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Effects of Dietary Addition of Baihu Decoction-Based Powder on Lactation Performance, Thermal and Immune Status in Lactating Dairy Cows During Hot Season

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Abstract: This study was conducted to evaluate the effects of Baihu Decoction-Based Powder (BDP) on lactation performance, thermal status and immune traits in lactating dairy cows during hot season. Twenty mid-lactating cows divided into two groups were fed the same basal diet and added without or with 120 g of BDP. Addition of BDP had no effect on dry matter intake and milk yield but significantly increased milk fat percent. Rectal temperature did not change in cows fed BDP while respiration rate and heart rate were significantly increased by BDP. No significant differences were found in the peripheral blood rheology and whole hemacyte indexes between two treatments. Concentration of plasma cGMP was significantly improved by BDP while the cAMP and its ratio to cGMP did not change. Addition of BDP tended to decrease IL-1β concentration but no differences were observed in IL-6 and prostaglandin E2 assay between two treatments. It is indicated that BDP could alleviate the negative effects of environment-induced hyperthermia on lactating cows.

Key words: Lactation performance, heat stress, baihu decoction, thermal status, immune traits

INTRODUCTION

Heat stress is considered as the most limiting factor in dairy production during hot climates. Climatic statistical characteristics in the East China are such that hot conditions are sudden and prolonged during the Summer, with the presence of high relative humidity (Shi *et al*., 2008; Bai and Liu, 2010). Thus, heat stress is chronic in nature there is often little relief from the heat during the evening hours and cows are less likely to acclimatize during hot season. Exposure of lactating dairy cows to heat stress ultimately produces a reduction in the rates of metabolism, feed intake and milk production and an increase in the respiratory rate and core body temperature that results in a significant loss of productivity during the Summer months (West, 2003).

It is generally accepted that reduced nutrient intake is primarily responsible for the diminished milk synthesis. However, a thermal load may directly affect milk yield by unknown mechanisms that are independent of reduced DMI. When overall heat stress (extent and duration) exceeds a given threshold (as yet unidentified) the cumulative thermal load disrupts the nutrient intake and milk production relationship and milk synthesis declines beyond expected levels (Rhoads *et al*., 2009). Moreover, duration and intensity of heat stress compromise immune function indicating that heat stress affect cow body temperature and consequently immune cell response (Lacetera *et al*., 2002, 2005). The precise mechanisms underlying reduced cellular immune function in cattle under higher temperatures remain undefined, particularly with regard to cytokine profiles that would be indicative of a regulatory and anti-inflammatory phenotype (Caroprese *et al*., 2009).

Baihu decoction, initially recorded in Treatise on Febrile Diseases (Shang Han Lun) is a curative for the syndrome with interior excessive heat. The typical symptoms that Baihu decoction could treat are high fever and big and strong pulse. Folium nelumbinis and Flos chrysanthemi are rich in flavonoid which has been demonstrated to possess immunoregulatory activities through antioxidant (Guabiraba *et al*., 2010). Taking into account that hyperthermia under heat stress is different from high fever, researchers modified the ingredients of Baihu decoction. New Baihu Decoction-based Powder (BDP) consists of Gypsum fibrosum, Folium nelumbinis, Radix isatidis, Flos chrysanthemi and Radix glycyrrhizae. It is assumed that the BDP may adjust the body's thermal status and modulate the immune response of cows in Summer. Therefore, the present study was carried out to investigate the changes in lactation performance, thermal and immune status of Chinese Holstein lactating cows reared during the hot season.

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MATERIALS AND METHODS

Animals and treatment: The experiment was carried out in the experiment herd of Zhejiang Academy of Agricultural Science. The herd is located in Jinhua Prefecture, China (30°25' North, 120°35' East). The cooling fans were turned on from 0600-2400 h in the whole period. Twenty mid-lactating cows (day in milk = 138±26, parity = 2.3±0.4) were used in a randomized block design for 8 weeks. Animals were divided into 10 blocks according to day in milk, milk yield and parity and then randomly allocated to two treatments (without or with addition of 120 g BDP per day). Cows were fed and milked at 06:30, 14:00 and 19:00 h in a tie-stall barn. All animals had free access to drinking water. The experiment lasted for 67 days (from June 28th to September 3rd, 2011) with 7 days for adaptation and 60 days for data collection.

