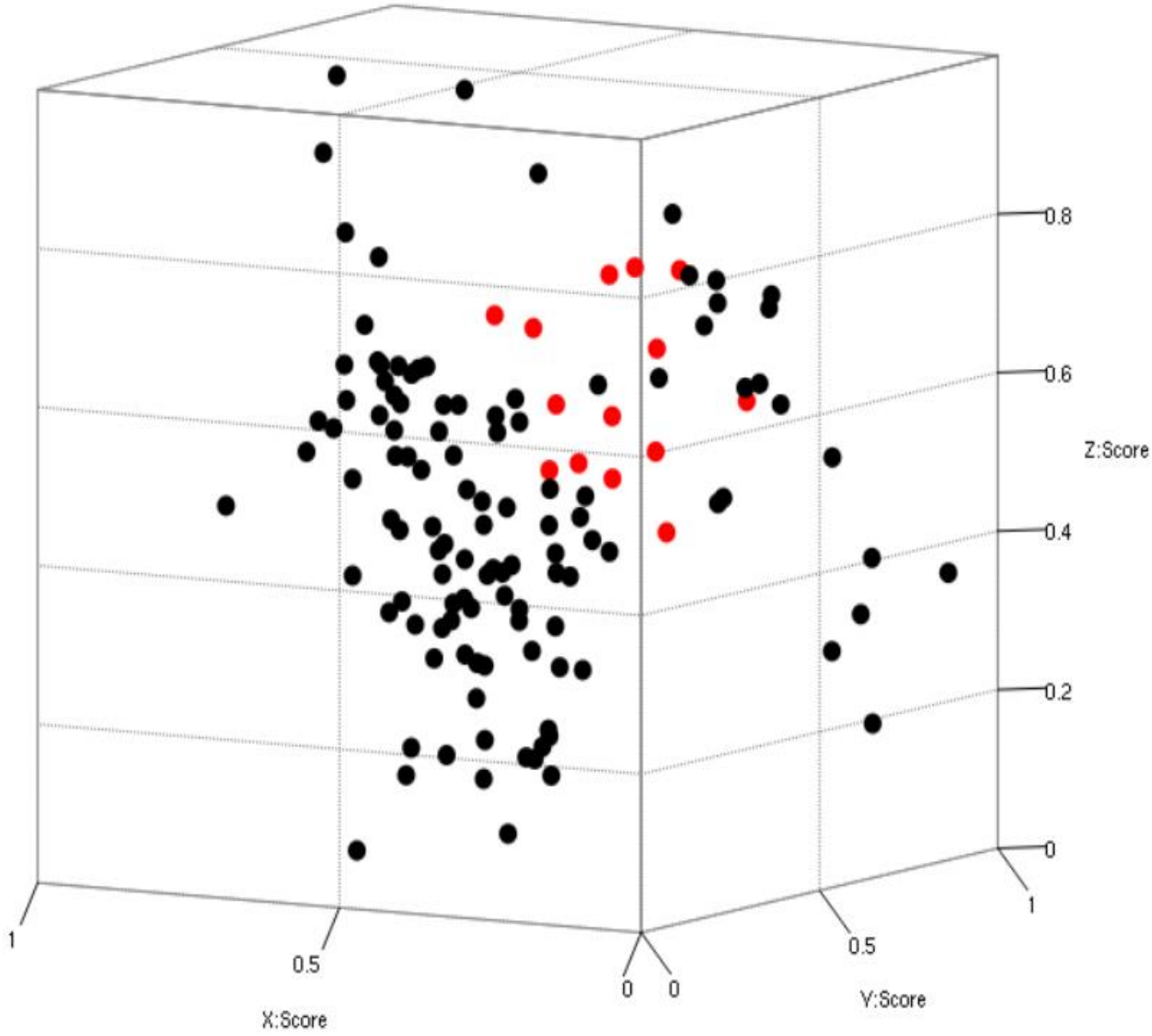

Figure 19. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Inside period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -5 to Day 1 during the Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

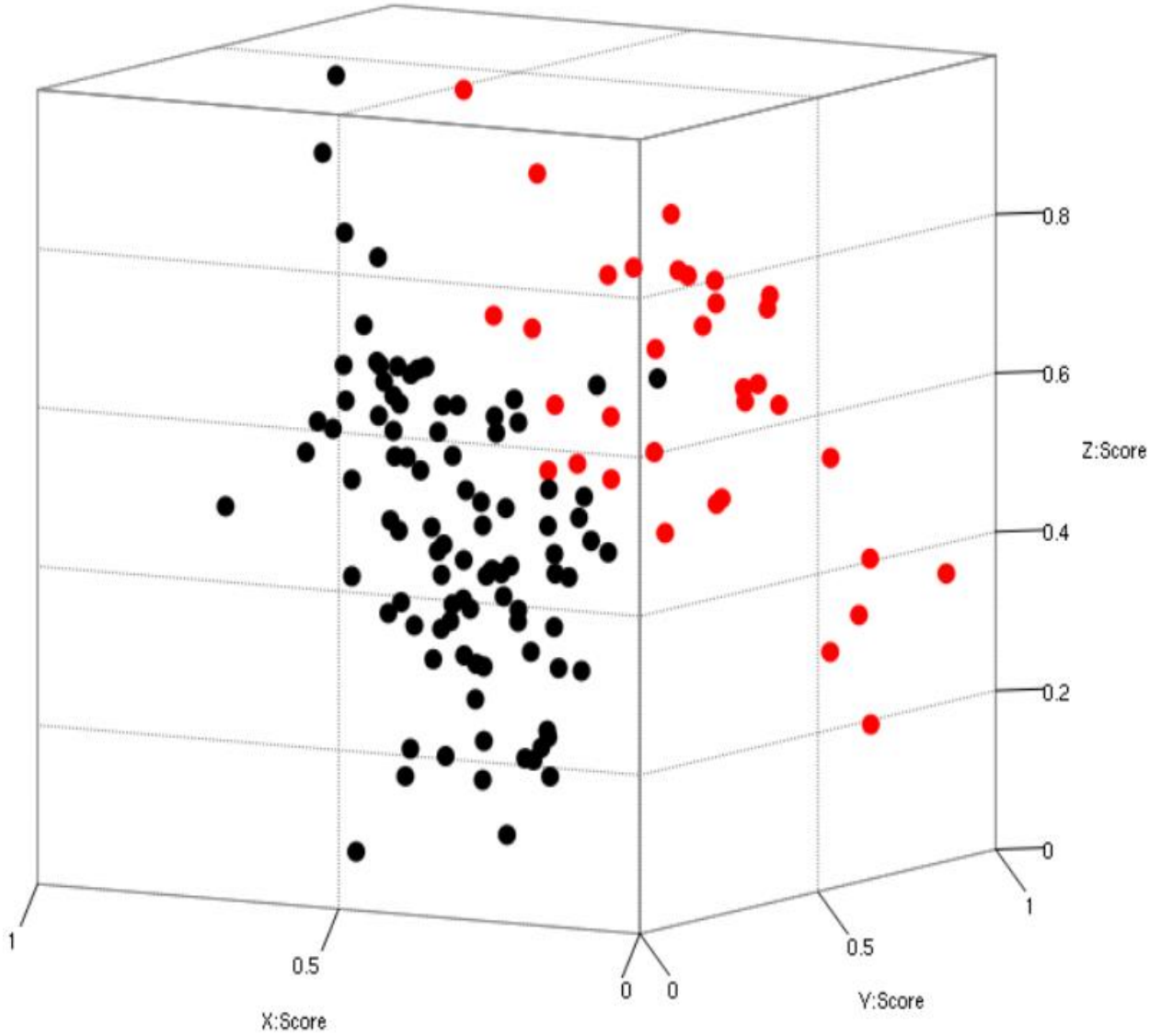

Figure 20. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside and Pre-infestation Inside period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day 1 during the Pre-infestation Outside and Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

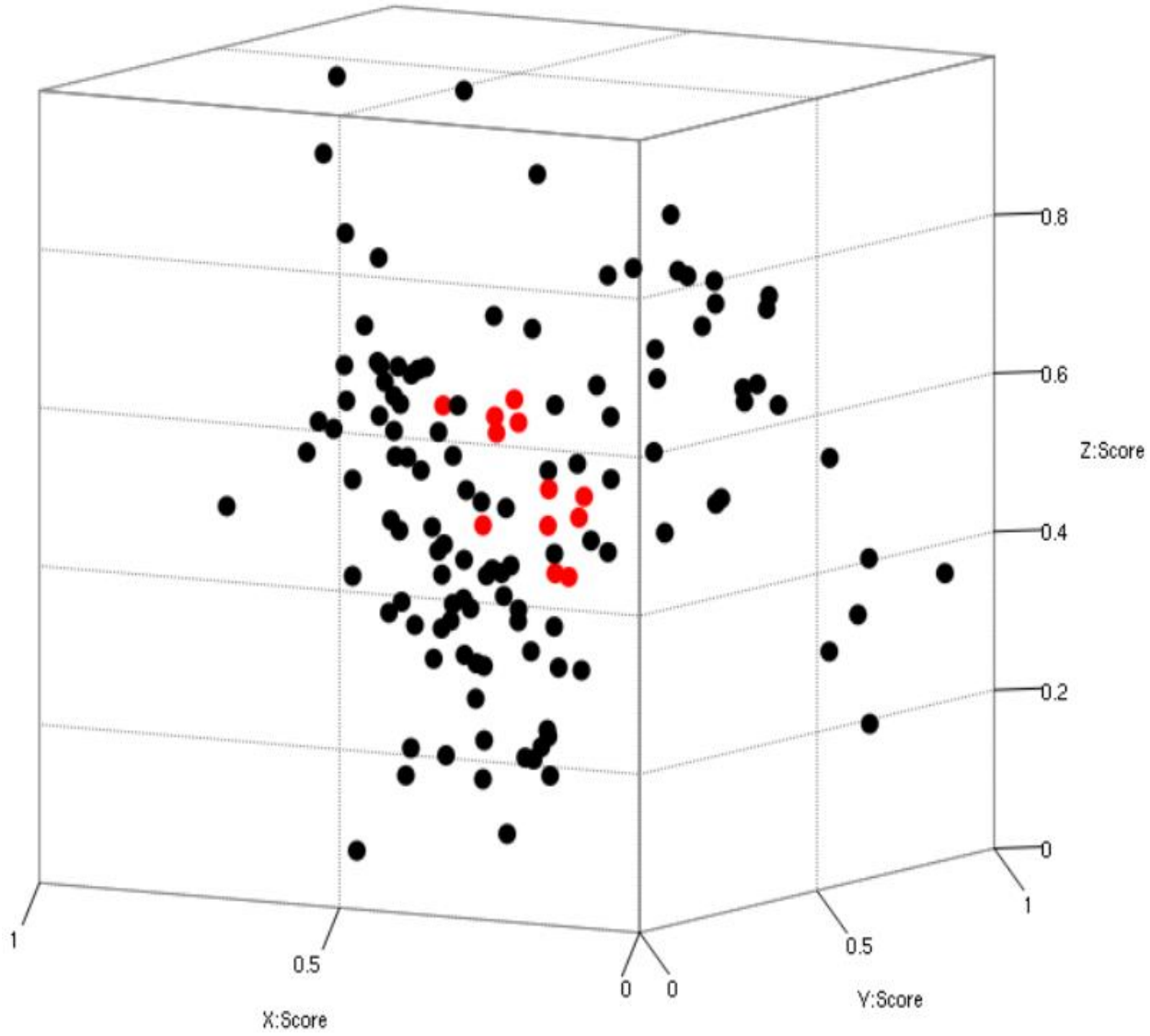

Figure 21. Three-dimensional cluster analysis of all daily fecal sample spectra for the Larval Feeding period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 2 to Day 7 during the Larval Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

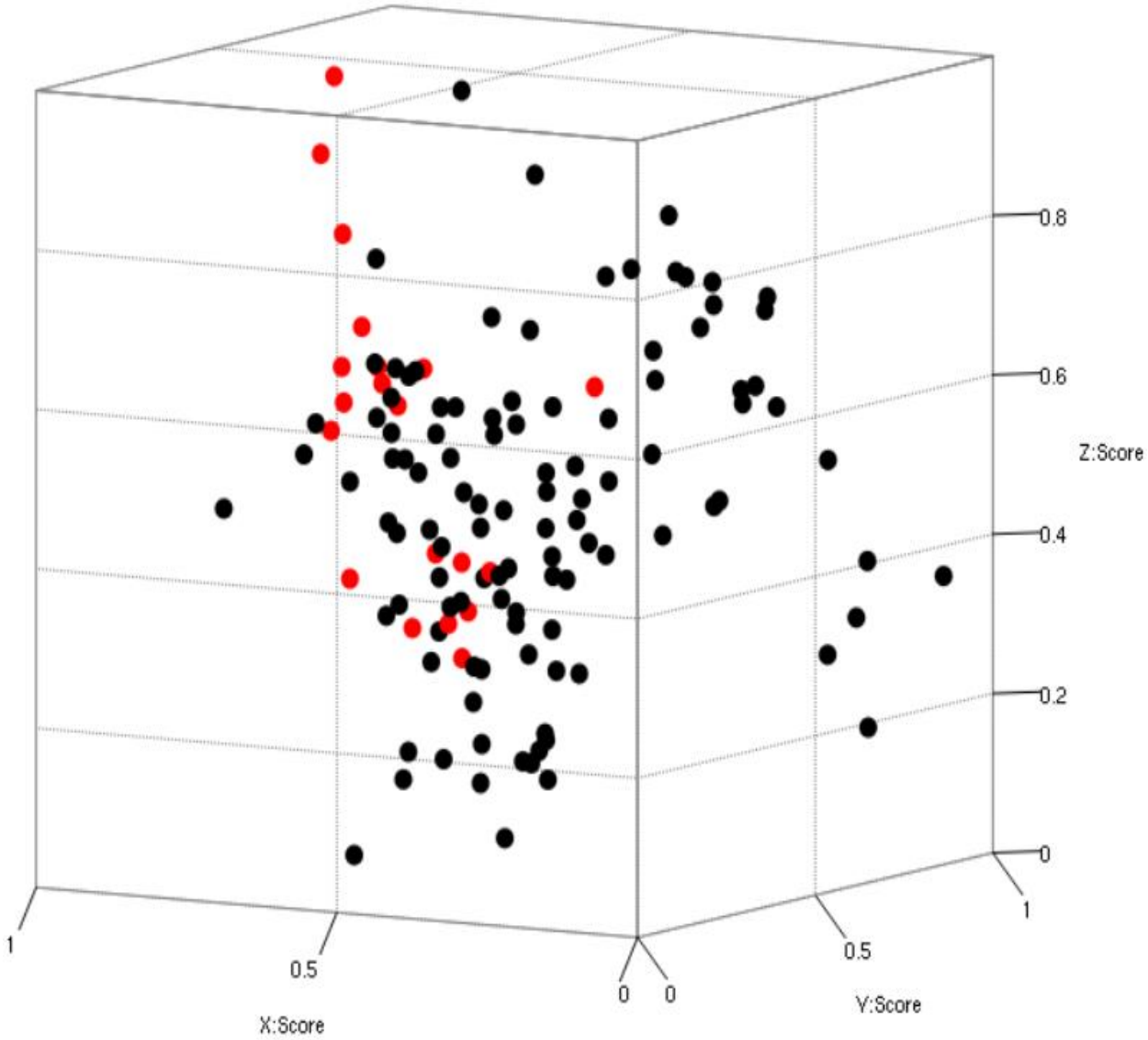

Figure 22. Three-dimensional cluster analysis of all daily fecal sample spectra for the Nymphal Feeding period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 8 to Day 17 during the Nymphal Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

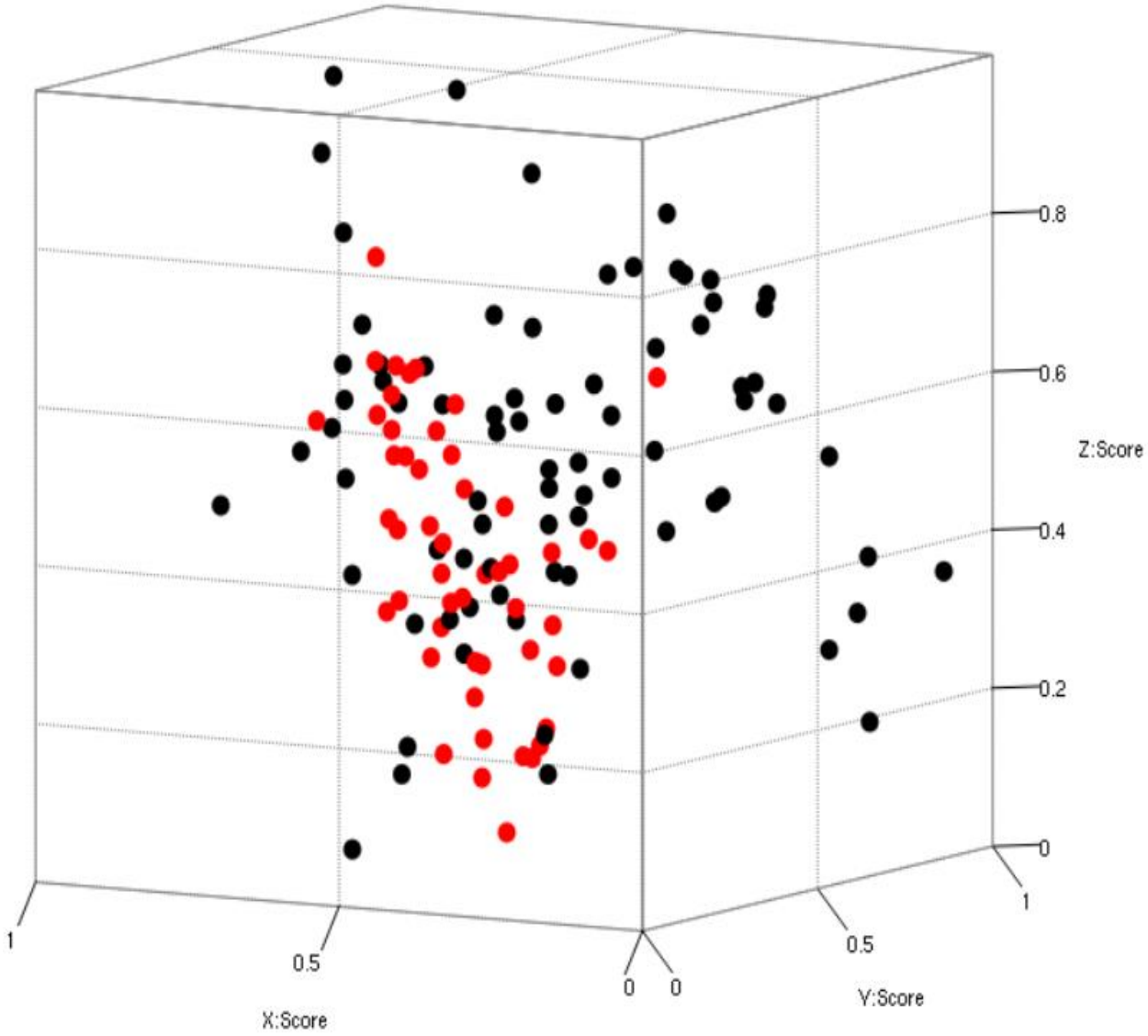

Figure 23. Three-dimensional cluster analysis of all daily fecal sample spectra for the Adult Feeding period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 18 to Day 41 during the Adult Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

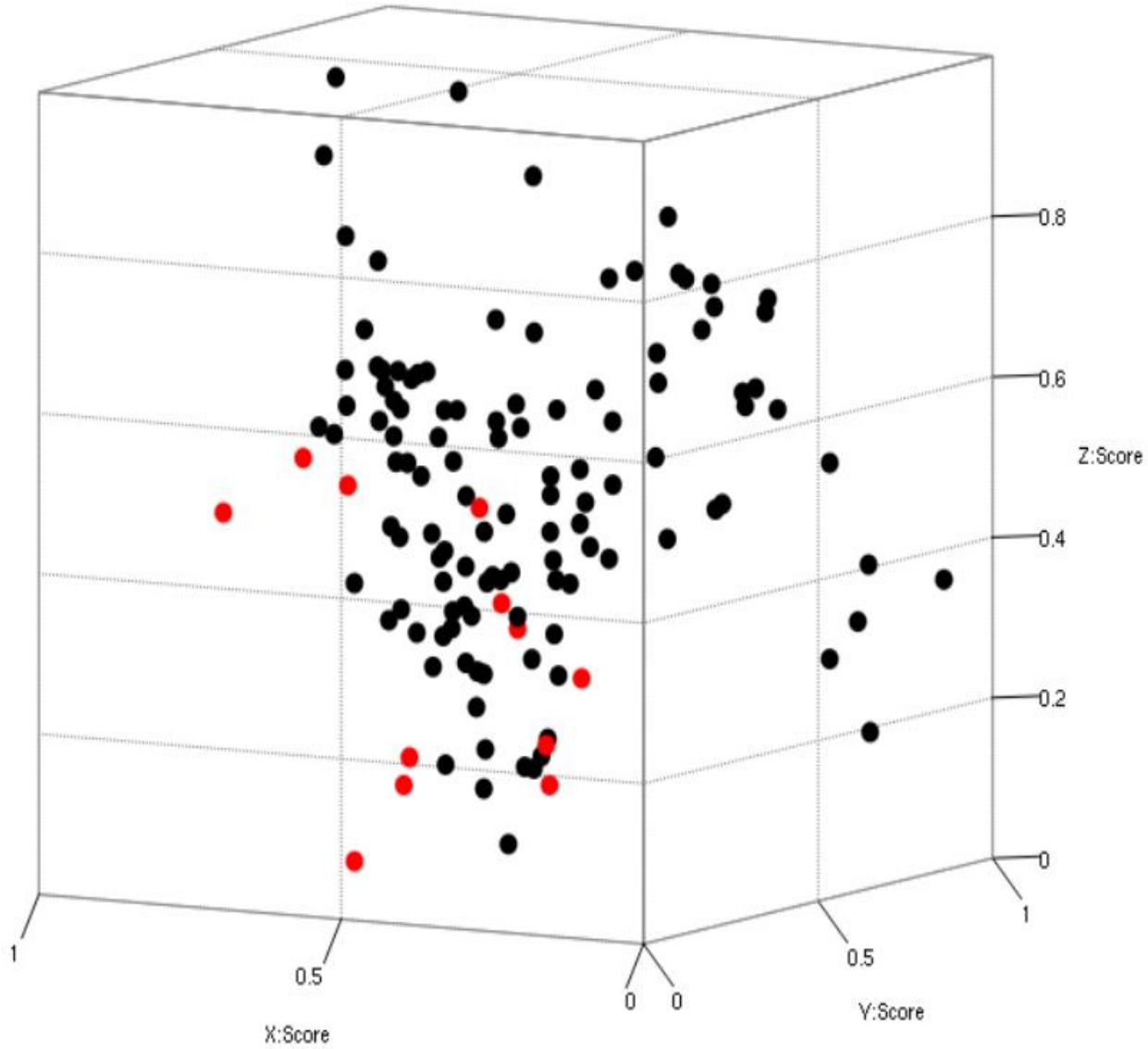

Figure 24. Three-dimensional cluster analysis of all daily fecal sample spectra for the Post-tick Recovery period for heifer numbers 2 and 6 in the medium infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 42 to Day 47 during the Post-tick Recovery period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 95.66% of total spectral variation.

Stepwise Cluster Analyses for the High Treatment Level Group

A discriminant analysis was conducted for raw spectral data from daily fecal samples for heifer numbers 4 and 8 in the high treatment level group from the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period (one fecal spectra per day per heifer). The analysis of spectra in the 576 nm – 1126 nm range produced 12 factors with the first three dominant spectral factors/variations representing 93.76% of the total variation among sample spectra for the medium infestation treatment level (see Figures 25 – 27).

The cluster analyses of the spectra from the pair of heifers in the high treatment level group resulted in a pattern of six clusters that depict shifts in fecal chemistry. Sample clusters were distinguishable for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period in the cluster analyses (see Figures 28 through 34). The first was comprised of samples originating from Day -15 (15 d prior to infestation) through Day -6 (6 d prior to infestation; allowing for a 48-h rumen passage time) (Figure 28). The second cluster was comprised of samples within the period from Day -5 (5 d prior to infestation) through Day 1 (1 day post infestation; allowing for a 48-h rumen passage time) (Figure 29). Figure 30 is the daily fecal spectra for the two pre-infestation acclimation periods in one cluster analysis, showing the entire experimental control period for the high treatment group. The third cluster was comprised of samples from Day 2 (2 d post infestation) through Day 7 (7 d post infestation), which is the period of attachment, feeding and molting of larvae (Figure 31). The fourth cluster originated from samples from Day 8 (8 d post infestation) to Day 17 (17 d post infestation), corresponding to attachment, feeding, and molting of nymphs (Figure 32). The fifth cluster includes samples

from Day 18 (18 d post infestation) to Day 41 (allowing for a 48-h rumen passage time), corresponding to the period of adult feeding and the period in which females complete their feeding and drop from the host (Figure 33). The sixth cluster was comprised of samples from Day 42 to Day 47, which consists of the period heifers were going through post-tick recovery (Figure 34). Spectral cluster shifts occurred representing time periods that are consistent with no tick feeding, low tick feeding, heavy feeding, and a period of post-tick recovery.

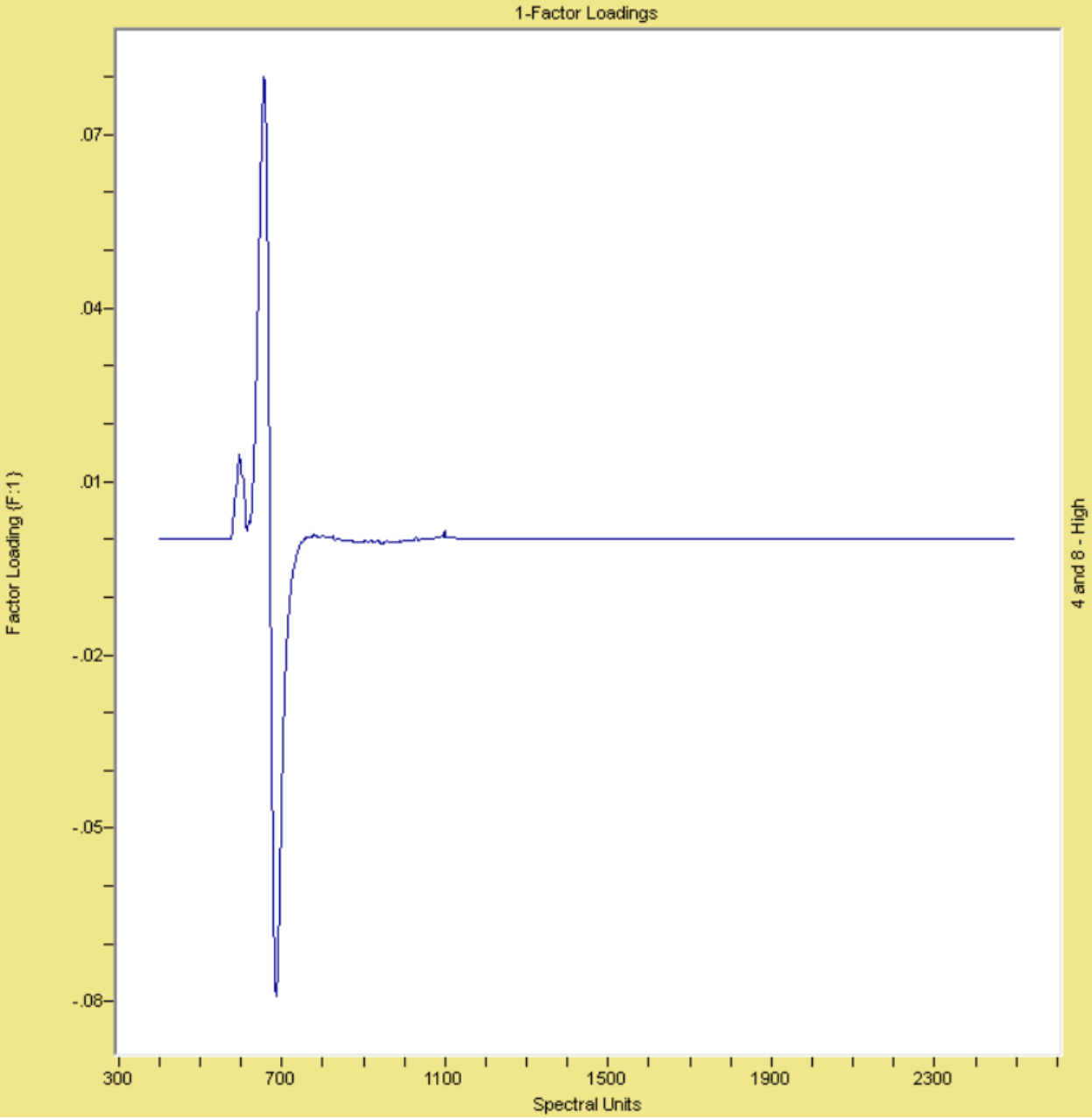

Figure 25. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the first most common spectral variation (factor) from the high infestation treatment level heifer numbers 4 and 8 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 83.66% of the total variation within this high infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

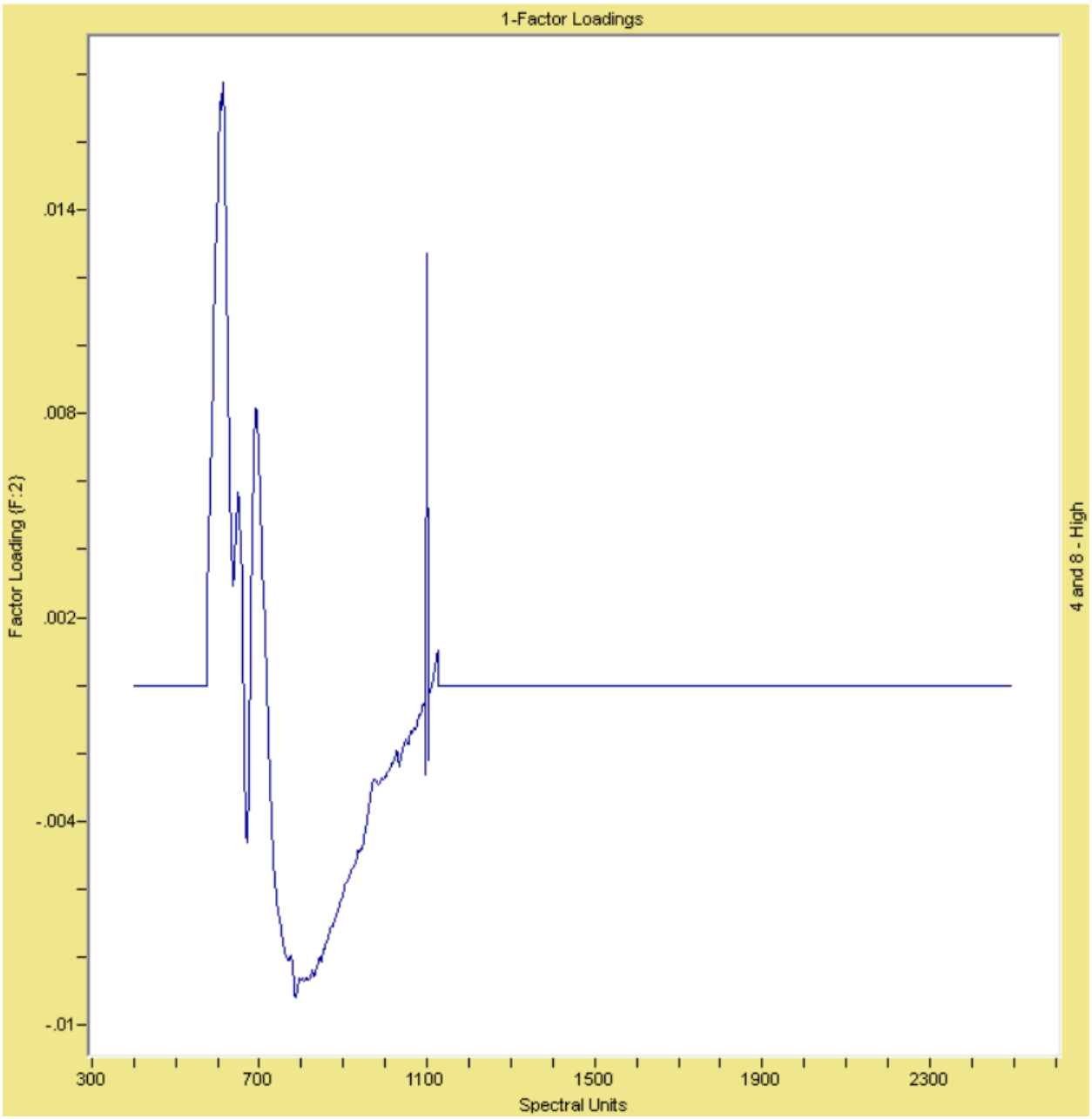

Figure 26. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the second most common spectral variation (factor) from the high infestation treatment level heifer numbers 4 and 8 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 8.02% of the total variation within this high infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

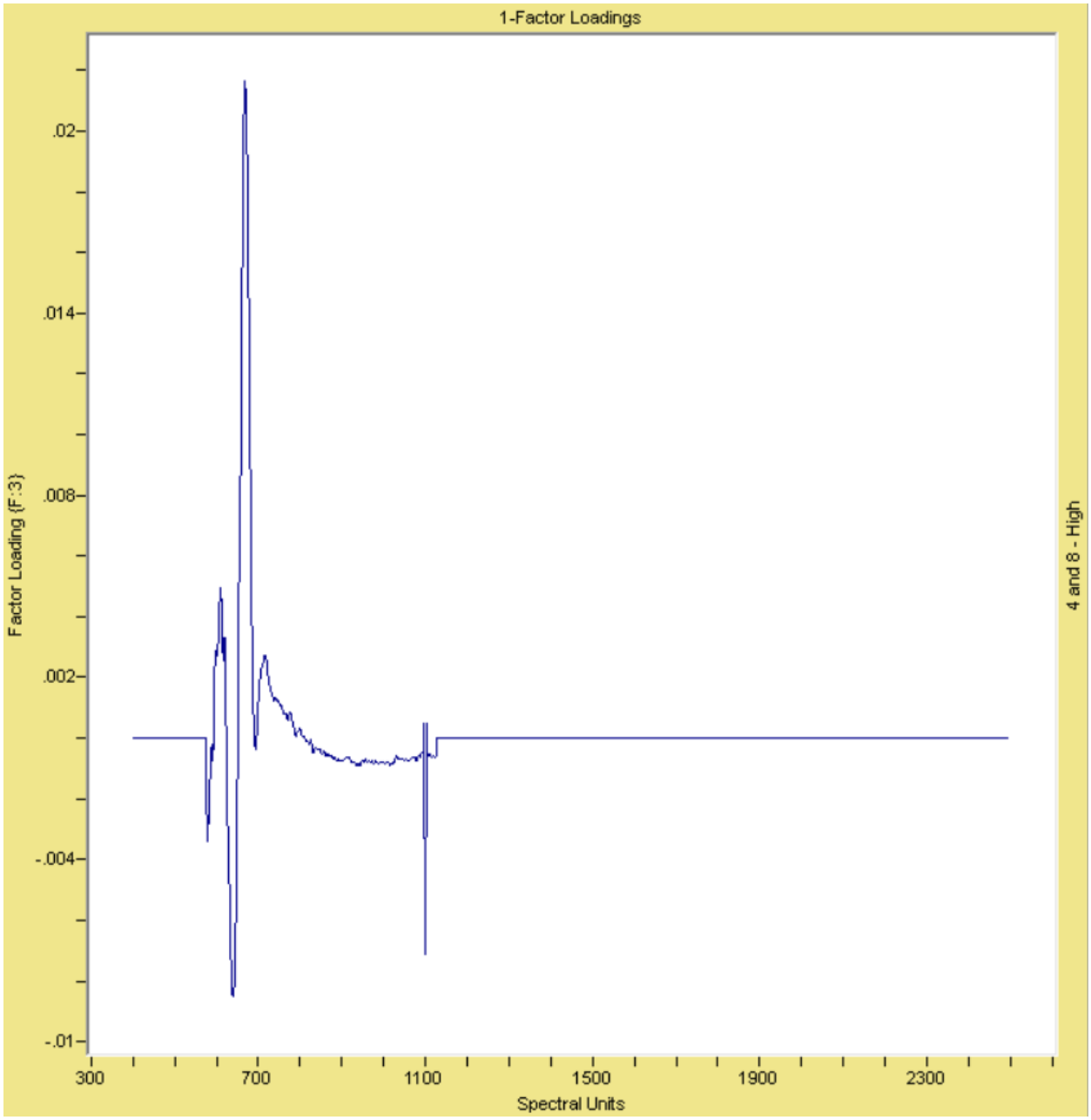

Figure 27. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the third most common spectral variation (factor) from the high infestation treatment level heifer numbers 4 and 8 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 2.08% of the total variation within this high infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

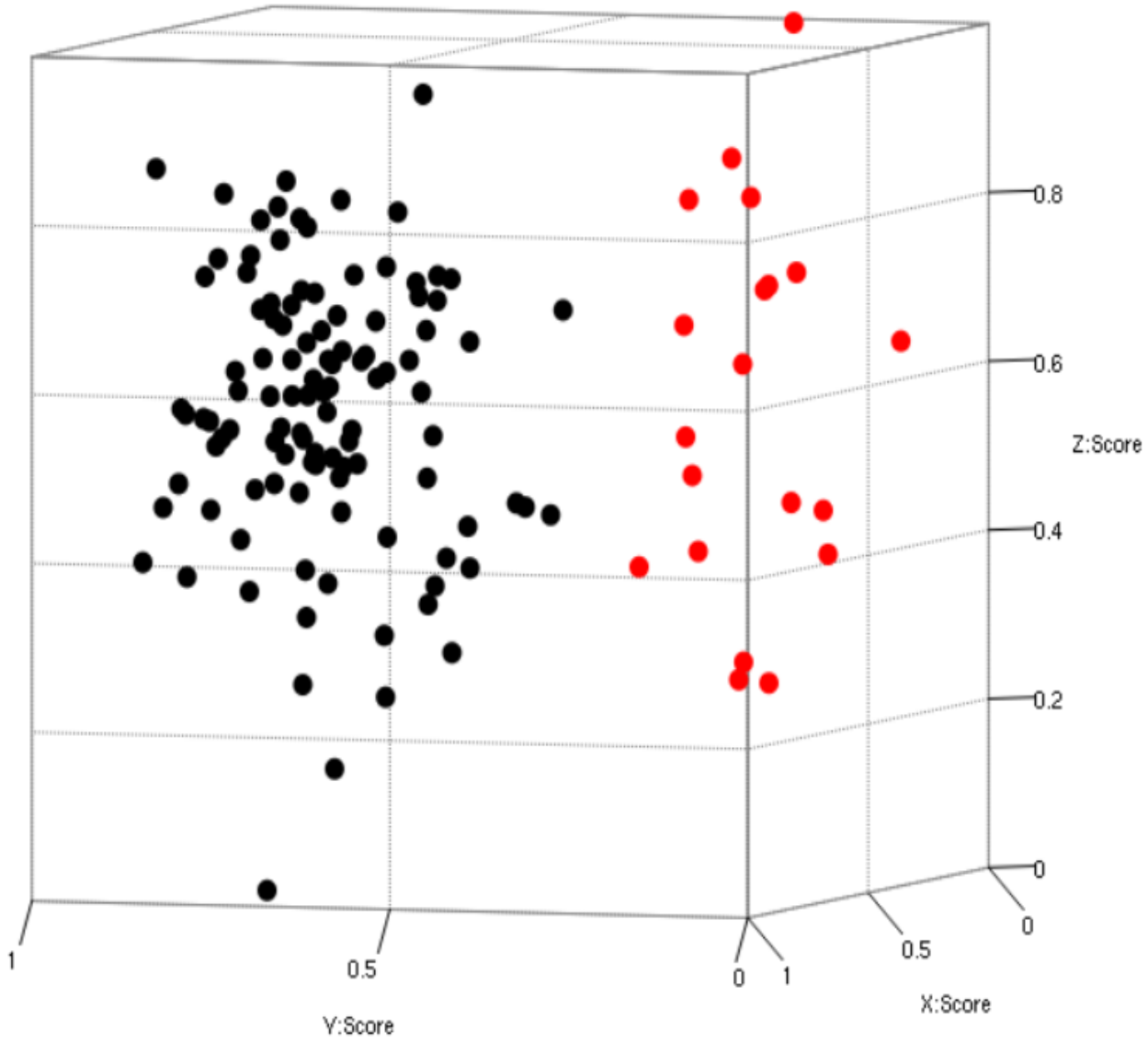

Figure 28. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day -6 during the Pre-infestation Outside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

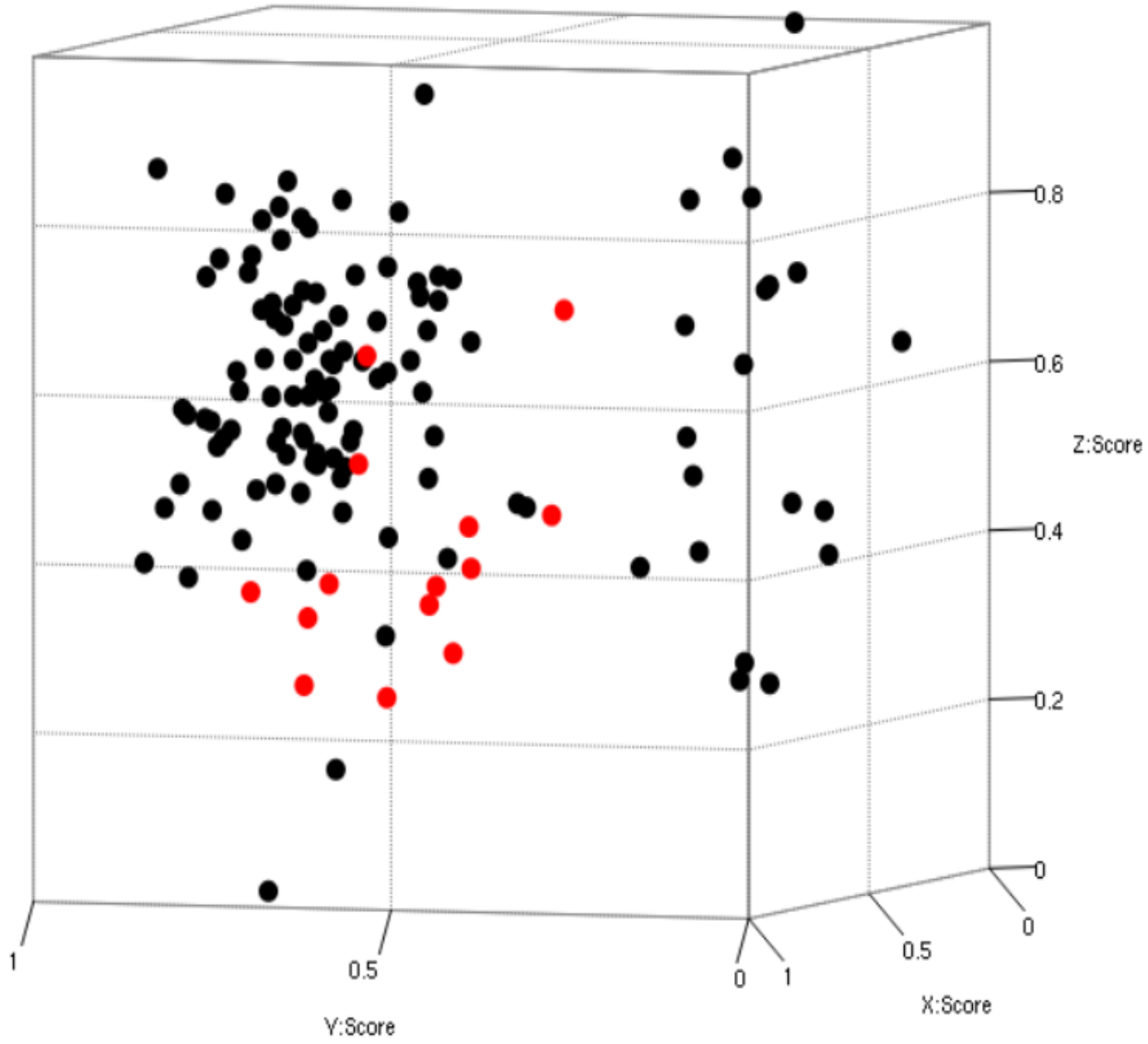

Figure 29. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Inside period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -5 to Day 1 during the Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

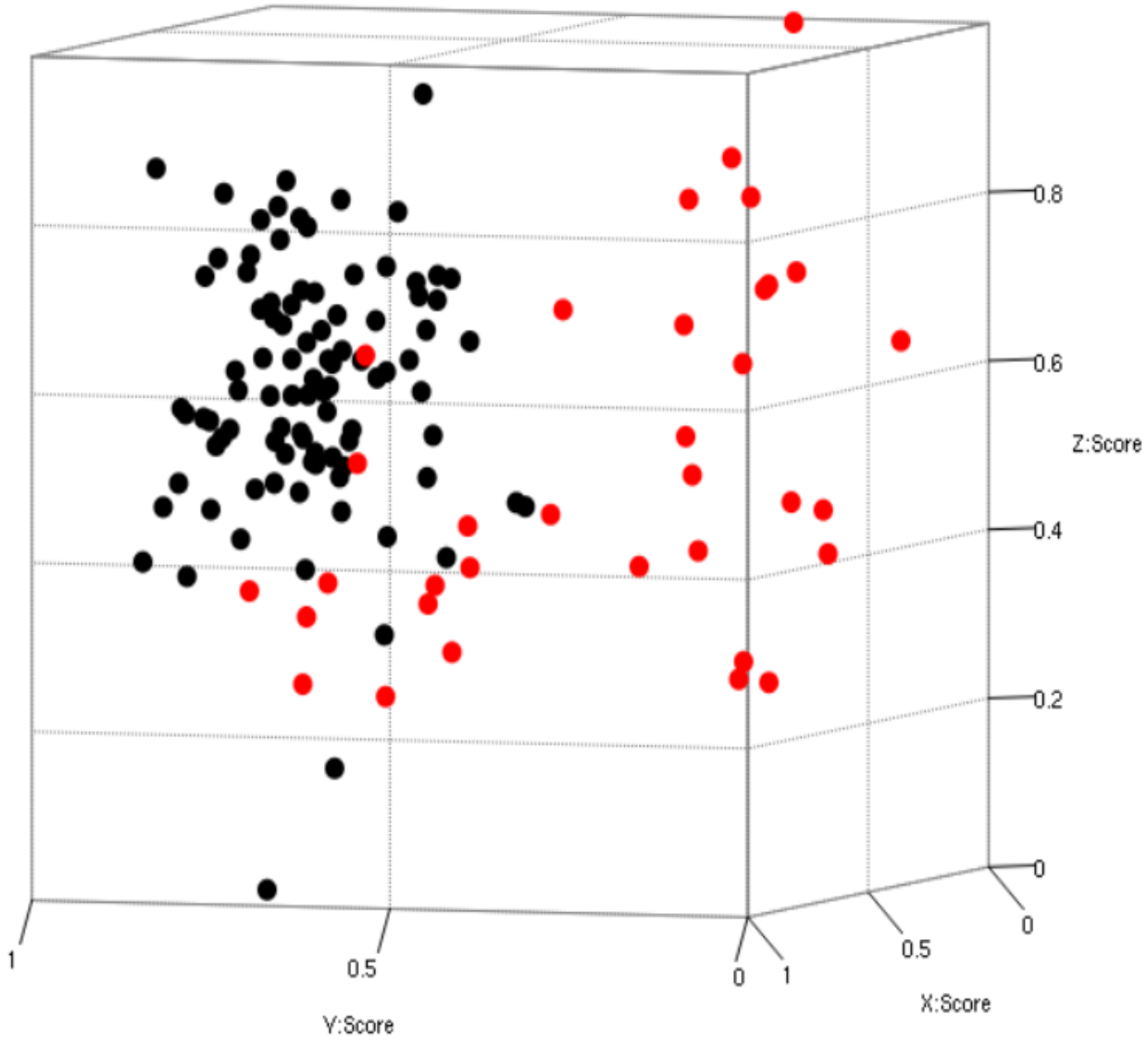

Figure 30. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside and Pre-infestation Inside period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day 1 during the Pre-infestation Outside and Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

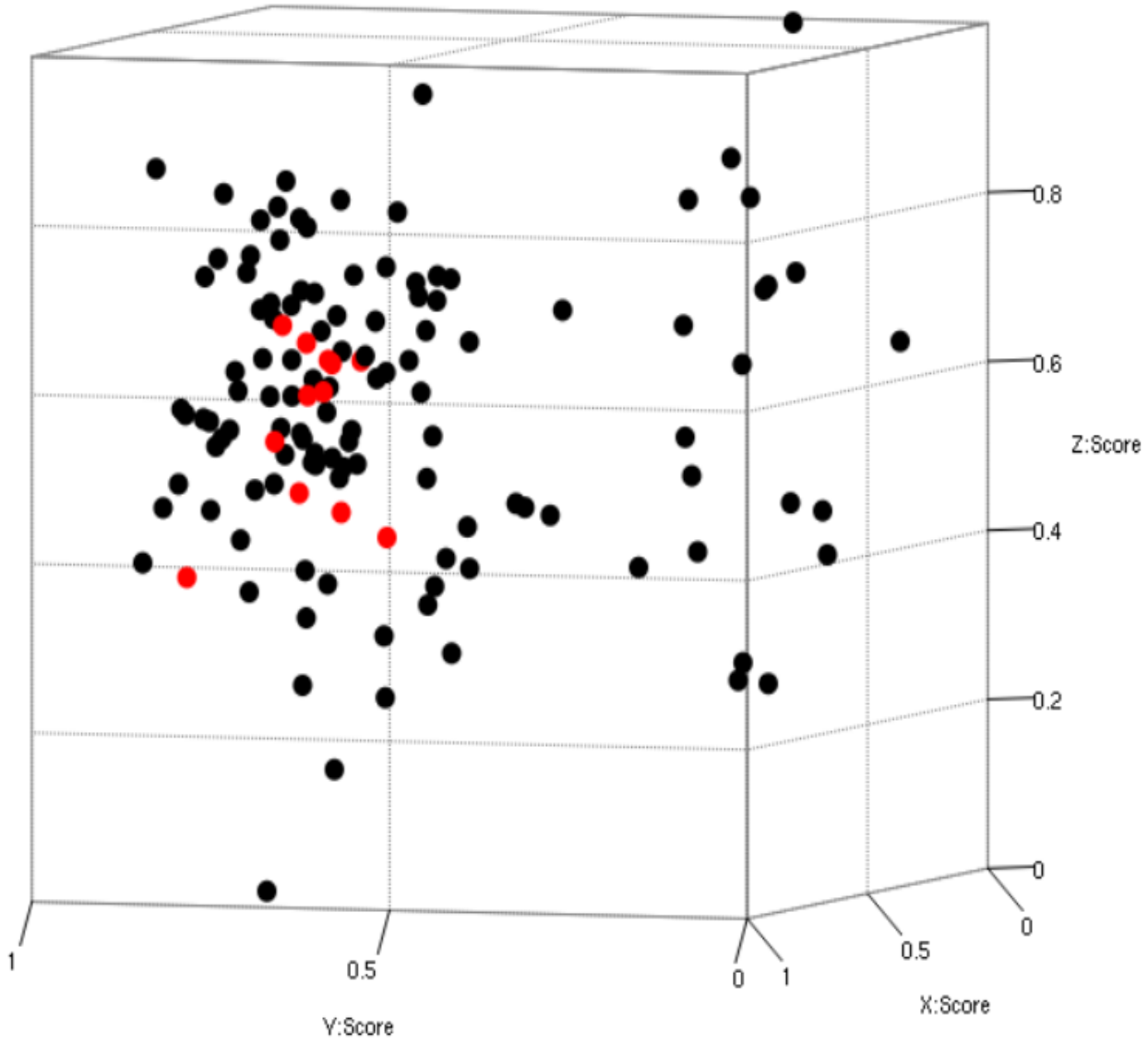

Figure 31. Three-dimensional cluster analysis of all daily fecal sample spectra for the Larval Feeding period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 2 to Day 7 during the Larval Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

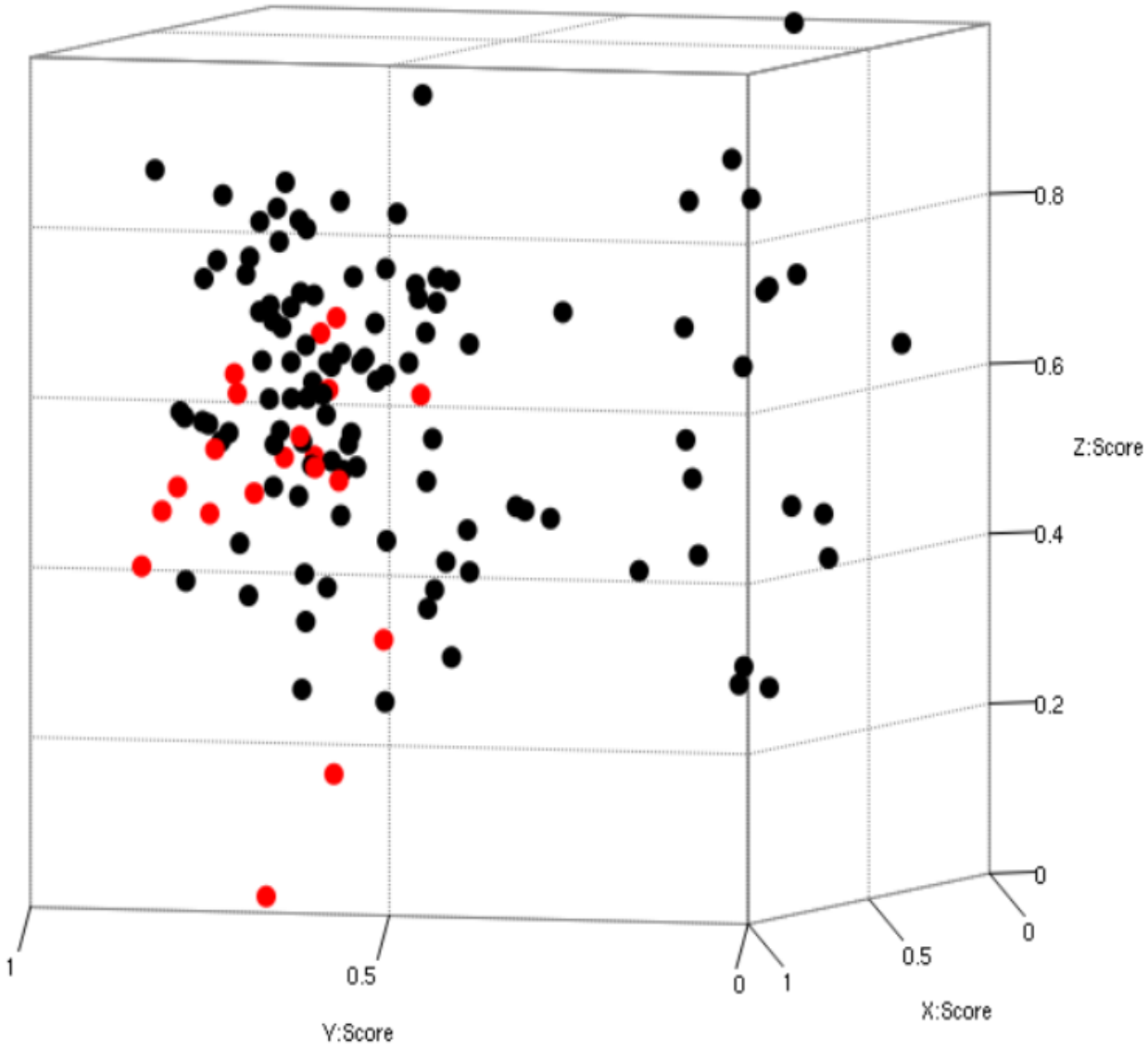

Figure 32. Three-dimensional cluster analysis of all daily fecal sample spectra for the Nymphal Feeding period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 8 to Day 17 during the Nymphal Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

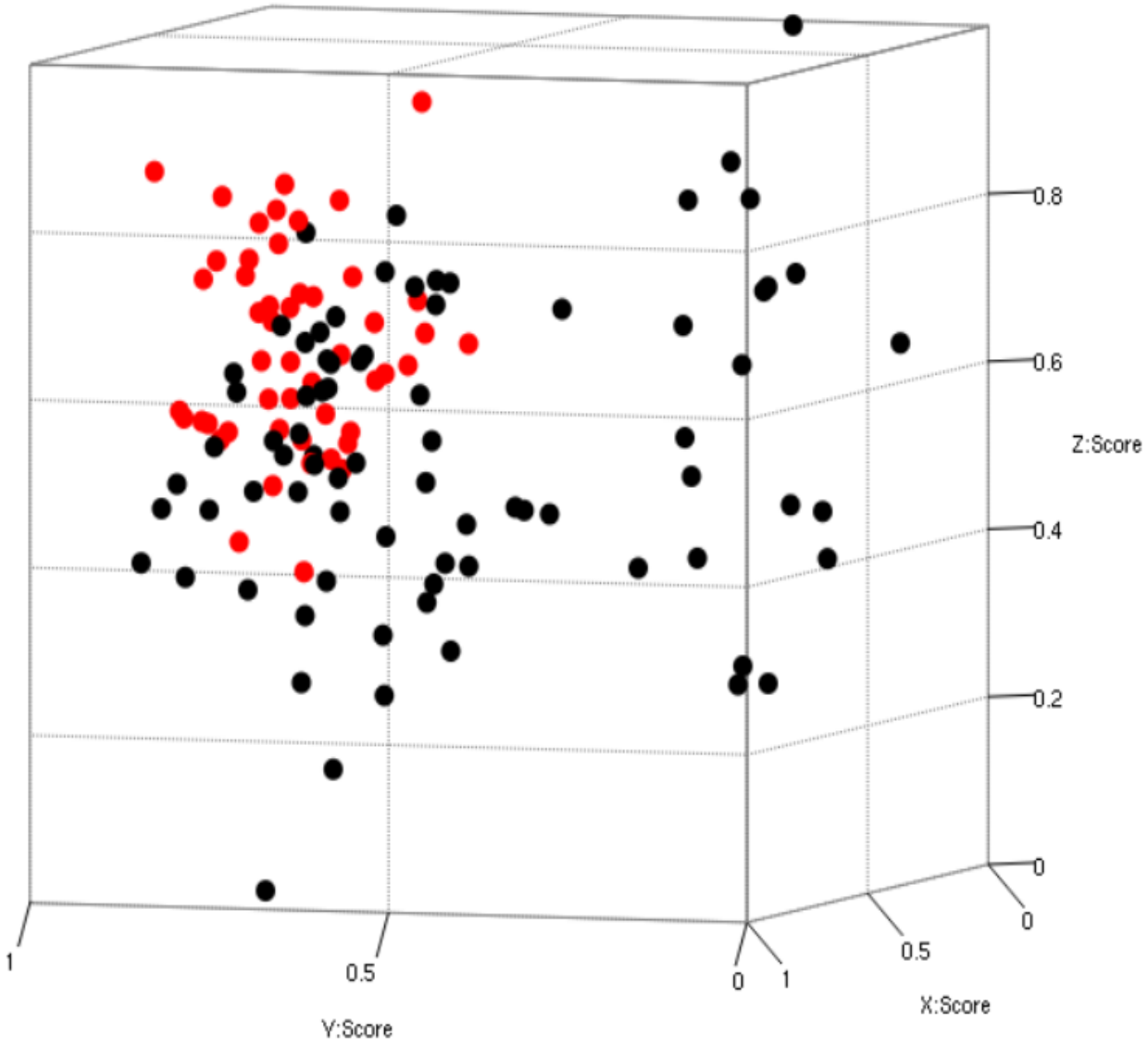

Figure 33. Three-dimensional cluster analysis of all daily fecal sample spectra for the Adult Feeding period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 18 to Day 41 during the Adult Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

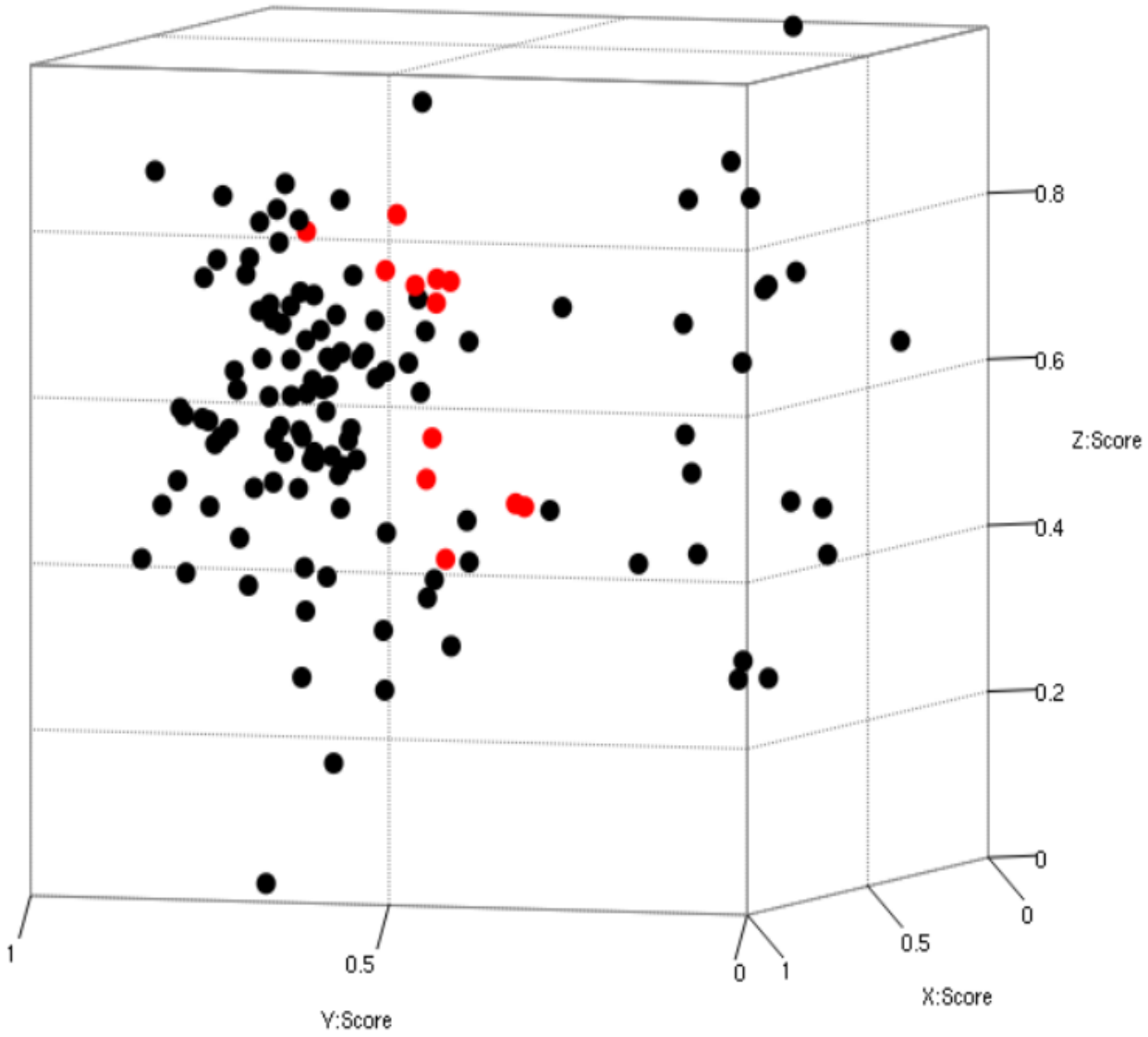

Figure 34. Three-dimensional cluster analysis of all daily fecal sample spectra for the Post-tick Recovery period for heifer numbers 4 and 8 in the high infestation level treatment group from Trial One shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 42 to Day 47 during the Post-tick Recovery period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 93.76% of total spectral variation.

