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Full Length Research Paper

Serum biochemical values and mineral contents of tissues in Przewalski's and Tibetan gazelles

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Reference values were established for some serum hematological and biochemical constituents in Przewaiski's and Tibetan gazelles in China. The contents of mineral elements in the blood, hair, liver, lung and muscle of Przewaiski's and Tibetan gazelles were also measured. All values are reported for the first time for Przewaiski's and Tibetan gazelles. Most hematological and serum biochemical values were similar to those of Tibet sheep, yaks, and camels, but the mean serum albumin concentration and the albumin/globulin ratio in Przewaiski's and Tibetan gazelles were significantly higher than those in other ruminants and the mean thyroxine (T_4) concentration was half that in Tibet sheep. Liver contains the highest concentrations of copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe). Hair contains higher amount of Sulphur (S). The concentrations of cobalt (Co), molybdenum (Mo), S and Mn from Przewaiski's and Tibetan gazelles in the blood, hair, liver, lung and muscle were within the reference range for other ruminants. The concentration of selenium (Se) from Przewaiski's gazelles in the blood and liver was significantly lower than that in Tibetan gazelles.

Key words: Przewaiski's gazelle, Tibetan gazelle, minerals, hematological values, biochemical constituents.

INTRODUCTION

Przewaiski's gazelle (*Procapra przewalskii*) and Tibetan gazelle (*Procapra picticaudata*) are two important wild ungulate on the Qinghai-Tibet Plateau. Historically, the species occurred in parts of the provinces of Gusu, Inner Mongolia, Ningxia and Qinghai. There have been no recent records of the species in western Inner Mongolia, adjacent Gansu and other areas of historical distribution. Przewalski's gazelle only occurs around Qinghai Lake and has about 300 individuals (Shen xiao yun, 2009). Przewalski's gazelle has been classified as critically endangered by the Species Survival Commission of IUCN-the World Conservation Union since 1996 and has been a Category I (Endangered in China) National Protected Wild Animal Species in China since 1989 (Baillie and Groombridge, 1996). Tibetan gazelle resides in fragmented habitat patches on the Qinghai-Tibet Plateau. The population is decreasing sharply and its listed as low risk in the IUCN red list of threatened species, Tibetan gazelle is a Category II (Threatened in China) National Protected Wild Animal Species in China (Mallon and Kingswood, 2003). Population decrease of the 2 gazelles is caused by multiple factors, such as habitat fragmentation, pasture fencing, nutrition, disease and over-hunting.

This study was designed to define reference hematological, serum biochemical values and mineral element concentrations, and further investigations to save these two kinds of animals in the Qinghai-Tibet Plateau, China.

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MATERIALS AND METHODS

We conducted our study in the areas around Qinghai Lake in the Qinghai province in the northwest part of Qinghai-Tibet Plateau, China. The total area was approximately 3,000 km^2 (35.9°-36.1°N; 99.9°-100.1°E). Elevations in our study area ranged from 3200 to 3500 m. The climate is marked by low humidity, wide range of temperature between day and night, strong ultraviolet radiation, low precipitation, and high evaporation. The annual precipitation is 360 mm, but the annual evapo-transpiration is 4 times greater. Annual temperatures range from -31°C in January to 28°C in July. June to mid-September is the plant-growing season, and late September to

May is the plant-withering season. Steppe and alpine meadow were the major vegetation types.

Twenty Przewaiski's gazelles (10 females and 10 males) and 20 Tibetan gazelles (10 females and 10 males) were selected for the study, of age between 3 and 5 years in the areas around Qinghai Lake in the Qinghai province in the northwest part of Qinghai-Tibet Plateau. All the animals were healthy, with no clinical signs of disease.