Diets for lactating cows consisted of a basic diet fed as Total Mixed Ratio (TMR). The TMR was formulated to meet the predicted requirements (NRC, 2001) of energy, protein, minerals and vitamins (Table 1). All diets were kept constant for the entire experimental period. The ingredient (%) of BDP consisted of Gypsum fibrosum 45.8, Folium nelumbinis 25.0, Radix isatidis 12.5, Radix glycyrrhizae 8.3 and Flos chrysanthemi 8.4. The herbs were ground through 40 mesh and total mixed before feeding. The BDP was delivered at 07:00 and 19:00 h along with TMR. Cows were observed daily for general health status.

Sample collection and measurements: The maximum, minimum and mean Temperature-Humidity Index (THI) was calculated according to McDowell *et al.* (1976). Respiration rate was measured daily at 06:00 and 14:00 h for all animals by flank movements and rectal temperature by means of clinical veterinary thermometers at the same time. Heart rate was measured by B-mode ultrasonic scanner at 14:00 h for all animals.

Table 1: Ingredients and chemical composition of diets

Items[1]	DM (%)
Ingredients	
Corn silage	22.10
Alfalfa hay	30.60
Dried distillers grains with solubles	9.60
Ground corn grain	23.40
Soybean meal	6.20
Calcium of long chain fatty acids	0.70
Supplement	1.50
Amino plus	5.90
Chemical analysis[1] (DM %)	
Crude protein	14.90
Neutral detergent fiber	32.40
Acid detergent fiber	25.00
NE_l[2] (Mcal kg^{-1} of DM)	1.55

[1]Calculated from the analyzed value of the dietary ingredientsl; [2]Calculated based on individual feedstuffs in China recommendations

Dry Matter Intake (DMI) body weights and backfat thickness were measured on all animals in the beginning, medium and end of the experiment period. The TMR was sampled weekly and analyzed for DM and crude protein (AOAC, 1990). The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined according to Van Soest *et al.* (1991). Cows were milked twice daily (0600 and 1800 h) with yields recorded every 5 days. Milk samples from each cow were collected every 10 days. Feed efficiency was calculated as the ratio of 3.5% Fat-Corrected Milk (FCM) to DMI.

Blood samples from cows were obtained from caudal vein every 10 days before morning feeding. Blood was transferred into EDTA vacutainers and centrifuged at 3500×g for 15 min to harvest plasma. Total white blood cells and red blood cells were counted using an automated blood analyzer (Hemavet cell counter 850, CDC Technologies, Inc., Centerville, USA) standardized for the analysis of bovine blood. Total leukocyte numbers together with differential counts for neutrophils, lymphocytes, monocytes, eosinophils and basophils were expressed as counts per microlitre of blood. Blood rheology test indexes were determined by automatic rheogeniometer (LBY-N6, Beijing Precil Instrument Co., Ltd.).

Plasma concentrations of cyclic 3',5'-Adenosine Monophosphate (cAMP), cyclic 3',5'-Guanosine Monophosphate (cGMP), Prostaglandin E_2 (PGE_2), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) were determined by sandwich capture ELISA using commercially available kits according to manufacturer's instruction. These procedures were conducted in 96 well microplates and read using a microplate photometer (Multiskan As-cent, Thermo Electron Corporation, Vantaa, Finland).

Statistical analysis: All dependent variable were analyzed by repeated measures using PROC MIXED (SAS Institute, 2005) with an autoregressive covariance structure and time of experiment as the repeated effect. Cows were the random effect and treatment was the fixed effect. For daily milk yield, DMI and feed efficiency, each animal's previous 305 days data record was used as a covariate. For physiological and plasma biochemical indexes, the data measured at the beginning of the experiment were used as covariates. All data were condensed into the treatment. Standard errors of the mean are reported and differences were considered significant at $p\leq 0.05$ and to have tendency at $0.05<p\leq 0.10$.

