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Study of stress in dairy cattle during student practical training on a farm

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Abstract

We investigated changes in cortisol (COR) concentration, which is well known as an index of stress in the serum of dairy cattle. The COR concentrations in serum obtained from dairy cattle were collected during practical training of first-year students on a farm attached to the Nippon Veterinary and Life Science University. Mean COR concentration in serum determined after practical training was significantly higher ($P < 0.001$) than that in serum collected before training. Discriminant analysis was used to classify the relation between COR concentration of serum collected before and after practical training. In conclusion, the data was bipartite according to the percentage of rise (rise rate) of COR concentration. Although the percentage of the rise was more than 300% in the high-rise-rate group, there was a significant negative correlation ($P < 0.05$) between age and COR concertation. It was thought that the high-rise-rate group has a chance to decrease stress after more experience. In contrast, the low-rise-rate group included 3 cattle indicating high COR concentration before and after practical training. Those 3 cattle were thought to be stressed easily. It is suggested that there was individual difference to stress.

Key Words: Animal welfare; Dairy cattle; Cortisol; Farm-based Education; Stress

Introduction

In farm-based education, participants over a wide age range are able to have an opportunity to have close contact with nature and agriculture. This type of activity has been focused on since the 1970s in Europe and the United States[18)]. In Japan, after the establishment of the Basic Act on Food Education in 2005, farm-based education has received increasing attention. This act is aimed at promoting activities related to food education through various experiences and activities related to food[15)]. An aim of the activities at stock farms is also to provide an understanding of animal husbandry due to the rising consumption of stock farm products. In

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Japan, the number of dairy farmers is on a declining trend due to reasons such as low consumer spending on drinking milk and rising costs of feed[9]. Since the self-sufficiency rate for food in Japan is low, the improvement of self-sufficiency for food products is an important issue. Reflecting this situation, the understanding of animal husbandry by consumers is thought to have an important role. Thus, Japan Dairy Council (JDC) and Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) are actively advancing education programs at stock farms where participants can experience dairy farming.

Other than understanding animal husbandry, the activities at stock farms are thought to offer a variety of benefits to participants. For example, the participants are reminded in the activity of the importance of the life of animals on farms where domestic animals are kept. Further, the psychological and/or physical beneficial changes due to contact with animals, including farm animals, has been reported[5]. Thus, the stock farms are also used as places for various activities, such as animal-assisted therapy[1,21] and education[16]. However, those activities on stock farms are carried out using food-producing animals. Thus, the quality of animal products has the potential to be influenced due to the stress to animals from human interactions. Grandin[7] suggested that novelty is a very strong stressor for animals. The intervention of many, unspecified persons on farms is thought to cause stress to food-producing animals. From a viewpoint of animal welfare, activities on farms that are less onerous for animals are thought to be an important subject for study. However, we could not find enough reports about the stress to farm animals during activities on a farm. The cortisol (COR) concentration in serum[3], urine[4] and hair[20] is used as an index of the stress in domestic animals. Thus, we investigated changes in COR concentration, which is well known as an index of stress in the serum of dairy cattle, during practical training of students. Further, since several biochemical markers, such as glucose (GLU), are also suggested as markers of stress[14], we also investigated the changes of several biochemical markers.

Materials and methods

Animals: A total of 29 dairy cattle (*Bos taurus*) including Holstein–Friesian (n = 26, average age, 52.72 ± 3.20 months), Brown Swiss (n = 1, 62.2 months), and Jersey (n = 2, average age, 59.97 months) was used in the present study. The animals were bred in a stall barn.

