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Effects of substitution of starch source with highly digestible fiber in
concentrate on energy utilization and recovery of ovarian function in early
lactating dairy cows

泌乳初期乳牛における濃厚飼料中デンプン源の高消化繊維への代替
がエネルギー利用および卵巣機能回復に及ぼす影響

北海道大学 大学院農学院

生物資源科学専攻 博士後期課程

Min Bo

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ABBREVIATIONS

ADF : Acid Detergent Fiber

ADL : Acid Detergent Lignin

BCS : Body Condition Score

BUN : Blood Urea Nitrogen

CL : Corpus Luteum

CP : Crude Protein

DIM : Day in Milk

DM : Dry Matter

DMI : Dry Matter Intake

FCM : Fat-converted Milk

GE : Gross Energy

GnRH: Gonadotrophin Releasing Hormone

LH : Lutenizing Hormone

ME : Metabolisable Energy

NDF : Neutral Detergent Fiber

NEB : Negative Energy Balance

NEFA: Non-esterified Fatty Acid

SARA: Sub Acute Ruminant Acidosis

VFA : Volatile Fatty Acid

Chapter 1

General introduction

1.1. Background

Over the last couple of decades, the use of the pure Holstein breed and intense genetic selection has significantly increased milk yields of dairy cows and, therefore, production efficiency of the dairy industry has also improved. However, it has now become evident that this increase in milk yield is accompanied by a reduction in reproductive performance of dairy cows (Webb *et al.*, 1999a).

Most recent dairy cows are not able to meet the energy requirements for growth, maintenance, and milk production in early lactation and are, therefore, in a negative energy balance (NEB) prior to peak milk production (Waltner *et al.*, 1993). The extend of NEB during the first weeks of lactation is highly correlated with the interval from calving to first ovulation (Beam and Butler, 1998; Butler, 2000), which may affect subsequent fertility and onset of luteal activity (Thatcher and Wilcox, 1973). Prolonged periods of NEB suppressed the pulsatile lutenizing hormone (LH) secretion, reduced ovarian responsiveness to LH stimulation, and reduced estradiol secretion by the dominant follicle (Butler, 2003). In turn, NEB in early lactating dairy cows is associated with elevated plasma non-esterified fatty acid (NEFA) and hepatic lipid concentrations (Beam and Butler, 1998; Butler, 2000). Plasma NEFA is typically released in the blood stream when glucose level falls and then reduced insulin to glucagons ratio due to low level of glucose leads to activation of the hormone sensitive

lipase (Veerkamp *et al.*, 2003). NEB in early lactating dairy cows resulted in loss of body condition score (BCS) as cows mobilized body fat reserves to support milk production. Large BCS loss was associated with delayed first ovulation postpartum and reduced conception rate (Butler, 2003). Extended postpartum anovulatory interval is a major source of reduced fertility of dairy cows (Rhodes *et al.*, 2003). The first postpartum ovulation is frequently associated with an absence of estrous behavior and is often followed by a luteal phase with short duration (Webb *et al.*, 1980; Murphy *et al.*, 1990; Mc Dougall *et al.*, 1995).

In an attempt to improve energy balance during the early postpartum period, carbohydrates such as grain starch of the ration at the expense of forage components are supplemented to dairy cow diets. Such strategy alters metabolic hormones, particularly insulin, which can influence the recovery of ovarian function (Boland *et al.*, 2001, Webb *et al.*, 2004, Garnsworthy *et al.*, 2008).

Plasma insulin concentration was decreased when dairy cows were under NEB (Beam and Butler, 1999; Butler, 2000). Plasma insulin plays a central role in metabolism by stimulating utilization of glucose in peripheral tissues such as muscle and adipose tissue and by promoting accumulation of glycogen and lipid reserves. Moreover, plasma insulin stimulated bovine follicular cells (Simpson *et al.*, 1994; Spicer *et al.*, 1995). Plasma insulin in dairy cows was increased by feeding diets with high starch content (Reynolds, 2006). Diets with high nonstructural carbohydrate from grain induce an increased glucose and insulin levels which enhances ovarian follicular development in dairy cows before ovulation (Garnsworthy *et al.*, 2009) and reduces postpartum anovulatory interval (Gong *et al.*, 2002).

Corn grain is a major source of concentrate for dairy cows in the world.

However, Corn grain is still a major staple food in several regions of the world: in some areas of North Africa and Near East, in the highlands of Central Asia, in the Horn of Africa, in the Andean countries and in the Baltic States. In several countries corn grain has been a renewed interest in human food. Therefore, corn grain is competitive food between human and animal. Thus, in spite of its high contents of energy in corn grain, to overuse for animal feed should be problem.

On the other hand, the feeding dairy cows of a highly digestible fiber such as beet pulp may encourage growth of the cellulolytic and hemicellulolytic microorganisms in the rumen. This, in turn, should increase the extent of digestion of the forage (Carey *et al.*, 1993). Among off-farm byproducts of pulses, chickpea husk and lablab bean husk are the preferred feed ingredients as sources of easily digested fiber for cattle in Myanmar, India, Egypt and other countries (Tin Ngwe *et al.*, 2011). Byproduct nature of the feedstuff and its comparative low price has made beet pulp an attractive ingredient in livestock rations (Bhattacharya *et al.*, 1975). The nutritive value of these by-products and its low costs has made them attractive to livestock producers (Weiss *et al.*, 1997). Adding nonforage neutral detergent fiber (NDF) to low-forage dairy diets might reduce the negative effects of increased starch fermentation. The responses of dry matter intake (DMI) to various nonforage fiber sources substituted for grain are not consistent (Allen, 2000). Beet pulp contains approximately 40% NDF and is unique in its high concentration of neutral-detergent soluble fiber, especially pectic substances. Clark and Armentano (1997) and O'Mara *et al.* (1997) reported the increase of DMI when beet pulp was substituted for corn grain in dairy cows. As insulin secretion is stimulated by increased plasma glucose concentration (Van Knegsel *et al.*, 2005), plasma glucose concentration may be increased by great gluconeogenesis

and by great availability of glucose-sparing fuels such as acetate which is the main fermentation end-product of beet pulp in the rumen (Voelker and Allen, 2003). It has been partly established that blood metabolites such as insulin and insulin-like growth factors, play an important role in the control of ovarian function in cattle (Gong *et al.*, 2002; Garnsworthy *et al.*, 2009). However, there were few studies concerning relationship between ruminal fermentation end products of volatile fatty acid (VFA) and reproductive performances of dairy cows during early lactating period. Maximizing energy intake of dairy cows in early lactation without causing sub acute ruminal acidosis (SARA) is a big challenge, but the effects of feeding highly digestible fiber as a substitute for grain on rumen fermentation, blood metabolites and reproduction of dairy cows in early lactation have not been extensively studied.

1.2. Objectives of the present study

The main objective of this study was to investigate effect of substitution of starch source with highly digestible fiber in concentrate on energy utilization and reproductive performances in early lactating dairy cows fed corn silage based diet. To attain this objective this study investigated following subjects.

- i) Effect of dietary carbohydrate source on changes in energy intake and nutrient utilization during early lactating period.
- ii) Relationship between dietary carbohydrate source and recovery of ovarian function.
- iii) Interaction between parity or body weight changes and dietary carbohydrate source on energy balance and recovery of ovarian function.

Results obtained were totally discussed in chapter 4.

Chapter 2

Effect of substitution of grain starch with highly digestible fiber of beet pulp in concentrate on nutrients utilization and energy balance in early lactating dairy cows

2.1. Introduction

After calving, high-producing dairy cows generally experience a period of NEB, the severity and duration of which are primarily related to DMI (Butler, 2000). In turn, NEB is associated with elevated plasma NEFA and hepatic lipid concentrations. Energy balance during the first weeks of lactation is highly correlated with the interval from calving to first ovulation (Beam and Butler, 1998; Butler, 2000), which may affect subsequent fertility and onset of luteal activity (Thatcher and Wilcox, 1973). NEB resulted in a loss of BCS as the cow mobilized body fat reserves to support milk production. An important goal in dairy cow management is the maximization of energy intake. One of approaches to reducing the degree of NEB increasing energy intake of dairy cows in early lactation is to increase the metabolizable energy (ME) density of diets by feeding more fermentable starch such as grain or fat components of the ration at the expense of forage components. Such strategy alters metabolic hormones, particularly insulin and glucose (Boland *et al.* 2001, Webb *et al.*, 2004, Garnsworthy *et al.* 2008), as well as rumen fermentation state.

Diets high in nonstructural carbohydrate induce an increased plasma insulin concentration before first ovulation of dairy cows (Garnsworthy *et al.*, 2009). Insulin

plays a central role in metabolism by stimulating utilization of glucose in peripheral tissues such as muscle and adipose tissue and by promoting accumulation of glycogen and lipid reserves. Insulin was increased by diets with high starch content (Reynolds, 2006) and was decreased by diets with high fat content (Choi and Palmquist, 1996), although increased insulin concentrations were found when supplementary fat increased energy intake (Palmquist and Moser, 1981). Plasma insulin was decreased in cows under NEB (Beam and Butler, 1999; Butler, 2000).

Dietary starch concentration such as steam flaked corn for dairy cows in early lactation is often increased in order to increase diet fermentability, but increasing the rate or the extent of ruminal fermentation do not necessarily result in optimal fermentation. Replacing feed ingredients of high cellulose and hemicellulose with ingredients of high starch usually increases ruminal production of VFA and alters the proportions of individual VFA produced. Increased absorption of propionate can reduce feed intake (Anil and Forbes, 1980) and may alter nutrient partitioning and milk production. Large VFA production can lead to reduced ruminal pH, which might increase the rate of VFA absorption from the rumen (Dijkstra *et al.*, 1993). Moreover, low ruminal pH inhibits fiber digestion (Ørskov and Fraser, 1975) due to the rapid fermentation rate of grain. Rumen acidosis in dairy cows is due to consumption of large amounts of fermented carbohydrates such as grain which causes the production of VFA. The production of vast amounts of VFA and lactic acid will drop the rumen pH and cause clinical forms of ruminal acidosis that can lead to peracute, acute, or SARA diseases (Tortosa, 2009). SARA frequently causes poor feed intakes and this reduction in DMI has serious consequences for production and energy balance of the cows (Kleen *et al.*, 2010). Moreover optimal ruminal fermentation for high-concentrate

diets especially during early lactation can be probably achieved by substitution of grain starch with a nonforage fiber source that is slowly fermented, produces low propionate in the rumen, and does not reduce ruminal pH.

The feeding of a highly digestible fiber such as beet pulp can increase the extent of digestion of the forage (Carey *et al.*, 1993). Nutritive value of beet pulp and its low cost have made them attractive to livestock producers (Weiss *et al.*, 1997). Substitution of beet pulp for corn grain increased DMI (Clark and Armentano, 1997; O'Mara *et al.*, 1997). The level of dietary fiber should be adequate to avoid rumen acidosis and metabolic difficulties (Lee *et al.*, 1999).

Beet pulp contains NDF approximately at 40% of DM and the NDF in beet pulp can be digested more quickly than forage NDF (Bhatti and Firkins, 1995), and pectin is degraded more rapidly than cellulose and hemicellulose (Marounek *et al.*, 1985). Using beet pulp as energy source in concentrate instead of the grain rich in starch, the highly digestible fiber of beet pulp could have adverse effects on the degradation of forage fiber in the rumen. Ferris and Mayne (1994) reported that an increased inclusion of beet pulp decreased lactic acid concentration when compared to grain starch. Therefore, using beet pulp instead of corn grain for cows just after calving should improve ruminal environment by its neutral detergent-soluble fiber, then it could improve DMI and/or hormone situation in early lactating dairy cows. However, there were a few studies concerning effect of inclusion of high amount of highly digestible fiber in diet on nutrient utilization and energy balance in early lactating dairy cows.

2.2. Objective

The main objective of this study was to investigate the effects of substitution of grain starch with highly digestible fiber in concentrate on DMI, energy status, rumen fermentation and plasma metabolites in early lactating dairy cows fed corn silage based diet.

2.3. Materials and methods

This study was undertaken at the Experimental Farm of Hokkaido University, Sapporo, Japan. Experimental period was from February to October, 2013.

2.3.1. Treatments

Sixteen lactating Holstein dairy cows were randomly separated into two groups, and two groups were assigned to one of the following dietary treatments (1) CN: feeding steam flaked corn (4kg FM/d) + commercial formula feed (4kg FM/d) + soybean meal (2kg FM/d) + grass hay (3kg FM/d) and corn silage (CS) (*ad lib*); (2) BP: feeding beet pulp (4kg FM/d) and amount of other feed was the same as CN. The experiment started immediately after calving.

2.3.2. Feeding and milking

Daily feed allowances were offered in two portions at 0730 h and 1600 h each day during the first 60 days in milk (DIM). All the animals had free access to fresh water and mineral salt blocks. Feed refusal of each cow was removed before a.m. and p.m feeding everyday and daily feed intakes were recorded. Cows were milked twice daily at 0900 h and 1530 h.

To prepare the marker for measuring amount of fecal excretion, $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ solution (62.5 g/L) was sprayed on beet pulp pellet (2 L/10 kg beet pulp). Then beet pulp pellet was placed in a forced air oven (60 °C, 72 h). Marker (100 g/d) was given equal portion to cows before am and pm feeding.

2.3.3. Sampling and analysis for feed

Feed samples were collected weekly and stored at -20 °C. All feeds were ground by a cutting mill to pass through 1-mm sieves for chemical analyses. The ground samples were analyzed for dry matter (DM), crude protein (CP), crude ash, NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL). DM, CP, and crude ash were determined as the method described by the AOAC (1990). For NDF, ADF and ADL, the procedure of Goering and Van Soest (1975) was used. Alpha amylase solution was used for the measuring of NDF in corn and mixed concentrate. Gross energy (GE) for feed samples was measured by auto-calculating bomb calorimeter (CA-4PJ, SHIMADZU, Tokyo).

2.3.4. Animal measurements

Body weight and BCS were recorded once a week (Monday). BCS was measured on a 0-5 scale with 0.25 intervals, where 0=thin and 5=very fat (Ferguson et al., 1994).

2.3.5. Milk sample collection and analysis

Milk yield were recorded everyday throughout the experiment. Milk samples were collected twice in a week at consecutive a.m and p.m milking and analyzed for

fat, protein and lactose contents by infrared analysis (Milko Scan S54A., Foss Electric, Denmark).

2.3.6. Fecal sample collection and analysis

Fecal samples were collected twice a day (0630 h, 1600 h) and pooled on weekly. These samples were dried in a forced air oven (60 °C) for 2 weeks and were ground by a cutting mill to pass through 1-mm sieves for chemical analyses. Fecal GE was measured by auto-calculating bomb calorimeter (CA-4PJ, SHIMADZU, Tokyo). Fecal and marker samples were digested with nitric acid and per-chloric acid.

2.3.7. Rumenal fluid collection

Rumenal fluid samples were collected three times a week via mouth by inserting catheter with vacuum pump (Monday, Wednesday and Friday) after morning milking throughout the experiment for analysis. The ruminal pH was measured immediately after sampling by using a digital pH meter (B-212; Horiba, Kyoto, Japan). After collection, samples were centrifuged (3000 rpm, 20 min) and stored at -80 °C until assays for VFA.

2.3.8. VFA analysis of ruminal fluid

VFA was analyzed by a gas liquid chromatograph (GC-2010, Shimazu, Kyoto, Japan). The rumen fluid (0.5 mL) was mixed with 0.1 ml of 25% metaphosphoric acid in 5 N H₂SO₄ and kept at 4 °C for overnight to precipitate protein. After removal of precipitates by centrifugation at 4 °C for 5 minutes at 10,000 rpm, 0.5 mL of supernatant fluid was taken and added with 0.5 mL of crotonic acid (3 mmol/dL) as an

internal standard. Then the mixture (0.5 μ L) was injected into a capillary column (ULBON HR-20M; Shinwa Chemical Ind., Ltd., Kyoto, Japan) with a column temperature of 150 °C, injection temperature of 150 °C, and helium as carrier gas.

2.3.9. Energy balance determination

Daily fecal DM output was calculated by dividing daily La intake from marker by La concentration in fecal DM. Gross energy digestibility was calculated by dividing differences between energy intake and fecal energy output by energy intake. Digestible energy intake was calculated by multiplying energy intake by energy digestibility. ME intake was calculated by multiplication of digestible energy intake by 0.82 (National Agriculture and Food Research Organization, NARO, 2006). ME requirement for each cow was calculated from measurements of body weight, milk yield, milk composition, parity according to NARO (2006). ME balance was calculated by the differences between ME requirement and ME intake.

2.3.10. Blood sample collection and analysis

Blood samples were collected every Monday, Wednesday and Friday after morning milking from calving to 60 DIM from jugular vein via jugular venipuncture into evacuated heparinized vacuum tubes. After collection, samples were centrifuged (3000 rpm, 4 °C, 20 min) to collect plasma. The plasma was frozen at -80 °C until determination of plasma insulin, glucose, NEFA and blood urea nitrogen (BUN).

Glucose was analyzed by Mutarotase-GOD method (Wako kit Glu CII, Wako Pure Chemical Ind., Ltd., Osaka, Japan). Samples (10 μ L) were dispensed to each well. Then color reagent (150 μ L) was added to each well. A plate was shaken about 30 min

at room temperature, and measured by plate reader (Viento 808 IU, Bio Tek Instruments, USA).

