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# A preliminary study on anti-hypoxia activity of yak milk powder in vivo

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**Abstract** Yak milk is the main source of dietary protein, fat, and micronutrients for Tibetan residents, who live at a high altitude and oxygen-deficient environment. The anti-hypoxia activity of yak milk powder (YMP) was investigated in mouse hypoxia models, using the normobaric hypoxia test, sodium nitrite toxicosis test, and hematological parameters analyses. The results showed that YMP could prolong the survival time under both normobaric hypoxia and sodium nitrite toxicosis conditions by 15.7%–17.3% and 13.9%–15.1%, respectively, and increase the levels of red blood cells (RBC) and blood hemoglobin (Hb). Furthermore, YMP was better than cow milk powder (CMP) in anti-hypoxia activity. YMP could be explored as a novel anti-hypoxia functional food ingredient.

**Keywords** Yak milk powder · Anti-hypoxia activity · Mouse hypoxia models

## 1 Introduction

China has the largest population of yaks in the world, approximately 14 million, that accounts for 95% of the total world population of yak (He et al. 2011). Yak milk ranks in third place after cow and buffalo milks in economic importance in China (Zhong et al. 2006). The yak is an iconic symbol of Tibet, which can survive under harsh environment, such as high altitude (2,500 to 5,000 m), deficient oxygen, and low temperature (–40 °C). Yak milk is the main source of dietary protein, fat, and micronutrients for Tibetan residents who adapt well at a high altitude and low oxygen environment (Dong et al. 2007). Yak milk contains high contents of total solids

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(16.9%–17.9%), protein (4.9%–5.9%), and fat (5.5%–7.5%) (Li et al. 2009; Wu et al. 2009; He et al. 2011). It has been speculated that adaptation of the Tibetan to the environment is related to their diet rich in yak milk.

The high-altitude stress and hypoxia symptom are very common for visitors, but not for the residents. Hypoxia, which occurs during acute and chronic vascular disease, cancer, and stroke, is defined as a decrease in the normal level of tissue oxygen tension (Xie et al. 2010). Some chemical drugs, such as acetazolamide, dexamethasone, and nimodipine, had a significant anti-hypoxia activity (Lee et al. 2007). However, most of these drugs also bring some side effects. Therefore, many studies began to investigate the anti-hypoxia activity of natural materials in recent years. Some plant polysaccharide and terpenoid extracts have been reported to have anti-hypoxia activity (Chen et al. 2009; Zhang and Dai 2011; Jia 2011). Although milk is one of the major foods in the world, there are no investigations on anti-hypoxia activity of milk, especially yak milk, which contains many nutrients and bioactive compounds. Here, we made a preliminary study on anti-hypoxia activity of yak milk powder (YMP) compared to cow milk powder (CMP), using mouse hypoxia models.

## 2 Materials and methods

### 2.1 Samples

Yak milk samples were manually milked from 120 yaks in three Tibetan areas, including Hong Yuan, Lu Qu, and Lin Xia, China, respectively, in June. Cow milk sample, as a positive control, was obtained from Beijing in June. All raw milk samples were pasteurized at 62.5 °C for 30 min, and then lyophilized and stored at –80 °C until use. The protein and fat contents (% g/g) are 26.5 and 32.8, respectively, in yak milk powder (YMP), and 24.4 and 31.7, respectively, in cow milk powder (CMP).

### 2.2 Animals

One hundred male BALB/c mice (16–18 g, special pathogen-free (SPF) grade, approval no. SCXK-2007-004) were purchased from the Academy of Military Medical Sciences (AMMS), Beijing, China. The mice were housed at five per cage in a room maintained at 23±1 °C, 50%–60% relative humidity, and air circulation all the time, with a 12/12-h light–dark cycle, in Beijing Centers for Diseases Control and Prevention. They had free access to distilled water and balanced murine diet provided by AMMS, containing (% g/g) crude protein (18%), crude fat (4%), crude fiber (5.0%), Ca (1–1.8%), P (0.6%–1.2%), moisture (10%), and ash (8%). All mice received humane care in compliance with the guide of Beijing Centers for Diseases Control and Prevention and also in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH, 2002).

