

—Original Article—

## Growth, Reproductive Performance, Carcass Characteristics and Meat Quality in F1 and F2 Progenies of Somatic Cell-Cloned Pigs

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**Abstract.** The objective of this study was to examine the health and meat production of cloned sows and their progenies in order to demonstrate the application of somatic cell cloning to the pig industry. This study compared the growth, reproductive performance, carcass characteristics and meat quality of Landrace cloned sows, F1 progenies and F2 progenies. We measured their body weight, growth rate and feed conversion and performed a pathological analysis of their anatomy to detect abnormalities. Three of the five cloned pigs were used for a growth test. Cloned pigs grew normally and had characteristics similar to those of the control purebred Landrace pigs. Two cloned gilts were bred with a Landrace boar and used for a progeny test. F1 progenies had characteristics similar to those of the controls. Two of the F1 progeny gilts were bred with a Duroc or Large White boar and used for the progeny test. F2 progenies grew normally. There were no biological differences in growth, carcass characteristics and amino acid composition among cloned sows, F1 progenies, F2 progenies and conventional pigs. The cloned sows and F1 progenies showed normal reproductive performance. No specific abnormalities were observed by pathological analysis, with the exception of periarteritis in the F1 progenies. All pigs had a normal karyotype. These results demonstrate that cloned female pigs and their progenies have similar growth, reproductive performance and carcass quality characteristics and that somatic cell cloning could be a useful technique for conserving superior pig breeds in conventional meat production.

**Key words:** Carcass, Cloned pig, Meat quality, Reproduction, Somatic cell clone

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Successful cloning of pigs from somatic cells has been reported [1–3]. Because of the repeatable and predictable potential of somatic cell cloning in pigs, application of this procedure to the pork industry can be expected. Several studies on pig cloning, including those on genetically modified cloned pigs, have been performed, although the success rate of cloning in pigs has been extremely low [4–8]. Furthermore, this research has been propelled by the need for animals for biomedical or experimental purposes [9–11]. However, to apply cloning to the pork industry, we must examine the growth and health of pigs along with the safety of meat products not only of cloned pigs but also their progenies [12]. Although several studies have been reported on cloned pigs and their progenies bred with cloned boars [13, 14], little is known about cloned female pigs and their progenies [15]. Somatic cell cloning is expected to be

successful in the production of superior commercial breeds and the conservation of superior economic traits. Clone-derived foods, such as meat or milk, have been analyzed in several nations, and their safety has been reported [16–18]. However, most nations still have restrictions on the entry of products from cloned animals into the food chain because little data exists on the safety of clone products. Thus, more research is required to apply somatic cell cloning for the conservation of superior genes and for pig meat production. To demonstrate an application of somatic cell cloning in conserving superior genes and pig meat production, we produced cloned pigs and compared the growth, reproductive performance, and meat quality characteristics of Landrace cloned female pigs with those of their progenies. In this paper, we discuss the normality of the clones and their progenies and note that abnormalities found in somatic cell clones do not transmit to their progenies.

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### Materials and Methods

#### *Animals and cloning*

Donor cell culture and nuclear transfer were carried out at PRIME

TECH (Tsuchiura, Japan), and each step of the nuclear transfer procedure was observed, as previously described [1]. A Landrace sow was used as a donor pig. We obtained somatic cells for nuclear transfer from the ear of a female purebred Landrace pig. The ear skin was cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 20% fetal bovine serum to prepare the fibroblasts, which would be used as nucleus donors. Cultures were established by plating cells for five passages. Cell culture was continued for 14 days without media replacement until confluent in DMEM. Cells were then removed from the plate using 0.25% trypsin and resuspended in DMEM until nuclear transfer. Oocytes from cross-bred gilts were collected from a slaughterhouse and used as recipients of nuclear transfer. Following oocyte maturation, cumulus cells were removed in Hepes-buffered TCM-199 medium (Gibco, Grand Island, NY, USA) supplemented with 0.1% hyaluronidase. Enucleation was performed by micromanipulation. Donor nuclei were injected into enucleated oocytes using piezo-actuated microinjection (PRIME TECH) in porcine zygote medium 3 [19]. The reconstructed pre-embryos were incubated at 38.5 C for 3 h before electroactivation. The electroactivation was performed with a single current pulse of 150 kV/cm for 99  $\mu$ sec in activation solution (0.28 M D-mannitol, 0.05 mM CaCl<sub>2</sub>, 0.1 mM MgSO<sub>4</sub> and 0.01% (w/v) bovine serum albumin).

