Effect of Sugar Supplementation on Rumen Protozoa Profile and Papillae Development in Retarded Growth Calves

Tomohiro SATO1,2, Kyosuke HIDAKA3, Takakibi MISHIMA4, Kazumi NIBE3, Go KITAHARA3, Yuichi HIDAKA6, Hiromu KATAMOTO4 and Shunichi KAMIMURA3

1) Department of Clinical Veterinary Science, United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi 785–8515,
2) Takaharu Veterinary Clinic, Takaharu, Nishimorokata, Miyazaki 889–4412, 3) Laboratories of Theriogenology, 4) Veterinary Internal Medicine and 5) Veterinary Surgery and 6) Division of the Project for Zoonosis Education and Research, University of Miyazaki, Miyazaki 889–2192, Japan

(Received 7 September 2009/Accepted 27 May 2010/Published online in J-STAGE 10 June 2010)

NOTE Internal Medicine

Effect of Sugar Supplementation on Rumen Protozoa Profile and Papillae Development in Retarded Growth Calves

Tomohiro SATO1,2, Kyosuke HIDAKA3, Takakibi MISHIMA4, Kazumi NIBE3, Go KITAHARA3, Yuichi HIDAKA6, Hiromu KATAMOTO4 and Shunichi KAMIMURA3*

1) Department of Clinical Veterinary Science, United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi 785–8515, 2) Takaharu Veterinary Clinic, Takaharu, Nishimorokata, Miyazaki 889–4412, 3) Laboratories of Theriogenology, 4) Veterinary Internal Medicine and 5) Veterinary Surgery and 6) Division of the Project for Zoonosis Education and Research, University of Miyazaki, Miyazaki 889–2192, Japan

(Received 7 September 2009/Accepted 27 May 2010/Published online in J-STAGE 10 June 2010)

ABSTRACT. The effect of sugar supplementation with 1 g/kg BW twice a week for eight weeks on rumen protozoa was determined in ten retarded growth calves. Rumen juice was sampled by abdominal paracentesis during the experiment. Papillae development of rumens excised by experimental laparotomy was macro- and micromorphologically determined before and after sugar supplementation in a selected calf. The numbers of Entodinium, Isotricha, Dasytricha and Epidinium protozoa increased by 3 to 12 folds after 1–3 wk of supplementation and subsequently decreased. The heights of the rumen papillae after sugar supplementation showed marked development compared with before supplementation (Post vs. Pre: 4.44 ± 0.43 vs. 1.36 ± 0.24 mm). Sugar supplementation accommodates the rumen protozoa profile and stimulates papillae development in retarded growth calves.

KEY WORDS: protozoa, retarded growth calf, rumen papillae, sugar supplementation.

The rumen of the neonatal calf occupies about 20% of the volume of the whole stomach, and development of the rumen is completed by 14 to 16 weeks of age. The constituents of rumen microflora are formulated in the early growth period. In general, the number of rumen protozoa in a calf exceeds more than 10⁹/ml, which is equivalent to 1.8% of the whole rumen contents, and a similar ratio of rumen bacteria is found in the cow [4]. Among the rumen protozoa, Entodinium, a representative of small-type protozoa is first observed in the newborn calf and is rather resistant to the acid rumen environment. Isotricha, a representative of large-type protozoa, appears with the elevation of rumen pH [5, 7]. The total volume of rumen protozoa doubles when large quantities of starch or carbohydrate materials are fed to the animal; however, small-type Entodinium, which occupies a greater portion of this transition, may not increase over a certain level [6, 16]. Development of rumen papillae is well correlated with calf growth; therefore, if a calf shows growth retardation or delayed weaning, the rumen papilla remains undeveloped [22], which decreases farm productivity. Therefore, early weaning or feeding of adequate concentrates for a calf is recommended to promote rumen development and successful calf growth [3, 18, 21, 23]. Completion of rumen development also depends on the intake of concentrate or hay and their ingredients [8, 11–13, 20]. Starch or carboysides in concentrates fed to calves is converted to volatile fatty acids (VFA), i.e., propionic acid or butyric acid, which shifts the rumen into an acidic condition [3] and promotes papillae growth or increase in the volume of rumen protozoa [9, 10, 12].

The simplest material for production of propionic acid by rumen fermentation is sucrose, the main component (95.4%) of table sugar, and the growth effect of periodic sugar supplementation on retarded growth calves, i.e., daily weight gain (DG) and the blood level of insulin-like growth factor-1 (IGF-1) has been clinically reported before [19]. Therefore, the authors speculated that supplementation of sugar directly moderates rumen development to some extent. In the present study, the effect of sugar supplementation on the rumen protozoa profile and papillae development was investigated in retarded growth calves.

A total of 10 retarded growth calves (5 Japanese Black heifers, 4 Japanese Black steers and a Holstein steer; 85.8 ± 14.6 days old, 73.0 ± 16.1 kg BW; average ± s.d.) with no critical symptoms were used. These calves were raised with their dams at 6 farms and fed rearing concentrates or hay (Italian ryegrass or clover) until weaning at 60–75 days old. These calves showed either retarded growth less than 70% of the Japanese Feeding Standard for Beef Cattle (2000) [2] or between 70 and 80% of the standard level accompanied with at least 2 of the following 5 clinical signs: rough coat, ventral descent, inertia, anorexia and chronic diarrhea. Commercial table sugar (Refined sugar; Daiichi-Togyo Co., Ltd., Hyuga, Miyazaki) was given per os to all ten retarded growth calves at 1 g/kg BW (about 5% of the non-fibrous carbohydrate requirement) twice a week for 8 weeks. About 3–5 hr after morning feeding at 10–12 a.m., rumen juice (0.5–2.0 ml) was sampled seven times during the experiment by abdominal paracentesis in the left middle abdominal region under local anesthesia, i.e., before supplementation (0 wk), 4 times during (1–4 wk), at the end...
(8 wk) and 8 weeks after supplementation (16 wk). The pH of the rumen juice was analyzed by pH meter (TWIN pH meter B-211, Horiba, Kyoto) immediately after sampling, 10% formalin solution was added into a 5-time dilution, and the sample was kept refrigerated in the dark until microscopic analysis of the protozoa profile.