### 2.3 Experimental design

The mice were randomized into five groups with 20 mice in each group after 1-week adaptation: control group (control, distilled water), cow milk powder group (CMP, 5.2

mg.  $\text{g}^{-1}$  bw.day $^{-1}$ ,  $\text{g}^{-1}$  bw.day $^{-1}$  = per gram body weight per day), low-dose yak milk powder group (YMP-L, 2.6 mg.g $^{-1}$  bw.day $^{-1}$ ), middle-dose yak milk powder group (YMP-M, 5.2 mg.g $^{-1}$  bw.day $^{-1}$ ), high-dose yak milk powder group (YMP-H, 7.8 mg.g $^{-1}$  bw.day $^{-1}$ ). The YMP and CMP were dissolved in distilled water. All mice were administrated at 0.2 mL.10  $\text{g}^{-1}$  bw.day $^{-1}$  by gavage. Body weights were measured weekly. The 20 mice in each group were divided into two subgroups. One group ( $n=10$ ) was firstly used to determine hematological parameters at 28 days and then used for normobaric hypoxia test at 30 days; the other group ( $n=10$ ) was used for sodium nitrite toxicosis test at 30 days.

#### 2.4 Normobaric hypoxia test

The normobaric hypoxia test was carried out according to Zhang and Dai (2011). One hour after last treatment, each mouse was put into a 250-mL wide-necked bottle with 5 g medical soda lime inside, and then the bottle was sealed with vaseline. When the mouse could not breathe, the survival time of oxygen deprivation was immediately recorded.

#### 2.5 Sodium nitrite toxicosis test

The sodium nitrite toxicosis test was carried out according to Xie et al. (2010). One hour after last treatment, the weights of mice were measured. Sodium nitrite was dissolved in distilled water. Then, each mouse was subcutaneously injected with sodium nitrite (220 mg.kg $^{-1}$ bw). The survival time of sodium nitrite toxicosis was immediately recorded after cardiac arrest.

#### 2.6 Hematological parameters analysis

One hour after last treatment, a 20- $\mu\text{L}$  volume of blood sample was precisely collected with a capillary tube, using the inner canthus bleeding method, and then was put into heparinized tube. The hematological parameters, including red blood cells (RBC) and the blood hemoglobin (Hb), were immediately measured by an automatic hematological analyzer (Nihon Kohden, Japan).

#### 2.7 Statistical analyses

The experimental data were expressed as mean $\pm$ SD. The data were subjected to one-way analysis of variance and Duncan's multiple range test was performed to determine the significant difference among samples within the 95% confidence interval, while Dunnett's T3 test was conducted if data have statistic heteroschedasticity.

### 3 Results and discussion

In this study, there were no significant differences among the control, CMP, and YMP groups in body weight after 4-week feeding ( $P>0.05$ ; Table 1). YMP prolonged significantly the survival time of oxygen deprivation mice by 15.7%–17.3% in the

**Table 1** Effect of YMP on BALB/c mice body weight

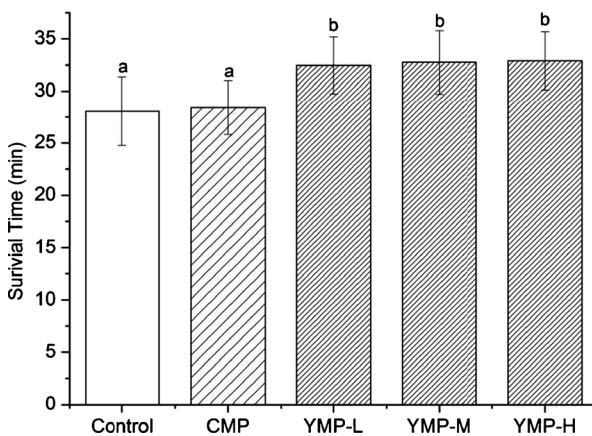
Group	Body weight (g)	
	Initial (week 0)	Final (week 4)
Control	19.53±2.85 <sup>a</sup>	27.24±2.02 <sup>b</sup>
CMP	19.61±2.44 <sup>a</sup>	27.18±2.37 <sup>b</sup>
YMP-L	19.88±2.09 <sup>a</sup>	27.15±1.38 <sup>b</sup>
YMP-M	19.71±2.16 <sup>a</sup>	27.03±2.03 <sup>b</sup>
YMP-H	19.98±2.07 <sup>a</sup>	26.97±1.16 <sup>b</sup>