Experimental animals were kept at the Ibaraki Prefecture Livestock Experiment Center, and standard experimental procedures were performed with the approval of the Institutional Research Committee.

#### *Embryo transfer and mating of offspring*

At 8–10 months of age, the estrus cycles of the recipient gilts were synchronized with hormone injections. They received 2 ml of prostaglandin F<sub>2</sub> $\alpha$  analogues containing 92  $\mu$ g/ml of cloprostenol sodium (Planate, Intervet, Osaka, Japan) on the first day of the early pregnancy period between 21 and 45 days of gestation. They received the same dosage of PGF<sub>2</sub> $\alpha$  analogues with 1,000 IU of equine chorionic gonadotropin (Serotropin, ASKA Pharmaceutical, Tokyo, Japan) on day 2 and 500 IU of human chorionic gonadotropin (Gonotropin, ASKA Pharmaceutical, Tokyo, Japan) on day 5. The onset of estrus was observed on day 6. Approximately 200 cloned embryos at the 2- to 16-cell stage were surgically transferred into the oviducts of seven anesthetized recipient gilts at 2 days after the onset of estrus. One recipient sow produced a litter of five cloned female piglets (clones A–E) via vaginal delivery. DNA microsatellite markers were used to confirm the genetic identity of the cloned piglets to the donor sow. The body weights from birth to 8 weeks of age of the clones and their progenies are presented in Table 1. We obtained five cloned piglets born alive from one litter. Three of the five cloned piglets were healthy. The cloned piglets grew similarly to the Landrace controls. The cloned pigs received the experimental barn's normal feeding program; that is, the piglets were weaned at about 3 weeks of age. They received creep feed until weaning. The weaned pigs were fed feed in mash form *ad libitum* until 5 months of age. At 9–10 months of age, two of the cloned gilts were bred with a Landrace boar. Because Clone C did not express a distinct estrus, we performed an anatomy examination. Clone C did not show any phenotypic abnormalities in the ovaries or uterus, which grew normally, and was diagnosed

instead with pituitary basophilism. The gilts consumed 2.5 to 3.0 kg of feed, which was restricted based on their live weight, until parturition. After parturition, F1 progenies were used for a progeny test. At 7–8 months of age, two of the F1 gilts were bred with a Duroc or Large White boar.

#### *Progeny test, carcass analysis, pathological analysis and chromosomal analysis*

We measured the body weight, growth rate and feed conversion and performed a pathological analysis of the anatomy for any abnormal pigs or pigs that died following birth. Three of the five cloned pigs were allocated to a growth test. F1 (8 males and 9 females) and F2 (9 males and 10 females) progenies were used for the growth test and a carcass quality test. All male piglets were castrated before the growth test. The progeny test was performed under the standard conditions for testing of pork products in pigs [20]. Pigs were slaughtered at the plant in the experimental center. Data collected included the carcass length, thickness of back fat and number of vertebrae. During carcass dissection into prime cuts, the ratio of the cuts and the loin eye area were measured. Dressing percentages were calculated from both the live and carcass measures of weight. The basic micronutrients in a loin sample were evaluated at the Tsukuba Food Evaluation Center (Tsuchiura, Japan), and the amino acid composition of the same sample was analyzed by ion-exchange chromatography by the Japan Frozen Foods Inspection Corporation (Tokyo, Japan). We compared the micronutrient and amino acid compositions of samples from clones and progenies to a national composite data source [21, 22]. Pathological analyses of all pigs were performed at the National Institute of Animal Health. Chromosome analysis was carried out on peripheral lymphocytes from cloned pigs (n = 2) and their offspring (6 F1s and 4 F2s) according to the method described previously [23].