In May 2016, the farm training program for first-year students at our school was conducted on the farm attached to our university located in Yamanashi Prefecture. A total of 51 students stayed at the farm from May 9 to May 11. The students were divided into 4 groups (a group of 12 students and 3 groups 13 students) and they joined several practical trainings on the farm. Before the beginning of the practical training on May 9, we collected approximately 20 ml of blood samples from the caudal vein of animals. Although the training program covered a wide range of topics, 2 programs were related to dairy cattle. In one program, the students had the experience of milking in the morning (05 : 00–07 : 00) or evening (15 : 45–17 : 30). In another program, the students had experiences such as handling, sampling, and analysis of rumen fluid. After finishing all practical trainings on the farm, blood samples were collected in the same way as the first day. The collection of samples started at 10 : 30 on the first and final day. The blood samples were transferred into vacuum blood collection tubes (Venoject II, Terumo Corporation, Tokyo, Japan). All samples were transported to the laboratory on the farm and then the samples were centrifuged at 3,000 rpm for 15 min. The serum samples in Eppendorf tubes were stored at −30°C in a deep freezer (Panasonic Healthcare Co. Ltd. Tokyo, Japan) until analysis.

This study was conducted following the ethical policies of the Nippon Veterinary and

Life Science University, Japan, for experimental animals (28K-20) and life ethics (S28K-20). The sampling was conducted by skilled veterinarians having a routine schedule to care for the dairy cattle on this farm.

Inspection item: The COR level in serum was determined using an enzyme immunoassay, according to the method by Nohara *et al*[19]. The concentrations of sodium, potassium, chlorine, total protein, albumin, total bilirubin, γ-glutamyltransferase, amylase, blood urea nitrogen (BUN), creatinine, total cholesterol, triglyceride, calcium, inorganic phosphorus, GLU, and magnesium and the activities of aspartate aminotransferase, alanine transaminase, and alkaline phosphatase were analyzed using a multi-purpose automatic dry-chemistry analyzer (Fuji Firm Corporation, Tokyo, Japan). The level of diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) were analyzed using a free radical analyzer (Free Carpe Diem, Diacron International srl., Grosseto, Italy).

Statistical analysis: Data were analyzed using the Japanese-language version of Excel 2016 software (Microsoft Japan, Tokyo) and SPSS Statistics 19 (IBM Japan, Tokyo, Japan). The results of all data of inspected items and year of animals were represented as the mean ± standard error of the mean (S.E.M.). The significant difference was tested by Student's T test. The significance of correlation was tested by Pearson's product-moment correlation. The data of COR concentration before and after praxis was divided using discriminant analysis.

Results

The mean COR concentration in serum before and after the practical training is shown in Table 1. As shown in the table, there was a significant difference ($P < 0.001$) in the comparison of COR concentration before and after the practical training. The relation between COR concentration of serum collected before and that of after training of students is shown in Fig. 1. After discriminant analysis, the data were grouped into 2 classes according to the percentage of rise [(100 × (COR concentration in serum after training/COR concentration in before training]. The identification rate of the discriminant analysis was 100%. The rise rate of COR concentration was 324.24% ± 24.72% in the high-rise-rate group (n = 11), although the COR concentration of the low-rise-rate group (n = 18) was 107.07% ± 6.72%. Thus, mean COR concentration in serum of after training was significantly higher ($P < 0.001$) than that before training in the high-rise-rate group. In contrast, there was no significant difference ($P = 0.276$) between COR concentration in serum before training and that after training in the low-rise-rate group. As shown in Fig. 2, a significant negative correlation between age and COR concentration in serum was obtained from only the high-rise-rate group. In the high-rise-rate group, the age-related significant correlation was observed in serum collected before ($r = -0.633$, $P < 0.05$) and after ($r = -0.712$, $P < 0.05$) practical training. A similar correlation was not obtained from cattle in the low-rise-rate group (data not shown).

As shown in the Table 1, there was a significant difference ($P < 0.01$) in the comparison of BUN concentration before and after the practical training. In the low-rise-rate group, the BUN levels before and after the practical training were 6.87 and 8.67 mg/dl, respectively (Fig. 3). A similar tendency was also obtained from the high-rise-rate group. In the low and high-rise-rate groups, the BUN level after the practical training was significantly higher ($P < 0.01$) than that before the practical training.

