EFFECT OF NUTRITION DURING PREGNANCY ON CALF BIRTH WEIGHTS AND VIABILITY AND FEAL MEMBRANE EXPULSION IN DAIRY CATTLE

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ABSTRACT

Neonatal mortality and retention of fetal membrane (RFM) are still important reproductive problems in cattle. It may be assumed that maternal nutrition during pregnancy affects the fetal growth and placental development in cattle. The objectives of this study were to show changes of blood parameters for nutrition during pregnancy and to examine the relationships between the blood parameters and calf birth weights, calf viability, time required for expulsion of fetal membrane, and the fetal membrane weights. Forty-one Holstein Friesian pregnant cows were used. A duration of dry period was 74±2 (SEM) days. The average milk yields (4% FCM) per cow per lactation for 305 days was 9,795±146 kg. Blood samples were collected via the tail vein between 90 days of pregnancy and the day of parturition. Plasma concentrations of glucose, non-esterified fatty acid (NEFA), total cholesterol, total protein (TP), calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) and blood urea nitrogen (BUN) were determined using a biochemical auto-analyzer. For each plasma parameters, cows were classified into three groups; low, mid, and high values, according to quantile (terciles) of the mean. Cotyledons were weighed after dissecting from the chorioallantoic membrane. All the calves delivered from the 41 cows were singletons. Mean gestation length, body weight of cows at parturition, and cotyledonary weight were 283.7 ± 0.5 days, 761 ± 11 kg, and $1562\pm 33g$, respectively. Mean birth weights were 47.4 ± 1.1 kg for femal and 49.8 ± 0.8 kg for male calves. Of the 41 cows 10 had weak calves and 13 had RFM. No significant effects of parity, bull, lactation length, milk yield, gestation length, cows' body weights, and gender of calves on the cotyledonary and calf birth weights, incidences of neonatal weakness and RFM were shown. Plasma NEFA started to increase during a late dry period towards a day of parturition, showing a drastic increase for a last few days. A rapid rise of plasma glucose was also shown for the last few days. On the contrary, plasma TP and cholesterol and BUN showed a gradual decrease during a late dry period. Cows showing low plasma glucose and TP concentrations 90 to 240 days of gestation had lower calf birth weights and cotyledonary weights, and a higher incidence of neonatal weakness. Likewise, cows with low glucose, cholesterol and Ca concentrations in plasma during 255 days of pregnancy or later had a higher incidence of RFM. The results indicate that low intake of energy and protein during the second trimester of pregnancy or a little later adversely affects feto-placental development, leading to neonatal weakness and RFM. Low intake of energy and Ca during dry period may also cause RFM.

KEYWORDS

Cattle, fetal growth, neonatal weakness, nutrition, placental development, retention of fetal membrane

INTRODUCTION

Weak calf syndrome characterized by neonatal weakness is one of the most important causes of calf mortality and neonatal diseases (10)). In cows RFM is still the biggest problem during periparturient period, since RFM is often followed by postpartum metritis, abomasal displacement, ketosis and mastitis. The authors recently reported that retardation of fetal growth as well as underdevelopment of placenta during pregnancy were predominant causes of neonatal weakness and RFM (13). A number of factors including maternal genetic influence, maternal nutrition and environmental influences have been considered to be involved in feto-placental development during pregnancy.

In dairy cows nutritional requirements for milk production increase rapidly after parturition, resulting in negative energy balance during early lactation period (3). During dry period, cows show rapid fetal growth requiring extra nutrition. Thus, nutritional deficiency is one of main challenges facing dairy cows, high-producing cows in particular. It is, therefore, assumed that maternal nutrition during pregnancy may affect fetal and placental growth and development, leading to neonatal weakness and RFM.

This paper reports effects of maternal nutrition assessed by blood metabolic profile on fetal growth (birth weight and viability) and placental development (placenta weight and expulsion) in high-producing dairy cows. **MATERILAS AND METHODS**

Forty-one pregnant Holstein-Friesian cows being reared at Rakuno Gakuen University Dairy Farm were used in this study. The cows were in their first to sixth lactations. The average milk yield (4 % FCM) per cow per lactation for 305 days in this herd was 9,795 \pm 146 kg. A duration of dry period was 74 \pm 2 days on the average.