Principal Components Analysis

To determine if there was a significant difference between the cluster shifts in the stepwise cluster analyses from Trial One, a PCA was performed on the spectra data from 384 observations from the six heifers over the six Stages in Trial One. The PCA resulted in 97.56% of the total variation in the 1050 frequencies being explained by the first three principal components (P_1, P_2, P_3). Results from the MANOVA and the Wilk's Lambda test showed highly significant evidence ($P < 0.0001$) of a difference in the means of the three principal components across the six Stages. There was significant evidence ($P = 0.0067$) of a difference between the means of the three principal components across the three levels of tick infestation. Results also revealed modest significant evidence ($P = 0.0222$) of an interaction between *Stage* and *Treatment* factors. Because there was significant interaction between *Stage* and *Treatment*, a comparison of treatment levels was made separately for each of the six Stages.

Principal Component 1: P_1. $P_1 = Heifer(Treatment) + Treatment + Stage + Treatment * Stage + Residuals$. Based on the *p*-values below in Table 5, there was not significant evidence of a difference between the three tick infestation levels with respect to the means of the first principal component for each of the six Stages in this trial. Least square means for *Stage*, *Treatment*, and *Stage*Treatment* for P_1 are shown in Figures 35 – 37.

Table 5. Comparison of Three Tick Infestation Levels across the six Stages for $\boldsymbol{P_1}$ in Trial One. Values with a *p*-value less than 0.0028 were declared to be significantly different.

	Stage					
	1 PIO	**2 PII**	**3 LF**	**4 NF**	**5 AF**	**6 PTR**
P*-values for Comparing *Low vs Medium	0.1566	0.1988	0.5955	0.7505	0.6574	0.0271
P*-values for Comparing *Medium vs High	0.1817	0.4282	0.0450	0.8883	0.4244	0.3514
P*-values for Comparing *Low vs High	0.9352	0.6215	0.1397	0.5890	0.2124	0.1992

*Denotes values that are significantly different with a *p*-value less than 0.0028. PIO = Pre-infestation Outside, PII = Pre-infestation Inside, LF = Larval Feeding, NF = Nymphal Feeding, AF = Adult Feeding, PTR = Post-tick Recovery.

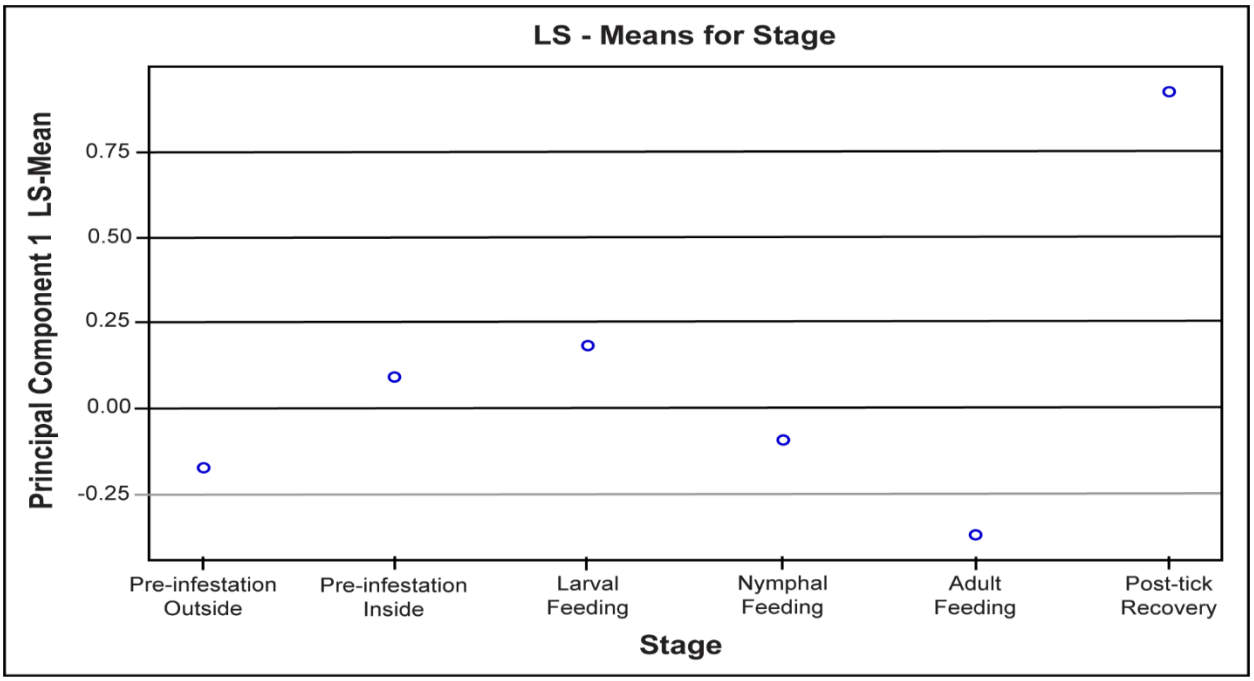

Figure 35. Least Squares Means for *Stage* of the first principal component in Trial One. The figure shows *Stage* (x-axis) by Principal Components 1 LS-Mean (y-axis).

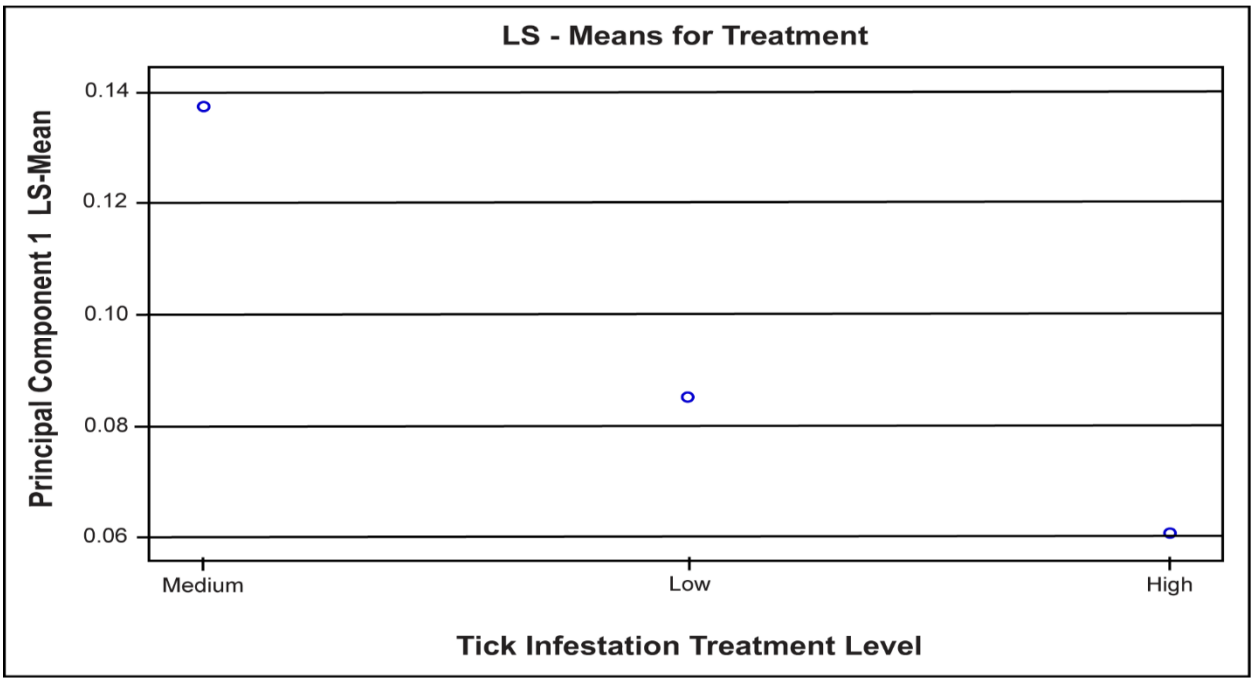

Figure 36. Least Squares Means for *Treatment* of the first principal component in Trial One. The figure shows Tick Infestation *Treatment* Level (x-axis) by Principal Components 1 LS-Mean (y-axis).

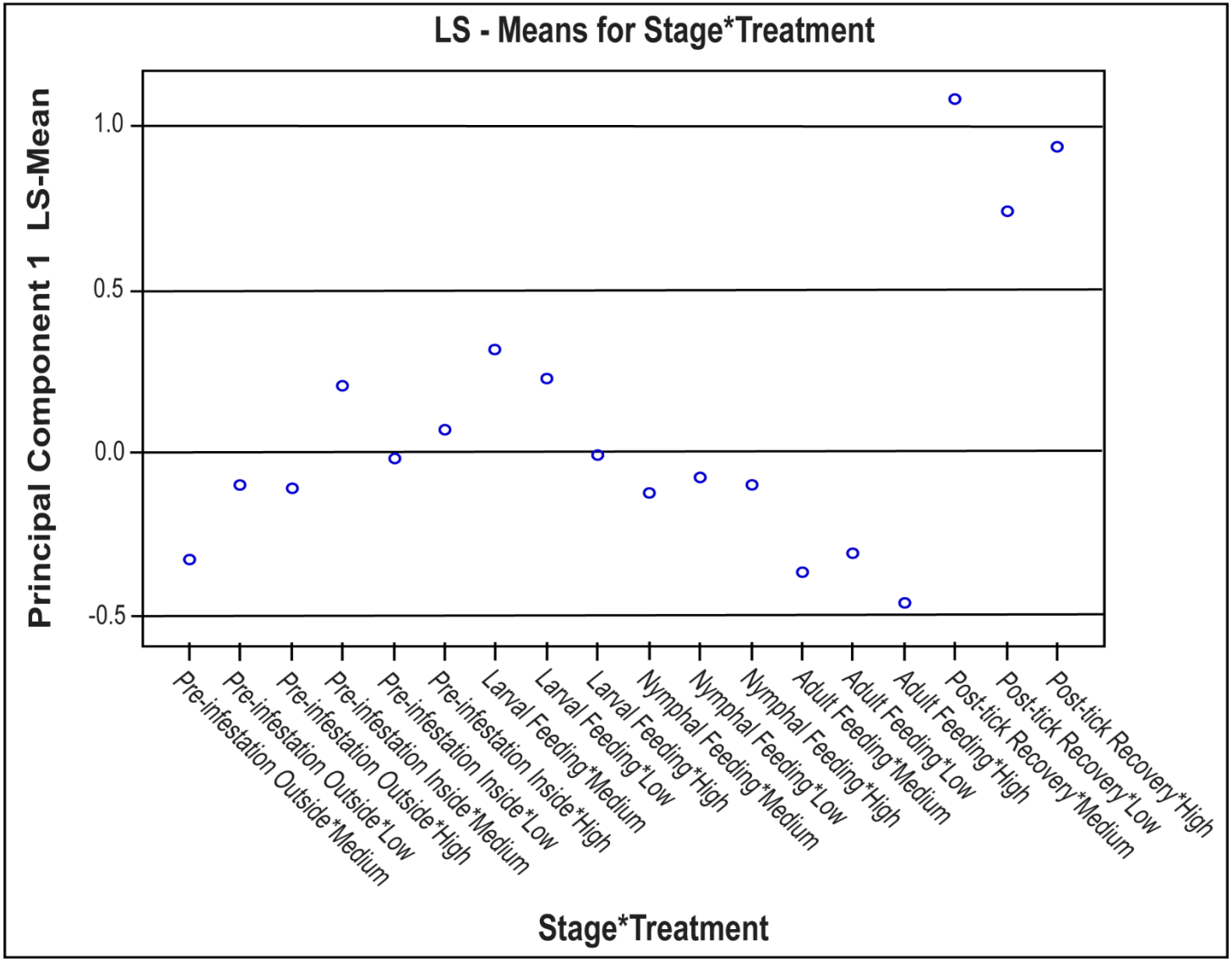

Figure 37. Least Squares Means for *Stage*Treatment* of the first principal component in Trial One. The figure shows *Stage*Treatment* (x-axis) by Principal Components 1 LS-Mean (y-axis).

Principal Component 2: P_2. $P_2 = Heifer(Treatment) + Treatment + Stage + Treatment * Stage + Residuals$. Based on the *p*-values below in Table 6, there was significant evidence of a difference between the following pairs of Treatments: Low *versus* Medium loadings during Larval Feeding and Medium *versus* High loadings during Larval Feeding. There was not significant evidence of differences for all the other combinations of the three tick infestation levels with respect to the means of the second principal component for each of the six Stages in this trial. Least square means for *Stage*, *Treatment*, and *Stage*Treatment* for P_2 are shown in Figures 38 – 40.

Table 6. Comparison of Three Tick Infestation Levels across the Six Stages for $\boldsymbol{P_2}$ in Trial One. Values with a *p*-value less than 0.0028 were declared to be significantly different.

	Stage					
	1 PIO	**2 PII**	**3 LF**	**4 NF**	**5 AF**	**6 PTR**
P*-values for Comparing *Low vs Medium	0.0618	0.7565	0.0025*	0.0791	0.0233	0.4524
P*-values for Comparing *Medium vs High	0.0153	0.9834	0.3152	0.8854	0.9337	0.5879
P*-values for Comparing *Low vs High	0.5743	0.7407	0.0001*	0.0576	0.0288	0.8339

*Denotes values that are significantly different with a *p*-value less than 0.0028. PIO = Pre-infestation Outside, PII = Pre-infestation Inside, LF = Larval Feeding, NF = Nymphal Feeding, AF = Adult Feeding, PTR = Post-tick Recovery.

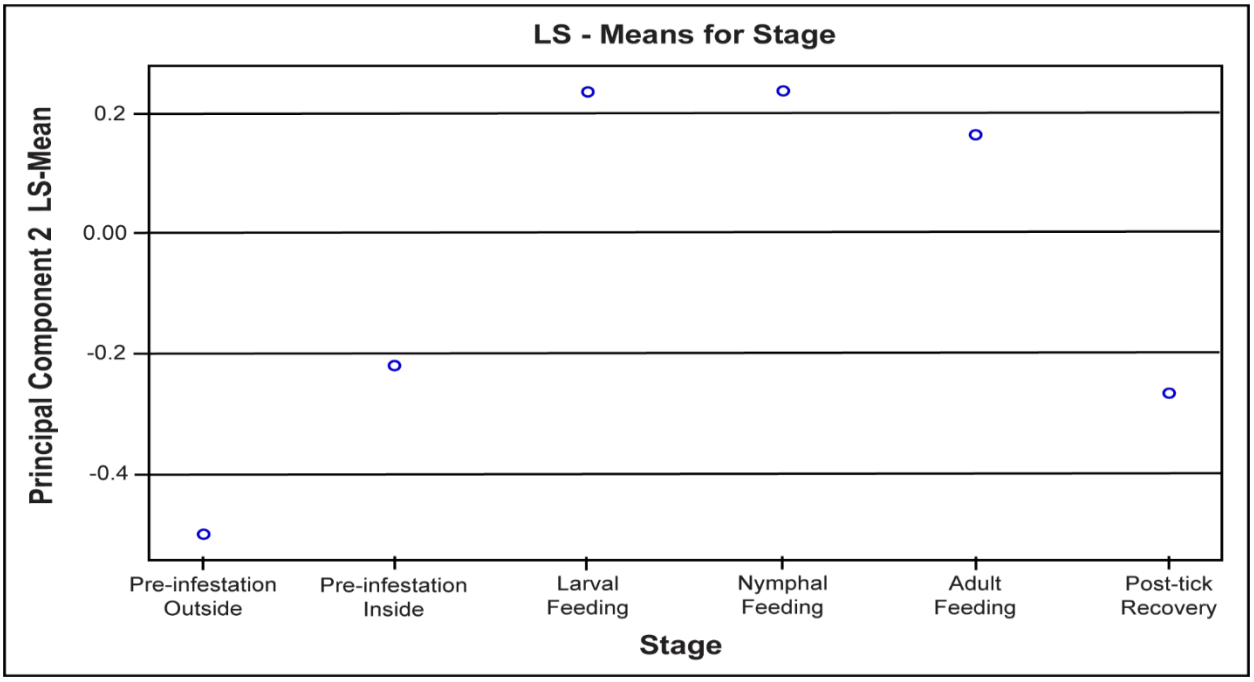

Figure 38. Least Squares Means for *Stage* of the second principal component in Trial One. The figure shows *Stage* (x-axis) by Principal Components 2 LS-Mean (y-axis).

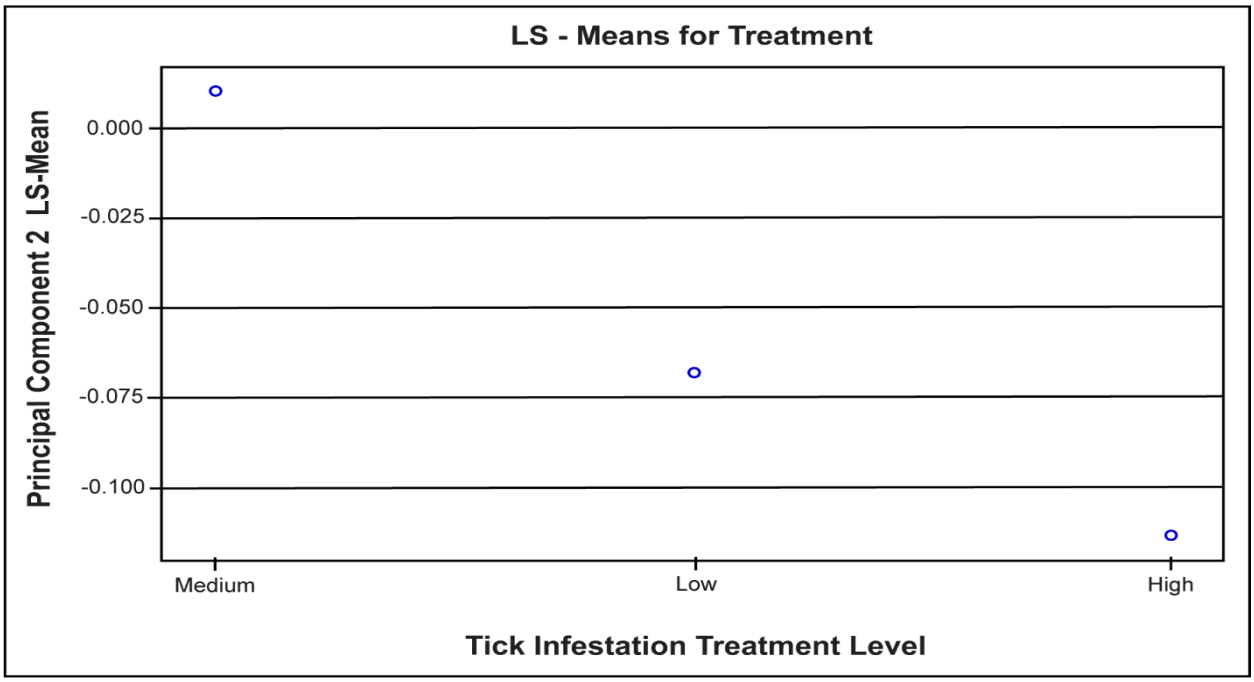

Figure 39. Least Squares Means for *Treatment* of the second principal component in Trial One. The figure shows Tick Infestation *Treatment* Level (x-axis) by Principal Components 2 LS-Mean (y-axis).

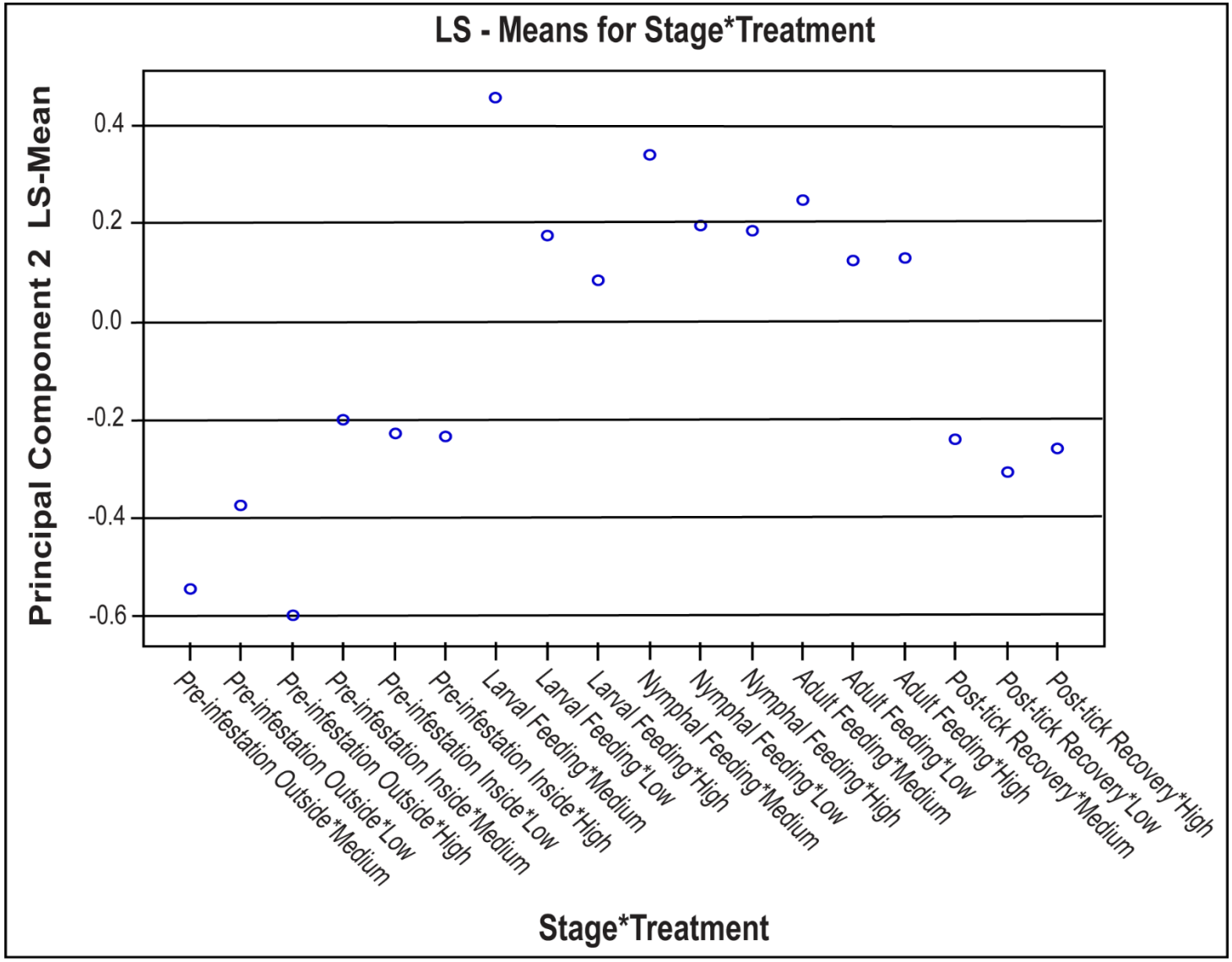

Figure 40. Least Squares Means for *Stage*Treatment* of the second principal component in Trial One. The figure shows *Stage*Treatment* (x-axis) by Principal Components 2 LS-Mean (y-axis).

Principal Component 3: P_3. P_3 = Heifer(Treatment) + Treatment + Stage + Treatment ∗ Stage + Residuals. Based on the *p*-values below in Table 7, there was not significant evidence of differences for all combinations of the three tick infestation levels with respect to the means of the second principal component for each of the six Stages in this trial. Least square means for *Stage*, *Treatment*, and *Stage*Treatment* for P_3 are shown in Figures 41 – 43.

Table 7. Comparison of Three Tick Infestation Levels across the Six Stages for $\boldsymbol{P_3}$ in Trial One. Values with a *p*-value less than 0.0028 were declared to be significantly different.

	Stage					
	1 PIO	**2 PII**	**3 LF**	**4 NF**	**5 AF**	**6 PTR**
P*-values for Comparing *Low vs Medium	0.2457	0.5975	0.2266	0.2652	0.1429	0.7123
P*-values for Comparing *Medium vs High	0.5873	0.4869	0.7405	0.7521	0.0893	0.4426
P*-values for Comparing *Low vs High	0.5360	0.8671	0.3796	0.4244	0.8140	0.6897

*Denotes values that are significantly different with a *p*-value less than 0.0028. PIO = Pre-infestation Outside, PII = Pre-infestation Inside, LF = Larval Feeding, NF = Nymphal Feeding, AF = Adult Feeding, PTR = Post-tick Recovery.

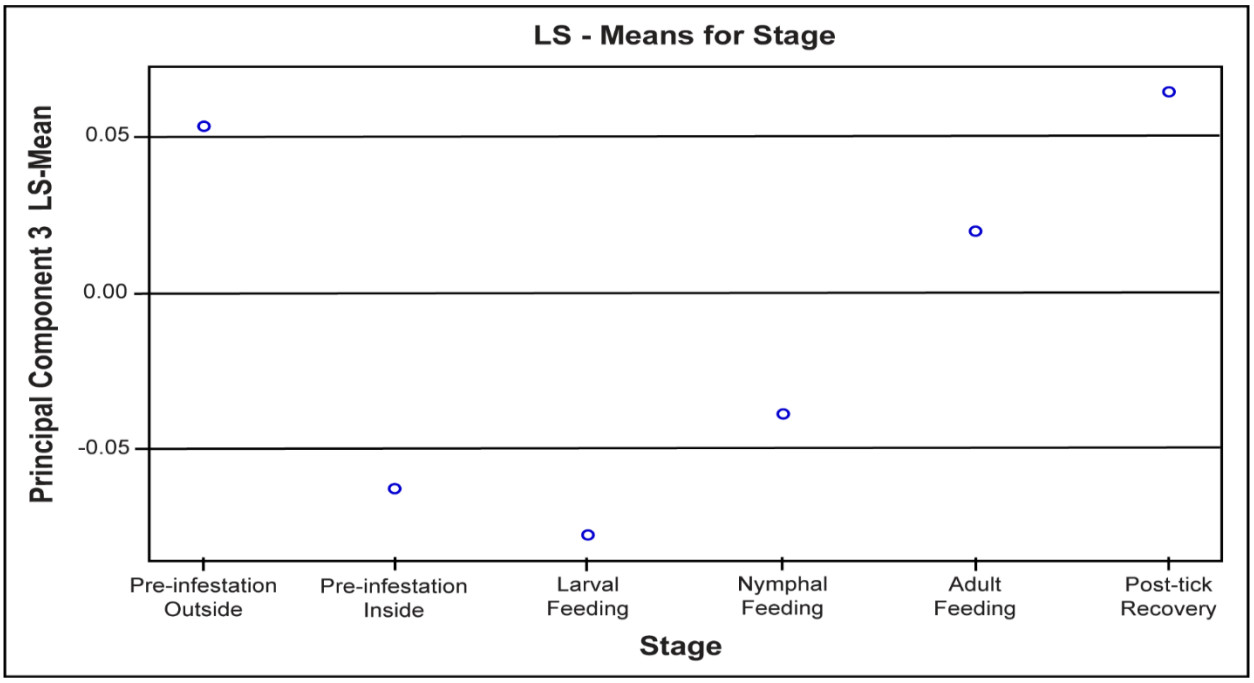

Figure 41. Least Squares Means for *Stage* of the third principal component in Trial One. The figure shows *Stage* (x-axis) by Principal Components 3 LS-Mean (y-axis).

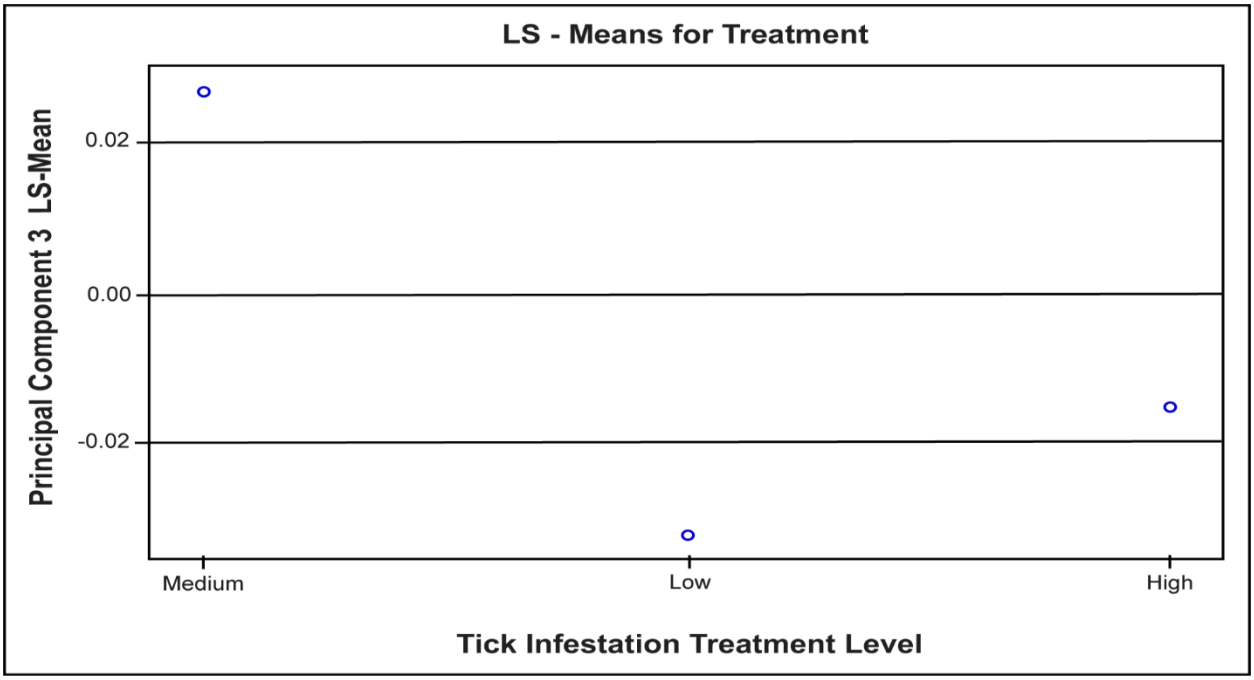

Figure 42. Least Squares Means for *Treatment* of the third principal component in Trial One. The figure shows Tick Infestation *Treatment* Level (x-axis) by Principal Components 3 LS-Mean (y-axis).

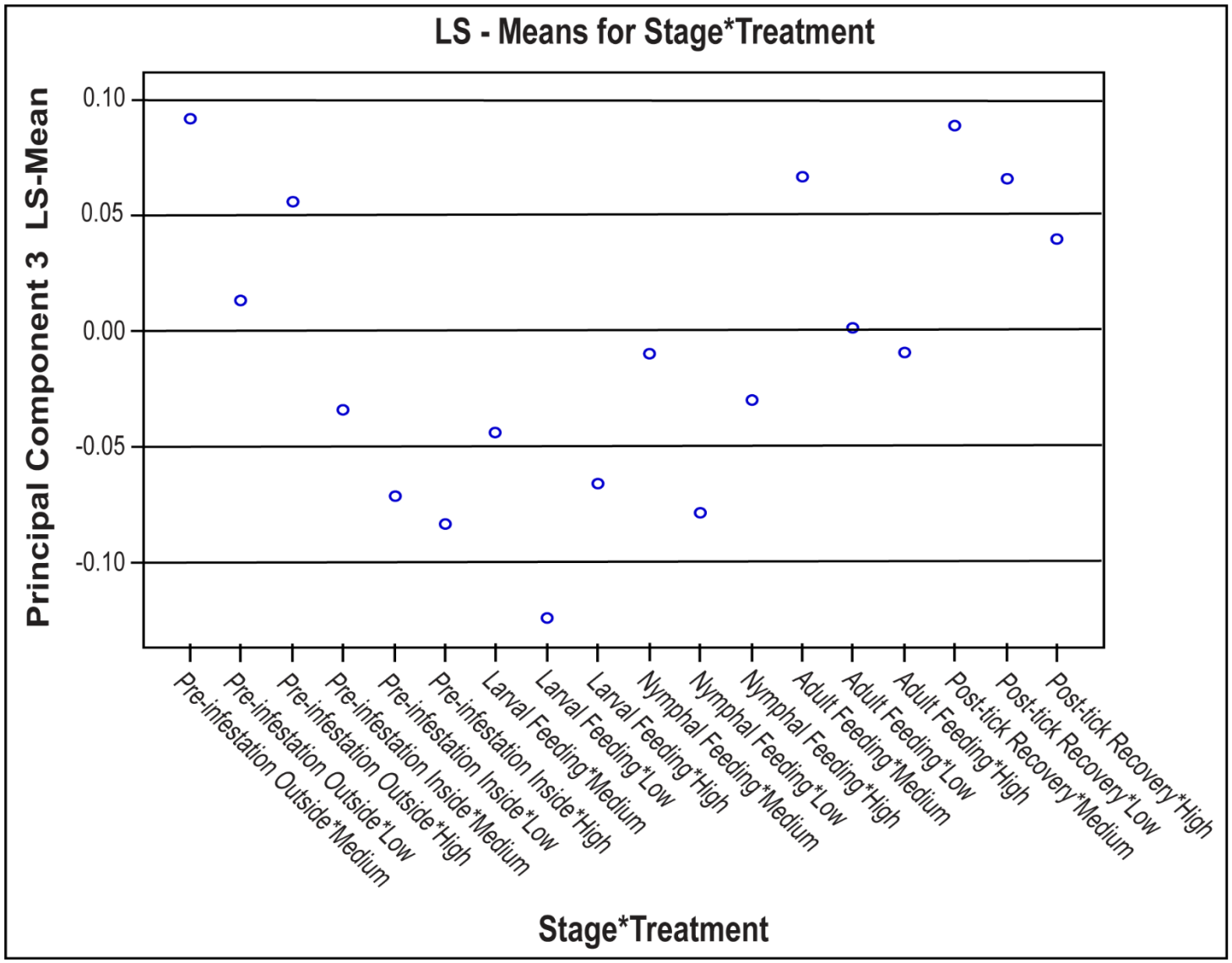

Figure 43. Least Squares Means for *Stage*Treatment* of the third principal component in Trial One. The figure shows *Stage*Treatment* (x-axis) by Principal Components 3 LS-Mean (y-axis).

Trial Two (Winter 2019)

Heifer Body Weights

The beginning and end weights by heifer and treatment are provided in Table 8. All six heifers gained weight during Trial Two. Weight gain ranged from 36.3 kg to 49.9 kg over the course of 57-d, which is equivalent to 1.4 kg to 1.93 kg per day. These are standard weight gains for heifers in their growing weight class (Ringwall 2012) regardless of tick infestation. The lowest weight gains were in the low and medium tick infestation level treatment groups.

Table 8. Comparison of beginning and ending heifer weights following infestation with *Dermacentor albipictus* larvae at three infestation level treatment groups over the course of 57-days in Trial Two.

Treatment Group Larval Infestation Level	Heifer Number	Beginning Body Weight (kg)	End Body Weight (kg)/ Weight Change (kg)
Low	4	190.5	229.1/38.6
	6	176.9	217.7/40.8
Medium	3	174.6	222.3/47.6
	8	174.6	217.7/43.1
High	2	186.0	222.3/36.3
	5	195.0	244.9/49.9

Engorged Female Drop

The result of each heifer's infestation was measured by determining the first date of engorged female tick drop, the daily and cumulative number of engorged females produced by each infestation, and the date of peak engorged female drop. Engorged *D. albipictus* females that dropped off the six heifers were collected three times daily. The approximate percentage of engorged female drop was calculated for each individual heifer based on a 50:50 sex ratio of

females to males for *D. albipictus* (Barker et al. 1990) is summarized in Table 9. Graphical representations of daily engorged female drop for the six heifers are shown in Figure 44.

Table 9. Trial Two engorged females drop data by heifer number for the three treatment groups. This table shows the number of engorged females, the percentage of engorged females collected from each heifer based on a 50:50 sex ratio of females to males, and the day(s) of peak female drop post-tick infestation.

Treatment Group	Heifer Number	Number of Engorged Females	Percent of Engorged Females Collected (50:50 sex ratio)	Peak Drop Day(s) Post-infestation
Low	4	9	1.8	27
	6	30	6.0	27 and 28
Medium	3	39	1.95	28
	8	14	0.7	28
High	2	149	3.75	27
	5	30	0.75	26 and 28

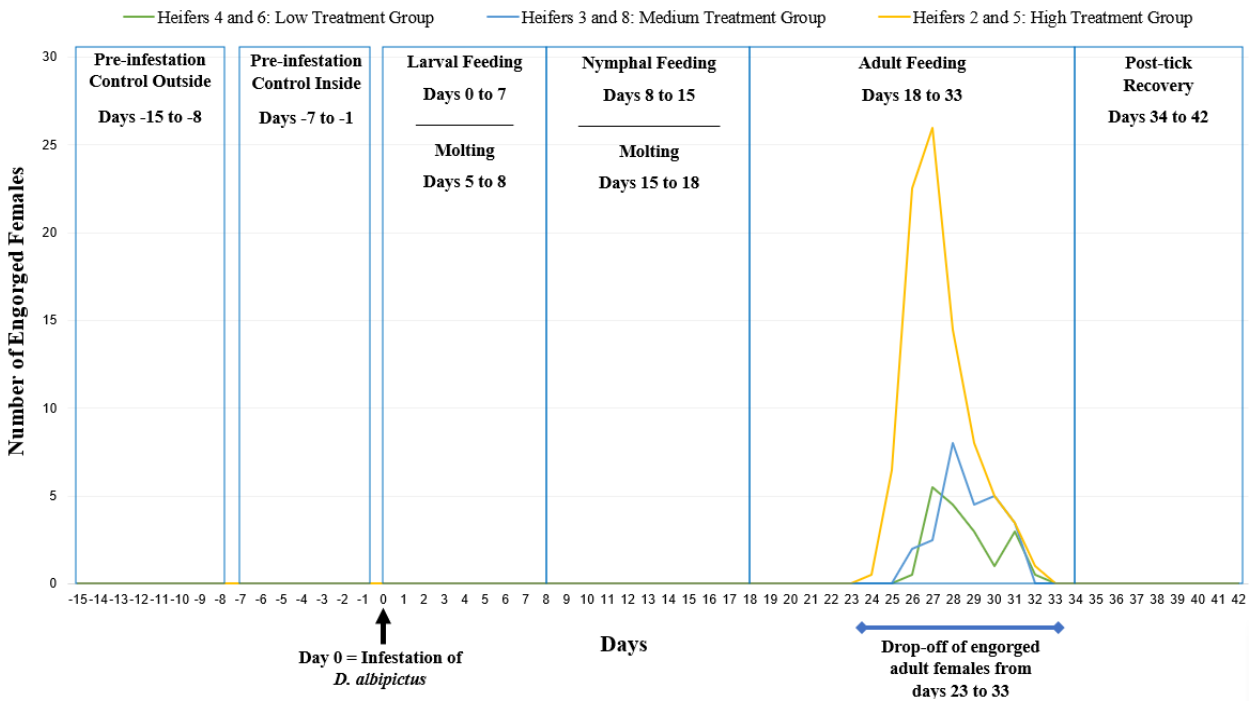

Figure 44. Number of engorged female *D. albipictus* collected per day from the start of female drop-off by heifer number for Trial Two. Days are shown on the x-axis and the number of females collected shown on the y-axis.

Stepwise Cluster Analyses for the Low Treatment Level Group

A discriminant analysis was conducted for raw spectral data from daily fecal samples for heifer numbers 4 and 6 in the low treatment level group from the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period (one fecal spectra per day per heifer). The analysis of spectra in the 576 nm – 1126 nm range produced 13 factors with the first three dominant spectral factors/variations representing 98.38% of the total variation among sample spectra for the medium infestation treatment level (see Figures 45 – 47). This result permitted the sample spectra to be analyzed by cluster analyses with each of the three dominant factors/variations plotted as “x”, “y” and “z”.

The cluster analyses of the spectra from the pair of heifers in the low treatment level group resulted in a pattern of six clusters that depict shifts in fecal chemistry. Sample clusters were distinguishable for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period in the cluster analyses (see Figures 48 through 54). The first was comprised of samples originating from Day -15 (15 d prior to infestation) through Day -6 (6 d prior to infestation; allowing for a 48-h rumen passage time) (Figure 48). The second cluster was comprised of samples within the period from Day -5 (5 d prior to infestation) through Day 1 (1 day post infestation; allowing for a 48-h rumen passage time) (Figure 49). Figure 50 is the daily fecal spectra for the two pre-infestation acclimation periods in one cluster analysis, showing the entire experimental control period for the low treatment group. The third cluster was comprised of samples from Day 2 (2 d post infestation) through Day 7 (7 d post infestation), which is the period of attachment, feeding and molting of larvae (Figure 51). The fourth cluster originated

from samples from Day 8 (8 d post infestation) to Day 17 (17 d post infestation), corresponding to attachment, feeding, and molting of nymphs (Figure 52). The fifth cluster includes samples from Day 18 (18 d post infestation) to Day 35 (allowing for a 48-h rumen passage time), corresponding to the period of adult feeding and the period in which females complete their feeding and drop from the host (Figure 53). The sixth cluster was comprised of samples from Day 36 to Day 42, which consists of the period heifers were going through post-tick recovery (Figure 54). Spectral cluster shifts occurred representing time periods that are consistent with no tick feeding, low tick feeding, heavy feeding, and a period of post-tick recovery.

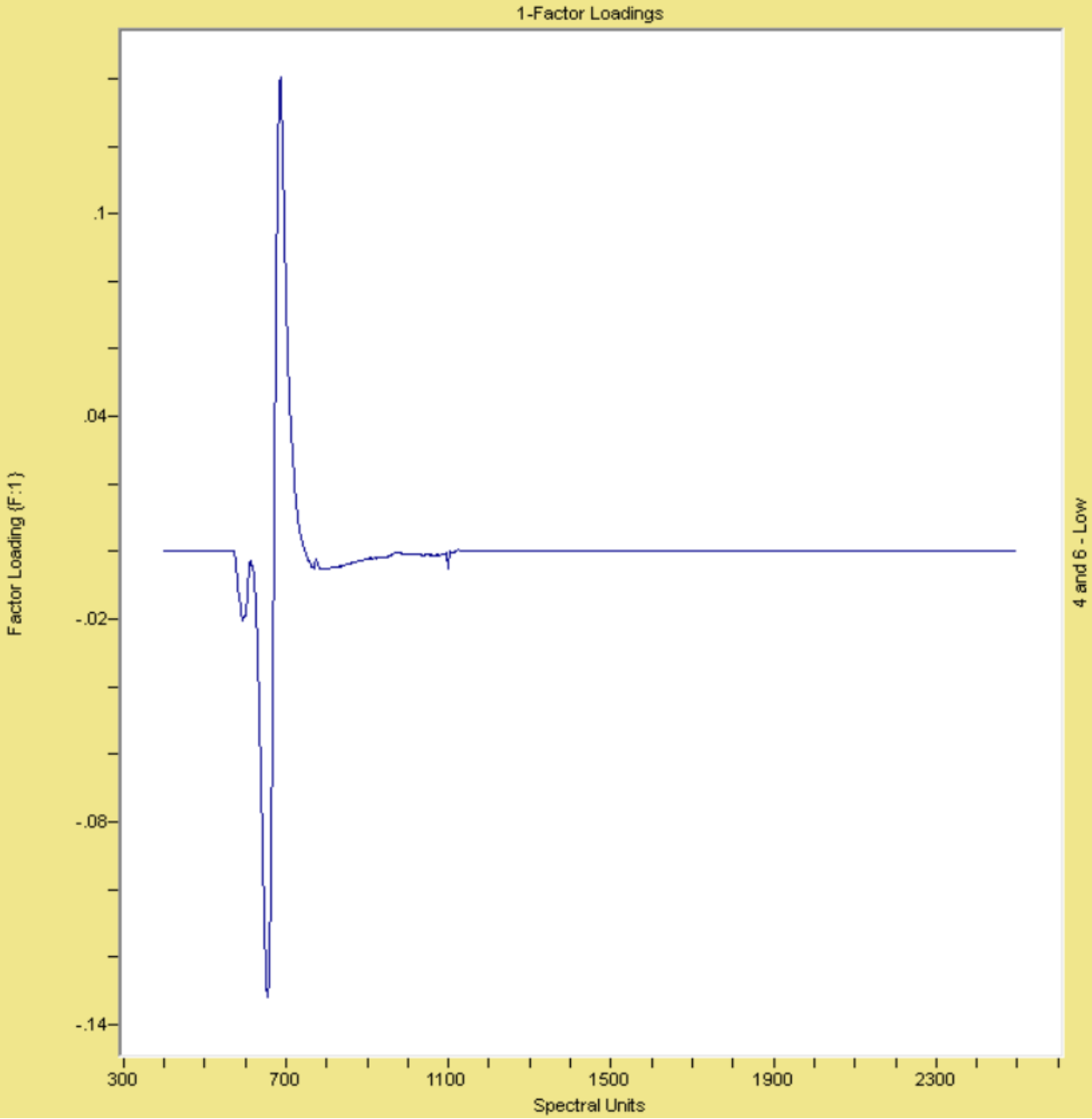

Figure 45. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the first most common spectral variation (factor) from the low infestation treatment level heifer numbers 4 and 6 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 95.86% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

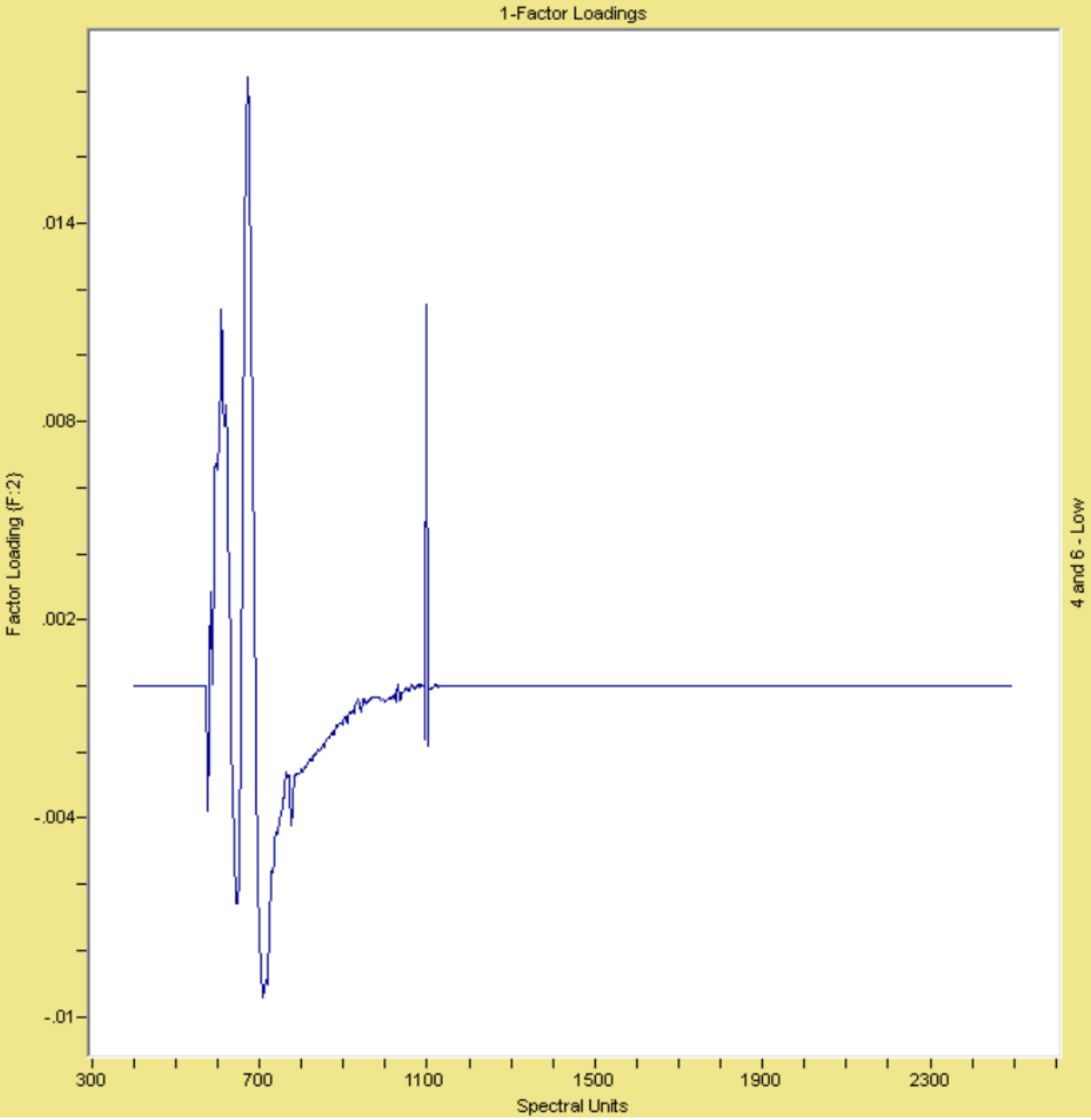

Figure 46. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the second most common spectral variation (factor) from the low infestation treatment level heifer numbers 4 and 6 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 1.51% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

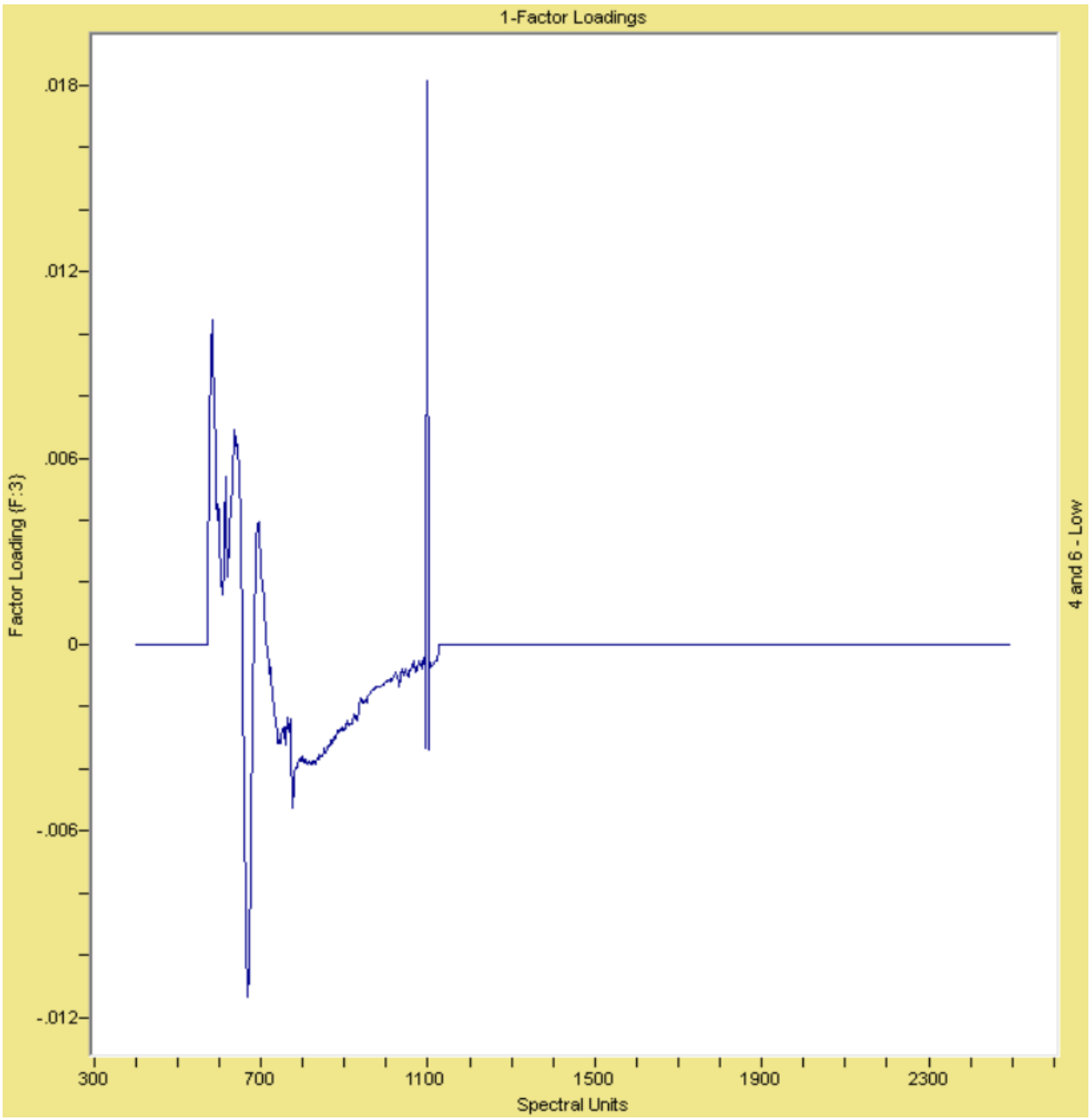

Figure 47. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the third most common spectral variation (factor) from the low infestation treatment level heifer numbers 4 and 6 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 1.01% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

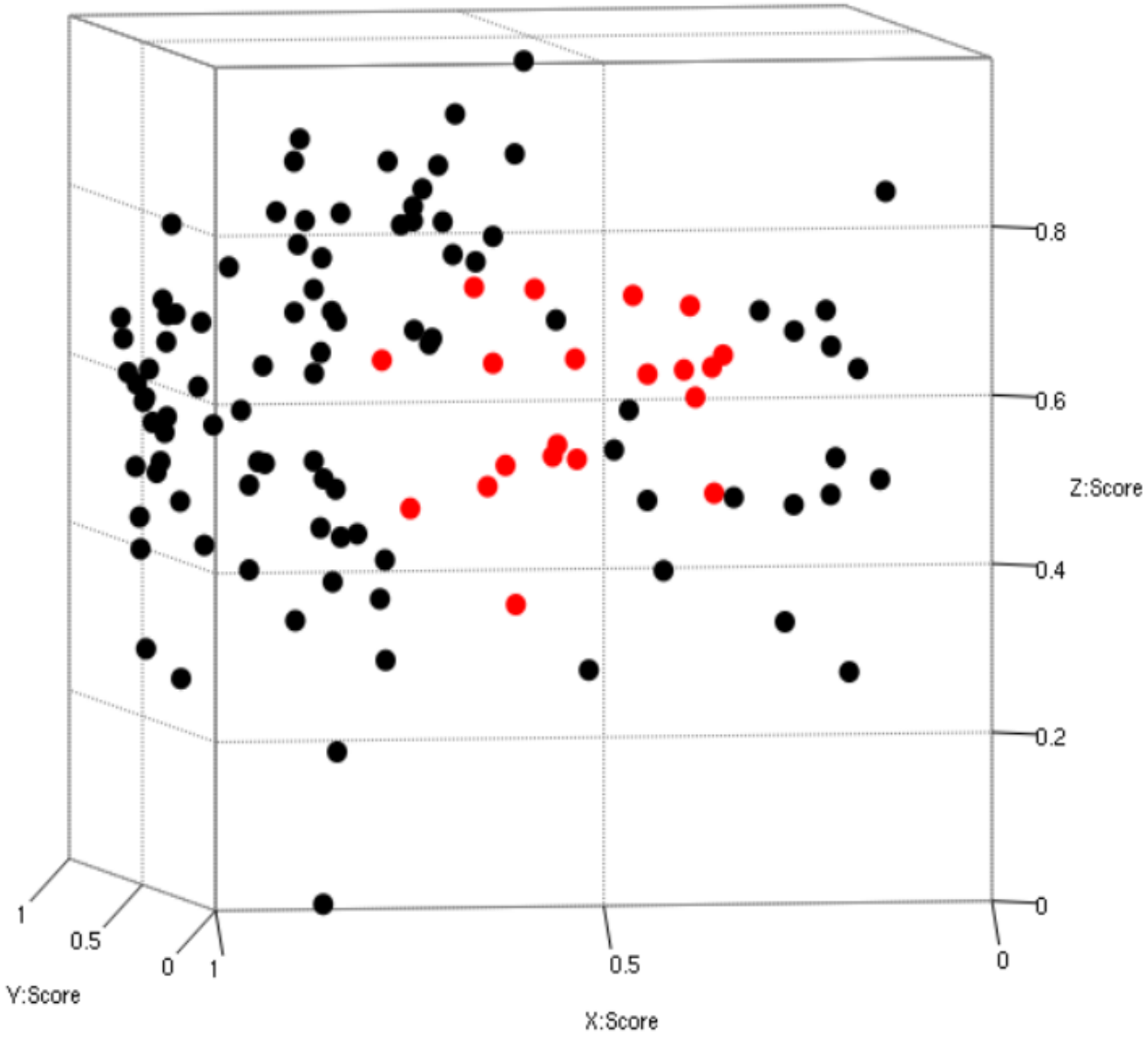

Figure 48. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day -6 during the Pre-infestation Outside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

