NOTE
Internal Medicine

Forestomach developmental failure in an 11-month-old Japanese Black steer with severely retarded growth and chronic ruminal tympany

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ABSTRACT. This study reports findings from the pathological examination of the forestomach of an 11-month-old Japanese Black steer with severely retarded growth (41% of expected weight) and chronic ruminal tympany. The ruminal papillae were weakly formed (0.3–0.5 cm long) and unevenly distributed. The cellulae and cristae reticuli were underdeveloped; the cristae were 0.4–0.7 cm in height and milky white. The keratinized layer in the stratified squamous epithelium was thickened. Ruminal pH was 5.25, and ruminal volatile fatty acid concentration was 11.7 mM. The steer's severely retarded growth was considered to be caused by malnutrition due to developmental and functional failure of the forestomach.

KEY WORDS: chronic ruminal tympany, developmental failure, Japanese Black steer, ruminal papillae

Growth retardation is a common symptom in young cattle with primary or secondary disorders [2, 13, 18]. It is well known that due to inbreeding, some Japanese Black cattle carry impaired genes that may cause diseases [2, 13, 18]. Studies monitoring the development of unthrifty young beef cattle are needed to illuminate the pathogenesis of growth retardation in these animals.

We report the case of an 11-month-old Japanese Black steer with severely retarded growth (41% of the expected weight for its breed and age) [17] and chronic ruminal tympany. Because of its unthrifty condition, far below the level of fattening expected for beef cattle, it became difficult to maintain the animal's welfare under normal rearing, and it was referred to the Veterinary Medical Hospital, Okayama University of Science, where clinical and pathological investigations were performed, including analysis of ruminal contents and function. This study describes the findings of these investigations.

Epidemiological and clinical background: The subject of this study was a Japanese Black steer that was reared on a beef cattle production farm (Fukuyama, Hiroshima, Japan) until 8 months of age, then moved to a beef cattle fattening center (Hiroshima, Japan). The steer was castrated at 5 months of age. At 8 months of age, the steer had a body weight of 135 kg (49% of the expected weight of age-matched Japanese Black cattle) [17] and a height of 92 cm. The farm did not record detailed information on the rearing, weight gain, and behavior of the steer before 8 months of age. In the fattening center, beef cattle were reared and fed together in age-matched groups of four to six. The subject was one of four calves sired by the same bull but was the only one of them that showed severely retarded growth and chronic ruminal tympany.

The diseased steer had been reared according to the Japanese Feeding Standard for Beef Cattle [3]. However, it had a poor appetite

that persisted over the subsequent 3 months at the fattening center, during which time it gained only 3 kg of body weight. In addition, the steer showed severe swelling of the left lateroabdominal area and was diagnosed with chronic ruminal tympany. The animal was treated by changing the feedstuff to include fewer pelleted concentrates and more good-quality hay, as well as introducing feed supplements. However, its ruminal condition did not improve.

On admission to the veterinary hospital at 11 months of age, the steer's body weight was 139 kg, measured with a Tru-Test EW5-1010 scale (Fujihira Industry, Tokyo, Japan); this value was 41% of the standard weight of age-matched Japanese Black cattle [17]. Its left lateroabdominal area was severely distended (Fig. 1); on percussion and auscultation of this area, a tympanic note was produced. The steer's gait did not display any overt abnormalities. Physical signs on admission were as follows: rectal temperature 38.9°C, heart rate 90 beats/min, respiratory rate 20 breaths/min, and ruminal motility 2 times/min. Nasal discharge, coughing, and diarrhea were not detected. The steer exhibited rumination after ingesting roughage; however, it did not emit ruminal gases by regular eructation. The feces were of normal shape and brownish-black in color.

Despite dietary changes, the steer's severely retarded growth and chronic ruminal tympany persisted, and its general condition continued to be unthrifty and inconsistent with fattening as a beef

Fig. 1. Caudodorsal view of an 11-month-old Japanese Black steer with severely retarded growth, showing chronic ruminal tympany.

steer. Based on animal welfare concerns and the steer's failure to gain weight, the decision was made to euthanize it and perform a necropsy. The steer was handled according to university guidelines for the care and use of animals (Animal Experiment Ethics Committee, Okayama University of Science) and was allowed *ad libitum* access to dry hay and water until euthanasia. Due to its recalcitrant chronic ruminal tympany, the steer was necropsied according to guidelines for the handling of diseased animals, and pathological examination was performed.

