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## Characteristics of Milk/Meat Derived from Progeny of Somatic Cell Cloned Cattle

## 体細胞クローン後代牛の生産物性状に関する調査報告書

独立行政法人
農業・食品産業技術総合研究機構
畜産草地研究所

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Livestock and Grassland Science

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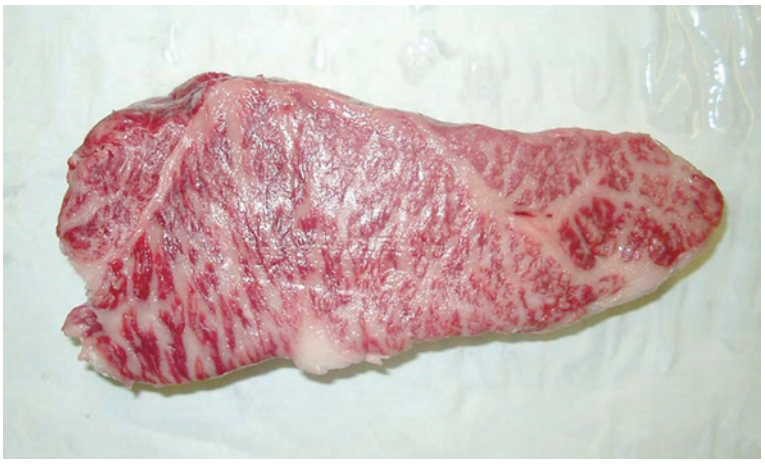
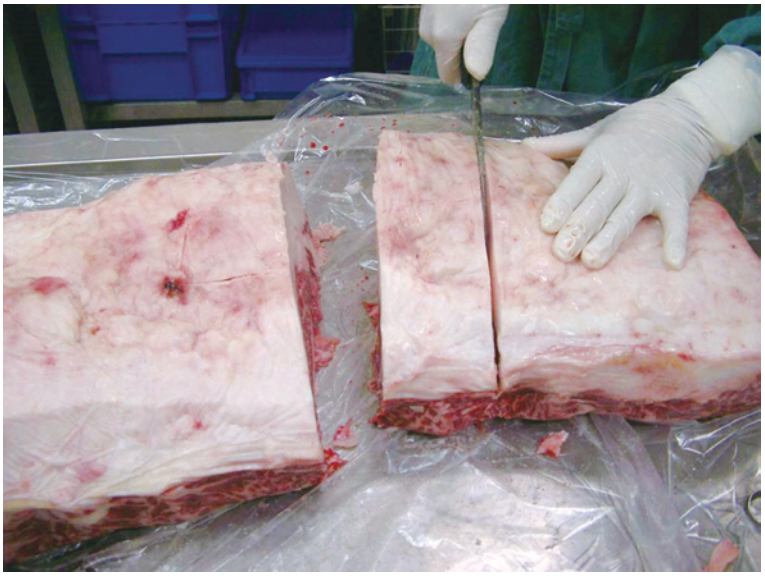


Loin

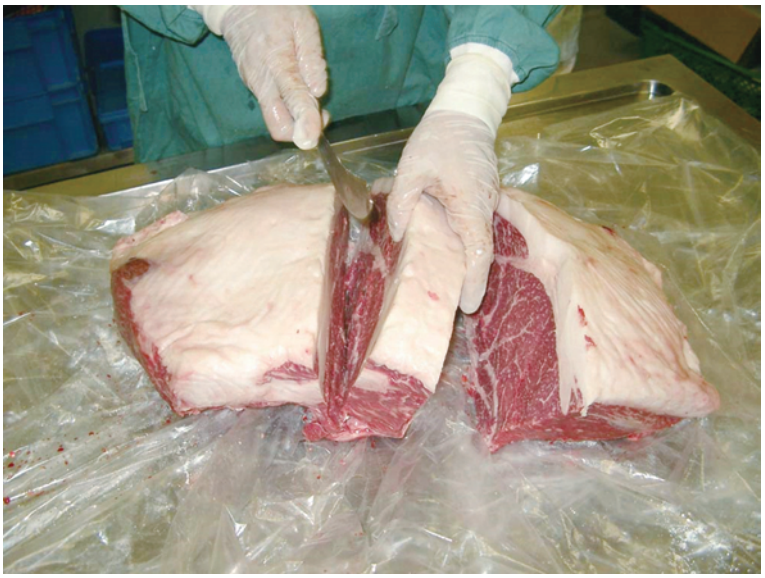
Round

Shoulder

Sliced meat of loin, round and shoulder derived from progeny of somatic cell cloned heifer used in the present investigation. The breed of heifer was Japanese Black beef cattle, Wagyu.



Loin



Shoulder

Preparation of retail cuts derived from somatic cell cloned heifer used in the present investigation. The breed of heifer was Japanese Black beef cattle, Wagyu.

# Preface

"The risk assessment based on current scientific findings showed that food derived from SCNT (somatic cell nuclear transfer) cloned cattle and pigs and their offspring would have equivalent safety as those derived from cattle and pigs produced by the ATRs (assisted reproductive technologies)". This is a conclusion of "Risk assessment report on foods derived from cloned cattle and pigs produced from somatic cell nuclear transfer and their offspring" issued by Food Safety Commission (FSC) of Japan in July 2009. FSC held committees for 16 times to have science-based discussions concerning safety of food products derived from these animals.

A report titled "Characteristics of milk/meat derived from progeny of somatic cell cloned cattle", which was the only reference concerning characteristics of animal products derived from progeny of clones, were used as a scientific data book on the committees and could found in "Reference" section of the risk assessment report by FSC. The report was published by National Institute of Livestock and Grassland Science (NILGS) of Japan in March 2008, as a part of "Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (#1602, 2004–2008)" from the Agriculture, Forestry and Fisheries Research Council, Ministry of Agriculture, Forestry and Fisheries, Japan.

The report contains plenty of data concerning characteristics of milk/meat derived from progeny of somatic cell cloned cattle including the nutritional constituents, digestion rate, allergenicity/mutagenicity potentials and twelve-month feeding study of rats. Few non-Japanese investigators recognize the presence of this report, since it is written in Japanese language. When this report is introduced to foreign visitors of NILGS who interested in animal cloning technology, everybody request its publication in English.

Responding to their requests, the English version of this report is published as No. 11 issue of "Memories of National Institute of Livestock and Grassland Science". The issue, which contains essence of the Japanese version, could be used as a precious data book concerning characteristics of milk/meat derived from progeny of somatic cell cloned cattle by foreign researches and administrators.

The risk assessments of somatic cell cloned animals and their progeny were carried out by Food and drug administration (USA) and European Food Safety Authority (European Commission). Following these cases, other organizations might perform such risk assessments. The present issue would be an instructive data book for their scientific assessments.

March 2011

Mitsuto Matsumoto Ph. D
Director general
National Institute of Livestock and Grassland Science
National Agriculture and Food Research Organization

# 刊行にあたって

「現時点における科学的知見に基づいて評価を行った結果，体細胞クローン牛及び豚並びにそれらの後代に由来する食品は，従来の繁殖技術による牛及び豚に由来する食品と比較して，同等の安全性を有すると考えられる」これが，わが国の食品安全委員会により，平成 21 年 6 月に公表された「新開発食品評価書：体細胞クローン技術を用いて産出された牛及び豚並びにそれらの後代に由来する食品」の結論である。この結論を導くため，食品安全委員会では 16 回にわたる各階層の委員会を開催し，科学的資料に基づく議論を重ねた。

その議論の際，世界唯一の体細胞クローン後代牛の生産物性状に関する資料として活用され，また，評価書の引用文献のひとつとして掲載されているものが，「体細胞クローン後代牛の生産物性状に関する調査報告書（平成 20 年 3 月）」である。この報告書は，平成 16 年度先端技術を活用した農林水産研究高度化事業の採択課題「産業利用に向けた体細胞クローン牛に関する技術開発と調査（課題番号：1602）」の一部として，畜産草地研究所が畜産生物科学安全研究所に再委託して実施した調査を取りまとめたものである。

この報告書には，体細胞クローン後代牛が生産した乳肉を対象に，栄養成分分析，ラットによる消化試験，マウス腹壁法によるアレルギー誘発試験，マウスを用いる小核試験，ラットを用いる 12 カ月間の飼養・生殖併合試験が収録されている。これらの内容は，一部が英文の学術論文として報告されているものの，和文で表記されている関係上，残念ながら，海外の研究者などの目に触れることはなかった。しかしながら，畜産草地研究所を訪問した海外の研究者や行政官にこの資料を紹介すると例外なく非常な関心を示し，英語版の刊行を熱望し，所を後にしていった。

そのような要望に応え，今回，「Characteristics of milk/meat derived from progeny of somatic cell cloned cattle」と題する上記報告書の英語版を作成し，畜産草地研究所研究資料第 11 号として刊行することにした。英語版の作成にあたっては，上記報告書の総括部分をもとにコンパクトにまとめた。これによって，体細胞クローン後代牛が生産した乳肉の安全性に関する稀少かつ貴重なデータを海外の研究者や行政官なども利用できるようになった。

体細胞クローン家畜やその後代のリスク評価は，わが国に先立って米国食品医薬品局（FDA）と欧州食品安全機関（EFSA）において行われている。今後，諸外国でも同様のリスク評価が行われることが予想され，その際，当資料の活用が期待される。

平成 23 年 3 月

独立行政法人　農業・食品産業技術総合研究機構
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ACKNOWLEDGEMENTS

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# Characteristics of Milk/Meat Derived from Progeny of Somatic Cell Cloned Cattle

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SUMMARY

The progeny of somatic cell cloned cattle might be appropriate candidates for commercial animals for milk/meat production; however, there are almost no available data for their safety as sources of food. This investigation was conducted to obtain data concerning the characteristics of milk/meat derived from these animals. To check the equivalence of their products to conventional milk/meat, the obtained data were compared with those of conventionally bred cattle. The investigated indices concerning the nutritional constituents of milk/meat were for general components, amino acids and fatty acids. After the milk/meat was processed to freeze-dried powder, digestion rate, allergenicity and mutagenicity potentials of the products were examined by *in vivo* digestion test, mouse abdominal wall method and mouse micronucleus test, respectively. No significant differences in these indices were found between products derived from the progeny and those derived from conventionally bred cattle. With regard to chronic toxicities of milk/meat derived from the progeny during the process of development and reproduction, a twelve-month feeding study was carried out with rat groups fed a diet supplemented with the milk/meat powder. The rats were subjected to clinical observations of general health condition and examinations such as sensory/reflex function, grip strength, motor activity, body weight, food consumption, ophthalmology and urinalysis. Moreover, sexually matured rats fed the test diets were mated and examined for indices such as the reproductive performances of the dams and health of their pups. After the feeding period, indices related to rat health status, based on the findings for hematology, clinical biochemistry, necropsy, organ weight and histology, were examined. There were no biologically significant differences in obtained indices between the rat groups fed meat/milk powder-supplemented diets derived from the progeny and those in rat groups fed meat/milk powder-supplemented diets derived from conventionally bred cattle. These findings suggest that milk/meat derived from the progeny of somatic cell cloned cattle might be equivalent to those derived from conventionally bred cattle.

Keywords: cattle, somatic cell clone, progeny, milk/meat, characteristics

## INTRODUCTION

The Research Institute for Animal Science in Biochemistry and Toxicology (RIAS) carried out a three-year project (1999–2002) concerning the characteristics of milk/meat derived from embryonic and somatic cell cloned cattle from the Japan Livestock Technology Association. In this project,

This investigation was adopted by the National Institute of Livestock and Grassland Science as a part of *Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries* (#1602, 2004–2008) from the Agriculture, Forestry and Fisheries Research Council, Ministry of Agriculture, Forestry and Fisheries, Japan.

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the following data obtained from milk/meat were compared between somatic cell cloned cattle and conventionally bred cattle: general components, amino acids and fatty acids, digestibility, allergenicity, mutagenicity and results of fourteen-week feeding test of rats. These results demonstrated that there were no significant differences in the obtained data between the clones and conventionally bred cattle. No harmful effects on rats were observed when they were fed with milk/meat derived from the clones[15,35,45].

Although somatic cell nuclear transfer technology has been considered as a promising innovatory technology for livestock agriculture[4,5,40,41], the productive efficiency of cloned cattle is insufficient for practical use. In contrast, no remarkable differences in health status and reproductive performance were found among conventionally bred cattle and progeny of somatic cell cloned cattle[39]. Therefore, progeny of clones might be appropriate candidates for commercial animals for milk/meat production; however, there are almost no available data for their safety as sources of food. This investigation was conducted to obtain data concerning the characteristics of milk/meat derived from the progeny. Most of the indices observed in the present investigation were designed in accordance with the previous project described above; however, the period of rat feeding test was prolonged to twelve months as a chronic toxicity test to obtain data concerning the consumption risks of these edible products. During the feeding period, sexually mature rats were mated in order to assess the toxicity of milk/meat derived from the progeny in terms of fertilization, embryo development and newborn growth. To check the substantial equivalence of the progeny and conventionally bred cattle, obtained data were compared between them.

## MATERIALS AND METHODS

### Progeny of cloned cattle and conventionally bred cattle

Three female progeny derived from dairy cattle (Holstein) were produced by artificial insemination (AI) of somatic cell cloned cows[47] with frozen-thawed semen derived from a conventionally bred bull. Three conventionally bred dairy cattle (Holstein) were also produced by AI as controls. These cattle were produced and raised at the National Livestock Breeding Center.

Three female progeny derived from beef cattle (Japanese Black) were produced by AI of conventionally bred cows with frozen-thawed semen derived from a somatic cell cloned bull[33]. They were produced and raised at Oita Prefectural Agriculture, Forestry and Fisheries Research Center. Three conventionally bred beef cattle (Japanese black) were also produced by AI as controls and were raised at the Shiga Prefectural Livestock Technology Promotion Center (Table 1). These cattle were fattened for meat production. To obtain reference data of blood properties in Japanese Black fattened heifers, an additional 33 heifers, which were under fattening trials at three institutions (Nagasaki Prefectural Livestock Experiment Station, Fukushima

Table 1. Somatic cell cloned cattle and conventionally bred cattle used for meat production and investigation of blood properties

| Heifer⌘ | Produced institution | Individual identification code | Name | Date of birth |
|---|---|---|---|---|
| Progeny of cloned cattle | Oita Prefectural Agriculture, Forestry and Fisheries Research Center | T1 | ITOHIKARI-1 | February 27, 2002 |
| | | T2 | ITOHIKARI-2 | March 9, 2002 |
| | | T3⌘⌘ | ITOHIKARI-3 | June 3, 2002 |
| | | T4 | ITOHIKARI-4 | April 23,2002 |
| Conventionally bred cattle | Shiga Prefectural Livestock Technology Promotion Center | B82 | YASUKO | January 26, 2002 |
| | | B89 | UMEHIROKO | March 21, 2002 |
| | | B90 | HANAYO | January 23, 2002 |

⌘: Heifers of fattened Japanese Black beef cattle.
⌘⌘ : T3 was used for investigation of blood properties but not for nutritional analysis and animal feeding studies of the meat.

Agricultural Technology Centre and Shiga Prefectural Livestock Technology Promotion Center), were also used for this study.

### Blood collection from cattle

In beef cattle, blood was collected from the jugular vein for hematological and clinical biochemistry analyses. Collections were carried out from the heifers at 22–28 months of age. Blood was collected 3–4 times from the progeny and twice from conventionally bred cattle.

### Preparation of milk/meat powder

Milk was collected independently from three progeny and three conventionally bred cows in the morning from the 3rd to 6th weeks of lactation and was stored at −25℃ as frozen milk plates. The frozen milk plates were freeze-dried below 133 Pascal for approximately 24 hours. The dry matter was passed through a 0.85 mm-mesh and mixed uniformly with a mixer. The samples were prepared as two pooled powders, progeny milk powder and control milk powder.

Meat derived from three progeny and three conventionally bred cattle was collected independently. Each sample was obtained as retail cuts such in *shoulder*, *loin* and *round* of the carcasses. The retail cuts were stored at −18℃ until use for the investigations. To prepare meat powder, excess fat was removed from the subcutis and intermuscle, and each meat sample was minced and freeze-dried below 133 Pascal for approximately 24 hours. The dry matter was passed through a 4 mm-mesh and mixed uniformly with a mixer. The samples were prepared as two pooled powders, progeny meat powder and control meat powder. They were stored at −25℃ until use.

### Preparation of test diets supplemented with milk/meat powder

Before supplementing the test diet with milk/meat powders, the nutritional values of each pooled powder were analyzed as described in previous reports[15,45]. The analyzed items included general components (crude protein, crude fat, carbohydrates, crude fiber, ash content and water content), vitamins (A, $B_1$, $B_2$, $B_6$, $B_{12}$, $D_3$, E, $K_2$, niacin, pantothenic acid, folic acid, biotin and choline) and minerals (Ca, P, K, Na, Mg, Fe, Zn, Cu, S, Mn, I, Se and Mo).

In accordance with the results of the analyses, the diet was supplemented with each pooled powder to the levels equivalent to the basal diet[1,2]. AIN93G-purified diet for rodents[31] was used and mouse micronucleus test, rat feeding study and AIN93M-purified diet for rodents[31] were carried out for a digestion test in rats.

Before the meat powder was added to the diet for the digestion test, fat in meat powder was removed, since it had high lipid content. For this, meat powder was processed in hot water for 10 minutes. The fat floating on the surface of the hot water was removed and prepared as freeze-dried powder. These powders were used as alternative protein and lipid sources for the test diet.

The percentage of milk/meat supplementation to test diet was decided according to results of a preliminary test (4 weeks) and the previous fourteen-week feeding study of rats[15,45]. Ten percent for milk powder and five percent for meat powder were found to be the maximum amounts of supplementation to test diets that do not affect feed intake and growth of rats. Therefore, these values were used as the higher supplementation levels of milk/meat powder for the test diets. As the lower supplementation levels of milk/meat powder for the test diets, 2% (w/w) for milk powder and 1% (w/w) for meat powder were used, namely, 1/5 of the higher supplementation levels. These were high-calorie diets owing to the 1 and 5% (w/w) meat or 2 and 10% (w/w) milk supplementation.

These test diets (meat powder diet derived from the progeny, meat powder diet derived from conventionally bred cattle, milk powder diet derived from the progeny and milk powder diet derived from conventionally bred cattle) were irradiated with γ-rays (10 kGy) and stored at −15℃ until use.

### Hematology and clinical biochemistry of cattle and rats

The blood collected from cattle and rats was

analyzed as shown below. The blood tests were preformed using a multi-item automatic blood analyzer ([E-4000 (for cattle) and XT-2000iV (for rats), Sysmex Corporation Kobe, Japan]) for the following: red blood cell count (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, white blood cell count (WBC), differential leucocyte count and platelet count; testing for prothrombin time and activated partial thromboplastin time was performed using a blood clot automatic measurement device (KC-10A, Amelung GmbH, Lemgo, Germany). The following clinical biochemistry tests were performed using an automatic biochemical analyzer (JCA-BM8 type Clinalyzer, JEOL Co., Ltd., Tokyo, Japan): lactate dehydrogenase (LDH), aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT), alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), creatine kinase (CK), alkaline phosphatase (ALP), γ-glutamyltransferase (γ–GTP), cholinesterase (ChE), total protein, albumin, globulin, A/G ratio, total cholesterol, triglyceride, phospholipid, glucose, total bilirubin, blood urea nitrogen, creatinine, calcium and inorganic phosphorous; testing for sodium, potassium and chloride was performed using an automated electrolyte analyzer (NAKL-132, PKK-TOA Corporation, Tokyo, Japan).

### Nutritional analysis of milk/meat

The general components (water content, protein, lipid, carbohydrate, ash content, calcium (only in milk) and cholesterol), amino acids (essential amino acids [isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine] and non-essential amino acids [histidine, arginine, alanine, aspartic acid, glutamic acid, glycine, proline and serine]) and fatty acids (essential fatty acids [linoleic acid, linolenic acid and arachdonic acid and others], decanoic acid, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, stearic acid, oleic acid, arachidic acid, icosenoic acid and icosatrienoic acid) were analyzed with milk/meat derived from the progeny and conventionally bred cattle. In milk samples, three other fatty acids (butyric acid, hexanoic acid and octanoic acid) were added. These analyses were carried out using Standard Tables of Food Composition in Japan[25]. As pretreatment of meat for the analyses, frozen meat was cut minutely and minced with a chopper. With regard to pretreatment of milk, each bulk of frozen milk, collected in the morning and at night, was thawed and mixed together at the ratio of the morning and night milk yield.

### Digestion test based on protein digestion rate in milk/meat with rats

#### 1) Animals, feeding condition and feces collection

Thirty-nine-week-old male SD rats [Crl:CD (SD)] (Charles River Japan Inc., Yokohama, Japan) weighing around 630 g, which could eat the prescribed amount of basal diet (AIN93M)[31] within seven days of pre-feeding test, reared under specific pathogen-free condition, were used for this study. Each test group consisting of five rats was fed one of the four test diets as shown above for eight days with water provided *ad libitum*. The rats were selected by stratified random sampling with their body weight. Each rat was kept in an individual stainless steel cage (260W mm × 380D mm × 180H mm) in an animal room with a barrier system, controlled temperature (22 ± 3℃)/humidity (55 ± 10%), ventilation about 10 times/hour (all fresh air) and a 12-hour light/dark cycle.

On the 4$^{th}$ and 7$^{th}$ days of the feeding period, test diets containing 0.1% carmine were fed to the rats for 24 hours. The feeding of a carmine-containing diet started at a fixed time. Food consumption was measured for 3 days. This measurement was also started on the 4$^{th}$ day and finished on the 7$^{th}$ day of the feeding period. The feces derived from diets ingested for these 3 days were collected on the basis of the red color of feces due to carmine.

#### 2) Measurement of digestion rates of protein in milk/meat and excreted feces

The amount of nitrogen in test diet containing milk/meat and the excreted feces was measured by the

modified macro-Kieldahl digestion[14]. The digestion rate was calculated with the following formula:
Digestion rate (%) = {[(total nitrogen in the consumed diet)−(total nitrogen in the excreted feces)]/(total nitrogen in the consumed diet)} × 100.

Significant difference between the sample group and the control group was calculated using Mann-Whitney's test.

### Detection of anaphylactic reaction in milk/meat samples by mouse abdominal wall method[17]

#### 1) Animals

Five-week-old ddy mice (Japan SLC, Inc., Hamamatsu, Japan) reared under specific pathogen-free conditions were used for this study. Each test group consisting of 10 mice was housed in a polycarbonate cage (210W mm × 320D mm, 130H mm) with wood chip bedding and fed commercial pellets (Lab. MR stock, Nihon Nosan Kogyo Ltd.) with water provided *ad libitum*. They were kept at 22 ± 3℃ and 55 ± 10% humidity in an animal room with a controlled barrier system.

Three mouse groups were assigned for detection of an anaphylactic reaction for milk samples with elicitation treatment. Another three mouse groups were also assigned for this detection without elicitation treatment. The contents of samples for the detection were milk, which was derived from the progeny of clones and conventionally bred cattle, and a positive control substance; therefore, six mouse groups were required for the detection of milk samples. The same assignments of mouse groups were also arranged for the detection of anaphylactic reaction in meat samples, which were derived from the progeny of clones and conventionally bred cattle, and a positive control substance.

#### 2) Preparation of milk/meat samples, sensitization solution and elicitation solution

For preparation of a sample solution, 5.0 g of milk/meat powder was added to 30 ml of saline. After the mixture was treated in a food mill, the total volume was adjusted to 50 ml with saline. The supernatant of the mixture was separated by centrifugation (3,000 rpm, 4℃, 20 minutes), filtrated under reduced pressure through #5A filter paper and sterilized with a 0.8 μm filter. After the protein concentration was adjusted to 1.3 mg/ml with saline, the filtrate was converted to emulsion by adding Freund's incomplete adjuvant (FIA) (Difco Laboratories Inc., Detroit, MI, USA) in equiponderance and used as a sensitization solution. Ten-fold diluted sensitization solution was used as the elicitation solution.

As the positive control substance, 2 mg/ml ovalbumin (OVA, Sigma-Aldrich, St. Louis, MI, USA) solution in saline was made into an emulsion by adding FIA. The elicitation solution for the positive control was prepared by diluting the ovalbumin solution to 0.1 mg/ml with saline.

#### 3) Sensitization and elicitation treatment

Sensitization was carried out by injecting 50 μl of sensitization solution intraperitoneally. Elicitation was performed after 14 days of sensitization. After 1% Evan's blue solution (100 μl/mouse) was injected through the tail vein, the abdominal wall was exposed under ether anesthesia. Five minutes after injection, elicitation solution (50 μl/site) was injected into the abdominal wall. The length and breadth of the dye leakage (forming a circular shape) on the abdominal wall was then measured 7 minutes after the abdominal wall injection.

#### 4) Evaluation of anaphylactic reaction and statistical analysis

An average of the minor and major axis of leakage was taken as the diameter. Anaphylactic reaction was evaluated by the diameter. Significant difference between the sample group and the control group was calculated using Student's *t*-test.

### Detection of mutagenicity in milk/meat by mouse micronucleus test[24]

#### 1) Animals, feeding condition and treatment

Eight-week-old ICR male mice ([Crj:CD-1 (ICR)] (Charles River Japan Inc., Yokohama, Japan) reared under specific pathogen-free conditions were used for this study. Each test group consisting of six mice was housed in a polycarbonate cage (210W mm × 320D mm, 130H mm) with wood chip bedding

and fed commercial pellets (Labo MR stock, Nosan Corporation, Kswasaki, Japan) with water provided *ad libitum*. They were kept at 22 ± 3℃ and 55 ± 10% humidity in an animal room with a controlled barrier system.

Six mouse groups were assigned for detection of mutagenicity in milk powder diets. Each of these groups was fed the test diet for 14 days as follows: basal diet (for negative control experiment), basal diet (for positive control experiment), diet supplemented with 2% (w/w) milk powder derived from the progeny, diet supplemented with 10% (w/w) milk powder derived from the progeny, diet supplemented with 2% (w/w) milk powder derived from conventionally bred cattle and diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle. For meat powder diets, another six mouse groups were prepared, each of which was fed the diet for 14 days as follows: basal diet (for negative control experiment), basal diet (for positive control experiment), diet supplemented with 1% (w/w) meat powder derived from the progeny, diet supplemented with 5% (w/w) meat powder derived from the progeny, diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle and diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle. For the positive control experiment groups, 2 mg/kg body weight mitomycin C (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was injected to mice intraperitoneally 24 hours before they were sacrificed.

#### 2) Preparation and examination of bone marrow smear

To prepare bone marrow smear, the mice were sacrificed by dislocation of the cervical vertebrae in the neck after the test diet feeding period. The femur bone was removed immediately and bone marrow cells were washed with fetal bovine serum (Nippon Biotest Laboratories Inc., Tokyo, Japan). The cells were collected by centrifugation at 1,000 rpm for 5 minutes, suspended in saline and smeared on a glass slide. After drying at room temperature, they were fixed with methanol for 5 minutes and stained using Giemsa solution.

These slides were observed through a microscope. The frequency of micronucleated cells was determined for one thousand polychromatic erythrocytes per specimen. Significant differences among the negative control group, test sample groups and positive control group were determined according to the Tables for Determining the Statistical Significance of Mutation Frequencies[16]. Simultaneously, the ratio of polychromatic erythrocytes to total erythrocytes was also calculated and analyzed by Kruskal-Wallis's rank test.

### Twelve-month feeding study combined with reproduction/development toxicity test in rats fed milk/meat powder-supplemented diets

#### 1) Animals and their feeding conditions

Five-week-old SD rats [Crl:CD (SD)] (Charles River Japan Inc., Yokohama, Japan) reared under specific pathogen-free condition were used for this study. Each rat group, which consisted of 12 females and 12 males, was fed one of the four test diets as shown above for 12 months with water provided *ad libitum*. Each rat was kept in an individual stainless steel mesh cage (260W mm × 380D mm × 180H mm) in an animal room with a barrier system, controlled temperature (22 ± 3℃) and humidity (55 ± 10%), ventilation about 10 times/hour (all fresh air) and a 12-hour light/dark cycle. These rats underwent the same observations and examinations (except for reproduction and development of newborns) as we described previously[15,45].

#### 2) Observations and examinations of rats during the feeding period

The animals were observed daily for clinical signs including examination of outer appearance, behavior, feces and general state. Detailed clinical observations were also carried out monthly.

Moreover, grip strength of forelimbs and hindlimbs (using a grip strength measuring device; MK380R/FR, Muromachi Kikai Co., Ltd., Tokyo, Japan), motor activity (using an auto-measuring system; Supermex, Muromachi Kikai Co., Ltd.) and sensory/reflex function (sound response, approach response, touch response, tail pinch response, pupil

reflex to light, pinna reflex, eyelid reflex, ipsilateral flexor reflex and righting reflex) were examined in the 3rd, 6th, 9th and 12th months of the feeding period [22].

To confirm normal growth, the rats were weighed at the beginning of the feeding period (on day 1 of the feeding period), every 7 days during the 52-week period and on the day of sacrifice. The 24-hour food consumption of the rats was also measured once a week. In females, these measurements were suspended during the reproduction/development test.

During the 12th month of feeding, examinations such as ophthalmology (anterior portion of the eye, chamber, optic media and ocular fundus) and urinalysis (color, pH, occult blood, protein, glucose, ketone body, bilirubin, urobilinogen, specific gravity and urine volume per 18 hours) were also performed.

**3) Hematology and clinical biochemistry of rats**

At the end of the feeding period, the rats were anesthetized and blood was collected from the abdominal aorta. The blood tests were performed as described above.

**4) Examinations of rats after autopsy**

After the rats were sacrificed by exsanguination, necropsy was performed and their organs were weighed. The weights are shown as absolute weights and relative weights (weight/100 g body weight). The organs investigated were as follows: brain, pituitary gland, thyroid gland, lung, heart, salivary glands (sublingual and submandibular), liver, spleen, kidney and adrenal gland for both sexes; testes, epididymis, prostrate and seminal vesicle for males; ovary and uterus for females. Histological examinations of the brain, pituitary gland, eyeball, Harderian gland, thyroid gland, parathyroid, spinal cord, heart, thymus, liver, kidney, spleen, trachea, lung, adrenal gland, salivary gland, tongue, esophagus, stomach, small intestine, large intestine, pancreas, urinary bladder, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, aorta, sciatic nerve, lymph nodes, bone, bone marrow, skeletal muscle, mammary gland and skin were also conducted.

**5) Reproduction of dams and observation of their pups**

The estrous cycles were examined by vaginal smear for 14 days comprising the 16th to 17th weeks of feeding in the female groups fed meat powder diets and the 11th to 12th weeks of feeding in the female groups fed milk powder diets. The females were mated with the same number of males for up to three weeks. After conceiving, the following items were examined in the dams: estrous cycle, copulation index, fertility index, gestation length and gestation index.

During the period from birth until weaning at 21 days, the following indices were examined in the pups: litter size, live birth index, sex ratio, body weight, viability index, lactation index, hair growth, pinna detachment, incisor eruption, eyelid opening, testicular descent, sensory response, reflex function tests, external abnormalities and visceral malformations.

**6) Statistical analyses**

For rats fed milk/meat powder diets derived from the progeny and conventionally bred cattle, statistical analyses of the origin of powders (the progeny and conventionally bred cattle) were carried out as follows. The F-test was conducted for parametric data (body weight, body weight gain, food intake, grip strength, mortar activity, estrous cycle, hematological data, clinical biochemistry and organ weight), Student's *t*-test was conducted for equal distribution data, Aspin-Welch's *t*-test was conducted for unequal distribution data, Mann-Whitney's U-test was conducted for non-parametric data (urine analyses) and Fisher's exact probability test (one-sided test)[8] or $\chi 2$ test for categorical data (clinical signs, autopsy and pathological examinations). The significance level was set to 5% in all analyses.

**Animal welfare and other guidelines**

Animal experimentation was carried out under approval of committee concerning animal care and ethics at RIAS. The study was also performed in accordance with procedures based on Good Laboratory Practice (GLP), the Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test of the Organization for Economic Co-operation and Development (OECD)[29] and the Guidelines for Designation of Food Additives and

for Revision of Standards for Use of Food Additives (Recommended Methods for One-year Toxicity Studies) of the government of Japan[26].

## RESULTS

### Blood properties in heifer produced meat (Hematology: Table 2, Clinical biochemistry: Tables 3-1, 3-2)

In four female progeny of somatic cell cloned cows (*T1*, *T2*, *T3* and *T4*) and three conventionally bred heifers (*B82*, *B89* and *B90*), platelet counts in *T1*, *T2* and *T4*, LDH in *T4* and Cl in *B90* were found to be higher than the reference ranges. Na and K in *B90* were within the reference ranges. Although occasionally high values were observed in leucocyte counts in *T1* and *T4*, AST in *T3* and ALP/γ-GTP in *T4*, their means were within the reference ranges. In the other parameters observed, almost all of the values were within the reference ranges. No remarkable abnormalities suggesting poor health status of the progeny were observed in the blood parameters investigated.

### Nutritional analysis of milk/meat

#### 1) General components

##### A) Milk (Fig. 1, Table 4)

Although protein and carbohydrate contents of milk derived from the progeny were found to be higher when compared with those of conventionally bred cattle, no significant differences in all components including water content, ash content, cholesterol and calcium due to the origin of milk were observed.