Statistical analysis: The Student's *t*-test was used for statistical analysis. A P value <0.05 was considered significant.

## **Results and Discussion**

#### *Pathological and chromosomal analyses*

Five cloned female piglets (clones A–E) were born after 115 days of gestation from a recipient Landrace sow. Three of the five cloned piglets (clones A, B and C) were used for the growth test. No specific abnormalities of pathological status were observed in clones A or B or in the F2 progenies. Clone C was diagnosed with pituitary basophilism. The details regarding the pathological anatomy of clone C are described in the section concerning reproductive performance. Clone D died on the day of birth and had an umbilical hernia with a deformed leg. Beckwith-Wiedemann syndrome is an epigenetic disorder usually present at birth that commonly includes abdominal wall defects such as hernia in humans [24]. Umbilical hernia is also reported in cloned cattle but is not always caused by epigenetic abnormalities peculiar to cloned animals [25]. Of the congenital abnormalities in pigs, umbilical hernia or ruptures are the most common and are considered to have very low heritability. No umbilical hernia was observed in the progenies in this study. It was not clear whether the umbilical hernia in clone D was caused by epigenetic or genetic disorder. However, it should be noted that

**Table 1.** Body weight from birth to 8 weeks of age in clones and their progenies

Animal	n	Day 0 (kg)	1 week (kg)	3 weeks (kg)	5 weeks (kg)	8 weeks (kg)
Clone <sup>a</sup>	3	1.34 ± 0.14	3.70 ± 0.34 <sup>h</sup>	8.30 ± 0.35 <sup>HI</sup>	14.55 ± 0.45 <sup>HI</sup>	26.37 ± 3.35
F1 (clone × L) <sup>b</sup>	17	1.48 ± 0.19	3.18 ± 0.40 <sup>i</sup>	6.18 ± 0.78 <sup>l</sup>	10.92 ± 1.41 <sup>l</sup>	23.03 ± 3.68
Landrace <sup>c</sup>	100	1.60 ± 0.31	2.94 ± 0.74	6.79 ± 1.40	11.91 ± 2.24 <sup>l</sup>	23.24 ± 4.34
F2 (F1 × D) <sup>d</sup>	11	1.62 ± 0.16 <sup>h</sup>	2.57 ± 0.26 <sup>HI</sup>	6.69 ± 0.26	11.01 ± 1.21 <sup>h</sup>	29.27 ± 2.56
LD <sup>e</sup>	7	1.87 ± 0.27 <sup>i</sup>	4.65 ± 0.69 <sup>l</sup>	7.53 ± 0.99	12.66 ± 1.75 <sup>i</sup>	29.07 ± 4.94
F2 (F1 × W) <sup>f</sup>	8	1.74 ± 0.18 <sup>HI</sup>	3.77 ± 0.56 <sup>HI</sup>	7.70 ± 1.03 <sup>HI</sup>	13.64 ± 1.34 <sup>HI</sup>	29.25 ± 2.58 <sup>HI</sup>
LW <sup>g</sup>	14	1.48 ± 0.19 <sup>l</sup>	2.51 ± 0.36 <sup>l</sup>	5.50 ± 0.93 <sup>l</sup>	8.95 ± 1.24 <sup>l</sup>	21.04 ± 2.31 <sup>l</sup>

Values are expressed as means ± SD. <sup>a</sup> Cloned females derived from Landrace donor cells. <sup>b</sup> F1 pigs from the cloned sows mated with a Landrace (L) boar. <sup>c</sup> Purebred Landrace pigs. <sup>d</sup> F2 pigs from the F1 sows mated with a Duroc (D) boar. <sup>e</sup> Landrace × Duroc crossbred pigs. <sup>f</sup> F2 pigs from the F1 sows mated with a Large White (W) boar. <sup>g</sup> Landrace × Large White crossbred pigs. <sup>h, i</sup> Values without common characters in the same row differ significantly (P<0.05). <sup>HI, l</sup> Values without common characters in the same row differ significantly (P<0.01).

