Feed Intake and Blood Metabolite-Profile in Pre-Weaning Calves Supplied with Different Amounts of Milk Replacer

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Abstract Intake patterns of concentrate and rice straw as well as fluctuation patterns of blood metabolite and insulin concentration were compared between the group (HMR) of 4 male Holstein calves receiving 600 g milk replacer/d and the group (LMR) of 4 calves receiving 200 g/d in each of duplicate experiments conducted on one day in May (Expt 1, 20-24°C in ambient temperature) and on another day in November (Expt 2, 12-13°C). Within 8 h after the supply of 300 or 100 g milk replacer and solid feeds in the morning on each experimental day, the intake of concentrate tended to be higher in LMR, while that of rice straw tended to reverse, and these tendencies were more obvious in Expt 2 than in Expt 1. The lower supply of energy from milk replacer in LMR was more or less compensated by increased intake of concentrate, and the compensation was more sufficient in Expt 2. Concentrations of glucose and insulin in plasma began to increase immediately after the supply of milk replacer, and reached a higher value in HMR than in LMR in both experiments, but began to decrease again after a few hours. The plasma concentration of β-hydroxybutyric acid showed a gradual increase especially in LMR in each experiment. Concentrations of non-esterified fatty acids in plasma decreased rapidly after the supply of milk replacer and solid feeds, and stayed at a lower level in Expt 2, but began to increase again after 30 min only in LMR in Expt 1. Concentrations of plasma urea-N were higher in the first few hours, and then decreased more remarkably in Expt 2 than in Expt 1, although there was little difference between groups in each experiment. These results suggest that an energostatic system would participate in a short-term regulation of the intake of concentrate in pre-weaning calves.


Key words: short-term regulation, feed intake, pre-weaning calf

Some differences may exist in the regulation system of voluntary feed intake between ruminants and non-ruminants depending on the structure and function of the digestive tract. A newborn calf is essentially a non-ruminant, but acquires the proportional volume of the rumen like an adult ruminant until 12 weeks of age when weaned at 5 weeks of age. It is, therefore, interesting to know if the voluntary feed intake is regulated by the same system before and after the early-weaning.

In our previous work1) using calves weaned at the end of 6 weeks of age, the intake of concentrate increased in response to the deterioration of energy status caused by the growth of calves, weaning, and reduced supply of milk replacer during the suckling period, suggesting that an energostatic system would participate in a long-term regulation of the intake of
concentrate. The intake of rice straw, however, increased almost irrespective of weaning as well as the daily amount of milk replacer during the suckling period1).

The present work aimed to ascertain the system involved in a short-term regulation of the voluntary feed intake in calves before the early-weaning by means of comparing the intake pattern of feeds and the fluctuation patterns of blood metabolite and insulin concentrations between groups different in energy supply from milk replacer.

Materials and Methods

Experiments and animals: Duplicate experiments were conducted on May 9th (Expt 1) and November 15th (Expt 2). In each experiment two groups of 4 male Holstein calves in individual pens were used, and each experimental day was within 1 week before the weaning at the end of 6 weeks of age. Ambient temperatures were 20–24°C in Expt 1 and 12–13°C in Expt 2.

Feeds: Until each experimental day, commercial milk replacer containing 28% CP and 105% TDN had been given 600 g/d to the HMR group or 200 g/d to the LMR group in two equal quantities at 8:30 and 16:30. A half of the daily milk replacer was suspended in 1.8l warm water irrespective of the amount, and given with open-buckets.

In addition to milk replacer, all calves had been given free access to concentrate pellet (3/16 inches or about 4.8mm in diameter) and rice straw chopped into 40mm in mean length. The concentrate consisted of 71% barley, 14.5% soybean meal, 10% wheat bran, 4.1% minerals and 0.4% vitamin A·D premix, and contained 16% CP and 70% TDN. Rice straw contained 3.9% CP by analysis and 38% TDN. On each experimental day, 3 l water was given to every calf at 12:30.

