Effects of 5'-uridylic acid feeding on postprandial plasma concentrations of GH, insulin and metabolites in young calves

K Katoh, K Yoshioka, H Hayashi, T Mashiko¹, M Yoshida, Y Kobayashi and Y Obara

Department of Animal Physiology, Graduate School of Agricultural Science, Tohoku University, Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan

¹Feed Functionality Research Laboratory, Meiji Feed Co., Kashima, Ibaraki 314-0103, Japan

(Requests for offprints should be addressed to K Katoh; Email:kato@bios.tohoku.ac.jp)

Abstract

Postprandial changes in plasma concentrations of GH, insulin, IGF-I, leptin and metabolites were compared between young Holstein bull calves fed with milk alone (control group) and with milk+5'-uridylic acid (UMP) (UMP group). UMP (2 g/day) was given with milk at 0830 h and 1530 h for 11 days from the 4th to the 14th day after birth. The perirenal fat weight was significantly lower in the UMP group than in the control group, but there was no significant difference in the weights of the liver, spleen and heart between the groups. Basal GH concentrations in the UMP group were slightly higher, but the postprandial increase in plasma insulin level and the area under the curve for insulin in the UMP group were significantly lower than those in the control group.

There was no significant difference in IGF-I levels between the groups. In addition, the postprandial glucose concentrations were lower in the UMP group as reflected by the insulin level, and nonesterified fatty acid concentrations were not different. In the muscle (*M. longissimus thoracis*) sampled at 14 days of age, the triacylglycerol (TAG) content was significantly greater but glycogen content was significantly lower in the UMP group than in the control group. From these results, we have concluded that feeding 5'-UMP at 2 g/day for 11 days significantly alters TAG accumulation in the body and plasma concentrations of GH and insulin in young bull calves.

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Introduction

After birth, young calves take milk for several weeks until weaning. During the first days of life, colostrum is believed to be essential for the healthy rearing of animals, because it contains such factors as immunoglobulins, hormones, growth factors and nucleic acids as well as all of the required nutrients (Gil 1981, Oda *et al.* 1989).

The biological roles of the active peptides in colostrum are still debated, however. In a previous study with neonatal calves, colostrum feeding (but not colostrum insulin-like growth factor (IGF-I)) increased plasma IGF-I levels by six times, but it did not otherwise affect the somatotrophic axis (Hammon & Blum 1997, 2002). It has been reported that IGF feeding increased the jejunal transporter expression and serum level of glucose in immature rats (Lane *et al.* 2002).

The composition and concentration of colostrum nucleic acids depends on the species of animal. For example, ruminant colostrum in particular contains a large amount of uridine and its derivatives such as uridine monophosphate (5'-uridylic acid; UMP) and uridine diphosphate. Concentrations of up to 1000, 2000 and

9000 µmol/l have been found in the colostrum of cattle, goats and sheep respectively (Gil 1981). These concentrations rapidly decline to the level of normal milk within weeks after parturition. Human colostrum contains several nucleotides (cytidine monophosphate, UMP, guanosine monophosphate and adenosine monophosphate), but at relatively low levels of between 1.0 and 23.0 µmol/l (Duchen & Thorell 1999). The biological actions of dietary nucleotides have been reported for the digestive system (Nunez *et al.* 1990, Uauy *et al.* 1990, Brunser *et al.* 1994, Bueno *et al.* 1994, Ortega *et al.* 1994), the immune system (Van Buren *et al.* 1983, 1985, 1994, Rudolph *et al.* 1986, Kulkarni *et al.* 1989, Carver *et al.* 1990, Matsumoto *et al.* 1995, Nagafuchi *et al.* 1997) and the microbial flora in the gut (Gil *et al.* 1986). However, to our knowledge, the effect of nucleotides on endocrine and metabolic levels has not yet been reported in the ruminant.

Our objective in this study was therefore to assess the effects of feeding UMP, the dominant nucleotide in bovine colostrum (Gil 1981), on endocrine and metabolic traits in young calves. The findings clearly demonstrated that UMP feeding significantly affects the endocrine system and metabolite levels.

Materials and Methods

Animals and milk feeding

Newborn Holstein bull calves were treated according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan), and the present experiment was approved by The Animal Care Committee of Tohoku University.

