PLASMA ALANTOIN CONCENTRATION IN RESPONSE TO CHANGES IN NUTRITIONAL STATUS OF CALVES

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Summary

Two experiments were conducted to search factor(s) affecting the plasma allantoin concentration in infant calves. In experiment 1, five male Holstein calves aged 1 week were given only milk replacer free from nucleic acids for 28 days following the regime of 500 g/d for the first 18 days, 250 g/d for the next 5 days, and 750 g/d for the last 5 days. Plasma allantoin concentration varied in a reverse proportion to daily amounts of milk replacer, and the concentration when calves received 750 g/d of milk replacer was significantly lower than that when they received 250 g/d. Contrary to plasma allantoin concentration, glomerular filtration rate (GFR) was directly proportional to daily amounts of milk replacer, leading to a constant filtration of allantoin across the glomeruli. Renal handling of allantoin was also unaffected by the amount of milk replacer, resulting in the constant urinary excretion of allantoin. These results suggested that GFR, which was affected by the nutritional status, could affect plasma allantoin concentration. In experiment 2, the effect of age-related changes in nutritional status after weaning on GFR was examined in eight calves weaned at 5 weeks of age. The GFR expressed as body weight basis was lower immediately after weaning, but linearly increased up to the 19th week post-weaning. The present results suggested that the changes in GFR in response to nutritional status would be one of the possible causes of atypical plasma allantoin concentration immediately after weaning. We conclude that plasma allantoin concentration would not be a proper estimator of intestinal flow of microbial protein in cattle.

(Key Words: Plasma Allantoin Concentration, Daily Amount of Milk Replacer, Glomerular Filtration Rate, Intestinal Flow of Microbial Protein, Calf)

Introduction

Nucleic acids in feed and salivary allantoin are completely decomposed by ruminal microbes, and, therefore, purines flowing to the small intestine are essentially the origin of ruminal microbes (McAllan and Smith, 1973; Chen et al., 1990a). Purines absorbed from the small intestine are rapidly degraded to purine derivatives (PD), hypoxanthine, xanthine, uric acid and allantoic, and quantitatively excreted into urine. In fact, the excretion of total PD or allantoic, the final metabolite of purine bases in most mammals except for primates, was correlated with the amount of nucleic acids infused postruminally to sheep and steers (Antoniewicz et al., 1980; Lindberg, 1985; Fujihara et al., 1987; Lindberg et al., 1989; Verbie et al., 1990). These results support the idea that urinary excretion of PD or allantoic by ruminants is a valid estimator of intestinal flow of microbial protein (Topps and Elliot, 1965; Matsumoto et al., 1990; Abe et al., 1993).

The estimation of microbial protein production from urinary PD excretion, however, requires a total collection of urine for several days, and would not be practical under farm conditions. Chen et al. (1992b) demonstrated that plasma allantoic concentration would be also applicable as a simple index of microbial protein supply in steers, because allantoic was rapidly removed from the blood, and because it was correlated with daily excretion of PD (Chen et al., 1991, 1992b). Since allantoic is not reutilized for the synthesis of purine bases (Chen et al., 1990b), the urinary excretion of allantoic will be proportional to its plasma concentration, if the clearance and filtration rate of allantoic are constant.

However, our previous study using calves indicated that plasma allantoic concentration was not so sensitive an index of the intestinal flow of microbial protein as urinary allantoic excretion (Iriki et al., 1994). Furthermore, age-related changes in plasma allantoic concentration in early-
weaned calves revealed a linear decrease with the peak immediately after weaning, in contrast to a steady increase in the urinary excretion of allantoin with advancing age (Funaba et al., 1995). This suggests that some factors other than the underdevelopment of the rumen at weaning (Leibholz, 1975; Quigley et al., 1985, 1991) would be concerned with the invalidity of plasma allantoin concentration as the index of intestinal flow of microbial protein. The renal excretion is not the sole route for eliminating allantoin from the blood. A part of allantoin will be excreted into the rumen via saliva and subsequently destructed by the microbes (Chen et al., 1990a). Therefore, either the altered allantoin clearance from the plasma or the changes in elimination route of allantoin, or both might be involved in our previous results.

