

Review Article

The Effect of Heat Stress on the Metabolism of Dairy Cows: Updates & Review

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Abstract

With the intensification of the greenhouse effect and global warming, heat stress has become one of the most important factors that affect the profit in dairy industry production. In recent years, investigators have performed plenty of researches concerning metabolism of dairy cows under the heat stress. This article has reviewed the effect of heat stress on metabolism of lipid, carbohydrate, protein and energy in dairy cows and described several nutritional strategies to alleviate heat stress to reveal the underlying metabolic mechanism during heat stress and to provide references for exploring more effective measures to alleviate heat stress.

Keywords: Dairy cows; Heat stress; Metabolism**Introduction**

Heat stress (HS), caused by a high ambient temperature, impacts animal health and hampers animal production, and thus is a significant economic issue in the dairy industry [1,2]. Heat stress leads to lower milk production, higher incidence of metabolic diseases (such as rumen acidosis), worse milk quality, and lower production performance [3-5]. In order to find more effective measures to alleviate HS, scientists devote to studying the occurrence and physiological mechanism of HS. Studies have shown that the suitable environment temperature for adult dairy cows is from 5°C to 25°C. Once the ambient temperature is above 25°C or the Temperature Humidity Index (THI) is higher than 72, then cows encounter HS and milk yield decreases [6]. For the sake of adapting to the environment, the metabolism of animals who suffer from HS varies to some extent.

Lipid metabolism

HS affects lipid metabolism significantly. Available data have suggested that the metabolism was changed by HS variously than would be anticipated derived from calculated energy balance. Compared with the paired HS-free group, HS did not decrease the weight of heifer [7], nor did the pregnant cows [8]. Studies reported that HS resulted in variations of intrauterine environment, which changed fatty acid metabolism of the fetus, and thereby affected adipose deposition of their offspring [8]. Ronchi, et al. [7] considered that HS reduced feed intake and the concentration of non esterified fatty acid (NEFA) in plasma. Baumgard, et al. [9] demonstrated that HS retarded the NEFA response in dairy cows that challenged with adrenaline. Because blood concentration of ketone reduced while urine ketone content remained constant under hyperthermia condition, the reduced NEFA was unlikely to derive from the strengthened oxidation or the accelerated conversion from NEFA to ketone [2,10]. In case of the increased lipoprotein lipase, which possesses the ability to uptake and store the triglycerides in the intestine and liver [11], animals suffered from HS have special capacity of lipogenesis but much blunted ability of lipolysis [2]. That is, the ability of breaking down fat in adipose tissue nearly diminished in HS animals, which may partly explain the reduction of plasma NEFA [12,13].

Studies concerning metabolomics show that HS increased the concentrations of acetoacetate, acetone and hydroxybutyric acid in blood, which might involve in energy delivery or synthesis of milk fat [14,15]. Tian, et al. [15] considered that the reduction of the energy intake and negative energy balance might be the adaptive performance as the results of the increased concentrations of relative metabolites. Compared to their pair fed thermal-neutral counterparts, acetoacetate decarboxylase and 3-hydroxybutyric acid dehydrogenase concentrations were significantly higher in dairy cows under hyperthermia. Simultaneously, cortisol, epinephrine and norepinephrine, the indicators reflecting catabolism, increased during HS which might stimulate lipid decomposition and adipose triglyceride mobilization to meet the body needs via β -oxidation to produce new free fatty acids for energy generation [16]. Tian, et al. [15] proved that lipid catabolism was strengthened by HS due to the increased plasma fatty acid, such as linoleic acid, oleic acid and arachidonic acid. In their study, the results of metabolomics and lipidomics as well as ELISA assays presented that HS increased epinephrine and norepinephrine. Compared with HS-free group, HS decreased phosphatidylcholine PC (16:0/14:0), PC (14:1/18:3), PC (12:0/22:2), PC (15:1/18:2), PC (20:2/12:0) and PC (18:1/18:3), but increased choline, lysoPC (0:0/18:0), lysoPE (18:0), lysoPC (16:0) and lysoPC (18:0). It is known that phospholipase A1, A2 and D regulated the catabolic metabolites of PC including choline, fatty acids and lecithin [17,18]. These findings reflect the alterations in lipid metabolism induced by hyperthermia.

