Short Communication

Nutritional parameters in the blood of dams during late gestation and immediately after calving, in the umbilical vein at calving, and in the blood of calves immediately following birth in Holstein heifers pregnant with either Holstein or beef breed fetuses

Chiho KAWASHIMA[1], Sakura KUME[1], and Norio YAMAGISHI[2, 3]

[1] Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

[2] Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

3 Division of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Izumisano, Osaka, Japan

Correspondence: Chiho Kawashima, Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555 Japan.

Phone: +81-155-49-5653; Fax: +81-155-49-5653

Email: kawasima@obihiro.ac.jp

ABSTRACT

Dairy cattle management lacks consideration of fetal breed, the effect of which on fetal growth and nutrition is unclear. We investigated blood parameters in 12 late-pregnant Holstein heifers with similar (Holstein, n = 5) or different (Japanese Black [n=4] or F1 cross [n=3]; Holstein × Japanese Black) fetus breeds and in their umbilical cords and calves. Samples were obtained from dams 1 week before calving (-1 week) and immediately after calving, from the umbilical vein at calving, and from calves immediately after birth. Dams with beef fetuses had higher serum glucose levels (-1 week; $p < 0.05$) than those with Holstein fetuses. Plasma total amino acid, total essential amino acid, total non-essential amino acid, and other amino acid concentrations were lower in the umbilical veins of dams with calves of the beef breeds than in those of the Holstein breeds ($p < 0.05$). Furthermore, serum glucose and plasma amino acid levels were lower in the beef calves than in the Holstein calves ($p < 0.05$). Overall, nutrient supply from dams to beef fetuses was lower than that to Holstein fetuses. Our findings may facilitate feeding management of dairy cattle pregnant with beef breeds for appropriate fetal growth and nutrition.

Key words: *Beef fetus, Blood parameter, Holstein fetus, Holstein dams, Umbilical veins*

Introduction

Recently, the ratio of Holstein cattle receiving artificial insemination or embryo transfer from the Japanese Black cattle population has been increasing in Japan. The Japanese feeding standard for dairy cattle (NARO, 2017) states that the nutritional requirements of dairy cattle pregnant with beef breeds are slightly higher than those of beef cattle pregnant with beef breeds; however, the differences between dairy cattle pregnant with similar dairy breeds and those pregnant with beef breeds are not discussed. In general, pregnant Japanese Black cattle receive individual management, whereas dairy cattle receive herd management. Therefore, dairy cattle are managed without considering fetal breeds, and the effect of this on fetal growth and nutrition is unclear.

The main fetal nutrients for growth are glucose and amino acids (Bell & Ehrhardt, 2002; Nishida, 2009). During late gestation, reduced insulin sensitivity in peripheral tissues ensures adequate transfer of glucose from the dam to the fetus, which is an insulin-independent process (Hayirli, 2006). Placental glucose transfer is dependent on the maternal–fetal plasma glucose concentration gradient, although it is also influenced by glucose transporters, maternal nutrition, and fetal growth capacity (Bell & Ehrhardt, 2002). Conversely, most amino acids are transported against a fetal–maternal concentration gradient by active transport processes (Bell & Ehrhardt, 2002). In a previous study on ewes, short-term fasting during late gestation had an insignificant effect on the umbilical net uptake of amino acids despite appreciable decreases in the maternal plasma concentrations of many amino acids (Lemons & Schreiner, 1983). This study concluded that the supply of amino acids from the dam to the fetus does not change dramatically during maternal fasting. Alternatively, Kwon et al. (2004) showed that long-term maternal nutrient restriction in ewes reduced blood amino acid concentrations in both fetuses and umbilical veins.

Pregnant heifers are still growing, which results in an increased need for nutrients beyond that needed for maintenance and pregnancy. As previously mentioned, pregnant Japanese Black cattle and dairy cattle receive individual and herd management, respectively. Therefore, we hypothesized that the supply of nutrients may differ between Holstein heifers pregnant with Holstein and beef fetus breeds. The aim of the present study was to investigate the blood glucose, total protein, and amino acid levels in late-pregnant Holstein heifers with similar (Holstein) or different (Japanese Black or F1 cross; Holstein × Japanese Black) breed fetuses, umbilical cords, and calves immediately after birth.

