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Short Communication: Early Detection of Mastitis Using Infrared Thermography in Dairy Cows¹

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ABSTRACT

Infrared thermography (IRT) absorbs infrared radiation and generates images based on the amount of heat generated. It has been used in human medicine for diagnosis of various cancers. This experiment was conducted to determine if IRT had merit for early detection of subclinical mastitis in dairy cows. Milk sample and skin surface temperature (SST) were simultaneously evaluated using the California Mastitis Test (CMT) and IRT for each quarter in 94 dairy cows (49 Brown Swiss and 45 Holstein). Average days in milk (DIM) and milk production were 93 ± 37 d and 16 \pm 2.2 kg (mean \pm SD) and their ages ranged from 4 to 8 yr. There was a strong correlation between SST and CMT score (r = 0.92). Average SST was 33.19, 34.08, 34.99, and 36.15°C for quarters with the CMT score of 0 (n = 156), +1 (n = 116), +2 (n = 80), and +3 (n = 24),respectively. This association was best described by a linear model as follows: y = 0.94x + 33.17, $R^2 = 0.85$, where y = SST and x = CMT score. Changes in rectal temperature (RT) due to the CMT score were minor (y = 0.09x + 38.39, $R^2 = 0.07$, where y = RT and x = average CMT score). In conclusion, RT may not confirm mastitis. However, IRT is sensitive enough to perceive changes in SST in response to varying degrees of severity of the mammary gland infection as reflected by the CMT score, suggesting that as a noninvasive tool, IRT can be employed for screening dairy cows for mastitis. **Key words:** mastitis, infrared thermography, California Mastitis Test, dairy cow

Infrared thermography (IRT) or thermovision was originally developed for military and industrial purposes. There is a growing interest in its usage in human and veterinary medicine (Mazur and Eugeniusz-

Herbut, 2006). All objects emit energy proportional to their temperature (the Stefan-Boltzmann law). This energy is lost in the form of heat via radiation, conduction, and convection. Radiation can be absorbed, emitted, reflected, or transmitted. The thermal camera is considered a state-of-the-art device. It absorbs infrared radiation and generates images based on the amount of heat generated rather than reflected (Eddy et al., 2001; Mazur and Eugeniusz-Herbut, 2006).

Infrared thermography has been successfully employed in various applications of animal production such as assessing meat quality in pigs (Schaefer et al., 1989) and feather cover in chickens (Cook et al., 2006). The IRT is sensitive to detect changes in body temperature, in cases of estrus (Hurnik et al., 1985), infection (Willard et al., 2007), and eating (Laue and Petersen, 1991). Moreover, it is noninvasive and does not cause radiation exposure (Eddy et al., 2001; Schaefer et al., 2004). Thus, IRT may also have merit for assessing welfare (Stewart et al., 2005) resulting from changes in body surface temperature associated with adaptation to microclimatic changes (Kimmel et al., 1992; Knizkova et al., 1996, 2002). This may also be useful in monitoring discomfort associated with some stressful managerial practices such as tail docking (Eicher et al., 2006), catheterization (Stewart et al., 2007), ear implantation (Spire et al., 1999), milking equipment (Kunc et al., 1999), and barn facilities (Schwartzkopf-Genswein and Stookey, 1997). The ability to detect thermal changes using IRT were also shown for bovine viral diarrhea infection in calves after facial scanning (Schaefer et al., 2004, 2007), for lameness in dairy cows after hoof scanning (Head and Dyson, 2001; Nikkhah et al., 2005), and for hyperthermia resulting from the tuberculin test administration on ruminants (Merkal et al., 1973; Lepper et al., 1974). Studies dealing with sperm quality and scrotal skin surface temperature (SST; Purohit et al., 1985; Lunstra and Coulter, 1997) and their responsiveness to environmental temperature (Kastelic et al., 1996, 1997) are available.

Early detection of mastitis is extremely important for the efficacy of treatment because it is associated with

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suppressed milk production, deteriorated milk quality, discarded milk, increased veterinary care, drug, and labor costs, shortened longevity, and increased culling rate (Sargeant et al., 1998). Temperature increases at the onset of inflammation. Using IRT, Hurnik et al. (1984) studied various health disorders in dairy cows and were able to detect 4 out of 6 mastitis cases. However, detection of more systemic infections such as pneumonia had a greater success rate. A more recent study by Scott et al. (2000) showed that endotoxin infusion into the mammary gland to induce an inflammatory response similar to mastitis resulted in a measurable increase (+2.3°C) in udder temperature as measured by IRT. It was hypothesized that mammary gland infection among quarters were variable and could be distinguished by SST. The objective of this study was therefore to evaluate changes in SST using IRT in conjunction with the California Mastitis Test (CMT) score in mammary gland lobes.