The mice in YMP-L, YMP-M, and YMP-H groups were treated with the YMP (2.6, 5.2, 7.8 mg·g<sup>-1</sup> bw·day<sup>-1</sup>). The mice in the CMP group were treated with CMP (5.2 mg·g<sup>-1</sup> bw·day<sup>-1</sup>), the mice in the control group were treated with distilled water (0.2 mL·10 g<sup>-1</sup> bw·day<sup>-1</sup>), once a day for 28 days. Values are means±SD (*n*=20). Significant differences between the five groups are noted by different letters within a column (*P*<0.05)

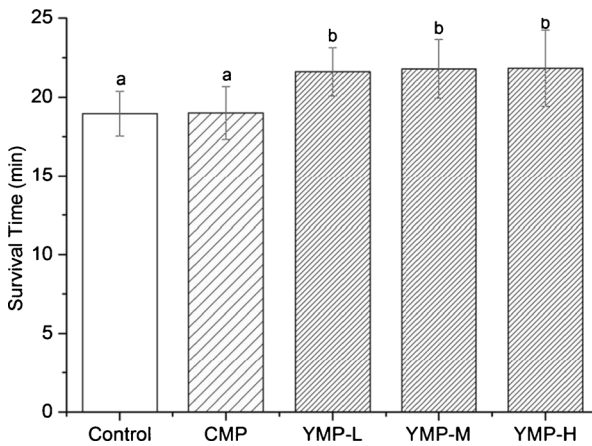
CMP cow milk powder, YMP yak milk powder

normobaric hypoxia test (Fig. 1). YMP also showed a significant effect on increasing the survival time of toxicosis mice in the sodium nitrite toxicosis test (Fig. 2). The survival time of YMP-M mice extended 15.3% and 14.7% compared to CMP mice in both normobaric hypoxia test and sodium nitrite toxicosis test. Both normobaric hypoxia test and sodium nitrite toxicosis test were widely used for the evaluation of anti-hypoxia activity (Chen et al. 2009; Zhang and Dai 2011). Sodium nitrite could translate bivalent iron into trivalent iron in hemoglobin, which induced that hemoglobin could not carry oxygen, and led to cell death (Gupta and Briyal 2004; Prabhakaran et al. 2004).

The levels of RBC and Hb in three YMP groups were higher than those in the control group (Table 2). In addition, the levels of RBC and Hb were 7.1% and 12.0% higher, respectively, in YMP-M group than in CMP group (Table 2). The levels of RBC and Hb are the major determinants of oxygen transport and utilization (Calbet et al.



**Fig. 1** Effect of YMP on survival time of BALB/c mice in normobaric hypoxia test. The mice in YMP-L, YMP-M, and YMP-H groups were treated with the YMP (2.6, 5.2, 7.8 mg·g<sup>-1</sup> bw·day<sup>-1</sup>). The mice in CMP group were treated with CMP (5.2 mg·g<sup>-1</sup> bw·day<sup>-1</sup>), the mice in the control group were treated with distilled water (0.2 mL·10 g<sup>-1</sup> bw·day<sup>-1</sup>), once a day for 30 days. CMP cow milk powder, YMP yak milk powder. Values are means±SD (*n*=10). Significant differences between the five groups are noted by *a* and *b* (*P*<0.05)



