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Comparison of meat composition from offspring of cloned and conventionally produced boars☆

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Abstract

This study compares the meat composition of the offspring from boars produced by somatic cell nuclear transfer ($n = 4$) to that of the offspring from conventionally produced boars ($n = 3$). In total, 89 commercial gilts were artificially inseminated and 61 progressed to term and farrowed. All of the resulting piglets were housed and raised identically under standard commercial settings and slaughtered upon reaching market weight. Loin samples were taken from each slaughtered animal and shipped offsite for meat composition analysis. In total, loin samples from 404 animals (242 from offspring of clones and 162 from controls) were analyzed for 58 different parameters generating 14,036 and 9396 data points from offspring of clones and the controls, respectively. Values for controls were used to establish a range for each parameter. Ten percent was then added to the maximum and subtracted from the minimum of the control range, and all results within this range were considered clinically irrelevant. Of the 14,036 data points from the offspring of clones, only three points were found outside the clinically irrelevant range, two of which were within the range established by the USDA National Nutrient Database for Standard Reference, Release 18, 2005; website: http://www.nal.usda.gov/fnic/foodcomp/search/. The only outlier was the presence of Eicosadienoic acid (C20:2) in one sample which is typically present in minute quantities in pork; no reference data were found regarding this fatty acid in the USDA National Nutrient Database. In conclusion, these data indicated that meat from the offspring of clones was not chemically different than meat from controls and therefore supported the case for the safety of meat from the offspring of clones.
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Keywords: Pigs; Cloning; Meat composition; Cloned offspring; Food safety

1. Introduction

Cloning by nuclear transfer has the potential to greatly enhance current agricultural practices but its application has been limited by producer observation of a voluntary moratorium until the United States Food and Drug Administration (FDA) releases a final risk assessment. The U.S. FDA released a draft executive summary for the risk assessment of food products from

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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cloned animals, which stated that meat and milk from animals produced by cloning do not present a significant risk increase over meat and milk products from animals produced by conventional methods [2]. This report was further supported by the National Academy of Sciences which stated that clones are not likely to pose a food consumption risk [3]. The FDA continues to gather information regarding the safety of products derived from cloned animals and their offspring.

Several reports have characterized the safety of products from clones. Both meat and milk from animals produced by nuclear transfer have been analyzed in a number of studies and in all instances have been shown to possess a similar composition to that from conventionally produced animals [4–8]. Furthermore, a feeding trial in rats demonstrated that the consumption of meat from cloned animals had no effect on body growth, food intake, general condition, locomotor activity, reflexes, sexual cycle, urinalysis, hematology, blood biochemistry, or histology [5,8].

Although these tests were conducted on products from cloned animals, the likelihood of human consumption of meat from clones is low. Cloning will likely be utilized to reproduce elite animals for greater dissemination of their genetics and food products will be derived from their conventionally produced offspring. As of yet, there has been no study comparing the meat composition of the offspring from individuals produced by nuclear transfer to that of the offspring of conventionally produced animals. This study was designed to address this matter. Cloned and conventionally produced boars were bred to commercial gilts and the resulting piglets were raised under commercial conditions and slaughtered at a target market weight. At slaughter, loin samples were obtained and shipped to Eurofins Scientific Inc. (ESI, Memphis, TN, USA) for compositional analysis of the meat. The composition of the meat was then compared between the offspring of clones and the controls utilizing previously described methods [9].

2. Materials and methods

2.1. General swine husbandry

Eighty-nine gilts were artificially inseminated with shipped boar semen resulting in the farrowing of 61 gilts, as previously described [10]. Many phenotypic differences were observed within the litters (Fig. 1) due to the genetic backgrounds of boars used in the study (terminal cross lines). Within 12–24 h after birth, each live pig was individually weighed, ear notched, needle teeth clipped, injectfed with 100 mg iron dextran and 300,000 units of procaine penicillin, tail docked and navel treated with iodine. Within each litter, the following characteristics were recorded: number born alive, number stillborn, and number of mummified fetuses. No differences were observed between the offspring of clones and controls [10]. Within 36 h after birth, the pigs within clone or control treatment were cross-fostered to adjust for litter size (target of 8–10 pigs/litter).

Fig. 1. A litter of offspring derived from a cloned boar.

A veterinarian observed the pigs weekly for the first 4 weeks after birth and monthly thereafter for abnormal health status and behavioral patterns. All treatments and medications were recorded for individual pigs. Any pigs that died post-weaning were necropsied to determine cause of death. No differences in the health status or mortality rates were observed between the offspring of clones or controls (data not shown).

