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Fecal NIRS: Detection of tick infestations in cattle and horses

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Abstract

Anti-tick treatments are often applied concurrent to routine livestock management practices with little regard to actual infestation levels. Prescription treatments against ticks on grazing cattle would be facilitated by non-invasive detection methods. One such method is fecal near infrared spectroscopy (NIRS). Four studies utilizing cattle (*Bos* spp.) and one with horses (*Equus caballus*) fed varying diets and infested with either *Amblyomma americanum*, *A. maculatum*, *A. cajennense* or *Dermacentor albipictus* were conducted to determine the ability of fecal NIRS to identify samples from animals with (High stress) and without (Low stress) a tick burden. Discriminant analysis of each individual trial resulted in $R^2 > 0.80$. Similar analyses utilizing all combinations of four studies, predicting group membership in the remaining study, yielded $R^2 > 0.80$, but correct determinations for Low and High tick stress samples of only 53.4 and 60.1%, respectively. All five trials were combined and a random 10 or 25% of the samples were removed from the calibration. As in the previous calibrations, a high degree of discrimination was achieved ($R^2 > 0.89$). The validation samples were correctly identified at 91.7% for Low stress and 96.3% for High stress, respectively. Difficulties in detecting differences in fecal samples due to confounding effects of trial were overcome by combining calibration sets. Overall, differences in fecal NIR spectra apparently due to tick stress were accurately detected across diet, host species, and tick species.

Keywords: *Amblyomma*; Cattle; Discriminant analyses; Fecal NIRS; Horses; IPM; Tick stress; Validation

1. Introduction

External parasites annually cost the US beef cattle industry over US$ 2.4 billion. Ticks cause harm to animals through: (1) blood loss; (2) general stress and irritation; (3) depression of immune function; (4) transmission of opportunistic pathogens. IPM strategies for ticks (Barnard et al., 1994; Schmidtmann, 1994) incorporate systematic or tactical use of acaricides. Producer adoption of IPM programs for ticks is made difficult because producers are expected to monitor for the presence and abundance of ticks to detect injury thresholds and make decisions regarding management tactics (Barnard, 1985). Timely gathering and inspection of animals is expensive and a disincentive to adoption of IPM programs. In lieu of making tick management decisions based on tick abundance data from cattle, producers often focus treatments on a seasonal schedule of ranching activities when cattle are

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"routinely" gathered for sorting, branding, and vaccinating. Treatments may thus be applied without regard for parasite presence or abundance. New and cost-effective methods of parasite detection are needed to improve decision-making, pest monitoring for overall animal health, and decreasing the development of resistance to acaricides.

Near infrared reflectance spectroscopy (NIRS) of feces has been used in nutritional monitoring of grazing livestock worldwide for over a decade (Coates, 2000). Interested readers are directed to Foley et al. (1998) for an excellent review of the subject of NIRS in natural resource research. Recently, the technique has also been expanded to physiological monitoring of herbivores. Godfrey et al. (2001) reported differences in fecal near infrared (NIR) spectra between male, pregnant, and lactating sheep (*Ovis aries*, L.) Similarly, differences in fecal NIR spectra were identified in ovariectomized, early (45–60 days), and late (>240 days) pregnant *Bos indicus* cows (Tolleson et al., 2000a). Tolleson et al. (2005) differentiated sex and species in red (*Cervus elaphus*, L.) and fallow (*Dama dama*, L.) deer using fecal NIRS. In these studies, apparent differences in reproductive or gastrointestinal physiology were discerned. Fecal NIRS has also been used to discriminate between samples from animals experiencing an external parasite burden versus those without parasites (Tolleson et al., 2000b). Additionally, the phase of external tick feeding cycle in synchronized single-cohort infestations affected fecal NIRS diet quality predictions in cattle (Tolleson et al., 2002).