Plasma insulin concentrations were measured using a time-resolved fluoroimmune assay (Sugino *et al.*, 2010). At 100 μ L of coating reagent was dispensed to each well and a plate-seal was covered on a plate and shaken overnight at room temperature (300 rpm). A plate was washed by the plate washer. Blocking buffer (200 μ L) was dispensed to each well. A plate-seal was placed on a plate and shaken for 2 h (300 rpm). A plate was washed and the primary antibody reagent (200 μ L) was dispensed to each well. A plate-seal was placed on a plate and shaken overnight at room temperature (300 rpm). A plate was washed and sample (100 μ L) was dispensed to each well (triplicate) as quickly as possible. Eu-insulin solution (100 μ L) was dispensed to each well and shaken for 3 h (300 rpm, 4 °C). A plate was washed and 100 μ L of Enhancer was dispensed to each well, shaken 5 min at room temperature (300 rpm) and measured Eu concentration by plate reader.

The plasma blood samples were analyzed for NEFA using a commercial diagnostic kit (Wako kit NEFA-C, Wako Pure Chemical Ind., Ltd., Osaka, Japan). Sample (10 μ L) was put in each well of micro-plate. Coloring solution I solution (100 μ L) was added to each well, shaken and left at room temperature for 30 min. Then coloring solution II solution (200 μ L) was added to each well, shaken and left at room temperature for 30 min. By placing micro-plate in absorbance microplate reader, results were measured and recorded.

For BUN analysis, the plasma sample (10 μ L) was put in each well of micro-plate. Coloring solution (200 μ L) was added to each well, shaken and left at 37 °C for 30 min. Ammonia solution A (150 μ L) and ammonia solution B (150 μ L) were

added to each well, shaken and left at 37 °C for 15 min. By placing micro-plate in absorbance microplate reader, results were measured and recorded (Saito *et al.*, 1964; Kanai and Kanai, 1983).

2.3.11. Statistical analysis

All data were analyzed using the general mixed procedure of SAS version 9.1.3 (2004). The model was included diet, week, diet x week as fixed effect, cow as random effect. Significant level was mentioned as $P < 0.05$ and tendency level as $P < 0.1$.

2.4. Results

2.4.1. Chemical compositions of feedstuffs

Chemical compositions of feedstuffs are shown in Table 1. The NDF and ADF contents of beet pulp were higher than those of corn. The CP and ADL contents of beet pulp were higher than those of corn.

2.4.2. Intake and digestibility

Table 2 shows DMI, digestibility and energy utilization between CN and BP. There was no significant difference between CN and BP for DMI of soybean meal, commercial formula feed and grass hay. DMI of CS in BP was significantly higher than that in CN. Total DMI of BP was significantly higher than that of CN. DM digestibility and GE digestibility tended to be higher for BP than for CN. GE and ME intake of BP was significantly higher than that of CN. There was no significant difference between BP and CN for energy balance. However, an interaction was

detected for energy balance indicating earlier recovery to positive energy balance for BP than for CN. Cows for BP attained positive energy balance at during wk 4 to 6, nevertheless those for CN attained during wk 7 to 9. Total DMI, GE intake and ME intake of CN and BP were significantly higher in wk 7 to 9 than in wk 4 to 6 than in wk 1 to 3.

2.4.3. Milk production, body weight and BCS

Milk production, body weight and BCS are shown in Table 3. There was no treatment effect on milk yield, FCM milk yield, milk fat, milk protein, milk lactose and SNF. However, milk yield increased from 4 to 6 wk to wk 7 to 9 after calving in BP although being constant milk yield in CN throughout the experimental period. There was no significant difference between two groups for body weight and BCS.

2.4.4. Rumen fermentation parameters

Rumen fermentation parameters are shown in Table 4. There was no significant difference between CN and BP for total VFA, ruminal pH and butyrate. The molar portion of acetic acid of BP was significantly higher than that of CN. The molar portion of acetic acid of BP was higher in wk 7 to 9 than in wk 4 to 6 than in wk 1 to 3. The molar portion of propionate of CN was significantly higher than that of BP. The molar portion of propionate of CN was higher in wk 1 to 3 than in wk 4 to 9.

2.4.5. Blood compositions

Blood compositions are shown in Table 5. No difference was observed in the plasma insulin, NEFA and BUN concentrations between CN and BP. Plasma glucose of

cows fed CN tended to be higher than that of cows fed BP. Plasma insulin concentrations of CN both treatments were higher in wk 7 to 9 than in wk 1 to 6. Plasma NEFA concentration of CN and BP was higher during wk 1 to 3 than during wk 3 to 9.

Table 1 Chemical composition of feeds.

Items	Soybean meal	Corn	Beet pulp	Mix feed	Grass hay	Corn silage
DM(%FM)	86.6	85.6	86.8	85.7	85.1	25.4
OM(%DM)	94.0	98.8	92.8	95.2	93.7	94.4
CP(%DM)	52.3	9.3	11.1	20.2	11.8	7.9
NDF(%DM)	15.1	15.1	45.1	25.0	73.0	48.8
ADF(%DM)	8.8	3.4	24.0	10.4	39.1	28.1
ADL(%DM)	0.4	0.7	1.1	2.5	4.2	3.3

Table 2 Dry matter intake, digestibility and energy utilization of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	CN (n=8)				BP (n=8)				SEM	P-value	
	wk 1 to 3	wk 4 to 6	wk 7 to 9		wk 1 to 3	wk 4 to 6	wk 7 to 9			TM	Wk
Dry matter intake, kg/day											TM*Wk
Soybean meal	1.7	1.7	1.7		1.7	1.7	1.7		0.02	0.837	0.079
Formula feed	3.3	3.4	3.4		3.4	3.4	3.4		0.03	0.730	0.056
Steam flaked corn	3.4	3.4	3.4		0.0	0.0	0.0		0.02	<0.0001	0.402
Beet pulp	0.0	0.0	0.0		3.4	3.4	3.5		0.03	<0.0001	0.162
Grass hay	2.0	2.0	1.9		2.0	2.0	2.2		0.10	0.521	0.813
Corn silage	6.4	7.0	8.1		8.5	9.3	9.9		0.58	0.013	0.001
Total diet	16.2	17.7	19.1		18.5	20.1	21.2		0.66	0.019	<0.0001
Digestibility, %											
Dry matter	65.0	66.4	65.8		70.1	69.3	65.9		1.47	0.086	0.222
GE	64.9	66.2	65.8		70.1	69.4	65.8		1.45	0.067	0.223
GE intake, MJ/day	308.1	335.5	361.2		346.6	374.7	395.2		12.50	0.035	<0.0001
ME intake, MJ/day	164.4	174.8	188.0		200.2	207.6	212.8		9.15	0.019	0.007
Energy balance, MJ/day	-41.5	-15.8	6.7		-12.4	5.8	12.0		14.77	0.371	<0.0001

GE = Gross energy
ME = Metabolizable energy
TM = Treatments
Wk = Weeks
TM*Wk = Interaction between treatments and weeks

Table 3 Milk production, body weight and body condition score of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	CN (n=8)			BP (n=8)			SEM	P-value	
	wk 1 to 3	wk 4 to 6	wk 7 to 9	wk 1 to 3	wk 4 to 6	wk 7 to 9		TM	Wk
Milk yield, kg/day	27.8	28.6	28.9	28.4	31.3	32.3	2.36	0.486	0.032
FCM yield, kg/day	29.7	29.5	27.9	30.9	31.9	31.5	2.68	0.514	0.561
Milk composition, %									
Fat	4.63	4.20	3.78	4.73	4.05	3.77	0.27	0.944	0.001
Protein	3.57	3.23	3.05	3.64	3.57	3.20	0.20	0.959	0.014
Lactose	4.39	4.49	4.55	4.30	4.48	4.56	0.05	0.587	0.000
SNF	8.96	8.71	8.66	8.96	8.65	8.65	0.17	0.950	0.102
Body weight, kg	656	651	658	649	651	659	31.36	0.963	0.162
BCS	3.35	3.35	3.35	3.23	3.23	3.23	0.67	0.216	1.000

FCM = Fat corrected milk

SNF = Solid not fat

BCS = Body condition score

TM = Treatments

Wk = Weeks

TM*Wk = Interaction between treatments and weeks

Table 4 Rumen fermentation of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	CN (n=8)			BP (n=8)			SEM		P-value	
	wk 1 to 3	wk 4 to 6	wk 7 to 9	wk 1 to 3	wk 4 to 6	wk 7 to 9			TM	Wk
pH	6.46	6.52	6.53	6.45	6.47	6.51	0.59		0.751	0.146
Total VFA, mmol/L	8.89	8.45	8.39	9.04	8.76	8.60	0.32		0.583	0.022
Individual VFA, mmol/100mmol										
Acetate	61.82	63.20	63.64	64.52	65.58	66.75	0.81		0.026	<0.0001
Propionate	22.06	20.96	20.34	19.88	18.95	18.01	0.71		0.034	<0.0001
Butyrate	12.40	12.08	12.20	12.43	12.39	12.11	0.40		0.872	0.418
									0.872	0.552

VFA = Volatile fatty acid

TM = Treatments

Wk = Weeks

TM*Wk = Interaction between treatments and weeks

Table 5 Blood compositions of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP) from 0 to 60 days after calving.

Items	CN (n=8)				BP (n=8)				SEM		P-value	
	wk 1 to 3	wk 4 to 6	wk 7 to 9		wk 1 to 3	wk 4 to 6	wk 7 to 9				TM	Wk
Insulin, ng/mL	3.61	3.79	4.20		3.68	3.96	3.93		0.23		0.980	0.016
Glucose, mg/dL	62.8	64.1	64.7		59.9	60.0	60.2		1.63		0.093	0.377
NEFA, μ Eq/L	285.7	202.2	136.5		329.5	216.1	161.6		36.69		0.559	<0.0001
BUN, mg/dL	10.3	9.8	9.3		8.7	9.2	9.8		0.64		0.280	0.493
												0.404

NEFA = Non esterified fatty acid

BUN = Blood urea nitrogen

TM = Treatments

Wk = Weeks

TM*Wk = Interaction between treatments and weeks

2.5. Discussion

2.5.1. Ruminal fermentation and blood metabolites

In this study, mean ruminal pH was above 6.4 for both treatments. Gozho *et al.* (2005) stated that SARA occurs when rumen pH is depressed < 5.6 for prolonged periods each day. All cows in this study were free from SARA. In this study, CN produced more propionate while BP produced more acetate in the rumen. Ruminal fermentation of starch produces more propionate than acetate. In contrast, fermentations of neutral detergent-soluble fiber carbohydrates, such as pectic substances, produce more acetate than propionate (Ben-Ghedalia *et al.*, 1989).

While no difference was observed in the plasma insulin concentrations between two treatments (Table 5), it would be due to rather small difference in milk fat, milk energy output and energy balance between two treatments (Table 2 and 3). Milk yield and milk fat are also related to energy balance through the effect on plasma metabolites such as insulin and glucose (Beam and Butler, 1999). High NEFA concentration generally reflects mobilization of body fat reserves and consequent perturbation of hepatic function and hormonal metabolism (Westwood *et al.*, 2002). Therefore elevated NEFA concentrations could be an important characteristic of the dairy cows indicating energy balance during the postpartum period. In the current experiment, however, no differences for plasma insulin, NEFA and BUN were found between two treatments. Plasma glucose concentrations were higher in CN than BP from calving to 9 week interval. It may be due to high ruminal propionate level in CN. Huntington *et al.* (2006) showed that there was great glucose production from the propionate derived from starch based concentrate.

2.5.2. DMI

In the current study, lower DMI for CN than BP throughout the experimental period might be associated with the higher propionate production in the rumen for CN (Table 4). Similarly feeding beet pulp in replace of corn grain at 15.7% of dietary DM increased DMI in dairy cows (Clark and Armentano, 1997). Some studies showed that VFA may be involved in the control of feed intake in ruminants. Ruminant propionate has greater hypophagic effects than acetate (Sheperd and Combs, 1998). Intraruminal infusion of propionate decreased DMI compared to acetate on both an isomolar (Farningham and Whyte, 1993) and isoenergetic basis (Sheperd and Combs, 1998). Excess starch fermentation may decrease DMI due to great propionate metabolism in the liver (Allen *et al.*, 2009). Feed intake might be controlled by a dominant mechanism related to the stimulation of tension receptors by ruminal fill until a mechanism possibly related to propionate begins to dominate on highly fermentable diets. Portal infusion of propionate acts through hepatic nerves to inhibit food intake in the sheep (Anil and Forbes, 1980). The satiety effects of portal infusions of propionate may be the result of administration of a hypertonic load (Grovum and Bignell, 1989). Therefore, DMI in CN decreased compared with BP was attributable to the greater propionate production in the rumen of cows fed CN diet.

2.5.3. Energy utilization

Alteration in glucogenic and lipogenic nutrient content in the diet would affect the energy balance in dairy cows and result in modifications in blood metabolic profiles and milk fat content or yield (van Knegsel *et al.*, 2005). However, in the current study, there was no significant difference in milk fat yield between CN and BP

(Table 3) even though acetic acid in BP was higher than that in CN (Table 4). Cows fed BP attained the recovery to the positive energy balance earlier than cows fed CN (Table 2). It would be due to higher total DMI of BP than that of CN. Moreover, gross energy digestibility and ME intake in BP were higher than that of CN (Table 2). The NDF in beet pulp could be digested more quickly than forage NDF (Bhatti and Firkins, 1995), and pectin was degraded more rapidly than cellulose and hemicellulose (Marounek *et al.*, 1985). By feeding beet pulp in dietary concentrate instead of steam flaked corn, ME supply could be fulfilled to improve NEB in dairy cows at early lactating stage and so beet pulp could serve as a good energy source in early lactating dairy cows.

2.5.4. Conclusion

According to this study, cows fed beet pulp in concentrates attained the recovery to the positive energy balance earlier than those fed corn in concentrates. Energy digestibility and ME intake in cows fed concentrate rich in beet pulp were higher than that rich in corn. Therefore substituting steam flaked corn with beet pulp has beneficial effects to improve negative balance that dairy cows face on during early lactating period. Therefore it was suggested that beet pulp could be used as an alternative source of energy in concentrate for early lactating dairy cows.

Chapter 3

Effects of carbohydrate source, parity and body weight changes on recovery of ovarian function in early lactating dairy cows

3.1. Introduction

Reproductive efficiency is one of the major factors affecting productive and economic efficiency. Energy balance during the first weeks of lactation is highly correlated with the interval from calving to first ovulation (Beam and Butler, 1998; Butler, 2000). Feeding management during the early postpartum period and metabolic conditions influences body condition and recovery of ovarian function. Great BCS loss was associated with delayed first ovulation postpartum (Butler, 2003). Parity can affect on reproductive parameters of the cow. Parity affected insulin like growth factor-I concentrations as primiparous cows had lower concentrations of this hormone than multiparous cows (Meikle *et al.*, 2004). However, Wathes *et al.* (2003) proposed that glucose and insulin concentrations were the higher in the younger animals. It has been suggested that low plasma insulin and glucose were the metabolic signals that delay ovulation (Beam and Butler, 1999).

Carbohydrates such as starch of the ration at the expense of forage components are supplemented to dairy cow diets in an attempt to improve energy balance during the early postpartum period. Diets high in nonstructural carbohydrate induce an increased glucose level and insulin profile which enhances ovarian follicular development before ovulation (Garnsworthy *et al.*, 2009) and reduces postpartum anovulatory interval (Gong *et al.*, 2002). Adding nonforage NDF to low-forage diets

might reduce the negative effects derived from the increased starch fermentation without increasing the filling effect of the diet to the same extent as forage NDF. The responses of DMI to various nonforage fiber sources substituted for grain are not consistent (Allen, 2000). Plasma glucose concentration may be increased by greater gluconeogenesis and by greater availability of glucose-sparing fuels such as acetate which is the main end-product of beet pulp (Voelker and Allen, 2003).

In chapter 2, cows fed beet pulp in concentrates attained the recovery to the positive energy balance earlier than those fed corn in concentrates. Energy digestibility and ME intake in cows fed rich in beet pulp concentrate were higher than CN. Therefore, the problem of NEB during early lactating period can be solved by feeding BP in concentrate to early lactating dairy cows. Nevertheless, there was not clear evidence regarding the effects of rumen fermentation products on plasma metabolites and recovery of ovarian function when substituted starch source with highly digestible fiber in early lactating dairy cows. Additionally, parity and body weight changes after calving can be important as covariate factors which can be affected by the carbohydrate alteration.

3.2. Objective

The objective of this study was to investigate the effect of diet on recovery of ovarian function in early lactating dairy cows by feeding two types of concentrates containing corn grain or beet pulp in the ration of corn silage based diet. Then the second objective was to test the effects of parity and body weight changes postpartum on recovery of ovarian function by the carbohydrate alteration.

3.3. Materials and methods

In this chapter, additional measurements were conducted in the experiment of chapter 2.

3.3.1. Additional blood samples collection and analysis

Blood samples were collected every Monday, Wednesday and Friday after morning milking after 60 DIM to 120 DIM via coccygeal venipuncture into evacuated heparinized vacuum tubes.

Plasma progesterone was determined using enzyme immuno assay as validated by Ishikawa *et al.* (2002). Assay buffer solution (200 μ L) was mixed with serum with vortex. Mixture solution (20 μ L) was dispensed to each well. The plate was covered with silver sheath and shaken with mixer (16 h, 300 rpm, 4 °C). A plate was washed by the plate washer. Component solution (150 μ L) was dispensed to each well and the plate was placed in incubator (37 °C, 40 min). Finally 50 μ L of sulfuric acid solution (4 N) was dispensed to each well, placed 5 min at room temperature and measured by plate reader.