Starting at 56–59 days of age, barrows and gilts were weighed on 28-day intervals (56–59, 84–87, 112–115, and 140–143 days of age). A slaughter weight projection was computed for a final weight of 123 kg, based on the two most recent weights. All pigs were slaughtered within ±7 days of their projected slaughter date. Animal management, including vaccinations and rations, is shown in Table 1.

2.2. Meat analysis

Following a 24-h chill at 0 °C, a sample (approximately 500 g) of Longissimus dorsi muscle anterior to the tenth rib was collected, cryovac packed, frozen and shipped overnight for nutrient analysis by Eurofins Scientific Inc. (ESI; Table 2). Once at ESI, the samples were logged in on the day of receipt and held at −13 °C until ready for homogenization. One day prior to homogenization, the samples were removed from the freezer and partially thawed overnight at 10–18 °C. The samples were then homogenized using a commercial grade meat grinder (NSF Model #MIN0012, (3/4) hp, #12 blade, (1/8) in. screen). Following homogenization,

Table 1
Management of cloned and control pigs

Day	Procedure
1 (12–24 h)	Piglets weighed, ear notched, needle teeth clipped, injected with iron dextran and 300,000 IU procaine penicillin G, tails docked, navel treated with iodine
Within 36 h of birth	Pigs within clone or control treatment were cross-fostered to adjust for litter size (target of 8–10 pigs per litter)
3–6	Vaccinated intranasally with 1cc of PRRS (Boehringer Ingelheim, St. Joseph, MO, USA)
10	23% crude protein commercial creep feed offered
12–15	Vaccinated for *Mycoplasma hyopneumonia* (Respisure, Pfizer, New York, NY, USA)
Approximately 14	Male piglets castrated
Approximately 18 (range 14–20 days)	Sows removed from farrowing stalls and piglets weaned. Pigs individually ear tagged and vaccinated with Strep Shield 2 (Novartis, Basil, Switzerland) and PRRS intramuscularly
24	Starter ration offered (21% crude protein)
28	Pigs removed from farrowing stalls to nursery. Vaccinated for *Mycoplasma hyopneumonia*
32	Nursery ration offered (20% crude protein)
35	Vaccinated with Strep Shield 2
56–59	Vaccinated for Erysipelas (Grand Labs, Larchwood, IA, USA), PRRS, and treated with Ivermectin (Durvet, Springfield, MO, USA)
66 (range 56–76 days)	Sorted by sex, moved to a single finishing building in pens of 13–20 pigs per pen, and switched to a grower ration (18% crude protein)
112–115	Switched to a finishing ration (16% crude protein)

samples were divided and frozen. One set of samples was sent to Des Moines for metals and cholesterol analysis; all remaining analysis was conducted at the Memphis location. Prior to testing, the samples were removed and allowed to thaw overnight. All samples were tested using recognized AOAC (Association of Official Analytical Chemists) methods. The specific AOAC methods utilized include amino acids profile (AOAC 982.30), metals by ICP (AOAC 965.17 and 985.01), cholesterol (AOAC 994.10), fatty acids profile (AOAC 996.06), niacin (AOAC 944.13), vitamin B12 (AOAC 952.20), and vitamin B6 (AOAC 961.15). All data were recorded as g/100 g or as percent of total with the following exceptions: niacin, vitamin B6 and cholesterol were reported in mg/100 g units and vitamin B12 was reported in μg/100 g units. At this point it was visually obvious that there was some sample-to-sample inhomogeneity, as some samples appeared to have more fat than others. No efforts were made to correct this by taking a sub-sample of the lean meat or measuring the