RESULTS

Meteorological data: During the whole experiment period, the minimum temperature was above 25°C (Fig. 1) suggesting that the cows were in heat stress all over the

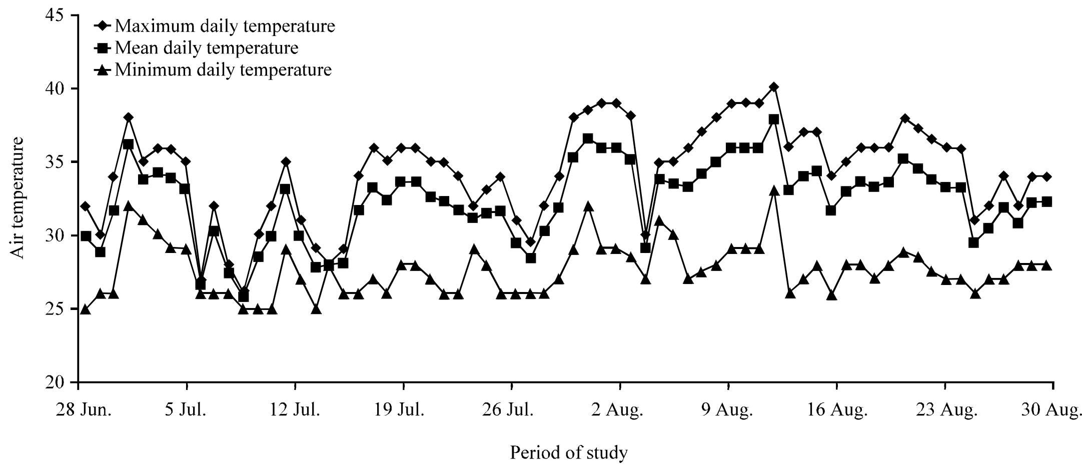

Fig. 1: Daily maximum, mean and minimum air temperature along the period of study

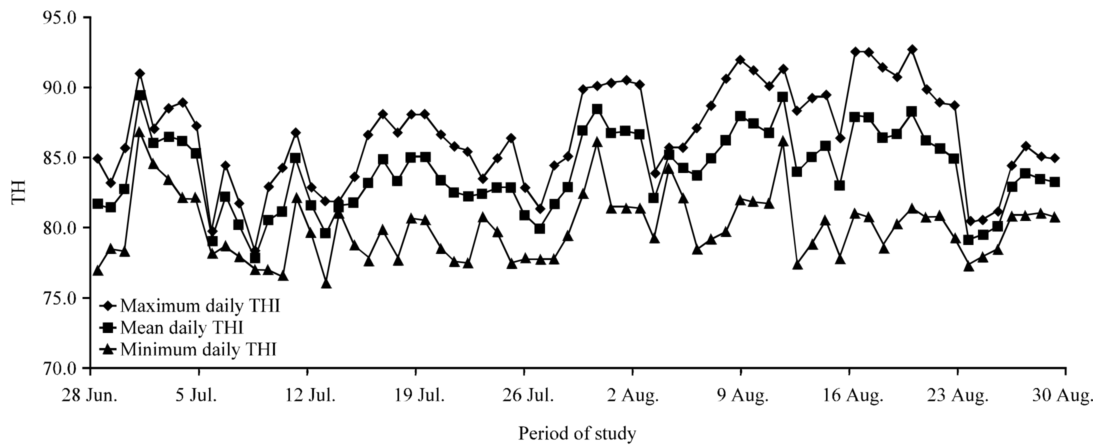

Fig. 2: Daily maximum, mean and minimum temperature-humidity index along the period of study

period. In addition, maximum air temperature in 61% of days was above 35°C, i.e., the cows endured severe heat exposure. Daily THI ranged from 77.8-89.4 with an average of 84.2 (±2.7). Maximum and minimum THI were 87.0 and 80.1 at 14:00 and 20:00 h, respectively (Fig. 2).