The present study was conducted to examine the effect of the nutritional status of calves on plasma allantoin concentration and renal allantoin clearance. The changes in nutritional status induced by varying the amount of milk replacer during the suckling period will be effective to examine the renal handling of plasma allantoin, with little influence on the salivary flow and the consequent salivary allantoin secretion. Only a part of data has been already shown in the report by Kagiymama et al. (1995).

Materials and Methods

Experiment 1

Five male Holstein calves aged about 1 week were used. They were given only nucleic acid-free milk replacer for 28 days without any solid feed. They were given 500 g/d of the milk replacer for the first 18 days, and 250 g/d for the next 5 days, and 750 g/d for the final 5 days. The milk replacer free from nucleic acids contained 67.0% skim milk powder, 4.0% dried whey, 8.0% lactose, 0.20% glucose, 18.4% tallow, 0.55% vitamins and minerals mixture, 1.6% emulsifier, and 0.25% Cr₂O₃. It contained 91.4% dry matter and provided 217 and 1,100 g/kg of crude protein and total digestible nutrients (TDN), respectively. The milk replacer was suspended in 3.61 warm water irrespective of the daily quantities, and nipple-fed three times a day (08:30, 12:30, 16:30 h) in three equal portions. A 24-hour urine of each calf was collected into a bottle added 100 ml of 20% (vol/vol) sulfuric acid on the last day of each dietary period, and blood plasma was collected by jugular vein puncture at 08:00 and 16:00 h. Urinary allantoin was determined by the method of Young and Conway (1942). Plasma concentration of allantoin was analyzed after deproteinization and defatted by the same procedure. Creatinine in urine and plasma was measured by the method of Peters (1942). Our previous studies indicated that urinary excretion of allantoin did not largely vary between days (Kagiymama et al., 1994, 1995), and a preliminary study showed that plasma creatinine and allantoin concentrations were quite constant within a day, substantially in agreement with the results of Chen et al. (1992b). Therefore, glomerular filtration rate (GFR) was estimated as creatinine clearance using the average concentrations in plasma. The filtered allantoin across the glomeruli was calculated by the multiplication of GFR and the average concentration of plasma allantoin. Apparent reabsorption of allantoin from the renal tubule was estimated as the difference between the filtration and the urinary excretion.

Experiment 2

As the results of experiment 1 suggested a significant relationship between GFR and nutritional status, age-related changes in GFR after weaning were examined using eight male Holstein calves weaned at 5 weeks of age. This experiment lasted for 19 weeks after weaning. During the suckling period, calves were given 600 g/d of commercial milk replacer, and offered commercial calf starter and rice straw freely. After weaning, commercial concentrate and rice straw were available on an ad libitum basis. Commercial milk replacer consisted of 74% milk products (skim milk, dried whey, and casein), and 26% animal fat, glucose, yeast, NaCl, and CaCO₃. It contained 93.5% dry matter and provided 267 and 1,050 g/kg of crude protein and TDN, respectively. Commercial calf starter consisted of 58% grains (corn, milo, and dextrin), 27% oil meal (soybean meal), 7% bran and by-product feed (wheat bran and corn gluten feed), 1% fish meal, and 7% glucose, animal fat, CaCO₃, and NaCl. It contained 86.5% dry matter and provided 187 and 780 g/kg of crude protein and TDN, respectively. Commercial concentrate consisted of 58% grains (corn, milo, and wheat), 26% bran and by-product feed (corn gluten feed and rice bran), 7% oil meals (rapeseed meal and safflower meal) and 9% molasses, CaCO₃, and NaCl. It contained 87.4% dry matter and provided 155 and 720 g/kg of crude protein and TDN, respectively. Furthermore, vitamins (A and D) were supplemented to these feeds. Rice straw contained 90.6% dry matter and provided 65 and 380 g/kg of crude protein and TDN, respectively. Crude protein contents in milk replacer, calf starter, and concentrate met or exceeded the nutrient requirements for growing cattle (NRC 1984).

Urine was collected for 5 to 7 days on the 1st, 6th, 11th, and 19th week after weaning. Blood was obtained on the last day of each sampling period at 08:00 and 12:00 h. Glomerular-filtration rate was estimated as
creatinine clearance mentioned above.

