STUDY ON FACTORS AFFECTING FEED INTAKE OF DAIRY COWS IMMEDIATELY AFTER CALVING

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分娩直後における乳牛の飼料摂取量に影響を及ぼす要因に関する研究

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DECLARATION

I hereby declare that the dissertation entitled "**Study on factors affecting feed intake of dairy cows immediately after calving**" for the degree of Doctor of Philosophy was compiled from the research works during 2015 April to 2019 September with my best ability and effort under the supervision of my academic advisors, wherein it was mainly guided by Prof. Dr. Masaaki HANADA, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Obihiro, 080-8555, Japan.

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Reshalaitihan Maimaiti

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SUMMARY

While annual milk yield of dairy cows has been increasing, the production life of dairy cows has been gradually decreasing. Shortening of production life leads to a decrease in earnings in dairy management. After calving, milk production increases rapidly but feed intake increases modestly, causing most cows to be malnourished. If the cows cannot meet their nutrients requirement after calving, they are prone to develop metabolic diseases such as hypocalcemia and ketosis and to reduce reproductive performance. These metabolic and reproductive disorders are main causes for culling of dairy cattle. Therefore, meeting the nutrients requirements by increasing feed intake immediately after calving is an important issue to improve the productivity and longevity of dairy cows. The overall goal of this study was to find influential factors for feed intake in dairy cows immediately after calving. In order to achieve the goal, three experiments were carried out at Obihiro University of Agriculture and Veterinary Medicine. The first experiment was done to investigate the effect of parity number on the dry matter intake (DMI) of cows immediately after calving. The second experiment was done to investigate the influence of calving difficulty on DMI in dairy cows immediately after calving. The third experiment was done to investigate the effects the oxidative status on the DMI of dairy cows immediately after calving.

[Experiment 1] I investigated the effect of parity number on the factors affecting the DMI of cows immediately after calving. Eighty-three cows were evaluated from 14 days before to 7 days after calving. The DMI and milk yield were measured for 7 days after calving, and the calving score was measured. Blood samples were collected throughout the experiment. The average DMI during the first week after calving was

reduced in first-lactation heifers and high parity number cows. A quadratic relationship between the parity number and DMI was observed. The first lactation heifers had lower precalving serum total protein (TP) concentrations and milk yield, higher precalving serum nonesterified fatty acid (NEFA) concentrations and calving scores than the multiparous cows. The recovery rate of serum calcium (Ca) after calving was slow in the cows with parity 6. The DMI was positively affected by the serum Ca concentration after calving; milk yield and precalving serum TP concentration and was negatively affected by the calving score and precalving serum NEFA concentration. It is concluded that the DMI immediately after calving tends to be lower in first lactation heifers and high parity number cows, but factors that reduce the DMI differ according to parity number.

[Experiment 2] I investigated the influence of calving difficulty on DMI in dairy cows immediately after calving using fifteen pregnant Holstein heifers and fifteen multiparous Holstein cows. DMI was measured 6 days after calving. Calving difficulty was evaluated with a calving score, and urinary cortisol concentration was measured. The calving score was higher in the first lactation heifers than in the multiparous cows. The average DMI 6 days after calving was lower in the first lactation heifers than in the multiparous cows. The urinary cortisol concentration at 4 days after calving were higher in the first lactation heifers than in the multiparous cows and were positively associated with the calving score. The average DMI was negatively associated with the calving score and the urinary cortisol concentration at 4 days after calving and was positively associated with the average milk yield 6 days after calving and the serum Ca concentrations at 3 days after calving. This experiment suggested that the low DMI immediately after calving in the first lactation heifers is

mainly due to the stress derived from their first experiences for example calving, milking and tying to the stall, and to the lower energy requirement compared with that of the multiparous cows.

[Experiment 3] The objective of experiment 3 was to investigate the effects the oxidative status on DMI of dairy cows immediately after calving. Sixty-two Holstein cows were monitored from calving to 21 days after calving. DMI was measured from 1 to 6 days after calving. Body weight (BW) was measured once per week, and milk yield was measured twice per day after calving. Blood samples were taken at 0, 7, and 21 days after calving to determine reactive oxygen metabolites (ROM), biological antioxidant potential (BAP), and metabolic indicators. Oxidative stress index (OSI) was calculated by dividing ROM by BAP * 100. The average DMI 6 days after calving of the first lactation heifers and the cows in parity 2, 3, and 4 or more were 86.1, 124.1, 124.1, and 117.3 g/BW0.75/d, respectively. Serum ROM concentrations tended to be lower in the first lactation heifers than multiparous cows and positively associated with average milk yield 6 days after calving. Serum BAP concentrations were lower in the first lactation heifers compared with multiparous cows through the experiment and were positively associated with average DMI and milk yield 6 days after calving. OSI was not affected by parity number of cows except for 21 days after calving, and did not differ due to the number of in days after calving. The DMI was not associated with OSI at 0 days after calving, but it was negatively associated with OSI at 7 days after calving. These results indicate that although the production of peroxides rose with the increase in milk production, the DMI and the antioxidant capacity also increased, so that the increase of milk production did not affect oxidative stress in this study. Therefore, the difference in the average DMI 6 days

after calving among the parity observed in this study might not be due to oxidative stress. It is concluded that the oxidative stress has little effect on DMI immediately after calving, but the low DMI would increase the oxidative stress of the cows after calving. Rapid increase in DMI immediately after calving together with an increase in milk yield must be an important issue for the prevention of the performance deterioration due to oxidative stress in cows after calving.

These experiments demonstrate that the DMI intake immediately after calving is affected by parity number of cows, and that the DMI is easy to be low in the first lactation heifers and cows with a high number of calving. In the case of the first lactation heifers, the DMI immediately after calving is likely to be suppressed by malnutrition in the late gestation period and stress around calving. On the other hand, delayed recovery of serum Ca concentration after calving is a limiting factor for the DMI in high parity cows. Since the influential factors affecting the DMI differ between the first lactation heifers and high parity cows, feeding management around calving according to the number of parity is required to enhance the DMI immediately after calving. Moreover, these experiments show that oxidative stress is unlikely to increase even with increased milk production, because the increase in milk yield increases the peroxides production and also increases the antioxidant capacity by increasing feed intake. This implies that the DMI of dairy cows immediately after calving is less susceptible to oxidative stress, and it suggests that increasing the feed intake immediately after calving can reduce the oxidative stress and the risk of metabolic and reproductive disorders due to oxidative stress. These results would contribute to the expand of longevity of dairy cows through reducing the risk of

metabolic and reproductive disorders by improving feed intake immediately after calving.

要約

乳牛の個体乳量は年々増加する一方で、徐々に乳牛の生産寿命は減少している。乳牛の生産寿命の短縮は、酪農経営における収益の減少につながる。分娩後、乳量は速やかに増加するが、飼料摂取量の増加は緩やかであり、ほとんどの乳牛が栄養不足に陥いる。分娩後、栄養要求量を満たすことができないと、代謝病や繁殖成績の低下を招きやすくなる。代謝病や繁殖障害は乳牛を淘汰する主な原因である。このため分娩後の飼料摂取量を増やして栄養要求量を満たすことは乳牛の生産寿命の増加にとって重要な課題である。この研究の目的は、乳牛の分娩直後における飼料摂取量に影響を及ぼす要因を明らかにすることであり、3 つの試験を実施した。試験 1 では乳牛の産次が分娩直後の飼料摂取量に及ぼす影響について検討した。試験 2 では分娩前後のストレスが分娩直後の飼料摂取量に及ぼす影響について検討し、試験 3 では酸化ストレスが分娩直後の飼料摂取量に及ぼす影響について検討した。

【試験 1】ホルスタイン種乳牛 83 頭を分娩前 14 日から分娩後 7 日目まで試験に用い、分娩後の飼料摂取量、乳量、血液成分、体重、分娩スコアを測定した。分娩後 7 日間の平均乾物摂取量は初産牛と産次数の多い牛で低くなり、摂取量と産次との間には二次曲線的関係が認められた。経産牛に比べ初産牛は分娩前の血清中のタンパク質濃度と乳量が低く、分娩前の血清遊離脂肪酸濃度と分娩スコアが高かった。6 産の牛は、分娩後の血清カルシウム濃度の回復が遅かった。分娩後の乾物摂取量は、血清カルシウム濃度、乳量、分娩前の血清タンパク質濃度と正の相関が、分娩スコア、分娩前の遊離脂肪酸濃度と負の相関が認

められた。これらのことから分娩直後の乾物摂取量は初産牛と産次数の多い牛で少なくなるが、その低下の要因は、初産牛と産次数の多い牛では異なることが明らかになった。

【試験 2】ホルスタイン種乳牛 30 頭を用いて分娩難易度が分娩後 6 日間の乾物摂取量におよぼす影響を検討した。分娩難易度は分娩スコアで評価し、ストレスの指標として尿中コルチゾール濃度を測定した。分娩スコアは経産牛よりも初産牛で高く、分娩後 6 日間の平均乾物摂取量は経産牛に比べ初産牛で少なかった。分娩後 4 日目の尿中コルチゾール濃度は経産牛よりも初産牛で高く、分娩スコアとの間に負の相関が認められた。分娩後の乾物摂取量は分娩スコアおよび分娩後 4 日目の尿中コルチゾール濃度との間に負の関係が、分娩後 3 日目の血清カルシウム濃度および乳量との間に正の関係が認められた。これらのことから初産牛の分娩直後の乾物摂取量の少なさは、主に分娩や搾乳などの初体験によるストレスの受けやすさと経産牛に比べ少ないエネルギー要求量に由来すると判断された。

【試験 3】ホルスタイン種乳牛 62 頭を用いて酸化ストレスが分娩直後の乾物摂取量に及ぼす影響を検討した。分娩後 6 日間の乾物摂取量を測定するとともに、体重および乳量を測定した。また、分娩後 0、7、21 日目に採血し、代謝物質を測定するとともに、過酸化物質(ROM)および抗酸化力(BAP)を測定して酸化ストレス度(OSI)を算出した。血清中 ROM 濃度は初産牛で低くなる傾向があり、分娩後 6 日間の平均乳量との間に正の相関が認められた。血清中の BAP 濃度は経産牛に比べ初産牛で低い値を示し、分娩後 6 日間の平均乾物摂取量や平均乳量との間に正の相関が認められた。OSI は分娩後 21 日目以外では、乳牛の産

次数の影響を受けず、分娩後日数の違いによる差もみられなかった。分娩日の OSI と分娩後 6 日間の乾物摂取量の平均値との間には有意な相関は認められなかったが、分娩後 7 日目の OSI と分娩後 6 日間の乾物摂取量の平均値との間には負の相関が認められた。これらの結果から、乳量の増加とともに過酸化物質の生成は増加したが、同時に DMI および抗酸化能力も増加したため、乳量が増加しても酸化ストレスは高まらなかったと推察された。このため産次の違いによる乾物摂取量の差は、酸化ストレス以外の要因によってもたらされたと判断された。また、酸化ストレスが分娩直後の乾物摂取量に及ぼす影響は小さいが、分娩後の乾物摂取量が減少すると抗酸化物質の摂取量が減少し、酸化ストレスが高まると推察された。

これらの研究の結果、分娩直後の乾物摂取量は初産牛と産次数の多い牛で少なくなり、初産牛では分娩前の低栄養や難産によって、産次数の多い牛では分娩後の血清カルシウム濃度の回復の遅延によって採食量が抑制されやすいことが示された。さらに乳量の増加とともに過酸化物質の生成は増加するが、同時に DMI および抗酸化能力も増加する。このため酸化ストレスが分娩直後の乾物摂取量に及ぼす影響は小さいが、分娩後の乾物摂取量が減少すると抗酸化物質の摂取量が減少し、酸化ストレスが高まることが示唆された。

LIST OF ABBREVIATIONS

ADFom	Ash-corrected acid detergent fiber
Alb	Albumin
BAP	Biological antioxidant potential
BCS	Body condition score
BHBA	β-hydroxybutyrate
BUN	Blood urea nitrogen
BW	Body weight
Ca	Calcium
CP	Crude protein
d	Day
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
MBW	kg of body weight power to 0.75
αNDFom	Ash-corrected neutral detergent fiber
NEFA	Serum non-esterified fatty acid
NEL	Net energy at actual intake
NFC	Non-fibrous carbohydrate
OSI	Oxidative stress index
P	Phosphorus
r	Correlation
R^2	Coefficient of determination

ROM	Reactive oxygen metabolites
ROS	Reactive oxygen species
SE	Standard error
TMR	Total mixed ration
TP	Total protein

CHAPTER I

General Introduction

1.1. Longevity of dairy cows

1.1.1. Necessity for increase in longevity of dairy cows

In constant conditions of herd size and reduced replacement rate, higher profitability of heifers are obtained by increasing the longevity of female cows (Berry et al. 2005). Therefore, longevity of dairy cows is currently one of the most important functional traits in dairy cattle breeding schemes. A longer productive life of dairy cows decreases the costs of herd replacement and leads to higher production from larger herds as older cows produce more milk (Vukasinovic et al., 1997). Therefore, longevity, or lifespan of dairy cattle, is an economically important trait for dairy farmers because increased longevity helps to increase profitability (Van Pelt et al. 2015). According to Ducrocq (1992), two types of longevity could be distinguished: real and functional.

1.1.2. Current situation of longevity of dairy cows

The annual milk yield of dairy cows has been increasing around world, but, longevity of dairy cow has been decreasing (Essl, 1998; Hare et al., 2006; Sewalem et al., 2008). Many countries include some measure of longevity in their national breeding objectives (VanRaden, 2002). The average number of parity in dairy cows in the United States of America (USA) and Japan was 2.4 (Tsuruta et al., 2005) and 2.7 (Livestock Improvement Corporation, 2012), respectively. In Hokkaido, Japan, the average numbers of parity in dairy cows declined from 3.1 in 1986 to 2.8 in 2011 (Livestock Improvement Corporation, 1987, 2012).

1.1.3. Reasons for culling

Real longevity of cows is determined by voluntary and involuntary culling decisions by individual farmers. In the process of making these decisions, the farmers or producers will take into account health, milk yield, fertility, and other factors such as milking speed, milking temperament, and calving difficulties. Usually, culling because of poor milk production is called voluntary culling, and culling for other reasons is called involuntary culling. Reducing the rate of involuntary culling allows a higher proportion of voluntary culling, which can increase profits for a dairy farm. According to a USDA (2007) report, main reasons for culling dairy cows were reproductive failure (26.3% of culled cows), mastitis, udder problems (23% of culled cows), lameness or injury (16%), other diseases (15.0%). and poor milk production (19.7%). Some studies have shown that the revenue of dairy farms might be increased by reducing involuntary culling (Chiumia et al., 2013; Sanjabi & Javanmardi, 2014). About 90% of the lifetime variation of cows is due to management, environmental factors, and diseases (Ghaderi-Zefrehei et al., 2017). Thus, changes in the environment, housing, and management facilities could affect most of the involuntary culling (Shahmoradi et al., 2008). Statistics of culling in many dairy herds (Caraviello et al., 2004) showed that low milk production, the main reasons for culling dairy cows are infertility and mastitis problems. Dystocia may be one of the fertility reasons for involuntary culling, because dystocia has negative effects on reproductive performance, causing stillbirth, cow death, retained placenta, or uterine infections (Abdela & Ahmed, 2016).

Most of reasons for voluntary culling are related to the nutritive status of the cows around calving. Lee & Kim (2006) reported that increase of milk yield, body condition loss during early lactation and periparturient disorders such as mastitis, milk fever and ketosis are lead to reproductive failure in dairy cows. Grohn & Rajala-Schultz (2000) reported that improvement of milk yield resulted in high incidences of reproductive and health problems. Large energy requirements in high producing dairy cows results in a severe negative energy balance during the early lactation period (Butler & Smith, 1989; Bell, 1995), which may adversely impact postpartum health and fertility (DeVries et al., 1999; Lucy, 2001).

Reproductive performance is another major factor affecting involuntary culling of dairy cows. Inadequate herd reproductive performance, manifested as prolonged calving intervals, increased involuntary culling, or both, can result in less milk and fewer calves per cow per year. Other consequences include more culling and, therefore, increased replacement costs and ultimately lower net returns. Until 10 to 15 years ago, most national dairy cattle research and breeding programs were mainly oriented toward yield traits (Leitch, 1994). However, functional traits, such as reproduction, longevity, and health traits are of increased interest to producers to improve herd profitability. Miglior et al. (2005), in their comparison of international selection indices, reported that selection indices have evolved worldwide, shifting their focus from primarily production to a more balanced breeding approach that includes longevity, udder health, conformation, and reproduction. Several reports have indicated that poor reproductive performance, manifested as prolonged calving intervals, can result in reduced milk yield and increased replacement cost (Pryce et al., 2000; Kadarmideen et al., 2003). Poor fertility causes many cows to be prematurely

culled, whilst suboptimal herd health can increase veterinary costs, reduce total production and affect cow longevity. Increased culling inevitably increases the number of heifer replacements required to maintain herd size, adding further costs to farmers (Lamming et al., 1998; Esslemont & Kossaibati, 1999). Although there are several studies that have investigated the association between fertility and production traits, there are some studies (Beaudeau et al., 1994; Schneider et al., 2005; Perez-Cabal et al., 2006; Sewalem et al., 2006) that examined the association of reproduction traits with longevity.

Milk fever, ketosis, retained fetal membranes, metritis, and displacement of the abomasum primarily affect cows within the first two weeks of lactation (Drackley, 1999). Milk fever generally occurs 12 to 24 hours after giving birth (Horst et al., 1997). Milk fever occurs when blood loss of calcium (Ca) during milk production occurs faster than it can be replaced from the diet, skeletal Ca stores, and renal conservation. The disease is characterized by an acute decline in blood Ca concentration to levels that no longer support nerve and muscle function. It has been reported that uterine involution is delayed in dairy cows with puerperal diseases (Mateus et al., 2002; Heppelmann et al., 2013). And in dairy cows with metabolic disorders (Paiano et al., 2019). Retention of the placenta is the inability of the fetal membrane to be expelled from 8 to 48 hours (average 8 hours) after parturition (Beagley at al., 2010). Research indicates that selenium supplementation reduces the incidence of retained placentas, cystic ovaries, mastitis, and metritis (Patterson et al., 2003). It is not surprising therefore that negative impacts of vitamin E and selenium deficiencies have been observed on various components of reproductive events, including ovulation rate (Goto et al., 1992) and uterine motility (Robinson, 1996). In

the early lactating cows, high levels of protein may exacerbate the negative energy balance and delay the return of normal ovarian function (Surai, 1999). All of these reports indicate that the relationship between nutrient deficiency and reproductive performance and yields more culling of dairy cows.

