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Short communication

Effect of lactation stage, season and parity on milk cortisol concentration in Holstein cows

Michiru Fukasawa *, Hideharu Tsukada, Takami Kosako, Akihisa Yamada

National Institute of Livestock and Grassland Science, 768 Senbonmatsu, Nasu-shiobara, Tochigi, 329-2793, Japan

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Abstract

This experiment was designed (1) to study the effects of lactation stage, season, and parity on milk cortisol concentrations in Holstein cows, and (2) to elucidate the relationships between milk yield, quality, and milk cortisol concentration. Subjects of this study were 24 dairy cows kept in a free stall barn. Milk samples were taken on test day in May, August, November, and February. Data of milk yield, quality, and cortisol concentration were collected. Random effects of animals and fixed effects of lactation stage, test day, and parity on milk yield, quality, and cortisol concentration were analyzed using mixed models. We estimated the best linear unbiased prediction of each trait (BLUP) which was an animal-specific value. Correlation among milk yield, quality, and cortisol concentration was calculated using raw data and BLUP. The effect of the lactation stage on milk cortisol concentration was significant: the value observed in early lactation was higher than in other stages. However, the correlation between milk yield and cortisol concentration was low. The beginning of lactation might be a strong stressor for every cow. Calculated with raw data, milk cortisol concentration had respectively significant negative correlation with milk protein contents and solid not-fat contents. Calculated with BLUP, milk cortisol concentration showed a significant and negative correlation with milk protein contents. The lactation stage and milk protein contents should be considered for measurement of milk cortisol concentrations.

Keywords: Dairy cow; Lactation stage; Milk cortisol concentration; Parity; Test day

1. Introduction

Recently, public interest in animal welfare is increasing. Reducing unnecessary or excessive stress is an important area for animal welfare improvement. Two strategies exist for reducing farm animals' stress: (1) Introduction of appropriate management and handling methods (Grandin, 1997). Stress under certain condition is assessed using behaviour and physiological responses. The level of plasma cortisol is typically used as a physiological indicator for comparing several new management systems (Ndibualonji et al., 1995; Abeni et al., 2005). (2) Genetic improvement for adaptability to the environment (Burrow, 1997). Certain behavioural and physiological responses to acute environmental challenges are regarded as potential selection criteria of genetic improvement for adaptability (Feaure and Mills, 1998). In addition, plasma cortisol levels are interrelated with individual differences in behavioural responses to acute stress, they might be an useful indicator for genetic improvement for adaptability (Van Reenen et al., 2005).

* Corresponding author. Tel./fax: +81 287 37 7245.
E-mail address: shakecat@affrc.go.jp (M. Fukasawa).

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However, plasma cortisol levels are easily affected by capture and blood collection (Hopster et al., 2005). For that reason, some researchers have investigated non-invasive sampling procedures such as the determination of cortisol in urine (Higashiyama et al., 2007), saliva (Negrao et al., 2004), and milk (Verkerk et al., 1998; Wenzel et al., 2003). For dairy cows, milk cortisol concentration is the most easily accessible and useful indicator for assessment of management systems and individual adaptability because milking is a daily procedure. In addition, it might reveal a genetic and phenotypic relationship between performance and adaptability, because evaluation systems for dairy cows are well established in many countries.

Verkerk et al. (1998) demonstrated ACTH challenge tests and showed that milk cortisol concentration was useful as an indicator of responses to acute stressors. However, little information exists about how the lactation stage, season and parity affect milk cortisol concentrations. Furthermore, the relationship between milk yield, quality, and milk cortisol concentration is unknown. This information will increase the reliability of assessing new management systems and individual adaptability using milk cortisol concentration. This experiment was designed (1) to study the effects of lactation stage, season, and parity on milk cortisol concentrations in Holstein cows, and (2) to elucidate the relationships between milk yield, quality, and milk cortisol concentration.

2. Animals, materials and methods

2.1. Animals and milk sampling

In all, 44 dairy cows were kept in the National Institution of Livestock and Grassland Science at Nasu, Tochigi, Japan. All were kept in a free stall barn in an open building equipped with large ventilation extractors on the ceiling. Cows in lactation (about 30 head) were separated into three sub-herds according to milk yield, each sub-herd (8–10 lactating cows) was kept in a separate compartment, which was about 200 m^2 concrete floor with 16 sand-bedded stalls. Every cow was milked twice a day (0830 h and 1730 h) in a tandem parlour. Each cow received a mixed ration (corn silage, grass silage, and commercial concentrate) six times per day (06:00, 08:50, 12:00, 17:30, 20:00, 22:00) and had free access to water and a mixture of minerals and vitamins.

2.2. Data collection and cortisol measurement

Milk samples from individual cows were taken on test days in May (spring), August (summer), November (autumn), and February (winter). On each test day, no special management or treatment existed for cows except milk collection. Climatic data are shown in Table 1.

After collection, milk samples were kept cool (4 °C) and worked up within 24 h after collection. Daily milk sample was prepared by mixing morning and evening milk according to each yield. Samples were centrifuged (20 min, 4 °C, 2000×*g*) and skim milk was stored at −35 °C until assay. Daily milk cortisol concentrations were measured using a commercial EIA kit for cortisol (Oxford Biomedical Research, Inc., Oxford, MI, USA). Details of this kit are available at http://www.oxford-biomed.com/ea65cortisol.html (last accessed on 10 May, 2007). Samples were prepared as follows: 100 µL of skim milk was mixed with 900 µL ethyl ether and shaken. The supernatant was moved into a tube and dried using a centrifugal evaporator. The residue was dissolved in 200 µL of extraction buffer supplied with the kit, and assayed 50 µL in duplicate. The inter- and intra-assay coefficients of variation were 7.8% and 15.2%, respectively.

Data of daily milk yield and quality, which were the milk fat contents (F%), milk protein contents (P%), milk solid-not-fat contents (SNF%), and somatic cell count (SCC) were collected from the monthly report of the dairy herd performance test.

2.3. Statistical analysis

For appropriate analyses, unsuitable-data were excluded from analyses according to the following three steps. First, cows with missing values of milk cortisol concentration or milk yield or quality were excluded. Secondly, cows treated for mastitis or in heat on a test day were excluded. Thirdly, cows from which samples had been collected only once during experiment were excluded from the analyses. As a result, 68 data of 24 cows were extracted from among 93 data of 44 cows for analysis.

Table 1
Monthly average of daily mean, maximum and minimum air temperature and humidity

Test day	Temperature (°C)			Humidity (%)
	Mean	Maximum	Minimum	
Feb	0.8	5.3	−3.7	65.2
May	13.8	19.4	8.1	67.7
Aug	24.4	29.0	21.0	84.8
Nov	10.6	15.2	5.7	72.0