Indian Journal of Animal Sciences **83** (5): 546–549, May 2013/Article

Effect of heat stress alleviation on plasma protein, metabolites and lipid profile in lactating Nili-Ravi buffaloes under tropical climate

K S DAS[1], J K SINGH[2], G SINGH[3] and V NAYAN[4]

Central Institute for Research on Buffaloes, Regional Station- Bir Dosanjh, Nabha, Patiala, Punjab 147 201 India

Received: 28 August 2012; Accepted: 3 November 2012

ABSTRACT

Nili-Ravi lactating buffaloes (42) of Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha, Patiala, Punjab were used to find out the effect of heat stress management on plasma protein, its metabolites and lipid profile during hot-dry (HD) and hot-humid (HH) seasons. All buffaloes were uniformly divided into 2 groups of 21 in each group. Out of which 10 buffaloes from each group were selected for blood sampling considering their lactation number, stage of lactation, body weight, dam's milk yield and milk yield in current lactation. The control (T_0) group buffaloes were kept in separate shed without any extra nutrient supplementation and modification in microclimate and management. The treatment (T_1) group was supplemented with niacin, yeast, edible oil and provided curtains and mist fans in the shed, and altered feeding time, frequency and type of ration. Four samplings from each season were done for plasma albumin, globulin, albumin globulin ratio, total protein, blood urea nitrogen (BUN), urea, uric acid, creatinine, cholesterol, HDL cholesterol and triglyceride. Total protein was noted to be slightly higher in control than treatment group during HD season. Almost similar values were recorded in both groups (8.13 g/dL vs. 8.14 g/dL) during HH seasons. The values did not significantly differ between the groups. The buffaloes under HD season had significantly higher plasma cholesterol and HDL cholesterol in T_1 (80.07 mg/dL and 31.63 mg/dL) than T_0 (62.83 mg/dL and 24.20 mg/dL) group. The HH season also showed similar result. The values of plasma albumin, globulin, albumin globulin ratio, BUN, triglyceride, urea, uric acid and creatinine were also found to be almost similar values in both groups during HD and HH seasons. It can be concluded that plasma total protein, BUN, metabolites and triglyceride are either very little affected or unaffected by the heat stress in buffaloes. The change in plasma cholesterol and HDL cholesterol might be due to either combine effect of management factors or only effect of nutrient supplementation that needs further investigation.

Key words: Buffalo, Heat stress, Lipid, Metabolite, Plasma, Protein

Summer of tropical and subtropical regions are very challenging to dairy buffaloes for maintaining production and reproduction. Moreover, due to dark skin and sparse coat/hair, buffaloes exhibit signs of great distress when exposed to direct solar radiation. Lactating buffaloes are most susceptible during summer as heat stress and lactation stress are combined together. Alterations of protein metabolism in buffaloes (Mehta *et al.* 1981) and different livestock species (Marai and Haeeb 2010) and lipid metabolism in2 lactating buffaloes (Mehta and Gangwar 1985) and in buffalo heifers (Hooda and Singh 2010) were reported due to heat stress. The biological changes in farm animals (Haeeb *et al.* 1992) and in cattle (Marai and Haeeb 1998) under hot climate were also studied by earlier researchers. However, reference related to the effect of heat stress amelioration on plasma metabolites and lipid profiles in lactating buffaloes is limited in the literature. Therefore, the experiment was designed to collect basic information on the effect of heat stress alleviation through using a combinations of alterations, supplementations and modifications on plasma protein metabolites and lipid profile in lactating Nili-Ravi buffaloes during hot-dry and hot-humid seasons under tropical region.

Present address: [1,3]Senior Scientist (kalyanlpm@gmail.com, jkphysiology@gmail.com, ghscirb@rediffmail.com). [4]Scientist (varij.biochem@gmail.com), Central Institute for Research on Buffaloes, Sirsa Road, Hisar 125 001, Haryana.

MATERIALS AND METHODS

Location of the study: The present study was conducted at Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha (latitude, 30° 22' 28" N and 76° 8' 54" E), Patiala, Punjab, India over hot-dry (HD; April to Mid June) and hot-humid (HH; Mid June to August) seasons. The minimum and maximum temperature within shed of T_0 and T_1 group were 17°C and 42°C, and 15°C and 38°C, respectively during HD period. The respective values during

HH period were 20°C and 40°C, and 19°C and 36°C.