3.3.2. Reproductive parameters

Visual observation of estrous behavior was monitored 1 hour per day (between 1100 h to 1200 h) throughout experimental period (calving to 120 DIM). Follicle size, ovulation and corpus luteum sizes of the cows were examined rectally three times a week by using an ultrasound scanner 3 times (Monday, Wednesday and Friday) a week (Convex scanner, HS-1500, Honda electronics, Aichi, Japan). Ultrasound examinations started about 7 day after calving.

3.3.3. Statistical analysis

All data were analyzed using the general mixed procedure of SAS version 9.1.3 (2004). For blood from 10 week to 18 weeks after calving, the model was included diet, week, diet x week as fixed effect, cow as random effect. Firstly, data was divided by parity; multiparous or primiparous and by body weight changes; normal type or increasing type or decreasing type. In each data set, the model was included diet as fixed effect, cows as random effect. Significant level was mentioned as $P < 0.05$ and tendency level as $P < 0.1$.

3.4. Results

3.4.1. Overall dietary effect

Blood compositions from 60 to 120 DIM are shown in Table 6. No difference was observed in the plasma insulin, glucose and NEFA between CN and BP. BUN of BP was tended to be higher than that of CN.

Days for first rise in progesterone and reproductive performances, and mean follicle diameter and number of follicle are shown in Table 7. The first rise in progesterone level of CN cows tended to be 2 weeks earlier than those of BP cows. CN cows also tended to be ovulated 2 weeks earlier than BP cows. However, there was no significant difference in days postpartum for first estrus between CN and BP. CN cows were inseminated about 10 days earlier than BP cows ($P = 0.107$).

Number of follicle from the day of emergence to ovulation of CN was tended to be higher than those of BP. There was no significant difference between CN and BP for follicle size.

Reproductive behaviors at first estrus are shown in Table 8. Standing heat was

observed only in CN group at first estrus. Moreover cows in CN group showed more estrous behaviors such as mounting and sign of sniffing vagina than those in BP although there was no significant difference between CN and BP.

Table 6 Blood compositions of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP) from 60 to 120 days after calving.

Items	CN (n=8)				BP (n=8)				P-value		
	wk 10 to 12	wk 13 to 15	wk 16 to 18		wk 10 to 12	wk 13 to 15	wk 16 to 18		SEM	TM	TM*Wk
Insulin, ng/mL	4.50	4.73	4.96		4.41	4.35	4.30		0.39	0.501	0.316
Glucose, mg/dL	62.0	62.4	64.2		63.0	63.2	61.6		2.16	0.932	0.929
NEFA, μ Eq/L	146.5	200.5	141.6		133.1	152.0	118.8		22.81	0.326	0.009
BUN, mg/dL	10.2	11.0	11.6		12.0	13.4	14.3		0.92	0.056	0.059
											0.835

NEFA = Non esterified fatty acid

BUN = Blood urea nitrogen

TM = Treatments

Wk = Weeks

TM*Wk = Interaction between treatments and weeks

Table 7 Day for first rise in progesterone and reproductive performances, and mean follicle diameter and number of follicles of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	CN (n=8)	BP (n=8)	P-value
Days postpartum for			
First progesterone rise	40.1	55.1	0.089
First ovulation	36.9	51.3	0.093
First estrus	64.4	68.4	0.797
First insemination	76.6	85.4	0.107
Number of follicles from day of emergence to ovulation	28.0	21.0	0.099
Follicle diameter, mm	13.8	14.7	0.336

Table 8 Reproductive behaviors at first estrus of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	CN (n=8)	BP (n=8)	P-value
St H, /h	0.9	0.0	–
M, /h	14.3	5.5	0.122
SnV, /h	11.8	4.9	0.289
Ch-re, /h	1.4	2.8	0.342

St H = Standing heat

M = Mounting

SnV = Sniffing vagina

Ch-re = Chin resting

3.4.2. Dietary effect by parity

DMI and energy utilization by parity were shown in Table 9. In multiparous cows, total DMI of BP cows tended to be higher than that of CN. GE and ME intake also tended to be higher in BP than CN. However, there was no significant difference between CN and BP for energy balance. In primiparous cows, there were no significant difference between CN and BP for total DMI, GE intake, ME intake and energy balance. Moreover, there was no significant difference between primiparous and multiparous cows for total DMI, GE intake, ME intake and energy balance.

Blood compositions (DIM < 60) and (DIM 60 to 120) by parity are shown in Table 10. In multiparous cows, glucose concentrations during DIM < 60 in CN tended to be higher than those in BP although there was no significant difference between CN and BP for insulin, NEFA and BUN. In primiparous cows, there were no significant differences between CN and BP for insulin, glucose, NEFA and BUN during DIM < 60. Plasma glucose concentration in multiparous cows was significantly higher than that in primiparous cows. BUN concentration in primiparous cows was significantly higher than that in multiparous cows. In multiparous cows, BUN concentration from DIM 60 to 120 in BP was significantly higher than that in CN although there was no significant difference between CN and BP for plasma insulin, glucose and NEFA. In primiparous cows, there was no significant difference between CN and BP for plasma insulin, glucose, NEFA and BUN from DIM 60 to 120. Moreover, there was no significant difference between multiparous and primiparous cows for plasma insulin, glucose, NEFA and BUN.

Table 9 Dry matter intake and energy utilization of early lactating multiparous and primiparous cows fed steam flaked corn (CN) or beet pulp (BP).

Items	Multi		P-value	Primi		P-value	P-value Primi & Multi
	CN (n=6)	BP (n=6)		CN (n=2)	BP (n=2)		
Total dry matter intake, kg/day	19.5	21.2	0.072	14.4	16.4	0.197	0.575
GE intake, MJ/day	369.0	396.6	0.095	274.2	306.1	0.251	0.622
ME intake, MJ/day	198.6	225.2	0.082	146.6	171.0	0.229	0.378
Energy balance, MJ/day	-5.1	14.5	0.465	-44.7	-47.1	0.917	0.500

GE = Gross energy

ME = Metabolisable energy

Primi = Primiparous cows

Multi = Multiparous cows

Table 10 Blood compositions of early lactating multiparous and primiparous cows fed steam flaked corn (CN) or beet pulp (BP).

Items	Multi		P-value	Primi		P-value	P-value Primi & Multi
	CN (n=6)	BP (n=6)		CN (n=2)	BP (n=2)		
DIM <60day							
Insulin, ng/mL	4.18	3.72	0.312	3.91	3.81	0.755	0.516
Glucose, mg/dL	70.2	61.0	0.091	63.1	61.9	0.625	0.037
NEFA, μ Eq/L	197.1	217.9	0.742	247.7	327.5	0.184	0.163
BUN, mg/dL	8.7	8.7	0.961	12.6	10.2	0.314	0.008
DIM 60 to 120							
Insulin, ng/mL	4.65	4.43	0.712	3.93	4.02	0.911	0.304
Glucose, mg/dL	62.8	61.4	0.697	67.0	62.2	0.419	0.436
NEFA, μ Eq/L	160.0	124.4	0.356	152.8	175.1	0.692	0.515
BUN, mg/dL	11.1	14.4	0.043	12.1	12.0	0.946	0.667

NEFA = Non esterified fatty acid

BUN = Blood urea nitrogen

DIM = Day in milk

Primi = Primiparous

Multi = Multiparous cows

Day for first rise in progesterone and reproductive performances, and mean follicle diameter and number of follicle are shown in Table 11. In multiparous cows, days postpartum for first progesterone rise and first ovulation in CN tended to be earlier than those in BP. Days for first estrus and first insemination in CN of multiparous cows was about 2 weeks earlier than those in BP. However, there was no significant difference between CN and BP for follicle diameter and number of follicle in multiparous cows. In primiparous cows, there were no significant difference between CN and BP for days of the first rise in progesterone and first ovulation, first estrus and first insemination, and follicle diameter and number of follicle. Moreover there were no significant differences between primiparous and multiparous cows for days of the first rise in progesterone and first ovulation, first estrus and first insemination, and follicle diameter and number of follicle.

Reproductive behaviors at first estrus by parity are shown in Table 12. Standing heat was seen only in CN of multiparous cows although there were no difference between CN and BP for mounting, sniffing vagina and chin resting in multiparous cows. In primiparous cows, there was no significant difference between CN and BP for reproductive behaviors. Moreover, there was no significant difference between multiparous and primiparous cows for reproductive behaviors.

Table 11 Day for first rise in progesterone and reproductive performances, and mean follicle diameter and number of follicles of early lactating multiparous and primiparous cows fed steam flaked corn (CN) or beet pulp (BP).

Items	Multi		P-value	Primi		P-value	P-value Primi & Multi
	CN (n=6)	BP (n=6)		CN (n=2)	BP (n=2)		
Days postpartum for							
First progesterone rise	39.0	55.3	0.086	43.5	59.5	0.558	0.570
First ovulation	35.7	52.2	0.076	40.5	53.5	0.624	0.649
First estrus	68.3	82.3	0.519	52.5	64.0	0.592	0.497
First insemination	77.2	93.0	0.222	75.0	74.0	0.901	0.629
Number of follicles from day of emergence to ovulation	29.53	21.78	0.158	23.45	18.50	0.395	0.361
Follicle diameter, mm	14.12	14.72	0.581	12.85	14.55	0.484	0.504

Primi = Primiparous

Multi = Multiparous cows

Table 12 Reproductive behaviors at first estrus of early lactating multiparous and primiparous cows fed steam flaked corn (CN) or beet pulp (BP).

Items	Multi		P-value	Primi		P-value	P-value Primi & Multi
	CN (n=6)	BP (n=6)		CN (n=2)	BP (n=2)		
St H, /h	3.5	0.0	–	0.0	0.0	–	0.461
M, /h	16.0	9.3	0.515	9.0	8.0	0.844	0.788
SnV, /h	14.8	20.0	0.794	5.0	9.0	0.725	0.662
Ch-re, /h	2.7	9.0	0.011	6.0	1.5	0.213	0.160

St H = Standing heat

M = Mounting

SnV = Sniffing vagina

Ch-re = Chin resting

Primi = Primiparous

Multi = Multiparous cows

3.4.3. Dietary effect by body weight changes

Based on the pattern of body weight changes from calving to 120 DIM, cows were divided into three types; (1) Normal type (N, Appendix 1), (2) Increasing type (I, Appendix 2) and (3) Decreasing type (D, Appendix 3). DMI and energy utilization by body weight changes were shown in Table 13. Total DMI, GE intake and ME intake of BP were higher than CN in type I although there was no significant difference between CN and BP in type I for energy balance. There was no significant difference between CN and BP in type N for total DMI, GE intake, ME intake and energy balance. There was also no significant difference between CN and BP in type D for total DMI, GE intake, ME intake and energy balance. Total DMI of type I tended to be higher than that of type N and type D. GE intake of type I was tended to be higher than that of type N and type D. ME intake of type I tended to be higher than that of type N and type D. There was no significant difference for energy balance among three types.

Blood compositions by three types are shown in Table 14 for DIM < 60. It tended to be different between CN and BP in type N and in type D for NEFA although there was no difference between CN and BP in those two types for plasma insulin, glucose and BUN concentrations. There was no significant difference between CN and BP in type I for plasma insulin, glucose and BUN. There were differences between type N and I, type N and D, and type I and D.

Blood compositions of three types during DIM 60 to 120 are shown in Table 14. There were no significant differences between CN and BP in each type for plasma insulin, glucose, NEFA and BUN concentrations. Cows in type I were significantly higher in insulin and tended to be lower in NEFA than those in type D.

Days for first rise in progesterone and reproductive performances, and mean

follicle diameter and number of follicle by body weight changes are shown in Table 15. In cows in type N, days postpartum for first progesterone rise and first ovulation of CN were significantly earlier than those of BP. In this type, there was no significant difference between CN and BP for days for first estrus and first insemination. In cows in type I, there was no significant difference between CN and BP for days of first progesterone rise and first ovulation. There were no significant differences in CN and BP in type D for days postpartum of first progesterone rise, first ovulation, first estrus and first insemination. There were no differences in three types for days postpartum of first progesterone rise, first ovulation, first estrus and first insemination. In all types, there were no significant differences between CN and BP for follicle size and number of follicle. There were no significant differences in three types for follicle size and number of follicle.

Reproductive behaviors at first estrus by body weight changes are shown in Table 16. Standing heat was seen in cows of CN in type N and I, while standing heat has never seen in cows of CN and BP in type D. Other estrous signs such as mounting and sniffing vagina were seen in type N and I of CN than BP.

Table 13 Dry matter intake and energy utilization of normal, increasing and decreasing types of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	N			I			D			P-value		
	CN (n=3)	BP (n=2)	P-value (n=2)	CN (n=3)	BP (n=4)	P-value (n=4)	CN (n=2)	BP (n=2)	P-value (n=2)	N vs I	N vs D	I vs D
Total dry matter intake, kg/day	18.6	17.4	0.694	19.3	21.9	0.039	16.1	18.7	0.265	0.073	0.636	0.0355
GE intake, MJ/day	353	325	0.636	367	410	0.072	305	351	0.301	0.066	0.629	0.0315
ME intake, MJ/day	192	178	0.745	199	236	0.087	157	195	0.141	0.0762	0.631	0.0359
Energy balance, MJ/day	4.7	-22.3	0.299	23.5	37.5	0.428	-64.8	-77.5	0.715	0.154	0.507	0.1009

GE = Gross energy

ME = Metabolisable energy

N = Normal type

I = Increasing type

D = Decreasing type

Table 14 Blood compositions of normal, increasing and decreasing types of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	N			I			D			P-value		
	CN	BP	P-value	CN	BP	P-value	CN	BP	P-value	N vs I	N vs D	I vs D
	(n=3)	(n=2)		(n=3)	(n=4)		(n=2)	(n=2)				
DIM <60day												
Insulin, ng/mL	4.10	3.92	0.744	3.97	3.92	0.928	3.50	3.29	0.673	0.888	0.137	0.142
Glucose, mg/dL	65.0	62.3	0.709	62.2	60.5	0.469	66.5	58.4	0.124	0.396	0.654	0.747
NEFA, μ Eq/L	206.9	315.8	0.053	158.2	146.9	0.858	291.5	371.6	0.081	0.025	0.091	0.001
BUN, mg/dL	10.0	10.1	0.985	9.4	8.3	0.361	9.6	9.7	0.986	0.275	0.774	0.466
DIM 60 to 120												
Insulin, ng/mL	4.48	4.53	0.842	5.23	4.47	0.420	3.32	3.87	0.326	0.555	0.123	0.037
Glucose, mg/dL	65.7	58.8	0.383	65.2	61.8	0.448	59.8	61.3	0.714	0.784	0.545	0.372
NEFA, μ Eq/L	141.3	179.2	0.325	106.2	125.1	0.624	204.1	157.7	0.684	0.152	0.603	0.069
BUN, mg/dL	12.0	14.3	0.368	9.7	14.3	0.012	12.0	12.4	0.912	0.871	0.886	0.982

NEFA = Non esterified fatty acid

BUN = Blood urea nitrogen

DIM = Day in milk

N = Normal type

I = Increasing type

D = Decreasing type

Table 15 Day for first rise in progesterone and reproductive performances, and mean follicle diameter and number of follicles of normal, increasing and decreasing types of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP).

Items	N			I			D			Type effect
	CN	BP	P-value	CN	BP	P-value	CN	BP	P-value	P-value
	(n=3)	(n=2)		(n=3)	(n=4)		(n=2)	(n=2)		
Days postpartum for										
First progesterone rise	30.0	53.5	0.041	43.0	57.3	0.396	51.0	57.5	0.729	0.656
First ovulation	26.7	50.0	0.035	39.0	54.0	0.364	49.0	52.0	0.856	0.654
First estrus	54.3	86.5	0.273	68.0	71.7	0.918	74.0	69.0	0.602	0.466
First insemination	69.7	96.5	0.165	89.5	82.5	0.577	74.0	69.0	0.602	0.355
Number of follicles from day of emergence to ovulation	29.3	24.2	0.609	30.9	19.9	0.162	21.8	20.0	0.800	0.567
Follicle diameter, mm	14.5	14.9	0.853	14.0	14.4	0.789	12.5	15.0	0.228	0.795

N = Normal type

I = Increasing type

D = Decreasing type

Table 16 Reproductive behaviors at first estrus of normal, increasing and decreasing types of early lactating dairy cows fed fed steam flaked corn (CN) or beet pulp (BP).

Items	N			I			D			Type effect
	CN (n=3)	BP (n=2)	P-value	CN (n=3)	BP (n=4)	P-value	CN (n=2)	BP (n=2)	P-value	P-value
St H, /h	1.0	0.0	–	1.8	0.0	–	0.0	0.0	–	0.241
M, /h	14.7	16.5	0.891	19.0	3.5	0.285	6.5	4.0	0.212	0.227
SnV, /h	12.3	17.0	0.622	17.7	0.0	–	1.8	1.8	–	0.477
Ch-re, /h	2.5	7.5	0.087	1.8	0.0	–	1.53	2.14	0.124	0.228

St H = Standing heat

M = Mounting

SnV = Sniffing vagina

Ch-re = Chin resting

N = Normal type

I = Increasing type

D = Decreasing type

3.4.3. Relationship between body weight changes per week or energy balance and recovery of ovarian function

Relationship between numbers of follicles and body weight changes per week within 1 month is shown in Figure 1. Within 1 month after calving, 2 cows in CN and 5 cows in BP gained body weight, and 6 cows in CN and 3 cows in BP lost weight. Relationship between numbers of follicles and energy balance is shown in Figure 2. Cows in 50% of each treatment were faced on NEB. Relationship between follicle size and body weight changes per week within 1 month is shown in Figure 3. Body weight changes per week and energy balance did not affect follicle numbers and size. Relationship between energy balance and follicle size is shown in Figure 4. Follicle sizes were nearly same between cows in weight loss and those in weight gain. Follicle sizes of cows in positive energy balance were also the same as those of cows in negative energy balance. Relationship between days of first ovulation and body weight changes per week within 1 month is shown in Figure 5. Weight loss within 1 month after calving did not affect on days of first ovulation in early lactating dairy cows. Relationship between energy balance and days of first ovulation is shown in Figure 6. Six cows in CN ovulated within 40 days postpartum although 50% in this group was faced on NEB. Cows in BP ovulated after 50 days postpartum.