Table 2
List of nutrients tested in cloned and control pigs

Alanine	C10:0 Decanoic (Capric)	C20:4 Eicosatetraenoic (Arachidonic)
Arginine	C11:0 Undecanoic (Hendecanoic)	C20:5 Eicosapentaenoic
Aspartic acid	C12:0 Dodecanoic (Lauric)	C21:5 Heneicosapentaenoic
Cystine	C14:0 Tetradecanoic (Myristic)	C22:0 Docosanoic (Behenic)
Glutamic acid	C14:1 Tetradecenoic (Myristoleic)	C22:1 Docosenoic (Erucic)
Glycine	C15:0 Pentadecanoic	C22:2 Docosadienoic
Histidine	C15:1 Pentadecenoic	C22:3 Docosatrienoic
Isoleucine	C16:0 Hexadecanoic (Palmitic)	C22:4 Docosatetraenoic
Leucine	C16:1 Hexadecenoic (Palmitoleic)	C22:5 Docosapentaenoic
Lysine	C17:0 Heptadecanoic (Margaric)	C22:6 Docosahexaenoic
Methionine	C17:1 Heptadecenoic Margaroleic	C24:0 Tetracosanoic (Lignoceric)
Phenylalanine	C18:0 Octadecanoic (Stearic)	C24:1 Tetracosenoic (Nervonic)
Proline	C18:1 Octadecenoic (Oleic)	Iron
Serine	C18:2 Octadecadienoic (Linoleic)	Niacin
Threonine	C18:3 Octadecatrienoic (Linolenic)	Phosphorus
Tyrosine	C18:4 Octadecatetraenoic	Vitamin B12
Valine	C20:0 Eicosanoic (Arachidic)	Vitamin B6
Calcium	C20:1 Eicosenoic (Gadoleic)	Zinc
Cholesterol	C20:2 Eicosadienoic	
C08:0 Octanoic (Caprylic)	C20:3 Eicosatrienoic	

Table 3
Nutrient analysis results in cloned and control pigs

	Control			Offspring of clones			No. of animals (offspring of clones) within			Results
	Minimum	Maximum	Average	Minimum	Maximum	Average	Range of controls	±10% of controls	Greater than ±10%	
Alanine	1.23	1.81	1.38	1.20	1.92	1.39	237	5	0	
Arginine	1.34	2.31	1.58	1.31	2.30	1.58	241	1	0	
Aspartic acid	1.62	3.02	2.29	1.63	3.33	2.30	241	0	1	3.33 (2.30)
Cystine	0.22	0.28	0.24	0.20	0.30	0.24	238	4	0	
Glutamic acid	3.24	4.87	3.71	3.08	5.31	3.75	230	12	0	
Glycine	0.86	1.69	1.11	0.87	1.84	1.13	240	2	0	
Histidine	0.82	1.29	0.97	0.80	1.65	0.97	239	2	1	1.65 (1.09)
Isoleucine	0.76	1.33	1.03	0.73	1.58	1.03	238	3	1	1.58 (1.08)
Leucine	1.63	2.45	1.89	1.60	2.80	1.90	240	1	1	2.8 (1.91)
Lysine	1.73	2.76	2.07	1.67	3.19	2.06	239	2	1	3.19 (2.08)
Methionine	0.52	0.73	0.62	0.51	0.74	0.61	237	5	0	
Phenylalanine	0.79	1.22	0.94	0.80	1.38	0.96	241	0	1	1.38 (0.79)
Proline	0.83	1.68	1.11	0.82	1.58	1.09	241	1	0	
Serine	0.78	1.25	0.95	0.70	1.33	0.96	240	2	0	
Threonine	0.92	1.45	1.08	0.75	1.61	1.09	239	1	2	1.61 (1.18) (0.75[a])
Tyrosine	0.69	1.05	0.81	0.67	1.19	0.81	237	4	1	1.19(.81)
Valine	0.81	1.43	1.10	0.77	1.70	1.09	238	3	1	1.7 (1.16)
Calcium	0.0021	0.018	0.0054	0.0037	0.034	0.006	240	0	2	0.034 (0.0042), 0.021 (0.0045)
Cholesterol	45.6	85.1	59.4	45.4	91.1	57.9	240	2	0	
C08:0 Octanoic (Caprylic)	0.01	0.01	0.01	0.00	0.00	0.00	242	0	0	
C10:0 Decanoic (Capric)	0.01	0.02	0.01	0.01	0.02	0.01	242	0	0	
C11:0 Undecanoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C12:0 Dodecanoic (Lauric)	0.01	0.01	0.01	0.01	0.01	0.01	242	0	0	
C14:0 Tetradecanoic	0.03	0.21	0.08	0.03	0.21	0.08	242	0	0	
C14:1 Tetradecenoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C15:0 Pentadecanoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C15:1 Pentadecenoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C16:0 Hexadecanoic	0.53	3.42	1.40	0.45	3.62	1.39	240	1	1	0.45 (0.99)
C16:1 Hexadecenoic	0.06	0.35	0.16	0.06	0.38	0.17	240	2	0	
C17:0 Heptadecanoic	0.01	0.02	0.01	0.01	0.02	0.01	242	0	0	
C17:1 Heptadecenoic	0.01	0.02	0.01	0.01	0.02	0.01	242	0	0	
C18:0 Octadecanoic (Stearic)	0.21	1.75	0.68	0.21	1.77	0.66	241	1	0	
C18:1 Octadecenoic (Oleic)	0.85	4.78	2.20	0.69	5.44	2.26	238	2	2	0.69 (1.76), 5.44 (5.67[b])
C18:2 Octadecadienoic	0.08	0.80	0.29	0.06	0.92	0.30	239	0	3	0.07 (0.29), 0.06 (0.33), 0.92 (0.39)
C18:3 Octadecatrienoic	0.01	0.03	0.01	0.01	0.05	0.02	240	0	2	0.04 (0.01) 0.05 (.01)
C18:4 Octadecatetraenoic	0.01	0.02	0.01	0.01	0.01	0.01	242	0	0	

