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Changes of β-carotene and retinol levels and BCO1 gene and protein expressions in yak tissues at different nutritional seasons

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ABSTRACT. In order to explore relationship between the yellow fat and β-carotene metabolism in yaks, contents of β-carotene and retinol in tissues, serum and digestive tract contents were analysed at periods of rich and deficient nutrition. Expressions of β-carotene 15, 15'-monooxygenase 1 (BCO1) gene and protein were also studied using real-time PCR and western blot. It was shown that contents of β-carotene in serum, liver, pancreas, duodenum, jejunum and contents of rumen and ileum were higher in the period of rich nutrition than in the period of deficient nutrition ($P < 0.05$). Content of retinol in liver was much higher in the period of rich nutrition than in the period of deficient nutrition ($P < 0.05$). Expression levels of BCO1 mRNA in kidney, jejunum, ileum, duodenum, muscle and rumen in the period of rich nutrition were significantly lower than in the period of deficient nutrition ($P < 0.05$), besides pancreas. Expressions of BCO1 protein in duodenum, jejunum, kidney, muscle, and rumen were lower in the period of rich nutrition than in the period of deficient nutrition ($P < 0.05$).

Introduction

Yaks (*Bos grunniens*) are important domestic animals for people living in Qinghai-Tibet Plateau. More than 14 mln domestic yaks provide for them basic resources, such as meat, milk, etc. necessary in the extremely harsh high-altitude region (Wiener et al., 2003). These animals are anatomically and physiologically adapted to live in high altitude region (Dolt et al., 2007; Shao et al., 2010; Wang et al., 2011). For example, yak yellow fat resulting from accumulation of carotenoids from diet in adipose tissue is a very important feature needed to keep metabolic balance of vitamin A throughout the year (Gou et al., 2016). As other animals, yaks are unable to directly synthesize vitamin A *de novo,* and therefore by metabolizing β-carotene and other carotenoids compounds produce vitamin A (Dag and Inger, 2010). The yellow fat in yaks, however, is genetically different than yellow fat in other domestic animals (occurred sporadically). The carotenoid metabolism is closely related with two key enzymes – β-carotene 15, 15'-monooxygenase 1 (BCO1) and β-carotene oxygenase 2 (BCO2).

BCO1 is responsible for the symmetric cleavage of β-carotene into two molecules of retinal (Wyss et al., 2000; Redmond et al., 2001), whereas BCO2 is responsible for the asymmetric cleavage of β-carotene into β-apo-10'-carotenal (C27) and β-ionone (C13) (Kiefer et al., 2001; Dag and Inger, 2010; Amengual et al 2013). β-carotene is not affected by ruminal fermentation (Van Soest, 1994) and is transported to the small intestine where it is absorbed. In the enterocyte, the enzyme BCO1 cleaves the central linkage of β-carotene to produce retinal or vitamin A (Glover, 1960). In cattle, the β-carotene that is not cleaved into retinal is incorporated into the chylomicrons in small intestine or bounded to high density lipoproteins and cleared from the circulation by the liver, where it can be cleaved by BCO1 and stored with other lipid compounds. Also, can be eventually transported to different tissues including the adipose tissue (Yang et al., 1992; Schweigert, 1998).

The amount of carotenoids in yak tissues changes along with seasons. The same is with fat deposition (Gou et al., 2016). Large amount of carotenoids are deposited in adipose tissue during summer and autumn, when there are plenty of green grasses in alpine meadow. The deposited carotenoids and fat are consumed during winter and spring due to the lack of green grasses in this area. It is obvious that the seasonal metabolism of carotenoids and fat results from long term adaptation to seasonal change of nutritional sources.

It is a very interesting scientific question how the deposition of carotenoids in fat tissues vary during rich nutrition period. It is apparent that the deposition of carotenoids is not possible if the expression and activity of degrading enzymes such as BCO1 and BCO2 are high. Nearly all absorbed β-carotene is converted to retinol in the intestine (Wang, 1994). In contrast, humans convert only a portion of ingested β-carotene into vitamin A, so up to 15%–30% of absorbed β-carotene remains intact and is delivered to tissues (Lemke et al., 2003; Burri and Clifford, 2004). The evidence suggests that intestinal BCO1 activity is subjected to transcriptional regulation (Bachmann et al., 2002). However, the mechanism of governing species-specific differences in efficiency of dietary β-carotene to retinoid cleavage remains unclear.