Materials and Methods

Animals, feeding, and management

The experimental procedures performed in the present study complied with the Guide for the Care and Use of Agricultural Animals of Obihiro University (approval number: #18-127). We examined 12 late-pregnant Holstein heifers with similar (Holstein, n = 5) or different (Japanese Black [n=4] or F1 cross [n=3]; Holstein × Japanese Black) fetal breeds. All dams were fed a limited total mixed ration (11.9 kg/day/head, dry matter basis: 133 g of crude protein/kg and a net energy of lactation of 1.54 MJ/kg) consisting of grass silage, maize silage, and concentrate for dry cows and were offered feed once daily at 11:30 hr. In addition, grass hay, minerals, and water were freely accessible.

Sampling

Body condition score (BCS) was assessed one week before the expected parturition, with the same operator using a 1 to 5 scale with 0.25 unit intervals according to Ferguson et al. (1994). Blood samples of dams were obtained via caudal venipuncture at approximately the same time of the day between 07:00 and 08:00 hr (approximately 3 h before feeding) one week before the expected parturition and immediately after calving. The umbilical vein at calving was clamped with forceps before cutting, and then bluntly cut between the forceps and calf. Blood from the umbilical vein was collected from the dam side. In addition, blood samples were collected from the jugular veins of calves immediately after birth before the first colostrum feeding. A non-heparinized and silicone-coated 9 mL tube (Venoject, Autosep, Gel + Clot. Act., VP-AS109K; Terumo Corporation, Tokyo, Japan) was used for glucose and total protein analyses and 5 mL tubes containing ethylenediaminetetraacetic acid (Venoject II, VP-NA050K; Terumo Corporation, Tokyo, Japan) were used for amino acid analyses. To obtain serum, blood samples were coagulated for 10 min at 38 °C in an incubator. All tubes were then centrifuged at 2,328 *g* for 15 min at 4 °C, and serum and plasma samples were stored at -30 °C until analysis. Newborn calves were cleaned and dried with a towel and weighed before the first colostrum feeding.

Measurement of glucose, total protein, and amino acid concentrations in blood

The serum concentrations of glucose and total protein were measured using a clinical chemistry automated analyzer (TBA120FR; Toshiba Medical Systems Co., Ltd., Tochigi, Japan). Plasma concentrations of amino acids were measured using an ultra-performance liquid chromatography–MS (Waters, Milford, MA, USA) with the derivatization method (AccQ-Tag Derivatization) provided by Waters.

Statistical analysis

Two samples from the umbilical veins of the dams with the Japanese Black fetuses and from the Japanese Black calves were not collected because of stillbirth, and two samples from the umbilical vein in a dam with the Holstein fetus and a dam with the F1 fetus were not collected because of umbilical cord cutting before being clamped with forceps. Therefore, these data were excluded from the analysis. The data between the dams pregnant with Holstein fetuses (DPH) and the dams pregnant with beef fetuses (DPB), the umbilical veins of dams pregnant with Holstein fetuses (UVH) and the umbilical veins of the dams pregnant with beef fetuses (UVB), and the Holstein calves (HC) and the beef calves (BC) were analyzed using Student's t-test or the Mann–Whitney U test after statistical testing for normality using the Shapiro–Wilk test (SigmaPlot® 13; Systat Software, Inc., San Jose, CA, USA). Results are reported as the mean ± standard error of the mean. p values < 0.05 were considered to indicate a statistically significant difference.

Results and Discussion

BCS at one week before the expected parturition in DPH and DPB was 3.50 ± 0.11 and 3.54 ± 0.08, respectively ($p = 0.76$). DPB had higher serum glucose levels (-1 week; $p < 0.05$) compared to those in DPH, although other parameters did not differ between DPH and DPB (Table 1). The body weights of BC (34.5 ± 2.2 kg) were lower than those of HC (42.2 ± 1.5 kg, $p < 0.05$). Figure 1 shows the serum glucose and total protein concentrations and plasma amino acid concentrations in dams after calving, the umbilical veins of dams at calving, and their calves immediately after birth. Plasma alanine concentrations in DPB were lower than those in DPH ($p < 0.05$). However, other parameters were not significantly different between DPH and DPB. In contrast, plasma total amino acid, total essential amino acid, total non-essential amino acid, leucine, histidine, lysine, methionine, glutamine, alanine, and proline concentrations were lower in UVB and BC than those in UVH and HC ($p < 0.05$). In addition, serum glucose and plasma isoleucine, valine, threonine, arginine, and glycine levels were lower in BC than those in HC ($p < 0.05$).