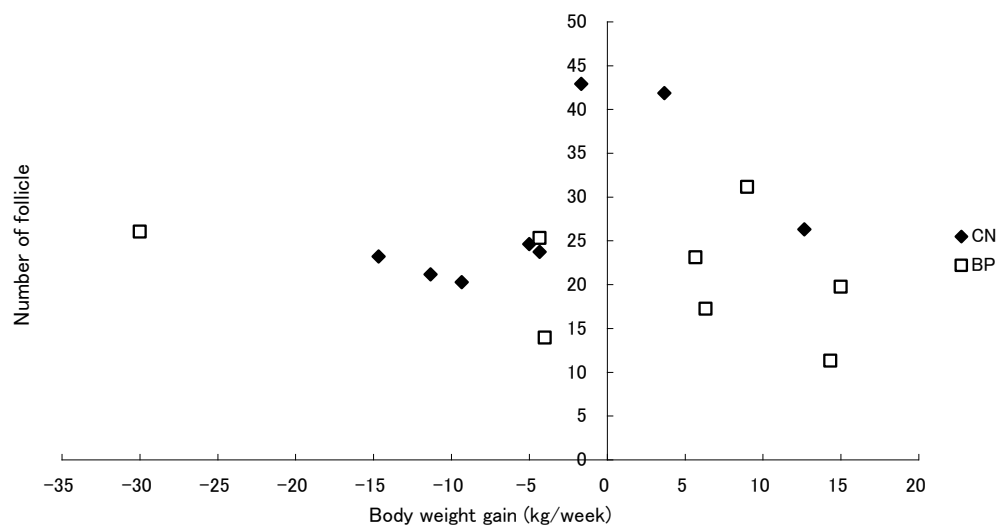


Figure 1. Body weight changes and numbers of follicles of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP)

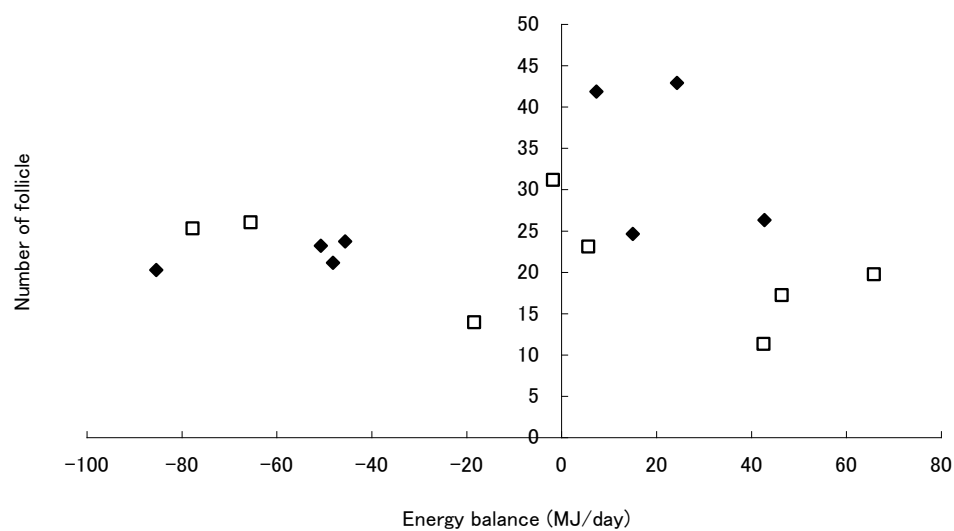


Figure 2. Energy balance and numbers of follicles of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP)

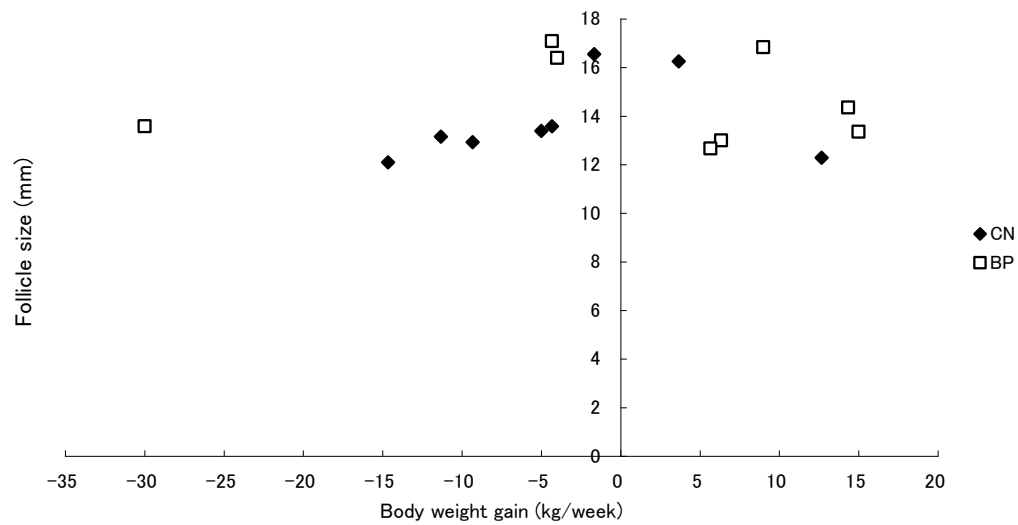


Figure 3. Body weight changes and follicle size of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP)

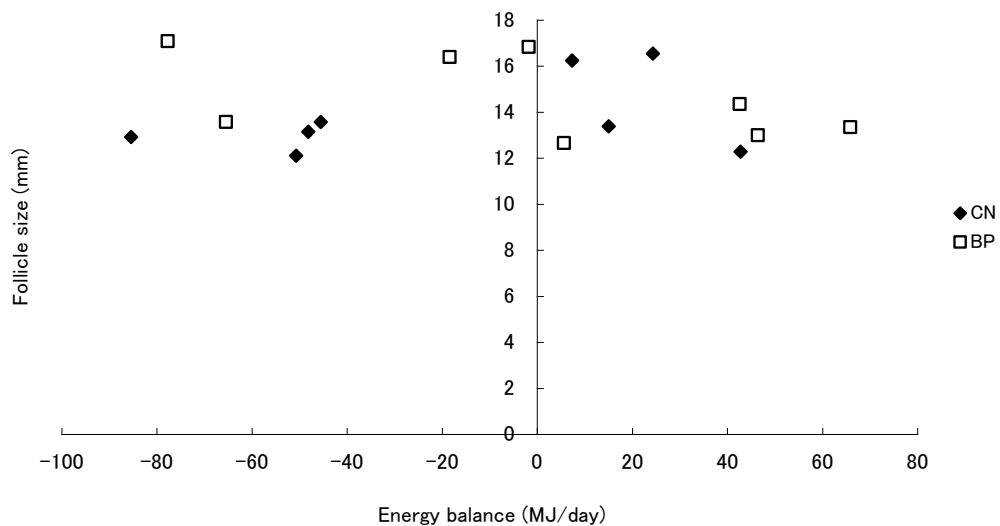


Figure 4. Energy balance and follicle size of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP)

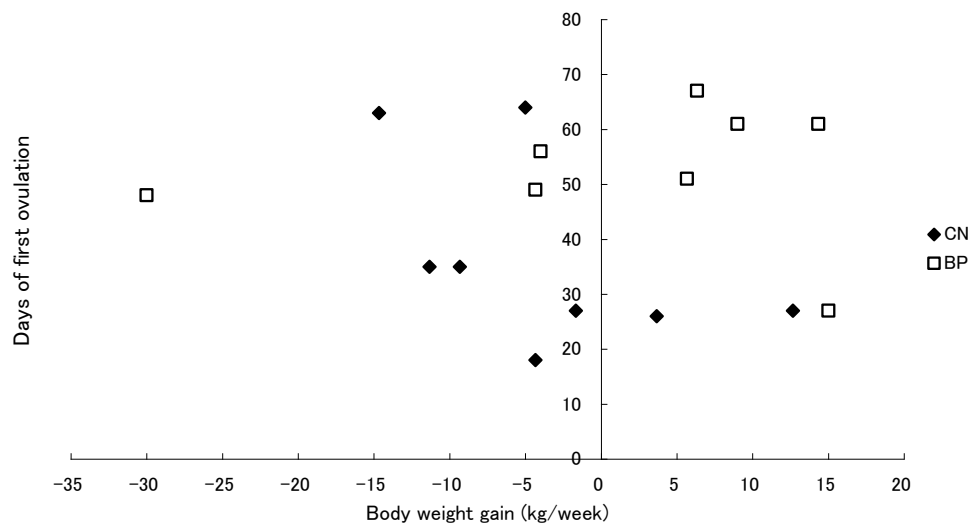


Figure 5. Body weight changes and days of first ovulation of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP)

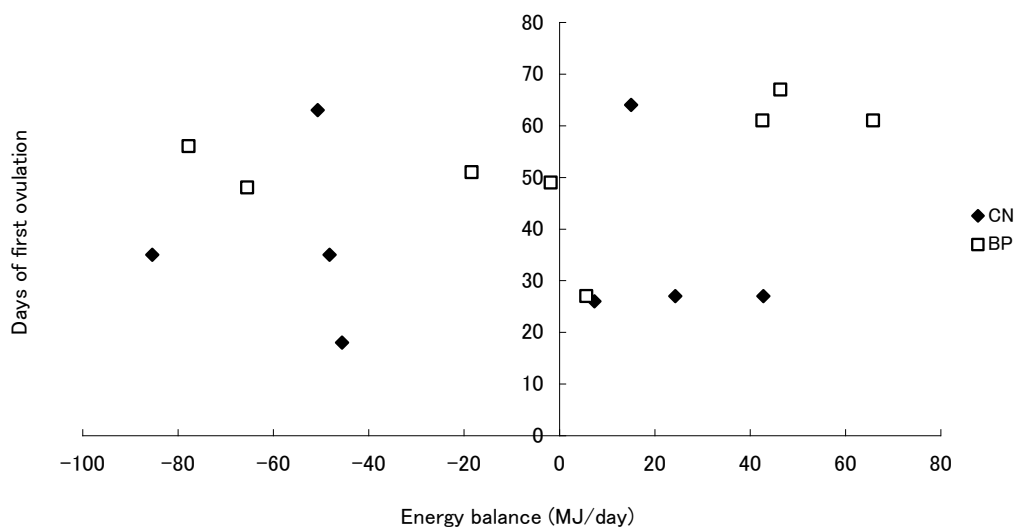


Figure 6. Energy balance and days of first ovulation of early lactating dairy cows fed steam flaked corn (CN) or beet pulp (BP)

3.5. Discussion

3.5.1. Dietary effects of corn or beet pulp in concentrates on ovarian functions

The metabolic and nutritional factors for post-partum cow to become pregnant are economically and scientifically important. High producing cows do not consume enough feed in the early stages of lactation to support their potential for milk production. Therefore, energy expenditures for milk production are contributed by body fat reserves (Villa-Godoy *et al.*, 1988). In this study, Total DMI, GE intake and ME intake of BP was higher than in those of CN. But glucose concentration was higher in CN than BP due to higher ruminal propionate production in CN than in BP.

When ultrasound scanning started about 7 days after calving, most of the cows had already developed a dominant follicle. Thereafter, continuous turnover in follicular waves (Appendix) was observed throughout the experimental feeding period except when ovulation occurred. Most of the cows did not ovulate the first dominant follicle of the postpartum period. Staples *et al.* (1990), Lucy *et al.* (1992), and Beam and Butler (1997, 1999) have shown that NEB status can significantly influence functional characteristics of ovarian follicular development during the early postpartum period and, therefore, affects the resumption of estrous cycles, interval from calving to the first ovulation and subsequent fertility. However, other studies have reported that the interval from calving to the first ovulation is not related to energy balance (Villa-Godoy *et al.*, 1988; Snijders *et al.*, 2001).

Gong *et al.* (2002) demonstrated that cows fed starch source in concentrate enhanced to ovulate before 50 days postpartum. The present study resulted days to first ovulation in CN and BP were 36.9 and 51.3 respectively (Table 7) although cows in two groups were under NEB within 3 weeks postpartum. As detected by

ultrasonography and confirmed by plasma progesterone measurements, profiles indicated that cows in CN ovulated about 40 days (ovulated before 50 days postpartum) but those in BP, about 55 days after calving (Table 7).

Cows in CN faced NEB during 1 to 6 weeks postpartum, but resumed early follicular function, and those in BP showed later follicular function despite positive energy balance from 4 to 6 weeks postpartum. High ruminal propionic acid resulted in increased concentrations of plasma glucose (Rutter *et al.*, 1983). Plasma glucose is a major source of energy for the ovary (Villa-Godoy *et al.*, 1988; Mc Dougall *et al.*, 1995 and Rabiee *et al.*, 1997), and a deficiency in glucose may impair ovarian function, resulting in follicles of poorer quality (Snijders *et al.*, 2001). Murahashi *et al.* (1996) found that a central sensor in the lower brain stem, the area postrema, could be an important glucosensor in the modulation of LH secretion. Moreover, plasma glucose is to be a metabolic signal providing information for the control of gonadotrophin releasing hormone (GnRH) secretion (Foster and Nagatani, 1999). Glucose appears to be centrally involved in the release of LH and this presumably reflects its role in modulating GnRH release to stimulate LH and follicle stimulating hormone and hence ovarian function. Therefore, the greater plasma glucose for CN in this study could be a cause why cows fed CN showed earlier ovulation early and more increase in intensity of estrous signs than cows fed BP. Standing heat could be observed only in cows fed CN. Standing immobile on being mounted is recognized as the primary and most reliable sign of estrus and the best indicator of the cow's preovulatory state (Hafez *et al.*, 1969). The present study indicates that glucose is the principal metabolic fuel of the ovary. Moreover, Gong *et al.* (2002) demonstrated that feeding high starch diet to dairy cows during early lactation reduced the interval from calving to the first

ovulation and high plasma glucose concentration was suggested as positive signal to the reproductive axis. Mc Clure *et al.* (1978) found that a glucose metabolic inhibitor (2-deoxy-D-glucose) blocked estrus and ovulation.

3.5.2. Interaction between carbohydrate source and parity

In multiparous cows, cows fed CN showed early resumption of ovarian cycle than cows fed BP. It may be due to glucose concentration of CN tended to be higher than that of BP although there was no significant difference between CN and BP in primiparous cows for plasma metabolites, recovery of ovarian function and reproductive behaviors. Furthermore, plasma insulin concentration was numerically, but not significantly, higher for cows fed CN than for cows fed BP in multiparous cow. Reproductive behaviors in multiparous were more intense than those in primiparous cows in the current study. It could be caused by the result that total DMI, GE intake, ME intake and glucose concentration in multiparous cows were higher than that of primiparous cows. Vasconcelos *et al.* (2003) stated that DMI and energy intake enhanced follicle development to secrete estrogen for estrus. Days of first ovulation and reproductive performances were not different between multiparous and primiparous cows. Parity does not affect on feed and energy intake and hence on ovarian function. Primiparous cows of both dietary treatments were under more severe NEB than multiparous cows in early lactating period because It might be DMI of primiparous cows was lower than that of multiparous cows. Moreover, dietary treatment could not affect on primiparous cows for recovery of ovarian function. In multiparous cows, cows fed CN were under more date NEB, however high in plasma glucose. Therefore, recovery of ovarian function was earlier in cows fed CN than BP.

3.5.3. Interaction between carbohydrate source and body weight changes

In type N, body weight loss was observed from calving to first week, and then gradually increased. In type I, body weight gain was observed throughout experimental period from calving. In type D, body weight loss was observed throughout experimental period from calving. In type N, cows in CN ovulated earlier than those in BP. This is probably because cows fed BP tended to be higher in plasma NEFA than cows fed CN. Elevated NEFA concentrations are an important characteristic of dairy cows during early lactating period because high NEFA reflect excessive mobilization of body fat reserves and consequent perturbation of hepatic function and hormonal metabolism which inhibit the ovarian function (Westwood *et al.*, 2002). In type I, feed and energy intake were high in BP than in CN. However, cows in CN were higher, numerically but not significantly, in glucose concentration than cows in BP. Therefore cows in CN ovulated earlier than cows in BP. In type D, all cows fed CN and BP was under NEB, and lower insulin level than in type I and type N. Therefore, those cows showed less intensity of estrous behaviors than type I and type N. During periods of NEB, blood concentrations insulin and glucose are low to enhance folliculogenesis, ovulation and steroid hormone such as estrogen (Villa-Godoy *et al.*, 1988).

3.5.4. Conclusion

Feeding beet pulp instead of corn in concentrate for cows during early lactation can not advance the recovery of ovulatory function by decreasing plasma glucose concentration in dairy cows. However, this dietary effect of steam flaked corn or beet pulp on the recovery of ovarian function was clearly evident in multiparous cows and dairy cows of normal type, not evident in primiparous cows and dairy cows

of increasing and decreasing types. In type N, cows fed CN was lower in NEFA than cows fed BP. In type N, recovery of ovarian function of CN was significantly earlier than that of BP. In type I, recovery of ovarian function of CN was about 2 weeks earlier than that of BP. In type D, two treatments were under NEB during early lactating period. Therefore, all cows in type D delayed recovery of ovarian function.

