



## Effect of a Copper, Selenium and Cobalt Soluble Glass Bolus Given to Grazing Yaks

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**ABSTRACT :** Two field trials were carried out to evaluate the performance of a soluble glass copper, cobalt and selenium bolus for maintaining adequate levels of the three trace elements in yaks. Forty yaks were used in trial 1 and 60 yaks were used in trial 2. In each trial two commercial soluble glass boluses were administered to half of the yaks. Blood samples were taken from the jugular vein at day 0, 30, 60, 90 in trial 1 and at day 0, 45, 75 and 105 in trial 2. The samples were analysed for copper status (serum caeruloplasmin activity and copper concentration), cobalt status (serum vitamin B<sub>12</sub> concentration and cobalt concentration), selenium status (erythrocyte glutathione peroxidase activity and selenium concentration) and serum zinc concentration. The erythrocyte glutathione peroxidase activities, serum caeruloplasmin activities and serum vitamin B<sub>12</sub> concentrations for trial 1 and 2 were all significantly increased for the bolused yaks ( $p < 0.001$  or  $p < 0.01$ ) on all sampling days. The bolused yaks had a significantly higher selenium and copper status in serum than the control yaks on all sampling days in trial 1 and 2 ( $p < 0.05$  or  $p < 0.01$ ). There were no significant differences in zinc and cobalt concentrations between the bolused yaks and the controls. (**Key Words :** Yak, Trace Elements, Controlled Release, Supplementation)

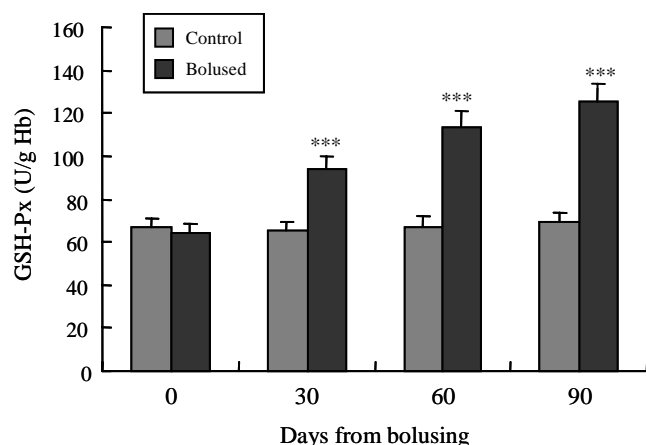
### INTRODUCTION

Yak (*Bos grunniens* or *poephagus grunniens*) is the prominent livestock breed in the Qinghai-Tibetan plateau where other livestock species can hardly live. Because of its large heart, lungs and a high erythrocyte count, the yak can tolerate low oxygen content in the air at high altitude. The yaks are distributed in China, Mongolia, Southern Russia and other places and estimated population worldwide is 14 million. The yak is indeed the only large domestic animal which populates the central Asian Highlands up to altitudes of 6,000 m. The yak is well adapted to extremely cold environments and to grazing at high altitudes on steep slopes. Chinese yaks are found on the pastoral plateau of western China, where they are the most important means of production and livelihood for local people and an economically important pillar of the animal husbandry industry.

Essential trace elements are integral components of certain enzymes and of other biologically important compounds which play important physiological and biochemical roles. Trace element deficiencies are common in ruminants, especially extensively grazed animals with

access only to grass and other naturally occurring plant material as their staple diet (McDowell, 1992; Liu et al., 1994; Lee et al., 1999; Khan et al., 2006). However, only a few authors have reported diseases caused by deficiencies of trace elements for yaks in China (Liu et al., 1995; Shen et al., 2005). Zhang (1983) reported that copper and selenium concentrations in soil and forage were low in Shandan county. Compared with normal values in cattle and sheep, the concentrations of iron, cobalt, manganese and calcium in the tissues of yaks in the area were within the normal range for ruminants, while the mean zinc concentration was half of that in sheep and cattle; the mean copper level ( $21.6 \pm 8.6$  mg/kg) in liver was very much lower than that in other ruminants (Liu et al., 1995). Zinc and copper deficiencies in yaks have not been reported in the area. Shen et al. (2005) reported molybdenum-induced copper deficiency in the yak in the North of the Qing Hai-Tibetan Plateau of China. It is very difficult to give supplements with trace elements for yaks on high mountain grassland areas, because of adverse natural environment and poor transport facilities. A soluble glass bolus has been developed for sustained release of cobalt, copper and selenium in ruminants (McDowell, 1992). Although the strategy has been used in cattle and sheep (Telfer et al., 1984; Kendall et al., 2000, 2001; Qian et al., 2003), there are no data for yaks.