the causes of death for the clones were widely known abnormalities that are common in conventionally bred pigs. Clone E died of pleuropneumonia 139 days after birth. Clone E was diagnosed as being infected with *Actinobacillus pleuropneumonia* and *Actinomyces pyogenes*. Park *et al.* suggested that cerebromeningitis and hemodynamic disorder are major risk factors for sudden early death in cloned piglets [26], but the piglets that survived to adulthood did not show any abnormalities. Although we cannot exclude the possibility that somatic cell-cloned pigs are susceptible to respiratory infections, the pathological data suggested that the death of clone E was because of common diseases. Although all F1 progenies were phenotypically normal, six of the 20 F1 progenies had periarteritis in the heart or spleen. Periarteritis was not observed in the clones or F2 progenies. Periarteritis is an inflammation of the outer membrane of an artery, which results in a mild lesion and is occasionally seen in the organs of pigs. It may be induced by autoimmune inflammation. Porcine Reproductive & Respiratory Syndrome (PRRS) is associated with periarteritis of the lung, heart and kidney [27]. However, since pigs usually are not subjected to precise pathological analysis under the meat production system, little is known about the incidence of periarteritis in conventional pigs. In this case, the F1 progenies were negative for PRRS and clinical infectious diseases. The cloned pigs showed no sign of the microscopic periarteritis lesion observed in F1 progenies. The periarteritis may have been caused by a recessive gene inherited from the donor sow and not by epigenetic factors. It has been reported that abnormalities found in clones may be removed by reprogramming in the following generations [28]. No disorders observed in clones were observed in the progenies in this study. Twelve piglets received a chromosomal analysis. They had a normal modal chromosome number, which showed a chromosome complement of 2n = 38, XY or 2n = 38, XX. In the present study, there were no chromosomally abnormal nuclei, such as near-triploid or near-tetraploid cells as observed previously in cloned cattle [23].

#### Growth performance

The clones were phenotypically normal and grew normally. The body weights of the clones were not different from those of the Landrace controls (Table 1). However, the body weights of the clones from 3 to 5 weeks of age were significantly greater than

those of the F1 progenies (clone × Landrace). This age coincides with the lactation period, and the daily intake of milk in clones was considered to be superior to the controls because of the small litter size. However, this difference was not observed at 8 weeks of age, perhaps because the nursing advantage for the cloned piglets was limited to the early period of growth.

With regard to the progenies, there were no differences between F1 progenies and Landrace controls in body weight until 8 weeks of age. Since the litter size for cloned sows was within the normal range for the breed [29], which ranged from 7 to 11, the nursing conditions can be assumed to be the same for the F1 progenies as for the Landrace controls. Martin *et al.* reported that progeny derived from the mating of cloned parents show normal growth performance in the pre-weaning period [30]. The data from their study support those reported by the Japan Pork Producers Association [29]. F2 progenies [F1 (clone × Landrace) × Large White] were significantly larger than the control LW (Landrace × Large White) at every week of age (P<0.01). F2 progenies reached approximately 30 kg at 8 weeks of age, with the exception of the LW crossbred control. Since the birth weight of the LW crossbred control was small (1.48 vs. 1.74 kg), it is possible that low birth weight and nursing problems may affect the growth of the piglets in the first 8-weeks after birth.

The daily gains and feed conversions of the progenies are presented in Table 2. The number of days to a body weight of 30 or 105 kg and the daily gain of the F1 progenies (clone × Landrace) were similar to those in the Landrace controls. However, feed conversion was lower in the F1 progenies than in the Landrace controls (P<0.01). In the F2 progenies, significant differences in daily gain (P<0.01) and feed conversion (P<0.05) were only observed between F2 progenies [F1 (clone × Landrace) × Large White] and LW (Landrace × Large White) controls. Daily gain and feed conversion were strongly related to the growth performance shown in Table 1. The same reason for the delayed growth in the LW controls caused the inferior daily gain and the feed conversion.