There is still little research on the nutritional parameters of dams and fetuses and/or calves, including the umbilical vein at calving in cattle. As the only study on this topic, to the best of our knowledge, Shen et al. (2019) showed a positive correlation between adipokine levels in umbilical veins and calf birth weights in Holstein dams and calves, although this study did not investigate the glucose or amino acid concentrations in umbilical veins. Thus, the lower body weight of BC may be the main cause of the lower concentrations of glucose and amino acids in the BC compared to those in HC. However, the birth weight of the F1 calves in BC (38.9 ± 2.7 kg; minimum: 33.6 kg, maximum: 42.8 kg) was not different from that in HC (minimum: 39.0 kg, maximum: 47.2 kg, $p = 0.286$). Therefore, it seems that the lower body weight of BC is not the only factor in the difference in blood glucose and amino acid concentrations between UVB and UVH, or BC and HC.

Although glucose is a principal energy substrate for fetal and placental metabolism in all mammals, glucose consumed by the placenta undergoes rapid conversion to lactate and fructose in the ewe (Bell & Ehrhardt, 2002), and fructose is the most abundant hexose sugar in fetal fluids of ungulate mammals including cows, sheep, and pigs (Kim et al., 2012). Moreover, Lucy et al. (2012) showed that a lower blood glucose level was associated with less glucose and fructose in placental fluids in dairy cows, because the fetus and placenta cannot sequester glucose against its concentration gradient. Therefore, the conversion rate of glucose to lactate in the placenta may differ between Holstein cattle pregnant with similar and different fetal breeds, and further research is needed. Paolini et al. (2001) showed that isoleucine, valine, and leucine of the essential amino acids are the branched-chain acids that are used as sources of muscle energy, and these amino acids, methionine, and phenylalanine are most rapidly transported from dam to the fetus, whereas tryptophan, threonine, histidine, and lysine of the essential amino acids are slowly transported from dam to the fetus in the ewe. In addition, they indicated that differences in transport rates of the branched-chain acids, methionine, and phenylalanine are determined primarily by differences in maternal plasma concentration, although maternal amino acid concentrations changes are not immediately translated into the fetal circulation because of transport and accumulation in the placenta (Paolini et al., 2001). However, the blood concentration of most amino acids in UVB and BC was lower than that in UVH and HC, even though there was no difference in the blood concentration of these amino acids between DPH and DPB in this study. In addition, for amino acids grouped in glucogenic amino acids (that can be converted into glucose through gluconeogenesis), and ketogenic amino acids (that can be degraded directly into acetyl-CoA), there was no consistency in the presence or absence of significant differences between UVB and UVH, or BC and HC. Thus, the reason for the differences in amino acid concentrations in the umbilical veins and in the calves due to differences in fetal breed cannot be clarified from the present study. Therefore, further study is needed on differences in placental clearance and transport systems of amino acids in Holstein cattle pregnant with similar and different breed fetuses.

In a previous study, Holstein cows inseminated with Holstein semen had greater placental weight than those of the Holstein and Japanese Black cows inseminated with Japanese Black semen (Isobe et al., 2003). In addition, the level and change in plasma concentration of estrone sulfate during late gestation, which is associated with placental function and calf birth weight, was different with the combination of the breed of bull and pregnant cow (Isobe et al., 2003). They suggest that the placental weight and function reflected by plasma estrone sulfate concentration is affected by the breed of pregnant cow and bull, and calf birth weight. Therefore, fetal breed and/or fetal body weight rather than maternal nutritional status in the Holstein late-pregnant heifers could influence the supply of amino acids and glucose from dams to fetuses. Overall, our data indicate that the supply of nutrition from dams to the beef fetuses was lower than that to the Holstein fetuses. This is the first study to show that the transfer of nutrients from dam to fetus differs depending on the fetal breed, and this finding may help to establish feeding management of dairy cattle pregnant with beef breeds for ensuring appropriate fetal growth and nutrition. Therefore, further detailed studies are needed to investigate the nutritional supply from dams to fetuses, including placental function.

