J. Dairy Sci. 98:1–11
http://dx.doi.org/10.3168/jds.2015-9791

Identifying alterations in metabolic profiles of dairy cows over the past two decades in Japan using principal component analysis

M. Kayano*† and K. Kida†[1]
*Global Agro-Medicine Research Center, and
†Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Inada-Cho, Obihiro, Hokkaido 080-8555, Japan

ABSTRACT

The aim of this study was to identify the metabolic changes that have occurred in Holstein-Friesian cows in Japan over the past 2 decades, based on long-term metabolic profiles. From 1987 to 2004, metabolic profile tests were conducted in 1,700 dairy herds containing ~50,000 cows. The cows were divided into 5 lactation stage groups: early (calving to 49 d in milk, DIM), peak (50–109 DIM), mid (110–209 DIM), and late (210 to dry-off) lactation and the dry period. Principal component analysis was applied to the metabolic profiles at each lactation stage separately to investigate changes in metabolic profiles across the multiyear periods 1987–1992, 1993–1998, and 1999–2004. We determined that cows have probably experienced increasing negative energy balance, energy deficiency, and liver dysfunction during the past 2 decades in Japan.
Key words: blood enzyme, blood metabolite, energy balance, liver dysfunction

INTRODUCTION

Because of the genetic improvement of dairy cows, annual milk yield has increased by more than 2,000 kg/cow over the past 2 decades in Japan (Statistics information, Ministry of Agriculture, Forestry and Fisheries of Japan; MAFF). This implies an increase in daily milk production of more than 10 kg/cow and DMI of more than 4 kg/d (NRC, 2001). However, the calving interval of dairy cows increased from 402 to 431 d in Japan from 1987 to 2006 (Livestock Improvement Association of Japan, LIAJ, Tokyo, Japan), and the conception rate at the first AI decreased from 59.1 to 49.7%, in Hokkaido, Japan , from 1987 to 2004 (Hokkaido Artificial Insemination Technician Association Inc., Hokkaido, Japan). The physiological status of dairy cows has also changed during the past 2 decades: for example, it is well known that the concentration of total serum cholesterol usually changes according to milk yield (Kaneko, 1989; Kida, 2003), and elevated free fatty acid content indicates a negative energy balance, which can be a cause of low fertility (Butler and Smith, 1989; Butler, 2005) in high-producing dairy cows.

The metabolic profile test (**MPT**) is a well-known method for evaluating the nutritional status of dairy cows (Payne et al., 1970; Payne, 1972; Lee et al., 1978; Rowlands, 1984; Eicher, 2003). Many applications exist for using MPT as a herd-screening tool (Adams et al., 1978; Kida, 2002b, 2003; Macrae et al., 2006) and for assessing disease conditions (Kudlác et al., 1995; Kida, 2002a; Holtenius et al., 2004; Stengärde et al., 2008). In particular, Kida (2002a,b, 2003) demonstrated the application of long-term MPT in Japan. In the current study, we applied principal component analysis (**PCA**) to a long-term MPT database that we compiled in Japan over the past 2 decades. The aim of applying PCA was to clarify the metabolic changes in Holstein-Friesian cows both statistically and visually.

MATERIALS AND METHODS

Study Population and Data Collection

From 1987 to 2004, MPT were conducted on 49,569 cows (average parity: 3.0 ± 1.5, range of 1 to 10) in 1,700 dairy herds in Hokkaido, Japan. The MPT was conducted as follows. Five to 6 apparently healthy cows were selected from each of 4 lactation stages and the dry period on each farm. The lactation stages were defined as early (calving to 49 DIM), peak (50–109 DIM), mid (110–209 DIM), and late (210 DIM to dry-off) lactation. Blood samples were collected from the jugular vein between 0900 and 1100 h, and 14 blood components (Table 1) were measured by automatic analyzers [VP-Super(R), Abbott Co. Ltd. (Chicago, IL) and TBA 70-R(R), Toshiba (Tokyo, Japan)]. This study focused on 12 components: hematocrit (**HT**, %), γ-glutamyl transferase (**GGT**, IU), aspartate amino transferase (**AST**, IU), BUN (mg/dL), blood glucose

Received May 7, 2015.
Accepted August 26, 2015.
[1]Corresponding author: kidak@obihiro.ac.jp

