

Evaluation of Meat Products from Cloned Cattle: Biological and Biochemical Properties

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ABSTRACT

Agricultural utilization of cloned livestock produced by nuclear transfer and their products for food will require public and governmental acceptance. A series of studies of properties of meat derived from cloned cattle was carried out to collect data for the safety assessment of cloned cattle products. Meat samples obtained from embryonic cloned, somatic cloned and non-cloned cattle were analyzed for chemical composition, as well as amino acids and fatty acids. Digestibility, allergenicity, and mutagenicity of meat were also examined. There were no significant differences in these properties among embryonic cloned, somatic cloned and non-cloned cattle. The analyses and tests revealed that there were no significant biological differences in meat from a non-cloned, an embryonic cloned, or a somatic cloned animal. A 14-week feeding trial in rats showed there were no abnormalities in body growth, general condition, locomotor activity, reflexes, sexual cycle, urinalysis, hematology, blood biochemistry, and histology. This study showed for the first time that the biological/biochemical properties of meat of cloned cattle are similar to those of non-cloned cattle.

INTRODUCTION

NUCLEAR TRANSFER TECHNOLOGY in domestic animals is being developed for agricultural and medical purposes. Agricultural applications currently focus on the reproduction of valuable animals for the purpose of broad distribution of their gametes, and also on the development of a clone performance test instead of a progeny test for beef sire selection. Further applications will include mass production of beef cattle from cells obtained from premium beef. For these applications, nuclear transfer using blastomeres has been developed, however, is utilized limitedly in cat-

tle production, because of its inefficient production of identical cloned calves.

In Japan since 1989, 682 embryonic cloned calves have been born in 41 laboratories belonging to a national institute, a prefectural institute, a private company or a university. The total number of embryonic cloned cattle shipped for beef is 233, and almost all of their meat products were labeled as cloned beef in the market. Although 340 somatic cloned calves have been born in Japan since 1998, no somatic cloned products have been allowed to enter the human food chain. The Ministry of Health, Labor and Welfare (MHLW) consider that embryonic cloned cattle

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have been produced by a method that is an extension of conventional animal production and thus there is no more risk than with products from non-cloned cattle. On the other hand, they consider that somatic cloned cattle are produced by a new technology, and thus a decision regarding the safety of this product is undecided. Therefore applications of bovine somatic clone technique will require public and governmental acceptance.

Regarding the safety of cloned animals, questions have been raised about the safety of food products from cloned animals and their offspring (Vandenbergh et al., 2002) even though they have existed for several years in animal breeding populations either as split-embryo twins or from reconstructed embryos using nuclear transfer (Wilson et al., 1994; Vandenbergh et al., 2002; Walsh et al., 2003). There is a report (Walsh et al., 2003) comparing the composition of milk produced from cloned animals with milk from non-cloned animals. This report concludes there are no obvious differences in milk composition produced from cloned cows compared to non-cloned cows (Walsh et al., 2003). However, there is no report about other food products, such as meat, from cloned animals.

The Japanese Ministry of Agriculture, Forest and Fisheries (MAFF) has studied the properties of cloned cattle and their products in order to collect data for the safety assessment of cloned cattle products. The Research Institute for Animal Science in Biochemistry and Toxicology (RIAS) investigated properties of cloned cattle products. This study included the nutrient composition, digestibility, allergenicity, and mutagenicity of cloned meat and milk, and also the effect on the growth and the physiological functions of the rat when supplied with food containing cloned cattle meat and milk. In this article we report a study of the properties of meat from cloned cattle.

MATERIALS AND METHODS

Clone and test samples

Japanese Black (JB) embryonic cloned calf and somatic cloned calf were produced at the Koiwai Farm, Ltd. (Morioka, Iwate) and the Nara Prefectural Animal Research Center (Ohuda, Nara), respectively, using nuclear transfer methods as

described elsewhere (Takano et al., 1996; Kato et al., 1998; Akagi et al., 2003). Test samples were prepared from meat derived from non-cloned JB cattle, embryonic cloned and somatic cloned cattle at the age of 27–28 months after fattening. For the *in vitro* digestion test, meat samples were prepared from another non-cloned and a somatic cloned newborn calf (postnatal days 1 and 4, respectively).