It was assumed that expressions of BCO1 gene and protein in yaks change along with the vitamin A level and carotenoid degradation products in tissues. Thus the changes of β-carotene, retinol and relating enzyme activities in yak tissues during spring (deficient nutrition period) and summer (rich nutrition period) were investigated. The expressions of BCO1 gene and protein were also studied with real-time PCR and western blot, respectively, to potentially explain possible relationship between BCO1 activity and accumulation of β-carotene.

Material and methods

Sample collection

Throughout the year, all animals freely grazed in alpine meadow pastureland belonging to private farms with an average altitude of 3000–3450 m. The climate in this region is described as continental for this plateau, with an average temperature of 3 °C. The average temperatures of the long winter and short summer are −13 °C and 14.4 °C, respectively. According to the nutritional characteristics of forage on Qinghai-Tibet Plateau, samples were collected on July 20, 2013 (rich nutrition period) and March 16, 2014 (deficient nutrition period) in Tong Pu Township of Wulan County, Qinghai Province, China.

Five healthy, 3 years old, male yaks were slaughtered in each nutritional season in order to collect samples. The animals were sacrificed after 12 h of feed deprivation. The tissues of thigh muscle, abdominal fat, duodenum, jejunum, ileum, lung, pancreas, kidney, liver, rumen and contents of intestine and rumen were collected. Intestine (50 cm long) was cut down near the pylorus as duodenum samples, and same length of intestine was cut down between duodenum and ileum as samples of jejunum. The part of the small intestine having almost parallel spiral valves between jejunum and the caecum was the ileum, and its middle 50 cm-section was taken for analyses. Ileal digesta and ruminal content were also taken. Each sample (5 g) was collected in 10 copies, and loaded into frozen tube. Tissue samples from the intestine and rumen were cleaned with cold 0.9% physiological saline. All samples were kept in liquid nitrogen. Blood samples of 10 ml were taken from the jugular veins of yaks before slaughter and the serum was collected by centrifugation at 8000 *g* (4 °C, 10 min) after coagulation. All samples have been taken back to laboratory and stored under −80 °C.

Reagents

Column RNA Gel Extraction Kit (UN1Q-10) was purchased from Shanghai Sangon Biological Engineering & Technology Co. Ltd (Shanghai, China). TaKaRa SYBR Premix Ex Taq II and Prime

Script™ RT Reagent Kit (Perfect Real Time) were purchased from TaKaRa Biotechnology (Beijing) Co. Ltd. (Beijing, China).

The β-carotene standard (C184250; all-trans-β-carotene) was purchased from Accustandard (New Haven, CT, USA), and retinol (200-683-7) was purchased from Sigma Chemical Co. (St Louis, Mo, USA). PVDF films were purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China); BCA protein assay kit was purchased from Thermo Fisher Scientific (China) Co. Ltd. (Shanghai, China).

Determination of β-carotene and retinol concentration

Methods of tissue sample treatment were based on Yao et al. (2008). The tissues and digestive tract contents (5 g) were weighed and adequately grinded, then placed into 100 ml centrifuge tubes, into which 30 ml anhydrous ethanol, 10 ml ascorbic acid ethanol solution (10 g/l) and 10 ml NaOH solution (0.5 g/ml) were added. Sample homogenate was mixed with all solutions. It was incubated on thermostatic water bath of 43 °C and vigorously stirred for 30 min which was a process of saponification. After the completion of saponification, the 30 ml of petroleum ether was added into centrifuge tube, and tube was sealed. It was put on whirlpool oscillator and vibrated for 5 min. The supernatant was collected after sedimentation. The β-carotene and retinol were repeatedly extracted 3 times by petroleum ether as extraction agent. All extracts were merged, and then they were dehydrated with anhydrous sodium sulphate and transferred into other containers. The butylated hydroxytoluene (BHT) was added into the container to prevent the oxidation of β-carotene and retinol. The petroleum ether was evaporated by nitrogen drying apparatus, and then extracts containing β-carotene and retinol were dissolved and the solution was set to scale of 10 ml in brown volumetric flask by petroleum ether. It was filtrated through 0.45 μm filter membrane and collected for detection of β-carotene and retinol using HPLC.