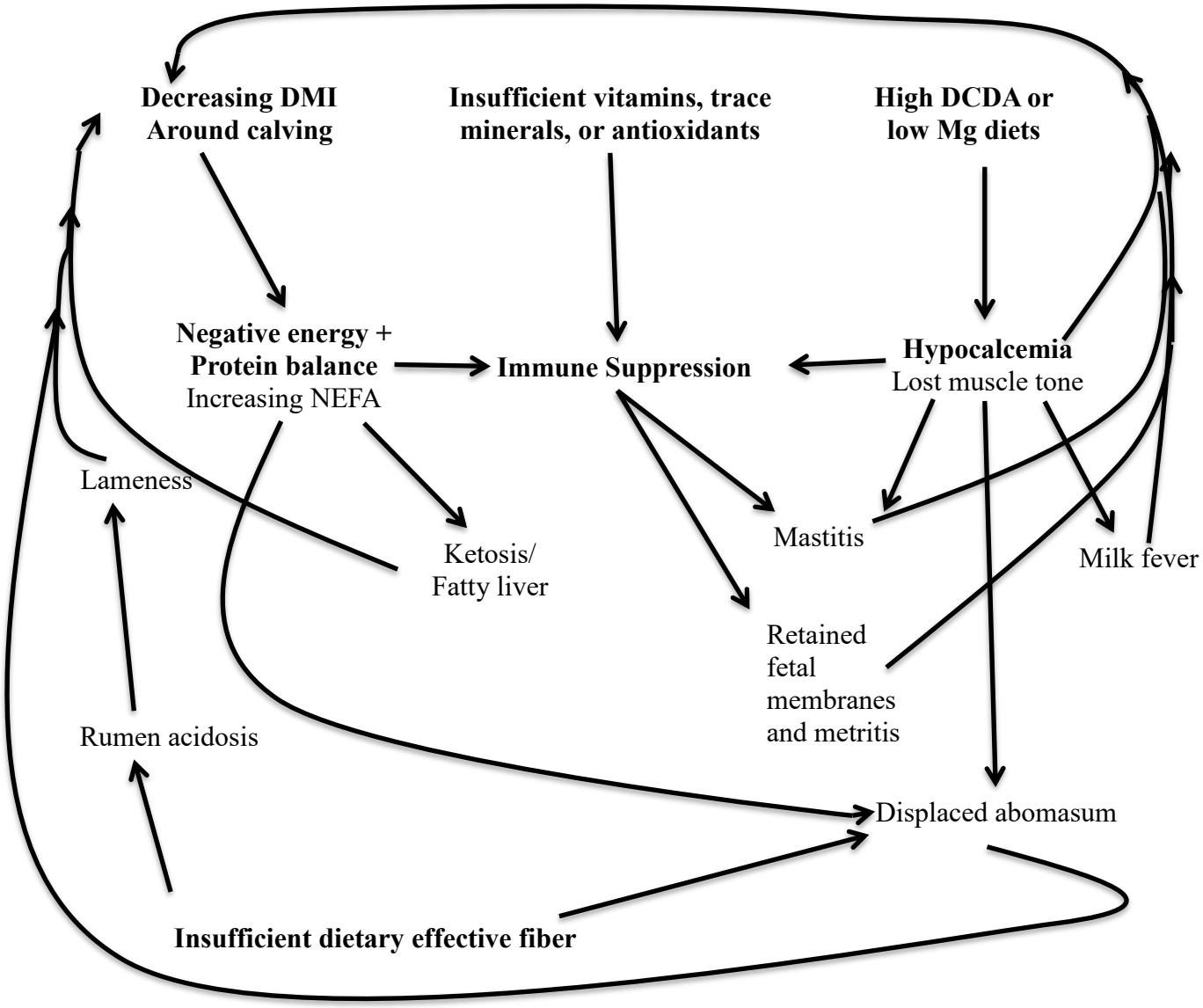

Figure 1.1. Interrelationships between nutrition and disease in the periparturient dairy cow (Goff, 2006).

The relationship between the dry matter intake (DMI), metabolic disease and impaired immune function leading to infectious disease, it seems more prudent than ever to do all that they can to reduce metabolic disease in the dairy cow (Goff, 2006) as shown in Figure 1.1. Nutrient deficiency, Ca deficiency, and insufficient fiber intake are considerable triggers for metabolic diseases, such as negative energy balance of early lactation develops insulin resistance in tissues (Bauman, 2000). Development of insulin resistance by negative energy balance increases the risk of ketosis and fatty liver. Those reports indicated that the metabolic and reproductive disorders are closely related to each other. Beever (2004, 2006) demonstrated that improvement of nutritional management might offer some effective solutions for current herd health and fertility problems. Therefore, meeting nutrient requirements such as protein and mineral nutrients by increasing feed intake of dairy cows as soon as possible after calving is one of the most important countermeasures to prevent those problems and to increase number of parity in a dairy cow's life.

1.2. Feed intake of dairy cows immediately after calving

After calving, nutrient requirements of cows, and consequently feed intake, increase quickly due to initiation of milk production and its increase. However, the increased rate of feed intake is less than the higher need nutrients (Bell, 1995). In general, milk yield of cows reaches its peak 4-8 weeks after calving, but feed intake peaks around 10-14 weeks after calving (NRC, 1989). Therefore, cows are prone to nutrient deficiencies which results in metabolic and reproductive disorders as mentioned above.

1.2.1. Factors affecting feed intake of dairy cows

Factors affecting and regulating the feed intake of lactating dairy cows are numerous and complex, and they span cellular to macro-environmental levels. Some can be controlled by humans and include animal factors (age, body condition, physiological stage, milk yield level, etc.) (Kononoff et al., 2006; Janovick & Drackley, 2010; Teramura et al., 2015), dietary factors (ingredient and nutrient composition of diet, physical and agronomic characteristics of feeds, etc.) (Minor et al., 1998; Beever, 2006; Dann et al., 2006), managerial factors (production, feeding system, housing system, etc.), and climatic factors (temperature, humidity, wind). Therefore, the determination of factors affecting DMI and quantification of their effects are important for developing new feeding strategies during the transition period (Hayirli et al., 2002).

a. Body condition

Gransworthy & Topps (1982) also demonstrated that cows with body condition score (BCS) of 4.0 at calving consumed less DMI and lost more BW and BCS during early lactation. Pregnant heifers had greater weekly non-esterified fatty acid concentrations than multiparous cows prepartum (Janovick et al., 2011).

b. Diets and feeding behavior

Feeding management is the important issues to improve the production level and longevity of dairy cows. Therefore diet and feeding behavior has focused on improving DMI of lactating dairy cows by changing the nutrient composition of feeds. Allen & Bardford (2007) reported that feed intake was reduced with increase of propionate from 0 to 100% of infuscate, a finding that indicates increased satiety. Diet NDF content was highly related with the DMI of diet compared to other chemical

measures in the sheep (Van Soest, 1965). Waldo (1986) also suggested that NDF content is the best single chemical predictor of DMI by ruminants.

The DMI of lactating dairy cows is also affected by the feeding behaviour of the cows with group-housed, which is modulated by the environment, management practices, and health conditions (Grant & Albright, 2001; DeVries et al., 2005). The majority of research on feeding behaviour has been completed with individually housed animals. First lactation heifers spend less time at the feed bunk compared with multiparous cows consequently, consume less diet (Grant & Albright, 1995). Restricting energy intake of first lactation heifers, whether intentional or not, might be detrimental to milk yield because these cows have requirements for growth in addition to pregnancy and lactation (NRC, 2001). Some studies that have focused on the effect of plane of nutrition on subsequent lactational performance in first lactation heifers have indicated that a higher plane of nutrition prepartum may confer advantages over restriction of nutrients prepartum (Broster & Tuck, 1967; Park et al., 1987).

c. Nutrient factors

Low serum Ca concentration decreases the movement of the digestive tract of cows because serum Ca has a role in maintaining muscular contractive activity (Johansson, 1987; Van Breemen & Saida, 1989). Cows with low serum Ca concentrations showed inactive digestive tract motility (Goff, 2004). Wynn et al. (2015) demonstrated that serum Ca concentrations after calving were positively associated with the rumen contraction frequency and DMI of multiparous Holstein cows immediately after calving.

Teramura et al. (2015) showed a decrease of serum Ca concentrations at calving according to the increase of parity number of the cow. Therefore, nutritional factors which influence on DMI rapidly after calving might differ with the parity number of the cows. In order to increase the parity number in dairy cow life, it might be necessary to find the factors which influence on DMI immediately after calving of cows for different parity number or age.

1.2.2. Influence of stress on dry matter intake

Stress is the natural response of living organisms to environmental perturbations. Acute or chronic exposure to stress (defined as an ongoing or anticipated threat to homeostasis or well-being) evokes a constellation of physiological and behavioral responses that markedly alter metabolic and behavioral state in humans and animals (Dallman et al., 2003). Stressors impact energy balance and affective state in a manner that depends on a multitude of biological and environmental factors, including temporal, genetic, social, contextual, species-specific, sex-dependent, nutritional, developmental, metabolic, and experience-dependent elements.

For most people, stress influences both the amount and types of food that they eat. For example, approximately 35–60% of people report eating more total calories when they are experiencing stress, whereas approximately 25–40% of people report eating less (Epel et al., 2004, Oliver and Wardle, 1999, Weinstein et al., 1997). Cortisol is a steroid hormone, in the glucocorticoid class of hormones. Cortisol is used as an indicator of stress and pain. Elevated serum cortisol has been shown in calves castrated without local anaesthesia (Thüer et al., 2007) and in surgical stress in dairy cows (Mudron et al., 1994). Previous study reported conclude that while moderate

increases in plasma cortisol can stimulate food intake slowly over days, larger catabolic doses of glucocorticoids may mask the appetite-stimulatory effects of cortisol (Bernier et al., 2004).

Oxidative stress plays a key role in the onset or progression of numerous human and animal diseases. Dairy cows undergo this deleterious process mainly during the transition period. The period of transition between late pregnancy and early lactation is associated with lipid and protein metabolic changes (Castillo et al., 2006). The energy reduction in the first weeks after calving results in increased fat mobilization, which is related to the generation of lipid peroxides and reactive oxygen species (ROS). These ROS are normally neutralized by sufficient antioxidant levels of living organisms. Imbalance between the production of ROS and the defense ability of biological systems to scavenge these reactive intermediates causes oxidative stress (Trevisan et al. 2001). In dairy cows increased intake and higher levels of nutritive antioxidants can efficiently reduce the level of lipid peroxidation (Brzezinska-Slebodzinska et al. 1993). Also several studies reported that oxidative stress rose with increase of milk yield in dairy cows (Castillo et al. 2003; Lohrke et al. 2004).

First lactation heifers are prone to dystocia and experience more stress at calving than the multiparous cows because pregnant heifers have a smaller pelvic area, lower live weight, and more frequent difficulty in calving than older cows (Bureš et al., 2008). Because dystocia is painful for cows (Huxley & Whay, 2006), stress from calving difficulty might contribute to the low DMI occurring immediately after calving, especially in the first lactation heifers. Kornmatitsuk et al. (2002) reported that the plasma cortisol concentration and calving score were higher in heifer with moderately difficult calving (required manual assistance) than in heifer with normal

calving. According to those results, I hypothesized that the factors affecting the DMI immediately after calving may differ among the parity number of cows and several types of stress might be affecting DMI of cows immediately after calving differs among the parity.

1.3. Objective of this study

The overall goal of this study was to find factors which affect the feed intake in dairy cows immediately after calving, and to improve the feed intake of cows immediately after calving. Three trails were carried out to achieve this goal. It was hoped I might find efficient methods to increase longevity of dairy cow's life.

The first trial was done to determine the effect of parity number on the factors affecting the DMI of cows immediately after calving. The second trial was done to investigate the influence of calving difficulty on DMI in dairy cows immediately after calving. Finally, the third trial was done to investigate the effects of oxidative status on dry matter intake of dairy cows immediately after calving.

CHAPTER II

[Experiment 1]

The Effect of Parity Number on the Differences in Factors Affecting the Dry Matter Intake of Dairy Cows Immediately After Calving

2.1. Introduction

The annual milk yield of dairy cows has been increasing, but the lifetime parity of dairy cows has been gradually decreasing (Essl, 1998; Hare et al., 2006). The average parity number in dairy cows was 2.7 in Japan in 2011 (Livestock Improvement Corporation, 2012) and 2.8 in the United States of America in 2004 (Tsuruta et al., 2005). The average parity number in dairy cows declined from 3.1 in 1986 to 2.8 in 2011 in Hokkaido, Japan (Livestock Improvement Corporation, 1987; 2012). Since the economic efficiency of dairy farming is mostly a result of achieved milk production and dairy cow longevity (Heins et al., 2012), a reduction in the lifetime parity number in dairy cows directly influences the profitability of dairy farming (Sewalem et al., 2005). The main reasons for dairy cow culling in the United States of America were reproductive failure, mastitis and udder problems, lameness or injury, other diseases, poor milk production (USDA, 2007), and reproductive disorders are the most common cause of dairy cow culling in Japan (Nakada, 2006).

Most of the aforementioned problems are associated with the nutritive status of the cows around the time of calving. Lee & Kim (2006) reported that a milk yield increase and body condition loss during early lactation as well as periparturient disorders caused reproductive failure in dairy cows. Grohn & Rajala-Schultz (2000) reported that an improvement in milk yield resulted in high incidence rates of reproductive health problems. The extensive energy requirements of high-producing dairy cows result in a severe negative energy balance, which may adversely impact postpartum health and fertility (DeVries et al., 1999; Lucy, 2001), during the early lactation period (Butler & Smith, 1989). Therefore, meeting nutrient requirements by increasing the feed intake of dairy cows as soon as possible after calving is one of the

most important countermeasures to prevent postpartum health and fertility problems and to increase parity number in dairy cows.

Dry matter intake (DMI) by dairy cows after calving is affected by many factors, such as diet composition (Beever, 2006; Dann et al., 2006), feeding behavior (Azizi et al.,2009), digesta flow dynamics in the digestive tract (Grandl et al., 2016), cow nutritional status (Gransworthy & Topps, 1982; Janovick & Drackley, 2010; Wynn et al., 2015), calving stress (Bareille et al., 2003; Reshalaitihan & Hanada, 2019) and milk yield (Reshalaitihan & Hanada, 2019). Azizi et al. (2009) observed an increased meal size and DMI in multiparous cows compared with first lactation heifers. Teramura et al. (2015) showed that the recovery of serum calcium (Ca) concentrations declined during calving to the normal range, and a reduced rate of decline was associated with an increase in the parity number of the cow. Reshalaitihan & Hanada (2019) reported a higher calving score in first lactation heifers than in multiparous cows. Since most of these factors change with the parity of cows, DMI immediately after calving and its limiting factors might also vary with the parity number. Jensen et al. (2015) suggested that the feed intake capacity in dairy cattle increases with age, and it has been reported that DMI after calving is lower in first lactation heifers than in multiparous cows (Azizi et al., 2009; Janovick & Drackley, 2010). However, there is little information on the factors affecting DMI during the first week after calving, even though DMI is considered to be closely associated with milk yield, health and reproductive performance after calving.

The objective of this study was to investigate the differences in factors affecting DMI in dairy cows during the first week after calving and their association with the parity number to support efforts to increase the production time in dairy cows.

2.2. Materials and methods

This study was performed from 2012 to 2014 at the Field Science Center of the Obihiro University of Agriculture and Veterinary Medicine in Hokkaido, Japan. Animal care and protocols for the experimental procedures were approved (No. 25-20 and 26-9) by the Animal Care and Use Committee of the Obihiro University of Agriculture and Veterinary Medicine.

Eighty-three Holstein cows were monitored from 14 days before to 7 days after calving. There were 15 pregnant heifers in the parity 1 group and 23, 15, 7, 13, and 10 cows in the parity 2, 3, 4, 5 and 6 groups, respectively. During the dry period, the experimental heifers and cows were kept in a free-stall barn with other pregnant cows. When calving signs were observed, the pregnant heifers and cows were moved into an individual maternity pen. On the second day after calving, they were confined to a stall barn for 7 days. The pregnant heifers and cows were offered 6.3 kg/day dry matter weight dry total mixed rations (TMRs) and were able to freely ingest hay, which consisted of timothy hay, orchard grass and white clover, from a hay rack placed in the paddock attached to the free-stall barn. After calving, all cows were offered lactation TMRs and grass hay ad libitum. The TMRs used before and after calving mainly consisted of grass and corn silages and a concentrate (Table 2.1). The cows had constant access to fresh water and mineral blocks (KNZ salt licks, Hengelo, the Netherlands) throughout the experiment.

The amount of feed offered and the feed residues were recorded daily from calving to 7 days after calving. Feed samples were taken once a week, and residue samples were taken every day. The samples were dried in a forced-air oven at 60 °C for 48 hours and then ground using a Wiley mill to pass a 1-mm screen (SM 2000;

Retsch, Haan, Germany). The moisture content in the ground sample was determined by drying at 135 °C for 2 hours (AOAC, 2003). The ash content was determined after placing the samples in a muffle furnace for 2 hours at 600 °C (AOAC, 2003). The ash-corrected neutral detergent fiber (αNDFom) content was determined with a heat-stable α-amylase and expressed exclusive of residual ash according to the method described by Van Soest et al. (1991). The ash-corrected acid detergent fiber (ADFom) was also analyzed according to methods of Van Soest et al. (1991). Ether extract content was measured according to the AOAC (2003). Nitrogen (N) content was measured according to the Kjeldahl method, and crude protein was calculated by multiplying the N content by 6.25 (AOAC, 2003). Dietary Ca and phosphorus (P) were analyzed by inductively coupled plasma emission spectroscopy analysis (ICPE-9000, Shimadzu Corporation, Tokyo, Japan). Actual net energy at intake (NEL) was calculated according to the National Research Council (NRC, 2001). The chemical compositions of the diets are shown in Table 2.2.