Hematological and biochemical findings: Peripheral blood collected from the diseased steer at admission was subjected to analysis of hematological and serum biochemical parameters using biochemistry analyzers (Celltac α MEK6558, Nihon Kohden, Tokyo, Japan; Hitachi 3100, Hitachi, Tokyo, Japan) (Table 1). Total serum protein was 5.83 g/dl, while total cholesterol was 59 mg/dl; these values were lower than reference values [12, 16]. In addition to low levels of serum protein and cholesterol, the steer also exhibited abnormally low levels of hemoglobin, albumin, and blood urea nitrogen (BUN), suggesting inadequate nutrient uptake. A relationship between markedly lowered BUN concentration and inadequate dietary nutritional content was previously observed in a breeding herd of Japanese Blacks [19]. Serum electrolytes (Na, K, Cl, Ca, and inorganic P) and liver function markers (aspartate aminotransferase, creatinine kinase, and lactate dehydrogenase) were within reference ranges [16].

Ruminal pH and volatile fatty acids: At necropsy, a plastic bottle was used to collect 50 ml of ruminal fluid from the interior of the rumen in order to analyze its pH and its volatile fatty acid (VFA) concentration. The collected fluid was filtered through two layers of cheesecloth and stored at −80°C until use. The purpose of the pH and VFA analysis was to evaluate the fermentation level and digestibility of the rumen contents. The pH was measured using a pH meter (Horiba D53, Hitachi, Tokyo, Japan), and the VFA concentration was analyzed with a gas chromatograph (GC9A, Shimadzu, Kyoto, Japan) according to a previously described procedure [5]. The ruminal pH of the diseased steer was 5.25, lower than the reference values reported in age-matched animals (mean 6.22, range 5.43–6.79) [12]. The reason for the abnormally low ruminal pH was not clear; contributing factors could include poor influx of saliva due to decreased rumination, failure to absorb VFAs due to undeveloped ruminal papillae, and easily degradable feed. As the steer had a ruminal pH below 5.6, it met the diagnostic threshold for rumen acidosis in cattle [11].

The total VFA concentration in the rumen contents was 11.7 mM (7.4 mM acetic acid, 2.1 mM propionic acid, and 1.2 mM lactic acid). This level was approximately tenfold lower than values reported for healthy cattle (112 mM in 6-month-old Holstein calves [1] and 131 mM in 10- to 14-month-old Japanese Black steers [12]). These markedly low VFA levels were likely due to the combination of a poorly developed forestomach and chronic ruminal tympany, with the latter making the steer reduce its dry feed intake, resulting in decreased ruminal fermentation.

Gross and microscopic anatomy: At necropsy, the steer seemed to have less subcutaneous adipose tissue than normal. The thoracic and peritoneal cavities did not display any overt abnormalities. There appeared to be an increased volume of pleural fluid in the pleural space, but fibrin deposition on the serous membrane was not found. Although the rumen was positioned normally within the abdominal cavity, it was expanded and its cranial sac was enlarged. The reticulum was highly dilated and thinned. The surface of the serous membrane of the forestomach and digestive tract appeared to be normal. The rumen was filled with foamy contents that were light green in color but lacked any abnormal odor (Fig. 2a).

