宮城大学食産業学部紀要　4(1):1－11（2010）
J. Miyagi Univ. Sch. Food Agri. Environ. Sci.　4(1):1－11（2010）

Earlier Distinction of Pregnant Goats Using Plasma Amino Acid Concentrations

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Abstract

Earlier distinction of the pregnancy status after mating is important for efficient production in domestic animals. The aim of this study was to differentiate pregnant animals from non-pregnant ones until the 47 days after mating (day 0 = day of estrus) by using a combination of plasma amino acid composition. At the Experimental Station for Bio-Animal Science, the University of Tokyo, female Shiba goats (n=10) randomly selected were synchronized their estrous cycle: A fertility proven male goat was introduced to 5 goats (pregnant group, PG), whereas a vasectomized goat was introduced to the remaining goats (non-pregnant group, NPG). All goats in PG were pregnant and delivered. Along with body weight measurements, weekly blood samples (10ml) from jugular vein were obtained, from which plasma was separated for measuring free amino acid concentrations. Analyses of amino acids were performed with duplicated samples by using High Speed Amino Acid Auto-analyzer. Analysis of variance was carried out and the weekly data was adjusted to average day after mating, 5^{th}, 12^{th}, 19^{th}, 26^{th}, 33^{th}, 40^{th} and 47^{th} day, by a linear regression analysis because the effect of days after mating on plasma amino acid concentrations was significant ($p<0.01$). Differences between PG and NPG animals over early pregnancy period were significant for amino acids of Ile, Glu and P-Ser. Interactions between pregnant status (PS) and adjusted day (AD) were significant for Ile ($P<0.01$) and P-Ser ($P<0.05$). Because a contribution of plasma amino acids to body weights (BW) was minimal during the experimental period, plasma amino acid concentrations would not be influenced by dietary amino acid intakes. To distinguish PG from NPG animals and select suitable amino acid family with high accuracy, a linear discriminant analysis was performed and examined for the usefulness of all detected amino acids and the family. Using results pooled form 5^{th} to 47^{th} day data, pregnant status was successfully determined with a mathematical model and plasma amino acids, Ile, Lue, Glu, Tau and P-Ser. Rather than analysis of a single factor, multivariate information such as plasma amino acid concentrations may represent an alternative method to diagnose the pregnancy outcome accurately.

（Received November 5, 2009 ; Accepted February 3, 2010）

Key Words : pregnancy, amino acids, shiba goat, discriminant analysis

Introduction

It is important to differentiate earlier the pregnant status after mating for efficient domestic animal production. A specific peptide present in early pregnancy has been searched in human (Cooper, 1963), horse (Bonte et al., 1981), pig (Yu et al., 1993), mouse (Born et al., 1973), rat (Sharma and Peel., 1973), sheep (Leslie et al. 1990) and cow (Szenci et al., 1998a, b). In ruminant ungulates, various factors such as glycoproteins, peptides, amino acids in urine and blood in the maternal system have been extensively studied to diagnose early pregnancy (Klima et al., 1987; Zoli et al., 1992a, b; Iniguez et al., 1995; Szenci et al., 1998a, b; Green et al., 2000). In addition, such

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factors as glycoprotein and peptides have been studied as "additives (supplements)" to enhance the development of bovine embryos fertilized in vitro (Ito et al., 1998). However, means to recognize the pregnant animals using such bio-markers as pregnancy associated proteins that have been identified to date are not always sufficient to differentiate early pregnancy in ruminant ungulates (Cordoba et al., 2001).

Instead of a single factor, multiple factors have been used to predict pregnancy status in humans. Deutinger et al. (1986) reported that, in early pregnancy, mathematical evaluation of the multiple biochemical parameters such as chorionic gonadotropin (CG), pregnancy-specific glycoprotein and progesterone is far superior for the determination of pregnancy status than that of the single factor. This observation suggests that rather than the detection of only one specific peptide in blood or tissues, multiple components of several amino acid concentrations to increase the accuracy to discriminate the pregnant status might give rise to a better way for pregnancy distinction. Therefore, use of the discriminant analysis with the plural biochemical variables should be considered as an effective method in the prediction of early pregnancy.