Measurement of feed intake: At 20:00 on days before each experiment, solid feeds were completely removed from the trough. At 8:30 on each experimental day, a half of the daily amount of milk replacer was supplied, and at the same time known quantities of concentrate and rice straw were set in the trough. Intakes were measured at 15 and 30 min and 1, 2, 4, 6 and 8 h after the supply of milk replacer and solid feeds. At every time residual feeds were replaced with known quantities of new ones as quickly as possible, and then residual feeds were weighed.

Blood samples: Blood samples were taken from jugular vein into heparinized centrifuge tubes immediately before the supply of milk replacer and solid feeds (0 h) and at every time when feed intakes were measured.

The plasma separated by centrifugation was divided into small test tubes according to the object of analysis. Analyses were executed as soon as possible especially when glucose was determined. If impossible, however, plasma samples were stored under the following conditions: Samples for quantifying glucose were stored at −4°C after adding sodium fluoride to inhibit glycolysis10), samples for non-esterified fatty acids (NEFA) at −20°C after adding N-(2-hydroxyethyl) ethylenedinitrilo-triacetic acid (EDTA-OH) to inhibit lipolysis11), samples for β-hydroxybutyric acid (BHBA) at −70°C, and samples for plasma urea-N and for insulin at −20°C.

Analyses: Plasma glucose was determined by o-toluidine-boric acid (OTB) method10), and NEFA by calorimetry using a copper reagent11). Plasma BHBA was determined by the p-nitrobenzene-diazonium fluoroborate method17) using a commercial kit (Ketonetest®; Sanwa Chemical Lab., Tokyo). Plasma urea-N was determined by the diacetylmonoxime method10), and immunoreactive insulin by enzyme-immunoassay17) using a commercial kit (Insulin-Mitsui-II®, Kainos lab., Tokyo). Results were subjected to analysis of variance.

Results

Table 1 shows the mean body weight as well
Table 1. Intakes of solid feeds and TDN within 8h after the supply of milk replacer and solid feeds

<table>
<thead>
<tr>
<th>Expt: Group</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>HMR(^1)</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>LMR(^2)</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>No. of calves</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Body weight (W; kg)</td>
<td>66.5 ± 4.4</td>
<td>59.3 ± 8.9</td>
</tr>
<tr>
<td>(W^{0.75})</td>
<td>23.3 ± 1.2</td>
<td>21.3 ± 2.3</td>
</tr>
<tr>
<td>Feed intake (g/kg W):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>9.6 ± 1.4</td>
<td>12.7 ± 1.0</td>
</tr>
<tr>
<td>Rice straw</td>
<td>3.3 ± 1.3</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>Total</td>
<td>12.9 ± 2.6</td>
<td>15.8 ± 2.2</td>
</tr>
<tr>
<td>Weight proportion of rice straw in the total (%):</td>
<td>25.8 ± 5.9</td>
<td>19.0 ± 8.1</td>
</tr>
<tr>
<td>TDN intake (g/(W^{0.75})):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk replacer</td>
<td>13.6**± 0.7</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>Solid feeds</td>
<td>22.7 ± 3.9</td>
<td>27.9 ± 3.1</td>
</tr>
<tr>
<td>Total</td>
<td>36.3 ± 4.1</td>
<td>32.9 ± 3.1</td>
</tr>
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</table>

\(^1\) Supplied with 300g milk replacer.
\(^2\) Supplied with 100g milk replacer.
*Significantly higher than the other group within the same experiment at P<0.05.
**Significantly higher than the other group within the same experiment at P<0.01.

as metabolic body size (\(W^{0.75}\)) of calves used, intakes of concentrate and rice straw per kg body weight, proportion by weight of rice straw in the total feed intake, and the TDN intake per \(W^{0.75}\) in each experiment.