The ruminal papillae were weakly developed on the ruminal mucosa (Fig. 2b and 2c). These papillae, which were 0.3–0.5 cm long and milky white in color, were observed to be poorly developed on the ventral floor of the cranial sac, in the caudoventral and caudodorsal blind sacs, on the left and right lateral walls, and along the cranial area of the roof of the dorsal sac (Fig. 2b); they

Table 1. Hematological and serum biochemical markers of the diseased steer at admission to the veterinary hospital

| Parameter (standard abbreviation, unit)	Measured value	Reference values [15, 16]
Packed cell volume (PCV, %)	25.5	22–33
Hydrogen ion concentration index (pH)	7.51	7.31–7.53
Red blood cell count (RBC, ×10^4/μl)	744	510–760
White blood cell count (WBC, /μl)	9,700	4,900–12,000
Hemoglobin (Hb, g/dl)	7.7↓	8.5–12.2
Platelet count (PLT, ×10^4/μl)	36.6	10–80
Sodium (Na, mmol/l)	142	132–152
Potassium (K, mmol/l)	4.8	3.9–5.8
Chloride (Cl, mmol/l)	107	97–111
Calcium (Ca, mg/dl)	9.9	9.7–12.4
Inorganic phosphorus (iP, mg/dl)	5.8	5.6–6.5
Magnesium (Mg, mg/dl)	1.7	1.8–2.3
Glucose (Glu, mg/dl)	69	45–75
Total protein (TP, g/dl)	5.83	6.74–7.46
Albumin (Alb, g/dl)	2.59	3.03–3.55
Blood urea nitrogen (BUN, mg/dl)	1.9	20–30
Total cholesterol (T-cho, mg/dl)	59	80–120
Total bilirubin (T-Bil, mg/dl)	0.12	0.01–0.5
Creatinine (Cre, mg/dl)	1.05	1.0–2.0
Aspartate aminotransferase (AST, U/l)	76	78–132
Creatinine kinase (CK, U/l)	115	44–211
Lactate dehydrogenase (LDH, U/l)	1,038	697–1,445
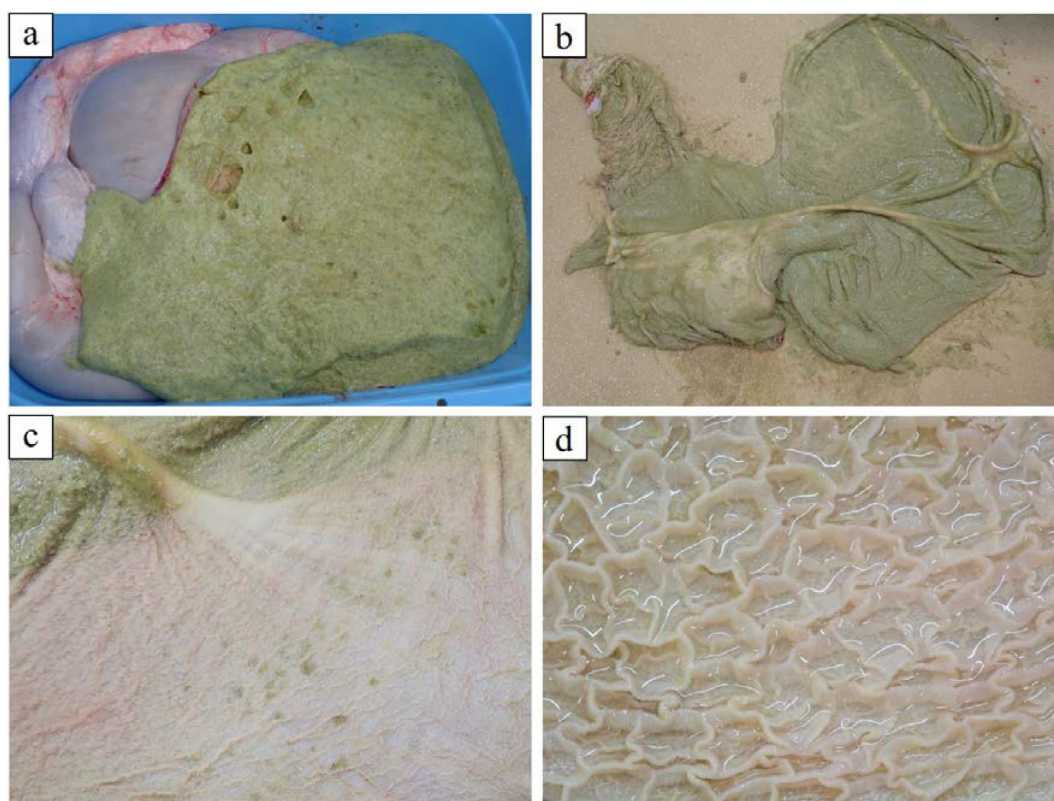
Fig. 2. Gross morphology of the rumen and reticulum of the diseased steer. a) Ruminal contents of the diseased steer. The rumen was filled with foamy contents that were homogeneous in nature and light green in color. b) Interior of the rumen after removal of ruminal contents. c) Interior of the rumen after washing out of the contents. Ruminal papillae on the mucosal surface were weakly developed and unevenly distributed. d) Interior of the reticulum after washing out of the contents. The cellulae and cristae reticuli were weakly formed, the latter being 0.4–0.7 cm in height.