Discussion

In our study, the mean COR concentrations

Table 1. The level of biochemical markers in the dairy cattle. The data presented were obtained from serum collected before praxis

Inspection Item	Unit	Before	After
Protein			
Total protein	g/dl	5.58 ± 0.16	5.67 ± 0.17
Albumin	g/dl	2.82 ± 0.06	2.88 ± 0.09
Hepatic system			
Aspartate aminotransferase	U/l	61.52 ± 2.31	62.97 ± 2.72
Alanine transaminase	U/l	35.21 ± 1.14	35.45 ± 1.12
Alkaline phosphatase	U/l	130.31 ± 7.38	140.10 ± 8.87
γ-glutamyltransferase	U/l	28.72 ± 1.35	29.66 ± 1.54
Renal system			
BUN	mg/dl	6.61 ± 0.28	8.31 ± 0.34**
Creatinine	mg/dl	0.60 ± 0.02	0.62 ± 0.02
Pancreas system			
Amylase	U/l	180.76 ± 14.44	190.93 ± 14.85
Blood lipid			
Triglyceride	mg/dl	8.38 ± 0.29	7.03 ± 0.31
Total cholesterol	mg/dl	183.34 ± 11.01	187.00 ± 11.01
Others			
Glucose	mg/dl	50.62 ± 1.62	52.17 ± 1.63
COR	ng/dl	6.95 ± 0.64	10.57 ± 0.81***
Electrolyte			
Sodium	mEq/l	119.00 ± 1.84	121.52 ± 2.15
Potassium	mEq/l	3.57 ± 0.08	3.61 ± 0.08
Chlorine	mEq/l	82.66 ± 1.66	84.17 ± 1.68
Magnesium	mg/dl	1.90 ± 0.06	1.99 ± 0.07
Calcium	mg/dl	7.99 ± 0.21	8.08 ± 0.25
Inorganic phosphorus	mg/dl	5.22 ± 0.14	5.49 ± 0.15
Oxidant stress marker			
d-ROMs	U.CARR	93.48 ± 3.42	97.59 ± 3.14
BAP	μmol/l	2622.09 ± 78.09	2588.57 ± 76.22

Results are expressed as mean ± SEM.
Abbreviations; BAP, biological antioxidant potential; BUN, blood urea nitrogen; d-ROMs, diacron-reactive oxygen metabolites. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicated differences between before and after the practical training.

in serum before and after the practical training were 6.95 and 10.57 ng/ml, respectively. The COR concentration in serum after the practical training was significantly higher ($P < 0.001$) than that before the practical training. Thus, it was thought that the practical training of students is a source of stress for dairy cattle. Grandin[7] summarized many studies describing COR concentration in serum of cattle and suggested that the COR levels in cattle fall into the following 3 categories; 1: baseline (2–9 ng/ml), 2: restraint in headgate (13–63 ng/ml), and 3: extreme value (>70 ng/ml). A similar baseline level of COR concentration in serum (mean 10.4 ng/ml) was also suggested in another study[17]. In this farm, the praxis of milking began at 05 : 00 in the early morning and 15 : 45 in the afternoon. Japan Livestock Technology Association