##### B) Meat (Fig. 2, Table 5)

The general components of meat differed

Table 2. Hematology parameters in progeny of somatic cell cloned cattle and conventionally bred cattle produced meat

| Heifer⌘ | Individual identification code | RBC ($10^4$ /μl) | Hemoglobin (g/dl) | Hematocrit (%) | MCV (fl) | MCH (pg) | MCHC (%) | Platelet ($10^4$ /μl) | PT (sec) | APTT (sec) |
|---|---|---|---|---|---|---|---|---|---|---|
| Progeny of cloned cattle | T1 | 806 | 15.4 | 42.5 | 53 | 19.1 | 36.1 | 45 | 14.8 | 69.4 |
| | T2 | 755 | 12.5 | 36.0 | 48 | 16.5 | 34.7 | 52 | 13.6 | 69.6 |
| | T3 | 775 | 14.3 | 41.5 | 54 | 18.5 | 34.6 | 36 | 14.4 | 66.9 |
| | T4 | 701 | 13.1 | 38.2 | 55 | 18.6 | 34.2 | 49 | 14.3 | 67.7 |
| Conventionally bred cattle | B82 | 643 | 11.6 | 33.0 | 52 | 18.1 | 35.2 | 33 | 13.2 | 86.3 |
| | B89 | 763 | 13.5 | 37.9 | 50 | 17.7 | 35.7 | 22 | 13.6 | 82.9 |
| | B90 | 647 | 10.7 | 30.4 | 47 | 16.5 | 35.2 | 21 | 13.2 | 91.8 |
| | Reference range⌘⌘ | 543-971 | 10.5-15.6 | 31.5-44.8 | 41-60 | 14.1-20.7 | 31.3-37.1 | 15-40 | 12.6-14.7 | 33.1-107.5 |

| Heifer⌘ | Individual identification code | WBC ($10^2$ /μl) | Differential leukocyte counts (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Neutrophil | | | |
| | | | Basophil | Eosinphil | Band | Segmented | Lymphocyte | Monocyte |
| Progeny of cloned cattle | T1 | 104 | 0 | 10 | 0 | 55 | 32 | 3 |
| | T2 | 65 | 1 | 8 | 0 | 26 | 62 | 2 |
| | T3 | 89 | 0 | 4 | 1 | 49 | 43 | 3 |
| | T4 | 140 | 1 | 8 | 4 | 47 | 38 | 3 |
| Conventionally bred cattle | B82 | 84 | 1 | 12 | 1 | 43 | 41 | 5 |
| | B89 | 84 | 0 | 6 | 1 | 32 | 59 | 3 |
| | B90 | 72 | 0 | 6 | 1 | 20 | 72 | 3 |
| | Reference range⌘⌘ | 50-112 | 0-1 | 0-16 | 0-2 | 19-63 | 30-69 | 0-7 |

Abbreviations : RBC, Red blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PT, Prothrombin time; APTT, Activated partial thromboplastin time; WBC, White blood cell

⌘: Heifers of fattened Japanese Black beef cattle.

⌘⌘: Reference ranges were calculated from background data obtained from 35 heifers of Japanese Black beef cattle.

Table 3-1. Clinical chemistry parameters in progeny of somatic cell cloned cattle and conventionally bred cattle produced meat

| Heifer[⌘] | Individual identification code | LDH (IU/l) | LDH-1 (%) | LDH-2 (%) | LDH-3 (%) | LDH-4 (%) | LDH-5 (%) | AST (IU/l) | ALT (IU/l) | ALP (IU/l) | γ-GTP (IU/l) | Creatine kinase (IU/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Progeny of cloned cattle | T1 | 5512 | 55.1 | 28.5 | 12.5 | 2.3 | 1.6 | 72 | 15 | 160 | 45 | 154 |
| | T2 | 5070 | 51.9 | 27.7 | 16.0 | 3.3 | 1.1 | 61 | 18 | 180 | 50 | 131 |
| | T3 | 6188 | 50.4 | 30.1 | 15.0 | 3.1 | 1.5 | 108 | 20 | 211 | 68 | 125 |
| | T4 | 7471 | 48.6 | 29.5 | 16.8 | 3.7 | 1.4 | 79 | 18 | 269 | 91 | 119 |
| Conventionally bred cattle | B82 | 4918 | 52.5 | 31.2 | 12.9 | 2.0 | 1.5 | 69 | 33 | 240 | 40 | 263 |
| | B89 | 4893 | 50.3 | 30.0 | 15.4 | 2.9 | 1.5 | 67 | 26 | 155 | 18 | 470 |
| | B90 | 3999 | 51.8 | 30.8 | 13.9 | 2.4 | 1.3 | 72 | 35 | 155 | 32 | 369 |
| | Reference range[⌘⌘] | 3042-6273 | 40.1-57.7 | 25.4-34.4 | 11.3-20.2 | 1.4-5.5 | 0.4-3.7 | 23-130 | 15-37 | 48-283 | 0-101 | 0-791 |

| Heifer[⌘] | Individual identification code | Choline esterase (IU/l) | Triglyceride (mg/dl) | Total cholesterol (mg/dl) | Phospholipid (mg/dl) | Total protein (g/dl) | Albumin (%) | α-Globulin (%) | β-Globulin (%) | γ-Globulin (%) | A/G ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Progeny of cloned cattle | T1 | 27 | 14 | 126 | 133 | 7.38 | 43.7 | 14.2 | 11.6 | 30.5 | 0.78 |
| | T2 | 29 | 16 | 83 | 91 | 6.55 | 49.1 | 14.1 | 12.3 | 24.5 | 0.97 |
| | T3 | 27 | 23 | 100 | 109 | 6.90 | 45.4 | 14.1 | 13.4 | 27.1 | 0.85 |
| | T4 | 32 | 23 | 99 | 110 | 7.11 | 40.0 | 14.7 | 13.8 | 31.4 | 0.67 |
| Conventionally bred cattle | B82 | 39 | 25 | 116 | 130 | 7.41 | 38.7 | 13.1 | 12.7 | 35.5 | 0.64 |
| | B89 | 38 | 32 | 112 | 122 | 7.16 | 35.8 | 14.9 | 12.9 | 36.5 | 0.56 |
| | B90 | 39 | 25 | 121 | 137 | 6.97 | 39.8 | 14.8 | 12.4 | 33.0 | 0.66 |
| | Reference range[⌘⌘] | 27-51 | 9-34 | 56-205 | 68-216 | 6.56-7.85 | 35.5-48.9 | 11.2-18.5 | 10.6-14.7 | 23.6-37.1 | 0.54-0.94 |

Abbreviations : LDH, Lactate dehydrogenase; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase γ-GTP, γ-Glutamyltranspeptidase; A/G, Albumin/globulin ratio

⌘: Heifers of fattened Japanese Black beef cattle.

⌘⌘: Reference ranges were calculated from background data obtained from 35 heifers of Japanese Black beef cattle.

Table 3-2. Clinical chemistry parameters in progeny of somatic cell cloned cattle and conventionally bred cattle produced meat (continued)

| Heifer[⌘] | Individual identification code | BUN (mg/dl) | Uric acid (mg/dl) | Glucose (mg/dl) | Creatinine (mg/dl) | Total bilirubin (mg/dl) | Calcium (mg/dl) | Inorganic phospharus (mg/dl) | Na (mEq/l) | K (mEq/l) | Cl (mEq/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Progeny of cloned cattle | T1 | 18.2 | 0.67 | 70 | 1.67 | 0.28 | 9.1 | 6.8 | 147 | 4.62 | 102 |
| | T2 | 16.5 | 0.57 | 67 | 1.58 | 0.28 | 9.2 | 6.6 | 146 | 4.53 | 105 |
| | T3 | 16.9 | 0.60 | 68 | 1.69 | 0.28 | 9.2 | 6.7 | 146 | 4.55 | 103 |
| | T4 | 16.8 | 0.65 | 69 | 1.51 | 0.28 | 9.0 | 7.1 | 145 | 4.66 | 103 |
| Conventionally bred cattle | B82 | 17.0 | 0.75 | 64 | 1.56 | 0.27 | 8.7 | 6.9 | 147 | 4.86 | 104 |
| | B89 | 20.9 | 0.88 | 68 | 1.71 | 0.26 | 8.5 | 7.8 | 147 | 4.79 | 104 |
| | B90 | 17.3 | 0.52 | 60 | 1.57 | 0.25 | 8.4 | 7.2 | 148 | 4.58 | 108 |
| | Reference range[⌘⌘] | 10.5-24.9 | 0.26-1.07 | 52-78 | 1.22-1.93 | 0.18-0.32 | 8.2-9.7 | 5.6-7.8 | 144-150 | 3.90-5.10 | 100-106 |

Abbreviations : BUN, Blood urea nitrogen; Na, Sodium; K, Potassium; Cl, Chlorine

⌘: Heifers of fattened Japanese Black beef cattle.

⌘⌘: Reference ranges were calculated from background data obtained from 35 heifers of Japanese Black beef cattle.

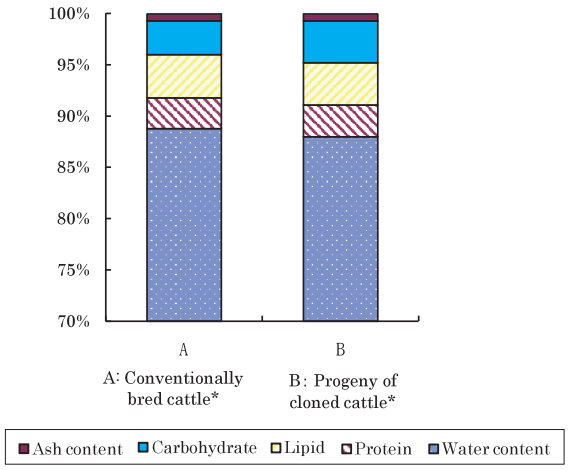


Fig. 1. Macronutrients of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle
*: Holstein dairy cattle
Columns represents the mean from six samples (two samples each in three cattle)

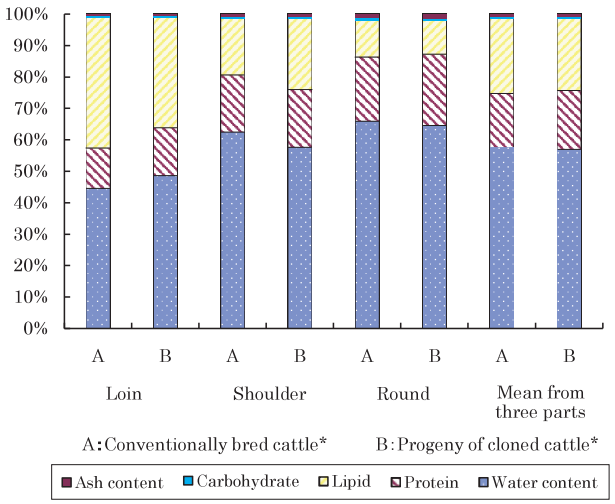


Fig. 2. Macronutrients of meat derived from conventionally bred cattle and progeny of somatic cell cloned cattle
*: Japanese Black beef cattle
Columns represents the mean from three carcasses

Table 4. General components of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle

| Sample | Individual identification code | | Water content | Protein | Lipid | Carbohydrate | Ash content | Calcium | Cholesterol |
|---|---|---|---|---|---|---|---|---|---|
| | | | g/100g | | | | | mg/100g | |
| Conventionally bred cattle⌘ | No.145 | I | 90.3 (87.7) | 2.8 (3.2) | 4.0 (3.7) | 2.2 (4.7) | 0.7 (0.7) | 116 (110) | 8 (12) |
| | | II | 89.3 | 2.9 | 4.1 | 3.0 | 0.7 | 117 | 9 |
| | No.152 | I | 87.8 | 2.7 | 3.8 | 5.0 | 0.7 | 99 | 9 |
| | | II | 87.6 | 3.1 | 4.4 | 4.2 | 0.7 | 109 | 9 |
| | No.153 | I | 89.0 | 3.1 | 3.6 | 3.6 | 0.7 | 110 | 9 |
| | | II | 88.4 | 3.4 | 5.5 | 2.0 | 0.7 | 118 | 8 |
| | Average | | 88.7 | 3.0 | 4.2 | 3.3 | 0.7 | 112 | 9 |
| Progeny of cloned cattle⌘ | No.148 | I | 88.1 (87.7) | 3.3 (3.2) | 4.0 (3.7) | 3.9 (4.7) | 0.7 (0.7) | 104 (110) | 7 (12) |
| | | II | 88.1 | 3.3 | 3.7 | 4.2 | 0.7 | 110 | 7 |
| | No.151 | I | 89.5 | 3.0 | 3.4 | 3.4 | 0.7 | 104 | 7 |
| | | II | 88.2 | 3.0 | 4.1 | 4.0 | 0.7 | 115 | 8 |
| | No.154 | I | 86.9 | 3.0 | 4.6 | 4.8 | 0.7 | 99 | 9 |
| | | II | 87.2 | 3.2 | 4.6 | 4.3 | 0.7 | 108 | 8 |
| | Average | | 88.0 | 3.1 | 4.1 | 4.1 | 0.7 | 107 | 8 |

⌘: Holstein dairy cattle
( ) : Standard value of milk composition of Holstein, quoted from " Standard Tables of Food Composition in Japan, (2005)[25]"
I : On 3 weeks after parturition
II : On 6 weeks after parturition

depending on the part of the carcass. Among *shoulder*, *loin* and *round*, the protein and water contents were lower in *loin* and higher in *round*. *Shoulder* showed intermediate levels. The micronutrients including carbohydrate, ash content and cholesterol in the meat were similar in the progeny and conventionally bred cattle.

### 2) Amino acid composition

#### A) Milk (Fig. 3, Table 6)

Glutamic acid was the most common amino acid in milk. The other major components in milk were proline, leucine, lysine and aspartic acid. Very small individual variances were observed in amino acid composition. No significant differences in amino acid composition due to the origin of milk were observed.

#### B) Meat (Fig. 4, Table 7-1, 7-2)

As was the case for milk, glutamic acid was the most common amino acid in meat. The other major components were lysine, aspartic acid, leucine and arginine. The amino acid constitutions of meat were

Table 5. General components of meat derived from conventionally bred cattle and progeny of somatic cell cloned cattle

| Sample | Region | Individual identification code | Water content | | Protein | | Lipid | | Carbohydrate | | Ash content | | Cholesterol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | g/100g | | | | | | | | | | mg/100g |
| Conventionally bred cattle⌘ | Shoulder | T1 | 63.6 | (66.3) | 18.0 | (20.2) | 17.4 | (12.2) | 0.4 | (0.3) | 0.9 | (1.0) | 66 (66) |
| | | T2 | 63.0 | | 17.7 | | 17.8 | | 0.5 | | 1.0 | | 69 |
| | | T4 | 61.8 | | 18.5 | | 18.9 | | 0.4 | | 1.0 | | 69 |
| | Loin | T1 | 38.5 | (55.9) | 10.6 | (17.1) | 50.5 | (25.8) | 0.3 | (0.4) | 0.5 | (0.8) | 74 (72) |
| | | T2 | 49.9 | | 14.4 | | 35.3 | | 0.3 | | 0.7 | | 71 |
| | | T4 | 45.9 | | 13.5 | | 39.4 | | 0.5 | | 0.7 | | 68 |
| | Round | T1 | 68.7 | (67.0) | 20.9 | (20.7) | 8.9 | (10.7) | 0.6 | (0.6) | 1.1 | (1.0) | 65 (68) |
| | | T2 | 66.8 | | 20.1 | | 11.3 | | 0.8 | | 1.3 | | 62 |
| | | T4 | 64.6 | | 20.4 | | 14.7 | | 0.6 | | 1.2 | | 76 |
| | Average | T1 | 56.9 | (63.1) | 16.5 | (19.3) | 25.6 | (16.2) | 0.4 | (0.4) | 0.8 | (0.9) | 68 (69) |
| | | T2 | 59.9 | | 17.4 | | 21.5 | | 0.5 | | 1.0 | | 67 |
| | | T4 | 57.4 | | 17.5 | | 24.3 | | 0.5 | | 1.0 | | 71 |
| Progeny of cloned cattle⌘ | Shoulder | B82 | 59.8 | (66.3) | 19.9 | (20.2) | 18.4 | (12.2) | 0.3 | (0.3) | 1.0 | (1.0) | 74 (66) |
| | | B89 | 52.2 | | 16.0 | | 29.6 | | 0.4 | | 0.9 | | 74 |
| | | B90 | 60.4 | | 19.0 | | 20.0 | | 0.3 | | 1.1 | | 72 |
| | Loin | B82 | 44.8 | (55.9) | 13.4 | (17.1) | 39.9 | (25.8) | 0.3 | (0.4) | 0.6 | (0.8) | 82 (72) |
| | | B89 | 46.0 | | 14.0 | | 39.6 | | 0.4 | | 0.7 | | 69 |
| | | B90 | 55.1 | | 18.2 | | 25.7 | | 0.4 | | 0.9 | | 66 |
| | Round | B82 | 66.6 | (67.0) | 23.8 | (20.7) | 9.3 | (10.7) | 0.5 | (0.6) | 1.5 | (1.0) | 72 (68) |
| | | B89 | 63.9 | | 21.5 | | 14.0 | | 0.7 | | 1.5 | | 70 |
| | | B90 | 67.1 | | 22.6 | | 9.2 | | 0.5 | | 1.2 | | 62 |
| | Average | B82 | 57.1 | (63.1) | 19.0 | (19.3) | 22.5 | (16.2) | 0.4 | (0.4) | 1.0 | (0.9) | 76 (69) |
| | | B89 | 54.0 | | 17.2 | | 27.7 | | 0.5 | | 1.0 | | 71 |
| | | B90 | 60.9 | | 19.9 | | 18.3 | | 0.4 | | 1.1 | | 67 |

⌘: Japanese Black beef cattle

( ) : Standard value of meat composition of Holstein, quoted from " Standard Tables of Food Composition in Japan, (2005)[25)]"

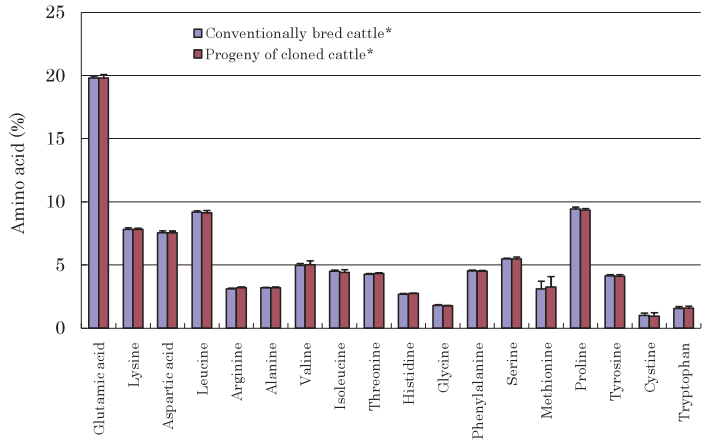


Fig. 3. Amino acid composition of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle

*: Holstein dairy cattle

Columns represents the mean±standard deviation from six samples (two samples each in three cattle)

Table 6. Amino acid composition of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle

(Upper: Amino acid composition (mg) per sample 100g; Sublevel:Amino acid composition (mg) per total nitrogen 1g;
Under: Amino acid composition (mg) per protein 1g)

| Sample | Individual identification code | Region | Protein | Isoleucine | Leucine | Lysine | Methionine | Cystine | Total | Phenylalanine | Tyrosine | Total | Threonine | Tryptophan | Valine | Histidine | Arginine | Alanine | Aspartic acid | Glutamic acid | Glycine | Proline | Serine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Sulphur Amino Acid | | | Aromatic Amino Acid | | | | | | | | | | | | | |
| | | | (g/100g) | | | | | | | | | | | | | | | | | | | | |
| Conventionally bred cattle※ | No.145 | I | 2.8 | 130 | 260 | 220 | 74 | 21 | 95 | 130 | 120 | 250 | 120 | 36 | 140 | 75 | 88 | 90 | 210 | 560 | 53 | 260 | 150 |
| | | | | 290 | 580 | 500 | 170 | 48 | 218 | 290 | 270 | 560 | 280 | 82 | 310 | 170 | 200 | 200 | 480 | 1,300 | 120 | 600 | 350 |
| | | | | 45 | 92 | 78 | 26 | 7.6 | 33.6 | 46 | 42 | 88 | 43 | 13 | 49 | 26 | 31 | 32 | 76 | 200 | 19 | 93 | 54 |
| | | II | 2.9 | 110 | 230 | 200 | 76 | 31 | 107 | 120 | 110 | 230 | 110 | 38 | 120 | 64 | 80 | 79 | 190 | 500 | 47 | 240 | 140 |
| | | | | 240 | 510 | 440 | 170 | 69 | 239 | 260 | 240 | 500 | 240 | 84 | 260 | 140 | 180 | 180 | 430 | 1,100 | 100 | 530 | 310 |
| | | | | 38 | 80 | 69 | 26 | 11 | 37 | 40 | 37 | 77 | 38 | 13 | 41 | 22 | 28 | 28 | 67 | 180 | 16 | 83 | 48 |
| | No.152 | I | 2.7 | 100 | 210 | 180 | 52 | 19 | 71 | 100 | 95 | 195 | 100 | 34 | 120 | 61 | 70 | 73 | 170 | 450 | 42 | 220 | 120 |
| | | | | 240 | 490 | 430 | 120 | 45 | 165 | 240 | 220 | 460 | 220 | 78 | 270 | 140 | 160 | 170 | 400 | 1,000 | 98 | 510 | 290 |
| | | | | 38 | 76 | 64 | 19 | 7.1 | 26.1 | 38 | 35 | 73 | 35 | 12 | 42 | 22 | 26 | 27 | 62 | 160 | 16 | 81 | 44 |
| | | II | 3.1 | 92 | 180 | 150 | 83 | 23 | 106 | 90 | 81 | 171 | 85 | 34 | 100 | 54 | 62 | 63 | 150 | 400 | 36 | 190 | 110 |
| | | | | 190 | 370 | 310 | 170 | 48 | 218 | 190 | 170 | 360 | 170 | 70 | 210 | 110 | 130 | 130 | 300 | 820 | 73 | 380 | 230 |
| | | | | 30 | 58 | 49 | 27 | 7.5 | 34.5 | 29 | 26 | 55 | 27 | 11 | 33 | 17 | 20 | 20 | 47 | 130 | 12 | 61 | 35 |
| | No.153 | I | 3.1 | 110 | 210 | 180 | 70 | 27 | 97 | 100 | 100 | 200 | 100 | 41 | 120 | 63 | 72 | 74 | 170 | 450 | 40 | 210 | 130 |
| | | | | 220 | 440 | 370 | 150 | 57 | 207 | 220 | 200 | 420 | 210 | 86 | 250 | 130 | 150 | 150 | 360 | 940 | 84 | 450 | 260 |
| | | | | 35 | 69 | 60 | 23 | 8.9 | 31.9 | 34 | 31 | 65 | 32 | 13 | 39 | 21 | 24 | 24 | 57 | 150 | 13 | 68 | 42 |
| | | II | 3.4 | 120 | 260 | 220 | 95 | 25 | 120 | 120 | 110 | 230 | 120 | 41 | 130 | 73 | 83 | 87 | 210 | 540 | 48 | 260 | 150 |
| | | | | 230 | 490 | 410 | 180 | 48 | 228 | 230 | 210 | 440 | 220 | 77 | 250 | 140 | 160 | 160 | 400 | 1,000 | 91 | 490 | 280 |
| | | | | 35 | 76 | 64 | 28 | 7.5 | 35.5 | 36 | 33 | 69 | 34 | 12 | 39 | 22 | 25 | 26 | 62 | 160 | 14 | 76 | 44 |
| Progeny of cloned cattle※ | No.148 | I | 3.3 | 100 | 230 | 200 | 95 | 27 | 122 | 120 | 110 | 230 | 110 | 40 | 110 | 71 | 81 | 83 | 200 | 510 | 47 | 240 | 140 |
| | | | | 190 | 450 | 380 | 180 | 52 | 232 | 220 | 200 | 420 | 210 | 77 | 220 | 140 | 160 | 160 | 380 | 980 | 89 | 470 | 270 |
| | | | | 30 | 70 | 59 | 28 | 8.1 | 36.1 | 35 | 31 | 66 | 33 | 12 | 34 | 21 | 24 | 25 | 60 | 150 | 14 | 73 | 42 |
| | | II | 3.3 | 140 | 290 | 240 | 81 | 21 | 102 | 140 | 130 | 270 | 130 | 44 | 160 | 83 | 96 | 99 | 230 | 610 | 54 | 290 | 170 |
| | | | | 260 | 550 | 460 | 160 | 41 | 201 | 270 | 240 | 510 | 250 | 84 | 300 | 160 | 180 | 190 | 440 | 1,200 | 100 | 550 | 320 |
| | | | | 41 | 86 | 72 | 24 | 6.4 | 30.4 | 42 | 38 | 80 | 40 | 13 | 47 | 25 | 29 | 30 | 70 | 180 | 16 | 86 | 50 |
| | No.151 | I | 3.0 | 120 | 230 | 210 | 66 | 23 | 89 | 120 | 110 | 230 | 120 | 39 | 130 | 71 | 84 | 83 | 200 | 520 | 47 | 250 | 150 |
| | | | | 250 | 500 | 440 | 140 | 50 | 190 | 250 | 230 | 480 | 250 | 84 | 280 | 150 | 180 | 180 | 420 | 1,100 | 100 | 530 | 320 |
| | | | | 39 | 79 | 69 | 22 | 7.8 | 29.8 | 39 | 36 | 75 | 39 | 13 | 45 | 24 | 28 | 28 | 66 | 170 | 16 | 82 | 50 |
| | | II | 3.0 | 110 | 220 | 190 | 85 | 21 | 106 | 110 | 100 | 210 | 110 | 40 | 130 | 67 | 80 | 76 | 180 | 490 | 42 | 220 | 140 |
| | | | | 230 | 470 | 400 | 180 | 45 | 225 | 230 | 210 | 440 | 220 | 84 | 270 | 140 | 170 | 160 | 380 | 1,000 | 89 | 470 | 290 |
| | | | | 36 | 73 | 63 | 28 | 7.0 | 35.0 | 36 | 33 | 69 | 35 | 13 | 42 | 22 | 26 | 25 | 60 | 160 | 14 | 74 | 45 |
| | No.154 | I | 3.0 | 85 | 170 | 150 | 91 | 29 | 120 | 87 | 77 | 164 | 82 | 38 | 100 | 54 | 60 | 61 | 140 | 380 | 34 | 180 | 100 |
| | | | | 180 | 370 | 320 | 190 | 62 | 252 | 180 | 160 | 340 | 170 | 80 | 210 | 120 | 130 | 130 | 310 | 800 | 72 | 390 | 220 |
| | | | | 28 | 58 | 50 | 30 | 9.6 | 39.6 | 29 | 26 | 55 | 27 | 12 | 32 | 18 | 20 | 20 | 48 | 130 | 11 | 61 | 35 |
| | | II | 3.2 | 140 | 280 | 240 | 79 | 21 | 100 | 140 | 130 | 270 | 130 | 42 | 160 | 84 | 97 | 98 | 230 | 600 | 53 | 280 | 160 |
| | | | | 280 | 570 | 480 | 160 | 43 | 203 | 280 | 260 | 540 | 260 | 85 | 320 | 170 | 200 | 200 | 460 | 1,200 | 110 | 570 | 330 |
| | | | | 44 | 89 | 76 | 25 | 6.7 | 31.7 | 44 | 41 | 85 | 41 | 13 | 51 | 27 | 31 | 31 | 73 | 190 | 17 | 89 | 51 |

※ : Holstein dairy cows
I : On 3 weeks after parturition
II : On 6 weeks after parturition

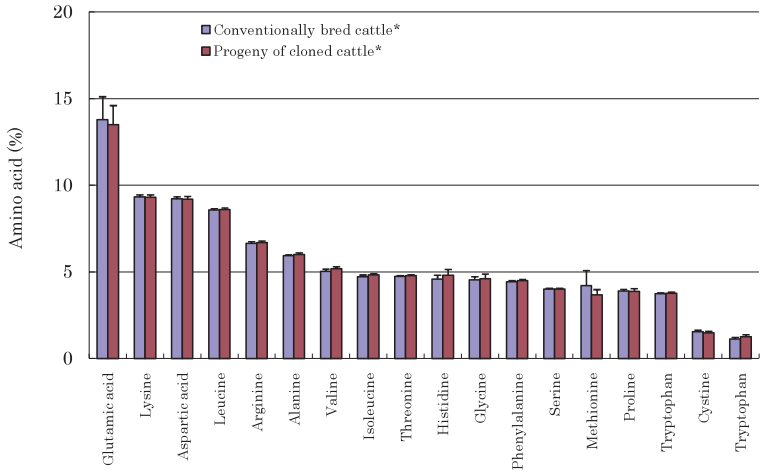


Fig. 4. Amino acid composition of meat derived from conventionally bred cattle and progeny of somatic cell cloned cattle
*: Japanese Black beef cattle
Columns represents the mean±standard deviation from nine samples (three parts each in three carcasses)

Table 7-1. Amino acid composition of meat derived from conventionally bred cattle⌘

(Upper: Amino acid composition (mg) per sample 100g; Sublevel: Amino acid composition (mg) per total nitrogen 1g; Under: Amino acid composition (mg) per protein 1g)

| Individual identification code | Region | Protein | Isoleucine | Leucine | Lysine | Methionine | Cystine | Total | Phenylalanine | Tyrosine | Total | Threonine | Tryptophan | Valine | Histidine | Arginine | Alanine | Aspartic acid | Glutamic acid | Glycine | Proline | Serine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (g/100g) | | | | Sulphur Amino Acid | | | Aromatic Amino Acid | | | | | | | | | | | | | |
| T1 | Shoulder | 18.0 | 810 | 1,500 | 1,600 | 660 | 260 | 920 | 760 | 640 | 1,400 | 820 | 210 | 870 | 770 | 1,200 | 1,000 | 1,600 | 2,400 | 830 | 700 | 690 |
| | | | 280 | 510 | 560 | 230 | 92 | 322 | 260 | 220 | 480 | 280 | 74 | 300 | 270 | 400 | 360 | 560 | 850 | 290 | 240 | 240 |
| | | | 45 | 82 | 90 | 37 | 15 | 52 | 42 | 36 | 78 | 45 | 12 | 48 | 43 | 64 | 58 | 89 | 140 | 46 | 39 | 39 |
| | Loin | 10.6 | 450 | 840 | 920 | 630 | 170 | 800 | 440 | 370 | 810 | 470 | 97 | 480 | 490 | 650 | 580 | 880 | 1,200 | 440 | 390 | 400 |
| | | | 260 | 500 | 540 | 370 | 98 | 468 | 260 | 220 | 480 | 280 | 57 | 290 | 290 | 380 | 340 | 520 | 680 | 260 | 230 | 240 |
| | | | 42 | 80 | 87 | 59 | 16 | 75 | 41 | 35 | 76 | 44 | 9.1 | 46 | 46 | 61 | 54 | 83 | 110 | 42 | 37 | 38 |
| | Round | 20.9 | 950 | 1,800 | 1,900 | 760 | 310 | 1,070 | 900 | 760 | 1,660 | 970 | 220 | 1,000 | 900 | 1,300 | 1,200 | 1,900 | 3,100 | 870 | 770 | 820 |
| | | | 280 | 520 | 570 | 230 | 93 | 323 | 270 | 230 | 500 | 290 | 66 | 300 | 270 | 400 | 360 | 580 | 930 | 260 | 230 | 240 |
| | | | 45 | 84 | 91 | 36 | 15 | 51 | 43 | 36 | 79 | 47 | 11 | 48 | 43 | 64 | 58 | 92 | 150 | 42 | 37 | 39 |
| T2 | Shoulder | 17.7 | 840 | 1,500 | 1,700 | 700 | 270 | 970 | 780 | 660 | 1,440 | 840 | 210 | 900 | 780 | 1,200 | 1,000 | 1,600 | 2,500 | 800 | 680 | 700 |
| | | | 300 | 540 | 590 | 250 | 95 | 345 | 280 | 230 | 510 | 300 | 75 | 320 | 280 | 420 | 370 | 580 | 890 | 280 | 240 | 250 |
| | | | 48 | 86 | 94 | 39 | 15 | 54 | 44 | 37 | 81 | 47 | 12 | 51 | 44 | 67 | 59 | 92 | 140 | 45 | 38 | 40 |
| | Loin | 14.4 | 660 | 1,200 | 1,300 | 590 | 230 | 820 | 620 | 520 | 1,140 | 660 | 150 | 710 | 670 | 930 | 810 | 1,200 | 1,600 | 630 | 540 | 550 |
| | | | 290 | 510 | 560 | 260 | 98 | 358 | 270 | 220 | 490 | 290 | 63 | 310 | 290 | 400 | 350 | 540 | 710 | 270 | 230 | 240 |
| | | | 46 | 82 | 90 | 41 | 16 | 57 | 43 | 36 | 79 | 46 | 10 | 50 | 47 | 64 | 56 | 86 | 110 | 44 | 37 | 38 |
| | Round | 20.1 | 960 | 1,700 | 1,900 | 740 | 290 | 1,030 | 900 | 750 | 1,650 | 960 | 240 | 1,000 | 910 | 1,300 | 1,200 | 1,900 | 3,000 | 960 | 800 | 810 |
| | | | 300 | 540 | 580 | 230 | 90 | 320 | 280 | 230 | 510 | 300 | 75 | 310 | 280 | 420 | 380 | 590 | 950 | 300 | 250 | 250 |
| | | | 47 | 86 | 93 | 37 | 14 | 51 | 45 | 37 | 82 | 48 | 12 | 50 | 45 | 67 | 61 | 95 | 150 | 48 | 40 | 40 |
| T4 | Shoulder | 18.5 | 850 | 1,500 | 1,600 | 740 | 280 | 1,020 | 780 | 660 | 1,440 | 840 | 200 | 900 | 770 | 1,200 | 1,100 | 1,600 | 2,600 | 840 | 700 | 700 |
| | | | 290 | 510 | 560 | 250 | 94 | 344 | 260 | 220 | 480 | 280 | 67 | 300 | 260 | 400 | 360 | 550 | 870 | 290 | 240 | 240 |
| | | | 46 | 82 | 89 | 40 | 15 | 55 | 42 | 35 | 77 | 45 | 11 | 49 | 42 | 64 | 58 | 89 | 140 | 46 | 38 | 38 |
| | Loin | 13.5 | 630 | 1,100 | 1,200 | 570 | 210 | 780 | 580 | 500 | 1,080 | 630 | 130 | 670 | 610 | 880 | 770 | 1,200 | 1,600 | 580 | 500 | 530 |
| | | | 290 | 520 | 570 | 270 | 97 | 367 | 270 | 230 | 500 | 290 | 58 | 310 | 280 | 410 | 360 | 550 | 760 | 270 | 230 | 240 |
| | | | 47 | 84 | 91 | 42 | 15 | 57 | 43 | 37 | 80 | 46 | 9.3 | 50 | 45 | 65 | 57 | 88 | 120 | 43 | 37 | 39 |
| | Round | 20.4 | 970 | 1,700 | 1,900 | 740 | 290 | 1,030 | 910 | 760 | 1,670 | 970 | 250 | 1,000 | 950 | 1,300 | 1,200 | 1,900 | 3,000 | 890 | 750 | 800 |
| | | | 300 | 530 | 580 | 230 | 89 | 319 | 280 | 230 | 510 | 300 | 76 | 310 | 290 | 410 | 370 | 580 | 930 | 270 | 230 | 250 |
| | | | 48 | 85 | 92 | 36 | 14 | 50 | 45 | 37 | 82 | 47 | 12 | 50 | 46 | 65 | 59 | 93 | 150 | 44 | 37 | 39 |