Carbohydrate metabolism

Available evidence supports that HS changes carbohydrate metabolism [19]. Sports in high temperature environment promote the creation of hepatic glucose, and the consumption of fat heighten carbohydrate oxidation. Furthermore, ingestion of carbohydrate cannot suppress the decrease of hepatic glucose [20]. That is, exogenous sugar cannot inhibit the output of hepatic glucose caused by HS [21]. Glycogenolysis [19] and gluconeogenesis [22] are the reason for the increased output of hepatic glucose. Wheelock, et al. [5] considered that HS reduced the milk lactose production by 200~400g daily compared with paired feeding HS-free group. The secretion amount

of lactose and glucose were often similar [23], but the underlying mechanism for the reduced lactose yield is still unclear. However, glucose tolerance test showed that cows consumed exogenous glucose faster in the HS group [5]. Furthermore, the expression of pyruvate carboxylase gene, which regulated the involvement of lactate and alanine into gluconeogenic pathway, had been proved to be up regulated in the liver of multiple animals during HS [24]. It has been presented that plasma lactate concentrations rise when growing steers suffer from HS, which maybe originated from skeletal muscle secretions [25], illustrating that the aerobic glycolysis in peripheral tissues increased, to some extent. Additionally, Monteiro, et al. [8] stated that maternal HS altered the metabolism of their offspring by reducing starter intake and growth but enhancing the insulin-independent glucose disposal [26].

Metabonomics method was applied to investigate the alterations in carbohydrate metabolic pathway by Tian, et al. who found that HS reduced blood glucose level, but increased pyruvate and lactate, as well as the activity of lactate dehydrogenase [15]. Reinforce of glycolysis and anaerobic respiration may be to maintain the body's energy balance during HS [27]. Meanwhile, researchers found that HS increased myocardial and muscle oxygen consumption, thereby reduced the oxygen supply and ultimately led to anaerobic fermentation. The changes in metabolic pathways deteriorated the negative energy balance that induced by the decrease in dry matter intake during HS [5].

Protein metabolism

HS affects protein metabolism by changing the carcass lean tissue [28,29]. Numerous investigations have presented that skeletal muscle catabolism is strengthened and plasma urea nitrogen content rises during HS, implying that HS may induce redistribution of nitrogen from protein to urea [5,30]. On the other hand, HS blunts the protein synthesis ability of mammary gland by reducing the contents of casein [31]. However, Rhoads, et al. [24] suggested that HS did not influence milk protein percentage, but reduced milk protein yield due to the declined milk yield [32].

Additionally, HS alters amino acid concentrations in the plasma of dairy cows [15]. To supply energy, phosphocreatine was mobilized in the muscle tissue resulting in the rise of creatine and creatinine in HS dairy cows [33,34]. Tian, et al. [15] found the concentrations of certain amino acids, such as proline, glycine, threonine, isoleucine and arginine, increased in the plasma of HS dairy cows. The reinforcement of amino acids mobilization might contribute to the enhanced urea in HS animals because the amino acids served as the precursors for glucose generation [35]. Simultaneously, HS induced the repartitioning of nitrogen from milk protein to milk urea in mid-lactation dairy cows [36], which agreed with the mechanism in skeletal muscles as mentioned above.

Energy metabolism

Energy metabolism of animal body is closely connected with substance metabolism, and the energy derives from carbohydrates, lipids and proteins, which release energy via oxidation process. The metabolic pathway of the substance varies as energy metabolism alters. HS results in reduced dry matter intake and negative energy balance, when energy for maintenance and lactation is scanty. It is the reduced feed intake that has been considered as the primary reason

for the decrease in milk yield due to the alterations of hormone levels and enzymatic activity involving in anabolism and catabolism of nutrients [37-42]. However, recent results obtained by Wheelock, et al. demonstrated that only part of the decrease in milk yield was attributed to the reduced nutrient intake caused by HS [43]. Previous literatures have shown that hyperthermia directly changes the nutrient partitioning which is energy intake-independent [5].

Carbohydrates are the main source of energy for dairy cows, which are mainly absorbed and utilized in two forms. Firstly, carbohydrates are fermented to produce volatile fatty acids in the rumen which are utilized after entry to the liver. On the other hand, carbohydrates are absorbed and applied as glucose in the small intestine. Abeni, et al. [44] reported that plasma glucose concentration in heat-stressed cows was significantly lower than that of thermal-neutral dairy cows. Hepatic glucose metabolism was influenced since HS altered the enzymes related to gluconeogenesis. Inhibition of nutrient absorption by the liver can effectively relieve HS in dairy cows with the increase of the ambient temperature.

