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Original Article

Differences in daily milk production during early pregnancy alter placental characteristics and neonatal metabolic amino acid levels in dairy cows

Riku MASHIMO[1)], Sayaka ITO[1)] and Chiho KAWASHIMA[1)]

[1)]*Field Centre of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan*

Abstract. We investigated the effects of differences in milk production during early pregnancy on placental characteristics at full term, calf birth weights, and their metabolic status. Thirty-four Holstein cows were categorized into three groups (Low, n = 9; Middle, n = 16; High, n = 9) based on the quartile of average daily 4% fat-corrected milk production during early pregnancy. The High group showed higher milk component production than the other groups ($P < 0.05$) during early and mid-pregnancy. Although most placental characteristics did not differ significantly among the groups, cows in the High group had larger individual cotyledons and fewer medium-sized cotyledons than those in the Low group ($P < 0.05$). Plasma amino acid concentrations of calves in the Low and High groups were significantly higher than those of calves in the Middle group, although calf birth weights were similar among the groups. Furthermore, cows in the Low group had longer dry periods than those in the High ($P = 0.004$) and Middle ($P = 0.058$) groups. This suggests that cows in the Low group may have provided more amino acids to the fetus because of low lactation and long dry periods. Conversely, cows in the High group required more energy for lactation during early pregnancy, which can reduce nutrient availability to the placenta and fetus; however, increasing individual cotyledonary sizes during late pregnancy may ensure that the same amounts of amino acids as those in cows in the Low group are supplied to the fetus, recovering the birth weights.

Key words: Calf birth weight, Cotyledons, Milk production, Plasma amino acid concentration

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In mammals, the placenta is the first organ to form during embryonic development; it exchanges respiratory gases, nutrients, and waste between the maternal and fetal systems throughout pregnancy [1, 2]. Therefore, proper placentation is critical for successful pregnancy [1]. In cattle, different growth patterns have been reported between the placenta and fetus. Although the bovine placenta grows throughout pregnancy [3], most growth occurs during early to mid-pregnancy [4–6]. In contrast, fetal exponential growth is limited to late pregnancy [4–6]; approximately 90% of the birth weight is obtained during this period [5]. However, as placental and fetal weights at full term are positively correlated in numerous species [5], placental growth during the first two-thirds of pregnancy plays a significant role in the subsequent trajectory of fetal growth [5, 7], thus directly influencing birth weight [5].

Studies in beef cattle have indicated that maternal nutritional status affects placental and fetal growth. Maternal undernutrition during early to middle pregnancy reduces the placental and fetal weights [8–10]. However, re-alimentation to an adequate nutritional plane in subsequent terms results in improved placental size [8], placental vascularity [9], uterine arterial blood flow [11, 12], and recovery of fetal weight to the same level as cows fed a sufficiently nutritious diet [8–11]. Furthermore, maternal protein restriction during early pregnancy increases the cotyledonary weight and trophectoderm volume observed at full term [13, 14]. These reports indicate that nutrient restriction during early to middle pregnancy and re-alimentation in the subsequent period may enhance transplacental nutrient exchange through dramatic placental growth, thus increasing fetal body weight.

In the dairy industry, feed composition is tightly controlled by optimizing production systems, and the diets provided have met or exceeded nutrient requirements [15, 16]. However, current production systems are not conducive for fetal development [15] because the nutritional requirements for placental and fetal growth must compete with those for milk production [16, 17]. During pregnancy, the mammary glands require more nutrients than the uterus [18]. Therefore, lactating cows experience a greater loss of nutrients to the uterus owing to the metabolic priority of lactation [15], which leads to a lighter placenta and fetus compared to non-lactating cows during early pregnancy [19]. Furthermore, high-lactating cows may have reduced nutrient availability for placental and fetal growth compared to low-lactating cows because of significant tissue mobilization and nutrient availability for milk production [20]. However, few studies have investigated the effects of differences in milk production on placental characteristics, fetal growth, and metabolic parameters at full term.

We hypothesized that high-lactating cows would improve fetal nutrient availability through compensatory placental growth (increased weight and surface area) during late pregnancy, producing calves with heavy birth weight. This study aimed to determine whether differences in maternal average daily milk production during early pregnancy affect placental characteristics at full term, the birth weight of calves, and their metabolic status.

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Correspondence: C Kawashima (e-mail: kawasima@obihiro.ac.jp)
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Materials and Methods

Experimental animals, feeding, and management

We investigated 34 Holstein-Friesian cows (parity: 1–4 at the onset of the experiment) that spontaneously expelled their complete placenta within 12 h of parturition and calved between April 2021 and October 2022 at the Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Japan.

