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Alteration of metabolic biomarkers and oxidative stress indices in pashmina (Changthangi) goats under climate change

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ABSTRACT

The main aim of this study was to evaluate climate change induced variation of metabolic biomarkers and oxidative stress indices in different age groups of pashmina goats. Adult animals in comparison to young and old animals had significantly higher levels of glucose, glycated haemoglobin (GHb), fructosamine and total protein during summer and significantly higher levels of glucose, fructosamine and haemoglobin (Hb) during winter. Significantly reduced levels of these biochemicals were noted in winter than summer in all age groups except for glucose in young and old, GHb in young and Hb in adult animals. Urea and acetoacetate levels were significantly higher in young animals than adults during winter. Higher betahydroxybutyrate and lower propionate levels were noted during winter than summer in all age groups. Significantly higher levels of insulin, T_3, T_4 and lower levels of TSH and cortisol in adult animals were noted in both seasons. Significant rise of insulin in adult, T_3 and T_4 in young and old, cortisol in young and TSH in all animals occurred during winter. Level of GSH in summer, CAT and SOD in winter were significantly higher in adult animals whereas, that of LPO were in young animals. Decrease in levels of antioxidants and increase of oxidants during winter was significant in all age groups. Hence, young and old age groups of pashmina goats are more vulnerable to metabolic alterations under climatic stress than the adult group.

Key words: Biomarker, Metabolism, Nutrient deficiency, Oxidative stress, Pashmina goats.

Climatic stress and nutrient deficiency are main reasons for low production of pashmina goats (Bhattacharya *et al.* 2004) and neonatal mortality in kids (Yatoo *et al.* 2014a), however molecular mechanisms for such losses are not known. Metabolic disturbance and oxidative stress can be of prime importance. Metabolic biomarkers and oxidative stress indices are vulnerable to change in different age groups under climatic and nutrient stress (Somvanshi *et al.* 1987, Celi *et al.* 2008, Bórnez *et al.* 2009). Hence, evaluation of biomarkers can be useful in determining the health and nutrient status of such animals and the level of stress (Kaneko *et al.* 1997, Celi *et al.* 2008, Bórnez *et al.* 2009) and accordingly strategies can be devised to overcome this stress.

MATERIALS AND METHODS

Animals and sampling: This study was carried under harsh climate of winter (December-March 2013) and relatively comfortable climate of summer (June-August 2014). For this purpose female pashmina goats (60) of different age groups, were selected randomly from farmers herd in Changthang. Animals were divided into three groups based on age viz. young (below 6 months, average weight 5 kg), adult (7 to 18 months, average weight 22 kg) and old age group (above 18 months, average weight 29 kg). Similar age groups were selected during winter and summer. Each group consisted of twenty animals (20) reared under similar managemental conditions with pasture grazing being the main feeding practice. In summer exposure temperature ranged from 18ºC to 28ºC (average 23ºC) and in winter it ranged from –10ºC to –40ºC (average –20ºC). In summer an adult and old animal consumed about 12.50–14.68 kg and a young one 3.57–5.21 kg of dry matter (DM) daily. In winter an adult and old animal consumed about 5.22–7.16 kg and a young one 1.53–3.21 kg of DM daily. Animals were considered stressed if having more than 45.00 nmol/l of cortisol levels in summer and more than 60.00 nmol/l in winter (Kaneko *et al*. 1997, Kannan *et al*. 2003), in summer LPO more than 0.60 nmol MDA/mg Hb and in winter more than 2.00 nmol MDA/mg Hb and in winter total antioxidant capacity (TAC) less than 50.00 nmol/µl and in winter less

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than 38.00 nmol/µl (Di Trana *et al.* 2006, Celi *et al.* 2010, Singh *et al.* 2014). Samples were taken at the same time of day and from same animals of herd, in case previous animal was not available or died then animal from similar age group and from same herd was chosen so as to minimise variation. Blood samples (10 ml) were collected from jugular venepuncture using vacutainer containing heparin sodium @ 0.2 mg/ml for biochemicals and sodium fluoride @ 2 mg/ml for glucose. Blood samples were immediately placed on ice. After centrifugation at 3,000 rpm for 10 min, plasma for biochemical estimation (plus TAC) and red blood cells (RBC) for oxidative stress indices, were harvested.

Biochemical estimation: Glucose, haemoglobin (Hb), total protein, total cholesterol, urea, total bilirubin and creatinine were estimated by standard methods. Glycated haemoglobin (GHb) by ion exchange chromatography method (Trivelli *et al.* 1971) and fructosamine by Nitroblue tetrazolium (NBT) reduction method (Sahu and Sarkar 2008). Volatile fatty acids were estimated by calorimetric methods including acetoacetate (Price *et al.* 1977), propionate (Hilliman 1978) and betahydroxybutyrate (Brashear *et al*. 1983). Insulin, triiodothyroxine (T_3), tetraiodothyroxine (T_4), cortisol and thyroid stimulating hormone (TSH) by radio immuno assay kits. Mineral estimation was done by calorimetric methods using commercial kits.