Lactation performance: Dietary treatment had no effect on DMI but BDP significantly increased milk fat percent ($p = 0.05$) with 1.11 kg day^{-1} more 3.5% FCM produced per head (Table 2). Feed efficiency, though not significant ($p>0.05$) was 7.8% higher for BDP group than for the control. No significant effects were observed on milk protein percentage by addition of BDP. At the start of the adaptation period, average daily milk yield was 26.9 (±4.7) kg day^{-1} and dropped to 16.9 (±2.5) kg day^{-1} at the end of the study. In the same time, milk fat and protein percent were increased from 3.18 (±0.11) and 2.80 (±0.17%) to 3.48 (±0.22) and 3.36 (±0.48%), respectively.

Table 2: Effects of supplementary Baihu Decoction-based Powder (BDP) on lactation performance in lactating Holstein dairy cows

Parameters[1]	Control	BDP	SEM	p-values
Body weight (kg)	501.90	514.60	12.640	0.488
DMI (kg day^{-1})	15.80	15.80	0.180	0.848
Milk yield (kg day^{-1})	16.40	17.00	0.810	0.627
3.5% FCM (kg day^{-1})	16.30	17.40	0.690	0.730
Milk fat (%)	3.46	3.65	0.061	0.050
Milk protein (%)	3.18	3.29	0.053	0.175
Feed efficiency	1.03	1.11	0.068	0.766
Backfat thickness (mm)	0.94	0.90	0.041	0.467

[1]DMI: Dry Matter Intake; FCM: Fat-Corrected Milk

Thermal status and immune traits: Dietary treatment had no effect on rectal temperature (Table 3) but respiration and heart rate were significantly higher ($p<0.05$) in BDP group. The rectal temperature at 06:00 h was nearly 39°C which implicate the heat strain was maintained for the entire 24 h of each day.

Addition of BDP had no effect on blood viscosity and aggregation index of red blood cell in peripheral blood

Table 3: Effects of supplementary Baihu Decoction-based Powder (BDP) on rectal temperature, respiration rate and heart rate in lactating Holstein dairy cows

Parameters	Control	BDP	SEM	p-values
Rectal temperature (°C)				
06:00 h	39.0	39.1	0.09	0.274
14:00 h	39.5	39.4	0.09	0.659
Respiration rate (breaths/min)				
06:00 h	61.1	66.2	1.70	0.067
14:00 h	68.4	76.7	1.14	0.003
Heart rate (beats/min)				
14:00 h	88.2	96.8	2.11	0.046

Table 4: Effects of supplementary Baihu Decoction-based Powder (BDP) on blood viscosity index in lactating Holstein dairy cows

Parameters	Control	BDP	SEM	p-values
In whole blood				
Low-shearing viscosity (mPa.sec), 1S	13.3	12.8	0.583	0.494
Middle-shearing viscosity (mPa.sec), 5S	6.14	5.92	0.208	0.483
Middle-shearing viscosity (mPa.sec), 30S	3.67	3.57	0.109	0.547
High-shearing viscosity (mPa.sec), 200S	2.82	2.76	0.086	0.635
High-shearing relative index	1.65	1.70	0.069	0.606
Low-shearing relative index	7.80	7.87	0.436	0.905
Plasma viscosity (mPa.sec)	1.72	1.65	0.059	0.391
Casson viscosity (mPa.sec)	2.34	2.30	0.077	0.711
Aggregation index of red blood cells	4.76	4.62	0.187	0.619

Table 5: Effects of supplementary Baihu Decoction-based Powder (BDP) on routine blood test in lactating Holstein dairy cows

Parameters	Control	BDP	SEM	p-values
White blood cell counts (10^3 mL^{-1})	11.46	9.51	0.967	0.172
Neutrophils counts (10^3 mL^{-1})	3.24	2.59	0.384	0.253
Lymphocytes counts (10^3 mL^{-1})	7.37	6.18	0.646	0.211
Monocytes counts (10^3 mL^{-1})	0.70	0.56	0.095	0.338
Neutrophils:Lymphocytes	0.50	0.56	0.078	0.659
Red blood cell counts (10^6 mL^{-1})	6.08	5.69	0.164	0.112
Hemoglobin (g mL^{-1})	82.00	80.90	1.812	0.688
Hematocrit (%)	25.40	25.00	0.594	0.667
Platelet count (10^5 mL^{-1})	3.38	3.51	2.739	0.730