Table 3 (*Continued*)

	Control			Offspring of clones			No. of animals (offspring of clones) within			Results
	Minimum	Maximum	Average	Minimum	Maximum	Average	Range of controls	±10% of controls	Greater than ±10%	
C20:0 Eicosanoic (Arachidic)	0.01	0.03	0.01	0.01	0.03	0.01	242	0	0	
C20:1 Eicosenoic (Gadoleic)	0.01	0.26	0.07	0.02	0.22	0.08	242	0	0	
C20:2 Eicosadienoic	0.01	0.03	0.02	0.01	0.04	0.02	240	0	2	0.04 (0.06[b]), 0.04 (0.03)
C20:3 Eicosatrienoic	0.00	0.00	0.00	0.01	0.01	0.01	241	0	1	0.01 (<0.01)
C20:4 Eicosatetraenoic	0.01	0.01	0.01	0.01	0.02	0.01	241	0	1	0.02 (<.01)
C20:5 Eicosapentaenoic	0.01	0.02	0.01	0.01	0.01	0.01	242	0	0	
C21:5 Heneicosapentaenoic	0.00	0.00	0.00	0.01	0.01	0.01	242	0	0	
C22:0 Docosanoic (Behenic)	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C22:1 Docosenoic	0.01	0.04	0.02	0.01	0.04	0.01	242	0	0	
C22:2 Docosadienoic	0.01	0.01	0.01	0.00	0.00	0.00	242	0	0	
C22:3 Docosatrienoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C22:4 Docosatetraenoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C22:5 Docosapentaenoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C22:6 Docosahexaenoic	0.01	0.08	0.02	0.01	0.06	0.02	242	0	0	
C24:0 Tetracosanoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
C24:1 Tetracosenoic	0.00	0.00	0.00	0.00	0.00	0.00	242	0	0	
Iron	0.0004	0.043	0.0009	0.0004	0.0057	0.001	242	0	0	
Niacin	8.15	13.00	10.64	7.34	19.10	10.68	233	8	1	19.1 (8.78)
Phosphorus	0.02	0.22	0.16	0.02	0.72	0.18	236	5	1	0.72 (0.21)
Vitamin B12	0.35	1.88	0.97	0.42	2.20	1.01	241	0	1	2.2 (1.2)
Vitamin B6	0.22	0.59	0.38	0.23	0.60	0.40	241	1	0	
Zinc	0.0011	0.0022	0.0015	0.0012	0.0046	0.002	239	1	2	0.0025 (0.0014), 0.0046 (0.0015)

The minimum, maximum and average values for 58 parameters are shown for the offspring of clones and controls. Additionally, the number of offspring of clones that were within the control range and within or >±10% of the control range is shown. The Results column shows initial values that were outside of the ±10% range and the retested values in parenthesis. All data are presented as g/100 g or as percent of total with the following exceptions: niacin, vitamin B6 and cholesterol are presented as mg/100 g and vitamin B12 is presented as μg/100 g.

[a] This result was not retested.

[b] These results were outside of clinically irrelevant range after retesting.

total fat and normalizing for the fat content which likely would have lead to greater consistency across groups.

2.3. Data analysis

The data were analyzed as described in a publication by the FDAs Center for Veterinary Medicine [9]. Briefly results from the control animals were utilized to establish a range representing the minimum and maximum value for each analyte. All results from the offspring of clones falling within this range were considered within the norm. Ten percent was then added to the maximum and subtracted from the minimum of the control range, and all results within this range were considered outside the comparison range but clinically irrelevant. Results falling outside the ±10% range were potentially clinically relevant and further evaluated by comparison to the USDA National Nutrient Database for Standard Reference [1].