During lactation, all cows were housed in a free-stall barn with a paddock and milked twice daily at 0500 and 1700 h. The animals were fed a mixed diet comprising grass silage, corn silage, and concentrate for dairy cows at 1500 h. Approximately 2 months before the expected calving date, cows were moved to the close-up dry period house with a paddock after the dry-off treatment and were fed a mixed ration comprising grass silage, corn silage, and concentrate for dry cows at 1500 h until calving. They also had free access to hay, minerals, and water. One week before the expected calving date, the rectal temperature of the cows was recorded every day at 1500–1600 h. When the rectal temperature of the cows decreased by 0.5°C compared to the previous day, the cows were moved to individual rooms and housed until calving. The calves were separated from their respective dams immediately after calving. The calves were cleaned and dried using a towel, and their umbilical cords were disinfected. The body weights of the calves were measured and they were subsequently housed in individual pens until the first colostrum feeding. The maternal body condition score (BCS) at pre-calving was measured by the same operator using a scale (1 to 5) at 0.25 intervals [21]. Furthermore, the lactation period, days open, gestation length, dry period, and level of calving difficulty were recorded for all cows.

All experimental procedures complied with the Guide for the Care and Use of Agricultural Animals of Obihiro University (approval numbers: #20-224, 20-226).

Sampling and measurement of milk production and milk components

We recorded the daily milk production of each cow from early pregnancy (days 1–90) to mid-pregnancy (days 91–180). Milk samples were collected monthly during the same period either in the morning or evening. Milk samples were stored at 4°C until further analysis and analyzed within 24 h of collection. Components such as lactose, milk fat, and milk protein were quantified at the Tokachi Federation of Agriculture Cooperatives (Hokkaido, Japan) using an infrared analyzer (MikoScanTMFT+, FOSS Analytical A/S, Hillerød, Denmark).

Placental collection and measurement

The total placenta was weighed immediately after expulsion. The cotyledons were then separated from the inter-cotyledonary membranes by pinching each cotyledon away from the inter-cotyledonary membranes and cutting them with scissors. Cotyledons weighing < 2.0 g were excluded. Individual cotyledons were then counted, weighed, flattened on a grid sheet, and photographed for surface area analysis using an image analysis software (ImageJ; https://imagej.net/software/fiji/). The weight of the inter-cotyledonary membrane was measured after the umbilical cords were cut. The total cotyledonary weight and surface area were calculated as the sum of all cotyledonary weights and surface areas, respectively. Additionally, we recorded the time interval between calving and placental expulsion.

Sampling and measurement of blood parameters

Blood samples from cows (dams) were collected by caudal venipuncture pre-calving (2–3 weeks before the expected calving date) and immediately after calving (within 1 h). Blood samples were collected from the jugular veins of the calves immediately after birth (within 1 h), before the first colostrum feeding. Non-heparinized, silicone-coated 9 ml tubes (Venoject, Autosep, Gel + Clot. Act., VP-AS109K; Terumo Corporation, Tokyo, Japan) were used for non-esterified fatty acid (NEFA), β-hydroxybutyrate (BHB), total protein, albumin, and glucose measurements; 5 ml tubes containing ethylenediaminetetraacetic acid (Venoject II, VP-NA050K; Terumo Corporation, Tokyo, Japan) were used for amino acid measurements. Furthermore, sterile 9 ml tubes containing a 200 µl stabilizer solution (0.3 mol/l EDTA-2 Na and 1% acetylsalicylic acid, pH 7.4) were used for insulin analysis. Blood samples were collected and coagulated for 10 min at 38°C in an incubator to obtain the serum. All tubes were then centrifuged at 2,328 × *g* for 15 min at 4°C, and the separated serum and plasma samples were stored at –30°C and –80°C, respectively, until they were subjected to NEFA, BHB, total protein, albumin, glucose, amino acid, and insulin analyses. Serum NEFA, BHB, total protein, albumin, and glucose concentrations were analyzed using an automated clinical chemistry analyzer (TBA120FR; Toshiba Medical Systems Co., Ltd., Tochigi, Japan). Plasma amino acid concentrations were analyzed at the NTDS Cooperation (Hokkaido, Japan) using ultra-performance liquid chromatography-mass spectrometry. Plasma insulin concentrations in pre-calving cows were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kits (Bovine Insulin ELISA 10-1201-01; Mercodia, Uppsala, Sweden). The mean intra- and inter-assay coefficients of variation were 6.27% and 3.32%, respectively.