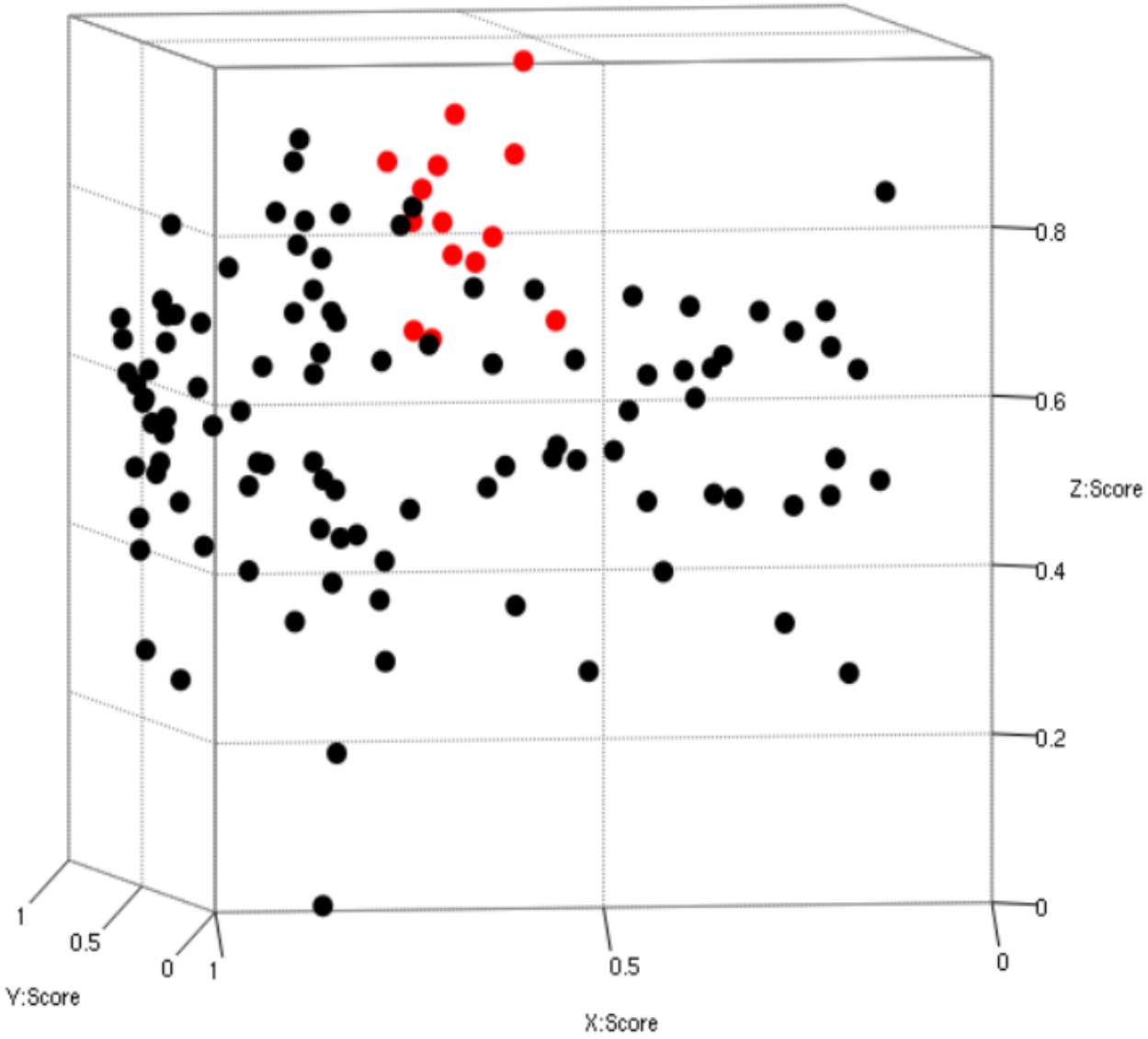

Figure 49. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Inside period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -5 to Day 1 during the Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

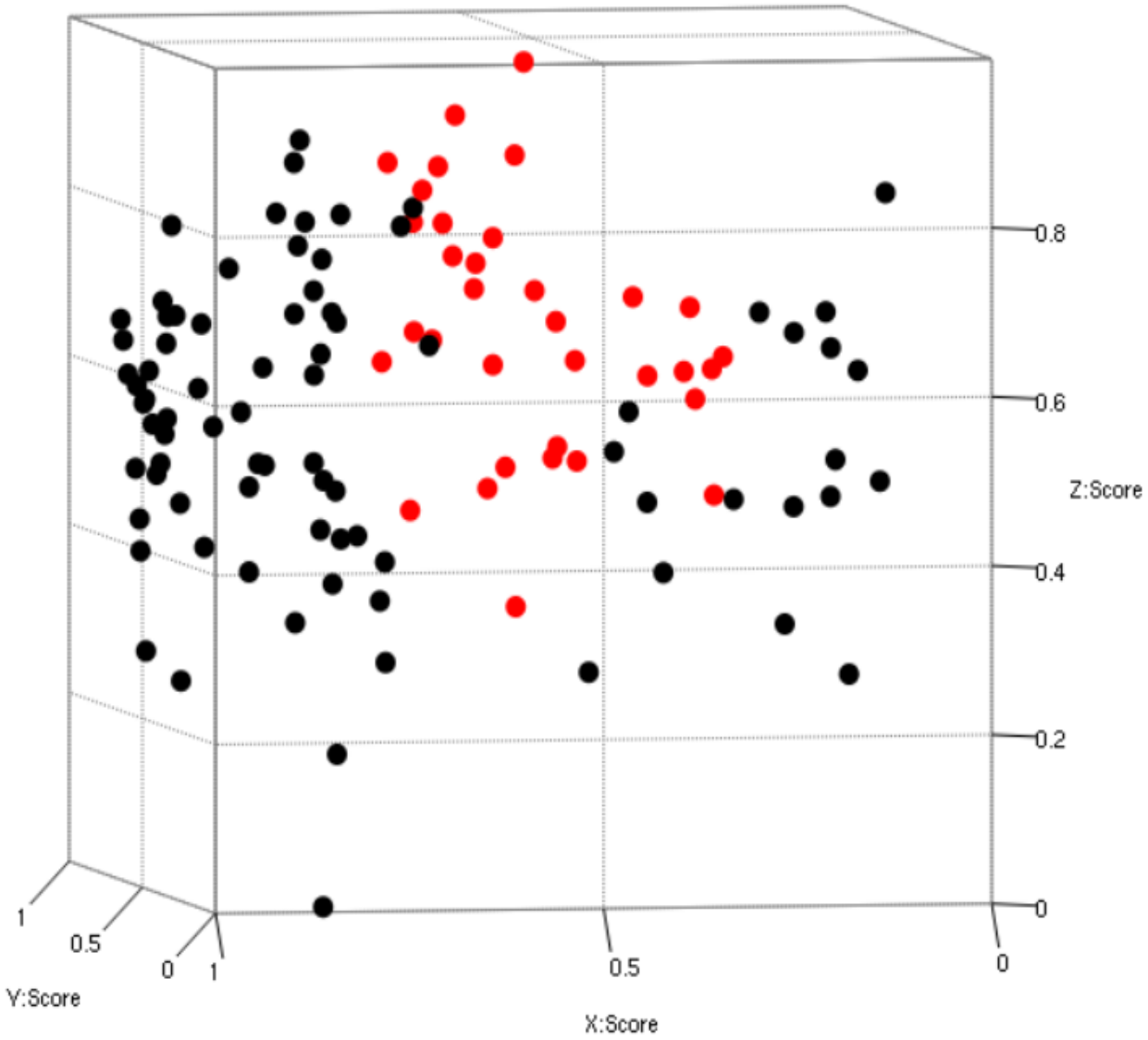

Figure 50. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside and Pre-infestation Inside period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day 1 during the Pre-infestation Outside and Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

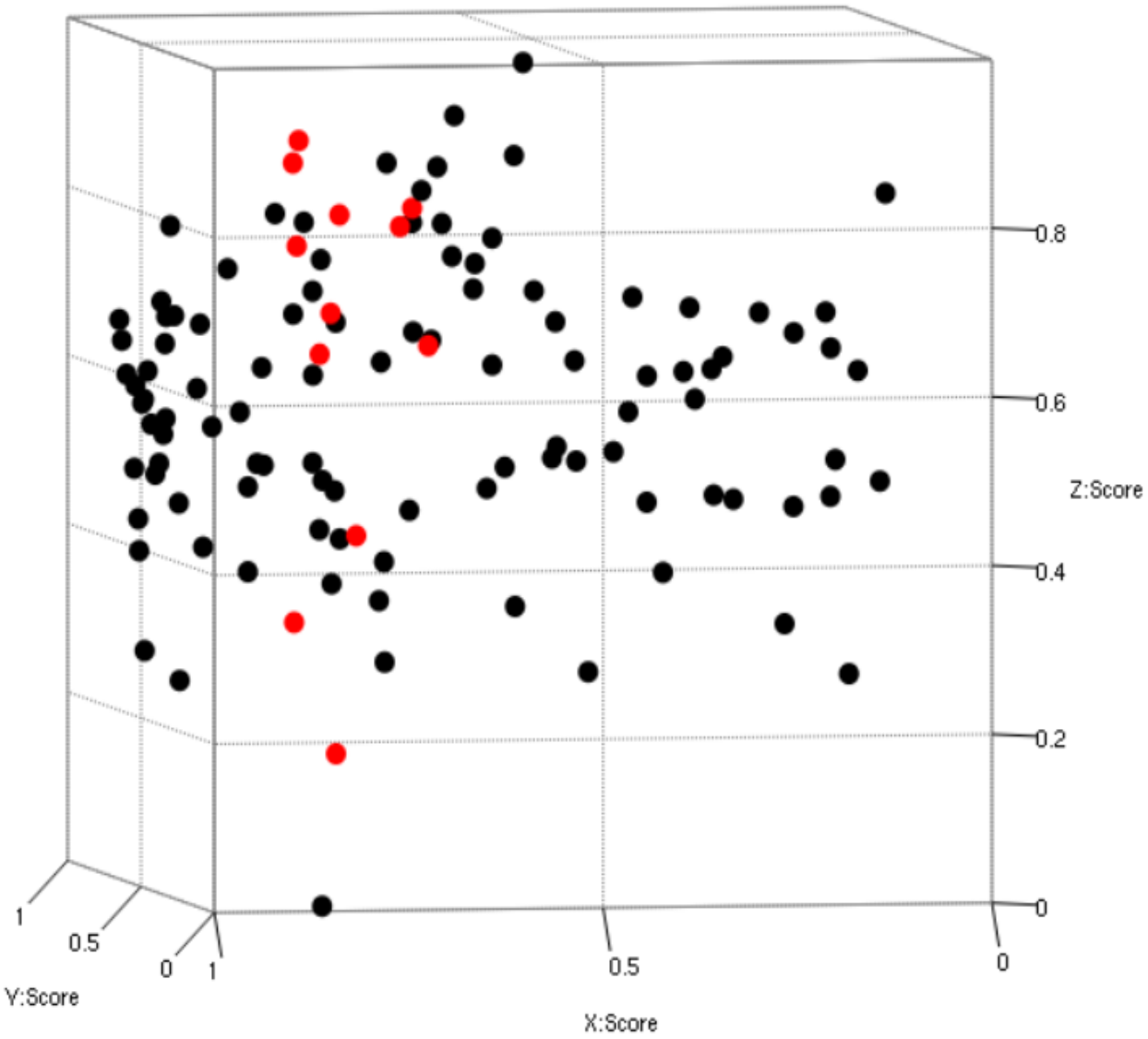

Figure 51. Three-dimensional cluster analysis of all daily fecal sample spectra for the Larval Feeding period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 2 to Day 7 during the Larval Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

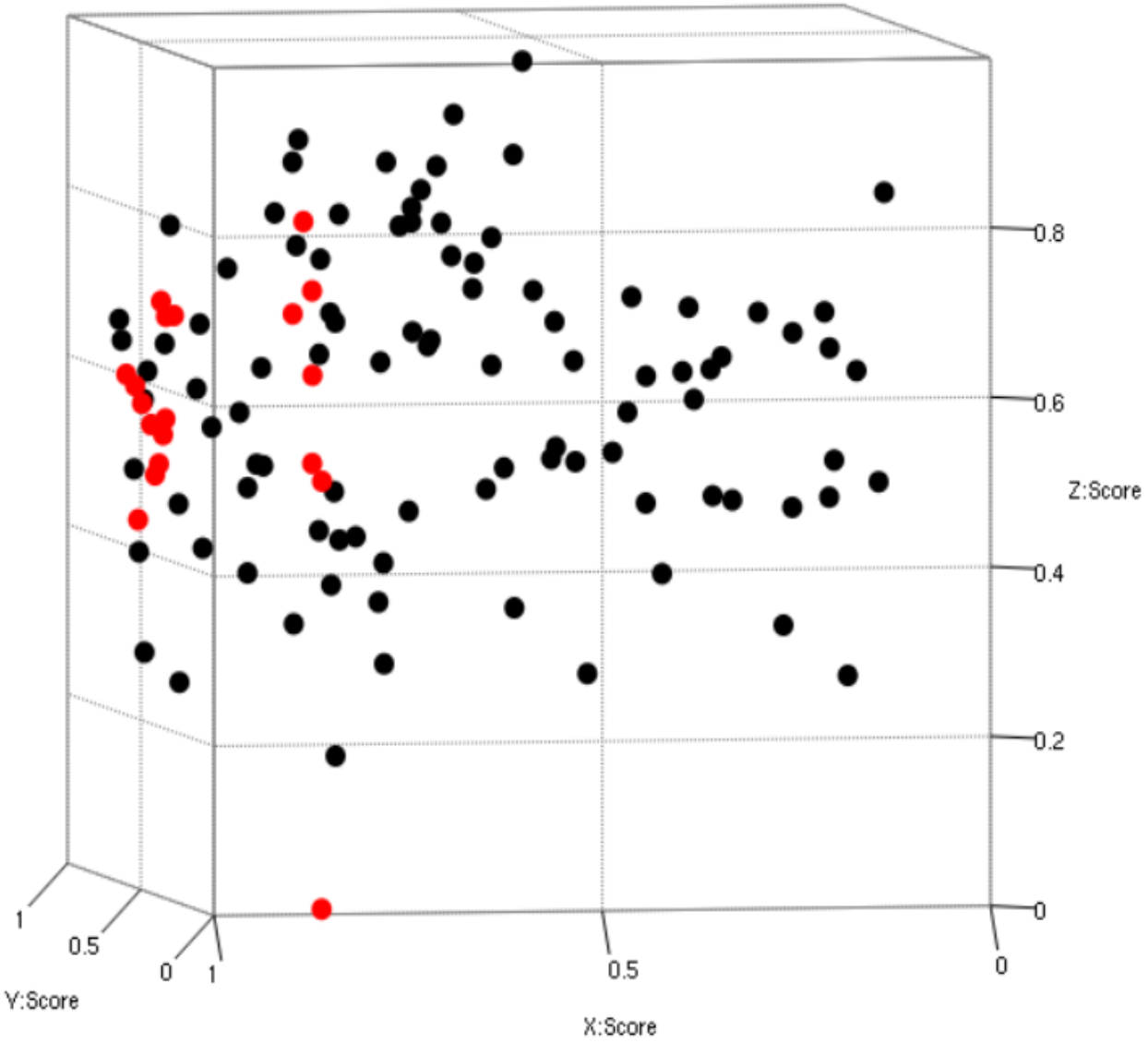

Figure 52. Three-dimensional cluster analysis of all daily fecal sample spectra for the Nymphal Feeding period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 8 to Day 17 during the Nymphal Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

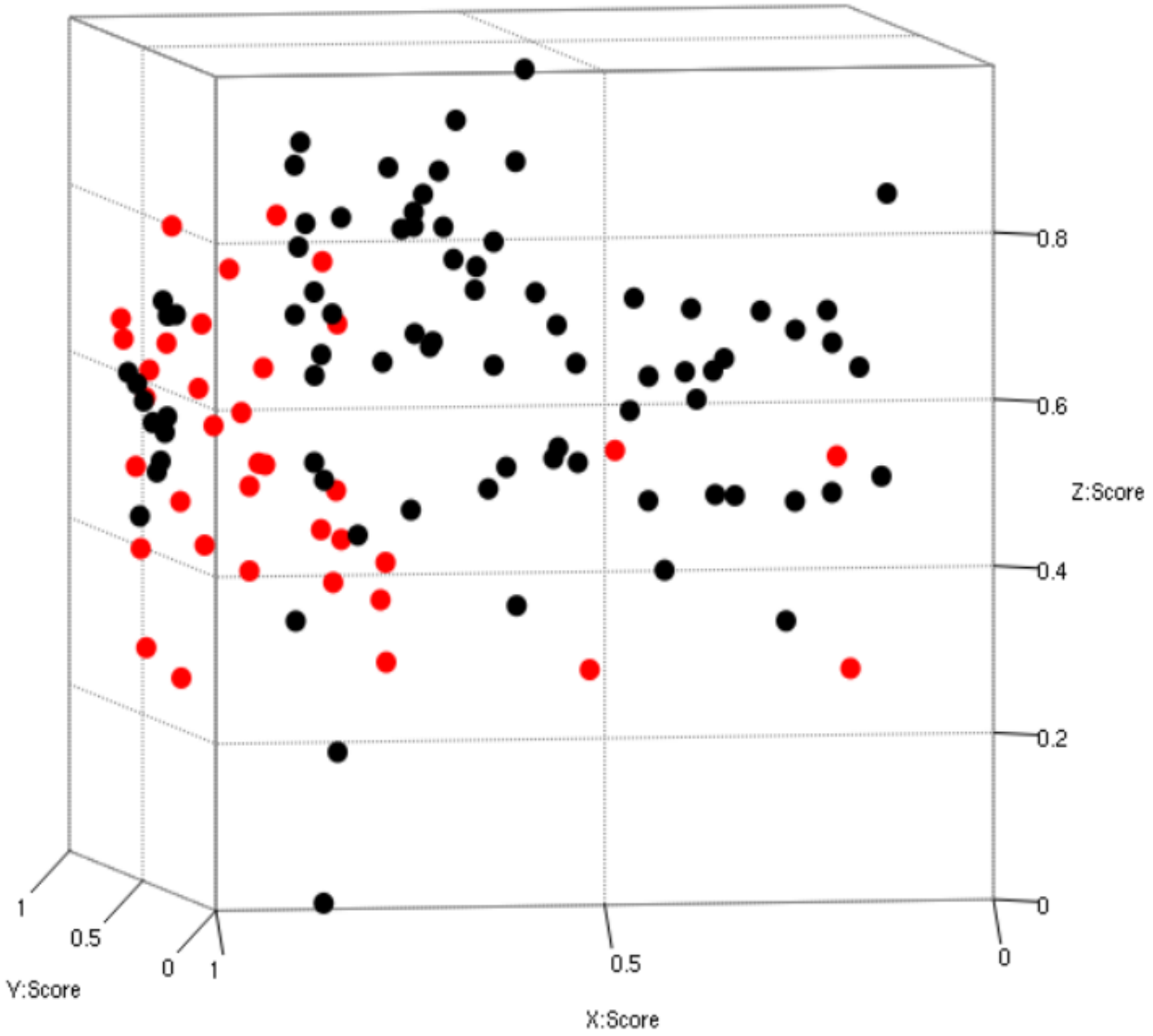

Figure 53. Three-dimensional cluster analysis of all daily fecal sample spectra for the Adult Feeding period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 18 to Day 35 during the Adult Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

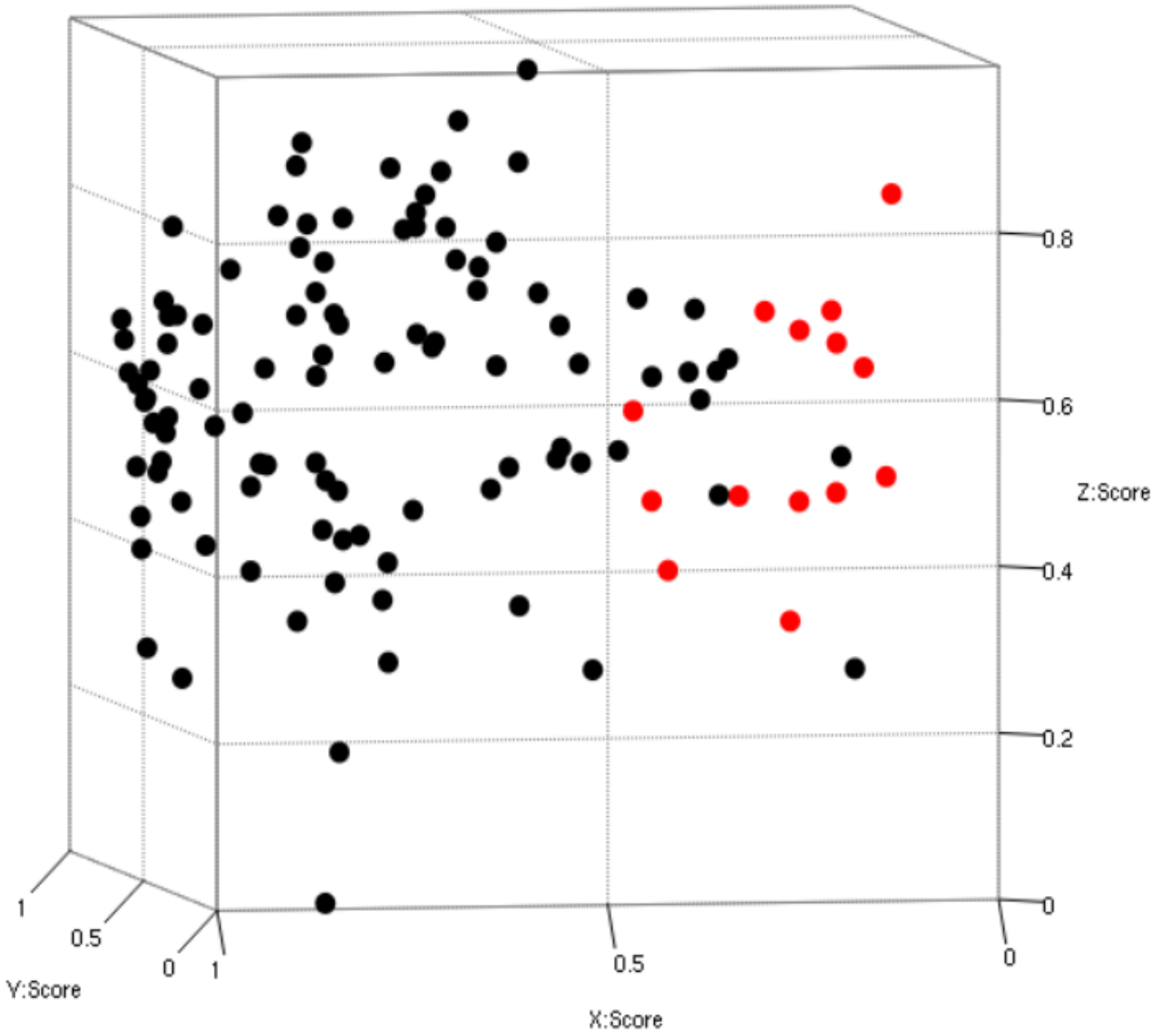

Figure 54. Three-dimensional cluster analysis of all daily fecal sample spectra for the Post-tick Recovery period for heifer numbers 4 and 6 in the low infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 36 to Day 42 during the Post-tick Recovery period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.38% of total spectral variation.

Stepwise Cluster Analyses for the Medium Treatment Level Group

A discriminant analysis was conducted for raw spectral data from daily fecal samples for heifer numbers 3 and 8 in the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period (one fecal spectra per day per heifer). The analysis of spectra in the 576 nm – 1126 nm range produced 12 factors with the first three dominant spectral factors/variations representing 98.26% of the total variation among sample spectra for the medium infestation treatment level (see Figures 55 – 57). This result permitted the sample spectra to be analyzed by cluster analyses with each of the three dominant factors/variations plotted as "x", "y" and "z".

The cluster analyses of the spectra from the pair of heifers in the medium treatment level group resulted in a pattern of six clusters that depict shifts in fecal chemistry. Sample clusters were distinguishable for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period in the cluster analyses (see Figures 58 through 64). The first was comprised of samples originating from Day -15 (15 d prior to infestation) through Day -6 (6 d prior to infestation; allowing for a 48-h rumen passage time) (Figure 58). The second cluster was comprised of samples within the period from Day -5 (5 d prior to infestation) through Day 1 (1 day post infestation; allowing for a 48-h rumen passage time) (Figure 59). Figure 60 is the daily fecal spectra for the two pre-infestation acclimation periods in one cluster analysis, showing the entire experimental control period for the medium treatment group. The third cluster was comprised of samples from Day 2 (2 d post infestation) through Day 7 (7 d post infestation), which is the period of attachment, feeding and molting of larvae (Figure 61). The fourth cluster originated from samples from Day 8 (8 d post infestation) to Day 17 (17 d post infestation),

corresponding to attachment, feeding, and molting of nymphs (Figure 62). The fifth cluster includes samples from Day 18 (18 d post infestation) to Day 35 (allowing for a 48-h rumen passage time), corresponding to the period of adult feeding and the period in which females complete their feeding and drop from the host (Figure 63). The sixth cluster was comprised of samples from Day 36 to Day 42, which consists of the period heifers were going through post-tick recovery (Figure 64). Spectral cluster shifts occurred representing time periods that are consistent with no tick feeding, low tick feeding, heavy feeding, and a period of post-tick recovery.

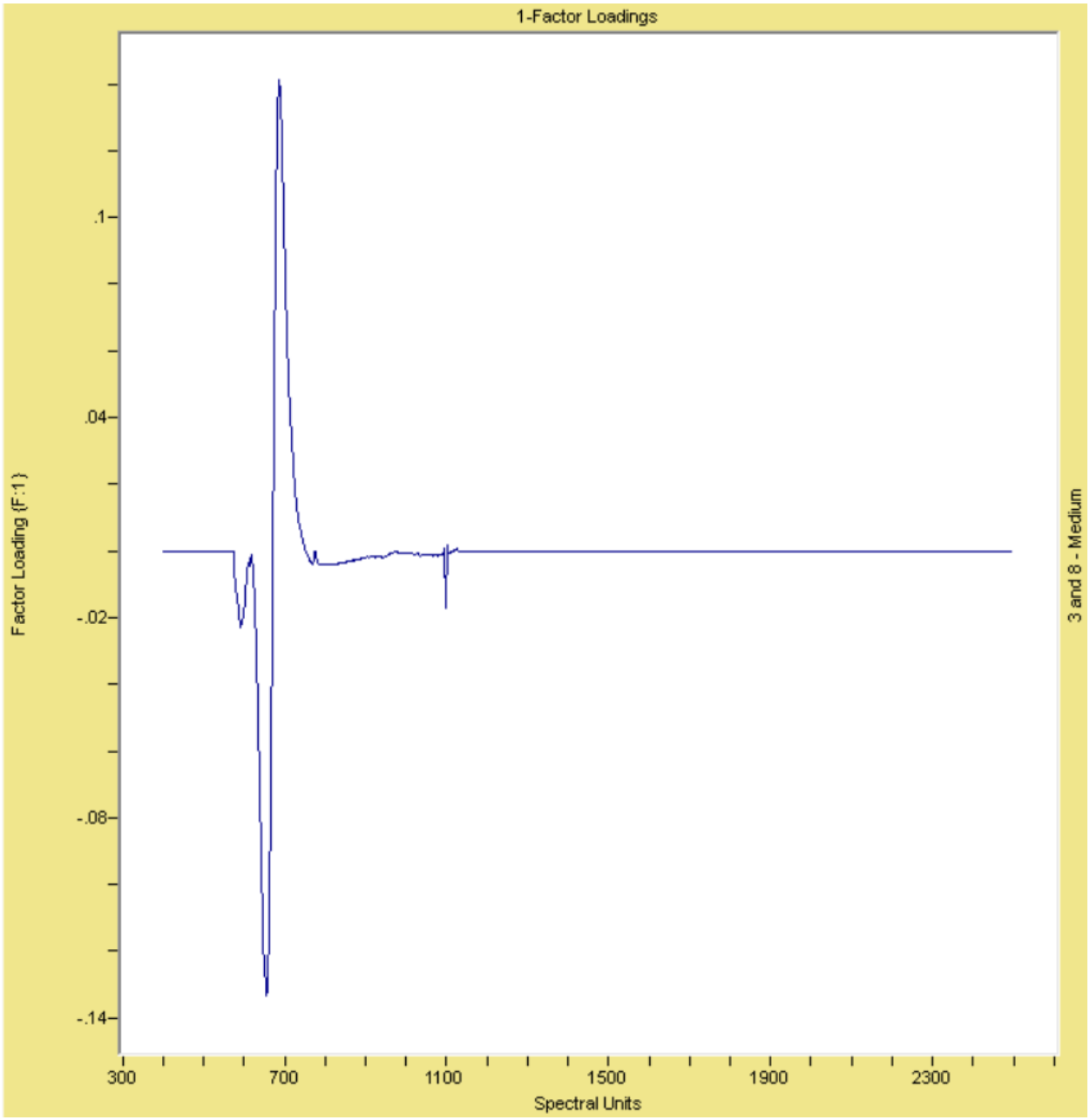

Figure 55. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the first most common spectral variation (factor) from the medium infestation treatment level heifer numbers 3 and 8 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 95.22% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

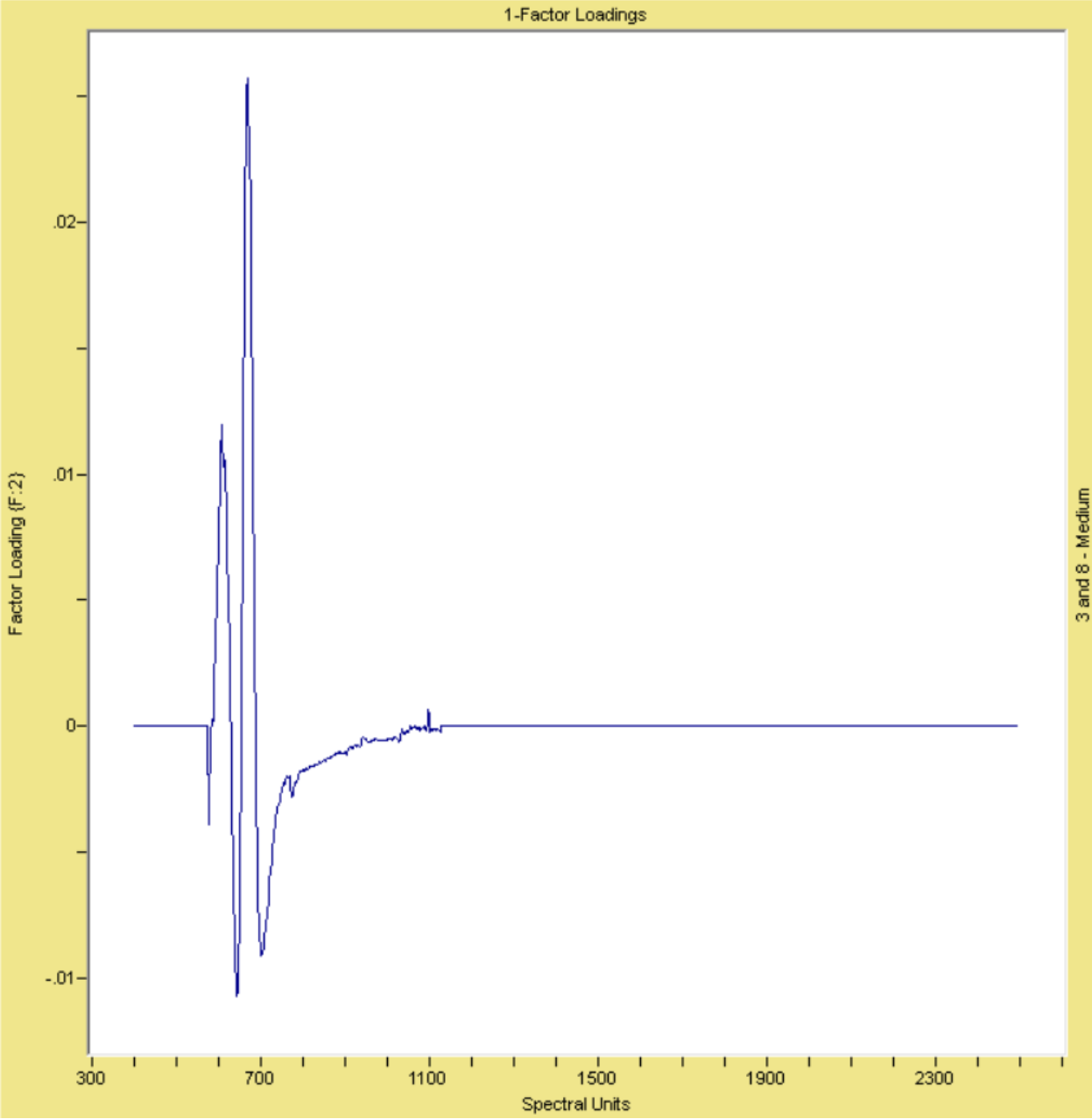

Figure 56. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the second most common spectral variation (factor) from the medium infestation treatment level heifer numbers 3 and 8 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 2.01% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

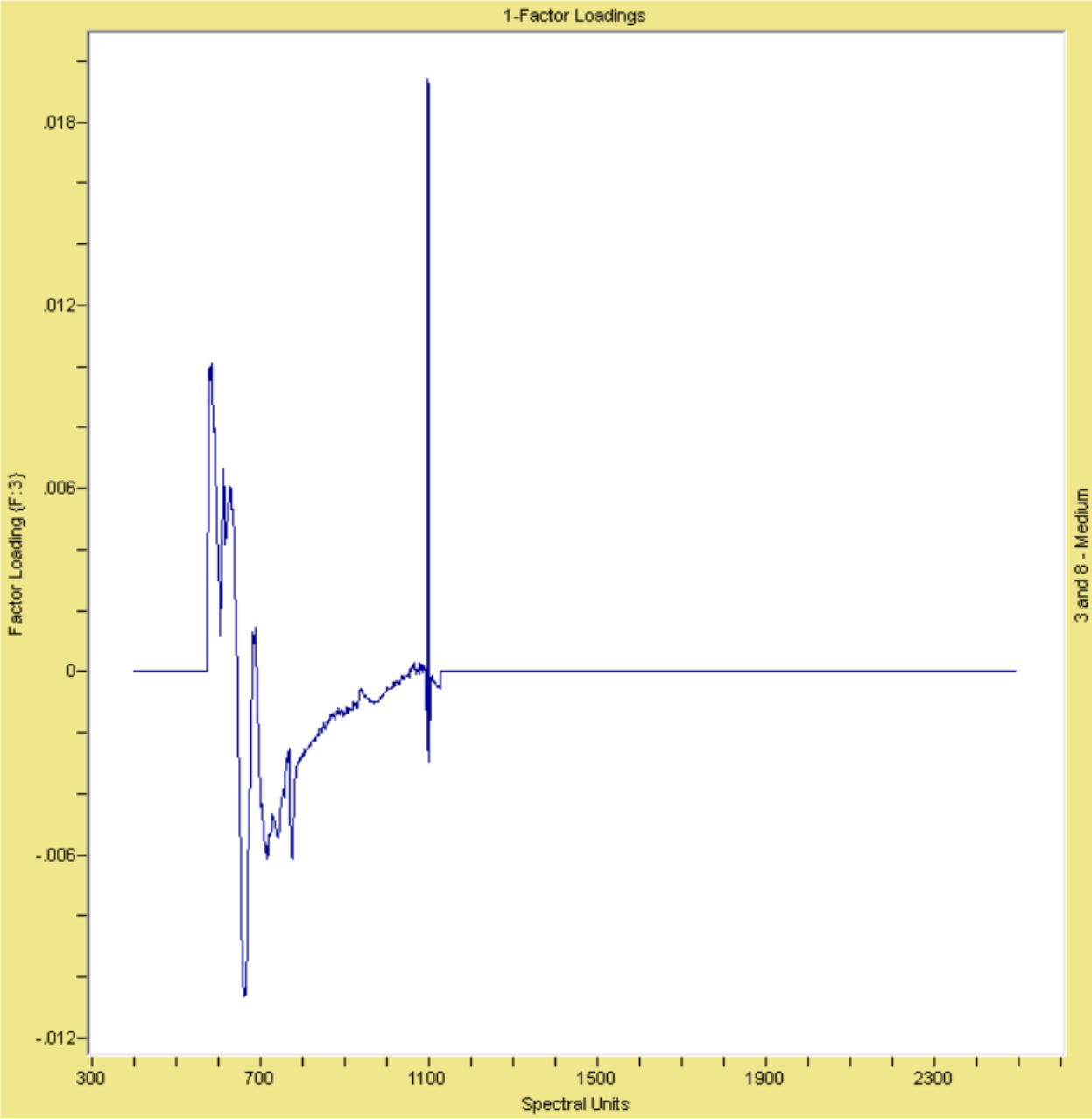

Figure 57. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the third most common spectral variation (factor) from the medium infestation treatment level heifer numbers 3 and 8 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 1.03% of the total variation within this low infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

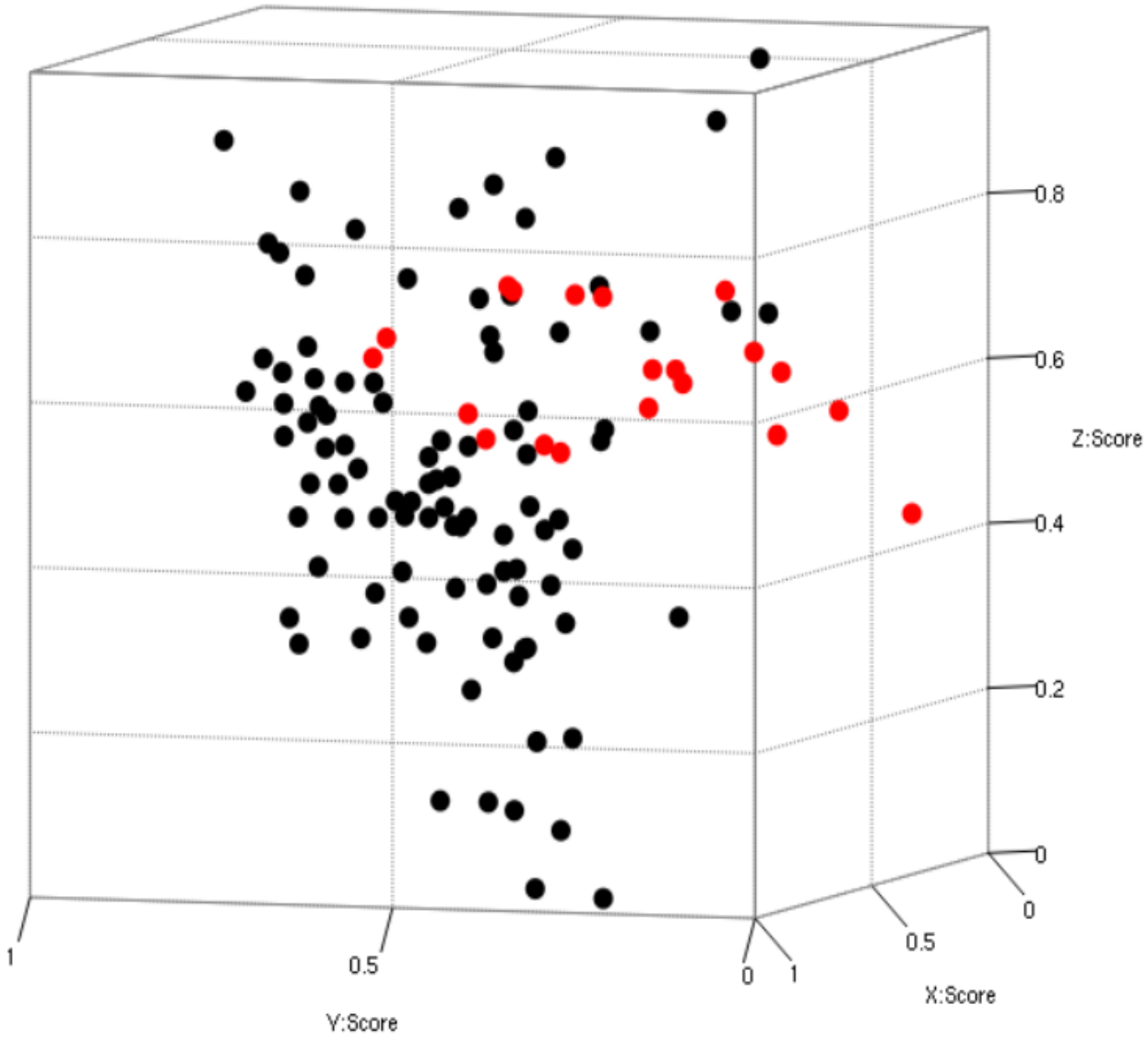

Figure 58. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day -6 during the Pre-infestation Outside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

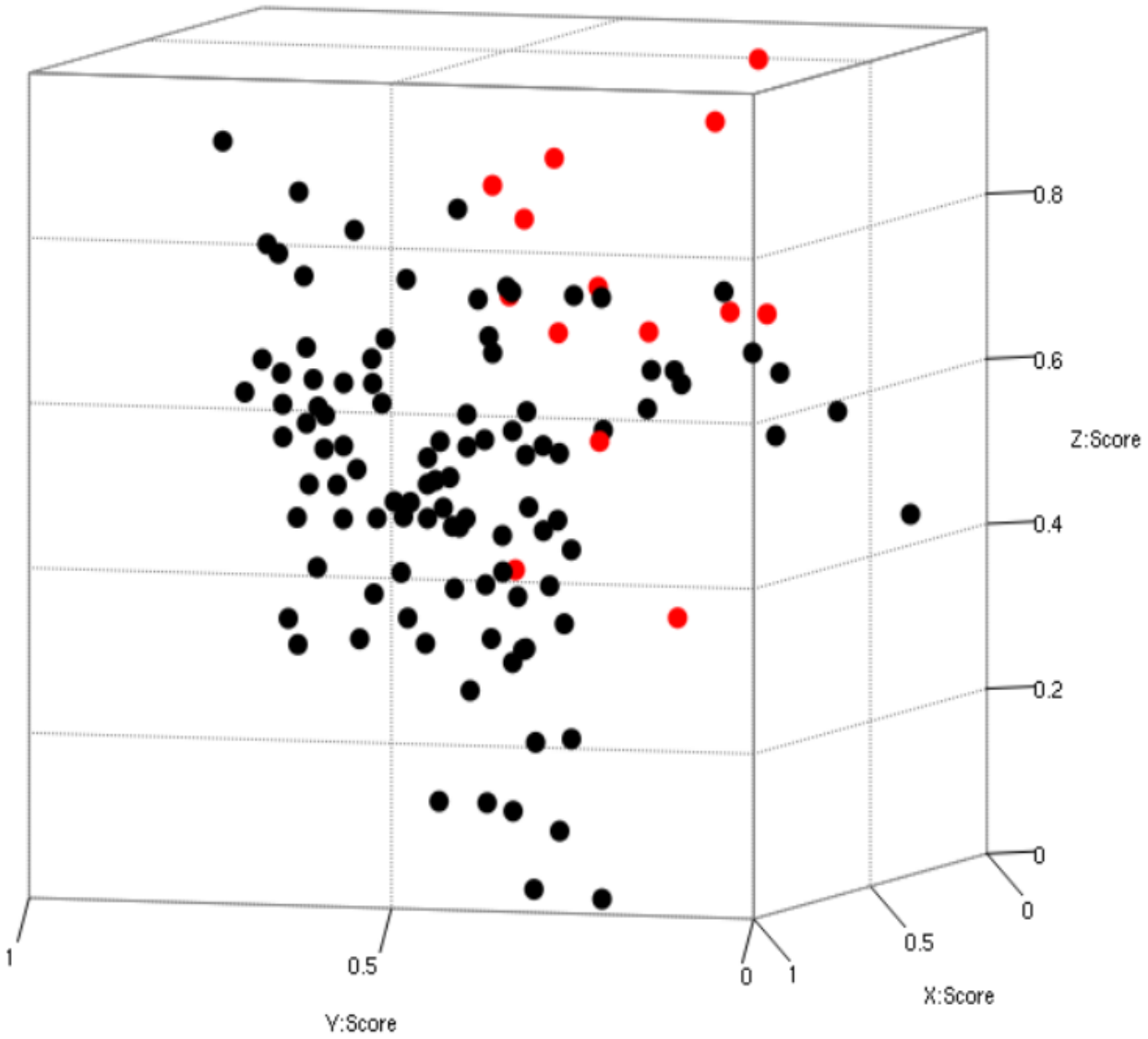

Figure 59. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Inside period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -5 to Day 1 during the Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

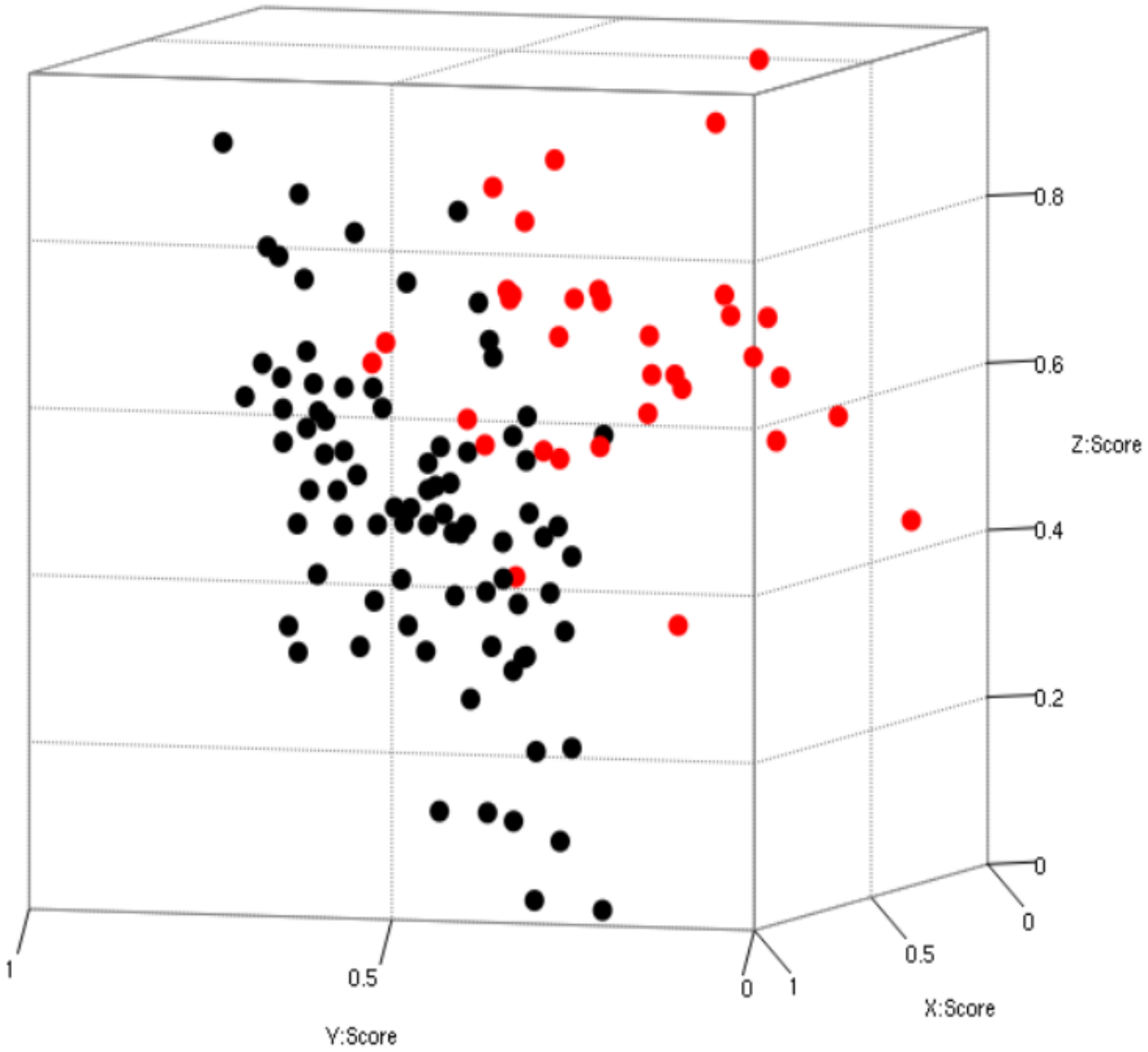

Figure 60. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside and Pre-infestation Inside period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day 1 during the Pre-infestation Outside and Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

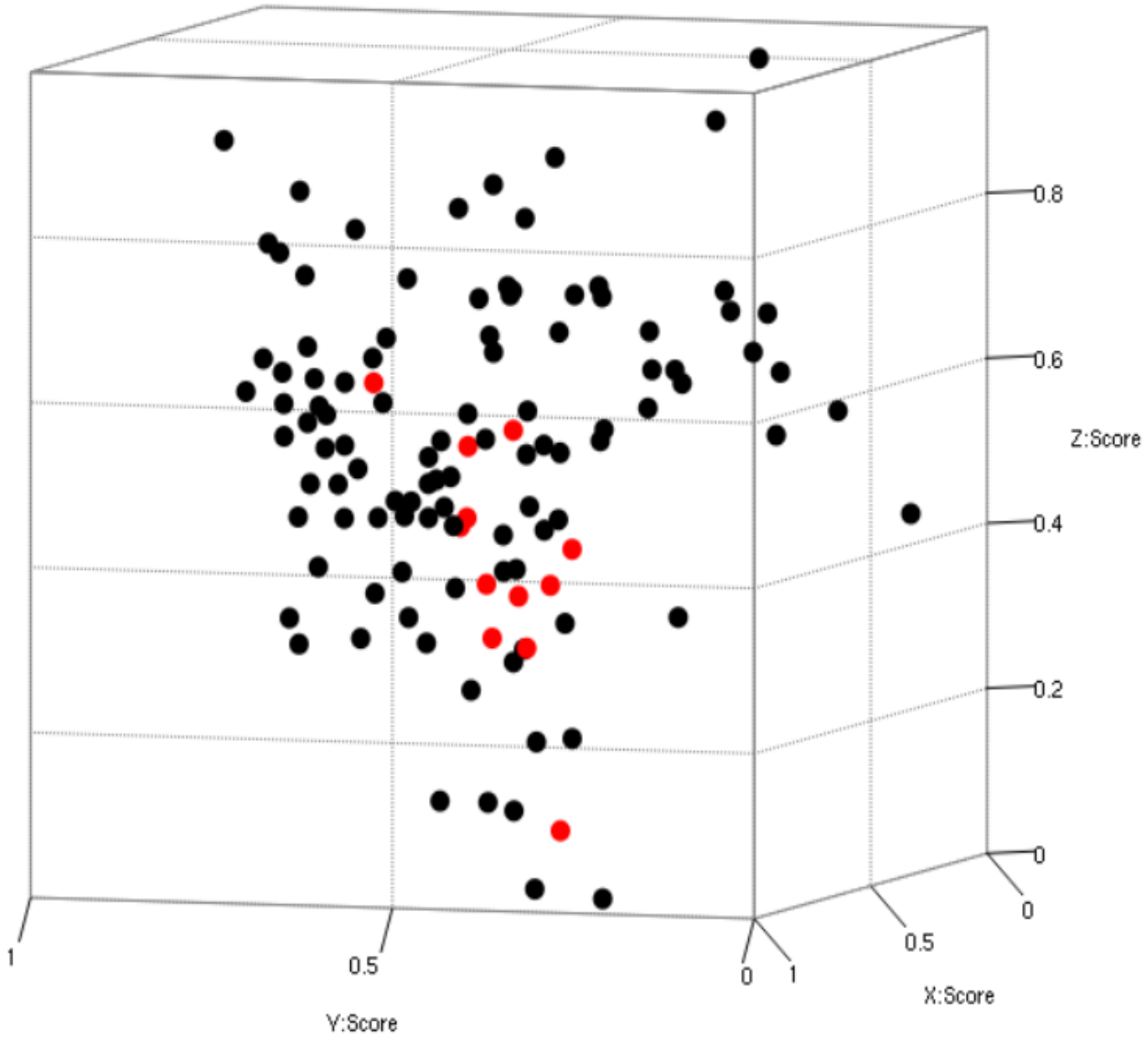

Figure 61. Three-dimensional cluster analysis of all daily fecal sample spectra for the Larval Feeding period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 2 to Day 7 during the Larval Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

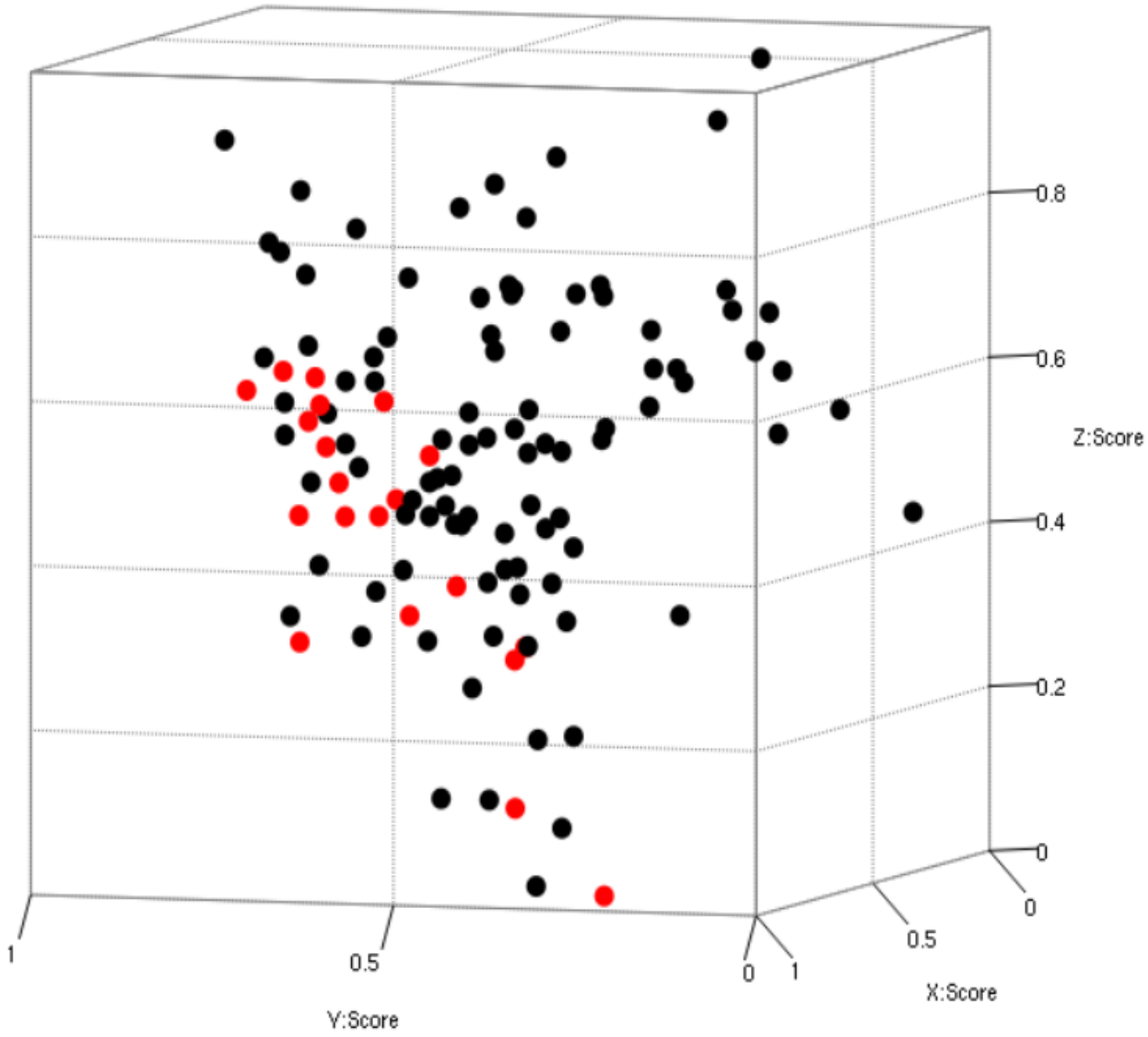

Figure 62. Three-dimensional cluster analysis of all daily fecal sample spectra for the Nymphal Feeding period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 8 to Day 17 during the Nymphal Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

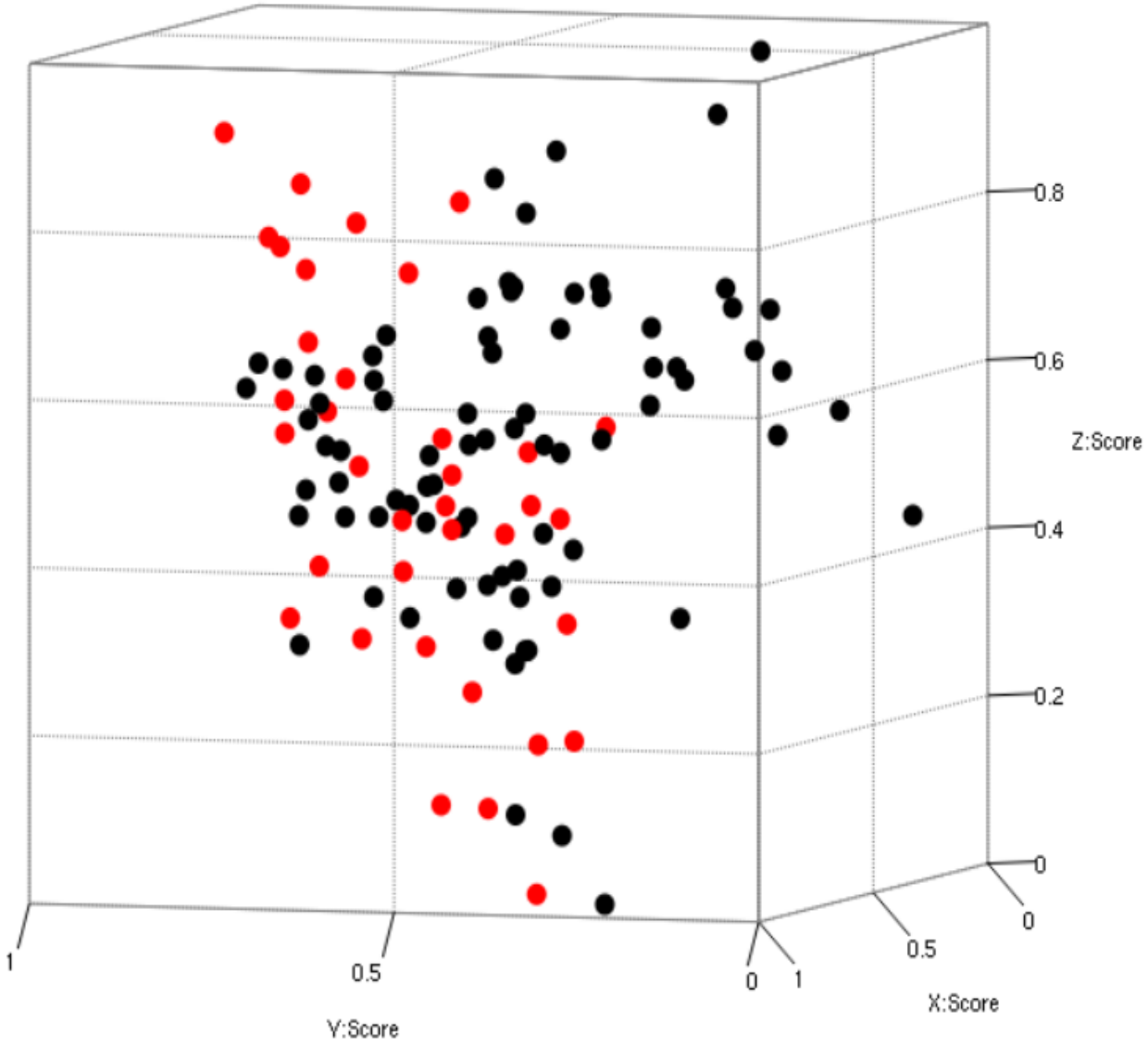

Figure 63. Three-dimensional cluster analysis of all daily fecal sample spectra for the Adult Feeding period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 18 to Day 35 during the Adult Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

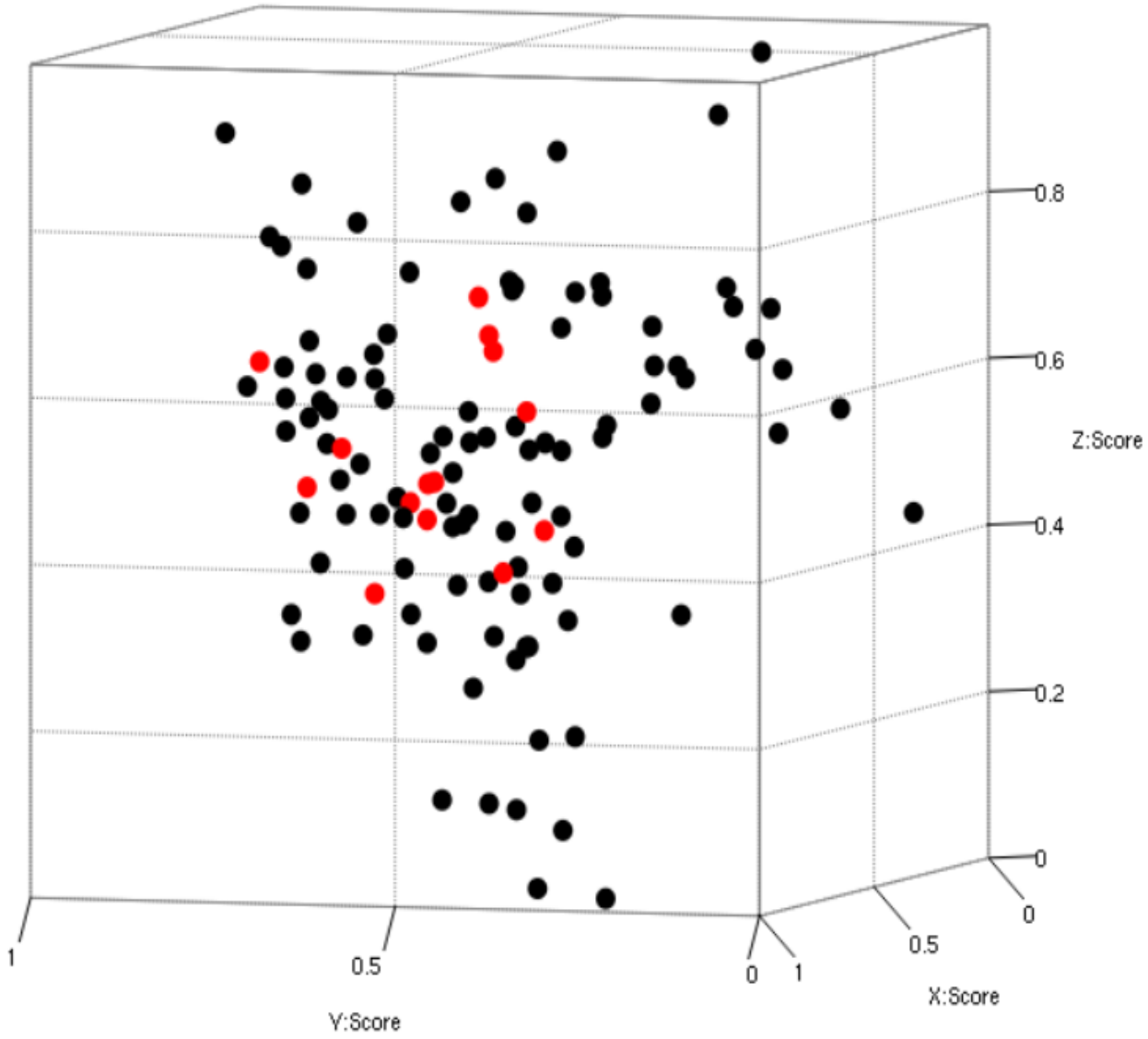

Figure 64. Three-dimensional cluster analysis of all daily fecal sample spectra for the Post-tick Recovery period for heifer numbers 3 and 8 in the medium infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 36 to Day 42 during the Post-tick Recovery period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 98.26% of total spectral variation.