Gross chemical composition, amino acids, and fatty acids

Meat samples were homogenized, and analyzed for protein, lipids, carbohydrates, ash, cholesterol, amino acids, and fatty acids by the methods described in the *Japanese Standard of Dietetic Information (JSD)*, 4th ed. (1986, 1989, 2000).

Digestibility test in vitro and in vivo

Meat samples obtained from a somatic cloned and a non-cloned calf were homogenized for *in vitro* digestion test. Simulated gastric juice and intestinal juice were prepared according to the method of the Japanese Pharmacopoeia. Meat homogenate (20 g) was incubated in 200 mL of simulated gastric or intestinal juice in a conical flask at 37°C. After a definite period of time, the mixture in the flask was neutralized and added with trichloroacetic acid. Then, the filtered mixture was diluted with purified water. Nitrogen was measured by Kieldahl method to obtain the digestive value of protein.

Feeding rations for rat *in vivo* digestion test were formulated as basal diet AIN93M (Reeves et al., 1993) using freeze-dried meat powder, instead of casein and soybean oil, with the same amount (13.09%) of protein as the original composition. SD rats (26 weeks old, Charles River Japan, Inc.) were used in this test. The administration period for test rations was 8 days, and carmine red was added to the test rations on days 4 and 7 only. Feces were collected from the first until the second carmine red excretion. Feed intake of rats was also recorded during this period. Digestibility of protein was obtained by measuring total nitrogen in feed and fecal samples by semi-micro Kieldahl method.

Allergenicity test

Allergenicity of embryonic/somatic cloned cattle meat was compared with the meat of non-

cloned cattle by the mouse abdominal wall method, because any foreign protein included in meat might have allergenicity. Extract fluid of meat samples mixed with Freund incomplete adjuvant (Difco Laboratories) was injected into ddy male mice (5 weeks old, Charles River Japan, Inc.) intraperitoneally as a sensitization. After 14 days, induction was made by injection of the meat sample extract fluid into the exposed abdominal wall of the mice following ether anesthesia and caudal intravenous injection of 1% Evans blue (100 μ L/mouse). Allergenicity was evaluated by the size of the blue stained spot in the injected site after 7 min of induction treatment. In the positive control group, egg albumin (0.1 mg/mL, Sigma) solution was used as a foreign protein instead of meat extract.

Mutagenicity test

Mutagenicity was tested by micronucleus test in mice. Artificial food containing 1%, 2.5%, or 5% meat powder, which was prepared to be equal to the basal diet AIN93G (Reeves et al., 1993) in nutritive qualities, was given to ICR mice (8 weeks old, Charles River Japan, Inc.) for 14 days. As a positive control, mitomycin C (Wako Pure Chemical Industries, Ltd.) was injected as a mutagen. After euthanasia a bone marrow smear was prepared for observation of micronuclei in the polychromatic erythrocytes.

Feeding test

To evaluate the general effects in animals fed meat products from cloned cattle, a feeding test in rats was carried out. Feeding rations were prepared as described above. SD rats (5 weeks old, Charles River Japan, Inc.) were fed and observed as to general condition, behavior, lethality, body growth, food intake, locomotor activity, 29 phys-

iological parameters, landing foot splay, grip strength, nine reflexes, and female sexual cycle for 14 weeks. Urine samples were collected at 4, 8, and 12 weeks of feeding for urinalysis. At the end of the feeding period, blood sampling and autopsy were carried out for further hematological, blood biochemical and histological examinations.

RESULTS AND DISCUSSION

The cattle used in this study had been produced independently at different institutes. While being raised, these cloned or non-cloned cattle were monitored for hematologic changes, serum chemistries, serum protein fraction, and LDH isoenzyme. There were no significant differences in any of the examinations, and all values were within normal limits (data not shown).