The contents of β-carotene and retinol were measured using HPLC (Wang and Huang, 2002; Moren et al., 2002) with Luna C_{18} column (4.6 mm × 150 mm). Mobile phase was acetonitrile:dichloromethane:methanol in a ratio (v/v) 60:20:20. Column temperature was 25 °C. The flow rate was 1.0 ml/min, and sample size was 20 μl. The detection wavelength of β-carotene was 450 nm and 325 nm for retinol. A dual channel detection wavelength was used. The limit of detection for β-carotene was 0.005mg/l and its spiked recovery was between 97.41% and 98.60%.

BCO1 mRNA expression

The gene sequence of BCO1 mRNA of *Bos mutus* (XM_005907884) and β-actin mRNA (DQ838049.1) were taken from National Center for Biotechnology Information (NCBI). The primers were designed by Primer 5.0 and synthesized by Shanghai Bioengineering Co., Ltd. (Shanghai, China). The following oligonucleotide primers were used to for gene expression analysis: 5'-AGAATGCAGAAGTGGGCTCC-3'(sense) and 5'-AGCAGCAAAGACATAGCGGT-3'(antisense) for BCO1;, 5'-CTTCCAGCCTTCCTTCCTG-3'(sense) and 5'-ACCGTGTTGGCGTAGAGGT-3' (antisense) for β-actin.

Total RNA was extracted with TRIzol™ reagent (Thermo Fisher Scientific, (China) Co. Ltd., Shanghai, China) following the instructions of the manufacturer. The isolated RNA was treated with DNase I (Applied Biosystems, Foster City, CA, USA). The concentration and purity of RNA were measured using the Germany Eppendorf nucleic acid protein detector. If the value of A260/A280 ratios was above 2 and A260/A230 ratios above 1.9, the RNA purity was considered satisfactory, otherwise total RNA extraction was repeated.

Synthesis of cDNA was performed using Prime Script RT reagent kit (TaKaRa Biotechnology (Beijing) Co., Ltd., China.) in a total reaction volume of 10 μl. RNA (100 ng) was added to each reaction. The reaction volume for fluorescence quantitative PCR reaction system was 25 μl, including: SYBR® Premix Ex Taq™ II 12.5 μl, and 1 μl upstream and downstream primer with concentration of 10 μm, template 2 μl, double-distilled nuclease-free water 8.5 μl. The qPCR program consisted of: 1 cycle of initial denaturation at 95 °C for 30 s, 40 cycles of denaturation at 95 °C for 5 s, annealing at 57 °C for 30 s and elongation at 72 °C for 30 s. Fluorescence signal data were taken during periods of elongation and annealing in each cycle. The melting curve analysis was done immediately at end of amplification.

Five to eight serial dilutions of purified and quantified PCR products were used as standard templates. The standard curve was given by the reaction system according to the linear relationship between the logarithm of template concentration and CT values.

BCO1 protein quantification

The monoclonal antibody against BCO1 was prepared by Beijing Protein Innovotion Co., Ltd. (China). The titers of antibody were evaluated by the method of enzyme-linked immunosorbent assay (ELISA). After detecting, the antibody met the requirements.

The western blot method was used to detect yak BCO1 protein expression in each group at different nutrition period. The concentration of protein was determined by the bicinchoninic acid (BCA) protein assay, and 50 μg cell lysate was fractionated by SDS-PAGE. Proteins were transferred to Immobilon-P membranes by semi-dry blotting. The membrane was treated according to a standard Western Blotting protocol with chemiluminescence detection. All antibodies were diluted from 1:500 to 1:1000 with PBS. The monoclonal antibody against BCO1 was added to the hybridization bags with PVDF membrane after diluting and removal of all air bubbles before an overnight incubation at 4 °C. The bags were washed 5 times, each time for 5 min. The horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was added to the hybridization bags after diluting and incubated at room temperature for 2 h. Then the bags were washed 10 times, each time for 6 min. The internal reference, i.e. β-actin, antibody followed the same steps.