Our previous studies (published and unpublished) of fecal NIRS and parasite status have been small and opportunistic. Although the results from these studies are similar, it is not known if the data sets are independent, if prediction models developed from any single data set or combination of data sets can accurately predict another, or if all the samples can be combined into an inclusive calibration for a more robust application of the technique. The need for specific calibrations based on animal species, vegetation/diet, or season would seriously hinder the development and adoption of fecal NIRS as a diagnostic tool for scientists, practitioners, or producers. Therefore, the objective of this manuscript was to synthesize previous data sets from fecal NIRS discrimination of pre-infested versus tick-infested animals, and to determine the ability of the technique to identify tick-infested animals across host species, tick species, and diet.

2. Materials and methods

2.1. Tick origins and maintenance

Ticks used in these studies were obtained from research and teaching colonies at the Tick Research Laboratory, College Station, TX 77843-2475. Experimental populations of *A. americanum* and *Dermacentor albipictus* (Packard) were established (and are periodically maintained) via progeny of gravid female ticks collected from cattle and horses at the Sonora Experiment Station and Hill Ranch, Sutton and Edwards Co., TX, respectively. Colonies of *A. maculatum* and *Amblyomma cajennense* (F.) originated from collections of engorged female specimens removed from cattle pastured at the Wallace Shay Ranch in Refugio Co., TX, and the La Copita Research Station, Jim Wells Co., TX. Between studies, colonies of tick species were maintained within separate glass humidity chambers where environmental conditions approximated 20 °C, 90% relative humidity, and 14-h light:10-h dark photoperiod.

2.2. Experimental animals and tick exposures

Five independent single group pretest–posttest response trials (Bodtker, 2001; Dangel et al., 1989) were performed during 1998–2003, where cattle (*Bos* spp.) and horses (*Equus caballus*) were artificially infested with different, trial specific numbers of 4 ixodid tick species (Table 1). In as much as possible, animals were obtained from sources known to have minimal risk of previous tick exposure. In studies where cattle were used to measure tick/host fecal responses, animals were confined within individual stanchions or gated paddocks, provided ad libitum access to water, and fed different commercially available grain and grain byproduct based rations (crude protein = 10.0 ± 0.5%). Animals were fed their respective rations for at least 7 days prior to tick exposure. Hair-coats covering the back regions of bovids were shorn to a length of 3–4 mm. In the shorn region, 6–8, 30-cm lengths of a 15-cm diameter surgical cotton stockinette were glued along each animal's midline using a commercially available adhesive (Nasco Livestock ID Tag Cement, Ft. Atkinson, WI, USA). Ticks were confined within these "socks" by twisting the open end of the fabric tightly into a pigtail and securing it closed with rubber bands. As blood feeding progressed and detachment from cattle hosts began, all fed female ticks were harvested daily from socks on each animal and recorded.

Table 1
Summary of trials using near infrared reflectance spectroscopy of feces to determine relative external parasite burden

Trial	Year	Host animal	No. of animals	Parasite species	No. of male/female ticks per animal	No. of spectra		R^2	SECV[c]
						Low Stress	High Stress		
1	1998	Cow	3	Aa, Am, Ac[a]	320/320	9	9	0.9569	0.2010
2	1999	Mare	20	Ac	50/50	4	4	NA	NA
3	2000	Heifer	4	Aa	250/250	12	12	0.9106	0.2619
4	2000	Steer	24	Aa, Da[b]	250/250	22	23	0.8209	0.2792
5	2003	Steer	7	Aa	300/300	35	35	0.8844	0.2166

Adult ticks were used in all trials but were used in combination with larvae in trial 4. Low stress was defined as either pre-exposure to ticks or pre-attachment/early tick feeding. High stress was defined as peak tick feeding for the respective species.

[a] Animal 1 received: 240 *Amblyomma americanum*, (Aa), and 80 *A. maculatum* (Am) adult tick pairs, animal 2 received: 240 Aa and 80 *A. cajennense* (Ac) adult tick pairs, and animal 3 received: 320 Aa adult tick pairs.