Acknowledgments

The authors would like to thank Mr. Yusuke Sugimoto (Ajinomoto Co., Inc.) for technical support of the amino acid analysis. This study was supported by the Ito Foundation (2018, Ken76).

Conflict of interest

Authors declare no Conflict of Interests for this article.

References

Bell, A. W., & Ehrhardt, R. A. (2002). Regulation of placental nutrient transport and implications for fetal growth. *Nutrition Research Reviews*, 15, 211–230. https://doi: 10.1079/NRR200239.

Ferguson, J. D., Galligan, D. T., & Thomsen, N. (1994). Principal descriptors of body condition score in Holstein cows. *Journal of Dairy Science*, 77, 2695–2703. https://doi: 10.3168/jds.S0022-0302(94)77212-X.

Hayirli, A. (2006). The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. *Veterinary Research Communications*, 30, 749–774. https://doi: 10.1007/s11259-006-3320-6.

Isobe, N., Nakao. T., Uehara, O., Yamashiro, H., & Kubota, H. (2003). Plasma concentration of estrone sulfate during pregnancy in different breeds of Japanese beef cattle. *Journal of Reproduction and Development*, 49, 369-374. https://doi: 10.1262/jrd.49.369.

Kim, J., Song, G., Wu, G., & Bazer, F. W. (2012). Functional roles of fructose. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E1619-1628. https://doi: 10.1073/pnas.1204298109.

Kwon, H., Ford, S. P., Bazer, F. W., Spencer, T. E., Nathanielsz, P. W., Nijland, M. J., … Wu, G. (2004). Maternal nutrient restriction reduces concentrations of amino acids and polyamines in ovine maternal and fetal plasma and fetal fluids. *Biology of Reproduction*, 71, 901-908. https://doi.org/10.1095/biolreprod.104.029645.

Lemons, J. A., & Schreiner, R. L. (1983). Amino acid metabolism in the ovine fetus. *American Journal of Physiology*, 244, E459–E466. https://doi.org/10.1152/ajpendo.1983.244.5.E459.

Lucy, M. C., Green, J. C., Meyer, J. P., Williams, A. M., Newsom, E. M., & Keisler, D. H. (2012). Short communication: Glucose and fructose concentrations and expression of glucose transporters in 4- to 6-week pregnancies collected from Holstein cows that were either lactating or not lactating. *Journal of Dairy Science*, 95, 5095-5101. http://dx.doi.org/ 10.3168/jds.2012-5456.

National Agriculture and Food Research Organization (NARO) (2017). *Japanese feeding standard for dairy cattle.* Tokyo, Japan: Japan Livestock Industry Association. (In Japanese).

Nishida, T. (2009). Nutrients and growth of fetus. In Y. Ogata, Z. Okamoto, N. Kimura, M. Koiwai, & S. Tsumagari (Eds.), *The calf: Management from the birth to first childbirth* (pp. 31-33). Tokyo: Midori Shobo Co., Ltd. (In Japanese).

Paolini, C. L., Meschia, G., Fennessey, P. V., Pike, A. W., Teng, C., Battaglia, F. C., & Wilkening, R. B. (2001). An in vivo study of ovine placental transport of essential amino acids. *American Journal of Physiology - Endocrinology and Metabolism*, 280, E31-39. https://doi: 10.1152/ajpendo.2001.280.1.E31.

Shen, L., Zhu, Y., Xiao, J., Deng, J., Peng, G., Zuo, Z., … Cao, S. (2019). Relationship of adiponectin, leptin, visfatin, and IGF-1 in cow's venous blood and venous cord blood with calf birth weight. *Polish Journal of Veterinary Sciences*, 22, 541-548. https://doi: 10.24425/pjvs.2019.129962.

Figure Legends

Figure 1.

Serum glucose, total protein, and plasma amino acid concentrations in dams after calving, in umbilical veins of dams at calving, and in calves immediately after birth. The number of dams pregnant with the Holstein and beef fetus breeds was 5 and 7 (Japanese Black, n = 4; F1, n = 3), respectively. The number of umbilical veins of the Holstein or beef fetus breeds was 4 and 3 (Japanese Black, n = 2; F1, n = 1), respectively. The number of Holstein and beef calves was 5 and 5 (Japanese Black, n = 2; F1, n = 3), respectively. Values are presented as mean ± standard error of the mean. ** Indicates differences of $p < 0.01$, and * indicates differences of $p < 0.05$ between the Holstein and beef breeds.