Ninety-four cows (49 Brown Swiss and 45 Holstein) averaging 93 ± 37 DIM and 16 ± 2.2 kg of milk production (mean \pm SD), and from 4 to 8 yr old were obtained from Atatürk University Research Farm to screen for subclinical mastitis using CMT. Rectal temperature (RT) was checked for the existence of systemic infec-

tions. Before the udder examination, all cows were allowed to rest for 30 min in a slightly dark room; room temperature was 18 to 23°C. After performing the CMT, each quarter was subjected to IRT (IR Flex-Cam S, Infrared Solutions Inc., Plymouth, MN) before milking. The thermograph resolution was calibrated to room temperature for each measurement. Scans were directed to areas where vascularization on skin surface was low to obtain accurate temperature in standing position and holding tail away from mammary gland (Berry et al., 2003; Figure 1). The Ethics Committee on Experimental Animal Use at Atatürk University reviewed and approved the experimental protocol.

The SST and CMT score for each quarter were analyzed using the UNIVARIATE, MEANS, REG, and CORR procedures (SAS Institute, 1999). The ANOVA procedure was used to determine differences in SST and CMT score by quarter. After averaging the CMT score and SST by cow, SST and RT were regressed on CMT score. Statistical significance was declared at P < 0.05.

Udder quarter SST was normally distributed (Table 1) and positively correlated with the CMT score when each quarter was considered separately (r = 0.92, P < 0.0001). As the CMT score increased, quarter SST

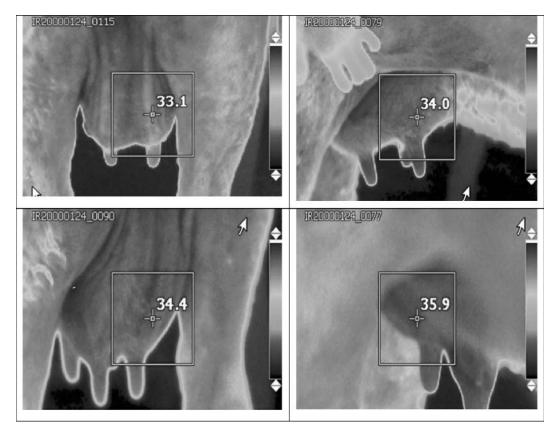


Figure 1. Measurement of the udder skin temperature by infrared thermography.

4246 COLAK ET AL.

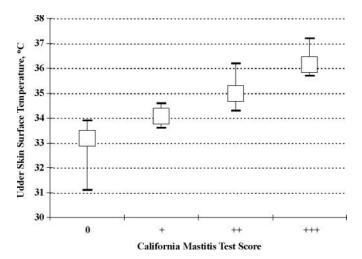


Figure 2. Relationship between the California Mastitis Test (CMT) score and skin surface temperature of mammary gland lobes determined by infrared thermography. Regression line was y=0.94x+33.17; $R^2=0.85$; P<0.0001 for slope and intercept, where y= udder skin surface temperature and x= CMT score. Number of quarters with the CMT score of 0, +1, +2, and +3 were 156, 116, 80, and 24, respectively.

increased linearly (y = 0.94x + 33.17; $R^2 = 0.85$; P < 0.0001 for slope and intercept; Figure 2).

The CMT score (0.96 vs. 0.89; P < 0.51) and SST (33.98 vs. 34.09°C; P < 0.26) for front quarters were not different from those for rear quarters. The CMT score and SST for the right-front, left-front, right-rear, and left-rear quarters were 0.95, 0.97, 0.93, and 0.86 (P < 0.88) and 33.99, 33.97, 34.13, and 34.05°C (P < 0.65), respectively (data not shown). When averaged by cow, there were a strong correlation between the CMT score and udder SST (r = 0.93; P < 0.0001) and weak correlations between the CMT score and RT (r = 0.27; P < 0.01) and between udder SST and RT (r = 0.24; P < 0.02).