Blood samples were collected via the tail vein using 21 gauge needles and heparinized vaccum test tubes between 90 days of pregnancy and the day of parturition; every month from days 90 to 180, every two weeks from days 181 to 270 and every day thereafter. Heparinized blood samples were kept at 4 degrees Celsius and centrifuged at 1700 xg within 30 minutes after collection. Plasma thus obtained was stored at -20 degrees Celsius until analysis.

Plasma concentrations of glucose, non-esterified fatty acid (NEFA), total cholesterol, total protein (TP), calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), asparte aminotransferase (AST), and gamma glutamyl transferase (GGT) and blood urea nitrogen (BUN) were determined using a biochemical auto-analyzer (TBA-80FR, Toshiba, Tokyo, Japan). For each cow a single mean value for each plasma biochemical parameter was calculated from values between days 90 to 210 of pregnancy (lactation period), days 225 to 240 (early dry period) and after day 255 (late dry period). Within each period, for each plasma parameter, cows were classified into 3 groups; low (n = 14), mid (n = 13) and high (n = 14) values, based upon quantile (terciles) of the means (12). The cotyledonary weights, calf birth weight, the occurrence of neonatal weakness and retained placenta were compared among the three groups showing low, mid or high values for the plasma parameters in order to demonstrate the effects of the nutritional parameters on the feto-placental function.

Calf birth weights were measured before colostrum was taken. The complete placenta was collected from 28 of the 41 cows within a few hours after the expulsion. The placenta was frozen at -20 degrees Celsius and then thawed at room temperature. The cotyledons dissected from the chorioallantoic membranes were weighed. The placenta was considered to be retained when it had not been expelled within 12 hours after the calf delivery.

Body condition was evaluated by a body condition score (five point scale with 0.25 increments) (6) at an interval of one month during the early and late dry periods.

Differences among means were tested using Least Significant Difference (LSD). Incidence of neonatal weakness and retained placenta among the three groups were compared by the Chi-square test. Variations in the data are presented as standard error of the mean (SEM).

Effects of factors, other than the nutritional factors, on the feto-placental parameters were also analized. Effects of parity, sires and fetal sex on the placenta and calf birth weights were analized by analysis of variance, and the effects of lactation length, milk yield, gestation length and body weight of cows after parturition by Pearson's correlation coefficient. The effects of the factors mentioned above on the occurrence of neonatal weakness and retention of placenta were analyzed by Chi-square test or LSD test.

RESULTS

Plasma Metabolic Profile during Pregnancy

Glucose, NEFA and cholesterol: Changes in plasma concentrations of glucose, NEFA and cholesterol in cows between 90 days of pregnancy and the day of parturition are shown in Fig. 1.

Plasma glucose remained stable until 3 days prepartum, then started to show an increase the following day and rose significantly (P<0.01) the day before calving. Plasma NEFA concentrations was almost consistent between 90 and 270 days of pregnancy and then gradually increased until two days prepartum, followed by a sharp increase the day before parturition (P<0.01). In contrast, plasma cholesterol showed a gradual decrease during pregnancy towards the day before calving.

TP and BUN: Profiles of plasma TP and BUN are presented in Fig. 2. Plasma TP did not show any fluctuating pattern between days 90 and 210 days of pregnancy and thereafter decreased gradually towards the end of pregnancy. BUN concentrations were consistent until 255 days of pregnancy, then decreased substantially by 10 days before calving and remained at low levels until the days before delivery.

Ca, **Pi and Mg**: Fig.3 shows changes in plasma concentrations of Ca, Pi and Mg during a gestation period. A high plasma Ca concentration was maintained until 3 days before calving, followed by a sharp decline towards the day before the parturition. The same trend was shown in plasma Pi profile. Plasma Mg tended to decrease gradually from 90 days of pregnancy, particularly after the 225 days, towards the day before delivery.

AST abd GGT: Plasma AST activity showed a gradual decline between 180 days and 225 days of pregnancy and the low activity was maintained until 8days prepartum, followed by a gradual increase towards parturition. The same fluctuating pattern was shown in plasma GGT activity.