Insulin resistance evaluation

The insulin resistance of pre-calving cows was assessed using the revised quantitative insulin sensitivity check index (RQUICKI), the revised quantitative insulin sensitivity check index β-hydroxybutyrate ($RQUICKI_{BHB}$), and the homeostasis model of insulin resistance (HOMA-IR) as insulin sensitivity indices [22].

RQUICKI was calculated from serum glucose, NEFA, and plasma insulin concentrations using the following equation [23]: RQUICK = 1/[log (glucose; mg/dl) + log (insulin; µIU/ml)] + log (NEFA; mmol/L)].

$RQUICKI_{BHB}$ was calculated from serum glucose, NEFA, BHB, and plasma insulin concentrations using the following equation [24]: $RQUICK_{BHB}$ = 1/[log (glucose; mg/dl) + log (insulin; µIU/ml) + log (NEFA; mmol/L) + log (BHB; mmol/L)].

HOMA-IR was calculated from serum glucose and plasma insulin concentrations using the following equation [25]: HOMA-IR = insulin (µIU/ml) × glucose (mmol/L)/22.

Statical analysis

Based on milk production and milk fat percentage, 4% fat-corrected milk (FCM, kg/day) production was calculated as [0.15 × milk fat (%) + 0.4] × milk production (kg) [26]. The cows were divided into three groups (Low group, 1–25%, n = 9; Middle group, 26–75%, n = 16; High group, 76–100%, n = 9) based on the quartile of average daily FCM production during early pregnancy (Fig. 1). Differences in average daily FCM production and milk components in early and middle pregnancy, placental measurements, maternal status (parity, maternal BCS, lactation period, days open, gestation length, and dry period), calving difficulty, birth weight of calves, blood parameters, and insulin resistance among the three groups were analyzed using Kruskal–Wallis analysis of variance on ranks (ANOVA on Ranks; SigmaPlot® 13; Systat Software, Inc., San Jose, CA, USA), and

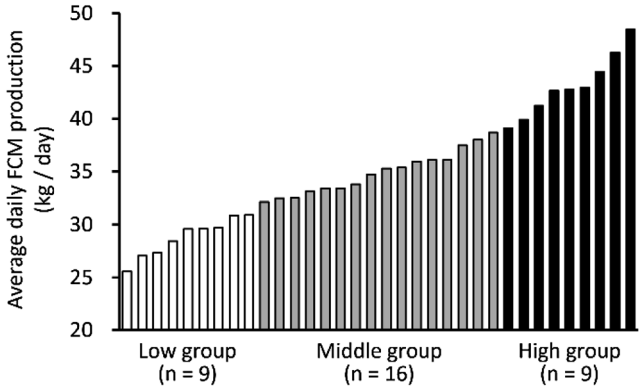

Fig. 1. A diagram showing groupingsbased on the quartile of average daily milk production during early pregnancy (conception to 90 days after conception) in the Low-, Middle-, and High-group cows. The average daily FCM in the Low, Middle, and High groups was 25.56–30.93, 32.13–38.70, and 39.11–48.43 kg, respectively.

multiple comparisons were performed using Holm-Šidák or Dunn's post-hoc test (SigmaPlot® 13). The calf sex ratio in each group was analyzed using Fisher's exact test (SigmaPlot® 13). To investigate the distribution of individual cotyledonary weights and surface areas among the three groups, all data were categorized into four stages by 25% in the order of heaviest or largest (first stage: 76–100%; second stage: 51–75%; third stage: 26–50%; fourth stage: 1–25%) based on the quartile. The chi-square test was used to compare the distribution of cotyledonary weight and surface area among groups (SigmaPlot® 13). Differences in the percentage of each stage among the groups were analyzed using ANOVA on Ranks (SigmaPlot® 13), and multiple comparisons were performed using Holm-Šidák or Dunn's post-hoc test (SigmaPlot® 13). All data were indicated as the mean ± standard error of the mean (SEM), and statistically significant differences were presented as $P < 0.05$.

Results

Milk production and milk components during early to middle pregnancy

The average daily FCM production in early and middle pregnancy differed significantly among the groups ($P < 0.001$), with the High group producing more FCM than the Low and Middle groups (Table 1). The percentage of milk components was not significantly different among the groups. However, the daily production of lactose, milk fat, and milk protein in early and middle pregnancy differed significantly among the groups ($P < 0.05$), except for lactose production between the Low and Middle groups in middle pregnancy ($P = 0.128$), with the High group producing more than the Low and Middle groups.

