Biochemical and Physiological Changes during Thermal Stress in Bovines: A Review

Review Article

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ABSTRACT

The variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species. Thermal environment is a major factor that can negatively affect both production and reproduction in dairy animals, especially in animals of high genetic merit. Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, sub-tropical and arid areas. There are few effective strategies for reducing the effects of heat stress on animal's health and performance. The major strategiesproviding elaborate housinginvolving shade, sprinklers, fans, air conditioner, etc. are capital intensive, not very efficient and are of limited use for small and medium-size dairies. Sweating, high respiration rate, vasodilation with increased blood flow to skin surface, elevated rectal temperature, reduced metabolic rate, decreased DM intake, efficiency of feed utilization and altered water metabolism are the physiological responses that are associated with negative impacts of heat stress on production and reproduction in dairy animals.This article aimed to review biochemical and physiological changes which occur during thermal stress in bovines.

KEY WORDS biochemical and physiological changes, stress, temperature.

INTRODUCTION

Climate change, defined as the long term imbalance of customary weather conditions such as temperature, radiation, wind and rainfall characteristics of a particular region, is likely to be one of the main challenges for mankind during the present century. The earth's climate has warmed in the last century (0.74±0.18 ˚C) with the 1990 s and 2000 s being the warmest on instrumental record (IPCC, 2007).

Furthermore, the earth's climate has been predicted to change continuously at rates unprecedented in recent human history (IPCC, 2007). Current climate models indicated an increase in temperature by 0.2 ˚C per decade and predicted that the increase in global average surface temperature would be between 1.8 ˚C to 4.0 ˚C by 2100 (IPCC, 2007). The variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species. Thermal environment is a major factor that can negatively affect production and reproduction in dairy animals, especially in animals of high genetic merit.

Stress has been defined by several workers. As per Dobson and Smith (2000), it is revealed by the inability of an animal to cope with its environment, a phenomenon which is often reflected in a failure to achieve genetic potential. Rosales (1994) defined stress as the cumulative detrimental effect of various factors on health and performance of animals. Stress represents the reaction of the body to stimuli that disturb normal physiological equilibrium or homeostasis, often with detrimental effects (David *et al.* 1990).

Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, subtropical and arid areas (Silanikove *et al.* 1997). The degree to which an animal resists change in body temperature varies in different species because of differences in their heat regulating mechanisms (Salah *et al.* 1995). Under thermal stress, a number of physiological and behavioral responses vary in intensity and duration in relation to the animal's genetic make-up and environmental factors through the integration of many organs and systems viz. behavioral, endocrine, cardio-respiratory and immune system (Altan *et al.* 2003). Sweating, high respiration rate, vasodilation with increased blood flow to skin surface, elevated rectal temperature, reduced metabolic rate, decreased DM intake, efficiency of feed utilization and altered water metabolism are the physiological responses that are associated with negative impacts of heat stress on production and reproduction in dairy animals (West *et al* 1999).

High metabolic demands during lactation can also impact the oxidative status of dairy cows. Researchers have reported higher oxidative stress in high-producing dairy cows (Lohrke *et al.* 2005) when compared to average-producing dairy cows. Stage of lactation has also been found to affect the oxidative status of the animal.

Buffaloes have poor heat tolerance capacity compared to other domestic ruminants, and are more prone to heat stress due to scarcely distributed sweat glands, dark body colour and sparse hair on the body surface (Das *et al.* 1999). The water buffalo has only 1 / 10th the number of sweat glands per unit area of skin compared to zebu cattle and must rely on wallowing or wetting to the skin during heat conditions to reduce the heat load. Air temperature (13-18 ˚C), RH (55-65%) and wind velocity (5-8 km/h) are the optimum conditions for buffaloes as suggested by Payne (1990). In terms of THI, the values of THI > 72 is considered as stressful and THI > 78 is considered very severe heat stress to this animal.