Calf Birth Weight, Calf Viability, Placental Weight and Placental Expulsion

All the calves delivered from the 41 cows were singletons, 14 female and 27 male calves. The birth weights for the female and male calves were 47.4 ± 1.1 (mean \pm SEM) kg and 49.8 ± 0.8 kg, respectively. Of the 41 newborns, 10 (24.4 %) showed neonatal weakness. The weight of the cotyledones obtained from 28 of the 41 cows averaged 1,562 \pm 33 kg. RFM was diagnosed in 13 (31.7 %) of the 41 animals/

Mean gestation length in the 41 cows was 283.7 ± 0.5 days. Body weight of the cows shortly after calving was 761 ± 11 kg on the average.



Fig. 1 Changes in plasma concentrations of glucose, NEFA and cholesterol in cows during gestation. Data shown as a mean \pm SEM.



Fig. 2 Changes in plasma concentration of total protein and BUN in cows during Gestation. Data shown as a mean \pm SEM.



Fig. 3 Changes in plasma concentrations of Ca, Pi and Mg in cows during gestation. Data shown as a mean \pm SEM.



Fig. 4 Changes in plasma GGT and AST activities in cows during gestation. Data Shown as a mean ± SEM.

The Effects of Metabolic Profile and BCS on Calf Birth Weight and Viability and Placental Weight and Expulsion

Plasma Glucose, NEFA and Cholesterol: Fig. 5 shows the effects of three different levels of plasma glucose; low, mid and high, 90 to 210 days, 225 to 240 days and 255 days or later of gestation on calf birth weight, occurrence of neonatal weakness, cotyledonary weight and time required for expulsion of fetal membrane. A group of cows showing relatively low plasma glucose 90 to 210 days as well as 225 to 240 days of gestation delivered calves with significantly lower birth weight and higher incidence of neonatal weakness as compared with another group of cows with higher plasma glucose (P<0.05). The low plasma glucose group also expelled the fetal membrane with significantly lower cotyledonary weight than the high plasma glucose group (P<0.05). Low plasma glucose 255 days or later of gestation resulted in significantly higher incidence of retained placenta (P<0.05).

No significant effects of plasma NEFA concentrations during gestation on cotyledonary weight and calf birth weight were observed (Fig. 6). The incidence of RFM was influenced by plasma NEFA concentrations 255days of pregnancy or later. A group of cows with relatively high plasma NEFA during a late dry period showed a significantly higher incidence of RFM than two other groups with mid and low plasma NEFA concentrations (P<0.05). There was a tendency that relatively high plasma NEFA concentrations during late dry period resulted in a higher incidence of neonatal weakness.

Plasma cholesterol concentrations in cows during pregnancy did not affect cotyledonary weight, calf birth weight and incidence of neonatal weakness (Fig. 7). Effect of plasma cholesterol 255 days of pregnancy or later (late dry period) on the incidence of RFM was shown. Low plasma cholesterol group showed a significantly higher incidence of RFM than the high cholesterol group (P<0.01).

Plasma TP and BUN: Effects of plasma TP concentrations during pregnancy on the calf birth weight and viability and the placental weight and expulsion were summarized in Fig. 8. A group of cows showing a relatively low plasma TP 90 to 210 days as well as 225 to 240 days of gestation had significantly lower cotyledanary weight and calf birth weight and had a significantly high incidence of neonatal weakness as compared with the high plasma TP group (P<0.05).

No significant effects of BUN during pregnancy on the feto-placental function was shown.

Plasma Ca, Pi and Mg: Cotyledonary weight, calf birth weight and calf viability were not affected by plasma Ca concentrations during gestation (Fig. 9). Plasma Ca 255 days of pregnancy or later showed an influence on RFM. A low Ca group showed a significantly higher incidence of RFM than another group of cows with high plasma Ca (P<0.05). There were no significant effects of plasma Pi and Mg profiles during pregnancy on the feto-placental function.

BCS: A group of cows with BCS 3.0 or lower during early as well as late dry periods showed a significantly higher incidence of RFM than another group of cows with BCS 3.25 to 3.5 during the same periods (P<0.05)(Fig. 10). Likewise, a group of cows with 3.75 or higher BCS during the dry periods also had a significantly higher incidence of RFM. There was a tendency that a group of cows with 3.25 to 3.5 BCS during the dry periods showed the lowest incidence of neonatal weakness.