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**Figure 1.** Mean erythrocyte glutathione peroxidase activities of the yaks in trial 1. \*\*\*  $p < 0.001$ .

The trials reported here were designed to study the effects of a copper, selenium and cobalt soluble glass bolus on the copper, selenium, cobalt and zinc status of yaks.

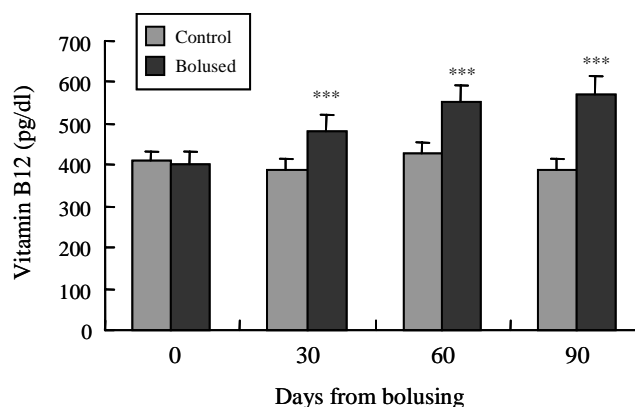
## MATERIAL AND METHODS

### Animals

In trial 1, 40 yaks aged 3-11 years and of both sexes in the Jianzha county of Qinghai province were tested from February to June in 2003. In trial 2, 60 yaks aged 3-10 years and of both sexes in the Shandan county of Gansu province were selected and tested from August to December in 2004. They were grazed on high mountain grassland at an elevation of 2,500-3,500 metres above sea level throughout the year. The basic features common to the environment where the yaks live were extreme cold, mountainous terrain, high altitudes with reduced oxygen content in the air and high solar radiation. In the Qinghai-Tibetan plateau, the weather was rather cold and humid, the average relative humidity is 55%-65%, the annual precipitation averages 350-650 mm, there are no absolute frost-free days in this area and the growth period of plants is only about 120 days a year. The annual average temperature was 0°C, in Jianzha county of Qinghai Province, the extreme lowest temperature was -41.8°C.

Yaks were allocated to two groups by randomisation based on sex and age (3-5 years and over 6 years) in trial 1 and trial 2. One group was dosed orally with two soluble glass sintered boluses (100 g for a bolus) containing 13.4% w/w copper, 0.3% w/w selenium and 0.5% w/w cobalt (manufactured by Pilkington Controlled Release Systems Ltd., UK) and the other group left untreated as a control group.

Blood samples were taken by jugular venipuncture using 1% sodium heparin as anticoagulant at day 0 (prior to bolus administration) and at day 30, 60 and 90 in trial 1 or at day 45, 75 and 105 in trial 2. Samples were stored at -



**Figure 2.** Mean serum vitamin B<sub>12</sub> concentrations of the yaks in trial 1. \*\*\*  $p < 0.001$ .

20°C for analysis of erythrocyte glutathione peroxidase activity. Serum samples for biochemical values were taken into anticoagulant free tubes and centrifuged at 1,000×g for 10 min. at room temperature. After centrifugation, samples were stored at -20°C until analysis.