Sampling

The animals utilized in these experiments were cared for by acceptable practices as outlined in Guide for the Care and Use of Wildlife Animals in Wildlife Research and Teaching Consortium (2003). All the samples were taken in may 2009. Blood samples, each of 15 ml, were obtained from the jugular vein, using 1% sodium heparin as anticoagulant, and stored at 4°C for hematological examination and at -20°C for analysis of mineral elements. Serum samples for biochemical values were taken without anticoagulant and were refrigerated until they arrived at the laboratory after less than 5 h, when the serum was separated by centrifugation and stored frozen in plastic vials until the laboratory determinations could be made. Hair was taken from the neck of all the animals, washed and degreased as described by Salmela and colleagues (1981). Liver, lung and muscle biopsies were also sampled by a trained technician using techniques previously described (Arthington and Corah, 1995) and approved by Institute of zoology, Chinese Academy of Sciences, Institutional Animal Care and Use Committee (Project＃A156). The liver, lung and muscle samples were dried at 80°C for 48 h.

Hematological and biochemical examination

Hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), packed cell volume (PCV), and red blood cell (RBC), white blood cell (WBC), Neutrophils, Lymphocytes, Eosinophils, Basophils and Monocytes were determined using an automatic hematology analyzer (SF-3000, Medical Electronic Instrument Co., Japan). The serum content of ceruloplasmin (Cp), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AKP), γ-glutamyl transferase (γ-GT), creatinine (CRT), cholesterol (Chol), blood urea nitrogen (BUN), glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), sodium (Na), potassium (K), magnesium (Mg), calcium(Ca), inorganic phosphorus (IP), total protein (TP), albumin (Alb) and globulin (Glob) were determined on an automatic analyzer (SF-1, Shanghai Medical Apparatus and Instruments Factory, Shanghai, China) using commercial test kits (Nanjing Medicine University Biochemical Co., Nanjing, China). Quality control serum (Shanghai Biochemical Co., Shanghai) was used to validate the blood biochemistry data. Serum protein electrophoretic studies were performed on cellulose acetate using the EA-4 electrophoresis apparatus (Shanghai Medical Apparatus and Instruments Factory). Serum triiodothyronine (T_3), thyroxine (T_4) and parathyroid hormone (PTH) concentrations were determined by radioimmunoassay (RIA) on a γ- counter (γ-Autocounter, Xian 262 Factory, Shanxi, China) using commercial test kits (Tianjing Medicine Biochemical Co., Tianjing, China). All the serum biochemical values were measured at room temperature.

Analysis of trace elements

Copper (Cu), iron (Fe), zinc (Zn), cobalt (Co), manganese (Mn) were determined by atomic absorption spectrophotometry (AAS) (AA-640, Shimadzu Co., Ltd, Tokyo, Japan). Molybdenum (Mo) contents were measured using flameless atomic absorption spectrophotometry with extra steps to produce reliable data. In brief, we extracted samples with isopentanol as soon as possible because the generated complex was not sufficiently stable. Selenium (Se) was assayed by hydride-generation atomic absorption spectrophotometry (Wang, 1999). Sulphur (S) was determined by nephelometry (Wen et al., 1983). The accuracy of the analytical values was checked by reference to certified values of elements in the National Bureau of Standards (NBS, Washington, USA) Standard Reference Material, bovine liver SRM1577a.

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA), and presented in the form of mean ± standard deviation. Significant differences between groups were assessed using Student's *t* test with least significant differences of 1 and 5%, respectively, ($P < 0.01$ and $P < 0.05$).

RESULTS AND DISCUSSION

Tables 1, 2, 3 and 4 summarize the hematological and biochemical values in the sera from Przewaiski's and Tibetan gazelles. The mean serum albumin concentration and the albumin/ globulin ratio in Przewaiski's and Tibetan gazelles were significantly higher than those in other ruminants ($P < 0.01$) and the mean T_4 concentration was half that in Tibet sheep. Activity of glutathione peroxidase (GSH-PX) from Przewaiski's gazelles was significantly lower than that in Tibetan gazelles ($P < 0.01$).

The concentrations of mineral elements in the blood, hair, liver, lung and muscle are given in Tables 5, 6, 7, 8 and 9. The liver contained the highest concentrations of Cu, Zn, Mn and Fe. The hair contained the higher contents of S. The concentration of Se from Przewaiski's gazelles in the blood and liver was significantly lower than that in Tibetan gazelles ($P < 0.01$).