Calving difficulty was evaluated with a calving score from 1 to 3 as follows: 1 = unassisted delivery (cow calved unassisted), 2 = minor assistance (with the assistance of a person), and 3 = major assistance (with the assistance of two or more persons and veterinary assistance) (Proudfoot et al., 2009a).

Blood samples were collected from the caudal vein of individual cows at –14, –7, 0, 2, and 7 days after calving using 9 ml evacuated tubes (VENOJECT II, Terumo Corporation, Tokyo, Japan). All tubes for serum collection were placed on ice immediately after sampling and were centrifuged at 1,500 × *g* for 10 minutes at room temperature by a centrifuge (05P-21, HITACHI) within 4 hours after collection. The supernatant was stored at –30 °C until analysis. Serum NEFAs, β-hydroxybutyric acid

(BHBA), albumin (Alb), total protein (TP), blood urea nitrogen (BUN), Ca and P concentrations were analyzed using an automatic clinical chemistry analyzer (TBA-12FR, Toshiba Medical Systems Corporation, Tochigi, Japan).

The body weight (BW) and body condition scores (BCSs) of the cows were measured before the morning feeding at –14, –7, 1, and 7 days after calving. The cows were milked at 04:00 and 17:00 hour, and milk yield was recorded twice per day.

All data were analyzed in SPSS Student Version for Windows 16.0 (SPSS Inc., Chicago, IL, US). One-way analysis of variance was used to analyze the effects of parity; the calving score; serum metabolites (NEFA, BHBA, TP, Alb, BUN, Ca, and P levels); BW; BW change; the BCS; the BW of the calf at birth; the ratio of calf BW at birth to the dam BW at calving; and milk yield on the DMI. Differences in the means among parity were analyzed by least significant difference (LSD) multiple comparison tests. Quadratic polynomial contrasts were used to determine the effect of parity on the average DMI during the first week after calving. A stepwise multiple regression analysis was performed to identify the factors affecting the average DMI in the week after calving by using the variables that had significant correlation coefficients with DMI. Pearson's correlation coefficients for the average DMI in the week after calving and the calving score, BW, BW change, BCS, milk yield, and blood metabolite levels were calculated. The differences in the DMI and milk yield means between 1 and 7 days after calving were analyzed with *t* tests. The data were considered significant at $P < 0.05$ unless otherwise noted.

Table 2.1. Ingredients of the total mixed rations (TMRs) offered to the cows before and after calving.

	TMRs	
	Before calving	After calving
	(% of dry matter weight basis)	
Corn silage	49.94	42.26
Grass silage	25.56	18.01
Grass hay	–	3.84
Beet pulp	–	5.27
Ear corn silage	–	1.35
Concentrate mix	20.19[†]	20.27[‡]
Soybean meal	2.18	5.33
Rice bran[§]	1.76	3.06
$CaCO_3$	–	0.19
MgO	–	0.02
Vitamin mineral mix[¶]	0.38	0.09
Molasses	–	0.31

[†] Dry Base 17 (Hokuren Kumiai Shiryo K. K., Hokkaido, Japan). [‡] Mo-Dairy 18 (Nippon Formula Feed Manufacturing Co. *Ltd*., Kanagawa, Japan). [§]Contents per gram: 5,000 IU of vitamin A, 1,000 IU of vitamin D, 2 mg of vitamin E, 0.2 mg of vitamin K_3, 0.5 mg of vitamin B_1, 1 mg of vitamin B_2, 0.1 mg of vitamin B_6, 1 ng of vitamin B_{12}, 6 mg of nicotinic acid, 2 mg of choline chloride, 10 mg of pantothenic acid, 156 µg of Mn ($MnSO_4$), 0.7 mg of Zn ($ZnCO_3$), 50 µg of Fe ($FeSO_4$), 139 µg of Cu ($CuSO_4$), 325 µg of I Ca $(IO_3)_2$, 38 µg of Co ($CoSO_4$), 1 mg of methionine, and 0.5 mg of lysine hydrochloride.

Table 2.2. Chemical compositions of the total mixed rations (TMRs) and grass hays offered to the heifers and the multiparous cows before and after calving.

Chemical compositions		TMRs		Grass hays	
		Before calving	After calving	Before calving	After calving
DM	%FM	34.30	39.83	87.90	85.74
CP	%DM	15.30	15.40	15.00	14.62
EE	%DM	4.20	3.78	3.00	2.50
Ash	%DM	8.00	8.07	11.20	9.20
αNDFom	%DM	42.30	41.32	59.50	63.34
ADFom	%DM	25.60	24.27	36.40	37.54
Ca	%DM	0.40	0.65	0.50	0.48
P	%DM	0.40	0.44	0.40	0.41
NEL[†]	Mcal/kgDM	1.50	1.51	1.20	1.19

TMR, total mixed ration; DM, dry matter; CP, crude protein EE, ether extract; αNDFom, ash-corrected neutral detergent fiber content was determined with a heat-stable α-amylase and expressed exclusive of residual ash; ADFom, ash-corrected acid detergent fiber; Ca, calcium; P, phosphorus; NEL, net energy at actual intake. [†]Calculated according to the NRC (2001).

2.3. Results

The results of the average DMI during the first week after calving are shown in Table 2.3 and Figure 2.1. The average DMI in the week after calving was lowest in the first lactation heifers and highest in the cows with a parity of 3. The DMI of the cows in the parity 6 group was also lower than that of the cows in the parity 2, 3, 4, and 5 groups ($P < 0.05$). A quadric relationship between parity and the average DMI during the first week after calving was observed and is as follows:

$$Y = -7.8X^2 + 58.9X + 37.6; R^2 = 0.478, P < 0.01$$

where Y = the average DMI during the first week after calving (kg/day), and X = the parity number.

It was estimated that the DMI was maximized in cows with a parity of 3.8 by differentiating the above equation. During the first week after calving, daily DMI in the multiparous cows increased significantly ($P < 0.05$), but the increase was not observed in the first lactation heifers (Figure 2.2a).

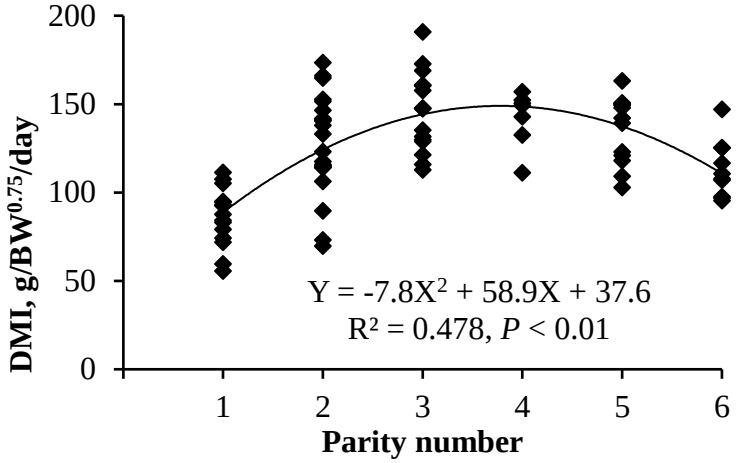

Figure 2.1. Relationship between parity number and average dry matter intake (DMI) of the cows (n=83) during the first week after calving. The regression equation is shown; Y = average DMI during the first week after calving and X = parity number.

The BW of the first lactation heifers was lower than that of the multiparous cows throughout the experiment ($P < 0.05$). The BW decreased at calving in all cows ($P < 0.05$), but the decrease was not affected by the parity number. The change in BW from –7 days to 0 days (r = 0.225, $P < 0.05$; Table 2.5) and from 0 days to 7 days (r = 0.397, $P < 0.01$; Table 2.5) after calving were positively associated with the average DMI in the week after calving. The BCS did not differ among parity groups throughout the experiment, and the BCS at 7 days before calving averaged 3.52. The average birth weight of the calves born to the first lactation heifers was lower than that of the calves born to the multiparous cows ($P < 0.05$). However, the average ratio of calf BW to dam BW at calving was not affected by the parity of the dam ($P > 0.05$; Table 2.5). The calving score was highest in the first lactation heifers (Table 2.4) and was negatively associated with the average DMI during the first week after calving (r = −0.386, $P < 0.01$).

The average milk yield during the first week after calving was the lowest in the first lactation heifers and increased according to an increase in parity number ($P < 0.05$; Table 2.3). The average milk yield was positively associated with the average DMI during the first week after calving (r = 0.593, $P < 0.01$; Table 2.5). During the first week after calving, daily milk yield increased in all groups ($P < 0.01$; Figure 2.2b).

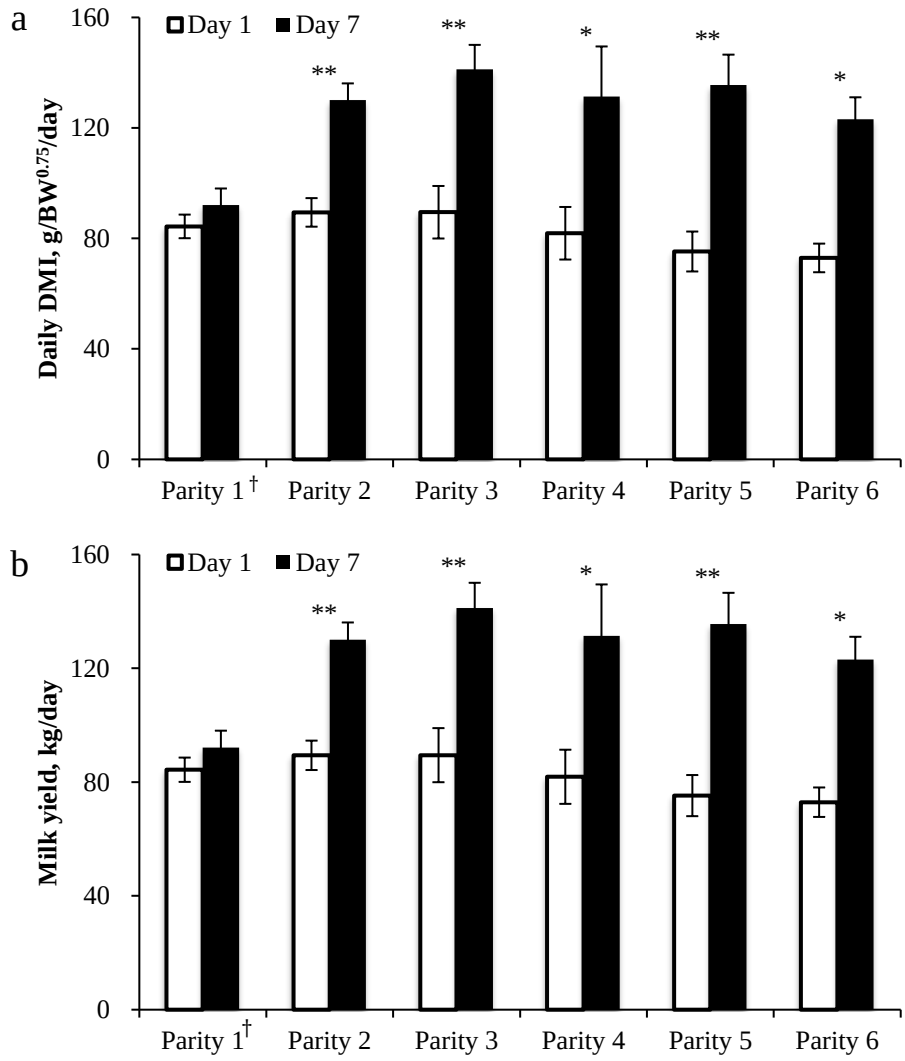

Figure 2.2. (a) Comparison of the daily dry matter intake (DMI) between 1 and 7 days after calving in cows in each parity number. **(b)** Comparison of the daily milk yield between 1 and 7 days after calving in cows in each parity number. †First lactation heifers; *, $P < 0.05$; **, $P < 0.01$.

The results of blood metabolites are shown in Table 2.4. The serum NEFA concentration at 14 days before calving was higher in the first lactation heifers than in the multiparous cows ($P < 0.05$) and negatively associated with the average DMI during the first week after calving ($r = -0.403$, $P < 0.01$; Table 2.5). Moreover, the serum NEFA concentration at 14 days before calving was positively associated with the calving score ($r = 0.260$, $P < 0.05$). The serum NEFA concentration after calving tended to be higher in the first lactation heifers than in the multiparous cows.

The serum TP concentration at 14 and 7 days before and at calving were lower in the first lactation heifers than in the multiparous cows ($P < 0.05$; Table 2.4) and 14 days before calving were positively associated with the average DMI during the first week after calving ($r = 0.408$, $P < 0.01$; Table 2.5). The serum TP concentration after calving tended to be lower in the first lactation heifers than in the multiparous cows.

The average serum Ca concentrations before calving in all groups were above 9.0 mg/dl. The serum Ca concentration decreased below 9.0 mg/dl at calving in all groups ($P < 0.05$), and a reduced Ca concentration was associated with an increased parity number ($P < 0.05$; Table 2.4). The decreased serum Ca concentration at calving recovered after calving, but the recovery rate was slow in the cows in the parity 6 group. Serum Ca concentrations at 0, 2 and 7 days after calving were lowest in the parity 6 group ($P < 0.05$). There was a positive relationship between the serum Ca concentration at 2 days after calving and the average DMI during the first week after calving ($r = 0.388$, $P < 0.01$; Table 5).

A stepwise multiple regression analysis was performed to compare the effectiveness of the variables on the average DMI during the first week after calving

using the variables that were significantly associated with the average DMI (i.e., the calving score, average milk yield during the first week after calving, serum Ca concentration at 2 days after calving, and serum NEFA concentrations at 7 and 14 days before calving). A significant multiple regression equation was obtained with the result of the analysis ($R^2 = 0.564$, $P < 0.01$), and the calving score, average milk yield during the first week after calving, serum Ca concentration at 2 days after calving and serum NEFA concentration at 7 days before calving were selected as explanatory variables (Table 2.6). The correlation coefficients among these four explanatory variables were not significant ($P > 0.05$).

Table 2.3. Dry matter intake (DMI), calving scores, body weight (BW) and milk yield in first lactation heifers and multiparous cows.

	Parity number						*P*-value
	1[†]	2	3	4	5	6	
Number of cows	15	23	15	7	13	10	
DMI[‡], g/BW$^{0.75}$/day							
Days after calving							
1	84	89	92	82	75	72	0.513
2	86^{c}	117^{b}	138^{a}	125^{ab}	119^{ab}	79^{c}	< 0.01
3	86^{d}	125^{bc}	147^{a}	142^{ab}	131^{abc}	111^{c}	< 0.01
4	82^{c}	131^{b}	151^{a}	147^{ab}	136^{ab}	127^{b}	< 0.01
5	87^{c}	129^{ab}	144^{a}	147^{a}	137^{ab}	121^{b}	< 0.01
6	92^{c}	130^{ab}	144^{a}	144^{a}	144^{a}	117^{b}	< 0.01
7	92^{c}	130^{ab}	149^{a}	147^{ap}	148^{a}	123^{b}	< 0.01
BW of dams, kg							
Days after calving							
–14	670^{c}	733^{b}	764^{b}	816^{a}	847^{a}	817^{a}	< 0.01
–7	681^{d}	744^{c}	775^{bc}	821^{ab}	857^{a}	827^{a}	< 0.01
1	611^{d}	672^{c}	718^{b}	761^{ab}	786^{a}	757^{ab}	< 0.01
7	583^{d}	653^{c}	701^{b}	739^{ab}	771^{a}	735^{ab}	< 0.01
BW change in dams, kg/day							
Period, days after calving							
From –14 to –7 days	1.69	1.52	1.49	0.78	1.41	1.43	0.913
From –7 to 1 days	–10.10	–10.21	–8.07	–8.59	–10.14	–10.09	0.475
From 1 to 7 days	–3.96	–2.71	–2.47	–3.06	–2.11	–3.07	0.816
BCS of dams, kg							
Days after calving							
–14	3.52	3.52	3.52	3.50	3.54	3.53	0.998
–7	3.52	3.52	3.50	3.50	3.54	3.53	0.992
1	3.42	3.46	3.48	3.39	3.50	3.43	0.677
7	3.30	3.36	3.37	3.36	3.35	3.28	0.811
BW of calves at birth, kg	41.4^{d}	44.9^{c}	47.2^{bc}	48.1^{abc}	51.6^{a}	48.6^{ab}	< 0.01
Calf BW/Dam BW[§], %	6.75	6.70	6.59	6.31	6.63	6.45	0.894
Calving score[¶]	1.93^{a}	1.26^{b}	1.27^{b}	1.29^{ab}	1.15^{b}	1.50^{ab}	0.064
Average milk yield[‖], kg/day	25.4^{d}	35.8^{c}	37.7^{b}	39.1^{ab}	39.9^{a}	41.0^{a}	< 0.01

[†]Pregnant or first lactation heifers. [‡]Average DMI 6 days after calving. [§]Ratio of calf BW at birth to dam BW at calving. [¶]Calving scores recorded according to the procedure of Proudfoot et al. (2009a). [‖]Average milk yield during the first week after calving. Means with different superscripts within a row indicate significant differences ($P < 0.05$).

Table 2.4. Changes in metabolic parameters from 14 days before to 7 days after calving by parity number.