Table 1. Blood parameters at 1 week before the expected parturition in dams[1]

	Dams with Holstein fetus (n=5)			Dams with beef fetus (n=7)			*P* value
Glucose (mg/dL)	69.8	±	2.2	92.7	±	14.6	0.018
Total protein (g/dL)	6.2	±	0.2	6.7	±	0.2	0.060
Total amino acid (µmol/L)	222.2	±	19.6	222.8	±	12.3	0.977
Total essential amino acid (µmol/L)	100.0	±	10.0	96.2	±	8.2	0.776
Total non-essential amino acid (µmol/L)	122.2	±	10.0	126.6	±	4.8	0.673
Isoleucine (µmol/L)	13.7	±	1.4	13.5	±	1.2	0.891
Valine (µmol/L)	26.9	±	2.9	25.7	±	1.9	0.709
Leucine (µmol/L)	16.5	±	1.9	15.7	±	1.4	0.750
Histidine (µmol/L)	6.1	±	0.8	5.9	±	0.3	0.741
Lysine (µmol/L)	8.6	±	1.0	8.7	±	1.2	0.960
Threonine (µmol/L)	8.8	±	1.5	7.7	±	0.8	0.477
Tryptophan (µmol/L)	2.9	±	0.3	3.0	±	0.5	0.910
Phenylalanine (µmol/L)	6.1	±	0.4	5.9	±	0.4	0.762
Methionine (µmol/L)	2.8	±	0.3	2.7	±	0.2	0.805
Arginine (µmol/L)	7.5	±	0.5	7.6	±	0.8	0.990
Asparagine (µmol/L)	3.6	±	0.5	3.7	±	0.4	0.912
Glutamine (µmol/L)	38.4	±	3.3	41.6	±	2.2	0.410
Serine (µmol/L)	8.7	±	0.9	9.8	±	0.3	0.233
Aspartic acid (µmol/L)	0.5	±	0.0	0.5	±	0.0	0.519
Glutamic acid (µmol/L)	6.5	±	0.9	7.1	±	0.5	0.533
Alanine (µmol/L)	21.6	±	2.5	20.8	±	1.9	0.805
Glycine (µmol/L)	30.9	±	4.8	31.0	±	1.9	0.986
Proline (µmol/L)	6.7	±	0.6	7.0	±	0.4	0.644
Tyrosine (µmol/L)	5.4	±	0.7	5.0	±	0.7	0.751

[1]Values are the mean ± standard error of the mean.