Stepwise Cluster Analyses for the High Treatment Level Group

A discriminant analysis was conducted for raw spectral data from daily fecal samples for heifer numbers 2 and 5 in the high treatment level group from the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period (one fecal spectra per day per heifer). The analysis of spectra in the 576 nm – 1126 nm range produced 14 factors with the first three dominant spectral factors/variations representing 97.81% of the total variation among sample spectra for the medium infestation treatment level (see Figures 65 – 67). This result permitted the sample spectra to be analyzed by cluster analyses with each of the three dominant factors/variations plotted as "x", "y" and "z".

The cluster analyses of the spectra from the pair of heifers in the high treatment level group resulted in a pattern of six clusters that depict shifts in fecal chemistry. Sample clusters were distinguishable for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period in the cluster analyses (see Figures 68 through 74). The first was comprised of samples originating from Day -15 (15 d prior to infestation) through Day -6 (6 d prior to infestation; allowing for a 48-h rumen passage time) (Figure 68). The second cluster was comprised of samples within the period from Day -5 (5 d prior to infestation) through Day 1 (1 day post infestation; allowing for a 48-h rumen passage time) (Figure 69). Figure 70 is the daily fecal spectra for the two pre-infestation acclimation periods in one cluster analysis, showing the entire experimental control period for the high treatment group. The third cluster was comprised of samples from Day 2 (2 d post infestation) through Day 7 (7 d post infestation), which is the period of attachment, feeding and molting of larvae (Figure 71). The fourth cluster originated

from samples from Day 8 (8 d post infestation) to Day 17 (17 d post infestation), corresponding to attachment, feeding, and molting of nymphs (Figure 72). The fifth cluster includes samples from Day 18 (18 d post infestation) to Day 41 (allowing for a 48-h rumen passage time), corresponding to the period of adult feeding and the period in which females complete their feeding and drop from the host (Figure 73). The sixth cluster was comprised of samples from Day 42 to Day 47, which consists of the period heifers were going through post-tick recovery (Figure 74). Spectral cluster shifts occurred representing time periods that are consistent with no tick feeding, low tick feeding, heavy feeding, and a period of post-tick recovery.

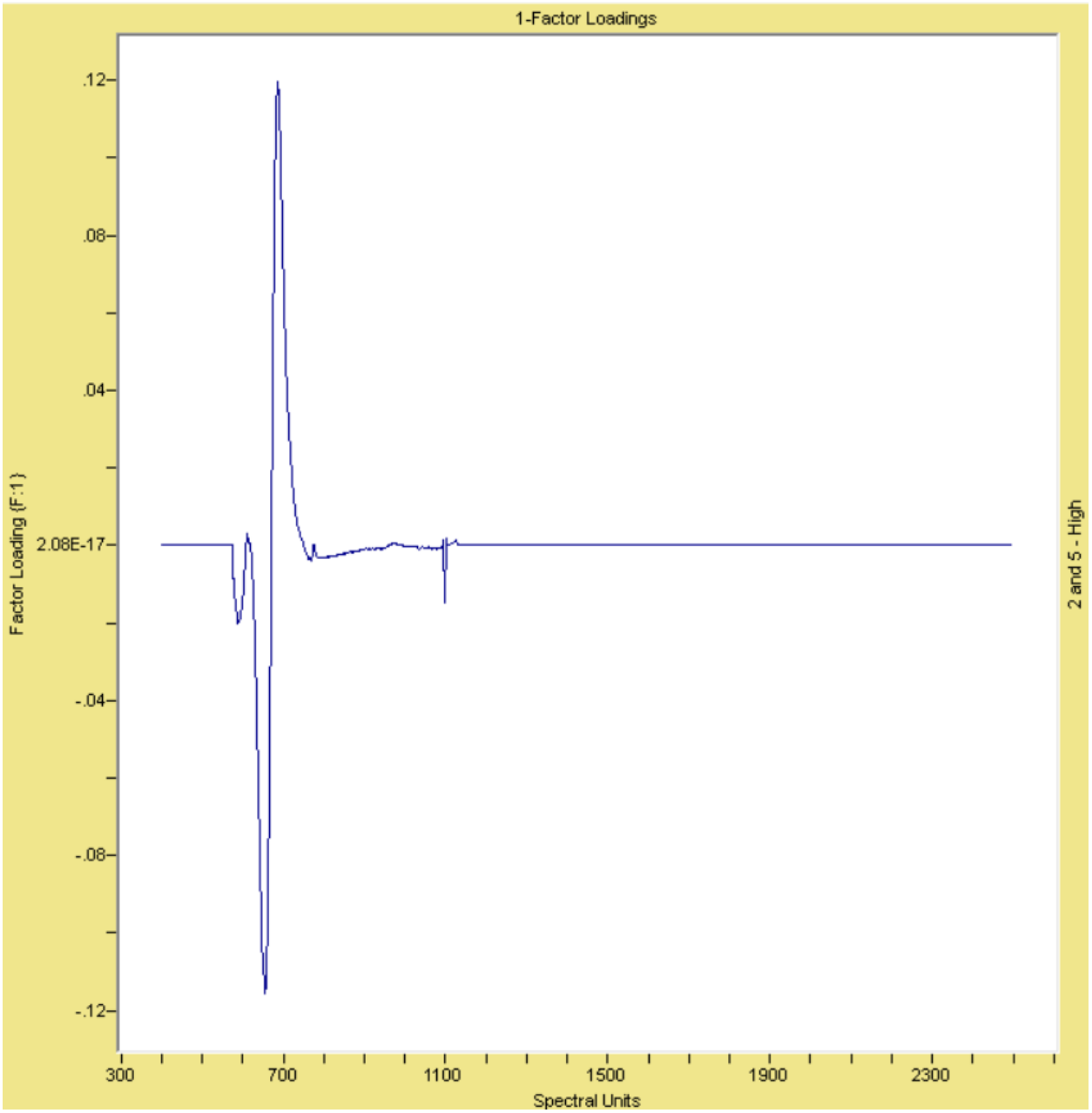

Figure 65. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the first most common spectral variation (factor) from the high infestation treatment level heifer numbers 2 and 5 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 95.08% of the total variation within this high infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

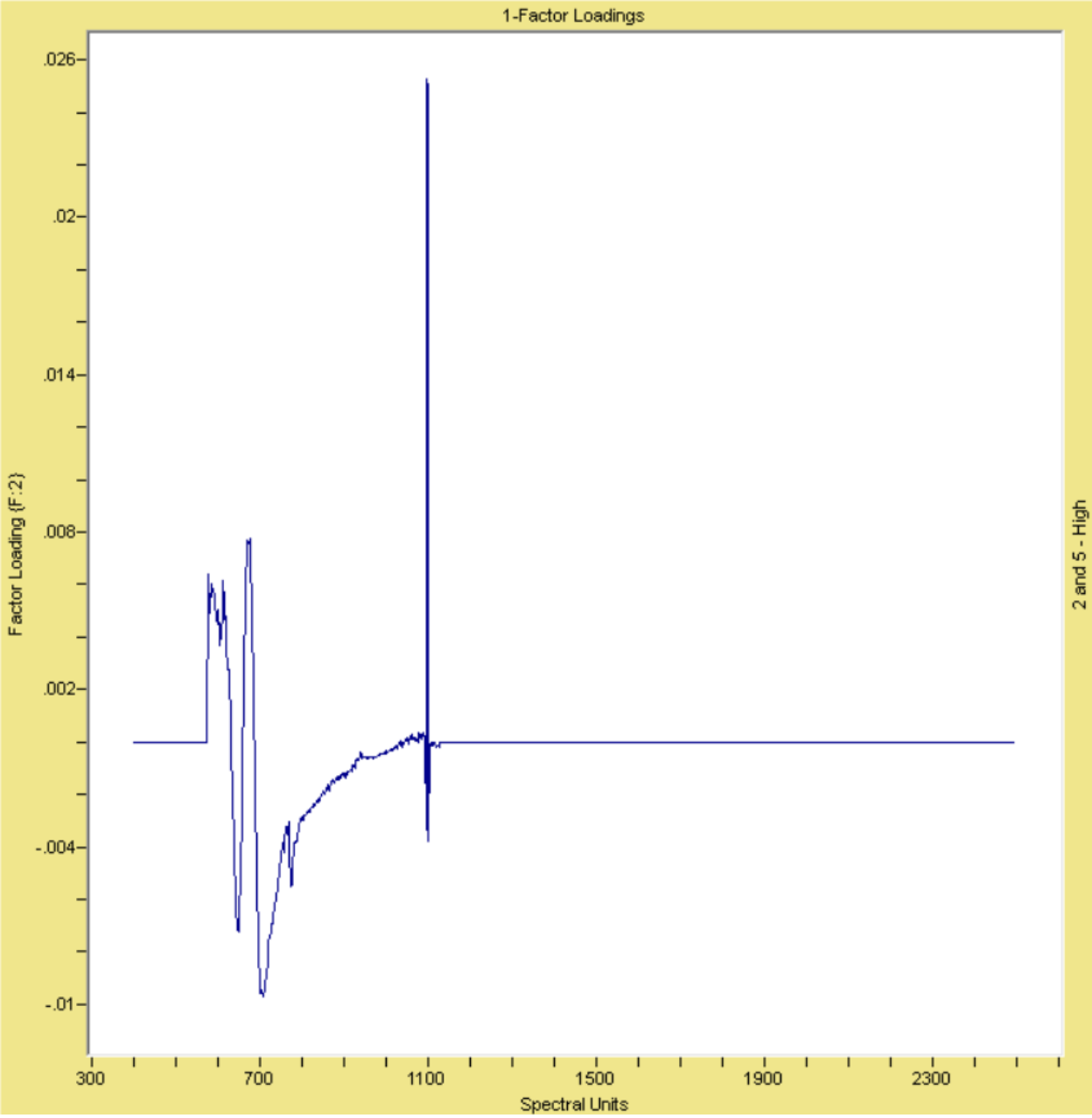

Figure 66. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the second most common spectral variation (factor) from the high infestation treatment level heifer numbers 2 and 5 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 1.68% of the total variation within this high infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

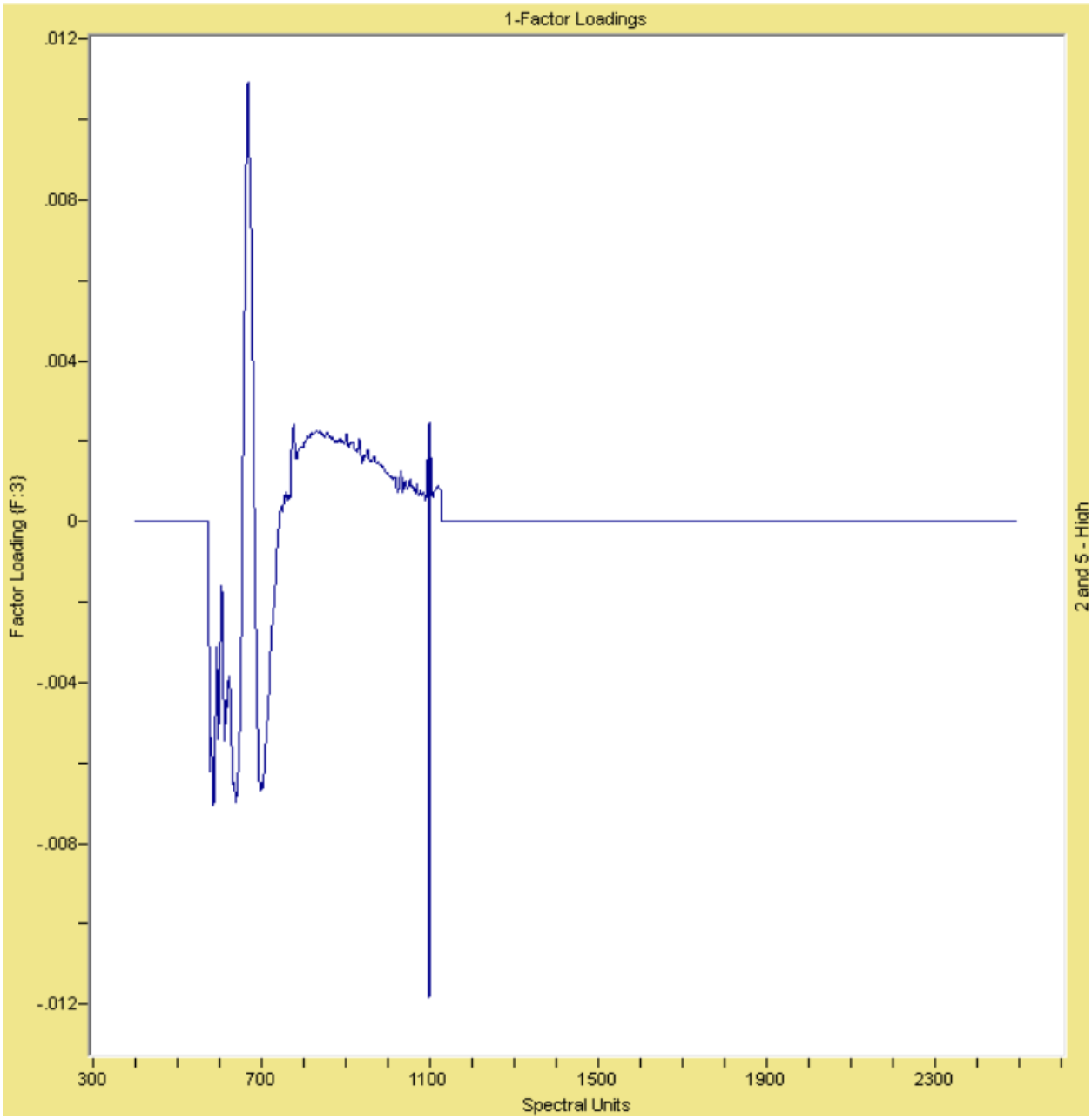

Figure 67. Near infrared reflectance spectroscopy fecal chemistry profile illustrated by spectrograph of the third most common spectral variation (factor) from the high infestation treatment level heifer numbers 2 and 5 using a narrowed spectrum (576nm-1126nm) to lessen the effect of noise and improve the contribution of each of the 25 factors. This spectral variation is representative of 1.05% of the total variation within this high infestation level treatment group. Each peak and valley represent a point of absorption or reflection with the factor loading as the "y" axis and the spectral units (in nanometers) as the "x" axis.

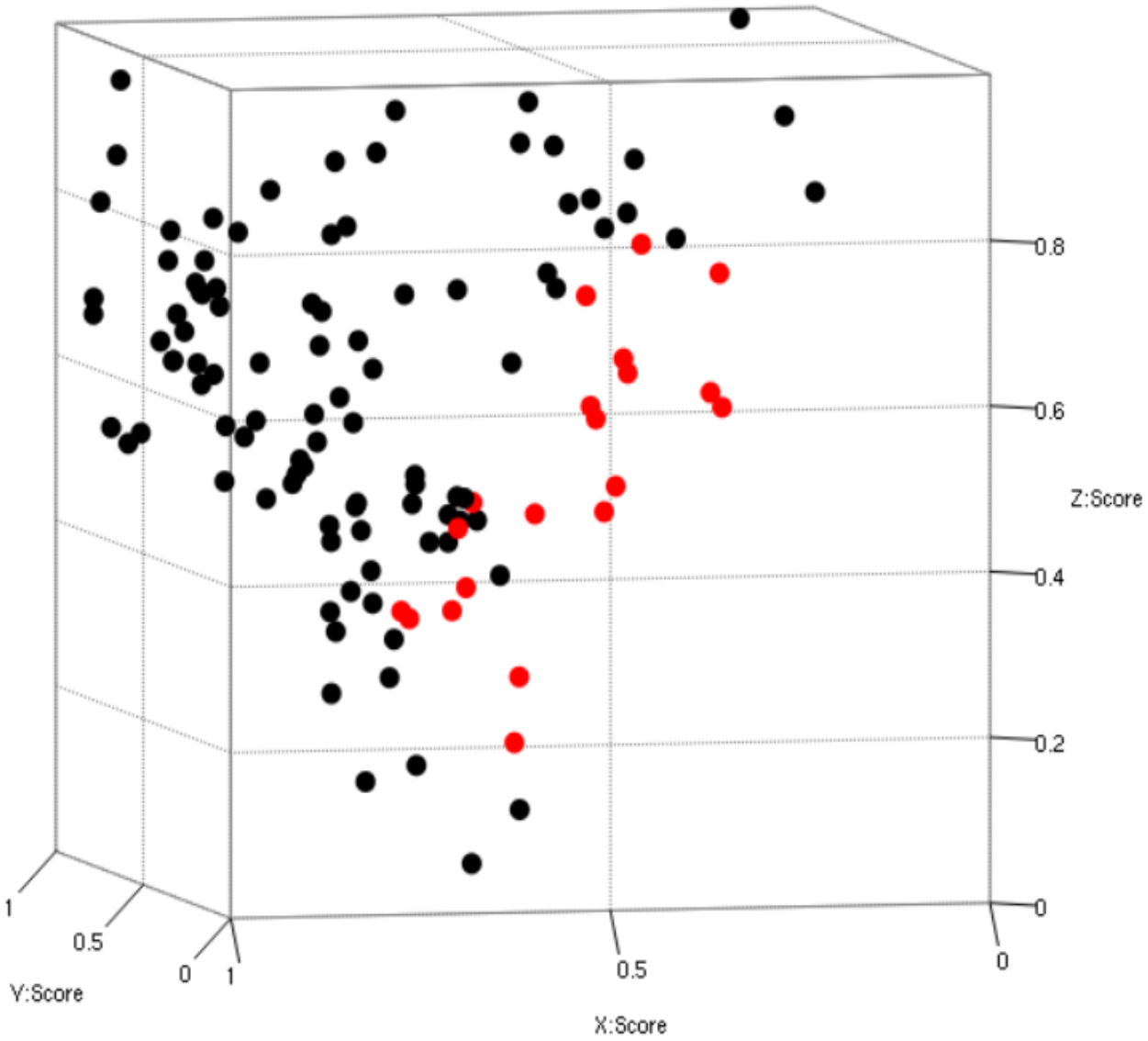

Figure 68. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day -6 during the Pre-infestation Outside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

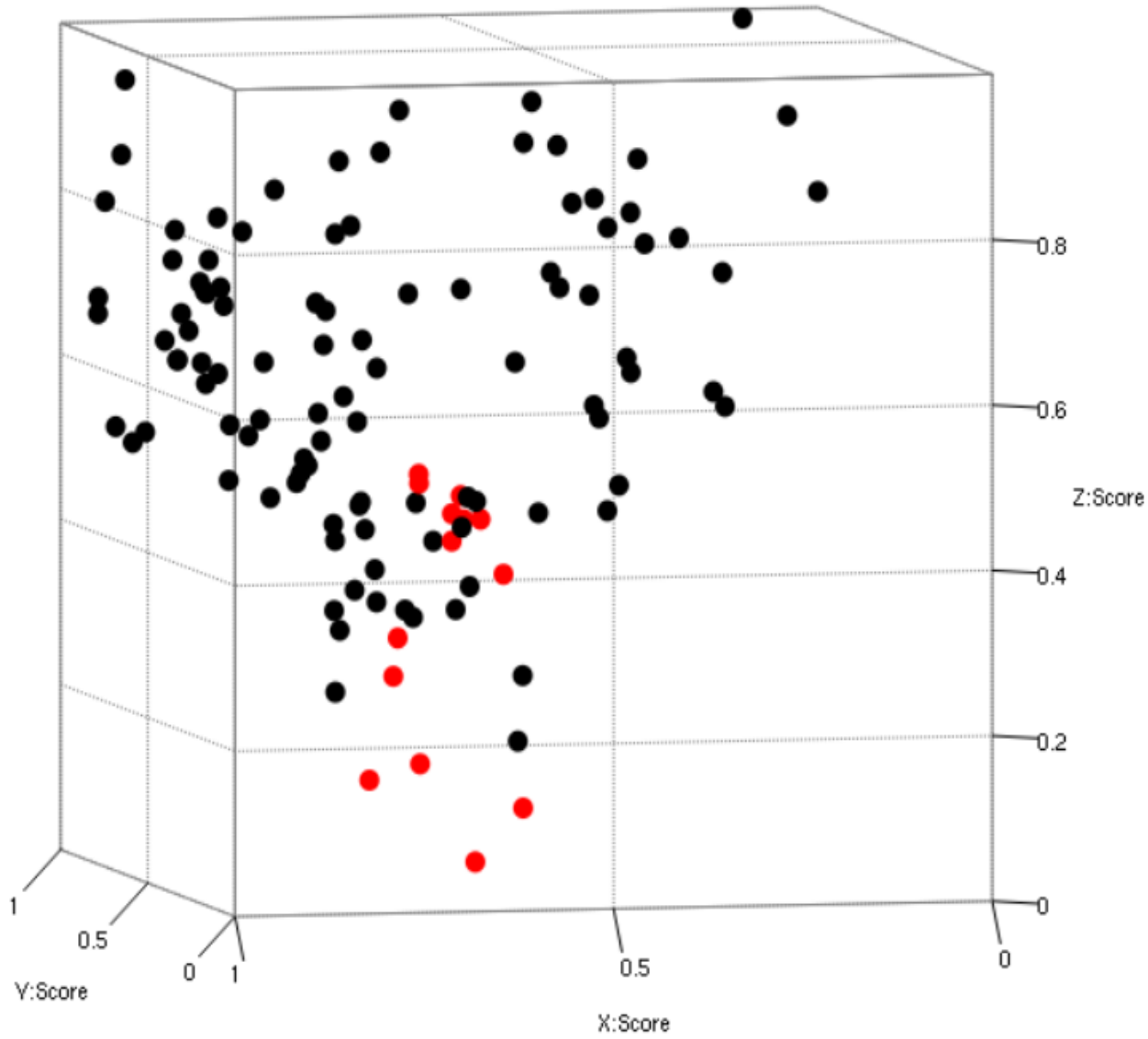

Figure 69. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Inside period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -5 to Day 1 during the Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

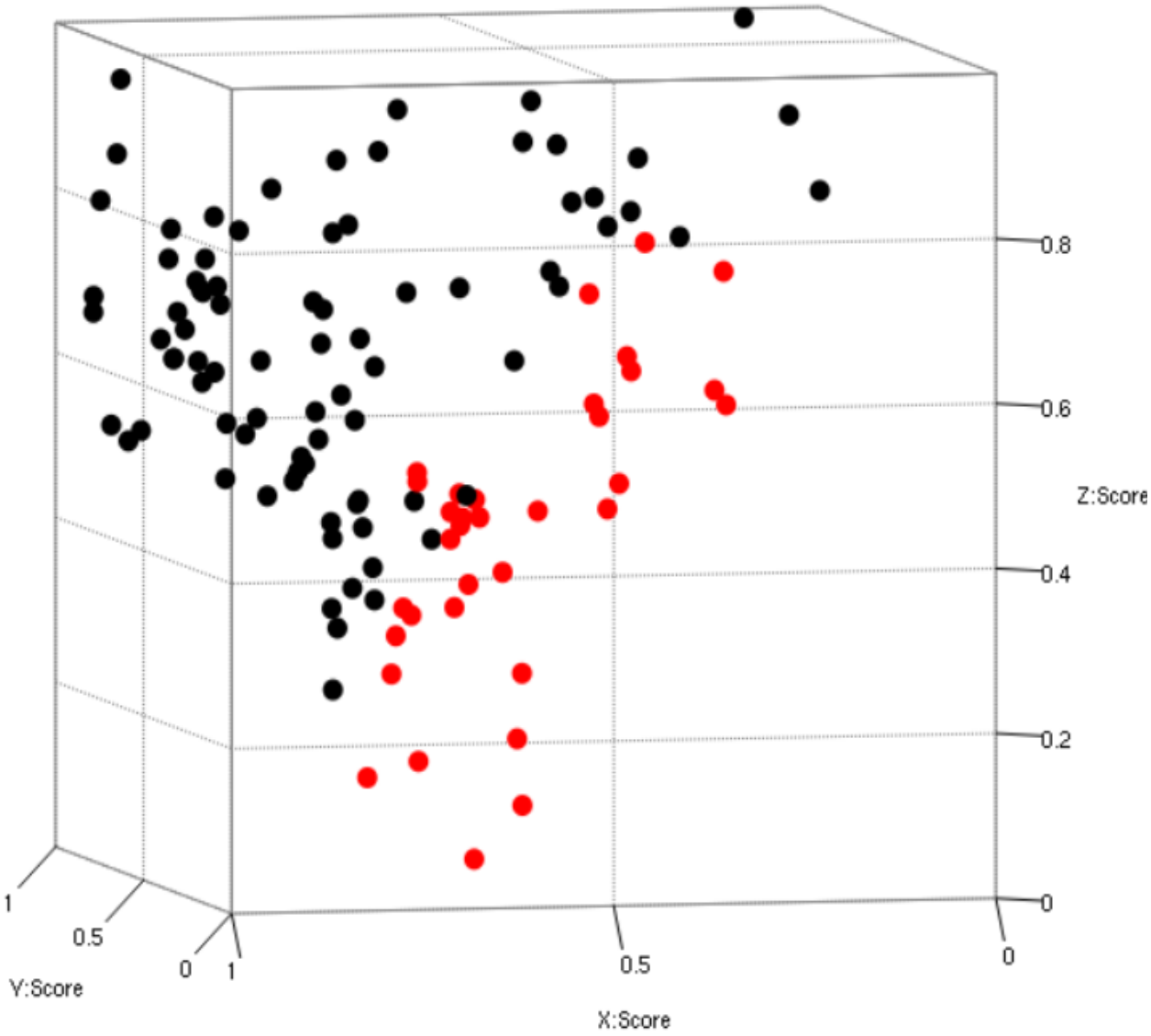

Figure 70. Three-dimensional cluster analysis of all daily fecal sample spectra for the Pre-infestation Outside and Pre-infestation Inside period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day -15 to Day 1 during the Pre-infestation Outside and Pre-infestation Inside period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

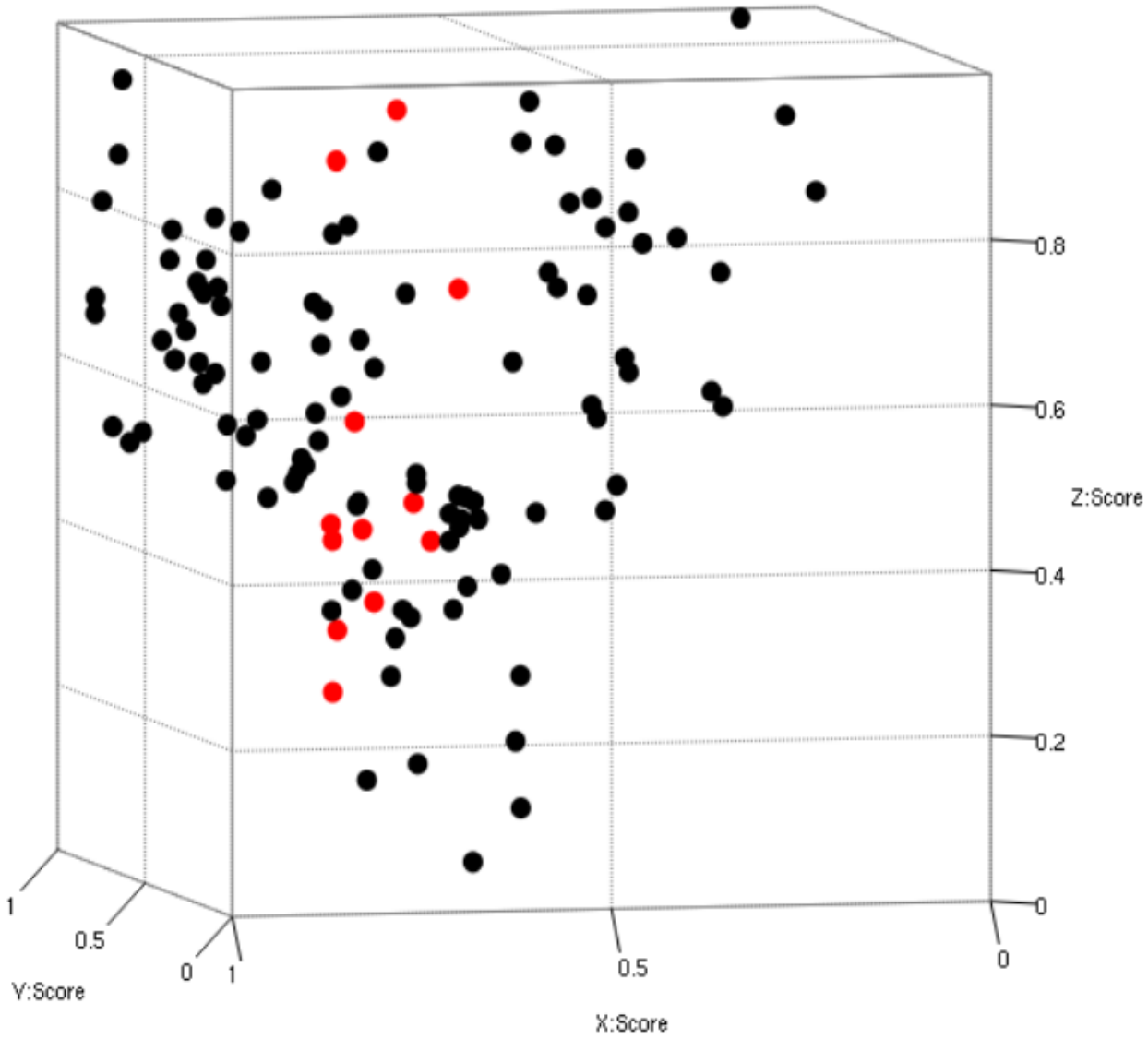

Figure 71. Three-dimensional cluster analysis of all daily fecal sample spectra for the Larval Feeding period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 2 to Day 7 during the Larval Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

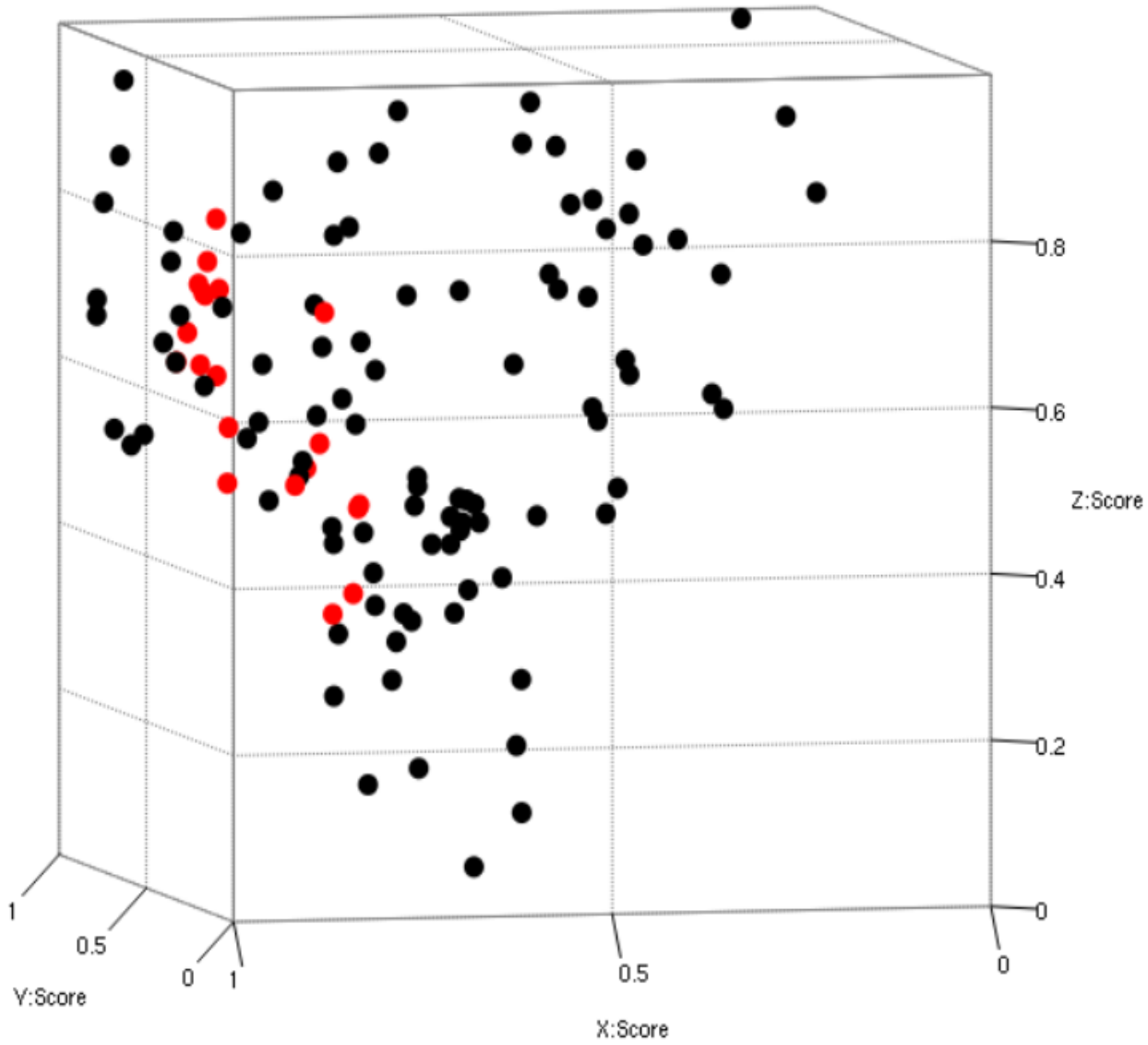

Figure 72. Three-dimensional cluster analysis of all daily fecal sample spectra for the Nymphal Feeding period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 8 to Day 17 during the Nymphal Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

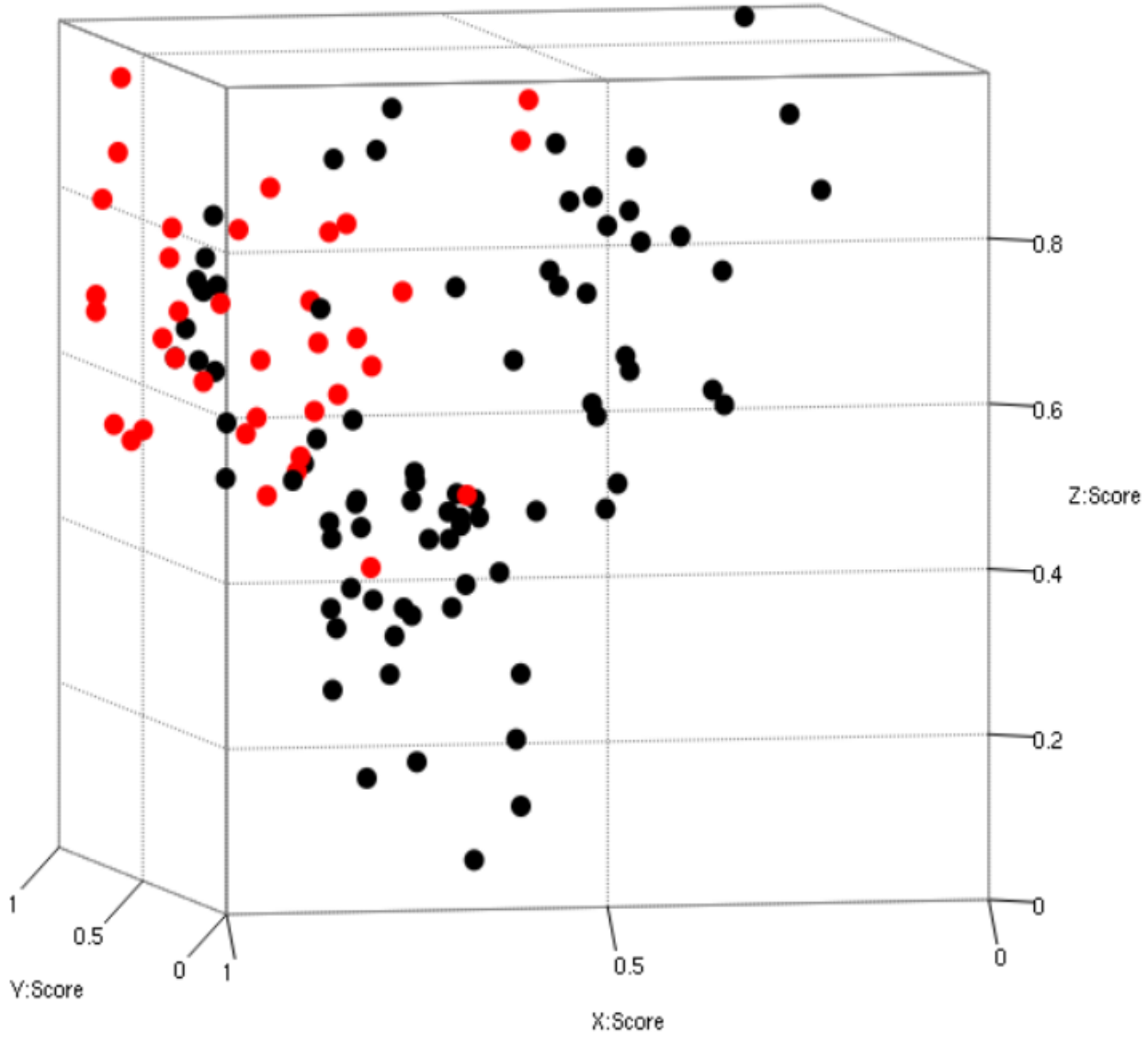

Figure 73. Three-dimensional cluster analysis of all daily fecal sample spectra for the Adult Feeding period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 18 to Day 35 during the Adult Feeding period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

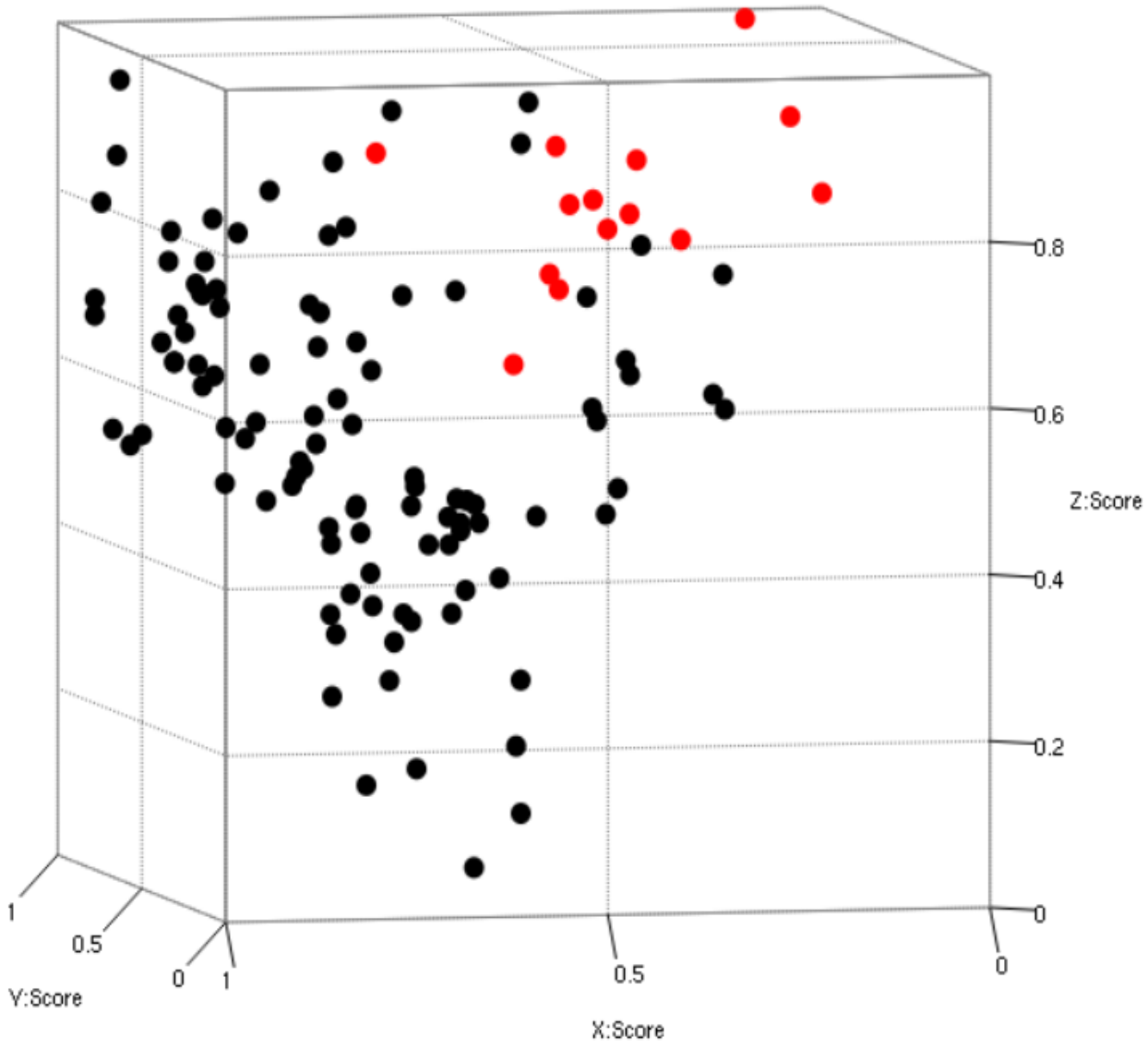

Figure 74. Three-dimensional cluster analysis of all daily fecal sample spectra for the Post-tick Recovery period for heifer numbers 2 and 5 in the high infestation level treatment group from Trial Two shown in both black and red. Fecal sample spectra indicated in red represent those collected from Day 36 to Day 42 during the Post-tick Recovery period. Figure axes correspond to the first three most common spectral variations (factors) and collectively represent 97.81% of total spectral variation.

Principal Components Analysis

To determine if there was a significant difference between the cluster shifts in the stepwise cluster analyses from Trial Two, a PCA was performed on the spectra data from 348 observations from the six heifers over the six Stages in Trial Two. The PCA resulted in 97.77% of the total variation in the 1050 frequencies being explained by the first three principal components (P_1, P_2, P_3). Results from the MANOVA and the Wilk's Lambda test showed highly significant evidence ($P < 0.0001$) of a difference in the means of the three principal components across the six Stages. There was significant evidence ($P < 0.0001$) of a difference between the means of the three principal components across the three levels of tick infestation. Results also revealed there was no significant evidence ($P = 0.0659$) of an interaction between *Stage* and *Treatment* factors. In the MANOVA analysis, the interaction between *Stage* and *Treatment* just missed being significant ($P = 0.0659$). In the individual repeated measures analysis of the three principal components (P_1, P_2, P_3), the results were mixed. For $P1$, *Stage*Treatment* was not significant, p-value = 0.1381. For $P2$, *Stage*Treatment* was significant, p-value = 0.0325. For $P3$, *Stage*Treatment* was not significant, p-value = 0.1756. Thus, for consistency, the pairwise analysis was considered of the *Treatment* levels separately for each *Stage*.

Principal Component 1: P_1. $P_1 = Heifer(Treatment) + Treatment + Stage + Treatment * Stage + Residuals$. Based on the *p*-values below in Table 10, there was not significant evidence of a difference between the three tick infestation levels with respect to the means of the first principal component for each of the six Stages in this trial. Least square means for *Stage*, *Treatment*, and *Stage*Treatment* for P_1 are shown in Figures 75 – 77.

Table 10. Comparison of Three Tick Infestation Levels across the Six Stages for $\boldsymbol{P_1}$ in Trial Two. Values with a *p*-value less than 0.0028 were declared to be significantly different.

	Stage					
	1 PIO	**2 PII**	**3 LF**	**4 NF**	**5 AF**	**6 PTR**
P*-values for Comparing *Low vs Medium	0.3019	0.7763	0.0429	0.8334	0.7913	0.5578
P*-values for Comparing *Medium vs High	0.2610	0.7602	0.3311	0.4693	0.7208	0.9461
P*-values for Comparing *Low vs High	0.0315	0.9936	0.2903	0.3505	0.9265	0.5156

*Denotes values that are significantly different with a *p*-value less than 0.0028. PIO= Pre-infestation Outside, PII = Pre-infestation Inside, LF = Larval Feeding, NF = Nymphal Feeding, AF = Adult Feeding, PTR = Post-tick Recovery.

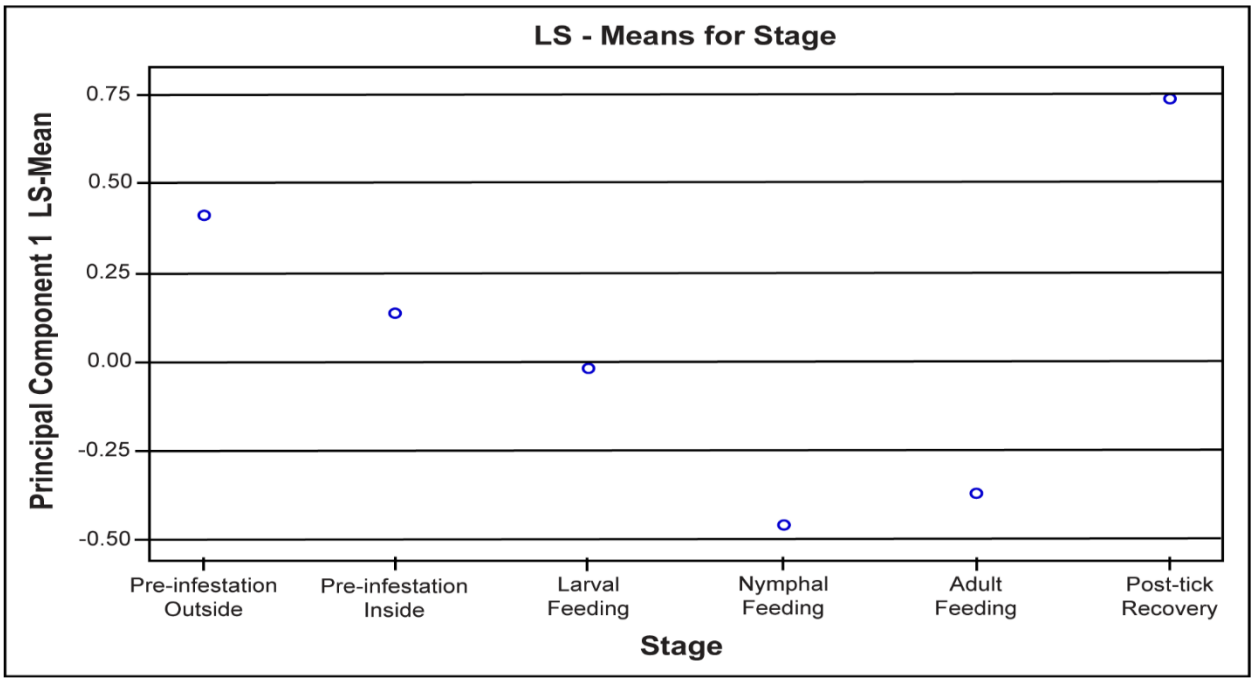

Figure 75. Least Squares Means for *Stage* of the first principal component in Trial Two. The figure shows *Stage* (x-axis) by Principal Components 1 LS-Mean (y-axis).

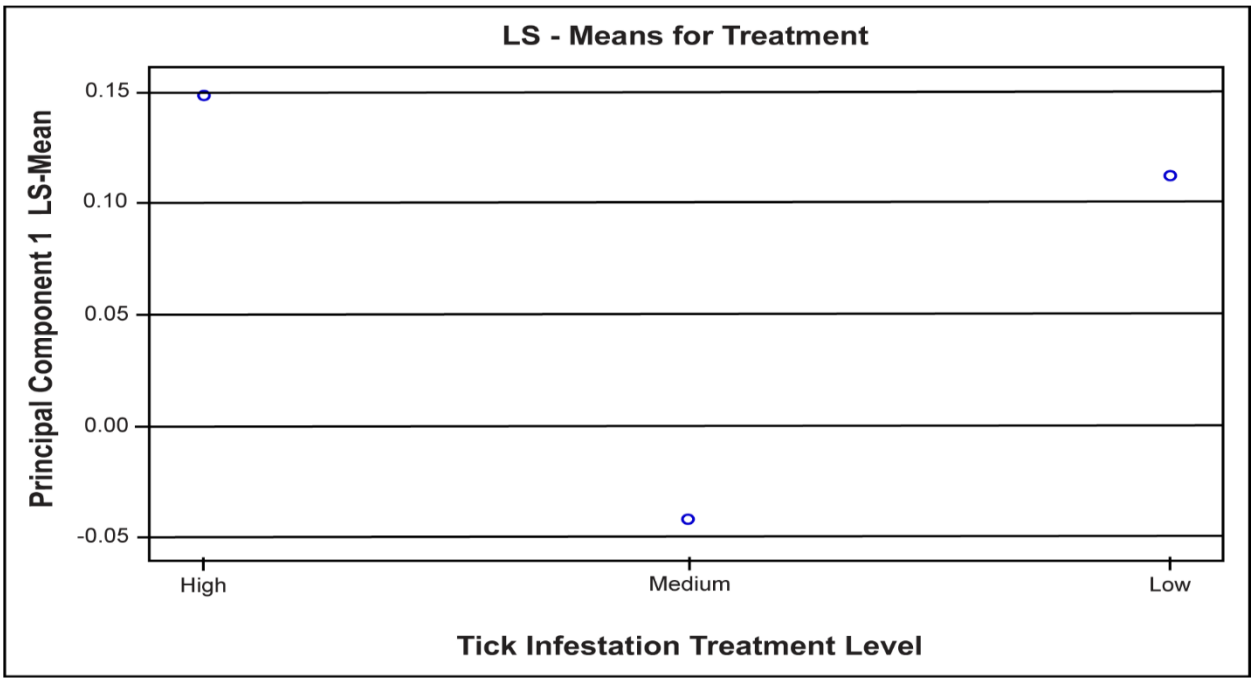

Figure 76. Least Squares Means for *Treatment* of the first principal component in Trial Two. The figure shows Tick Infestation *Treatment* Level (x-axis) by Principal Components 1 LS-Mean (y-axis).

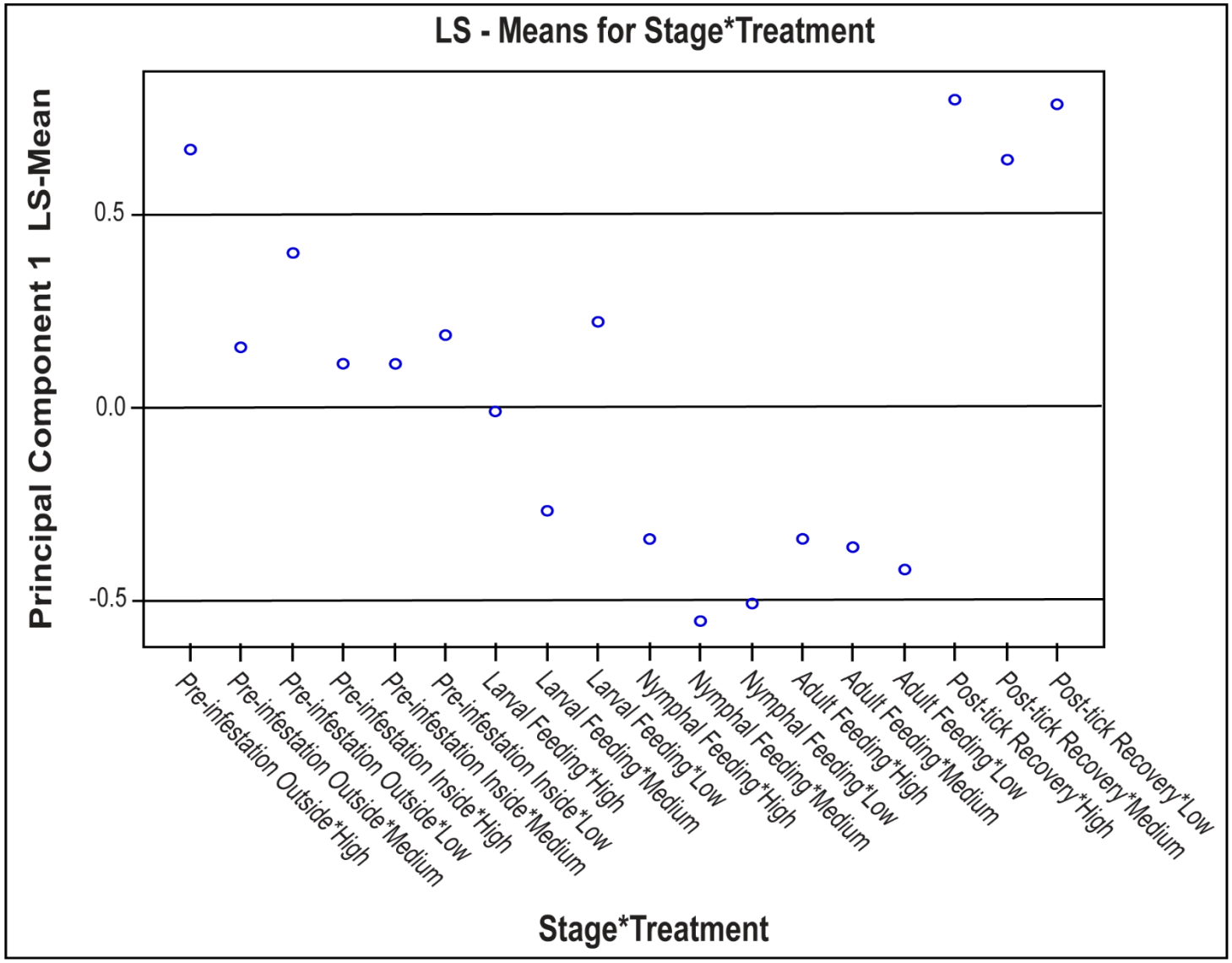

Figure 77. Least Squares Means for *Stage*Treatment* of the first principal component in Trial Two. The figure shows *Stage*Treatment* (x-axis) by Principal Components 1 LS-Mean (y-axis).

Principal Component 2: P_2. $P_2 = Heifer(Treatment) + Treatment + Stage + Treatment * Stage + Residuals$. Based on the *p*-values below in Table 11, there is significant evidence of a difference between the following pairs of Treatments: Low *versus* Medium loadings during Pre-infestation Outside, Low *versus* High loadings during Pre-infestation Inside, Medium *versus* High loadings during Pre-infestation Inside. There was not significant evidence of differences for all the other combinations of the three infestation rates with respect to the means of the second principal component for each of the six Stages in this trial. Least square means for *Stage*, *Treatment*, and *Stage*Treatment* for P_2 are shown in Figures 78 – 80.

Table 11. Comparison of Three Tick Infestation Levels across the Six Stages for $\boldsymbol{P_2}$ in Trial Two. Values with a *p*-value less than 0.0028 were declared to be significantly different.