Days after calving	Parity number						SEM	Parity number						SEM
	1[†]	2	3	4	5	6		1	2	3	4	5	6	
	NEFA, μEq/L							BHBA, μmol/L						
–14	350^{a}	160^{b}	167^{b}	183^{b}	167^{b}	201^{b}	19.1	461	408	472	482	397	400	18.7
–7	330^{a}	215^{b}	177^{b}	217^{ab}	231^{ab}	275^{ab}	20.2	484	432	446	431	441	356	18.4
0	842^{a}	564^{b}	537^{b}	647^{ab}	801^{a}	717^{ab}	31.0	772^{a}	482^{b}	464^{b}	509^{ab}	568^{ab}	591^{ab}	42.6
2	783^{a}	507^{bc}	387^{c}	405^{bc}	645^{ab}	665^{ab}	34.8	890^{a}	612^{b}	554^{b}	520^{b}	828^{a}	777^{ab}	33.1
7	880^{a}	547^{b}	664^{b}	569^{b}	877^{a}	642^{ab}	38.1	1297^{a}	677^{b}	755^{b}	626^{b}	946^{ab}	891^{b}	58.4
	TP, g/dL							Alb, g/dL						
–14	6.3^{c}	7.2^{b}	7.3^{ab}	7.6^{ab}	7.4^{ab}	7.6^{a}	0.07	3.4	3.5	3.5	3.6	3.5	3.5	0.02
–7	6.1^{c}	7.0^{b}	7.1^{ab}	7.2^{ab}	7.2^{ab}	7.4^{a}	0.07	3.3^{c}	3.4^{bc}	3.5^{a}	3.5^{ab}	3.5^{a}	3.5^{abc}	0.02
0	6.5^{c}	6.6^{c}	6.7^{bc}	7.1^{ab}	7.3^{a}	7.5^{a}	0.08	3.4^{bc}	3.3^{c}	3.5^{b}	3.6^{ab}	3.7^{a}	3.6^{ab}	0.03
2	6.5^{b}	6.7^{b}	6.7^{b}	6.9^{ab}	7.2^{a}	7.3^{a}	0.06	3.3^{cd}	3.3^{d}	3.4^{bc}	3.4^{abc}	3.6^{a}	3.5^{ab}	0.02
7	7.0^{b}	7.1^{ab}	7.4^{ab}	7.2^{ab}	7.4^{a}	7.4^{ab}	0.06	3.4^{b}	3.3^{b}	3.6^{a}	3.5^{ab}	3.6^{a}	3.4^{b}	0.03
	BUN, mg/dL							Ca, mg/dL						
–14	13.1	13.1	13.3	13.0	12.2	11.5	0.32	9.6	9.7	9.6	9.8	9.7	9.8	0.04
–7	12.5^{a}	11.7^{ab}	11.9^{ab}	12.3^{ab}	10.5^{b}	11.1^{ab}	0.26	9.7^{ab}	9.6^{ab}	9.5^{ab}	10.0^{a}	9.7^{ab}	9.4^{b}	0.05
0	13.2^{a}	10.6^{b}	10.4^{b}	11.7^{ab}	11.4^{ab}	10.9^{ab}	0.36	8.8^{a}	8.2^{b}	8.1^{bc}	7.3^{cd}	7.1^{de}	6.3^{e}	0.13
2	9.9	9.5	9.0	8.8	9.2	10.5	0.36	9.2^{ab}	8.9^{b}	9.4^{ab}	9.6^{ab}	9.5^{a}	8.1^{c}	0.11
7	12.1^{a}	9.4^{b}	10.6^{ab}	10.2^{ab}	10.0^{ab}	10.3^{ab}	0.36	9.7^{a}	9.6^{a}	9.6^{a}	9.8^{a}	9.4^{a}	8.6^{b}	0.08
	P, mg/dL													
–14	6.1	5.7	5.9	5.7	5.7	6.0	0.08							
–7	5.9	6.0	5.8	5.6	5.6	5.7	0.09							
0	4.8^{ab}	5.1^{a}	4.4^{abc}	3.3^{bc}	3.8^{bc}	3.7^{bc}	0.17							
2	5.1	5.6	5.7	6.0	6.0	5.1	0.14							
7	5.5^{a}	5.1^{ab}	5.3^{ab}	4.8^{ab}	4.8^{ab}	4.5^{b}	0.11							

SEM, Standard error of means; NEFA, serum nonesterified fatty acid; BHBA, β-hydroxybutyrate; TP, total protein; Alb, albumin; BUN, blood urea nitrogen; Ca, calcium; P, phosphorus. [†]Pregnant or first lactation heifers. Means with different superscripts within a row indicate significant differences ($P < 0.05$).

Table 2.5. Correlation coefficients for the average DMI during the first week of lactation, parity number, serum metabolic parameters, BW, BW change, BCS and milk yield around calving.

	Days after calving									
	–14		–7		0†		2		7	
	DMI	Parity	DMI	Parity	DMI	Parity	DMI	Parity	DMI	Parity
Serum metabolic parameters										
NEFA, μEq/L	–0.403**	–0.191	–0.369**	–0.089	–0.281*	0.049	–0.421**	–0.029	–0.201	–0.019
BHBA, μmol/L	0.031	0.078	0.118	–0.184	–0.306**	–0.072	–0.385**	0.018	–0.388**	–0.101
TP, g/dL	0.408**	0.578**	0.359**	0.589**	0.062	0.560**	0.124	0.506**	0.062	0.246*
Alb, g/dL	0.354**	0.131	0.363**	0.291*	0.192	0.409**	0.387**	0.434**	0.445**	0.213
BUN, mg/dL	–0.027	–0.169	–0.163	–0.201	–0.140	–0.103	–0.178	0.003	–0.084	–0.089
Ca, mg/dL	0.018	0.131	0.051	–0.053	–0.195	–0.649**	0.388**	–0.120	0.057	–0.333**
P, mg/dL	–0.025	–0.026	0.140	–0.161	0.009	–0.363**	0.329**	0.062	–0.045	–0.286*
BW, kg	0.263*	0.717**	0.254*	0.710**	0.316**	0.690**	–	–	0.419**	0.684**
BW change‡, kg/day	–		–0.070	–0.067	0.225*	–0.016	–	–	0.397**	–0.085
BCS	0.141	0.025	0.133	0.026	0.252*	0.131	–	–	0.294**	0.033
Average milk yield§, kg/day	–	–	–	–	0.317**	0.401**	–	–	0.593**	0.548**

DMI, average dry matter intake during the first week; NEFA, nonesterified fatty acid; BHBA, β-hydroxybutyrate; TP, total protein; Alb, albumin; BUN, blood urea nitrogen; Ca, calcium; P, phosphorus; BW, body weight; BCS, body condition score. †BW and BCS measurements for day 0 were performed at a day after calving. ‡BW changes from –7 days to 1 days after calving. §Average milk yield during the first week after calving. * $P < 0.05$; ** $P < 0.01$.

Table 2.6. Results of the multiple regression analysis for the average dry matter intake (DMI) during the first week after calving ($R^2 = 0.564$, $P < 0.01$).

Variables	r[†]	Partial regression coefficient	Standard partial regression coefficient	*P*-value[‡]
Serum Ca[§], mg/dL	0.388	8.10	0.265	< 0.001
Average milk yield[¶], kg/day	0.593	2.18	0.482	< 0.001
Calving score[‖]	–0.386	–8.26	–0.217	0.007
Serum NEFA[††]	–0.369	–0.05	–0.250	0.002

[†]r single correlation between the dependent variable and each explanatory variable. [‡]*P*-value for the partial regression coefficient from multiple regression analysis. [§]Serum calcium concentration at 2 days after calving. ¶Average milk yield during the first week after calving. [‖]Calving scores recorded according to the procedure of Proudfoot et al. (2009a). [††]Serum nonesterified fatty acid concentration at 7 days before calving.

2.4. Discussion

In this study, the average DMI during the week after calving ranged from 87 to 138 g/$BW^{0.75}$/day. According to the prediction equation for the DMI of lactating Holstein cows (NRC, 2001), the predicted DMI for the first week after calving is 100 g/$BW^{0.75}$/d when the BW and fat corrected milk yield of a cow are 650 kg and 25 kg/d, respectively. It seemed that the DMI during the first week after calving in this study was within the standard DMI range for Holstein cows.

A quadratic relationship between the number of parities and the average DMI during the first week after calving was observed in this study (Figure 2.1). It was

reported that the DMI after calving was lower in first lactation heifers than in multiparous cows (Azizi et al., 2009; Janovick & Drackley, 2010), but the studies did not analyze the DMI during the first week after calving. The results of the present study also indicated that the DMI during the first week after parturition was lower in the first lactation heifers than in the multiparous cows. Moreover, the results of the present study showed a decrease in the DMI during the first week after parturition in the cows in the parity 6 groups. Marquardt et al. (1977) reported that paretic aged cows with reduced serum Ca presented a lower feed intake on calving days than nonparetic aged cows with increased serum Ca at calving. Grandl et al. (2016) reported that NDF intake was related to age in a linear curve and suggested a decrease in the fiber degradation ability in aged cows. While milk yield increases with increasing parity number, factors such as increased morbidity and decreased digestive performance, which negatively affect DMI, might have a strong impact on DMI. Regardless, the results of the current study indicated that the influence of parity number on the DMI during the first week after calving might be curvilinear and estimated that the DMI would be maximized at a parity of 3.7. It is important to promote DMI, especially in first lactation heifers and high parity number cows, to extend the longevity of dairy cows. According to a survey in the United States of America (Hare et al., 2006), the number of cows without subsequent lactation was highest in those with a parity of 1, and the survival rates to a 2 and 3 parities were 73.3% and 50.3%, respectively.

The average DMI during the first week after calving was negatively associated with the prepartum serum NEFA concentration and positively associated with the prepartum serum TP concentration, and the first lactation heifers showed a higher

serum NEFA concentration and lower serum TP concentration before calving than the multiparous cows (Table 2.4). This implied that a poor nutritional status before calving suppressed the DMI after calving. The high serum NEFA and low serum TP concentrations before calving might have indirectly influenced the DMI after calving. Hammon et al. (2006) and Ribeiro et al. (2013) reported that the serum NEFA concentration before calving was positively associated with the prevalence of uterine diseases. In the present study, the serum NEFA concentration at 14 days before calving was positively associated with the calving score, and the calving score was negatively associated with the DMI after calving (Table 2.5). If the serum NEFA concentration is high before calving, calf delivery is likely to be difficult, and as a result, the DMI after calving might be decreased.

On the other hand, a high serum NEFA concentration and low serum TP concentration were observed in the first lactation heifers compared with the multiparous cows (Table 2.4), and the average DMI during the first week after calving was lower in the first lactation heifers than in the multiparous cows (Figure 2.1). The first lactation heifers were more likely to be restricted in their feeding behavior before and after calving than the multiparous cows. A lower DMI in first lactation heifers during the transition period than in multiparous cows has already been reported (Proudfoot et al., 2009b; Grandl et al., 2016; Neave et al., 2017). It is speculated that multiparous cows consume more feed than first lactation heifers because of the relatively large rumen capacity in multiparous cows. The average DMI during the first week after calving in the first lactation heifers was 87 g/$BW^{0.75}$/day (Table 2.5), and the estimated DMI of the pregnant heifers was 86 g/$BW^{0.75}$/day (NRC, 2001). It might be difficult for first lactation heifers to increase their rumen capacity immediately

after calving. Moreover, Neave et al. (2017) showed that first lactation heifers had a lower DMI, spent more time feeding, ate more slowly, visited the feeder more frequently, explored their feeding environment more, and laid down more frequently but for shorter periods than multiparous cows. Beauchemin & Rode (1994) also reported that first lactation heifers ate more slowly than multiparous cows during peak lactation. There are several stressful events, such as diet changes, calving, the onset of lactation, and recovering, associated with calving. These events might be more difficult for first lactation heifers that have not had these experiences previously.

It was shown that the prepartum nutritional status affected the DMI during the first week after calving, especially in the first lactation heifers; however, the causal relationship remains controversial. Moreover, I could not explain the reasons for the poor nutritional status before calving in the first lactation heifers in this study. It is necessary to clarify these factors to increase the DMI of first lactation heifers in the first week after calving.

Calving is a stressful event. The average DMI during the first week after calving was negatively associated with the calving score (Table 2.5), and the first lactation heifers had more calving difficulty than the cows in the parity 2, 3, and 5 groups. A higher incidence of calving difficulty in first lactation heifers than in multiparous cows has already been reported (Berry et al., 2007; Civelek et al., 2008). Reshalaitihan & Hanada (2019) reported that the average DMI 6 days after calving was negatively associated with the calving score and urinary cortisol concentration at 4 days after calving and that the calving score and the urinary cortisol concentration were higher in first lactation heifers than in multiparous cows. It has also been reported that the DMI after calving was lower in cows with dystocia than in cows that

calved normally (Bareille et al., 2003). The prevention of dystocia is necessary for increasing the DMI immediately after calving, especially in first lactation heifers.

In the present study, the calving score was positively associated with the serum NEFA concentration at 14 days before calving. Dyk et al. (1995) reported that an elevated prepartum NEFA concentration is a risk factor for dystocia, retained placenta, ketosis, displaced abomasum, and mastitis in the prepartum period. Several immune cell dysfunctions (Ster et al., 2012), such as peripheral blood neutrophil and uterine disorders (Hammon et al., 2006; Ribeiro et al., 2013), appear to be affected by the precalving NEFA concentration. Therefore, a higher precalving NEFA concentration might increase the calving difficulty by suppressing the immunity of the cows. The difficulty in calving might also be affected by the size of the cow and its calf (Price & Wiltbank, 1978); Berry et al., 2007; Bureš et al., 2008; Civelek et al., 2008). Bureš et al. (2008) demonstrated that first lactation heifers were more prone to dystocia at calving than multiparous cows because they had a smaller pelvic area and lower live weight than multiparous cows. Price & Wiltbank (1978) also reported that pelvic area was highly associated with the calving score in first lactation heifers. The calving score was higher in the first lactation heifers than in the multiparous cows, but the ratio of calf BW to dam BW at calving did not differ between the firstlactation heifer–calf pairs and the multiparous cow–calf pairs, which averaged 6.6% (Table 2.3). It might be necessary to decrease the ratio below 6.6% to prevent dystocia in first lactation heifers.

The average DMI during the first week after calving was positively associated with the serum Ca concentration after calving ($P < 0.01$). These results agreed with those obtained by Wynn et al. (2015) and Reshalaitihan & Hanada (2019). Wynn et al.

(2015) demonstrated that serum Ca concentration during the first week after calving was positively associated with the rumen contraction frequency and the DMI after calving. Goff (2004) reported that cows with lower serum Ca concentrations developed inactive digestive tract motility. The serum Ca concentration considerably affects the DMI immediately after calving because Ca has a role in maintaining muscular contraction activity (Van Breemen & Saida, 1989).

The serum Ca at 0 days after calving was negatively associated with the parity number of the cows (Table 2.5), but the decreased serum Ca concentration at calving recovered to the standard level of 9.0 mg/dl (NRC, 2001) at 2 days after calving in all cows except for the cows in the parity 6 group. Even if the serum Ca concentration decreases at calving, if it recovers quickly, the influence on the DMI after calving might be minimal. However, if the recovery of the Ca concentration is delayed, the DMI might be decreased. Teramura et al. (2015) reported that a slow recovery of the decreased serum Ca concentration at calving to the standard level was associated with increased parity number. An early recovery of the declined serum Ca concentration at calving is important for improving the DMI immediately after calving.

The serum Ca concentration is maintained by bone absorption, intestinal absorption through the transcellular and paracellular pathways, and kidney reabsorption. However, the Ca supply from bone absorption and intestine absorption through the transcellular pathway immediately after calving seemed to be minimal (Teramura et al., 2015). It has already been reported that bone resorption is inactive during the early postpartum period (Moreira et al., 2009; Taylor et al., 2009; Teramura et al., 2015). Moreover, the Ca supply from born resorption and intestinal absorption through the transcellular pathway seemed to decline with age (Horst et al.,

1997; Kurosaki et al., 2007; Yamagishi et al., 2009). Kurosaki et al. (2007) demonstrated that biomarkers of bone metabolic activities were markedly higher in first lactation heifers than in multiparous cows. Horst et al. (1990) reported that the number of vitamin D_3 receptors within the intestinal epithelial cells declined with aging. Yamagishi et al. (2009) reported that the expression of genes for transepithelial calcium-transporting proteins in the bovine duodenum decreased with increasing age. On the other hand, Teramura et al. (2015) reported the promotion of Ca absorption through the paracellular pathway in the intestine and an increase in serum Ca concentration after parturition by difructose anhydrate III supplementation in multiparous cows. The paracellular pathway is a passive absorption pathway that does not involve any carriers; promoting the absorption of Ca through the paracellular pathway could prevent a reduction in the DMI immediately after calving in high parity number cows.

The average DMI during the first week after calving was positively associated with the average milk yield during the first week after calving ($P < 0.01$; Table 2.5), and the average milk yield was positively associated with parity number ($P < 0.01$; Table 2.5). It was speculated that milk yield increased the DMI and not that the DMI increased the milk yield because the daily milk yield increased even though the daily DMI did not increase during the first week after calving in the first lactation heifers. An increasing daily milk yield promoted DMI immediately after calving (Figure 2.2), but it did not contribute to improving the energy balance of the cows. Rather, it was feared that the energy balance might deteriorate as the milk production increased. From the results of the multiple regression analysis (Table 2.6), it was shown that the average DMI during the first week after calving increased by 2.18 g/$BW^{0.75}$/day with

an increase of 1 kg of milk. When the BW was 700 kg, an increase of 1 kg of daily milk yield increased the intake by 0.297 kg of TMR. Since the NEL content of the TMR in this study was 1.51 Mcal/kgDM (Table 2.2), the increase in NEL intake due to the increase of 1 kg of milk was approximately 0.448 Mcal/day. However, the increase in NEL intake was less than the increase in the NEL requirement associated with an increase of 1 kg of milk yield (0.690 Mcal/day, NRC, 2001). These results suggest that a rapid increase in milk yield immediately after parturition is undesirable, especially in first lactation heifers. The response to the increase in the DMI due to the increase in milk production immediately after calving was small in the first lactation heifers (Figure 2.2). The milk yield was positively associated with the DMI immediately after calving, and cows that gradually increase their milk yield after calving would be preferable to cows that produce high milk quantities immediately after parturition to prolong the production time of dairy cows.

2.5. Conclusion

It can be conclude that the influence of parity number on the DMI immediately after calving might be curvilinear, as it tends to be lower in first lactation heifers and high parity number cows, but factors that reduce the DMI differ according to parity number. The results of this study showed the importance of improving the nutritional status of cows before calving to increase the DMI during the first week after calving in first lactation heifers. However, the causal relationship remains controversial, and I cannot explain the reasons for the low nutritional status before calving in the first lactation heifers observed in this study. Moreover, this study showed the importance

of an early serum Ca concentration recovery from its level at calving to increase the DMI during the first week after calving in high parity number cows.