Maternal status, calving difficulty, calf birth weight, and calf sex

The parity, maternal BCS, lactation period, days open, gestation length, dry period, calving difficulty, birth weight, and sex of the calves in the Low, Middle, and High groups were recorded (Table 2). Cows in the High group had a higher parity at the onset of the experiment than those in the Low group ($P = 0.002$), and the Middle group had intermediate parity ($P = 0.066$). Maternal BCS at pre-calving was significantly higher in the Low group than in the Middle group ($P = 0.019$). Furthermore, the dry period was significantly longer in the Low group than in the High group ($P = 0.004$), and longer than that in the Middle group ($P = 0.058$). The dry period was also longer in the Middle group than in the High group ($P = 0.098$). The lactation period, gestation length, levels of calving difficulty, birth weight of calves, and calf sex ratio did not differ significantly among the groups.

Table 1. 4% FCM production and milk components in early and middle pregnancy in the Low-, Middle-, and High-group cows

	Low group (n = 9)	Middle group (n = 16)	High group (n = 9)
Early pregnancy [1]			
FCM production (kg/day)	30.0 ± 0.6 [c]	36.0 ± 0.5 [b]	45.3 ± 1.0 [a]
Lactose (%)	4.57 ± 0.03	4.60 ± 0.03	4.52 ± 0.05
Lactose (kg/day)	136.7 ± 5.6 [c]	166.4 ± 3.9 [b]	203.8 ± 8.6 [a]
Milk fat (%)	3.80 ± 0.17	3.84 ± 0.10	3.71 ± 0.11
Milk fat (kg/day)	112.2 ± 1.2 [c]	138.9 ± 3.0 [b]	165.9 ± 4.2 [a]
Milk protein (%)	3.59 ± 0.08	3.49 ± 0.07	3.34 ± 0.08
Milk protein (kg/day)	106.4 ± 2.5 [c]	126.3 ± 2.9 [b]	149.2 ± 3.1 [a]
Milk fat to protein ratio	1.06 ± 0.03	1.10 ± 0.02	1.12 ± 0.02
Middle pregnancy [2]			
FCM production (kg/day)	26.8 ± 0.7 [c]	30.9 ± 0.5 [b]	38.2 ± 1.0 [a]
Lactose (%)	4.57 ± 0.03	4.56 ± 0.03	4.51 ± 0.04
Lactose (kg/day)	121.0 ± 4.6 [b]	138.1 ± 3.3 [b]	172.3 ± 8.1 [a]
Milk fat (%)	3.93 ± 0.17	4.08 ± 0.11	4.16 ± 0.15
Milk fat (kg/day)	103.0 ± 3.2 [c]	124.2 ± 3.8 [b]	158.0 ± 6.3 [a]
Milk protein (%)	3.68 ± 0.10	3.61 ± 0.08	3.59 ± 0.07
Milk protein (kg/day)	96.8 ± 2.4 [c]	109.4 ± 2.3 [b]	136.7 ± 4.9 [a]
Milk fat-to-protein ratio	1.10 ± 0.03	1.10 ± 0.02	1.16 ± 0.03

Values are presented as the means ± SEM. [1] Terms between conception (day 1) and day 90 after conception. [2] Terms between day 91 and day 180 after conception. [abc] Different letters indicate statistical differences among the groups ($P < 0.05$). FCM, milk fat-corrected milk.

Placental characteristics

The duration of placental expulsion, total placental weight, inter-cotyledonary membrane weight, total cotyledonary weight, total cotyledonary surface area, and the number of cotyledons were not significantly different among the groups (Table 3). The percentages of the four stages (first to fourth) of cotyledonary weight (A) and cotyledonary surface area (B) were observed in the Low, Middle, and High groups (Fig. 2). The cotyledonary weight ranges from the first to the fourth stage were 35.86–136.54, 22.76–35.85, 11.54–22.75, and 2.00–11.53 g, respectively. The cotyledonary surface area ranges from the first to the fourth stage were 87.55–282.20, 58.06–87.54, 32.40–58.05, and 5.16–32.39 cm^2, respectively. The distributions of cotyledonary weight and surface area differed significantly among the groups ($P < 0.001$). When comparing each stage among the groups, the first stage of cotyledonary weight and surface area in the High group were significantly higher than those in the Low group ($P = 0.016$ and $P = 0.015$, respectively), and were higher than those in the Middle group ($P = 0.076$ and $P = 0.085$, respectively). Furthermore, the third stage of cotyledonary weight in the Low group was significantly higher than that in the High group ($P = 0.031$) and higher than in the Middle group ($P = 0.096$).