Twelve calves were fed colostrum, of which the mean UMP concentration was 60 µmol/l, from their dams once after birth. They were then fed artificial colostrum (Meiji Feed Co., Tokyo, Japan), which was pooled from the colostrum from several dams and supplemented with several vitamins, for 3 days before starting the experimental feeding. The artificial colostrum contained 57.4% crude protein (15.5% IgG), 4.2% crude fat and 10 µmol/l UMP. At 4 days of age, they were divided into two groups: the 5'-UMP-fed ($n=6$) and the control (no 5'-UMP; $n=6$) groups. The control animals were fed only milk replacer for 11 days between 4 and 14 days of age. The 5'-UMP concentration of the milk replacer was less than 1 µmol/l. The 5'-UMP-fed (UMP) group was fed milk replacer diet containing 5'-UMP derived from yeast (Meiji Feed Co.; 0.5 g/l milk) between 4 and 14 days of age. Milk replacer (250 g) diluted to 2 liters was given to each animal in the morning (0830 h) and afternoon (1530 h). The total 5'-UMP given per day per calf was 2 g. In a preliminary (K Kato, K Yoshioka and Y Obara, unpublished observations) study in which calves were given 1 g/day 5'-UMP, no significant effects on the animals' metabolism or organ weights were demonstrated. We therefore employed the dosage of 2 g/day in the present study.

The milk replacer contained skim milk, whey protein, casein, corn products, minerals and vitamins (24% crude protein, 20% crude fat, 10% mineral and non-detectable concentrations of UMP) (Meiji Feed Co.), and was given according to the manufacturer's instructions as described in our previous paper (Kitade *et al.* 2002).

Experimental design

At 13 days of age, on the day prior to the blood sampling, calves were fitted with a catheter (type V-1; Top, Tokyo, Japan) in the right jugular vein under local anesthesia. The catheter was filled with sterile iso-osmotic sodium citrate (3.8%; w/v) solution before and during the sampling. The blood samples (6 ml each) were taken on the following day every 15 min from 30 min before until 270 min after the morning feed (0800–1300 h). During the blood sampling, calves were fed a milk replacer (2 liters with or without 5'-UMP) at 0830 h. Blood samples were centrifuged at 8000 g for 15 min, and blood plasma was divided into five portions and stored at -30 °C until analyzed for hormones and metabolites.

Sampling and weighing of the organs

The animals were slaughtered at 15–21 days of age after being maintained as described above, and the organ weights of liver, spleen and heart were measured. The perirenal fat was weighed after being removed with the kidney from the body cavity and separated from the kidney. A part of the longissimus thoracic muscle at the 6th–7th rib was sampled for the measurement of the triacylglyceride (TAG) and glycogen concentrations.

Analyses

Plasma concentrations of growth hormone (GH), insulin and IGF-I were measured by RIA as described previously (Kuhara *et al.* 1991). The glucose, nonesterified fatty acid (NEFA) and TAG concentrations were determined using commercial kits (Glucose CII-Test, NEFA C-Test and Triglyceride E-Test respectively; Wako Pure Chemicals, Osaka, Japan). Glycogen as well as TAG concentrations were determined after homogenization of the tissues. Glycogen and protein concentrations were determined by methods described previously (Lowry & Passonneau 1972, Hespel & Richter 1990).

Statistics

The data are expressed as means $\pm$ S.E.M. For GH and insulin, the area under the curve (AUC) and incremental area (IA) (area over the basal release before feeding) were calculated and expressed as ng/min per ml and µU/min per ml respectively.

The significance of the difference between the treatments was determined by Student's *t*-test (Zar 1984).

Results

All the animals of both groups were healthy, and there was no significant difference in the body weight (47.9 ± 1.5 and 46.8 ± 1.3 kg for the control and UMP group respectively, $P=0.557$).

Effects on the organ weights

The weights of the organs are shown in Table 1. The weight of perirenal fat was significantly lower in the UMP group than in the control group ($P<0.05$), although there was no significant difference between the two groups in the weights of the liver, spleen and heart.

Effects on plasma GH and insulin concentrations

Basal plasma GH levels in the UMP group were about 50% higher than those of the control group (10.1 ± 3.0 vs 6.7 ± 1.7 ng/ml at time 0 min for the UMP and the

Table 1 Weights (g) of the organs and perirenal fat in the control ($n=6$) and UMP-fed ($n=6$) group. Values are means $\pm$ S.E.M. Values in parentheses are organ weight (g)/body weight (kg)

	Control	UMP fed	P
Liver	859 $\pm$ 35 (17.3 $\pm$ 0.9)	894 $\pm$ 41 (19.1 $\pm$ 2.2)	0.535 0.127
Spleen	110 $\pm$ 11	110 $\pm$ 10	0.974
Heart	333 $\pm$ 13	325 $\pm$ 16	0.718
Perirenal fat	142 $\pm$ 10	94 $\pm$ 16	0.028

control group respectively; Fig. 1, upper panel). The peak level at 45 min after milk feeding was similar in the two groups. However, there was no significant difference in the AUC for GH ($P=0.275$; Fig. 1, lower panel). In addition, the IA for GH was not different between the two groups ($P=0.523$; not shown).