**Fig. 2** Effect of YMP on survival time of BALB/c mice in sodium nitrite toxicosis test. The mice in YMP-L, YMP-M, and YMP-H groups were treated with the YMP (2.6, 5.2, 7.8 mg. g<sup>-1</sup> bw. day<sup>-1</sup>). The mice in CMP group were treated with CMP (5.2 mg. g<sup>-1</sup> bw. day<sup>-1</sup>), the mice in the control group were treated with distilled water (0.2 mL. 10 g<sup>-1</sup> bw. day<sup>-1</sup>), once a day for 30 days. *CMP* cow milk powder, *YMP* yak milk powder. Values are means±SD (*n*=10). Significant differences between the five groups are noted by *a* and *b* (*P*<0.05)

2006). High levels of Hb and RBC could enhance the endurance of hypoxia (Xie et al. 2010), which could be the mechanism of anti-hypoxia activity of YMP.

The contents of fat and protein between YMP and CMP are at the same levels, which ensured mice in YMP-M and CMP groups received the same levels of fat and protein. The results of anti-hypoxia activity of YMP were better than CMP, which could be associated with higher proportions of branched chain amino acid, conjugated linoleic acid (CLA) and Fe in yak milk (Li et al. 2011; He et al. 2011; Liu et al. 2011). These nutrients could exert synergetic effects for the anti-hypoxia activity of YMP. It is well known that Fe could contribute to the synthesis of RBC, which may explain the high levels of RBC and Hb in YMP groups. CLA inhibited prolyl hydroxylase (PHD) protein expression and elevated the expression of hypoxia inducible factor (HIF) related transcriptional factors, which could improve hypoxia-dependent process (Zhang and Li 2012). The inhibition of PHD could increase the expression of HIF which could

**Table 2** Effect of YMP on RBC and HB levels in BALB/c mice

Group	RBC ( $1 \times 10^{12} \text{ L}^{-1}$ )	HB (g.L <sup>-1</sup> )
Control	9.70±0.37 <sup>a</sup>	137.8±8.8 <sup>a</sup>
CMP	9.72±0.50 <sup>a</sup>	140.4±10.6 <sup>ab</sup>
YMP-L	10.27±0.44 <sup>b</sup>	147.6±4.5 <sup>bc</sup>
YMP-M	10.28±0.61 <sup>b</sup>	149.2±10.0 <sup>c</sup>
YMP-H	10.39±0.62 <sup>b</sup>	154.4±7.0 <sup>c</sup>

The mice in YMP-L, YMP-M, and YMP-H groups were treated with the YMP (2.6, 5.2, 7.8 mg. g<sup>-1</sup> bw. day<sup>-1</sup>). The mice in the CMP group were treated with CMP (5.2 mg. g<sup>-1</sup> bw. day<sup>-1</sup>), the mice in the control group were treated with distilled water (0.2 mL. 10 g<sup>-1</sup> bw. day<sup>-1</sup>), once a day for 28 days. Values are means±SD (*n*=10). Significant differences between the five groups are noted by different letters within a column (*P*<0.05)

*CMP* cow milk powder, *YMP* yak milk powder, *RBC* red blood cells, *Hb* blood hemoglobin

enhance oxygen availability by promoting erythropoiesis and angiogenesis (Appelhoff et al. 2004; Lee et al. 2004). Thus, CLA may be one of the bioactive compounds for the anti-hypoxia activity of YMP. Therefore, YMP could be explored as a novel anti-hypoxia agent.

## 4 Conclusions

YMP improved the survival time of mice under hypoxia conditions by increasing the oxygen-delivery capacity. Furthermore, YMP was better than CMP in anti-hypoxia activity. This study could contribute to the application of yak milk powder as a functional ingredient for hypoxia related disease. However, further research needs to be carried out to evaluate the mechanism at cellular and molecular levels.

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**Conflict of interest** There is no conflict of interest concerning this manuscript.

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