All hematological values are reported for the first time for Przewaiski's and Tibetan gazelles in China. The hematological results for Przewaiski's and Tibetan gazelles were within the reference ranges for other ruminants, including cattle (Shi, 1990), yaks (Shen et al., 2006), sheep (Abdelgadir et al., 1984) and camels (Liu, 2003), with the exception of the Hb concentration, which was significantly ($P < 0.01$) less than the value for cattle (103 ± 23 g/L) (Shi, 1990), yaks (108 ± 6.51 g/L) (Shen et al., 2006), camel(109 ± 24 g/L) (Liu, 2003) and sheep (107 ± 19 g/L) (Abdelgadir et al., 1984).

All biochemical values are reported for the first time for Przewaiski's and Tibetan gazelles in China. Most of these values were similar to the reference values for cattle (Liu et al., 1995; Shen et al., 2006), sheep (Shi, 1990) and camels (Abdelgadri et al., 1984; Bengoumi et al., 1999; Liu, 2005). However, the mean serum albumin concentration and albumin/globulin ratio were significantly ($P < 0.01$) higher than those in other ruminants, the albumin/

Table 1. Mean hematological value in Przewaiski's and Tibetan gazelles (n = 20).

Blood index	Przewaiski's gazelles	Range	Tibetan gazelles	Range
RBC (10^{12}/L)	12.3 ± 2.6	7.53 - 14.13	13.1 ± 3.6	6.97 - 13.98
Hb (g/L)	92.8 ± 26	67.8 - 126.7	93.5 ± 27	71.8 - 123.1
PCV (%)	31.2 ± 4.2	23.5 - 41.6	32.1 ± 3.1	21.5 - 43.6
MCH (pg)	7.55 ± 2.7	5.31 - 11.21	7.14 ± 2.1	5.12 - 12.31
MCV (fl)	25.4 ± 5.9	18.6 - 36.1	24.5 ± 6.2	17.6 - 37.3
MCHC (%)	29.8 ± 3.9	21.5 -43.9	29.1 ± 4.5	20.3 - 41.2
WBC (10^{9}/L)	6.38 ±1.97	4.56 - 9.68	6.56 ± 2.13	4.71 - 9.37
Neutrophils (%)	56.9 ± 9.8	35.6 - 69.7	57.5 ± 9.1	36.3 - 69.9
Lymphocytes (%)	29.8 ± 6.31	22.1 to 39.1	30.1 ± 6.1	23.1 - 39.7
Eosinophils (%)	6.12 ± 2.98	3.1 - 16.2	6.56 ± 2.37	3.9 - 17.2
Basophils (%)	0.48 ± 0.23	0.21 - 0.79	0.51 ± 0.32	0.23 - 0.81
Monocytes (%)	0.71 ± 0.34	0.52 - 0.95	0.69 ±0.27	0.43 - 0.92

Result between Przewaiski's and Tibetan gazelles are no significantly difference (P > 0.05).

Table 2. Serum biochemical values in Przewaiski's and Tibetan gazelles (n = 20).

Parameter	Przewaiski's gazelles	Range	Tibetan gazelles	Range
Cp (mg/L)	41.3 ± 7.6	23.2 - 83.7	39.7 ± 7.2	21.2 - 73.7
LDH (μmol.s $^{-1}$/L)	3.51 ± 0.32	2.32 - 4.47	3.67 ± 0.43	2.22 - 4.83
AKP(IU/L)	276 ± 83	187 - 383	287 ± 76	201 - 365
AST(IU/L)	38.7 ± 11.3	23.6 - 51.3	37.9 ± 13.5	22.6 - 57.3
ALT(IU/L)	12.3 ± 2.9	7.7 - 17.3	12.8 ± 3.1	7.6 - 18.3
γ -GT(IU/L)	18.9 ± 3.5	11.2 - 26.3	17.9 ± 3.8	10.2 - 28.1
BUN (mmol/L)	5.77 ± 1.37	3.26 - 8.87	6.07 ± 1.76	3.16 - 9.86
SOD (μmol.s $^{-1}$/L)	16.3 ± 2.7	11.2 - 21.3	15.6 ± 2.6	11.6 - 23.7
GSH -PX (μmol.s $^{-1}$/L)	13.7 ± 2.3**	5.2 - 21.8	19.5 ± 2.7	13.2 - 25.8
CRT (μmol/L)	317 ± 63	252 - 436	348 ± 71	263 - 456
Chol (mmol/L)	2.77 ± 0.31	1.23 - 3.89	2.87 ± 0.36	1.35 - 3.98
K (mmol/L)	3.89 ± 0.37	2.21 - 5.53	4.03 ± 0.58	2.31 - 5.83
Na (mmol/L)	137 ± 35	61 - 203	141 ± 28	65 - 213
Ca (mmol/L)	2.28 ± 0.21	1.41 - 3.23	2.31 ± 0.23	1.48 - 3.52
IP (mmol/L)	1.89 ± 0.32	0.98 - 2.76	1.81 ± 0.26	0.93 - 2.83
Mg (mmol/L)	0.87 ± 0.23	0.49 - 1.27	0.92 ± 0.21	0.51 - 1.39