Table 6: Effects of supplementary Baihu Decoction-based Powder (BDP) on plasma biochemical parameters in lactating Holstein dairy cows

Parameter[1]	Control	BDP	SEM	p-values
cAMP (nmol L^{-1})	106.60	112.90	3.120	0.447
cGMP (nmol L^{-1})	19.70	21.60	0.650	0.030
cAMP:cGMP	5.66	5.26	0.199	0.727
IL-1 (ng L^{-1})	153.30	134.50	7.140	0.069
IL-6 (ng L^{-1})	166.30	166.80	5.480	0.357
PGE_2 (ng L^{-1})	1141.50	1198.20	29.700	0.325

[1]cAMP = plasma cyclic 3', 5'-adenosine Monophosphate; cGMP = cyclic 3',5'-Guanosine Monophosphate; PGE_2 = Prostaglandin E2; IL = cytokine

(Table 4, p>0.05). In agreement with the blood rheology test, white and red blood cell counts of heat-stressed cows were not influenced by feeding BDP, although almost all the indices numerically decreased (Table 5, p>0.05).

Concentration of cGMP was significantly improved by addition of BDP (Table 6, p<0.05) but ratio of cAMP to cGMP did not change. Addition of BDP tended to decrease concentration of IL-1β. No difference was observed for IL-6 and PGE_2 assay between the two groups.

DISCUSSION

Lactation performance: In the current study, BDP had no effect on DMI and milk yield but feed efficiency was 7.8% higher for BDP group than the control indicating that other factors besides insufficient DMI contribute to the decrease in milk synthesis. McGuire *et al.* (1989, 1991) determined that the heat stress induced reduction in feed intake was sufficient to explain most (if not all) of the reduction in milk yield. The key difference between the studies by McGuire *et al.* (1989, 1991) and the current study is the diurnal heat-load pattern. Specifically, their overnight temperature was 19°C and ours was earlier 25°C. The marked reduction in evening ambient temperature allowed their cows to completely dissipate their heat load (based on morning rectal temperatures). Thus, the close association between DMI and milk production remained intact in the studies by McGuire *et al.* (1989, 1991). The results in agreement with previous data (Rhoads *et al.*, 2009) suggest that heat stress for the whole day disrupts the nutrient intake and milk production relationship and milk synthesis declines beyond expected levels.

In the current study, milk fat and protein contents were higher in the test period than in adaptation and addition of BDP significantly increased milk fat percent. Although, the increase in milk fat during heat stress agrees with a previous report (Rhoads *et al.*, 2009) it was slightly surprising as milk fat is typically depressed during the Summer months on commercial dairies (Huber, 1996; Kadzere *et al.*, 2002). What need to point out is that temperature did not affect milk production and the lactose percentage in milk in those studies. Moreover, the effect of experimental heat stress on milk fat is thought dependent upon the interaction between dietary forage: concentrate ratios and temperature (Morrison, 1983). It is currently unclear whether heat stress and BDP affects milk component synthesis directly or indirectly by influencing the delivery of component precursors.

Thermal status: Because the primary non-evaporative means of cooling for the cow (radiation, conduction, convection) become less effective with rising ambient temperature, the cow becomes increasingly reliant upon evaporative cooling in the form of sweating and panting. In the current study, respiration rate and heart rate were significantly higher in BDP group indicating that the cows try to dissipate heat through respiration and sweat. Lemerle and Goddard (1986) reported that although rectal temperature only increased when THI was >80, the respiration rate would begin to increase at a THI value of 73 and probably increase steeply at a THI of 80. Richards (1985) measured an increase of the heart rate of lactating Friesian cows after a diurnal exposure of the cows to high ambient temperature (38°C at an RH of 80% for up to 7 h day^{-1}) for 3 weeks.