⌘: Japanese Black beef cattle

Table 7-2. Amino acid composition of meat derived from progeny of somatic cell cloned cattle※ (continued)

(Upper: Amino acid composition (mg) per sample 100g; Sublevel:Amino acid composition (mg) per total nitrogen 1g;
Under: Amino acid composition(mg) per protein 1g)

| Individual identification code | Region | Protein | Isoleucine | Leucine | Lysine | Methionine | Cystine | Total | Phenylalanine | Tyrosine | Total | Threonine | Tryptophan | Valine | Histidine | Arginine | Alanine | Aspartic acid | Glutamic acid | Glycine | Proline | Serine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (g/100g) | | | | Sulphur Amino Acid | | | Aromatic Amino Acid | | | | | | | | | | | | | |
| B82 | Shoulder | 19.9 | 890 | 1,600 | 1,700 | 620 | 260 | 880 | 830 | 690 | 1,520 | 890 | 240 | 970 | 830 | 1,300 | 1,200 | 1,700 | 2,600 | 990 | 790 | 760 |
| | | | 280 | 510 | 540 | 200 | 82 | 282 | 260 | 220 | 480 | 280 | 75 | 300 | 260 | 400 | 370 | 550 | 830 | 310 | 250 | 240 |
| | | | 45 | 81 | 87 | 31 | 13 | 44 | 42 | 35 | 77 | 45 | 12 | 48 | 42 | 65 | 59 | 87 | 130 | 50 | 40 | 38 |
| | Loin | 13.4 | 640 | 1,100 | 1,200 | 480 | 190 | 670 | 590 | 490 | 1,080 | 620 | 140 | 700 | 660 | 880 | 790 | 1,200 | 1,500 | 630 | 500 | 530 |
| | | | 300 | 520 | 570 | 220 | 88 | 308 | 270 | 230 | 500 | 290 | 64 | 320 | 310 | 410 | 370 | 550 | 690 | 290 | 230 | 240 |
| | | | 47 | 84 | 91 | 36 | 14 | 50 | 44 | 36 | 80 | 46 | 10 | 52 | 49 | 65 | 59 | 87 | 110 | 47 | 37 | 39 |
| | Round | 23.8 | 1,100 | 2,000 | 2,200 | 860 | 350 | 1,210 | 1,000 | 880 | 1,880 | 1,100 | 330 | 1,200 | 1,100 | 1,600 | 1,400 | 2,200 | 3,500 | 1,100 | 890 | 930 |
| | | | 300 | 530 | 570 | 220 | 93 | 313 | 270 | 230 | 500 | 290 | 88 | 320 | 280 | 410 | 370 | 580 | 910 | 280 | 230 | 240 |
| | | | 48 | 84 | 91 | 36 | 15 | 51 | 44 | 37 | 81 | 47 | 14 | 50 | 44 | 65 | 59 | 93 | 150 | 44 | 37 | 39 |
| B89 | Shoulder | 16.0 | 740 | 1,300 | 1,400 | 610 | 240 | 850 | 680 | 580 | 1,260 | 740 | 190 | 800 | 700 | 1,000 | 920 | 1,400 | 2,000 | 690 | 580 | 620 |
| | | | 290 | 520 | 560 | 240 | 94 | 334 | 270 | 230 | 500 | 290 | 74 | 310 | 270 | 410 | 360 | 560 | 780 | 270 | 230 | 240 |
| | | | 47 | 83 | 90 | 38 | 15 | 53 | 43 | 36 | 79 | 46 | 12 | 50 | 44 | 65 | 57 | 89 | 130 | 43 | 36 | 39 |
| | Loin | 14.0 | 630 | 1,100 | 1,200 | 560 | 200 | 760 | 590 | 490 | 1,080 | 630 | 150 | 670 | 660 | 880 | 780 | 1,200 | 1,700 | 610 | 510 | 530 |
| | | | 280 | 500 | 540 | 250 | 89 | 339 | 260 | 220 | 480 | 280 | 66 | 300 | 300 | 390 | 350 | 520 | 770 | 270 | 230 | 240 |
| | | | 45 | 80 | 87 | 40 | 14 | 54 | 42 | 35 | 77 | 45 | 11 | 48 | 47 | 63 | 56 | 84 | 120 | 43 | 36 | 38 |
| | Round | 21.5 | 1,000 | 1,800 | 2,000 | 750 | 310 | 1,060 | 960 | 800 | 1,760 | 1,000 | 260 | 1,100 | 1,000 | 1,400 | 1,300 | 2,000 | 3,000 | 950 | 810 | 840 |
| | | | 300 | 540 | 580 | 220 | 89 | 309 | 280 | 230 | 510 | 300 | 77 | 330 | 290 | 420 | 370 | 580 | 880 | 280 | 240 | 240 |
| | | | 49 | 86 | 82 | 35 | 14 | 49 | 45 | 37 | 82 | 48 | 12 | 52 | 47 | 67 | 59 | 93 | 140 | 44 | 38 | 39 |
| B90 | Shoulder | 19.0 | 900 | 1,600 | 1,700 | 660 | 280 | 940 | 840 | 690 | 1,530 | 880 | 250 | 960 | 880 | 1,200 | 1,100 | 1,700 | 2,600 | 820 | 700 | 740 |
| | | | 300 | 530 | 570 | 220 | 93 | 313 | 280 | 230 | 510 | 290 | 83 | 320 | 290 | 410 | 370 | 570 | 840 | 270 | 230 | 240 |
| | | | 47 | 85 | 92 | 35 | 15 | 49 | 44 | 37 | 81 | 47 | 13 | 51 | 46 | 65 | 59 | 91 | 130 | 43 | 37 | 39 |
| | Loin | 18.2 | 790 | 1,400 | 1,500 | 620 | 260 | 880 | 760 | 650 | 1,410 | 790 | 230 | 850 | 900 | 1,100 | 970 | 1,500 | 2,100 | 740 | 670 | 670 |
| | | | 270 | 480 | 530 | 210 | 91 | 301 | 260 | 220 | 480 | 270 | 77 | 290 | 310 | 380 | 330 | 510 | 700 | 250 | 230 | 230 |
| | | | 43 | 77 | 84 | 34 | 14 | 48 | 41 | 35 | 76 | 44 | 12 | 46 | 49 | 60 | 53 | 81 | 110 | 41 | 37 | 37 |
| | Round | 22.6 | 1,100 | 2,000 | 2,100 | 770 | 310 | 1,080 | 1,000 | 850 | 1,850 | 1,100 | 300 | 1,200 | 1,100 | 1,500 | 1,400 | 2,100 | 3,500 | 1,000 | 850 | 910 |
| | | | 300 | 540 | 580 | 210 | 86 | 296 | 280 | 230 | 510 | 300 | 83 | 320 | 290 | 420 | 380 | 590 | 950 | 280 | 230 | 250 |
| | | | 49 | 86 | 92 | 34 | 14 | 48 | 45 | 38 | 83 | 48 | 13 | 51 | 47 | 67 | 60 | 95 | 150 | 44 | 37 | 40 |

※: Japanese Black beef cattle

different from those of milk; proline was not a major component. Small variances in amino acid constitution were observed in individuals and among meat regions. No significant differences in amino acid composition due to the origin of meat were observed.

### 3) Fatty acid composition

#### A) Milk (Fig. 5, Table 8)

Palmitic acid and oleic acid were major components of milk. It was also rich in myristic acid and stearic acid. Individual differences were observed in the fatty acid composition of milk. The individual differences in fatty acid composition were larger than those in amino acid composition. The proportions of fatty acids in milk were similar between the progeny and conventionally bred cattle.

#### B) Meat (Fig. 6, Tables 9-1, 9-2)

The contents of palmitic acid and oleic acid were higher than those of other components. Although individual and regional differences were observed in fatty acid content, the proportions of fatty acids in meat were similar between the progeny and conventionally bred cattle.

### 4) Nutritional analysis of freeze-dried milk/meat powder (Tables 10, 11)

For the freeze-dried milk/meat powder that was prepared for test diets, no differences in vitamin and ash contents due to the origin of meat were found.

### 5) Conclusions

No differences in milk/meat compositions due to the origin of milk/meat were observed.

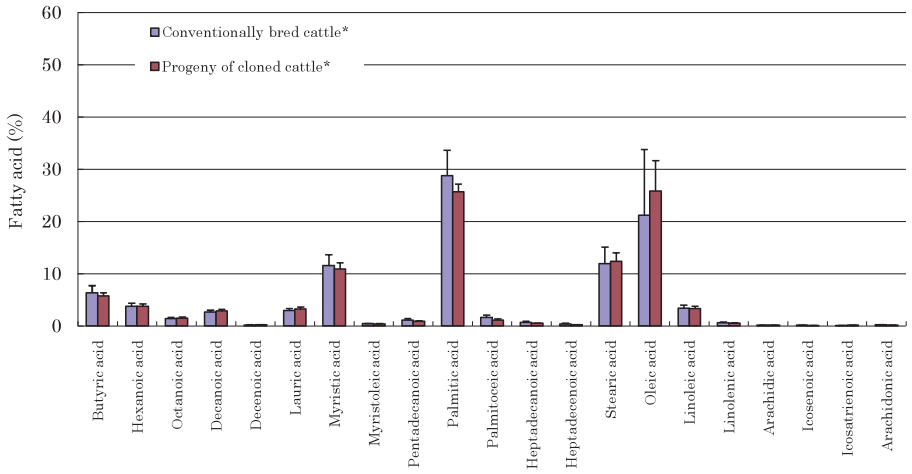


Fig. 5. Fatty acid composition of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle
*: Holstein dairy cattle
Columns represents the mean±standard deviation from six samples (two samples each in three cattle)

Table 8. Fatty acid composition of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle

(Upper: Fatty acid (mg) in sample 100g; Under: Fatty acid (g) per fatty acid gross 100g)

| Sample | Individual identification code | | Lipid quantity (g/100g) | Fatty acid composition (%) Saturated | Unsaturated | 4:0 Butyric acid | 6:0 Hexanoic acid | 8:0 Octanoic acid | 10:0 Decanoic acid | 10:1 Decenoic acid | 12:0 Lauric acid | 14:0 Myristic acid | 14:1 Myristoleic acid | 15:0 Pentadecanoic acid | 16:0 Palmitic acid | 16:1 Palmitoleic acid | 17:0 Heptadecanoic acid | 17:1 Heptadecenoic acid | 18:0 Stearic acid | 18:1 Oleic acid | 18:2 Linoleic acid | 18:3 Linolenic acid | 20:0 Arachidic acid | 20:1 Icosenoic acid | 20:3 Icosatrienoic acid | 20:4 Arachidonic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conventionally bred cattle⌘ | No.145 | I | 3.6 | 87.0 | 13.0 | **223** | **113** | **39** | **72** | **4** | **74** | **291** | **13** | **32** | **843** | **63** | **29** | **18** | **452** | **78** | **108** | **25** | **6** | **6** | **3** | **7** |
| | | | | | | 8.9 | 4.5 | 1.6 | 2.9 | 0.2 | 3.0 | 11.6 | 0.5 | 1.3 | 33.7 | 2.5 | 1.2 | 0.7 | 18.1 | 3.1 | 4.3 | 1.0 | 0.2 | 0.2 | 0.1 | 0.3 |
| | | II | 4.1 | 58.1 | 41.9 | **248** | **139** | **53** | **102** | **6** | **109** | **416** | **16** | **42** | **1070** | **67** | **31** | **17** | **601** | **1750** | **129** | **27** | **9** | **8** | **5** | **9** |
| | | | | | | 5.1 | 2.9 | 1.1 | 2.1 | 0.1 | 2.2 | 8.6 | 0.3 | 0.9 | 22.0 | 1.4 | 0.6 | 0.4 | 12.4 | 36.1 | 2.7 | 0.6 | 0.2 | 0.2 | 0.1 | 0.2 |
| | No.152 | I | 3.6 | 73.3 | 26.7 | **226** | **138** | **55** | **100** | **7** | **110** | **453** | **15** | **51** | **1050** | **60** | **24** | **10** | **334** | **699** | **102** | **18** | **5** | **5** | **5** | **7** |
| | | | | | | 6.5 | 4.0 | 1.6 | 2.9 | 0.2 | 3.2 | 13.0 | 0.4 | 1.5 | 30.2 | 1.7 | 0.7 | 0.3 | 9.6 | 20.1 | 2.9 | 0.5 | 0.1 | 0.1 | 0.1 | 0.2 |
| | | II | 3.8 | 78.7 | 21.3 | **213** | **141** | **55** | **106** | **9** | **120** | **487** | **18** | **48** | **1140** | **59** | **22** | **10** | **391** | **481** | **126** | **18** | **6** | **4** | **6** | **7** |
| | | | | | | 6.1 | 4.1 | 1.6 | 3.1 | 0.3 | 3.5 | 14.0 | 0.5 | 1.4 | 32.9 | 1.7 | 0.6 | 0.3 | 11.3 | 13.9 | 3.6 | 0.5 | 0.2 | 0.1 | 0.2 | 0.2 |
| | No.153 | I | 3.5 | 73.3 | 26.7 | **263** | **165** | **64** | **115** | **11** | **135** | **522** | **21** | **47** | **1260** | **58** | **29** | **13** | **439** | **800** | **158** | **26** | **7** | **7** | **6** | **11** |
| | | | | | | 6.3 | 4.0 | 1.5 | 2.8 | 0.3 | 3.2 | 12.6 | 0.5 | 1.1 | 30.3 | 1.4 | 0.7 | 0.3 | 10.6 | 19.2 | 3.8 | 0.6 | 0.2 | 0.2 | 0.1 | 0.3 |
| | | II | 3.7 | 59.3 | 40.7 | **227** | **141** | **56** | **106** | **9** | **117** | **425** | **16** | **36** | **1030** | **41** | **23** | **9** | **431** | **1530** | **134** | **22** | **6** | **7** | **5** | **8** |
| | | | | | | 5.2 | 3.2 | 1.3 | 2.4 | 0.2 | 2.7 | 9.7 | 0.4 | 0.8 | 23.5 | 0.9 | 0.5 | 0.2 | 9.8 | 34.9 | 3.1 | 0.5 | 0.1 | 0.2 | 0.1 | 0.2 |
| Progeny of cloned cattle⌘ | No.148 | I | 3.6 | 66.6 | 33.4 | **223** | **146** | **59** | **110** | **10** | **123** | **384** | **17** | **29** | **929** | **47** | **19** | **10** | **449** | **1030** | **95** | **16** | **6** | **6** | **4** | **7** |
| | | | | | | 6.0 | 3.9 | 1.6 | 3.0 | 0.3 | 3.3 | 10.3 | 0.5 | 0.8 | 25.0 | 1.3 | 0.5 | 0.3 | 12.1 | 27.7 | 2.6 | 0.4 | 0.2 | 0.2 | 0.1 | 0.2 |
| | | II | 3.5 | 62.1 | 37.9 | **197** | **131** | **52** | **99** | **10** | **110** | **379** | **20** | **30** | **1000** | **59** | **20** | **12** | **397** | **1230** | **109** | **17** | **6** | **6** | **5** | **8** |
| | | | | | | 5.1 | 3.4 | 1.3 | 2.5 | 0.3 | 2.8 | 9.7 | 0.5 | 0.8 | 25.7 | 1.5 | 0.5 | 0.3 | 10.2 | 31.6 | 2.8 | 0.4 | 0.2 | 0.2 | 0.1 | 0.2 |
| | No.151 | I | 2.4 | 73.8 | 26.2 | **132** | **92** | **38** | **71** | **6** | **81** | **259** | **8** | **21** | **545** | **18** | **10** | **3** | **271** | **406** | **79** | **13** | **4** | **2** | **3** | **4** |
| | | | | | | 6.4 | 4.5 | 1.8 | 3.4 | 0.3 | 3.9 | 12.5 | 0.4 | 1.0 | 26.4 | 0.9 | 0.5 | 0.1 | 13.1 | 19.7 | 3.8 | 0.6 | 0.2 | 0.1 | 0.1 | 0.2 |
| | | II | 4.2 | 72.8 | 27.2 | **247** | **156** | **59** | **114** | **8** | **125** | **473** | **12** | **40** | **1100** | **35** | **22** | **7** | **613** | **858** | **142** | **20** | **9** | **4** | **8** | **9** |
| | | | | | | 6.1 | 3.8 | 1.5 | 2.8 | 0.2 | 3.1 | 11.6 | 0.3 | 1.0 | 27.1 | 0.9 | 0.5 | 0.2 | 15.1 | 21.1 | 3.5 | 0.5 | 0.2 | 0.1 | 0.2 | 0.2 |
| | No.154 | I | 3.8 | 71.4 | 28.6 | **217** | **142** | **57** | **108** | **9** | **123** | **414** | **16** | **34** | **954** | **43** | **20** | **7** | **443** | **762** | **134** | **22** | **6** | **4** | **5** | **8** |
| | | | | | | 6.2 | 4.0 | 1.6 | 3.1 | 0.3 | 3.5 | 11.7 | 0.5 | 1.0 | 27.0 | 1.2 | 0.6 | 0.2 | 12.6 | 21.6 | 3.8 | 0.6 | 0.2 | 0.1 | 0.1 | 0.2 |
| | | II | 3.9 | 60.2 | 39.8 | **204** | **131** | **53** | **100** | **9** | **115** | **392** | **16** | **34** | **951** | **48** | **21** | **10** | **459** | **1370** | **140** | **21** | **6** | **5** | **5** | **8** |
| | | | | | | 5.0 | 3.2 | 1.3 | 2.4 | 0.2 | 2.8 | 9.6 | 0.4 | 0.8 | 23.2 | 1.2 | 0.5 | 0.2 | 11.2 | 33.4 | 3.4 | 0.5 | 0.1 | 0.1 | 0.1 | 0.2 |

⌘: Holstein dairy cattle
I : On 3 weeks after parturition
II : On 6 weeks after parturition

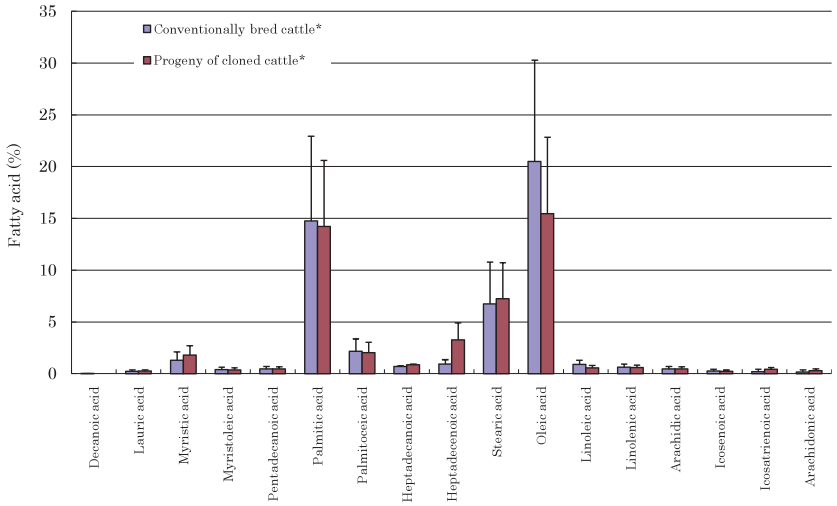


Fig. 6. Fatty acid composition of meat derived from conventionally bred cattle and progeny of somatic cell cloned cattle
*: Japanese Black beef cattle
Columns represents the mean±standard deviation from nine samples (three parts each in three carcasses)

**Digestion test based on protein digestion rate in milk/meat with rats (Table 12)**

The digestion rates of milk were 88.8% for that of conventionally bred cattle and 86.8% for that of the progeny. With regard to meat, the rates were 90.3% for that of conventionally bred cattle and 89.5% for that of the progeny. No significant differences in digestibility due to the origin of milk/meat were found.

**Detection of anaphylactic reaction in milk/meat samples by mouse abdominal wall method[17)]**

**1) Milk (Table 13)**

**A) Positive control substance**

The diameter of the dye leakage spot for ovalbumin as a positive control substance (10.0 mm) was significantly larger than that of the negative control (3.6 mm). The result showed that the present detection system could detect the anaphylactic reactions of the protein antigen.

**B) Milk powder**

For milk powder derived from conventionally bred cattle, the diameters of dye leakage spots for negative control and test dosages were 3.7 and 8.9 mm, respectively. For milk powder derived from the progeny, the diameters of dye leakage spots for negative control and test dosages were 4.0 and 8.5 mm, respectively. Significant differences in anaphylactic reactions were observed between test dosages and negative controls; however, no significant differences in anaphylactic reactions due to the origin of milk powder were observed.

**2) Meat (Table 14)**

**A) Positive control substance**

As in the detection with milk, ovalbumin was used as the positive control substance. Positive detections with ovalbumin also showed that the present detection system could detect the anaphylactic reactions of the protein antigen.

**B) Meat powder**

For meat powder derived from conventionally bred cattle and the progeny, the diameters of dye leakage spots in the test dosage were 8.8 mm and 10.8 mm, respectively. For the negative control of conventionally bred cattle and progeny, spot diameters were 3.3 mm and 3.5 mm, respectively. Significant differences in anaphylactic reactions were observed between test dosages and negative controls; however, no significant differences in anaphylactic reactions due to the origin of meat powder were observed.

**3) Conclusions**

To detect anaphylactic reactions to milk/meat derived from the progeny, the mouse abdominal wall method was applied by injecting the test dosage intraperitoneally. In the present investigation, no significant differences in anaphylactic reactions due to the origin of milk/meat were found.

Table 9-1. Fatty acid composition of meat derived from conventionally bred cattle※

(Upper: Fatty acid (mg) in sample 100g; Under: Fatty acid (g) per fatty acid gross 100g)

| Individual identification code | Region | Lipid quantity (g/100g) | Fatty acid composition(%) Saturated | Unsaturated | 10 : 0 Decanoic acid | 12 : 0 Lauric acid | 14 : 0 Myristic acid | 14 : 1 Myristoleic acid | 15 : 0 Pentadecanoic acid | 16 : 0 Palmitic acid | 16 : 1 Palmitoleic acid | 17 : 0 Heptadecanoic acid | 17 : 1 Heptadecenoic acid | 18 : 0 Stearic acid | 18 : 1 Oleic acid | 18 : 2 Linoleic acid | 18 : 3 Linolenic acid | 20 : 0 Arachidic acid | 20 : 1 Icosenoic acid | 20 : 3 Icosatrienoic acid | 20 : 4 Arachidonic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 | Shoulder | 17.4 | 41.9 | 58.1 | | | **343** | **212** | **61** | **4144** | **695** | **317** | **251** | **1655** | **7430** | **388** | **36** | **5** | **22** | | |
| | | | | | 0.0 | 0.0 | 2.2 | 1.4 | 0.4 | 26.6 | 4.5 | 2.0 | 1.6 | 10.6 | 47.8 | 2.5 | 0.2 | 0.0 | 0.1 | 0.0 | 0.0 |
| | Loin | 50.5 | 45.3 | 54.7 | | **11** | **1045** | **649** | **134** | **11460** | **1983** | **655** | **568** | **4551** | **17250** | **887** | **77** | **26** | **143** | | |
| | | | | | 0.0 | 0.0 | 2.6 | 1.6 | 0.3 | 29.1 | 5.0 | 1.7 | 1.4 | 11.5 | 43.7 | 2.2 | 0.2 | 0.1 | 0.4 | 0.0 | 0.0 |
| | Round | 8.9 | 43.2 | 56.8 | | | **151** | **109** | **22** | **2046** | **332** | **116** | **105** | **790** | **3344** | **184** | **13** | **3** | **20** | | **2** |
| | | | | | 0.0 | 0.0 | 2.1 | 1.5 | 0.3 | 28.3 | 4.6 | 1.6 | 1.5 | 10.9 | 46.2 | 2.5 | 0.2 | 0.0 | 0.3 | 0.0 | 0.0 |
| T2 | Shoulder | 17.8 | 42.5 | 57.5 | | | **346** | **215** | **60** | **4831** | **695** | **349** | **246** | **1959** | **8591** | **391** | **41** | **5** | **51** | | |
| | | | | | 0.0 | 0.0 | 1.9 | 1.2 | 0.3 | 27.2 | 3.9 | 2.0 | 1.4 | 11.0 | 48.3 | 2.2 | 0.2 | 0.0 | 0.3 | 0.0 | 0.0 |
| | Loin | 35.3 | 52.5 | 47.5 | | **9** | **681** | **336** | **117** | **6830** | **897** | **396** | **394** | **3462** | **8233** | **460** | **25** | **21** | **87** | **3** | **2** |
| | | | | | 0.0 | 0.0 | 3.1 | 1.5 | 0.5 | 31.1 | 4.1 | 1.8 | 1.8 | 15.8 | 37.5 | 2.1 | 0.1 | 0.1 | 0.4 | 0.0 | 0.0 |
| | Round | 11.3 | 56.7 | 43.3 | | **2** | **178** | **98** | **33** | **2434** | **231** | **143** | **104** | **1259** | **2487** | **133** | **7** | **8** | **32** | **1** | **1** |
| | | | | | | 0.0 | 2.5 | 1.4 | 0.5 | 34.0 | 3.2 | 2.0 | 1.5 | 17.6 | 34.8 | 1.9 | 0.1 | 0.1 | 0.4 | 0.0 | 0.0 |
| T4 | Shoulder | 18.9 | 37.1 | 62.9 | | | **362** | **250** | **39** | **4203** | **985** | **186** | **225** | **1412** | **8610** | **403** | **41** | | **22** | | |
| | | | | | 0.0 | 0.0 | 2.2 | 1.5 | 0.2 | 25.1 | 5.9 | 1.1 | 1.3 | 8.4 | 51.4 | 2.4 | 0.2 | 0.0 | 0.1 | 0.0 | 0.0 |
| | Loin | 39.4 | 55.7 | 44.3 | | **8** | **708** | **351** | **113** | **7957** | **819** | **447** | **367** | **3916** | **8342** | **431** | **23** | **30** | **129** | **3** | **1** |
| | | | | | 0.0 | 0.0 | 3.0 | 1.5 | 0.5 | 33.7 | 3.5 | 1.9 | 1.6 | 16.6 | 35.3 | 1.8 | 0.1 | 0.1 | 0.5 | 0.0 | 0.0 |
| | Round | 14.7 | 53.4 | 46.6 | | **3** | **249** | **152** | **49** | **3070** | **374** | **237** | **173** | **1520** | **3528** | **212** | **9** | **9** | **30** | **1** | **1** |
| | | | | | 0.0 | 0.0 | 2.6 | 1.6 | 0.5 | 31.9 | 3.9 | 2.5 | 1.8 | 15.8 | 36.7 | 2.2 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |

※: Japanese Black beef cattle

Table 9-2. Fatty acid composition of meat derived from progeny of somatic cell cloned cattle※ (continued)

(Upper: Fatty acid (mg) in sample 100g; Under: Fatty acid (g) per fatty acid gross 100g)

| Individual identification code | Region | Lipid quantity (g/100g) | Fatty acid composition(%) Saturated | Unsaturated | 10 : 0 Decanoic acid | 12 : 0 Lauric acid | 14 : 0 Myristic acid | 14 : 1 Myristoleic acid | 15 : 0 Pentadecanoic acid | 16 : 0 Palmitic acid | 16 : 1 Palmitoleic acid | 17 : 0 Heptadecanoic acid | 17 : 1 Heptadecenoic acid | 18 : 0 Stearic acid | 18 : 1 Oleic acid | 18 : 2 Linoleic acid | 18 : 3 Linolenic acid | 20 : 0 Arachidic acid | 20 : 1 Icosenoic acid | 20 : 3 Icosatrienoic acid | 20 : 4 Arachidonic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B82 | Shoulder | 18.4 | 57.6 | 42.4 | **6** | **8** | **892** | **195** | **53** | **4237** | **583** | **735** | **201** | **2190** | **4681** | **247** | **11** | **12** | **65** | **2** | **2** |
| | | | | | 0.0 | 0.1 | 6.3 | 1.4 | 0.4 | 30.0 | 4.1 | 5.2 | 1.4 | 15.5 | 33.2 | 1.7 | 0.1 | 0.1 | 0.5 | 0.0 | 0.0 |
| | Loin | 39.9 | 52.6 | 47.3 | **8** | **12** | **212** | **394** | **98** | **7616** | **1208** | **1524** | **407** | **3888** | **9365** | **487** | **21** | **22** | **135** | **4** | **2** |
| | | | | | 0.0 | 0.0 | 0.8 | 1.6 | 0.4 | 30.0 | 4.8 | 6.0 | 1.6 | 15.3 | 36.9 | 1.9 | 0.1 | 0.1 | 0.5 | 0.0 | 0.0 |
| | Round | 9.3 | 57.2 | 42.8 | **1** | **3** | **485** | **108** | **28** | **2105** | **303** | **359** | **110** | **1006** | **2290** | **145** | **6** | **6** | **24** | **2** | **1** |
| | | | | | 0.0 | 0.0 | 6.9 | 1.5 | 0.4 | 30.1 | 4.3 | 5.1 | 1.6 | 14.4 | 32.8 | 2.1 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |
| B89 | Shoulder | 29.6 | 58.9 | 41.1 | **8** | **12** | **907** | **358** | **120** | **7180** | **938** | **1734** | **373** | **3773** | **7428** | **391** | **17** | **20** | **73** | **4** | **2** |
| | | | | | 0.0 | 0.1 | 3.9 | 1.5 | 0.5 | 30.8 | 4.0 | 7.4 | 1.6 | 16.2 | 31.8 | 1.7 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |
| | Loin | 39.6 | 58.9 | 41.1 | **12** | **15** | **1054** | **379** | **130** | **7897** | **1061** | **1899** | **389** | **4024** | **8226** | **378** | **14** | **20** | **69** | **2** | |
| | | | | | 0.0 | 0.1 | 4.1 | 1.5 | 0.5 | 30.9 | 4.1 | 7.4 | 1.5 | 15.7 | 32.2 | 1.5 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |
| | Round | 14.0 | 59.7 | 40.3 | **4** | **5** | **377** | **164** | **54** | **3507** | **403** | **837** | **173** | **1810** | **3480** | **199** | **8** | **9** | **28** | **2** | **1** |
| | | | | | 0.0 | 0.0 | 3.4 | 1.5 | 0.5 | 31.7 | 3.6 | 7.6 | 1.6 | 16.4 | 31.5 | 1.8 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |
| B90 | Shoulder | 20.0 | 57.6 | 42.4 | | **9** | **564** | **228** | **82** | **4560** | **649** | **1046** | **232** | **1994** | **4664** | **254** | **11** | **10** | **34** | **1** | **1** |
| | | | | | 0.0 | 0.1 | 3.9 | 1.6 | 0.6 | 31.8 | 4.5 | 7.3 | 1.6 | 13.9 | 32.5 | 1.8 | 0.1 | 0.1 | 0.2 | 0.0 | 0.0 |
| | Loin | 25.7 | 57.1 | 42.9 | **7** | **11** | **750** | **295** | **100** | **5554** | **829** | **1329** | **297** | **2619** | **6012** | **312** | **15** | **14** | **51** | **2** | **1** |
| | | | | | 0.0 | 0.1 | 4.1 | 1.6 | 0.5 | 30.5 | 4.6 | 7.3 | 1.6 | 14.4 | 33.0 | 1.7 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |
| | Round | 9.2 | 56.4 | 43.6 | **2** | **3** | **227** | **106** | **33** | **2063** | **299** | **432** | **108** | **908** | **2183** | **122** | **5** | **5** | **20** | **1** | **1** |
| | | | | | 0.0 | 0.0 | 3.5 | 1.6 | 0.5 | 31.7 | 4.6 | 6.6 | 1.7 | 13.9 | 33.5 | 1.9 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |

※: Japanese Black beef cattle

Table 10. Nutritional analysis of freeze-dried milk powder[⌘] derived from conventionally bred cattle and progeny of somatic cell cloned cattle

| Macronutrient | (%) | | Vitamin | (ppm) | | Mineral | (ppm) | |
|---|---|---|---|---|---|---|---|---|
| | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] | | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] | | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] |
| Crude protein | 24.6 | 22.8 | A(retinol) | 5.4 | 3.9 | Ca | 8770 | 7660 |
| Crude fat | 24.6 | 31.9 | $B_1$ | 3.5 | 4.4 | P | 7860 | 7180 |
| Carbohydrate | 39.1 | 34.7 | $B_2$ | 10.0 | 9.0 | K | 17200 | 15800 |
| Crude fiber | <0.1 | <0.1 | $B_6$ | 3.2 | 4.2 | Na | 4570 | 4310 |
| Ash content | 5.7 | 5.1 | $B_{12}$ | 0.032 | 0.037 | Mg | 829 | 673 |
| Water content | 6.0 | 5.5 | $D_3$ | 0.005 | 0.003 | Fe | 3.1 | 2.8 |
| | | | E | 5 | 6 | Zn | 30.1 | 32.4 |
| | | | $K_2$ | 0.07 | 0.11 | Cu | <0.5 | 0.7 |
| | | | Niacin | 11.3 | 7.8 | S | 0.23 % | 0.21 % |
| | | | Pantotheinic acid | 33.1 | 38.4 | Mn | <0.5 | <0.5 |
| | | | | | | I | 0.8 | 0.6 |
| | | | Folic acid | 0.71 | 0.62 | Se | 0.1 | 0.1 |
| | | | Biotin | 0.22 | 0.33 | Mo | <0.5 | <0.5 |
| | | | Choline | 0.09 % | 0.08 % | | | |

⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried.
⌘⌘: Holstein dairy cattle.