CHAPTER III

[Experiment 2]

Influence of Calving Difficulty on Dry Matter Intake of Dairy Cows Immediately After Calving

3.1 Introduction

The annual milk yield of dairy cows has been increasing, and, at that time, longevity during dairy cow life has been gradually decreasing (Essl, 1998; Hare et al., 2006; Sewalem et al., 2008). In the United States of America, the main reasons for culling dairy cows are reproductive failure, mastitis and udder problems, lameness or injury, other diseases, and poor milk production (USDA, 2007). After calving, dairy cows often have nutrient deficiencies, which lead to reproductive failure and/or metabolic diseases (Yavas & Walton, 2000; Marquezini et al., 2013). Dry matter intake (DMI) after calving in the first lactation heifers is often lower than that in the multiparous cows (Iwama et al., 2004; Janovick & Drackley, 2010). Thus, meeting the nutrient requirements by increasing feed intake immediately after calving, especially in the first lactation heifers, is an important issue to improve the productivity and longevity of dairy cows.

First lactation heifers are prone to dystocia and experience more stress at calving than the multiparous cows because pregnant heifers have a smaller pelvic area, lower live weight, and more frequent difficulty in calving than older cows (Bureš et al., 2008). Because dystocia is painful for cows (Huxley & Whay, 2006), stress from calving difficulty might contribute to the low DMI occurring immediately after calving, especially in the first lactation heifers. Kornmatitsuk et al. (2002) reported that the plasma cortisol concentration and calving score were higher in heifer with moderately difficult calving (required manual assistance) than in heifer with normal calving.

Cortisol has been used as an indicator of stress and pain (Watts & Stookey, 2000). Cortisol can be measured in the saliva, blood, milk, feces and urine to evaluate

acute stress in animals. Artificial stress to animals, such as holding and manipulation, must be minimized when cortisol is used as an index of stress (Cook et al., 2000). Because urine samples can be collected without manipulation of animals, measuring urine cortisol concentrations might be a suitable method for evaluating the stress from calving difficulty. Morrow et al. (2000) reported that monitoring urinary corticosteroid concentration was a valid method for studying and evaluating adrenal activity and acute stress in cattle. Several studies have used urinary cortisol concentration as an index of stress in cows (Morrow et al., 2000; Higashiyama et al., 2005). The aim of this study was to investigate the influence of calving difficulty on the DMI immediately after calving, and to clarify whether urinary cortisol can be used as a stress indicator of cows around calving.

3.2. Materials and Methods

This study was performed from 2015 to 2016 at the Field Science Center of the Obihiro University of Agriculture and Veterinary Medicine in Hokkaido, Japan. Animal care and protocols for the experimental procedures were approved (Approved no. 28-88) by the Animal Care and Use Committee of the Obihiro University of Agriculture and Veterinary Medicine.

3.2.1. Cows and diets

I used 15 pregnant Holstein heifers and 15 multiparous Holstein cows in this study from 35 days before the expected calving date to 7 days after calving. The average parity number in the multiparous cows before calving was 2.53 ± 2.0 (mean ± SD). Before calving, the heifers and the multiparous cows were kept in a free stall barn

with other pregnant cows and were moved into a maternity pen just before calving. On the second day after calving, they were tied in a stall barn for 6 days.

Before calving, the heifers and the multiparous cows were offered 6.2 kg of total mixed ration (TMR, dry matter weight) at 15:00 h and could freely ingest hay, consisting timothy, orchard grass and white clover from a hay rack placed in the paddock attached to the free stall barn. After calving, all cows were offered another TMR and hay ad libitum at 11:00 and 16:00 h. The ingredients of these TMRs are shown in Table 3.1. After calving, the cows were milked at 08:00 and 16:00 h with a bucket milker.

3.2.2. Sampling and laboratory analysis

Amounts of the offered feeds and residues were recorded every day. Feed samples were collected once a week, and the residue samples were collected daily. These samples were dried in an air-forced oven at 60°C for 48 h and then ground using a Wiley mill to pass through a 1-mm screen (SM 2000; Retsch, Haan, Germany). The moisture content in the ground sample was determined by drying at 135°C for 2 h (AOAC, 2003). The ash-corrected neutral detergent fiber content was determined with heat-stable α amylase according to the method described by Van Soest et al. (1991). The ash-corrected acid detergent fiber and acid detergent lignin were also analyzed according to methods of Van Soest et al. (1991). The nitrogen content was determined using the Kjeldahl method (AOAC, 2003), and the crude protein was calculated by multiplying the nitrogen content by 6.25. The ether extract and ash contents were determined by methods described in AOAC (2003). The chemical

compositions of the TMRs and the hays that were offered to cows before and after calving are shown in Table 3.2.

Calving difficulty was evaluated with a. calving score ranged from 1 to 3 as follows: 1 = unassisted delivery (cow calved unassisted), 2 = easy assistance (with the assistance of a person), and 3 = difficult assistance (with the assistance of two or more persons and/or veterinary assistance) (Proudfoot et al., 2009a). Urinary samples were collected from the first urination after 05:30 h at −21, −15, −11, −8, −4, 0, 4, 8, 13, and 21 days after calving. I used a dipper to collect spontaneously voided urine samples without holding the cows. The collected urine was filtered with filter paper to remove debris and stored at −30°C until being assayed for cortisol and creatinine concentration. Cortisol concentration in the urine was determined using a commercial enzyme immunoassay kit (salivary cortisol ELISA Kit, Salimetrics LLC., PA, USA), and the urinary creatinine concentration was determined using a commercial enzyme immunoassay kit (LabAssay^{TM} Creatinine, Wako Pure Chemical Industries, *Ltd.*, Wako, Japan). The cortisol concentration in the urine was divided by the urinary creatinine concentration to correct for urine dilution (Klante et al., 1997).

Blood samples were collected from the caudal vein at –35, 0, 3 and 7 days after calving in evacuated tubes (VENOJECT II, Terumo Corporation, Tokyo, Japan). The serum was separated via centrifugation (Hitachi Centrifuge 05P-21; Hitachi Koki Co., *Ltd*., Tokyo, Japan) at 1,500 × *g* for 10 min and stored at −80 ºC until analysis of the serum calcium (Ca) and the non-esterified fatty acid (NEFA) using an Automatic Clinical Chemistry Analyzer (TBA-12FR, Toshiba Medical Systems Corporation, Tochigi, Japan).

The milk yield was recorded every day, and the body weight (BW) of the cows was measured at −14, 1, and 7 days after calving. The average milk yield 6 days after calving and the average BW for 1 and 7 days after calving were used to estimate net energy requirement for the lactation cows with software in NRC (2001).

3.2.3. Statistical analysis

All data were analyzed in SPSS Student Version for Windows 16.0 (SPSS Inc., Chicago, IL, US). The results obtained from the individual groups are presented as the mean (± SE). The differences in the means between the first lactation heifers and the multiparous cows for calving score, DMI, age of dams at calving, BW of calf at birth, ratio of calf BW at birth to the dam BW at calving, urinary cortisol concentration, blood metabolite concentrations, milk yield, and BW were analyzed with t-tests. One-way ANOVA followed by LSD multiple comparison test was performed to evaluate the effects of sampling time on urinary cortisol concentration and blood metabolite concentrations in each group (the first lactation heifers and the multiparous cows). Correlation coefficients between the average DMI 6 days after calving and the age of dams at calving, BW of calf at birth, ratio of calf BW at birth to the dam BW at calving, calving score, milk yield, urinary cortisol concentration, serum NEFA and Ca levels were calculated with Pearson's procedure by analysis of all first lactation heifers and the multiparous cows. The data were considered significant at $P < 0.05$ unless otherwise noted.

Table 3.1. Ingredients of the total mixed rations offered to the cows before and after calving

Ingredients (Based on fresh weight, %)	Before calving	After calving
Corn silage	52.10	55.10
Grass silage	27.20	27.91
Grass hay	1.07	–
Corn grain	2.00	2.52
Beet pulp	2.32	–
Ear corn silage	0.69	–
Concentrate mix	12.67†	11.29‡
Soybean meal	1.17	2.44
Rice bran	0.51	0.56
Rolled barley	0.16	–
CaCO3	0.03	0.03
MgO	0.02	0.01
Vitamin mineral mix§	0.05	0.14
Molasess	0.05	–

† Dry Base 17 (Hokuren Kumiai Shiryo K. K., Hokkaido, Japan). ‡ Mo-Dairy 18 (Nippon Formula Feed Manufacturing Co. *Ltd*., Kanagawa, Japan). §Contents per gram: 5,000 IU of vitamin A, 1,000 IU of vitamin D, 2 mg of vitamin E, 0.2 mg of vitamin K_3, 0.5 mg of vitamin B_1, 1 mg of vitamin B_2, 0.1 mg of vitamin B_6, 1 ng of vitamin B_{12}, 6 mg of nicotinic acid, 2 mg of choline chloride, 10 mg of pantothenic acid, 156 µg of Mn ($MnSO_4$), 0.7 mg of Zn ($ZnCO_3$), 50 µg of Fe ($FeSO_4$), 139 µg of Cu ($CuSO_4$), 325 µg of I Ca $(IO_3)_2$, 38 µg of Co ($CoSO_4$), 1 mg of methionine, and 0.5 mg of lysine hydrochloride.

Table 3.2. Chemical compositions of the total mixed rations and hay offered to cows before and after calving (mean ± SE; n = 16)

Compositions		TMR		Hay	
		Before calving	After calving	Before calving	After calving
DM	%FM	30.6 ± 1.08	32.1 ± 1.01	83.9 ± 0.88	82.7 ± 1.28
Ash	%DM	6.7 ± 0.14	6.2 ± 0.05	10.4 ± 0.45	10.9 ± 0.19
CP	%DM	14.0 ± 0.23	14.4 ± 0.19	11.7 ± 0.41	11.0 ± 0.40
αNDFom	%DM	41.2 ± 2.79	39.6 ± 1.44	60.4 ± 2.56	60.2 ± 0.69
ADFom	%DM	23.5 ± 1.81	23.1 ± 0.98	35.5 ± 1.50	36.5 ± 0.51
ADL	%DM	3.1 ± 0.39	3.1 ± 0.31	4.5 ± 0.30	4.0 ± 0.09
NFC	%DM	34.9 ± 1.23	36.4 ± 1.09	14.6 ± 0.65	15.0 ± 0.86
EE	%DM	3.2 ± 0.25	3.4 ± 0.23	2.9 ± 0.17	2.9 ± 0.03

DM, dry matter; CP, crude protein; αNDFom, ash-corrected neutral detergent fiber content was determined with a heat-stable α-amylase and expressed exclusive of residual ash; ADFom, ash-corrected acid detergent fiber; ADL, acid detergent lignin; NFC, non-fibrous carbohydrate; NFC = 100 − (CP + EE + αNDFom + Ash); EE, ether extract.

3.3. Results and Discussion

3.3.1. DMI and calving difficulties

The average DMI 6 days after calving was lower in the first lactation heifers than in the multiparous cows ($P < 0.01$). Previous studies have also reported lower feed intake in the first lactation Holstein heifers than in the multiparous Holstein cows (Janovick & Drackley, 2010; Halli et al., 2015).

Table 3.3. Means (± SE) of dry matter intake (DMI) of dams after calving, age of dams at calving, body weight (BW) of calf at birth and their dams at calving, ratio of calf BW at birth to the dam BW at calving, calving score and milk yield 6 days after calving

	Cows		*P*-value
	Heifers	Multiparous	
Number of head	15	15	—
DMI[†], g/$BW^{0.75}$/day	87.1± 4.91	109.1 ± 4.10	< 0.01
Age of dams at calving, month	23.9 ± 0.63	59.2 ± 5.89	< 0.01
BW of dams at calving, kg	559.7 ± 11.52	741.1 ± 14.98	< 0.01
BW of calf at birth, kg	39.0 ± 1.61	44.3 ± 1.20	0.031
Calf BW/Dam BW[‡]	0.07 ± 0.004	0.06 ± 0.002	0.039
Calving score[§]	2.3 ± 0.27	1.4 ± 0.21	< 0.01
Milk yield[¶], kg/day	19.6 ± 0.83	31.2 ± 1.12	< 0.01

[†]Average DMI 6 days after calving.

[‡]Ratio of calf body weight at birth to the dam body weight at calving.

[§]Calving score was recorded according to the procedure of Proudfoot et al. (2009a).

[¶]Average milk yield 6 days after calving.

The average BW of dams at calving was lower in the first lactation heifers than in the multiparous cows (Table 3.3). The average birth weight was lower in calf born from the first lactation heifers than the calf born from multiparous cows ($P < 0.05$). The average ratio of the calf BW to the dam BW at calving was higher in the

first lactation heifer-calf pair than in the multiparous cow-calf pair ($P < 0.05$). The calving score was higher in the first lactation heifers than in the multiparous cows ($P < 0.01$). A higher incidence of calving difficulty in the first lactation heifers with a higher calf birth weight compared to those of multiparous cows has been already reported (Berry et al., 2007; Civelek et al., 2008). The average DMI 6 days after calving was negatively associated with the ratio of calf BW at birth to the dam BW at calving ($P < 0.05$; Table 3.5) and the calving score ($P < 0.01$; Table 3.5; Figure 3.1), but the age and BW of dams at calving and the birth weight of calf had no significant relationship with the average DMI 6 days after calving.

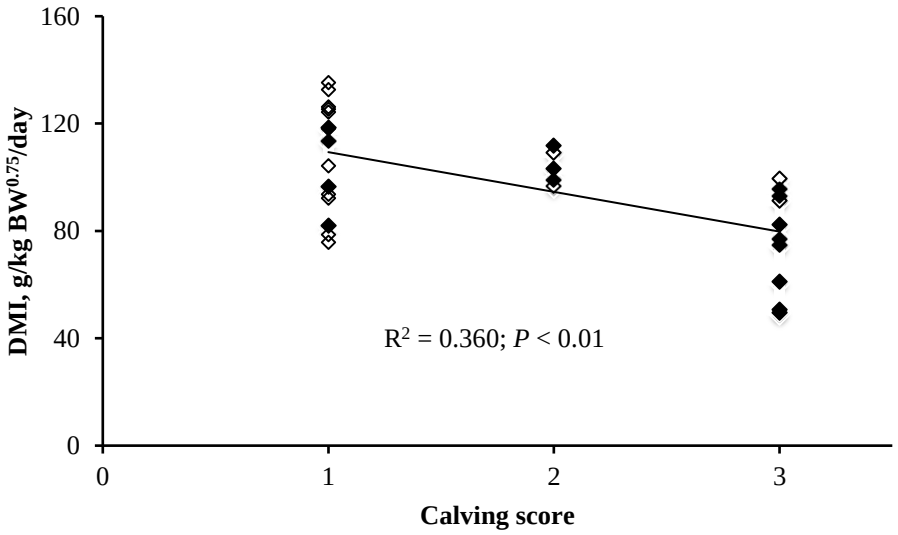

Figure 3.1. Relationship between the average dry matter intake (DMI) 6 days after calving and the calving score in the first lactation heifers (◆, n = 15) and the multiparous cows (◇, n = 15). R^2, correlation of determination; *P*, significant correlation at 0.01; n, number of cows.

3.3.2. Urinary cortisol concentration

The average dates for urine sampling before calving were −22, −16, −11, −8, and −4 days before calving, and the average time for urine sampling on the calving day was 7 hours after calving. The urinary cortisol concentration sharply increased just after calving in all cows (Figure 3.2). The urinary cortisol concentration at 7 hours after calving did not differ between the first lactation heifers and the multiparous cows ($P > 0.05$; Figure 3.2) and was not associated with the calving score ($P > 0.05$; Table 3.5). After peaking, the urinary cortisol concentration declined quickly in the multiparous cows, but it gradually declined in the first lactation heifers. At 4 days after calving, the urinary cortisol concentration was higher in the first lactation heifers than in the multiparous cows ($P < 0.01$). The urinary cortisol concentration at 4 days after calving was positively associated with the calving score ($P < 0.05$; Table 3.5) and was negatively associated with the average DMI 6 days after calving ($P < 0.01$; Table 3.5; Figure 3.3). Thereafter, the urinary cortisol concentration did not differ between the first lactation heifers and the multiparous cows ($P > 0.05$).

Some reports have demonstrated that cows with premature delivery, stillborn calves, and dystocia show high plasma cortisol concentrations at calving (Hudson et al., 1975; Patel et al., 1996; Kornmatitsuk et al., 2002). However, it is challenging to evaluate calving difficulty with urinary or blood cortisol concentration at calving because cortisol concentration at calving is affected not only by stress derived from caving but also by cortisol derived from the fetus (Adams & Wanger, 1970). Fetal cortisol production is elevated from 7 to 9 days before birth and further increases in the last week before birth (Taverne et al., 1988). However, the urinary cortisol concentrations at 4 days after calving were not affected by cortisol derived from the fetus because the half-life of cortisol in blood is 1.55 ± 0.33 h (Lassourd et al., 1996).

Instead, the urinary cortisol concentration at 4 days after calving might be affected not only by calving stress but also by milking and tying the cows to the stall.