Protein-antibody interactions were detected by enhanced chemiluminescence. The gray scale values were quantified by using Image Quantifier 5.2 software, which represented the level of protein expression. A semi-quantitative analysis was carried out with β-actin as an internal reference, and the final relative expression of BCO1 protein was calculated.

Statistical analysis

The data are presented as means ± SD. Student's t test was used to analyse data between rich and deficient nutrition periods. SPSS software version 22.0 was used for statistical analysis. *LSD* was used for multiple comparisons to analyse data among the tissues and intestine contents after analysis of variance (ANOVA). The differences were considered statistically different at $P < 0.05$.

Results

Comparison of β-carotene and retinol content in yak tissues and ileal and rumen digesta in regard to nutrition periods

The contents of β-carotene and retinol in different samples at different periods of yak nutrition are shown in Table 1. In the period of rich nutrition, the content of β-carotene in serum was the highest, followed by the rumen. In the liver higher content of β-carotene than in fat, kidney, pancreas, duodenum, jejunum and ileum content was shown ($P < 0.05$), and there was no difference between fat, kidney, pancreas, duodenum and content of ileum ($P > 0.05$). Trace amounts of β-carotene were detected in muscle, lung, ileum and rumen. Retinol was detected in all samples. Interestingly, liver had the highest content of retinol, which was significantly higher than that in other tissues ($P < 0.05$). In the serum retinol content was higher than in internal organs, but far lower as compared with the liver ($P < 0.05$).

In the period of deficient nutrition only small amount of β-carotene was detected in liver, fat and kidney, while trace existed in all other samples. Trace amount of β-carotene was detected in the whole digestive tract. Liver was characterised by the highest content of retinol among all samples, much higher than that in others samples ($P < 0.05$). In the serum there was a small amount of retinol, but it was higher than in organs and ileal and rumen contents. There was no obvious difference between muscle and fat ($P > 0.05$).

Effect of nutrition period on β-carotene and retinol content in yak tissues and ileal and rumen digesta

As shown in Table 1, the content of β-carotene in most tissues of yaks was high, besides rumen, lung, ileum and muscle at period of rich nutrition, while no β-carotene was detected in most tissues of yak, besides liver, fat and kidney at period of deficient nutrition. Interestingly, obvious difference of β-carotene content was shown in liver ($P < 0.05$), and no significant difference was detected in fat and kidney ($P > 0.05$) between the two periods. There was an obvious difference of β-carotene content in serum between two periods, which indicated that the amount of β-carotene in serum had a close relationship with the intake of β-carotene from forage. Retinol was detected in each tissue at both periods. Significant difference in retinol content between two periods in liver, contents of ileum and rumen was also shown ($P < 0.05$), while no difference in serum, fat, muscle, rumen and duodenum between the two periods was found ($P > 0.05$), which indicated that retinol content was stable in serum, fat, muscle, duodenum and rumen during two periods.

Relative expression of BCO1 gene in different tissues of yaks at different nutrition periods

As shown in Table 2, the expression level of BCO1 mRNA in yak duodenum was the highest ($P < 0.05$) among all tissues at period of deficient nutrition, followed by kidney, muscle, jejunum and rumen. Low gene expression was in lung and liver ($P < 0.05$).

Table 1. Contents of β-carotene and retinol in different samples from yaks at different nutrition periods