Results of gross composition analysis of embryonic cloned, somatic cloned and non-cloned meat showed higher lipid content than the standard JSD value (Table 1). This tendency was clearer in embryonic and somatic cloned cattle, because their meat quality was high (class 4) according to the Japanese Meat Grading Standard by which meat is rated from class 1 (poor) to class 5 (premium) based on the marbling score. Results of amino acids and fatty acids analyses were similar among the three samples (Figs. 1 and 2) and also similar to the standard JSD value.

Results comparing the *in vitro* digestibility of somatic cloned versus non-cloned meat are shown in Figure 3. The digestibility of somatic cloned meat differed slightly from non-cloned meat at the early period of digestion in the simulated gastric or intestinal juice; however, at the late stage of digestion there was no significant difference between groups. *In vivo* digestibility of

TABLE 1. CHEMICAL PROPERTIES OF MEAT FROM CLONED AND NON-CLONED CATTLE^a

	Water (g/100 g)	Protein (g/100 g)	Lipids (g/100 g)	Carbohydrates (g/100 g)	Ash (g/100 g)	Cholesterol (mg/100 g)
Non-clone 1	64.8	19.6	13.8	0.8	1.0	50
Non-clone 2	59.6	17.9	21.1	0.6	0.9	58
Non-clone 3	58.0	17.8	22.9	0.4	0.9	68
Embryonic clone	60.2	17.4	21.2	0.4	0.9	56
Somatic clone	57.9	16.8	23.8	0.5	0.9	68
JSD	(59.8)	(17.9)	(21.1)	(0.4)	(0.9)	(73)

^aValues indicate the mean from nine parts of the carcass.

Values in parentheses cited from Japanese Standard Dietetic Information (JSD), 5th ed.

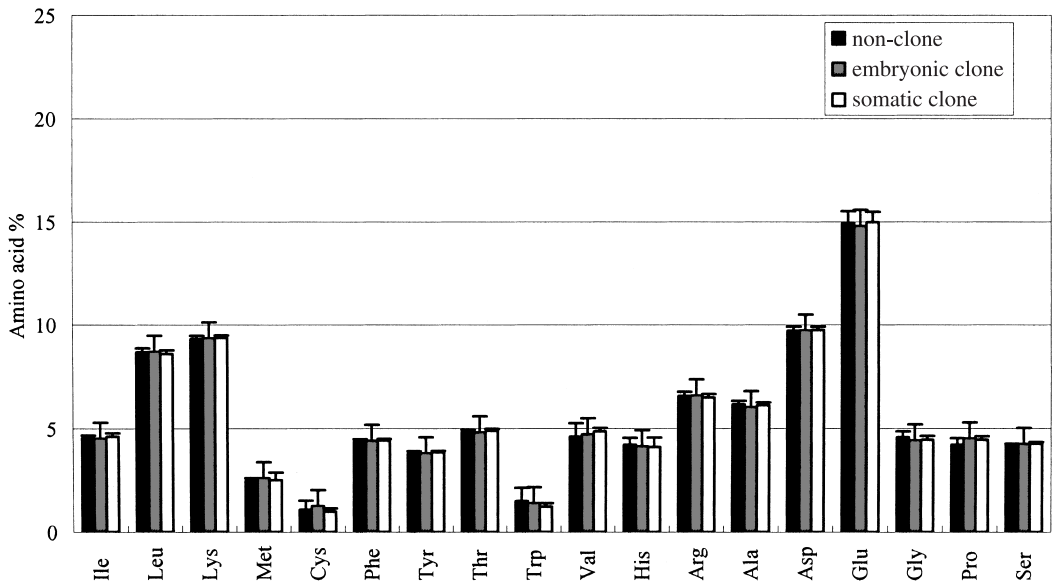


FIG. 1. Amino acid composition of meat from embryonic cloned, somatic cloned, and non-cloned cattle.

embryonic, somatic and non-cloned meat was 82.3%, 84.9%, and 83.8%, respectively. These results indicate that there is no significant difference in the digestibility of meat derived from cloned or non-cloned cattle. A previous report on the digestibility of Mexican cattle meat in the rat