were flattened and fragile in appearance. The reticular mucosa was milky white in color; the cellulae reticuli were weakly formed, exhibiting a pentagonal or hexagonal shape, and the cristae reticuli were also poorly developed (0.4–0.7 cm in height) (Fig. 2d).

For microscopic examination of the forestomach, samples taken from the rumen and reticulum were dehydrated and embedded in paraffin. Sections 6 µm thick were cut and stained with hematoxylin and eosin or with Masson's trichrome. Light microscope examination revealed poorly developed ruminal and reticular papillae (Figs. 3a and 4a). The samples from the diseased steer were compared with a normal control, which showed well-developed ruminal papillae and cristae reticuli (Fig. 5). In the diseased steer, a thickened stratum corneum was observed in the stratified squamous epithelium of the rumen and reticulum (Figs. 3b and 4b); however, no morphological abnormalities were observed in the stratum granulosum, stratum spinosum, or stratum basale of the ruminal papillae. The muscle layers in the rumen and reticulum were weakly developed. No leukocyte infiltration or microabscesses were observed in the ruminal papillae or reticulum. No overt pathologies of the omasum and abomasum were found.

McGavin and Morill [10] studied the development of ruminal papillae in 4- to 6-week-old male Holstein calves maintained under different diet regimens and reported that the intake of roughage favored the development of tongue-shaped papillae covered by a thin layer of parakeratotic cells. In 6-week-old Holstein calves fed a concentrated diet as well as hay, the ventral floor of the cranial sac was observed to be covered by well-developed papillae that were pale yellow to brown in color and exhibited a soft, flexible, and tongue like appearance.

Regarding endocrinological factors affecting growth and development, the relationship among growth hormone (GH), ghrelin [6], and GH secretagogue receptor (GHS-R) [9] has been found to be pivotal with regard to GH secretion, enhancement of feed intake, and increased adiposity in Japanese Black cattle [7, 8]. Whether genetic impairment of these factors in this breed might be associated with the severely retarded growth and poorly developed ruminal papillae observed in this study remains to be elucidated in further studies.
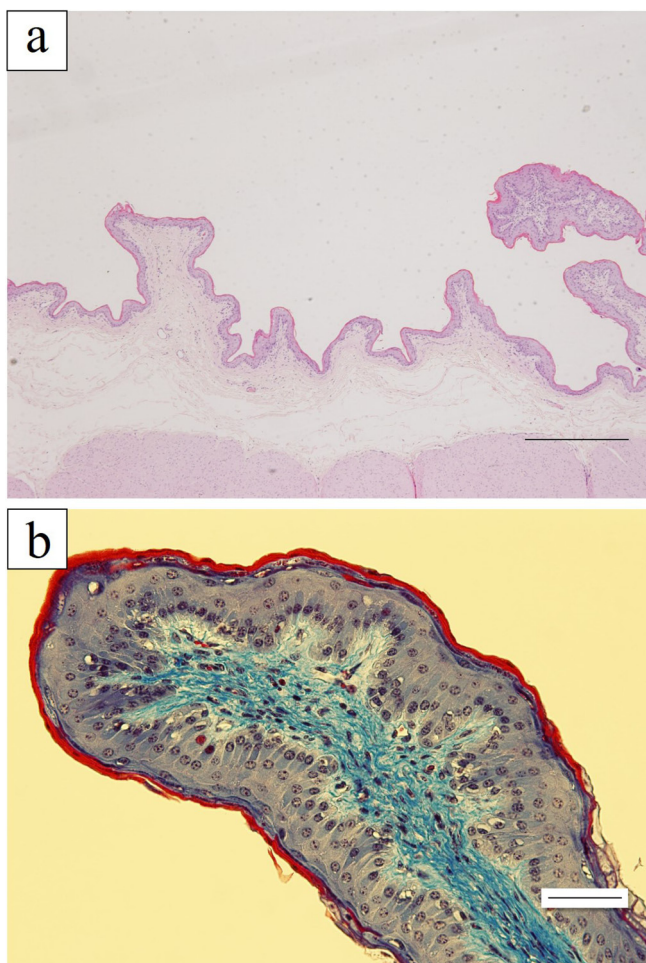