Tissues	β-carotene (mg/100 g)		Retinol (mg/100 g)	
	rich periods	deficient periods	rich periods	deficient periods
Serum	7.000 ± 0.163^{a}	Trace	4.000 ± 0.408^{b}	3.750 ± 0.500^{b}
Liver	2.200 ± 0.231^{Ac}	1.650 ± 0.100^{Ba}	61.350 ± 17.845^{Aa}	30.300 ± 1.281^{Ba}
Fat	1.500 ± 0.115^{d}	1.350 ± 0.191^{b}	0.700 ± 0.115^{ef}	0.650 ± 0.100^{cd}
Muscle	Trace	Trace	0.800 ± 0.086^{de}	0.550 ± 0.191^{cd}
Lung	Trace	Trace	0.360 ± 0.046^{g}	0.405 ± 0.034^{d}
Kidney	1.410 ± 0.147^{d}	1.400 ± 0.082^{b}	1.000 ± 0.231^{cd}	1.100 ± 0.115^{c}
Pancreas	1.250 ± 0.100^{de}	Trace	0.450 ± 0.100^{fg}	0.405 ± 0.010^{d}
Duodenum	1.500 ± 0.115^{d}	Trace	0.450 ± 0.100^{fg}	0.400 ± 0.033^{d}
Jejunum	1.050 ± 0.100^{e}	Trace	0.200 ± 0.033^{g}	0.205 ± 0.010^{d}
Ileum	Trace	Trace	0.700 ± 0.115^{ef}	0.210 ± 0.020^{d}
Ileum content	1.400 ± 0.098^{d}	Trace	0.700 ± 0.115^{Aef}	0.405 ± 0.010^{Bd}
Rumen	Trace	Trace	0.205 ± 0.010^{g}	0.210 ± 0.060^{d}
Rumen content	3.000 ± 0.462^{b}	Trace	1.100 ± 0.115^{Ac}	0.350 ± 0.100^{Bd}

^{AB} – means within rows with different uppercase letters are significantly different at $P < 0.05$; $^{a–g}$ – means within columns with different lowercase letters are significantly different at $P < 0.05$

Table 2. Relative expression of β-carotene 15, 15'-monooxygenase 1 mRNA in yak tissues at different nutrition periods

Tissues	$2^{-\Delta\Delta C_T}$ relative value to lung	
	rich period	deficient period
Lung	0.96^{f}	0.88^{f}
Liver	1.25^{f}	2.86^{f}
Kidney	38.18^{Bc}	397.17^{Ab}
Jejunum	3.62^{Bf}	126.23^{Ac}
Ileum	2.22^{Bf}	26.99^{Ae}
Duodenum	107.16^{Ba}	2330.49^{Aa}
Muscle	27.10^{Bd}	81.65^{Acd}
Pancreas	79.12^{Ab}	53.95^{Bde}
Rumen	1.90^{Bf}	126.64^{Ac}

^{AB} – means within rows with different uppercase letters are significantly different at $P < 0.05$; $^{a–g}$ – means within columns with different lowercase letters are significantly different at $P < 0.05$

Table 3. Relative expression of β-carotene 15, 15'-monooxygenase 1 (BCO1) protein in yak tissues at different nutrition periods

Tissues	Rich period (BCO1/β-actin)	Deficient period (BCO1/β-actin)
Lung	1.702 ± 0.441^{c}	0.916 ± 0.040^{d}
Liver	2.430 ± 0.521^{a}	0.698 ± 0.043^{e}
Kidney	1.063 ± 0.170^{d}	1.128 ± 0.037^{c}
Jejunum	1.802 ± 0.345^{bc}	1.545 ± 0.006^{b}
Ileum	1.808 ± 0.377^{bc}	1.694 ± 0.023^{b}
Duodenum	2.095 ± 0.426^{b}	2.062 ± 0.011^{a}
Muscle	0.282 ± 0.097^{e}	0.945 ± 0.006^{d}
Rumen	0.218 ± 0.054^{e}	1.127 ± 0.012^{c}

^{AB} – means within rows with different uppercase letters are significantly different at $P < 0.05$; $^{a–g}$ – means within columns with different lowercase letters are significantly different at $P < 0.05$

At period of rich nutrition, the expression level of BCO1 mRNA in yak duodenum was highest ($P < 0.05$), followed by pancreas, kidney and muscle. The expression level of BCO1 mRNA was not significantly different among jejunum, ileum, liver, lung and rumen tissues ($P > 0.05$).

BCO1 mRNA was expressed in every tissue at different periods of nutrition. The expression levels in kidney, jejunum, duodenum, ileum, muscle, rumen were much lower at period of rich nutrition than at the period of deficient nutrition ($P < 0.05$). On contrary, the mRNA expression level in pancreas was higher at period of rich nutrition than at the period of deficient nutrition.