Oxidative stress indices: CAT activities were measured as per Bergmeyer (1983) and SOD as per Madesh and Balasubramanian (1998). Reduced glutathione (GSH) was measured by dithio-bis–2 nitro benzoic acid (DTNB) method (Prins and Loos 1969). Lipid peroxidation was measured in terms of malondialdehyde (MDA) production as per Placer *et al.* (1966). Total antioxidant capacity (TAC) was estimated by modified method as described by Singh *et al.* (2014).

Statistical analysis: Data were analyzed by two way analysis of variance (ANOVA) using statistical software package SPSS 16.0. A General Linear Model was used to examine the effects of age (young vs adult vs old) and season (summer vs winter) and their interactions on biomarkers of metabolism and stress, to determine the differences, if any. Tukey's test at a significance level of P<0.05 was carried to find differences between pairs of groups in different seasons. Graphs were prepared by GraphPad Prism4.

RESULTS AND DISCUSSION

Seasonal and age related variation of biochemicals, VFAs, hormones, oxidative stress indices and minerals is given in Tables 1– 5, respectively. In summer, glucose, glycated haemoglobin, fructosamine and total protein levels were significantly (P<0.05) higher in adult animals than young and old animals. In winter, young animals had significantly lower levels of glucose, fructosamine, haemoglobin, total protein and higher urea levels than adults. Difference in levels of biochemicals between young and old animals was nonsignificant (P≥0.05) except for significantly lower levels of haemoglobin in former. Significantly (P<0.05) lower levels of glucose, glycated haemoglobin, fructosamine, total protein and haemoglobin was found in all age groups in winter than summer except glucose in young and old animals, GHb in young animals and haemoglobin in adult animals. Significantly (P<0.05) higher urea levels were noted in young animals during winter. Higher level of glucose, glycated haemoglobin, fructosamine, total protein, haemoglobin and cholesterol in adult animals in both seasons can be attributed to better availability of pasture in summer, adaptability and consumption capability of adult animals. Reduction in levels of glucose, glycated haemoglobin (GHb), fructosamine, cholesterol and propionate may be due to inadequate feed intake due to fodder scarcity, low energy content of diet[1] and increased energy demands under severe cold climate besides the physiological effects. Glucose level in goats is affected by age, environment and plane of nutrition (Mbassa and Poulsen 1991a, 1991d, Celi *et al.* 2008, 2010). Deepa *et al.* (2014) have reported a similar glycated haemoglobin (3.4±0.6 to 10.1±0.5%) and fructosamine level (2 to 4.7 mmol/l) in cattle. Significantly, decreased levels of glycated haemoglobin and fructosamine during winter indicated a prolonged deficiency of energy or carbohydrates in diet whereas that of glucose represented instantaneous deficiency. In general and particularly in winter, reduction in levels of total protein and haemoglobin in young and old age groups is related to low intake of diet due to shortage and lesser content of crude protein in poor quality of available fodders. Reduced haemoglobin levels may also be due to Fe, Cu and vitamin B complex deficiency especially due to their low content in local fodders (Unpublished data ; supplementary Table 6 and 7; *available online*) besides age related requirements. Mbassa and Poulsen (1991a, 1991b) have also found age and environment related variation of total serum proteins in goats. Bornez *et al.* (2009) found lower total protein levels in older lambs after stress. Lower haemoglobin levels in young pashmina goats were also noted by Somvanshi *et al.* (1987). According to Kaneko *et al.* (1997), diminution in the serum concentration of total protein may also be as a result of cortisol due to its catabolic effect. Though non significant but lower cholesterol in young ones especially during winter may indicate age and environment effect. Cholesterol level is affected by age and environment in goats (Mbassa and Poulsen 1991a, 1991d). Cholesterol level increased with age in goats (Hussein and Azab 1998). Higher levels of urea, creatinine and total bilirubin in young and old animals may be due to excess protein metabolism in relevance to age, lesser adaptability or poor nutrient resource utilization under scarcity or deficiency of crude protein in diet. This may also be a stress induced metabolic alteration. Higher levels of urea, creatinine and bilirubin in young and old groups are in corroboration with Mbassa and Poulsen (1991a, 1991d). Celi *et al.* (2008) have also reported similar age related trend in urea levels in goats and kids. Higher levels of urea were also reported by Kannan *et al.* (2003) in feed restricted goats. Serum urea and

Table 1. Biochemical levels (Mean±SE) in different age groups of pashmina goats in summer and winter.