Ingestion of various proteins and free amino acids are important for proper development of conceptuses. During pregnancy, the amino acid are transferred from the mother to the fetus (Palou et al., 1977). Requirement of amino acids for proper conceptus development might have a close relationship between protein synthesis at the placenta and the transfer of amino acids (Carrol and Young, 1983). During conceptus development, it is possible that the maternal system may have specific profiles of amino acid compositions, resulting from active transfer and placental synthesis. Therefore, even in the early stage of pregnancy, maternal plasma amino acid composition might give an indication of pregnancy status if such changes could be analyzed (Suda et al., 2002).

The objectives of the present investigation were to evaluate maternal amino acid composition valance and differentiate pregnant animals from non-pregnant animals for pregnant prediction using plasma amino acid concentrations. With a computer-aided, the discriminant analysis, mathematical models with plasma amino acid concentrations were developed, which differentiated pregnant animals from non-pregnant ones.

Materials and Methods

Animals

Female Shiba goats (n=10) were randomly assigned to one of two treatment groups at Experimental Station for Bio-Animal Science, The University of Tokyo. These animals have been kept at this location as a closed colony for over 30 years and thus are genetically homogeneous. All animals were raised together in a free stall with sufficient room (10 m^2/head) during the experimental period and had free access to haylages composed of Orchard grass and Italian ryegrass. A universal concentrate (60 g/head/day) consisted of cracked corn, soybean meal and alfalfa hay was fed to each animal (Table 1). All animals were treated with 3 mg of prostaglandin $F_2\alpha$(Panacelan-Hi, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) to synchronize their estrous cycle (day 0=day of estrus). On day 0, a proven fertile male goat was introduced to 5 female goats (pregnant group, PG), whereas a vasectomized male goat was placed with the remaining female goats (non-pregnant group,

Table 1. Composition of concentrate diet

Ingredient	% of Diet DM[1]
Cracked corn	55. 0
Soybean meal	17. 0
Alfalfa hay	14. 0
Molasses and others	14. 0
Nutrient analysis	
Crude protein	14. 6
Crude lipid	2. 0
Crude fiver	12. 0
TDN[2]	73. 0
Calcium carbonate	0. 6
Crude ash content	10. 0

[1]DM : dry matter.
[2]TDN : total digestible nutrients.

NPG). All goats in PG were pregnant and delivered. The means of initial body weight (BW) on PG and NPG animals were 19.9 and 21.3kg, respectively. In addition, the mean age at the initiation of the study and number of pregnancies were 20.5 months and 0.6, respectively. On approximately 30th day after mating, pregnant status was determined by using ECHO VISION (SSD-500EV, ALOKA Co., Ltd., Tokyo Japan).

Blood samples and body weight measurement

From the beginning day of this experiment, blood samples (10 ml) from jugular vein were obtained weekly to a tube containing heparin sodium (90 units) while taking weekly body weight measurement. Plasma was separated from its bloods by centrifugation and stored at -20℃ until the measurement of free amino acid concentrations.

Free amino acid concentrations measurement

Plasma was deproteinised by centrifugation following the addition of an equal volume of 5% trichloroacetic acid loading buffer, from which the supernatant was separated. The deproteinised plasma was analyzed for free amino acid concentrations by using High Speed Amino Acid Auto-analyzer (L-8500A, HITACHI Co. Ltd., Tokyo, Japan) according to the manufacture's instructions. All determinations were done by using duplicated samples.

Statistical analysis

Linear multiple regression analysis of plasma amino acid concentrations on BW

The degrees of relative contribution of plasma amino acid concentrations to changes in BW were tested by a linear multiple regression analysis. The value of R2 as degree of decision for fitting to a linear function with plasma amino acid concentrations was also calculated during pregnant periods examined. The linear regression model was:

$$y = \sum_{k=1}^{n} a_k x_k + e (n = 25)$$

where, a_k = (a_1 ・・・・ a_n : n=25) : vectors weighted by standard deviation of 25 of amino acid concentrations as standardized partial regression coefficients (SPRC) in order.

$$x_k = \begin{pmatrix} x_1 \\ \vdots \\ x_{25} \end{pmatrix}$$: vectors of observation of 25 of amino acids in order.

e: intercept.

In this analysis, REG procedure of SAS (SAS Institute, 1989) was used.

For selection amino acids to discriminate PG from NPG, the degrees of variances for plasma amino acid concentrations collected from PG and NPG animals were tested by using Bartlett's chi-square test (χ^2).