Table 11. Nutritional analysis of the freeze-dried meat powder[⌘] derived from conventionally bred cattle and progeny of somatic cell cloned cattle

| Macronutrient | (%) | | Vitamin | (ppm) | | Mineral | (ppm) | |
|---|---|---|---|---|---|---|---|---|
| | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] | | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] | | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] |
| Crude protein | 41.5 | 43.3 | A(retinol) | 0.04 | 0.08 | Ca | 80 | 95 |
| Crude fat | 54.6 | 52.3 | $B_1$ | 1.9 | 2.1 | P | 3830 | 3930 |
| Carbohydrate | 1.2 | 1.2 | $B_2$ | 6.8 | 4.9 | K | 5230 | 5740 |
| Crude fiber | <0.1 | <0.1 | $B_6$ | 6.2 | 7.5 | Na | 1710 | 1920 |
| Ash content | 1.9 | 2.1 | $B_{12}$ | 0.029 | 0.022 | Mg | 449 | 475 |
| Water content | 0.8 | 1.1 | $D_3$ | <0.01 | <0.01 | Fe | 47.3 | 45.1 |
| | | | E | 2 | 3 | Zn | 101 | 114 |
| | | | $K_2$ | 0.14 | 0.08 | Cu | 2.1 | 1.5 |
| | | | Niacin | 107 | 120 | S | 3000 | 3100 |
| | | | Pantotheinic acid | 17.7 | 14.4 | Mn | <0.5 | <0.5 |
| | | | | | | I | 0.2 | 0.3 |
| | | | Folic acid | 0.13 | 0.16 | Se | 0.3 | 0.3 |
| | | | Biotin | 0.035 | 0.038 | Mo | <1 | <1 |
| | | | Choline | 0.13 % | 0.13 % | | | |

⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried.
⌘⌘: Japanese Black beef cattle.

### Detection of mutagenicity by mouse micronucleus test[24)]

#### 1) Milk (Table 15)

##### A) Positive control

The frequency of micronucleated cells in bone marrow polychromatic erythrocytes upon mitomycin C treatment was 6.17%. The result obtained in the negative control was 0.28%. Significant differences in the frequency were observed between these groups. The result verified the sensitivity of the mutagen in the present system. In addition, the polychromatic cell rate as a proportion of total erythrocytes in the negative control was 52.7%. This rate decreased significantly in the positive control to 36.3%. The

Table 12. Digestion rates[⌘] obained with rats for milk/meat derived from conventionally bred cattle and progeny of somatic cell cloned cattle

| Sample | Test group | Number of rats | Digestion rate (%, mean ± standard deviation) |
|---|---|---|---|
| Milk | Conventionally bred cattle[⌘⌘] | 5 | 88.8 ± 2.9 |
| | Progeny of cloned cattle[⌘⌘] | 5 | 86.8 ± 2.8 |
| Meat | Conventionally bred cattle[⌘⌘⌘] | 5 | 90.3 ± 0.7 |
| | Progeny of cloned cattle[⌘⌘⌘] | 5 | 89.5 ± 0.8 |

Milk/meat was derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each powder was supplemented to a test diet.

[⌘]: The digestion rate shows the protein digestion rate.

[⌘⌘]: Holstein dairy cattle

[⌘⌘⌘]: Japanese Black beef cattle

Table 13. Allergen study of milk derived from conventionally bred cattle and progeny of somatic cell cloned cattle (by mouse abdominal wall method)

| Derivation of milk | Group | Number of animals | Diameter of dye leakage (mm) (mean±standard deviation) |
|---|---|---|---|
| Conventionally bred cattle[⌘] | Control group | 10 | 3.7 ± 1.4 |
| | Test group | 10 | 8.9 ± 4.1 ** |
| Progeny of cloned cattle[⌘] | Control group | 10 | 4.0 ± 1.3 |
| | Test group | 10 | 8.5 ± 3.1 ** |
| Ovalbumin (positive control substance) | Control group | 10 | 3.6 ± 1.1 |
| | Test group | 10 | 10.0 ± 3.9 ** |

Significantly different from control group (** : $p<0.01$)

Milk were freeze-dried and the extracts were used as samples. The test groups underwent sensitization treatment and elicitation, while the control groups underwent elicitation only.

[⌘]: Holstein dairy cattle

Table 14. Allergen study of meat derived from conventionally bred cattle and progeny of somatic cell cloned cattle (by mouse abdominal wall method)

| Derivation of milk | Group | Number of animals | Diameter of dye leakage (mm) (mean±standard deviation) |
|---|---|---|---|
| Conventionally bred cattle[⌘] | Control group | 10 | 3.3 ± 0.9 |
| | Test group | 10 | 8.8 ± 3.3 ** |
| Progeny of cloned cattle[⌘] | Control group | 10 | 3.5 ± 0.9 |
| | Test group | 10 | 10.8 ± 3.9 ** |
| Ovalbumin (positive control substance) | Control group | 10 | 3.8 ± 1.2 |
| | Test group | 10 | 11.2 ± 3.9 ** |

Significantly different from control group (** : $p<0.01$)

Meat were freeze-dried and the extracts were used as samples. The test groups underwent sensitization treatment and elicitation, while the control groups underwent elicitation only.

[⌘]: Japanese Black beef cattle.

Table 15. Mutagenicity of milk[⌘] derived from conventionally bred cattle and progeny of somatic cell cloned cattle (by mouse micronucleus test)

| Test group | Number of mice | Body weight (g) (Min-max) | Incidence (%) of micronucleus appearance (Min-max) | Polychromatic erythrocyte rate (%) (Min-max) | Assessment |
|---|---|---|---|---|---|
| Negative control (basal diet) | 6 | 37.3 ± 2.6 (32.9-40.2) | 0.28 ± 0.08 (0.20-0.40) | 52.7 ± 6.3 (46.3-63.8) | Negative |
| Conventionally bred cattle[⌘] | | | | | |
| 2% supplementation to diet | 6 | 35.7 ± 1.9 (32.6-37.7) | 0.23 ± 0.13 (0.00-0.40) | 54.8 ± 8.4 (42.7-64.3) | Negative |
| 10% supplementation to diet | 6 | 37.2 ± 2.3 (34.9-40.1) | 0.27 ± 0.14 (0.05-0.45) | 51.7 ± 4.7 (45.2-58.7) | Negative |
| Progeny of cloned cattle[⌘] | | | | | |
| 2% supplementation to diet | 6 | 37.4 ± 3.4 (32.5-41.3) | 0.34 ± 0.10 (0.20-0.45) | 50.0 ± 3.8 (46.3-56.2) | Negative |
| 10% supplementation to diet | 6 | 37.5 ± 1.6 (35.7-39.6) | 0.25 ± 0.07 (0.15-0.35) | 50.9 ± 6.7 (44.3-63.1) | Negative |
| Positive control[⌘⌘⌘] (Mitomycin C) | 6 | 36.8 ± 2.0 (34.1-39.5) | 6.17 ± 1.51** (4.10-7.70) | 36.3 ± 6.2* (27.6-43.7) | Positive |

Values were shown as mean±standard deviation.
[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet. Each test diet was fed to a mouse group for 14 days.
[⌘⌘]: Holstein dairy cattle.
[⌘⌘⌘]: The positive control group was administered a single dose of 2mg/kg of mitomycin C intraperitoneally.
*: Significantly different from negative control group ($p<0.05$)
**: Significantly different from negative control group ($p<0.01$)

result demonstrated the cell toxicity of mitomycin C as a positive control.

**B) Milk powder**

Milk powders derived from the progeny and conventionally bred cattle were investigated. In test diets supplemented with milk powder at 2 or 10% (w/w), frequencies of micronucleated cells were 0.23–0.34%. No significant differences were observed with the frequency of 0.23% in the negative control. In addition, polychromatic cell rate as a proportion of total erythrocytes was 50.0–54.8%, which was not significantly different from the 52.7% observed in the negative control group.

**2) Meat (Table 16)**

**A) Positive control**

The sensitivity for mutagenicity in the detection system based on the frequency of micronucleated cells was also verified using mitomycin C as a positive substrate.

**B) Meat powder**

Meat powders derived from progeny and conventionally bred cattle were investigated. In the test diet supplemented with meat powder at 1 or 5% (w/w), frequencies of micronucleated cells were 0.18–0.24%. No significant differences were observed with the frequency of 0.23% in the negative control. In addition, the mean of the polychromatic cell rate as a proportion of total erythrocytes was 48.3–57.3%, which was not significantly different from the 54.4% observed in the negative control group.

**3) Conclusions**

It was concluded that milk/meat derived from the progeny and conventionally bred cattle was negative for mutagenicity.

## Twelve-month feeding study combined with reproduction/development toxicity test in rats

**1) Milk**

**A) Effects of milk intake on health status in rats**

***a) Clinical signs***

***i) Dead cases***

In the group fed a diet supplemented with 2% (w/w) milk powder derived from the progeny, a male rat (No. 019) lost weight continuously from the 36$^{th}$ month

Table 16. Mutagenicity of meat[⌘] derived from conventionally bred cattle and progeny of somatic cell cloned cattle (by mouse micronucleus test)

| Test group | Number of animals | Body weight (g) (Min-max) | Incidence (%) of micronucleus appearance (Min-max) | Polychromatic erythrocyte rate (%) (Min-max) | Assessment |
|---|---|---|---|---|---|
| Negative control (basal diet) | 6 | 38.5 ± 1.4 (36.6-40.6) | 0.23 ± 0.13 (0.00-0.40) | 54.4 ± 3.4 (50.8-59.8) | Negative |
| Conventionally bred cattle[⌘] | | | | | |
| 1% supplementation to diet | 6 | 36.4 ± 1.2 (34.4-37.9) | 0.21 ± 0.12 (0.00-0.35) | 57.3 ± 2.3 (54.8-60.4) | Negative |
| 5% supplementation to diet | 6 | 38.0 ± 4.1 (32.7-44.4) | 0.18 ± 0.12 (0.00-0.35) | 48.3 ± 7.6 (35.6-57.2) | Negative |
| Progeny of cloned cattle[⌘] | | | | | |
| 1% supplementation to diet | 6 | 37.0 ± 1.3 (35.5-38.5) | 0.20 ± 0.14 (0.00-0.40) | 54.6 ± 3.5 (48.0-57.4) | Negative |
| 5% supplementation to diet | 6 | 37.0 ± 2.1 (33.9-39.5) | 0.24 ± 0.08 (0.10-0.30) | 52.3 ± 4.1 (46.0-57.4) | Negative |
| Positive control (Mitomycin C) | 6 | 37.6 ± 1.8 (35.5-40.1) | 8.17 ± 0.76** (7.20-9.20) | 38.1 ± 3.2* (33.4-41.4) | Positive |

Values were shown as mean±standard deviation.
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet. Each test diet was fed to a mouse group for 14 days.
[⌘⌘]: Holstein dairy cattle.
[⌘⌘⌘]: The positive control group was administered a single dose of 2mg/kg of mitomycin C intraperitoneally.
*: Significantly different from negative control group ($p<0.05$)
**: Significantly different from negative control group ($p<0.01$)

of the feeding period. On the 282nd day of feeding, it was euthanized as it had reached a moribund stage.

Another male (No. 035) in the group fed 10% (w/w) milk powder diet from conventionally bred cattle, which showed no abnormal clinical signs, died on the 327th day of feeding. Pathological examinations of these rats revealed that the probable cause of death was hepatic failure, since multifocal necrosis was found in the liver.

***ii) Changes in external appearance***

Subcutaneous masses were found in two females (Nos. 503 and 508) fed 2% (w/w) milk powder diet derived from conventionally bred cattle (from the 357th day of feeding for No. 503, and the 306th day of feeding for No. 508) and a female (No. 539) fed 10% (w/w) milk powder diet derived from the progeny (from the 301st day of feeding). Pathology revealed that these masses were benign fibroadenoma of the mammary gland. Sporadic changes, such as chromodacryorrhea, crushing of teeth, alopecia/scab formation, rough fur and soiling around the anus were also observed. These changes were not attributed to the diets.

***b) Body weights (Figs. 7-1, 7-2)***

In terms of the body weights of female/male rat groups fed milk powder diet (2 or 10% (w/w)) derived from the progeny or conventionally bred cattle, no significant differences due to the origin of milk powder were observed.

***c) Food consumption (Figs. 8-1, 8-2)***

In food consumption of female rat groups fed 2% (w/w) milk powder diet derived from the progeny or conventionally bred cattle, no significant differences due to the origin of milk powder were observed. Similar results were obtained for female/male rat groups fed 10% (w/w) milk powder diet. In female rat groups fed diet supplemented with 2% (w/w) milk powder derived from the progeny, significantly lower food consumption was found in the 6th and 39th weeks of feeding compared with those fed diet supplemented with 2% (w/w) milk powder derived from

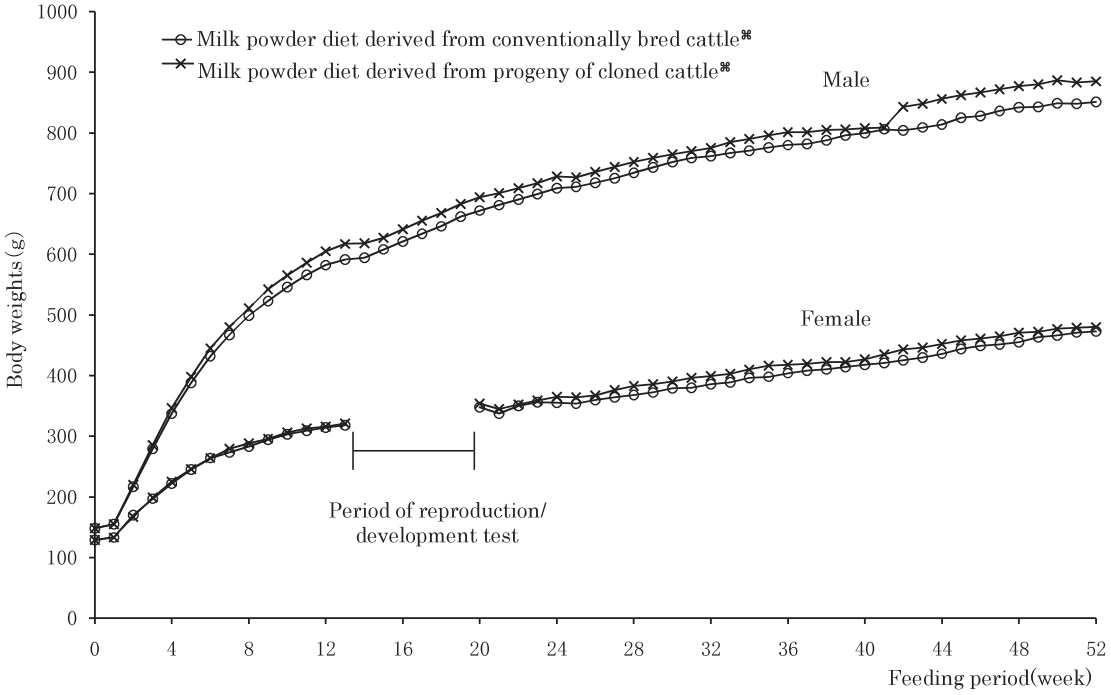


Fig. 7-1. Body weight curves of rats fed diet supplemented with 2% milk powder ⌘⌘

⌘: Holstein dairy cattle.

⌘⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

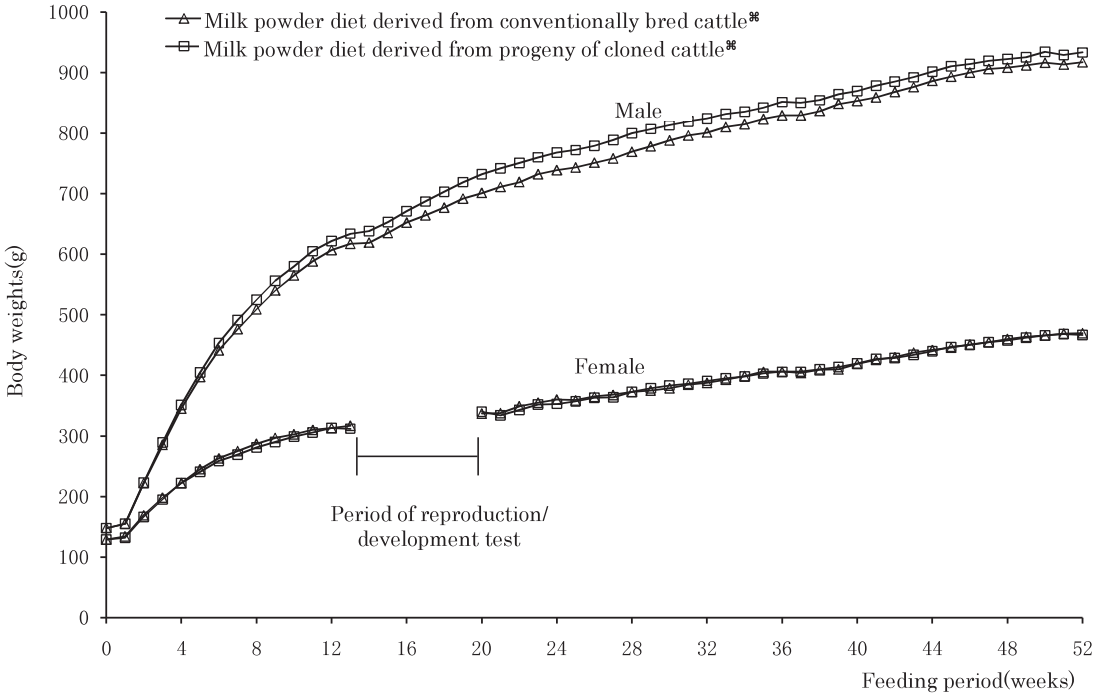


Fig. 7-2. Body weight curves of rats fed diet supplemented with 10% milk powder ⌘⌘

⌘: Holstein dairy cattle.

⌘⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

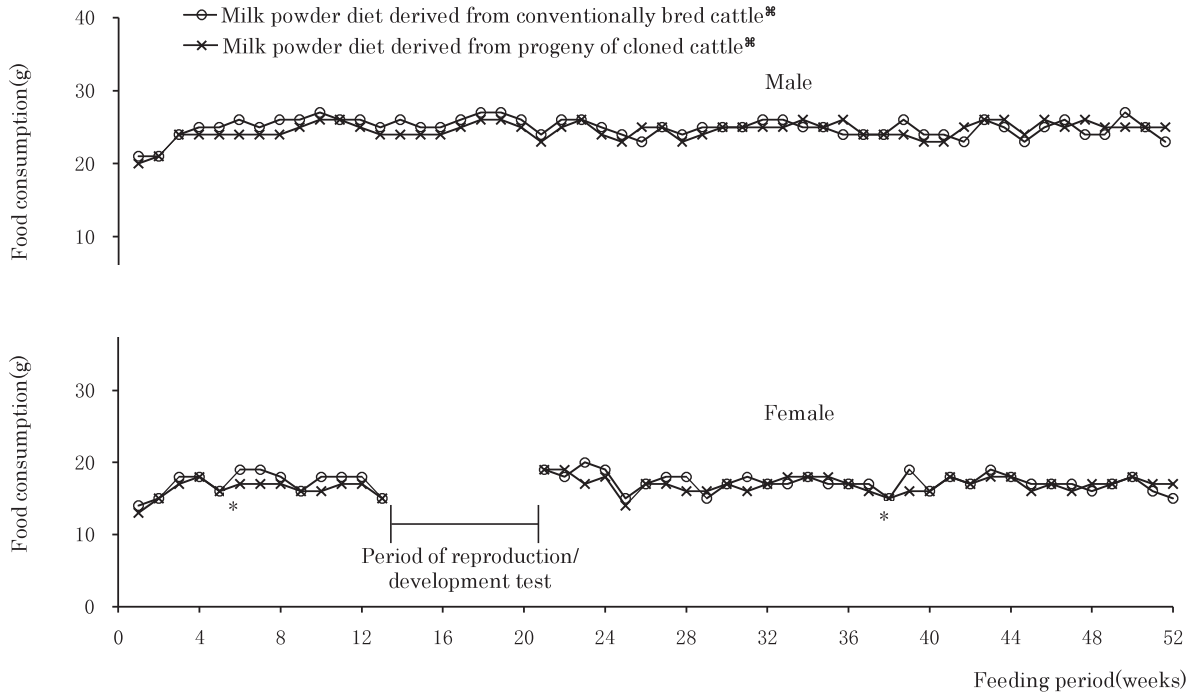


Fig. 8-1. Food consumption curves of rats fed diet supplemented with 2% milk powder⌘⌘

⌘: Holstein dairy cattle.

⌘⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was prepared independently and freeze-dried. Each milk powder was supplemented to a test diet.

Significant difference between two test diets (* : $p<0.05$).

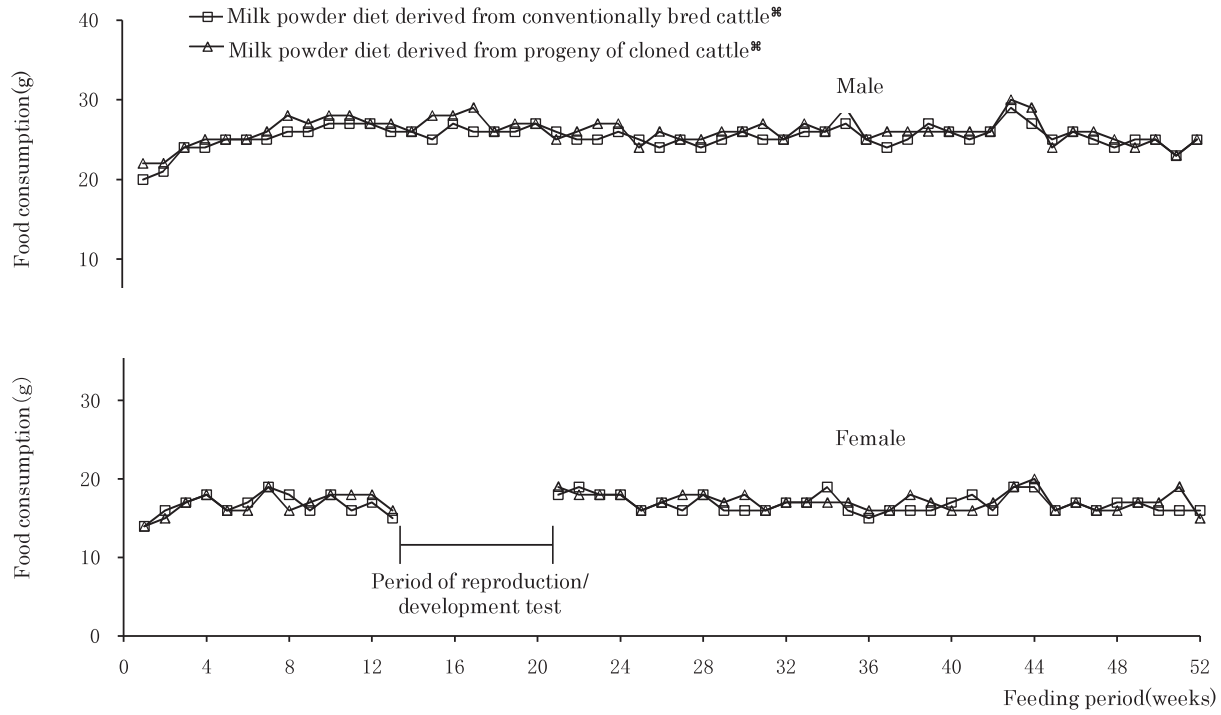


Fig. 8-2. Food consumption curves of rats fed diet supplemented with 10% milk powder⌘⌘

⌘: Holstein dairy cattle.

⌘⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was prepared independently and freeze-dried. Each milk powder was supplemented to a test diet.

conventionally bred cattle; however, no significant differences in food consumption due to the origin of milk powder were observed at other observation points.

The average food consumption per body weight of rats was observed throughout the twelve-month feeding period, except for pregnant females. In rat groups fed diet supplemented with 2% (w/w) milk powder derived from conventionally bred cattle, it was 832 mg/kg/day in males and 1,018 mg/kg/day in females. In the rat groups fed diet supplemented with 2% (w/w) milk powder derived from the progeny, it was 793 mg/kg/day in males and 963 mg/kg/day in females. In rat groups fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle, it was 4,024 mg/kg/day in males and 4,960 mg/kg/day in females. In rat groups fed diet supplemented with 10% (w/w) milk powder derived from the progeny, it was 4,054 mg/kg/day in males and 5,044 mg/kg/day in females.

**B) Effects of milk intake on physiological functions in rats**

***a) Sensory and reflex functions***

In observations in the 3rd, 6th, 9th and 12th months of the feeding period, no abnormalities in sound response, approach response, touch response, tail pinch response, pupil reflex to light, pinna reflex, eyelid reflex, ipsilateral flexor reflex and righting reflex due to the origin of milk powder were observed in rat groups. In an observation in the 12th month of feeding, one or two males had less touch response, tail pinch response, eyelid reflex and righting reflex in rat groups fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle.

***b) Grip strength and motor activity (Tables 17–19)***

In the 3rd, 6th, 9th and 12th months of the feeding period, grip strength of the forelimbs and hindlimbs and motor activity of rat groups fed diet supplemented with 2 or 10% (w/w) milk powder derived from the progeny or conventionally bred cattle were examined. No significant differences in these indices due to the origin of milk powder were found.

***c) Reproductive function (Table 20)***

In terms of the reproductive function in rat groups fed 2 or 10% (w/w) milk powder diet derived from the progeny or conventionally bred cattle, the estrous cycle, copulation index, fertility index, gestation period and delivery index were determined. No significant differences in these indices due to the origin of milk powder were observed.

***d) Ophthalmology***

In rat groups fed diet supplemented with 2 or 10% (w/w) milk powder derived from conventionally bred cattle or the progeny, no abnormalities in the anterior portion of the eye, optic media and ocular

Table 17. Grip strength of male rats fed diet supplemented with freeze-dried milk powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test (g)

| | Rat group for milk powder diet | | Point of the measurement | | | |
|---|---|---|---|---|---|---|
| | Milk powder in test diet | | | | | |
| | Origin | Content (%) | 3rd month | 6th month | 9th month | 12th month |
| Forelimb | Conventionally bred cattle⌘⌘ | 2 | 806 ± 194 (12) | 959 ± 152 (12) | 869 ± 269 (12) | 931 ± 133 (12) |
| | Progeny of cloned cattle⌘⌘ | 2 | 864 ± 99 (12) | 899 ± 248 (12) | 969 ± 205 (12) | 819 ± 279 (11) |
| | Conventionally bred cattle⌘⌘ | 10 | 901 ± 258 (12) | 1121 ± 282 (12) | 892 ± 343 (12) | 817 ± 158 (11) |
| | Progeny of cloned cattle⌘⌘ | 10 | 862 ± 166 (12) | 1013 ± 235 (12) | 906 ± 293 (12) | 853 ± 244 (12) |
| Hindlimb | Conventionally bred cattle⌘⌘ | 2 | 430 ± 85 (12) | 517 ± 152 (12) | 485 ± 134 (12) | 523 ± 102 (12) |
| | Progeny of cloned cattle⌘⌘ | 2 | 462 ± 100 (12) | 550 ± 106 (12) | 497 ± 150 (12) | 500 ± 134 (11) |
| | Conventionally bred cattle⌘⌘ | 10 | 464 ± 128 (12) | 576 ± 134 (12) | 589 ± 144 (12) | 567 ± 121 (11) |
| | Progeny of cloned cattle⌘⌘ | 10 | 443 ± 92 (12) | 516 ± 143 (12) | 483 ± 154 (12) | 481 ± 125 (12) |

Mean±standard deviation (number of animals)

⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

⌘⌘: Holstein dairy cattle.

Table 18. Grip strength of female rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test (g)

| | Rat group for milk powder diet | | Point of the measurement | | | |
|---|---|---|---|---|---|---|
| | Milk powder in test diet | | | | | |
| | Origin | Content (%) | 3rd month | 6th month | 9th month | 12th month |
| Forelimb | Conventionally bred cattle[⌘⌘] | 2 | 585 ± 95 (12) | 718 ± 101 (12) | 713 ± 207 (12) | 626 ± 194 (12) |
| | Progeny of cloned cattle[⌘⌘] | 2 | 546 ± 83 (12) | 766 ± 173 (12) | 695 ± 179 (12) | 688 ± 161 (12) |
| | Conventionally bred cattle[⌘⌘] | 10 | 537 ± 78 (12) | 732 ± 152 (12) | 754 ± 155 (12) | 753 ± 154 (12) |
| | Progeny of cloned cattle[⌘⌘] | 10 | 588 ± 62 (12) | 845 ± 147 (12) | 804 ± 190 (12) | 643 ± 139 (12) |
| Hindlimb | Conventionally bred cattle[⌘⌘] | 2 | 334 ± 55 (12) | 376 ± 60 (12) | 517 ± 98 (12) | 541 ± 70 (12) |
| | Progeny of cloned cattle[⌘⌘] | 2 | 355 ± 57 (12) | 367 ± 55 (12) | 462 ± 116 (12) | 494 ± 146 (12) |
| | Conventionally bred cattle[⌘⌘] | 10 | 304 ± 50 (12) | 409 ± 97 (12) | 499 ± 89 (12) | 576 ± 157 (12) |
| | Progeny of cloned cattle[⌘⌘] | 10 | 309 ± 44 (12) | 395 ± 55 (12) | 512 ± 140 (12) | 564 ± 159 (12) |

Mean±standard deviation (number of animals)

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

Table 19. Motor activity of rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test (Counts/60minuts)

| Sex of rats fed test diet | Rat group for milk powder diet | | Point of the measurement | | | |
|---|---|---|---|---|---|---|
| | Milk powder in test diet | | | | | |
| | Origin | Content (%) | 3rd month | 6th month | 9th month | 12th month |
| Male | Conventionally bred cattle[⌘⌘] | 2 | 14572 ± 2707 (12) | 10169 ± 3009 (12) | 4373 ± 2472 (12) | 5201 ± 2637 (12) |
| | Progeny of cloned cattle[⌘⌘] | 2 | 13554 ± 3070 (12) | 10830 ± 1905 (12) | 5098 ± 2340 (12) | 4374 ± 1740 (12) |
| | Conventionally bred cattle[⌘⌘] | 10 | 14666 ± 2547 (12) | 10182 ± 3014 (12) | 4683 ± 2487 (12) | 5592 ± 1021 (12) |
| | Progeny of cloned cattle[⌘⌘] | 10 | 13562 ± 2540 (12) | 10902 ± 2595 (12) | 4525 ± 1808 (12) | 4717 ± 1299 (12) |
| Female | Conventionally bred cattle[⌘⌘] | 2 | 14810 ± 2939 (12) | 13205 ± 2389 (12) | 8134 ± 2970 (12) | 5502 ± 1937 (12) |
| | Progeny of cloned cattle[⌘⌘] | 2 | 13823 ± 3200 (12) | 12208 ± 3835 (12) | 8556 ± 3616 (12) | 5446 ± 2043 (12) |
| | Conventionally bred cattle[⌘⌘] | 10 | 14964 ± 3838 (12) | 12410 ± 3564 (12) | 11509 ± 3041 (12) | 5745 ± 3396 (12) |
| | Progeny of cloned cattle[⌘⌘] | 10 | 14452 ± 3570 (12) | 12801 ± 3364 (12) | 10740 ± 3963 (12) | 5369 ± 3531 (12) |

Mean±standard deviation (number of rats investigated)

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

Table 20. Reproductive function of female rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | Estrous cycle (days, Mean±SD) | Copulation index (%) | Fertility index (%) | Gestation length (days, Mean±SD) | Gestation index (%) |
|---|---|---|---|---|---|---|
| Milk powder in test diet | | | | | | |
| Origin | Content (%) | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 4.0 ± 0.0 (12)[a)] | 100 (12/12) | 100 (12/12) | 22.6 ± 0.9 (12) | 100 (12/12) |
| Progeny of cloned cattle[⌘⌘] | 2 | 4.1 ± 0.3 (12) | 91.7 (11/12) | 90.9 (10/11) | 23.2 ± 0.6 (10) | 100 (10/10) |
| Conventionally bred cattle[⌘⌘] | 10 | 4.2 ± 0.2 (12) | 100 (12/12) | 83.3 (10/12) | 22.8 ± 0.4 (10) | 100 (10/10) |
| Progeny of cloned cattle[⌘⌘] | 10 | 4.1 ± 0.2 (11) | 100 (12/12) | 91.7 (11/12) | 22.4 ± 0.7 (11) | 100 (11/11) |

Copulation index = (Number of pairs with successful copulation / Number of pairs mated)×100

Fertility index = (Number of pregnant female / Number of pairs with successful copulation)×100

Gestation index = (Number of females with live pups / Number of pregnant females)×100

SD : Standard deviation

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

[a)] : Number of rats investigated

fundus due to the origin of milk powder were found.

***e) Urinalysis (Tables 21-1–22-2)***

In urinalysis observations of female/male rat groups fed diet supplemented with 2 or 10% (w/w) milk powder derived from the progeny, no significant differences in each index due to the origin of milk were observed.

***f) Blood analysis***

***i) Hematology (Tables 23, 24)***

In female rat groups fed diet supplemented with 2% (w/w) milk powder derived from the progeny, significantly high values in RBC ($798 \times 10^4$/μl), hemoglobin (14.7 g/dl) and hematocrit (43.7%) were found compared with those in rat group fed milk powder derived from conventionally bred cattle (RBC: $736 \times 10^4$/μl, hemoglobin: 13.5 g/dl and hematocrit: 40.8%). These data in females were within the ranges of the reference data obtained in our laboratory (RBC: $683–814 \times 10^4$/μl, hemoglobin: 13.5–15.4 g/dl and hematocrit: 40.1–46.0%).