Parameter	Group	Summer	Winter	P value		
				(between age groups in summer)	(between age groups in winter)	[(between seasons, Summer (S) *Winter (W)]
Glucose (mmol/l)	Young (Y)	3.137±0.750^{a} (1.53–5.82)	1.550±0.488^{Aa} (0.88–3.98)	Y*A=0.000	Y*A =0.013	YS*YW=0.569
	Adult (A)	8.373±0.955^{b} (5.17–12.17)	4.983±0.699^{B} (3.47–7.69)	A*O=0.000	A*O=0.009	AS*AW=0.015
	Old (O)	3.656±0.659^{ac} (1.97–5.49)	1.390±0.328^{ACc} (0.91–3.02)	Y*O=0.994	Y*O =1.000	OS*OW=0.199
Glycated haemoglobin (%)	Young (Y)	3.175±0.113^{a} (2.87–3.66)	1.910±0.035^{Aa} (1.79–2.01)	Y*A=0.000	Y*A=0.476	YS*YW=0.186
	Adult (A)	7.670±0.750^{b} (5.64–10.27)	2.860±0.044^{AB} (2.68–2.99)	A*O=0.000	A*O=0.607	AS*AW=0.000
	Old (O)	3.808±0.502^{ac} (2.87–5.91)	2.021±0.049 ^{ABC} (1.89–2.22)	Y*O=0.831	Y*O=1.000	OS*OW=0.021
Fructosamine (mmol/l)	Young (Y)	2.780±0.095 ^{a} (2.39–3.01)	1.565±0.166 ^{A} (1.02–1.99)	Y*A=0.000	Y*A=0.000	YS*YW=0.002
	Adult (A)	4.478±0.327^{b} (3.76–5.92)	3.090±0.240^{B} (2.22–3.87)	A*O=0.001	A*O=0.001	AS*AW=0.000
	Old (O)	3.151±0.184^{ac} (2.77–4.02)	1.806±0.084^{AC} (1.47–2.02)	Y*O=0.778	Y*O=0.995	OS*OW=0.001
Protein (g/dl)	Young (Y)	7.920±0.174^{a} (7.09–8.29)	6.876±0.097^{A} (6.58–7.21)	Y*A=0.002	Y*A=0.027	YS*YW=0.000
	Adult (A)	8.832±0.144^{b} (8.47–9.46)	7.576±0.147^{B} (7.19–8.12)	A*O=0.028	A*O=0.341	AS*AW=0.000
	Old (O)	8.135±0.211^{ac} (7.32–8.55)	7.143±0.087^{ABC} (6.87–7.44)	Y*O=0.909	Y*O=0.804	OS*OW=0.001
Haemoglobin (g/dl)	Young (Y)	9.889±0.232^{a} (8.99–10.74)	7.048±0.143^{A} (6.79–7.72)	Y*A=0.102	Y*A=0.000	YS*YW=0.000
	Adult (A)	11.082±0.44^{ab} (9.82–12.94)	9.827±0.315^{Bb} (8.56–10.75)	A*O=0.209	A*O=0.046	AS*AW=0.075
	Old (O)	10.053±0.255^{abc} (9.48–11.23)	8.477±0.381^{C} (7.58–9.93)	`Y*O=0.999	Y*O=0.031	OS*OW=0.013
Cholesterol (mmol/l)	Young (Y)	1.198±0.319^{a} (0.64–2.77)	0.761±0.094^{Aa} (0.51–1.14)	Y*A=0.679	Y*A=0.962	YS*YW=0.760
	Adult (A)	1.680±0.419^{ab} (0.88–3.38)	1.028±0.092^{ABb} (0.76–1.42)	A*O=0.709	A*O=0.997	AS*AW=0.364
	Old (O)	1.215±0.136^{abc} (0.97–1.72)	0.873±0.061^{ABCc} (0.73–1.13)	Y*O=1.000	Y*O=0.999	OS*OW=0.897
Urea (mmol/l)	Young (Y)	7.715±0.516^{a} (5.99–9.62)	10.40±0.585^{A} (8.38–12.73)	Y*A=0.885	Y*A=0.019	YS*YW=0.013
	Adult (A)	6.907±0.757^{ab} (4.66–9.74)	10.17±1.545^{Bb} (6.88–16.58)	A*O=0.999	A*O=0.852	AS*AW=0.882
	Old (O)	6.616±0.440^{abc} (4.98–7.86)	8.688±0.313^{ABCc} (7.69–9.54)	Y*O=0.685	Y*O=0.229	OS*OW=0.090
Creatinine (mmol/l)	Young (Y)	0.596±0.123^{a} (0.14–0.98)	1.166±0.186^{Aa} (0.76–1.83)	Y*A=1.000	Y*A=1.000	YS*YW=0.921
	Adult (A)	0.412±0.086^{ab} (0.22–0.79)	1.033±0.198^{ABb} (0.46–1.83)	A*O=1.000	A*O=0.506	AS*AW=0.890
	Old (O)	0.565±0.105^{abc} (0.19–0.93)	2.056±0.952^{ABCc} (0.82–6.78)	Y*O=1.000	Y*O=0.648	OS*OW=0.138
Total bilirubin (mmol/l)	Young (Y)	0.044±0.005^{a} (0.031–0.059)	0.080±0.009^{Aa} (0.059–0.121)	Y*A=1.000	Y*A=1.000	YS*YW=0.993
	Adult (A)	0.043±0.007^{ab} (0.021–0.067)	0.082±0.005^{ABb} (0.062–0.099)	A*O=0.480	A*O=1.000	AS*AW=0.991
	Old (O)	0.160±0.111^{abc} (0.027–0.713)	0.097±0.007^{ABCc} (0.085–0.129)	Y*O=0.484	Y*O=1.000	OS*OW=0.924

^{abc} Values with different superscript differ significantly (P<0.05) between age groups in summer. ^{ABC} Values with different superscript differ significantly (P<0.05) between age groups in winter. ^{abcABC} Values with different superscript differ significantly (P<0.05) between seasons within age group. Variables represented by respective abbreviations Young (Y), Adult (A), Old (O), Summer (S), Winter (W). * Represents comparison between two variables. Values in parenthesis are minimum to maximum range of biochemical.