**Statistical analysis**

Data were analyzed by analysis of variance using the GLM procedures of SAS (1985). The model included sampling period (500, 250, or 750 g/d of milk replacer for experiment 1, and weeks after weaning for experiment 2), and animal. Orthogonal polynomial contrasts were used to detect linear and quadratic effects of time for experiment 1, and linear, quadratic and cubic effects of weeks after weaning for experiment 2. Furthermore, the difference from the period of 500 g/d milk replacer in experiment 1 was examined by orthogonal contrasts. The effects and differences were considered significant at $p < 0.10$.

**Results and Discussion**

The amount of milk replacer caused a small but significant changes in daily gain, i.e., 0.24 kg/d for 500 g/d, $-0.30$ kg/d for 250 g/d, and 0.46 kg/d for 750 g/d, indicating that the reduction of milk replacer led to the state of nutritional restriction. As shown in fig. 1, plasma allantoin concentration exhibited the reverse change against the amount of milk replacer. A 50% reduction of milk replacer given to the calves increased the plasma allantoin level, although the difference at 16:00 h was not significant. On the contrary, the increase in milk replacer from 250 g/d to 750 g/d resulted in a clear decrease in plasma allantoin concentration, and the concentration was significantly lower than in the calves that received 500 g/d milk replacer at both sampling times. Because the calves were fed only milk replacer and not given any solid feed, it is considered that the changes in plasma allantoin concentration would reflect neither those in intestinal flow of purines nor those in salivary allantoin secretion caused by altered salivary flow. In addition, a trace of PD possibly contained in skim milk, a major constituent of milk replacer, could not be considered to have a significant effect on the changes in plasma allantoin concentration. Basically, allantoin concentration in the blood of ruminants indicates the balance between the absorption of purines and the subsequent catabolism, and the removal from the blood to urine and saliva. Therefore, the changes in plasma allantoin concentrations in response to the amounts of milk replacer might imply the altered clearance in the kidney of suckling calves.

![Figure 1](image)

**Figure 1.** (Experiment 1) Plasma allantoin concentration in response to the daily amounts of milk replacer. Data are the means of five calves $\pm$ SEM. Plasma allantoin concentrations indicated linear and quadratic effects with advancing age and increased with advancing age, i.e., linear and quadratic effects were $P = 0.06$ and $P = 0.03$ at 08:00 h, and $P = 0.007$ and $P = 0.05$, respectively, at 16:00 h. Orthogonal contrasts revealed that the periods of 250 g/d ($P = 0.08$) and 750 g/d ($P = 0.06$) at 08:00 h and the period of 750 g/d ($P = 0.007$) at 16:00 h were significantly different from the period of 500 g/d of milk replacer at the same sampling time.

Table 1 shows GFR and the renal clearance of allantoin in the suckling calves. The GFR indicated the quadratic changes in response to the amount of milk replacer. When milk replacer given to the calves was changed from 500 g/d to 250 g/d and from 250 g/d to 750 g/d, GFR exhibited a 16% decrease and a 32% increase, respectively. The GFR in calves fed 750 g/d of milk replacer tended to be higher (11%) than that in calves fed 500 g/d of milk replacer, though not significant. Even if GFR was expressed as body weight basis, the tendency was not changed because of only a slight weight gain during the suckling period (data not shown). The changes in GFR were the reverse to those in plasma allantoin concentration, and, in fact, these two negatively related as shown in fig. 2. As a result, the daily amount of allantoin filtered across the glomeruli was almost constant (table 1), irrespective of the amount of milk replacer. These data suggest that the changes in plasma allantoin concentration, would relate to the alteration of GFR caused by nutritional states. It has been well documented that GFR was increased by the higher protein intake in dogs, cats, and sheep (Massry and Kleeman, 1972; Funaba et al., 1991; Hashimoto et al., 1995).
Figure 2. (Experiment 1) Relationships between glomerular filtration rate (GFR) and plasma allantoin concentration at 08:00 h (left) and 16:00 h (right).