Expression of BCO1 protein in yak tissues at different nutrition period

The expression of BCO1 protein in yak tissues analysed using western blot is shown in Table 3. The expression of BCO1 protein in duodenum was the highest at period of deficient nutrition ($P < 0.05$). There was no significant difference in protein expression between jejunum and ileum tissue ($P > 0.05$), but it was higher than in kidney, rumen, lung, liver and muscle ($P < 0.05$). Liver showed the lowest expression among all tissues.

Western blot results showed that BCO1 protein expression in liver was significantly higher than in other tissues in rich nutrition period ($P < 0.05$). There was no difference about BCO1 protein expressions among duodenum, jejunum and ileum tissues ($P > 0.05$), but it was higher in duodenum than in lung ($P < 0.05$). The expression of BCO1 protein in lung was higher than that in kidney, muscle and rumen ($P < 0.05$), and no significant difference between muscle and rumen ($P > 0.05$) existed in rich nutrition period. However, BCO1 protein expression in duodenum was significantly higher than in other tissues

in deficient nutrition period ($P < 0.05$). There was no difference about BCO1 protein expressions between jejunum and ileum ($P > 0.05$), but it was higher in kidney and rumen than in lung and muscle ($P < 0.05$), and it was the lowest in liver in deficient nutrition period ($P < 0.05$).

Discussion

The special metabolic pattern of vitamin A is very important for yaks to keep balance of vitamin A. There are some evidences showing that intake of β-carotene cannot directly influence its content in tissues (Hickenbottom et al., 2002; Wang et al., 2004). In this study, it was demonstrated that the content of β-carotene in serum of yaks changed along with its intake, as the content of β-carotene in serum was far higher at period of rich nutrition than that at period of deficient nutrition. Summer is the time of forage abundance on plateau, and it is a good time for yaks to get all nutrients, thus it is easy to understand why high contents of β-carotene in serum and contents of rumen are high during rich nutrition period. But the content of retinol in serum has no relationship with the intake of β-carotene. In this study, there is no difference in the content of retinol in serum between the two periods. The β-carotene stored in fat during summer and autumn provides a sustained source of retinol in winter and spring, thus there have been no evidence of lack retinol in yaks at any time. Retinol is also largely stored in the liver during the summer-autumn period in order to maintain a steady-state retinol status at the level of the whole body, as shown in Table 1.

Although the fat content of yak is different, there is no difference in β-carotene content is shown in fat between the two periods ($P > 0.05$). The body weight of yak decreases 25%–30% during deficient nutrition period and the most of loss weight results from fat consumption (Xue et al., 2005). Through this way, adipose tissue of yaks is the greatest storage bank of nutrients, which is not available in the hay during nutrition deficiency period namely winter and spring. So β-carotene stored in fat may be degraded or transported to other tissues to meet the body's needs in response to nutrient deprivation, especially vitamin A. Fat tissue of yaks is the largest repository of β-carotene. Yaks have a sharp increase in weight of fat tissue during rich nutrition period and content of subcutaneous fat could achieve 10% of body weight during this period (Xue et al., 2005). At this time, large amounts of β-carotene are stored in adipose tissue. Yak fat is gradually consumed during deficient nutrition period, and is almost exhausted during the season of extremely deficient nutrition. Consumption of fat provides lots of energy and metabolites of nutrients including β-carotene. β-carotene is degraded into vitamin A, involved in the fat metabolism, and ensures that vitamin A is present in the period of nutrient deficiency. Results showed that there is no difference in the content of β-carotene in fat between different periods, indicating that β-carotene and fat are consumed almost at the same time during deficient nutrition period.