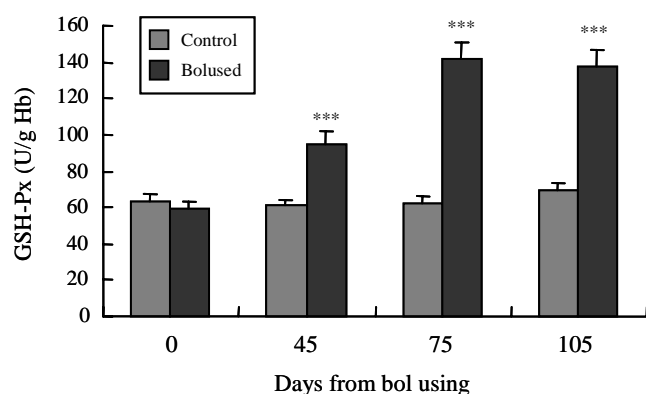
The concentrations of copper, zinc and cobalt in serum were determined using atomic absorption spectrophotometry (AA-640, Shimadzu Co., Ltd, Tokyo, Japan), while the concentration of selenium was assayed by hydride generation atomic absorption spectrophotometry (Liu et al., 1995). The accuracy of the analytical values was checked by reference to certified values of elements in the National Bureau of Standards (NBS) Standard Reference Material, bovine liver SRM 1577a.

The serum content of caeruloplasmin (Cp) and erythrocyte glutathione peroxidase activity (GSH-Px) 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.). Serum vitamin B<sub>12</sub> concentration was determined by radioimmunoassay (RIA) using commercial test kits (Tianjing Medicine Biochemical Co.).

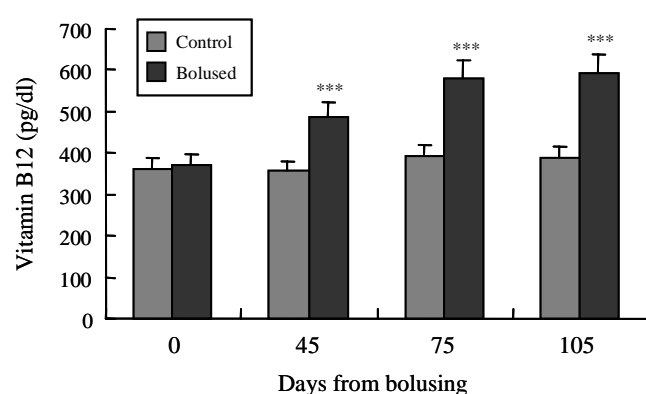
The data are presented as means ± standard deviation. The differences between mean values in treated animals and controls were analyzed statistically using SPSS 10.0.7 for Windows, and we used a level of significance of 0.05.

## RESULT

The erythrocyte glutathione peroxidase activities and serum vitamin B<sub>12</sub> concentrations for trial 1 and 2 were all significantly increased for the bolused yaks ( $p < 0.001$ ) on all sampling days (Figures 1, 2, 3 and 4). The serum caeruloplasmin activities were significantly higher for the bolused yaks on days 30, 60 and 90 ( $p < 0.01$ ) in trial 1 (Figure 5) and days 45 ( $p < 0.01$ ), 75 and 105 ( $p < 0.001$ ) (Figure 6). However, status of these values in untreated



**Figure 3.** Mean erythrocyte glutathione peroxidase activities of the yaks in trial 2. \*\*\*  $p < 0.001$ .



**Figure 4.** Mean serum vitamin B<sub>12</sub> concentrations of the yaks in trial 2. \*\*\*  $p < 0.001$ .

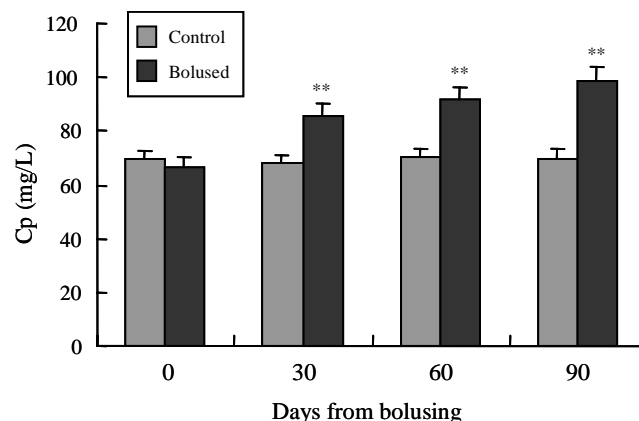
yaks remained remarkably stable.