Basal plasma insulin levels were not significantly different between the two groups, but the insulin levels after the morning feeding had a tendency to be lower in the UMP group than in the control group (peak value 4.9 ± 1.1 vs 2.0 ± 0.3 μ U/ml at 90 min after feeding for the control and the UMP groups respectively, $P=0.037$; Fig. 2, upper panel). The AUC for insulin in the UMP group was significantly lower than that for the control group ($P=0.048$; Fig. 2, lower panel). There was also a significant difference in IA between the two groups ($P=0.012$; not shown).

Effects on plasma IGF-I levels

There was no significant difference in plasma IGF-I levels between the two groups just before and after the morning feeding (Fig. 3).

Effects on plasma glucose and NEFA levels

Although the basal glucose levels were not significantly different between the two groups, the postprandial glucose level in the UMP group tended to be lower ($P<0.10$) than that of the control group (Fig. 4, upper panel). This is reflected in the plasma levels of insulin (Fig. 2). However, NEFA concentrations were not significantly different between the two groups (Fig. 4, lower panel).

Effects on intramuscular TAG and glycogen levels

The intramuscular TAG level, when expressed as mg/mg soluble protein, was significantly higher in the UMP group than in the control group ($P=0.007$; Table 2). The intramuscular glycogen level in the UMP group was significantly lower than that in the control group ($P=0.002$; Table 2). In the liver, however, no significant difference was demonstrated in TAG (0.6015 vs 0.6278 mg/mg protein for the UMP and the control

Figure 1 Postprandial changes in (upper panel) plasma GH concentrations and (lower panel) GH AUC in 14-day-old calves ($n=6$ for each group). In the upper panel, animals were given a milk replacer diet alone (Control) or a milk replacer diet containing 1 g 5'-UMP (UMP) at time 0. The values are means $\pm$ S.E.M. * $P<0.05$ (control vs UMP).

group respectively, $P=0.825$) and glycogen (0.2240 vs 0.1750 mg/mg protein for the UMP and the control group respectively, $P=0.468$) levels (not shown).

Discussion

The present study has, for the first time, demonstrated that dietary 5'-UMP, when supplied at 2 g/day with milk to young calves for 11 days, significantly alters perirenal fat

Figure 2 Postprandial changes in (upper panel) plasma insulin concentrations and (lower panel) insulin AUC in 14-day-old calves ($n=6$ for each group). In the upper panel, animals were given a milk replacer diet alone (Control) or a milk replacer diet containing 1 g 5'-UMP (UMP) at time 0. The values are means $\pm$ S.E.M. * $P<0.05$ (control vs UMP).

weight, and the endocrine and metabolic status. In our preliminary study in which 5'-UMP was given at 1 g/day for 11 days, no significant effects were seen in the endocrine and metabolic traits and in the organ weights. Therefore, in young Holstein bull calves, 2 g/day 5'-UMP should be fed in order to cause fat mobilization and hormonal changes. The dosage is about 1.6 times greater compared with the calculated amount of 5'-UMP in 4 liters of milk at the reported concentration for bovine colostrum (1000 $\mu\text{mol/l}$) (Gil 1981).

In the calf intestine, as well as in the rodent, pyrimidine nucleosides are transported by an N2-type transporter,

Figure 3 Postprandial changes in plasma IGF-I concentrations in 14-day-old calves ($n=6$ for each group). Animals were given a milk replacer diet alone (Control) or a milk replacer diet containing 1 g 5'-UMP (UMP) just after 0 min. The values are means $\pm$ S.E.M.

whereas purine nucleosides are transported by a N1-type transporter, in a Na^+ -dependent manner (Balcells *et al.* 1992, Theisinger *et al.* 2002). Interestingly, although the reason is not known, nucleoside transport into brush border membrane vesicles of the proximal intestine is inhibited by glucose (Theisinger *et al.* 2002).