Blood parameters of the dams and their calves

The RQUICKI (Low, 0.58 ± 0.04; Middle, 0.59 ± 0.03; High, 0.53 ± 0.03, respectively), $RQUICKI_{BHB}$ (Low, 0.72 ± 0.08; Middle, 0.74 ± 0.05; High, 0.67 ± 0.05, respectively), and HOMA-IR (Low, 1.98 ± 0.34; Middle, 1.91 ± 0.39; High, 2.29 ± 0.39, respectively) indices of the dams at pre-calving were similar among the groups. Serum total protein, albumin, glucose, and plasma amino acid concentrations of the dams before calving, dams immediately after calving, and calves immediately after birth in the Low, Middle, and High groups were recorded (Fig. 3), and no differences were observed in the pre-calving blood parameters of the dams among the groups. However, serum total protein concentrations in dams immediately after calving in the Low group were lower than those in the High group ($P = 0.065$). Serum concentrations of total protein, albumin, and glucose in calves immediately after birth were similar among the groups. However, the plasma amino acid concentrations in the calves differed among the three groups. The concentrations of plasma total amino acids ($P < 0.05$), total essential amino acids ($P < 0.05$), branched-chain amino acids ($P < 0.05$), isoleucine ($P < 0.05$), and leucine ($P < 0.01$) were significantly higher in the Low and High groups than in the Middle group. Furthermore, plasma non-essential amino acid concentrations in Low-group calves were significantly higher than those in Middle-group calves ($P = 0.003$), and valine concentrations in Low-group calves were higher than in the Middle-group calves ($P = 0.088$). Similarly, plasma valine concentrations were significantly higher ($P = 0.019$), and non-essential amino acid concentrations were higher ($P = 0.064$) in the High group than in the Middle group.

Table 2. Parity, maternal BCS, lactation period, days open, gestation length, dry period, calving difficulty, body weight of the calves at birth, and sex of the calves in the Low-, Middle-, and High-group cows

	Low group (n = 9)	Middle group (n = 16)	High group (n = 9)
Parity at the onset of experiment	1.00 ± 0.00 [b]	1.56 ± 0.18 [ab]	2.56 ± 0.29 [a]
Maternal BCS at pre-calving [1]	3.39 ± 0.04 [a]	3.17 ± 0.05 [b]	3.25 ± 0.06 [ab]
Lactation period (day)	367.0 ± 19.2	335.8 ± 12.8	341.0 ± 19.2
Days open (day)	147.8 ± 19.1	111.1 ± 11.3	107.4 ± 19.1
Gestation length (day)	279.7 ± 1.2	278.0 ± 0.9	278.9 ± 1.3
Dry period (day)	60.6 ± 1.4 [a]	52.7 ± 2.8 [ab]	44.6 ± 3.5 [b]
Calving difficulty [2]	1.56 ± 0.20	1.44 ± 0.20	1.22 ± 0.15
Birth weight of the calves (kg)	46.6 ± 1.2	45.8 ± 0.7	46.6 ± 2.1
Sex of the calves (male/female)	3/6	9/7	4/5

Values are presented as means ± SEM. [1] 2–3 weeks before the expected calving date. [2] score 1, unassisted birth (natural, without human assistance); score 2, easy calving with human assistance; score 3, difficult calving with few humans; score 4, dystocia (requiring much more force than normal); and score 5, surgical treatment or death of cow. [ab] Different letters indicate statistical differences among the groups ($P < 0.05$). BCS, body condition score.

Table 3. Placental characteristics in the Low-, Middle-, and High-group cows

	Low group (n = 9)	Middle group (n = 16)	High group (n = 9)
Placental characteristics			
Duration of placenta expulsion after calving (min) [1]	406.4 ± 50.7	346.3 ± 39.8	330.4 ± 14.9
Total placental weight (kg) [2]	5.87 ± 0.53	6.08 ± 0.32	6.30 ± 0.65
Inter-cotyledonary membrane weight (kg)	2.93 ± 0.39	3.23 ± 0.23	3.15 ± 0.51
Total cotyledonary weight (g) [3]	2389 ± 139.6	2205 ± 115.9	2442 ± 69.7
Total cotyledonary surface area (cm^2) [3]	6109 ± 305.0	5528 ± 190.7	5862 ± 218.6
Number of cotyledons (n) [3]	101.6 ± 7.7	88.9 ± 5.5	80.0 ± 7.7

Values are presented as the means ± SEM. [1] Intervals from calving to placental expulsion. [2] Indicates placental weight that was spontaneously expelled within 12 h of parturition. [3] Excluded less than 2.0 g of cotyledons from the experiment.

