Changes in Activities of Enzymes Related to Energy Metabolism in Peripheral Leukocytes of Fattening Steers

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ABSTRACT

Glucose, triglyceride, cholesterol and immunoreactive insulin (IRI) concentrations, some enzyme activities in plasma, and activities of enzymes related to energy metabolism in peripheral leukocytes were measured in fattening Japanese Black Wagyu × Holstein steers fed on different diets at 8, 12, 16, 20 and 24 months of age. The plasma IRI concentrations at 20 and 24 months of age were significantly higher than those at 8 months of age. Activities of hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), aspartate aminotransferase (AST), and malate dehydrogenase (MDH) in cytosolic fractions, and glutamate dehydrogenase (GLDH), MDH and AST in mitochondrial fractions in peripheral leukocytes of steers at 24 months of age were significantly higher than those at 8 months. Increasing plasma insulin concentration was considered to induce acceleration of glucose utilization in leukocytes of fattening steers. The cytosolic ratio of MDH/lactate dehydrogenase (LDH) activity in leukocytes increased significantly in the fattening process and was considered to be a useful indicator for evaluating changes in energy metabolism in steers.

Keywords: fattening steer, malate dehydrogenase, plasma insulin

Abbreviations: AST, aspartate aminotransferase; CP, crude protein; DMI, dry matter intake; GLDH, glutamate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; HK, hexokinase; IRI, immunoreactive insulin; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; TC, total cholesterol; TDN, total digestible nutrients; TG, triglyceride

INTRODUCTION

As the Japanese Black Wagyu, which is very popular as fattening beef cattle in Japan, is fed on different diets in the process of fattening, various changes in energy metabolism may be induced in the animals’ tissues. On the other hand, the activities of certain enzymes related to energy metabolism in the peripheral leukocytes are considered to reflect the metabolic states in animal tissues (Washizu et al., 1998; Arai et al., 2001). In particular, the activity of malate dehydrogenase (MDH) in the malate–aspartate shuttle, which has a very important role in the transfer from cytosolic NADH
to mitochondrial NADH, with no loss of potential for producing ATP except that resulting from a possible proton exchange (MacDonald, 1982; Eto et al., 1999), is specific to each animal species (Washizu et al., 2002) and varies significantly according to changes in metabolic conditions in diabetic dogs (Arai et al., 2002) and in race horses undergoing continuous training (Arai et al., 2001). The activity of lactate dehydrogenase (LDH) as the cytosolic marker enzyme is relatively stable in various metabolic conditions. Therefore, the cytosolic ratio of MDH/LDH activities (ML ratio) is considered to be a useful indicator for evaluating metabolic states in animal tissues (Washizu et al., 2002).

In the present study, glucose, triglyceride, total cholesterol and immunoreactive insulin concentrations and some enzyme activities in plasma and activities of enzymes related to energy metabolism in leukocytes of Japanese beef cattle (fattening steers) at various ages were measured to investigate the usefulness of the ML ratio as indicator for evaluating the changes in energy metabolism in the process of fattening with different diets.

MATERIALS AND METHODS

Animals

Twenty-five fattening Japanese Black Wagyu x Holstein steers of various ages (five steers for each age of 8, 12, 16, 20 and 24 months) at a farm in Shimane Prefecture, Japan, were used. The steers were fed ad libitum on three kinds of diets according to their month of age: stage I (6–12 months), stage II (13–20 months) and stage III (21–24 months). Ingredients and chemical composition of supplemented diets are shown in Table I.

Collection and preparation of blood samples

Blood samples (5 ml each) were withdrawn from the jugular vein into heparinized tubes, with the animals at rest and unfasted, between 12:00 and 14:00. Generally, the steers showed no excitement or fear and they were not forcibly restrained. The plasma (0.5–1 ml) was recovered from 2 ml of blood by centrifugation at 4°C. Leukocytes were isolated from 3 ml of blood by gradient centrifugation with a lymphocyte-isolating solution (ICN Biochemical Inc., Aurora, OH, USA). Cytosolic and mitochondrial fractions in leukocytes were prepared by the method described previously (Washizu et al., 1998).

Assay of enzyme activities

The activities of lactate dehydrogenase (LDH) as a cytosolic marker enzyme, hexokinase (HK) as a rate-limiting enzyme of glycolysis, glucose-6-phosphate dehydrogenase (G6PD) as a rate-limiting enzyme of the pentose phosphate pathway, malate dehydro-