Chapter 4

General discussion

The objective of the current study was to fulfill energy level and to evaluate how to affect on rumen fermentation, blood metabolites and reproductive outcomes by replacing steam flaked corn with beet pulp in concentrates in the diets during the early postpartum period. The main problem of feeding BP to early lactating dairy cows is being low ruminal propionic acid production that stimulates to increase plasma glucose level although it can fulfill energy level to decrease NEB during early lactating period. The low level of ruminal propionic acid could reduce reproductive performances indirectly. A decrease in glucose, induced by a decrease in ruminal propionic acid production, would increase lipolysis and increase plasma NEFA concentration in cows in BP than those in CN. Inadequate energy intake causes mobilization of body lipids, which increases the concentration of NEFA in serum, and, in turn, can cause hepatic lipolysis if mobilization is excessive (Gerloff *et al.*, 1986; Grummer, 1995). Hepatic lipolysis decreases the glucogenic capacity of hepatocytes (Cadorniga *et al.*, 1997). After 6 weeks postpartum, the plasma NEFA had returned to lower level that indicates that cows in two treatments may resume positive energy balance nevertheless ME balance showed negative for CN. Evidence from several species highlights the importance of glucose as a mediator of nutritional effects on reproduction. Unlike monogastric animals, blood concentrations of glucose are much more stable in cattle. Plasma concentrations of glucose are inversely related to energy intake (Yelich *et al.*,

1996). Medina *et al.* (1998) has established that glucose availability influences both tonic and surge modes of LH secretion, presumably through its effects on GnRH to develop follicles and to secrete estrogen hormones for showing reproductive behaviors. Moreover LH is essential in ovarian follicular development postpartum and estrous behavior (Ferguson, 1996). Therefore the cows fed highly digestible fiber such as beet pulp show much less intensity of reproductive behaviors and delay the recovery of ovarian function than those fed starch source carbohydrates such as steam flaked corn nevertheless cows fed beet pulp in concentrates attained the recovery to the positive energy balance earlier than those fed corn in concentrates.

An animal in estrus starts with sniffing and chin resting, then starts to mount other animals and lastly displays standing heat. Sniffing and chin resting are not useful as predictors for time of ovulation; these signs were displayed by all animals in every estrus, but were not exclusive for estrus, and mounting seems to be an accurate sign of estrus (Van Eerdenburg *et al.*, 1996). Van Eerdenburg *et al.* (1996) found that less than 50% of the animals displayed standing heat during estrus. In the present study, standing heat was displayed in 25% of cows in CN in estrous periods.

Energy deprivation reduces the frequency of pulses of LH; thereby impairing follicle maturation and ovulation. Furthermore, undernutrition inhibits estrous behaviors by reducing responsiveness of the central nervous system to estradiol by reducing the estrogen receptor and content in the brain (Hileman *et al.*, 1999). Postpartum body condition associated with high NEFA is an important indicator for energy balance of early lactating dairy cows. Cows in type D faced NEB ovulated after 50 days postpartum. However, only cows of BP in type N and type I ovulated after 50 days postpartum although these cows did not face NEB. This could be caused by the lower

plasma glucose concentration in cows of BP compared with those for CN. Snijders *et al.* (2001) reported that the interval from calving to the first ovulation is not related to energy balance of cows, but directly related to glucose concentration (Lucy *et al.*, 1999). Villa-Godoy *et al.* (1988) also presented that glucose is a major source of energy for the ovary and that a deficiency in glucose can impair ovarian function, resulting in follicles of poor quality. NEB during early lactation for CN cows may be not severe because and body weight loss BCS loss was not observed in this study. Changes in BCS during early lactation indicate changes in energy balance (Villa-Godoy *et al.*, 1988). Thus, cows losing more BCS early postpartum should show a more severe NEB, which could result in reduced reproductive performance. Indeed, Butler and Smith (1989) observed that only severe body condition losses postpartum were associated with lower first service conception rate. Therefore, severe NEB in early lactating dairy cows could decrease BCS which inhibits recovery of ovarian function. However, moderate NEB in early lactating dairy cows could maintain BCS which does not affect on recovery of ovarian function.

Conclusion

This study clearly showed substituting steam flaked corn (starch source diets) with beet pulp (highly digestible fiber) in the diets of dairy cows had beneficial effects to improve NEB that dairy cows face on during early lactating period. Therefore, beet pulp could be a satisfactory source of energy in rations to recover NEB in early stage for early lactating dairy cows. However, feeding beet pulp in concentrate to early lactating dairy cows delayed the recovery of ovarian function and also showed less intensity of reproductive behaviors by decreasing plasma glucose concentration

compared with feeding steam flaked corn in concentrate. Moreover, this study indicated that NEB in dairy cows during early lactating period was not the primary factor for early resumption of ovarian function but high plasma glucose concentration altered by carbohydrate source in concentrate is a positive signal for the recovery of ovarian function.

SUMMARY

The objective of this study was to decrease NEB at early lactating period and to affect on rumen fermentations, plasma metabolites and reproductive performances by feeding carbohydrates. Sixteen lactating Holstein dairy cows were randomly separated into two groups, and two groups were assigned to one of the following dietary treatments (1) CN: feeding steam flaked corn (4kg FM/d) + commercial formula feed (4kg FM/d) + soybean meal (2kg FM/d) + grass hay (3kg FM/d) and corn silage (CS) (*ad lib*); (2) BP: feeding beet pulp (4kg FM/d) and amount of other feed was the same as CN. The experiment started immediately after calving. Total DMI of BP was significantly higher than that of CN. DM digestibility and GE digestibility tended to be higher for BP than for CN. GE and ME intake of BP was significantly higher than that of CN. An interaction was detected for energy balance indicating earlier recovery to positive energy balance for BP than for CN. There was no treatment effect on milk yield, FCM milk yield, milk fat, milk protein, milk lactose and SNF. The molar portion of acetic acid of BP was significantly higher than that of CN. The molar portion of propionate of CN was significantly higher than that of BP. Plasma glucose of cows fed CN tended to be higher than that of cows fed BP, nevertheless, no difference was observed in the plasma insulin, NEFA and BUN concentrations between CN and BP. Day postpartum for first progesterone rise in CN tended to be 2 weeks earlier than that in BP. CN also tended to be ovulated 2 weeks earlier than BP. Standing heat was observed only in CN at first estrus. Moreover cows in CN showed more intensity of reproductive behaviors, but not significantly, such as mounting and sign of sniffing vagina than those in BP. In multiparous cows, dietary treatments did not affect

on energy balance of early lactating period. However, plasma glucose level in CN during DIM < 60 in CN tended to be higher than that in BP. Days postpartum for first progesterone rise and first ovulation in CN tended to be earlier and days for first estrus and first insemination in CN was about 2 weeks earlier than those in BP in multiparous cows. In primiparous cows, there were no significant difference between CN and BP energy balance and plasma glucose during DIM < 60. In primiparous cows, there was no significant difference between CN and BP for the recovery of ovarian function. There was no significant difference between CN and BP in type I and type D for energy balance and plasma glucose. No significant difference for days of first progesterone rise and first ovulation was observed between CN and BP in type I and type D. There was difference between CN and BP in type N and in type D for plasma NEFA. Days postpartum for first progesterone rise and first ovulation of CN were significantly earlier than those of BP in type N. Standing heat was seen in cows of CN in type N and type I. In conclusion, substituting steam flaked corn with beet pulp in the dietary ration of dairy cows could be a satisfactory source of energy in rations for early lactating cows. However, feeding beet pulp in concentrate to early lactating cows delayed the resumption of ovarian function by decreasing plasma glucose concentration. Moreover, moderate NEB in early dairy cows is not the primary factor for early resumption of ovarian function and high plasma glucose concentration influenced the recovery of ovarian function.

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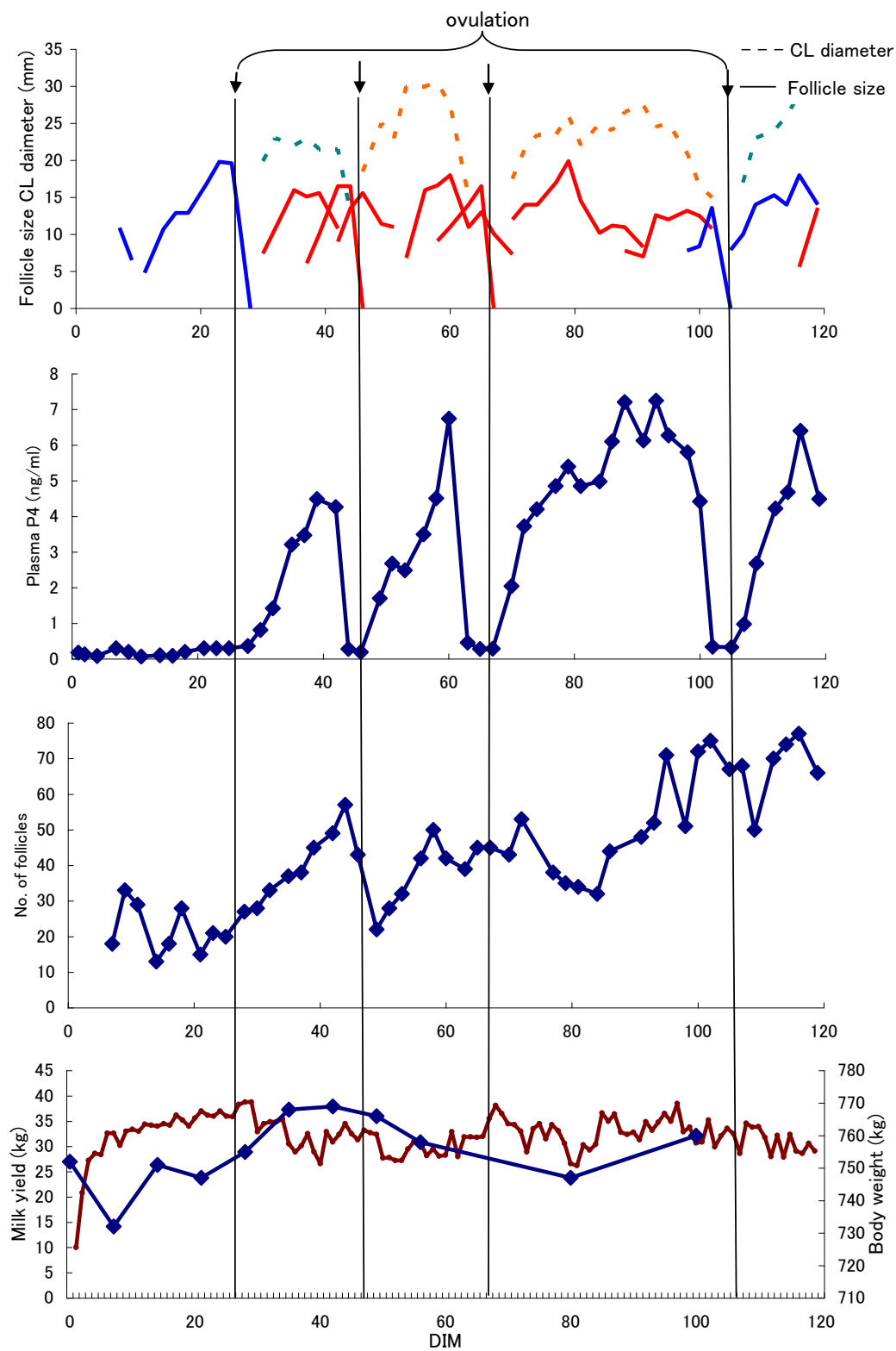
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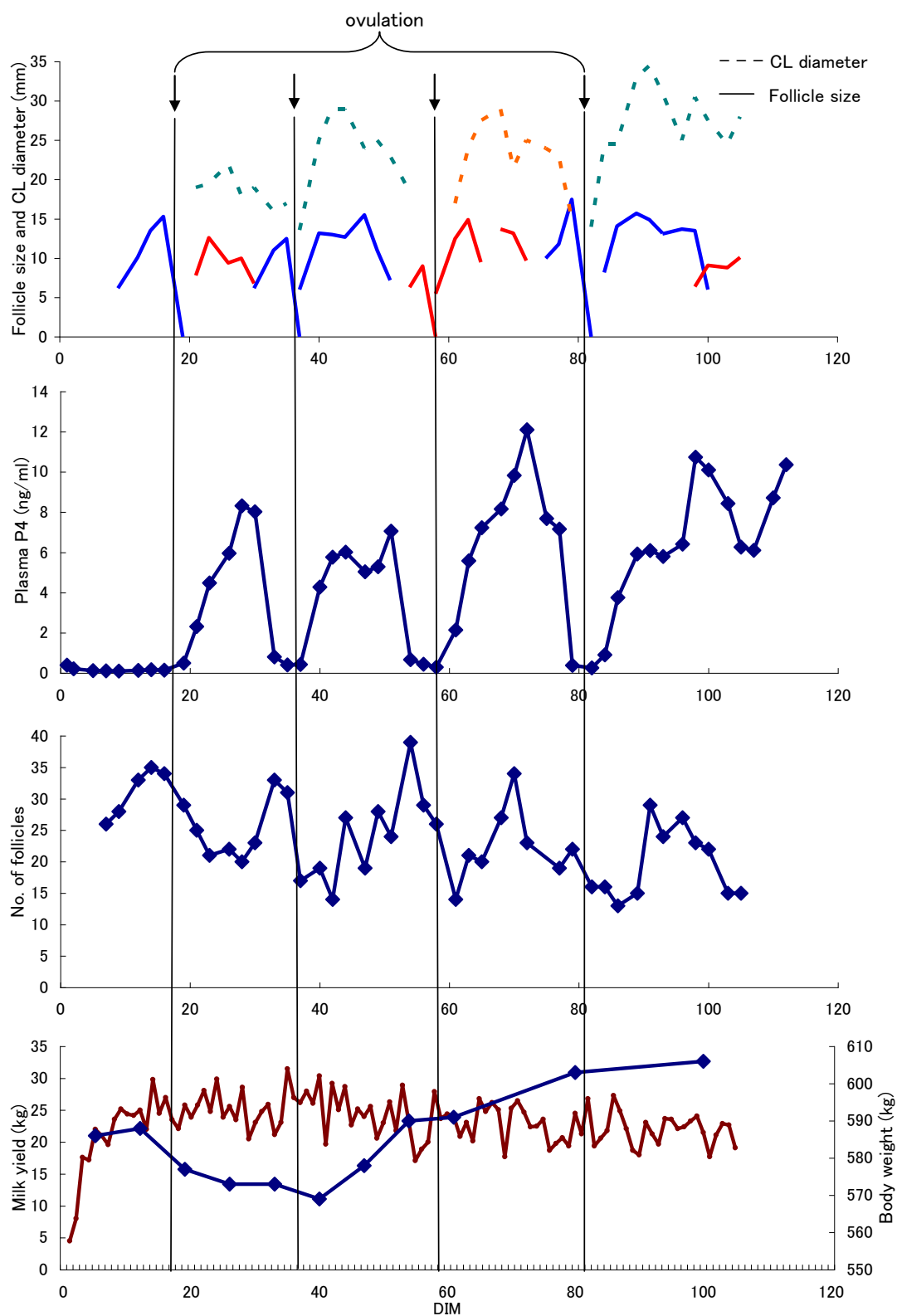
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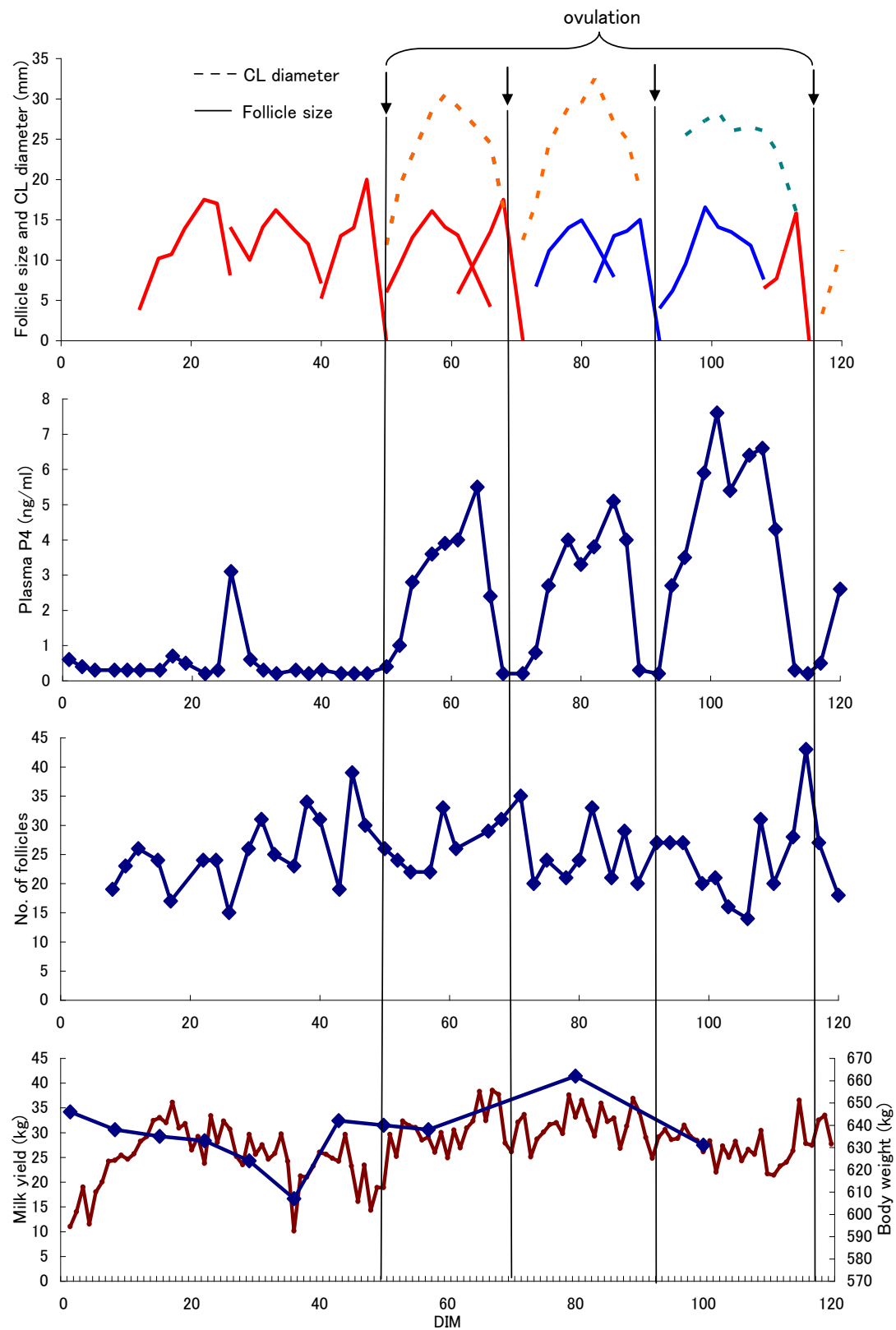
APPENDIX