The average udder SST was lower than RT (34.04 vs. 38.47°C; P < 0.0001; Figure 3). When averaged by cow, the CMT score had a stronger relationship with udder SST (y = 0.97x + 33.14; $R^2 = 0.87$; P < 0.0001 for both slope and intercept) than RT (y = 0.09x + 38.39; $R^2 = 0.07$; P < 0.01 for slope and P < 0.0001 for intercept;

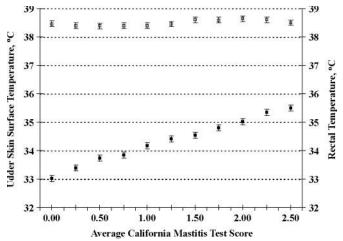


Figure 3. Changes in the skin surface temperature of mammary gland determined by infrared thermography (■) and rectal temperature (□) due to average California Mastitis Test (CMT) score. Standard error of a mean was 0.11 and 0.10 for skin surface temperature and rectal temperature, respectively. Regression line for the udder skin surface was y = 0.97x + 33.14; $R^2 = 0.87$; P < 0.0001 for both slope and intercept and for the rectal temperature was y = 0.09x + 38.39; $R^2 = 0.07$; P < 0.0001 for intercept and P < 0.01 for slope, where x = average CMT score.

Figure 3). However, SST did not explain variability in RT (y = 0.08x + 35.76; R² = 0.06; P < 0.02 for slope and P < 0.0001 for intercept, where y = RT and x = average udder SST; Figure 3).

The idea of IRT use in veterinary diagnostic to determine changes in SST resulting from administration of pharmaceutical agents and intervention of surgical procedures, which are accompanied by alterations in vascularization or blood flow as both systemic (fever) and local (inflammatory) responses, is not new (Stephan and Görlach, 1971; Clark and Cena, 1977; Cockcroft et al., 2000). Despite being expensive and lacking specificity regarding the etiology, thermography provides useful information for the presence of pathology in a region of interest (Purohit and McCoy, 1980; Hurnik et al., 1984; Schaefer et al., 2000). Moreover, the equipment is becoming less expensive each year, whereas the prevalence of mastitis remains high. Living organs transfer more heat associated with blood

Table 1. Descriptive statistics of the skin surface temperature of mammary gland lobes measured by infrared thermography¹

Descriptive measure								
Median	Mode	SE	SD	Variance	Minimum	Maximum	Kurtosis	Skewness
33.30	33.60	0.04	0.52	0.22	31.10	33.90	3.10	-1.42 0.33
34.90	35.20	0.04	0.25	0.10	34.30	36.20	1.28	0.61 1.45
	33.30 34.10	33.30 33.60 34.10 33.90 34.90 35.20	33.30 33.60 0.04 34.10 33.90 0.02 34.90 35.20 0.04	Median Mode SE SD 33.30 33.60 0.04 0.52 34.10 33.90 0.02 0.29 34.90 35.20 0.04 0.25	Median Mode SE SD Variance 33.30 33.60 0.04 0.52 0.22 34.10 33.90 0.02 0.29 0.07 34.90 35.20 0.04 0.25 0.10	Median Mode SE SD Variance Minimum 33.30 33.60 0.04 0.52 0.22 31.10 34.10 33.90 0.02 0.29 0.07 33.60 34.90 35.20 0.04 0.25 0.10 34.30	Median Mode SE SD Variance Minimum Maximum 33.30 33.60 0.04 0.52 0.22 31.10 33.90 34.10 33.90 0.02 0.29 0.07 33.60 34.60 34.90 35.20 0.04 0.25 0.10 34.30 36.20	Median Mode SE SD Variance Minimum Maximum Kurtosis 33.30 33.60 0.04 0.52 0.22 31.10 33.90 3.10 34.10 33.90 0.02 0.29 0.07 33.60 34.60 -0.90 34.90 35.20 0.04 0.25 0.10 34.30 36.20 1.28

¹Linearity test, P < 0.0001.

²California Mastitis Test; n = the number of quarters.

flow than do dead tissues (Bhattacharya and Mahajan, 2003). Redness due to vascularization or blood flow, pain (hypersensitivity), swelling, and hyperthermia are major signs in the early stage of inflammation and infection (Cheville, 1999). Skin surface temperature reflects the underlying circulation and tissue metabolism (Berry et al., 2003), which is under the control of the sympathetic nervous system and noradrenergic sympathetic neurons in mammary gland (Paulrud et al., 2005). These factors suggest that determination of SST using IRT may assess mammary gland health status. As can be seen in Figure 1, color pattern changes by thermal gradients. The warmest areas appear white or red, whereas the coolest regions appear blue or black (Eddy et al., 2001).