Blazquez *et al*. (1994) reported that increased blood flow to the skin is positively correlated to the sweating rate. Signal-induced relaxation of vascular smooth muscle is mediated by cGMP-activated protein kinase G. Addition of BDP significantly improved cGMP (Table 6) indicating that BDP may be beneficial for vascular relaxation and vasodilation. Although not significant, the blood rheology test indices and blood cell counts were all numerically lower in BDP-added cows than the control. Overall, it is assumed that BDP may improve the blood fluidity to help dissipate heat through sweat. Certainly, it is apparent that improved blood fluidity means more than that. Further study is needed to investigate BDP effects on blood biochemistry.

Although, it is generally accepted that environmentally induced hyperthermia is different from fever, we detected the classic fever signal pathway. The PGE2 has been considered to be the proximal mediator of fever, acting directly in the Preoptic region of the Anterior hypothalamus (POA) to increase the thermoregulatory set point (Blatteis and Sehic, 1997). It has been previously suggested that an increase in cAMP levels, especially cAMP to cGMP ratio in the POA is associated with fever. In the current study, BDP significantly improved cGMP, though cAMP versus cGMP ratio was not changed. It seems that BDP may positively help the cow maintain thermal equilibrium.

Immune function: Addition of BDP tended to have a lower IL-1β concentration than the control (Table 6). Innate immune traits and disease resistance attributes also impact upon animal performance. Several innate traits in particular, levels of circulating monocytes, natural killer cells and B cells are indicative of growth performance in Large White pigs (Clapperton *et al*., 2005). In addition, production of pro-inflammatory cytokines is linked to decreased productivity (Johnson, 1997; Fossum, 1998; Colditz, 2002). In fact, the cows endured oxidative stress in hot season (Padilla *et al*., 2006; Calamari *et al*., 2011). A variety of reactive oxygen species are produced by normal metabolic processes and by activated neutrophils and macrophages during defense against disease (Babior *et al*., 2002). A better understanding of how antioxidants may prevent immune dysfunction and oxidative damage to host tissues may lead to more effective strategies to alleviate heat stress (Sordillo and Aitken, 2009).

CONCLUSION

Extended periods of high ambient temperature coupled with high relative humidity compromise the ability of the lactating dairy cow to dissipate excess body heat. Addition of BDP had no effect on DMI but significantly increase milk fat percent resulting in more fat corrected milk produced. Respiration and heart rate were significantly increased by BDP. Concentration of cGMP was significantly improved by addition of BDP. In conclusion, addition of BDP could ameliorate the negative effects of environment-induced hyperthermia on lactating dairy cows.

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REFERENCES

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Arlington, VA., pp: 69-90.

Babior, B.M., L.D. Lambeth and W. Nauseef, 2002. The neutrophil NADPH oxidase. Arch. Biochem. Biophys., 397: 342-344.

Bai, A.J. and X.D. Liu, 2010. Characteristics of rainfall variation over East China during the last 50 years and relationships with droughts and floods. J. Trop. Meteorol., 26: 194-200.

Blatteis, C.M. and E. Sehic, 1997. Prostaglandin E: A Putative Fever Mediator. In: Fever: Basic Mechanisms and Management, Mackowiak, P.A. (Ed.). Lippincott-Raven, Philadelphia, PA., pp: 117-145.

Blazquez, N.B., S.E. Long, T.M. Mayhew, G.C. Perry, N.J. Prescott and C.M. Wathes, 1994. Rate of discharge and morphology of sweat glands in the perineal, lumbodorsal and scrotal skin of cattle. Res. Vet. Sci., 57: 277-284.

Calamari, L., F. Petrera, F. Abeni and G. Bertin, 2011. Metabolic and hematological profiles in heat stressed lactating dairy cows fed diets supplemented with different selenium sources and doses. Livestock Sci., 142: 128-137.

Caroprese, M., A. Marzano, G. Entrican, S. Wattegedera, M. Albenzio and A. Sevi, 2009. Immune response of cows fed polyunsaturated fatty acids under high ambient temperatures. J. Dairy Sci., 92: 2796-2803.