TABLE 1. GLOMERULAR FILTRATION RATE (GFR) AND RENAL HANDLING OF ALLANTOIN IN RESPONSE TO THE AMOUNT OF MILK REPLACER FED TO THE SUCKLING CALVES (EXPERIMENT 1)

<table>
<thead>
<tr>
<th>Milk replacer (g/d)</th>
<th>SEM</th>
<th>Orthogonal contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFR (l/d)</td>
<td>Filtered allantoin (mmol/d)</td>
</tr>
<tr>
<td>500</td>
<td>69.7</td>
<td>12.00</td>
</tr>
<tr>
<td>250</td>
<td>58.3*</td>
<td>10.64</td>
</tr>
<tr>
<td>750</td>
<td>77.2</td>
<td>10.58</td>
</tr>
</tbody>
</table>

L : Linear effect.
Q : Quadratic effect.
* : Significantly different from the period of 500 g/d \( (p = 0.08) \).

Apparent reabsorption of allantoin from the renal tubule was not significantly affected by the changes in nutritional status. It was near zero or rather negative, indicating that the net secretion of allantoin from the renal tubule occurred in calves. Greger et al. (1976) suggested that there was neither reabsorption nor secretion of allantoin along the renal tubule in rats. In contrast, Chen et al. (1991) reported that in sheep allantoin in glomerular filtrate was reabsorbed, although the capacity was limited and saturated by a tubular load equivalent to endogenous allantoin production. The reasons of these discrepant suggestions are not clear, but one could infer that the differences among species in renal handling of allantoin might depend on the activity of xanthine oxidase, an enzyme responsible for the oxidation of xanthine to uric acid. The xanthine oxidase activity was not detected in the serum of sheep, but was higher in cattle, and further the activity was two thirds of cattle in rats (Al-Khalidi and Chaglassian, 1965; Chen et al., 1990b). It has been reported that xanthine oxidase activity is an important determinant of the ratio of allantoin to total PD in urine (Chen et al., 1990b). If allantoin is a predominant PD, allantoin secretion from the renal tubule might not be neglectable, which would lead to the net secretion of allantoin.

Because both filtered allantoin and the apparent reabsorption were relatively constant, urinary allantoin excretion was also unaffected by the nutritional status (table 1), in good agreement with the results by Fujihara et al. (1987) using steers. In contrast, Chen et al. (1992a) observed that urinary excretion of allantoin was varied in sheep in response to the amount of protein supply. These results might imply the difference between species in respect of the relationship between urinary allantoin excretion and nutritional status, which might also relate to the difference of xanthine oxidase activity.
Many studies have revealed the underdevelopment of the rumen immediately after weaning from not only the qualitative aspect but also the quantitative aspect (Leibholz, 1975; Qingley et al., 1985, 1991; Funaba et al., 1994). However, our previous study showed that plasma allantoin concentration was unexpectedly higher immediately after weaning, and decreased with advancing age suggesting that an unknown factor other than intestinal flow of microbial purines would participate in the atypical changes in the plasma allantoin concentration (Funaba et al., 1995). Because the results of experiment 1 indicated the negative relationship between plasma allantoin concentration and the GFR, which was concerned with nutritional status of calves. Therefore, the following hypothesis was supposed, i.e., the transient nutrient restriction induced by the underdevelopment of the rumen immediately after weaning would cause the decrease in GFR, leading to the increase in plasma allantoin concentration.

Therefore, the changes in GFR with age after weaning were examined in experiment 2. As shown in table 2, GFR expressed as body weight basis was lower immediately after weaning and linearly increased with age up to week 19 post-weaning. The deleterious nutrition of calves might result in the lower GFR immediately after weaning, leading to the decrease in clearance of blood allantoin and the subsequent higher plasma allantoin concentration. Alternatively, age-related maturation of the kidney should be also concerned with it. In rats and guinea pigs, it has been reported that the increase in GFR with age is caused not only by postnatal nephrogenesis but also by the increase in glomerular pressure and glomerular capillary surface area (Spitzer and Brandis, 1974; Aperia and Herin, 1975; Soloman, 1977). Anyhow, present data suggest that plasma allantoin concentration is not solely determined by intestinal flow of microbial purines but in part by GFR, which is dependant of nutritional status. Consequently, plasma allantoin concentration does not seem to be a proper estimator of intestinal flow of microbial protein in cattle.

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