In the present study, we used a CMT scoring system that is frequently applied for screening dairy cows for IMI (Sargeant et al., 2001). This CMT score increases as SCC increases (Goyache et al., 2005). The sensitivities for detecting IMI with any pathogen, IMI with a major pathogen, and IMI with a minor pathogen were 56.7, 66.7, and 49.5%, respectively (Sargeant et al., 2001). Moreover, the sensitivity and specificity of CMT compared with culture and SCC methods were reported to be 84.1 and 97.5% and 61.22 and 79.6%, respectively (Gharagozloo et al., 2003). A CMT score of +1 reflects subclinical mastitis, which slightly exceeds the physiological threshold of 100,000 cells/mL (Redetzky et al., 2005). Gharagozloo et al. (2003) reported that SSC exponentially decreased with lowered CMT score, with SSC of 7,190,000, 1,930,000, 1,240,000, and 154,000 for +3, +2, +1, and negative CMT, respectively. Udder temperature is closely correlated to body temperature (body and udder temperatures were 38.8 ± 0.1 °C) in cows without mastitis implanted with thermistors (Bitman et al., 1984). Changes due to mastitis may be detected using IRT (Schaefer et al., 2000; Berry et al., 2003) as seen in the present experiment (Figures 1, 2, and 3). Evaluating udder inflammation by IRT, Barth (2000) reported that mean surface temperature of teats increased from 30.1°C at the tip to 35.1°C at the udder base and that SST was greater for quarters with SCC >100,000 than those <100,000 (34.1 vs. 33.6°C). Paulrud et al. (2005) confirmed asymmetry in the integrity of teat channel and SST of rear quarters exposed to tension by different type of liner (extended vs. soft) and level of milking (normal vs. excessive) compared with those at prechallenge and adjacent quarters using ultrasound and IRT.

Despite significance, the relationship between udder SST and RT was negligible (Figure 3), because it was within physiological range (Kahn, 2005). This may suggest an absence of systemic effect of positive CMT.

Numerous factors that are not taken into account may affect udder skin temperature such as humidity of environment and skin, physiological state and production level of the cow, and time relative to feeding and milking. However, this experiment showed that IRT was sensitive to detect differences in udder skin temperature with respect to CMT score. In conclusion, IRT is a noninvasive and rapid method and can be of great value in early detection for optimum response from treatment. Further studies should study thermographic evaluation of udder of cows under different conditions.