The intake of concentrate tended to be higher in LMR than in HMR, and the tendency was more obvious in Expt 2. On the other hand, the intake of rice straw tended to be higher in HMR than in LMR, and also the tendency was more evident in Expt 2. The proportion of rice straw in the total feed intake tended to be higher in HMR than in LMR, and the difference between the two groups in Expt 2 was significant (P<0.05). The TDN intake from solid feeds tended to be higher in LMR, and the tendency was more marked in Expt 2 than in Expt 1. The lower supply of energy from milk replacer in LMR was more or less compensated by the increased intake of concentrate in both experiments, but the compensation was more sufficient in Expt 2.

Fig. 1 shows cumulative intakes of concentrate and rice straw during the experimental period as well as changes in the intake rate, which means the quantity of solid feeds (g) ingested per unit time (min).

In Expt 2, two third of the total intake of concentrate was consumed in the first 1h on account of the intake rate being high within the first 30 min in HMR or even 1h in LMR. In Expt 1, on the other hand, only a half of the total intake of concentrate was consumed in the first 1h, because calves in both groups ingested concentrate at a high intake rate only in the first 15 min, and in the next 15 min they eagerly ingested rice straw.

Fig. 2 shows the fluctuation of plasma metabolite and insulin concentrations. Plasma glucose and insulin began to increase in concentration immediately after the supply of milk replacer and solid feeds, and reached a higher value in HMR than in LMR in both experiments, but began to decrease again after a few
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Fig. 1. Cumulative intakes of concentrate and rice straw per kg body weight, and their intake rates within 8 h after the supply of 300 g milk replacer to the HMR group (○) or 100 g to the LMR group (●) with solid feeds. Vertical bars denote SD, and asterics represent means different significantly between groups (P < 0.05).

Concentrations of BHBA in plasma gradually increased within 4 or 6 h especially in LMR, resulting in a tendency to be higher in LMR than in HMR at least in the first 4 h. Plasma NEFA concentrations were relatively higher immediately before the beginning of each experiment, but decreased rapidly within 30 min (Expt 1) or 1 h (Expt 2) after the supply. The plasma NEFA concentration was lower in Expt 2 than in Expt 1, but began to increase again only in LMR in Expt 1. Concentrations of plasma urea-N were relatively higher immediately before and in a few hours after the beginning of each experiment, but began to decrease especially in Expt 2, although there was little difference between groups in each experiment.

Discussion

In common with each experiment, the intake of concentrate tended to be higher in LMR than in HMR. Also in our previous work\(^1\), the intake of concentrate during the suckling period of 5 weeks was significantly higher when calves received 300 g milk replacer/d than when received 600 g/d. Calves in LMR ingested more concentrate probably to compensate the reduced supply of energy from milk replacer, suggesting that the intake of concentrate would depend on the energy status of calves.

The compensation was more sufficient in Expt 2 than in Expt 1, and the difference may be attributed to different ambient temperatures.
between the experiments. The thermal environment in Expt 1 (20-24°C) was near or even above the upper limit of thermoneutral zone of calves (10-20°C)\(^4\). At an ambient temperature above the thermoneutral zone, it seems that the compensatory intake of concentrate would hardly occur to preserve the consistency of deep body temperature. In Expt 2, however, it is considered that calves consumed enough concentrate to preserve the homeostasis of energy balance and bodily function without disturbing the consistency of deep body temperature under the lower ambient temperature (12-13°C).

The rapid increase in plasma glucose and insulin concentrations would be attributed primarily to the supply of milk replacer into the abomasum through the esophageal groove, while the gradual increase in plasma BHBA concentration would be due to the fermentation of solid feeds directed into the rumen. An inverse relationship was observed between the plasma concentration of glucose or insulin and the intake of concentrate, suggesting that the intake regulation system like a mono-gastric animal\(^5,9,14\) might participate in the pre-weaning calf. However, the possibility of glucose or insulin being in itself a negative feedback signal seems doubtful, because decrease in glucose and insulin concentrations after 4 h did not affect the intake of concentrate in the present study, and additionally because the glucostatic theory has not been verified entirely even in mono-gastric animals\(^6\). The possibility of BHBA being itself a negative feedback signal to regulate the intake of concentrate would be deniable, because the concentration of plasma BHBA tended to be higher in LMR than in HMR in accordance with the intake of concentrate. Furthermore, the possibility of plasma NEFA concentration being a single positive feedback signal would also be neglected, because there was little difference in its concentration between groups in the first 1 h when calves ingested a lot of concentrate.