More than 500 somatic cell-cloned cattle have been produced for animal science research in Japan. However, high rates of pregnancy failure, postnatal death and lethal abnormality are still common [31]. Although efficiency is low, cloned cattle surviving to adulthood have growth and reproductive performance similar to those of cattle

**Table 2.** Daily gain and feed conversion of the progenies

Animal	n	Days to 30 kg of body weight	Days to 105 kg of body weight	Daily gain (g)	Feed conversion
F1 (clone × L) <sup>a</sup>	10	66.2 ± 4.7	144.7 ± 3.5	956.0 ± 49.8	2.89 ± 0.31 <sup>G</sup>
Landrace <sup>b</sup>	22	66.9 ± 4.7	148.5 ± 9.1	933.5 ± 121.0	3.50 ± 0.50 <sup>H</sup>
F2 (F1 × D) <sup>c</sup>	4	61.3 ± 2.1	142.5 ± 9.1	934.2 ± 103.4	3.70 ± 0.07
LD <sup>d</sup>	4	65.5 ± 11.0	149.0 ± 15.0	919.4 ± 54.4	3.41 ± 0.47
F2 (F1 × W) <sup>e</sup>	4	68.3 ± 3.3 <sup>G</sup>	146.3 ± 2.4	968.3 ± 51.4 <sup>G</sup>	3.10 ± 0.25 <sup>S</sup>
LW <sup>f</sup>	3	77.3 ± 2.3 <sup>H</sup>	166.0 ± 16.5	646.7 ± 78.3 <sup>H</sup>	3.60 ± 0.04 <sup>h</sup>

Values are expressed as means ± SD. <sup>a</sup> F1 pigs from the cloned Landrace sows mated with a Landrace (L) boar. <sup>b</sup> Purebred Landrace females. <sup>c</sup> F2 pigs from the F1 sows mated with a Duroc (D) boar. <sup>d</sup> Landrace × Duroc crossbred pigs. <sup>e</sup> F2 pigs from the F1 sows mated with a Large White (W) boar. <sup>f</sup> Landrace × Large White crossbred pigs. <sup>S, h</sup> Values without common characters in the same row differ significantly ( $P < 0.05$ ). <sup>G, H</sup> Values without common characters in the same row differ significantly ( $P < 0.01$ ).

**Table 3.** Gestation period and litter size of the clones and F1 progenies

Gilts	n	Bred boar	Gestation period	Litter size	Born alive
Clone <sup>a</sup>	2	Landrace	115.5	10.5	8.5
F1 <sup>b</sup>	1	Duroc	116	11	11
F1 <sup>b</sup>	1	Large White	113	8	8
Landrace <sup>c</sup>	48	Landrace	117 ± 1.3	10.9 ± 3.2	10.1 ± 2.9

<sup>a</sup> Cloned females derived from Landrace donor cells. <sup>b</sup> F1 females from the cloned sows mated with a Landrace boar. <sup>c</sup> Purebred Landrace females.

produced by artificial insemination or natural breeding [31]. In contrast, although the efficiency of pregnancy is extremely low in somatic cell clones in pigs, cloned piglets have fewer abnormalities in the postnatal period [32]. From these results, we inferred that the cloned sows and their live-born progenies showed no abnormalities and had normal growth performance.

#### Reproductive performance

Clones A and B reached puberty at 4–5 months of age, and they conceived by natural breeding with a Landrace boar at 9–10 months of age. The gestation periods and litter sizes of the clones and the F1 progenies are presented in Table 3. The clones and F1 progenies were born after 115–116 and 113–116 days of gestation, respectively. Their litter sizes and numbers of progenies born alive ranged from 8 to 11 and were similar to those of Landrace controls. This result supports observations from the literature in which gestation period and average litter size were similar to those of non-cloned pigs [15, 33]. Basophilism is a syndrome caused by increased production of ACTH from a tumor of the adrenal cortex or the anterior lobe of the pituitary gland. In the present study, no microscopic lesions or tumors were observed. It was unclear as to whether the basophilism in clone C was related to epigenetic abnormalities of cloned animals. However, we may conclude that cloned female pigs and their F1 progenies are capable of normal reproductive performance.