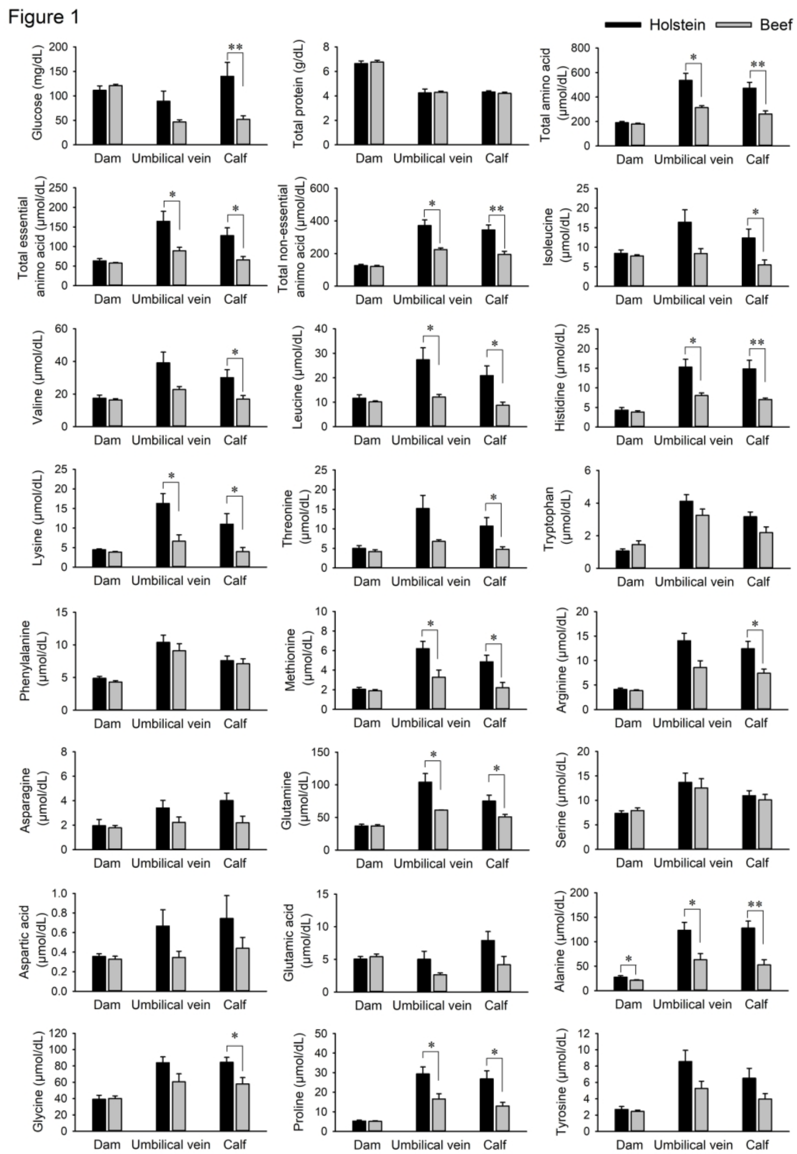

Figure 1. Serum glucose, total protein, and plasma amino acid concentrations in dams after calving, in umbilical veins of dams at calving, and in calves immediately after birth. The number of dams pregnant with the Holstein and beef fetus breeds was 5 and 7 (Japanese Black, n = 4; F1, n = 3), respectively. The number of umbilical veins of the Holstein or beef fetus breeds was 4 and 3 (Japanese Black, n = 2; F1, n = 1), respectively. The number of Holstein and beef calves was 5 and 5 (Japanese Black, n = 2; F1, n = 3), respectively. Values are presented as mean ± standard error of the mean. ** Indicates differences of $p < 0.01$, and * indicates differences of $p < 0.05$ between the Holstein and beef breeds.

209x296mm (300 x 300 DPI)

ホルスタイン種または肉用種を妊娠したホルスタイン種未経産牛における妊娠後期と分娩直後，分娩時の臍静脈血および出生直後の産子の血液中栄養パラメーター

川島千帆[1]，条咲良[1]，山岸則夫[2]

[1]帯広畜産大学畜産フィールド科学センター，帯広市　080-8555

[2]帯広畜産大学グローバルアグロメディシンセンター，帯広市　080-8555

一般的な乳用牛の飼育管理下では, 肉用種を妊娠させた場合でも乳用種妊娠と同じ群飼育が行われており, 胎子品種はほとんど考慮されないため, 胎子の成長や栄養への影響は不明である．そこで, ホルスタイン種または肉用種（黒毛和種またはホルスタイン種と黒毛和種の交雑種；F1）を妊娠したホルスタイン種未経産牛に対し，妊娠後期と分娩直後, 分娩時の臍静脈および産子の出生直後の血液から栄養パラメーターを比較した．試験は，ホルスタイン種（$n = 5$）または肉用種（黒毛和種；$n = 4$，F1；$n = 3$）を妊娠した12頭のホルスタイン種未経産牛を対象に実施した．分娩予定1週間前と分娩直後の母牛，娩出時の臍静脈，出生直後の子牛から採血し，血中グルコース，総タンパク質およびアミノ酸濃度を測定した．ホルスタイン種を妊娠している場合に比べて，肉用種妊娠の母牛は，分娩予定1週間前の血中グルコース濃度が高かった（$p < 0.05$）．血中総アミノ酸，総必須アミノ酸，総非必須アミノ酸および7種類のアミノ酸濃度は，ホルスタイン種よりも肉用種妊娠の臍静脈血と肉用子牛で低かった（$p < 0.05$）. さらに，血中グルコース濃度および5種類のアミノ酸濃度は，ホルスタイン種子牛よりも肉用子牛で低かった（$p < 0.05$）．以上より，母牛から肉用種胎子への栄養供給は，ホルスタイン種妊娠の場合より少ないことが示された．本結果は, 肉用種妊娠の乳牛に対する適切な飼養管理構築の一助になるだろう.