Linear discriminant analysis for pregnant status with plasma amino acid concentrations

In order to distinguish PG animals from NPG ones using the pooled data from 5th to 47th days, the two-group discriminant analysis was preformed. The first group included the data from pregnant animals resulting in delivery and the second group used the data from non-pregnant animals. The discriminant model and functional value (Z) were:

$$z = a_0 + \sum_{k=1}^{n} a_k x_k (n = 25)$$

where, a_k = (a_1 ・・・・ a_n : n=25) : weighted vectors of amino acid composition in order.

$$x_k = \begin{pmatrix} x_1 \\ \vdots \\ x_{25} \end{pmatrix}$$: vectors of observation of 25 amino acid concentrations in order.

a_0 : intercept.

Variance-covariance matrixes of PG (Σ_{PG}) and NPG (Σ_{NPG}) to calculate parameters on discriminant model was given by

$$\sum_{PG} = \begin{pmatrix} \sigma_{11PG} & \cdots & \sigma_{1nPG} \\ \vdots & \vdots & \vdots \\ \sigma_{n1PG} & \cdots & \sigma_{nnPG} \end{pmatrix} \text{ and } \sum_{NPG} = \begin{pmatrix} \sigma_{11PG} & \cdots & \sigma_{1nPG} \\ \vdots & \vdots & \vdots \\ \sigma_{n1PG} & \cdots & \sigma_{nnPG} \end{pmatrix},$$

respectively. In this analysis, STEPWISE of SAS (SAS Institute, 1989) was used.

For selection amino acids to discriminate PG from NPG, the degrees of variances for plasma amino acid

concentrations collected from PG and NPG animals were tested by using Bartlett's chi-square test (χ^2).

Analysis of variance

All data were analyzed by using least squares analysis with the General Linear Model (GLM) procedure of SAS (SAS Institute, 1989). In PG animals, weekly measurements of plasma amino acid concentrations and BW were evaluated on each days that had been adjusted by using linear regression analysis to 5th, 12th, 19th, 26th, 33rd, 40th and 47th days after mating. In NPG animals, the same data sets were evaluated on each day that had been adjusted by using same analysis with those of PG to 5th through 47th days after the observation of behavioral estrus. Suitable amino acids, isoleucine (Ile), leucine (Leu), glutamic acid (Glu), taurine (Tau) and phosphoserine (P-Ser) were selected by considering the results of a discriminant analysis. The mathematical function for analysis of variance that included interactions among pregnant status, number of pregnancy and adjusted days after mating was:

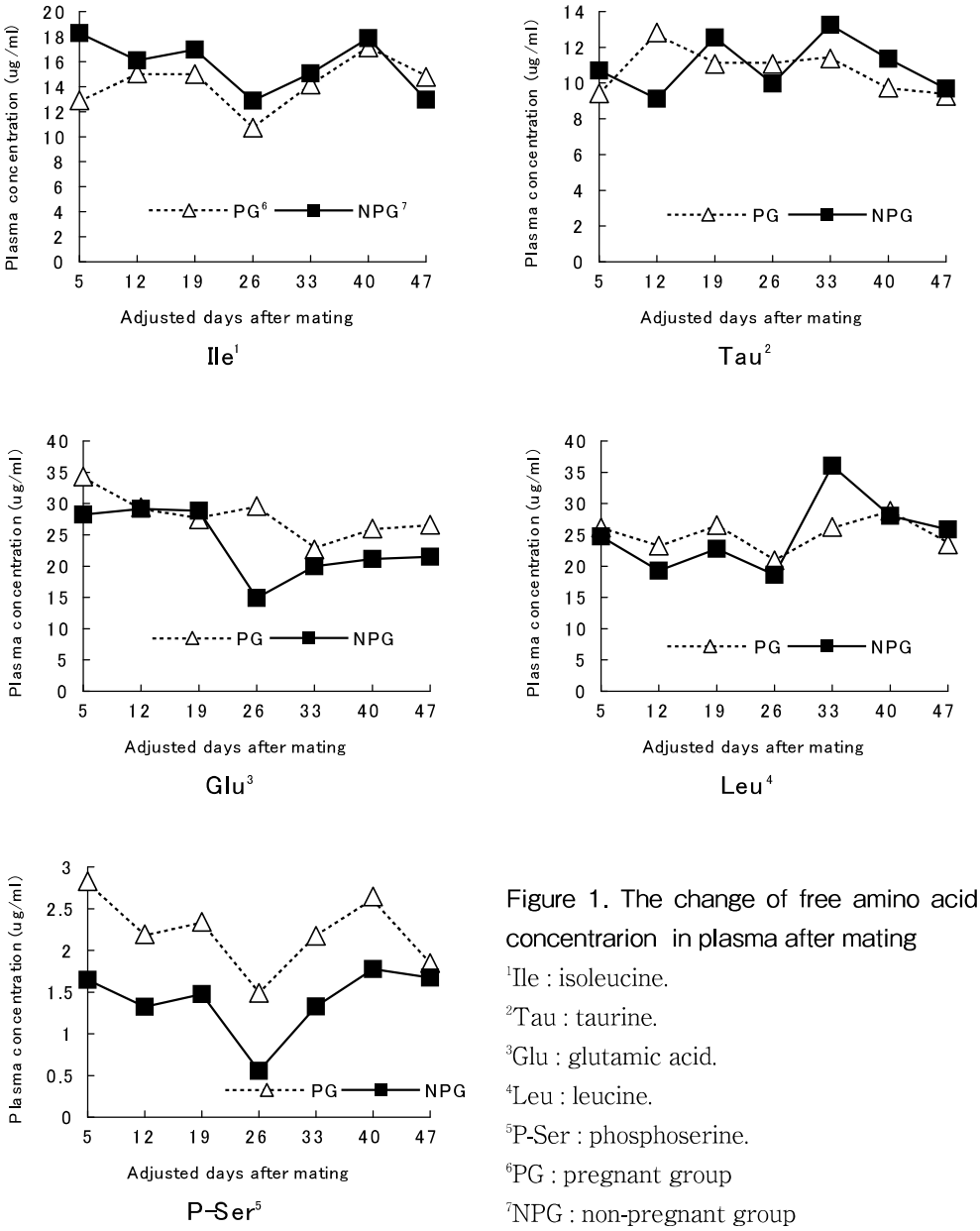

Figure 1. The change of free amino acid concentrarion in plasma after mating

[1]Ile : isoleucine.

[2]Tau : taurine.

[3]Glu : glutamic acid.

[4]Leu : leucine.

[5]P-Ser : phosphoserine.

[6]PG : pregnant group

[7]NPG : non-pregnant group

Table 2. Analysis of variance for BW, and plasma Ile, Leu, Glu, Tau and P-Ser concentrations in early pregnancy

Item	BW[1]	Ile[2]	Leu[3]	Glu[4]	Tau[5]	P-Ser[6]
PN[7]	P<0.01	P<0.05	N.S	N.S	N.S	P<0.01
PS[8]	P<0.01	P<0.01	N.S	P<0.05	N.S	P<0.01
AM[9]	P<0.01	P<0.05	N.S	N.S	N.S	P<0.01
AD[10]	N.S	P<0.01	P<0.01	N.S	N.S	P<0.01
PS×AD[11]	P<0.05	P<0.01	P<0.01	N.S	N.S	P<0.05
PS×PN[12]	P<0.05	N.S	N.S	N.S	N.S	N.S
PN×AD[13]	P<0.05	P<0.05	P<0.01	N.S	P<0.05	P<0.05

[1]BW : body weight (kg).
[2]Ile : isoleucine (ug/ml).
[3]Leu : leucine (ug/ml).
[4]Glu : glutamic acid (ug/ml).
[5]Tau : taurine (ug/ml).
[6]P-Ser : phosphoserine (ug/ml).
[7]PN : pregnant number.
[8]PS : pregnant status.
[9]AM : age of month.
[10]AD : days adjusted to average days after mating in each PS.
[11]PS×AD : interaction between PS and AD.
[12]PS×PN : interaction between PS and PN.
[13]PN×AD : interaction between PN and AD, N.S: not significant.