#### Carcass characteristics and micronutrient and amino acid compositions

The carcass characteristics of the progenies are presented in Table 4. There were no significant differences in carcass traits between F1

or F2 progenies and their controls, with the exception of dressing percentage ( $P < 0.01$ ), back fat thickness at the shoulder ( $P < 0.05$ ) and loin length ( $P < 0.01$ ). The reasons for these differences were unclear, but the differences have no biological significance and resulted in pigs with values in the normal range. The micronutrient and amino acid compositions of the loin samples of the clones and progenies are presented in Table 5. There were no differences in basic micronutrients between clones and their progenies. However, the levels of energy and fat tended to be lower both in the clones and the progenies compared with the controls. These values depend on the sampling regions, and the sample sites may have been different in the controls.

With regard to the amino acid composition, no abnormal values were observed in the clones or their progenies. The compositions of essential amino acids were within the normal range in both clones and progenies [21, 22]. Although only a very small number of animals were examined, the basic micronutrient and amino acid compositions showed no unusual nutrient values in either the clones or the progenies.

To facilitate the integration of cloning techniques into the livestock industry, the food products from these cloned animals were closely examined for safety. In a risk assessment of the products derived from cloned animals, the FDA and EFSA reported that edible products from healthy clones pose no increased food consumption risks relative to comparable products from non-cloned animals [16, 17]. A similar assessment was reported for the progeny of cloned cattle in Japan [18]. However, most nations still restrict entry of cloned products into the food chain because the data are insufficient and the experiments have only been conducted over a short term.



**Table 4.** Carcass characteristics of the progenies

Animal	F1 <sup>a</sup> (Clone × L) (n = 10)	L <sup>b</sup> (Cont) (n = 22)	F2 <sup>c</sup> (F1 × D) (n = 4)	L × D <sup>d</sup> (Cont) (n = 4)	F2 <sup>e</sup> (F1 × W) (n = 4)	L × W <sup>f</sup> (Cont) (n = 3)
Dressing percentage (%)	70.1 ± 1.2 <sup>l</sup>	72.9 ± 1.4 <sup>j</sup>	72.7 ± 1.9	72.4 ± 0.6	74.3 ± 0.8	73.4 ± 0.4
Carcass length <sup>g</sup> (cm)	69.7 ± 2.0	68.8 ± 3.0	66.2 ± 2.6	69.3 ± 2.5	64.6 ± 1.8	68.3 ± 0.6
Back fat thickness (cm)						
Shoulder	4.4 ± 0.3 <sup>i</sup>	4.0 ± 0.5 <sup>j</sup>	3.8 ± 0.8	3.5 ± 0.2	4.0 ± 0.7	4.0 ± 0.6
Rib	2.3 ± 0.3	2.2 ± 0.5	2.0 ± 0.6	1.8 ± 0.3	2.1 ± 0.3	2.2 ± 0.2
Lumbar	3.7 ± 0.4	3.3 ± 0.5	3.3 ± 0.4	2.6 ± 0.9	3.5 ± 0.5	3.1 ± 0.1
Number of vertebrae	21.6 ± 0.5	21.2 ± 0.8	21.0 ± 0	NA <sup>h</sup>	21.3 ± 0.5	NA
Weight ratio of prime cuts (%)						
Shoulder	30.7 ± 1.6	28.7 ± 2.4	30.4 ± 1.4	31.3 ± 0.3	31.4 ± 0.7	31.4 ± 0.6
Loin	39.7 ± 1.4	41.4 ± 3.4	38.7 ± 1.4	37.3 ± 1.9	37.7 ± 1.4	39.3 ± 0.9
Ham	29.7 ± 1.0	29.9 ± 1.8	30.9 ± 1.7	31.4 ± 1.7	30.9 ± 1.6	29.4 ± 0.7
Loin length (cm)	55.9 ± 1.1 <sup>l</sup>	58.8 ± 2.5 <sup>j</sup>	56.1 ± 2.3	55.1 ± 2.1	55.6 ± 1.3	56.6 ± 1.3
Loin eye area (cm <sup>2</sup> )	22.8 ± 2.6	21.3 ± 4.1	20.4 ± 3.7	16.5 ± 3.9	24.5 ± 2.2	20.8 ± 1.9