(Hernandes et al., 1996) shows results similar to those of the present study. Though tropomyosin is known as an allergen in beef, many points are still unclear. For studying a food allergen, antibody reactivity of the specific material (e.g., a gene product in GMO) is

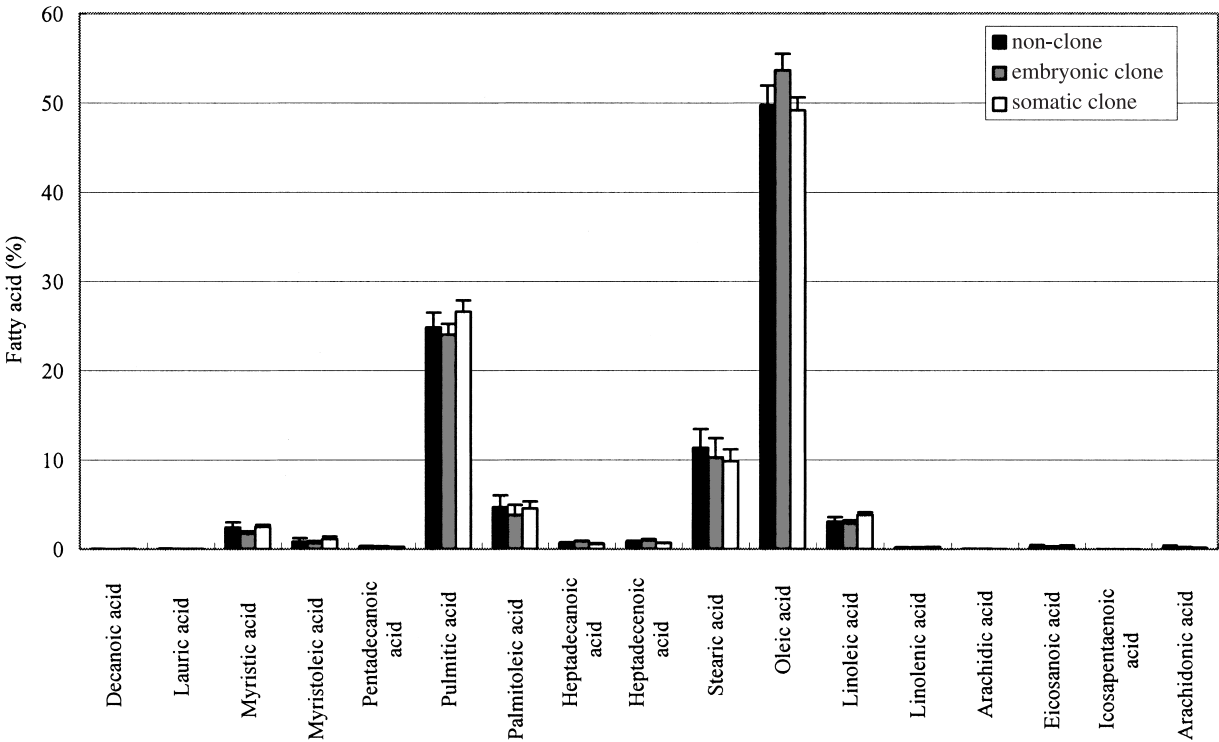


FIG. 2. Fatty acid composition of meat from embryonic cloned, somatic cloned, and non-cloned cattle.