REFERENCES

- Barth, K. 2000. Basic investigations to evaluate a highly sensitive infrared-thermograph technique to detect udder inflammation in cows. Milk Sci. Int. 55:607–609.
- Berry, R. J., A. D. Kennedy, S. L. Scott, B. L. Kyle, and A. L. Schaefer. 2003. Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: Potential for mastitis detection. Can. J. Anim. Sci. 83:687–693.
- Bhattacharya, A., and R. L. Mahajan. 2003. Temperature dependence of thermal conductivity of biological tissues. Physiol. Meas. 24:769–783.
- Bitman, J., A. Lefcourt, D. L. Wood, and B. Stroud. 1984. Circadian and ultradian temperature rhythms of lactating dairy cows. J. Dairy Sci. 67:1014–1023.
- Cheville, N. F. 1999. Inflammation and healing. Pages 101–102 in Introduction to Veterinary Pathology. 2nd ed. Iowa State University Press, Ames.
- Clark, J. A., and K. Cena. 1977. The potential of infra-red thermography in veterinary diagnosis. Vet. Rec. 100:402–404.
- Cockcroft, P. D., F. M. D. Henson, and C. Parker. 2000. Thermography of a septic metatarsophalangeal joint in a heifer. Vet. Rec. 146:258–260.
- Cook, N. J., A. B. Smykot, D. E. Holm, G. Fasenko, and J. S. Church. 2006. Assessing feather cover of laying hens by infrared thermography. J. Appl. Poult. Res. 15:274–279.
- Eddy, A. L., L. M. van Hoogmoed, and J. R. Snyder. 2001. Review: The role of thermography in the management of equine lameness. Vet. J. 162:172–181.
- Eicher, S. D., H. W. Cheng, A. D. Sorrells, and M. M. Schutz. 2006. Behavioral and physiological indicators of sensitivity or chronic pain following tail docking. J. Dairy Sci. 89:3047–3051.
- Gharagozloo, F., M. Blourchi, A. M. Tabatabaee, H. G. Nava, and M. Vojgani. 2003. Sensitivity and specificity of CMT to detect subclinical mastitis in dairy cows. Anim. Fishery Sci. 59:59–62. (in Farsi)
- Goyache, F., J. Diez, S. Lopez, G. Pajares, B. Santos, I. Fernandez, and M. Prieto. 2005. Machine Learning as an aid to management decisions on high somatic cell counts in dairy farms. Arch. Anim. Breed. 48:138–148.
- Head, M. J., and S. Dyson. 2001. Talking the temperature of equine thermography. Vet. J. 162:166–167.
- Hurnik, J. F., S. DeBoer, and A. B. Webster. 1984. Detection of health disorders in dairy cattle utilizing a thermal infrared scanning technique. Can. J. Anim. Sci. 64:1071–1073.
- Hurnik, J. F., A. B. Webster, and S. DeBoer. 1985. An investigation of skin temperature differentials in relation to estrus in dairy cattle using a thermal infrared scanning technique. J. Anim. Sci. 61:1095–1102.
- Kahn, C. M. 2005. Reference guides. Page 2582 in The Merck Veterinary Manual. 9th ed. Merck & Co. Inc., Whitehouse Station, NJ.
- Kastelic, J. P., R. B. Cook, and G. H. Coulter. 1997. Contribution of the scrotum, testes, and testicular artery to scrotal/testicular

4248 COLAK ET AL.

thermoregulation in bulls at two ambient temperatures. Anim. Reprod. Sci. 45:255–261.

- Kastelic, J. P., R. B. Cook, G. H. Coulter, G. L. Wallins, and T. Entz. 1996. Environmental factors affecting measurement of bovine scrotal surface temperature with infrared thermography. Anim. Reprod. Sci. 41:153–159.
- Kimmel, E., H. Arkin, and A. Berman. 1992. Evaporative cooling of cattle: Transport phenomena and thermovision. Page 14 in Papers of the Am. Soc. Agric. Eng., St. Joseph, MI.
- Knizkova, I., P. Kunc, M. Koubkova, J. Flusser, and O. Dolezal. 2002. Evaluation of naturally ventilated dairy barn management by a thermographic method. Livest. Prod. Sci. 77:349–353.
- Knizkova, I., P. Kunc, Z. Novy, and J. Knizek. 1996. Evaluation of evaporative cooling on the changes of cattle surface body temperatures with use of thermovision. Zivocisna Vyroba 41:433–439. (in Czech)
- Kunc, P., I. Knizkova, and M. Koubkova. 1999. The influence of milking with different vacuum and different design of liner on the change of teat surface temperature. Czech J. Anim. Sci. 44:131–134.
- Laue, H. J., and U. Petersen. 1991. Relations between temperature changes in the rumen and roughage intake of dairy cows. Zuchtungskunde 63:282–293. (in German)
- Lepper, A. W. D., M. R. Meharry, and P. M. Outterri. 1974. Measurement of infrared radiation from tuberculin skin-test sites in cattle. Aust. Vet. J. 50:192–198.
- Lunstra, D. D., and G. H. Coulter. 1997. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. J. Anim. Sci. 75:767-774.
- Mazur, D., and J. W. Eugeniusz-Herbut. 2006. Infrared thermography as a diagnostic method. Roczniki Naukowe Zootechniki 33:171–181. (in Polish)
- Merkal, R. S., A. B. Larsen, H. A. Nelson, and A. C. Pier. 1973. Thermography of tuberculin reactions in cattle. Infect. Immun. 7:805–808.
- Nikkhah, A., J. C. Plaizier, M. S. Einarson, R. J. Berry, S. L. Scott, and A. D. Kennedy. 2005. Infrared thermography and visual examination of hooves of dairy cows in two stages of lactation. J. Dairy Sci. 88:2749–2753.
- Paulrud, C. O., S. Clausen, P. E. Andersen, and M. D. Rasmussen. 2005. Infrared thermography and ultrasonography to indirectly monitor the influence of liner type and overmilking on teat tissue recovery. Acta Vet. Scand. 46:137–147.
- Purohit, R. C., R. S. Hudson, M. G. Riddell, R. L. Carson, D. F. Wolfe, and D. F. Walker. 1985. Thermography of the bovine scrotum. J. Am. Vet. Med. Assoc. 186:2388–2392.
- Purohit, R. C., and M. D. McCoy. 1980. Thermography in the diagnosis of inflammatory processes in the horse. Am. J. Vet. Res. 41:1167–1174.
- Redetzky, R., J. Hamann, N. T. Grabowski, and G. Klein. 2005. Diagnostic value of the California Mastitis Test in comparison to electronically-counted somatic cells in bovine milk. Pages 487–494 in Mastitis in Dairy Production. 4th IDF International