**Result between Przewaiski's and Tibetan gazelles are significantly different (P < 0.01).

Table 3. Serum protein concentrations in Przewaiski's and Tibetan gazelles (n=20).

Parameter	Przewaiski's gazelle	Range	Tibetan gazelles	Range
Total protein (g/L)	80.7 ± 7.2	57.3 - 95.9	81.6 ± 6.9	55.3 - 98.5
Albumin (g/L)	65.0 ± 5.3	49.3 - 83.8	65.8 ± 6.7	48.9 - 85.8
α-Globulin (g/L)	2.79 ± 0.56	1.82 - 4.16	2.76 ± 0.22	1.75 - 4.23
β- Globulin (g/L)	4.23 ± 1.33	2.58 - 6.38	4.25 ± 1.52	2.48 - 6.78
γ- Globulin (g/L)	8.71 ± 2.68	5.12 - 12.23	8.78 ± 2.35	5.32 - 13.12
A/ G	4.13 ± 0.31	2.53 - 6.21	4.17 ± 0.23	2.51 - 6.18

Result between Przewaiski's and Tibetan gazelles are no significantly difference (P > 0.05).

Table 4. Concentration of parathyroid hormone, triiodothyronine and thyroxine in serum from Przewaiski's and Tibetan gazelles (n=20).

Parameter	Przewaiski's gazelle	Range	Tibetan gazelles	Range
PTH (ng/L)	113.2 ± 23.6	63.7 - 147.8	112.5 ± 42.7	61.7 - 143.8
T_3 (nmol/L)	1.73 ± 0.31	1.35 - 2.37	1.82 ± 0.21	1.37 - 2.57
T_4 (nmol/L)	46.7 ± 18.7	25.7 - 66.7	45.8 ± 12.7	23.7- 62.3

Result between Przewaiski's and Tibetan gazelles are no significantly difference (P > 0.05).

Table 5. Concentrations of mineral element in blood from Przewaiski's and Tibetan gazelles (n=20).

Element	Przewaiski's gazelle	Range	Tibetan gazelles	Range
Se (µg/g)	0.047 ± 0.07**	0.026 - 0.063	0.067 ± 0.17	0.033 - 0.091
Fe (µg/g)	319 ± 87	219 - 427	317 ± 86	213 - 416
Zn (µg/g)	26.8 ± 2.9	16.8 - 35.7	27.1 ± 2.7	18.2 - 43.9
Cu (µg/g)	0.34 ± 0.12	0.23 - 0.52	0.39 ± 0.13	0.25 - 0.53
Mn (µg/g)	0.38 ± 0.13	0.24 - 0.43	0.37 ± 0.12	0.23 - 0.49
Mo (µg/g)	0.36 ± 0.07	0.21 - 0.52	0.32 ± 0.05	0.21 - 0.47
Co (µg/g)	0.71 ± 0.21	0.32 - 1.13	0.73 ± 0.23	0.37 - 1.12
S (mmol/l)	32.3 ± 7.9	23.3 - 43.3	33.1 ± 6.3	22.7 - 45.1

**Result between Przewaiski's and Tibetan gazelles are significantly different (P < 0.01).

Table 6. Concentrations of mineral element in hair from Przewaiski's and Tibetan gazelles (n=20).