Experimental animals: Lactating Nili-Ravi buffaloes (42), maintained at Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha, Patiala, Punjab, were taken to find out the effect of heat stress management on plasma protein, metabolites and lipid profile during HD and HH season. These buffaloes were uniformly divided into 2 groups i.e. 21 in each group based on their lactation number, stage of lactation, body weight, dam's highest milk yield and milk yield in current lactation. One group was considered as treatment (T_1) and another as control (T_0). From each group 10 buffaloes were also uniformly selected for blood sampling considering the above factors.

Management of animals: A similar but separate conventional tail- to-tail house containing concrete roof with widely opened window (wall height- 4ft) shed were used for keeping both T_1 and T_0 group buffaloes. The height of shed at roof was 12 ft. The windows of T_1 group buffalo shed were covered with curtains to prevent entry of hot air. Additional mist fans and ceiling fans were also fitted to modify microclimate for animals' comfort. Windows of T_0 group buffalo shed were opened. Only few ceiling fans but no mist fans were used. All buffaloes of T_1 and T_0 group were tied individually within the shed throughout 24 h except at the time of wallowing. As a routine practice of the farm, during summer, all experimental buffaloes were allowed to the wallowing tank for 10–15 min twice daily i.e. around 7 AM and 2.30 PM.

Both experimental group animals were individually fed. The farm prepared balance concentrate mixture was offered to all experimental buffaloes. The T_1 group was supplemented with feed grade niacin @6 g/buffalo/day, feed grade yeast @10 g/buffalo/day to reduce heat arising from fermentation, and edible oil (mustard oil) @150g/buffalo/day to increase energy density to meet nutrient requirements. The total amount of required concentrate ration for treatment group was divided into 2 equal parts. One part was mixed with nutrient supplements. The supplemented ration was distributed among buffaloes twice in a day i.e. in the morning and evening before milking. Another part was mixed with the little amount of chopped green fodder and wheat straw (used as partially mixed ration) to encourage complete consumption. It was offered to the animals around 12 noon. The remaining required amount of green fodder was supplied twice i.e. around 6 AM and 7 PM. Fresh, cool and *ad lib.* drinking water was supplied throughout the period. The T_0 group buffaloes were not supplemented any nutrient additives. The total amount of required concentrate ration was divided into 2 equal parts and was distributed among lactating buffaloes twice i.e. in the morning and evening before milking as per standard practice of the farm. The green fodder was chopped and total required amount of was supplied to the buffaloes twice daily i.e. around 10 AM and 3 PM. *Ad lib.* drinking water was available for 24 h.

Blood collection and sampling: After proper restraining and humane handling, blood was collected from jugular vein in 10 ml BD vacutainer tube containing sodium heparin as anticoagulant. Four samples during HD period particularly when ambient temperature exceeded 40°C and 4 samples during HH period when ambient temperature exceeded 30°C were collected for analysis of parameters. Samples from both groups were collected between 6 to 7 AM.

Parameters estimation: Plasma albumin, total protein, urea, uric acid, creatinine, cholesterol, HDL cholesterol, and triglyceride were estimated by blood biochemistry analyser using kits. Plasma globulin was calculated by deducting the plasma albumin concentration from total plasma protein. Albumin globulin ratio was also calculated. Blood urea nitrogen (BUN) concentration in plasma was calculated from plasma urea concentration after dividing by 2.14.

Statistical analyses: All data of T_0 and T_1 group during HD and HH seasons are reported as means ± SEM. Data were analysed by the method of analysis of contrast variables using the GLM (generalized linear model) procedures on analysis of variance for repeated measures using the Greenhouse-Geisser adjusted univariate significance tests as described by earlier procedure (Little *et al.* 1998). The differences between treatment means were considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