Table 3. Least squares mean (±SEM[1]) of BW, and plasma Ile, Leu, Glu, Tau and P-Ser concentration

Status	BW[4]	Ile[5]	Leu[6]	Glu[7]	Tau[8]	P-Ser[9]
PG[2]	21.5[a](0.13)	14.2[b](0.50)	25.1(0.87)	28.3[a](1.66)	10.7(0.48)	2.2[a](0.12)
NPG[3]	20.2[b](0.12)	15.8[a](0.50)	25.0(0.80)	23.4[b](1.82)	10.9(0.68)	1.3[b](0.10)

[1]SEM : standard error of mean.
[2]PG : pregnant status.
[3]NPG : non-pregnant status.
[4]BW : body weight (kg).
[5]Ile : isoleucine (ug/ml).
[6]Leu : leucine (ug/ml).
[7]Glu : glutamic acid (ug/ml).
[8]Tau : taurine (ug/ml).
[9]P-Ser : phosphoserine (ug/ml).
Significance between different superscripts in the same column was regarded at 5 % level.

$$Y_{hijkl} = \mu + \alpha_h + \beta_i + \gamma_j + a_j(\delta_{hijkl} - \delta) + \varepsilon_k + a(\beta)_{hi} + \beta(\gamma)_{ij} + \alpha(\gamma)_{hj} + e_{hijkl}$$

where,

Y_{hijkl}=Observations of dependent variable, BW (kg), plasma concentrations (ug/ml) of isoleucine(Ile), leucine(Leu), glutamic acid(Glu) taurine(Tau) and phosphoserine (P-Ser) for the l^{th} individual in the k^{th} age of month (AM), the j^{th} pregnant or non-pregnant, the i^{th} number of pregnancy (PN) and h^{th} adjusted day (AD).

μ=Overall mean with unequal subclass numbers.

α_h= effect of the hth AD as deviation from the overall mean.

β_i= effect of the ith PN as deviation from the overall mean.

γ_j= effect of the jth PS as deviation from the overall mean.

δ_{hijkl}= days after mating on lth individual for the kth age of month, the jth pregnant status, the ith number of pregnancy and the hth adjusted days.

δ= means of days after mating.

ε_k= effect of the kth AM as deviation from the overall mean.

a_j= the jth first-degree regression efficient of each amino acid to day after mating in each PS.

$a(\beta)_{hi}$= interaction between AD and PN.

$\beta(\gamma)_{ij}$= interaction between PN and PS.

$\alpha(\gamma)_{hj}$= interaction between AD and PS.

e_{hijkl}= random error.

On the result of analysis of variance for plasma amino acid concentrations, mean differences for each dependent variable were tested for significance by Student's t-test.

Results

Analysis of variance and least squares mean for BW and plasma amino acid concentrations

A result of analysis of variance, and least squares

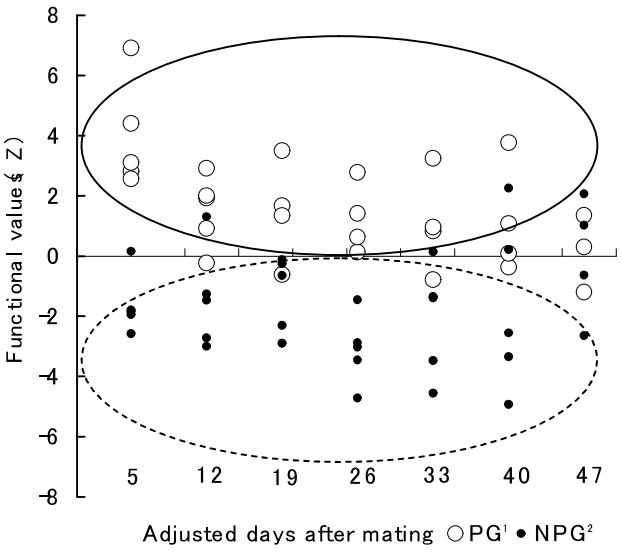

Figure 2. Disinction of functional values (Z) predicted by a linear discriminant model with pooled plasma amino acid family concentration in the early pregnancy,
——:area of PG, - - - - -:area of NPG, [1]PG : pregnant group, [2]NPG : non-pregnant group.

