Measurement of milk D-3-hydroxybutyrate with a simple UV spectrophotometer method: an alternative assay method

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Abstract

The measurement of D-3-hydroxybutyrate (D-BHBA) in milk samples is an important tool for diagnosis of subclinical/clinical ketosis in dairy cows. We describe a simple UV spectrophotometric method for measuring the concentration of D-BHBA in milk of dairy cows. From two herds, 119 milk samples were taken from dairy cows. The standard-curve equation was $y = 0.2582x + 0.0269$ ($R^2 = 0.9967$). The assay was highly specific with a minimum detection limit of 0.01 mmol/L and measuring range of up to 5 mmol/L. The recovery was between 99.35% and 100.22% and repeatability was 99.8%. The comparison between the spectrophotometric method and the fluorometric method revealed a close correlation ($r = 0.9939$). These results show that the spectrophotometric method can be successfully used as an alternative method to measure D-BHBA content in milk.

Key words: dairy cows, D-3-hydroxybutyrate assay, milk

Introduction

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period. It is a metabolic condition characterized by increased levels of ketone bodies in blood, urine and milk. The major ketone body is D-3-hydroxybutyrate (D-BHBA). Determination of D-BHBA in milk samples is an important tool in the diagnosis of subclinical/clinical ketosis in dairy cows. In this report, we describe a novel method based on the traditional spectrophotometric approach for measuring D-BHBA levels in milk of dairy cows (Williamson and Mellanby 1974).

Materials and Methods

From two commercial dairy farms located in Changchun City, Jilin Province, China, 119 milk samples were taken from dairy cows within 2 months after lactation.

The spectrophotometric approach involved two reactions. D-BHBA, in the presence of NAD, was oxidized by D-BHBA dehydrogenase to AcAc and NADH. Then NADH and nitroblue tetrazolium (NBT) were reduced by diaphorase to formazan and NAD, respectively. The amount of formazan was measured photometrically at 563 nm. All chemicals