Fig. 5 Effects of three different levels of plasma glucose 90 to 210 days, 225 to 240 days and 255 days or later of gestation on the cotyledonary weight (g), calf birth weight (kg), occurrences of neonatal weakness (NW)(%) and retained placenta (RP) (%) in cows

Plasma glucose

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Fig. 6 Effects of three different levels of plasma NEFA 90 to 210 days, 225 to 240 days and 255 days or later of gestation on the cotyledonary weight (g), calf birth weight (kg), occurrences of neonatal weakness (NW)(%) and retained placenta (RP) (%) in cows

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Fig. 7 Effects of three different levels of plasma cholesterol 90 to 210 days, 225 to 240 days and 255 days or later

of gestation on the cotyledonary weight (g),

calf birth weight (kg),

occurrences of neonatal weakness (NW)(%) and

retained placenta (RP) (%) in cows



Fig. 8 Effects of three different levels of plasma total protein (TP) 90 to 210 days, 225 to 240 days and 255 days or later of gestation on the cotyledonary weight (g), calf birth weight (kg), occurrences of neonatal weakness (NW) (%) and retained placenta (RP) (%) in cows



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Fig. 9 Effects of three different levels of plasma Ca 90 to 210 days, 225 to 240 days and 255 days or later of gestation on the cotyledonary weight (g), calf birth weight (kg), occurrences of neonatal weakness (NW)(%) and retained placenta (RP) (%) in cows



Fig. 10 Effect of body condition 225 to 240 days (early dry period) and 255 days or later of gestation (late dry period) on the cotyledonary weight (g), calf birth weight (kg), occurrences of neonatal weakness (NW)(%) and retained placenta (RP) (%) in cows

DISCUSSION

Plasma concentrations of NEFA, glucose and cholesterol have been considered to be indicators of energy balance in cows (2,7). In dairy cows with high milk production negative energy balance begins a few days before parturition (1,4). A drastic increase in plasma NEFA the day before calving observed in this study may indicate that the cows were already having a shortage of energy. A sharp rise of plasma glucose the day before parturition might have been caused by a rise of plasma glucocorticoids.

Among the nutritional factors investigated in the present study, plasma glucose and TP concentrations 90 to 240 days of gestation showed significant effects on feto-placental development estimated by cotyledonary weight, calf birth weight and calf viability. Low plasma glucose and TP levels during a 90 to 240 days gestation period resulted in a low birth weight, more weak calves and a small placenta. The placenta exerts its effects on the growth of the fetus from the beginning of pregnancy via metabolic and endocrine mechanisms. The placenta exchanges wide array of nutrients, endocrine signals, cytokines and growth factors between the dam and fetus, modulating or programming fetal growth and development (8). Recently, we reported that placental function estimated by plasma estrone sulfate concentrations was closely associated with fetal body weight and calf viability (13). Therefore, it may be postulated that a low supply of glucose as well as protein to the dam adversely affected the placental development and as a consequence fetal growth was retarded.

A lot of nutritional factors are also involved in RFM in cows. It was shown in this study that plasma metabolic profiles 90 to 210 days and 225 to 240 days of pregnancy did not influence the incidence of RFM, while the metabolic profile 255 days of pregnancy or later (late dry period) significantly affected RFM. A relatively low plasma glucose and high NEFA levels in cows during late dry period were associated with an increased incidence of retention of fetal membrane (P<0.05). The similar findings have previously been reported by Kaneene et al. (8) and Chassagne et al. (5). High plasma NEFA concentrations during a periparturient period may cause reduction in leukocyte counts and activity, which in turn leads to RFM (5,8).

The expulsion of the matured placenta following the delivery of a calf is a physiological process, involving the loosening of the chorionic villi from maternal crypts, together with uterine muscular contraction which physically expel the placenta (9). Ca is known to play an important role in the uterine contraction. A relatively low plasma Ca concentration during late dry period was associated with an increased incidence of RFM in this experiments.

BCS can be used as indirect assessment of energy balance. Optimum BCS during a dry period is around 3.5 at the beginning of the dry period, which is maintained throughout the period. A group of cows showing BCS of 3.25 to 3.5 during early as well as late dry periods had no incidence of retention of fetal membrane in the present study, while other groups of cows with under (3.0 or lower) or over conditions (3.75 or higher) had significantly higher incidence of the retention. Over or under conditions during dry period may cause metabolic and hormonal disturbance around parturition, leading to delayed expulsion of the placenta after the calf delivery.

In conclusion maternal nutrition during the second trimester of pregnancy and a little later influence the feto-placental growth and development, while nutritional state during late pregnancy has effects on RFM in dairy cattle.

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