The bolused yaks had a significantly higher selenium and copper status in serum on all sampling days than the control yaks in trial 1 and 2 ( $p < 0.05$  or  $p < 0.01$ ) (Tables 1 and 2). The selenium and copper status of all the bolused yaks was in the normal range for cattle. There were no significant differences in zinc and cobalt concentrations between the bolused yaks and the controls.

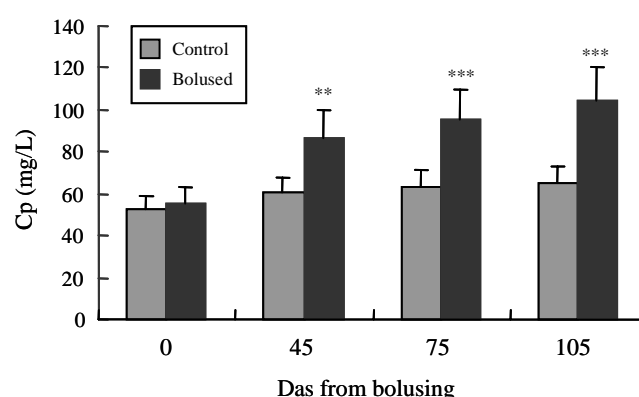
No differences between the sexes or ages were observed with regard to the serum biochemical values and trace elements.

## DISCUSSION

The erythrocyte glutathione peroxidase activities, serum caeruloplasmin activities and serum vitamin B<sub>12</sub> concentrations are reported for the first time for yaks in China. In the control yaks and prior to bolus administration, serum vitamin B<sub>12</sub> concentrations were significantly higher than in cattle (Kincaid et al., 2003; Tiffany et al., 2003) and lower than normal level of 0.2  $\mu\text{g/ml}$  in cattle (Radostits et al., 2005). The mean serum caeruloplasmin activities in the control yaks were very much lower than in cattle (Radostits



**Figure 5.** Mean serum caeruloplasmin activities of the yaks in trial 1. \*\*  $p < 0.01$ .



**Figure 6.** Mean serum caeruloplasmin activities of the yaks in trial 2. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

et al., 2005). Normal levels of serum caeruloplasmin activity and erythrocyte glutathione peroxidase activity in cattle are respectively 120-200 mg/L and 60 U/g Hb (Radostits et al., 2005). However, clinical signs of copper deficiencies in these yaks were not found in the present study.

Yaks that received the glass bolus had significantly increased selenium (GSH-Px), copper (Cp) and cobalt (vitamin B<sub>12</sub>) status in trials 1 and 2. This was in agreement with the findings of Kendall et al. (2000, 2001) in sheep. The boluses negate the need to supply these trace elements in free-access minerals, which can have variable intakes from zero to excessive (McDowell, 1992), or in supplemental feed when only mineral supplementation is required. It is very difficult to give mineral supplementation for yaks on high mountain grassland areas, because of adverse natural environment and poor transport facilities.

Serum trace elements levels may have good diagnostic value. Normal levels of zinc, selenium and copper in serum are respectively 0.8-1.2 mg/L, 0.07-0.15 mg/L and 0.7-1.3 mg/L in sheep and cattle (Radostits et al., 2005). The concentrations of copper in serum were marginally lower in

**Table 1.** Concentrations of trace elements in serum in yaks in trial 1 (mean±SD) (µmol/L)

	Day 0		Day 30		Day 60		Day 90	
	Bolused (20)*	Control (20)	Bolused (20)	Control (20)	Bolused (20)	Control (20)	Bolused (20)	Control (20)
Selenium	0.10±0.01	0.13±0.02	0.22±0.02 <sup>a</sup>	0.11±0.01	0.28±0.05 <sup>a</sup>	0.11±0.09	0.48±0.16 <sup>b</sup>	0.15±0.05
Copper	0.68±0.16	0.74±0.15	0.92±0.20 <sup>a</sup>	0.75±0.17	1.10±0.20 <sup>b</sup>	0.81±0.21	1.24±0.20 <sup>b</sup>	0.80±0.19
Cobalt	0.0058±0.001	0.0065±0.002	0.0068±0.001	0.0066±0.002	0.0071±0.002	0.0070±0.001	0.0075±0.001	0.0066±0.002
Zinc	16.1±3.5	17.3±2.6	16.8±3.9	17.4±3.8	15.9±2.2	17.1±4.4	14.9±3.3	16.2±1.5

\* Number of yaks. <sup>a</sup> p<0.05. <sup>b</sup> p<0.01.