Reactions of homeotherms to moderate climatic changes are compensatory and are directed at restoring thermal balance (West *et al.* 1999). However, when the environmental temperature becomes near the animal's body temperature, high ambient relative humidity reduces evaporation, overwhelms the animal's cooling capacity and the body temperature rises. The increasing concern of thermal discomfort of farm animals is debatable not only for countries of tropical zones, but also for nations of temperate zones in which ambient temperature is increasing due to climate change (Nardone *et al.* 2010). In terms of adaptation measures, it is generally faster to improve welfare, production and reproduction performances of animals by altering their micro-environment (West, 2003; Mader *et al.* 2006). Despite its importance, there are few effective strategies for reducing the effects of heat stress on animal's health and performance. The major strategies providing elaborate housing involving shade, sprinklers, fans, air conditioner, etc. are capital intensive, not very efficient and are of limited use for small and medium-size dairies. There is thus needed for research in developing alternative approaches to reduce thermal stress.

Oxidative stress

Oxidative stress results from increased production of free radicals and reactive oxygen species, and a decrease in antioxidant defense (Trevisan *et al.* 2001). Williams *et al.* (2002) reported that oxidation is essential to nearly all cells in the body to provide energy for vital functions. Approximately, 95 to 98% of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative by-products-reactive oxygen species (ROS), that may damage the DNA of genes and contribute to degenerative changes. One of the main reasons for oxidative stress in animals during summer in tropics is heat stress. Heat stress occurs when the core body temperature of a given species exceeds its range specified for normal activity resulting from a total heat load (internal heat production and heat gained from environment) exceeding the capacity for heat dissipation.

Antioxidants

Antioxidants are those nutrients which are required to cleanse cells of ROS. Vitamin E, vitamin A and vitamin C are classic examples of antioxidants. Antioxidant in low concentrations significantly delays or inhibits oxidation of oxidisable substrates. The endogenous antioxidant capacity of the animal consists of three main groups of antioxidants (Miller *et al.* 1993). The first group, enzymatic antioxidants, is represented by superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). They are the chief intracellular antioxidant defense system and the first defense system against reactive oxygen metabolites (ROM) such as peroxides (ROO$^-$) and hydroperoxides (ROOH). SOD enzymes are Mn, Zn and Cu dependent and GSH-Px is Se dependent. The second group consists of protein antioxidants in the intracellular fluids such as sulfhydryl groups of albumin, cysteine and homocysteine. The third group consists of low molecular weight chain breaking antioxidants such as water-soluble vitamin C, glutathione and the lipid soluble vitamin E.

Tissue defense mechanisms against free-radical damage generally include vitamin C, vitamin E, and β-carotene as the major vitamin antioxidant sources. In addition, several metalloenzymes which include glutathione peroxidase (Se), catalase (Fe) and superoxide dismutase (Cu, Zn, and Mn) are also critical in protecting the internal cellular constituents from oxidative damage (Weiss, 2006).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) was first isolated by Mann and Keilin (1938) and thought to be a copper storage protein. Subsequently, the enzyme was named as erythrocuprein, indophenol oxidase, and tetrazolium oxidase until its catalytic function was discovered by Mc Cord and Fridovich (1969). SOD is now known to catalyse the dismutation of superoxide to hydrogen peroxide and oxygen.

$$^{\circ}O^{-}_{2} + {}^{\circ}O^{-}_{2} + 2H^{+} \longrightarrow H_2O_2 + O_2$$

The major defense in detoxification of superoxide anion and hydrogen peroxide, are superoxide dismutase (SOD), catalase and glutathione peroxidase (Mc Cord and Fridovich, 1969; Chance *et al.* 1979).