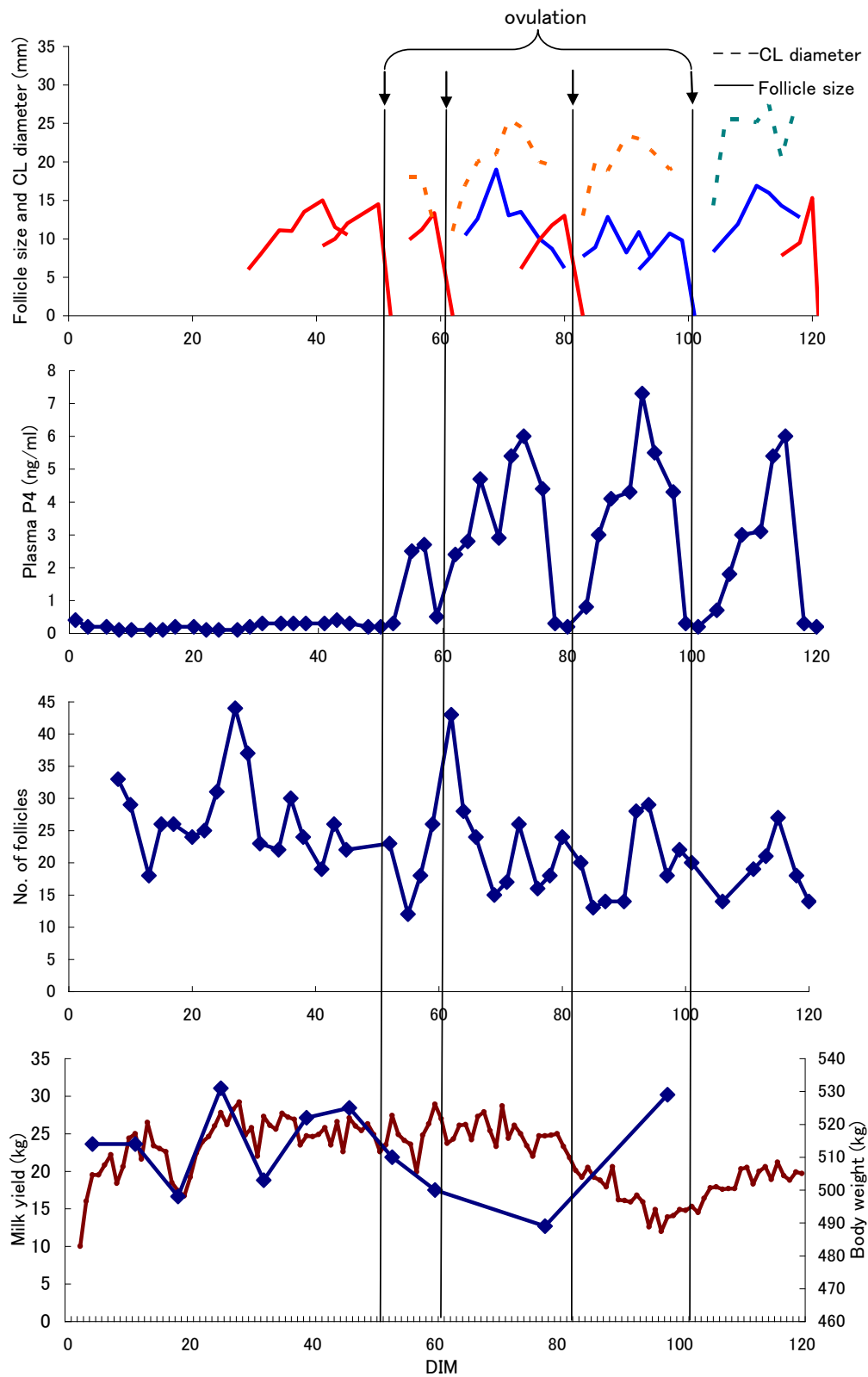
Appendix 1 a. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of normal type after calving to 120 DIM of dairy cow (no. 1190) fed steam falked corn



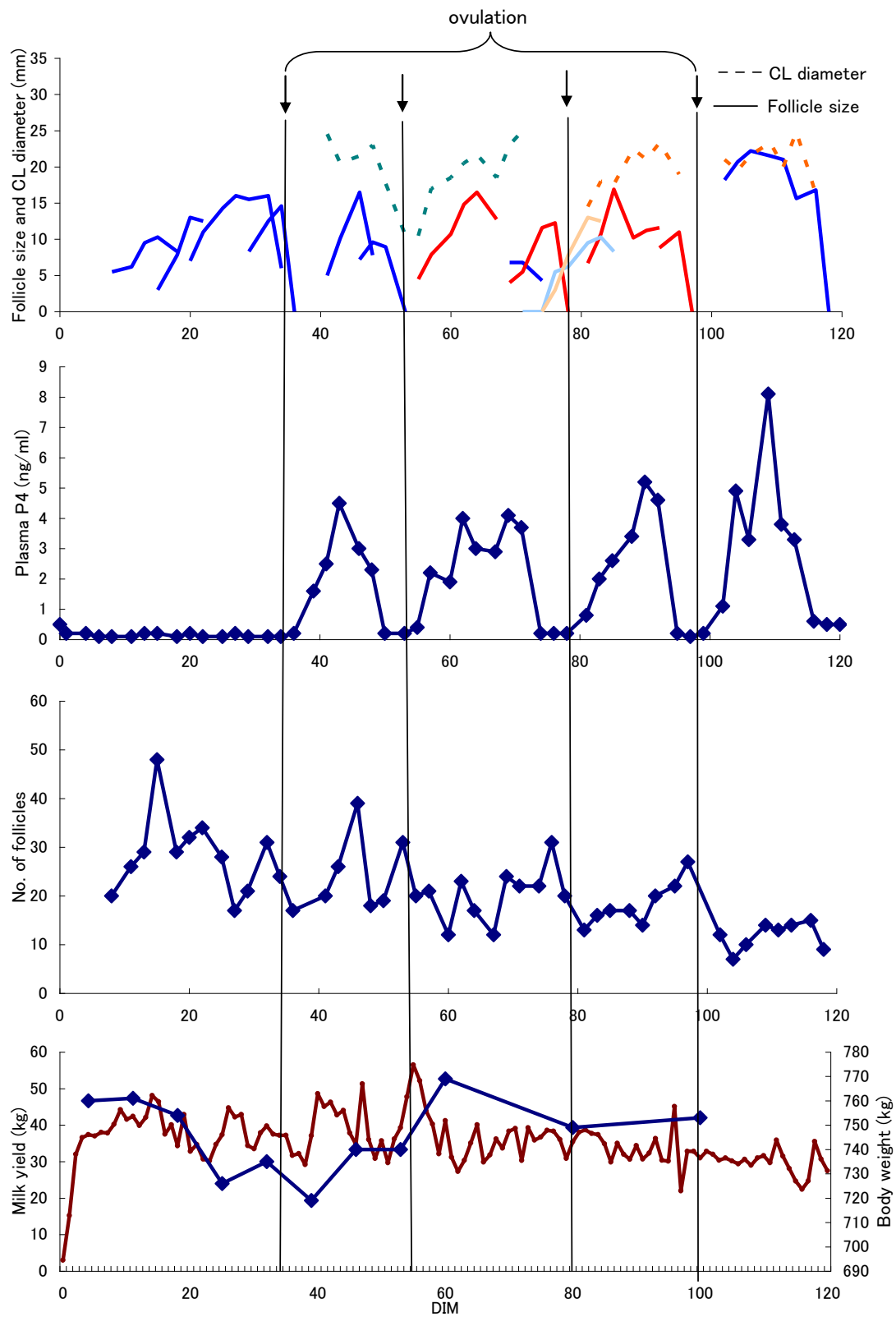
Appendix 1 b. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of normal type after calving to 120 DIM of dairy cow (no. 1232) fed steam falked corn



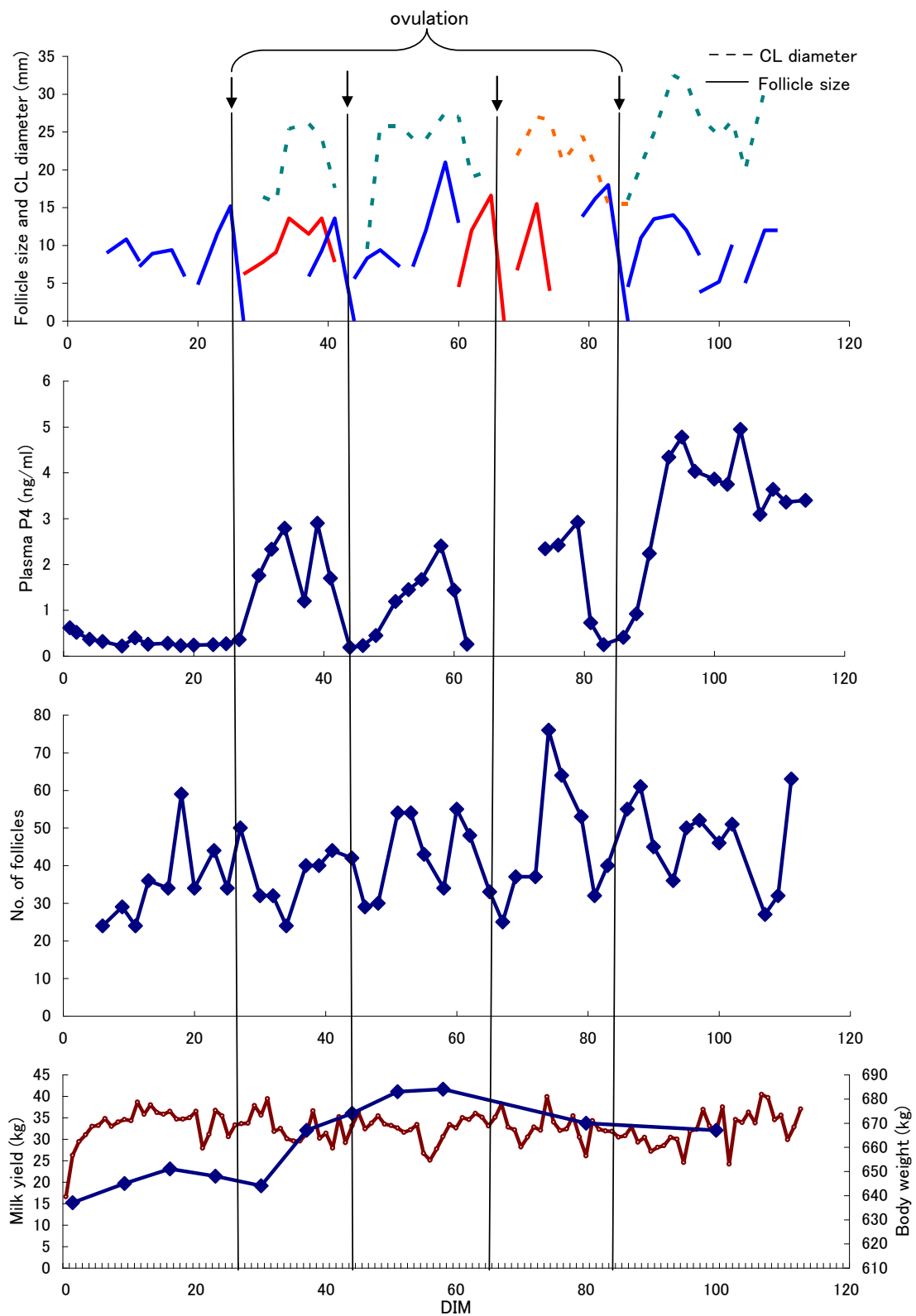
Appendix 1 c. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of normal type after calving to 120 DIM of dairy cow (no. 1211) fed beet pulp



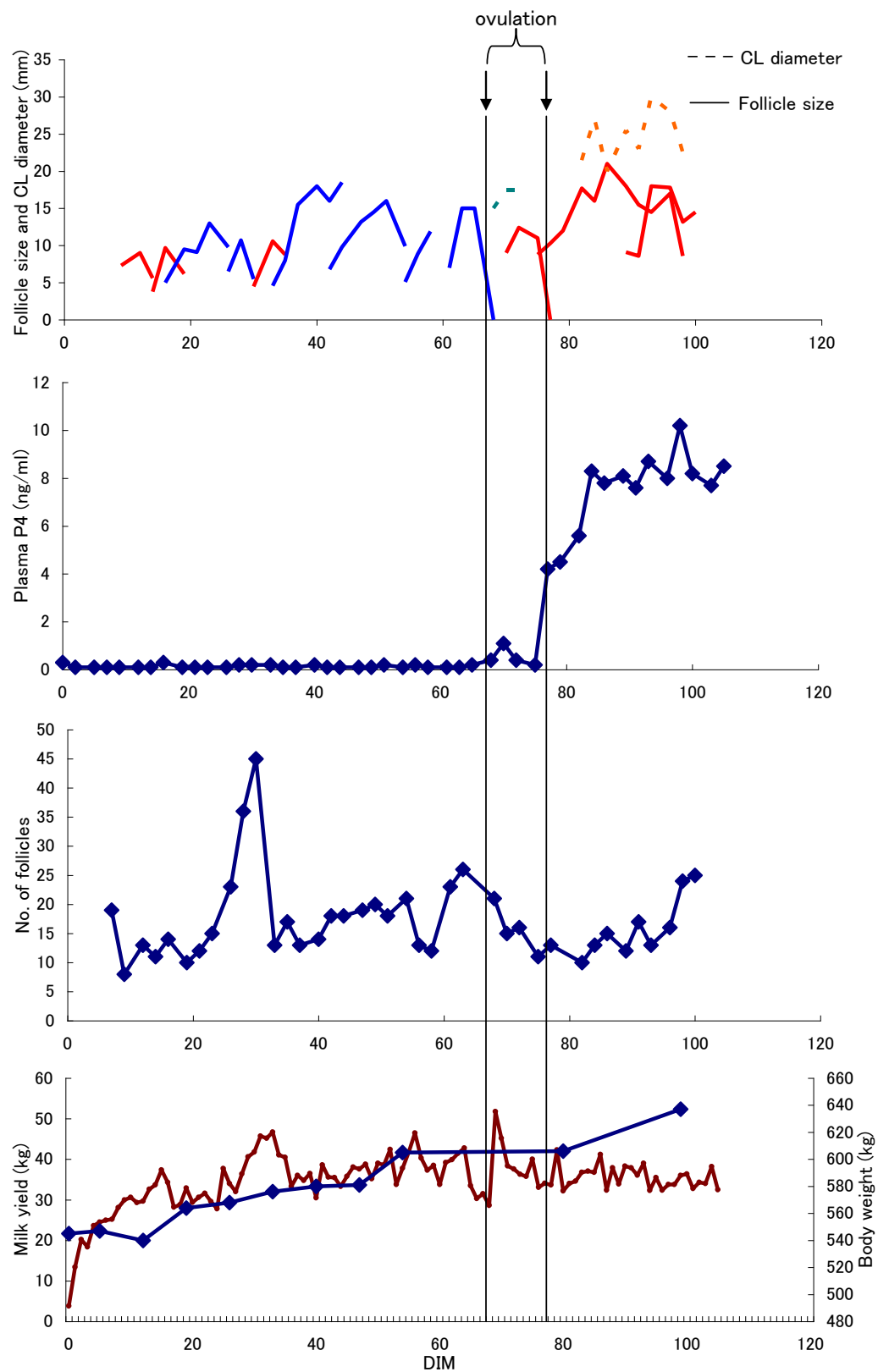
Appendix 1 d. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of normal type after calving to 120 DIM of dairy cow (no. 1234) fed beet pulp