[b] In addition to the 250 male/female Aa adult tick pairs, approximately 300 *Dermacentor albipictus* (Da) larvae were infested per animal.

[c] SECV: standard error of cross validation.

Alternatively, a single trial involving horses that were maintained outdoors (in 4 groups of 5 animals) within sheltered, concrete-floored paddocks was also conducted. A commercially available horse and mule ration (crude protein = 10.0%) mixed with chopped alfalfa (*Medicago sativa*) hay (crude protein = 16.0%) and augmented with bermuda grass (*Cynodon dactylon*) hay (crude protein = 10.0–12.0%) was fed 90 days prior to, and throughout tick exposure. Ticks infested on these animals were free-released near the mane/wither regions of each horse; not confined within stockinette socks as they were on cattle. To maximize recovery of detached ticks and to impede lateral movement by ticks between pens, the perimeter fencing of each horse paddock was completely encircled by doubled lengths of rubber garden hose. This strategy had proved successful in previous free-release experiments where it was discovered that the hoses posed a formidable physical barrier to tick escape. Detached female ticks were collected from the floors of each horse pen during daily pen cleanings, and during morning and evening feedings. An unoccupied paddock was also maintained between each pen of infested horses to enhance tick recovery efforts and to maximize the accuracy of daily tick tallies from each horse group.

The total number of ticks attaching, feeding to repletion, and then detaching as engorged females, was recorded by date and animal in each of the 5 trials to provide a unique temporal profile of tick stress across tick/host species for comparison to results of NIR fecal spectra. Counts of male ticks were made only for trials using three-host species and male ticks were allowed to remain on their respective hosts as would occur in the field.

2.3. Fecal sampling and NIRS procedures

Fecal samples were obtained daily from the stall floor of individual animals starting 3 days prior to tick exposure through completion of tick feeding and detachment in each of the cattle trials. Fresh, non-urine or feed contaminated feces were selected. Non-invasive fecal sampling was the same for horses except that samples collected represented a daily composite from the five animals within each pen. The horse samples were collected in conjunction with morning feeding, at which time the animals routinely defecated in anticipation of feeding. An attempt was made to equally represent each animal in each sample. Samples were stored at −20 °C until processed for NIRS using the method of Lyons and Stuth (1992), briefly this involved drying at 60 °C in a forced air oven, grinding to 1 mm particle size and re-drying at 60 °C. Spectra (400–2500 nm) were collected on a Foss® NIRS 6500 scanning monochrometer with spinning cup attachment.

2.4. Statistical analyses

Five unique calibration data sets were generated from these trials by comparing the differing numbers and species of ticks with the corresponding host group's fecal responses using NIR procedures. Three-host tick species *A. americanum*, *A. cajennense*, and *A. maculatum* were able to complete their respective feeding cycles in 10–21 days (Fig. 1), whereas female *D. albipictus* required 21–30 days (following their release on steers in Trial 4 as one-host larvae) to feed to completion. Low tick stress samples were defined as those obtained from animals before exposure, or during pre-attachment/early tick feeding. High tick stress