Clapperton, M., S.C. Bishop, N.D. Cameron and E.J. Glass, 2005. Associations of weight gain and food intake with leukocyte sub-sets in Large White pigs. Livestock Prod. Sci., 96: 249-260.

Colditz, I.G., 2002. Effects of the immune system on metabolism: Implications for production and disease resistance in livestock. Livest. Product. Sci., 75: 257-268.

Fossum, C., 1998. Cytokines as markers for infections and their effect on growth performance and well-being in the pig. Domestic Anim. Endocrinol., 15: 439-444.

Guabiraba, R., A.L. Campanha-Rodrigues, A.L.S. Souza, H.C. Santiago and C. Lugnier *et al*., 2010. The flavonoid dioclein reduces the production of pro-inflammatory mediators *in vitro* by inhibiting PDE4 activity and scavenging reactive oxygen species. Eur. J. Pharmacol., 633: 85-92.

Huber, J.T., 1996. Amelioration of Heat Stress in Dairy Cattle. In: Progress in Dairy Science, Philips C.J.C. (Ed.). CAB International, Wallingford, pp: 211-243.

Johnson, R.W., 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci., 75: 1244-1255.

Kadzere, C.T., M.R. Murphy, N. Silanikove and E. Maltz, 2002. Heat stress in lactating dairy cows: A review. Livestock Prod. Sci., 77: 59-91.

Lacetera, N., U. Bernabucci, B. Ronchi, D. Scalia and A. Nardone, 2002. Moderate summer heat stress does not modify immunological parameters of Holstein dairy cows. Int. J. Biometeorol., 46: 33-37.

Lacetera, N., U. Bernabucci, D. Scalia, B. Ronchi, G. Kuzminsky and A. Nardone, 2005. Lymphocyte functions in dairy cows in hot environment. Int. J. Biometeorol., 50: 105-110.

Lemerle, C. and M.E. Goddard, 1986. Assessment of heat stress in dairy cattle in Papua New Guinea. Trop. Anim. Health Prod., 18: 232-242.

McDowell, R.E., N.W. Hooven and J.K. Camoens, 1976. Effect of climate on performance of Holsteins in first lactation. J. Dairy Sci., 59: 965-971.

McGuire, M.A., D.K. Beede, M.A. Delorenzo, C.J. Wilcox, G.B. Huntington, C.K. Reynolds and R.J. Collier, 1989. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. J. Anim. Sci., 67: 1050-1060.

McGuire, M.A., D.K. Beede, R.J. Collier, F.C. Buonomo and M.A. DeLorenzo *et al*., 1991. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, Insulin-like Growth Factor (IGF)-I and IGF-II and thyroid hormones in plasma of lactating Holstein cows. J. Anim. Sci., 69: 2050-2056.

Morrison, S.R., 1983. Ruminant heat stress: Effect on production and means of alleviation. J. Anim. Sci., 57: 1594-1600.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn., National Academy of Science, Washington, DC., USA.

Padilla, L., T. Matsui, Y. Kamiya, M. Kamiya, M. Tanaka and H. Yano, 2006. Heat stress decreases plasma vitamin C concentration in lactating cows. Livestock Sci., 101: 300-304.

Rhoads, M.L., R.P. Rhoads, M.J. VanBaale, R.J. Collier and S.R. Sanders *et al*., 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism and aspects of circulating somatotropin. J. Dairy Sci., 92: 1986-1997.

Richards, J.I., 1985. Milk production of friesian cows subjected to high daytime temperatures when allowed food *Eitherad lib* or at night-time only. Trop. Anim. Health Prod., 17: 141-152.

SAS Institute, 2005. SAS/STAT Users Guide. Release 9.3. SAS Institute Inc., Cary, NC.

Shi, J., Y. Ding and L. Cui, 2008. Climatic characteristics and their changing law during Summer high-temperature times in East China. Acta Geog. Sin., 63: 237-246.

Sordillo, L.M. and S.L. Aitken, 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol., 128: 104-109.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods of dietary fiber, NDF and nonstarch polysaccharides in realation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

West, J.W., 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci., 86: 2131-2144.