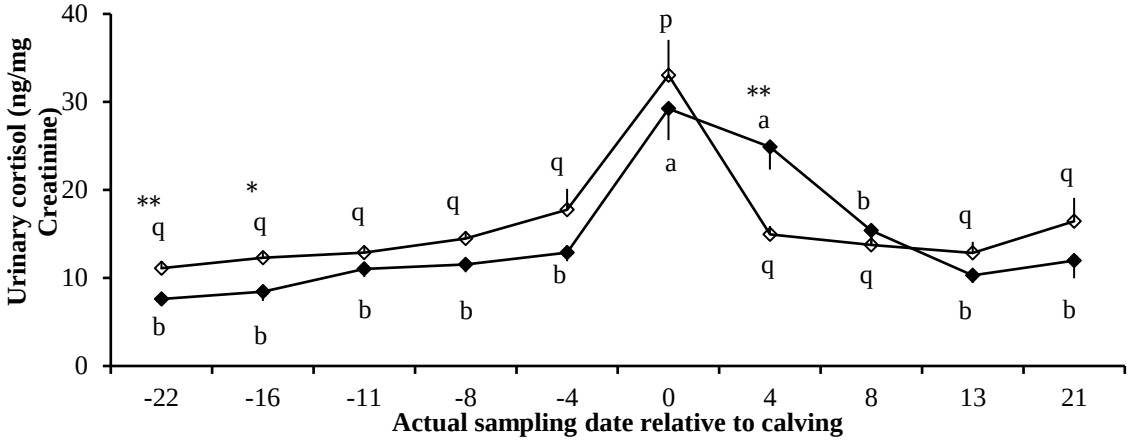

Figure 3.2 Changes in urinary cortisol concentrations from 22 days before to 21 days after calving in the first lactation heifers (◆, n = 15) and the multiparous cows (◇, n = 15). The error bars in time-point measurements indicate the standard errors of the mean. Sampling on day 0 was performed at 7 hours after calving.

[a-b] different superscripts indicate significant differences in the means in the first lactation heifers ($P < 0.05$).

[p-q] different superscripts indicate significant differences in the means in the multiparous cows ($P < 0.05$).

* and ** indicate significant differences between the first lactation heifers and the multiparous cows at $P < 0.05$ and $P < 0.01$, respectively.

Neary et al. (2002) reported that measuring urinary cortisol was a valid method for evaluating the cortisol production rate because it was positively associated with the serum and saliva cortisol concentrations. Civelek et al. (2008) also reported that plasma cortisol concentration was significantly elevated in Holstein heifers with

dystocia at calving. Monitoring urinary cortisol concentration after calving could be a valid method for evaluating the stress derived from calving.

These findings suggest that high urinary cortisol concentration at 4 days after calving in the first lactation heifers is probably due to the stress derived from their first experiences, such as calving, milking by machine and tying to the stall, and these experiences must be one of the reasons for the lower DMI immediately after calving in first lactation heifers compared with multiparous cows.

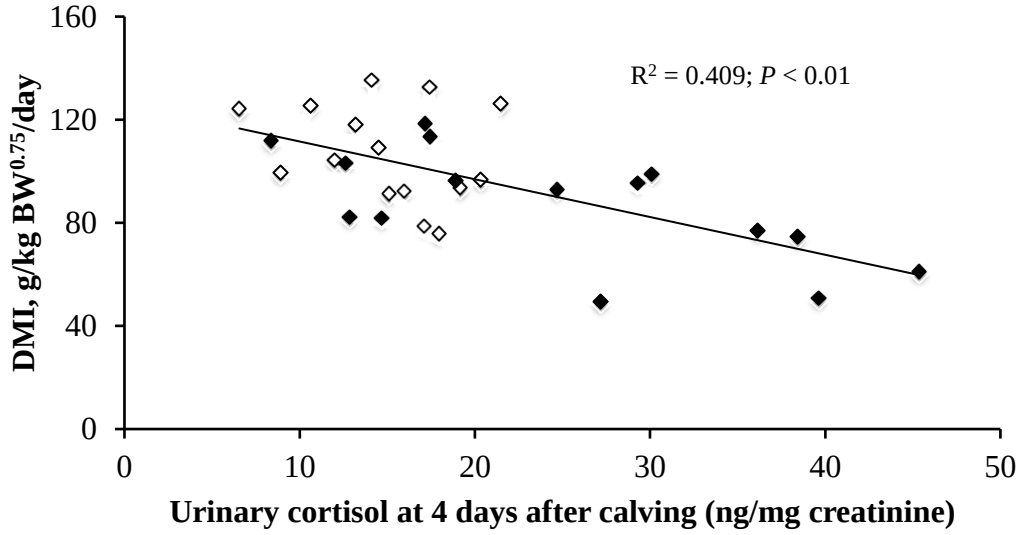

Figure 3.3. Relationship between the average dry matter intake (DMI) 6 days after calving and the urinary cortisol concentration at 4 days after calving in the first lactation heifers (◆, n = 15) and the multiparous cows (◇, n = 15). R^2, correlation of determination; *P*, significant correlation at 0.01; n, number of cows.

3.3.3. Milk yield

The average milk yield 6 days after calving was lower in the first lactation heifers than in the multiparous cows ($P < 0.01$; Table 3.3). The net energy requirement of the cows for lactation calculated by NRC (2001) was higher in the multiparous cows

(21.6 Mcal/day) than in the first lactation heifers (13.5 Mcal/day). The average DMI 6 days after calving was positively associated with the average milk yield 6 days after calving ($P < 0.01$; Table 3.5). Instead of DMI affecting the milk yield, the milk yield would have influenced the DMI because the increase in DMI is generally slower than the increase in milk yield just after calving (NRC, 2001). The low net energy requirement of cows may be one of the reasons for the low DMI immediately after calving in the first lactating heifers compared with the multiparous cows.

Table 3.4. Means (± SE) of serum NEFA and serum Ca concentrations in the first lactation heifers (n = 15) and the multiparous cows (n = 15).

		Days relative to calving			
		-33	0	3	7
NEFA,	First lactation heifers	$330^{b} \pm 80.3$	$300^{b} \pm 36.9$	$512^{a} \pm 65.0$	$558^{a} \pm 83.1$
μEq/L	Multiparous cows	$66^{b} \pm 4.8$	$552^{a} \pm 80.1$	$436^{a} \pm 42.5$	$586^{a} \pm 61.0$
	P-value	0.018	0.020	0.405	0.814
Ca,	First lactation heifers	9.9 ± 0.29	9.0 ± 0.29	8.9 ± 0.31	9.0 ± 0.30
mg/100 ml	Multiparous cows	$9.9^{a} \pm 0.24$	$8.0^{b} \pm 0.45$	$9.4^{a} \pm 0.39$	$9.8^{a} \pm 0.30$
	P-value	0.920	0.114	0.462	0.100

Ca, calcium; NEFA, non-esterified fatty acid. $^{a-b}$ within a row, different superscripts indicate significant differences in means ($P < 0.05$).

3.3.4. Blood metabolites

The average date for blood sampling before calving was 33 days before calving. The results for blood metabolites concentration are shown in Table 3.4. The average

serum NEFA concentration at 33 days before calving was higher in the first lactation heifers than in the multiparous cows ($P < 0.05$). However, the serum NEFA concentrations at calving were lower in first lactation heifers than in multiparous cows ($P < 0.05$). The serum NEFA concentration did not differ between the two groups at 3 and 7 days after calving ($P > 0.05$). The serum NEFA concentrations were not associated with the average DMI 6 days after calving, except for the NEFA concentration at 3 days after calving, which was negatively associated with DMI ($P < 0.05$; Table 3.5).

The serum Ca concentration in multiparous cows decreased at calving ($P < 0.05$) and recovered to the pre-calving values on the third day after calving. However, a significant decline in the serum Ca concentration at calving was not observed in the first lactating heifers.

Serum Ca concentration was positively associated with the DMI at 3 ($P < 0.01$) and 7 ($P < 0.05$) days after calving (Table 3.5). The low serum Ca concentration decreased the movement of the digestive tract of cows because serum Ca has a role in maintaining muscular contractive activity (Johansson, 1987; Van Breemen & Saida, 1989). Cows with low serum Ca concentrations showed inactive digestive tract motility (Goff, 2004). Wynn et al. (2015) demonstrated that serum Ca concentrations after calving were positively associated with the rumen contraction frequency and DMI after calving. Although there was no significant difference in the serum Ca concentration at 3 days after calving between the first lactation heifers and the multiparous cows, the serum Ca concentration generally tends to be low in high parity cows (Teramura et al., 2015; Wynn et al., 2015). First lactation heifers can maintain serum Ca at steady concentrations without any treatments after calving because low

parity cows absorb Ca efficiently, owing to active mechanisms of Ca homeostasis, including Ca absorption from the intestine and bone resorption (Goff et al., 1991; Bronner, 1992; Kamiya et al., 2005). Therefore, low serum Ca concentration is unlikely to cause the low DMI immediately after calving in the first lactation heifers but might affect the DMI in the multiparous cows.

Table 3.5. Correlation coefficients between average DMI 6 days after calving and age of dams at calving, body weight (BW) of calf at birth, ratio of calf BW at birth to the dam BW at calving, calving score, urinary cortisol, milk yield, BW, serum non-esterified fatty acid (NEFA) and calcium (Ca) level in each sampling time (n = 30).

		DMI[†], g/kg $BW^{0.75}$/day	BW of dams at calving, kg	Age of dams at calving, month	BW of calf at birth, kg	Calf BW /Dam BW[‡]	Calving score[§]	Milk yield[¶], kg/day	Urinary cortisol, ng/mg Creatinine			Serum metabolites						
												NEFA, μEq/L				Ca, mg/100 ml		
Days[‖]									0[††]	4	8	-33	0	3	7	-33	0	3
BW of dams at calving		0.295																
Age of dams at calving		0.048	0.645**															
BW of calf at birth		-0.117	0.338	0.284														
Calf BW/Dam BW		-0.419*	-0.562**	-0.314	0.580**													
Calving score		-0.665**	-0.223	-0.267	-0.033	0.228												
Milk yield		0.658**	0.722**	0.393*	0.274	-0.377	-0.383											
Urinary cortisol	0	-0.109	0.104	0.022	0.154	0.114	0.130	-0.045										
	4	-0.669**	-0.427*	-0.267	-0.045	0.378	0.551*	-0.603**	0.184									
	8	-0.482*	-0.086	-0.017	0.202	0.355	0.402*	-0.314	0.198	0.644**								
NEFA	-33	0.256	-0.364	-0.335	-0.034	0.270	0.041	-0.011	-0.109	-0.089	0.029							
	0	-0.157	0.400*	0.470*	0.351	-0.054	-0.123	0.232	0.245	-0.103	0.083	-0.038						
	3	-0.482*	-0.242	0.008	0.168	0.348	0.307	-0.132	-0.044	0.067	0.187	0.082	0.125					
	7	-0.309	0.201	0.435*	0.037	-0.134	0.041	-0.063	0.132	0.299	0.382	-0.150	0.121	-0.091				
Ca	-33	-0.029	-0.025	0.065	0.345	0.281	0.109	-0.064	-0.137	-0.028	0.221	0.032	0.005	-0.106	-0.075			
	0	0.329	-0.227	-0.294	-0.063	0.164	0.138	0.009	-0.100	0.033	0.116	0.480*	-0.453*	-0.241	-0.127	0.25		
	3	0.683**	0.073	-0.126	0.067	-0.031	-0.059	0.322	0.018	-0.103	0.367	0.312	-0.175	-0.189	0.015	0.131	0.391	
	7	0.505*	0.115	0.060	0.139	0.032	-0.360	0.314	-0.035	-0.341	-0.060	0.252	-0.145	-0.397	-0.036	0.277	0.329	0.438*

[†]Average DMI 6 days after calving. [‡]Ratio of calf body weight at birth to the dam body weight at calving. [§]Calving score was recorded according to the procedure of Proudfoot et al. (2009a). [¶]Average milk yield 6 days after calving. [‖]Days after calving. [††]The urine sampling on day 0 was performed at 7 hours after calving. * and ** indicate significant correlations between the factors at $P < 0.05$ and $P < 0.01$, respectively.

3.4. Conclusion

Dry matter intake immediately after calving is influenced not only by nutrient requirements of cows but also stress caused by calving difficulty, milking or tying to the stall. The low dry matter intake in the first lactation heifers compared with multiparous cows is due to the lower energy requirement and high susceptibility to the stress in the first lactation heifers. Moreover, this study shows that monitoring urinary cortisol concentration after calving is a valid method for evaluating the stress derived from calving.

CHAPTER IV

[Experiment 3]

Effect of Oxidative Status on Dry Matter Intake of Dairy Cows Immediately After Calving

4.1. Introduction

The annual milk yield of cows has been increased, in opposite direction of the decreasing productive lifetime of cows in the world. The average productive lifetime has declined from about 3.5 in 1970 to currently between 2.5 and 3.0 lactations in the world (Martens & Bange, 2013). That means many cows leave the herd already after 2 or 3 calving, before reaching the maximum of their production capacity. As I explained in previous experiment meeting nutrient requirements by increasing the feed intake of dairy cows as immediately as possible after calving is one of the most important countermeasures to prevent postpartum health and fertility problems and to increase parity number in dairy cows. The transition from gestation to lactation is the most critical moment in the lactating cycle of dairy cows. This period is characterized by several orchestrated metabolic and endocrine changes as a consequence of the increased metabolic demands that aim to support the milk production (Bell, 1995).

In human, it has been demonstrated that oxidative stress was implicated in the progression of major health problems by inactivating the metabolic enzymes and damaging important cellular components, oxidizing the nucleic acids, leading to cardiovascular diseases, eye disorders, joint disorders, neurological diseases (Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis), atherosclerosis, lung and kidney disorders, liver and pancreatic diseases, cancer, ageing, disease of the reproductive system including the male and female infertility etc. (Rahman et al., 2012). Recent studies in dairy cattle support the concept that oxidative stress is a significant underlying factor to dysfunctional host immune and inflammatory responses that can increase the susceptibility of cattle to a variety of

health disorders, particularly during the transition period (Allison & Laven, 2000; Bernabucci et al., 2005; Castillo et al., 2005; Sordillo, 2005; Wilde, 2006).

The considerable increase in oxygen requirements due to increase of metabolic demands after calving results in augmented production of reactive oxygen species (ROS). An imbalance between production of ROS and the availability of antioxidant defenses are needed to reduce oxidative stress during the periparturient period when cows are exposed to a lot of oxidative stress (Bernabucci et al., 2002). Konvičná et al. (2015) reported that the mean malondialdehyde, indicator of oxidative stress, concentration was significantly higher in the cows 1 week after calving compared to the cows 3, 6, and 9 weeks after calving. Failure to adequately control the accumulation of ROS will result in oxidative stress and possibly increased health disorders in high producing dairy cattle (Sordillo & Aitken, 2009). LeBlanc et al. (2004) demonstrated that the oxidative stress might contribute to an impaired immune function and an enhanced susceptibility to periparturient diseases in dairy cows. Mastitis, metritis, and incidence of retained placenta are three common diseases linked to a compromised immune system. Castillo et al. (2003) reported that the cows with a high milk yield present higher plasma lipid hydroperoxides levels than the other group. Cows with high milk yields have higher levels of oxidative stress than low milk yield cows, resulting in a higher incidence of health problem and/or reproductive disorders (Castillo et al. 2003; Lohrke et al. 2004; Bernabucci et al. 2005). Sordillo & Aitken (2009) suggested that oxidative stress during the periparturient and early lactation period might contribute to a number of health disorders in dairy cattle. From these facts it is expected that the intake of dairy cows will also be affected by oxidative stress, but few studies have examined the effects of

oxidative stress on DMI of dairy cows immediately after calving. The objective of the experiment 3 was to investigate the relationship between the oxidative status and DMI immediately after calving of dairy cows.

4.2. Materials and Methods

This study was performed from 2012 to 2016 at the Field Science Center of the Obihiro University of Agriculture and Veterinary Medicine in Hokkaido, Japan. Animal care and protocols for the experimental procedures were approved (No. 25-20, 26-9 and 28-88) by the Animal Care and Use Committee of the Obihiro University of Agriculture and Veterinary Medicine.

4.2.1. Cows and diets

Sixty-two Holstein cows were monitored from the calving to 21 days after calving. The numbers of the cows in first calving heifers and parity 2, 3, and 4 or more were 20, 18, 7 and 17 heads, respectively. When calving signs were observed, the cows were moved into an individual maternity pen and kept in the maternity pen up to 6 days after calving then moved to another free stall barn for milking cows. After calving, the all cows were offered total mixed ration (TMR) and grass hay ad libitum. The TMRs mainly consisted with grass and corn silages and a concentrate (Table 4.1). The cows could always access to fresh water and mineral blocks (KNZ salt licks, Hengelo, the Netherlands) freely through the experiment.

4.2.2. Sampling and laboratory analysis

The amount of feed offered and residues were recorded daily from the calving to 6

days after calving. The feed samples were taken once a week and the residues samples were taken every day. These samples were dried in an air-forced oven at 60 °C for 48 h, then ground using a Wiley mill to pass 1-mm screen (SM 2000; Retsch, Haan, Germany). The moisture content in the ground sample was determined by drying at 135°C for 2 h (AOAC, 2003). The ash-corrected neutral detergent fiber (αNDFom) content was determined with a heat-stable α-amylase and expressed exclusive of residual ash according to the method described by Van Soest et al. (1991). The ash-corrected acid detergent fiber (ADFom) was also analyzed according to methods of Van Soest et al. (1991). The nitrogen content was determined using the Kjeldahl method (AOAC, 2003), and crude protein (CP) was calculated by multiplying the nitrogen content by 6.25. Chemical compositions of the diets are shown in Table 4.2. Calving difficulty was evaluated with a calving score ranged from 1 to 3 as follows: 1 = unassisted delivery (cow calved unassisted), 2 = easy assistance (with the assistance of a person), and 3 = difficult assistance (with the assistance of two or more persons and/ or veterinary assistance) (Proudfoot et al., 2009a). Body weight (BW) and body condition score of the cows was measured before the morning feeding 1, 7, and 21 days after calving. The cows were milked at 04:00 and 17:00 h, and milk yield was recorded twice per day.

Blood samples were taken from caudal vein of individual cow at 0, 7, and 21 days after calving using 9 ml evacuated tubes (VENOJECT II, Terumo Corpration, Tokyo, Japan). All tubes for serum collection were placed on ice immediately after the sampling, and were centrifuged at 1,500 × *g* for 10 min at room temperature by a centrifuge (05P-21, HITACHI) within 4 hours after collection. The supernatant was stored at –80 °C until analysis. Blood metabolites were analyzed using Automatic

Clinical Chemistry Analyzer (TBA-12FR, Toshiba Medical Systems Corporation, Tochigi, Japan). Reactive oxygen metabolites (ROM), biological antioxidant potential (BAP) were measured by use Free Radical Evaluator (WISMERLL) and oxidative stress index (OSI) was calculated by the formula ROMs/BAP * 100 = OSI (Celi, 2011). The ratio of increase rate of DMI to increase rate of milk yield during 6 days after calving were calculated as follows:

The ratio of increase rate of DMI to increase rate of milk yield during 6 days after calving = ((DMI a day after calving － DMI 6 days after calving) / 5) / ((Milk yield a day after calving － Milk yield 6 days after calving) / 5)

Serum nonesterified fatty acid, β-hydroxybutyric acid, glucose, calcium, phosphors, total protein, albumin, blood urea nitrogen, gamma-glutamyl transpeptidase and glutamic-oxalacetic transaminase were analyzed using an automatic clinical chemistry analyzer (TBA-12FR, Toshiba Medical Systems Corporation, Tochigi, Japan).