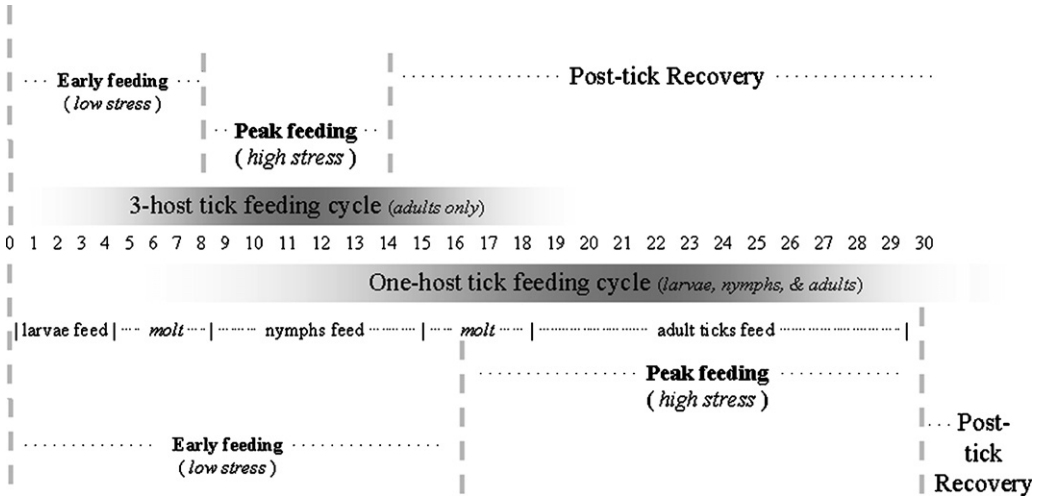

Fig. 1. Comparative sequences, intensities, and durations of three-host and one-host tick feeding cycles by calendar day. Note: day "0" represents the day that ticks are placed onto hosts to commence feeding.

samples were defined as those obtained from animals during peak adult female tick feeding, and is associated with highest blood loss and tick salivary secretion (Sonenshine, 1991). Teel et al. (unpublished data) have determined that fecal NIR spectra of tick treated and non tick treated animals are undistinguishable at 28 and 35 days post tick infestation.

Discriminant analyses to determine if differences exist in NIR spectra of samples associated with low versus high tick stress were performed using a 2-block partial least squares procedure (Martens and Martens, 2001) in WinISI® v. 1.04a software. Calibration sets were developed for: (1) each individual trial, (2) each combination of four trials, and (3) with all five trials combined. Acceptable discriminant equation performance was defined as $R^2 > 0.80$ and standard error of cross validation (SECV) < 0.33. Validation was accomplished by: (1) cross validation (Westerhaus, 1985), (2) prediction of the remaining calibration set, and (3) predicting a random 10 or 25% of samples removed from the combined calibration sets.

In order to determine if group membership of samples was based on biological characteristics, a calibration set created by dividing samples into groups with odd versus even numbers was employed (Tolleson et al., 2005). As with the previous discriminant data, a random 10 or 25% of the calibration data set was also removed for validation purposes.

3. Results

Calibration equation performance for each individual trial is found in Table 1. There was a high degree of discrimination ($R^2 > 0.82$; SECV < 0.28) in each individual trial indicating physico-chemical differences between fecal samples from Low versus High tick stress. This relationship existed regardless of ration, host species, and tick species/number. In order to determine if these differences were consistent across trials, each combination of four trials was combined. The resulting discriminant equations were then used to predict group membership for samples from the remaining trial (Table 2). Again, R^2 and SECV values indicate generally acceptable discrimination between calibration samples. Prediction of group membership in the validation sets however, yielded varying degrees of success. The mean percentage of correct determinations for Low and High tick stress samples was only slightly better than random chance (53.4 and 60.1, respectively). This would indicate little chance for practical application of discriminant equations with fecal NIRS if the unknowns were from a sample population decidedly different from the training set. When discriminant equations from only those trials conducted with cattle and one-host ticks (trials 1,3,5) were compared in a similar fashion (i.e. use two data sets to predict the third), similar results were obtained. Values for R^2 and SECV ranged from 0.66 and 0.33, to 0.92 and 0.20, respectively. Correct determinations ranged from 0 to 89% for Low stress, and from 44 to 100% for High stress.

Tolleson et al. (2005), using fecal NIRS, were unable to discriminate sex and species in cervids across years due to differences in diet quality. When years were combined in that study, greater than 90% of validation samples were correctly identified. Diet

Table 2
Tick stress/fecal NIRS discriminant equation results of all combinations of 4 individual trials using 5th trial as an independent validation set

Calibration groups	No. of spectra		R^2	SECV[a]	Validation group	% Correct	
	Low stress	High stress				Low stress	High stress
1,2,3,4	47	48	0.8667	0.3596	5	94.3	100
1,2,3,5	60	60	0.8779	0.3121	4	72.7	26.1
1,2,4,5	70	71	0.8450	0.3450	3	0.0	75.0
1,3,4,5	78	79	0.8814	0.3154	2	0.0	100
2,3,4,5	73	74	0.8713	0.3106	1	100	22.2

Adult ticks were used in all trials. Low stress was defined as either pre-exposure to ticks or pre-attachment/early tick feeding. High stress was defined as peak female tick feeding for the respective species.
[a] SECV: standard error of cross validation.