	Stage					
	1 PIO	**2 PII**	**3 LF**	**4 NF**	**5 AF**	**6 PTR**
P*-values for Comparing *Low vs Medium	0.0002*	0.0297	0.0328	0.7517	0.9618	0.1563
P*-values for Comparing *Medium vs High	0.2431	0.0001*	0.0172	0.0754	0.4926	0.2728
P*-values for Comparing *Low vs High	0.0112	0.0001*	0.8019	0.1433	0.4627	0.7608

*Denotes values that are significantly different with a *p*-value less than 0.0028. PIO = Pre-infestation Outside, PII = Pre-infestation Inside, LF = Larval Feeding, NF = Nymphal Feeding, AF = Adult Feeding, PTR = Post-tick Recovery.

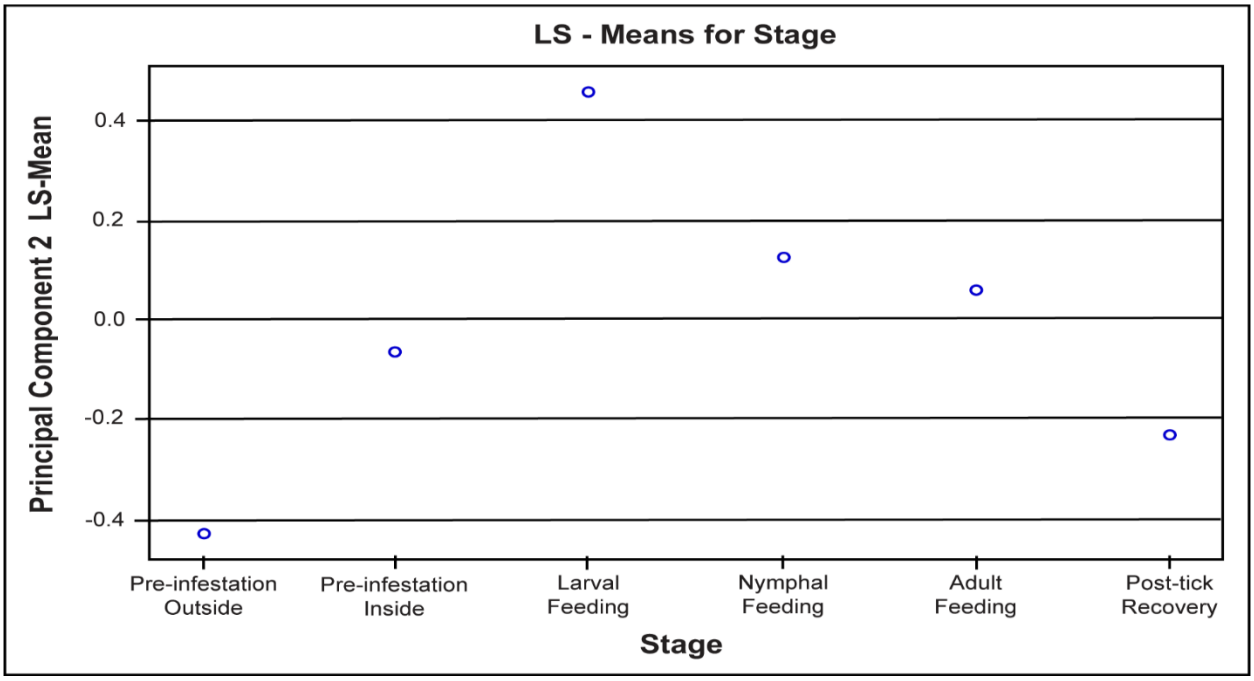

Figure 78. Least Squares Means for *Stage* of the second principal component in Trial Two. The figure shows *Stage* (x-axis) by Principal Components 2 LS-Mean (y-axis).

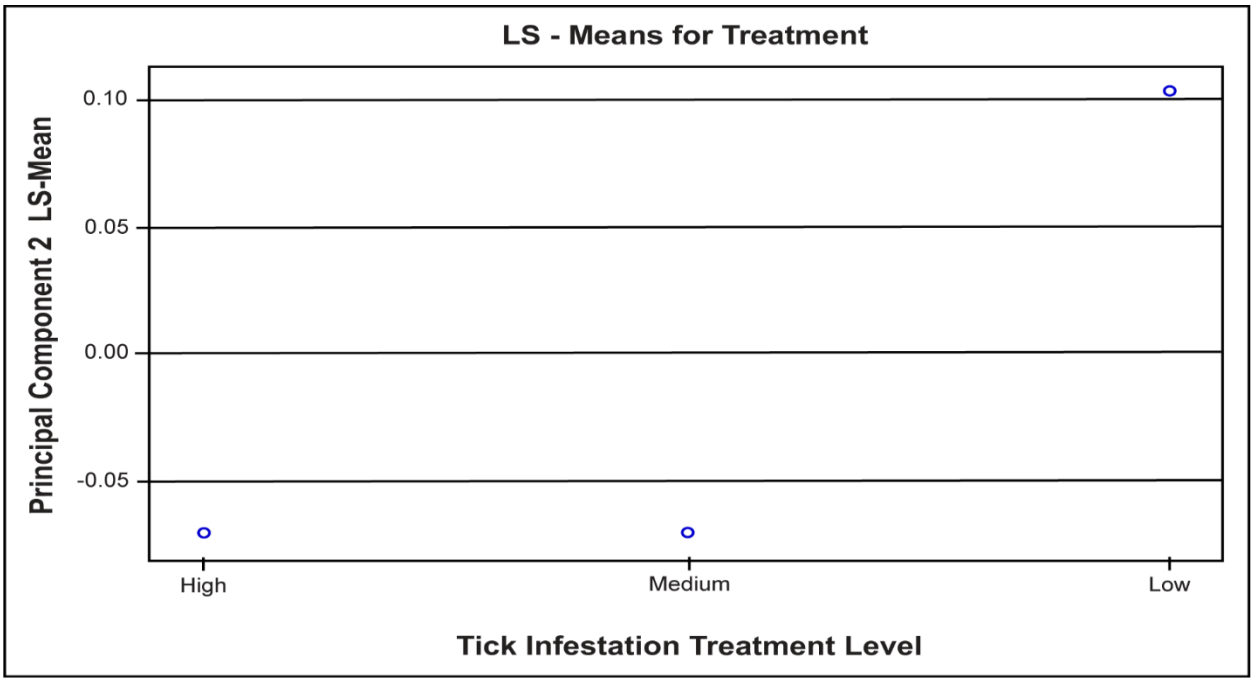

Figure 79. Least Squares Means for *Treatment* of the second principal component in Trial Two. The figure shows Tick Infestation *Treatment* Level (x-axis) by Principal Components 2 LS-Mean (y-axis).

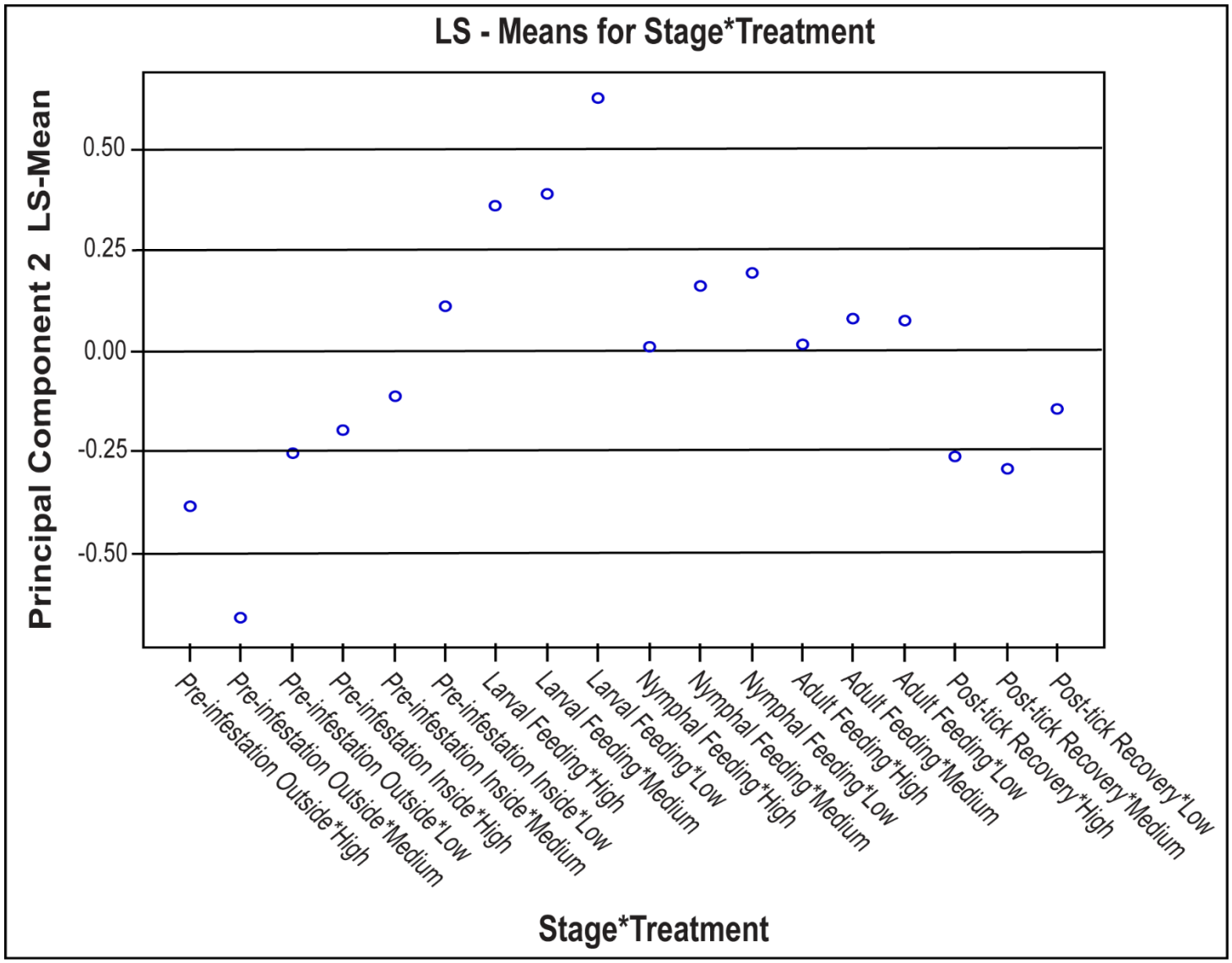

Figure 80. Least Squares Means for *Stage*Treatment* of the second principal component in Trial Two. The figure shows *Stage*Treatment* (x-axis) by Principal Components 2 LS-Mean (y-axis).

Principal Component 3: P_3. P_3 = Heifer(Treatment) + Treatment + Stage + Treatment ∗ Stage + Residuals. Based on the *p*-values below in Table 12, there was not significant evidence of differences for all combinations of the three tick infestation levels with respect to the means of the second principal component for each of the six Stages in this trial. Least square means for *Stage*, *Treatment*, and *Stage*Treatment* for P_3 are shown in Figures 81 – 83.

Table 12. Comparison of Three Tick Infestation Levels across the Six Stages for $\boldsymbol{P_3}$ in Trial Two. Values with a *p*-value less than 0.0028 were declared to be significantly different.

	Stage					
	1 PIO	**2 PII**	**3 LF**	**4 NF**	**5 AF**	**6 PTR**
P*-values for Comparing *Low vs Medium	0.0498	0.1093	0.2919	0.6219	0.6010	0.0747
P*-values for Comparing *Medium vs High	0.1847	0.8454	0.0331	0.1854	0.5405	0.8529
P*-values for Comparing *Low vs High	0.5228	0.1593	0.2790	0.4052	0.9318	0.1154

*Denotes values that are significantly different with a *p*-value less than 0.0028. PIO = Pre-infestation Outside, PII = Pre-infestation Inside, LF = Larval Feeding, NF = Nymphal Feeding, AF = Adult Feeding, PTR = Post-tick Recovery.

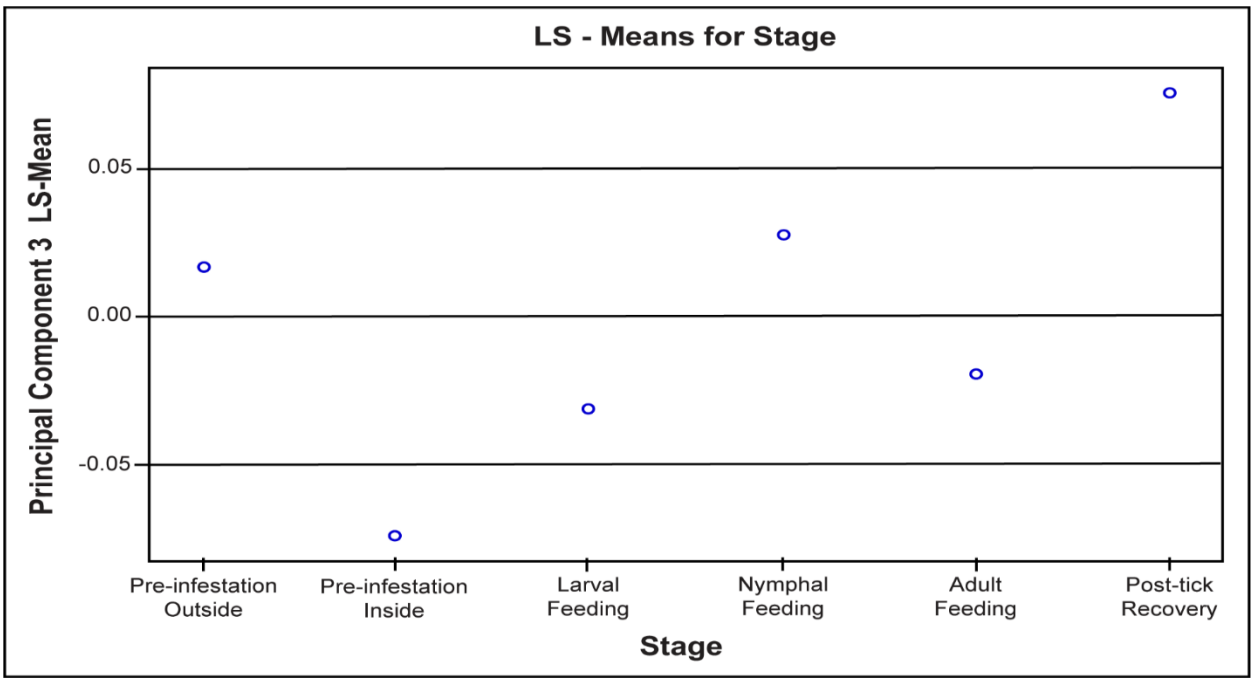

Figure 81. Least Squares Means for *Stage* of the third principal component in Trial Two. The figure shows *Stage* (x-axis) by Principal Components 3 LS-Mean (y-axis).

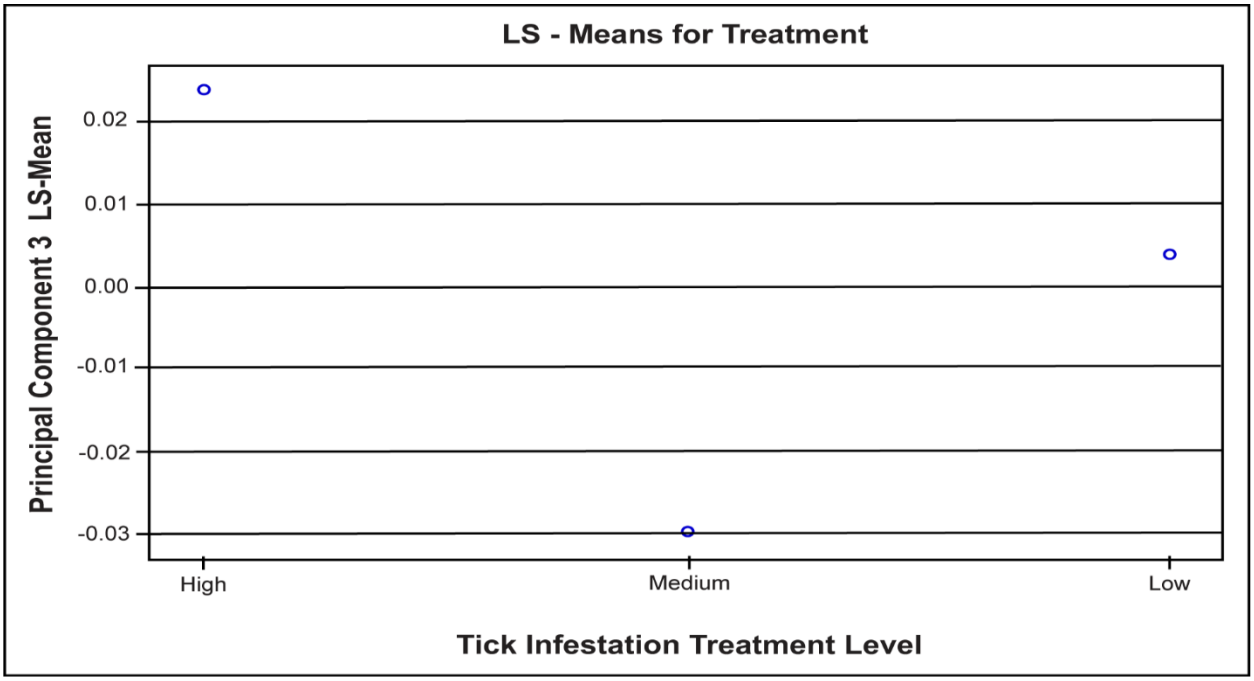

Figure 82. Least Squares Means for *Treatment* of the third principal component in Trial Two. The figure shows Tick Infestation *Treatment* Level (x-axis) by Principal Components 3 LS-Mean (y-axis).

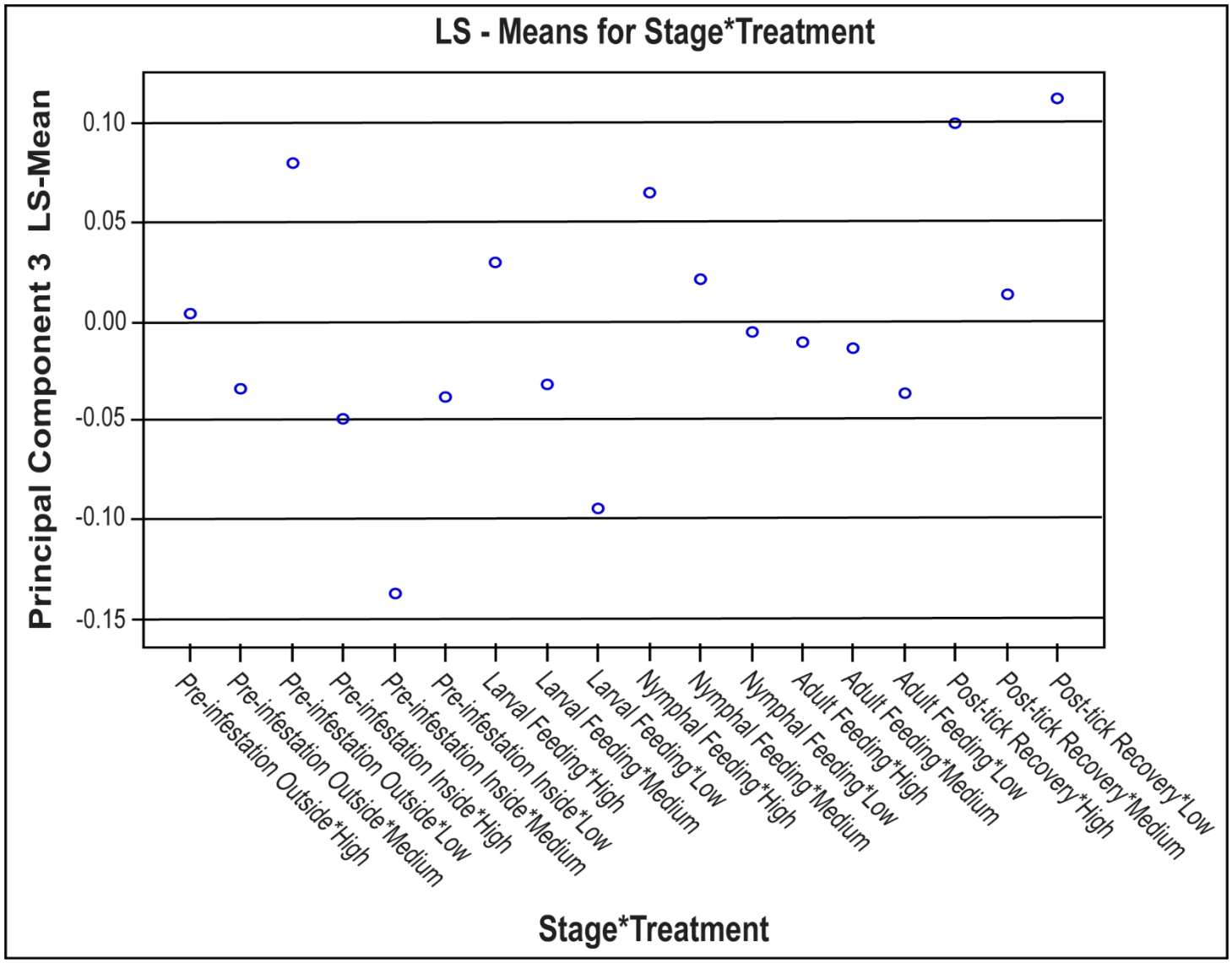

Figure 83. Least Squares Means for *Stage*Treatment* of the third principal component in Trial Two. The figure shows *Stage*Treatment* (x-axis) by Principal Components 3 LS-Mean (y-axis).

Discussion

Fecal NIRS detected spectral changes in daily fecal samples from the infestation period (larval, nymphal, and adult feedings) as different from the two pre-infestation control periods (outside and inside) for each heifer pair in the three-tick infestation level treatment groups in Trials One and Two. Cluster shifts occurred in the stepwise cluster analyses between the two periods of pre-infestation (outside and inside), the three stages of tick development (larval, nymphal, and adult feedings), and the post-tick recovery period. The PCAs conducted on raw daily spectral data from both trials provided evidence that the five cluster shifts displayed in the stepwise cluster analyses were significantly different. Results from the PCAs also provided evidence that three levels of tick infestation were successfully achieved in both trials.

The single-cohort artificial tick infestations from both trials were representative of the typical host-parasite interaction for *D. albipictus*. Drummond et al. (1968) concluded that engorged female drop of *D. albipictu*s is expected to begin 22 to 23 d post-tick infestation and that drop should cease by 40-d post-tick infestation. The engorged female drop period in Trial One began 23 d post-tick infestation with peak drop occurring from the range of days 26 to 34 post-tick infestation. The engorged female drop period in Trial Two started 24 d post-tick infestation with peak drop occurring from the range of days 26 to 28 post-tick infestation. Thus, the on-host biology of the ticks artificially infested on the six heifers in both trials was typical for *D. albipictus*.

Artificial infestations of *Dermacentor albipictus* initiated with different levels of larvae were detected in cattle using fNIRS. In Trial One, based on engorged female drop during the adult feeding period, we successfully achieved low, medium, and high tick infestation levels. In Trial Two, based on engorged female drop during the adult feeding period, we did not

successfully achieve low, medium, and high tick infestation levels. During the beginning stages of Trial Two, an unexpected situation occurred on Day 3 (3 d post-infestation). It was found that all six heifers were not only artificially infested with the *D. albipictus* larvae used in the study but also with a naturally occurring infestation of cattle biting lice, *Bovicola bovis* (Phthiraptera: Trichodectidae). Both the tick and chewing lice infestations caused the heifers to rub on their head stanchions resulting in the heifers losing clumps of hair. Therefore, it is reasonable to assume the rubbing may have dislodged or killed a number of ticks on each heifer at a critical time in the tick's life cycle, reducing each heifer's tick burden and initial tick infestation level. We must note that *B. bovis* is a chewing/biting louse, not a blood-feeding ectoparasite. Since they are not long-term blood feeders and modulating the immune system like ticks do, it is yet to be determined if *B. bovis* would contribute to any changes in fecal chemistry. Both *D. albipictus* and *B. bovis* are winter ectoparasites, so it would be common for cattle under field conditions to be parasitized simultaneously with both. Count data of ticks destroyed by each heifer was not obtained in this study. Thus, one point of fact may be that we did achieve low, medium, and high tick infestation levels on the heifers in Trial Two, and at some point, that was disrupted by the louse infestation and associated irritation. Nevertheless, despite what occurred in Trial Two, fNIRS was still able to detect *D. albipictus* infestations for each heifer pair in the three treatment groups.

Fecal near infrared reflectance spectroscopy was sensitive to the phase of on-host stage-specific tick development (larval, nymphal, and adult feedings). In both trials, raw spectral data from daily fecal samples for heifer pairs in each treatment group appear to follow the parasitic phase of the life cycle of *D. albipictus* with cluster shifts occurring between the two periods of pre-infestation (outside and inside), larval feeding, nymphal feeding, adult feeding, and post-tick

recovery. The first cluster shift from pre-infestation outside (Days -15 to -6) to pre-infestation inside (Days -5 to Day 1) suggests that the shift may be related to the heifers being moved from an outside paddock with a less strict diet ration to inside head stanchions where heifers were kept on a uniform diet ration comprising of creep feed and alfalfa cubes. The second cluster shift from pre-infestation inside (Days -5 to Day 1) to larval feeding (Days 2 to 7) suggests that the shift may be related to the onset of larval feeding (considered a low stress period) initiating the modulation of the heifer's immune systems (Tolleson et al. 2007). The third cluster shift from larval feeding (Days 2 to 7) to nymphal feeding (Days 8 to 17) suggests that the shift may be related to the change from larval feeding and molting to the commencement of nymphal tick feeding (considered a low stress period) (Tolleson et al. 2007). The fourth cluster shift from nymphal feeding (Days 8 to 17) to adult feeding (Trial One: Days 18 to 41; Trial Two: Days 18 to 35) suggests that the shift may be related to the change from nymphal feeding to adult feeding where there is the highest volume of salivary secretion from the ticks and when females rapidly engorge resulting in more blood loss from the host, causing high stress to the host (Sonenshine and Roe 2014, Tolleson et al. 2007). The fifth cluster shift from adult feeding (Trial One: Days 18 to 41; Trial Two: Days 18 to 35) to post-tick recovery (Trial One: Days 42 to 47; Trial Two: Days 36 to 42) suggests that the shift may be correlated to a slowing or cessation of blood feeding and a period when ticks were no longer present on the host and the heifers were trying to recover from tick infestation (Tolleson et al. 2007).

Female hard ticks might ingest blood more than 100-times their initial body weight over a period of days to weeks (Sauer et al. 1995), thus the time period of host recovery following a tick infestation may be variable depending on the size of infestation the host experienced and host physiology. In Trial One, the cluster analyses from all three treatment groups revealed that daily

fecal spectra from the post-tick recovery period were grouped with daily fecal spectra from the adult feeding period. In Trial Two, only the medium treatment groups daily fecal spectra from post-tick recovery period were grouped with daily fecal spectra from the adult feeding period. For the low and high treatment groups in Trial Two, there was a distinct cluster shift from adult feeding to post-tick recovery when there were no ticks present on the host and the heifers were trying to recover from tick infestation. Findings from the stepwise cluster analyses indicate that the time allotted for post-tick recovery of each heifer pair may need to be extended as the animals are attempting to get back to a state of homeostasis. There may be a "cost of fitness" (drain on available host energy) as a host attempts to combat ectoparasite burden (Tolleson et al. 2012). The drain on available host energy (protein-energy malnourishment) may have endocrine, metabolic, and immune consequences with respect to parasitism (Tolleson et al. 2012). As the ectoparasite burden decreases, so should the drain on the hosts available energy and resources. This would not only have the effect of lessening the blood imbibed by the ticks, but also the saliva and pharmacologically active compounds that the ticks secrete into the host to counteract host defense systems such as inflammation and immune responses.

The spectral changes detected by fNIRS provide a visual representation of cluster shifts. The PCA conducted on raw daily fecal spectra separately for Trial One and Trial Two was used to determine if there was significant difference between cluster shifts shown in the stepwise cluster analyses for the two pre-infestation control periods (outside and inside), the three stages of tick development (larval feeding, nymphal feeding, adult feeding), and the post-tick recovery period. For Trials One and Two, the PCA results provide evidence that the five cluster shifts shown in the stepwise cluster analyses were significantly different. Furthermore, results from the

PCA showed that three levels of tick infestation were successfully achieved in Trials One and Two.

Each tick infestation level treatment group (low, medium, and high) triggered a change in fecal spectra detectable by fNIRS technology. Changes in fecal chemistry indicated by NIR spectra were consistent with the on-host stage-specific feeding of *D. albipictus*. Future work will include the continuation of testing the sensitivity and feasibility of fNIRS technology to detect animals infested with *D. albipictus*, improve IPM adoption, decision-making, and efficacy for tick management.

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CHAPTER III

CATTLE PRODUCER ADOPTION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY TECHNOLOGY IN GRAZING CATTLE SYSTEMS

The southern region of the United States (US) is comprised of 13 states including: Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, and Texas. Among the top five agricultural commodities in seven of 13 southern region states are cattle and calves where cash receipts by state range from $122,162 (South Carolina) to $8,424,033 (Texas) (USDA ERS 2021). The southern region cow-calf inventory is more than 40% of the entire US inventory, with Texas ranking first in the US in total number of cattle and calves at 12.7% of the total nation's inventory (USDA NASS 2020, USDA ERS 2021). Of all the fed cattle in the US, about 1/3 originate on ranches in the southern region that are operated by about 400,000 cattle producers which represent 49% of all US cow-calf producers (McBride and Mathews 2011). Consumer demand for beef produced under alternative systems (e.g., grass-fed; organic) is growing and creates special challenges, including that animals be maintained on pastures longer, increasing exposure to production and health risks (Mathews and Johnson 2013).

External parasites have been estimated to annually cost the US beef cattle industry $2.4 billion (Drummond 1987, inflation corrected Friedman 2008) through the direct effects of parasitism, and an even greater cost when animal handling and treatment expenses are included. Two tick species, *Amblyomma americanum* L. (Acari: Ixodidae), and *A. maculatum* Koch (Acari: Ixodidae), are among annual species attacking range cattle in the southern region and direct losses to cattle producers are estimated to be more than $218 million (Drummond 1987, inflation

corrected Friedman 2008). Recent geographic expansions of *A. maculatum* (Teel et al. 2010) and *A. americanum* (Springer et al. 2014) have exposed more producers to the impacts of tick parasitism. Direct production costs accrued from tick parasitism include irritation, blood loss, weight loss, loss of body condition, and reduced reproductive capacity (Barnard 1985, Teel et al. 1990, Williams 2010).

Tick parasitism in range cattle can occur year-round. Most tick species are seasonally active in the spring and summer months, certain species such as *Dermacentor albipictus* Packard (Acari: Ixodidae), are active in fall (October through November) and winter (December through February). *Dermacentor albipictus* infestations are easily unnoticed due to winter hair coats and occur on animal hosts when annual forage quality and quantity are low (Bishopp and Tremley 1945, Teel et al. 1990). Integrated pest management (IPM) strategies have been developed for ticks (Barnard et al. 1994, Williams 2010). These strategies rely on habitat management, grazing rotations, fencing, wildlife management, and cattle treatments with acaricides (Barnard et al. 1994, Williams 2010). However, the on-animal application of acaricides has become the principle recommended tactic to control ticks. Pesticide application to food animals for tick control is common to many states including Texas (Hoelscher et al. 2000), Oklahoma (Barker et al. 1998), and Florida (Kaufman et al. 2009).

Infestations by native tick species risk production and economic losses in young-growing and mature animals (Barnard 1985, Drummond 1987). Five tick species with different seasonal activities provide year-round risk of tick infestation in the Southern Region: *A. americanum*, *A. maculatum*, *D. albipictus*, *D. variabilis* Say (Acari: Ixodidae), and *Ixodes scapularis* Say (Acari: Ixodidae) (http://tickapp.tamu.edu; Teel et al. 2011). For tick management, producers are required to gather and physically inspect animals on a regular basis to determine tick presence

and abundance and make informed decisions regarding management tactics (Barnard 1985, Williams 2010). Gathering and physically inspecting cattle results in animal stress, time, labor, facilities wear, and expense that are disincentives to IPM adoption. Instead, anecdotal information suggests producers treat for ticks when it is convenient to gather cattle to accomplish other management tasks such as sorting calves/cows, branding, and vaccinating. Treatments for ectoparasites applied during general management tasks for prophylactic value are likely ineffective and costly.

Hands-on inspection of cattle for ticks has many challenges. Humans are only able to detect objects greater than or equal to 8 mm in size (Palmer et al. 1976), which is equivalent to the approximate size of most unfed adult ticks, or the size of engorged nymphs. Thus, count data for tick larvae and nymphs are rarely detected. Reliable animal inspection is subject to many biophysical and human factors making probabilities of detection less than satisfactory (Teel et al. 2003). These factors include quality and state of handling facilities, animal behavior and experience, environmental conditions, and skills/experience of inspectors. Prior research indicates that near infrared reflectance spectroscopy of bovine feces (fNIRS) offers a non-invasive (not dependent on inspecting cattle), economical, and efficient means of detecting tick infested animals and monitoring effectiveness of tactics for tick suppression (Tolleson et al. 2015).

Near infrared reflectance spectroscopy (NIRS) is recognized as playing a key role in the diagnostic surveillance framework for agricultural and environmental management (Shepherd and Walsh 2007). Near infrared reflectance spectroscopy applications extend from soils, to plants, to water, to crop and livestock product quality and processing, and to livestock health. These applications include biosecurity, bio-forensics, quality assessment and quality assurance.

Global applications of NIRS for evaluating herbivore nutrition and physiology include parasite stress (Dixon and Coates 2015). Near infrared reflectance spectroscopy analysis of bovine feces coupled with a computer-aided, decision support system (NUTBAL) has led to a diagnostic surveillance method for monitoring the nutritional status of grazing animals such as cattle (Artiodactyla: Bovidae; *Bos* spp.), sheep (Artiodactyla: Bovidae; *Ovis aries*), goats (Artiodactyla: Bovidae; *Capra aegagrus hircus*), and white-tailed deer (Artiodactyla: Cervidae; *Odocoileus virginianus*) (Lyons and Stuth 1992, Cook 1999). The method of fNIRS coupled with NUTBAL was the basis for establishment of the Grazing Animal Nutrition Laboratory (GANLAB) by Texas A&M AgriLife Research (http://cnrit.tamu.edu/ganlab/) that offers a fee-based service for livestock owners for forage analysis. This service served approximately 4,200 clients and processed about 17,500 fecal samples in 2016. The value of this service is recognized and recommended by the United States Department of Agriculture (USDA), Natural Resources Conservation Service (NRCS) as part of its Conservation Stewardship Program having nutrition monitoring as a practice. The NRCS participation has driven an estimated 25 to 30% increase in GANLAB clients and samples each year. Research into the further development of NIRS applications is also part of the GANLAB mission (https://cnrit.tamu.edu/).

Near infrared reflectance spectroscopy has been investigated to monitor tick infestations in grazing beef cattle, provide evidence of tick suppression to acaricide treated cattle, and aide in decisions for acaricide retreatment in response to tick re-infestation (Teel et al. 2004, Tolleson et al. 2015). Controlling and reducing damages caused by arthropods in grazing cattle systems remains challenging due to the life-histories of the arthropods, the interactions of the landscape and grazing cattle system, and the operators' willingness to adopt new management techniques and technology for their system.

There are a variety of technologies available for adoption by cattle producers. This can include advanced breeding technologies (e.g., embryo transfer, artificial insemination, sexed semen), nutritional testing technologies (e.g., forage quality testing, NIRS for dietary diagnostic analysis and decision support system to be used as a nutritional monitoring system for grazing livestock), animal identification systems and record-keeping systems (e.g., GPS and RFID ear tags for animal identification and tracking, computerized record-keeping systems), implants to potentially increase weight gains, and veterinary services (e.g., bull breeding soundness exams, ultrasound) (Johnson et al. 2010, Pruitt et al. 2012, Selk et al. 2006, USDA 2009, Ward et al. 2008). Other technologies could include soil and water health associated with forage/grassland management and stewardship, grazing strategies, and marketing. There are several factors that have been shown to affect technology adoption including: farm size, off-farm employment, risk assessment, and farm location (Dorfman 1996, Fernandez-Cornejo 2007, Fernandez-Cornejo et al. 2005, Ward et al. 2008). More factors that have been identified to influence technology adoption include human attributes such as education, years of experience, and age (Johnson et al. 2010, Ward et al. 2008).

Operator/farm size is considered a factor that fundamentally affects technology adoption and whether the operation can afford the initial cost of adopting the technology (Dorfman 1996, Johnson et al. 2010, Pruitt et al. 2012, Ward et al. 2008). For cow-calf operations, small-size operations can be categorized as managing 1-49 head of cattle with medium-size managing 50-199 head, and large-size managing greater than 200 head (USDA 2020). In relation to stocker/feeder cattle operations, Johnson et al. (2010) divided operation size into small (less than 100 head managed each year), medium (100-500 head managed each year), and large (greater than 500 head managed each year). Thus, depending on operation type, cattle herd size

characterizations may vary. The probability and speed of adoption is also hypothesized to be positively related to the size of the operation (Gafsi and Roe 1979, Perrin and Winkelmann (1976), Wozniak 1987). Operation management goals are expected to affect technology adoption. A factor motivating technology adoption by large-size operations is the profitability of the technology (Johnson et al. 2010).

The importance cattle producers place on choosing technology is if it provides immediate economic benefits, reduces general labor, and whether their operation is producing sufficient income to avoid hiring off-farm employment. Through prior surveys, the primary disincentives of technology adoption by cattle producers include the overall cost and lack of labor, time, and facilities (Elliott et al. 2013, Pruitt et al. 2012, Ward et al. 2008). These deterrents suggest that the operation type (e.g., stocker/background operation, cow-calf operation) and management characteristics (e.g., on-farm and off-farm employment, facilities) influence the adoption of technology in cattle production systems. Further investigation into the different types of cattle operations and the operations management characteristics should permit extension service personnel to identify cattle producers that would profit from educational and/or incentive programs.

Educational and/or incentive programs might encourage the adoption of new technology in grazing cattle systems. Technology adoption by cattle producers could greatly benefit the future of beef production and IPM tactics. The objective of this study was to assess producer adoption of NIRS technology in grazing cattle systems. Producer adoption of NIRS technology can potentially serve as a beneficial tool for grazing cattle systems to improve production management decision-making, and efficacy of management tactics for tick suppression on pastured cattle.

Materials and Methods

Assessment of Producer Adoption

All data used in the analyses were obtained from three audiences including the Texas A&M Annual Beef Cattle Short Course (BCSC), Producer Meetings (PM), and Veterinarian Meetings (VM) that were provided a survey with questions determined by the author. The analysis examined producer adoption of NIRS technology in grazing cattle systems including applications for beef cattle nutrition and tick control in accordance with the Institutional Review Board (IRB)-approved a 29-question survey (IRB ID: IRB2017-0259) entitled 'Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems'. The survey was a paper hand-out questionnaire (B-1 in Appendix B) designed to be completed in approximately 5-10 minutes. The survey focused on four content areas: 1) background of cattle producers, 2) beef production characteristics of the respondents' operation, 3) ectoparasite control including ticks, and 4) use of fecal NIRS technology for forage assessment and nutritional balance. The breakdown of survey questions assigned to each of the four content areas are provided in B-2 in Appendix B. Survey questions answer formats are shown in B-3 in Appendix B.

Texas A&M Annual Beef Cattle Short Course (BCSC)

Each year the BCSC offers a seminar series on "Ticks, Flies and Gnats" that provides Texas Department of Agriculture-approved Continuing Education Units (CEUs) for producers maintaining a Pesticide Applicator License in Texas. The BCSC is regional in scope often drawing attendees from many states. Attendance at this seminar has been 175-200 producers annually. The survey was provided to this audience in years 2017, 2018, and 2019 (Table 13). Presentations provided to this audience included subject matters such as tick biology, impacts of

ticks on livestock, economic effects, fNIRS applications for tick control, findings from research studies using fecal NIRS technology, and the NIRS-based nutritional balance service provided by the GANLAB.

Producer Meetings (PM)

The PM audience was surveyed in years 2017 and 2018 through Texas A&M AgriLife Extension Programs (Table 13). Five annual producer meetings were surveyed for this audience and the meetings spanned from one to 21 counties per meeting. The first producer meeting surveyed was an annual seminar where general property management and pests of pasture and range vegetation were the main focuses, and this survey was provided to producers after receiving a presentation on Bermudagrass stem maggots, armyworms, sugarcane aphids and fire ants. The second producer meeting surveyed was an annual conference providing Texas Department of Agriculture-approved CEUs for Laws & Regulations, IPM, and General CEUs, with sessions provided on beef, grain, cotton, forage, horses, wildlife, and rural land management. Producers surveyed at this annual conference were in the beef session and received a presentation on herd health, vector-borne diseases of livestock, bovine anaplasmosis and ticks specifically. Producer meetings three, four and five primarily focused on herd health, disease management, and overall cattle production. Producers in attendance at meeting number three were given a presentation on internal and external parasites of cattle. For meeting number four, producers surveyed received a presentation on vector-borne diseases of livestock, bovine anaplasmosis and ticks specifically. Lastly, producers surveyed at meeting number five were provided with a presentation on tick species commonly found in Texas and the impacts of ticks on cattle.

Veterinarian Meetings (VM)

The VM audience consisted of veterinary practitioners with large animal practices and some of whom also have beef cattle enterprises. The two annual veterinarian meetings representing this audience were surveyed in year 2018 (Table 13). Both meetings received presentations on vector-borne diseases of livestock, bovine anaplasmosis, and ticks specifically. The surveys were administered to this audience with the acknowledgement that the VM respondents may answer the survey questions from the view of their clientele base, veterinary procedures, their own operations, or a combination of the two. Since respondents in this audience could have varied points of view, the VM audience for the remainder of this chapter will be referred to as practitioners/producers.

Table 13. Summary of number of respondents from the three audiences and the year data were obtained in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems.

Audience	Year	Number of Respondents
Beef Cattle Short Course	2017	96
Producer Meeting	2017	214
Producer Meeting	2018	45
Producer Meeting	2018	34
Producer Meeting	2018	15
Veterinarian Meeting	2018	10
Veterinarian Meeting	2018	42
Beef Cattle Short Course	2018	108
Producer Meeting	2018	65
Beef Cattle Short Course	2019	101
Total	**All**	**730**

Results

The summary of respondent answers for the three surveyed audiences are provided as either count data or frequencies and percentages for each of the 29-questions. Three audiences

(BCSC, PM, and VM) were surveyed during the years 2017, 2018, and 2019, resulting in a sample size of 785. Of 316 survey responses from the BCSC, 11 were removed from the analysis as they did not contain pertinent descriptive information permitting their operation to be categorized (n = 305). Of 405 survey responses from PM audiences, 37 were removed from the analysis as they did not contain pertinent descriptive information permitting their operation to be categorized (n = 368). Of 64 survey responses from VM audiences, seven were removed from the analysis as they did not contain pertinent descriptive information permitting their operation to be categorized (n = 57). The final sample size for all three audiences combined totaled 730 respondents. Some surveys received from all three audiences had questions that were not answered; therefore, the sample size for the results may not equal the numbers stated above.

The characterization of the three audiences is provided Tables 14 through 17. In Table 14 is the summarized data for the gender of the three audiences. Males (> 70%) were more present at the meetings than females (< 27%). The general age of respondents in the BCSC and PM were greater than 46 years old, where in the VM the top three age groups were 31 to 35, 36 to 40, and > 60 (Table 15). The respondent's highest education level for the BCSC and PM ranged from high school graduate or equivalent to a professional degree (Table 16). The VM respondent's highest education level was professional degree which is standard as a Doctor of Veterinary Medicine is a professional degree (Table 16). In Table 17 is the summarized data for the ethnicity of the three audiences. White/Caucasian was the predominant response of ethnicity (> 83.6%) for all three audiences, with Hispanic American or Latino origin coming in second, and African American coming in third.

Table 14. Summary of respondent answers to gender by audience in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the three surveyed audiences in years 2017, 2018, and 2019.

	Gender					
	Male		Female		No response	
Audience	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Beef Cattle Short Course	223	73.1	76	24.9	6	2.0
Producer Meetings	327	88.9	36	9.8	5	1.4
Veterinarian Meetings	41	71.9	15	26.3	1	1.8

Note: Frequencies may not total stated *n* for Beef Cattle Short Course, Producer Meetings, and Veterinarian Meetings because of missing data.

Table 15. Summary of respondent answers to age by audience in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the three surveyed audiences in years 2017, 2018, and 2019.

	Audience					
	Beef Cattle Short Course		Producer Meetings		Veterinarian Meetings	
Responses	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
> 26	9	3.0	4	1.1	1	1.8
26 to 30	7	2.3	3	0.8	2	3.5
31 to 35	7	2.3	10	2.7	7	12.3
36 to 40	13	4.3	7	1.9	6	10.5
41 to 45	14	4.6	6	1.6	5	8.8
46 to 50	22	7.2	25	6.8	3	5.3
51 to 55	24	7.9	26	7.1	2	3.5
56 to 60	38	12.5	50	13.6	5	8.8
> 60	156	51.1	220	59.8	24	42.1
No response	15	4.9	17	4.6	2	3.5

Note: Frequencies may not total stated *n* for Beef Cattle Short Course, Producer Meetings, and Veterinarian Meetings because of missing data.

Table 16. Summary of respondent answers to highest education level by audience in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the three surveyed audiences in years 2017, 2018, and 2019.

	Highest Education Level													
	Some high school		High school graduate or equivalent		Some college, no degree		Associates or Bachelor's degree		Post-graduate degree		Professional degree		No response	
Audience	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Beef Cattle Short Course	2	0.7	21	6.9	50	16.4	120	39.3	57	18.7	44	14.4	11	3.6
Producer Meetings	4	1.1	60	16.3	72	19.6	145	39.4	49	13.3	28	7.6	10	2.7
Veterinarian Meetings	0	0	0	0	0	0	2	3.5	4	7.0	50	87.7	1	1.8

Note: Frequencies may not total stated *n* for Beef Cattle Short Course, Producer Meetings, and Veterinarian Meetings because of missing data.

Table 17. Summary of respondent answers to ethnicity by audience in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the three surveyed audiences in years 2017, 2018, and 2019.

	Ethnicity													
	African American		Asian American		Hispanic American or Latino Origin		Native American		White/ Caucasian		Other, Please Specify		No response	
Audience	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Beef Cattle Short Course	9	3.0	3	1.0	18	5.9	5	1.6	255	83.6	3	1.0	12	3.9
Producer Meetings	4	1.1	0	0	3	0.8	2	0.5	340	92.4	6	1.6	13	3.5
Veterinarian Meetings	0	0	0	0	2	3.5	0	0	52	91.2	1	1.8	2	3.5

Note: Frequencies may not total stated *n* for Beef Cattle Short Course, Producer Meetings, and Veterinarian Meetings because of missing data.

Texas A&M Annual Beef Cattle Short Course (BCSC)

Background of Cattle Producers

Respondents from three separate years at the BCSC (*n* = 305), ranging from 96 to 108 producers per year, indicated their place of residence by state was 96.4% from Texas and the remaining 3.6% were from Arizona (0.7%), Kansas (0.7%), Louisiana (0.7%), Oklahoma (0.3%), Tennessee (0.3%), and Utah (0.3%), and 0.7 did not respond (Question #1, Part 2). Within the state of Texas, this producer audience represented 115 out of 254 counties across the state (Question #1, Part 1) (B-4 in Appendix B). Across all states, 31.1% of respondents indicated being a cattle producer (Question #25) less than 10 years, 26.2% responded 11 – 20 years, 39.7% responded over 20 years, and 3.0% chose not to answer. The producer's primary role for the cattle portion of their operation (Question #4) is presented in Figure 84. Over 87.5% of 305 responses to question #4 were owner and owner/manager.

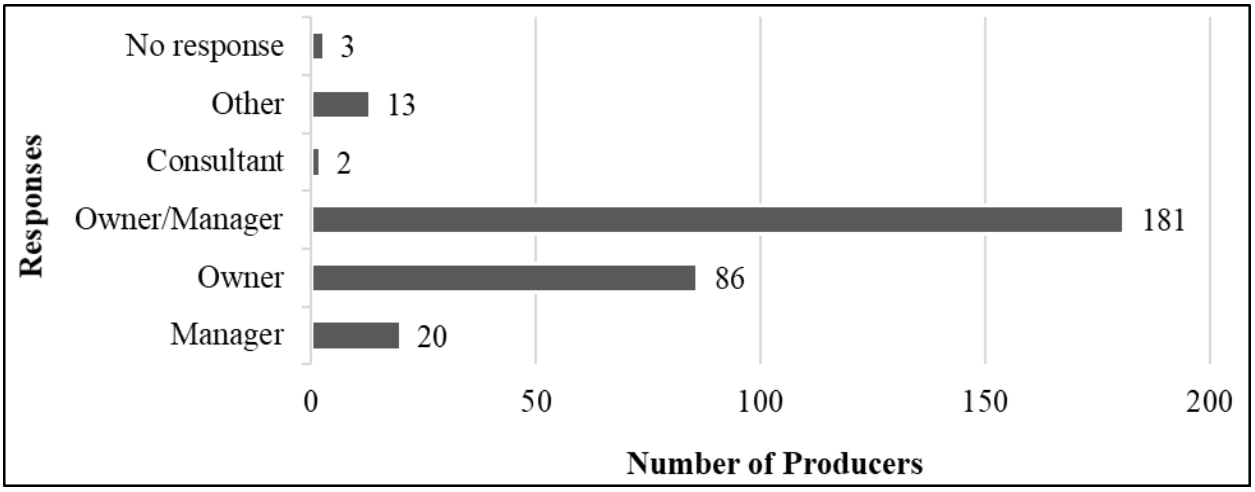

Figure 84. Summary of producer responses to the primary role of respondents for the cattle portion of the operation (Question #4) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. *n* = 305.

Beef Production Characteristics of the Respondents' Operation

Beef Cattle Short Course producer responses to the primary location of cattle by state was 96.7% from Texas with the remaining 3.3% were from Arizona (0.3%), California (0.3%), Kansas (0.7%), Louisiana (0.7%), Oklahoma (0.3%) and 1.0% did not respond (Question #2, Part 2). Within the state of Texas, this producer audience represented 121 out of 254 counties across the state (Question #2, Part 1) (B-5 in Appendix B). Responses to indicate the approximate number of each type of free-ranging animals (cattle, other livestock, wildlife, and exotics) managed on their property (Question #3) are provided in B-6 – B-11 in Appendix B. From our findings, some beef cattle production systems might manage other animals on the same properties where cattle are located. These animals may include goats/sheep, horses/mules/ donkeys, white-tailed deer, other cervids and exotics, and poultry.

Figure 85 summarizes audience responses to select the approximate number of cattle they manage by category (Question #5). Most producer respondents stated to manage 1 to 500 head of cattle. The type of pastured cattle production system that most reflected the respondent's operation (Question #6) was 81.6% for commercial/cow-calf (n = 249), with seed stock or replacement at 21.3% (n = 65), stocker/backgrounder at 6.2% (n = 19) and show stock at 5.6% (n = 17). Respondents characterized their cattle working facilities (Question #7) as 92.5% stationary facilities (n = 282), 13.8% portable facilities (n = 42), 18.0% weigh station/scales (n = 55), and 2.6% stated to have no facilities (n = 8).

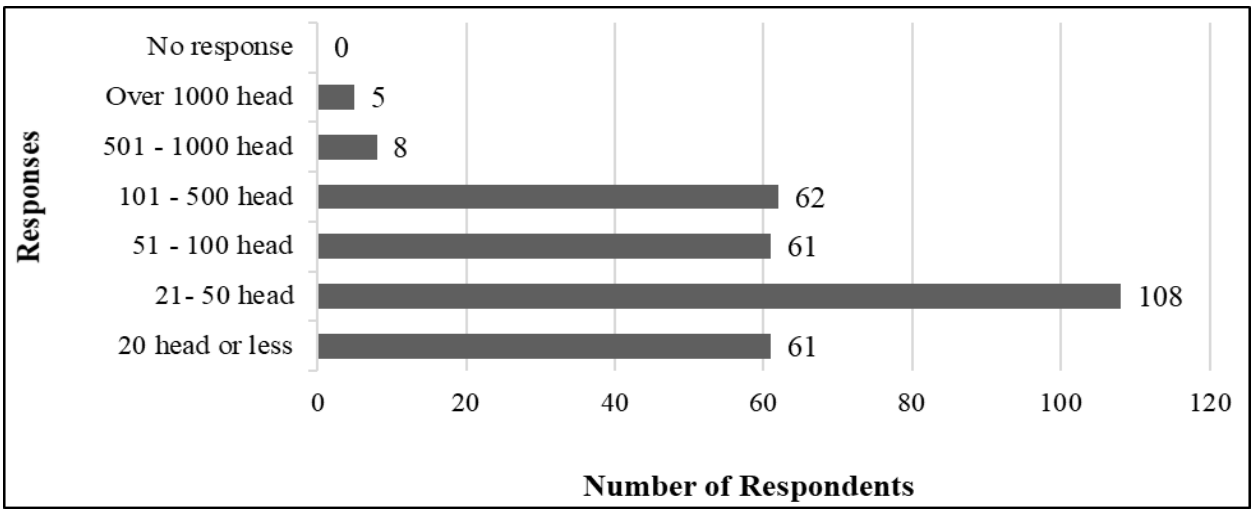

Figure 85. Summary of producer responses to the approximate number of cattle managed (Question #5) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. *n* = 305.

The approximate length of breeding seasons (Question #8) was 34.8% of 305 respondents for 1 – 3 months, 23.3% for 4 – 6 months, 38.4% for 7 – 12 months, and 3.6% did not respond. The types of pasture on which cattle are grazed during each season (Question #11) is summarized in Table 18. Producers were asked to fill out a table indicating the timing of certain management practices (Question #14). Responses to question #14 are summarized in Tables 19 and 20. From our findings, producer responses to the timing of certain management practices shows that ectoparasite control is typically conducted when it is convenient to gather cattle for other routine management practices such as castration, vaccination, and endoparasite control. The top two responses to how cattle are evaluated for proper nutrition (Question #19) were body condition of the cattle and pasture conditions with forage/hay testing and weighing animals at a close tie for the top third response (Table 21).

Table 18. Summary of producer responses to the type of pasture on which cattle are grazed during each season (Question #11) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

	Season							
	Spring		Summer		Fall		Winter	
Type of Pasture	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Brushy/Shrubland	50	16.4	43	14.1	43	14.1	47	15.4
Improved	132	43.3	126	41.3	121	39.7	93	30.5
Mixed	99	32.5	102	33.4	103	33.8	86	28.2
Native	76	24.9	72	23.6	75	24.6	71	23.3
Annual Forages	30	9.8	22	7.2	27	8.9	50	16.4
Other (Please explain)	3	1.0	4	1.3	3	1.0	6	2.0

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 19. Summary of producer responses to the timing of following cattle management practiced for months January through June (Question #14) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

	Months											
	January		February		March		April		May		June	
Type of Cattle Management	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Castration	22	7.0	26	8.2	62	19.6	62	19.6	57	18.0	33	10.4
Vaccination	29	9.2	31	9.8	79	25.0	93	29.4	66	20.9	37	11.7
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*	23	7.3	19	6.0	72	22.8	90	28.5	86	27.2	84	26.6
Endoparasite Control *(ex. intestinal worms)*	17	5.4	14	4.4	64	20.3	71	22.5	48	15.2	29	9.2
Weaning Cattle	13	4.1	13	4.1	25	7.9	41	13.0	35	11.1	28	8.9
Weighing Cattle	13	4.1	17	5.4	22	7.0	23	7.3	25	7.9	12	3.8
Culling	14	4.4	16	5.1	29	9.2	34	10.8	29	9.2	18	5.7
Other (Please explain)	5	1.6	4	1.3	4	1.3	4	1.3	4	1.3	4	1.3

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 20. Summary of producer responses to the timing of following cattle management practiced for months July through December (Question #14) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

	Months											
	July		August		September		October		November		December	
Type of Cattle Management	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**
Castration	23	7.3	18	5.7	40	12.7	23	7.3	18	5.7	40	12.7
Vaccination	32	10.1	26	8.2	51	16.1	32	10.1	26	8.2	51	16.1
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*	92	29.1	80	25.3	92	29.1	92	29.1	80	25.3	92	29.1
Endoparasite Control *(ex. intestinal worms)*	28	8.9	26	8.2	54	17.1	28	8.9	26	8.2	54	17.1
Weaning Cattle	31	9.8	48	15.2	79	25.0	31	9.8	48	15.2	79	25.0
Weighing Cattle	17	5.4	17	5.4	31	9.8	17	5.4	17	5.4	31	9.8
Culling	24	7.6	23	7.3	57	18.0	24	7.6	23	7.3	57	18.0
Other (Please explain)	4	1.3	4	1.3	4	1.3	4	1.3	4	1.3	4	1.3

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 21. Summary of producer responses to how are cattle evaluated for proper nutrition (Question #19) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	***f***[a]	**%**
Body condition	284	93.1
Forage/hay testing	62	20.3
Manure/fecal testing	23	7.5
Pasture conditions	163	53.4
Weigh animals	36	11.8
Other	3	1.0

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Ectoparasite Control Including Ticks

Questions 9, 10, 12, 13, and 15-18 address factors related to ectoparasite-host-pasture/range interactions and decision making for ectoparasite control. Producers responded (*n*

= 303) that 45.2% had a regular schedule for moving cattle between pastures, 54.1% did not, and 0.7% chose not to respond (Question #9). Producer responses (*n* = 304) show that 71.5% have a majority of pastures in their cattle production system with some type of shrub/brush coverage, 28.2% stated they did not, and 0.3% had no response (Question #10). Table 22 summarizes responses to whether brush control had been used as means of pasture management in the past two years (Question #12). Responses show that 68.2% (*n* = 208) have used brush control as a means of pasture management with 17.7% (*n* = 54) stating they have not. In reply to methods practiced for brush control (Question #13), producers responded with 9.2% for fire (*n* = 28), 70.2% for herbicide (*n* = 214), 53.1% for mechanical (*n* = 162), and 2.3% for other (*n* = 7).

Producer responses indicate that flies (296), ticks (102), lice (90), grubs (27), and other (2) were the ectoparasites causing problems in local cattle operations (Question #15). More than half of the respondents indicated (Figure 86) they gather cattle specifically to treat for ectoparasites (Question #16). The high-to-low order of responses to methods of ectoparasite treatment used in the previous year (Question #17) were pour-on, spray, ear tags, vet gun, other and dip (Figure 87). Producer responses are summarized in Table 23 to question #18 on how the need for ectoparasite treatment of cattle is determined. These data show that just over half make decisions by inspecting animals and/or observing ectoparasites on their animals.

Table 22. Summary of producer responses to has brush control been used as means of pasture management in the past two years (Question #12) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. *n* = 305.