It was also demonstrated that 5'-UMP feeding significantly reduced the insulin level (Fig. 2), which coincided with the reduction in the postprandial glucose level (Fig. 4). Although the detailed mechanism remains to be clarified, the reduced levels of plasma glucose and enhanced TAG (reduced glycogen) in the longissimus muscle may indicate an enhanced insulin-sensitive glucose uptake by the skeletal muscles. This is because the major mechanism of insulin-sensitive glucose uptake by glucose transporter-4 (GLUT-4) (Baron *et al.* 1988, Rose *et al.* 1997) negatively correlates with muscle glycogen content in the rat (Derave *et al.* 2000). A possible explanation, therefore, is that the reduced glycogen content in the skeletal muscles caused by 5'-UMP feeding enhanced insulin-dependent glucose uptake via increased GLUT-4 activity in young calves. This assumption, however, requires another assumption that increased glucose uptake resulted in TAG, but not glycogen, accumulation in the skeletal muscle. Alternatively, it has been shown that nucleotides enhance gut growth and maturation (Uauy *et al.* 1990), possibly causing the reduced post-absorptive glucose levels. However, the possibility of reduced glucose uptake in the small intestine of the 5'-UMP-fed calves may be small, because there was no difference in the

Figure 4 Postprandial changes in (upper panel) plasma glucose and (lower panel) NEFA concentrations in 14-day-old calves ($n=6$ for each group). Animals were given a milk replacer diet alone (Control) or a milk replacer diet containing 1 g 5'-UMP (UMP) at time 0. The values are means $\pm$ S.E.M. * $P<0.05$ (control vs UMP).

ileal glucose transporter (sodium-dependent glucose transporter-1) expression between the two groups in our preliminary experiment (K. Katoh, K Yoshioka and

Y Obara, unpublished observations). In addition, urea N level in the 5'-UMP-fed calves was slightly reduced (K Katoh, K Yoshioka and Y Obara, unpublished observations), suggesting that protein metabolism may not be enhanced in the UMP group.

Feeding 5'-UMP caused the redistribution of fat accumulation in calves: reduced perirenal fat weight (Table 1) and enhanced TAG content in the longissimus muscle (Table 2). We do not know the precise reason for the heterogeneous fat distribution at present, but the enhanced GH and reduced insulin level may be involved in the mechanism. This is because the action of GH is catabolic and that of insulin is anabolic, and fat redistribution is determined by an interaction of these hormones.

Interestingly, however, it has also been demonstrated in the present study that the levels of plasma GH and insulin were simultaneously increased after milk feeding. In our recent study (Katoh *et al.* 2004), we also reported that postprandial GH responses were reduced by aging even though calves were maintained on milk until 12 weeks of age. It is usual that feeding reduces plasma GH levels in calves (Moseley *et al.* 1988) and sheep (Bassett 1974a,b, Driver & Forbes 1981, Trenkle 1989, Thomas *et al.* 1991, Matsunaga *et al.* 1998, 1999) except that some reports suggest a postprandial increase in lambs (Driver & Forbes 1981) and steers (Moseley *et al.* 1988). Feeding-induced reduction in plasma GH levels is more apparent in adult animals under restricted feeding or at low feeding frequency during the day because these conditions are known to raise basal GH levels and pulse amplitude (Thomas *et al.* 1990, 1991) and to reduce somatostatin release from the hypothalamus (Thomas *et al.* 1991, Henry *et al.* 2001). The mechanism underlying the difference in the somatotrophic axis between pre- and post-weaning animals remains to be clarified. We have postulated a physiological role of short-chain fatty acids produced by microbes in the forestomachs, and have demonstrated their involvement in the inhibitory mechanism for GH levels *in vivo* and *in vitro* (Matsunaga *et al.* 1998, 1999, Katoh *et al.* 1999, Ishiwata *et al.* 2000, Katoh & Obara 2001).

In summary, we found that feeding 5'-UMP at 2 g/day to young bull calves for 11 days significantly reduced perirenal fat accumulation, but increased intramuscular fat content. Coincident with the change in fat accumulation, the feeding also enhanced GH but reduced insulin levels.

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Table 2 TAG and glycogen concentrations in the longissimus muscle (mg/mg protein). Values are means $\pm$ S.E.M.; $n=6$ per group

	Control	UMP fed	P
TAG	0.563 $\pm$ 0.066	0.959 $\pm$ 0.097	0.007
Glycogen	0.949 $\pm$ 0.052	0.622 $\pm$ 0.056	0.002

and IGF-I provided by NIDDK. We also acknowledge K Sakai and T Hakamazuka for their help with the animal feeding. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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