Table 3
Tick stress/fecal NIRS discriminant equation results of all trials combined using cross validation or, either 10 or 25% of samples randomly selected and removed from calibration as a validation set

Calibration	No. of spectra		R^2	SECV[a]	Validation	% Correct	
	Low stress	High stress				Low stress	High stress
Full	82	83	0.9023	0.3128	CV[b]	90.2	94.0
90%	74	75	0.9562	0.3379	10%	100	100
75%	62	63	0.9585	0.3563	25%	85.0	95.0

Adult ticks were used in all trials. Low stress was defined as either pre-exposure to ticks or pre-attachment/early tick feeding. High stress was defined as peak female tick feeding for the respective species.
[a] SECV: standard error of cross validation.
[b] CV: cross validation.

quality was thus a greater distinguishing feature in the feces than any differences due to sex or species. A similar occurrence could be at play in the current study given the variety in animals and diets that exist. All five trials in the current study were combined and either 10 or 25% of the samples were randomly selected and removed from the calibration set (Table 3). As in the previous calibrations, a high degree of discrimination was achieved ($R^2 > 0.89$; SECV < 0.36). The validation samples were correctly identified at 91.7% for Low stress and 96.3% for High stress, respectively.

Table 4
Non-biological (odd versus even sample numbers)/fecal NIRS discriminant equation results of all trials combined using cross validation or, either 10 or 25% of samples randomly selected and removed from calibration as a validation set

Calibration	No. of spectra		R^2	SECV[a]	Validation	% Correct	
	Odd	Even				Odd	Even
Full	83	82	0.1761	0.5542	CV[b]	54.2	47.6
90%	75	74	0.2258	0.5566	10%	50.0	50.0
75%	63	62	0.2103	0.6103	25%	40.0	60.0

[a] SECV: standard error of cross validation.
[b] CV: cross validation.

To determine if the differences in fecal spectra noted above were indeed related to biological characteristics, the entire calibration set was arranged numerically by sample number and divided into ''odd'' versus ''even'' numbered samples. As described in the previous section, calibration equations with 10 or 25% of samples removed for validation were developed. Results of this exercise are presented in Table 4. Poor discrimination ($R^2 < 0.23$) and imprecision (SECV > 0.55) were obtained from the odd versus even data set. Correct identification of group membership was approximately 50%. Performance of this calibration supports the biological significance of the Low versus High tick stress calibrations described herein.

4. Discussion

Near infrared spectroscopy of feces has been used successfully to identify animals of different species, consuming varied diets, undergoing disparate tick stress burdens. Stress is often defined as an interruption in, or perceived threat to homeostasis. Parasitism by definition is a stressful situation for the host. The interrelationships between stress, gut motility, digestion, metabolism, endocrinology, and immunology are

well established (Glaser and Kiecolt-Glaser, 2005; Mulak and Bonaz, 2004; Hart and Kamm, 2002; Tache et al., 2001). In particular, ticks are known to modulate the immune system of their hosts as part of long-term blood-feeding strategies (Brossard and Wikel, 1997; Sauer et al., 1994; Jaworski et al., 2001). Feces contain the end products, by products or other evidence of these various processes (Church, 1969) and are a repository of metabolic information. Fecal NIRS can thus be utilized as a ''bio-forensic'' approach and its obvious advantages exploited in the study of free-ranging animals.