It is generally accepted that NEFA are apt to accumulate in the circulating blood owing to the mobilization of depot fat when animals are deficient in energy\(^13,16\). The rapid decrease in the plasma concentration of NEFA (Fig. 2) suggests that the supply of milk replacer temporarily improved the energy status of calves. The improvement lasted for a longer time in Expt 2 than in Expt 1, probably because more concentrate was consumed in Expt 2. Because the intake of concentrate was suppressed by a higher ambient temperature in Expt 1, energy status shifted to negative balance in a short while and the NEFA concentration began to increase again after 30 min in LMR which was supplied with only a limited amount of milk replacer. Additionally remarking, the higher level of NEFA in Expt 1 than in Expt 2 might be due to the chronic deficiency of energy caused by hot weather lasting for the preceding few days.

Energy status above stated was reflected also on the fluctuation of plasma urea-N concentrations. Protein is utilized as an energy source when animals are lacking energy, resulting in the rise of urea-N in the circulating blood. Changes in the concentration of plasma urea-N shown in Fig. 2 suggest that negative energy balance lasted for a longer period in Expt 1 than in Expt 2. Because the concentration of plasma urea-N varies also with the quantity and the quality of protein supplied, the difference between groups seems not so obvious as the case of NEFA.

Consequently, it is considered that the intake of concentrate depended on the energy status of calves. Probably this suggests that energostasis or homeostatic regulation of energy balance\(^2,3,6,12\) would well apply to concentrate as a short-term intake regulation system. Of the manifold functions which are assumed to be monitored by brain in this regulation system\(^6\), it was assumed that preserving the consistency of deep body temperature might have a priority over other functions, although
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Further studies will be needed to verify this hypothesis. In addition, the intake of rice straw seemed to be affected by the intake of concentrate in the present study, suggesting that the system regulating its intake might differ from that regulating the intake of concentrate. Further studies will be needed also for this matter.

References

代用乳給与量の異なる離乳前子牛における
採食量および血液代謝像

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5月に実施した実験1（舎内温度 20-24℃）と11月実施の実験2（同 12-13℃）のそれぞれにおいて、代用乳給与量が 600 g/日のホルスタイン種離乳前子牛4頭（HMR 区）と 200 g/日の同種離乳前子牛4頭（LMR 区）との間で濃厚飼料と稲穂の採食パターン, および血中代謝産物とインスリン濃度の変動パターンを比較した。いずれの場合も、朝 300 または 100 g の代用乳と固形飼料を給与してから 8 時間以内においては、濃厚飼料摂取量は HMR 区より LMR 区の方が多く、稲穂摂取量には逆の傾向が見られたが、これらの傾向は実験1より実験2の方がより顕著であった。LMR 区における代用乳からのエネルギー供給の低さは濃厚飼料摂取量の増加により多少とも代償されたが、その代償の程度も実験2の方が完全であった。血漿グルコースおよびインスリン濃度は代用乳給与直後から増加しはじめ、両実験において HMR 区の方が LMR 区より高い値に達したが、数時間後からふたび減少しはじめた。血漿 β-ヒドロキシ酪酸濃度は両実験とも特に LMR 区において徐々に増加する傾向を示した。血漿 NEFA 濃度は両実験とも開始直後に減少し、実験2ではその後低濃度を維持したが、実験1ではその濃度がLMR 区においてのみ 30 分後から増加しはじめた。血漿炭素態 N 濃度は、最初の数時間は比較的高く、その後は特に実験2において目立って減少したが、両実験とも区間にほとんど差がなかった。

以上の結果、離乳前子牛における濃厚飼料摂取量の短期調節にはエネルギー恒常性機構が関与していることが示唆された。

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