In addition, heat-stressed cows would increase the mobilization of peripheral adipose tissue to make up for the energy deficiency due to inadequate intake of nutrients. In the liver of dairy cows suffered from HS, the amount of ketone elevates through β -oxidation by fatty acids. Because of the relative lack of oxaloacetate, ketone bodies cannot be oxidized timely via the citric acid cycle oxidation, leading to the increased ketone bodies in the blood to develop high ketosis. Flamenbaum, et al. [45] and Ronchi, et al. [7] found that NEFA concentration decreased in the plasma of dairy cows under HS, but β -hydroxybutyric acid concentration increased during HS, indicating that the utilization of NEFA as a source of energy increased in peripheral tissues and liver, resulting in reduction of plasma NEFA. Some scholars [46] believed that the decomposition of protein was accelerated when body fat was used in the body, which, to some extent, alleviated the glucose deficiency because glycogenic amino acid provided energy via the tricarboxylic acid cycle or by glucose synthesis through gluconeogenesis.

Investigation performed by Monteiro, et al. [8] has presented that maternal HS changed the preference of energy source in calves. HS calves had a noninsulin-dependent glucose disposal and utilized glucose prior to fatty acid or ketone. HS calves before weaning had lower blood glucose levels but higher utilization of glucose than HS-free calves. The rate of insulin clearance after insulin gavage was decreased in HS dairy cows, which indicated that the insulin resistance was enhanced in the peripheral tissues, such as muscle and fat tissues, and this limits the insulin-mediated glucose into peripheral tissues.

Nutritional strategies to alleviate HS

In the past decades, people have devoted to investigate effective feed additives aimed to alleviate heat stress [6,36,47]. Previous studies have proved that some feed additives, such as lipid substances, neurotransmitters, Chinese herbal medicine preparation can relieve the negative effects of heat stress by maintaining homeostasis and preventing nutrient deficiency [6,36,47,48].

As HS always induces energy deficit, enhancement of dietary energy density may be an alternative way that helps to mitigate HS [49,50]. Considering the supplementation of unsaturated fatty acid

may negatively impact rumen fermentation [51,52], Wang, et al. [6] supplemented saturated fatty acids (SFA) in the diet of mid-lactating dairy cows and found that SFA decreased the rectal temperature but increased milk yield and milk fat content and yield. Wang, et al. explained that the decrease of body temperature might be due to the lower heat increment of the diets with SFA, in which metabolic heat was saved. Similar results were also observed by Chan, et al. [49], who noted that fatty acids could reduce rectal temperature. On the other hand, supplementation of high SFA might improve the energy status in heat stressed cows, as found by Wang, et al. who proposed that plasma NEFA in dairy cows fed 3% SFA were slightly lower than those fed no SFA [6]. Furthermore, plasma NEFA in dairy cows fed SFA was similar [53] or lower [54,55] than those fed unsaturated fatty acids.

γ -Aminobutyric acid (GABA) is a kind of inhibitory neurotransmitter [56] which has the function of regulating body temperature and feed ingestion. Cheng, et al. [36] demonstrated that rumen protected GABA in the diet could be well absorbed into the blood and reduced the rectal temperature of dairy cows. Simultaneously, GABA increased the feed intake of HS dairy cows, because it irritated gastrin and digestive enzyme release, restrained cholecystokinin oxytocin release and weakened the negative feedback effect of alimentary canal chime on feed intake. Consequently, not only milk production, but milk protein and lactose concentrations were elevated as the ingestion of diets increased with GABA supplementation, which indirectly reflected the alleviation of HS.

Chinese herbal medicine has some special functions for mitigation of heat stress without toxic side effects, which has been verified in live stocks [47,57]. Compared with traditional feed additives, herbal feed additives have nutritional and medicinal values but no residues, which have been regarded as the potential effective alleviation for HS [58,59]. Pan, et al. reported that dietary supplemented *Radix Bupleuri* extract (RBE) reduced the rectal temperature of HS dairy cows and increased dry matter intake, which, at least partly promoted the milk production, but had no effect on milk composition, apparent digestibility and rumen fermentation [47]. Meanwhile, Chinese herbal medicine declined the respiration rate of HS animals which, in turn, increased the comfort and finally improved the health of the HS dairy cows. This might explain why lactation performance was promoted with increased milk yield but decreased somatic cell count in milk after RBE supplementation [47].