One of the ten retarded growth animals was selected (#07, 101 days old, 81.7 kg BW, DG 0.51 kg/day, Holstein steer), and the development of rumen papillae excised by experimental laparotomy was microscopically determined before sugar supplementation. Under general anesthesia, a rumenotomy was performed, and specimens in the fore-ventral sac and left-longitudinal groove were excised in a 3 × 3-cm square shape and put into the 10% formalin solution for histological analysis. The heights of the rumen papillae at 5 spots in each sample were determined. After 8 weeks of sugar supplementation, a 2nd rumenotomy was performed to collect rumen mucosa specimens, and the papillae development was evaluated. Another retarded growth calf (#011, 129 days old, 60 kg BW, DG 0.23 kg/day, Japanese Black steer) was subjected to an experimental laparotomy to compare the heights of the rumen papillae with those of the supplemented calf. The calf was 4 weeks older than #07 and received no supplementation with sugar after birth. Papillae development was determined in the same manner as for a supplemented case (#07).

Protozoa profile: The contents of the rumen juice were stained with methyl green-formalin-saline solution, and the protozoa profile was classified under microscope by the method of Ogimoto and Imai [15] and Mishima [14]. The composition of the rumen protozoa was determined based on the counts of 300 cells in each sample.

Rumen mucosa pathology: Rumen mucosa tissues (3 × 3 cm) were fixed in 10% formalin solution, and the heights of papillae were measured at 5 points in the respective specimen using a micrometer. After observation, tissue specimens were dehydrated and embedded in paraffin for routine histological examination. Sections that were 4-μm thick were stained with hematoxylin and eosin solution and then morphologically observed under a microscope.

The number of Entodinium protozoa increased rapidly to almost 4 folds at 1 wk after sugar supplementation (initial volume: 1.43 × 10^6/ml) and then decreased but maintained a higher level than at wk 0. With a little delay, the large type of Protozoa Isotricha increased by almost three folds (initial volume: 6.49 × 10^3/ml), and Dasytricha increased by twelve folds (initial: 1.52 × 10^4/ml) at 2 wk after supplementation. Ultimately, Epidinium increased by almost six folds (initial: 6.24 × 10^3/ml) at 3 wk after supplementation (Fig. 1). These results indicate the variations of acid resistance in protozoa, which were similar to those in previous reports [1, 8, 17, 22]. The pH of rumen juice showed a lower level in the early period (wk 0, 1 and 2: 5.89 ± 0.41, 5.85 ± 0.55 and 5.92 ± 0.47, respectively) and increased to 6.27 ± 0.34 at 8 wk. Epidinium showed lower resistance to acidity and delayed acclimation to the rumen environment. These results indicated that these protozoa consumed sugar, produced VFA and lowered pH.

The heights of rumen papillae increased significantly in the fore-ventral sac and left-longitudinal groove in a retarded growth calf (#07) after supplementation (1.36 ± 0.24 in wk 0 vs. 4.44 ± 0.43 mm in wk 8 for the fore-ventral sac; 1.14 ± 0.17 in wk 0 vs. 2.44 ± 0.43 mm in wk 8 for the left-longitudinal groove). On the other hand, calf #011, which was 4 weeks older than calf #07, showed lower heights of rumen papillae (fore-ventral sac and left-longitudinal groove: 1.30 ± 0.16 mm and 1.34 ± 0.11 mm, respectively) that were similar to those of calf #07 before sugar supplementation. These results indicated that sugar supplementation stimulates the development of rumen papillae. No histological differences were observed in rumen papillae before and after sugar supplementation (Fig. 2), which indi-

![Fig. 1. Changes of rumen protozoa number and rumen juice pH in retarded growth calves before and after sugar supplementation (1 g/kg BW, twice a week for 8 weeks during 0 to 8 wk). The variations in protozoa number showed comparative indexes compared to before sugar supplementation (0 wk). The initial amounts of the protozoa were 1.43 × 10^6/ml for Entodinium, 6.24 × 10^3/ml for Epidinium, 6.49 × 10^3/ml for Isotricha and 1.52 × 10^4/ml for Dasytricha, respectively. Rumen juice was collected by abdominal paracentesis.](image-url)
cates that VFA and lowered pH stimulate development of rumen papillae, lead to extension of the rumen mucosa area and simply upgrade the VFA absorption rate.

The growth effect of antibiotic monensin added to diet has been reported [20], whereas the increase of propionic acid resulted in unbalanced microflora constituents; therefore, the effect would be rather temporary and would last only during the prescription period. However, the growth effect of sugar would continue for more than the supplementation period. However, the growth effect of sugar would continue for more than the supplementation period, since sucrose, the main component (97%) of table sugar, promotes VFA production by rumen fermentation, papillae development and VFA absorption.

In conclusion, sugar supplementation accommodates the rumen protozoa profile and stimulates papillae development in retarded growth calves.

REFERENCES

11. Kaufmann, W., Hagemeister, H. and Dirksen, G. 1980. Adaptation to changes in dietary composition, level and frequency...