Table 2. Volatile fatty acids (Mean±SE) in different age groups of pashmina goats in summer and winter

Parameter	Season	Young group (Y)	P value (Y*A)	Adult group (A)	P value (A*O)	Old group (O)	P value (O*Y)
Acetoacetate (mmol/l)	Summer (S)	0.047 ± 0.013^{a} (0.023–0.109)	0.987	0.036 ± 0.008^{ab} (0.015–0.071)	0.818	0.057 ± 0.014^{abc} (0.012–0.108)	0.990
	Winter (W)	0.117 ± 0.016^{A} (0.078–0.181)	0.013	0.055 ± 0.007^{Bb} (0.041–0.079)	0.972	0.068 ± 0.012^{ABCc} (0.011–0.098)	0.078
	P value (S*W)	0.004		0.879		0.988	
Propionate (mmol/l)	Summer (S)	0.382 ± 0.033^{a} (0.29–0.49)	0.491	0.437 ± 0.017^{ab} (0.39–0.51)	0.905	0.405 ± 0.029^{abc} (0.29–0.48)	0.973
	Winter (W)	0.218 ± 0.007^{A} (0.19–0.24)	0.973	0.242 ± 0.008^{AB} (0.22–0.27)	0.999	0.252 ± 0.022^{ABC} (0.21–0.35)	0.885
	P value (S*W)	0.000		0.000		0.000	
Beta hydroxy-butyrate (mmol/l)	Summer (S)	0.470 ± 0.027^{a} (0.39–0.58)	0.928	0.689 ± 0.134^{ab} (0.32–1.22)		1.025 ± 0.173^{abc}0.682 (0.68–1.84)	0.178
	Winter (W)	1.923 ± 0.199^{A} (1.38–2.69)	0.824	1.645 ± 0.179^{AB} (0.99–2.22)	0.347	2.110 ± 0.190^{ABC} (1.66–2.99)	0.962
	P value (S*W)	0.000		0.003		0.001	

^{abcABC} Values with different superscript differ significantly ($P<0.05$) within column and between rows of a particular volatile fatty acid. Variables represented by respective abbreviations young (Y), adult (A), Old (O), summer (S), winter (W). *Represents comparison between two variables. Values in parenthesis are minimum to maximum range of volatile fatty acids.

Table 3. Mean±SE of hormones in different age groups of pashmina goats in summer and winter.

	Season	Young group (Y)	P value (Y*A)	Adult group (A)	P value (A*O)	Old group (O)	P value (O*Y)
Insulin (pmol/l)	Summer (S)	60.38 ± 0.328^{a} (59.43–61.33)	0.000	66.18 ± 1.075^{b} (63.92–70.11)	0.000	57.48 ± 0.629^{ac} (55.02–59.38)	0.177
	Winter (W)	63.57 ± 0.343^{Aa} (62.64–64.74)	0.000	75.28 ± 1.488^{B} (70.44–79.54)	0.000	60.74 ± 0.541^{ACc} (59.12–62.64)	0.199
	P value (S*W)	0.113		0.000		0.099	
T_3 (nmol/l)	Summer (S)	3.008 ± 0.057^{a} (2.87–3.24)	0.000	4.368 ± 0.177^{b} (3.92–5.12)	0.000	3.552 ± 0.114^{c} (3.22–3.87)	0.022
	Winter	1.830 ± 0.084^{A} (1.44–2.01)	0.000	3.950 ± 0.040^{Bb} (3.86–4.12)	0.000	2.557 ± 0.144^{C} (1.99–2.92)	0.001
	P value (S*W)	0.000		0.127		0.000	
T_4 (nmol/l)	Summer (S)	46.44 ± 1.196^{a} (41.87–49.43)	0.811	48.23 ± 0.508^{ab} (45.94–49.43)	0.890	46.69 ± 0.901^{abc} (44.21–49.41)	1.000
	Winter (W)	39.46 ± 0.967^{A} (34.78–41.12)	0.000	46.73 ± 1.566^{Bb} (43.21–53.78)	0.013	41.55 ± 0.569^{AC} (39.23–42.92)	0.699
	P value (S*W)	0.000		0.898		0.014	
TSH (mU/l)	Summer (S)	57.83 ± 0.565^{a} (56.19–59.28)	0.000	33.08 ± 0.687^{b} (30.92–35.23)	0.000	59.62 ± 0.545^{ac} (57.38–61.36)	0.993
	Winter (W)	81.88 ± 4.822^{A} (60.18–94.16)	0.000 0.000	$54.51\pm1.\ 241^{B}$ (50.92–59.41)	0.000	77.65 ± 2.275^{AC} (70.72–85.16)	0.774
	P value (S*W)	0.000		0.000		0.000	
Cortisol (nmol/l)	Summer (S)	50.14 ± 3.111^{a} (38.95–60.17)	0.434	41.29 ± 3.692^{ab} (33.12–57.01)	0.000	66.80 ± 4.265^{c} (57.01–85.52)	0.015
	Winter (W)	85.76 ± 2.260^{A} (79.26–92.17)	0.000	$55.\ 38\pm1.804^{Bb}$ (49.98–60.17)	0.000	80.80 ± 4.074^{ACc} (69.97–96.17)	0.896
P value (S*W)0.000			0.056		0.058		