Response	***f*[a]**	**%**
Yes	208	68.2
No	54	17.7
Not applicable to my operation	16	5.2
No response	27	8.9

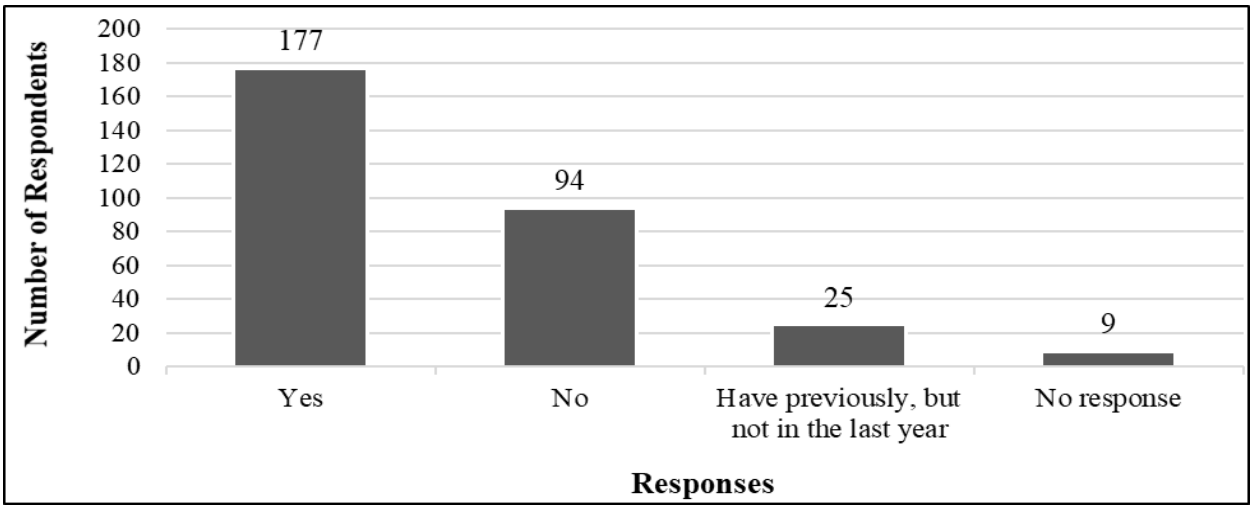

Figure 86. Summary of producer responses to are cattle ever gathered specifically to treat for ectoparasites (Question #16) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. *n* = 305.

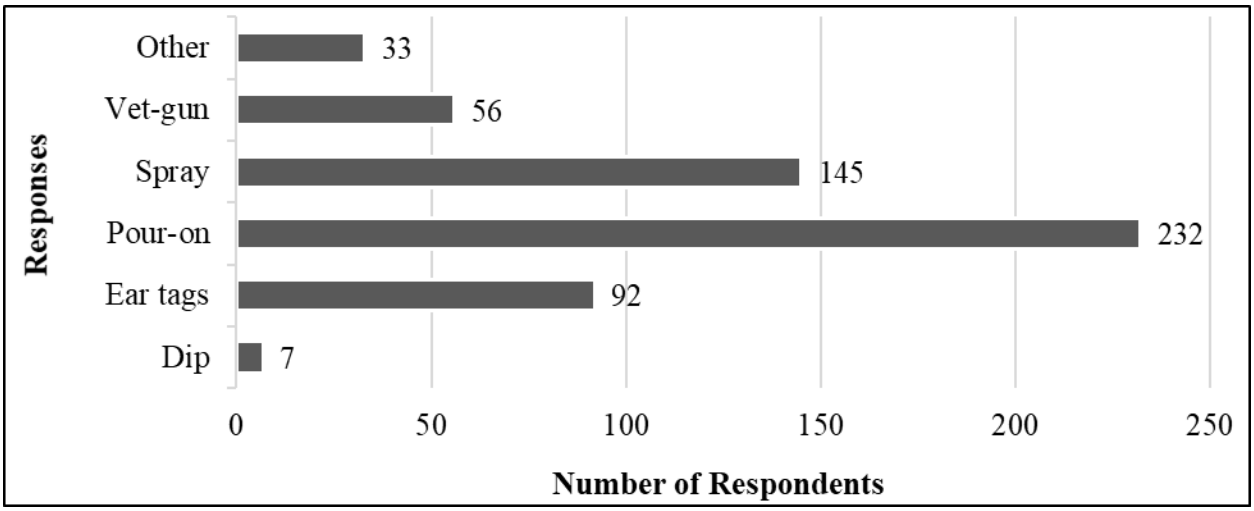

Figure 87. Summary of producer responses to what methods of ectoparasite treatment have been used in the past year (Question #17) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. Note: Responses may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 23. Summary of producer responses to how is the need for ectoparasite treatment of cattle determined (Question #18) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	***f*[a]**	**%**
Changes in behavior	54	17.7
Changes in body condition	49	16.1
Convenience	97	31.8
Physical examination of cattle	153	50.2
Observe ectoparasites on cattle	156	51.1
Time of the year	82	26.9

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Use of Fecal NIRS Technology for Forage Assessment and Nutritional Balance

Producer responses (*n* = 291) indicated that 41.6% believe a manure/fecal test that could diagnose a tick infestation in cattle would be useful (Question #20), 14.1% stated no, 39.7% stated maybe, and 4.6% did not respond. In response to producer's awareness of services available to them to test forage and hay for nutritional value (Question #21), producers (*n* = 305) responded yes at 72.8%, 18.7% stated no, and 8.5% chose not to answer. Figure 88 summarizes producer responses to "if the option of submitting forage/hay and/or manure/fecal samples for nutritional testing would be considered"? (Question #22). Producer responses to question #22 were forage/hay (210 producers) and manure/fecal (136 producers) for yes and maybe responses were forage/hay (14 producers) and manure/fecal (22 producers). Furthermore, Figure 89 summarizes producer responses to "if cost shares were available *(ex., from the Natural Resources Conservation Service's Program)*, would testing services be useful for forage/hay and/or manure/fecal samples?" (Question #23). Producer responses to question #23 were

forage/hay (212 producers) and manure/fecal (173 producers) for yes and maybe responses were forage/hay (14 producers) and manure/fecal (18 producers). Responses to "if forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used?" (Question #24) are provided in Table 24. Respondents to question #24 indicated that to rotate animals on pastures, supplemental feed purchase and supplemental hay purchase were the top three uses of information from forage testing (*n* = 35, *n* = 60, and *n* = 36, respectively) and hay testing (*n* = 14, *n* = 87, and *n* = 48, respectively). The top three uses of information from manure testing were culling animals (*n* = 15), rotating animals on pastures (*n* = 12), and supplemental feed purchase (*n* = 17).

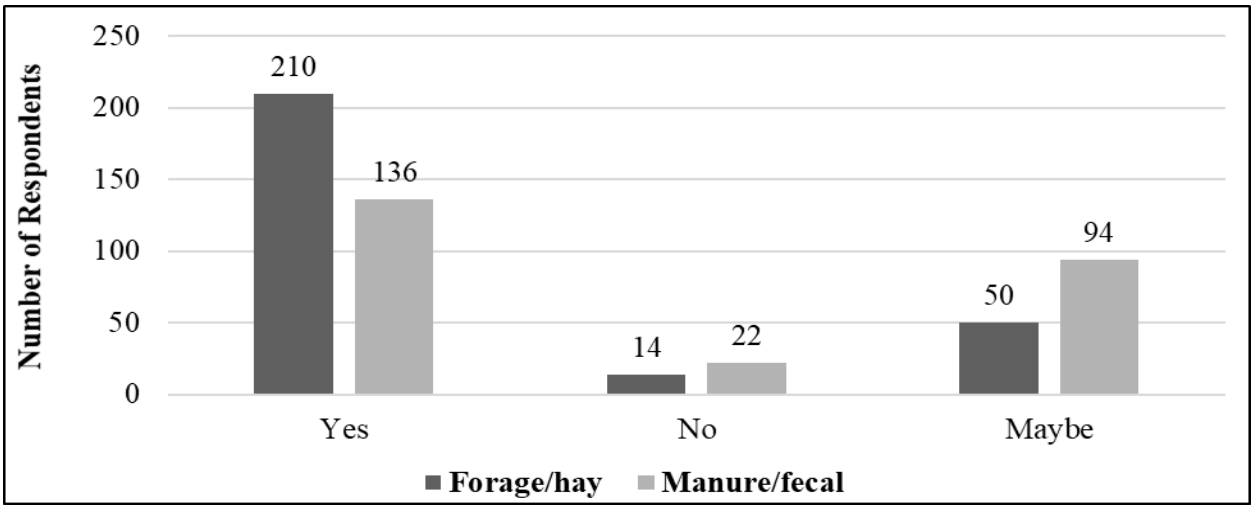

Figure 88. Summary of producer responses to would the option of submitting the following samples for nutritional testing be considered (Question #22) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. Forage/hay: *n* = 274, Manure/fecal: *n* = 252. Note: Responses may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

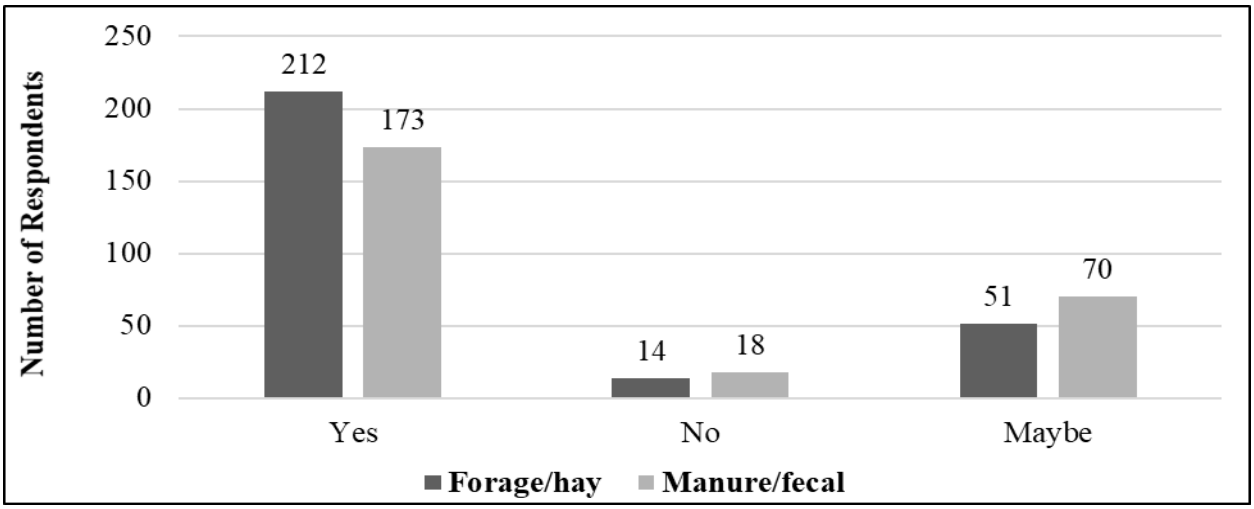

Figure 89. Summary of producer responses to if cost shares were available *(ex., from the Natural Resources Conservation Service's Program)*, would the following testing services be useful (Question #23) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019. Forage/hay: *n* = 277, Manure/fecal: *n* = 261. Note: Responses may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 24. Summary of producer responses to if forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used (Question #24) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

	Response					
	Forage Testing		**Hay Testing**		**Manure Testing**	
Use of Information	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Cull animals	10	3.3	9	3.0	15	4.9
Move animals to a different pasture	35	11.5	14	4.6	12	3.9
Supplemental feed purchase	60	19.7	87	28.5	17	5.6
Supplemental hay purchase	36	11.8	48	15.7	8	2.6
Did not use it for management decision	24	7.9	21	6.9	20	6.6
Other (Please explain):	3	1.0	4	1.3	1	0.3

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Producer Meetings (PM)

Background of Cattle Producers

Respondents from the five meetings surveyed for the PM audience (*n* = 386), ranging from 15 to 214 producers per meeting, indicated their place of residence by state was 99.7% from Texas and the remaining 0.3% were from Mississippi (Question #1, Part 2). Within the state of Texas, this producer audience represented 50 out of 254 counties across the state (B-12 in Appendix B). Across all states, 13.0% of respondents indicated being a cattle producer (Question #25) less than 10 years, 22.3% responded 11 – 20 years, 63.3% responded over 20 years, and 1.4% chose not to answer. The producer's primary role for the cattle portion of their operation (Question #4) is presented in Figure 90. Over 88.5% of responses to question #4 were owner and owner/manager.

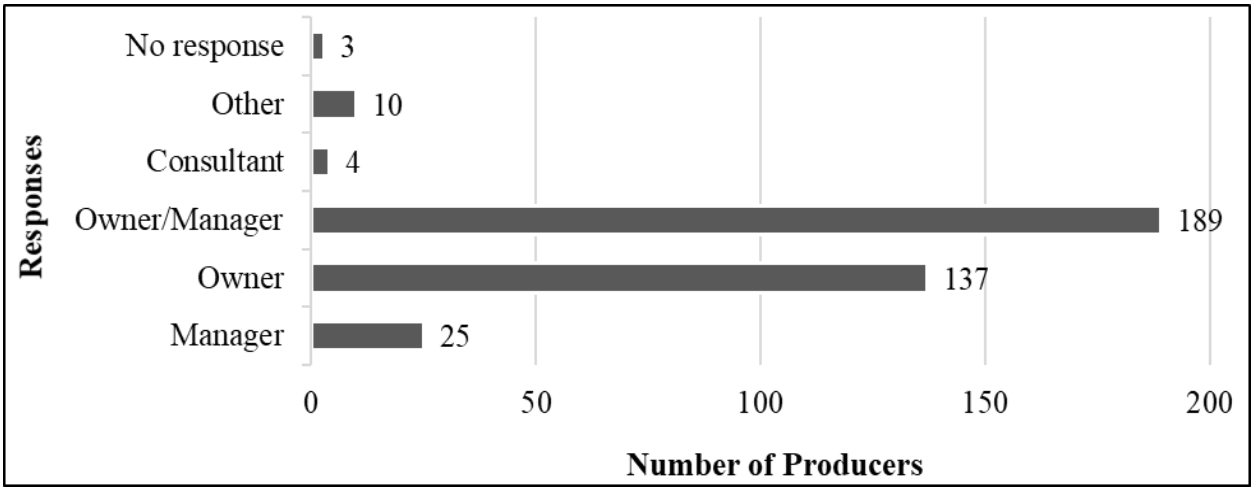

Figure 90. Summary of producer responses to the primary role for the cattle portion of the operation (Question #4) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. *n* = 368.

Beef Production Characteristics of the Respondents' Operation

Producer Meetings producer responses to the primary location of cattle by state was 98.9% from Texas and 1.1% did not respond (Question #2, Part 2). Within the state of Texas, this producer audience represented 55 out of 254 counties across the state (B-13 in Appendix B). Responses to indicate the approximate number of each type of free-ranging animals (cattle, other livestock, wildlife and exotics) managed on their property (Question #13) and these data are provided in B-14 – B-19 in Appendix B. From our findings, some beef cattle production systems might manage other animals on the same properties where cattle are located. These animals may include goats/sheep, horses/mules/donkeys, white-tailed deer, other cervids and exotics, and poultry.

Figure 91 summarizes audience responses to select the approximate number of cattle they manage by category (Question #5). Most producer respondents stated to manage 1 to 500 head of cattle. The type of pastured cattle production system that most reflected the respondent's operation (Question #6) was 80.2% for commercial/cow-calf (n = 295), with seed stock or replacement at 20.7% (n = 76), stocker/backgrounder at 7.1% (n = 26) and show stock at 5.2% (n = 19). Respondents characterized their cattle working facilities (Question #7) as 90.8% stationary facilities (n = 334), 12.0% portable facilities (n = 44), 6.3% weigh station/scales (n = 23), and 3.5% stated to have no facilities (n = 13).

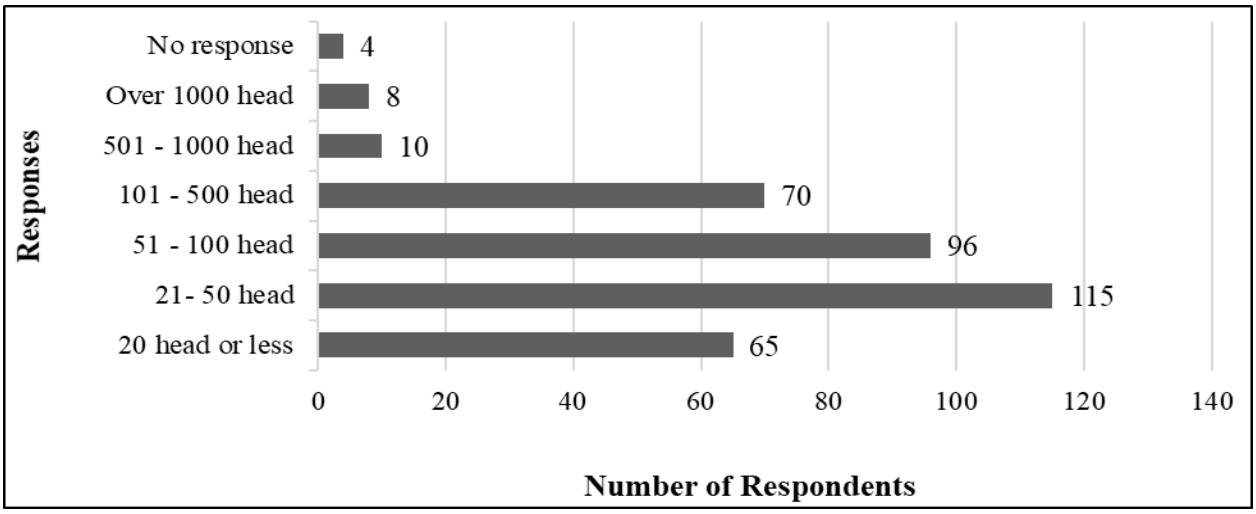

Figure 91. Summary of producer responses to the approximate number of cattle managed (Question #5) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. *n* = 368.

The approximate length of breeding seasons (Question #8) was 23.6% of 368 respondents for 1 – 3 months, 26.1% for 4 – 6 months, 47.6% for 7 – 12 months and 2.7% did not respond. The types of pasture on which cattle are grazed during each season (Question #11) is summarized in Table 25. Producers were asked to fill out a table indicating the timing of certain management practices (Question #14). Responses to question #14 are summarized in Tables 26 and 27. From our findings, producer responses to the timing of certain management practices shows that ectoparasite control is typically conducted when it is convenient to gather cattle for other routine management practices such as castration, vaccination, and endoparasite control. The top two responses to how cattle are evaluated for proper nutrition (Question #19) were body condition of the cattle and pasture conditions with forage/hay testing and weighing animals at a close tie for the top third response (Table 28).

Table 25. Summary of producer responses to the type of pasture on which cattle are grazed during each season (Question #11) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

	Season							
	Spring		Summer		Fall		Winter	
Type of Pasture	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Brushy/Shrubland	52	14.1	38	10.3	41	11.1	46	12.5
Improved	161	43.8	151	41.0	135	36.7	97	26.4
Mixed	133	36.1	113	30.7	112	30.4	107	29.1
Native	125	34.0	109	29.6	110	29.9	100	27.2
Annual Forages	47	12.8	16	4.3	28	7.6	58	15.8
Other (Please explain)	1	0.3	0	0	0	0	5	1.4

Note: Frequencies may not total stated *n* for Producer Meetings because respondents were asked to "answer all that apply".

Table 26. Summary of producer responses to the timing of following cattle management practiced for months January through June (Question #14) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

	Months											
	January		February		March		April		May		June	
Type of Cattle Management	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Castration	43	10.6	46	11.4	77	19.0	67	16.5	51	12.6	33	8.1
Vaccination	41	10.1	43	10.6	93	23.0	96	23.7	70	17.3	39	9.6
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*	20	4.9	22	5.4	72	17.8	81	20.0	87	21.5	78	19.3
Endoparasite Control *(ex. intestinal worms)*	20	4.9	16	4.0	72	17.8	74	18.3	45	11.1	34	8.4
Weaning Cattle	21	5.2	29	7.2	45	11.1	45	11.1	44	10.9	40	9.9
Weighing Cattle	5	1.2	4	1.0	12	3.0	12	3.0	11	2.7	12	3.0
Culling	27	6.7	25	6.2	45	11.1	39	9.6	43	10.6	30	7.4
Other (Please explain)	1	0.2	0	0	0	0	0	0	0	0	0	0

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 27. Summary of producer responses to the timing of following cattle management practiced for months July through December (Question #14) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

	Months											
	July		August		September		October		November		December	
Type of Cattle Management	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Castration	24	5.9	25	6.2	38	9.4	49	12.1	47	11.6	33	8.1
Vaccination	30	7.4	31	7.7	53	13.1	73	18.0	61	15.1	38	9.4
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*	71	17.5	60	14.8	80	19.8	68	16.8	45	11.1	21	5.2
Endoparasite Control *(ex. intestinal worms)*	21	5.2	19	4.7	46	11.4	67	16.5	46	11.4	19	4.7
Weaning Cattle	35	8.6	44	10.9	72	17.8	73	18.0	48	11.9	23	5.7
Weighing Cattle	7	1.7	11	2.7	20	4.9	20	4.9	9	2.2	6	1.5
Culling	25	6.2	27	6.7	60	14.8	70	17.3	50	12.3	24	5.9
Other (Please explain)	0	0	0	0	0	0	0	0	1	0.2	0	0

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 28. Summary of producer responses to how are cattle evaluated for proper nutrition (Question #19) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	***f*[a]**	**%**
Body condition	341	92.7
Forage/hay testing	43	11.7
Manure/fecal testing	20	5.4
Pasture conditions	213	57.9
Weigh animals	32	8.7
Other	3	0.8

Note: Frequencies may not total stated *n* for Producer Meetings because respondents were asked to "answer all that apply".

Ectoparasite Control Including Ticks

Questions 9, 10, 12, 13, and 15-18 address factors related to ectoparasite-host-pasture/range interactions and decision making for ectoparasite control. Producers responded (*n*

= 368) that 41.6% had a regular schedule for moving cattle between pastures, 54.3% did not, and 4.1% chose not to respond (Question #9). Producer responses (*n* = 368) show that 62.5% have a majority of pastures in their cattle production system with some type of shrub/brush coverage, 36.7% stated they did not, and 0.8% had no response (Question #10). Table 29 summarizes responses to whether brush control had been used as means of pasture management in the past two years (Question #12). Responses show that 64.9% (*n* = 239) have used brush control as a means of pasture management with 22.6% (*n* = 83) stating they have not. In reply to methods practiced for brush control (Question #13), producers responded with 4.1% for fire (*n* = 15), 68.8% for herbicide (*n* = 253), 54.3% for mechanical (*n* = 200), and 2.7% for other (*n* = 10).

Producer responses indicate that flies (352), ticks (54), lice (123), grubs (37), and other (3) were the ectoparasites causing problems in local cattle operations (Question #15). More than half of the respondents indicated (Figure 92) they gather cattle specifically to treat for ectoparasites (Question #16). The high-to-low order of responses to methods of ectoparasite treatment used in the previous year (Question #17) were pour-on, spray, ear tags, vet gun, other and dip (Figure 93). Producer responses are summarized in Table 30 to question #18 on how the need for ectoparasite treatment of cattle is determined. These data show that just over half make decisions by inspecting animals and/or observing ectoparasites on their animals.

Table 29. Summary of producer responses to has brush control been used as means of pasture management in the past two years (Question #12) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. *n* = 368.

Response	***f*[n]**	**%**
Yes	239	64.9
No	83	22.6
Not applicable to my operation	10	2.7
No Response	36	9.8

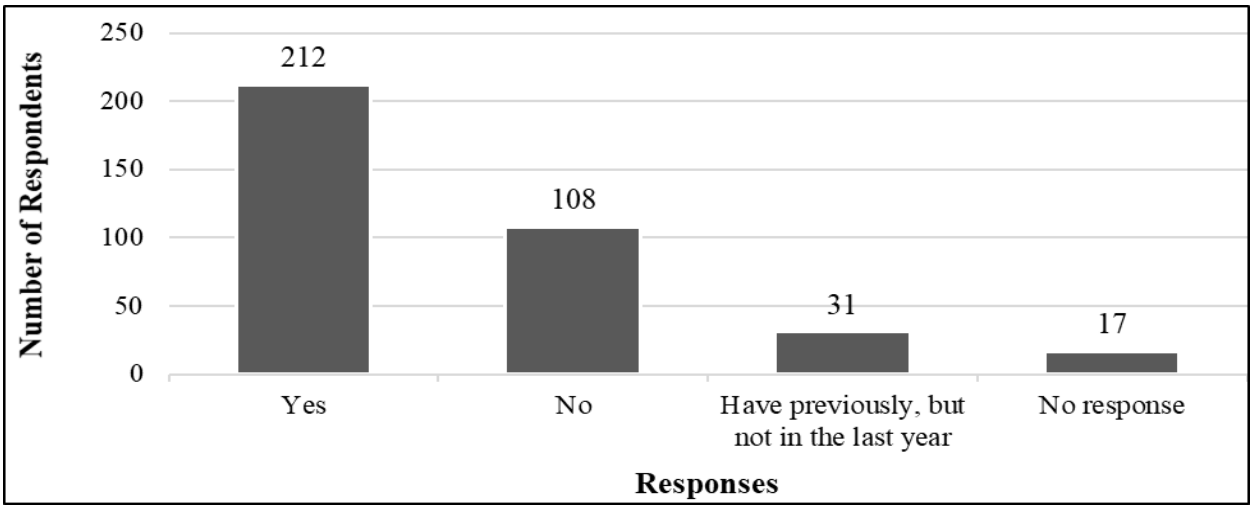

Figure 92. Summary of producer responses to are cattle ever gathered specifically to treat for ectoparasites (Question #16) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. *n* = 368.

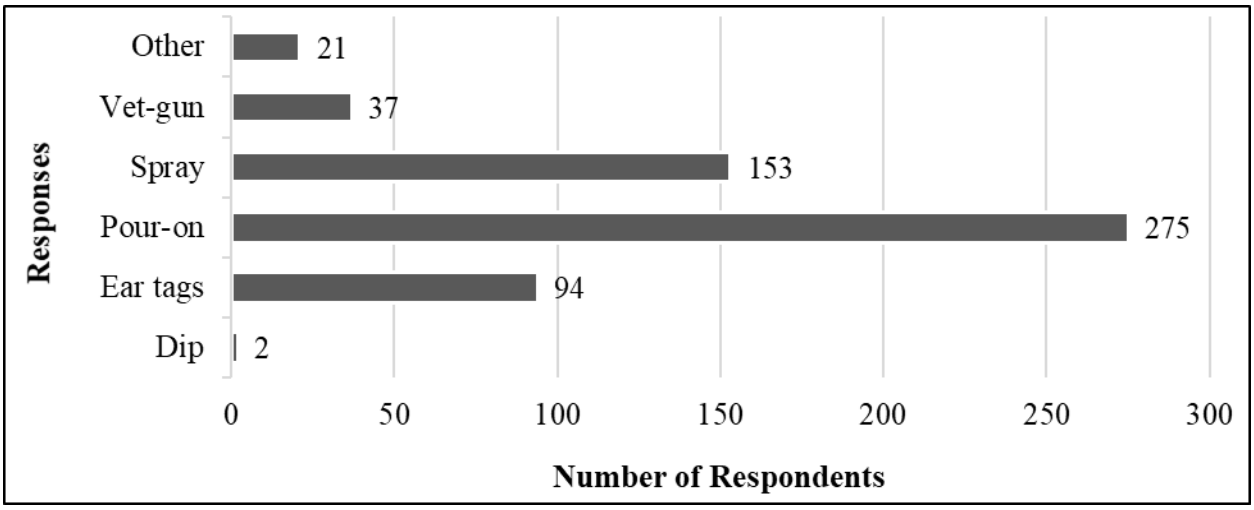

Figure 93. Summary of producer responses to what methods of ectoparasite treatment have been used in the past year (Question #17) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. Note: Responses may not total stated *n* for Producer Meetings because respondents were asked to "answer all that apply".

Table 30. Summary of producer responses to how is the need for ectoparasite treatment of cattle determined (Question #18) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	***f*[a]**	**%**
Changes in behavior	53	14.4
Changes in body condition	73	19.8
Convenience	100	27.2
Physical examination of cattle	149	40.5
Observe ectoparasites on cattle	166	45.1
Time of the year	87	23.6

Note: Frequencies may not total stated *n* for Producer Meetings because respondents were asked to "answer all that apply".

Use of Fecal NIRS Technology for Forage Assessment and Nutritional Balance

Producer responses (*n* = 368) indicated that 28.3% believe a manure/fecal test that could diagnose a tick infestation in cattle would be useful (Question #20), 20.1% stated no, 47.3% stated maybe, and 4.3% did not respond. In response to if producer's awareness of services available to them to test forage and hay for nutritional value (Question #21), producers (*n* = 386) responded yes at 68.5%, 23.1% stated no, and 8.4% chose not to answer. Figure 94 summarizes producer responses to "if the option of submitting forage/hay and/or manure/fecal samples for nutritional testing would be considered"? (Question #22). Producer responses to question #22 were forage/hay (213 producers) and manure/fecal (109 producers) for yes and maybe responses were forage/hay (94 producers) and manure/fecal (111 producers). Furthermore, Figure 95 summarizes producer responses to "if cost shares were available *(ex., from the Natural Resources Conservation Service's Program)*, would testing services be useful for forage/hay and/or manure/fecal samples?" (Question #23). Producer responses to question #23 were

forage/hay (218 producers) and manure/fecal (146 producers) for yes and maybe responses were forage/hay (85 producers) and manure/fecal (94 producers). Responses to "if forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used?" (Question #24) are provided in Table 31. Respondents to question #24 indicated that to rotate animals on pastures, supplemental feed purchase and supplemental hay purchase were the top three uses of information from forage testing (*n* = 24, *n* = 50, and *n* = 18, respectively) and hay testing (*n* = 12, *n* = 65, and *n* = 34, respectively). The top three uses of information from manure testing were culling animals (*n* = 7), rotating animals on pastures (*n* = 9), and supplemental feed purchase (*n* = 14).

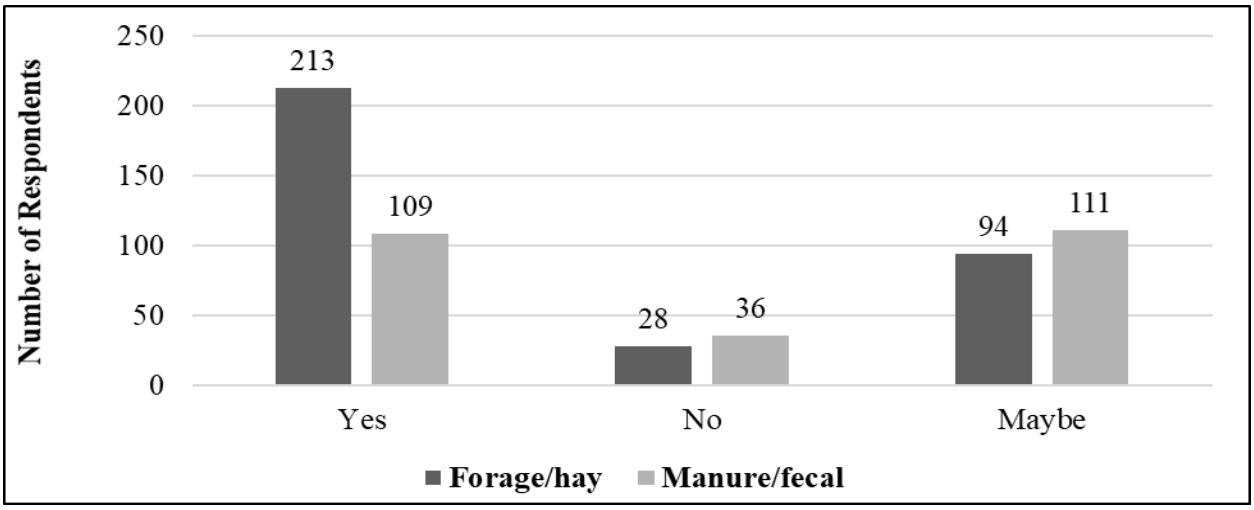

Figure 94. Summary of producer responses to would the option of submitting the following samples for nutritional testing be considered (Question #22) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. Forage/hay: *n* = 335, Manure/fecal: *n* = 256. Note: Responses may not total stated *n* for Producer Meetings because respondents were asked to "answer all that apply".

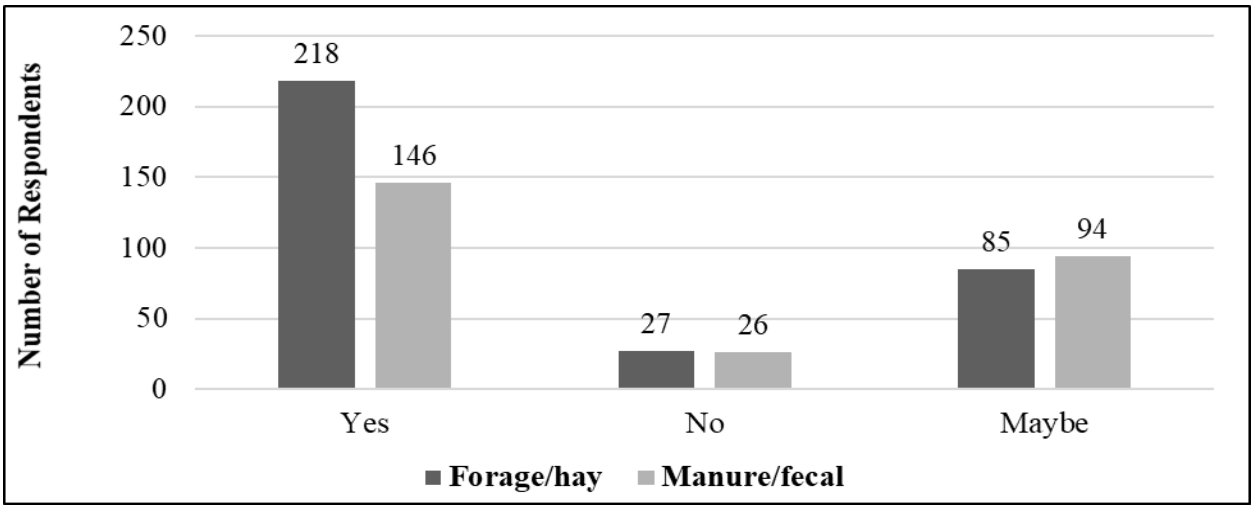

Figure 95. Summary of producer responses to if cost shares were available *(ex., from the Natural Resources Conservation Service's Program)*, would the following testing services be useful (Question #23) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018. Forage/hay: n = 330, Manure/fecal: n = 266. Note: Responses may not total stated n for Producer Meetings because respondents were asked to "answer all that apply".

Table 31. Summary of producer responses to if forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used (Question #24) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

	Response					
	Forage Testing		**Hay Testing**		**Manure Testing**	
Use of Information	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Cull animals	11	3.0	3	0.8	7	1.9
Move animals to a different pasture	24	6.5	12	3.3	9	2.4
Supplemental feed purchase	50	13.6	65	17.7	14	3.8
Supplemental hay purchase	18	4.9	34	9.2	3	0.8
Did not use it for management decision	28	7.6	26	7.1	22	6.0
Other (Please explain):	4	1.1	5	1.4	3	0.8

Note: Frequencies may not total stated n for Producer Meetings because respondents were asked to "answer all that apply".

Veterinarian Meetings (VM)

Background of Practitioners/Cattle Producers

Respondents at VM (*n* = 57) from two separate meetings ranging from 10 to 42 practitioners/producers indicated their place of residence by state was 94.7% from Texas and Oklahoma (5.3%) (Question #1, Part 2). Within the state of Texas, this audience represented 41 out of 254 counties across the state (Question #1, Part 1) (B-20 in Appendix B). Across all states, 19.3% of respondents indicated being a cattle producer (Question #25) less than 10 years, 24.6% responded 11 – 20 years, 49.1% responded over 20 years, and 7.0% chose not to answer. The practitioners/producers primary role for the cattle portion of their operation (Question #4) is presented in Figure 96. Responses to question #4 were primarily owner, owner/manager, consultant and other.

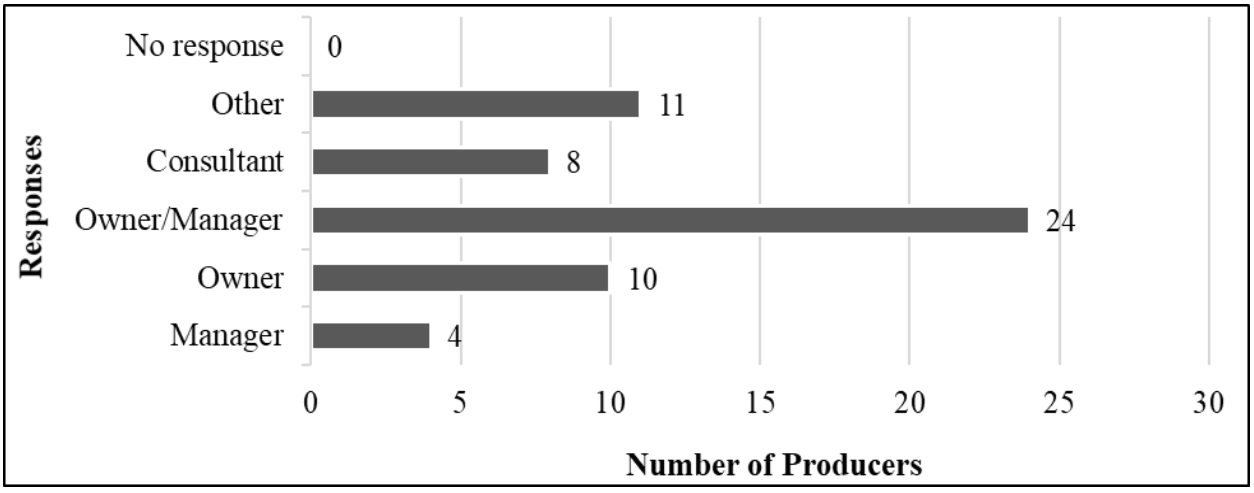

Figure 96. Summary of practitioner/producer responses to the primary role for the cattle portion of the operation (Question #4) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. *n* = 57.

Beef Production Characteristics of the Respondents' Operation

Veterinarian Meetings responses to the primary location of cattle by state was 93.0% from Texas and Oklahoma (7.0%) and 0% did not respond (Question #2, Part 2). Within the state of Texas, this audience represented 44 out of 254 counties across the state (Question #2, Part 1) (B-21 in Appendix B). Responses to indicate the approximate number of each type of free-ranging animals (cattle, other livestock, wildlife and exotics) managed on their property (Question #3) and these data are provided in B-22 – B-27 in Appendix B. From our findings, some beef cattle production systems might manage other animals on the same properties where cattle are located. These animals may include goats/sheep, horses/mules/donkeys, white-tailed deer, other cervids and exotics, and poultry.

Figure 97 summarizes audience responses by category to select the approximate number of cattle they manage (Question #5). Most practitioner/producer respondents stated to manage 1 to 500 head of cattle, with 22.8% responding over 1000 head. The type of pastured cattle production system that most reflected the respondent's operation (Question #6) was 66.7% for commercial/cow-calf (n = 38), with seed stock or replacement at 24.6% (n = 14), stocker/backgrounder at 28.1% (n = 16) and show stock at 22.8% (n = 13). Respondents characterized their cattle working facilities (Question #7) as 84.2% stationary facilities (n = 48), 33.3% portable facilities (n = 19), 19.3% weigh station/scales (n = 11), and 10.5% stated to have no facilities (n = 6).

Figure 97. Summary of practitioner/producer responses to the approximate number of cattle managed (Question #5) for the Veterinarian Meetings response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. *n* = 57.

The approximate length of breeding seasons (Question #8) was 40.4% of 57 respondents for 1 – 3 months, 19.3% for 4 – 6 months, 26.3% for 7 – 12 months and 14.0% did not respond. The types of pasture on which cattle are grazed during each season (Question #11) is summarized in Table 32. Practitioners/producers were asked to fill out a table indicating the timing of certain management practices (Question #14). Responses to question #14 are summarized in Tables 33 and 34. From our findings, practitioner/producer responses to the timing of certain management practices shows that ectoparasite control is typically conducted when it is convenient to gather cattle for other routine management practices such as castration, vaccination, and endoparasite control. The top two responses to how cattle are evaluated for proper nutrition (Question #19) were body condition of the cattle and pasture conditions with forage/hay testing and weighing animals at a close tie for the top third response (Table 35).

Table 32. Summary of practitioner/producer responses to the type of pasture on which cattle are grazed during each season (Question #11) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

	Season							
	Spring		Summer		Fall		Winter	
Type of Pasture	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Brushy/Shrubland	12	21.1	11	19.3	9	15.8	12	21.1
Improved	20	35.1	18	31.6	18	31.6	17	29.8
Mixed	14	24.6	13	22.8	11	19.3	10	17.5
Native	19	33.3	22	38.6	21	36.8	19	33.3
Annual Forages	7	12.3	3	5.3	8	14.0	9	15.8
Other (Please explain)	2	3.5	2	3.5	2	3.5	3	5.3

Note: Frequencies may not total stated *n* for Veterinarian Meetings because respondents were asked to "answer all that apply".

Table 33. Summary of practitioner/producer responses to the timing of following cattle management practiced for months January through June (Question #14) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

	Months											
	January		February		March		April		May		June	
Type of Cattle Management	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Castration	7	10.9	10	15.6	15	23.4	19	29.7	12	18.8	12	18.8
Vaccination	6	9.4	7	10.9	16	25.0	17	26.6	15	23.4	12	18.8
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*	4	6.3	6	9.4	13	20.3	18	28.1	18	28.1	18	28.1
Endoparasite Control *(ex. intestinal worms)*	3	4.7	4	6.3	16	25.0	18	28.1	11	17.2	13	20.3
Weaning Cattle	4	6.3	3	4.7	5	7.8	7	10.9	7	10.9	11	17.2
Weighing Cattle	7	10.9	7	10.9	11	17.2	9	14.1	9	14.1	10	15.6
Culling	5	7.8	5	7.8	12	18.8	7	10.9	7	10.9	9	14.1
Other (Please explain)	1	1.6	1	1.6	1	1.6	1	1.6	2	3.1	1	1.6

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 34. Summary of practitioner/producer responses to the timing of following cattle management practiced for months July through December (Question #14) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

	Months											
	July		August		September		October		November		December	
Type of Cattle Management	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**	***f***[a]	**%**
Castration	7	10.9	7	10.9	14	21.9	9	14.1	11	17.2	10	15.6
Vaccination	8	12.5	10	15.6	15	23.4	13	20.3	11	17.2	10	15.6
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*	11	17.2	12	18.8	19	29.7	15	23.4	11	17.2	10	15.6
Endoparasite Control *(ex. intestinal worms)*	7	10.9	8	12.5	18	28.1	15	23.4	12	18.8	8	12.5
Weaning Cattle	7	10.9	6	9.4	20	31.3	15	23.4	9	14.1	5	7.8
Weighing Cattle	10	15.6	9	14.1	15	23.4	13	20.3	8	12.5	6	9.4
Culling	6	9.4	9	14.1	14	21.9	14	21.9	10	15.6	9	14.1
Other (Please explain)	1	1.6	1	1.6	1	1.6	3	4.7	1	1.6	1	1.6

Note: Frequencies may not total stated *n* for Beef Cattle Short Course because respondents were asked to "answer all that apply".

Table 35. Summary of practitioner/producer responses to how are cattle evaluated for proper nutrition (Question #19) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	***f***[a]	**%**
Body condition	55	96.5
Forage/hay testing	14	24.6
Manure/fecal testing	4	7.0
Pasture conditions	35	61.4
Weigh animals	15	26.3
Other	1	1.8

Note: Frequencies may not total stated *n* for Veterinarian Meetings because respondents were asked to "answer all that apply".

Ectoparasite Control Including Ticks

Questions 9, 10, 12, 13, and 15-18 address factors related to ectoparasite-host-pasture/range interactions and decision making for ectoparasite control. Practitioners/producers

responded (*n* = 57) that 38.6% had a regular schedule for moving cattle between pastures, 56.1% did not, and 5.3% chose to not respond (Question #9). Practitioner/producer responses (*n* = 57) show 66.7% have a majority of pastures in the cattle production system with some type of shrub/brush coverage, 26.3% stated no, and 7.0% had no response (Question #10). Table 36 summarizes response to whether brush control had been used as means of pasture management in the past two years (Question #12). Responses show that 52.6% (*n* = 30) have used brush control as a means of pasture management with 21.1% (*n* = 12) stating they have not. In reply to methods practiced for brush control (Question #13), this audience responded with 1.8% for fire (*n* = 1), 49.1% for herbicide (*n* = 28), 38.6% for mechanical (*n* = 22), and 1.8% for other (*n* = 1).

Practitioner/producer responses indicate that flies (53), ticks (26), lice (33), grubs (11), and other (0) were the ectoparasites causing problems in local cattle operations (Question #15). More than half of the respondents indicated (Figure 98) they gather cattle specifically to treat for ectoparasites (Question #16). The high-to-low order of responses to methods of ectoparasite treatment used in the previous year (Question #17) were pour-on, ear tags, spray, other, vet gun, and dip (Figure 99). Practitioner/producer responses are summarized in Table 37 to question #18 on how the need for ectoparasite treatment of cattle is determined. These data show that just over half make decisions by inspecting animals and/or observing ectoparasites on their animals.

Table 36. Summary of practitioner/producer responses to has brush control been used as means of pasture management in the past two years (Question #12) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. *n* = 57.

Response	***f*[a]**	**%**
Yes	30	52.6
No	12	21.1
Not applicable to my operation	9	15.8
No Response	6	10.5

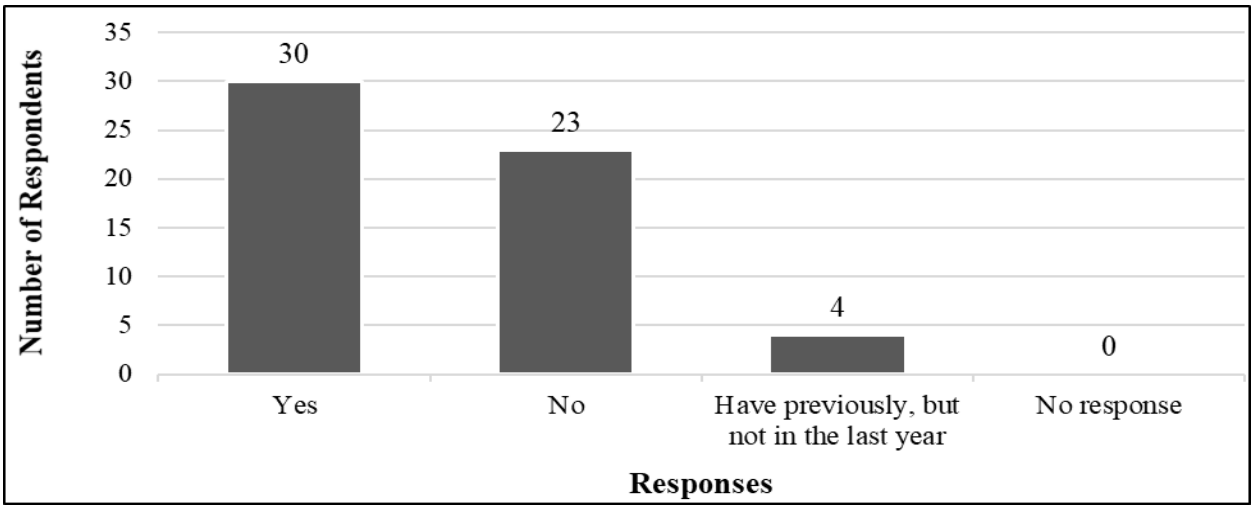

Figure 98. Summary of practitioner/producer responses to are cattle ever gathered specifically to treat for ectoparasites (Question #16) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. *n* = 57.

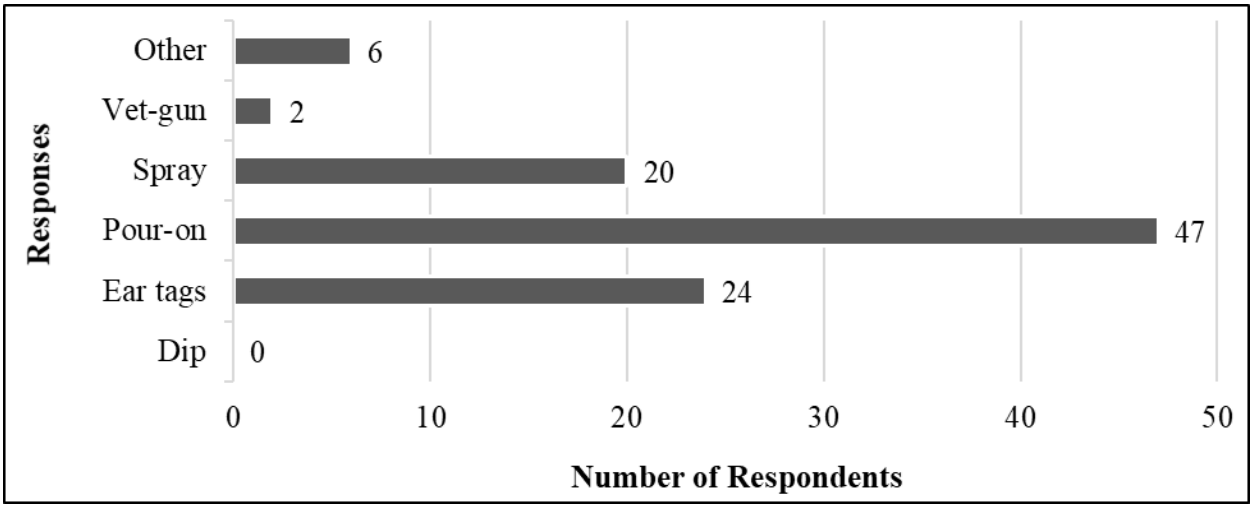

Figure 99. Summary of practitioner/producer responses to what methods of ectoparasite treatment have been used in the past year (Question #17) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. Note: Responses may not total stated *n* for Veterinarian Meetings because respondents were asked to "answer all that apply".

Table 37. Summary of practitioner/producer responses to how is the need for ectoparasite treatment of cattle determined (Question #18) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	***f*[a]**	**%**
Changes in behavior	8	14.0
Changes in body condition	7	12.3
Convenience	25	43.9
Physical examination of cattle	25	43.9
Observe ectoparasites on cattle	32	56.1
Time of the year	16	28.1

Note: Frequencies may not total stated *n* for Veterinarian Meetings because respondents were asked to "answer all that apply".

Use of Fecal NIRS Technology for Forage Assessment and Nutritional Balance

Practitioner/producer responses (*n* = 57) indicated that 31.6% believe a manure/fecal test that could diagnose a tick infestation in cattle would be useful (Question #20), 19.3% stated no, 43.9% stated maybe, and 5.3% did not respond. In response to if practitioners/producers awareness of services available to them to test forage and hay for nutritional value (Question #21), practitioners/producers (*n* = 57) responded yes at 84.2%, 10.5% stated no, and 5.3% chose not to answer. Figure 100 summarizes practitioner/producer responses to "if the option of submitting forage/hay and/or manure/fecal samples for nutritional testing would be considered"? (Question #22). Practitioner/producer responses to question #22 were forage/hay (39 respondents) and manure/fecal (24 respondents) for yes and maybe responses were forage/hay (9 respondents) and manure/fecal (22 respondents). Furthermore, Figure 101 summarizes practitioner/producer responses to "if cost shares were available *(ex., from the Natural Resources Conservation Service's Program),* would testing services be useful for forage/hay and/or

manure/fecal samples?" (Question #23). Practitioner/producer responses to question #23 were forage/hay (37 respondents) and manure/fecal (34 respondents) for yes and maybe responses were forage/hay (15 respondents) and manure/fecal (15 respondents). Responses to "if forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used?" (Question #24) are provided in Table 38. Respondents to question #24 indicated that to rotate animals on pastures, supplemental feed purchase and supplemental hay purchase were the top three uses of information from forage testing (n = 5, n = 12, and n = 5, respectively) and hay testing (n = 3, n = 15, and n = 9, respectively). The top use of information from manure testing was rotating animals on pastures (n = 2).

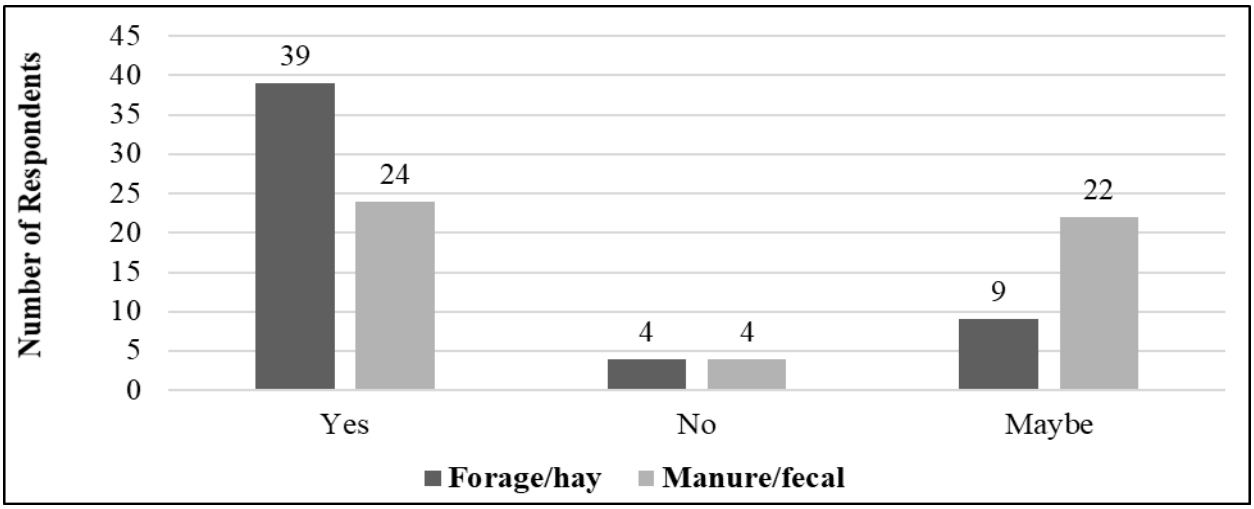

Figure 100. Summary of practitioner/producer responses to would the option of submitting the following samples for nutritional testing be considered (Question #22) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. Forage/hay: n = 52, Manure/fecal: n = 50. Note: Responses may not total stated n for Veterinarian Meetings because respondents were asked to "answer all that apply".

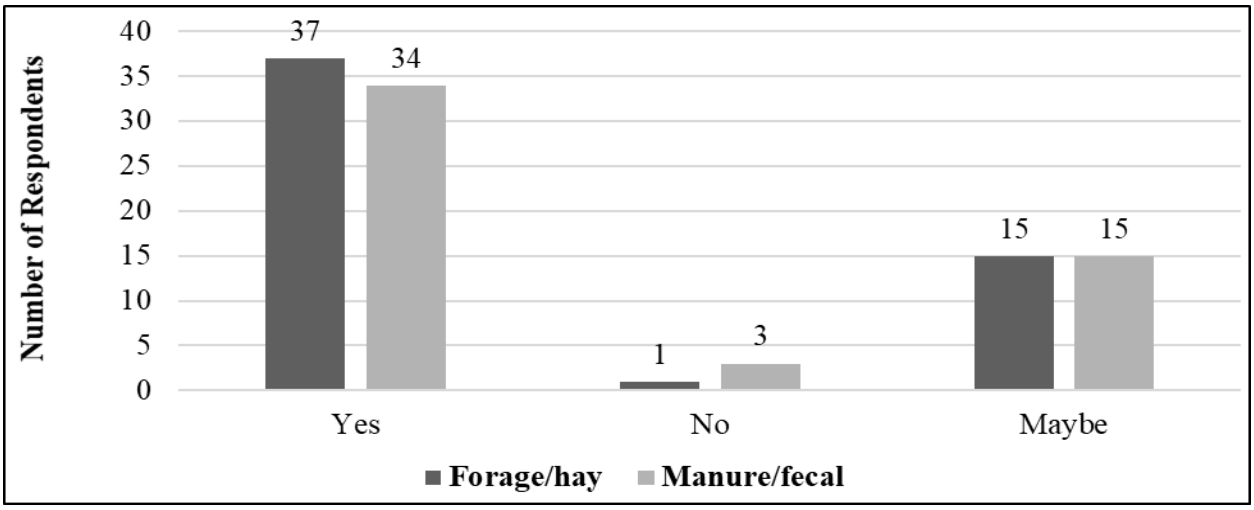

Figure 101. Summary of practitioner/producer responses to if cost shares were available *(ex., from the Natural Resources Conservation Service's Program)*, would the following testing services be useful (Question #23) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018. Forage/hay: *n* = 53, Manure/fecal: *n* = 52. Note: Responses may not total stated *n* for Veterinarian Meetings because respondents were asked to "answer all that apply".

Table 38. Summary of practitioner/producer responses to if forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used (Question #24) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

	Response					
	Forage Testing		**Hay Testing**		**Manure Testing**	
Use of Information	***f*[a]**	**%**	***f*[a]**	**%**	***f*[a]**	**%**
Cull animals	2	3.5	2	3.5	1	1.8
Move animals to a different pasture	5	8.8	3	5.3	2	3.5
Supplemental feed purchase	12	21.1	15	26.3	0	0
Supplemental hay purchase	5	8.8	9	15.8	0	0
Did not use it for management decision	4	7.0	4	7.0	6	10.5
Other (Please explain):	1	1.8	2	3.5	1	1.8

Note: Frequencies may not total stated *n* for Veterinarian Meetings because respondents were asked to "answer all that apply".

Discussion

There are a wide range of herd sizes, types of pastured production systems, ectoparasites, strategies for ectoparasite control, and use of adoptable technologies in beef cattle production systems. These factors, along with others, result in differences of a cattle producer's willingness to adopt technology in their operation. Data obtained from survey question responses from the BCSC, PM, and VM audiences provided valuable insights by using count data to explore the background of Texas beef cattle production systems and the willingness to adopt NIRS technology.

There was a broad geographical representation of Texas cattle producers that helped describe the different types of beef cattle production systems across the state. The primary location of cattle in Texas from all three survey audiences represented 136 out of the 254 counties in the state. Findings from this study indicate that some beef cattle production systems manage other animals on the same properties where cattle are located. The other animals may include goats, sheep, horses, mules, donkeys, poultry, white-tailed deer, and other cervids and exotics. While managing multiple animal species on the same properties is a common management technique, the diversity and abundance of tick hosts can positively influence tick populations, furthering the potential spread of ticks across cattle production systems.

The number head of cattle managed in a beef production system, also known as operation size, is a factor that may influence technology adoption (Johnson et al. 2010, Ward et al. 2008). Most respondents to this survey stated to manage 1 to 500 head of cattle, except in the VM audience, where 22.8% of respondents manage over 1000 head of cattle. Veterinarian Meeting respondents may have been using knowledge of their clientele base to answer survey questions, and where appropriate, their own operation. The basic characteristics of the cattle operations

provided through survey questions improved the understanding of how cattle producers may consider adopting technologies in their operations and whether it will benefit their overall production goals.

The type of pastured cattle production system may also influence willingness to adopt technologies (Elliott et al. 2013, Pruitt et al. 2012). The most frequent type of pastured production system in response to this survey was commercial/cow-calf operations, with seedstock or replacement and stocker/backgrounder being the next two most frequent. All of these production systems rely on working facilities to help maintain cattle herds for sorting, branding, castrations, vaccinations, and other routine management practices. From the three surveyed audiences, respondents stated to mainly have stationary facilities with a few reporting to have portable facilities and weigh stations/scales. Stationary facilities could make working cattle more difficult if the operation manages cattle that must be moved over long distances to the facilities. Movement of cattle causes additional stress on the animals aside from the stress already imposed on them during times of being worked through the facilities. Portable facilities and weigh stations/scales are costly to producers and could be considered a type of technology available for adoption. Cattle producers may benefit from the purchase of portable facilities and weigh stations/scales if it increases productivity and decreases the stress imposed on the animals.