Two potential applications of fecal NIRS as a non-invasive monitoring technique are in: (1) ranching and livestock production and (2) surveillance against bio- or agro-terrorism. If spectral signatures in feces associated with economic thresholds of parasitism can be established, livestock producers could target treat only those individuals or herds affected. This would help avoid unnecessary use of pesticides and handling of animals and in turn reduce input costs per unit of meat, milk or fiber since current recommendations for tick management in livestock production depends on physical inspection of individual animals for attached ticks. Parasite detection with fecal NIRS would complement existing diet quality monitoring by fecal NIRS (Stuth and Tolleson, 2000) and contribute to overall herd health programs. Ticks are vectors of numerous disease pathogens both native and foreign to US livestock and humans. Heartwater, babesiosis, and Crimean Congo Haemorragic Fever are among the list of important foreign animal diseases important to US livestock economic and biosecurity (USAHA,1992). Strategic collection of geo-referenced fecal samples from transported and free-ranging livestock and wildlife could provide early detection and surveillance of emerging tick or disease outbreaks similar to those produced for drought or forage conditions (Stuth et al., 2005). Fecal NIRS predictions could also be combined with environmental conditions (temperature, humidity, forage availability, previous winter kill, etc.) to develop surveillance indices and risk assessment of vulnerability to unintended introductions or bio-terrorist attack.

5. Conclusion

Difficulties in detecting differences in fecal samples due to confounding effects of trial were overcome by combining calibration sets. Overall, differences in fecal NIR spectra apparently due to tick stress were accurately detected across diet, host species, and tick species. There is however, insufficient information to determine what biological event or events might be causing the differences in fecal spectra observed in this study. Ongoing laboratory studies are examining interactions of the immune- and endocrine systems of tick infested cattle on different planes of nutrition to clarify the basis for fecal chemistry changes as reflected by NIRS profiles. Complementary field studies will evaluate fecal NIRS profiles of natural tick infestations of range cattle in the presence of other ectoparasites, particularly the horn fly. These studies should help elucidate the capabilities and or limitations of fecal NIRS as either an animal management or bio-surveillance technique.

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References

Barnard, D.R., 1985. Injury thresholds and production loss functions from the lone star tick, *Amblyomma americanum* (Acari: Ixodidae), on pastured, pre-weaner beef cattle *Bos taurus*. J. Econ. Entomol. 78, 852–855.

Barnard, D.R., Mount, G.A., Haile, D.G., Daniels, E., 1994. Integrated management strategies for *Amblyomma americanum* (Acari: Ixodidae) on pastured beef cattle. J. Med. Entomol. 31, 571–585.

Bodtker, A., 2001. Conflict education and special-needs students, part two: Improving conflict competence and emotional competence. Mediation Q. 18, 377–396.

Brossard, M., Wikel, S.K., 1997. Immunology of interactions between ticks and hosts. Med. Vet. Entomol. 11, 270–276.

Church, D.C., 1969. Digestive Physiology and Nutrition of Ruminants, second ed. O&B Books, Inc., Corvallis, OR, 350 pp.

Coates, D.B., 2000. Faecal NIRS—what does it offer today's grazier? Trop. Grasslands 34, 230–239.

Dangel, R.F., Deschner, J.P., Rasp, R.R., 1989. Anger control training for adolescents in residential treatment. Behav. Modification 13, 447–458.

Foley, W.J., McIlwee, A., Lawler, I.R., Aragones, L., Woolnough, A., Berding, N., 1998. Ecological applications of near-infrared spectroscopy: a tool for rapid, cost effective prediction of the composition of plant tissues and aspects of animal performance. Oecologia 116, 293–305.

Glaser, R., Kiecolt-Glaser, J.K., 2005. Stress-induced immune dysfunction: Implications for health. Nat. Rev. Immunol. 5, 243–251.

Godfrey, R.W., Dodson, R.E., Bultman, J.K., Tolleson, D.R., Stuth, J.W., Norman, A.J., 2001. Use of near infrared reflectance spectroscopy to differentiate pregnancy status and gender of hair sheep in the tropics. J. Anim. Sci. 79, 26.

