Factors Affecting Plasma Glucose, Insulin and Cortisol Levels and the Reliability of their Repeated Measurements in Cattle

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Abstract Factors affecting plasma glucose, insulin and cortisol levels were surveyed and the reliability of their repeated measurements was examined, using twenty nine progeny (steers and heifers) of two different bulls of the Japanese Black Cattle. Blood samples were taken at 10:00 a.m. in three consecutive days within one month before the end of performance testing. Plasma glucose, insulin and cortisol levels were determined and the reliability for these traits was calculated using the variance components. From the results of analysis of variance, the effect of sire was significant for plasma glucose and insulin levels and the effect of progeny within sire was significant for all traits. The reliabilities of the mean of three repeated measurements for plasma glucose, insulin and cortisol were 0.801, 0.435 and 0.612, respectively. From the results of this study, it is suggested that an inherent value exist in these plasma levels and the inherent level seems to be confirmed by the mean of three repeated measurements, under the presented sampling procedure.


Key words: reliability, plasma hormone levels, sampling procedure, inherent level

So far performace traits such as carcass traits, milk production etc. can not be measured directly in the bull itself. Therefore, progeny test has been used to evaluate the bull in terms of these traits, but it takes long time for the evaluation.

In relation to this problem, some trials have been carried out to investigate the relationships between plasma hormone levels and some economically important traits\(^1\)\(^{-}^\)\(^4\). Recently, SEJERSEN et al. reported the genetic relationship between milk yield and plasma insulin and thyroxine levels in young bulls\(^1\). If such relationship could be established, genetic evaluation of bulls for sex-limited or hidden traits can be
Moriya, Tsuki, Murayama and Sasaki carried out early in life by means of the plasma hormones and metabolites level1).

On the other hand, plasma hormone levels are affected by various factors, so that a procedure is required to obtain a reliable measurement of the inherent level of an individual5).

The objectives of this study are to survey factors affecting plasma glucose, insulin and cortisol levels and to examine reliability of the repeated measurements in cattle.

Materials and Methods

Twenty nine progenies (steers and heifers) aged between 322 and 384 days (Mean 353 days), of two different bulls. Cattle at the Livestock Experimental Farm, Kyoto University were used in this study. Numbers of the animals by sire and sex are summarized in Table 1. They were fed in the same manner as the official performance test procedure6).

Blood samples were taken by jugular venipuncture at 10:00 a.m. of the three consecutive days within one month before the end of performance testing. Plasma was separated by centrifugation and stored at -20°C until assayed. Plasma glucose level was determined by the glucose oxidase method using a commercial assay kit (Latro-Chrom GLU-LQ, IATRON LABORATORIES, INC., Tokyo). Plasma insulin level was determined by the radioimmunoassay method using polyethylene glycol to separate free and antibody-bound insulin7,8). Plasma cortisol level was determined by the high pressure liquid chromatography method9).

The data were analyzed by the least-squares analysis of variance10) with unequal subclass number, using LSML 76 program written by Harvey11). In preliminary analysis of variance, sex and season effects were not significant in all traits, so that the following mathematical model was adopted for the final analysis of variance and estimation of variance components.

Mathematical Model:

\[ y_{ijkl} = \mu + S_i + P : S_{ij} + D_k + S \times D_{ik} + e_{ijkl} \]

where \( y_{ijkl} \) : concentration of plasma hormone or plasma glucose, 
\( \mu \) : overall mean, 
\( S_i \) : effect of \( i \) th sire, 
\( P : S_{ij} \) : effect of \( j \) th progeny within \( i \) th sire, 
\( D_k \) : effect of \( k \) th sampling date, 
\( S \times D_{ik} \) : interaction effect of \( i \) th sire by \( k \) th sampling date,

<table>
<thead>
<tr>
<th>Table 1. Numbers of progeny by sire and sex</th>
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<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Steer</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
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</table>
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e_{ijk} : error,

In addition, the reliability of repeated measurements of these traits was calculated using the estimated variance components\(^{12}\).

The reliability of the average measurements of \(k\) times, \(\rho_k\) is defined as

\[
\rho_k = \frac{\hat{\sigma}_p^2}{\hat{\sigma}_p^2 + (\hat{\sigma}_e^2/k)}
\]

where, \(\rho_k\) : the reliability of the mean of \(k\) measurements,
\(\hat{\sigma}_p^2\) : the estimated variance component of progeny within sire,
\(\hat{\sigma}_e^2\) : the estimated variance component of error,
\(k\) : number of measurements.

When \(k = 1\), \(\rho_1\) becomes \(\hat{\sigma}_p^2/(\hat{\sigma}_p^2 + \hat{\sigma}_e^2)\)

That is, \(\rho_1\) means the repeatability of each traits. If one defines \(\theta = \hat{\sigma}_p^2/\hat{\sigma}_e^2\), then the expression of \(\rho_k\) may be cast in the form

\[
\rho_k = \frac{k\theta}{1 + k\theta}
\]

and

\[
\rho_1 = \frac{\theta}{1 + \theta}
\]

With (2) and (3), \(\rho_k\) becomes \(k\rho_1/(1 + (k - 1)\rho_1)\)

(4).