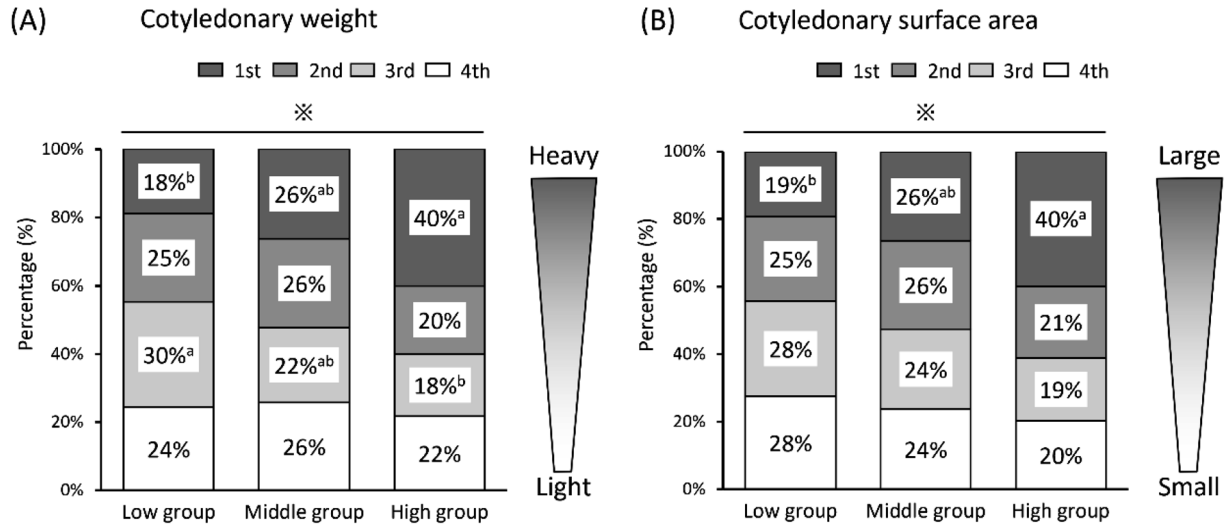

Fig. 2. Percentages of four stages of cotyledonary weight (A) and surface area (B) in three groups (Low, n = 9; Middle, n = 16; High, n = 9). ※ Indicates statistical differences in the distributions of cotyledonary weight and surface area among the three groups ($P < 0.001$). ab Different letters indicate statistical differences in the percentage of each stage among the groups ($P < 0.05$).