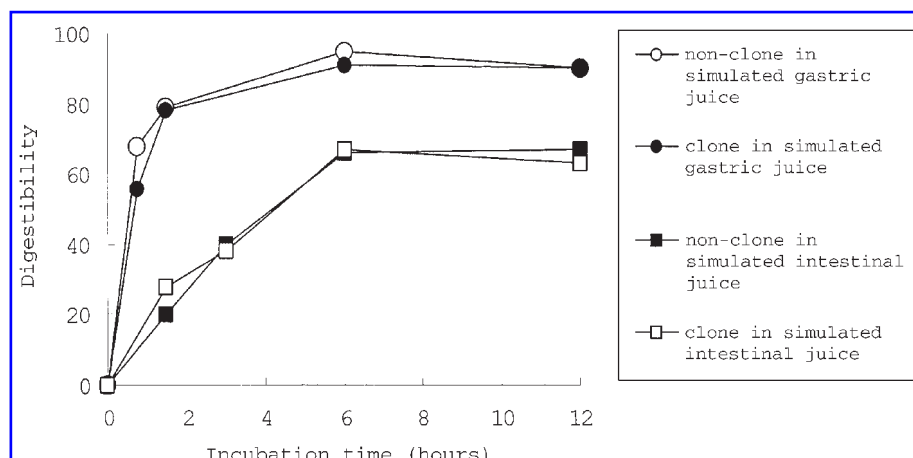


FIG. 3. Changes of digestibility of meat incubated in simulated gastric or intestinal juice. Meat samples were incubated in simulated gastric or intestinal juice at 37°C.

examined by Western blotting and ELISA. However, all proteins are potential allergens, thus it is inappropriate to apply this method for investigating allergenicity of the whole food. Therefore, allergenicity of non-cloned, embryonic and somatic cloned cattle meat was studied by the mouse abdominal wall method. The results of these tests are shown in Table 2. In the positive control group, in which egg albumin was administered to the mice, the diameter of the dye spot that resulted from the allergic reaction was significantly larger than in the negative control group. In all test groups administered embryonic, somatic, and non-cloned cattle derived meat extract, respectively, the second meat extract injection increased the allergic reaction. However, there was no significant difference in dye spot diameter among groups. These results indicate that allergenicity of embryonic and somatic cloned meat is equivalent to that of non-cloned meat.

To evaluate mutagenicity of cloned meat, a mi-

cronucleus test was carried out (Table 3). In the positive control group in which mitomycin C was injected into mice, the frequency of micronuclei in bone marrow polychromatic erythrocytes was dramatically higher than in the negative control group. On the other hand, in the test groups in which mice were given food containing meat powder derived from embryonic, somatic cloned or non-cloned cattle, the frequency of micronuclei was similar to the negative control. Also micronuclei frequency did not tend to increase dose dependently. Therefore, it was concluded that mutagenicity of meat from embryonic and somatic clones was negative, as was the meat of non-cloned cattle. Changes in the proportion of polychromatic erythrocyte counts to total erythrocytes counts were not observed. When bone marrow cells are exposed to chemical substances absorbed by animals, polychromatic erythrocytes are reduced by the cytotoxic effect. In this study, although a high concentration (maximum 5%) of

TABLE 2. RESULTS OF ALLERGENICITY TEST OF MEAT USING THE MOUSE ABDOMINAL WALL METHOD

Test group	Subject administered	Number of animals	Diameter of spot (mm)
Non-clone	Nothing	7	5.3 ± 5.0
	Meat powder	10	13.0 ± 5.9*
Embryonic clone	Nothing	7	7.0 ± 4.9
	Meat powder	10	12.5 ± 3.5*
Somatic clone	Nothing	7	5.7 ± 4.2
	Meat powder	10	13.1 ± 5.0**
Positive control	Nothing	7	3.3 ± 3.3
	Egg albumin	10	9.9 ± 6.7*

* $p < 0.05$; ** $p < 0$.

TABLE 3. FREQUENCY OF MICRONUCLEUS AND POLYCHROMATIC ERYTHROCYTES IN THE BONE MARROW CELLS OF MOUSE GIVEN A DIET CONTAINING MEAT POWDER FOR 14 DAYS