Table 1. The 14 blood components analyzed in the metabolic profile test

Abbreviation	Description
HT	Hematocrit
GGT	Gamma-glutamyl transferase
AST	Aspartate amino transferase
BUN	Blood urea nitrogen
GLU	Blood glucose
IP	Inorganic phosphate
T-CHOL	Total cholesterol
F-CHOL[1]	Free cholesterol
Mg	Magnesium
NEFA	Nonesterified fatty acid
Ca	Calcium
TP[1,2]	Total protein
ALB	Albumin
GLOB	Globulin (TP – ALB)

[1]Not studied in our analysis.

[2]Total protein was used only for showing yearly change of protein in Figures A1, A2 and A3.

level (**GLU**, mg/dL), inorganic phosphate (**IP**, mg/dL), total cholesterol (**T-CHOL**, mg/dL), Mg (mg/dL), NEFA (μEq/L), Ca (mg/dL), albumin (**ALB**, g/dL), and globulin (**GLOB**, g/dL). Indicators used to evaluate (1) protein, (2) energy metabolism, (3) liver function, and (4) mineral metabolism were (1) HT, BUN, ALB, and GLOB; (2) GLU, T-CHOL, and NEFA; (3) AST and GGT, and (4) Ca, IP, and Mg, respectively (e.g., Payne and Payne, 1987; Kida, 2002a).

In this study, we investigated changes in the metabolic profile at each lactation stage across 3 multiyear periods: 1987–1992, 1993–1998, and 1999–2004. The cows were allotted into 3 and 5 groups based on period and lactation stage, respectively.

Statistical Analysis 1: Summary and Descriptive Statistics

We first removed data for individual cows that included outliers or missing values, according to the procedure below: (1) data for cows that had missing values for at least one component were removed ; (2) for each component, the following procedure was repeated up to 3 times: cows outside the range of mean ± 3 SD for that component were excluded. Procedures (1) and (2) removed 2,440 and 4,923 cows from the original data set, respectively. The remaining data set included 42,206 cows, which were utilized in the analyses in this study. Tables 2 and 3 summarize the number of cows, milk yield (kg/d), and average age (mo) of the cows in each period and lactation stage group. The mean value of the 12 components in each year and lactation stage was summarized in a table. The early and peak lactation stages and dry period were the main focus in this study, because these 3 stages are important in dairy cattle performance; in addition, change in milk yield in mid and late lactation were related to that at early and peak lactation stages among 3 multiyear periods. Yearly boxplots of milk yield, AST, NEFA, and TP at peak lactation stage are shown in the Appendix.

Statistical Analysis 2: PCA

Principal component analysis is a dimension-reduction technique for multivariate data. For metabolic profile data in this study, PCA summarized the 12 components into a few scores (i.e., principal components, **PC**) by summation of the components: for example, the first score (PC1) may be given by 0.5 HT + 0.5 GGT − 0.5 AST + 0.5 BUN, and the second score (PC2) may be obtained by −0.5 GLU − 0.5 IP + 0.5 T-CHOL + 0.5 Mg, where the coefficients such as 0.5 and −0.5 (loadings) are important to interpret the meaning of each PC. Each PC may show a specific feature of cows such as negative energy balance or liver dysfunction according to how the PCA adds the components in each PC. A contribution rate of each PC in all 12 components can be evaluated in terms of variance; for example, when PC1 has variance of 30 out of 100 for all components, the contribution of PC1 in the 12 components is 30%.

A PCA based on a correlation matrix was conducted on the metabolic profile data from each lactation stage separately. The use of a correlation matrix of the components relaxes the scale difference among the components to the result of PCA. The purpose of applying PCA is to investigate changes in metabolic profiles over time using summarized components; that is, PC. In examining the resulting PCA plots, we investigated the

Table 2. The number of cows in each period and lactation stage group

	Lactation stage					
Period	Early	Peak	Mid	Late	Dry period	Total
1987–1992	1,452	2,387	3,748	3,307	1,257	12,151
1993–1998	2,256	3,423	4,926	4,488	3,208	18,301
1999–2004	1,436	2,217	3,091	2,983	2,027	11,754
Total	5,144	8,027	11,765	10,778	6,492	42,206