3. Results

In this study, 404 loin samples, 242 samples from the offspring of clones and 162 from the controls, were analyzed for 58 different parameters (Table 2) generating 23,432 data points. A total of 14,036 results were obtained for the offspring of the clones; 13,936 were determined to be within the range established for the controls (falling between minimum and maximum of control). Seventy-one results fell outside the control range but were within ±10% of it and were therefore not considered to be clinically relevant. The remaining 29 results fell outside ±10% of the control range (Table 3).

Twenty-eight samples corresponding to these results were submitted for retesting. One sample from pig 200437509 was not retested due to an oversight. Only two of the new results fell outside the clinically irrelevant range (Table 3).

4. Discussions

The classic approach to the compositional analysis of food is a targeted one; rather than analyzing every single constituent, which would be impractical, the aim is to analyze only those constituents most relevant to the safety of the food or that may have an impact on the overall diet. The base set of constituents commonly analyzed includes the key nutrients that may vary from food to food. Analysis of elements other than the key constituents is generally not considered necessary [11]. The question of the appropriate comparator for meats may be approached from two perspectives. In order to determine whether cloning results in potential food consumption hazards, one approach is to compare animals that are matched as closely as possible by age, genetics, husbandry and environment. The second approach is to compare meat samples from clones and their offspring to the national herds by using composite data sources [9].

Of the 14,036 data points from the offspring of clones, 29 points were initially found to be outside the control range. Twenty-eight samples corresponding to these points were retested and 26 of the new results fell within the clinically irrelevant range. One sample was not retested due to an oversight; pig 200437509 had a Threonine result of 0.75% which was outside the clinically irrelevant range of the controls (0.828–1.595%) for this study, but within the Threonine range for pork as reported in the USDA National Nutrient Database [1], in which pork samples ranged from 0.265 to 4.581% (NDB Nos. 10218 and 10048). The change in results from the 26 samples could be due to multiple factors, including the low concentration of the nutrient tested, the lack in sensitivity of the test at these lower levels, and/or inhomogeneity of the samples tested. From visual observation it was clear that some samples contained more fat than others. As no attempt was made to trim the fat to increase homogeneity, it is likely that sample-to-sample variation was high, which likely caused a high level of variation in the results.

Of the two retested samples that were outside the clinically irrelevant range, one was from pig 200438107, which had an original C18:1 Octadecenoic (Oleic) result of 5.44% and a retest value of 5.66% (Fig. 2). Both values were outside the clinically irrelevant range for the controls of 0.765–5.258% but within the range for pork loin as reported in the USDA National Nutrient Database [1]; C18:1 in pork can range from 0.130 to 23.315% of total (NDB Nos. 10020 and 10225). As stated above, this

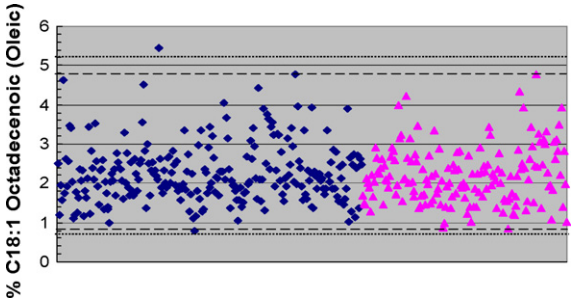

Fig. 2. Distribution of Octadecenoic acid in loin samples of offspring of cloned and control pigs. Offspring of ◆, clones; ▲, controls; — — —, denotes range of controls; · · ·, denotes range of controls ±10%; each point represents the result from an individual animal (404 total). USDA National Nutrient Database for Standard Reference, Octadecenoic acid range from 0.130 to 23.315% (NDB Nos. 10020 and 10225).

value appears to be highly correlated to the level of fat in the sample as the USDA National Nutrient Database for Standard Reference samples containing fat showed a much higher level of C18:1 than the samples lacking fat. The other retested sample was from pig 200430710 which had an original C20:2 Eicosadienoic result of 0.04% and a retest of 0.06%, which were both outside the clinically irrelevant range of the controls 0.009–0.033%. Meat by definition contains several tissue types and each varies according to the genetics, nutrition, and environment of the food animal. There are no full chemical characterizations for meats [9]. No data were found regarding expected C20:2 levels in pork, likely due to its low concentration within the samples.

These data demonstrated that meat from the offspring of cloned swine was not chemically different than meat from conventionally produced animals. Combined with previous research regarding the safety of meat and milk products derived directly from cloned animals [4–8], these results expanded the body of scientific knowledge on which governmental agencies across the world are basing their decisions regarding the approval of human consumption of food products derived from clones and their offspring.

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