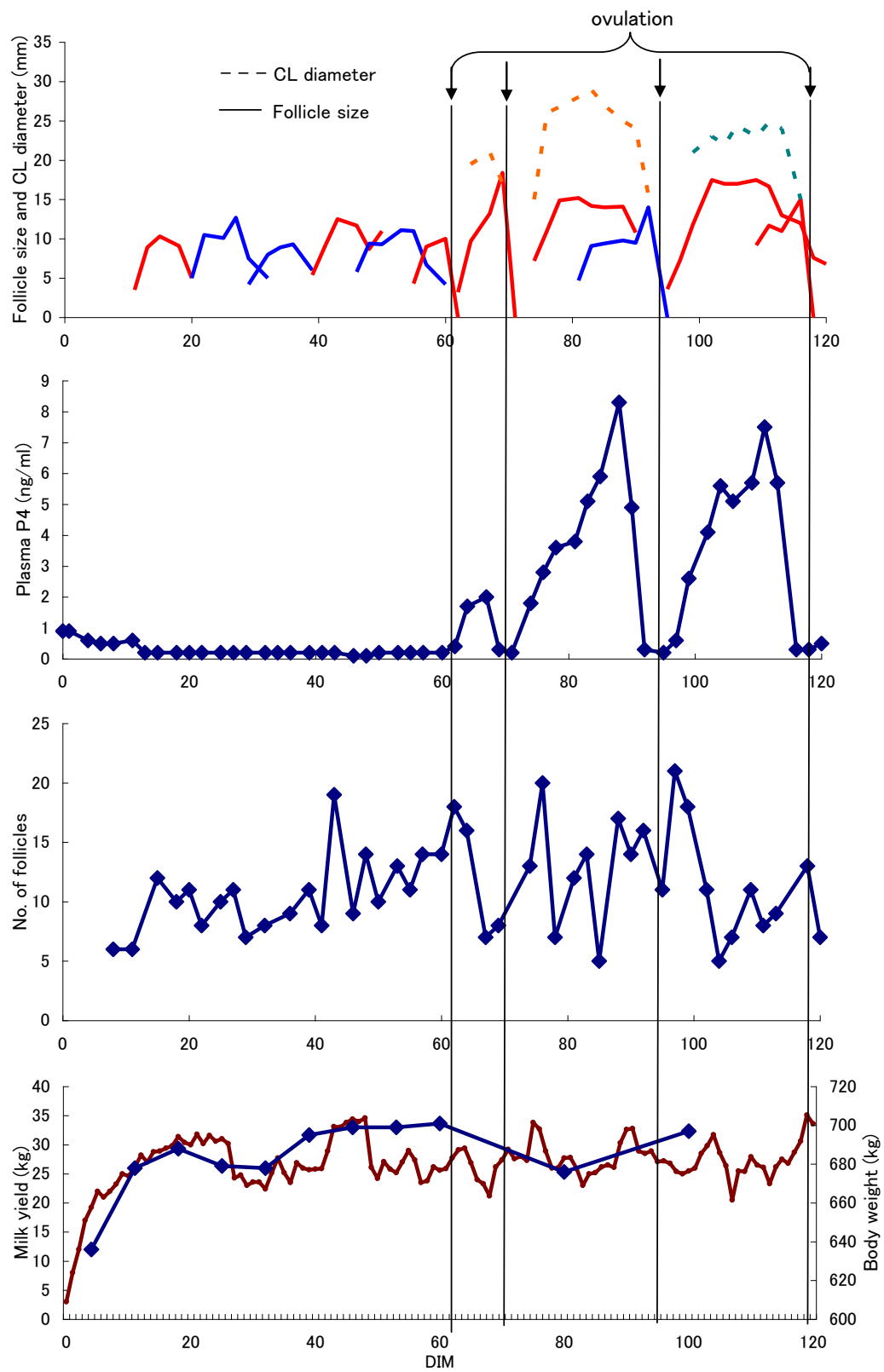
Appendix 1 e. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of normal type after calving to 120 DIM of dairy cow (no. 1239) fed steam falked corn



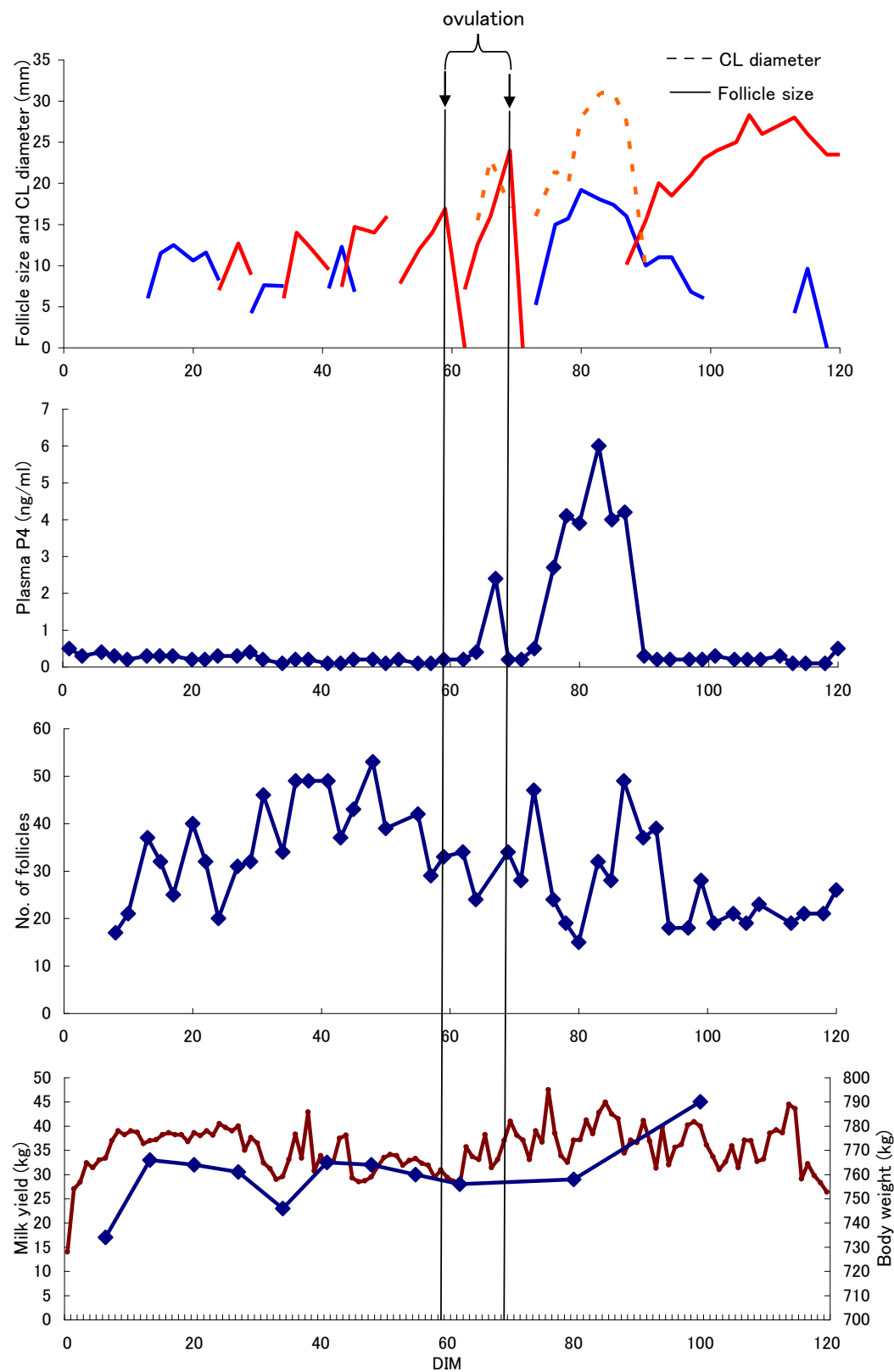
Appendix 2 a. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1179) fed steam flaked corn



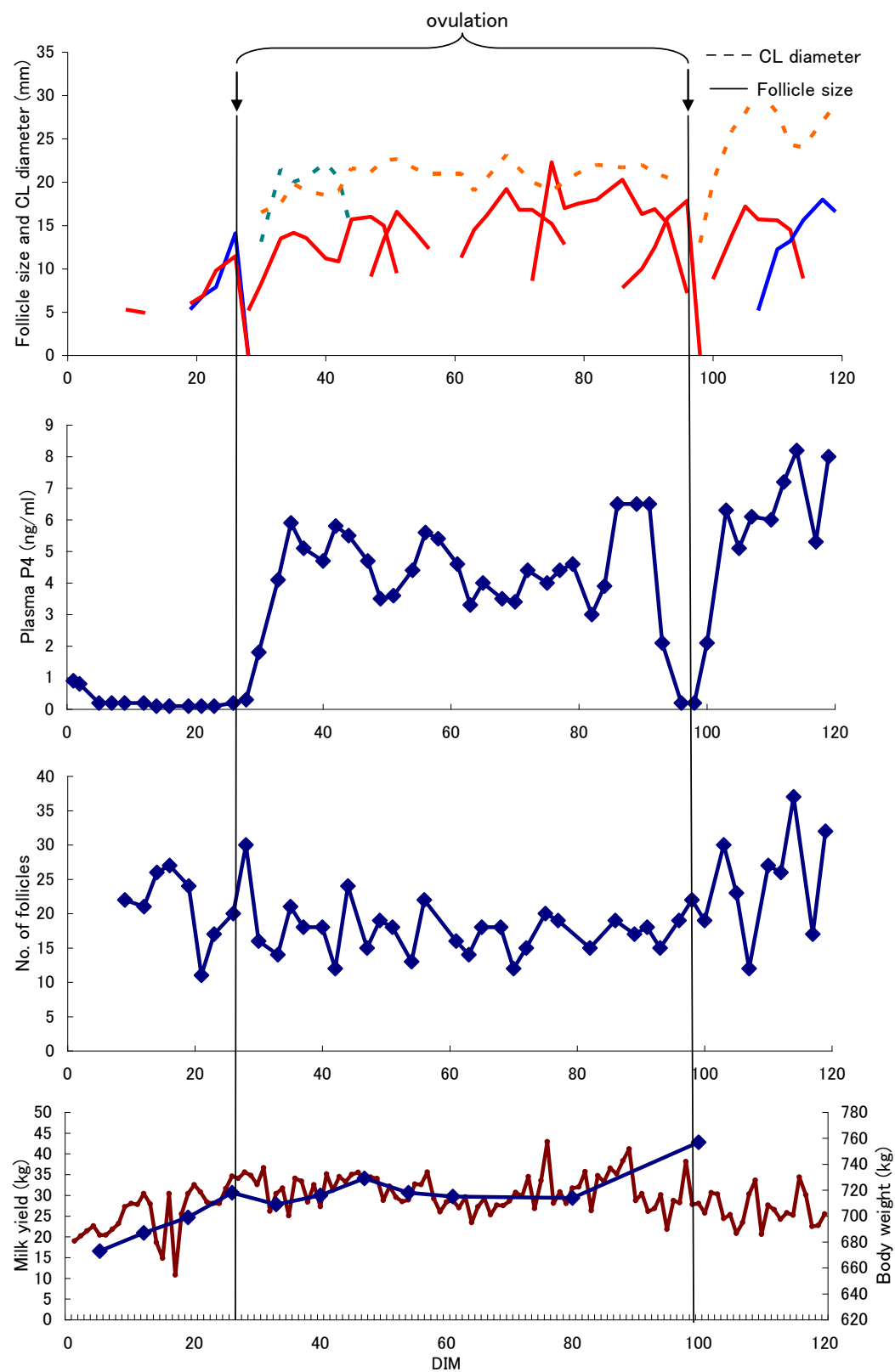
Appendix 2 b. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1241) fed beet pulp



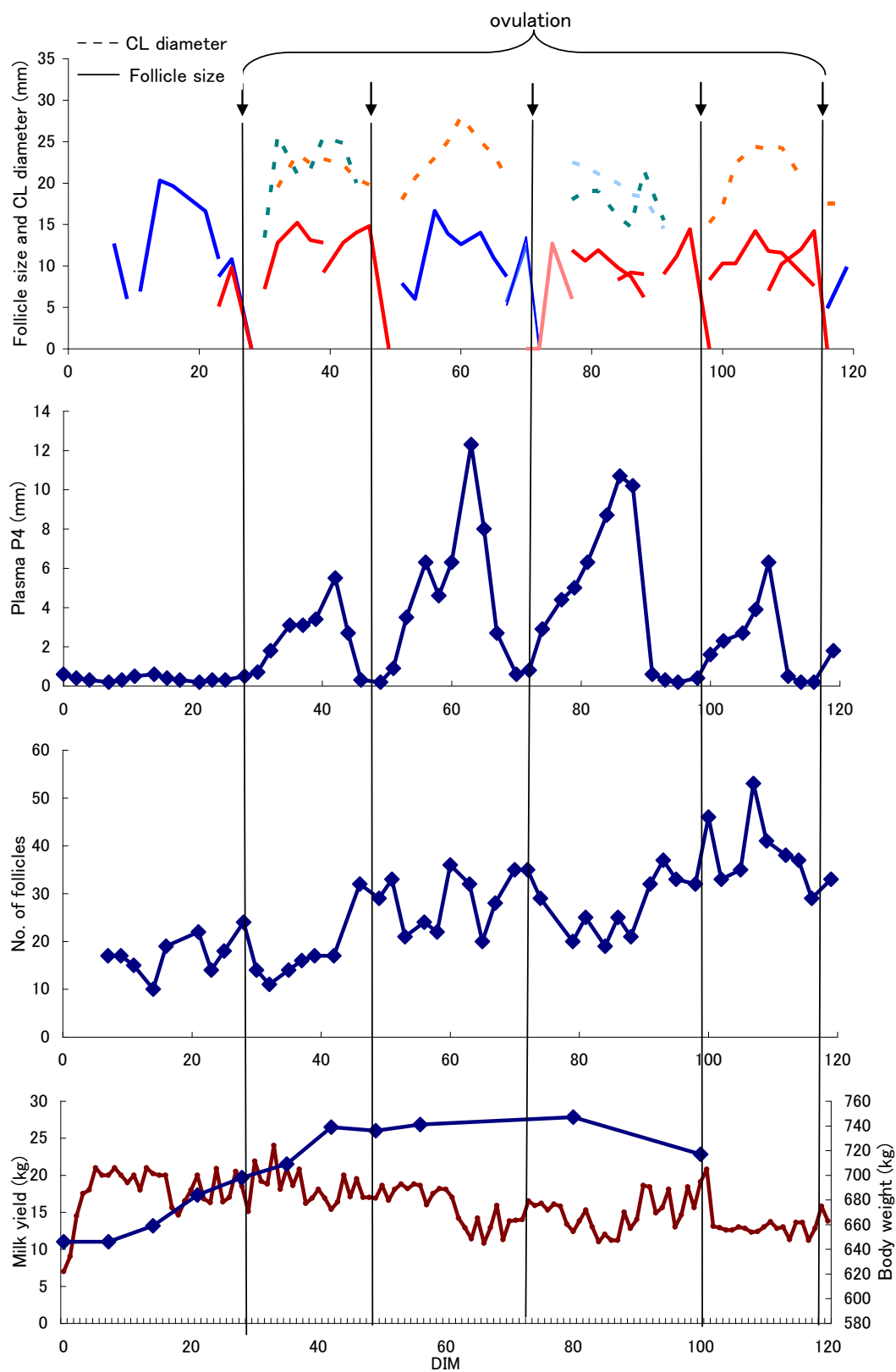
Appendix 2 c. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1242) fed beet pulp



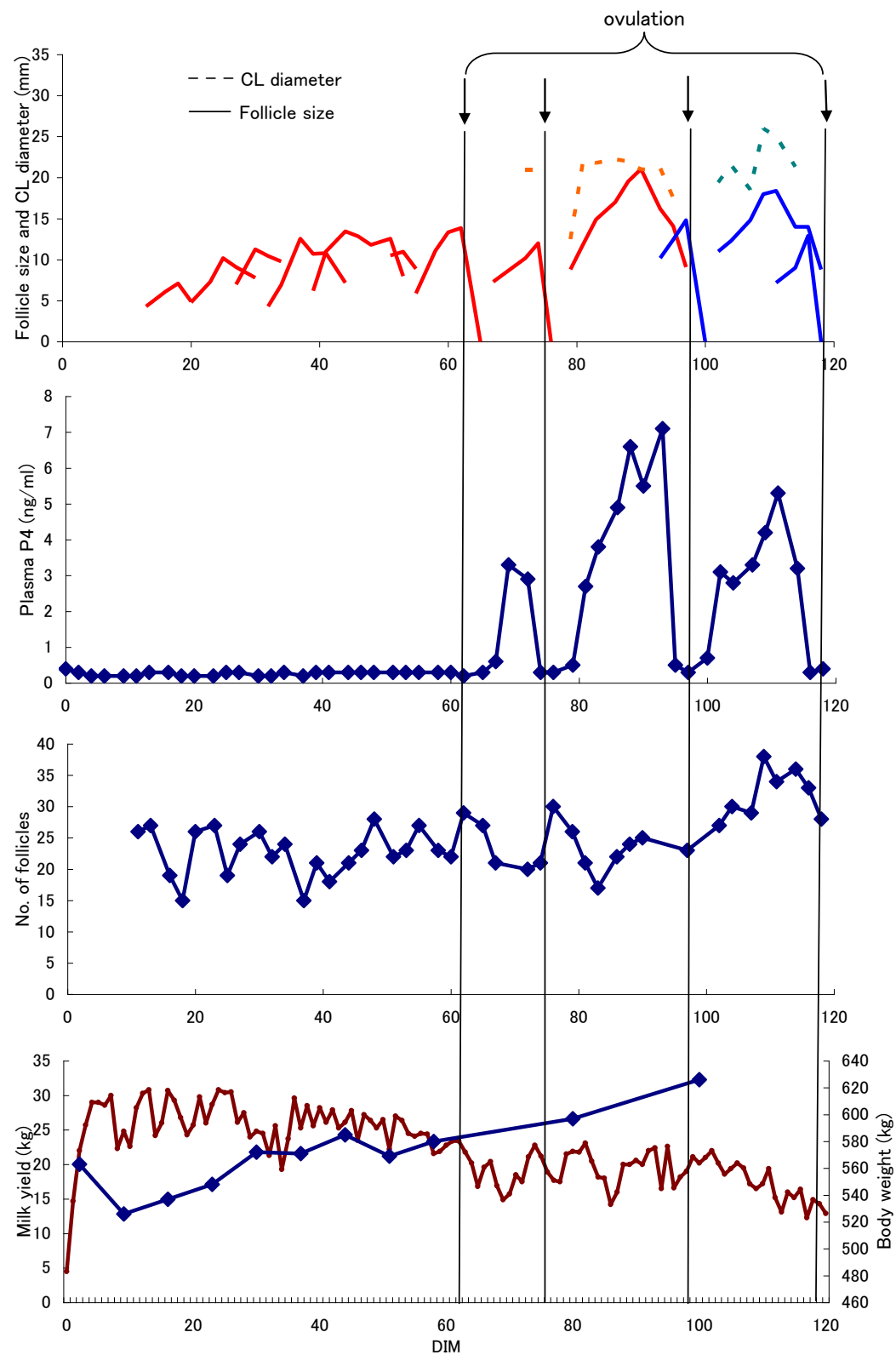
Appendix 2 d. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1214) fed beet pulp