^{abcABC} Values with different superscript differ significantly ($P<0.05$) within column and between rows of a particular hormone.

Variables represented by respective abbreviations young (Y), adult (A), old (O), summer (S), winter (W). *Represents comparison between two variables. Values in parenthesis are minimum to maximum range of hormone.

Table 4. Mean±SE of oxidative stress indices in different age groups of pashmina goats in summer and winter

	Season	Young group (Y)	P value (Y*A)	Adult group (A)	P value (A*O)	Old group (O)	P value (O*Y)
CAT	Summer (S)	163.94±3.576^{a}	0.690	171.15±3.835^{ab}	0.958	167.01±4.316^{abc}	0.989
(µmol H_2O_2		(155.32– 180.32)		(160.43–184.32)		(154.92–182.83)	
decomposed	Winter (W)	69.67±2.834^{A}	0.000	95.59±3.864^{B}	0.001	72.46±1.981^{AC}	0.993
/min/mg Hb)		(59.32–78.71)		(84.92–110.73)		(66.32–79.38)	
	P value (S*W)	0.000		0.000		0.000	
SOD	Summer (S)	0.873±0.040^{a}	0.381	0.991±0.057^{ab}	0.175	0.845±0.060^{abc}	0.997
(µmol MTT		(0.765–1.021)		(0.762–1.126)		(0.699–1.101)	
formazan	Winter (W)	0.476±0.024^{A}	0.026	0.675±0.022^{B}	0.145	0.524±0.033^{ABC}	0.967
/mg Hb)		(0.402–0.539)		(0.601–0.753)		(0.401–0.638)	
		P value (S*W)	0.000		0.000		0.000
GSH	Summer (S)	0.723±0.102^{a}	0.056	1.076±0.118^{ab}	0.039	0.704±0.072^{ac}	1.000
(µmol conjugate		(0.481–1.021)		(0.769–1.561)		(0.501–1.022)	
/ml packed	Winter (W)	0.319±0.081^{A}	0.134	0.624±0.020^{AB}	0.376	0.390±0.073^{ABCc}	0.990
RBC)		(0.069–0.579)		(0.581–0.699)		(0.109–0.576)	
	P value (S*W)	0.021		0.007		0.115	
LPO	Summer (S)	0.717±0.066^{a}	0.428	0.339±0.028^{ab}	0.718	0.622±0.067^{abc}	0.997
(nmol MDA		(0.498–0.952)		(0.285–0.475)		(0.399–0.821)	
/mg Hb)	Winter (W)	2.654±0.236^{A}	0.000	1.442±0.137^{B}	0.002	2.323±0.189^{AC}	0.571
		(1.998–3.421)		(0.998–1.844)		(1.921–3.165)	
	P value (S*W)	0.000		0.000		0.000	
Total	Summer (S)	49.20±2.895^{a}	0.958	52.25±2.640^{ab}	0.572	46.23±2.818^{abc}	0.963
antioxidant		(38.79–57.82)		(45.12–62.56)		(39.46–58.25)	
capacity	Winter (W)	33.04±1.194^{A}	0.226	41. 41±3.247^{ABb}	0.578	36.52±2.112^{ABCc}	0.928
(nmol/µl)		(30.62–38.17)		(31.92–53.17)		(29.73–43.21)	
	P value (S*W)	0.001		0.058		0.111	

^{abcABC} Values with different superscript differ significantly (P<0.05) within column and between rows of particular oxidative stress indices. Variables represented by respective abbreviations young (Y), adult (A), old (O), summer (S), winter (W). *Represents comparison between two variables. Values in parenthesis are minimum to maximum range of oxidative stress indices.

creatinine concentrations can increase during stress (Bórnez *et al.* 2009) due to muscular activity and renal vasoconstriction produced by catecholamines (Finco 1997, Kannan *et al.* 2003).