The results related to the effect of heat stress amelioration on plasma metabolites and lipid profile of lactating Nili-Ravi buffaloes under different seasons are presented in Table 1. The findings envisaged that total protein was estimated to be slightly higher in T_0 than T_1 group during HD season. Almost similar values were recorded in both groups during HH season. However, values did not differ significantly between 2 experimental groups. Albumin concentration, globulin concentration and albumin globulin ratio of T_0 and T_1 group in HD and HH seasons were almost similar and there were no statistically significant difference between values of both groups. Plasma total protein level tended to be lower ($P<0.05$) during maximum heat load (Gudev *et al.* 2007). The authors described that heat exposure caused initial haemoconcentration followed by haemodilution. Therefore, the observed decline of plasma total protein in their studies indicated that animals were in the stage of haemodilution. In lactating buffaloes, El-Khashab (2010) noted that total protein, albumin and globulin were improved significantly by cooling methods. Joshi and Tripathy (1991) in buffalo calves and Hooda and Singh (2010) in buffalo heifers found that in heat stressed group, blood plasma total protein was significantly higher than control group. In one experiment, Mehta *et al.* (1981) showed decrease plasma protein concentration in showered and wallowed group buffaloes than untreated (control) group. Abou-Zeina *et al.* (2009) supplemented anti-heat stress preparations like zinc, sodium

Table 1. Plasma protein metabolites and lipid profile of lactating Nili-Ravi buffaloes under hot dry and hot humid seasons

Parameters	Season	Control group	Treatment group	SEM	P value
Albumin (g/dL)	HD	3.81	3.72	0.095	0.223
	HH	3.54	3.51	0.058	0.684
Globulin (g/dL)	HD	4.60	4.62	0.103	0.805
	HH	4.59	4.64	0.106	0.805
Albumin: Globulin	HD	0.85	0.83	0.046	0.411
	HH	0.85	0.85	0.068	0.909
Total protein (g/dL)	HD	8.41	8.35	0.104	0.491
	HH	8.13	8.14	0.096	0.944
BUN (mg/dL)	HD	19.07	19.05	2.670	0.990
	HH	16.86	16.97	1.370	0.901
Urea (mg/dL)	HD	40.80	40.76	5.70	0.99
	HH	36.09	36.31	2.94	0.90
Uric acid (mg/dL)	HD	0.82	0.92	0.12	0.56
	HH	1.21	1.04	0.44	0.36
Creatinine (mg/dL)	HD	1.34	1.33	0.05	0.93
	HH	1.69	1.69	0.17	0.96
Cholesterol (mg/dL)	HD	62.83	80.07	4.11	0.01
	HH	58.78	74.38	1.74	0.02
HDL Cholesterol	HD	24.20	31.63	1.04	0.00
(mg/dL)	HH	23.45	32.27	0.79	0.01
Triglyceride (mg/dL)	HD	38.63	45.37	6.68	0.10
	HH	19.38	20.60	1.42	0.39

HD, Hot dry; HH, hot humid.

selenite and vitamin E to lactating, non-lactating and pregnant buffaloes and observed significant ($P<0.05$) increase of total serum proteins and total globulins in the supplemented group. Similar decrease of total protein concentration under heat stress condition was also recorded by Haeeb *et al.* (2007). Another experiment of Koubkova *et al.* (2002) also pointed out that total protein values increased significantly ($P<0.05$) during hot period followed by further significant reduction in hot period with cooling. On the contrary, Aboulnaga *et al.* (1989) demonstrated that sprinkling increased total protein levels. The significant ($P<0.05$) difference of blood protein, albumin and globulin concentrations between heat ameliorative measured and non-measured (control) buffalo heifer groups was also found in the studies of Vijaykumar *et al.* (2011).

The buffaloes under HD season had significantly higher plasma cholesterol ($P<0.01$) and HDL cholesterol ($P<0.001$) in treatment (80.07 mg/dL and 31.63 mg/dL) than control (62.83 mg/dL and 24.20 mg/dL) group. The HH season also showed similar result. Blood cholesterol was lower during summer than during winter in lactating buffaloes (Verma *et al.* 2000). Haeeb *et al.* (1996) showed that cholesterol concentration decreased markedly with the increase in environmental temperature. Marai and Haeeb *et al.* (2010) explained that the decrease might be due to dilution resulting from increase of total body water or to the decrease in acetate concentration, a primary precursor for the synthesis of cholesterol. The report of Hooda and Singh (2010) envisaged that when animals were exposed at 40°C for 4 h for 16 days in a psychrometric chamber, blood total cholesterol decreased significantly at day 8 and 16 after exposure, whereas, no significant change was observed in unexposed (control) group. Similar reports were put forth by Sang-Runzi *et al.* (2002). In another experiment, Gudev *et al.* (2007) noted that plasma cholesterol tended to be lower during the exposure to direct solar radiation compared to the morning levels.