**Table 2.** Concentrations of trace elements in serum in yaks in trial 2 (mean±SD) (µmol/L)

	Day 0		Day 45		Day 75		Day 105	
	Bolused (30)*	Control (30)	Bolused (30)	Control (30)	Bolused (30)	Control (30)	Bolused (30)	Control (30)
Selenium	0.17±0.06	0.18±0.02	0.27±0.10 <sup>a</sup>	0.17±0.05	0.32±0.05 <sup>b</sup>	0.19±0.11	0.36±0.09 <sup>b</sup>	0.20±0.05
Copper	0.83±0.22	0.89±0.16	1.08±0.27 <sup>a</sup>	0.88±0.09	1.18±0.32 <sup>a</sup>	0.94±0.25	1.33±0.32 <sup>b</sup>	0.85±0.16
Cobalt	0.0092±0.002	0.0085±0.003	0.0099±0.002	0.0087±0.002	0.0102±0.003	0.0094±0.002	0.0109±0.003	0.0095±0.003
Zinc	17.4±1.8	19.1±3.8	19.6±2.5	17.9±3.3	18.9±1.6	20.5±1.9	17.8±1.5	18.9±2.3

\* Number of yaks. <sup>a</sup> p<0.05. <sup>b</sup> p<0.01.

control yaks than the normal for other ruminants (McDowell, 1992; Liu et al., 1995; Radostits et al., 2005). This agreed with the results of serum caeruloplasmin activities in control yaks. Copper deficiencies in yaks and other animal species have not been reported in these areas. The concentrations of molybdenum in herbage and soil samples in these areas were within the normal ranges (less than 3 mg/kg DM) (Zhang, 1983), although Shen et al. (2005) have reported molybdenum-induced copper deficiency in yaks in the North of the Qing Hai-Tibetan Plateau of China. The selenium and copper status indicators in bolused yaks indicated adequate selenium and copper concentrations. This agreed with the results of other investigators who have reported that copper concentrations in soil and forage were low in Shandan county (Zhang, 1983; Liu et al., 1995). There were no effects of the bolus on concentrations of cobalt in serum. This is because cobalt absorption and tissue retention are very low in ruminants (Kincald et al., 2003). Notwithstanding this, the analysis of cobalt by AAS is insensitive. The failure to find a significant effect of supplementation on serum cobalt to match the response in vitamin B<sub>12</sub> is due solely to the large error associated with the cobalt values. Thus, further research appears to be needed on the effects of cobalt supplementation on ruminal fermentation and the production of vitamin B<sub>12</sub> by rumen microbes.

Trace element deficiencies are common in many countries and affect animal health, productivity and welfare. Deficiency diseases may be manifested as a consequence of single or multiple element deficiency. Deficiency in any of the above trace elements can result in an increased disease susceptibility and a decreased immune function. The zinc, cobalt and selenium soluble glass bolus has previously been demonstrated to give increased humoral immune response in grazing lambs (Kendall et al., 1997). Yaks are grazed at high altitudes on steep slopes and the only effective way to prevent trace elements deficiencies is by providing an easy

supplementation. The bolus has demonstrated a capacity to raise low selenium and copper status of the yaks to higher reference ranges. Yaks with health problems likely to be caused or exacerbated by low selenium and copper should benefit from receiving this type of bolus.

The controlled release bolus route should provide each animal with a consistent dose in line with its requirements sustained over a long period of time, such that one treatment should ensure adequate trace element cover for a number of months. Previous work has shown that the monolithic and sintered bolus gave excellent results in field trails and has been previously shown to supply copper, cobalt and selenium in extensively grazed cattle and sheep for up to 345-days (Telfer, 1984; Kendall et al., 2001; Mackenzie and Telfer, 2001).

In conclusion, the copper, selenium and cobalt soluble glass bolus increased the status of all three trace elements consistently for a period of at least 90 days. The bolus proved to be an appropriate method of increasing the selenium, copper and cobalt status of extensively grazed yaks.

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