In summer and winter, significantly (P<0.05) higher acetoacetate levels were noted in young animals than adults. Significantly (P<0.05) higher levels of betahydroxybutyrate and lower levels of propionate were noted during winter than summer in all age groups. Lower acetoacetate and BHB levels in adult age group may be due to better adaptability or utilization of available fodders and more consumption than other age groups. These findings are in corroboration with Hussein and Azab (1998) who also reported significant decrease of fatty acids (NEFA) with age. Drop in propionate levels may be due to nutrient deficiency. Higher levels of fatty acids in old age group are in agreement with the findings of Solaiman *et al.* (2012) who reported increase in levels of serum fatty acids in goats with increase in age. This may be due to aging effect as in old age fatty acids rise. Dietary deficiency of energy content of diet may also induce this change. Elevated betahydroxybutyrate and acetoacetate levels during winter indicate utilization of fatty reserves of body under negative energy balance or climatic/ nutritional stress. Hence, fatty acids can reflect nutritional and health status of animal. The plasma NEFA concentrations can be used as indices of nutritional status during pregnancy in goats (Khan and Ludri 2002a).

In summer, levels of insulin, T_3 (both higher) and TSH (lower) in adult animals differed significantly (P<0.05) from those of young and old animals. Significantly (P<0.05) higher T_3 levels in old animals were noted. Summer cortisol levels of old animals were significantly (P<0.05) higher from those of young and adult animals. In winter, levels of insulin, T_3, T_4 (all higher) and TSH, cortisol (both lower) in adult animals differed significantly (P<0.05) from those of young and old animals. Difference in summer and winter levels of all these hormones was significant (P<0.05) in most of the age groups. Higher levels of cortisol and TSH and lower levels of insulin T_3 and T_4 in young and old animals than adults particularly in winter indicate more stress and lesser adaptability in former groups than latter. Elevation in levels of insulin during winter may be partly as a physiological response to hasten uptake of glucose under negative energy balance, low energy content of diet

Table 5. Mean±SE of minerals in different age groups of pashmina goats in summer and winter.

	Season	Young group (Y)	P value (Y*A)	Adult group (A)	P value (A*O)	Old group (O)	P value (O*Y)
Calcium (mmol/l)	Summer (S)	2.255±0.129^{a} (2.01–2.87)	0.027	2.950±0.258^{b} (2.01–3.99)	0.048	2.305±0.110^{ac} (2.07–2.75)	1.000
	Winter (W)	1.902±0.095^{Aa} (1.47–2.15)	0.053	2.537±0.148^{ABb} (1.99–2.92)	0.061	1.915±0.092^{ABCc} (1.58–2.12)	1.000
	P value (S*W)	0.556		0.386		0.450	
Phosphorus (mmol/l)	Summer (S)	1.917±0.203^{a} (1.11–2.37)	0.035	2.627±0. 203^{b} (2.17–3.49)	0.569	2.258±0.079^{abc} (2.01–2.49)	0.644
	Winter (W)	1.348±0.163^{Aa} (0.96–1.93)	0.002	2.332±0.163^{Bb} (1.99–2.88)	0.663	1.997±0.096^{BCc} (1.72–2.37)	0.066
	P value (S*W)	0.140		0.768		0.844	
Magnesium (mmol/l)	Summer (S)	1.162±0.078^{a} (0.81–1.33)	0.484	1.298±0.068^{ab} (0.98–1.42)	0.688	1.187±0.038^{abc} (1.03–1.32)	0.999
	Winter (W)	1.043±0.039^{Aa} (0.93–1.15)	0.165	1.232±0.037^{ABb} (1.11–1.32)	0.085	1.014±0.047^{ABCc} (0.87–1.18)	0.999
	P value (S*W)	0.634		0.950		0.263	
Sodium Summer (S) (mmol/l)	138.36±1.88^{a}	0.001 (133.29–145.82)	155.92 ±1.33^{b}	0.393 (150.47–159.72)	145.64 ±4.17^{abc}	0.093 (132.96–157.56)	
	Winter (W)	134.38±1.58^{Aa} (130.28–140.47)	0.000	153.09±2.43^{Bb} (145.72–160.36)	0.016	139.92±3.27^{Cac} (130.37–150.36)	0.676
	P value (S*W)	0.891		0.972		0.647	
Potassium (mmol/l)	Summer (S)	3.257±0.145^{a} (2.87–3.86)	0.000	5.328±0.360^{b} (3.99–6.19)	0.001	3.602±0.373^{ac} (2.12–4.72)	0.938
	Winter (W)	2.502±0.179^{Aa} (2.09–3.11)	0.047	3.65±0.1949^{B} (2.98–4.16)	0.704	3.112±0.245^{ABCc} (2.22–4.01)	0.586
	P value (S*W)	0.357		0.001		0.778	
Copper (µmol/l)	Summer (S)	14.47±1.742^{a} (9.49–20.63)	0.076	19.68±1.316^{ab} (15.28–23.64)	0.173	15.22±1.525^{abc} (10.46–21.15)	0.998
	Winter (W)	10.23±1.018^{Aa}	0.231	14.41±1.079^{ABb}	0.506	11.20±0.844^{ABCc}	0.995
	P value (S*W)	(7.97–14.12) 0.217		0.070	(11.23–17.97)	(8.99–14.63) 0.267	
Iron (µmol/l)	Summer (S)	29.53±0.648^{a} (27.19–31.68)	0.018	35.86±2.320^{b} (27.47–40.12)	0.021	29.63±1.387^{ac} (27.02–36.13)	1.000
	Winter (W)	25.89±1. 102^{Aa} (22.17–29.16)	0.023	32.03±0.725^{Bb} (29.88–33.92)	0.008	25.12±0.692^{ACc} (22.17–27.11)	0.998
	P value (S*W)	0.368		0.313		0.163	
Zinc (µmol/l)	Summer (S)	13.33±1.242^{a} (9.94–18.84)	0.107	17.14±1.530^{ab} (11.79–22.74)	0.806	15.36±1.047^{abc} (11.82–18.47)	0.707
	Winter (W)	9.870±0.591^{Aa} (8.12–12.43)	0.811	11.63±0. 578^{AB} (9.78–13.21)	0.982	10.66±0.589^{ABC} (8.91–12.89)	0.993
	P value (S*W)	0.172		0.006		0.026	