Summary

With global warming, HS will be one of the most important issues that threaten the profits of the future dairy industry. Numerous investigations have witnessed that HS influences the health and performance of dairy cows including inhibited reproductive performance, suppressed growth, increased metabolic diseases, reduced milk yield and compromised milk quality. Although great advances have been achieved in environmental cooling systems, dairy production losses are still remarkable. Therefore, it is of great importance to reveal the underlying mechanism of the variations of metabolism in hyperthermia and investigate the pathway for HS alleviation. New strategies that can help maintain dairy cow health and improve milk performance are under investigation. Further studies are still needed to elucidate the variations of energy distribution and

material metabolism of dairy cattle during HS. Effective additives in parallel with the advanced technology in the construction design of animal housing facilities as well as the cooling systems may contribute to minimize economic losses caused by HS in the future.

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References

1. Wrinkle SR, Robinson PH, Garrett JE. Niacin delivery to the intestinal absorptive site impacts heat stress and productivity responses of high producing dairy cows during hot conditions. *Anim Feed Sci Technol.* 2012; 175: 33-47.
2. Baumgard LH, Rhoads RP. Effects of heat stress on post absorptive metabolism and energetics. *Annu Rev Anim Biosci.* 2013; 1: 311-337.
3. Collier RJ, Beede DK, Thatcher WW. Influences of environment and its modification on dairy animal health and production. *J Dairy Sci.* 1982; 65: 2213-2227.
4. West JW. Nutritional strategies for managing the heat-stressed dairy cow. *J Anim Sci.* 1999; 2: 21-35.
5. Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH. Effects of heat stress on energetic metabolism in lactating Holstein cows. *J Dairy Sci.* 2010; 93: 644-655.
6. Wang JP, Bu DP, Wang JQ, Huo XK, Guo TJ, Wei HY, et al. Effect of saturated fatty acid supplementation on production and metabolism indices in heat-stressed mid-lactation dairy cows. *J Dairy Sci.* 2010; 93: 4121-4127.
7. Ronchi B, Bernabucci U, Lacetera N, VeriniSupplizi A, Nardone A. Distinct and common effects of heat stress and restricted feeding on metabolic status in Holstein heifers. *ZootecNutr Anim.* 1999; 25:71-80.
8. Monteiro AP, Guo JR, Weng XS, Ahmed BM, Hayen MJ, Bernard JK, et al. Effect of maternal heat stress during the dry period on growth and metabolism of calves. *J Dairy Sci.* 2016; 99: 3896-3907.
9. Baumgard LH, Wheelock JB, Sanders SR, Moore SR, Green HB, Waldron MR, et al. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. *J Dairy Sci.* 2011; 94: 5620-5633.
10. Dale HE, Goberdhan CK, Brody S. A comparison of the effects of starvation and thermal stress on the acid-base balance of dairy cattle. *Am J Vet Res.* 1954; 15: 197-201.
11. Sanders SR, Cole LC, Flann KL, Baumgard LH, Rhoads RP. Effects of acute heat stress on skeletal muscle gene expression associated with energy metabolism in rats. *FASEB J.* 2009; 23.
12. Bauman DE, Peel CJ, Steinhour WD, Reynolds PJ, Tyrrell HF, Brown AC, et al. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. *J Nutr.* 1988; 118:1031-1040.
13. Dunshea FR, Bell AW, Trigg TE. Non-esterified fatty acid and glycerol kinetics and fatty acid re-esterification in goats during early lactation. *British J Nutr.* 1990; 64: 133-145.
14. Peterson SE, Rezamand P, Williams JE, Price W, Chahine M, McGuire MA. Effects of dietary betaine on milk yield and milk composition of mid-lactation Holstein dairy cows. *J Dairy Sci.* 2012; 95: 6557-6562.
15. Tian H, Wang WY, Zheng N, Cheng JB, Li SL, Zhang YD, et al. Identification of Diagnostic Biomarkers and Metabolic Pathway Shifts of Heat-Stressed Lactating Dairy Cows. *J Proteomics.* 2015; 125: 17-28.
16. Dunning KR, Russell DL, Robker RL. Lipids and oocyte developmental