It is essential for all cattle operations to conduct certain management practices to maintain a healthy herd. The three audiences surveyed in this study showed that the certain management practices provided in question #14 are conducted year-round rather than on a strict schedule for most of the operations. From the responses, ectoparasite control appears to be conducted when it is convenient for producers to gather cattle for routine management practices such as castration, vaccination, and endoparasite control. Treatments for ectoparasites applied

during general management tasks for prophylactic value are likely ineffective and costly. Survey responses from the three audiences show that cattle are evaluated for proper nutrition primarily by two means including assessing the body condition of cattle and pasture conditions; however, some respondents stated to also use forage/hay testing and the practice of weighing animals. These responses indicate that some survey respondents have already adopted forage/hay testing technology in their cattle production system. From these findings, producers and practitioners/producers may be willing to continue adopting more technologies such as NIRS in their cattle operations. Data suggest there is a willingness to actively engage in collecting, submitting, and interpret samples from forage/hay testing. The respondents who are already actively engaged in forage/hay testing may also being willing to actively engage in manure/fecal testing. Findings from Vestal et al. (2006) showed that larger producers (14%; herd sizes of 100 or more breeding females; percentage of household income from the beef enterprise in 2003 was greater than 40%) were significantly more engaged in forage testing for purchased hay than smaller producers (8%; herd sizes of 1-99 breeding females; percentage of household income from the beef enterprise in 2003 was between 1 to 40%). Vestal et al. (2006) concluded that the results may be related to costs, knowledge, and availability about forage testing, and that some producers might not know how to use the test results. Passive techniques such as observing pasture conditions and body condition of cattle requires less work and time. Active techniques such as forage/hay testing and manure/fecal testing requires the proper collection of samples, submission, interpretation of the findings, and a willingness to do something with the findings.

Regular schedules of pasture rotation can benefit cattle producers practicing ectoparasite control, as it reduces the host-finding rate of ectoparasites like ticks by changing host-density and availability. From all three audiences, over 38% of respondents stated to have a regular

schedule for moving cattle between pastures and more than 50% stated they have shrub/brush coverage in their production system. Tick habitat is dependent on shrub/brush coverage, and integrated pest management methods for ticks indicates that managing the covered habitats where ticks reside in the environment is important (Barnard et al. 1994, Williams 2010). Producer and practitioner/producer responses to how brush control is practiced showed that herbicides or mechanical means were the preferred methods over the option of fire. Previous studies have shown that the method of fire, also known as prescribed burning, is a known advantage of controlling some tick species (Drew and Samuel 1985, Gleim et al. 2014, Polito et al. 2013). Through incineration when a prescribed burn occurs, ticks can be killed directly (Polito et al. 2013). In a study conducted by Willis et al. (2012), the number of ticks collected one-year following a prescribed burn was relatively low (total of 25 ticks collected over six months), but tick numbers quickly increased within 2-5 years post-burn (60-110 ticks collected over six months). However, all three methods of brush control are viable options for removing dense vegetation, where suitable tick habitats and populations can be reduced (Teel et al. 2011).

External parasitism is a well-known issue in the livestock industry (Tolleson et al. 2015) and external parasites can cost livestock owners billions of dollars each year (Swiger 2012). Effects of ectoparasites documented on animal agriculture include loss of productivity and energy, stress and irritation, and the potential for pathogen transmission (Swiger 2012, Tolleson et al. 2015). Results from the three audiences surveyed in this study showed that the top three ectoparasites causing problems in local cattle operations were flies, ticks, and lice. Only one of these ectoparasites are easily noticed on animals (flies), the others (ticks and lice) require physical inspection of animals. More than half of all respondents from the three audiences indicated they gather cattle specifically to treat for ectoparasites which costs the producers in

time, labor, and facilities wear, not including the cost of animal stress. However, regular gathering and inspection of animals is unfeasible and expensive which costs cattle producers in time and money, impeding the adoption of IPM (Tolleson et al. 2015). Surveys from all audiences revealed that the top three responses to how the need for ectoparasite treatment is determined were convenience, physical examination of cattle, and observe ectoparasites on cattle, with time of year coming in fourth. All these responses are crucial in determining when to treat cattle and what ectoparasites to treat for to maximize efficacy of treatments and return on investment.

Collateral effects of ectoparasites include the cost of detection and treatment (Tolleson et al. 2007, 2015). Responses from all audiences indicated that ectoparasite treatment was mainly conducted with pour-on, spray, and ear tags. Method of treatment is particularly important as it can depend greatly on what ectoparasite is needing to be controlled and greatly depends on the time of year it is applied to correspond directly when the ectoparasites are seasonally active. Otherwise, treating for ectoparasites when it is convenient rather than when the ectoparasites are seasonally active would be ineffective and costly, and the treatments may also add to drug resistance (Whalon et al. 2008).

Adoption of technology, like non-invasive technologies such as NIRS, could help producers save overall cost, time, labor, and facilities wear. From the three audiences, responses to questions regarding the use of existing NIRS approaches for forage/hay and manure/fecal testing for nutritional decisions and proposed fNIRS for manure/fecal testing to detect tick infestations were positive. Majority of responses to these questions were either yes or maybe, indicating that there may be a willingness to engage in NIRS technology. The willingness might be gauged by the current usage of similar technologies that require producers to do sample

collection, submission, and interpretation of results, and the potential of cost support and tick control. As indicated by responses, producers may benefit from cost-share programs to aide in the initial cost of adopting NIRS technology and other technologies available to them. The respondents to this survey have previously adopted newer technologies for ectoparasite treatment and for forage or hay analysis. Adoption of technologies could be beneficial for cattle producers and their overall production goals.

The survey data provide the basis for future assessments to more completely identify the characteristics of those respondents who indicated "yes" or "maybe" to questions 20, 22, and 23. What is the background of the cattle producers who responded yes and maybe to the NIRS technology related questions? Are they cattle producers who stated to already use forage/hay and/or manure/fecal testing? Do they manage large (> 100 head) or small (< 100 head) herds of cattle? What type of beef cattle production system do they operate; commercial/ cow-calf, seedstock or replacement, stocker/backgrounder, show stock? Would one production system benefit from NIRS technology adoption over another? The future survey data assessments and pondered questions stated above will further help determine the need for educational programs and teaching seminars to get cattle producers actively engaged with NIRS technology, and the need for cost-share programs like the one provided by the USDA, NRCS as part of its Conservation Stewardship Program having nutrition monitoring as a practice. Educational programs and teaching seminars could be utilized through Texas A&M AgriLife Extension Services and the Texas A&M Annual Beef Cattle Short Course. Using these services could provide information to producers on the proper protocol for sampling as established by the GANLAB (https://cnrit.tamu.edu/cnrit/wp-content/uploads/2019/04/nutbal-info-packet-2018-

with-sample-sheets.pdf), how to interpret results, and what producers can do with the results in their production systems.

Future endeavors will be needed to move fNIRS technology to detect tick infestations from research to practice. Continuing to develop and demonstrate best practices for manure/fecal sampling will be important, as well as, educating individuals of the practice and application of the results. Near infrared reflectance spectroscopy technology could be an alternative method to improve IPM programs and surveillance, decision-making, and efficacy of acaricides for on-animal tick management. To conclude, responses from the three audiences to the 29-question survey entitled "Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems", suggest that producers are willing to adopt NIRS technology in their grazing cattle systems including a potential for the detection of tick infested animals.

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CHAPTER IV

LARVAL SURVIVORSHIP OF THE WINTER TICK, *Dermacentor albipictus* (ACARI: IXODIDAE), EXPOSED TO VARYING SATURATION DEFICITS IN THE LABORATORY

The winter tick, *Dermacentor albipictus* Packard (Acari: Ixodidae), is broadly distributed in North America (United States (US) and Canada) and can be found in the northern ranges of Mexico (Bishopp and Tremley 1945, Levin 2020). The winter tick parasitizes large ungulates including livestock, such as horses (Perissodactyla: Equidae; *Equus spp.*) and cattle (Artiodactyla: Bovidae; *Bos* spp.), and wildlife, such as moose (Artiodactyla: Cervidae; *Alces alces*), elk (Artiodactyla: Cervidae; *Cervus canadensis*), and white-tailed deer (Artiodactyla: Cervidae; *Odocoileus virginianus*). This tick species is active during fall (October-November), winter (December-February), and early spring (March) (Parish and Rude 1946, Strickland et al. 1976). The winter tick is recognized as one of approximately 12 species in Ixodidae that display a one-host life cycle in which all three developmental life stages blood-feed on a single large host (Guglielmone et al. 2010, Sonenshine and Roe 2014). The majority of these one-host tick species produce multiple generations each year, including *Dermacentor nitens* Neumann (Acari: Ixodidae), *Rhipicephalus annulatus* Say (Acari: Ixodidae) and *R. microplus* Canestrini (Acari: Ixodidae) (Barbosa et al. 1995, Cruz et al. 2020, Labruna and Faccini 2020, Walker et al. 2003), which are all found in Texas. *Dermacentor albipictus* has been described to complete one generation per year under natural conditions (Strickland et al. 1976).

Under field conditions in Texas, adult female winter ticks will fall off their host and lay eggs on the ground in protected micro-habitats during winter (December-February) or early spring (March) (Wright 1971). Larval ticks hatch in late winter and early spring (Wright 1969a).

Larvae remain inactive in clusters through the summer (Howell 1939; Wilkinson et al. 1982) to avoid desiccating environmental conditions (Yoder et al. 2016). The effects of short-day photoperiod and cool fall temperatures activate larvae to quest (ascend vegetation) for hosts in late September and October (Wright 1969b). The abundance of winter ticks to quest for hosts in the fall relies heavily on larval tick survival throughout the summer period, especially in warm southern climates like Texas.

Ixodid tick larvae are the most susceptible developmental stage to the effects of desiccating environments (Knülle and Rudolph 1982, Needham and Teel 1991, Ogden and Lindsay 2016, Teel et al. 2010, Trout Fryxell et al. 2015). Tick larvae have the highest surface area-to-volume ratio compared to nymph and adult ticks (Needham and Teel 1991, Yoder and Knapp 1999, Yoder et al. 2012), and respiration is trans-cuticular (no spiracles are present to regulate respiration rate) (Lees 1952). Water moves passively across the cuticle with respect to the surrounding environment; when the surrounding environment is water-vapor rich, water can be absorbed, but when it is water-vapor poor, there is net body water loss (greater out flow than in flow) (Belozerov and Seravin 1960; Browning 1954; Knülle 1966; Lees 1946, 1947, 1948; Needham and Teel 1991; Sauer and Hair 1971). *Dermacentor albipictus* larvae have the ability to absorb water vapor in moisture-rich environments as shown by results in Yoder et al. (2016) where larvae were able to survive for several weeks when exposed to 25°C and 85% relative humidity (RH). Concluded from mouth-blocking experiments in Yoder et al. (2016), *D. albipictus* larvae lack the ability to actively recuperate water from the air using salivary fluid (like the active water uptake mechanisms of nymphs, adults, and some larvae of other tick species), and they observed winter tick larvae exposed to water-stress environments would not "drink" liquid water when offered. Maintaining body water is essential to tick longevity during

the off-host period and ticks vary in abilities to compartmentalize water sources among different tissues and vary in abilities to recuperate water losses through active water update mechanisms (Needham and Teel 1991). The spatial and temporal distributions of habitats with suitable micro-environments combined with behavioral strategies to minimize water loss during off-host periods favor tick survivorship and population dynamics (Klompen et al. 1996; Needham and Teel 1986, 1991; Yoder et al. 2012; Yoder et al. 2016).

Body water in tick larvae is carried from that of the egg and is protected by the cuticle which acts as a passive barrier to water loss to the surrounding environment. Therefore, length of larval life is in part dependent upon maintaining water balance through all available means. Yoder et al. (2016) found that exposure to long-day (16 light (L):8 dark (D)) and short-day (8L:16D) photoperiod at 25°C and 93% RH impacted the ability of winter tick larvae to maintain water balance by measuring weight changes of individual larvae. From their findings, winter tick larvae held under a long-day photoperiod lost water at about half the rate as larvae held under a short-day photoperiod (Yoder et al. 2016). In the same study, winter tick larvae in long-day photoperiod switched to a short-day photoperiod, triggered to them become active from their dormant state and resulted in a higher water loss rate (Yoder et al. 2016). The conclusion from Yoder et al. (2016) was that *D. albipictus* larvae use dormancy (quiescence) in summer to reduce water loss and they will also utilize aggregations to conserve water. Forming aggregations increases the within cluster relative humidity and reduces water loss for individuals in the cluster (Benoit et al. 2007, Yoder et al. 1993), which could vary based on cluster size. Yoder et al. (2016) estimated that a cluster size of 50 individual winter tick larvae or greater is needed to accomplish this. The ability of *D. albipictus* larvae to conserve water through dormancy and

aggregation, as supported by Yoder et al. (2016), may increase their chance of survival in the more extreme environmental conditions during Texas summers.

There are two basic types of dormancy that are primary strategies in response to environmental stress (Belozerov 2008). The first is quiescence, which is considered an immediate response to unfavorable environmental conditions that ceases with the disappearance of the unfavorable environmental conditions (Gray et al. 2016, Kostal 2006). The second type is diapause, which can be characterized as a period of arrested development occurring seasonally before unfavorable environmental conditions ensue (Gray et al. 2016). Unlike quiescence, diapause is a fixed dormancy period that does not abruptly cease once unfavorable conditions disappear, thus it must be completed before resuming development (Bale and Hayward 2010, Tauber et al. 1986).

The most diverse type of dormancy is diapause. In ticks, diapause has been divided into behavioral diapause and developmental (morphogenetic) diapause (Belozerov 1971). Behavioral diapause, characterized for unfed ticks, involves an absence of activity and aggressiveness of unfed ticks to host-seeking (Belozerov 1971, 1982). Developmental diapause, characterized for engorged ticks, designates a delay in the development or morphogenetic processes of engorged ticks at a predetermined time which blocks essential steps in the development process that are presumed to be under hormonal control (Belozerov 1971, 1982). Behavioral diapause is the most common and is prevalent in association with a ticks' ability to survive long periods of time.

Dermacentor albipictus larvae have been categorized to enter a facultative behavioral diapause during summer months by Wright (1969b). The facultative behavioral diapause may be utilized by *D. albipictus* larvae to avoid host-seeking during an environmentally unfavorable time of year and has been noted to be induced and maintained by the influence of

photoperiodicity (Wright 1969b). In the laboratory, Wright (1969b) found that *D. albipictus* larvae kept at a long-day photoperiod of 16L:8D at 27°C and 80% RH, will enter diapause and refuse to attach to a host until they are six (four larvae), eight (four larvae), 10 (eight larvae), and 12 (31 larvae) weeks old. In contrast, *D. albipictus* larvae kept at a short-day photoperiod of 8L:16D for four weeks (49 larvae) at 27°C and 80% RH, will readily attach and feed on a host (Wright 1969b). Photoperiod plays an important role in the larval inactive period of *D. albipictus*, but it may also directly or indirectly influence the ability of larvae to maintain water. The summer inactive period has been given names including: a resting period (Bishopp and Wood 1913), a state of inactivity (Howell 1939, Yoder et al. 2016), diapause (Wright 1969b), dormancy (Cameron and Fulton 1926, Yoder et al. 2016), and quiescence (Drummond 1967, Yoder et al. 2016).

The *D. albipictus* inactive period may be regulated primarily by temperature and photoperiod. Findings from Holmes et al. (2018), suggest that *D. albipictus* larval survival is associated with hardiness at both low (-10 to -25°C) and high (35-46°C) temperatures. Temperature and photoperiod ultimately impact both the seasonal active period of questing and the willingness to attach and blood feed on an animal, and the inactive period where winter tick larvae reside in the litter layer and vegetation for months during summer months. Drew and Samuel (1985) outlined that under field conditions, the duration of inactivity *D. albipictus* larvae exhibit during the summer period can vary based upon geographical location: 2 weeks in Alberta (Drew 1984), 2-3 months in British Columbia (Wilkinson 1967), 3-6 months in Texas (Bishopp and Wood 1913), 4-5 months in Oklahoma (Patrick and Hair 1975), and 5-8 months in California (Howell 1939). Most recently, Terry (2015) stated that *D. albipictus* larvae remain inactive for three months in northeast Minnesota. These different durations of inactivity have various

physiological explanations as *D. albipictus* larvae react to the environmental conditions associated with their geographical locations. More research is needed to explain the relationships between photoperiod and environmental conditions *D. albipictus* larvae may be exposed to in Texas summer months, determine how the relationships might explain the ecology of this species, and whether they provide a basis for developing integrated tick management strategies for control of *D. albipictus*. The objective of this study was to compare the survivorship of *D. albipictus* larvae in two photoperiod groups (long-day and short-day) exposed to a range of saturation deficits.

Materials and Methods

Source of Larval Ticks

Larvae of *D. albipictus* (F_3 generation in colony) originated from 60 fed females (F_2 generation in colony) that were collected from six *Bos taurus* cattle artificially infested with *D. albipictus* during routine colony maintenance at the Tick Research Laboratory, Texas A&M AgriLife Research, College Station, Texas, US. This colony was established with ticks collected from cattle and horses in Dickens, King, and Knox counties, Texas, in 2016 and 2017. Colony maintenance and rearing on cattle for this study was in accordance with the Institutional Animal Care and Use Committee (IACUC)-approved Animal Use Protocol (AUP) No. 2017-0345.

Larval Tick Collection

The 60 fed females were placed together in a large petri dish and kept at 14L:10D, 25.0 ± 3.0°C and 80-85% relative humidity (RH) in sealed glass chambers for oviposition, egg hatching, and larval storage. One-week post-onset of oviposition, individual egg batches were carefully weighed on a Mettler electrobalance (Mettler AE163, Mettler Instrument Corporation, Highstown, New Jersey, US) to approximately 32.8 mg each. The weight of individual egg

batches was based on the average weight of a freshly laid *D. albipictus* egg equaling 65.6 µg (Wright 1971) and needing approximately 500 eggs per batch. Each individual egg batch was placed in a 4-dram glass vial (25 by 52 mm) with 25.4^2 mm polyethylene mesh placed at the open end to allow for proper air flow, and then all vials were returned to the sealed glass chamber environment to wait for larval hatch. For this experiment, larvae used were nine weeks post-hatch.

Experimental Treatments

Vials of *D. albipictus* larvae in the sealed glass chambers were randomly selected to be placed into two photoperiod groups (long-day and short-day) and six saturation deficit (SatDf) treatments with ten vial replicates per treatment and approximately 500 larvae per vial (Table 39). The two photoperiod groups used in this study were: Long-day (LD; 16L:8D) and Short-day (SD; 8L:16D). The LD and SD photoperiods were chosen for this study based on Wright (1969b), who previously demonstrated that 16L:8D is a "diapausing" photoperiod and 8L:16D is a "non-diapausing" photoperiod for *D. albipictus* larvae. The six SatDf treatments chosen for this study include: 1.88, 3.14, 6.28, 9.35, 11.68, and 14.02 mm of mercury (mmHg). Saturation deficits were calculated with the formula: *saturation deficit* = $\left(1 - (\frac{RH}{100})\right) \times 4.9463e^{(0.0621T)}$, as defined by Randolph and Storey (1999). Temperatures and RH used to obtain the six SatDf were: 15°C and 50, 75, and 85% RH, and 25°C and 40, 50, and 60% RH. These temperatures and RH were selected because preliminary range-finding experiments conducted personally in the Tick Research Laboratory at higher temperatures of 27°C and 37°C using the same RH (27°C and 50, 75, and 85% RH, and 37°C and 40, 50, and 60% RH) (SatDf ranging from 3.97 to 29.53 mmHg), resulted in larval survival times ranging from 14 days to 4 months. The lower range of SatDf treatment conditions provided by temperatures at 15°C and 25°C were selected to better assess

longer survival. A personal field study was conducted at the Texas A&M AgriLife Research Station in Sonora, Texas, USA, revealing that winter tick larvae can survive for eight months in open and closed canopy habitats exposed to a range of saturation deficits from 0.05 to 38.31 mmHg. Thus, the saturation deficits used in this laboratory experiment were comparable to those *D. albipictus* larvae were exposed to in Texas rangeland habitats.

Vials of larvae designated to the six SatDf in the SD photoperiod group were placed in a temperature-RH incubator (CMS EQUATHERM AMBI-HI-LO CHAMBER, CMS No. 213-330, Thermo Fisher Scientific, Waltham, Massachusetts, US) at 15°C, 85% RH, and short-day photoperiod (8L:16D) for 10 d before beginning the study to activate them from quiescence/ behavioral diapause (Wright 1969b). Similarly, vials of larvae dedicated to the six SatDf treatments in the LD photoperiod group were placed in a different temperature-RH incubator (CMS EQUATHERM AMBI-HI-LO CHAMBER, CMS No. 213-330, Thermo Fisher Scientific, Waltham, Massachusetts, US) at 25°C, 85% RH, and long-day photoperiod (16L:8D) for the same 10 d before beginning the study. Density.

Experimental Conditions and Instrumentation

Treatment conditions were achieved through a combination of twelve sealed plastic humidity containers (29.21L x 21.59W x 16.51H cm; 0.006 m^3 WEATHERTIGHT® Storage Box, IRIS USA, Surprise, Arizona, US) placed in temperature- and light-controlled incubators (CMS EQUATHERM AMBI-HI-LO CHAMBER, CMS No. 213-330, Thermo Fisher Scientific, Waltham, Massachusetts, US). Relative humidity was achieved and maintained using glycerol-distilled water solutions in the sealed plastic humidity containers (500 mL per container) (Johnson 1940). To inhibit microbial growth, twenty drops of a saturated copper sulfate ($CuSO_4$) solution was added to each 500 mL glycerol-distilled water solution (4 drops/100 mL) (ASTM

1983). Temperature-RH atmospheres inside the sealed plastic humidity containers were monitored with HOBO Temperature/RH Data Loggers (HOBO MX2301A, Onset Computer Corporation, Bourne, Massachusetts, US). Sealed plastic humidity containers were kept at a constant temperature of either 15°C or 25°C and allowed to reach thermal equilibrium for three d before the introduction of larval ticks in four temperature incubators (CMS Equatherm Ambi-Hi-Lo Chamber, CMS No. 213-330, Thermo Fisher Scientific, Waltham, Massachusetts, US) (Table 39). During this three-day period, all containers were monitored to assure we achieved the correct RH. The day that ticks were introduced into the twelve treatments was considered Day-0, where the number of dead larvae in each vial was recorded prior to placing the larval ticks in their respective treatments. Larvae in each of the twelve treatments were assessed for survivorship over time. Counts of dead larvae were assessed in response to human breath under a stereoscope (Olympus Corporation, Tokyo, Japan) every two weeks following Day-0 until all larvae were pronounced dead in each treatment. Indication of death included curled legs, lack of movement, and deflated opisthosoma (Yoder et al. 2018).

Table 39. Experimental conditions used in the study to measure *D. albipictus* larval survivorship including the comparative range of saturations deficits, temperature-relative humidities, photoperiod groups, number of replicates, approximate number of larvae per replicate and incubator number.

Saturation deficit (mmHg)	Temp (± 2°C) / RH (± 5%)	Photoperiod		Number of Replicates		Approximate Number of Larvae Per Replicate		Incubator Number	
		LD	SD	LD	SD	LD	SD	LD	SD
1.88	15 / 85	16L:8D	8L:16D	10	10	500	500	1	3
3.14	15 / 75	16L:8D	8L:16D	10	10	500	500	1	3
6.28	15 / 50	16L:8D	8L:16D	10	10	500	500	1	3
9.35	25 / 60	16L:8D	8L:16D	10	10	500	500	2	4
11.68	25 / 50	16L:8D	8L:16D	10	10	500	500	2	4
14.02	25 / 40	16L:8D	8L:16D	10	10	500	500	2	4

mmHg = millimeters of mercury, Temp = temperature, RH = relative humidity, LD = Long-day photoperiod group, SD = Short-day photoperiod group.

Statistical Analyses

All data analyses were obtained using the SAS v.9.4 software system (SAS Institute Inc., Cary, North Carolina, US) and non-parametric analyses. Statistical analyses were only conducted on larval survivorship in the four lowest SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) for both LD and SD groups because the larvae in the two highest SatDf treatments (11.68 and 14.02 mmHg) in both LD and SD groups died before the first observation on Day-14. The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in three separate analyses for the four lowest SatDf treatment groups in the LD and SD groups. The three separate analyses were: 1) comparison of all eight combinations of (LD group *versus* SD group) and the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg), 2) comparison of the four curves for the SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) separately for LD group and then SD group, and 3) comparison of the LD group *versus* SD group separately for each of the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg). Then, the Wilcoxon test of the equality of the eight curves ($P < 0.05$), resulting from the Kaplan-Meier product-limit estimator, was used to compare all eight combinations of (LD group *versus* SD group) and the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg). To evaluate the differences between Days to Death for LD and SD groups at the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg), the following four summary statistics were computed for each vial: 1) Days to 50% death, 2) Days to 75% death, 3) Days to 85% death, and 4) Average Days to Death. This yielded 10 replications for each of the eight combinations of LD *versus* SD and the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg). The 80 values for each of the four summary statistics were then analyzed using analysis of variance procedures ($P < 0.05$).

Results

Observations of Larval Activity

Dermacentor albipictus larvae in LD and SD groups in the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) were found in aggregations at either the top or bottom of the vials during every two-week observation. Once larvae were stimulated by human breath, they would disperse from the aggregation and begin moving around inside the vials. Counts of dead larvae were conducted while live larvae were actively moving around in the vial. When the stimulus of human breath was no longer present, the larvae were observed to go back into an aggregation. Over time, the longer larvae were exposed to the SatDf, the harder it was to stimulate them to become active.

Larval Survivorship Comparison of Long-day versus Short-day Photoperiod Groups

In both LD and SD groups at SatDf treatments 11.68 and 14.02 mmHg, no larvae survived to the first observation at Day-14. Statistical analyses were conducted on tick larval data from the four lowest SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) and both LD and SD groups (eight combinations). The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare all eight combinations of (LD group *versus* SD group) and the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) (Figure 102). The Wilcoxon test of the equality of the eight curves resulted in a *p*-value of less than 0.0001, which indicated highly significant differences in the eight curves. The product-limit survival estimates of the eight curves shows that as SatDf decreases, survival increases. The LD group had a higher survival time over the SD group for SatDf treatments 1.88, 6.28, and 9.35 mmHg. However, in the SatDf treatment 3.14 mmHg, there was a reversal, with the SD group resulting in a higher survival time over the LD group.

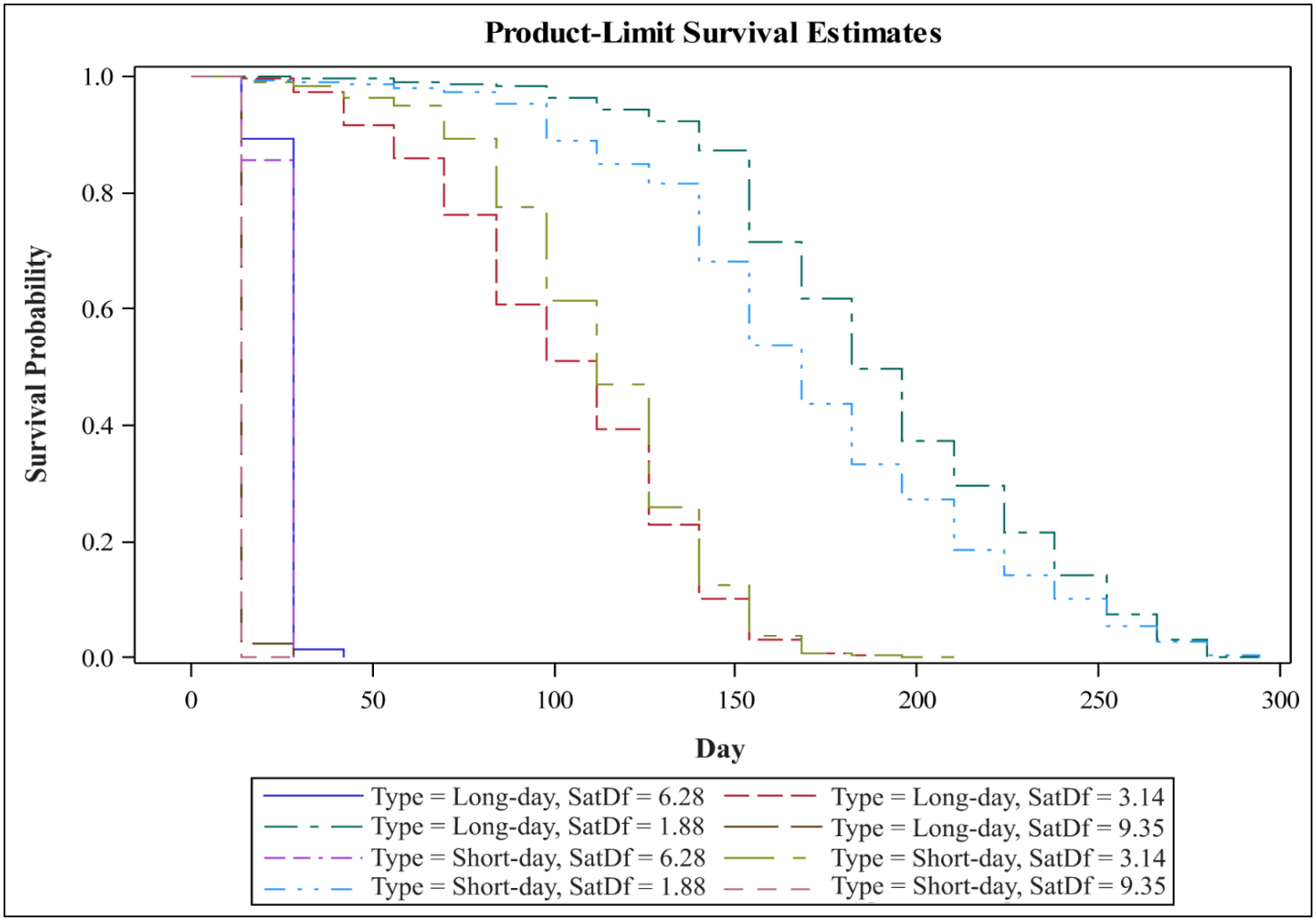

Figure 102. Comparison of survivorship of *D. albipictus* larvae subjected to either Long-day (16L:8D) or Short-day (8L:16D photoperiod and the four saturation deficit treatments (1.88, 3.14, 6.28, and 9.35 mmHg) placed in incubators 1, 2, 3, and 4. This figure shows survival probability by day for the eight curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per saturation deficit treatment.

Larval Survivorship Comparison of Long-day versus Short-day Photoperiod Groups Separately for the 1.88 mmHg Saturation Deficit Treatment

The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare LD *versus* SD groups separately for the SatDf treatment 1.88 mmHg (Figure 103). The Wilcoxon test of the equality was used to compare LD *versus* SD groups for the 1.88 mmHg SatDf treatment. The comparison had a *p*-value of less than 0.0001 which indicated highly significant evidence of a difference in the two curves. The LD (Long-day) curve is shifted to the right of the SD (Short-day) curve.

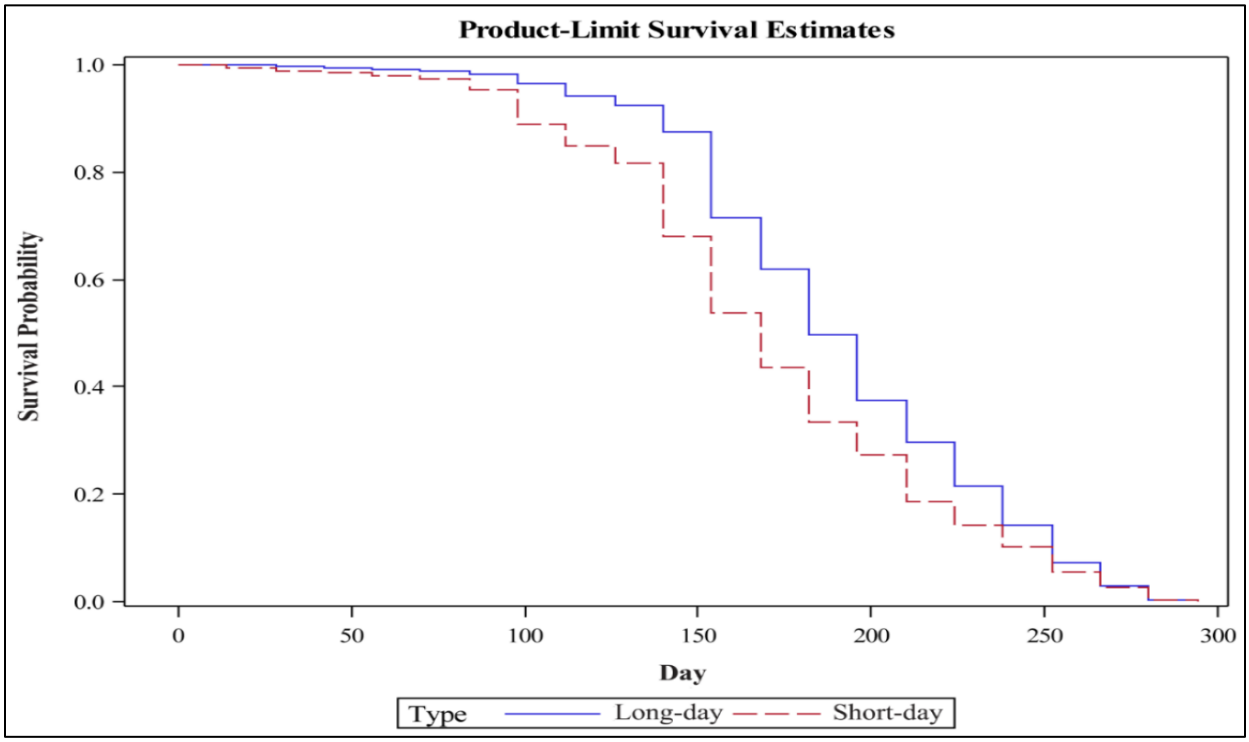

Figure 103. Comparison of survivorship of *D. albipictus* larvae subjected to either Long-day (16L:8D) or Short-day (8L:16D photoperiod in the 1.88 mmHg saturation deficit treatment placed in incubators 1 and 3, respectively. The figure shows survival probability by day for the two curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per photoperiod group.

Larval Survivorship Comparison of Long-day versus Short-day Photoperiod Groups Separately for the 3.14 mmHg Saturation Deficit Treatment

The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare LD *versus* SD groups separately for the SatDf treatment 3.14 mmHg (Figure 104). The Wilcoxon test of the equality was used to compare LD *versus* SD groups for the 3.14 mmHg SatDf treatment. The comparison had a *p*-value of less than 0.0001 which indicated highly significant evidence of a difference in the two curves although the plots do not reveal much of a difference. There is a more apparent difference in the two curves with the SD group appearing to have a higher survival time than the LD group larvae. That is, the SD (Short-day) curve is shifted to the right of the LD (Long-day) curve.

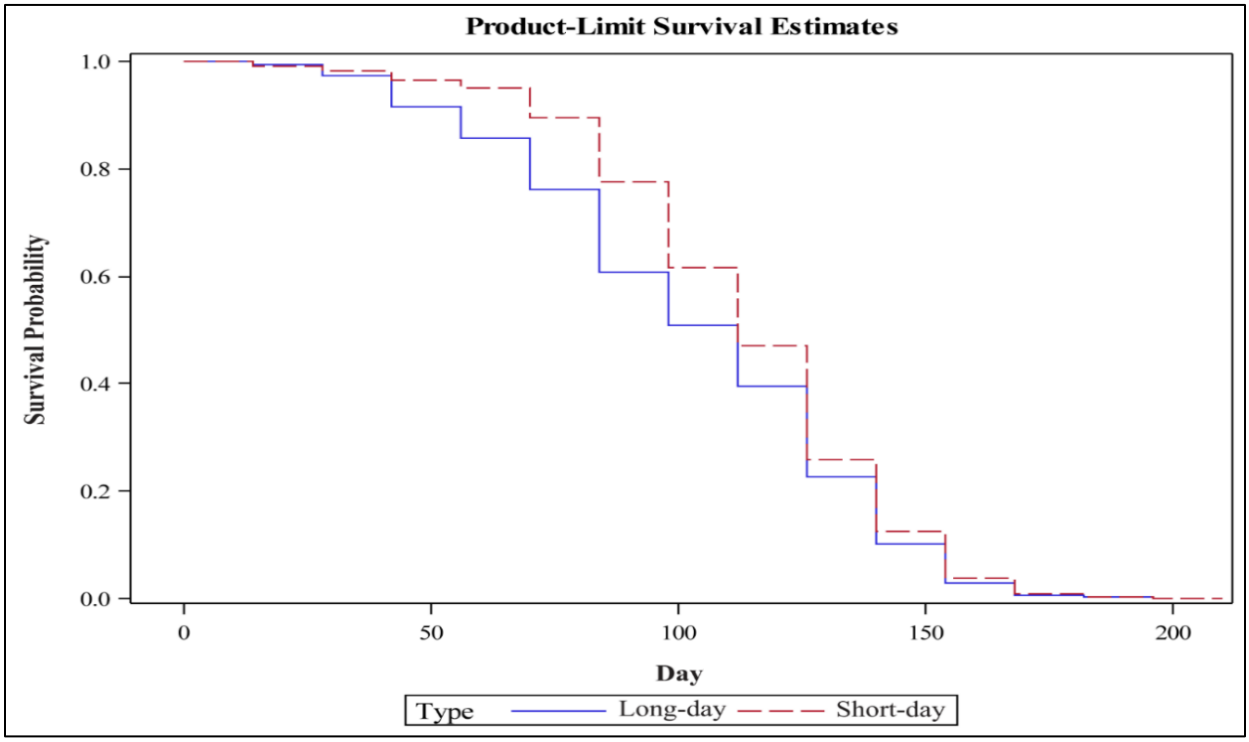

Figure 104. Comparison of survivorship of *D. albipictus* larvae subjected to either Long-day (16L:8D) or Short-day (8L:16D photoperiod in the 3.14 mmHg saturation deficit treatment placed in incubators 1 and 3, respectively. The figure shows survival probability by day for the two curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per photoperiod group.

Larval Survivorship Comparison of Long-day versus Short-day Photoperiod Groups Separately for the 6.28 mmHg Saturation Deficit Treatment

The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare LD *versus* SD groups separately for the SatDf treatment 6.28 mmHg (Figure 105). The Wilcoxon test of the equality was used to compare LD *versus* SD groups for the 6.28 mmHg SatDf treatment. The comparison had a *p*-value of less than 0.0001 which indicated highly significant evidence of a difference in the two curves although the plots do not reveal much of a difference. The LD (Long-day) curve is shifted to the right of the SD (Short-day) curve.

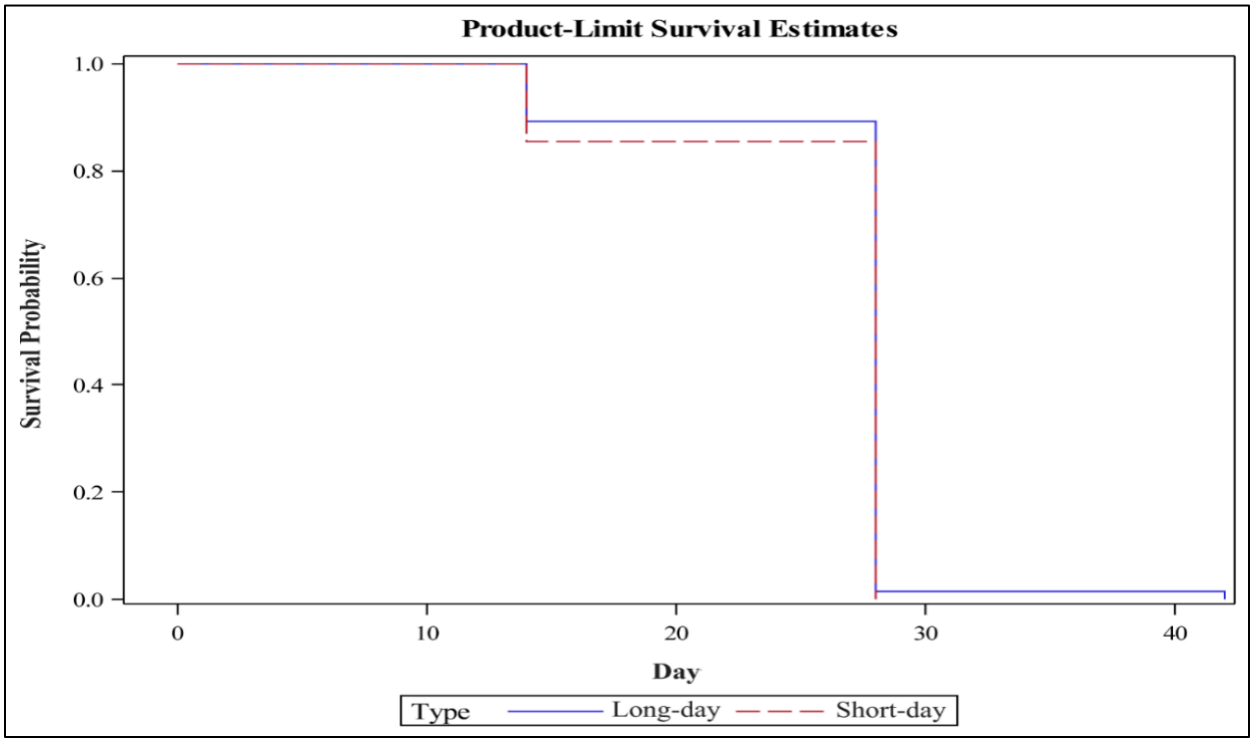

Figure 105. Comparison of survivorship of *D. albipictus* larvae subjected to either Long-day (16L:8D) or Short-day (8L:16D photoperiod in the 6.28 mmHg saturation deficit treatment placed in incubators 1 and 3, respectively. The figure shows survival probability by day for the two curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per photoperiod group.

Larval Survivorship Comparison of Long-day versus Short-day Photoperiod Groups Separately for the 9.35 mmHg Saturation Deficit Treatment

The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare LD *versus* SD groups separately for the SatDf treatment 9.35 mmHg (Figure 106). The Wilcoxon test of the equality was used to compare LD *versus* SD groups for the 9.35 mmHg SatDf treatment. The comparison had a *p*-value of less than 0.0001 which indicated highly significant evidence of a difference in the two curves although the plots do not reveal much of a difference. The LD (Long-day) curve is shifted to the right of the SD (Short-day) curve.

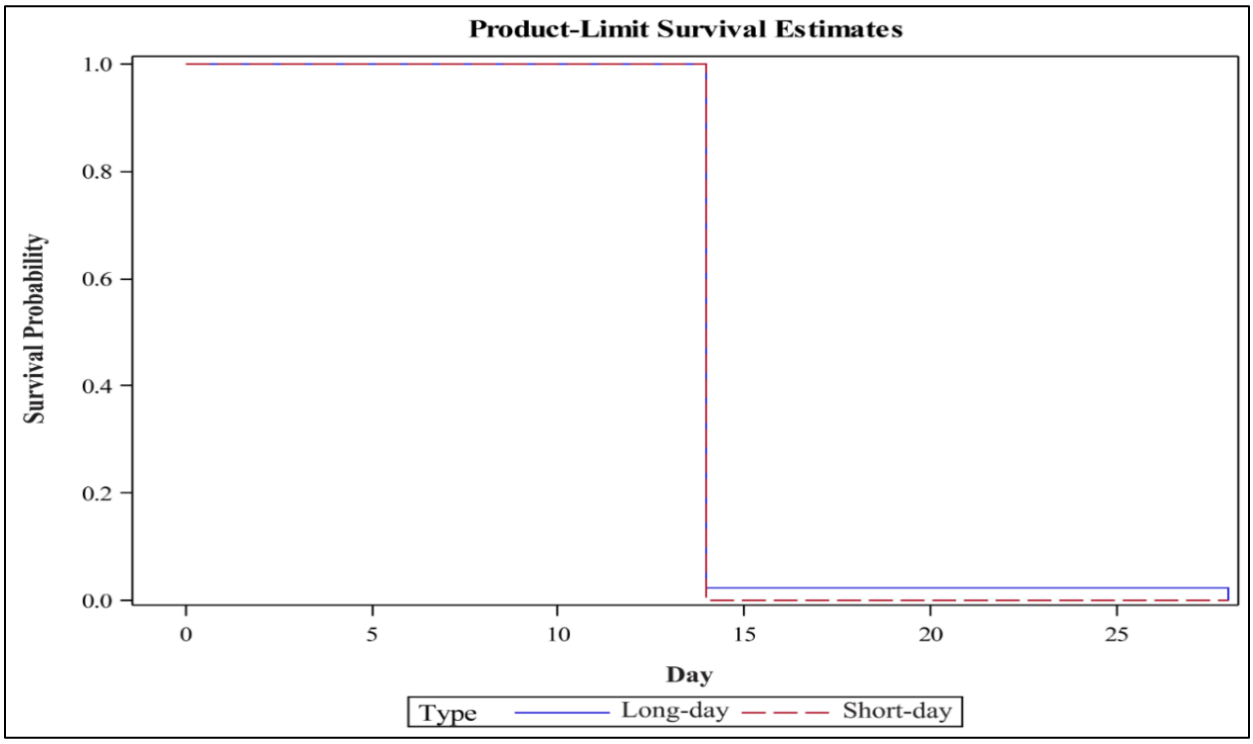

Figure 106. Comparison of survivorship of *D. albipictus* larvae subjected to either Long-day (16L:8D) or Short-day (8L:16D) photoperiod in the 9.35 mmHg saturation deficit treatment placed in incubators 2 and 4, respectively. The figure shows survival probability by day for the two curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per photoperiod group.

Larval Survivorship Comparison of the Four Saturation Deficit Treatments for the Long-day Photoperiod Group

The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare the four curves for the SatDf treatments 1.88, 3.14, 6.28, and 9.35 mmHg separately for the LD group (Figure 107). The Wilcoxon test of the equality was used to compare the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) curves for the LD group. The four SatDf treatment curves for the LD group had a *p*-value of less than 0.0001 which indicated highly significant evidence of difference in the four curves with the 9.35 mmHg SatDf treatment (25°C - 60%) curve showing a very short survival time. The three curves representing the SatDf treatments 1.88, 3.14, and 6.28 mmHg (temperature 15°C at 85, 75, and 50% RH, respectively) demonstrated higher survival as the temperature decreased and RH increased.

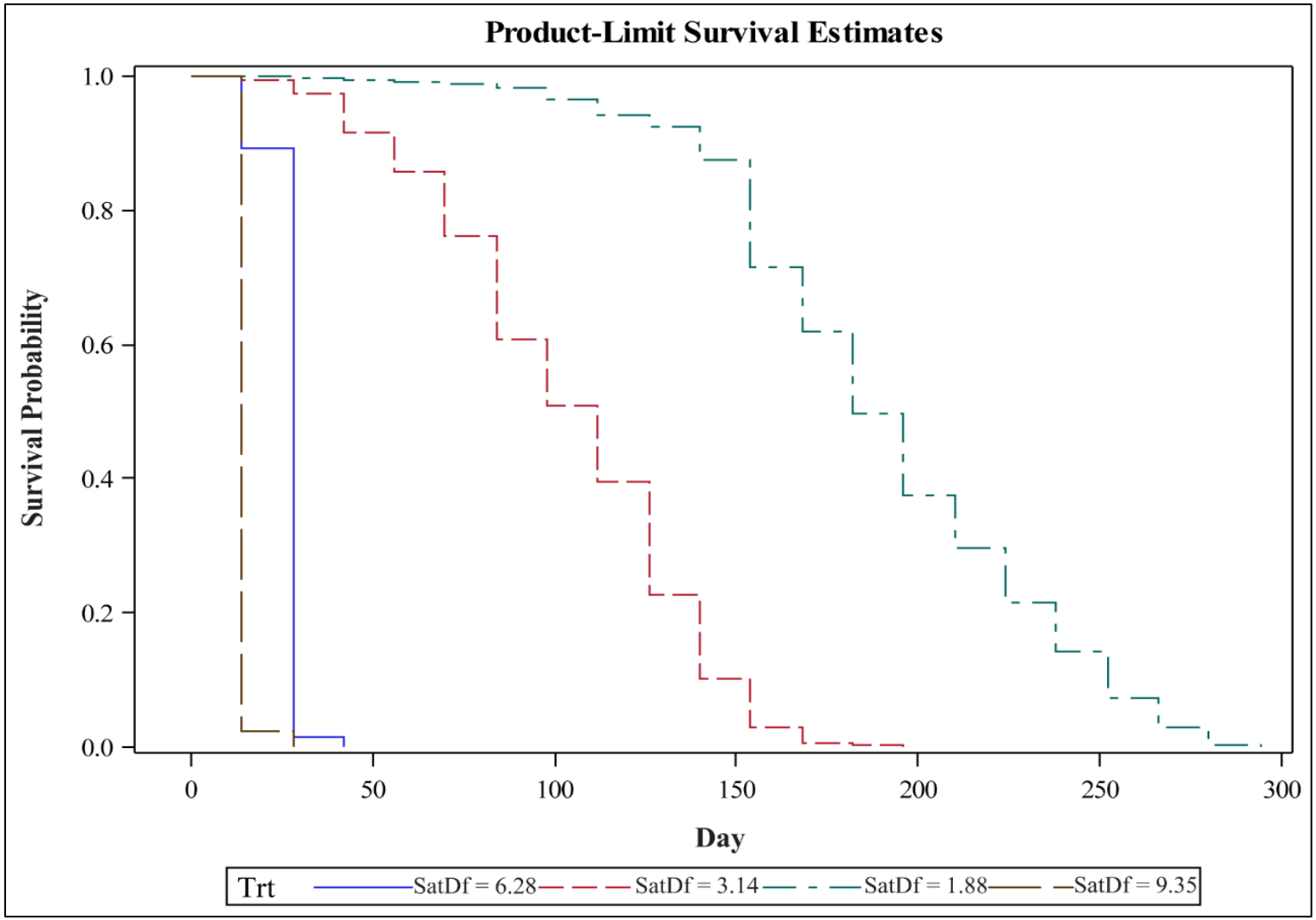

Figure 107. Comparison of survivorship of *D. albipictus* larvae subjected to Long-day (16L:8D) photoperiod and the four saturation deficit treatments (1.88, 3.14, 6.28, and 9.35 mmHg) placed in incubators 1 and 2. The figure shows survival probability by day for the four curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per saturation deficit treatment.

Larval Survivorship Comparison of the Four Saturation Deficit Treatments for the Short-day Photoperiod Group

The Kaplan-Meier product-limit estimator of the survival curve was applied to the tick larval data in the analysis to compare the four curves for the SatDf treatments 1.88, 3.14, 6.28, and 9.35 mmHg separately for the SD group (Figure 108). The Wilcoxon test of the equality was used to compare the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) curves for the SD group. The four SatDf treatment curves for the SD group had a *p*-value of less than 0.0001 which indicated highly significant evidence of difference in the four curves with the 9.35 mmHg SatDf treatment (25°C - 60%) curve showing a very short survival time. The three curves representing the SatDf treatments 1.88, 3.14, and 6.28 mmHg (temperature 15°C at 85, 75, and 50% RH, respectively) demonstrated higher survival as the temperature decreased and RH increased.

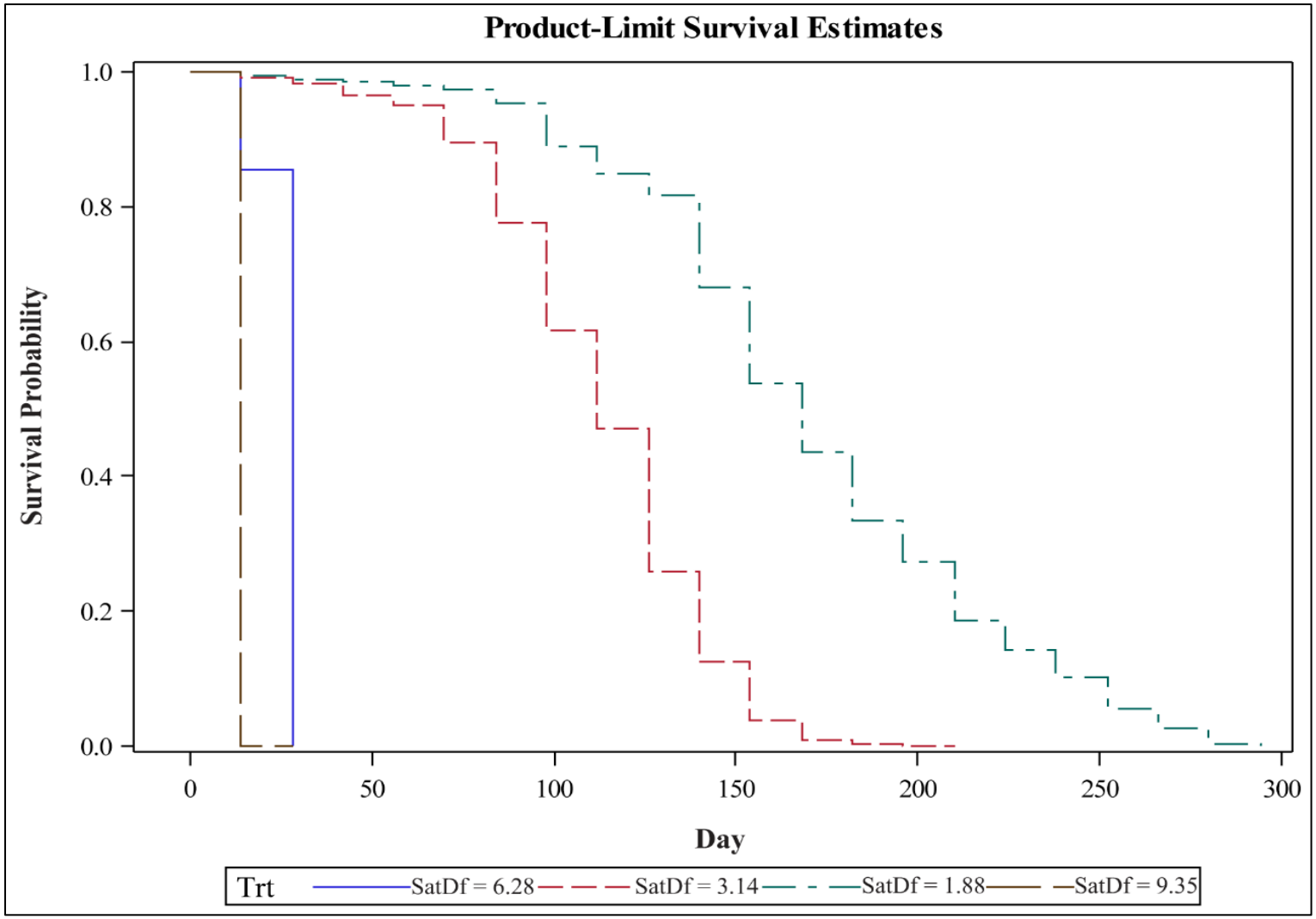

Figure 108. Comparison of survivorship of *D. albipictus* larvae subjected to Short-day photoperiod (8L:16D) and the four saturation deficit treatments (1.88, 3.14, 6.28, and 9.35 mmHg) placed in incubators 3 and 4. The figure shows survival probability by day for the four curves. Survival curves were the result of the Kaplan-Meier product limit estimator and the Wilcoxon test of the equality. Data are the mean of 10 vial replicates of approximately 500 larvae per saturation deficit treatment.

Summary Statistics of Larval Survivorship for Long-day versus Short-day Photoperiod Groups and the Four Saturation Deficit Treatments

Summary statistics were obtained for the survival times separated for each of the eight conditions: LD and SD groups at the four SatDf treatments (1.88, 3.14, 6.28, and 9.35 mmHg) (Table 40). The values obtained for the summary statistics are extrapolated from the Kaplan-Meier curves. The 1.88 SatDf treatment for the LD group resulted in 50% Death at 182 days, 75% Death at 224 days, and Max Death at 294 days. Whereas the 1.88 SatDf treatment for the SD group resulted in 50% Death at 168 days, 75% Death at 210 days, and Max Death at 294 days. In the 3.14 mmHg SatDf treatment, 50% Death occurred at 112 days for both LD and SD groups, while 75% Death occurred at 126 and 140 days and Max Death occurred at 196 and 210 days for the LD and SD groups, respectively. The 50 and 75% Deaths occurred at 28 days for the 6.28 SatDf treatment and both LD and SD groups, but Max Death for the SD group occurred at 28 days and 42 days for in LD group. For the 9.35 mmHg SatDf treatment, 50 and 75% Death occurred at 14 days and Max Death occurred at 28 days for both LD and SD groups.

Table 40. Summary statistics obtained for the survival times separately for Long-day (16L:8D) and Short-day (8L:16D) photoperiod groups and the four saturation deficit treatments (1.88, 3.14, 6.28, and 9.35 mmHg). The values in this table are extrapolated from the Kaplan-Meier curves. Data are the survival times of the 10 vial replicates of approximately 500 larvae per saturation deficit treatment in both Long-day and Short-day photoperiod groups. In this table, Days to 50% Death = 50% Death, Days to 75% Death = 75% Death, Days to 100% Death = Max Death, and Average Days to Death = Average.

Condition (Photoperiod Group)	Saturation Deficit (mmHg)	Temp (± 2°C) / RH (± 5%)	50% Death	75% Death	Max Death	Average
Long-day	1.88	15 / 85	182	224	294	190.71
Long-day	3.14	15 / 75	112	126	196	103.41
Long-day	6.28	15 / 50	28	28	42	26.71
Long-day	9.35	25 / 60	14	14	28	14.34
Short-day	1.88	15 / 85	168	210	294	170.83
Short-day	3.14	15 / 75	112	140	210	113.08
Short-day	6.28	15 / 50	28	28	28	25.96
Short-day	9.35	25 / 60	14	14	28	14.01

Discussion

The objective of this study was to compare the survivorship of *D. albipictus* larvae in two photoperiod groups (long-day and short-day) exposed to a range of saturation deficits. Data indicate that larval survivorship was influenced by photoperiod where larvae exposed to the LD photoperiod (16L:8D) tended to have a higher survival time than larvae exposed to the SD photoperiod (8L:16D). Data also indicate that survival is highly associated with saturation deficit or drying power of the environment. As saturation deficit decreased, larval survivorship increased regardless of photoperiod treatment. The 50% mortality for the 3.14, 6.28, and 9.35 mmHg saturation deficit treatments in both LD and SD groups occurred on days 112, 28, and 14, respectively. For the 1.88 mmHg saturation deficit treatment, the 50% mortality occurred on days 182 and 168 for the LD and SD groups, respectively. Average days to death tended to be longer for larvae in the LD group compared to larvae in the SD group except for the 6.28 mmHg saturation deficit treatment, where SD group larvae survived roughly 10 d longer than the LD group larvae.

Dermacentor albipictus larvae have a characteristic to protect them from high temperatures and low RH during the 3-6 month off-host period while in quiescence or behavioral diapause in summer. Survival at low and high temperatures offers potential benefits for survival during Texas summers, but is also heavily influenced by RH. One point to make is that this study was conducted in the laboratory, where larvae had no escape from the environmental conditions in which they were exposed. Under field conditions, the larvae can utilize the litter layer and vegetation as protection from unfavorable conditions as compared to this laboratory experiment where larvae were in glass vials and had no source of protection. Though this experiment was

conducted in the laboratory, the results showed that these larvae can survive extended periods of time in extreme environmental conditions.