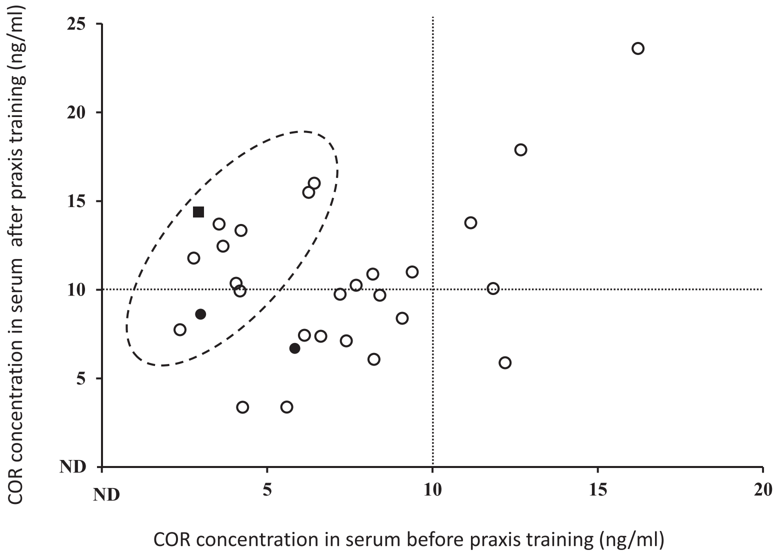

Fig. 1. The relation between COR concentration in serum before praxis and that after praxis. Empty circle: Holstein–Friesian, filled circle: Jersey, and filled square: Brown Swiss. The data inside the dotted line circle indicate the cattle in which the rate of increase in COR concentration was high (high-rise-rate group, n = 11). Other data belong to cattle of the low-rise-rate group (n = 28). ND: not detected.

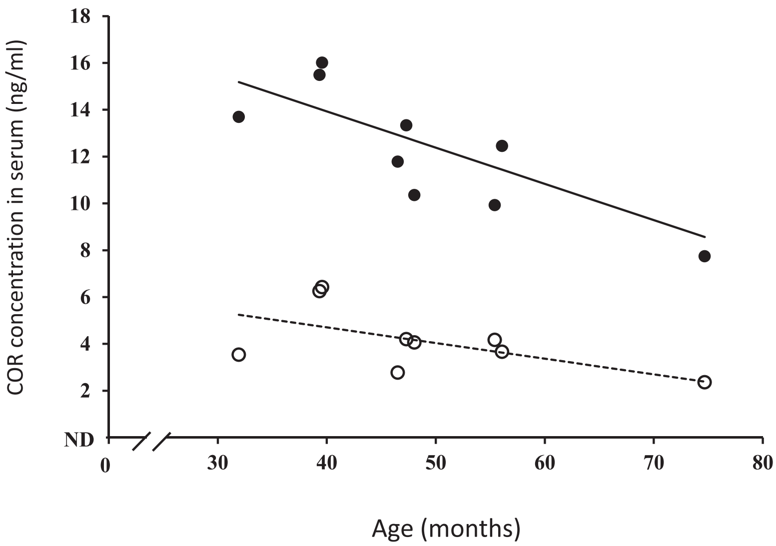

Fig. 2. The relation between age and COR concentration in serum of cattle classified into high-rise-rate group (n=11). Empty symbols: before praxis; Filled symbols: after praxis; Circle: Holstein–Friesian, Triangle: Jersey, and Square: Brown Swiss. ND: not detection.

(JLTA)[10] as announced guidelines for the feeding system of domestic animals providing for animal welfare. According to this guideline, a change in the interval of time and the number of times for milking are sources of stress for dairy cattle. During the stay of the students on the farm, milking and feeding began at a set time. It was thought that the COR concentration in serum of cattle stayed at the baseline due to the regular schedule.

On the other hand, in a study using Limousin cattle, it was suggested that there is an individual difference for the immobilization stress[12]. The individual differences for other stresses have also been suggested in various studies, such as isolation stress[2,8] and heat stress[24]. Since there