In terms of the WBC of the female rat group fed diet supplemented with 10% (w/w) milk powder derived from the progeny, a significantly lower value ($31 \times 10^2$/μl) was observed compared with that of the rat group fed diet supplemented with 10% (w/w) milk powder derived from progeny ($42 \times 10^2$/μl). The values were within the range of our reference data ($19–55 \times 10^2$/μl). No significant differences in hematology parameters due to the origin of milk powder were observed in male groups. Therefore, the findings observed in female rat groups might have occurred spontaneously.

Table 21-1. Urinalysis of male rats fed diet supplemented with freeze-dried milk powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Color | | | Cloudy | | Volume[a)] | Specific[a)] | pH | | | | | | | Protein | | | | |
| Origin | Content (%) | of rats | PY | Y | C | − | 1+ | (ml/18hr) | gravity | 5.0 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | − | ± | 1+ | 2+ | 3+ |
| Conventionally bred cattle⌘⌘ | 2 | 12 | 12 | | | 12 | | 4.9 ± 2.2 | 1.066 ± 0.013 | | | 8 | 4 | | | | | | 4 | 4 | 4 |
| Progeny of cloned cattle⌘⌘ | 2 | 11 | 9 | 2 | | 11 | | 5.9 ± 2.0 | 1.063 ± 0.008 | | 5 | 3 | 2 | | 1 | | | | 1 | 4 | 6 |
| Conventionally bred cattle⌘⌘ | 10 | 11 | 10 | 1 | | 11 | | 7.1 ± 2.4 | 1.056 ± 0.013 | | 6 | 2 | | 2 | 1 | | | | | 3 | 8 |
| Progeny of cloned cattle⌘⌘ | 10 | 12 | 11 | 1 | | 12 | | 6.2 ± 2.3 | 1.060 ± 0.011 | | 5 | 4 | 3 | | | | | 1 | | | 11 |

| Rat group for milk powder diet | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Glucose | | | | Ketone body | | | | Occult blood | | | | | Urobilinogen | | | | Bilirubin | | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | ± | 1+ | 2+ | − | ± | 1+ | 2+ | 3+ | 0.1 | 1 | 2 | 4 | − | 1+ | 2+ | 3+ |
| Conventionally bred cattle⌘⌘ | 2 | 12 | 12 | | | | 9 | 3 | | | 2 | 8 | 2 | | | 12 | | | | 12 | | | |
| Progeny of cloned cattle⌘⌘ | 2 | 11 | 11 | | | | 7 | 4 | | | 6 | 4 | | | 1 | 11 | | | | 11 | | | |
| Conventionally bred cattle⌘⌘ | 10 | 11 | 11 | | | | 8 | 3 | | | 9 | 2 | | | | 11 | | | | 11 | | | |
| Progeny of cloned cattle⌘⌘ | 10 | 12 | 12 | | | | 10 | 2 | | | 7 | 3 | 1 | | 1 | 12 | | | | 12 | | | |

[a)]: Mean±standard deviation
Color : PY(pale yellow); Y(yellow); C(color less)
Cloudy : −(negligible); 1+(cloudy)
Protein : −(negligible); ±(15~30mg/dl); 1+(30mg/dl); 2+(100mg/dl); 3+(300mg/dl)
Glucose : −(negligible); ±(0.1g/dl), 1+(0.25g/dl); 2+(0.5g/dl)
Ketone body : −(negligible); ±(5mg/dl); 1+(15mg/dl); 2+(40mg/dl)
Occult blood : −(negligible); ±(trace); 1+(slight); 2+(moderate); 3+(marked)
Urobilinogen : Ehrlich unit/dl
Bilirubin : −(negligible), 1+(slight), 2+(moderate), 3+(marked).
⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.
⌘⌘: Holstein dairy cattle.

Table 21-2. Urinalysis of male rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | Erythrocytes | | | | Leukocytes | | | | Crystals | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number of rats | | | | | | | | | ammonium magnesium phosphate | | | | calcium carbonate | | | amorphous | | |
| Origin | Content (%) | | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 8 | 4 | | | 7 | 3 | 2 | | 12 | | | | 12 | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 9 | 2 | | | 8 | 2 | 1 | | 11 | | | | 11 | | | 11 | | |
| Conventionally bred cattle[⌘⌘] | 10 | 11 | 11 | | | | 10 | 1 | | | 9 | 2 | | | 11 | | | 11 | | |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 9 | 3 | | | 9 | 3 | | | 12 | | | | 12 | | | 12 | | |

| Rat group for milk powder diet | | | Epithelial cells | | | | | | | | | | Casts | | | | | | Fat globules | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number of rats | squamous | | | | round | | | spindle | | | granule | | hyaline | | waxy | | | | |
| Origin | Content (%) | | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ | − | 1+ | − | 1+ | − | 1+ | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 2 | 10 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 4 | 7 | | | 11 | | | 11 | | | 11 | | 11 | | 11 | | 11 | | |
| Conventionally bred cattle[⌘⌘] | 10 | 11 | 5 | 6 | | | 11 | | | 11 | | | 11 | | 11 | | 11 | | 11 | | |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 6 | 6 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |

− : Not observed; 1+ : A few(1-10 counts) in some fields; 2+ : A few(1-10 counts) in all fields; 3+ : Many(over 11 counts) in all fields

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

***ii) Clinical biochemistry (Tables 25, 26)***

In clinical biochemistry of the female rat group fed a diet supplemented with 2% (w/w) milk powder derived from the progeny, significantly high levels of total cholesterol (128 mg/dl) and phospholipids (218 mg/dl) were observed compared with those in the rat group fed 2% (w/w) milk powder from conventionally bred cattle (108 mg/dl for total cholesterol and 185 mg/dl for total cholesterol). With regard to the male rat group fed the same diet shown above, significantly high levels of total bilirubin (0.26 mg/dl) were found compared with those in the rat group fed 2% (w/w) milk powder from conventionally bred cattle (0.21 mg/dl). Such high values were never observed in other rat groups; these high values were within the reference ranges obtained in our laboratory (0.19–0.33 mg/dl for total bilirubin in males, 66–164 mg/dl for total cholesterol in females and 137–278 mg/dl for phospholipids in females).

In the male rat group fed diet supplemented with 10% (w/w) milk powder derived from the progeny, a significantly high level of inorganic phosphorous (5.7 mg/dl) was found compared with that of the rat group fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle (5.1 mg/dl). These values were within the reference range (4.4–6.1 mg/dl). In female groups fed diet supplemented with 10% (w/w) milk powder derived from progeny or conventionally bred cattle, no significant differences in clinical biochemistry parameters due to the origin of milk powder were observed.

Although some clinical biochemistry parameters showed significant differences due to the origin of milk powder, all values were within the reference ranges. Therefore, the present investigation suggested that there were no vital functional abnormalities in rats fed diets supplemented with milk powder derived from the progeny.

**C) Effects of milk intake on morphology in rats**

***a) Autopsy***

The subcutaneous masses found as clinical signs were confirmed by necropsy in two females fed diet

Table 22-1. Urinalysis of female rats fed diet supplemented with freeze-dried milk powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Color | | | Cloudy | | Volume[a)] | Specific[a)] | pH | | | | | | | Protein | | | | |
| Origin | Content (%) | of rats | PY | Y | C | − | 1+ | (ml/18hr) | gravity | 5.0 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | − | ± | 1+ | 2+ | 3+ |
| Conventionally bred cattle⌘⌘ | 2 | 12 | 11 | | 1 | 12 | | 6.8 ± 2.2 | 1.060 ±0.014 | | 6 | 3 | 1 | 2 | | | 2 | 2 | 5 | 2 | 1 |
| Progeny of cloned cattle⌘⌘ | 2 | 12 | 12 | | | 12 | | 7.4 ± 2.6 | 1.057 ±0.015 | | 3 | 5 | 1 | 1 | 2 | | 2 | 2 | 1 | 5 | 2 |
| Conventionally bred cattle⌘⌘ | 10 | 12 | 11 | 1 | | 12 | | 7.2 ± 3.0 | 1.060 ±0.016 | | 7 | 3 | 2 | | | | 1 | 1 | 6 | 1 | 3 |
| Progeny of cloned cattle⌘⌘ | 10 | 12 | 11 | 1 | | 12 | | 6.4 ± 2.4 | 1.063 ±0.015 | | 8 | 2 | 2 | | | | 1 | 2 | | 5 | 4 |

| Rat group for milk powder diet | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Glucose | | | | Ketone body | | | | Occult blood | | | | | Urobilinogen | | | | Bilirubin | | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | ± | 1+ | 2+ | − | ± | 1+ | 2+ | 3+ | 0.1 | 1 | 2 | 4 | − | 1+ | 2+ | 3+ |
| Conventionally bred cattle⌘⌘ | 2 | 12 | 12 | | | | 9 | 3 | | | 11 | | | 1 | | 12 | | | | 12 | | | |
| Progeny of cloned cattle⌘⌘ | 2 | 12 | 12 | | | | 12 | | | | 12 | | | | | 12 | | | | 12 | | | |
| Conventionally bred cattle⌘⌘ | 10 | 12 | 12 | | | | 7 | 5 | | | 12 | | | | | 12 | | | | 12 | | | |
| Progeny of cloned cattle⌘⌘ | 10 | 12 | 12 | | | | 8 | 3 | 1 | | 10 | | 1 | | 1 | 12 | | | | 12 | | | |

[a)]: Mean±standard deviation
Color : PY(pale yellow); Y(yellow); C(color less)
Cloudy : −(negligible); 1+(cloudy)
Protein : −(negligible); ±(15~30mg/dl); 1+(30mg/dl); 2+(100mg/dl); 3+(300mg/dl)
Glucose : −(negligible); 1+(0.1g/dl); 2+(0.25g/dl); 3+(0.5g/dl)
Ketone body : −(negligible); ±(5mg/dl); 1+(15mg/dl); 2+(40mg/dl)
Occult blood : −(negligible); ±(trace); 1+(slight); 2+(moderate); 3+(marked)
Urobilinogen : Ehrlich unit/dl
Bilirubin : −(negligible); 1+(slight); 2+(moderate); 3+(marked)
⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.
⌘⌘: Holstein dairy cattle.

Table 22-2. Urinalysis of female rats fed diet supplemented with freeze-dried milk powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | Crystals | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Erythrocytes | | | | Leukocytes | | | | ammonium magnesium phosphate | | | | calcium carbonate | | | amorphous | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ |
| Conventionally bred cattle⌘⌘ | 2 | 12 | 11 | 1 | | | 11 | 1 | | | 10 | 2 | | | 12 | | | 12 | | |
| Progeny of cloned cattle⌘⌘ | 2 | 12 | 12 | | | | 12 | | | | 11 | 1 | | | 12 | | | 12 | | |
| Conventionally bred cattle⌘⌘ | 10 | 12 | 12 | | | | 12 | | | | 12 | | | | 12 | | | 12 | | |
| Progeny of cloned cattle⌘⌘ | 10 | 12 | 10 | 2 | | | 11 | 1 | | | 12 | | | | 12 | | | 12 | | |

| Rat group for milk powder diet | | | Epithelial cells | | | | | | | | | | Casts | | | | | | Fat globules | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | squamous | | | | round | | | spindle | | | granule | | hyaline | | waxy | | | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ | − | 1+ | − | 1+ | − | 1+ | − | 1+ | 2+ |
| Conventionally bred cattle⌘⌘ | 2 | 12 | 2 | 10 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Progeny of cloned cattle⌘⌘ | 2 | 12 | 3 | 9 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Conventionally bred cattle⌘⌘ | 10 | 12 | 5 | 7 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Progeny of cloned cattle⌘⌘ | 10 | 12 | 3 | 8 | 1 | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |

− : Not observed; 1+ : A few(1-10 counts) in some fields; 2+ : A few(1-10 counts) in all fields; 3+ : Many(over 11 counts) in all fields
⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.
⌘⌘: Holstein dairy cattle.

Table 23. Hamatological data of male rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | RBC | Hemoglobin | Hematocrit | MCV | MCH | MCHC | Reticulocyte | PT | APTT |
| Origin | Content (%) | of rats | ($10^4$/μl) | (g/dl) | (%) | (fl) | (pg) | (%) | (‰) | (sec) | (sec) |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 875 ± 46 | 14.9 ± 0.7 | 44.5 ± 2.2 | 51 ± 3 | 17.0 ± 0.8 | 33.5 ± 0.7 | 18.1 ± 4.1 | 13.1 ± 0.5 | 17.2 ± 2.0 |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 845 ± 43 | 14.7 ± 0.6 | 44.6 ± 1.3 | 53 ± 3 | 17.4 ± 0.7 | 32.9 ± 0.7 | 21.4 ± 4.0 | 12.7 ± 0.4 | 16.8 ± 1.3 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 844 ± 42 | 14.8 ± 0.8 | 44.5 ± 2.1 | 53 ± 2 | 17.6 ± 0.5 | 33.3 ± 0.6 | 19.1 ± 4.4 | 12.6 ± 0.7 | 16.4 ± 1.5 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 841 ± 52 | 14.7 ± 0.9 | 44.7 ± 2.7 | 53 ± 3 | 17.5 ± 0.9 | 32.9 ± 0.5 | 21.3 ± 5.5 | 12.7 ± 0.8 | 16.6 ± 2.1 |

| Rat group for milk powder diet | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Platelet | WBC | Differential leukocyte counts (%) | | | | | |
| Origin | Content (%) | of rats | ($10^4$/μl) | ($10^2$/μl) | Basophil | Eosinophil | Neutrophil | Lymphocyte | Monocyte | Others |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 116 ± 11 | 76 ± 25 | 0.0 ± 0.0 | 1.9 ± 0.8 | 24.0 ± 7.8 | 71.6 ± 8.9 | 2.6 ± 1.0 | 0.0 ± 0.0 |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 123 ± 16 | 86 ± 15 | 0.0 ± 0.0 | 1.8 ± 0.6 | 21.7 ± 6.2 | 73.8 ± 6.5 | 2.6 ± 1.2 | 0.0 ± 0.0 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 121 ± 14 | 84 ± 22 | 0.0 ± 0.0 | 1.8 ± 0.9 | 20.7 ± 4.2 | 74.8 ± 5.2 | 2.7 ± 1.0 | 0.0 ± 0.0 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 126 ± 18 | 87 ± 25 | 0.0 ± 0.0 | 1.7 ± 0.7 | 21.4 ± 6.9 | 74.0 ± 7.6 | 2.9 ± 1.5 | 0.0 ± 0.0 |

Mean±standad deviation
Abbreviations : RBC, Red blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PT, Prothrombin time; APTT, Activated partial thromboplastin time; WBC, White blood cell
[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.
[⌘⌘]: Holstein dairy cattle.

Table 24. Hamatological data of female rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | RBC | Hemoglobin | Hematocrit | MCV | MCH | MCHC | Reticulocyte | PT | APTT |
| Origin | Content (%) | of rats | ($10^4$/μl) | (g/dl) | (%) | (fl) | (pg) | (%) | (‰) | (sec) | (sec) |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 736 ± 81 | 13.5 ± 1.4 | 40.8 ± 3.7 | 56 ± 3 | 18.4 ± 0.7 | 33.1 ± 0.5 | 19.8 ± 4.0 | 12.3 ± 0.3 | 16.3 ± 1.5 |
| Progeny of cloned cattle[⌘⌘] | 2 | 12 | 798* ± 50 | 14.7* ± 0.7 | 43.7* ± 1.9 | 55 ± 2 | 18.4 ± 0.6 | 33.6 ± 0.6 | 16.8 ± 3.1 | 12.4 ± 0.5 | 16.4 ± 1.2 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 778 ± 39 | 14.3 ± 0.6 | 42.5 ± 2.1 | 55 ± 2 | 18.4 ± 0.5 | 33.7 ± 0.6 | 16.6 ± 4.6 | 12.4 ± 0.3 | 16.3 ± 1.1 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 771 ± 40 | 14.4 ± 0.9 | 43.1 ± 2.7 | 56 ± 2 | 18.6 ± 0.5 | 33.4 ± 0.3 | 19.4 ± 3.9 | 12.4 ± 0.5 | 15.8 ± 1.8 |

| Rat group for milk powder diet | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number | Platelet | WBC | Differential leukocyte counts (%) | | | | | |
| Origin | Content (%) | of rats | ($10^4$/μl) | ($10^2$/μl) | Basophil | Eosinophil | Neutrophil | Lymphocyte | Monocyte | Others |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 98 ± 17 | 35 ± 11 | 0.0 ± 0.0 | 2.6 ± 1.5 | 33.0 ± 11.8 | 61.6 ± 12.7 | 2.9 ± 1.2 | 0.0 ± 0.0 |
| Progeny of cloned cattle[⌘⌘] | 2 | 12 | 97 ± 14 | 41 ± 18 | 0.0 ± 0.0 | 2.3 ± 0.9 | 30.9 ± 11.8 | 63.1 ± 11.6 | 3.7 ± 1.0 | 0.0 ± 0.0 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 94 ± 13 | 42 ± 12 | 0.0 ± 0.0 | 2.1 ± 0.6 | 26.0 ± 6.5 | 68.8 ± 7.0 | 3.1 ± 1.1 | 0.0 ± 0.0 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 95 ± 14 | 31* ± 9 | 0.0 ± 0.0 | 2.2 ± 0.9 | 27.3 ± 7.9 | 67.6 ± 7.6 | 2.9 ± 0.8 | 0.0 ± 0.0 |

Mean±standad deviation
Significant difference between two rat groups fed test diets containing milk powder derived from conventionally bred cattle or progeny of cloned cattle in the same milk powder content (*: $p<0.05$).
Abbreviations : RBC, Red blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PT, Prothrombin time; APTT, Activated partial thromboplastin time; WBC, White blood cell
[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.
[⌘⌘]: Holstein dairy cattle.

Table 25. Clinical chemistry data of male rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number of rats | LDH (IU/l) | AST (IU/l) | ALT (IU/l) | ALP (IU/l) | γ-GTP (IU/l) | CK (IU/l) | ChE (IU/l) | T.P. (g/dl) | Alb. (g/dl) | Glob. (g/dl) | A/G | T-Cho. (mg/dl) |
| Origin | Content (%) | | | | | | | | | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 547 ± 509 | 123 ± 179 | 50 ± 67 | 231 ± 41 | 0.71 ± 0.48 | 87 ± 25 | 122 ± 39 | 6.65 ± 0.21 | 2.92 ± 0.22 | 3.73 ± 0.23 | 0.79 ± 0.10 | 114 ± 26 |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 725 ± 769 | 101 ± 62 | 52 ± 43 | 228 ± 77 | 0.84 ± 0.39 | 105 ± 48 | 108 ± 66 | 6.60 ± 0.26 | 2.93 ± 0.24 | 3.67 ± 0.27 | 0.80 ± 0.10 | 123 ± 19 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 343 ± 196 | 87 ± 76 | 41 ± 46 | 178 ± 59 | 0.72 ± 0.29 | 82 ± 21 | 120 ± 86 | 6.60 ± 0.30 | 3.02 ± 0.33 | 3.59 ± 0.19 | 0.85 ± 0.13 | 143 ± 42 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 742 ± 898 | 109 ± 99 | 37 ± 20 | 222 ± 59 | 0.99 ± 0.44 | 114 ± 57 | 116 ± 75 | 6.61 ± 0.30 | 2.89 ± 0.30 | 3.72 ± 0.19 | 0.78 ± 0.10 | 127 ± 20 |

| Rat group for milk powder diet | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number of rats | T.G. (mg/dl) | PL (mg/dl) | Glu. (mg/dl) | BUN (mg/dl) | Crea. (mg/dl) | T-Bil. (mg/dl) | Ca (mg/dl) | P (mg/dl) | Na (mEq/l) | K (mEq/l) | Cl (mEq/l) |
| Origin | Content (%) | | | | | | | | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 149 ± 66 | 161 ± 26 | 170 ± 16 | 15.2 ± 4.2 | 0.34 ± 0.05 | 0.21 ± 0.03 | 10.2 ± 0.3 | 5.1 ± 0.5 | 149 ± 2 | 4.74 ± 0.36 | 105 ± 2 |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 127 ± 44 | 168 ± 22 | 175 ± 19 | 13.6 ± 2.9 | 0.34 ± 0.06 | 0.26** ± 0.03 | 10.3 ± 0.3 | 5.2 ± 0.5 | 150 ± 3 | 4.83 ± 0.58 | 105 ± 2 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 194 ± 66 | 199 ± 50 | 173 ± 14 | 13.2 ± 2.6 | 0.36 ± 0.10 | 0.29 ± 0.04 | 10.5 ± 0.3 | 5.1 ± 0.3 | 150 ± 1 | 4.60 ± 0.35 | 105 ± 1 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 148 ± 56 | 175 ± 21 | 177 ± 17 | 12.5 ± 3.2 | 0.35 ± 0.04 | 0.30 ± 0.05 | 10.5 ± 0.5 | 5.7* ± 0.6 | 151 ± 4 | 4.82 ± 0.33 | 106 ± 2 |

Mean±standard deviation

Significant difference between two rat groups fed test diets containing milk powder derived from conventionally bred cattle or progeny of cloned cattle in the same milk powder content (*: $p<0.05$, **: $p<0.01$).

Abbreviations : LDH, Lactate dehydrogenase; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase
γ-GTP, γ-Glutamyltranspeptidase; CK, Creatine kinase; ChE : Cholinesterase; T.P., Total protein; Alb., Albumin; Glb., Globulin
A/G, Albumin/globulin ratio; T-Cho., Total cholesterol; T.G., Triglyceride; PL, Phospholipid; Glu., Glucose; BUN, Blood urea nitrogen
Crea., Creatinine; T-Bil., Total bilirubin; Ca, Calcium; P, Inorganic phosphorus; Na, Sodium; K, Potassium; Cl, Chloride

⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

⌘⌘: Holstein dairy cattle.

supplemented with 2% (w/w) milk powder derived from conventionally bred cattle and one female fed diet supplemented with 10% (w/w) milk powder derived from the progeny. Scattered white spots on the liver were found in two rats: one euthanized male fed diet supplemented with 2% (w/w) milk powder derived from the progeny and one dead male fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle. A black area on the pituitary gland was observed in 11 rats: one male and two females fed diet supplemented with 2% (w/w) milk powder derived from progeny; two males and three females fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle; and one male and 3 females fed diet supplemented with 10% (w/w) milk powder derived from the progeny.

Other necropsy findings were as follows: enlarged adrenal gland in one male fed diet supplemented with 1% (w/w) milk powder derived from conventionally bred cattle; enlarged kidney (characteristically edematous) in one male fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle; white area on the spleen in one female fed diet supplemented with 2% (w/w) milk powder derived from conventionally bred cattle; localized red/black spot on the liver in one female fed diet supplemented

Table 26. Clinical chemistry data of female rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number of rats | LDH (IU/l) | AST (IU/l) | ALT (IU/l) | ALP (IU/l) | γ-GTP (IU/l) | CK (IU/l) | ChE (IU/l) | T.P. (g/dl) | Alb. (g/dl) | Glb. (g/dl) | A/G | T-Cho. (mg/dl) |
| Origin | Content (%) | | | | | | | | | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 230 ± 86 | 66 ± 9 | 24 ± 9 | 73 ± 35 | 0.62 ±0.22 | 93 ± 21 | 439 ± 130 | 7.14 ±0.49 | 3.84 ±0.48 | 3.30 ±0.35 | 1.18 ±0.21 | 108 ± 15 |
| Progeny of cloned cattle[⌘⌘] | 2 | 12 | 344 ±389 | 87 ± 66 | 34 ± 34 | 79 ± 18 | 0.71 ±0.17 | 92 ± 17 | 540 ± 177 | 7.42 ±0.22 | 4.15 ±0.22 | 3.27 ±0.26 | 1.28 ±0.15 | 128 ** ± 17 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 306 ±135 | 77 ± 34 | 26 ± 12 | 58 ± 10 | 0.51 ±0.13 | 101 ± 29 | 533 ± 97 | 7.32 ±0.36 | 4.14 ±0.39 | 3.18 ±0.20 | 1.31 ±0.17 | 105 ± 14 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 277 ±125 | 75 ± 32 | 23 ± 8 | 75 ± 55 | 0.65 ±0.43 | 97 ± 30 | 518 ± 179 | 7.15 ±0.54 | 3.91 ±0.55 | 3.24 ±0.27 | 1.22 ±0.22 | 101 ± 25 |

| Rat group for milk powder diet | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | Number of rats | T.G. (mg/dl) | PL (mg/dl) | Glu. (mg/dl) | BUN (mg/dl) | Crea. (mg/dl) | T-Bil. (mg/dl) | Ca (mg/dl) | P (mg/dl) | Na (mEq/l) | K (mEq/l) | Cl (mEq/l) |
| Origin | Content (%) | | | | | | | | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 76 ± 51 | 185 ± 21 | 152 ± 24 | 12.4 ± 3.0 | 0.42 ±0.05 | 0.29 ±0.04 | 10.4 ± 0.3 | 3.9 ± 1.0 | 142 ± 1 | 4.62 ±0.60 | 108 ± 3 |
| Progeny of cloned cattle[⌘⌘] | 2 | 12 | 89 ± 43 | 218 ** ± 19 | 148 ± 9 | 11.0 ± 2.7 | 0.40 ±0.04 | 0.31 ±0.04 | 10.4 ± 0.4 | 4.0 ± 1.2 | 143 ± 2 | 4.62 ±0.46 | 108 ± 2 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 70 ± 44 | 189 ± 30 | 148 ± 14 | 10.8 ± 1.5 | 0.40 ±0.04 | 0.37 ±0.06 | 10.6 ± 0.5 | 4.5 ± 0.7 | 143 ± 1 | 4.64 ±0.40 | 109 ± 2 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 54 ± 30 | 178 ± 34 | 146 ± 14 | 11.0 ± 3.0 | 0.40 ±0.05 | 0.37 ±0.05 | 10.6 ± 0.4 | 4.9 ± 1.0 | 143 ± 2 | 4.78 ±0.90 | 110 ± 2 |

Mean±standard deviation

Significant difference between two rat groups fed test diets containing milk powder derived from conventionally bred cattle or progeny of cloned cattle in the same milk powder content (**: $p<0.01$).

Abbreviations : LDH, Lactate dehydrogenase; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase γ-GTP, γ-Gutamyltranspeptidase; CK, Creatine kinase; ChE : Cholinesterase; T.P., Total protein; Alb., Albumin; Glb., Globulin A/G, Albumin/globulin ratio; T-Cho., Total cholesterol; T.G., Triglyceride; PL, Phospholipid; Glu., Glucose; BUN, Blood urea nitrogen Crea., Creatinine; T-Bil., Total bilirubin; Ca, Calcium; P, Inorganic phosphorus; Na, Sodium; K, Potassium; Cl, Chloride

⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

⌘⌘: Holstein dairy cattle.

with 2% (w/w) milk powder derived from the progeny and one female fed diet supplemented with 10% (w/w) milk powder derived from the progeny; ovarian cyst in one female fed diet supplemented with 2% (w/w) milk powder derived from the progeny and uterine mass in two females fed diet supplemented with 10% (w/w) milk powder derived from the progeny.

***b) Organ weights (Table 27)***

In terms of the organ weights of female/male rat groups fed 2 or 10% (w/w) milk powder diets derived from the progeny or conventionally bred cattle, no significant differences in the values due to the origin of milk powder were observed.

***c) Histology (Table 28)***

In terms of the histological findings of rat groups fed diet supplemented with 10% (w/w) milk powder derived from progeny or conventionally bred cattle, non-neoplastic lesions found in males/females were as follows: artery mineralization; accumulation of foam cells in the lungs; myocardial degeneration/fibrosis in the heart; congestion and increased extramedullary hematopoiesis in the spleen; fatty change of hepatocytes in the liver; fatty change in the parotid gland; squamous hyperplasia of the forestomach; fatty change, deposit of brown pigment, fibrosis and focal atrophy of acinar cells in the pancreas;

Table 27. Organ weights of rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

Male

| Rat group for milk powder diet | | Number of rats | Body weight | Brain | Salivary gland | Heart | Lung | Liver | Kidney | Adrenal gland | Spleen | Pituitary gland | Thyroid gland | Prostate | Seminal vesicle | Testis | Epididymis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | | | | | | | | | | | | | | | | |
| Origin | Content (%) | | (g) | (g) | (g) | (g) | (g) | (g) | (g) | (mg) | (g) | (mg) | (mg) | (g) | (g) | (g) | (g) |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 842 ±138 | 2.22 ±0.11 | 0.95 ±0.13 | 1.83 ±0.25 | 2.03 ±0.20 | 22.16 ±4.18 | 4.04 ±0.44 | 76.7 ±64.0 | 1.12 ±0.17 | 17.3 ±2.6 | 36.6 ±8.0 | 0.59 ±0.19 | 3.04 ±0.56 | 3.75 ±0.37 | 1.49 ±0.16 |
| Progeny of cloned cattle[⌘⌘] | 2 | 11 | 883 ±135 | 2.23 ±0.09 | 0.95 ±0.09 | 1.87 ±0.18 | 1.99 ±0.20 | 23.06 ±3.90 | 4.32 ±0.36 | 65.6 ±11.4 | 1.27 ±0.31 | 20.9 ±14.6 | 38.4 ±7.1 | 0.59 ±0.15 | 3.08 ±0.47 | 3.64 ±0.24 | 1.63 ±0.14 |
| Conventionally bred cattle[⌘⌘] | 10 | 11 | 909 ±70 | 2.20 ±0.10 | 0.98 ±0.13 | 1.91 ±0.13 | 2.08 ±0.21 | 21.99 ±2.81 | 4.77 ±1.66 | 68.4 ±10.7 | 1.19 ±0.23 | 19.1 ±4.5 | 38.5 ±7.3 | 0.71 ±0.17 | 3.02 ±0.55 | 3.76 ±0.31 | 1.56 ±0.21 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 927 ±126 | 2.23 ±0.10 | 0.93 ±0.15 | 2.05 ±0.24 | 2.16 ±0.30 | 23.77 ±4.82 | 4.37 ±0.39 | 72.6 ±9.4 | 1.23 ±0.24 | 17.4 ±2.7 | 45.0 ±10.1 | 0.65 ±0.21 | 2.84 ±0.47 | 3.70 ±0.27 | 1.53 ±0.23 |

Female

| Rat group for milk powder diet | | Number of rats | Body weight | Brain | Salivary gland | Heart | Lung | Liver | Kidney | Adrenal gland | Spleen | Pituitary gland | Thyroid gland | Ovary | Uterus |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | | | | | | | | | | | | | | |
| Origin | Content (%) | | (g) | (g) | (g) | (g) | (g) | (g) | (g) | (mg) | (g) | (mg) | (mg) | (mg) | (g) |
| Conventionally bred cattle[⌘⌘] | 2 | 12 | 459 ±88 | 2.01 ±0.09 | 0.58 ±0.05 | 1.23 ±0.14 | 1.43 ±0.17 | 10.97 ±3.91 | 2.48 ±0.29 | 69.3 ±11.6 | 0.67 ±0.16 | 29.4 ±8.9 | 30.1 ±5.4 | 72.1 ±30.3 | 0.93 ±0.23 |
| Progeny of cloned cattle[⌘⌘] | 2 | 12 | 472 ±70 | 1.97 ±0.11 | 0.59 ±0.05 | 1.27 ±0.15 | 1.39 ±0.11 | 10.83 ±2.23 | 2.62 ±0.40 | 75.9 ±14.3 | 0.61 ±0.12 | 32.4 ±12.4 | 33.7 ±5.4 | 67.7 ±41.4 | 0.92 ±0.25 |
| Conventionally bred cattle[⌘⌘] | 10 | 12 | 459 ±69 | 1.99 ±0.09 | 0.59 ±0.07 | 1.24 ±0.18 | 1.43 ±0.14 | 9.85 ±1.19 | 2.56 ±0.24 | 66.8 ±10.3 | 0.64 ±0.10 | 44.6 ±40.4 | 31.4 ±7.5 | 60.2 ±17.4 | 0.82 ±0.18 |
| Progeny of cloned cattle[⌘⌘] | 10 | 12 | 450 ±61 | 2.00 ±0.09 | 0.55 ±0.06 | 1.23 ±0.10 | 1.44 ±0.16 | 9.73 ±1.71 | 2.42 ±0.21 | 65.9 ±11.1 | 0.81 ±0.59 | 38.3 ±36.0 | 31.2 ±4.7 | 70.8 ±23.4 | 0.99 ±0.37 |

Each value is shown as mean±standard deviation

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

chronic nephrosis, pelvic inflammation and tubular mineralization in the kidney; lymphocytic infiltration of submucosa in the urinary bladder; focal hyperplasia of the anterior lobe of the pituitary gland; C-cell hyperplasia and remnant of ultimobranchial body in the thyroid gland; focal hyperplasia of the cortex and angioectasis in the adrenal gland. Among these lesions, the rates of occurrence of fatty change in the parotid gland, pancreas lesions and chronic nephrosis in the kidney were higher in males than in females. No significant differences in the occurrence of these histological findings in rats due to the origin of milk powder were observed. Some lesions observed in a rat (No. 035) were severe multifocal necrosis in the liver and slight congestive edema in the lung.

Other lesions that were observed sporadically on histological observation were as follows: osseous metaplasia; mineralization of tunica media of aorta in the lung; increased hematopoiesis in bone marrow; increased deposit of brown pigmentation in the spleen; hematoma in the liver; lymphocytic infiltration in the parotid gland; gastric gland dilatation in the glandular stomach; fibrosis of lamina propria, suppurative inflammation in the cecum; solitary cyst, cystic kidney (unilateral) and lymphocytic infiltration of the cortex in the kidney; lymphoctytic infiltration in the prostate gland; endometrial hyperplasia in the uterus; cyst of anterior lobe, angioectasis and aberrant craniopharyngeal tissue in the pituitary gland; lymphocytic infiltration in the thyroid gland; and sporadic circular lymphocytic infiltration in the Harderian gland.