^{abcABC}Values with different superscript differ significantly ($P<0.05$) within column and between rows of a particular mineral.

Young (Y), adult (A), old (O), summer (S), winter (W) represent respective abbreviations. *Represents comparison between two variables. Values in parenthesis are minimum to maximum range of mineral.

and partly due to rise of volatile fatty acid levels and cortisol or in response to increased energy demands under severe cold. Fall in T_3 and T_4 levels may be related to their excessive consumption due to higher metabolic demands under harsh climate, as they are particularly essential in metabolism. TSH levels rise in response to fall in T_3 and T_4 levels. Higher cortisol levels indicate stress in animals. Inter-hormonal interactions can also be a reason for these variations in addition to age and seasonal effect. Similar hormonal levels were noted by Khan and Ludri (2002a, 2002b) in goats. Magistrelli and Rosi (2014) have reported higher insulin in primiparous goats. We have found significant fall in T_3 and T_4 levels in lambs and kids in our previous study (Yatoo *et al.* 2014b). Seasonal variation of T_3, T_4 and TSH were also noted by Zarei *et al.* (2009) in goats. They reported fall in TSH, T_3 and T_4 level in goats with decrease in temperature. T_4 and TSH showed significant correlation with temperature but not T_3. Rise in cortisol levels has been noted by Khan and Ludri (2002b) in goats. Increase in cortisol may be due to nutritional and environmental stress. This elevation may also be in response to maintaining homeostatic levels of various biochemicals

Table 6. Mean±SE of nutrients in common fodders/roughages of Changthang

Fodder	DM	CP	EE	CF	Ash
Oat straw	88.62±5.52	3.46±0.92	0.94±0.13	34.93±4.47	7.59±1.92^{a}
Wheat straw	89.41±4.03	3.59±1.27	1.03±0.45	41.52±3.99	11.04±3.67
Barley straw	89.93±7.91	3.78±1.06	0.92±0.09^{a}	47.45±6.81^{a}	9.11±0.58
Alfa alfa	71.05±9.06^{a}	16.44±2.99^{a}	1.48±0.52	29.44±3.13	12.73±2.22^{b}
Local pea	88.32±4.89	11.98±1.68^{b}	1.95±0.55	31.85±2.54	9.83±1.03
Pasture grass	88.56±6.77	4.47±1.22	1.52±0.79	30.93±5.59	11.56±2.25
Salix leaves	55.54±12.99^{b}	12.86±2.27^{b}	2.96±0.12^{b}	27.84±1.88^{b}	9.98±0.94

^{abc} Values with different superscript differ significantly P<0.05 within a column.

Table 7. Mean±SE of minerals in common fodders/roughages of Changthang.

Fodder	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Cu (ppm)	Fe (ppm)	Zn (ppm)
Oats	0.193±0.016	0.177±0.0092	0.157± 0.0158	1.253±0.034	0.793±0.016	14.23±0.29*	149.54±9.84	12.21±2.251
Straw	(0.154–0.255)	(0.157–0.214)	(0.088–0.302)	(1.174–1.395)	(0.753–0.852)	(10.68–19.73)	(120.86–174.29)	(9.94–15.09)
Wheat	0.136±0.011	0.1683±0.016	0.168±0.01662	0.955±0.022	1.376±0.091	3.57±0.456	331.42±17.44	10.25±2.693
straw	(0.112–0.184)	(0.113–0.235)	(0.113–0.235)	(0.89–1.04)	(0.92–1.51)	(1.49–5.21)	(297.67–419.56)	(7.75–14.18)
Barley	0.242±0.012	0.215±0.011	0.136±0.02931	1.113±0.024	1.288±0.031	4.87±0.385	195.99±25.73	6.591±0.735
straw	(0.217–0.297)	(0.188–0.252)	(0.092–0.276)	(1.01–1.17)	(1.19–1.38)	(2.57–7.34)	(123.76–296.99)	(4.76–8.81)
Alfa	1.330±0.027*	0.216±0.017	0.301±0.03321	2.466±0.048*	2.073±0.051*	8.79±0.531	200.72±11.27	13.59±1.124
alfa	(1.233–1.429)	(0.189–0.281)	(0.241–0.473)	(2.31–2.62)	(1.92–2.25)	(6.12–10.98)	(155.43–271.93)	(9.97–16.82)
Local	1.246±0.156	0.2300±0.032	0.389±0.04224*	2.323±0.344	1.276±0.266	9.21±0.672	571.16±27.17*	16.36±3.342
pea	(0.953–1.993)	(0.124–0.362)	(0.153–.562)	(1.12–3.76)	(0.57–2.51)	(6.33–17.98)	(395.79–631.93)	(13.39–21.22)
Pasture	0.487±0.091	0.482±0.091*	0.179±0.03613	2.235±0.338	0.766±0.144	3.95±0.541	194.31±12.38	19.74±2.193*
grass	(0.191–0.832)	(0.191–0.832)	(0.099–0.329)	(1.09–3.67)	(0.36–1.43)	(1.52–5.79)	(145.66–281.21)	(15.82–24.16)
Salix	0.675±0.092	0.419±0.312	0.386±0.25	1.122±0.424	0.696±0.215	7.79±2.12	164.33±12.24	13.17±2.284
leaves	(0.32–1.89)	(0.21–0.64)	(0.16–0.74)	(0.55–2.18)	(0.23–1.19)	(3.92–10.47)	(142.25–197.12)	(5.99–22.26)