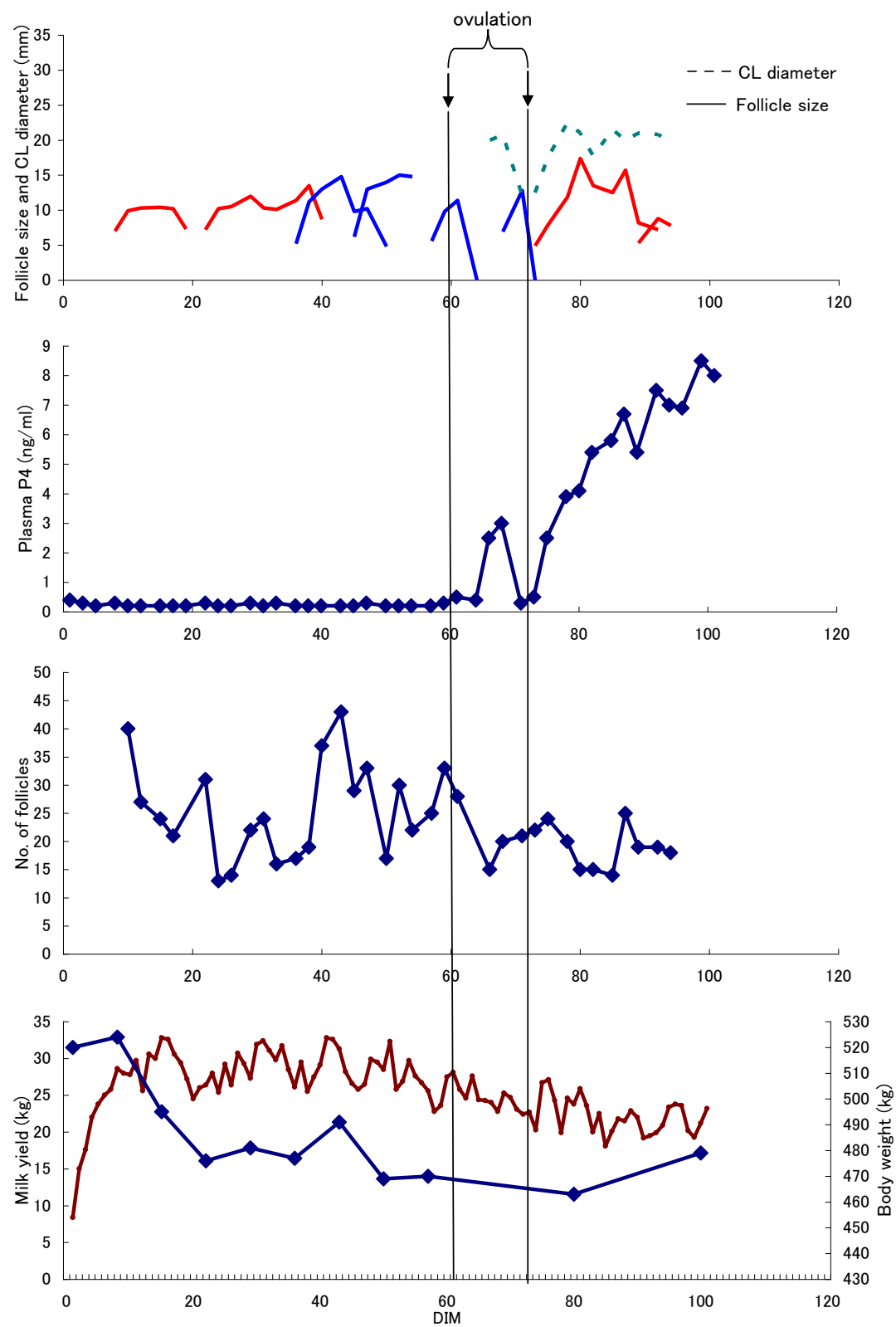
Appendix 2 e. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1209) fed beet pulp



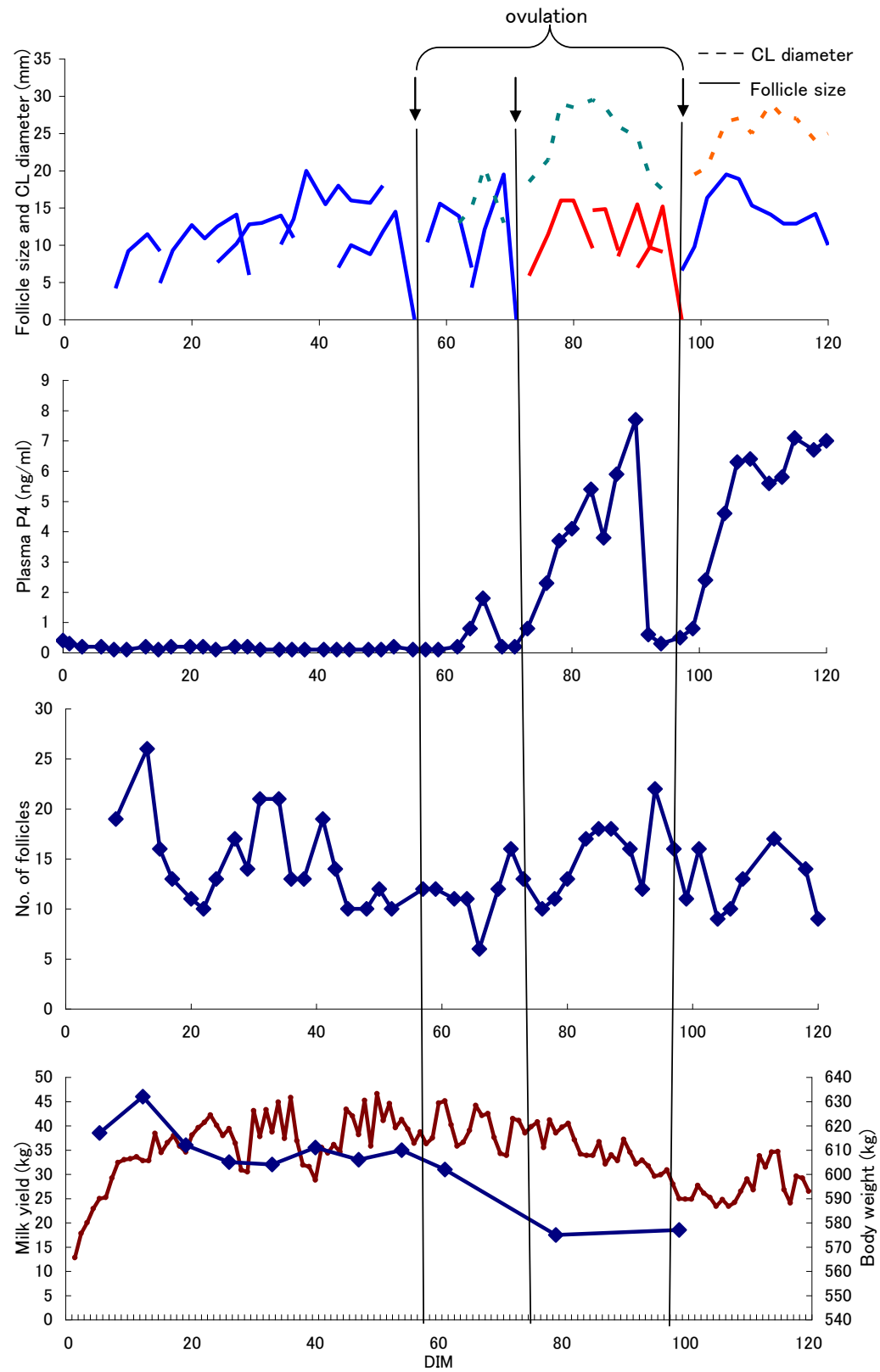
Appendix 2 f. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1222) fed steam flaked corn



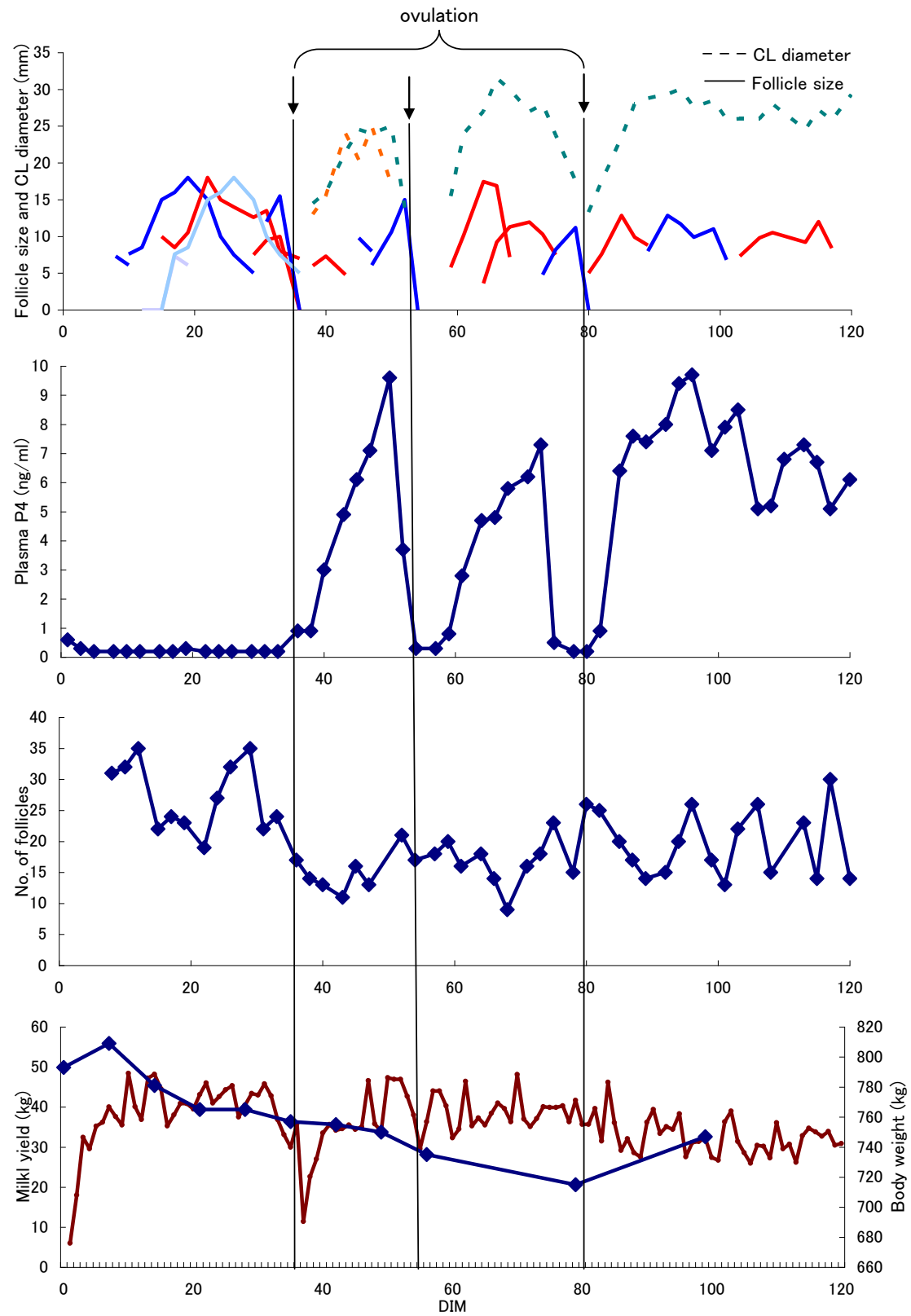
Appendix 2 g. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of increasing type after calving to 120 DIM of dairy cow (no. 1189) fed steam flaked corn



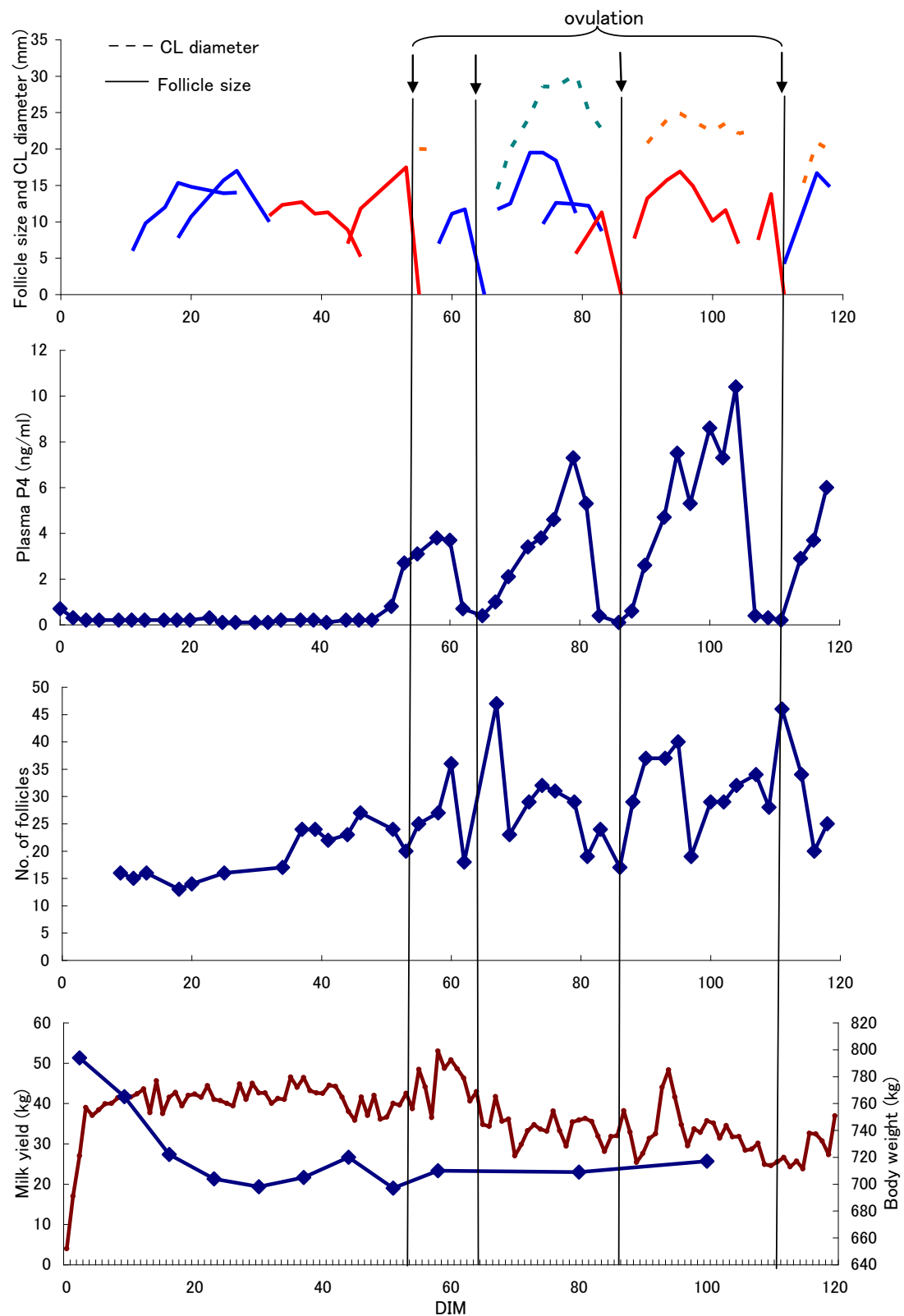
Appendix 3 a. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of decreasing type after calving to 120 DIM of dairy cow (no. 1235) fed steam flaked corn



Appendix 3 b. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of decreasing type after calving to 120 DIM of dairy cow (no. 1233) fed beet pulp



Appendix 3 c. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of decreasing type after calving to 120 DIM of dairy cow (no. 1210) fed steam flaked corn



Appendix 3 d. Follicle and corpus luteum (CL) size, progesterone level, number of follicles, milk yield and body weight of decreasing type after calving to 120 DIM of dairy cow (no. 1221) fed beet pulp