Values are expressed as means ± SD. <sup>a</sup> F1 pigs from the cloned Landrace sows mated with a Landrace (L) boar. <sup>b</sup> Purebred Landrace females. <sup>c</sup> F2 pigs from the F1 sows mated with a Duroc (D) boar. <sup>d</sup> Landrace × Duroc crossbred pigs. <sup>e</sup> F2 pigs from the F1 sows mated with a Large White (W) boar. <sup>f</sup> Landrace × Large White crossbred pigs. <sup>g</sup> Length from the first rib to the last lumbar vertebra. <sup>h</sup> NA means lack of data. <sup>i,j</sup> Values without common characters in the same row differ significantly (P<0.05). <sup>l,j</sup> Values without common characters in the same row differ significantly (P<0.01).

**Table 5.** Micronutrient and amino acid compositions of the clones and progenies

Nutrients	Clone <sup>a</sup> (n = 2)	F1 <sup>b</sup> (n = 2)	F2 <sup>c</sup> (n = 2)	Controls <sup>d</sup>
Basic micronutrient*				
Energy (kcal in 100 g)	116.5	117.5	118.0	150.0
Water (%)	73.4	73.0	74.7	70.3
Protein (%)	22.0	22.8	19.7	22.7
Fat (%)	3.0	2.7	4.2	5.6
Carbohydrate (%)	0.6	0.5	0.3	0.3
Ash (%)	1.1	1.2	1.1	1.1
Amino acid composition (mg/100 g)*				
Alanine	1270	1255	1125	1300
Arginine	1435	1400	1315	1500
Asparagic acid	2210	2175	1890	2200
Cysteine	260	270	230	260
Glutamic acid	3545	3405	2985	3400
Glycine	965	960	895	1100
Histidine	1245	1160	855	1000
Isoleucine	955	995	930	1000
Leucine	1860	1845	1635	1800
Lysine	2110	2070	1840	2000
Methionine	675	645	565	620
Phenylalanine	1035	1020	865	910
Proline	810	770	805	920
Serine	990	925	810	940
Threonine	1100	1070	920	1100
Tryptophan	275	290	240	280
Tyrosine	820	760	685	810
Valine	1000	1055	920	1100

\* Meat samples collected from the loin. <sup>a</sup> Cloned females derived from Landrace donor cells. <sup>b</sup> F1 females from the cloned sows mated with a Landrace (L) boar. <sup>c</sup> F2 females from the F1 sows mated with a Large White (W) boar. <sup>d</sup> Loin, Fresh, Lean and Raw as listed in Standard Tables of Food Composition in Japan. Fifth Revised and Enlarged Edition. 2010. Ministry of Education, Culture, Sports, Science and Technology, Japan.

Foot-and-mouth disease (FMD) has recently appeared in many countries, particularly in East Asia. FMD is an extremely contagious disease of cloven-hoofed animals, notably in pigs. The disease is dreadful, causing economic disaster, including loss of superior genetic resources, and thus FMD is the target of international regulations and tightened trade restrictions. Cloning technology has the potential for conserving superior genetic traits, which are beneficial to the pig industry, including provision against FMD. Thus, further long-term study or more data will be required for the application of cloning to the pig industry. It is expected that, in future years, additional analysis may be conducted to elucidate the safety of products from cloned animals given the importance of cloning technology for food production.

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