mean and standard error of the mean for BW and plasma amino acid concentrations are shown in Table 2 and 3, respectively. And Figure 2 shows the change of free amino acid concentrations after mating. Interactions among PN, PS and AD were examined to know complicated relationships between their factors. In BW, the effects of all factors ($P<0.01$) and interactions ($P<0.05$) were significant except for AD effect. Least squares mean of BW was 21.5kg in PG and 20.2kg in NPG, respectively ($P<0.05$). Variances among PNs were significant in Ile ($P<0.05$) and P-Ser ($P<0.01$). Least squares mean of Ile (14.2μg/ml) in PG was lower than that (15.8μg/ml) in NPG ($P<0.05$). Least squares means of Glu (28.3μg/ml) in PG was higher than Glu (23.4μg/ml) in NPG ($P<0.05$). Least squares mean of P-Ser in PG was 2.2μg/ml, which was higher than that (1.3μg/ml) in NPG ($P<0.05$). The effect of AM was significant in Ile ($P<0.05$) and P-Ser ($P<0.01$). Plasma concentrations of Ile ($P<0.01$), Leu ($P<0.01$) and P-Ser ($P<0.01$) changed significantly from 5th to 47th day of pregnancy or estrus cycle. Interactions of PS×AD were significant in Ile ($P<0.01$), Leu ($P<0.01$) and P-Ser ($P<0.05$). Interactions of PN×AD were significant in all amino acid concentrations except for Glu whereas PS×PN interaction for all amino acid concentrations was not significant.

Relationship among BW and plasma amino acid concentrations in PG and NPG

To examine relative relationship among BW and plasma amino acid concentrations in PG and NPG, a linear multiple regression analysis was performed on BW and plasma amino acid concentrations during this experimental term (Table 4). SPRC as the degree of relative contribution to BW on plasma amino acid concentrations were calculated. In PG, SPRCs in all amino acid concentrations exhibited small, and they were not statistically significant and R^2 was also very low at 0.07. In NPG, they tended to show same relationship. And, when pooled data were used for the calculation of their parameters, relative contribution in P-Ser was middle level at 0.49 ($P<0.01$), however, R^2 was very low at 0.25.

Discriminant analysis for pregnant status with plasma amino acid concentrations as explanatory variables

Plasma amino acid concentrations on each adjusted days were analyzed by using a linear discriminant analysis, followed by Bartlett's chi-square test (χ^2) (Table 5). To estimate the frequency to misdiagnose pregnant status, probability of erroneous assessment (EDR) was also calculated. On the 5th day after mating, PG animals could be distinguished from NPG ones at 0.05% of EDR. The models from 12th to 47th day after mating were formed at very low EDR levels, however, χ^2 values of 12th and 40th day were large ($P<0.01$), and the values of 5th, 19th, 26th, 33th, and 47th could not be calculated. Instead of examinations on each adjusted days, pooled data from 5th to 47th days were used to form a linear model, resulting in discrimination of PG from NPG at high level, 1%. The linear model with the pooled data also made EDR low level at 18.24% and the value small, indicating that amino acid concentrations in PG and NPG animals had similar variance. The distribution of functional values, discrimination scores (Z) was calculated by using the formed model with the pooled data (Figure

Table 4. Linear multiple regression analysis of plasma amino acid concentrations on BW

	Pool[1]			PG			NPG		
Variables	SPRC[2]	SEM	P values	SPRC	SEM	P values	SPRC	SEM	P values
Ile[3]	−0.20	0.04	0.11	0.01	0.08	0.98	0.07	0.04	0.76
Leu[4]	−0.12	0.02	0.34	−0.19	0.04	0.41	−0.05	0.01	0.84
Tau[5]	0.10	0.04	0.42	−0.04	0.08	0.85	0.20	0.03	0.37
Glu[6]	0.05	0.01	0.67	−0.05	0.02	0.79	−0.30	0.01	0.80
P-Ser[7]	0.49	0.16	0.01	0.24	0.34	0.29	0.20	0.21	0.40
R^2 [8]	0.25			0.07			0.11		

[1]Pool : total data pooled PG and NPG.
[2]SPRC : standardized partial regression coefficient.
[3]Ile : isoleucine (ug/ml).
[4]Leu : leucine (ug/ml).
[5]Glu : glutamic acid (ug/ml).
[6]Tau : taurine (ug/ml).
[7]P-Ser : phosphoserine (ug/ml).
[8]R^2 : coefficients of decision.