In neoplastic lesions of rat groups fed milk powder diet, a mammary gland tumor (cf. 1)-A)-*a)-ii)* of feeding study section; p.21) was observed. Other neoplastic lesions in rats were as follows: benign

Table 28. Histopathological findings of rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| | | | | Male rat group fed diet supplemented with milk powder derived from; | | Female rat group fed diet supplemented with milk powder derived from; | |
|---|---|---|---|---|---|---|---|
| | | | | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] |
| Organ | : | Findings | Number of rats | 12 | 12 | 12 | 12 |
| ***NON-NEOPLASTIC LESIONS*** | | | | | | | |
| Lung | : | Mineralization, artery | | 8 | 8 | 3 | 5 |
| | | Accumulation, foam cell | | 3 | 3 | 2 | 1 |
| | | Metaplasia, osseous | | 0 | 0 | 1 | 0 |
| | | Congestive edema | | 1 | 0 | 0 | 0 |
| Heart | : | Myocardial degeneration/fibrosis | | 8 | 9 | 4 | 4 |
| Aorta | : | Mineralization, tunica media | | 0 | 0 | 1 | 0 |
| Bone Marrow | : | Increased hematopoiesis | | 0 | 0 | 0 | 1 |
| Spleen | : | Congestion | | 2 | 1 | 1 | 1 |
| | | Increased extramedullary hematopoiesis | | 0 | 0 | 2 | 2 |
| | | Increased deposit, brown pigment | | 0 | 0 | 1 | 1 |
| Liver | : | Fatty change, hepatocyte | | 7 | 8 | 5 | 4 |
| | | Necrosis, focal | | 1 | 0 | 0 | 0 |
| | | Hematoma | | 0 | 0 | 0 | 1 |
| Parotid gland | : | Fatty change | | 12 | 10 | 2 | 1 |
| | | Lymphocytic infiltration | | 0 | 0 | 1 | 0 |
| Forestomach | : | Squamous hyperplasia | | 0 | 2 | 1 | 1 |
| Glandular stomach | : | Dilatation, gastric gland | | 0 | 1 | 0 | 1 |
| Cecum | : | Fibrosis, lumina propria | | 1 | 0 | 0 | 0 |
| | | Inflammation, suppurative | | 0 | 0 | 0 | 1 |
| Pancreas | : | Fatty change | | 7 | 8 | 2 | 2 |
| | | Deposit, brown pigment | | 6 | 6 | 1 | 0 |
| | | Fibrosis | | 8 | 5 | 0 | 0 |
| | | Atrophy, aciner cell, focal | | 2 | 2 | 0 | 0 |
| Kidney | : | Cyst, solitary | | 0 | 0 | 2 | 0 |
| | | Cystic kidney, unilateral | | 1 | 0 | 0 | 0 |
| | | Mineralization, tubular | | 0 | 0 | 1 | 2 |
| | | Lymphocytic infiltration, cortex | | 0 | 0 | 1 | 0 |
| | | Chronic nephrosis | | 12 | 8 | 4 | 4 |
| | | Lymphocytic infiltration, pelvis | | 2 | 0 | 0 | 0 |
| | | Inflammation, pelvis | | 1 | 2 | 2 | 3 |
| Urinary bladder | : | Lymphocytic infiltration, submucosa | | 0 | 0 | 1 | 1 |
| Prostate | : | Lymphocytic infiltration | | 0 | 1 | – | – |
| Uterus | : | Hyperplacia, endometrial | | – | – | 0 | 1 |
| Pituitary gland | : | Focal hyperplasia, anterior lobe | | 5 | 2 | 4 | 1 |
| | | Cyst, anterior lobe | | 2 | 0 | 1 | 0 |
| | | Aberrant craniopharyngeal tissue | | 1 | 0 | 0 | 0 |
| | | Angioectasis, anterior lobe | | 0 | 0 | 2 | 0 |
| Thyroid gland | : | Remnant, ultimobranchial body | | 3 | 1 | 0 | 2 |
| | | C-cell hyperplasia | | 1 | 2 | 2 | 1 |
| | | Lymphocytic infiltration | | 0 | 1 | 0 | 0 |
| Adrenal gland | : | Focal hyperplasia, cortex | | 3 | 3 | 0 | 0 |
| | | Angioectasis | | 0 | 0 | 3 | 1 |
| | | Cyst, hemorrhagic | | 0 | 1 | 0 | 0 |
| Harderian gland | : | Lymphocytic infiltration | | 1 | 0 | 0 | 0 |
| ***NEOPLASTIC LESIONS*** | | | | | | | |
| Pituitary gland | : | Adenoma | | 2 | 2 | 2 | 2 |
| Thyroid gland | : | Adenoma, follicular cell | | 0 | 1 | 0 | 0 |
| Mammary gland | : | Fibroadenoma | | 0 | 0 | 0 | 1 |
| Uterus | : | Polyp, endometrial stromal | | – | – | 0 | 1 |

No abnormalities were detected in the brain spinal cord, sciatic nerve, trachea, sublingnal and submandibular glands, parathyroid, lymph node, thymus, bone marrow, tongue, esophagus, small intestine, eye ball, skeltal muscle, skin, testis, epididymis, seminal vesicle and vagina.

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

adenoma in the pituitary gland (two males and two females in group fed diet supplemented with 10% (w/w) milk powder derived from the progeny; three males and two females in group fed diet supplemented with 10% (w/w) milk powder derived from conventionally bred cattle); benign follicular cell adenoma in the thyroid gland (one male fed diet supplemented with 10% (w/w) milk powder derived from the progeny); and benign endometrial stromal polyp of the uterus (one female fed diet supplemented with 10% (w/w) milk powder derived from the progeny).

In the rat group fed diet supplemented with 2% (w/w) milk powder derived from the progeny, findings obtained from histological examination of the gross lesions were as follows: severe multifocal necrosis in the liver with scattered white spots of a male (No. 019), which was euthanized as it had reached the moribund stage; angioectasis in the liver, which showed red spots; follicular cysts in the ovary; and benign anterior lobe adenoma in the pituitary grand with a black area.

With regard to the rat group fed diet supplemented with 2% (w/w) milk powder derived from conventionally bred cattle, findings obtained from histological examination of the gross lesions were as follows: angioectasis of the cortex in the enlarged adrenal gland and inflammation in the spleen with a white area; benign anterior lobe adenoma in the pituitary grand with a black area; and benign fibroadenoma of the mammary gland.

These lesions observed in the present investigation were assumed to be spontaneous lesions[13]. In rat groups fed diets supplemented with milk powder derived from the progeny or conventionally bred cattle, no significant differences in the rate of occurrence of these lesions due to the origin of milk powder were observed.

#### *d) Observation of pups ($F_1$) produced by rats fed diets supplemented with milk powder (Tables 29–31)*

In pups delivered from rat groups fed diets supplemented with milk powder (2 or 10% (w/w)) derived from the progeny, no significant differences in litter size, delivery index, sex ratio, viability index (day 4 of lactation) and lactation index (day 21 of lactation) due to the origin of milk powder were observed.

In terms of the body weights of female pups produced by female groups fed 2% (w/w) diet supplemented with milk powder from the progeny, a significantly high value was obtained on day 0 of lactation compared with that of pups delivered from female groups fed diet supplemented with 2% (w/w) milk powder derived from conventionally bred cattle. However, no significant differences in body weights due to the origin of milk powder were found. Thus, the differences in body weights were considered to be coincidences.

In terms of the external appearance of pups delivered from rat groups fed milk powder diets derived from the progeny or conventionally bred cattle, no differences in hair growth, pinna detachment, incisor eruption, eyelid opening and testicular descent due to the origin of milk powder were observed. All pups delivered from rat groups fed milk powder diet showed normal performance in the sensory response/reflex function test. There were no pups with any external abnormalities or visceral malformations.

In terms of the body weights and food consumption of rat groups fed milk powder diets derived from the progeny or conventionally bred cattle, no significant differences due to the origin of milk powder were observed during pregnancy and lactation.

### 2) Meat

#### A) Effects of meat intake on health status in rats

##### *a) Clinical signs*

###### *i) Dead cases*

In the group fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle, a female rat (No. 511) died. In this case, a subcutaneous mass was found in the right axillary region on the 162$^{nd}$ day of feeding, which became larger over time and ulcerated. It caused pale skin due to hemorrhage and hypothermia with increased pallor. Thus, this female was euthanized on the 191$^{st}$ day of the feeding period. The subcutaneous mass observed in this case was benign adenoma of the mammary gland.

A subcutaneous mass was also found in 2 females (Nos. 542 and 547) fed diet supplemented with

Table 29. Observation of pups ($F_1$) from rats fed diet supplemented with freeze-dried milk powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Item | | Dams of pups investigated | | | |
|---|---|---|---|---|---|
| | | Fed diet supplemented with 2% milk powder derived from; | | Fed diet supplemented with 10% milk powder derived from; | |
| | | Conventionally bred cattle⌘⌘ | Progeny of cloned cattle⌘⌘ | Conventionally bred cattle⌘⌘ | Progeny of cloned cattle⌘⌘ |
| On day 0 of lactation | | | | | |
| Litter size | | 12.4 ± 5.0[a)] | 10.4 ± 4.5 | 12.4 ± 4.1 | 13.3 ± 1.9 |
| Live birth index(%) | | 98.7 | 94.2 | 99.2 | 99.3 |
| Sex ratio(Male/Female) | | 0.863 | 0.891 | 1.138 | 1.246 |
| Body weights(g) | Male | 6.9 ± 0.5 | 7.3 ± 0.7 | 6.9 ± 0.5 | 6.8 ± 0.9 |
| | Female | 6.4 ± 0.5 | 6.8 ± 0.5* | 6.5 ± 0.6 | 6.5 ± 0.9 |
| On day 4 of lactation | | | | | |
| Viability index on day 4(%) | | 95.2 | 96.9 | 98.4 | 97.9 |
| Body weights(g) | Male | 11.4 ± 1.5 | 12.7 ± 1.7 | 11.6 ± 1.1 | 11.1 ± 2.0 |
| | Female | 10.2 ± 2.0 | 11.9 ± 1.2 | 10.7 ± 1.1 | 10.9 ± 1.9 |
| On day 7 of lactation | | | | | |
| Body weights(g) | Male | 18.8 ± 2.9 | 20.6 ± 2.4 | 19.2 ± 1.7 | 18.2 ± 2.6 |
| | Female | 20.9 ±14.4 | 20.0 ± 1.4 | 18.2 ± 2.4 | 18.0 ± 2.4 |
| On day 14 of lactation | | | | | |
| Body weights(g) | Male | 40.0 ± 4.7 | 42.3 ± 3.3 | 40.2 ± 3.4 | 39.1 ± 3.8 |
| | Female | 39.1 ± 3.8 | 42.3 ± 2.5 | 39.4 ± 3.9 | 38.4 ± 3.5 |
| On day 21 of lactation | | | | | |
| Lactation index(%) | | 98.8 | 100 | 100 | 100 |
| Body weights(g) | Male | 70.5 ± 5.9 | 75.6 ± 5.2 | 72.2 ± 5.0 | 69.8 ± 5.3 |
| | Female | 68.7 ± 5.3 | 72.8 ± 3.1 | 69.3 ± 5.0 | 67.2 ± 4.7 |
| Sensory response / reflex function test[b)] | | NAD | NAD | NAD | NAD |
| External abnormalities(%) | | 0.0 (0/149) | 0.0 (0/104) | 0.0 (0/124) | 0.0 (0/146) |
| Visceral malformations(%) | | 0.0 (0/149) | 0.0 (0/104) | 0.0 (0/124) | 0.0 (0/146) |

Live birth index = (Number of live pups on day 0 / Number of pups born)×100
Viability index on day 4 = (Number of live pups on day 4 / Number of pups on day 0)×100
Lactation index = (Nunber of live pups on day 21 / Number of pups on day 4)×100
NAD : No abnormalities were detected
a) : Mean±standard deviation
b) : Responses to sound, approach, touch and tail pinch, pupil reflex to light, and pinna, ipsilateral flexor, eyelid and righting reflexes
Significant difference between two rat groups fed test diets containing milk powder derived from conventionally bred cattle or progeny of cloned cattle in the same milk powder content (*: $p<0.05$).
⌘: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.
⌘⌘: Holstein dairy cattle.

5% (w/w) meat powder derived from the progeny. The masses appeared on the 351st day of the feeding period in the right axillary region in No. 542 and on the 260th day of feeding in the right inguinal region in No. 547. The masses in Nos. 542 and 547 were benign adenoma and benign fibroadenoma, respectively.

Another female (No. 515) in the group fed diet supplemented with 5% (w/w) meat powder from conventionally bred cattle showed chromodacryorrhea (from the 279th day of feeding) and pale skin (from the 322nd day of feeding), and its general condition deteriorated very quickly. On the last day of the feeding period (364th day), hypothermia, ataxic gait and accelerated respiration were observed. This rat was diagnosed with myelogenous leukemia by pathological examination. In addition to these symptoms, chromodacryorrhea, crushing of incisor, crust formation and abdominal distention were also observed sporadically.

These cases found in the present investigation

Table 30. Developmental observation of pups from rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | Number of dams | Developmental observation (day) | | | | |
|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | | Hair growth | Pinna detachiment | Incisor eruption | Eyelids opening | Testicular descent |
| Origin | Content (%) | | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 11 | 4.0 ± 0.0 | 4.0 ± 0.0 | 9.5 ± 1.7 | 13.3 ± 0.8 | 18.4 ± 1.0 |
| Progeny of cloned cattle[⌘⌘] | 2 | 8 | 4.0 ± 0.0 | 4.0 ± 0.0 | 10.0 ± 0.7 | 12.6 ± 0.5 | 17.2 ± 0.8 |
| Conventionally bred cattle[⌘⌘] | 10 | 10 | 4.0 ± 0.0 | 4.0 ± 0.0 | 10.1 ± 1.2 | 12.9 ± 0.7 | 18.0 ± 0.9 |
| Progeny of cloned cattle[⌘⌘] | 10 | 11 | 4.0 ± 0.0 | 4.0 ± 0.0 | 10.3 ± 0.7 | 13.3 ± 0.7 | 17.8 ± 1.1 |

Mean±standard deviation

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

Table 31. Body weight, food consumption and milk powder intake during gestation and lactation periods of female rats fed diet supplemented with freeze-dried milk powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for milk powder diet | | | Days of pregnancy | | | | Days of lactation | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Milk powder in test diet | | | | | | | | | | |
| Origin | Content (%) | | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 |
| Conventionally bred cattle[⌘⌘] | 2 | Body weight (g) | 318±38 (12) | 351±33 (12) | 381±36 (12) | 434±34 (11) | 358±37 (12) | 362±34 (11) | 361±30 (10) | 338±31 (10) |
| | | Food consumption (g/day) | 22±3 | 24± 4 | 25± 3 | 22± 4 | 23± 8 | 41± 9 | 55± 5 | 41± 6 |
| | | Milk powder intake (mg/kg/day) | 1383 | 1368 | 1312 | 1014 | 1285 | 2265 | 3047 | 2426 |
| Progeny of cloned cattle[⌘⌘] | 2 | Body weight (g) | 316±26 (10) | 345±30 (10) | 375±36 (10) | 426±56 (10) | 363±37 (9) | 365±30 (8) | 353±21 (8) | 334±18 (8) |
| | | Food consumption (g/day) | 19± 5 | 24± 2 | 24± 3 | 23± 4 | 23± 9 | 39± 5 | 60±11 | 40± 6 |
| | | Milk powder intake (mg/kg/day) | 1203 | 1391 | 1280 | 1080 | 1267 | 2137 | 3399 | 2395 |
| Conventionally bred cattle[⌘⌘] | 10 | Body weight (g) | 317±32 (10) | 349±29 (10) | 375±31 (10) | 435±45 (10) | 357±37 (10) | 359±25 (10) | 350±18 (10) | 334±16 (10) |
| | | Food consumption (g/day) | 21± 5 | 24± 6 | 23± 6 | 23± 6 | 25±10 | 42±12 | 64±18 | 43±14 |
| | | Milk powder intake (mg/kg/day) | 6625 | 6877 | 6133 | 5287 | 7003 | 11699 | 18286 | 12874 |
| Progeny of cloned cattle[⌘⌘] | 10 | Body weight (g) | 310±31 (11) | 343±31 (11) | 378±32 (11) | 446±34 (11) | 359±35 (11) | 357±31 (11) | 352±22 (11) | 332±24 (11) |
| | | Food consumption (g/day) | 23± 6 | 23± 6 | 25± 5 | 22± 9 | 23± 7 | 40± 4 | 58± 6 | 42± 7 |
| | | Milk powder intake (mg/kg/day) | 7419 | 6706 | 6614 | 4933 | 6407 | 11204 | 16477 | 12651 |

Values represent mean±standard deviation or mean

(n): Number of animals available

[⌘]: Milk derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each milk powder was supplemented to a test diet.

[⌘⌘]: Holstein dairy cattle.

could also generally occur during long-term raising of rats; therefore, the symptoms were not considered to be due to feeding on diets supplemented with meat powder derived from progeny.

***b) Body weights (Figs. 9-1, 9-2)***

In terms of the body weights of rat groups fed diets supplemented with 1 or 5% (w/w) meat powder derived from the progeny, a fluctuating tendency of heavier body weight was observed in male groups fed diet supplemented with 1% (w/w) meat powder and female groups fed diet supplemented with 5% (w/w) meat powder when compared with rats of the same sex fed the same amount of meat powder diet from conventionally bred cattle. However, no significant differences in body weights due to the origin of meat powder were observed in all observation points.

***c) Food consumption (Figs. 10-1, 10-2)***

In terms of the food consumption of female rat groups fed diet supplemented with 1% (w/w) meat powder diet derived from the progeny, a significantly lower value in the 37$^{th}$ week of the feeding period was observed compared with that in female groups fed diet supplemented with meat powder derived from conventionally bred cattle. In other rat groups fed diet supplemented with meat powder derived from the progeny, no significant differences in food consumption due to the origin of meat powder were found.

The average food consumption per body weight of rats was observed throughout the twelve-month feeding period, except for pregnant females. In rat groups fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle, it was 434 mg/kg/day in males and 531 mg/kg/day in females. In rat groups fed diet supplemented with 1% (w/w) meat powder derived from progeny, it was 415 mg/kg/day in males and 528 mg/kg/day in females. In rat groups fed diets supplemented with 5% (w/w) meat powder derived from conventionally bred cattle, it was 2,057 mg/kg/day in males and 2,623 mg/kg/day in females. In rat groups fed diet supplemented with 5% (w/w) meat powder derived from the progeny, it was 2,090 mg/kg/day in males and 2,549 mg/kg/day in females.

**B) Effects of meat intake on physiological functions in rats**

***a) Sensory and reflex functions***

Slight hypersensitivity in touch response was observed in one male fed diet supplemented with 1% (w/w) meat powder derived from the progeny. In this case, slight hypersensitivity at each observation point was shown at 3$^{rd}$, 6$^{th}$, 9$^{th}$ and 12$^{th}$ months of the feeding period; these observations were regarded as characteristics peculiar to this individual. The other rat groups fed 1 or 5% (w/w) meat powder diet derived from the progeny or conventionally bred cattle showed normal sound response, approach response, touch response, tail pinch response, pupil reflex to light, pinna reflex, eyelid reflex, ipsilateral flexor reflex and righting reflex.

***b) Grip strength and motor activity (Tables 32–34)***

In terms of the grip strength of the male group fed diet supplemented with 1% (w/w) meat powder derived from progeny, significantly high values were observed in hindlimb grip on the 3$^{rd}$ month of feeding and forelimb grip strength on the 6$^{th}$ month compared with those observed in the rat group fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle. In other rat groups fed diet supplemented with meat powder derived from the progeny or conventionally bred cattle, no significant differences in hindlimb and forelimb grip due to the origin of meat powder were observed. Although significant differences in grips were observed, they were considered irrelevant and coincidental findings. Therefore, these significant differences were not attributed to the origin of meat powder.

In terms of the motor activity of male rat group fed diet supplemented with 1% (w/w) meat powder derived from the progeny, significantly reduced activities at the 3$^{rd}$ month of the feeding period were observed when compared with those in rat group fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle. However, no significant differences in motor activity due to the origin of meat powder were observed in other female/male groups. The significant differences in motor

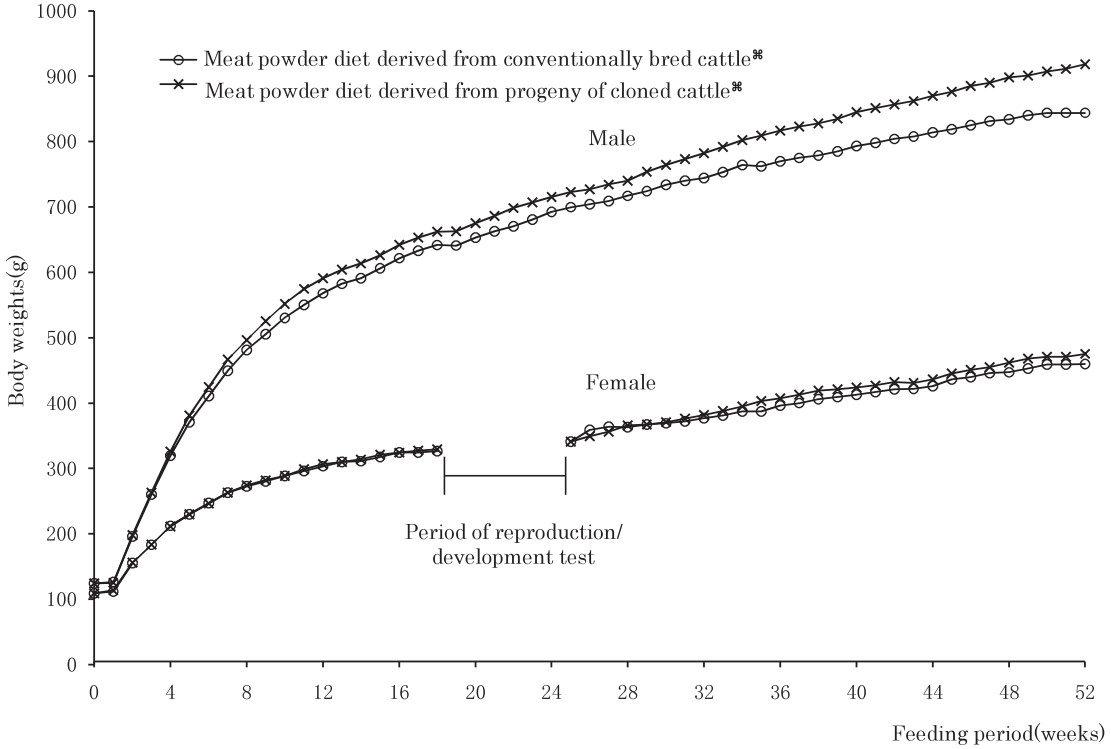


Fig. 9-1. Body weight curves of rats fed diet supplemented with 1% meat powder ⌘⌘

⌘: Japanese Black beef cattle.

⌘⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was prepared independently and freeze-dried. Each meat powder was supplemented to a test diet.

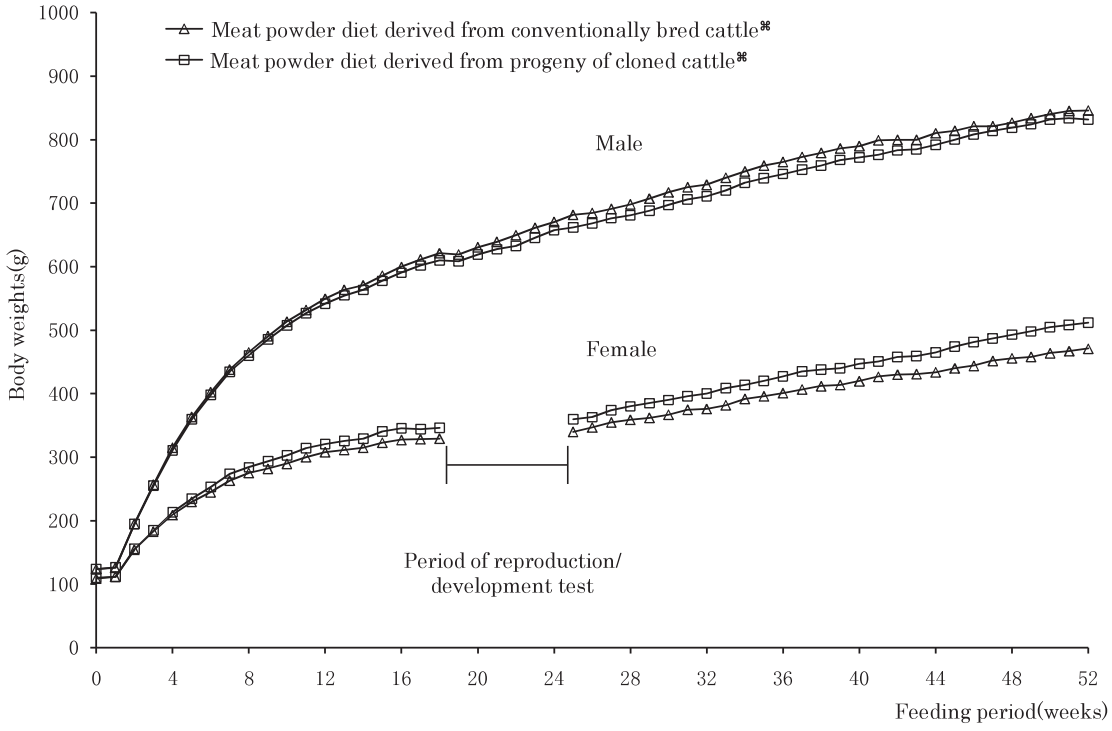


Fig. 9-2. Body weight curves of rats fed diet supplemented with 5% meat powder ⌘⌘

⌘: Japanese Black beef cattle.

⌘⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was prepared independently and freeze-dried. Each meat powder was supplemented to a test diet.

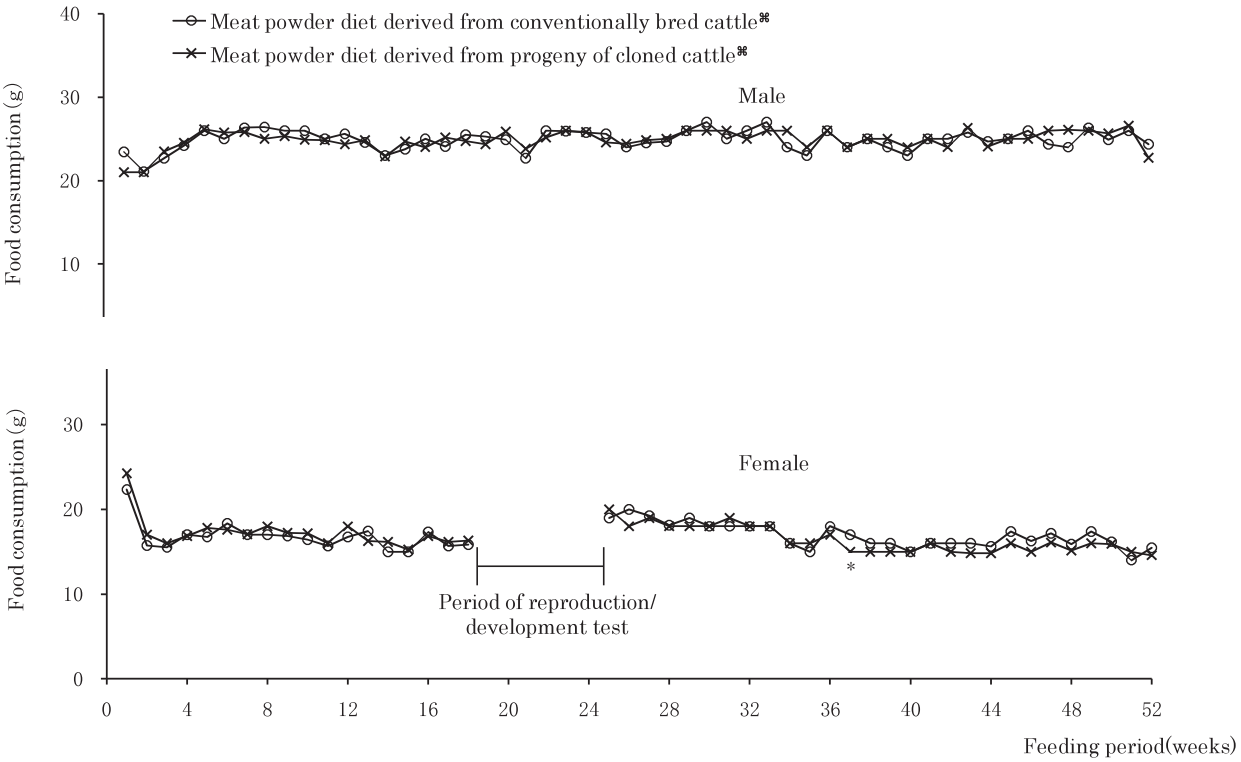


Fig. 10-1. Food consumption curves of rats fed diet supplemented with 1% meat powder⌘⌘

⌘: Japanese Black beef cattle.

⌘⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was prepared independently and freeze-dried. Each meat powder was supplemented to a test diet.

Significant difference between two test diets (* $p<0.05$).

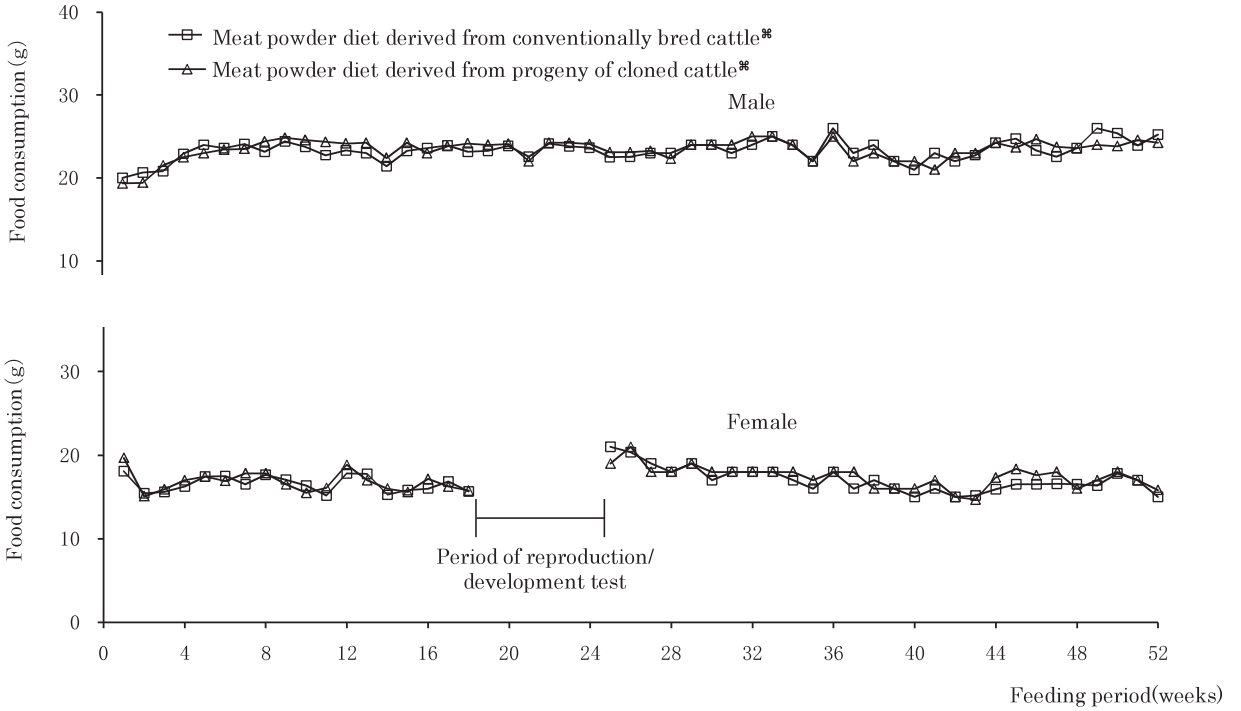


Fig. 10-2. Food consumption curves of rats fed diet supplemented with 5% meat powder⌘⌘

⌘: Japanese Black beef cattle.

⌘⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was prepared independently and freeze-dried. Each meat powder was supplemented to a test diet.