There is an increased production of superoxide radicals from threshold concentration due to stress conditions imposed either by thermal stress or physiological status (pregnancy/lactation) of an animal. Pathan *et al.* (2009) observed increase in the concentration of SOD with the advancement of pregnancy. The SOD concentration was significantly higher on the day of parturition (3.03±0.13 U/mL) compared to day 30 before parturition (2.13±0.19 U/mL). Bernabucci *et al.* (2002) also observed increase in SOD and GPX concentration in prepartum cows with peaks around calving during summer month. The SOD and GPX levels were 176.4 ± 19.8 and 65.7 ± 3.9 U/g Hb, respectively at day -21, and 215.3 ± 21.3 and 69.4 ± 4.2 U/g Hb, respectively at day 1 after parturition. These authors suggested that this increase in SOD and GPx around calving might be the result of a possible homeostatic control. Megahed *et al.* (2008) examined the effects of heat stress in Egyptian buffaloes in summer and winter seasons and reported that superoxide dismutase (SOD) activities were significantly lower in the summer (3.17±0.13 U/mL) compared to winter (3.8±0.16 U/mL) season. Chandra (2009) also reported higher SOD levels in prepartum crossbred cows during summer (3.83±13.59 m*M*/L) compared to winter (3.39±16.50 m*M*/L) season, indicating the effect of hot summer season on the oxidative status of transition dairy cows. Lallawmkimi *et al.* (2009) studied the effect of winter and summer seasons on antioxidant status of growing calves, heifers and lactating Murrah buffaloes and reported significantly higher SOD levels during summer compared to winter in all the three experimental groups. Significantly higher concentration of SOD in goats during summer (THI>79) compared to pre-summer months (THI=69.5).

Catalase (CAT)

Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. In peroxisomes, catalase takes care of the cytosolic and mitochondrial peroxides formed during urate oxidation (Oshino and Chance, 1977). Mitochondrial SOD readily converts the bulk of mitochondrial superoxide ions to H_2O_2. Thus, SOD and catalase protect the cell from the damage due to the secondary generation of the highly reactive hydroxyl group from superoxide ion to H_2O_2 (Miyazaki *et al.* 1991).

Hydrogen peroxide production was found to increase due to augmented SOD activity during heat stress (Bernabucci *et al.* 2002) and this in turn resulted in a coordinated increase in plasma catalase and glutathione peroxidase concentrations. Thus, a positive and significant correlation exists between catalase and SOD activities. Kumar (2005) observed a significant positive correlation of THI with the erythrocyte catalase activity in Murrah buffalo and KF cattle. The highest increase in catalase activity was registered in KF followed by Murrah buffaloes. Lallawmkimi *et al.* (2009) studied the effect of winter and summer seasons on antioxidant status of growing, heifer and lactating Murrah buffaloes and reported significantly higher catalase activity during summer compared to winter in all the three experimental groups. Further, Chandra (2009) also reported higher catalase activity in prepartum crossbred cows during summer (159.94±0.10 µmol/min/mg Hb) compared to winter (153.85±0.08 µmol/min/mg Hb) season.

Glutathione peroxidase (GPX)

GPx is a selenium dependent antioxidant enzyme. It converts H_2O_2 to water. The increased production of H_2O_2 due to increased activity of SOD during heat stress resulted in a coordinated increase in GPX.

Bisla *et al.* (2000) reported that GSH concentration increased due to oxidative stress caused by diaphragmatic herniorrhaphy in buffaloes. The concentration of erythrocytic GSH-PX increased with the advancement of pregnancy in buffaloes, and its concentration was significantly higher on the day of parturition (912.59±17.46 U/mL) compared to day 30 before parturition (819.41±22.33 U/mL) (Pathan *et al.* 2009). Bernabucci *et al.* (2002) also reported an increase in serum SOD and GPx concentrations during summer in prepartum cows with peaks reaching around calving. The serum SOD and GPX levels were 176.4 ± 19.8 and 65.7 ± 3.9 units respectively, at day -21, and 215.3 ± 21.3 and 69.4 ± 4.2 units, respectively, at day 1 after parturition. Lallawmkimi *et al.* (2009) studied the effect of winter and summer seasons on antioxidant status of growing calves, heifers and lactating Murrah buffaloes and reported significantly higher GPX concentrations during summer compared to winter in all the three experimental groups.

The view that GPX concentration in buffaloes increases during thermal stress conditions.