Fig. 3. Histopathology of the ruminal papillae of the diseased steer. a) Ruminal papillae and muscle layer, both weakly developed, stained with hematoxylin and eosin. Scale bar: 500 µm. b) Stratified squamous epithelium of the rumen, stained with Masson's trichrome. The stratum corneum was thickened. Cell differentiation appeared to be normal. Scale bar: 50 µm.
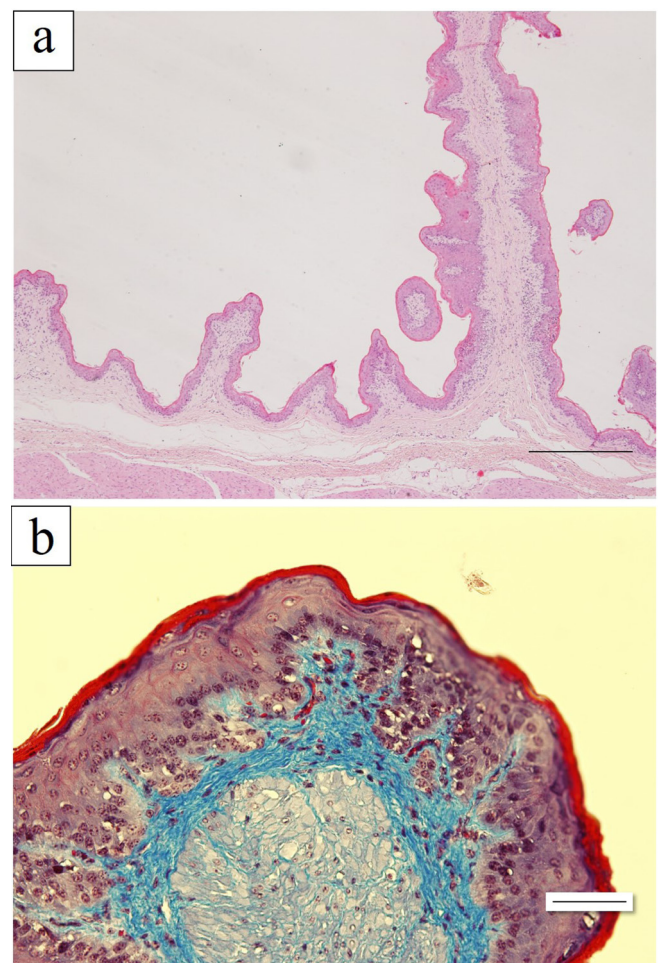