Hart, A., Kamm, M.A., 2002. Review article: mechanisms of initiation and perpetuation of gut inflammation by stress. Aliment. Pharmacol. Ther. 16, 2017–2028.

Jaworski, D.C., Jasinskas, A., Metz, C.N., Bucala, R., Barbour, A.G., 2001. Indentification and characterization of a homologue, of the pro-inflammatory cytokine macrophage migration inhibitory factor in the tick, *Amblyomma americanum*. Insect Mol. Biol. 10, 323–331.

Lyons, R.K., Stuth, J.W., 1992. Fecal NIRS equations for predicting diet quality of free-ranging cattle. J. Range Manage. 45, 238–244.

Martens, H., Martens, M., 2001. Multivariate Analysis of Quality: An Introduction. Wiley, J. and Sons Ltd., Chichester, England, 445 pp.

Mulak, A., Bonaz, B., 2004. Irritable bowel syndrome: a model of the brain–gut interactions. Med. Sci. Monit. 10, 55–62.

Sauer, J.R., McSwain, J.L., Essenberg, R.C., 1994. Cell membrane receptors and regulation of cell function in ticks and blood-sucking insects. Int. J. Parasitol. 24, 33–52.

Schmidtmann, E.T., 1994. Ecologically based strategies for controlling ticks. In: Sonenshine, D.E., Mather, T.N. (Eds.), Ecological Dynamics of Tick-Borne Zoonoses. Oxford Univ. Press, pp. 240–282.

Sonenshine, D.E., 1991. Biology of ticks, vol. I. Oxford University Press, New York, pp. 141–158.

Stuth, J.W., Tolleson, D.R., 2000. Monitoring the nutritional status of grazing animals using near infrared spectroscopy. Compend. Contin. Ed. Pract. Vet. 22, S108–S115.

Stuth, J.W., Angerer, J., Kaitho, R., Jama, A., Marambii, R., 2005. Livestock early warning system for Africa's rangelands. In: Boken, V.K., Cracknell, A.P., Heathcote, R.L. (Eds.), Monitoring and predicting agricultural drought: A global study. Oxford University Press, Oxford, pp. 283–294.

Tache, Y., Martinez, V., Million, M., Wang, L., 2001. Stress and the gastrointestinal tract III. Stress-related alterations of gut motor function: role of brain corticotropin-releasing factor receptors. Am. J. Physiol. Gastrointest. Liver Physiol. 280, 173–177.

Tolleson, D.R., Wilson, T.W., Randel, R.D., Neuendorff, D.A., Lewis, A.W., Stuth, J.W., 2000a. Discrimination between physiologically different groups of cattle via near infrared reflectance spectroscopy of feces. J. Anim. Sci. 78 (14 (Suppl. II)).

Tolleson, D.R., Teel, P.D., Stuth, J.W., Strey, O.F., 2000b. Discrimination of parasite burden in livestock via near infrared reflectance spectroscopy of feces. J. Anim. Sci. 78 (14 (Suppl. II)).

Tolleson, D.R., Teel, P.D., Stuth, J.W., Strey, O.F., 2002. Phase of external parasite feeding cycle affects NIRS predicted diet quality in cattle. J. Anim. Sci. 80 (8 (Suppl. II)).

Tolleson, D.R., Randel, R.D., Stuth, J.W., Neuendorff, D.A., 2005. Determination of sex and species in red and fallow deer by near infrared reflectance spectroscopy of the faeces. Small Ruminant Res. 57, 141–150.

USAHA, 1992. Foreign animal diseases. Their prevention, diagnosis and control. Committee on Foreign Animal Diseases. USAHA, p. 381.

Westerhaus, M.O., 1985. Validation. In: Marten, G.C., Shenk, J.S., Barton, II, F.E. (Eds.), Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality. USDA Agric. Handbook No. 643, Washington, DC, pp. 40–41.