Thiobarbituric acid reactive substance (TBARS)

Lipid peroxidation is commonly measured in terms of TBARS. TBARS is known to be one of the oxidative stress markers in the plasma. However, the thiobarbituric acid test would be considered to be a good general indicator of oxidative stress rather than a marker of lipid peroxidation. Oxidative stress can lead to increase in TBARS (Halliwel and Chirico, 1993). Unsaturated fatty acid oxidation by ROS in the presence of iron generates additional lipid centric radicals through the Fenton's reaction that leads to the formation of lipid hydro-peroxides (Trevisan *et al.* 2001). This resulted in TBARS induced reduction of membrane fluidity and increased erythrocyte membrane fragility (Chen and Yu, 1994). ROS status in animals is often determined using the TBARS assay that measures acetaldehyde generated from products of lipid peroxidation. The erythrocyte TBARS concentration increased in heat exposed cattle and buffalo (Ashok *et al.* 2007). Similar increase in TBARS concentration was observed in heat exposed Holstein cows by Bernabucci *et al.* (2002). Dietary supplementation of ascorbic acid resulted in lower TBARS concentration (Tanaka *et al.* 2007). This is because ascorbic acid acts as a chain blocker of lipid peroxidation. The increase of thiobarbituric acid reactive substance (TBARS) immediately before and after calving confirmed that cows during the transition period were under higher oxidative stress (Bernabucci *et al.* 2002). These results indicated that mild heat stress could induce higher oxidative stress in transition cows. Further, Bernabucci *et al.* (2005) evaluated the oxidative stress on cows calving during spring or summer (39.5 ˚C; 73 THI). Levels of TBARS and SOD in blood erythrocytes were higher in cows calving during summer than in spring. *Bos taurus* cows showed a reduction of plasma lipid soluble antioxidants (vitamin E and β-carotene) and an increase of plasma TBARS under moderately heat stressed during summer were apparent, particularly in mid lactation. TBARS concentration was also higher during summer (6.23±0.02 nmol/mL) in prepartum crossbred cows compared to winter season (5.37±0.07 nmol/mL) as reported by Chandra (2009). The TBARS concentration increased from 8.3±0.4 nmol/ml at day -21 to 10.2 ± 0.4 nm/mL at day 1 after calving.

Aengwanich *et al.* (2011) studied the effect of hot summer on cattle maintained under artificial shade, tree shade and no shade conditions. The authors observed that there was a significantly higher concentration of TBARS in no shade (33.29±9.40 µmol/L) compared to artificial (25.64±8.35 µmol/L) and tree shade (25.25±2.05 µmol/L) animals. However, Chaiyabutr *et al.* (2011) reported no significant difference between cooled (Mist-fan system) and non-cooled cows with or without recombinant bovine somatotropin (rbST) supplementation.

Plasma protein

Asignificant increase in the serum protein of sheep exposed to heat stress. The increase in serum protein could be a physiological attempt to maintain extended plasma volume. Observed little variation in serum protein concentration in buffaloes during spring and summer seasons. Serum total protein concentrations were 44 g/L and 51 g/L in summer and winter seasons, respectively, as reported by El-Masery and Marai (1991), while Yousef (1990) reported slightly higher protein concentrations of 7.4 and 9.5 g/dL in the same seasons, respectively, in Egyptian buffalo calves. High environmental temperature caused an increase in total plasma protein in lactating cattle (Podar and Oroian, 2003). Serum protein concentration decreased significantly during summer season in lactating cattle and buffaloes (Verma *et al.* 2000). Ahmed (1990) reported that the total protein levels decreased from 7.7 g/dL in winter to 6.4 g/dL in summer in cattle. However, Rasooli *et al.* (2004) found a significant increase in plasma total protein from 63.88 ± 0.77 g/L in winter to 69.26 ± 0.70 g/L in hot summer in non-pregnant Holstein heifers. In cattle, heat exposure and dehydration during heat stress resulted in a sharp increase in ADH level, which was associated with a significant decrease in urine output and a significant increase in plasma protein (El-Nouty *et al.* 1980). Shafie and Badreldin (1962) reported that plasma total protein content decreased by 11.9% in buffaloes and increased by 4.4% in Baladi cattle when exposed to direct solar radiations.