Table 32. Grip strength of male rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test (g)

| | Rat group for meat powder diet | | Point of the measurement | | | |
|---|---|---|---|---|---|---|
| | Meat powder in test diet | | | | | |
| | Origin | Content (%) | 3rd month | 6th month | 9th month | 12th month |
| Forelimb | Conventionally bred cattle[⌘⌘] | 1 | 898 ± 248 (12) | **740 ± 237** (12) | 646±117 (12) | 623 ± 65 (12) |
| | Progeny of cloned cattle[⌘⌘] | 1 | 965 ± 204 (12) | **987 ± 195** (12) * | 622±122 (12) | 849 ± 201 (12) |
| | Conventionally bred cattle[⌘⌘] | 5 | 1014 ± 277 (12) | 893 ± 174 (12) | 644±101 (12) | 726 ± 215 (12) |
| | Progeny of cloned cattle[⌘⌘] | 5 | 908 ± 266 (12) | 763 ± 189 (12) | 669±109 (12) | 845 ± 215 (12) |
| Hindlimb | Conventionally bred cattle[⌘⌘] | 1 | **253 ± 104** (12) | 412 ± 151 (12) | 542±142 (12) | 467 ± 175 (12) |
| | Progeny of cloned cattle[⌘⌘] | 1 | **405 ± 99** (12) ** | 509 ± 111 (12) | 595±107 (12) | 499 ± 96 (12) |
| | Conventionally bred cattle[⌘⌘] | 5 | 408 ± 128 (12) | 514 ± 95 (12) | 603±114 (12) | 504 ± 117 (12) |
| | Progeny of cloned cattle[⌘⌘] | 5 | 386 ± 66 (12) | 463 ± 101 (12) | 615±148 (12) | 522 ± 167 (12) |

Mean±standard deviation (number of rats investigated)
Significant difference between two rat groups fed test diets containing meat powder derived from conventionally bred cattle or progeny of cloned cattle in the same meat powder content (*: $p<0.05$, **: $p<0.01$).
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

Table 33. Grip strength of female rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test (g)

| | Rat group for meat powder diet | | Point of the measurement | | | |
|---|---|---|---|---|---|---|
| | Meat powder in test diet | | | | | |
| | Origin | Content (%) | 3rd month | 6th month | 9th month | 12th Month |
| Forelimb | Conventionally bred cattle[⌘⌘] | 1 | 788 ± 150 (12) | 778 ± 82 (12) | 721±175 (11) | 742 ± 154 (11) |
| | Progeny of cloned cattle[⌘⌘] | 1 | 761 ± 76 (12) | 709 ± 153 (12) | 666±122 (12) | 627 ± 116 (12) |
| | Conventionally bred cattle[⌘⌘] | 5 | 694 ± 125 (12) | 760 ± 148 (12) | 832±150 (12) | 610 ± 150 (12) |
| | Progeny of cloned cattle[⌘⌘] | 5 | 794 ± 127 (12) | 801 ± 141 (12) | 734±167 (12) | 621 ± 164 (12) |
| Hindlimb | Conventionally bred cattle[⌘⌘] | 1 | 273 ± 129 (12) | 440 ± 113 (12) | 387±106 (11) | 435 ± 74 (11) |
| | Progeny of cloned cattle[⌘⌘] | 1 | 250 ± 61 (12) | 436 ± 122 (12) | 497±104 (12) | 477 ± 116 (12) |
| | Conventionally bred cattle[⌘⌘] | 5 | 268 ± 95 (12) | 366 ± 75 (12) | 493±107 (12) | 450 ± 79 (12) |
| | Progeny of cloned cattle[⌘⌘] | 5 | 219 ± 57 (12) | 397 ± 100 (12) | 490± 95 (12) | 540 ± 158 (12) |

Mean±standard deviation (number of rats investigated)
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

activity shown above were considered as coincidental findings and were not attributed to the origin of meat powder.

***c) Reproductive function (Table 35)***

In terms of the reproductive function of rat groups fed diets supplemented with 1 or 5% (w/w) meat powder derived from progeny, no significant differences in the estrous cycle, copulation index, fertility index, gestation period and delivery index due to the origin of meat powder were observed.

***d) Ophthalmology***

In rat groups fed diet supplemented with 1 or 5% (w/w) meat powder derived from conventionally bred cattle or the progeny, no abnormalities in ophthalmology findings due to the origin of meat powder were found.

***e) Urinalysis (Tables 36-1–37-2)***

In urinalysis observations of female/male rat groups fed diet supplemented with 1 or 5% (w/w) meat powder derived from the progeny, no significant

Table 34. Motor activity of rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

(Counts/60minuts)

| Sex of rats fed test diet | Rat group for meat powder diet | | Point of the measurement | | | |
|---|---|---|---|---|---|---|
| | Meat powder in test diet | | | | | |
| | Origin | Content (%) | 3rd month | 6th month | 9th month | 12th month |
| Male | Conventionally bred cattle[⌘⌘] | 1 | 13729 ± 2403 (12) | 9171 ± 2847 (12) | 5173 ± 1968 (12) | 4888 ± 1584 (12) |
| | Progeny of cloned cattle[⌘⌘] | 1 | 9623 ± 2263 (12) ** | 8651 ± 2323 (12) | 4562 ± 2423 (12) | 4299 ± 1731 (12) |
| | Conventionally bred cattle[⌘⌘] | 5 | 11991 ± 2228 (12) | 10370 ± 3118 (12) | 5884 ± 1652 (12) | 3826 ± 2413 (12) |
| | Progeny of cloned cattle[⌘⌘] | 5 | 9514 ± 3511 (12) | 8244 ± 1781 (12) | 2770 ± 1020 (12) | 4659 ± 1245 (12) |
| Female | Conventionally bred cattle[⌘⌘] | 1 | 11882 ± 3304 (12) | 9696 ± 2023 (12) | 8721 ± 4141 (11) | 8652 ± 2965 (11) |
| | Progeny of cloned cattle[⌘⌘] | 1 | 13099 ± 3111 (12) | 8126 ± 2929 (12) | 9482 ± 2613 (12) | 7363 ± 2894 (12) |
| | Conventionally bred cattle[⌘⌘] | 5 | 10909 ± 3407 (12) | 6726 ± 2917 (12) | 9346 ± 2445 (12) | 6288 ± 1486 (12) |
| | Progeny of cloned cattle[⌘⌘] | 5 | 10472 ± 3549 (12) | 8666 ± 2882 (12) | 9630 ± 2383 (12) | 5094 ± 1761 (12) |

Mean±standard deviation (number of rats investigated)

Significant difference between two rat groups fed test diets containing meat powder derived from conventionally bred cattle or progeny of cloned cattle in the same meat powder content (**: $p<0.01$).

[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

[⌘⌘]: Japanese Black beef cattle.

Table 35. Reproductive function of female rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | Estrous cycle (days, Mean±SD) | Copulation index (%) | Fertility index (%) | Gestation length (days, Mean±SD) | Gestation index (%) |
|---|---|---|---|---|---|---|
| Meat powder in test diet | | | | | | |
| Origin | Content (%) | | | | | |
| Conventionally bred cattle[⌘⌘] | 2 | 4.4 ± 0.8 (12)[a)] | 100 (12/12) | 100 (12/12) | 22.6 ± 0.5 (12) | 100 (12/12) |
| Progeny of cloned cattle[⌘⌘] | 2 | 4.5 ± 1.8 (11) | 91.7 (11/12) | 100 (11/11) | 22.6 ± 0.5 (11) | 90.9 (10/11) |
| Conventionally bred cattle[⌘⌘] | 10 | 4.1 ± 0.2 (12) | 100 (12/12) | 100 (12/12) | 22.6 ± 0.5 (12) | 100 (12/12) |
| Progeny of cloned cattle[⌘⌘] | 10 | 4.1 ± 0.2 (12) | 91.7 (11/12) | 100 (11/11) | 22.5 ± 0.7 (11) | 90.9 (10/11) |

Copulation index = (Number of pairs with successful copulation / Number of pairs mated)×100

Fertility index = (Number of pregnant female / Number of pairs with successful copulation)×100

Gestation index = (Number of females with live pups / Number of pregnant females)×100

SD : Standard deviation

[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

[⌘⌘]: Japanese Black beef cattle.

[a)] : Number of rats investigated

differences in each index due to the origin of meat powder were observed.

***f) Blood analysis***

***i) Hematology (Tables 38, 39)***

In male rat groups fed diet supplemented with 1% (w/w) meat powder derived from the progeny, a significant increase in monocyte percentage (5.6%) in the differential leucocyte count was obtained compared with that in rat group fed diet supplemented with 1% (w/w) meat powder from conventionally bred cattle (4.3%). However, in other rat groups fed diet supplemented with meat powder derived from the progeny, no significant differences in hematology parameters due to the origin of meat powder were observed.

Furthermore, myelogenous leukemia was found in one female (No. 515) fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle. The blood in this case showed a markedly increased level of granulated leucocytes and decreased

Table 36-1. Urinalysis of male rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Color | | Cloudy | | Volume[a)] | Specific[a)] | pH | | | | | | | Protein | | | | | |
| Origin | Content (%) | of rats | PY | Y | − | 1+ | (ml/18hr) | gravity | 5.0 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | − | ± | 1+ | 2+ | 3+ |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 8 | 4 | 12 | | 5.9 ± 2.6 | 1.060 ± 0.013 | | 5 | 3 | 1 | 3 | | | | | 3 | 9 | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 8 | 4 | 12 | | 5.7 ± 0.8 | 1.055 ± 0.012 | | 4 | 4 | 2 | 1 | 1 | | | | 1 | 11 | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 7 | 5 | 12 | | 6.9 ± 2.0 | 1.052 ± 0.010 | | 3 | 8 | 1 | | | | | | 2 | 10 | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 9 | 3 | 12 | | 5.6 ± 1.9 | 1.056 ± 0.010 | | 3 | 4 | 3 | | 1 | 1 | | 1 | 2 | 8 | 1 |

| Rat group for meat powder diet | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Glucose | | | | Ketone body | | | | Occult blood | | | | | Urobilinogen | | | | Bilirubin | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | ± | 1+ | 2+ | − | ± | 1+ | 2+ | 3+ | 0.1 | 1 | 2 | 4 | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 12 | | | | 5 | 7 | | | 8 | 2 | 2 | | | 12 | | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 12 | | | | 8 | 4 | | | 10 | 1 | 1 | | | 12 | | | | 12 | | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 12 | | | | 6 | 6 | | | 11 | | | | 1 | 12 | | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 12 | | | | 6 | 6 | | | 8 | 2 | 2 | | | 12 | | | | 12 | | |

[a)]: Mean±standard deviation
Color : PY(pale yellow); Y(yellow)
Cloudy : −(negligible); 1+(cloudy)
Protein : −(negligible); ±(15~30mg/dl); 1+(30mg/dl); 2+(100mg/dl); 3+(300mg/dl)
Glucose : −(negligible); ±(0.1g/dl); 1+(0.25g/dl); 2+(0.5g/dl)
Ketone body : −(negligible); ±(5mg/dl); 1+(15mg/dl); 2+(40mg/dl)
Occult blood : −(negligible); ±(trace); 1+(slight); 2+(moderate); 3+(marked)
Urobilinogen : Ehrlich unit/dl
Bilirubin : −(negligible); 1+(slight); 2+(moderate); 3+(marked)
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

Table 36-2. Urinalysis of male rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | | Crystals | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Erythrocytes | | | | Leukocytes | | | | ammonium magnesium phosphate | | | | calcium carbonate | | | amorphous | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 10 | 2 | | | 11 | 1 | | | 12 | | | | 12 | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 11 | 1 | | | 11 | 1 | | | 12 | | | | 12 | | | 12 | | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 11 | | 1 | | 11 | 1 | | | 12 | | | | 12 | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 9 | 2 | 1 | | 10 | 2 | | | 12 | | | | 12 | | | 12 | | |

| Rat group for meat powder diet | | | Epithelial cells | | | | | | | | | | Casts | | | | | | Fat | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | squamous | | | | round | | | spindle | | | granule | | hyaline | | waxy | | globules | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ | − | 1+ | − | 1+ | − | 1+ | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 2 | 9 | 1 | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 3 | 8 | 1 | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 1 | 10 | 1 | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | | 12 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |

− : Not observed; 1+ : A few(1-10 counts) in some fields; 2+ : A few(1-10 counts) in all fields; 3+ : Many(over 11 counts) in all fields
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

Table 37-1. Urinalysis of female rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Color | | Cloudy | | Volume[a)] | Specific[a)] | pH | | | | | | | Protein | | | | |
| Origin | Content (%) | of rats | PY | Y | − | 1+ | (ml/18hr) | gravity | 5.0 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | − | ± | 1+ | 2+ | 3+ |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 11 | | 11 | | 6.0 ± 2.3 | 1.061 ± 0.015 | | 5 | 2 | 1 | 2 | 1 | | | 2 | 5 | 4 | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 10 | 2 | 12 | | 7.5 ± 2.7 | 1.057 ± 0.015 | | 5 | 3 | 2 | 1 | | 1 | | 5 | 5 | 2 | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 10 | 2 | 12 | | 7.4 ± 2.7 | 1.057 ± 0.011 | | 9 | 2 | 1 | | | | | 4 | 4 | 4 | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 12 | | 12 | | 7.4 ± 3.0 | 1.057 ± 0.013 | | 5 | 5 | 1 | 1 | | | | 1 | 8 | 3 | |

| Rat group for meat powder diet | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Glucose | | | | Ketone body | | | | Occult blood | | | | | Urobilinogen | | | | Bilirubin | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | ± | 1+ | 2+ | − | ± | 1+ | 2+ | 3+ | 0.1 | 1 | 2 | 4 | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 11 | | | | 10 | 1 | | | 10 | | | | 1 | 11 | | | | 11 | | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 12 | | | | 11 | 1 | | | 11 | | | | 1 | 12 | | | | 12 | | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 12 | | | | 8 | 4 | | | 11 | | 1 | | | 12 | | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 12 | | | | 7 | 5 | | | 11 | | 1 | | | 12 | | | | 12 | | |

[a)]: Mean±standard deviation
Color : PY(pale yellow); Y(yellow)
Cloudy : −(negligible); 1+(cloudy)
Protein : −(negligible); ±(15~30mg/dl); 1+(30mg/dl); 2+(100mg/dl); 3+(300mg/dl)
Glucose : −(negligible); ±(0.1g/dl); 1+(0.25g/dl); 2+(0.5g/dl)
Ketone body : −(negligible); ±(5mg/dl); 1+(15mg/dl); 2+(40mg/dl)
Occult blood : −(negligible); ±(trace); 1+(slight); 2+(moderate); 3+(marked)
Urobilinogen : Ehrlich unit/dl
Bilirubin : −(negligible); 1+(slight); 2+(moderate); 3+(marked)
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

Table 37-2. Urinalysis of female rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | | Crystals | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Erythrocytes | | | | Leukocytes | | | | ammonium magnesium phosphate | | | | calcium carbonate | | | amorphous | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 10 | | 1 | | 10 | 1 | | | 10 | 1 | | | 11 | | | 11 | | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 11 | | 1 | | 11 | 1 | | | 12 | | | | 12 | | | 12 | | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 11 | 1 | | | 12 | | | | 12 | | | | 12 | | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 11 | 1 | | | 12 | | | | 11 | 1 | | | 12 | | | 12 | | |

| Rat group for meat powder diet | | | Epithelial cells | | | | | | | | | | Casts | | | | | | Fat | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | squamous | | | | round | | | spindle | | | granule | | hyaline | | waxy | | globules | | |
| Origin | Content (%) | of rats | − | 1+ | 2+ | 3+ | − | 1+ | 2+ | − | 1+ | 2+ | − | 1+ | − | 1+ | − | 1+ | − | 1+ | 2+ |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 5 | 6 | | | 11 | | | 11 | | | 11 | | 11 | | 11 | | 11 | | |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 5 | 7 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 6 | 6 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 6 | 6 | | | 12 | | | 12 | | | 12 | | 12 | | 12 | | 12 | | |

− : Not observed; 1+ : A few(1-10 counts) in some fields; 2+ : A few(1-10 counts) in all fields; 3+ : Many(over 11 counts) in all fields
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

Table 38. Hamatological data of male rats fed diet supplemented with freeze-dried meat powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | RBC | Hemoglobin | Hematocrit | MCV | MCH | MCHC | Reticulocyte | PT | APTT |
| Origin | Content (%) | of rats | ($10^4$/μl) | (g/dl) | (%) | (fl) | (pg) | (%) | (‰) | (sec) | (sec) |
| Conventionally bred cattle⌘⌘ | 1 | 12 | 848[a)] ± 72 | 14.3 ± 0.8 | 42.9 ± 2.1 | 51 ± 4 | 16.9 ± 1.3 | 33.2 ± 0.7 | 22.7 ± 10.0 | 13.4 ± 0.5 | 17.1 ± 0.8 |
| Progeny of cloned cattle⌘⌘ | 1 | 11 | 831 ± 52 | 14.2 ± 0.9 | 43.0 ± 2.0 | 52 ± 3 | 17.1 ± 0.8 | 32.9 ± 0.7 | 27.0 ± 11.7 | 13.1 ± 0.6 | 16.6 ± 1.2 |
| Conventionally bred cattle⌘⌘ | 5 | 12 | 881 ± 80 | 14.8 ± 1.5 | 44.4 ± 3.7 | 51 ± 5 | 16.8 ± 1.6 | 33.2 ± 0.9 | 23.2 ± 16.8 | 13.1 ± 0.5 | 17.8 ± 1.8 |
| Progeny of cloned cattle⌘⌘ | 5 | 12 | 870 ± 49 | 14.9 ± 0.8 | 44.6 ± 2.0 | 51 ± 2 | 17.1 ± 0.5 | 33.4 ± 0.6 | 19.6 ± 8.7 | 13.2 ± 0.8 | 17.5 ± 1.6 |

| Rat group for meat powder diet | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Platelet | WBC | Differential leukocyte counts (%) | | | | | |
| Origin | Content (%) | of rats | ($10^4$/μl) | ($10^2$/μl) | Basophil | Eosinophil | Neutrophil | Lymphocyte | Monocyte | Others |
| Conventionally bred cattle⌘⌘ | 1 | 12 | 123[a)] ± 18 | 86 ± 37 | 0.0 ± 0.0 | 1.7 ± 0.5 | 29.2 ± 10.7 | 64.9 ± 11.3 | **4.3 ± 1.6** | 0.0 ± 0.0 |
| Progeny of cloned cattle⌘⌘ | 1 | 11 | 123 ± 22 | 97 ± 33 | 0.0 ± 0.0 | 2.1 ± 1.5 | 28.3 ± 5.8 | 64.0 ± 6.7 | **5.6* ± 1.4** | 0.0 ± 0.0 |
| Conventionally bred cattle⌘⌘ | 5 | 12 | 128 ± 21 | 74 ± 16 | 0.0 ± 0.0 | 1.7 ± 0.7 | 27.5 ± 6.8 | 67.0 ± 6.5 | 3.8 ± 1.0 | 0.0 ± 0.0 |
| Progeny of cloned cattle⌘⌘ | 5 | 12 | 120 ± 16 | 82 ± 23 | 0.0 ± 0.0 | 1.4 ± 0.6 | 25.9 ± 8.2 | 69.4 ± 8.5 | 3.3 ± 1.1 | 0.0 ± 0.0 |

[a)] : Mean±Standad deviation
Significant difference between two rat groups fed test diets containing meat powder derived from conventionally bred cattle or progeny of cloned cattle in the same meat powder content (*: $p<0.05$).
Abbreviations : RBC, Red blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PT, Prothrombin time; APTT, Activated partial thromboplastin time; WBC, White blood cell
⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
⌘⌘: Japanese Black beef cattle.

red blood cells, platelets and lymphocytes.

***ii) Clinical biochemistry (Tables 40, 41)***

In terms of the clinical biochemistry of male rat group fed diet supplemented with 1% (w/w) meat powder derived from the progeny, significantly higher values of sodium (149 mEq/l in males) and γ-glutamyl transpeptidase (0.25 IU/l in females) were observed compared with those in rat group fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle (147 mEq/l for sodium in males and 0.10 IU/l for γ-glutamyl transpeptidase in females).

In male rat groups fed diet supplemented with 5% (w/w) meat powder derived from the progeny, significant differences in lactate dehydrogenase (266 IU/l), creatine kinase (64 IU/l), glucose (176 mg/dl) and sodium (150 mEq/l) were found compared with those in rat group fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle (521 IU/l for dehydrogenase, 96 IU/l for creatine kinase, 161 mg/dl for glucose and 148 mEq/l for sodium).

In female rat group fed diet supplemented with 5% (w/w) meat powder derived from the progeny, significant differences in aspartate aminotransferase (70 IU/l), blood urea nitrogen (13.3 mg/dl) and inorganic phosphorous (4.3 mg/dl) were observed compared with those in rat group fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle (56 IU/l for aspartate aminotransferase, 10.5 mg/dl for blood urea nitrogen and 3.6 mg/dl for inorganic phosphorous).

When these values were compared with our reference data of males (155–810 IU/l for lactate dehydrogenase, 57–171 IU/l for creatine kinase, 141–185 mg/dl for glucose and 144–149 mEq/l for sodium) and of females (0.24–3.25 IU/l for γ-glutamyl transpeptidase, 43–282 IU/l for aspartate aminotransferase and 3.00–5.34 mg/dl for inorganic phosphorous), the present data were within the

Table 39. Hamatological data of female rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | RBC | Hemoglobin | Hematocrit | MCV | MCH | MCHC | Reticulocyte | PT | APTT |
| Origin | Content (%) | of rats | ($10^4$/μl) | (g/dl) | (%) | (fl) | (pg) | (%) | (‰) | (sec) | (sec) |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 780 ± 33 | 14.5 ± 0.5 | 43.4 ± 1.9 | 56 ± 2 | 18.5 ± 0.6 | 33.3 ± 0.6 | 26.7 ± 11.8 | 12.7 ± 0.3 | 17.1 ± 1.5 |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 771 ± 37 | 14.6 ± 0.9 | 43.5 ± 2.1 | 56 ± 2 | 18.9 ± 0.9 | 33.4 ± 0.6 | 22.9 ± 6.1 | 12.6 ± 0.4 | 17.2 ± 1.6 |
| Conventionally bred cattle[⌘⌘] | 5 | 11 | 803 ± 41 | 14.9 ± 0.6 | 44.4 ± 2.3 | 55 ± 2 | 18.6 ± 0.6 | 33.5 ± 0.6 | 19.4 ± 2.8 | 12.8 ± 0.3 | 17.0 ± 0.6 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 785 ± 59 | 14.5 ± 0.6 | 43.9 ± 1.9 | 56 ± 4 | 18.6 ± 1.2 | 33.2 ± 0.6 | 23.7 ± 7.8 | 12.9 ± 0.5 | 16.3 ± 0.9 |

| Rat group for meat powder diet | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | Platelet | WBC | Differential leukocyte counts (%) | | | | | |
| Origin | Content (%) | of rats | ($10^4$/μl) | ($10^2$/μl) | Basophil | Eosinophil | Neutrophil | Lymphocyte | Monocyte | Others |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 105 ± 14 | 45 ± 11 | 0.0 ± 0.0 | 1.7 ± 0.7 | 21.6 ± 4.4 | 73.5 ± 4.5 | 3.2 ± 1.0 | 0.0 ± 0.0 |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 103 ± 12 | 39 ± 10 | 0.0 ± 0.0 | 1.8 ± 0.6 | 24.1 ± 5.9 | 70.7 ± 6.6 | 3.4 ± 1.4 | 0.0 ± 0.0 |
| Conventionally bred cattle[⌘⌘] | 5 | 11 | 107 ± 13 | 35 ± 13 | 0.0 ± 0.0 | 2.1 ± 0.9 | 22.7 ± 4.1 | 71.9 ± 4.7 | 3.3 ± 1.3 | 0.0 ± 0.0 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 104 ± 12 | 43 ± 16 | 0.0 ± 0.0 | 2.4 ± 0.9 | 24.6 ± 9.4 | 69.5 ± 8.7 | 3.5 ± 1.2 | 0.0 ± 0.0 |

[a)] : Mean±standad deviation
Abbreviations : RBC, Red blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PT, Prothrombin time; APTT, Activated partial thromboplastin time; WBC, White blood cell
[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
[⌘⌘]: Japanese Black beef cattle.

reference ranges, with the exception of sodium in males.

Although a significant difference in sodium was found, all parameters except for this were within the reference ranges. Therefore, no clinical biochemistry parameters obtained in the present investigation suggested the presence of vital functional abnormality in rats fed diets supplemented with meat powder derived from the progeny.

### C) Effects of meat intake on morphology in rats

#### *a) Autopsy*

A subcutaneous mass found as a clinical sign was confirmed by necropsy in one female (No. 515) fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle. This rat was diagnosed as having leukemia with enlarged organs. The other specific features of this case were greenish-gray-colored spleen, kidney and fat tissue. Among 12 males/females in each group, a black area on the pituitary gland was found in one or two rats. Other autopsy findings were as follows: softening of the testis in one male fed diet supplemented with 1% (w/w) meat powder derived from the progeny; red spot on the liver of one male fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle; ovarian cyst in one female fed diet supplemented with 1% (w/w) meat powder derived from progeny and in one female fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle; and bursa follicular cyst of the ovary in one female fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle.

#### *b) Organ weights (Table 42)*

In terms of the organ weights of female/male rat groups fed diet supplemented with 1% (w/w) meat powder derived from the progeny, significant differences in male liver (21.74 g) and female spleen (0.71 g) were found compared with those in rat groups fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle (19.01 g for

Table 40. Clinical chemistry data of male rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | LDH | AST | ALT | ALP | γ-GTP | CK | ChE | T.P. | Alb. | Glb. | A/G | T-Cho. |
| Origin | Content (%) | of rats | (IU/l) | (IU/l) | (IU/l) | (IU/l) | (IU/l) | (IU/l) | (IU/l) | (g/dl) | (g/dl) | (g/dl) | | (mg/dl) |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 665[a)] ±593 | 142 ± 93 | 70 ± 67 | 230 ± 110 | 0.79 ±0.37 | 114 ± 55 | 87 ± 38 | 6.50 ± 0.21 | 3.06 ±0.23 | 3.44 ±0.19 | 0.90 ±0.11 | 107 ± 37 |
| Progeny of cloned cattle[⌘⌘] | 1 | 11 | 550 ±414 | 155 ±124 | 78 ± 77 | 207 ± 51 | 0.82 ±0.38 | 89 ± 33 | 120 ± 53 | 6.43 ± 0.20 | 3.00 ±0.24 | 3.43 ±0.23 | 0.88 ±0.12 | 106 ± 31 |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | **521** **±358** | 160 ±155 | 80 ± 77 | 194 ± 48 | 0.63 ±0.41 | **96** ± **28** | 78 ± 42 | 6.66 ± 0.33 | 3.16 ±0.29 | 3.50 ±0.26 | 0.91 ±0.12 | 108 ± 28 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | **266*** ± **94** | 91 ± 42 | 56 ± 53 | 193 ± 63 | 0.53 ±0.17 | **64**** ± **14** | 105 ± 52 | 6.67 ± 0.26 | 3.22 ±0.21 | 3.45 ±0.18 | 0.94 ±0.09 | 109 ± 24 |

| Rat group for meat powder diet | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number | T.G. | PL | Glu. | BUN | Crea. | T-Bil. | Ca | P | Na | K | Cl |
| Origin | Content (%) | of rats | (mg/dl) | (mg/dl) | (mg/dl) | (mg/dl) | (mg/dl) | (mg/dl) | (mg/dl) | (mg/dl) | (mEq/l) | (mEq/l) | (mEq/l) |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 141[a)] ± 42 | 157 38 | 159 ± 12 | 12.4 ± 2.0 | 0.45 ± 0.10 | 0.22 ± 0.04 | 10.7 ± 0.3 | 4.8 ± 0.3 | **147** ± **1** | 4.80 ±0.43 | 106 ± 2 |
| Progeny of cloned cattle[⌘⌘] | 1 | 11 | 123 ± 35 | 154 33 | 170 ± 17 | 10.7 ± 2.6 | 0.40 ± 0.09 | 0.22 ± 0.02 | 10.6 ± 0.3 | 4.8 ± 0.5 | **149**** ± **2** | 4.59 ±0.34 | 106 ± 2 |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 131 ± 45 | 158 ± 32 | **161** ± **9** | 10.4 ± 1.4 | 0.39 ± 0.04 | 0.21 ± 0.04 | 10.7 ± 0.2 | 5.0 ± 0.4 | **148** ± **1** | 4.87 ±0.24 | 107 ± 1 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 122 ± 55 | 160 ± 31 | **176*** ± **19** | 10.6 ± 1.4 | 0.40 ± 0.07 | 0.24 ± 0.02 | 10.8 ± 0.3 | 5.4 ± 0.7 | **150**** ± **1** | 4.51 ±0.56 | 107 ± 2 |

[a)] : Mean±standard deviation

Significant difference between two rat groups fed test diets containing meat powder derived from conventionally bred cattle or progeny of cloned cattle in the same meat powder content (*: $p<0.05$, **: $p<0.01$).

Abbreviations : LDH, Lactate dehydrogenase; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase γ-GTP, γ-Glutamyltranspeptidase; CK, Creatine kinase; ChE : Cholinesterase; T.P., Total protein; Alb., Albumin; Glb., Globulin A/G, Albumin/globulin ratio; T-Cho., Total cholesterol; T.G., Triglyceride; PL, Phospholipid; Glu., Glucose; BUN, Blood urea nitrogen Crea., Creatinine; T-Bil., Total bilirubin; Ca, Calcium; P, Inorganic phosphorus; Na, Sodium; K, Potassium; Cl, Chloride

[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

[⌘⌘]: Japanese Black beef cattle.

male liver, 0.62 g for female spleen). The final body weights of female/male groups fed diet supplemented with 1% (w/w) meat powder derived from progeny were higher than those fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle. However, in rat groups fed diet supplemented with 1% (w/w) meat powder derived from the progeny, no significant difference was observed in male liver weight per 100 g body weight (2.39 ± 0.33 g/100 g body weight, mean ± standard deviation) and female spleen weight per 100 g body weight (0.15 ± 0.02 g/100 g body weight) compared to those in rat group fed diet supplemented with 1% (w/w) meat powder derived from conventionally bred cattle (2.30 ± 0.23 g/100 g body weight for male liver and 0.14 ± 0.02 g/100 g body weight for female spleen). These findings suggested that the significant differences in these organ weights were due to the body weights of the rat groups.

With regard to organ weights in female/male rat groups fed diet supplemented with 5% (w/w) meat powder, no significant differences in organ weights due to the origin of meat powder were found out, including for liver and spleen.

In the leukemic female (No. 515) fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred cattle, liver, kidney and ovary were markedly heavier than those of healthy females.

Table 41. Clinical chemistry data of female rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number of rats | LDH (IU/l) | AST (IU/l) | ALT (IU/l) | ALP (IU/l) | γ-GTP (IU/l) | CK (IU/l) | ChE (IU/l) | T.P. (g/dl) | Alb. (g/dl) | Glb. (g/dl) | A/G | T-Cho. (mg/dl) |
| Origin | Content (%) | | | | | | | | | | | | | |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 223[a)] ± 111 | 51 ± 10 | 19 ± 7 | 163 ± 214 | 0.10 ±0.08 | 65 ± 31 | 498 ± 75 | 7.37 ±0.25 | 4.40 ± 0.28 | 2.97 ±0.23 | 1.49 ±0.18 | 104 ± 16 |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 339 ± 232 | 73 ± 35 | 23 ± 12 | 117 ± 111 | 0.25* ±0.18 | 70 ± 36 | 528 ± 72 | 7.15 ±0.27 | 4.19 ± 0.23 | 2.96 ±0.23 | 1.43 ±0.14 | 112 ± 29 |
| Conventionally bred cattle[⌘⌘] | 5 | 11 | 240 ± 109 | 56 ± 10 | 20 ± 4 | 103 ± 32 | 0.23 ±0.15 | 63 ± 21 | 440 ± 107 | 6.96 ±0.26 | 3.91 ± 0.29 | 3.04 ±0.20 | 1.29 ±0.15 | 99 ± 27 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 269 ± 92 | 70* ± 17 | 24 ± 8 | 125 ± 76 | 0.27 ±0.23 | 65 ± 26 | 421 ± 131 | 6.91 ±0.44 | 3.83 ± 0.28 | 3.07 ±0.19 | 1.25 ±0.07 | 101 ± 26 |

| Rat group for meat powder diet | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | Number of rats | T.G. (mg/dl) | PL (mg/dl) | Glu. (mg/dl) | BUN (mg/dl) | Crea (mg/dl) | T-Bil. (mg/dl) | Ca (mg/dl) | P (mg/dl) | Na (mEq/l) | K (mEq/l) | Cl (mEq/l) |
| Origin | Content (%) | | | | | | | | | | | | |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 127[a)] ± 44 | 192 ± 23 | 146 ± 16 | 12.0 ± 1.7 | 0.51 ± 0.03 | 0.25 ±0.04 | 10.8 ± 0.2 | 3.4 ± 0.6 | 141 ± 2 | 4.05 ±0.51 | 106 ± 2 |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 148 ± 90 | 207 ± 48 | 146 ± 8 | 12.0 ± 2.5 | 0.50 ± 0.06 | 0.26 ±0.04 | 10.6 ± 0.3 | 3.3 ± 0.8 | 142 ± 1 | 4.12 ±0.38 | 106 ± 3 |
| Conventionally bred cattle[⌘⌘] | 5 | 11 | 111 ± 87 | 190 ± 44 | 160 ± 14 | 10.5 ± 2.8 | 0.48 ± 0.03 | 0.21 ±0.03 | 10.2 ± 0.3 | 3.6 ± 0.5 | 141 ± 2 | 4.47 ±0.49 | 107 ± 1 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 105 ± 59 | 188 ± 44 | 163 ± 16 | 13.3* ± 2.5 | 0.52 ± 0.08 | 0.22 ±0.04 | 10.5 ± 0.5 | 4.3* ± 0.8 | 142 ± 1 | 4.48 ±0.50 | 106 ± 3 |

[a)] : Mean±standard deviation

Significant difference between two rat groups fed test diets containing meat powder derived from conventionally bred cattle or progeny of cloned cattle in the same meat powder content (*: $p<0.05$).

Abbreviations : LDH, Lactate dehydrogenase; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase γ -GTP, γ-Gutamyltranspeptidase; CK, Creatine kinase; ChE : Cholinesterase; T.P , Total protein; Alb., Albumin; Glb., Globulin A/G, Albumin/globulin ratio; T-Cho., Total cholesterol; T.G., Triglyceride; PL, Phospholipid; Glu., Glucose; BUN, Blood urea nitrogen Crea., Creatinine; T-Bil., Total bilirubin; Ca, Calcium; P, Inorganic phosphorus; Na, Sodium; K, Potassium; Cl, Chloride

[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

[⌘⌘]: Japanese Black beef cattle.

***c) Histology (Table 43)***

In terms of the histological findings of rat groups fed diet supplemented with 5% (w/w) meat powder derived from the progeny or conventionally bred cattle, the non-neoplastic lesions found in males and/or females were as follows: artery mineralization, accumulation of foam cells in the lungs; myocardial degeneration/fibrosis in the heart; congestion, increased extramedullary hematopoiesis in the spleen; increased hematopoiesis of bone marrow; fatty change of hepatocytes and focal necrosis in the liver; fatty change in the parotid gland; squamous hyperplasia of the forestomach; fatty change, deposit of brown pigment, fibrosis and atrophy of acinar cells in the pancreas; chronic nephrosis and pelvic inflammation with lymphocytic infiltration of the pelvis in the kidney; focal hyperplasia of anterior lobe and anterior lobe cyst in the pituitary gland; C-cell hyperplasia and remnant of the ultimobranchial body in the thyroid gland; focal hyperplasia of the cortex; and hemorrhagic cyst and angioectasis in the adrenal gland. Among these lesions, no significant differences in the occurrence of these histological findings due to the origin of meat powder were observed.