- competence: The role of fatty acids and β -oxidation. *Reproduction*. 2014; 148: R15-27.
17. Glunde K, Serkova NJ. Therapeutic targets and biomarkers identified in cancer choline phospholipid metabolism. *Pharmacogenomics*. 2006; 7: 1109-1123.
18. Dong J, Cai XM, Zhao LL, Xue XY, Zou LJ, Zhang XL, et al. Lysophosphatidylcholine profiling of plasma: discrimination of isomers and discovery of lung cancer biomarkers. *Metabolomics*. 2010; 6: 478-488.
19. Streffer C. Aspects of metabolic change after hyperthermia. *Recent Results Cancer Res*. 1988, 107:7-16.
20. Febbraio MA. Alterations in energy metabolism during exercise and heat stress. *Sports Med*. 2001; 31: 47-59.
21. Angus DJ, Febbraio MA, Lasini D, Hargreaves M. Effect of carbohydrate ingestion on glucose kinetics during exercise in the heat. *J Appl Physiol*. 2001; 90: 601-605.
22. Collins FG, Mitros FA, Skibba JL. Effect of palmitate on hepatic biosynthetic functions at hyperthermic temperatures. *Metabolism*. 1980; 29: 524-531.
23. Kronfeld DS. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. *J Dairy Sci*. 1982; 65: 2204-2212.
24. Rhoads RP, LaNoce AJ, Wheelock JB, Baumgard LH. Alterations in expression of gluconeogenic genes during heat stress and exogenous bovine somatotropin administration. *J Dairy Sci*. 2011; 94: 1917-1921.
25. Yaspelkis BB, Scroop GC, Wilmore KM, Ivy JL. Carbohydrate metabolism during exercise in hot and thermoneutral environments. *Int J Sports Med*. 1993; 14:13-19.
26. Chen XC, Fahy AL, Green AS, Anderson MJ, Rhoads RP, Limesand SW. β 2-Adrenergic receptor desensitization in perirenal adipose tissue in fetuses and lambs with placental insufficiency-induced intrauterine growth restriction. *J Physiol*. 2010; 588: 3539-3549.
27. Tao S, Dah GE. Invited review: heat stress effects during late gestation on dry cows and their calves. *J Dairy Sci*. 2013; 96: 4079-4093.
28. Close WH, Mount LE, Start IB. The influence of environmental temperature and plane of nutrition on heat losses from groups of growing pigs. *Animal Prod*. 1971; 13: 285-294.
29. Lu Q, Wen J, Zhang H. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poult Sci*. 2007; 86: 1059-1064.
30. Hall GM., Lucke JN, Lovell R, Lister D. Porcine malignant hyperthermia. VII: Hepatic metabolism. *Br J Anaesth*. 1980; 52:11-17.
31. Bernabucci U, Lacetera N, Ronchi B, Nardone A. Effects of the hot season on milk protein fractions in Holstein cows. *Anim Res*. 2002; 51: 25-33.
32. Tissieres A, Mithell HK, Tracy VM. Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J Mol Biol*. 1974; 84: 389-398.
33. Koubkova M, Knizkova I, Kunc P, Hartlova H, Flusser J, Dolezal O. Influence of high environmental temperatures and evaporative cooling on some physiological hematological and biochemical parameters in high-yielding dairy cows. *Czech J Anim Sci*. 2002; 47: 309-318.
34. Scharf B, Carroll JA, Riley DG, Chase CC Jr, Coleman SW, Keisler DH, et al. Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heatsusceptible (Angus) *Bos taurus* cattle during controlled heat challenge. *J Anim Sci*. 2010; 88: 2321-2336.
35. Li LO, Grevengoed TJ, Paul DS, Ilkayeva O, Koves TR, Pascual F, et al. Compartmentalized acyl-CoA metabolism in skeletal muscle regulates systemic glucose homeostasis. *Diabetes*. 2015; 64: 23-25.
36. Cheng JB, Bu DP, Wang JQ, Sun XZ, Pan L, Zhou LY, et al. Effects of rumen-protected γ -aminobutyric acid on performance and nutrient digestibility in heat-stressed dairy cows. *J Dairy Sci*. 2014; 97: 5599-5607.
37. Bernabucci U, Lacetera N, Baumgard LH, Rhoad RP, Ronchi B, Nardone A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal*. 2010; 4: 1167-1183.