Fig. 4. Histopathology of the reticulum of the diseased steer. a) Epithelium and muscle layer of the reticulum, both weakly developed, stained with hematoxylin and eosin. Scale bar: 500 µm. b) Stratified squamous epithelium of the reticulum, stained with Masson's trichrome. The stratum corneum was thickened. Cell differentiation appeared to be normal. Scale bar: 50 µm.
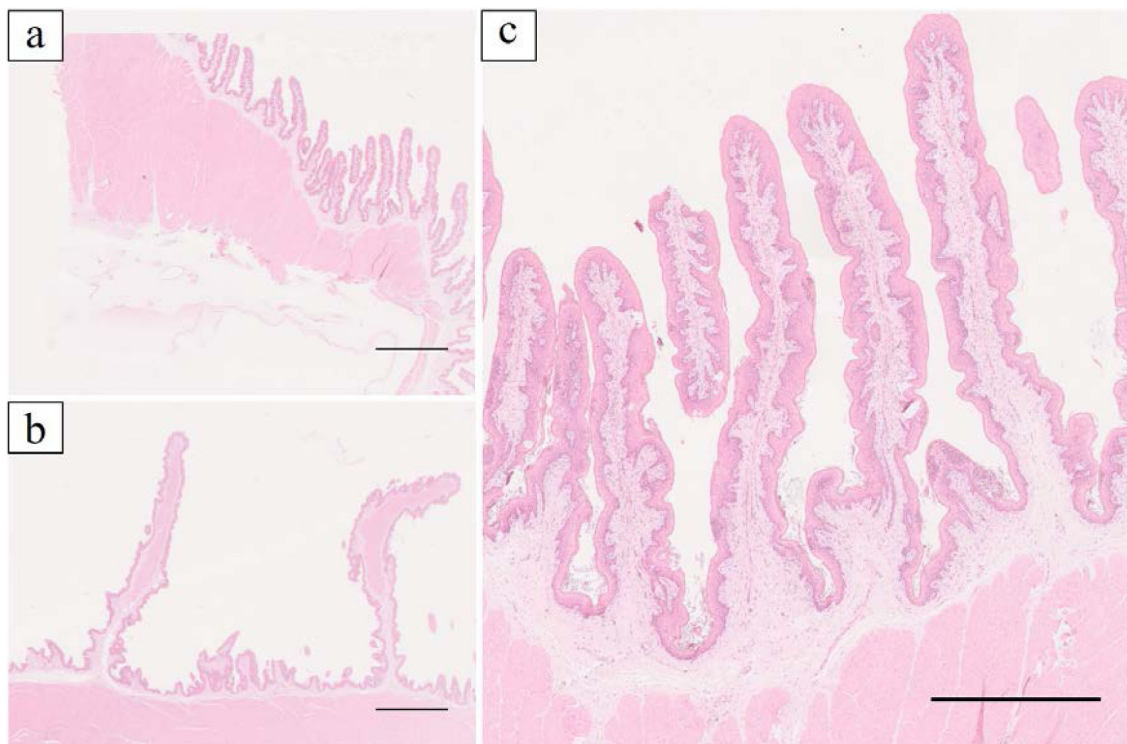
Fig. 5. Morphology of the rumen and reticulum of the control animal. a) Rumen of the control animal (2-year-old heifer), stained with hematoxylin and eosin. Ruminal papillae and muscle layer were well developed. Scale bar: 3 mm. b) Reticulum of the control animal (2-year-old heifer), stained with hematoxylin and eosin. Cristae reticuli and muscle layer were well developed. Scale bar: 3 mm. c) Ruminal papillae of the control animal, stained with hematoxylin and eosin. Papillae were well developed and distributed. Scale bar: 1 mm.

Our study confirmed that the diseased steer's chronic foamy ruminal tympany was associated with developmental failure of the rumen and reticulum, as revealed by gross and microscopic morphology. In addition, the thickened keratinized layer of the ruminal stratified squamous epithelium suggested possible impairment of VFA absorption from the ruminal epithelium, indicating a potential cause of the animal's malnutrition. Thus, pathological analysis of the forestomach indicated the origin of the major clinical signs of chronic ruminal tympany observed in the animal.

The disturbances in ruminal fermentation in the diseased steer appeared to be associated with developmental failure of the mucosa of the rumen and reticulum, particularly the ruminal papillae and epithelial keratinized layer. The cause of this developmental failure remains unclear. It may have been due to vagal indigestion, which may cause dysfunction of the digestive tract in ruminants [4, 14], or to a primary genetic disorder, related to the high level of inbreeding and selection in the Japanese Black breed. Further studies are required to elucidate the responsible factors.

In conclusion, the present study revealed that the diseased steer's forestomach showed poorly developed mucosal membranes in the rumen and reticulum. The morphological characteristics of the undeveloped forestomach and the resulting pathological effects may have led to reduced VFA synthesis and decreased absorption by the ruminal epithelium. The steer's severely retarded growth and chronic ruminal tympany were considered to be associated with malnutrition due to developmental and functional failure of the forestomach.

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