This formula is known as the Spearman-Brown prediction formula\(^{12}\).

**Results**

In preliminary analysis of variance, sex and season effects were not significant in all traits. As shown in Table 2, in addition, the result of analysis of variance using the final mathematical model indicated that the interaction effect of sire by sampling date was not significant for all traits. On the other hand, the effect of sire was significant for both plasma glucose (P<.01) and insulin (P<.01) levels but not significant for plasma cortisol level. However, the effect of sampling date was significant for only plasma cortisol level. The effect of progeny within sire was significant for all traits.

Variance components of progeny within sire and error, and the reliability of the

<table>
<thead>
<tr>
<th>Table 2. Least-squares analysis of variance for plasma glucose, insulin and cortisol levels</th>
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<tr>
<td><strong>Source of variance</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Sire (S)</td>
</tr>
<tr>
<td>Sampling Date (D)</td>
</tr>
<tr>
<td>Progeny within Sire</td>
</tr>
<tr>
<td>S × D</td>
</tr>
<tr>
<td>Error</td>
</tr>
</tbody>
</table>

** : P<0.01, * : P<0.05
mean of $k$ measurements ($k = 1, 2$ and 3) were shown in Table 3. The reliabilities of the single measurements, namely repeatabilities of plasma glucose, insulin and cortisol levels were 0.572, 0.204 and 0.344, respectively, while the reliabilities of the mean of three measurements for these traits were 0.801, 0.435 and 0.612, respectively.

Discussion

As pointed out by HART et al.\textsuperscript{13), it is necessary to assess factors affecting the variability of plasma hormone levels in order to use plasma hormone levels as an indicator of the economically important traits\textsuperscript{13). Nutritional condition, time of feeding, time of day, stress and season etc. can affect on plasma hormone levels\textsuperscript{5,13). However, most of these factors were controlled as uniform as possible in this study, because all the individuals were managed under the similar environment and fed in the same manner as the official performance-testing procedure of Japanese Black Cattle\textsuperscript{6).}

Furthermore, the effects of sampling date except for plasma cortisol level and the interaction effects of sire by sampling date for all traits were not significant, while the effect of progeny within sire was significant for all traits. So, it is suggested that an inherent plasma level of individual exists.

On the other hand, sire's effect was also significant and the interaction effect of sire by sampling date was not significant for plasma glucose and insulin levels. Therefore, it may reflect genetic differences among animals for these two traits, although the results were obtained from only two different sires.

TILAKARATNE et al. reported that the repeatability which is the same as the reliability of single measurement as described previously of plasma glucose level was ranged from 0.201 to 0.368\textsuperscript{14). The repeatability of plasma glucose level in this study, was higher than their values. However, the repeatabilities of plasma insulin and cortisol were similar to those of the glucose level in their study.

Theoretically, the more repeated measurements are obtained, the higher the reliability of the mean. It was pointed out that frequent blood samplings within a day were necessary to obtain the inherent levels of plasma hormones and metabolites levels of each animals\textsuperscript{5). However, getting measurements of many times is expensive in terms of both labor and cost.

If the reliability necessary to obtain an inherent value of these plasma levels is assumed to be 0.5 or more, repeated of measurements for plasma glucose and cortisol levels were 1 and 2, respectively. But the reliability of plasma insulin level even with

<table>
<thead>
<tr>
<th>Traits</th>
<th>Variance ratio of progeny/error</th>
<th>Reliability of the mean of $k$ measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.339</td>
<td>0.572, 0.728, 0.801</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.296</td>
<td>0.204, 0.290, 0.435</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.525</td>
<td>0.344, 0.512, 0.612</td>
</tr>
</tbody>
</table>
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the mean of three measurements was somewhat lower than 0.5.

From the results of this study, it is suggested that an inherent value exist in these plasma levels and the inherent level seems to be obtained by the mean of three repeated measurements using, the presented sampling procedure.

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References

ウシの血糖値、血漿中インスリンおよびコルチゾール濃度に影響する要因と反復測定値の信頼性について

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ウシの血糖値、血漿中インスリンおよびコルチゾール濃度に影響する要因、並びにそれらの反復測定値の信頼性について検討するために、2頭の黒毛和種稚牛の後代牛をもっていて3日間、連続して、午前10時に採血を行なった。

分散分析の結果、血糖値および血漿中インスリン濃度は種雄牛の効果に有意な変動が認められた。更に、血糖値、血漿中インスリンおよびコルチゾール濃度のいずれにおいても、種雄牛内後代牛の効果に有意な変動が認められた。一方、採血日と種雄牛との交互作用についてはいずれの形質においても有意な変動は認められなかった。3回の測定値より算出した信頼性（Reliability）は、血糖値、血漿中インスリンおよびコルチゾール濃度で、各々0.801，0.435，0.612となった。

以上の結果から、これらの血漿中濃度には個体固有のレベルが存在し、かつ、今回の採血法により、3回の測定値の平均を用いれば個体の固有値を把握することができるものと推察される。

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