Table 5. Linear discriminant analysis for pregnant status with plasma amino acid family concentrations in increasing adjusted days after mating

	Adjusted days after mating							
Item	5th	12th	19th	26th	33th	40th	47th	Pool[1]
	Discriminant coefficients							
Ile[2]	−5.25	−0.25	−1.38	−3.26	0.01	4.81	2.55	−0.39
Leu[3]	1.24	1.02	0.61	−0.22	−0.57	2.11	−2.60	−0.05
Tau[4]	1.12	0.48	−1.36	0.48	0.15	−4.81	0.52	0.09
Glu[5]	0.28	−0.80	−0.20	−0.28	0.32	7.47	0.92	0.07
P-Ser[6]	11.31	9.15	20.06	23.77	5.33	25.98	−2.54	2.74
Const[7]	4.73	-15.61	−9.36	19.77	−0.06	−327.22	6.11	−0.51
F value	10.90*	3.48	4.70	4.77	2.04	12.36	1.02	9.61**
χ^2value	----[9]	50.15 **	----	----	----	35.11**	----	11.04
EDR[8]	0.05	3.10	0.65	0.62	5.06	0.00	2.63	18.24

[1]Pool : total data pooled from 5th to 47th day after mating.
[2]Ile : isoleucine (ug/ml).
[3]Leu : leucine (ug/ml).
[4]Glu : glutamic acid (ug/ml).
[5]Tau : taurine (ug/ml).
[6]P-Ser : phosphoserine (ug/ml).
[7]Const : constant value.
[8]EDR : probability (%) of erroneous assessment.
[9] ---- : calculation impossibility.
** Significance of variance ($P<0.01$).
* Significance of variance ($P<0.05$).

2). Each of Z values was placed in the positive and negative areas for PG and NPG animals ($P<0.01$), respectively.

Discussion

In the ruminant ungulates, most of dietary protein components consumed is used to produce several amino acids by rumen protozoa (Wallace et al., 1997), and a part of which are absorbed in the small intestine. In this examination, no direct relationships among changes in BW, uptake volume of the feed and plasma amino acid concentrations were recognized because R^2 values of multiple regression analysis were 0.07 and 0.11 for PG and NPG, respectively. So, these results indicated that measurements of BW were not able to differentiate PG with NPG using plasma amino acid concentrations, because their relationship was very small. Though more data would increase the accuracy of observations, the maternal plasma amino acids concentration in the early stage of pregnancy might show a specific valance.

In the present investigation, pregnant animals were successfully differentiated from the non-pregnant ones with the use of plasma amino acid concentrations that had been weighted with mathematical models. In our preliminary study (Suda et al., 2002), many amino acids were detected in the

plasma. Among those, five amino acids of Ile, Leu, Glu, P-Ser and Tau were selected as explanatory variables for the mathematical model, because the degree to fit formed model ($P<0.01$) and accuracy to discriminate pregnancy status with these 5 factors were statistically higher than other amino acid family detected and examined. Thus, a combination of these amino acids was the best to form the linear model for the discrimination of pregnancy status. Significance of the model explained by just 5 variables in PG and NPG on the 5th day after mating and pooled period were high, respectively, and possibility to statistically discriminate pregnancy status using a linear model was useful. In addition, the χ^2 value of data in pooled period was not significant, suggesting a possibility that each variance within two groups of PG and NPG had similar variances. These allowed the linear model formation with just 5 amino acids in the pooled period at 18.24% level of EDR.