In previous studies, the expression and distribution of BCO1 mRNA gene in different species were studied with the methods of western blot hybridization, fluorescence quantitative *in situ* hybridization and immunohistochemistry. In the study by Wyss et al. (2001) it was shown that BCO1 mRNA expression level was the highest in duodenum of chicken, followed by liver, testis, lung and ileum. The expression levels of BCO1 mRNA in skin and intestine were relatively lower than that in mouse testis, liver and kidney (Wyss et al., 2001). Research by Lindqvist and Andersson (2002) showed that BCO1 mRNA in human digestive tract, from stomach to rectum, showed higher expression level in jejunum and ileum than that in kidney, liver, testis, ovary, prostate and skeletal muscle. Histochemical analysis of human tissues demonstrated that BCO1 gene was expressed in kidney, lung, skin, testis and retinal pigment epithelial cells, besides the small intestine and liver (Van Helden et al., 2010). Morales et al. (2007) used *in situ* hybridization and real-time quantitative methods to study the expression of BCO1 mRNA in liver and duodenum of yellow and white fat cattle. Results suggested that there was no difference of BCO1 mRNA expressions in duodenal epithelial cells and mucosal crypt between yellow fat and white fat cattle, but the expressions of BCO1 mRNA in liver were higher in yellow fat cattle than in white fat cattle. However it was not high enough to prevent the accumulation of β-carotene in yellow fat cattle (Morales, et al., 2007). We have shown that BCO1 mRNA was expressed in yak lung, liver, kidney, intestine, muscle, pancreas and rumen, and its expression was the highest in duodenum ($P < 0.05$), which is similar to the results of Wyss et al. (2001). The BCO1 mRNA expressions in duodenum, kidney, rumen and jejunum of yak were significantly lower at period of rich nutrition than at period of deficient nutrition. This may results from high level of retinoic acid and vitamin A in yak during rich nutrition period. Some researchers have provided retinoic acid as a regulatory factor that can inhibit

the expression of BCO1 mRNA (Kam et al., 2012). Results of inter-tissue difference of BCO1 mRNA showed that the BCO1 mRNA gene was intensively expressed in small intestine of yak, so the small intestine may be the main site of β-carotene changing into vitamin A. In BCO1 knockout mice, disruption of BCO1 leads to hypercarotenemia on a provitamin A rich and vitamin A deficient diet and to changes in lipid metabolism and susceptibility to diet-induced obesity (Hessel et al., 2007). Pathological effect of mutation in BCO1 has also been described in humans (Lindqvist et al., 2007). All results showed that BCO1 controlled the process of converting provitamin A carotenoids into vitamin A.

β-carotene can be converted into vitamin A, thus it is a source of vitamin A. In the previous study it was found that β-carotene is transformed into vitamin A mainly in mammalian small intestine, and transformation requires participation of a key enzyme (BCO1) (Dag and Inger, 2010). Expression of BCO1 in human tissues is proved by RNA blot and western blotting. Von Lintig and Wyss (2001) concluded that BCO1 is mainly expressed in mucosal cells of stomach, small intestine and colon and glandular epithelial cells, also in liver cells, pancreatic exocrine gland cells, prostate cells, tissue of uterus and breast, adrenal cells and skin keratinocytes. Unlike earlier studies, some experiments have shown that the cleavage of β-carotene is not restricted to the digestive tract. In this study, BCO1 protein expressions were studied by western blot in different tissues of yaks at different nutrition periods, and results showed that BCO1 protein was expressed in each tissue at different nutrition periods. The expression of BCO1 protein was the highest in duodenum of yak, while the lowest in the liver in deficient nutrition period. Expression of BCO1 protein in liver increased during the period of rich nutrition. We presumed that the increase of BCO1 expression in liver was caused by increase of intake of β-carotene, as it is known that liver is the main organ for metabolism of β-carotene. However, the expressions of BCO1 protein in other tissues decreased during the period of rich nutrition, which may result from abundance of vitamin A from degradation of β-carotene. The expression of BCO1 protein is inhibited in some tissues because of high level of vitamin A and provitamin A. It may be due to adequate supplement of vitamin A at period of rich nutrition, which causes the feedback regulation to inhibit the expression of BCO1 protein in these tissues.

Conclusions

β-carotene content in yak body is lower at period of deficient nutrition than at the period of rich nutrition, however the level of vitamin A is stable in most tissues except for liver. It means that β-carotene deposited in fat is degraded at period of deficient nutrition. Vitamin A in yak originates from degradation of β-carotene deposited in yak subcutaneous fat. This special metabolic mechanism prevents yaks from β-carotene deficiency at period of deficient nutrition.

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