* Values with different superscript differ significantly P<0.05 within a column.

and hormones involved in metabolism (e.g. insulin, T_3, T_4) which are directly or indirectly related to it.

In summer, Ca, K and Fe levels in adult animals were significantly (P<0.05) higher than young and old animals. Significantly (P<0.05) higher levels of P and Na were noted in adults than young ones. Winter levels of Na and Fe were significantly (P<0.05) higher in adult animals than young and old animals. In winter, P and K levels in young and adult animals differed significantly (P≥0.05). Rest all combinations were non significant (P≥0.05). Significant (P>0.05) reduction in K levels in adult, Zn levels in adult and old animals were noted in winter. In general and particularly in winter, lower levels of minerals are attributed to fodder scarcity and poor quality of available fodders or pasture grasses besides their lower mineral content. Lower levels of Ca, P, Na, K and Fe in young animals can also be attributed to either lower concentration in milk or more requirements in young animals. Similarly lower levels of Ca, Na, K and Fe in old animals may be due to production loss, lower intestinal absorption in older animals, an old age effect. Fall of mineral levels during winter may also be due to physiological and production demands, utilization as antioxidants under oxidative stress and age effect. Mbassa and Poulsen (1991c, 1991d) have also reported similar age and environment related variation of minerals in goats. Higher levels of Na and K were noted in well fed lambs by Bornez *et al.* (2009). They also noted rise in Na level after transport, a form of stress but K was higher at farm *i.e.* at rest.

Oxidative stress indices

In summer significantly (P<0.05) higher levels of GSH in adult animals than old animals were noted. In winter significantly (P<0.05) higher catalase and lower LPO levels were noted in adult animals than young and old animals. Significantly (P<0.05) higher levels of SOD in adults were noted than young animals. In all age groups antioxidants decreased and oxidants increased significantly (P<0.05) in winter than summer except for non significant (P>0.05) change in GSH in old and TAC in adult and old animals. Lower levels of CAT, GSH, SOD, TAC and higher levels LPO in young and older age groups in both seasons indicate more vulnerability to oxidative stress of these two groups. Lower levels of antioxidants and higher levels of oxidants during winter indicates oxidative stress in animals. This may be due to both nutritional and climatic stress besides metabolic disturbances. Our findings are in corroboration with Singh *et al.* (2014) who have noted similar summer and winter levels of CAT, SOD, TAC and LPO/MDA in goats. In our previous study we found higher CAT, GSH, SOD, LPO levels in cattle and buffalo during summer than winter (Yatoo *et al.* 2014c). Pedernera *et al.* (2010) reported that negative energy balance is related to high oxidative stress. Hence nutritional deficiency and climatic stress can induce oxidative stress especially in young and old age groups.

Metabolic and deficiency diseases predisposed by harsh climate are main reasons for low production and neonatal mortality in pashmina goats and for their prevention and control, early prediction based on species specific biomarkers is essential. We evaluated glycated haemoglobin, fructosamine and glucose as biomarkers of energy metabolism; haemoglobin, total protein, urea, creatinine and total billirubin of protein metabolism; VFAs (acetoacetate, propionate, betahydroxybutyrate) and cholesterol of fat metabolism; serum minerals of mineral status, antioxidants (CAT, SOD, GSH, TAC) and oxidants (LPO/MDA) of oxidative stress in pashmina goats. Lower levels of metabolic biomarkers may be due to nutrient deficiency and higher level of stress biomarkers may be due to both nutritional and climatic stress. Nutrient deficiency is related to low feed intake due to less availability of pasture grasses and scarcity of fodders in winter whereas metabolic alterations are related to both deficiency and inactivity. These metabolic alterations along with nutrient deficiency aggravated by climatic stress leads to oxidative stress in animals. Identifying reference range of specific biomarkers of metabolism and oxidative stress is a prerequisite for diagnosing any abnormality. Hence, present study can be a preliminary one for Pashmina goats.

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