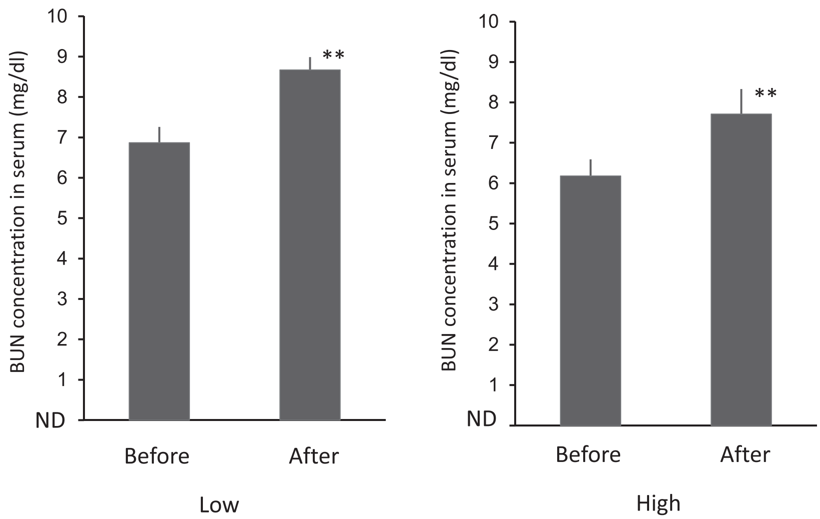

Fig. 3. The BUN concentration in serum of dairy cattle. Before: the result of serum collected before the practical training on the farm, After: the result of serum collected after the practical training, Low: low-rise-rate group, High: high-rise-rate group, ND: not detected. $^{**}P < 0.01$ indicated differences between before and after the practical training.

is also individual variability in the change of stress in this study, we investigated the relation between COR concentration in serum before and after the training of students in the next study. After discriminant analysis, the cattle population was classified into 2 groups with respect to the rate of increase of COR concentration. Although the percentage of the rise was more than 300% in the high-rise-rate group, there was no great change in the low-rise-rate group. Thus, a significant difference between COR concertations before and after training was not obtained in the low-rise-rate group. However, this group included the 5 dairy cattle in which the COR concentration in serum was more than 10 ng/ml before the practical training. The sampling of blood is thought to have been a novel stimulus. It is well known that stimulation due to novel stimuli of animals is relieved over time and with experience. Especially, the COR concentration of 3 dairy cattle was high after praxis. Those cattle were thought to be individuals that stressed easily. It is interesting to note that a significant negative correlation ($P < 0.05$) between age and COR concentration in serum was obtained from only the high-rise-rate group. A similar significant correlation was not obtained from the cattle of the low-rise-rate group. It was thought that the cattle classified into the high-rise-rate group have a better chance to decrease stress with experience.

We also investigated changes in the level of biochemical markers in serum. Biochemical markers, such as GLU, are known to be changed by stress in domestic animals[14] but that was not changed in our study. However, Probst *et al.*[22] suggested a longer stay in a stunning box led to increased COR concentrations ($P < 0.05$), but had no influence on GLU concentrations. Thus, COR was thought to be a more sensitive means of detecting stress in animals. In this study, only the concentration of BUN showed a marked change. The BUN concentration obtained after the training of students was 8.67 (low-rise-rate group) and 7.72 (high-rise-rate group) mg/dl, respectively. In both groups, the BUN concentration after training was significantly higher ($P < 0.01$) than that before training. Protein metabolism in lactating cows is influenced by stresses, such as heat stress[6]. We could not find the reports describing the relation between stress and BUN level in dairy cattle. However, Liu *et al.*[13] reported the effects of lairage time after 8 hours road transport on BUN level of sheep. The result of 48 hours lairage group was significant higher than that of other groups. Kannan *et al.*[11] also reported that the transportation causes an elevation of BUN level in goat. Thus, BUN concentration in serum may be a marker of stress for cattle. Since urinary urea nitrogen (UUN) and milk urea nitrogen (MUN) are also indexes for nitrogen metabolism[23], a more detailed study including UUN and MUN will be also necessary.

The public health in veterinary medicine includes the field of symbiosis with animals, animal assisted interventions. However, it was thought that there were not enough studies related with this field. Underpinning in this field according to the current trend of animal welfare, the studies related with stress of used animals in the activities will be key issue.

In conclusion, it is suggested that there was individual difference to stress of dairy cattle during student practical training on a farm in the present study. Further, it was also suggested that BUN concentration in serum may be a marker of stress for cattle.

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