The values of plasma urea nitrogen, urea, uric acid and triglyceride were also found to be almost similar in both experimental groups during HD and HH seasons. There were no statistically significant differences between 2 groups. The reports available in the literature related to these parameters resulting from heat stress are conflicting. However, the urinary nitrogen excretion under hot climatic condition in ruminants is well discussed by Marai and Haeeb (2010). Blood urea increased significantly in high yielding dairy cows (Koubkova *et al.* 2002). Contrary to those reports, blood urea was lower in lactating Murrah buffaloes during hot summer than winter (Verma *et al.* 2000) and in Friesian heifers when exposed to heat stress (85.9 THI) from 10.00 to 18.00 h for 4 weeks (Ronchi *et al.* 1995). Bertoni *et al.* (1997) explained that urea was normally high when protein was in excess in the diet or there was low energy/protein ratio or during lactation. Montmurro *et al.* (1995) pointed out that high level urea might be due to low energy/protein ratio and to gluconeogenesis by protein degradation in conditions of insufficient energy for growth.

Blood urea nitrogen (BUN) in Friesian calves and lactating cows decreased when they were exposed to heat (Ahmed 1990). In contrast, increase level of blood urea nitrogen under heat stress conditions was reported by Haeeb *et al*. (2007) in Egyptian buffalo calves and Gudev *et al.* (2007) in lactating buffaloes. Nessim (2004) found no change of plasma urea nitrogen level between animals of acute heat exposed and non exposed groups. This result supported the findings of present experiment. However, the decrease level associated with heat stress might be due to more reabsorption of urea nitrogen from blood to the rumen to compensate for the decrease in rumen ammonia nitrogen as a result of reduction of feed intake (Yousef 1990) or digestible nitrogen consumption or increase nitrogen excretion as indicated by a negative nitrogen balance leading to low serum urea level (Kamal and Johnson 1970).

No change of plasma creatinine concentration in T_0 and T_1 group under HD and HH seasons were observed (Table 1). This result also supported the finding of Nessim (2004) who conducted experiment on 6- and 12– month-old Egyptian buffalo calves. Whereas, the results of Haeeb *et al.* (2007) in Egyptian buffalo male calves, El-Masry *et al.* (1989) in Friesian cows, and Mcveign and Tarrent (1982) in Friesian bulls recorded significant increase of creatinine concentration in blood of heat stressed animals.

It can be concluded from the experiment that plasma protein, metabolites and triglyceride are either very little affected or unaffected by the heat stress in buffaloes. The change in plasma cholesterol and HDL cholesterol might be due to either combine effect of management factors or only effect of nutrient supplementation that needs further investigation.

REFERENCES

Aboulnaga A I, Kamal T H, El-Masry K A and Marai I F. 1989. Short-term response of spray cooling and drinking cold water for improving milk production of heat stressed Friesian cows. *In: Proceedings of the third Egyptian British Conference*, Alexandria, Egypt 2, pp 607–12.

Abou-Zeina A A A, Hassan S G, Sabra H A and Hamam A M. 2009. Trials for elevating adverse effect of heat stress in buffaloes with emphasis on metabolic status and fertility. *Global Veterinaria* **3** (1): 51–62.

Ahmed H I A. 1990. 'Studies on alleviation of thermal stress in imported Friesian cattle under Egyptian environmental conditions.' Ph.D. Thesis. Faculty of Agriculture, Zagazig University Zagazig, Egypt.

Bertoni G, Bartocci S, Cappelli F P and Mici A A. 1997. Blood metabolites and hormone changes in lactating buffaloes fed diets different for energy content and protein degradability. *Proceedings of 5th World Buffalo Congress*, Caserta, Italy.

El-Khashab M A. 2010. Physiological and productive responses to amelioration of heat stress in lactating buffaloes under hot summer conditions in Egypt. (http://www.spsa-egy.org/?p=835).

EL-Masry K A, Kamal T H, Aboulnaga A I and Marai I F M. 1989. Daily body weight gain, total body solids and biochemical responses of Friesian calves to mineral mixture and urea during heat stress. *Proceedings of the 3rd Egyptian British Conference on Animals, Fish and Poultry Production*. Alexandria, Egypt, pp. 643–48.