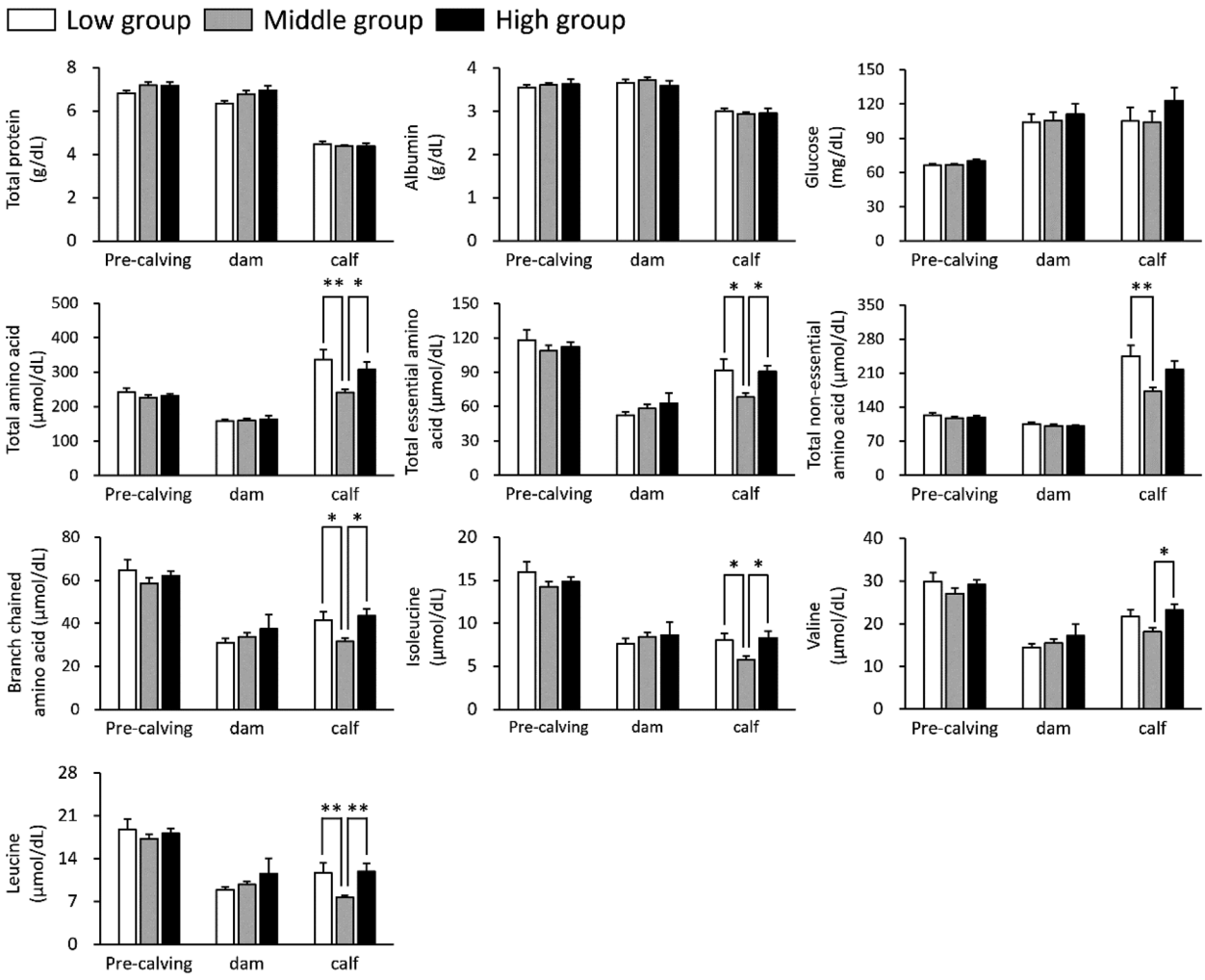

Fig. 3. Concentrations of serum protein, albumin, glucose, and plasma amino acid of dams at pre-calving (2–3 weeks before the expected calving date), immediately after calving, and immediately after birth before the first colostrum feeding in three groups (Low, n = 9; Middle, n = 16; High, n = 9). Values are described as the mean ± SEM. * $P < 0.05$; ** $P < 0.01$.

Discussion

In high-lactating cows, more energy is preferentially directed to the mammary glands, which can reduce the nutrient supply to the placenta and fetus. Therefore, we hypothesized that dairy cows with high lactation during early pregnancy have a larger placental size at full term and enhanced fetal nutritional availability than cows with low lactation, leading to the production of calves with heavier birth weights.

Milk components are indicators of dietary energy intake and nutritional status [27]. The ratio of milk fat to protein (F/P) ranges from 1.35 to 1.5, indicating a higher probability of negative energy balance, metabolic disease, and other conditions [27]. In our study, the percentage of milk components was similar among the groups, and the F/P ratio in each group was less than 1.35–1.5. However, cows in the High group produced more milk components than those in the Low group during early to middle pregnancy, with the Middle group being intermediate. These findings suggest that the nutritional requirements of the mammary gland increase as milk production increases; thus, nutrient delivery to the placenta and fetus could be reduced from early to middle pregnancy.