Group	Concentration of meat powder	Number of animals	Micronucleus frequency (%) ^a	Polychromatic erythrocyte frequency (%) ^a
Negative control ^b		6	0.20 ± 0.18 (0.0–0.5)	47.7 ± 9.7 (30.2–59.7)
Non-clone	1%	6	0.17 ± 0.12 (0.1–0.4)	50.0 ± 9.1 (37.9–61.3)
	2.5%	6	0.13 ± 0.08 (0.0–0.2)	47.3 ± 13.1 (22.3–60.2)
	5%	6	0.12 ± 0.15 (0.0–0.3)	46.8 ± 10.5 (37.2–63.5)
Embryonic clone	1%	6	0.20 ± 0.06 (0.1–0.3)	51.0 ± 7.3 (41.3–59.3)
	2.5%	6	0.23 ± 0.14 (0.0–0.4)	47.1 ± 4.3 (40.6–51.1)
	5%	6	0.12 ± 0.08 (0.1–0.2)	49.6 ± 9.6 (37.9–61.9)
Somatic clone	1%	6	0.18 ± 0.10 (0.1–0.3)	48.3 ± 8.4 (35.6–55.1)
	2.5%	6	0.22 ± 0.10 (0.1–0.4)	51.7 ± 7.3 (44.3–63.9)
	5%	6	0.22 ± 0.08 (0.1–0.3)	48.4 ± 8.1 (38.4–58.1)
Positive control ^c		6	6.95 ± 1.56** (4.1–8.4)	25.7 ± 6.5 (19.4–36.4)

^aValues indicate mean ± SD. ** $p < 0.01$. Numbers in parentheses indicate min–max value.

^bIn negative control group, mice were fed basic diet only.

^cIn positive control group, mice were injected with mitomycin C.

meat powder was administered to animals, polychromatic erythrocytes were not reduced. This is because there was no cytotoxic substance in the test sample.

During the rat feeding test, there were no significant differences in general condition, lethality, growth (body weight and food intake), physiological functions, reflexes, landing foot splay length, grip strength, locomotor activity, and female sexual cycle among test groups given food containing meat powder derived from embryonic cloned, somatic cloned or non-cloned cattle. Also there were no significant differences in urinalysis, hematological examination, blood biochemical examination, autopsy, organ weight, and histological examination among the test groups.

From the results of this study, we concluded that the biological/biochemical properties of meat of cloned cattle are similar to those of non-cloned cattle. The use of somatic cells taken from adults or fetuses by natural breeding has raised concerns that genetic mutations or epigenetic alterations would alter the food composition of products taken from cloned animals and make

them unsafe for human consumption (Vandenbergh et al., 2002; Walsh et al., 2003). In addition, the generation of heteroplasmic animals, in which it is unclear if food properties are altered, is also a clone peculiar phenomenon that is rarely observed in non-cloned animals (Steinborn et al., 1998, 2000; Hiendleder et al., 1999; Takeda et al., 1999, 2003). However, it is difficult to examine individually whether these problems hurt the safety of food. In this study we examined chemical properties of meat and the effect on animals fed “whole” meat from cloned cattle and non-cloned cattle. It is considered that these results are useful for further research on the risk assessment of products from cloned cattle.

This year, Kumagai (2003), commissioned by the MHLW, submitted a report on the safety of cloned products according to an RIAS study and data by other research groups. The report concluded “From present knowledge, it is difficult to consider that the safety of bovine products as a food is hurt by a peculiar factor involved in cloned cattle and not in cattle produced by conventional technology. However, since cloning

technology is new, prudent consideration is necessary on declaration of the safety of food from cloned cattle" (Kumagai, 2003). Furthermore, the Food Safety Commission of the Japanese government was established last July. This committee has the authority to advise MAFF and MHLW will decide on the safety of products derived from cloned cattle from the viewpoint of risk assessment.

ACKNOWLEDGMENTS

We thank M. Yamaguchi, S. Yamamoto, and N. Fukuda (RIAS) for investigation of a series of examinations and analyses. We thank Drs. Y. Shioya (National Institute of Livestock and Grassland Science) and T. Nagai (National Institute of Agrobiological Sciences) for their helpful discussions. This study was carried out as part of Operation of Urgent Research for Utilization of Clone Technology (supported by Ministry of Agriculture, Forest and Fisheries and Japanese Livestock Technology Association (JLTA)); a document including this study was published as a Business Report of Operation Study for Properties of Products from Cloned Cattle (2002) by JLTA (in Japanese).

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