Other lesions that were found sporadically on histological observation were as follows: osseous metaplasia in the lungs; necrosis in the spleen; angiectasis and hyperplasia of bile duct in the

Table 42. Organ weights of rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

Male

| Rat group for meat powder diet | | Number of rats | Body weight | Brain | Salivary gland | Heart | Lung | Liver | Kidney | Adrenal gland | Spleen | Pituitary gland | Thyroid gland | Prostate | Seminal vesicle | Testis | Epididymis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | | | | | | | | | | | | | | | | |
| Origin | Content (%) | | (g) | (g) | (g) | (g) | (g) | (g) | (g) | (mg) | (g) | (mg) | (mg) | (g) | (g) | (g) | (g) |
| Conventionally bred cattle[⌘⌘] | 1 | 12 | 827 ±85 | 2.17 ±0.06 | 1.00 ±0.08 | 1.91 ±0.17 | 2.01 ±0.11 | **19.01 ±2.80** | 3.95 ±0.55 | 62.3 ±3.7 | 1.13 ±0.28 | 16.5 ±2.0 | 38.8 ±7.9 | 0.43 ±0.11 | 2.93 ±0.52 | 3.69 ±0.45 | 1.41 ±0.13 |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 914 ±127 | 2.23 ±0.09 | 0.94 ±0.12 | 1.94 ±0.28 | 1.99 ±0.20 | **21.74* ±3.29** | 4.21 ±0.57 | 63.6 ±10.2 | 1.34 ±0.78 | 15.7 ±2.2 | 45.0 ±7.8 | 0.44 ±0.20 | 2.66 ±0.51 | 3.47 ±0.40 | 1.39 ±0.14 |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 834 ±102 | 2.23 ±0.08 | 0.97 ±0.13 | 1.80 ±0.16 | 1.94 ±0.11 | 17.71 ±3.45 | 3.84 ±0.36 | 61.0 ±8.3 | 1.05 ±0.35 | 16.4 ±2.9 | 41.2 ±4.3 | 0.45 ±0.12 | 2.69 ±0.60 | 3.62 ±0.28 | 1.44 ±0.15 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 825 ±80 | 2.20 ±0.10 | 0.95 ±0.09 | 1.81 ±0.14 | 1.92 ±0.22 | 18.21 ±2.31 | 3.83 ±0.55 | 64.0 ±10.5 | 0.99 ±0.16 | 17.7 ±6.9 | 41.4 ±4.5 | 0.43 ±0.13 | 2.77 ±0.48 | 3.56 ±0.22 | 1.51 ±0.18 |

Female

| Rat group for meat powder diet | | Number of rats | Body weight | Brain | Salivary gland | Heart | Lung | Liver | Kidney | Adrenal gland | Spleen | Pituitary gland | Thyroid gland | Ovary | Uterus |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | | | | | | | | | | | | | | |
| Origin | Content (%) | | (g) | (g) | (g) | (g) | (g) | (g) | (g) | (mg) | (g) | (mg) | (mg) | (mg) | (g) |
| Conventionally bred cattle[⌘⌘] | 1 | 11 | 443 ±35 | 1.98 ±0.09 | 0.58 ±0.07 | 1.23 ±0.11 | 1.39 ±0.11 | 10.05 ±0.83 | 2.45 ±0.20 | 68.5 ±16.2 | **0.62 ±0.08** | 28.7 ±7.8 | 34.4 ±5.4 | 78.7 ±40.4 | 1.02 ±0.24 |
| Progeny of cloned cattle[⌘⌘] | 1 | 12 | 465 ±72 | 1.96 ±0.10 | 0.59 ±0.06 | 1.20 ±0.14 | 1.36 ±0.11 | 10.48 ±2.15 | 2.52 ±0.38 | 68.9 ±17.7 | **0.71* ±0.11** | 29.4 ±6.9 | 33.0 ±7.5 | 68.7 ±21.5 | 0.98 ±0.32 |
| Conventionally bred cattle[⌘⌘] | 5 | 12 | 456 ±96 | 1.96 ±0.10 | 0.57 ±0.05 | 1.21 ±0.15 | 1.37 ±0.19 | 10.19 ±3.12 | 2.59 ±1.11 | 63.7 ±17.9 | 2.00 ±4.85 | 24.1 ±8.1 | 33.5 ±4.8 | 124.7 ±171.1 | 0.87 ±0.25 |
| Progeny of cloned cattle[⌘⌘] | 5 | 12 | 503 ±60 | 2.00 ±0.13 | 0.59 ±0.06 | 1.29 ±0.14 | 1.37 ±0.17 | 11.45 ±2.58 | 2.66 ±0.68 | 70.9 ±14.6 | 0.74 ±0.29 | 29.9 ±9.8 | 32.9 ±7.0 | 73.8 ±26.4 | 0.87 ±0.26 |

Each value is shown as mean±standard deviation

Significant difference between two rat groups fed test diets containing meat powder derived from conventionally bred cattle or progeny of cloned cattle in the same meat powder content (*: $p<0.05$).

[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

[⌘⌘]: Japanese Black beef cattle.

liver; lymphocytic infiltration in the parotid gland; dilatation of gastric gland in the glandular stomach; mucosal mineralization and fibrosis of lamina propria in the cecum; lymphocytic infiltration in the pancreas; hyaline droplet degeneration, lymphocytic infiltration of the cortex and cortical fibrosis in the kidney; atrophy of somniferous tubules in the testis; inflammation in the prostate; inflammation of endometrium in the uterus; lymphocytic infiltration in the thyroid gland; focal fatty change in the adrenal gland; lymphocytic infiltration in the Harderian gland; and inflammation in the mammary gland.

In terms of neoplastic lesions of rat groups fed diet supplemented with 5% (w/w) meat powder derived from the progeny or conventionally bred cattle, mammary gland tumor and leukemia were shown. Other neoplastic lesions in rats fed such a diet were as follows: benign adenoma in the pituitary gland (two males and one female in groups fed diet supplemented with meat powder from the progeny; two males and two females in groups fed diet supplemented with meat powder from conventionally bred cattle) and benign C-cell hyperplasia in the thyroid gland (one male fed diet supplemented with meat powder derived from conventionally bred cattle).

These lesions observed in the present investigation were assumed to be spontaneous[8]. In rat groups fed diets supplemented with meat powder derived from the progeny or conventionally bred cattle, no significant differences in the rate of occurrence of these lesions due to the origin of meat powder were observed.

Table 43. Histopathological findings of rats fed diet supplemented with freeze-dried meat powder[⌘] in twelve-month feeding study combined with reproduction/development toxicity test

| | | | Male rat group fed diet supplemented with meat powder derived from; | | Female rat group fed diet supplemented with meat powder derived from; | |
|---|---|---|---|---|---|---|
| | | | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] | Conventionally bred cattle[⌘⌘] | Progeny of cloned cattle[⌘⌘] |
| Organ | : Findings | Numer of rats | 12 | 12 | 12 | 12 |
| ***NON-NEOPLASTIC LESIONS*** | | | | | | |
| Lung | : Mineralization, artery | | 10 | 11 | 5 | 6 |
| | Accumulation, foam cell | | 2 | 3 | 0 | 1 |
| | Metaplasia, osseous | | 0 | 1 | 0 | 1 |
| Heart | : Myocardial degeneration/fibrosis | | 1 | 4 | 5 | 4 |
| Bone Marrow | : Increased hematopoiesis | | 0 | 0 | 0 | 1 |
| Spleen | : Congestion | | 3 | 0 | 2 | 1 |
| | Increased extramedullary hematopoiesis | | 0 | 1 | 0 | 2 |
| | Necrosis | | 0 | 0 | 1 | 0 |
| Liver | : Fatty change, hepatocyte | | 8 | 9 | 4 | 6 |
| | Necrosis, focal | | 3 | 3 | 0 | 0 |
| | Angiectasis | | 1 | 0 | 0 | 0 |
| | Hyperplasia, bile duct | | 0 | 0 | 1 | 0 |
| Parotid gland | : Fatty change | | 12 | 9 | 2 | 2 |
| | Lymphocytic infiltration | | 0 | 1 | 0 | 0 |
| Forestomach stomach | : Squamous hyperplasia | | 2 | 3 | 0 | 0 |
| Glandular stomach | : Dailatation, gastric gland | | 1 | 0 | 0 | 0 |
| Cecum | : Mineralization, mucosa | | 1 | 0 | 0 | 0 |
| | Fibrosis, lumina propria | | 1 | 0 | 0 | 0 |
| Pancreas | : Fatty change | | 6 | 9 | 1 | 2 |
| | Deposit, brown pigment | | 4 | 4 | 1 | 0 |
| | Fibrosis | | 2 | 2 | 0 | 1 |
| | Atrophy, aciner cell, focal | | 7 | 4 | 0 | 0 |
| | Lymphocytic infiltration | | 1 | 0 | 0 | 0 |
| Kidney | : Degeneration, hyaline droplet | | 0 | 0 | 1 | 0 |
| | Lymphocytic infiltration, cortex | | 0 | 1 | 0 | 0 |
| | Fibrosis, cortex | | 1 | 0 | 0 | 0 |
| | Chronic nephrosis | | 9 | 8 | 1 | 1 |
| | Lymphocytic infiltration, pelvis | | 0 | 1 | 1 | 1 |
| | Inflammation, pelvis | | 4 | 2 | 1 | 1 |
| Testis | : Atrophy, seminiferous tubule | | 1 | 0 | - | - |
| Prostate | : Inflammation | | 1 | 0 | - | - |
| Uterus | : Inflammation, endometrium | | - | - | 1 | 0 |
| Pituitary gland | : Focal hyperplasia, anterior lobe | | 2 | 2 | 4 | 4 |
| | Cyst, anterior lobe | | 0 | 1 | 1 | 1 |
| Thyroid gland | : Remnant, ultimobranchial body | | 3 | 1 | 3 | 0 |
| | C-cell hyperplasia | | 2 | 2 | 3 | 4 |
| | Lymphocytic infiltration | | 1 | 0 | 1 | 0 |
| Adrenal gland | : Focal hyperplasia, cortex | | 3 | 3 | 1 | 0 |
| | Angioectasis | | 0 | 0 | 1 | 1 |
| | Cyst, hemorrhagic | | 0 | 0 | 3 | 4 |
| | Fatty change, focal | | 0 | 1 | 0 | 0 |
| Harderian gland | : Lymphocytic infiltration | | 0 | 0 | 1 | 0 |
| Mammary gland | : Inflammation | | 1 | 0 | 0 | 0 |
| ***NEOPLASTIC LESIONS*** | | | | | | |
| Pituitary gland | : Adenoma | | 2 | 2 | 2 | 1 |
| Thyroid gland | : Adenoma, C-cell | | 1 | 0 | 0 | 0 |
| Mammary gland | : Fibroadenoma | | 0 | 0 | 0 | 1 |
| | Adenoma | | 0 | 0 | 0 | 1 |
| Bone Marrow | : Luekemia, myelogeneous [a)] | | 0 | 0 | 1 | 0 |

No abnormalities were detected in the brain spinal cord, sciatic nerve, trachea, sublingnal and submandibular glands, parathyroid, aorta, lymph node, thymus, tongue, esophagus, glandular stomach, small intestine, eye ball, skeltal muscle, skin, epididymis, seminal vesicle and vagina.

[a)] : The tumor cell infiltration was also observed in almost organs examined.

[⌘]: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

[⌘⌘]: Japanese Black beef cattle.

***d) Observation of pups ($F_1$) produced by rats fed diets supplemented with milk powder (Tables 44–46)***

In pups delivered from rat groups fed diets supplemented with 1 or 5% (w/w) meat powder derived from the progeny, no significant differences in litter size, delivery index, sex ratio, viability index (day 4 of lactation), lactation index (day 21 of lactation) and body weight due to the origin of meat powder were found.

In terms of the external appearance of pups delivered from rat groups fed diets with meat powder derived from the progeny or conventionally bred cattle, no differences in hair growth, pinna detachment, incisor eruption, eyelid opening and testicular descent due to the origin of meat powder were observed. All pups delivered from rat groups fed a meat powder-supplemented diet showed normal performance in the sensory response/reflex function test.

From external observation, no pups with any external abnormalities or visceral malformations, except one rudimentary tail, were observed among 136 pups produced by rats fed diet supplemented with 5% (w/w) meat powder derived from conventionally bred

Table 44. Observation of pups ($F_1$) from rats fed diet supplemented with freeze-dried meat powderz in twelve-month feeding study combined with reproduction/development toxicity test

| Item | | Dams of pups investigated | | | |
|---|---|---|---|---|---|
| | | Fed diet supplemented with 1% meat powder derived from; | | Fed diet supplemented with 5% meat powder derived from; | |
| | | Conventionally bred cattle⌘⌘ | Progeny of cloned cattle⌘⌘ | Conventionally bred cattle⌘⌘ | Progeny of cloned cattle⌘⌘ |
| On day 0 of lactation | | | | | |
| Litter size | | 11.5 ± 4.5[a)] | 10.6 ± 4.1 | 11.3 ± 3.3 | 10.9 ± 3.6 |
| Live birth index(%) | | 96.8 | 98.3 | 97.1 | 96.7 |
| Sex ratio(Male/Female) | | 1.625 | 1.127 | 1.345 | 1.000 |
| Body weights(g) | Male | 6.1 ± 1.1 | 6.7 ± 0.7 | 6.8 ± 0.7 | 6.8 ± 0.7 |
| | Female | 6.0 ± 0.9 | 6.5 ± 0.8 | 6.6 ± 0.7 | 6.4 ± 0.7 |
| On day 4 of lactation | | | | | |
| Viability index on day 4(%) | | 89.3 | 100.0 | 97.7 | 94.0 |
| Body weights(g) | Male | 10.0 ± 2.9 | 11.7 ± 1.9 | 11.4 ±2.2 | 11.3 ± 1.5 |
| | Female | 9.9 ± 2.4 | 11.4 ± 1.5 | 11.2 ± 2.0 | 10.8 ± 1.6 |
| On day 7 of lactation | | | | | |
| Body weights(g) | Male | 16.5 ± 4.8 | 19.0 ± 2.9 | 18.6 ± 3.4 | 19.0 ± 2.5 |
| | Female | 16.3 ± 4.1 | 18.9 ± 2.4 | 18.3 ± 2.9 | 18.1 ± 2.5 |
| On day 14 of lactation | | | | | |
| Body weights(g) | Male | 35.7 ± 7.3 | 39.0 ± 3.4 | 39.0 ± 4.2 | 39.7 ± 3.2 |
| | Female | 35.2 ± 6.4 | 38.7 ± 2.2 | 38.3 ± 3.7 | 38.5 ± 2.8 |
| On day 21 of lactation | | | | | |
| Lactation index(%) | | 98.6 | 100 | 98.9 | 98.7 |
| Body weights(g) | Male | 64.5 ± 11.3 | 70.0 ± 6.1 | 69.5 ± 8.1 | 70.7 ± 4.6 |
| | Female | 62.8 ± 9.9 | 68.5 ± 4.4 | 67.0 ± 6.2 | 67.5 ± 4.1 |
| Sensory response / reflex function test[b)] | | NAD | NAD | NAD | NAD |
| External abnormalities(%) | | 0.0 (0/126) | 0.0 (0/117) | 0.7 (1[c)] /136) | 0.0 (0/120) |
| Visceral malformations(%) | | 0.0 (0/126) | 0.0 (0/117) | 0.0 (0/136) | 0.0 (0/120) |

Live birth index = (Number of live pups on day 0 / Number of pups born)×100
Viability index on day 4 = (Number of live pups on day 4 / Number of pups on day 0)×100
Lactation index = (Nunber of live pups on day 21 / Number of pups on day 4)×100
NAD : No abnormalities were detected
[a)] : Mean±standard deviation
[b)] : Responses to sound, approach, touch and tail pinch, pupil reflex to light, and pinna, ipsilateral flexor, eyelid and righting reflexes
[c)] : One pup had a external abnormality, rudimentary tail
⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.
⌘⌘: Japanese Black beef cattle.

cattle.

In terms of the body weights and food consumption of rat groups fed diet with meat powder derived from the progeny or conventionally bred cattle, no significant differences due to the origin of meat powder were observed during pregnancy and lactation.

## DISCUSSION

### Blood properties in heifer produced meat

In somatic cell cloned cattle, placental/fetal edema in the perinatal period [11,30,43] and high mortality rate due to large offspring syndrome[3,7,19,21]

Table 45. Developmental observation of pups from rats fed diet supplemented with freeze-dried meat powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | Number of dams | Developmental observation (day) | | | | |
|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | | Hair growth | Pinna detachiment | Incisor eruption | Eyelids opening | Testicular descent |
| Origin | Content (%) | | | | | | |
| Conventionally bred cattle⌘⌘ | 1 | 11 | 4.1 ± 0.3 | 4.1 ± 0.3 | 9.5 ± 0.6 | 13.3 ± 0.7 | 18.5 ± 1.1 |
| Progeny of cloned cattle⌘⌘ | 1 | 10 | 4.0 ± 0.0 | 4.0 ± 0.0 | 9.4 ± 0.4 | 13.1 ± 0.7 | 17.9 ± 0.6 |
| Conventionally bred cattle⌘⌘ | 5 | 12 | 4.1 ± 0.3 | 4.1 ± 0.2 | 9.5 ± 0.8 | 13.1 ± 1.3 | 18.1 ± 0.7 |
| Progeny of cloned cattle⌘⌘ | 5 | 10 | 4.0 ± 0.0 | 4.0 ± 0.1 | 9.7 ± 0.8 | 13.7 ± 0.8 | 18.1 ± 0.5 |

Mean±standard devision

⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

⌘⌘: Japanese Black beef cattle.

Table 46. Body weight, food consumption and meat powder intake during gestation and lactation periods of female rats fed diet supplemented with freeze-dried meat powder⌘ in twelve-month feeding study combined with reproduction/development toxicity test

| Rat group for meat powder diet | | | Days of pregnancy | | | | Days of lactation | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat powder in test diet | | | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 |
| Origin | Content (%) | | | | | | | | | |
| Conventionally bred cattle⌘⌘ | 1 | Body weight (g) | 333±20 (12) | 365±18 (12) | 398 ±15 (12) | 446 ±23 (12) | 370±32 (11) | 368±25 (11) | 367±23 (11) | 352±22 (11) |
| | | Food consumption (g/day) | 19± 2 | 23± 3 | 22± 3 | 21± 4 | 21± 6 | 38±12 | 54±11 | 44±17 |
| | | Meat powder intake (mg/kg/day) | 571 | 630 | 553 | 471 | 568 | 1033 | 1471 | 1250 |
| Progeny of cloned cattle⌘⌘ | 1 | Body weight (g) | 334±37 (11) | 365±41 (11) | 393±40 (11) | 449±44 (11) | 368±46 (10) | 370±39 (10) | 365±30 (10) | 353±27 (10) |
| | | Food consumption (g/day) | 19± 3 | 22± 3 | 22± 5 | 23± 6 | 23± 3 | 39± 5 | 56± 6 | 42±12 |
| | | Meat powder intake (mg/kg/day) | 569 | 603 | 560 | 512 | 625 | 1054 | 1534 | 1190 |
| Conventionally bred cattle⌘⌘ | 5 | Body weight (g) | 340±36 (12) | 367±35 (12) | 399±36 (12) | 453±42 (12) | 379±34 (12) | 371±29 (12) | 364±23 (12) | 344±20 (12) |
| | | Food consumption (g/day) | 18± 3 | 21± 3 | 22± 3 | 21± 4 | 20± 7 | 37±12 | 54±11 | 42± 9 |
| | | Meat powder intake (mg/kg/day) | 2647 | 2861 | 2757 | 2318 | 2639 | 4987 | 7418 | 6105 |
| Progeny of cloned cattle⌘⌘ | 5 | Body weight (g) | 351±32 (11) | 376±33 (11) | 407±34 (11) | 466±36 (11) | 391±39 (10) | 376±36 (10) | 367±29 (10) | 351±26 (10) |
| | | Food consumption (g/day) | 18± 3 | 21± 3 | 21± 3 | 23± 4 | 23± 3 | 38± 5 | 55± 9 | 40±10 |
| | | Meat powder intake (mg/kg/day) | 2564 | 2793 | 2580 | 2468 | 2941 | 5053 | 7493 | 5698 |

Values represent mean±standard deviation or mean

(n): Number of animals available

⌘: Meat derived from three progeny of somatic cell cloned cattle and three conventionally bred cattle was collected independently and freeze-dried. Each meat powder was supplemented to a test diet.

⌘⌘: Japanese Black beef cattle.

are well-known abnormal findings. These abnormalities seems to occur due to epigenetic errors[32,46]. With regard to the somatic cell cloned cattle that survived the prenatal period, it is hard to find any differences compared with conventionally bred cattle[12,33,42]. Studies concerning blood properties in somatic cell cloned cattle also support these findings[6,15,20].

With regard to progeny of somatic cell cloned cattle, it is also hard to find any abnormal findings including high mortality rate in the perinatal period[12,33,42]. In the present investigation, 47 blood parameters concerning hematology and clinical biochemistry were examined. No remarkable abnormalities suggesting poor health status of the progeny were observed in the blood parameters investigated. This result agrees with a finding from blood parameter analysis carried out in New Zealand[42].

The findings suggesting normality of the progeny could be supported by a model mouse system; it is assumed that there is no risk increase in terms of induction of abnormalities due to epigenetic errors in the progeny of cloned animals since any epigenetic errors that exist in a clone's genome would be erased during the process of gamete genesis and fertilization[9].

### Nutritional components and digestibility of milk/meat used in the present investigation

In the previous investigation, we analyzed micronutrients, amino acids and fatty acids in milk/meat derived from somatic cell cloned cattle[15,45]. The results revealed that there were no differences in these nutritional values between the progeny and conventionally bred cattle. After the investigation, Welsh *et al.*[38] analyzed lipids, proteins, protein fractions, lactose, pH, nitrogen, solids, somatic cells and minerals in milk derived from somatic cell cloned cattle. They concluded that there were no differences in these values between samples derived from somatic cell cloned cattle and those from conventionally bred cattle. Moreover, Tome *et al.*[37] and Tian *et al.*[36] analyzed the composition of milk/meat derived from somatic cell cloned cattle. They also concluded that there were no differences in these compositions between samples derived from somatic cell cloned cattle and conventionally bred cattle.

In the present investigation, we analyzed nutritional values including general components, 18 amino acids and 17 fatty acids of milk/meat derived from progeny of somatic cell cloned cattle. Although individual differences were observed in some indices, no differences in milk/meat compositions due to the origin of milk/meat were observed.

When the analyzed results were compared with the Standard Tables of Food Composition in Japan[25], higher lipid and lower carbohydrate compositions were observed in milk derived from progeny and conventionally bred cattle, and higher lipid composition was observed in meat derived from progeny and conventionally bred cattle. These findings suggest that the progeny and conventionally bred cattle used for this study produced high-quality milk/meat.

In order to compare the digestibility of milk/meat derived from the progeny and conventionally bred cattle, we carried out *in vivo* digestion tests based on protein digestion rate with rats by supplying diets supplemented with milk/meat powders. The digestion rates in all samples analyzed were more than 85%. No differences in digestion rates due to the origin of milk/meat were found.

### Anaphylactic reaction for milk/meat samples

Food allergy is a harmful reaction due to the intake of a particular food. In livestock food products, the reaction is well known in infants that ingest milk or dairy products, but it diminishes with age. Therefore, food allergy is not due to toxicity of food but instead develops due to individual sensitivity to allergen.

It is well known that milk contains allergens such as casein, ß-lactoglobulin and α-lactoalbumin[34]. In milk fraction analyses, no significant differences in the amounts of these proteins were found between samples derived from somatic cell cloned cattle and conventionally bred cattle[6,36,38]. With regard to allergy induced by meat, it seems to occur in countries with a

high level of meat consumption. Few allergens in meat are known, although one is tropomyosin.

Since animal cloning technology does not include a gene manipulation process, an increase of existing allergen and the generation of new types of allergen seem unlikely. However, to confirm this, we evaluated the anaphylactic reaction from milk/meat derived from the progeny by the mouse abdominal wall method[17]. If a reaction protein is present in the test dosage, an antigen that depends on IgE induces an anaphylactic reaction. As a result of the reaction, chemical mediators such as histamine from fat/mast cells increase blood vessel wall permeability. If Evan's blue solution is injected into the vein before inducing the reaction, pigment leakage spots due to increased blood vessel wall permeability are seen on the abdominal wall. The allergenicity of test dosage can thus be evaluated by comparing the size of the pigment leakage between the test group and a negative control group.

The present investigation revealed that there were significant differences in the size of pigment leakage with milk/meat derived from the progeny and conventionally bred cattle compared with that of negative controls; the results demonstrated the presence of allergens in the milk/meat evaluated here. However, no significant differences in anaphylactic reactions due to the origin of milk/meat powder were observed. Therefore, it can be concluded that the anaphylactic reaction from milk/meat derived from the progeny is equivalent to that derived from conventionally bred cattle.

### Mutagenicity of milk/meat derived from the progeny

Since many carcinogens induce mutations in DNA, the mutagenicity of novel foods has been investigated as a method of screening for carcinogens. The mouse micronucleus test based on chromosomal abnormality is known as an *in vivo* assay of mutagenicity. Therefore, mutagenicity of milk/meat derived from the progeny was investigated with the mouse micronucleus test[24].

After chromosomal abnormality of the erythroblasts in the marrow is induced, a smaller independent nucleus (micronucleus) will form owing to the absence of spindle formation after cell division. Since the micronucleus remains in the red blood corpuscle even after the erythroblast differentiates into red blood corpuscle, the micronucleus test has been used as an index of chromosomal abnormality[27].

When the marrow cells are exposed to chemical substances by absorption, the rate of multistained red blood corpuscles decreases owing to cell toxicity of the substances. Therefore, the rate of multistained red blood corpuscles could be used as an index of exposure of marrow cells to a positive agent[34]. In the present investigation, milk powder (maximum 10% (w/w)) and meat powder (maximum 5% (w/w)) in diets were fed to mice for 14 days; however, the rate of multistained red blood corpuscles did not decrease. This result suggests that the substance causing cell toxicity was not contained in the milk/meat powder.

### Twelve-month feeding study combined with reproduction/development toxicity test in rats

In a previous investigation, we carried out a fourteen-week feeding study of rats with milk/meat derived from embryonic and somatic cell cloned cattle[15,45]. This investigation demonstrated that there were no significant differences in indices of rats concerning health status, development, physiological functions and morphology compared to those in rats fed diet supplemented with milk/meat derived from conventionally bred cattle. We developed the feeding study as a "progeny version" with a twelve-month feeding period.

It is known that the balance and content of protein, lipid, fiber, minerals and vitamins in the nutritional composition of a diet influence growth, physiological function and the occurrence of cancer in rats[10,18,23,28,44]. Therefore, the nutritional compositions including macronutrients, vitamins and essential minerals of test diets supplemented with milk/meat powder were adjusted to equivalence with the basal diet[31].

The highest diet supplementation that does not negatively affect food consumption was estimated to

be 5% (w/w) for meat powder and 10% (w/w) for milk powder. In diets supplemented with 5% (w/w) meat powder and 10% (w/w) milk powder, the calorific values of these powders were 8.2% for meat and 13% for milk. The average meat/milk powder consumption of rats (per kg body weight) in this feeding study was comparable to that in humans (60 kg for men, 50 kg for women) of daily intakes of 300 g of meat (65% water content) and 1,600 g of milk (89% water content). Although these amounts were still below those for conventional toxicological tests using a 100-fold dose of a single substance, they exceeded the normal human dietary intake.

In the present twelve-month feeding study, there were no biologically significant differences in most of the growth- and reproduction-related indices between the rat groups fed the meat/milk diet derived from the progeny and conventionally bred cattle; however, abnormal findings were observed in terms of clinical signs and histology. These included sporadic symptoms that are not uncommon during the long-term raising of rats. As for the lesions found in the histological examinations, these were assumed to be spontaneous[13]; therefore, we concluded that the symptoms and lesions observed in the rats were not due to feeding on the meat/milk powder diets derived from the progeny. These data concerning chronic toxicity show that milk/meat derived from the progeny is equivalent to those derived from conventionally bred cattle in use as dietary supplements for rats.

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# 体細胞クローン後代牛の生産物性状に関する調査報告書

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**摘　　要**

体細胞クローン後代牛には，乳肉生産のための商業利用に利用できる可能性が指摘されているが，これらの動物が生産した乳肉の生産物性状に関する知見はほとんど得られていなかった。そこで，本研究ではこれらの知見を得るため，体細胞クローン後代牛が生産した乳肉の生産物性状と一般牛で得られた対応データとを比較した。まず，後代牛および一般牛が生産した乳肉の一般成分，アミノ酸および脂肪酸を分析した。次に，これらの乳肉を凍結乾燥粉末に加工した後，消化試験（ラット），アレルギー誘発試験（マウス腹壁法試験）および変異原試験（小核試験）を実施した。これらの調査項目において，後代牛と一般牛との間で有意差は認められなかった。さらに，後代牛が生産した乳肉を摂取した場合の慢性毒性を解明するため，ラット（雌雄）を用いた 12 カ月間の飼養・生殖併合試験を行った。その結果，試験区ラットの各種データ（動物の一般状態と詳細な臨床観察，体重・飼料摂取量の測定，自発運動量・前後肢握力・感覚反射機能等の機能検査，眼科検査，尿検査，雌の繁殖性と分娩した子ラットの健康状態）と対照区ラット（一般牛由来試験飼料を給与）のデータとの間に生物学的な差異は認められなかった。以上の結果は，体細胞クローン後代牛が生産した乳肉と一般牛が生産した乳肉とが同等であることを示唆している。

**キーワード：**牛，後代，体細胞クローン，乳肉，生産物性状

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# 畜産草地研究所研究報告及び畜産草地研究所研究資料投稿要領

13畜草Ｂ第43号
平成13年4月1日

（目的）
第１条　畜産草地研究所研究報告及び畜産草地研究所研究資料への投稿については，独立行政法人農業･食品産業技術総合研究機構刊行物著作権取扱規程（14規程56号）に定めるもののほかこの要領の定めるところによる。

（投稿者の資格）
第２条　投稿者は原則として，畜産草地研究所職員（以下「職員」という。）及び流動研究員，依頼研究員，日本学術振興会特別研究員，日本学術振興会外国人特別研究員等（以下「他の職員」という。）とする。
一　職員が投稿する内容は，主として畜産草地研究所（以下「研究所」という。）で行った研究とする。
二　他の職員が投稿する内容は，研究所で行った研究とする。

（投稿原稿の内容）
第３条　投稿原稿の内容は次のとおりとする。
１　畜産草地研究所研究報告（Bulletin of National Institute of Livestock and Grassland Science / 略誌名：Bull. Natl. Inst. Livest. Grassl. Sci.）
一　原著論文：研究所において行った試験研究及び研究所以外の者に委託して行った試験研究の成果に関わる論文とする。
二　短　　報：一以外の研究の予報，速報などの短報とする。
三　技術論文：新しい技術や技術の組立，実証などを主体とする報告。
四　総　　説：畜産草地研究に関わるものとする。総説は投稿のほか，編集委員会が依頼したものを含む。
五　学位取得論文：研究所において主として行った試験研究による学位取得論文とする。
２　畜産草地研究所研究資料（Memoirs of National Institute of Livestock and Grassland Science / 略誌名：Mem. Natl. Inst. Livest. Grassl. Sci.）
調査資料･技術資料･研究資料：研究所において行った試験研究及び研究所が研究所以外のものに委託して行った試験研究のうち，学術的･産業的に有用な未発表の資料とする。

（原稿の執筆）
第４条　原稿の執筆にあたっては，別に定める畜産草地研究所研究報告及び畜産草地研究所研究資料執筆要領（13畜草Ｂ第44号）に基づくものとする。使用する言語は日本語又は英語とする。

（原稿の提出）
第５条　次の手続きにより原稿及び原稿提出票を事務局に提出する。
一　職員は原稿提出票に必要事項を記載し，所属研究チーム長及び担当する研究管理監等の校閲を受ける。
二　他の職員は原稿提出票に必要事項を記載し，所属研究チーム長及び研究チームを担当する研究管理監等の校閲を受ける。

（受付）
第６条　原稿及び原稿提出票を事務局が受け取った日を受付日とする。受理日は編集委員会の審査の結果，掲載が妥当と認められた日とする。

（審査）
第７条　編集委員会は次の手続きにより論文を審査する。ただし，学位取得論文については審査を省略することができる。
一　編集委員会は論文の内容により審査員正副をそれぞれ１名決定し，論文審査を依頼する。審査員は研究所内及び研究所外の研究者等とし，その氏名は公表しない。
二　審査員は論文審査票により審査を行う。また必要に応じて指摘事項を書き出し提出する。
三　事務局は審査員と著者の間のやり取りの対応にあたる。
四　編集委員会は審査員の審査結果を参考にして掲載の可否を判断する。
審査の内容によっては著者に原稿の訂正を求めることができる。
五　著者は審査結果を受領後，編集委員会が指定する期日までに修正原稿を事務局に提出する。

（校正）
第８条　著者による校正は原則として初校のみとする。校正は誤植の訂正程度にとどめる。やむを得ず大きな変更等を行う場合には編集委員会の承認を得なければならない。

（別刷り）
第９条　別刷りは次のとおりとする。
一　100部とし，筆頭著者が代表で受け取る。
二　別刷りの追加を希望する場合は研究チーム負担で印刷する。

附　　則
この規定は，平成14年４月１日から施行する。

附　　則
この規定は，平成15年10月１日から施行する。

附　　則
この規定は，平成18年４月１日から施行する。

附　　則
この要領は，平成20年４月１日から施行する。