Element	Przewaiski's gazelle	Range	Tibetan gazelles	Range
Se (µg/g)	0.36 ± 0.11	0.25 - 0.51	0.38 ± 0.12	0.23 - 0.56
Fe (µg/g)	285 ± 52	187 - 421	278 ± 137	192 - 412
Zn (µg/g)	89 ± 12	58 - 141	87 ± 13	52 - 139
Cu (µg/g)	2.37 ± 1.23	1.31 - 4.83	2.43 ± 1.17	1.43 - 4.97
Mn (µg/g)	7.89 ± 1.47	5.31 - 11.63	7.69 ± 1.36	5.38 - 12.13
Mo (µg/g)	0.37 ± 0.12	0.21 - 0.63	0.36 ± 0.13	0.19 - 0.65
Co (µg/g)	1.12 ± 0.31	0.61 - 1.51	1.02 ± 0.28	0.56 - 1.48
S (%)	3.56 ± 0.79	2.31 - 5.87	3.63 ± 0.72	2.13 - 5.91

Result between Przewaiski's and Tibetan gazelles are no significantly difference (P > 0.05).

Table 7. Concentrations of mineral element in liver from Przewaiski's and Tibetan gazelles (n=20).

Element	Przewaiski's gazelle	Range	Tibetan gazelles	Range
Se (µg/g)	1.13 ± 0.82**	0.57 - 1.53	1.43 ± 0.57	0.77 - 2.17
Fe (µg/g)	462 ± 26	337 - 576	471 ± 26	349 - 586
Zn (µg/g)	97 ± 28	56 - 151	95 ± 21	53 - 158
Cu (µg/g)	72 ± 13	48 - 112	76 ± 12	49 - 115
Mn (µg/g)	5.41 ± 1.13	3.82 - 7.15	5.26 ± 1.21	3.28 - 7.85
Mo (µg/g)	5.87 ± 1.17	3.36 - 6.98	5.23 ± 1.18	3.56 - 7.17
Co (µg/g)	1.21 ± 0.37	0.71 - 1.81	1.17 ± 0.29	0.61 - 1.83
S (%)	0.23 ± 0.08	0.12 - 0.35	0.22 ± 0.07	0.11 - 0.37

**Result between Przewaiski's and Tibetan gazelles are significantly different (P < 0.01).

Table 8. Concentrations of mineral element in lung from Przewaiski's and Tibetan gazelles (n=20).

Element	Przewaiski's gazelle	Range	Tibetan gazelles	Range
Se (µg/g)	1.21 ± 0.12	0.77 - 1.69	1.13 ± 0.82	0.76 - 1.57
Fe (µg/g)	362 ± 62	216 - 513	387 ± 78	258 - 517
Zn (µg/g)	77 ± 28	46 - 113	81 ± 28	41 - 121
Cu (µg/g)	2.21 ± 0.41	1.32 - 3.13	2.32 ± 0.53	1.37 - 3.26
Mn (µg/g)	1.41 ± 1.13	0.87 - 1.93	1.38 ± 1.13	0.86 - 1.91
Mo (µg/g)	3.81 ± 0.87	2.31 - 4.97	3.87 ± 0.85	2.12 - 4.98
Co (µg/g)	0.81 ± 0.12	0.51 - 1.26	0.83 ± 0.11	0.49 - 1.35
S (%)	0.16 ± 0.03	0.11 - 0.23	0.17 ± 0.02	0.09 - 0.24

Result between Przewaiski's and Tibetan gazelles are no significantly difference (P > 0.05).

Table 9. Concentrations of mineral element in muscle from Przewaiski's and Tibetan gazelles (n = 20).

Element	Przewaiski's gazelle	Range	Tibetan gazelles	Range
Se (µg/g)	0.61 ± 0.12	0.37 - 0.89	0.63 ± 0.17	0.36 - 0.91
Fe (µg/g)	232 ± 59	167 - 315	241 ± 53	172 - 323
Zn (µg/g)	137 ± 28	93 - 176	142 ± 27	95 - 181
Cu (µg/g)	3.61 ± 1.12	2.15 - 5.12	3.73 ± 1.32	2.25 - 5.31
Mn (µg/g)	1.41 ± 0.33	0.98 - 1.95	1.38 ± 0.32	0.92 - 1.87
Mo (µg/g)	2.87 ± 0.78	1.86 - 3.87	2.69 ± 0.67	1.73 - 3.79
Co (µg/g)	0.46 ± 0.17	0.21 - 0.65	0.48 ± 0.19	0.23 - 0.67
S (%)	0.13 ± 0.04	0.08 - 0.17	0.14 ± 0.05	0.09 - 0.19