Plasma albumin

A significant increase in plasma albumin levels was reported in cows (El-Masery and Marai, 1991) and buffalo calves (Koubkova *et al.* 2002) during heat stress. This finding is quite relevant considering that albumin is the major extracellular source of thiols, which are scavengers of free radicals allowing albumin to function as an antioxidant (Halliwel, 1998). Rasooli *et al.* (2004), reported an increase in plasma albumin concentrations in summer (40.23±0.38 g/L) compared to winter (35.09±0.42 g/L) in non pregnant Holstein heifers and suggested that the increase in plasma protein concentrations might be due to the loss of extracellular fluid due to heat exposure. However, the plasma albumin concentration did not differ between cooled (mist-fan system) (0.60 µmol/L) and non-cooled (0.61 µmol/L) cows with or without rbST supplementation (Chaiyabutr *et al.* 2008). Celi *et al.* (2008) found significantly lower plasma albumin concentration near parturition (36.06 g/L) compared to day -21 before parturition (39.28 g/L) in goats.

This decrease in albumin levels further indicated that goats were subjected to oxidative stress during the peripartum period.

Physiological responses to heat stress

Increasing air temperature above the critical threshold is related with reduced feed intake (Holter *et al.* 1997), decreased activity, milk yield (Umphrey *et al.* 2001) and a deleterious effect on the physiologic status (West, 2003) of farm animals. Physiological parameters like respiration rate, heart rate, body temperature and skin temperature gives an immediate response to the climatic stress and consequently, the level of discomfort/comfort to the animal. These responses have been used as a measure of dairy cow comfort and adaptability to an adverse environment or as a sensitive physiologic measure of environmental modification. Physiological responses like rectal temperature, pulse rate and respiration rate reflect the degree of stress imposed on animals by climatic parameters. The ability of an animal to withstand the rigors of climatic stress under warm conditions has been assessed physiologically by changes in body temperature, respiration rate and pulse rate (Leagates *et al.* 1991; Sethi *et al.* 1994).

Core (Rectal) body temperature

Change in rectal temperature has been considered an indicator of heat storage in animal's body and may be used to assess the adversity of thermal environment, which can affect growth, lactation and reproduction of dairy animals (Hansen and Arechiga, 1999; West *et al.* 1999). The rectal temperature is recognized as an important measure of physiological status as well as the ideal indicator for assessment of stress in animals. Rectal temperature and skin temperature have been reported to fluctuate much more in buffaloes than in tropical cattle under increased ambient temperature (Koga *et al.* 2004). Even a rise of less than 1 ˚C in rectal temperature was enough to reduce performance in most livestock species (Shebaita El-Banna, 1982) which makes the body a sensitive indicator of physiological response to heat stress because it is nearly constant under normal conditions. RT is generally considered to be a useful measure of body temperature and changes in RT indicates changes of a similar magnitude in deep body temperature.

The plasma concentration of vitamin C is ruminants is an oxidative stress indicator which is affected by heat stress. A negative correlation between rectal temperature and ascorbic acid concentrations of *Bos taurus* cattle in the hot season was reported by Tanaka *et al.* (2007) and Chaiyabutr *et al.* (2011). Chaiyabutr *et al.* (2011) exposed the Holstein cows to normal shade (NS) and mist-fan (MF) system and reported that both respiration rate and rectal temperature were higher in cows under normal shade (NS) compared to mist fan (MF) system, which indicated a partial alleviation of heat stress by MF system. High ambient temperature in NS barn increased the rectal temperature of cows up to 39.9 ˚C in mid lactation.

Joshi and Tripathy, (1991) noticed an increase in rectal temperature from 102.0 ˚F to 103.8 ˚F when buffalo calves were exposed to 40.5 ˚C for eight hours daily for three months. Sethi *et al.* (1994) recorded 2.6 ˚C rises in rectal temperature in buffaloes when exposed to direct sun rays in the months of June and July. The rectal temperature and respiration rates of buffaloes were significantly higher during direct sun exposure than the values obtained when the animals were kept under shade in the barn (Gudev *et al.* 2007). Therefore, when kept in the barn the buffaloes maintained their RT within the thermoneutral zone at the expense of higher respiratory rate. The rectal temperature was higher during summer (39.83 ˚C) than autumn (38.30 ˚C) in lactating cows (Padilla *et al.* 2006).

High relative humidity reduced the effectiveness of the evaporative cooling, and the high relative humidity coupled with high environmental temperature apparently overwhelmed the capacity of the cow to maintain normal body temperature (West *et al.* 1999). The coefficient of correlation indicated that body temperature of buffaloes had highly significant correlation with seasonal changes of air temperature (Chikamune and Shimizu, 1983).