Survival at varying saturation deficits offers potential benefits for survival during Texas summers. Data indicate winter tick larvae did not tolerate the two higher saturation deficit treatments 11.68 and 14.02 mmHg in both LD and SD photoperiod groups. This response is evident as all larvae in these saturation deficit and photoperiod groups failed to survive to the first observation on Day-14. At such low RH, desiccation would be very rapid regardless of temperature. In the 9.35 mmHg saturation deficit treatment the larvae held at both LD and SD photoperiods survived longer than 14 days but did not survive to 28 days. The saturation deficit treatment 6.28 mmHg (15°C - 50% RH) resulted in larvae exposed to LD photoperiod surviving longer than 28 days as compared to the SD photoperiod where all larvae died before Day-28. The saturation deficit treatment 1.88 mmHg showed a higher survival time for larvae exposed to the LD photoperiod over the larvae exposed to SD photoperiod. However, there was a reversal in survival time in the saturation deficit treatment 3.14 mmHg. The larvae in the SD photoperiod had a higher survival time over the larvae exposed to LD photoperiod. Despite temperature and photoperiod in the saturation deficit treatment 3.14 mmHg, it seems that larvae in both LD and SD photoperiods were simply trying to survive in an environment with a lower relative humidity (75% RH) that may promote desiccation for this tick species. Regardless of photoperiod group and temperature, when the RH was 60% or less, larval survivorship was reduced. This suggest that the determining factor in larval survival at the low RH was the humidity itself. Furthermore, larvae exposed to both LD and SD photoperiod in the saturation deficit treatments 1.88 and 3.14 mmHg, survived more than 280 d (comparable to approximately 10 months) and 182 d (comparable to approximately 6.5 months), respectively. Both of these survival times are longer

than the previously stated 3–6 month summer quiescent period utilized by winter tick larvae in Texas. Overall, the general result was that larvae exposed to the LD photoperiod generally promoted higher survival times over larvae exposed to the SD photoperiod. This may be due to what Yoder et al. (2016) has previously stated about *D. albipictus* larvae where exposure to a SD photoperiod increases the physical activity of larvae resulting in water loss and exposure to a LD photoperiod results in larval quiescence during summer promoting survival and water conservation.

Winter tick larvae are reliant on the micro-environment in the habitats where they reside in Texas to maintain water balance and survive throughout their summer quiescent period. Larvae of the three-host tick species, *Amblyomma americanum* L. (Acari: Ixodidae), are seasonally active during the summer period when winter tick larvae are quiescent. They have been compared to have one of the closest water loss rates in eggs and larvae to those of *D. albipictus* (Yoder et al. 2012). Both tick species require moisture-rich environments as larvae and are known to co-occur on the same landscapes in Texas (Teel et al. 1990). From the findings in this study and reports from Koch and Dunn (1980), unfed larvae of both *D. albipictus* and *A. americanum* have the ability to survive for weeks and months at varying saturation deficits in the laboratory.

Winter tick larvae must be able to sustain water balance when exposed to variable saturation deficits during both their inactive periods in the summer (LD photoperiod) and seasonally active periods when they are questing on vegetation (SD photoperiod). To maintain water balance when they quest on vegetation (SD photoperiod), winter tick larvae will use clumping/aggregation behavior (Wilkinson et al. 1982) to prevent desiccation (Yoder et al. 2016). Larval aggregation behavior in this experiment was observed in both LD and SD

photoperiod groups at all saturation deficit treatments that survived to longer than the first observation at Day-14. Once larval ticks were stimulated by human breath, they would disperse from the aggregation and begin moving around inside the vials and would soon go back into an aggregation after the stimulus was no longer present. Forming aggregations increases the within cluster relative humidity and reduces water loss for individuals in the cluster (Benoit et al. 2007, Yoder et al. 1993). The aggregation behavior observed in this study may have been a tactic the winter tick larvae used to prevent desiccation. However, whether the aggregations in the LD group was to prevent water loss, and aggregations in the SD group was like the behavior they exert during questing, is not known. The aggregations in the SD group could have also been used to prevent water loss as well but there is no way to quantify this; therefore, it remains an untested hypothesis.

The winter ticks larval quiescent period appears to begin before the onset of unfavorable environmental conditions where they will cease activity until favorable conditions resume. This is a common characteristic of behavioral diapause where unfed ticks remain inactive to avoid host-seeking during unfavorable conditions (Belozerov 1971, 1982) and relates back to a ticks' ability to survive long periods of time. Therefore, it cannot be ruled out that the larval quiescent period during summer could also be categorized as a facultative behavioral diapause. This behavioral response has also been shown to occur in the three-host tick, *A. mixtum* Koch (formerly known as *A. cajennense* Fabricius; Acari: Ixodidae), where unfed larvae will enter a behavioral diapause under LD photoperiods (>12 h) to avoid host-seeking during unfavorable conditions in summer (Labruna et al. 2003). It was stated that the larval behavioral diapause of *A. mixtum* may ensure that the subsequent adult ticks will actively seek a host during periods of higher rainfall rates and temperature; therefore, promoting more favorable micro-environments

for oviposition and egg incubation (Labruna et al. 2003). The same characteristics of *A. mixtum* must be employed by *D. albipictus* to ensure survival of the next generation. Nevertheless, the findings from this study, these larvae can survive for six to 10 months to a range of saturation deficits comparable to environmental conditions *D. albipictus* larvae may be exposed to in the different vegetational habitats they reside in during Texas summers. Overall, findings from this study and future studies conducted on *D. albipictus* larvae could provide a basis for developing integrated tick management strategies for control of this tick species.

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CHAPTER V

CONCLUSIONS AND FUTURE WORK

Research findings related to each objective provided foundational information related to *D. albipictus* biology and ecology with respect to environment-host-parasite interactions, and background information on Texas grazing cattle systems. Collectively, these results may guide future studies to determine the best integrated pest management (IPM) strategies for control of *D. albipictus* and the use of fecal near infrared reflectance spectroscopy (fNIRS) to detect tick infestations in grazing cattle systems. Results may also guide extension program development to demonstrate best practices on new approaches and technologies that improve tick control.

Fecal NIRS technology was used to detect spectral changes in daily fecal samples from *D. albipictus* infestation periods (larval, nymphal, and adult feedings) as different from two pre-infestation control periods (outside and inside) for heifer pairs in three-tick infestation level treatment groups. The principal components analysis conducted on raw daily spectral data from both trials provided evidence that the five cluster shifts displayed in the stepwise cluster analyses were significantly different between the two pre-infestation control periods (outside and inside), the three tick infestation periods (larval, nymphal, and adult feedings), and the post-tick recovery period. Future work will include the continuation of testing the sensitivity and feasibility of fNIRS technology to detect animals infested with *D. albipictus*, improve IPM adoption by using fNIRS technology to detect tick-infested livestock, decision-making, and efficacy for tick management.

Beef cattle producer audience surveys to assess producer adoption of NIRS technology will be further evaluated to more completely identify the characteristics of those respondents

who indicated either “yes” or “maybe” to questions about potential adoption. Future research will examine producer characteristics that make them more likely to adopt NIRS technology. Characteristics such as past use of feed testing, herd size, type of cattle, production system, and demographic factors will be examined as important factors for adoption. Research results may help design best practices for delivering fNIRS technology and complimentary educational programs and teaching seminars supporting producer engagement and practices. Educational programs and teaching seminars could be utilized through Texas A&M AgriLife Extension Services and the Texas A&M Annual Beef Cattle Short Course. Using these services could provide information to producers on the proper protocol for sampling as established by the GANLAB, how to interpret results, and what producers can do with the results in their production systems. Continuing to develop and demonstrate best practices for manure/fecal sampling will be important, as well as, educating individuals of the practice and application of the results.

Over summer survivorship of *D. albipictus* larvae is a critical link in year-to-year infestations of this tick species to ensure the survival of future generations. Project results showed that these larvae can survive for six to 10 months to a range of saturation deficits comparable to environmental conditions they might be exposed to in the different vegetation habitats during Texas summers. To further understand how winter tick larvae prevent desiccation and survive during Texas summers, future work should include studies conducted under field conditions to provide more information on the biology, life cycle, and habits of *D. albipictus*.

Future Needed Research

The research in the dissertation suggests a number of future research needs. Several ongoing projects were not included in the dissertation but are related and conclusions will further

extend the research. Ongoing work is being conducted tracking the efficacy of acaricide treatments on grazing cattle using fNIRS technology with field collected data from two geographical locations in Texas and one in Oklahoma. Under real world conditions, animal hosts acquire ticks throughout the year and depending upon grazing behavior through tick-infested habitats an animal may also acquire multiple tick species and accumulate multiple stages of three-host ticks feeding at the same time. Therefore, future work to test the specificity of fNIRS technology to single and multiple tick species infestations and their interactions with dietary changes in grazing animals. Further research will also be conducted to assess the use of gas chromatography-mass spectroscopy to investigate the relative increase and decrease of long-chain fatty acids and sterols discovered during a preliminary investigation of the daily fecal samples collected from the fNIRS study conducted in this dissertation.

A field study was conducted from April 2019 through March 2020 observing *D. albipictus* engorged females placed in open and closed canopy in range habitats at the Texas A&M AgriLife Research Station, Sonora, Texas. Data from this field study were collected once a month and include: 1) female oviposition, 2) egg hatch, 3) larval survival, 4) monthly larval activity, 5) fall date of first larval questing behavior, and 6) micro- and macro-environmental data. Forthcoming analysis and interpretations are expected to show which Texas rangeland vegetation communities are more suitable for *D. albipictus* survival and when they become seasonally active to quest for animal hosts. Conducting research experiments on larval survivorship in field settings and observing tick abundance and seasonal activity on animal hosts, could benefit grazing production systems and the owners/managers of the operations by providing control options for *D. albipictus*.

On-host animal observation studies over two years at the Texas A&M AgriLife Research Station, Sonora, Texas have been conducted to determine abundance and seasonal activity of winter ticks. Animals observed in these studies included: heifers, sheep, white-tailed deer, and axis deer. A second study examined *D. albipictus* abundance and seasonal activity on bulls for two years in northwest Texas in the Rolling Plains region. It was found that this tick species is very abundant through the months October to April in both locations on the animal hosts surveyed. It may be advantageous to employ tick traps and conduct tick dragging near vegetation communities in both locations where on-host animal observations have been conducted to look for residing *D. albipictus* larvae or determine how ticks are redistributed by animal hosts during the active season. Overall, studies conducted on *D. albipictus* larvae could provide a basis for developing integrated tick management strategies for control of this tick species.

Integrated Pest Management for Control of the Winter Tick

A holistic management approach (Walker and Stachecki 1996, Prokopy and Kocan 2003) that addresses the entire tick system would benefit IPM tactics for *D. albipictus*. The holistic approach should include five important tactics: 1) biosecurity, 2) habitat/brush management, 3) forage/pasture management, 4) wildlife management, and 5) on-animal application of acaricides (Barnard et al. 1994, Williams 2010). First, biosecurity involves the isolation of new animals that were purchased to add to or rebuild a herd as they present a risk to the introduction of ticks and tick-borne pathogens. Measures for biosecurity should be exercised at the purchase or introduction of livestock to an operation including considerations of treating livestock for internal and external parasites including ticks, and even taking measures to quarantine newly purchased animals before introducing them into a herd (Russell et al. 1981, Walker and Stachecki 1996).

Second, habitat/brush management can negatively impact tick populations by limiting vegetation habitats that support tick development and survivorship. Principal goals of brush management are to increase forage production and control invasive woody species (Archer et al. 2011, Russell et al. 1981). This tactic decreases soil moisture and tick micro-habitat humidity and decreases wildlife host utilization. The three main types of brush management include herbicides, mechanical removal, and prescribed fire. Herbicide application removes woody plant species and herbaceous vegetation inherently reducing the off-host survival of some tick life stages (Teel et al. 2011, Wigley et al. 2002). Mechanical removal of brush clears the dense vegetation helping reduce tick habitat and populations. Also, trimming tree branches and shrubs allows more sunlight into the environment which can reduce suitable tick habitats and tick survival. Prescribed fire, also known as prescribed burning, is a known advantage of controlling some tick species and internal parasites (Drew and Samuel 1985, Gleim et al. 2014, Polito et al. 2013, Teel et al. 2011, Willis et al. 2012). When a prescribed burn in implemented, ticks can be killed directly through incineration (Polito et al. 2013). Prescribed burns that are slow moving can generate temperatures necessary to physically damage or kill ticks on improved pastures and rangelands (Teel et al. 2011). Additional benefits of prescribed fire include the removal of undergrowth, leaf-litter, and shrub vegetation which renders the soil-vegetation interface less hospitable for tick survival while off the host (Teel et al. 2011, Willis et al. 2012).

Third, forage/pasture management should include grazing or pasture rotations and fencing (Russell et al. 1981, Williams 2010). Grazing or pasture rotations changes the availability of animal hosts to ticks actively searching for a host. Fencing can be used to prevent or limit access by stray animals and certain wildlife species onto properties that can serve as tick hosts (Eisen and Stafford 2020). Furthermore, property owners should monitor the perimeters of

their fences to observe any breaches that may offer wildlife entry onto the property (Teel et al. 2011). Fourth, wildlife management would include the control of wildlife hosts of certain species from the livestock environment. Managing wildlife populations (e.g., white-tailed deer and feral swine) to recommended levels for the owners' operation would minimize the availability of them to serve as tick hosts.

Lastly, the application of acaricides on-animal hosts focuses on tick suppression during a brief period of the tick life cycle when seasonally active ticks are obtaining a blood meal on the host animal. Acaricide applications should not be used as a cure all solution for tick control and management. There are many important decisions to be considered to achieve maximum value in tick suppression using acaricides. These decisions involve: 1) choice of acaricide active ingredient (chemical class), 2) formulation, 3) method of delivery, and 4) timing of application. Active ingredients (chemical class) include Organophosphates, Carbamates, Pyrethrins/synthetic Pyrethroids/Amadines (Peter et al. 2005, Swiger 2012, Teel et al. 2011, Williams 2010). Formulations and application type for cattle consist of sprays (use enough water to cover the animal thoroughly to run-off), dips (effective, ensures good coverage by wetting the animal thoroughly), pour-on (applied down the backline; chemical absorbed and circulated through animal's system), insecticide-impregnated ear tags (plastic device in animal's ear that dispenses acaricide over time), dusting powder (hand shakers or self-treatment dust bags), wettable powder (can be formulated into a spray), and aerosol spray (spray onto ticks in/outside of ear) (Swiger 2012, Teel et al. 2011, Williams 2010). It is important for cattle producers to always read and follow the manufacturer's label recommendations concerning safety restrictions, dosage, and application when working with acaricides. The timing of application relies on what tick species needed to be controlled and when that tick species is seasonally active (obtaining a bloodmeal

from the host). Frequent and continuous application of chemicals on animal hosts is not sustainable on disease, environmental, and economic grounds (Peter et al. 2005).

Integrated tick management should include multiple tactics in an integrated strategy consistent with, and supportive of livestock production goals. Cattle producers should manage local populations of ticks for minimal influence on the surrounding environment and non-target organisms. There is not a cure all for controlling ticks on livestock (or any external or internal parasites), thus cattle producers should use a holistic tick management approach.

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APPENDIX A

A-1. Basic components of pelleted creep feed from three samples of the pelleted feed from the same lot with components listed on the right. Percentages are given where applicable. Megacalories per pound (Mcal/lb) and parts per million (ppm) are used where appropriate.

	Percentages and International Units per Pound (IU/lb)			
Components	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Crude Protein (%)	18.3	19.4	18.3	18.2
Acid Detergent Fiber (%)	21.8	23.1	21.3	23.8
TDN-based on ADF (%)	73.5	72.3	74.0	71.7
Net Energy Lactation (Mcal/lb)	0.76	0.75	0.77	0.74
Phosphorus (%)	0.62	0.87	0.84	1.22
Potassium (%)	1.09	1.23	1.27	1.54
Calcium (%)	1.81	1.22	2.38	1.87
Magnesium (%)	0.38	0.41	0.39	0.46
Sodium (ppm)	3077.00	3148.00	3512.00	3091.00
Zinc (ppm)	64.00	116.00	136.00	145.00
Iron (ppm)	177.00	62.00	61.00	108.00
Copper (ppm)	10.00	16.00	29.00	28.00
Manganese (ppm)	148.00	211.00	221.00	252.00
Sulfur (ppm)	3032.00	2017.00	1890.00	2028.00
Boron (ppm)	3.06	1.42	2.48	3.82

A-2. Basic components of alfalfa cubes from three samples of the alfalfa cubes from the same lot with components listed on the right. Percentages are given where applicable. Megacalories per pound (Mcal/lb) and energy concentration in the feed (therm/cwt) are used where appropriate.

	Percentages and International Units per Pound (IU/lb)		
Components	**Sample 1**	**Sample 2**	**Sample 3**
Crude Protein (%)	20.6	20.6	19.0
Dig. Crude Protein (%)	15.7	15.6	14.2
Acid Detergent Fiber (%)	31.6	30.7	32.2
Neutral Detergent Fiber (%)	37.2	36.1	38.4
TDN-based on ADF (%)	64.5	65.1	63.4
Net Energy Lactation (Mcal/lb)	0.66	0.67	0.65
Net Energy Maintenance (Mcal/lb)	0.72	0.73	0.70
Net Energy Gain (Mcal/lb)	0.39	0.40	0.38
Energy Est. (therms/cwt)	54.8	55.4	53.8
IVTD (in vitro true digestible) (%)	79.1	79.2	78.7
Ash (%)	9.5	9.6	10.1
Relative feed value (RFV)	160.6	167.6	154.6
Phosphorus (%)	0.25	0.25	0.24
Potassium (%)	2.70	2.54	2.56
Calcium (%)	1.36	1.38	1.29
Magnesium (%)	0.26	0.26	0.27

A-3. Environmental data from the animal use room in Trial One where the six heifers were maintained during the pre-infestation control period inside and tick infestation period.

Date	Maximum Temperature °C	Minimum Temperature °C	Maximum Relative Humidity %	Minimum Relative Humidity %
14-Sept-2018	33.08	26.82	86.61	76.15
15-Sept-2018	34.20	27.80	91.45	64.29
16-Sept-2018	32.79	27.73	89.54	56.72
17-Sept-2018	33.84	28.39	91.71	66.51
18-Sept-2018	34.31	28.69	94.37	62.70
19-Sept-2018	32.56	28.54	96.68	59.29
20-Sept-2018	32.61	28.52	96.70	66.92
21-Sept-2018	29.99	27.11	96.90	68.67
22-Sept-2018	28.47	26.33	100.00	76.32
23-Sept-2018	30.85	26.50	100.00	74.92
24-Sept-2018	31.71	27.24	100.00	74.89
25-Sept-2018	30.93	26.65	100.00	74.19
26-Sept-2018	27.97	24.56	100.00	76.13
27-Sept-2018	29.67	24.80	95.05	69.87
28-Sept-2018	27.24	25.84	100.00	76.60
29-Sept-2018	30.55	25.50	100.00	86.89
30-Sept-2018	28.69	25.62	100.00	75.83
01-Oct-2018	31.00	25.84	100.00	86.63
02-Oct-2018	31.31	26.65	100.00	71.19
03-Oct-2018	28.27	26.77	100.00	72.62
04-Oct-2018	31.89	26.21	100.00	26.79
05-Oct-2018	31.59	26.50	100.00	73.11
06-Oct-2018	30.37	26.62	100.00	73.76
07-Oct-2018	30.44	25.79	100.00	73.59
08-Oct-2018	29.59	24.10	100.00	77.99
09-Oct-2018	26.30	23.11	100.00	85.28
10-Oct-2018	26.23	21.29	100.00	75.10
11-Oct-2018	28.87	22.49	100.00	60.21
12-Oct-2018	29.02	24.97	100.00	66.58
13-Oct-2018	30.29	25.74	100.00	82.26
14-Oct-2018	27.70	14.31	100.00	74.81
15-Oct-2018	18.84	14.94	100.00	71.35
16-Oct-2018	20.17	17.18	100.00	80.32
17-Oct-2018	21.63	17.56	100.00	81.60
18-Oct-2018	24.61	20.53	100.00	75.96
19-Oct-2018	23.26	20.27	100.00	82.08
20-Oct-2018	24.32	19.60	100.00	84.54
21-Oct-2018	22.03	19.10	100.00	53.52
22-Oct-2018	21.08	19.06	100.00	57.21
23-Oct-2018	21.75	19.70	100.00	72.61
24-Oct-2018	20.67	18.60	100.00	76.96
25-Oct-2018	23.28	17.92	99.03	78.50
26-Oct-2018	27.01	18.99	97.06	65.29
27-Oct-2018	27.73	20.29	98.18	61.01
28-Oct-2018	28.02	21.46	100.00	57.76
29-Oct-2018	26.30	22.18	100.00	67.56
30-Oct-2018	26.30	22.18	100.00	78.42

A-4. Environmental data from the animal use room in Trial Two where the six heifers were maintained during the pre-infestation control period inside and tick infestation period.

Date	Maximum Temperature °C	Minimum Temperature °C	Maximum Relative Humidity %	Minimum Relative Humidity %
15-Feb-2019	25.01	19.39	99.61	80.42
16-Feb-2019	21.79	18.40	98.21	70.35
17-Feb-2019	0.11	-2.52	78.33	65.15
18-Feb-2019	-1.17	-2.82	86.71	63.65
19-Feb-2019	0.60	-2.88	86.61	76.15
20-Feb-2019	1.22	-2.33	91.45	64.29
21-Feb-2019	0.44	-2.37	89.54	56.72
22-Feb-2019	1.02	-2.00	91.71	66.51
23-Feb-2019	1.28	-1.84	94.37	62.70
24-Feb-2019	0.31	-1.92	96.68	59.29
25-Feb-2019	0.34	-1.93	96.70	66.92
26-Feb-2019	-1.12	-2.72	96.90	68.67
27-Feb-2019	-1.96	-3.15	100.00	76.32
28-Feb-2019	-0.64	-3.06	100.00	74.92
01-Mar-2019	-0.16	-2.65	100.00	74.89
02-Mar-2019	-0.60	-2.97	100.00	74.19
03-Mar-2019	-2.24	-4.14	100.00	76.13
04-Mar-2019	-1.30	-4.00	95.05	69.87
05-Mar-2019	-2.65	-3.42	100.00	76.60
06-Mar-2019	-0.81	-3.61	100.00	86.89
07-Mar-2019	-1.84	-3.54	100.00	75.83
08-Mar-2019	-0.55	-3.42	100.00	86.63
09-Mar-2019	-0.39	-2.97	100.00	71.19
10-Mar-2019	-2.07	-2.91	100.00	72.62
11-Mar-2019	-0.06	-3.22	100.00	26.79
12-Mar-2019	-0.23	-3.06	100.00	73.11
13-Mar-2019	-0.91	-2.99	100.00	73.76
14-Mar-2019	-0.86	-3.45	100.00	73.59
15-Mar-2019	-1.34	-4.39	100.00	77.99
16-Mar-2019	-3.16	-4.94	100.00	85.28
17-Mar-2019	-3.21	-5.95	100.00	75.10
18-Mar-2019	-1.74	-5.28	100.00	60.21
19-Mar-2019	-1.66	-3.91	100.00	66.58
20-Mar-2019	-0.95	-3.48	100.00	82.26
21-Mar-2019	-2.39	-9.83	100.00	74.81
22-Mar-2019	-7.31	-9.48	100.00	71.35
23-Mar-2019	-6.57	-8.24	100.00	80.32
24-Mar-2019	-5.76	-8.02	100.00	81.60
25-Mar-2019	-4.11	-6.37	100.00	75.96
26-Mar-2019	-4.86	-6.52	100.00	82.08
27-Mar-2019	-4.27	-6.89	100.00	84.54

A-5. Daily Observation Log used at the Tick Research Laboratory.

STANCHION #: _____ **ANIMAL ID #:** _____ **SEX (S = steer; H = heifer:** _____

TAMU TICKLAB ANIMAL CARE PROGRAM
LARGE ANIMAL DAILY OBSERVATIONS

P.I. NAME: Pete D. Teel, Ph.D. MONTH / YR: _____

PROTOCOL NUMBER: AUP 2017 - 0345 PHONE No: 979-255-3013 or 979-268-2714

PROCEDURE: Adult tick feeding for tick colony maintenance / research EMAIL: pteel@tamu.edu; ostrey@tamu.edu

(*Include emergency numbers and email addresses*)

DAY	APPT	Stool/Waste	Injuries	Responsiveness	OBSERVATIONS/COMMENTS	INITIALS
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						

CODES: Appetite (APPT) (food & water intake) N = Normal P = Poor O = None

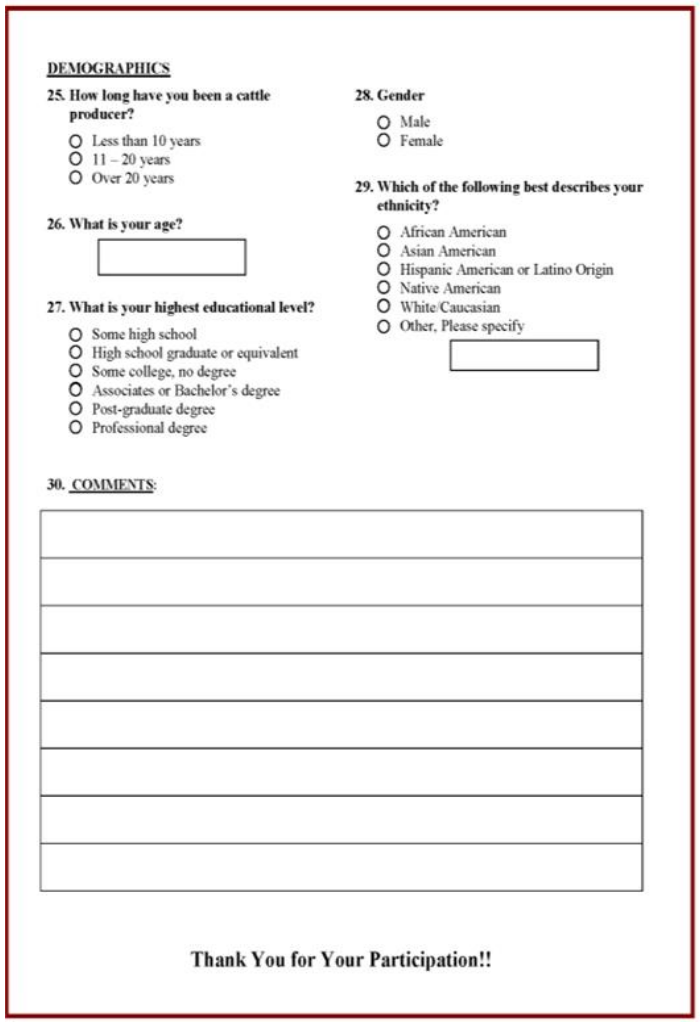
Injuries N = None Y = Yes (describe)

Responsiveness N = Normal (bright & alert) A = Abnormal (lethargic, lameness, feverish; describe)

Stool / Waste N = Normal A = Abnormal (describe) O = None D = Diarrhea

APPENDIX B

B-1. Paper hand-out survey questionnaire provided to the three producer audiences Beef Cattle Short Course, Producer Meetings, and Veterinarian Meetings in years 2017, 2018, and 2019.



DEMOGRAPHICS

25. How long have you been a cattle producer?

- ○ Less than 10 years
- ○ 11 – 20 years
- ○ Over 20 years

26. What is your age?

27. What is your highest educational level?

- ○ Some high school
- ○ High school graduate or equivalent
- ○ Some college, no degree
- ○ Associates or Bachelor's degree
- ○ Post-graduate degree
- ○ Professional degree

28. Gender

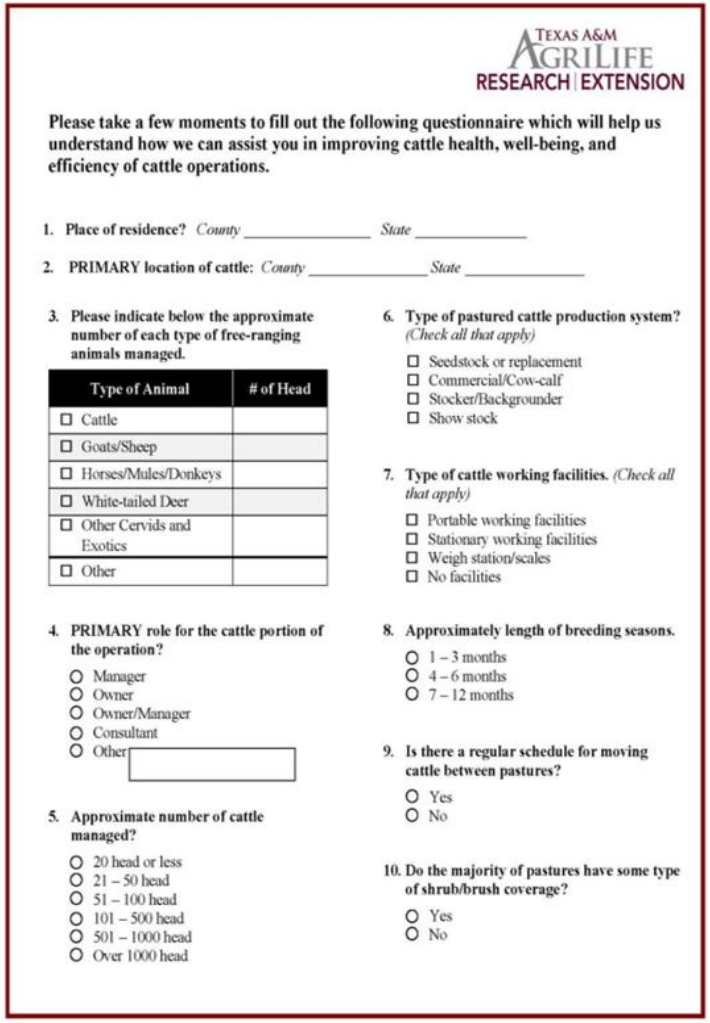
- ○ Male
- ○ Female

29. Which of the following best describes your ethnicity?

- ○ African American
- ○ Asian American
- ○ Hispanic American or Latino Origin
- ○ Native American
- ○ White/Caucasian
- ○ Other, Please specify

30. COMMENTS:

Thank You for Your Participation!!



TEXAS A&M AGRILIFE RESEARCH | EXTENSION

Please take a few moments to fill out the following questionnaire which will help us understand how we can assist you in improving cattle health, well-being, and efficiency of cattle operations.

1. **Place of residence?** *County* _____ *State* _____
2. **PRIMARY location of cattle:** *County* _____ *State* _____
3. **Please indicate below the approximate number of each type of free-ranging animals managed.**

Type of Animal	# of Head
☐ Cattle	
☐ Goats/Sheep	
☐ Horses/Mules/Donkeys	
☐ White-tailed Deer	
☐ Other Cervids and Exotics	
☐ Other	

4. **PRIMARY role for the cattle portion of the operation?**
   - ○ Manager
   - ○ Owner
   - ○ Owner/Manager
   - ○ Consultant
   - ○ Other
5. **Approximate number of cattle managed?**
   - ○ 20 head or less
   - ○ 21 – 50 head
   - ○ 51 – 100 head
   - ○ 101 – 500 head
   - ○ 501 – 1000 head
   - ○ Over 1000 head
6. **Type of pastured cattle production system?** *(Check all that apply)*
   - ☐ Seedstock or replacement
   - ☐ Commercial/Cow-calf
   - ☐ Stocker/Backgrounder
   - ☐ Show stock
7. **Type of cattle working facilities.** *(Check all that apply)*
   - ☐ Portable working facilities
   - ☐ Stationary working facilities
   - ☐ Weigh station/scales
   - ☐ No facilities
8. **Approximately length of breeding seasons.**
   - ○ 1 – 3 months
   - ○ 4 – 6 months
   - ○ 7 – 12 months
9. **Is there a regular schedule for moving cattle between pastures?**
   - ○ Yes
   - ○ No
10. **Do the majority of pastures have some type of shrub/brush coverage?**
    - ○ Yes
    - ○ No

B-1 continued.

11. Please indicate below the type of pasture on which cattle are grazed during each season.

Type of Pasture	Season			
	Spring	Summer	Fall	Winter
Brushy/Shrubland				
Improved				
Mixed				
Native				
Annual Forages				
Other (please explain)				

12. Has brush control been used as means of pasture management in the past two years?

- ○ Yes
- ○ No
- ○ Not applicable to my operation

13. How is brush control practiced? *(Check all that apply)*

- ☐ Fire
- ☐ Herbicide
- ☐ Mechanical
- ☐ Other ____

14. Please indicate below timing of the following cattle management practices.
(Check all months that apply)

Type of Cattle Management	Months											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Castration												
Vaccination												
Ectoparasite Control *(ex. flies, ticks, lice, etc.)*												
Endoparasite Control *(ex. intestinal worms)*												
Weaning Cattle												
Weighing Cattle												
Culling												
Other (Please explain)												

15. Which ectoparasites are a problem in local cattle operations?
(Check all that apply)

- ☐ Flies
- ☐ Grubs
- ☐ Lice
- ☐ Ticks
- ☐ Other ____

16. Are cattle ever gathered specifically to treat for ectoparasites?

- ○ Yes
- ○ No
- ○ Have previously, but not in the last year

17. What methods of ectoparasite treatment have been used in the past year?
(Check all that apply)

- ☐ Dip
- ☐ Ear tags
- ☐ Pour-on
- ☐ Spray
- ☐ Vet-gun
- ☐ Other ____

18. How is the need for ectoparasite treatment of cattle determined?
(Check all that apply)

- ☐ Changes in behavior *(ex. kicking, rubbing/scratching, lethargic)*
- ☐ Changes in body condition
- ☐ Convenience *(When cattle are worked)*
- ☐ Physical examination of cattle
- ☐ Observe ectoparasites on cattle
- ☐ Time of the year

19. How are cattle evaluated for proper nutrition?
(Check all that apply)

- ☐ Body condition
- ☐ Forage/hay testing
- ☐ Manure/fecal testing
- ☐ Pasture conditions
- ☐ Weigh animals
- ☐ Other ____

20. Would a manure/fecal test that can diagnose a tick infestation in cattle be useful?

- ○ Yes
- ○ No
- ○ Maybe

21. Are services available to test forage and hay for nutritional value?

- ○ Yes
- ○ No

22. Would the option of submitting the following samples for nutritional testing be considered?

Type of Sample	Yes	No	Maybe
Forage/hay			
Manure/fecal			

23. If cost shares were available *(ex., from the Natural Resources Conservation Service's programs)*, **would the following testing services be useful?**

Type of Sample	Yes	No	Maybe
Forage/hay			
Manure/fecal			

24. If forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used.

Use of Information	Forage Testing	Hay Testing	Manure Testing
Cull animals			
Move animals to a different pasture			
Supplemental feed purchase			
Supplemental hay purchase			
Did not use it for management decision			
Other (Please explain):			

B-2. The breakdown of survey questions assigned to each of the four content areas for the survey entitled "Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems".

Content Area	Question Number	Question
Background of Cattle Producers	1, Part 1	Place of residence? County
	1, Part 2	Place of residence? State
	25	How long have you been a cattle producer?
	4	Primary role for the cattle portion of the operation.
Beef Production Characteristics of Respondents Operation	2, Part 1	Primary location of cattle: County
	2, Part 2	Primary location of cattle: State
	3	Please indicate below the approximate number of each type of free-ranging animals managed.
	5	Approximate number of cattle managed.
	6	Type of pastured cattle production system?
	7	Type of cattle working facilities?
	8	Approximately length of breeding seasons.
	11	Please indicate below the type of pasture on which cattle are grazed during each season.
	14	Please indicate below timing of the following cattle management practices.
	19	How are cattle evaluated for proper nutrition?

B-2 continued.

Content Area	Question Number	Question
Ectoparasite Control including Ticks	9	Is there a regular schedule for moving cattle between pastures?
	10	Do the majority of pastures have some type of shrub/brush coverage?
	12	Has brush control been used as means of pasture management in the past two years?
	13	How is brush control practiced?
	15	Which ectoparasites are a problem in local cattle operations?
	16	Are cattle ever gathered specifically to treat for ectoparasites?
	17	What methods of ectoparasite treatment have been used in the past year?
	18	How is the need for ectoparasite treatment of cattle determined?
Use of Fecal NIRS Technology for Forage Assessment and Nutritional Balance	20	Would a manure/fecal test that can diagnose a tick infestation in cattle be useful?
	21	Are services available to test forage and hay for nutritional value?
	22	Would the option of submitting the following samples for nutritional testing be considered?
	23	If cost shares were available (ex., from the Natural Resources Conservation Service's programs), would the following testing services be useful?
	24	If forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used.

B-3. The answer format of survey questions for the survey entitled "Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems".

Questions	**Answer Format**
Place of residence?	Fill in the blank
Primary location of cattle.	Fill in the blank
Please indicate below the approximate number of each type of free-ranging animals managed.	Table/Fill in the blank
Primary role for the cattle portion of the operation?	Multiple choice
Approximate number of cattle managed	Multiple choice
Type of pastured cattle production system?	Check all that apply
Type of cattle working facilities?	Check all that apply
Approximate length of breeding seasons.	Multiple choice
Is there a regular schedule for moving cattle between pastures?	Multiple choice
Do the majority of pastures have some type of shrub/brush coverage?	Multiple choice
Please indicate below the type of pasture on which cattle are grazed during each season.	Table/Fill in the blank
Has brush control been used as means of pasture management in the past two years?	Multiple choice
How is brush control practiced?	Check all that apply
Please indicate below timing of the following cattle management practices.	Table/Fill in the blank
Which ectoparasites are a problem in local cattle operations?	Check all that apply
Are cattle ever gathered specifically to treat for ectoparasites?	Multiple choice
What methods of ectoparasite treatment have been used in the past year?	Check all that apply
How is the need for ectoparasite treatment of cattle determined?	Check all that apply
How are cattle evaluated for proper nutrition?	Check all that apply

B-3 continued.

Questions	Answer Format
Would a manure/fecal test that can diagnose a tick infestation in cattle be useful?	Multiple choice
Are services available to test forage and hay for nutritional value?	Multiple choice
Would the option of submitting the following samples for nutritional testing be considered?	Table/Fill in the blank
If cost shares were available *(ex., from the Natural Resources Conservation Service's programs)*, would the following testing services be useful?	Table/Fill in the blank
If forage, hay, or manure has been sent for nutritional testing, please indicate below how the information was used.	Table/Fill in the blank
How long have you been a cattle producer?	Multiple choice
What is your age?	Fill in the blank
What is your highest educational level?	Multiple choice
Gender.	Multiple choice
Which of the following best describes your ethnicity?	Multiple choice

B-4. Summary of producer responses to the place of residence: County (Question #1, Part 1) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	***f*[a]**	**%**
Anderson	3	1.0
Atascosa	2	0.7
Austin	4	1.4
Bandera	1	0.3
Bastrop	5	1.7
Bee	1	0.3
Bell	5	1.7
Bexar	2	0.7
Blanco	2	0.7
Bosque	1	0.3
Bowie	2	0.7
Brazoria	2	0.7
Brazos	14	4.7
Brazos/Burleson	1	0.3
Brooks	1	0.3
Burleson	9	3.0
Burnet	1	0.3
Caldwell	3	1.0
Cass	4	1.4
Cherokee	3	1.0
Collin	3	1.0
Colorado	2	0.7
Comal	2	0.7
Coryell	1	0.3
Denton	2	0.7
Dewitt	5	1.7
Dimmit	1	0.3
Eastland	2	0.7
Edwards	1	0.3
Ellis	4	1.4
Erath	1	0.3
Falls	5	1.7
Fayette	4	1.4
Fort Bend	1	0.3
Franklin	1	0.3
Galveston	1	0.3
Gonzales	2	0.7
Grayson	1	0.3
Gregg	1	0.3
Grimes	6	2.0
Guadalupe	2	0.7
Hamilton	1	0.3
Harris	15	5.1
Harris/Fayette	1	0.3
Harrison	2	0.7
Hays	3	1.0
Henderson	1	0.3
Hidalgo	1	0.3
Hill	4	1.4
Hopkins	1	0.3
Houston	6	2.0
Hunt	2	0.7
Jack	2	0.7
Jackson	1	0.3
Jefferson	1	0.3
Jim Wells	6	2.0
Johnson	1	0.3
Kaufman	3	1.0
Kerr	1	0.3

B-4 continued.

Response	*f*[a]	%
Kleberg	4	1.4
Lamar	1	0.3
Lampasas	1	0.3
Lavaca	2	0.7
Lee	3	1.0
Leon	6	2.0
Liberty	2	0.7
Limestone	1	0.3
Live Oak	2	0.7
Llano	1	0.3
Madison	1	0.3
Mason	1	0.3
Matagorda	1	0.3
McLennan	1	0.3
Medina	2	0.7
Milam	5	1.7
Mills	1	0.3
Montague	2	0.7
Montgomery	8	2.7
Nacogdoches	1	0.3
Orange	1	0.3
Panola	2	0.7
Polk	3	1.0
Rains	2	0.7
Randall	1	0.3
Red River	1	0.3
Roberts	1	0.3
Robertson	2	0.7
Runnels	2	0.7
Rusk	2	0.7
San Augustine	2	0.7
San Patricio	2	0.7
San Saba	2	0.7
Shackelford	1	0.3
Shelby	2	0.7
Smith	2	0.7
Tarrant	3	1.0
Tom Green	3	1.0
Travis	4	1.4
Tyler	1	0.3
USA	1	0.3
Uvalde	1	0.3
Van Zandt	4	1.4
Victoria	5	1.7
Walker	5	1.7
Waller	4	1.4
Washington	2	0.7
Webb	2	0.7
Webster	1	0.3
Wharton	4	1.4
Williamson	5	1.7
Williamson/Lee	1	0.3
Wilson	2	0.7
Wood	1	0.3
Young	2	0.7
Zapata	3	1.0
No Response	2	0.7

B-5. Summary of producer responses to the primary location of cattle: county (Question #2, Part 1) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	***f*[a]**	**%**
Anderson	3	1.0
Archer	1	0.3
Atascosa	2	0.7
Austin	8	2.7
Bandera	1	0.3
Bastrop	5	1.7
Bee	1	0.3
Bell	7	2.4
Bell/Bastrop	1	0.3
Bexar	3	1.0
Blanco	2	0.7
Bosque	1	0.3
Bowie	2	0.7
Brazoria	4	1.4
Brazos	7	2.4
Brazos/Burleson	1	0.3
Brooks	1	0.3
Burleson	12	4.1
Burleson/Milam	1	0.3
Burnet	3	1.0
Caldwell	3	1.0
Cass	4	1.4
Cherokee	3	1.0
Coleman	1	0.3
Collin	3	1.0
Collin/Grayson	1	0.3
Colorado	2	0.7
Comal	1	0.3
Coryell	1	0.3
Dewitt	5	1.7
Duval	1	0.3
Eastland	2	0.7
Edwards	1	0.3
Ellis	2	0.7
Erath	1	0.3
Falls	4	1.4
Fayette	5	1.7
Fort Bend	2	0.7
Franklin	1	0.3
Galveston	1	0.3
Gonzales	7	2.4
Grayson	1	0.3
Grimes	8	2.7
Guadalupe	5	1.7
Hamilton	1	0.3
Harris	4	1.4
Harrison	2	0.7
Henderson	2	0.7
Hidalgo	1	0.3
Hill	5	1.7
Hopkins	1	0.3
Houston	6	2.0
Hunt	2	0.7
Jack	2	0.7
Jackson	1	0.3
Jackson/Lavaca	1	0.3
Jefferson	1	0.3
Jim Wells	6	2.0
Jim Wells/Live Oak	2	0.7

B-5 continued.

Response	*f*[a]	%
Johnson	1	0.3
Kaufman	1	0.3
Kerr	1	0.3
Kleberg	2	0.7
Kleberg/Bee	1	0.3
Lamar	1	0.3
Lampasas	1	0.3
Lavaca	4	1.4
Lee	4	1.4
Leon	10	3.4
Liberty	1	0.3
Limestone	2	0.7
Live Oak/Bee	1	0.3
Llano	3	1.0
Madison	2	0.7
Mason	1	0.3
Matagorda	2	0.7
Maverick/Webb	1	0.3
McLennan	1	0.3
Medina	1	0.3
Milam	5	1.7
Mills	1	0.3
Montague	2	0.7
Montgomery	1	0.3
Nacogdoches	2	0.7
Navarro	1	0.3
Panola	4	1.4
Polk	1	0.3
Rains	1	0.3
Rains/Runnels	1	0.3
Randall	1	0.3
Red River	1	0.3
Roberts	1	0.3
Robertson	2	0.7
Runnels	2	0.7
Rusk	1	0.3
San Augustine	2	0.7
San Patricio	1	0.3
San Saba	2	0.7
Shackelford	1	0.3
Shelby	2	0.7
Smith	1	0.3
Tarrant	1	0.3
Tom Green	1	0.3
Trinity	1	0.3
Tyler	1	0.3
USA	1	0.3
Uvalde	1	0.3
Van Zandt	4	1.4
Victoria	6	2.0
Walker	7	2.4
Waller	4	1.4
Washington	2	0.7
Webb	2	0.7
Webster	1	0.3
Wharton	4	1.4
Williamson	4	1.4
Williamson/Lee	1	0.3
Wilson	2	0.7
Wood	2	0.7
Young	2	0.7
Zapata	2	0.7
No Response	2	0.7

B-6. Summary of producer responses to the approximate number of free-ranging animals managed: Cattle (Question #3) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	*f*[a]	%
1	1	0.3
3	3	1.0
5	2	0.7
6	5	1.6
7	1	0.3
8	3	1.0
10	9	3.0
11	1	0.3
12	1	0.3
13	2	0.7
15	10	3.3
16	2	0.7
18	2	0.7
19	2	0.7
20	12	3.9
21	1	0.3
22	3	1.0
23	2	0.7
24	1	0.3
25	13	4.3
27	1	0.3
28	2	0.7
29	1	0.3
30	14	4.6
31	1	0.3
33	2	0.7
34	2	0.7
35	9	3.0
37	1	0.3
38	1	0.3
40	16	5.2
41	1	0.3
45	5	1.6
50	22	7.2
52	1	0.3
55	3	1.0
59	1	0.3
60	6	2.0
65	2	0.7
66	1	0.3
70	4	1.3
75	8	2.6
77	1	0.3
78	1	0.3
80	3	1.0
85	2	0.7
87	1	0.3
100	19	6.2
108	1	0.3
109	1	0.3
110	1	0.3
120	5	1.6
125	1	0.3
127	1	0.3
130	1	0.3
135	1	0.3
140	2	0.7
150	7	2.3
160	2	0.7

B-6 continued.

Response	*f*[a]	%
175	2	0.7
180	1	0.3
200	12	3.9
248	1	0.3
250	3	1.0
275	1	0.3
300	4	1.3
350	2	0.7
500	4	1.3
600	3	1.0
725	1	0.3
1200	1	0.3
2000	1	0.3
10000	1	0.3
15000	1	0.3
20000	1	0.3
Yes	29	9.5
No Response	6	2.0

B-7. Summary of producer responses to the approximate number of free-ranging animals managed: Goats/Sheep (Question #3) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	*f*[a]	%
1	5	1.6
2	2	0.7
4	1	0.3
5	1	0.3
15	1	0.3
16	1	0.3
30	1	0.3
35	1	0.3
40	2	0.7
70	1	0.3
75	1	0.3
200	1	0.3
294	1	0.3
340	1	0.3
400	1	0.3
Yes	1	0.3
No Response	283	92.8

B-8. Summary of producer responses to the approximate number of free-ranging animals managed: Horses/Mules/Donkeys (Question #3) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	*f*[a]	%
1	16	5.2
2	19	6.2
3	10	3.3
4	14	4.6
5	9	3.0
6	5	1.6

B-8 continued.

Response	*f*[a]	%
7	5	1.6
8	1	0.3
9	3	1.0
10	7	2.3
12	1	0.3
13	1	0.3
15	3	1.0
20	2	0.7
25	1	0.3
30	1	0.3
40	1	0.3
75	1	0.3
Yes	10	3.3
No Response	195	63.9

B-9. Summary of producer responses to the approximate number of free-ranging animals managed: White-tailed Deer (Question #3) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	*f*[a]	%
1	2	0.7
3	2	0.7
5	3	1.0
10	1	0.3
15	1	0.3
20	5	1.6
30	2	0.7
50	5	1.6
60	1	0.3
100	1	0.3
125	1	0.3
150	1	0.3
200	2	0.7
250	1	0.3
500	1	0.3
Many	1	0.3
Yes	19	6.2
No Response	256	83.9

B-10. Summary of producer responses to the approximate number of free-ranging animals managed: Other Cervids and Exotics (Question #3) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	*f*[a]	%
1	1	0.3
2	1	0.3
20	1	0.3
50	2	0.7
55	1	0.3
400	1	0.3
Axis	1	0.3
Yes	2	0.7
No Response	295	96.7

B-11. Summary of producer responses to the approximate number of free-ranging animals managed: Other (Question #3) for the Beef Cattle Short Course in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Beef Cattle Short Course audience in years 2017, 2018, and 2019.

Response	*f*[a]	%
1	3	1.0
15	1	0.3
100	1	0.3
200	1	0.3
30 poultry	1	0.3
40 chickens	1	0.3
5 dogs; 5 barn cats	1	0.3
Hogs	1	0.3
Many	1	0.3
No Response	294	96.4

B-12. Summary of producer responses to the place of residence: County (Question #1, Part 1) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
Anderson	1	0.3
Austin	4	1.1
Bastrop	2	0.5
Bell	14	3.8
Bosque	4	1.1
Brazoria	1	0.3
Brazos	19	5.2
Burleson	6	1.6
Burnet	4	1.1
Cherokee	1	0.3
Colorado	4	1.1
Comanche	4	1.1
Coryell	3	0.8
Dallas	2	0.5
Dewitt	2	0.5
Eastland	1	0.3
Ellis	6	1.6
Erath	21	5.7
Falls	11	3.0
Fayette	4	1.1
Freestone	9	2.4
Gaines	1	0.3
Grimes	2	0.5
Guadalupe	1	0.3
Hamilton	2	0.5
Harris	6	1.6
Hill	17	4.6
Hood	11	3.0
Jack	1	0.3
Johnson	2	0.5
Kendall	1	0.3
Lampasas	5	1.4
Leon	17	4.6
Limestone	13	3.5
Madison	1	0.3
McLennan	31	8.4
Milam	21	5.7
Navarro	26	7.1

B-12 continued.

Response	*f*[a]	%
Nueces	1	0.3
Palo Pinto	1	0.3
Robertson	24	6.5
San Saba	2	0.5
Somervell	1	0.3
Stephens	1	0.3
Tarrant	4	1.1
Travis	2	0.5
Van Zandt	1	0.3
Walker	1	0.3
Washington	36	9.8
Williamson	13	3.5

B-13. Summary of producer responses to the primary location of cattle: County (Question #2, Part 1) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
Anderson	1	0.3
Austin	6	1.6
Bastrop	1	0.3
Bell	11	3.0
Bosque	5	1.4
Brazos	11	3.0
Burleson	6	1.6
Burleson/Washington	1	0.3
Burnet	4	1.1
Burnet/Blanco	2	0.5
Cherokee	2	0.5
Colorado	2	0.5
Comanche	4	1.1
Coryell	4	1.1
Dewitt	2	0.5
Eastland	1	0.3
Ellis	7	1.9
Erath	18	4.9
Falls	11	3.0
Fayette	9	2.4
Fayette/Live Oak/Bee	1	0.3
Freestone	9	2.4
Gaines	1	0.3
Gonzales	2	0.5
Grimes	2	0.5
Guadalupe	1	0.3
Hamilton	7	1.9
Hamilton/Brazos	1	0.3
Harris	2	0.5
Hill	18	4.9
Hood	10	2.7
Houston	1	0.3
Jack	1	0.3
Jim Wells	1	0.3
Johnson	2	0.5
Lampasas	6	1.6
Leon	19	5.2
Limestone	14	3.8
Limestone/Navarro	1	0.3

B-13 continued.

Response	*f*[a]	%
Llano	1	0.3
Madison	1	0.3
McLennan	24	6.5
Milam	24	6.5
Navarro	27	7.3
Palo Pinto	1	0.3
Robertson	25	6.8
Robertson/Limestone	1	0.3
San Saba	2	0.5
Somervell	1	0.3
Stephens	1	0.3
Van Zandt	2	0.5
Waller	1	0.3
Washington	31	8.4
Williamson	11	3.0
Wise	1	0.3
No Response	7	1.9

B-14. Summary of producer responses to the approximate number of free-ranging animals managed: Cattle (Question #3) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
2	1	0.3
3	1	0.3
4	2	0.5
5	2	0.5
6	1	0.3
8	2	0.5
9	3	0.8
10	6	1.6
11	5	1.4
12	5	1.4
13	2	0.5
14	2	0.5
15	8	2.2
16	2	0.5
17	2	0.5
18	2	0.5
20	12	3.3
21	1	0.3
23	1	0.3
24	2	0.5
25	17	4.6
27	1	0.3
28	1	0.3
30	15	4.1
32	2	0.5
35	9	2.4
40	23	6.3
45	4	1.1
46	1	0.3
50	25	6.8
55	1	0.3
60	17	4.6
65	3	0.8
70	9	2.4

B-14 continued.

Response	*f*[a]	%
80	11	3.0
88	1	0.3
90	4	1.1
99	1	0.3
100	21	5.7
105	1	0.3
110	3	0.8
115	1	0.3
120	3	0.8
125	3	0.8
130	2	0.5
135	1	0.3
140	5	1.4
150	5	1.4
160	1	0.3
175	2	0.5
200	14	3.8
210	1	0.3
230	2	0.5
250	3	0.8
300	3	0.8
400	5	1.4
450	1	0.3
500	2	0.5
550	1	0.3
600	1	0.3
700	1	0.3
800	1	0.3
1000	1	0.3
1150	1	0.3
1200	2	0.5
5000	1	0.3
Yes	60	16.3
No Response	7	1.9

B-15. Summary of producer responses to the approximate number of free-ranging animals managed: Goats/Sheep (Question #3) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
0	3	0.8
1	2	0.5
2	2	0.5
4	1	0.3
5	1	0.3
6	1	0.3
10	2	0.5
11	1	0.3
20	2	0.5
25	4	1.1
30	1	0.3
40	2	0.5
60	1	0.3
100	1	0.3
150	1	0.3
156	1	0.3
250	1	0.3
Yes	3	0.8

B-15 continued.

Response	*f*[a]	%
No Response	338	91.8

B-16. Summary of producer responses to the approximate number of free-ranging animals managed: Horses/Mules/Donkeys (Question #3) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
0	2	0.5
1	15	4.1
2	17	4.6
3	15	4.1
4	9	2.4
5	8	2.2
6	1	0.3
8	1	0.3
9	2	0.5
10	2	0.5
11	1	0.3
12	1	0.3
14	1	0.3
15	1	0.3
20	1	0.3
45	1	0.3
Yes	8	2.2
No Response	282	76.6

B-17. Summary of producer responses to the approximate number of free-ranging animals managed: White-tailed Deer (Question #3) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
5	1	0.3
8	1	0.3
15	1	0.3
20	1	0.3
24	1	0.3
25	1	0.3
30	1	0.3
40	2	0.5
70	1	0.3
100	1	0.3
150	1	0.3
200	2	0.5
Yes	17	4.6
No Response	337	91.6

B-18. Summary of producer responses to the approximate number of free-ranging animals managed: Other Cervids and Exotics (Question #3) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
0	1	0.3
2	1	0.3
4	1	0.3
20	1	0.3
25	1	0.3
30	1	0.3
1200	1	0.3
Yes	3	0.8
No Response	358	97.3

B-19. Summary of producer responses to the approximate number of free-ranging animals managed: Other (Question #3) for the Producer Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Producer Meetings audience in years 2017 and 2018.

Response	*f*[a]	%
3	1	0.3
17	1	0.3
25	1	0.3
100	1	0.3
Hogs	1	0.3
Wild Hogs	1	0.3
Yes	1	0.3
No Response	361	98.1

B-20. Summary of practitioner/producer responses to the place of residence: county (Question #1, Part 1) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
Austin	1	1.8
Bastrop	1	1.8
Beaver	1	1.8
Bell	1	1.8
Bexar	1	1.8
Brazos	4	7.0
Burleson	1	1.8
Burnet	1	1.8
Carson	1	1.8
Castro	1	1.8
Coleman	1	1.8
Comanche	1	1.8
Dawson	1	1.8
Falls	1	1.8
Fayette	3	5.3
Freestone	1	1.8
Guadalupe	1	1.8
Harris	2	3.5
Houston	1	1.8
Jones	1	1.8

B-20. Summary of practitioner/producer responses to the place of residence: county (Question #1, Part 1) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
Kerr	1	1.8
Kinney	1	1.8
Leon	1	1.8
Mason	1	1.8
McLennan	1	1.8
Oldham	1	1.8
Polk	1	1.8
Potter	1	1.8
Randall	9	15.8
Rockwall	1	1.8
Runnels	1	1.8
Shackelford	1	1.8
Stephens	1	1.8
Swisher	1	1.8
Tom Green	1	1.8
Tulsa	1	1.8
Waller	1	1.8
Washington	3	5.3
Wichita	1	1.8
Wilbarger	1	1.8
Williamson	1	1.8
No Response	0	0

B-21. Summary of practitioner/producer responses to the primary location of cattle: county (Question #2, Part 1) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
Austin	1	1.8
Bastrop	1	1.8
Beaver	1	1.8
Brazos	1	1.8
Burleson	2	3.5
Burnet	1	1.8
Carson	1	1.8
Castro	1	1.8
Coleman	2	3.5
Comanche	1	1.8
Concho	1	1.8
Dawson/Borden	1	1.8
Deaf Smith	1	1.8
Falls	1	1.8
Fayette	2	3.5
Freestone	1	1.8
Guadalupe	2	3.5
Harmon	1	1.8
Harris	1	1.8
Houston	1	1.8
Kerr	1	1.8
Kinney	1	1.8
Leon	1	1.8
Mason	1	1.8
McLennan	1	1.8
Milam	1	1.8

B-21 continued.

Response	*f*[a]	%
Montgomery	1	1.8
Montgomery/San Jacinto/Liberty	1	1.8
Polk	1	1.8
Potter	1	1.8
Randall	8	14.0
Rockwall	1	1.8
Runnels	1	1.8
San Saba	1	1.8
Shackelford	2	3.5
Stephens	1	1.8
Swisher	1	1.8
Tulsa	1	1.8
Waller	1	1.8
Washington	3	5.3
Wichita	2	3.5
Wilbarger	1	1.8
No Response	0	0

B-22. Summary of practitioner/producer responses to the approximate number of free-ranging animals managed: Cattle (Question #3) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
1	2	3.5
10	2	3.5
20	3	5.3
25	4	7.0
30	3	5.3
35	2	3.5
40	1	1.8
45	1	1.8
50	2	3.5
56	1	1.8
60	1	1.8
80	1	1.8
100	3	5.3
110	1	1.8
150	1	1.8
200	4	7.0
300	2	3.5
400	1	1.8
600	2	3.5
799	1	1.8
1000	2	3.5
1600	1	1.8
2500	2	3.5
300000	1	1.8
Yes	9	15.8
No Response	4	7.0

B-23. Summary of practitioner/producer responses to the approximate number of free-ranging animals managed: Goats/Sheep (Question #3) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
10	1	1.8
15	1	1.8
60	2	3.5
125	1	1.8
1500	1	1.8
Yes	4	7.0
No Response	47	82.5

B-24. Summary of practitioner/producer responses to the approximate number of free-ranging animals managed: Horses/Mules/Donkeys (Question #3) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
1	1	1.8
2	4	7.0
3	4	7.0
5	5	8.8
6	1	1.8
9	1	1.8
15	2	3.5
19	1	1.8
20	2	3.5
30	1	1.8
45	1	1.8
50	2	3.5
1000	1	1.8
1500	1	1.8
Yes	4	7.0
No Response	26	45.6

B-25. Summary of practitioner/producer responses to the approximate number of free-ranging animals managed: White-tailed Deer (Question #3) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
10	1	1.8
30	1	1.8
50	1	1.8
100	1	1.8
200	1	1.8
3000	1	1.8
Yes	6	10.5
No Response	45	78.9

B-26. Summary of practitioner/producer responses to the approximate number of free-ranging animals managed: Other Cervids and Exotics (Question #3) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
5	1	1.8
30	1	1.8
36	1	1.8
200	1	1.8
400	1	1.8
Yes	1	1.8
No Response	51	89.5

B-27. Summary of practitioner/producer responses to the approximate number of free-ranging animals managed: Other (Question #3) for the Veterinarian Meetings in response to a survey for the Economic Assessment of Working Cattle and Technology Adoption in Grazing Cattle Systems. Data were obtained from all states and the Veterinarian Meetings audience in year 2018.

Response	*f*[a]	%
1000	1	1.8
No Response	56	98.2