Result between Przewaiski's and Tibetan gazelles are no significantly difference (P > 0.05).

globulin ratio in Przewaiski's and Tibetan gazelles being 1.5 to 2.6 times higher than that in cattle, yaks, and sheep, which probably makes it possible to maintain the high colloid osmotic pressure needed for storing water in the blood (Liu et al., 1995; Shen et al., 2006; Shi, 1990).

Essential trace elements are integral components of certain enzymes and of other biologically important compounds that have major physiological and biochemical roles, for example Se in glutathione peroxidase, Cu and Zn in superoxide dismutase, Fe in hemoglobin, and Co in vitamin B_{12}. It is well known that dromedaries have some physiological peculiarities in trace element metabolism due to their adaptation to arid conditions and poor feeding resources (Faye and Bengoumi, 1994). We think that the Przewaiski's and Tibetan gazelles have some physiological peculiarities in trace element metabolism maybe due to geographic (altitude, latitude, climate) and dietary factors. The previous study showed Przewaiski's gazelle ate 40 plant species from 14 families and Tibetan gazelle fed on 43 plant species from 16 families, whereas Tibetan domestic sheep ate 39 plant species from 15 families in around Qinghai Lake, China (Li and Jiang, 2008). In the present study, the concentration of Zn in whole blood was significantly higher ($P < 0.01$) in Przewaiski's and Tibetan gazelles than in dairy cattle (10.91 ± 3.86 mg/L) (Liu et al., 1994) or sheep (10.06 ± 3.04 mg/L) (Liu et al.,1994) in the same area. The concentration of Se in whole blood was significantly lower ($P < 0.01$) in Przewaiski's gazelles than in yaks (0.088 ± 0.036 µg/g) (Shen et al., 2006), sheep (0.097 ± 0.04 µg/g) (Liu et al., 1992) or Tibetan gazelles. The mean Fe concentrations from Przewaiski's and Tibetan gazelles in the liver were significantly higher than those in other ruminants ($P < 0.01$) (Faye and Bengoumi, 1994; Faye et al., 1992; Liu et al., 1995; Shen et al., 2006; Shi, 1990). The concentration of Cu from Przewaiski's and Tibetan gazelles in the blood, hair, liver, lung and muscle was significantly lower than that in other ruminants ($P < 0.01$) (Georaie-vaskii et al., 1982; Liu et al., 1995; Shen et al., 2006; Shi, 1990). Under normal conditions, most of the Cu in serum is present as ceruloplasmin (Cp), which plays an essential role in promoting the rate of Fe saturation of transferring, and so in the absorption and transport of Fe and in the utilization of Fe by the bone marrow. For this reason, Cu deficiency not only markedly reduces the content of Cp and accompanied by anemia. Cu is an essential dietary element for ruminants. Cu deficiency has a direct impact on the ability of ruminants to mount a normal response to viral infection. This alteration in immune competence may result in failure to respond to vaccination along with increase energy losses (Arthington et al., 2002). The low Cu content in the blood, hair, liver, lung and muscle maybe is a reason for the endangerment of Przewaiski's and Tibetan gazelles in the Qinghai Plateau, China. Further study is needed to determine contents of Cu in soils and forages. Hence, increasing concentration of Cu in forage maybe is an effective measure to save the endangered Przewaiski's and Tibetan gazelles in the Qinghai Plateau, China. The concentrations of the other elements in the blood were within the reference ranges for cattle and sheep (Georaie-vaskii et al., 1982; Huang and Chen, 2001; Liu et al., 1995; Shen et al., 2006; Shi, 1990).

The hematological and serum biochemical values and mineral contents in the tissues of wildlife may vary according to geographic (altitude, latitude, climate) and dietary factors. Too little information is available to permit conclusions on the effects of these factors on Przewaiski's and Tibetan gazelles in China. Further studies will also be needed to the effects of diet, regional differences, season of year and the reproductive and physiological status of the animal.

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