Alfredo *et al.* (2008) exposed the four cattle breeds (Frisian, Limousine, Alentejana and Mertolenga) to different climatic conditions and observed that during the thermically stressful period, there was an increase of the RT mean values, compared to the ones measured in thermoneutrality, in all breeds: 2.0%, 1.1%, 1.8% and 0.2% with highest mean values observed at 15:0 hours: 40.03 ˚C, 39.77 ˚C, 39.47 ˚C and 38.76 ˚C for the Frisian, Limousine, Alentejana and Mertolenga breeds, respectively. Although all breeds have developed tachypnea and raised the RT during thermal stress period (THI=75-85), a slight hyperthermia (40.03 ˚C) was observed in the Frisian. The intense respiratory evaporative cooling, together with sweating (not measured) were not enough to prevent hyperthermia (Brown-Brandl *et al.* 2001).

The high rectal temperature observed for the heat stressed animals was the indicator of disturbance in the homoeothermic status of the animals which was not being effectively countered by the enhanced heat loss by physical and physiological processes of thermolysis (Joshi and Tripathy, 1991).

Respiration rate

Respiration rate was the indicator of heat stress in the hot environment and gave significant correlations with circulating corticoids concentration (Vijay Kumar, 2005). Normal respiration rate is approximately 10 - 30 breaths/minute. The respiration rate increased when the environmental temperature increased. An evaporative heat loss from the respiratory tract is regarded as one of the primary mechanisms for maintenance of heat balance.

This respiratory response arises from direct heat stimulation of peripheral receptors, which transmit nervous impulses to the thermal centre in the hypothalamus. A high RR in most cases did not necessarily indicate that the animal is successful in keeping its body temperature constant, but rather indicated that it is already overheated and trying to restore normal heat balance. A higher respiration rate of 71.5/minute during summer compared to 38.8/minute in winter was recorded in lactating cows by Padilla *et al.* (2006). Respiration rate increased from 29 to 59/minute when male buffalo calves exposed to 40.5 ˚C (Joshi and Tripathy, 1991).

The increase in respiratory frequency was two and half times in heat stressed animals than control animals (Joshi and Tripathy, 1991). Das *et al.* (1999) observed an increase in respiration rate from 14 to 70/minute in the month of June in Murrah buffalo calves when exposed to direct sunlight for six hours.

Alfredo *et al.* (2008) exposed four different breeds of cattle (two Algerian and two exotic breeds) to different environmental conditions and studied the respiratory frequency and rectal temperature of the animals. They found that RF means values increased by more than twice in all breeds (2.7, 2.8, 2.5, and 2.9 in Alentejana, Frisian, Limousine and Mertolenga, respectively) during the stress period. The highest values were found in the Frisian, with a mean of 105 costal movements per minute for the entire stress period.

Pulse rate

Pulse rate did not exhibit consistent and a definite trend with changing environmental conditions. Observed a decrease in pulse rate whereas, and Seath and Miller (1946) observed an increase in pulse rate with the increase in environmental temperature. Chikamune and Shimizu (1983) reported a negative correlation between air temperature and pulse rate in swamp buffaloes. Joshi *et al.* (1982) reported that pulse rate increased moderately during exposure to hot environment in buffaloes. There was a higher pulse rate during summer months and lower during winter months in Indian buffalo bulls. Chikamune (1983) reported an increase in pulse rate with an increase in air temperature in swamp buffaloes. This increasing trend in pulse rate continued even when the ambient temperature declined indicating that the physiological responses of animals returned to its normal levels only after a definite period when animals were brought to comfort zone. Environmental temperature has significant relation to the variation in the pulse rate. The result of their studies indicated that average values of pulse rate were higher during summer and lower during winter. Heart rate of calves increased during exposure to severe heat. Observed a positive correlation (r=0.234-0.768) between ambient temperature and respiration and pulse rates in buffaloes. The seemingly contradictory finding that heart rate responds to heat exposure either by a rise or by a fall may be largely explained by the fact that heart rate is positively correlated with metabolic rate.

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