Contrary to our hypothesis, no differences in total placental weight, inter-cotyledonary membrane weight, total cotyledonary weight, total cotyledonary surface area, or the number of cotyledons due to differences in milk production were observed during early pregnancy. However, a difference in cotyledonary size was observed, and the High group had larger cotyledons than the Low group. Rapid fetal growth occurs during late pregnancy, with a 60% increase in birth weight, especially during the last 2 months of pregnancy [28–30]. Morphologically, the bovine placenta is fully developed during middle to late pregnancy [31]; however, the microvilli of the cotyledon continue to grow by branching and rearranging until full term, increasing the cotyledonary surface area, which responds to fetal nutritional demands [31–33]. Studies on beef cattle have shown that maternal undernutrition during early to middle pregnancy decreases placental and fetal weight [8–10]. However, re-alimentation to an adequate nutritional requirement during subsequent pregnancy recovered fetal weight to the same levels as the cows fed a sufficiently nutritious diet [8–11]. This has been attributed to an increase in placentome vascular density [10] and individual placentome surface area [8]. Increased placental vascularity is associated with increased placental (uterine and umbilical) blood flow, which continues to grow with fetal growth by increasing the placental transport capacity [6]. Although there are limited studies on the relationship between individual placental size and function in cattle, a study on pregnant sheep demonstrated that individual placental size may be an indicator of vascular function [34] and, ultimately, placental blood flow and nutrient exchange capacity [35]. These findings indicate that the enlargement of individual cotyledonary sizes in High-group cows could enhance placental vascular function and nutrient exchange between the dam and fetus as an adaptive response to high milk production during early to middle pregnancy. In this study, we could not eliminate the effect of parity in the Low- and High groups as milk production increases with parity. Yoon *et al*. [36] reported that milk production is lower at first parity than in multiparous cows, increases until the fifth parity, and then decreases. In beef cows, no differences were observed in the total placental weight, total cotyledonary weight, and average cotyledon weight at full term between primiparous and multiparous cows [37]. To the best of our knowledge, few studies have investigated the effects of parity on placental characteristics in dairy cows, including individual cotyledonary distribution. Therefore, further research is needed to determine the effect of differences in milk production on placental characteristics by selecting cows with similar parity.

Because the dry period overlaps with rapid fetal development [29, 30] and owing to the cessation of nutrient supply to the mammary glands, cows with longer dry periods can provide greater amounts of energy and amino acids to the fetus, thus increasing calf birth weight [38]. Our data showed that Low-group cows had longer dry periods than Middle-group cows and statistically higher plasma amino acid concentrations in Low-group calves than those in Middle-group calves, although there was no difference in calf birth weight between the groups. The BCS of the cows in the Low group was higher than that in the Middle group; however, the scores of both groups were within the ideal pre-calving BCS range (3.0–3.5) [39], suggesting less influence on the differences in plasma amino acid concentrations and calf birth weight. A dry period of 51–60 days is widely adopted in dairy farms [40], which is consistent with the means of the dry period in the Low and Middle groups. Therefore, although a longer dry period in the Low group may have increased the amino acid supply to the fetus and fetal amino acid availability, the difference in length was not significant enough to influence calf birth weight. Kamal *et al*. [38] reported that dams with short dry periods (3–44 days) produced lighter calves than dams with long dry periods (55–275 days). The authors also observed that high-lactating cows had a shorter dry period than low-lactating cows [38]. Consistent with previous studies, this study revealed that cows in the High group had shorter dry periods than those in the Low group. The mean duration of the dry period in the High group (44.6 days) was less than the typical dry period length [40], and 6 of 9 cows had a dry period shorter than 44 days, which was expected to result in lower calf birth weights compared to the Low group. However, the birth weights and plasma amino acid concentrations of the calves in the High group were similar to those in the Low group. This could be due to the enlargement of individual cotyledonary sizes in the High group, which enhances the delivery of amino acids to the fetus and fetal amino acid availability, ultimately achieving the same calf birth weights as those in the Low group, regardless of the shorter dry period. Notably, our data showed that the plasma valine, leucine, and isoleucine concentrations of the calves were similar in the Low and High groups, and were higher in both groups than in the Middle group. Branched-chain amino acids such as valine, leucine, and isoleucine [41] are known activators of mammalian targets of rapamycin (mTOR) signaling [42], which regulates protein synthesis [43, 44]. Therefore, inadequate fetal amino acid supply caused by maternal malnutrition downregulates muscle mTOR signaling, reduces protein synthesis [45], and promotes protein degradation, resulting in decreased fetal muscle fiber number and muscle mass [42, 43]. This study demonstrates that a sufficient supply of branched-chain amino acids to a High group fetus leads to fetal muscle development, ultimately increasing calf birth weight.

In conclusion, high-lactating cows require more energy for milk production, which can reduce nutrient availability to the placenta and fetus. However, increasing individual cotyledonary sizes can support the amino acid supply to the fetus, resulting in calf birth weights similar to those of low-lactating cows. Therefore, it is believed that high milk production during early pregnancy does not affect fetal development. However, we could not eliminate the effects of parity on the different milk production levels. Recent studies have reported that parity and milk production by dams may have a negative effect on the longevity and lactation ability of their calves [15, 16, 46–48]. Further research is required to produce healthy, productive, and long-lived dairy calves.

Conflict of interests: The authors declare no conflicts of interest regarding this manuscript.

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