Gudev D, Popova-Ralcheva S, Moneva P, Aleksiev Y, Peeva T, Ilieva Y and Penchev P. 2007. Effect of heat stress on some physiological and biochemical parameters in buffaloes. *Italian Journal of Animal Science* **6** (Supple. 2): 1325–28.

Habeeb A A M, Marai I F M and Kamal T H. 1992. Heat stress. *Farm Animals and the Environment.* pp. 27–47. (Eds) Phillips C and Piggins D. CAB International, UK.

Habeeb A A M, EL-Masry K M, Aboulnaga A I and Kamal T H. 1996. The effect of hot summer climate and level of milk yield on blood biochemistry and circulating thyroid and progesterone hormones in Friesian cows. *Arabian Journal of Nuclear Science Applications* **29**: 161–73.

Haeeb A A M, Fatma F I T, Osman S F. 2007. Detection of heat adaptability using heat shock proteins and some hormones in Egyptian buffalo calves. *Egyptian Journal of Applied Science* **22** (2A): 28–53.

Hooda O K and Singh S. 2010. Effect of thermal stress on feed intake, plasma enzymes and blood biochemicals in buffalo heifers. *Indian Journal of Animal Nutrition* **27** (2): 122–27.

Joshi B C and Tripahi K C. 1991. Heat stress effect on weight gain and related physiological responses of buffalo calves. *Journal of Veterinary Physiology and Allied Science* **9**: 33–43.

Kamal T H and Johnson H D. 1970. Whole body ^{40}K loss as a predictor of heat tolerance in cattle. *Journal of Dairy Science* **53**: 1734–38.

Koubkova M, Knizkova I, Kunc P, Hartlova H, Flusser J and Dolezal O. 2002. Influence of high environmental temperature and evaporative cooling on some physiological, haematological and biochemical parameters in high-yielding dairy cows. *Czech Journal of Animal Science* **47**: 309–18.

Little R C, Henry P R and Ammerman C B. 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* **76**: 1216–31.

Marai I F M and Habeeb A A M. 1998. Adaptability of Bos taurus cattle under hot arid conditions. *Annals of Arid Zone* **37** (3): 253–81.

Marai I F M and Haeeb A A M. 2010. Buffalo's biological functions as affected by heat stress- a review. *Livestock Science* **127**: 89–109.

McVeign J M and Tarrent P V. 1982. Behavioral stress and skeletal muscle glycogen metabolism in young bulls. *Journal of Animal Science* **54**: 790.

Mehta S N and Gangwar P C. 1985. Effect of season in the concentration of blood plasma cholesterol and free fatty acids in lactating buffaloes. *Indian Journal of Animal Sciences* **55**: 590–95.

Mehta S N, Gangwar P C, Srivastava R K and Dhingra D P. 1981. Effect of cooling on certain blood constituents in relation to reproductive behaviour i buffaloes. *International Journal of Biometeriology* **25** (3): 199–203.

Montmurro N, Pacelli C and Borghese A. 1995. Metabolic profiles in buffalo heifers bred in two farms with different feeding and climatic conditions. *Egyptian Journal of Animal Production* **32**: 1–12.

Nessim M G. 2004. 'Heat-induced biological changes as heat tolerance indices related to growth performance in buffaloes.' Ph.D. Thesis. Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Ronchi B, Bernabucci U, Lacetera N G, Nardine A and Bertoni G. 1995. Effects of heat stress on metabolic status of Friesian heifer. *Zootechnica-e- Nutizione-Animale* **21**: 209–21.

Sang-Runzi, Tian, Shuzan, Li-Junjie, Ni-Junging, Yong H J Zaho-Cunxia, Wu-Pei, Ma-Yabin, Jhang-Junhui, Hu-Yuechao and Hai W Y. 2002. Influence of heat stress on semen quality of Holstein bull and approach to its mechanism. *Chinese Journal of Veterinary Science* **22**: 407–10.

Verma D N, Lal S N, Singh S P, Parkash O M and Parkash O. 2000. Effect of season on biological responses and productivity of buffalo. *International Journal of Animal Science* **15** (2): 237–44.

Vijaykumar P, Dutt T, Singh M and Pandey H N. 2011. Effect of heat amelioration measures on biochemical and hormonal responses of buffalo heifers. *Journal of Applied Animal Research* **39 (3)**: 181–84.

Yousef H M. 1990. 'Studies on adaptation of Friesian cattle in Egypt.' Ph.D. Thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.