Ile and Leu are essential amino acids that are metabolized mainly in muscle to compose several proteins and to produce energy whereas Glu is non-essential amino acid and is known as a neurotrans mitter. Significant interactions between PS and AD, and PN and AD in Ile and Leu, and significant effect of PS in Glu indicated that pregnancy would influence maternal nutrition and body condition. And serine exists in vivo as phosphrous serine (P-Ser) (Murray et al., 1996) and is one of amino acids that composes of P-Ser prolactin (PPRLS) peptide. Generally, PRL is known as a hormone necessary for lactation in ruminants (Maciejewski et al., 1995). Significant effects of AD and interaction between PS and AD in P-Ser would reflect an indirect response in pregnancy. These data suggest that the concentration change of each amino acid in plasma could be observed detected in early pregnancy, however, relative and integrated evaluation using some amino acids must increase the accuracy of discrimination. Identification of pregnancy at the early stage often influences on the efficiency of animal production. Ito et al. (1998) suggested that a specific protein, early pregnancy factor (EPF), existed in the bovine serum from 24 to 48 hr after insemination, and its activity could be determined experimentally in vitro. Green et al. (2000) reported that pregnancy status could be diagnosed with the use of pregnancy-associated glycoprotein family at the 25th day of bovine and ovine pregnancies. Despite of these developments, useful and efficient means to diagnose pregnancy status in a cost effective manner have not been developed for ruminant ungulates. Carrol and Young (1983) have indicated that close relationships exist between transfer of plasma amino acids from the mother to fetus and placental protein synthesis. Observations made by Kalhan et al. (1998) suggest that relative changes in transamination of branched-chain amino acids and urea synthesis may determine pregnancy status in humans. These suggest that qualitative and/or quantitative changes in plasma amino acid concentrations may exist over pregnant period, particularly middle to late pregnancy. In the early pregnant period, it might be difficult to detect amino acid transfer from the mother to developing conceptus because placental tissues are not functioning fully. However, differences in plasma amino acid concentrations could still be detected due to physiological changes in the preparation for lactation (Baumrucker, 1984), formation of immune system (Denison et al., 1997) and maternal recognition of pregnancy through biochemical signals from the conceptus (Roberts et al., 1992). In addition, Deutinger et al. (1986) have attempted to predict early pregnancy by using the mathematical model that has been applied to the beta subunit of human chorionic gonadotropin, pregnancy-specific glycoprotein (Sp-1) and progesterone. Thus, a combination of mathematical model and biochemical factors used in the present investigation should provide an alternative approach to diagnose pregnancy outcome.

In conclusion, pregnant animals were discriminated from non-pregnant ones even at 5th day after mating. This was based on 5 amino acid concentrations analyzed by using a linear model and the subsequent χ^2 value. Although more data are required to increase the accuracy of distinction, plasma amino acid concentrations in pregnant ruminants might keep a specific valance. Further studies are required

to understand potential functions of these amino acids, individually or in combination, in the context of early pregnancy.

Acknowledgements

A subsidy program of Livestock Improvement Association of Japan, Inc. and Kamiasahi Beef Cattle Farm, Inc. funded this research. We are graceful to Dr. H. Shinohara (Tohoku University) for helpful advice and criticism and Mr. A. Sakai (Graduate School of Agriculture and Life Science, The University of Tokyo) for preparation and supports of measurements of amino acid concentrations in plasma.

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日本シバヤギにおける血漿アミノ酸組成を用いた早期妊娠識別

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反芻家畜における交配後早期に妊娠の有無を判断することは生産性向上のために重要である。本研究の目的は、交配後47日間で妊娠成立の有無を判断するために、母体における血漿アミノ酸濃度のバランス変化を評価することを目的とした。

供試動物には、長期的に閉鎖集団で無作為交配によって生産・維持された日本シバヤギ雌10頭を用いた。全ての個体は発情の同期化を施された。成熟発情した雄1頭を雌5頭に交配し妊娠群(PG)とし、精管結さつした雄1頭と同居させた雌5頭を非妊娠群（NPG）とした。毎週の体重測定とともに頸静脈より採血を行った。そして速やかに血漿分離後、遊離アミノ酸濃度分析（L-8500A, HITACHI Co. Ltd., Tokyo, Japan）を行った。PGの5頭は全て妊娠し分娩に至った。統計分析はSASコンピュータープログラム（SAS Institute, 1989）で行った。全てのデータを、線形一次回帰補正で交配後5、12、19、26、33、40、47日目に補正した。結果、イソロイシン（Ile）、グルタミン（Glu）そしてセリン（P-Ser）についてPGとNPGの間で有意な差異が認められた($P<0.05$)。特に、Ile（$P<0.01$）とP-Ser（$P<0.05$）については妊娠の有無と補正交配後日数との間で有意な交互作用が認められた。本実験期間における血漿アミノ酸濃度の体重への寄与は小さく、血漿アミノ酸濃度が飼料摂取量の違いによって影響されていないと考えられた。PGとNPGの個体を高い正確度で区別するための血漿アミノ酸を選択するのに2群線形判別分析を行った。説明変量としてIle、Lue、Glu、TauそしてP-Serを用いることで、補正交配後5日目から47日目の間でPGとNPGを有意に判別できる数学モデルを作成できた。単一の血漿アミノ酸濃度の変化で判別するよりも、Ile、Lue、Glu、Tau、P-Serの血漿濃度バランスを評価し得点化することによって判別できる可能性が示唆された。

キーワード：妊娠、アミノ酸、日本シバヤギ、判別分析