38. Collier RJ, Stiening CM, Pollard BC, Baumgard LH, Gentry PC, Coussens PM. Use of gene expression microarrays for evaluating environmental stress tolerance at the cellular level in cattle. *J Anim Sci*. 2006.
39. Baumgard LH, Rhoads RP. Ruminant Nutrition Symposium: ruminant production and metabolic responses to heat stress. *J Anim Sci*. 2012; 90: 1855-1865.
40. West JW. Effects of heat-stress on production in dairy cattle. *J Dairy Sci*. 2003; 86: 2131-2144.
41. Fuquay JW. Heat stress as it affects animal production. *J Anim Sci*. 1981; 52: 164-174.
42. Silanikove N, Shamay A, Shinder D, Moran A. Stress down regulates milk yield in cows by plasmin induced beta-casein product that blocks K⁺ channels on the apical membranes. *Life Sci*. 2000; 67: 2201-2212.
43. Rhoads ML, Rhoads RP, Vanbaale MJ, Collier RJ, Sanders SR, Weber WJ, et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *J Dairy Sci*. 2009; 92: 1986-1997.
44. Abeni F, Calamari L, Stefanini L. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. *Int J Biometeorol*. 2007; 52: 87-96.
45. Flamenbaum I, Wolfenson D, Kunz PL, Maman M, Berman A. Interactions between body condition at calving and cooling of dairy cows during lactation in summer. *J Dairy Sci*. 1995; 78: 2221-2229.
46. Sarr O, Yang K, Regnault TR. In utero programming of later adiposity: the role of fetal growth restriction. *J Pregnancy*, 2012; 2012: 1-10.
47. Pan L, Bu DP, Wang JQ, Cheng JB, Sun XZ, Zhou XZ, et al. Effects of Radix Bupleuri, extract supplementation on lactation performance and rumen fermentation in heat-stressed lactating Holstein cows. *Animal Feed Science & Technology*. 2014; 187: 1-8.
48. Das R, Sailo L, Verma N, Bharti P, Saikia J, Imtiwati, et al. Impact of heat stress on health and performance of dairy animals: A review. *Vet World*. 2016; 9: 260-268.
49. Chan SC, Huber JT, Chen KH, Simas JM, Wu Z. Effects of ruminally inert fat and evaporative cooling on dairy cows in hot environmental temperatures. *J Dairy Sci*. 1997; 80:1172-1178.
50. Drackley JK, Cicela TM, LaCount DW. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. *J Dairy Sci*. 2003; 86:1306-1314.
51. Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows: A review. *Livest Prod Sci*. 2002; 77: 59-91.
52. Maia MR, Chaudhary LC, Figueres L, Wallace RJ. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Antonie van Leeuwenhoek*. 2007; 91: 303-314.
53. Harvatine KJ, Allen MS. Effects of fatty acid supplements on milk yield and energy balance in lactating dairy cows. *J DairySci*. 2006; 89:1081-1091.
54. Harvatine KJ, Allen MS. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. *J Dairy Sci*. 2005; 88: 4018-4027.
55. Relling AE, Reynolds CK. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. *J Dairy Sci*. 2007; 90:1506-1515.
56. Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki T. GABA and GABA receptors in the central nervous system and other organs. *Int. Rev*. 2002; 213:1-47.
57. Wang DM, Wang C, Liu HY, Liu JX, Ferguson JD. Effect of rumen-protected γ -aminobutyric acid on feed intake, lactation performance, and antioxidative status in early lactating dairy cows. *J Dairy Sci*. 2013; 96: 3222-3227.

58. Dong H, Zhong YG, Liu FH, Yang K, Yu J, Xu JQ. Regulating effects and mechanisms of Chinese medicine decoction on growth and gut hormone expression in heat stressed pigs. *Livest Sci.* 2012; 143: 77-84.
59. Wang L, Piao XL, Kim SW, Piao XS, Shen YB, Lee HS. Effects of forsythia suspensa extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. *Poult Sci.* 2008; 87: 1287-1294.