- Mastitis Conference, Maastricht, the Netherlands. H. Hogeveen, ed. Wageningen Acad. Press, Wageningen, the Netherlands.
- Sargeant, J. M., K. E. Leslie, J. E. Shirley, B. J. Pulkrabek, and G. H. Lim. 2001. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. J. Dairy Sci. 84:2018–2024.
- Sargeant, J. M., H. M. Scott, K. E. Leslie, M. J. Ireland, and A. Bashiri. 1998. Clinical mastitis in dairy cattle in Ontario: Frequency of occurrence and bacteriological isolates. Can. Vet. J. 39:33–38.
- SAS Institute. 1999. User's Ğuide. Statistics, Version 8. SAS Inst. Inc., Cary, NC.
- Schaefer, A. L., N. J. Cook, J. S. Church, J. Basarab, B. Perry, C. Miller, and A. K. W. Tong. 2007. The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. Res. Vet. Sci. 83:376–384.
- Schaefer, A. L., N. Cook, S. V. Tessaro, D. Deregt, G. Desroches, P.L. Dubeski, A. K. W. Tong, and D. L. Godson. 2004. Early detection and prediction of infection using infrared thermography. Can. J. Anim. Sci. 84:73–80.
- Schaefer, A. L., S. D. M. Jones, A. P. Murray, A. P. Sather, and A. K. W. Tong. 1989. Infrared thermography of pigs with known genotypes for stress susceptibility in relation to pork quality. Can. J. Anim. Sci. 69:491–495.
- Schaefer, A. L., S. V. Tessaro, D. Deregt, G. Desroches, N. G. Cook, P. Lepage, J. J. Colyn, P. L. Dubeski, and D. L. Godson. 2000. Early detection of infection using infrared thermography. Proc. CSAS Annual Meeting, Winnipeg, Manitoba, Canada. Univ. Manitoba, Winnipeg, Canada.
- Schwartzkopf-Genswein, K. S., and J. M. Stookey. 1997. The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle. Can. J. Anim. Sci. 77:577–583
- Scott, S. L., A. L. Schaefer, A. K. W. Tong, and P. Lacasse. 2000. Use of infrared thermography for early detection of mastitis in dairy cows. In Proc. CSAS Annual Meeting, Winnipeg, MB, Canada.
- Spire, M. F., J. S. Drouillard, J. C. Galland, and J. M. Sargeant. 1999. Use of infrared thermography to detect inflammation caused by contaminated growth promotant ear implants in cattle. J. Am. Vet. Med. Assoc. 215:1320-1324.
- Stephan, E., and A. Görlach. 1971. Measuring of surface temperatures using infrared thermography in veterinary medicine. Preliminary report. Dtsch. Tierarztl. Wochenschr. 78:330–332. (in German).
- Stewart, M., J. R. Webster, A. L. Schaefer, N. J. Cook, and S. L. Scott. 2005. Infrared thermography as a non-invasive tool to study animal welfare. Anim. Welf. 14:319–325.
- Stewart, M., J. R. Webster, G. A. Verkerk, A. L. Schaefer, J. J. Colyn, and K. J. Stafford. 2007. Non-invasive measurement of stress in dairy cows using infrared thermography. Physiol. Behav. 92;520–525.
- Willard, S., S. Dray, R. Farrar, M. McGee, S. Bowers, A. Chromiak, and M. Jones. 2007. Use of infrared thermal imaging to quantify dynamic changes in body temperature following lipopolysaccharide (LPS) administration in dairy cattle. J. Anim. Sci. 85(Suppl. 2):26. (Abstr.)