4.2.3. Statistical analysis

All data were analyzed in SPSS Student Version for Windows 16.0 (SPSS Inc., Chicago, IL, US). One-way analysis of variance was used to analyze effect of the parity, BW of dam, BW of calf at birth, ratio of calf BW at birth to the dam BW at calving, calving score, milk yield ROM, BAP, and OSI on the DMI and difference of the means among the parity was analyzed by LSD multiple comparison tests. Correlation coefficients between the average DMI 6 days after calving, BW of dam, BW of calf at birth, ratio of calf BW at birth to the dam BW at calving, calving score, average milk yield 7 days after calving, ROM, BAP, and OSI were calculated with

Pearson's procedure. The data were considered significant at $P < 0.05$ unless otherwise noted.

Table 4.1. Ingredients of the total mixed ration (TMR) offered to the cows after calving.

Ingredients (Based on dry matter weight, %)	TMR
Corn silage	49.27
Grass silage	28.04
Corn grain	1.26
Concentrate mix	16.89†
Soybean meal	2.52
Rice bran	1.72
$CaCO_3$	0.03
MgO	0.04
Vitamin mineral mix‡	0.26

† Mo-Dairy 18 (Nippon Formula Feed Manufacturing Co. *Ltd*., Kanagawa, Japan). ‡Contents per gram: 5,000 IU of vitamin A, 1,000 IU of vitamin D, 2 mg of vitamin E, 0.2 mg of vitamin K_3, 0.5 mg of vitamin B_1, 1 mg of vitamin B_2, 0.1 mg of vitamin B_6, 1 ng of vitamin B_{12}, 6 mg of nicotinic acid, 2 mg of choline chloride, 10 mg of pantothenic acid, 156 µg of Mn ($MnSO_4$), 0.7 mg of Zn ($ZnCO_3$), 50 µg of Fe ($FeSO_4$), 139 µg of Cu ($CuSO_4$), 325 µg of I Ca $(IO_3)_2$, 38 µg of Co ($CoSO_4$), 1 mg of methionine, and 0.5 mg of lysine hydrochloride.

Table 4.2. Chemical composition of the total mixed ration (TMR) and grass hay offered to cows after calving.

Compositions		TMR	Hay
DM	%FM	36.0	84.2
CP	%DM	14.9	12.8
αNDFom	%DM	40.5	61.8
ADFom	%DM	23.7	37.0
Lignin	%DM	3.3	4.2
EE	%DM	3.6	2.7
Ash	%DM	7.1	10.1
NFC	%DM	33.9	12.7

DM, dry matter; CP, crude protein EE, ether extract; αNDFom, ash-corrected neutral detergent fiber content was determined with a heat-stable α-amylase and expressed exclusive of residual ash; ADFom, ash-corrected acid detergent fiber; NFC, non-fibrous carbohydrate; NFC = 100 − (CP + EE + αNDFom + Ash).

4.3. Results and Discussion

The average DMI 6 days after calving of the first lactation heifers and the cows in parity 2, 3, and 4 or more were 86.1, 124.1, 124.1, and 117.3 g/$BW^{0.75}$/d, respectively ($P < 0.05$; Table 4.3), a quadric relationship between parity number and the average DMI during the first week after calving was observed ($P < 0.01$; Table 4.4) as in case of experiment 1. The average DMI 6 days after calving was lower in the first lactation heifers than in the multiparous cows ($P < 0.05$). The lower DMI in first lactation

heifers than in multiparous cows immediately after calving was also reported by Halli et al. (2015) and Janovick & Drackley (2010).

The average birth weight of calf at birth and the ratio of calf BW to the dam BW at calving were no difference according to parity number ($P > 0.05$; Table 4.3), However, the birth weight of calf at birth was negatively associated with the average DMI 6 days after calving ($P < 0.01$; Table 4.4). The ratio of calf BW to the dam BW at calving was also negatively associated with the average DMI 6 days after calving ($P < 0.01$; Table 4.4) as in case of experiment 2. The calving score was higher in the first lactation heifers than the cows in parity 2 and 4 or more groups ($P < 0.05$; Table 4.3), and positively associated with the average DMI 6 days after calving ($P < 0.01$; Table 4.4). A higher incidence of calving difficulty in the first lactation heifers with a higher calf birth weight compared to those of multiparous cows has been already reported (Berry et al., 2007; Civelek et al., 2008). The high ratio of calf weight to dam's body weight might be one of the reasons of lower DMI immediately after calving in the first lactation heifers, because it positively associated with the calving difficulty ($P < 0.01$; Table 4.4). Although intake of antioxidants was not measured in this study, it was probably high in the multiparous cows.

The average milk yield 7 days after calving was higher in the multiparous cows than in the first lactation heifers ($P < 0.01$; Table 4.3), and it was positively associated with average DMI immediately after calving ($P < 0.01$; Table 4.4). Synthesis of peroxide in cows was probably high in the multiparous cows compared with the first lactation heifers, because peroxide production is enhanced as milk yield increases (Aoki et al., 2010).

Table 4.3. Dry matter intake (DMI), calving scores, body weight (BW), body condition score (BCS), milk yield, and oxidative stress indicators in first lactation heifers and multiparous cows.

		Parity number				SEM	*P*-value
Days after calving		1[†]	2	3	4 or more		
Number of cows		20	18	7	17		
DMI[‡], g/BW$^{0.75}$/day		86.1^{b}	124.1^{a}	124.2^{a}	117.3^{a}	3.86	0.342
BW of dams, kg							
	0	563.7^{c}	686.5^{b}	739.1^{a}	761.5^{a}	12.29	0.060
	7	541.6^{c}	661.0^{b}	711.6^{a}	734.6^{a}	11.84	0.058
	21	537.7^{c}	627.9^{b}	665.3^{ab}	700.5^{a}	10.57	0.038
BCS of dams							
	0	3.0^{b}	3.3^{a}	3.5^{a}	3.4^{a}	0.04	0.263
	7	2.9^{b}	3.3^{a}	3.3^{a}	3.3^{a}	0.04	0.348
	21	2.9^{c}	3.0^{bc}	3.3^{a}	3.2^{ab}	0.04	0.124
BW of calves at birth, kg		39.1	37.2	37.5	38.8	0.99	0.710
Calf BW/Dam BW[§], %		7.1	5.4	5.1	5.1	0.20	0.335
Calving score[¶]		2.3^{a}	1.3^{b}	1.6^{ab}	1.4^{b}	0.11	0.341
Milk yield[‖], kg/day		16.7^{b}	35.1^{a}	39.6^{a}	40.6^{a}	1.81	0.243
Oxidative status indicators							
ROM[††],	0	73.0^{b}	95.7^{a}	83.9^{ab}	86.4^{ab}	3.51	0.337
U CARR	7	90.5	96.8	104.7	106.9	5.61	0.605
	21	80.5	98.5	101.6	90.1	3.94	0.391
BAP[‡‡],	0	1868.9$^{b/p}$	2462.9^{a}	2506.1^{a}	2204.5$^{ab/q}$	91.64	0.291
µ mol/L	7	1539.3$^{b/q}$	2507.0^{a}	2805.9^{a}	2622.9$^{a/pq}$	113.48	0.303
	21	1861.2$^{b/p}$	2924.1^{a}	2546.8^{a}	2935.3$^{a/p}$	129.52	0.305
OSI[§§],	0	3.9^{q}	3.9	3.3	3.9^{p}	0.01	0.753
U CARR L/µ mol	7	5.9^{p}	3.9	3.7	4.1^{p}	0.38	0.482
	21	4.3$^{a/q}$	3.4^{ab}	4.0^{ab}	3.1$^{b/q}$	0.01	0.303

[†]First lactation heifers. [‡]Average DMI 6 days after calving. [§]Ratio of calf BW at birth to dam BW at calving. [¶]Calving scores recorded according to the procedure of Proudfoot et al. (2009a). [‖]Average milk yield during the first week. [††]Reactive oxygen metabolites. [‡‡]Biological antioxidant potential. [§§]Oxidative stress index. Means with different superscripts within a row indicate significant differences ($P < 0.05$).

Serum ROM concentration ranged from 73 to 106 U.CARR in this study, and the range was almost the same as the values of Mirzad et al. (2018). They reported that serum ROM concentration ranged from 77.2 to 101.6 U. CARR in the Holstein Friesian cows sampled from 4 weeks before to 4 weeks after calving. Serum ROM concentration were lower in first lactation heifers than in multiparous cows at 0 days after calving ($P < 0.05$). Serum ROM concentration at 7 and 21 days after calving did not differ among the parities ($P > 0.05$; Table 4.3), but it was numerically lower in the first lactation heifers compared with the multiparous cows. There were positive relationships between the serum ROM concentrations and the average milk yield 6 days after calving ($P < 0.05$, Table 4.4). Jóźwik et al. (2012) reported that the increased production of milk led to an increase of energy demand of cows and increase of ROS generation. The high milk productivity associates with oxidative stress due to the increase of cellular metabolism (Castillo et al., 2003; Lohrke et al., 2005). It was recognized in this study that the synthesis of peroxide increased with the increase of milk production.

Serum BAP concentration ranged from 1539 to 2935 μ mol/L, and the first lactation heifers showed the lowest serum BAP concentration through the experiment ($P < 0.05$; Table 4.3). Mirzad et al. (2018) reported that serum BAP concentration ranged from 2506.8 to 3055.9 μ mol/L in the Holstein Friesian cows sampled from 4 weeks before to 4 weeks after calving. The serum BAP concentrations of the multiparous cows in this experiment was within the range of report of Mirzad et al. (2018), but the serum BAP concentrations of the first lactation heifers were lower than that range. It was suggested that the serum BAP concentration after calving was lower in first lactation heifers than in multiparous cows. Serum BAP concentrations

positively associated with the average DMI and milk yield 6 days after calving ($P < 0.01$, Table 4.4). Antioxidant defenses are diverse, and can be either synthesized in vivo or derived from the diet. Cytosolic glutathione peroxidase (GPX1) is the selenoenzyme most often associated with antioxidant functions in cattle (Smith et al., 1997; Wichtel, 1998). Glutathione peroxidase is present in the cytoplasm of cells throughout the body, and its antioxidant capacity is strongly dependent on selenium. The non-enzymatic antioxidants are represented by tocopherols, ascorbic acid, carotenoids, and lipoic acid (Halliwell, 2007; Papas, 1999). Feed intake might be an influential factor for the capacity both of enzymatic and non-enzymatic antioxidant, because cattle can only obtain selenium and those antioxidants except for ascorbic acids from feed. Considering this and the positive correlation between milk yield and BAP, it seems that serum BAP concentration increased with increased intake.

OSI was no significant difference among the parties at 0 and 7 days after calving ($P > 0.05$; Table 4.3), and it was higher in fist lactation heifers than the cows in parity 4 or more at 21 days after calving ($P < 0.05$; Table 4.3). The DMI was not associated with OSI at 0 days after calving, but it was negatively associated with OSI at 7 days after calving. Although the serum ROM concentrations increased with the increase in milk production, the OSI did not change because the serum BAP concentration also increased at the same time. It was suggested that increase in milk yield results in an increase in the production of peroxides, but oxidative stress might not increase if antioxidant capacity of cows is enhanced by increase of feed intake with increased milk yield. Therefore, the difference in the average DMI 6 days after calving among the number of parity observed in this study might not be due to oxidative stress.

Although Mirzad et al. (2018) also reported no significant was found in serum values of oxidative status biomarker after calving, oxidative stress in the first lactation heifers might increase after calving. OSI in multiparous cows did not change after calving except the parity 4 or more group, but OSI in the first lactation heifers was increased at 7 days after calving than the 0 days after calving ($P < 0.05$). The ratio of increase rate of DMI to increase rate of milk yield during 6 days after calving tended to be lower in the first lactation heifers compared with multiparous cows and it was positively associated with parity number ($P < 0.01$; Figure 4.1). If the rate of increase in DMI due to the increase in milk yield is low, the cows might receive more oxidative stress. Although the first lactation heifers had lower milk yield than the multiparous cows, but might be more susceptible to oxidative stress due to modest increases in DMI immediately after calving.

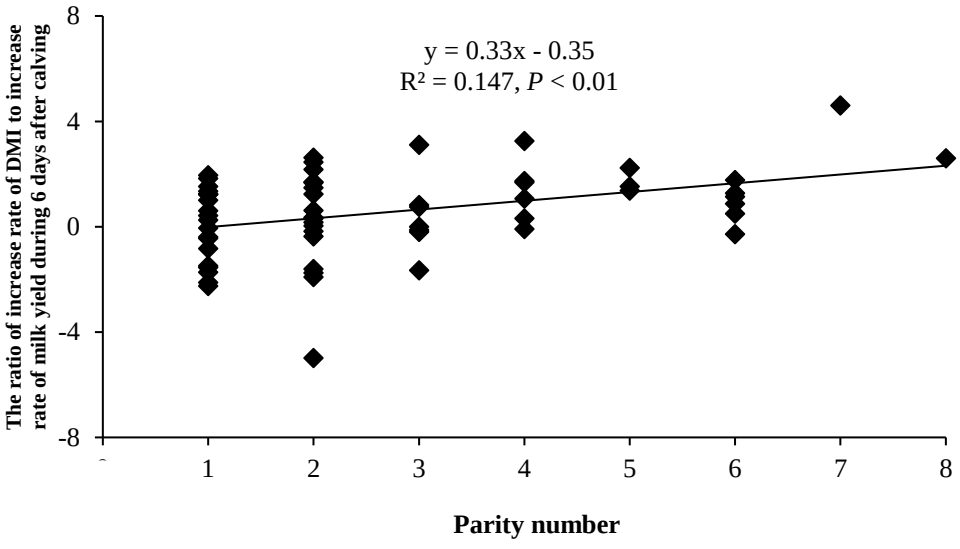

Figure 4.1. Relationship between the ratio of increase rate of DMI to increase rate of milk yield during 6 days after calving and parity number. R^2, correlation of determination.

Table 4.4. Correlation coefficients between the dry matter intake (DMI), body weight (BW), milk yield and oxidative status indicators.

		Parity number	DMI†	BW of dams	BW of calf	Calf BW/ Dam BW	Calving score	Average milk yield
DMI, g/$BW^{0.75}$/day		0.547**§§						
BW of dams, kg		0.664**	0.445**					
BW of calf, kg		0.022	−0.418**	0.010				
Calf BW/ Dam BW‡, %		−0.364*	−0.613**	−0.617**	0.778**			
Calving score§		−0.289*	−0.551**	−0.315*	0.242	0.405**		
Average milk yield¶, kg/day		0.531**	0.623**	0.590**	−0.471**	−0.694**	−0.356*	
	Days after calving							
ROM¶, U CARR	0	0.103	0.251*	0.128	−0.273*	−0.284*	0.030	0.440**
	7	0.054	0.070	0.227	−0.348*	−0.398**	0.003	0.288*
	21	0.010	0.312*	0.175	−0.361*	−0.323*	−0.092	0.429**
BAP††, µ mol/L	0	0.098	0.425**	0.259*	−0.336*	−0.417**	−0.137	0.481**
	7	0.392*	0.540**	0.460**	−0.308*	−0.501**	−0.430**	0.643**
	21	0.236	0.521**	0.284*	−0.503**	−0.526**	−0.311*	0.714**
OSI‡‡, U CARR L/µ mol	0	0.013	−0.120	−0.133	0.107	0.165	0.064	−0.126
	7	−0.131	−0.333*	−0.064	0.010	0.033	0.328*	−0.214
	21	−0.272*	0.198	−0.218	0.157	0.271*	0.246	0.311*

†Average DMI 6 days after calving. ‡Ratio of calf BW at birth to dam BW at calving. §Calving scores recorded according to the procedure of Proudfoot et al. (2009a). ¶Average milk yield during the first week. ‖Reactive oxygen metabolites. ††Biological antioxidant potential. ‡‡Oxidative stress index; §§ A quadratic relationship between the parity number and the DMI was observed. * $P < 0.05$; ** $P < 0.01$.

The results for blood metabolites concentration are shown in Table 4.5. Some blood metabolites differed among the parity number, but the OSI had little effect on the biochemical properties of blood measured in this experiment (Table 4.6). It has been demonstrated that oxidative stress during the transition period might be a major underlying cause of inflammatory and immune dysfunctions in dairy cattle (Sordillo & Aitken, 2009). However, I did not measure the index of the inflammatory and immune dysfunctions such as incidence of peripartum diseases and functional capabilities of leukocyte populations. Within the OSI range observed in this

experiment, the impact of the oxidative stress on DMI seems to be small, but further investigation is needed to determine whether oxidative stress affects the health of livestock within the range of the OSI.

Table 4.5. Changes of metabolic parameters among the parities from 0 day to 21 days after calving.

Days after calving		Parity number				SEM	P-value
		1†	2	3	4 or more		
NEFA, μEq/L	0	306.1^{b}	542.7^{a}	556.9^{a}	707.8^{a}	37.91	0.222
	7	530.3	560.9	705.7	619.0	36.85	0.451
	21	285.4	301.9	382.3	432.1	25.36	0.340
BHBA, μmol/L	0	684.7	549.4	661.6	688.7	28.82	0.475
	7	707.9	677.6	639.7	693.7	55.18	0.789
	21	538.9^{ab}	524.1^{b}	736.7^{a}	541.4^{b}	25.42	0.442
Glu, mg/dl	0	77.0^{b}	94.8^{ab}	99.9^{a}	90.2^{ab}	3.85	0.363
	7	62.5	61.6	62.3	58.6	1.33	0.592
	21	65.9	63.1	59.6	62.7	1.26	0.448
Ca, mg/dl	0	9.1^{a}	7.8^{b}	7.7^{b}	7.4^{b}	0.21	0.345
	7	9.1	9.8	9.8	9.5	0.14	0.395
	21	9.8	9.8	9.9	10.4	0.15	0.596
P, mg/dl	0	5.1^{a}	5.2^{a}	3.8^{b}	3.5^{b}	0.20	0.258
	7	4.7^{b}	4.9^{ab}	6.2^{a}	4.7^{b}	0.16	0.358
	21	5.1	4.9	4.5	4.6	0.12	0.429
TP, mg/dl	0	6.2^{b}	6.6^{b}	6.7^{ab}	7.2^{a}	0.11	0.255
	7	6.6	7.5	7.6	7.3	0.15	0.351
	21	7.2^{b}	7.4^{b}	7.6^{ab}	9.0^{a}	0.24	0.269
Alb, mg/dl	0	3.3	3.4	3.3	3.6	0.05	0.330
	7	3.2	3.6	3.5	3.5	0.06	0.402
	21	3.3	3.4	3.4	3.9	0.07	0.265
BUN, mg/dl	0	10.0	10.5	9.8	11.6	0.52	0.543
	7	6.3^{b}	7.6^{a}	6.2^{b}	7.7^{a}	0.26	0.365
	21	6.2^{b}	7.6^{a}	5.6^{ab}	7.4^{a}	0.33	0.286
γGTP, Iμ/L	0	23.2	23.1	24.9	27.4	1.98	0.665
	7	15.2^{b}	20.7^{ab}	27.3^{a}	31.2^{a}	1.98	0.173
	21	18.3	24.7	22.9	33.4	1.62	0.182
GOT, Iμ/L	0	82.3	76.3	77.6	70.1	2.52	0.494
	7	108.7	107.9	110.8	83.9	5.37	0.481
	21	85.9	94.5	76.0	88.2	4.78	0.586

†First lactation heifers. NEFA, nonesterified fatty acid; BHBA, β-hydroxybutyrate; Glu, glucosw; Ca, calcium; P, phosphorus; TP, total protein; Alb, albumin; BUN, blood urea nitrogen; γGTP, gamma-glutamyl transpeptidase; GOT, glutamic-oxalacetic transaminase. Means with different superscripts within a column indicate significant differences ($P < 0.05$).

Table 4.6. Correlation coefficients between the oxidative status indicators and metabolic parameters.

		OSI, U CARR L/μ mol		
Days after calving		0	7	21
NEFA, μEq/L	0	0.035	0.081	−0.230
	7	0.018	0.115	0.039
	21	−0.195	−0.226	−0.103
BHBA, μmol/L	0	0.182	0.276*	0.003
	7	0.096	0.120	0.190
	21	0.146	0.008	0.094
Glu, mg/dl	0	0.012	−0.094	−0.247
	7	0.020	0.007	0.023
	21	−0.087	−0.086	0.172
Ca, mg/dl	0	0.011	0.029	0.055
	7	0.149	−0.220	−0.207
	21	0.015	0.092	−0.241
P, mg/dl	0	0.032	0.139	0.064
	7	0.096	0.069	0.030
	21	0.176	0.016	0.129
TP, mg/dl	0	0.005	−0.198	−0.295*
	7	0.116	0.249	0.220
	21	0.070	0.145	0.228
Alb, mg/dl	0	−0.029	−0.188	−0.308*
	7	0.015	−0.292*	−0.169
	21	0.147	0.058	−0.305*
BUN, mg/dl	0	0.083	0.026	0.208
	7	0.015	0.201	0.027
	21	0.020	0.243	0.319*
γGTP, Iμ/L	0	0.005	0.059	−0.115
	7	−0.067	−0.120	−0.126
	21	0.112	0.059	−0.044
GOT, Iμ/L	0	0.123	0.147	−0.117
	7	0.228	−0.126	0.079
	21	0.025	0.156	0.005

DMI, Average DMI 6 days after calving. NEFA, nonesterified fatty acid; BHBA, β-hydroxybutyrate; Glu, glucose; Ca, calcium; P, phosphorus; TP, total protein; Alb, albumin; BUN, blood urea nitrogen; γGTP, gamma-glutamyl transpeptidase; GOT, glutamic-oxalacetic transaminase; OSI, oxidative stress index. * $P < 0.05$; ** $P < 0.01$.

4.4. Conclusion

It is concluded that the oxidative stress has little effect on DMI immediately after calving, but the low DMI would increase the oxidative stress of the cows after calving. Rapid increase in DMI immediately after calving synchronized with increase in milk yield must be important issue for the prevention of the performance deterioration due to oxidative stress in cows after calving.

Chapter V

General Discussion and Conclusion

5.1. General discussion

While the annual milk yield of dairy cows has been increasing, their production life has been gradually decreasing. Shortening of production life leads to a decrease in earnings in dairy management. After calving, milk production increases rapidly, but feed intake increases only modestly, causing most cows to be malnourished. If the cows cannot meet their nutrients requirements after calving, they are prone to develop metabolic diseases such as hypocalcemia and ketosis and to reduced reproductive performance. These metabolic and reproductive disorders are the main causes for culling of dairy cattle. One of the issues that must be addressed to prevent these problems and to expand the production life of dairy cows is meeting the nutrients requirements by increasing feed intake as soon as possible after calving. Therefore, I focused on the DMI immediately after calving and carried out three experiments to find factors which affect the feed intake in dairy cows immediately after calving and improve feed intake of cows immediately after calving.

Exp. 1) Assessment of the effect of parity number on the factors affecting the dry matter intake (DMI) of dairy cows immediately after calving

Exp. 2) Assessment of the influence of calving difficulty on the DMI immediately after calving, and to clarify whether urinary cortisol can be used as a stress indicator of cows around calving, and

Exp. 3) Assessment of the effects the oxidative status on DMI of dairy cows immediately after calving.

It seems that a significant part of this goal has been achieved, and several findings were obtained that contributed to the improvement of the DMI after parturition. However, there are still many challenges to increase postpartum feeding and longevity of dairy cows.

At first, I investigated the changes of feed intake immediately after calving according to parity number and concluded that the DMI immediately after calving tends to be lower in first lactation heifers and high parity number cows, but factors that reduce the DMI differ according to parity number. This implies that first lactation heifers and high parity cows are more easily suffer from a negative energy balance, and that the feeding management according to the cow's age is necessary to increase the DMI immediately after calving and extend the longevity of dairy cows.

The main factors which reduce the DMI immediately after calving is malnutrition before calving, calving stress, and low energy requirement compared with multiparous cows. It has been reported that excessive nutritional status before calving reduces feed intake after calving, but my study shows that malnutrition before calving also reduces the intake, especially in first lactation heifers. There are many reasons for low feed intake of first lactation heifers before calving, such as development of gastrointestinal, social order in a herd, and stress from the first pregnancy experience. Rabelo et al. (2005) reported that a higher energy density diet resulted in lower NEFA compared with a diet with moderate energy density prepartum in first lactation heifers. This is probably related to increased needs for growth in primiparous cows occurring simultaneously with the demands of lactation and their lower feed intake capacity as described previously (Bačić at al., 2007). This means is under the normal conditions of feeding, first lactation heifers might be find it

difficult to get enough energy from their diet and become malnourished. Because of at late gestation, first lactation heifers need energy not only for maintains and also for growth. However, at that time cows approach calving, and nutrient demands by the near-term foetus and reproductive tissues reach a certain maximum level. It might be necessery to improve the energy density of the diet to prevent the malnutrition before calving in first lactation heifers. However, as I could not determine the reason for the malnutrition before calving in the first pregnant heifers in this study, further research be required.

The stress derived from calving is another factor that reduces the DMI immediately after calving of first lactation heifers. I evaluated calving stress with a calving score and urinary cortisol concentration. First lactation heifers have no experience of gestation, calving, and milking, and are more prone to dystocia at calving than multiparous cows, because pregnant heifers have a smaller pelvic area, lower live weight, and more frequent difficulty in calving than older cows (Bureš et al., 2008). My study shows that the calving scores were higher in first lactation heifers than the multiparous cows. This was negatively associated with average DMI immediately after calving. A higher incidence of calving difficulty in first lactation heifers with a higher calf birth weight compared to those of multiparous cows has been already reported (Berry et al., 2007; Civelek et al., 2008). Some reports have demonstrated that cows with premature delivery, stillborn calves, and dystocia show high plasma cortisol concentrations at calving (Hudson et al., 1975; Patel et al., 1996; Kornmatitsuk et al., 2002). Civelek et al. (2008) also reported that plasma cortisol concentration was significantly elevated in Holstein heifers with dystocia at calving. In my study, urinary cortisol concentration was higher in first lactation heifers than in

multiparous cows 4 days after calving. This was negatively associated with the average DMI immediately after calving (experiment 2). Those findings suggested that monitoring urinary cortisol concentration after calving might be a valid method for evaluating the stress derived from calving. This is important because calving stress is a considerable factor which affects the DMI immediately after calving in dairy cows, especially in first lactation heifers. Increased risk of mortality with an increasing calving difficulty score is supported by previous studies. Dematawewa and Berger (1998) observed that cows with calving difficulty score 5 (extreme calving difficulty) had 4% higher mortality than cows with score 1 (calving without assistance). Similarly, Bicalho et al. (2007) found that cows with calving difficulty scores 3 and 4 had 20% higher risk of death or culling than cows with calving difficulty scores 1 and 2. Thus, it has been shown that the stress of calving is a considerable factor in reducing the DMI of the first lactation heifers immediately after calving and in increasing longevity of dairy cows, but this study could not examine how the stress could be reduced. This is also an issue to be addressed in the future.

Previous studies reported that calving difficulty is affected by two categories of factors: (1) those attributed to the dam, and (2) those attributed to the calf (Bellows et al., 1969; Xu & Burton, 2003). High calf birth weights are the main cause of dystocia (Bellows et al, 1969). Pelvic area of the dam must be large enough to accommodate the calf (Bellows et al, 2003). Those reports suggest that a decrease of BW of calf at birth and an increase of pelvic area of the dam by increasing BW of first lactation heifers at artificial insemination might be efficient methods to reduce the calving difficulty in first lactation heifers.

On the other hand, a considerable factor to consider for reducing the DMI of high parity cows immediately after calving is the delayed recovery of serum Ca concentrations after calving. These considerably affect the DMI immediately after calving, because serum Ca has a role in maintaining muscular contractive activity (Van Breemen and Saida, 1989). Cows with lower serum Ca concentrations develop inactive digestive tract motility (Goff, 2004). Wynn et al. (2015) have demonstrated that serum Ca concentrations after calving are positively associated with the rumen contraction frequency and DMI after calving. First lactation heifers can maintain serum Ca at steady concentrations without any treatments after calving because low parity cows absorb Ca efficiently, owing to active mechanisms of Ca homeostasis, including Ca absorption from the intestine and bone resorption (Goff et al., 1991; Bronner, 1992; Kamiya et al., 2005). My results showed serum Ca concentrations just after calving were lower in high parity cows than in young cows, and the serum Ca at 2 or 3 days after calving was positively associated with the average DMI immediately after calving. Therefore, low serum Ca concentration is unlikely to cause the low DMI immediately after calving in first lactation heifers, but it can affect the DMI immediately after calving in multiparous cows. It is known that the ability to absorb Ca from the intestine decreases with the age of cows, and it is thought that the delayed recovery of the serum Ca concentration is due to the decrease in ability to absorb calcium. There are intracellular and paracellular pathways for Ca absorption in the digestive tract (Bronner, 1998). The previously mentioned methods of preventing hypocalcemia by limiting Ca supply and injecting exogenous vitamin D before calving promote Ca absorption via the transcellular pathway by stimulating the production of 1,25-$(OH)_2$D. However, the Ca absorption capacity through the

intracellular pathway decreases with aging (Horst et al., 1990; Goff et al., 1991). Methods to prevent the occurrence of low serum Ca have been proposed that involve limiting the Ca supply before calving (Goings et al., 1974), feeding or injecting exogenous vitamin D at 10 to 14 d before calving (Hibbs & Pounden, 1955), and adjusting the DCAD by supplying anions (Block, 1984). Teramura et al. (2015) has also reported the enhancement of paracellular Ca absorption and improvement of the recovery rate of serum Ca concentration by feeding difructose anhydrate III (DFA III), a kind of oligosaccharide processed from inulin in chicory. Wynn et al. (2015) reported improvement of rumen mobility by feeding with the DFA III. The enhancement of Ca absorption through the paracellular pathway is an effective way to prevent reduction of the DMI of high parity cows immediately after calving.

Before starting the experiments, I thought that oxidative stress would reduce feed intake of dairy cows, because milk productivity is associated with oxidative stress due to the increase of cellular metabolism (Castillo et al., 2003; Lohrke et al., 2005). However, the DMI was not associated with OSI at 0 days after calving in my study. The production of peroxides increased with the increase of milk production, the DMI and the antioxidant capacity also increased, as increase of milk yield. Therefore, the increase of milk production did not affect oxidative stress in this study. The current study shows that average DMI immediately after calving was positively associated with the serum biological antioxidant potential (BAP) concentration at 7 days after calving. The serum BAP and reactive oxygen metabolites (ROM) concentrations through the experimental period were positively associated with average milk yield immediately after calving. It is suggested that oxidative stress can be reduced if the amount of antioxidant intake increases even if the amount of

peroxide production increases, and that the difference in the average DMI 6 days after calving among the number of parity observed in this study might not be due to oxidative stress. Vitamins A and E and selenium are known antioxidants that play important roles in animal health and production. Previous research has been shown that the supplementation of antioxidant nutrients, that is, vitamin E (Al-Mabruk et al., 2004; Sympoura et al., 2009) and trehalose supplement (Aoki et al., 2010), in the diets of dairy cows could result in an increase in the intake and milk yield and high antioxidant activity. Moreover, these results indicate that a rapid increase in DMI immediately after calving together with an increase in milk yield must be an important issue for the prevention of the performance deterioration due to oxidative stress in cows after calving, because the low DMI would decrease antioxidant intake and increase the oxidative stress of the cows after calving. The DMI immediately after calving increased as milk production increased, but the ratio of increase in the DMI to the increase in milk production was smaller in first lactation heifers. First lactation heifers might be more susceptible to oxidative stress than multiparous cows. Further studies are needed on how feed intake and oxidative stress are involved in prolonging the production life of dairy cows.

The correlation between the feed intake and milk yield is often used as an animal factor in feed intake predictions. Milk yield was positively associated with the DMI immediately after calving in this study. Milk yield has also typically been lower in first lactation heifers than in multiparous cows. Therefore, we considered the low milk yield might be one of the reasons for low DMI in first lactation heifers, because according to the net energy requirement of the cows for lactation that was calculated

by National Research Council (NRC) (2001) was higher in the multiparous cows than in first lactation heifers.

Research has shown that dietary factors are the reasons effects on DMI soon after calving. However, all cows in my study are the same stage of lactation and were fed the same diet. Therefore, we can confirm that the effect of feeding management on DMI is same for all cows in this study.

Many researches have focused on changing the nutrient composition of feed to improving the DMI of lactating dairy cows. However, grouping, feeding behaviour, environment, management practices, health, and social interactions are also affect on DMI of lactating dairy cows (Grant & Albright, 2001; DeVries et al., 2005). As we knows, to provide the balance of nutrients that ruminants need to maintain a stable the feeding a TMR is the more optimal way. However, the availability of feed over time and the distribution of intake over the course of the day contribute to the maintenance of a stable ruminal microbial population, which is important to reduce the risk of cows developing subacute ruminal acidosis (Nocek & Braund, 1985). The time spent eating, and the pattern of meals, can obviously have important effects on the total daily intake of dairy cattle (Grant & Albright, 2000). The majority of research on feeding behavior has been completed with individually housed animals. In modern free-stall dairy operations, cows are group-housed. In my study, first lactation heifers were grouped with multiparous cows before calving. This social environment can play a major role in the modulation of feeding behavior (DeVries & Von Keyserlingk, 2006). When cows are kept in individual cubicles, free from the effects of social interaction, those with higher feed intakes take fewer meals during the day. Furthermore, meal size (quantity and length), but not meal number, is positively

related to milk production (Dado & Allen, 1994). Competition at the feed bunk is highest when cows return from milking and when fresh feed is offered. At these times, dominant cows will demand priority for feeding. Thus, those cows that are less dominant may be limited in their access to the feed bunk at these times, forcing them to eat less, or at times where competition at the feed-bunk is reduced (DeVries & Von Keyserlingk, 2006). In group housing, eating behavior of dairy cows during the day, eating time, or both vary with social dominance. During periods when many cows are eating (after milking and feeding), cows with a lower social rank may have to wait (Metz, 1985).

According to those reports we can conclude that to improve the DMI soon after calving, we have to consider more suitable ingredients and nutritional value of diet, feeding behavior, feed bunk, and grouping of animals.

5.2. General Conclusions

The present PhD thesis investigated the effect of nutritional status, parity, and oxidative and calving stress on feed intake of dairy cows immediately after calving. These experiments demonstrate that the DMI intake immediately after calving is affected by parity number of cows, and that the DMI is easy to be low in first lactation heifers and cows with a high number of calving. In the case of the first lactation heifers, the DMI immediately after calving is likely to be suppressed by malnutrition in the late gestation period and by stress around calving. On the other hand, delayed recovery of serum Ca concentration after calving is a limiting factor for the DMI in high parity cows. Since the influential factors affecting the DMI differ between the first lactation heifers and high parity cows, feeding management around

calving according to the number of parity is required to enhance the DMI immediately after calving. Moreover, these experiments show that oxidative stress is unlikely to increase even with increased milk production, because the increase i+n milk yield increases peroxide production and also increases the antioxidant capacity by increasing feed intake. This implies that the DMI of dairy cows immediately after calving is less susceptible to oxidative stress, and it suggests that increasing the feed intake immediately after calving can reduce the oxidative stress and the risk of metabolic and reproductive disorders due to oxidative stress. These results would contribute to expanding longevity of dairy cows through reducing the risk of metabolic and reproductive disorders by improving feed intake immediately after calving.

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