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Author	Shah Ahmed Belal[1,2], Jong-Hyun Jung[3,*]
Affiliation	1 Department of Animal Biotechnology, Jeonbuk National University, Jeonju 54896, Korea 2 Department of Poultry Science, Sylhet Agricultural University, Sylhet-3100, Bangladesh 3 Executive Director, Jung P&C Institute Inc., Yongin 16951, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Shah Ahmed Belal (https://orcid.org/0000-0001-5353-2439) Jong-Hyun Jung (https://orcid.org/0000-0003-3667-7710)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jong-Hyun Jung
Email address – this is where your proofs will be sent	jhjjh7@snu.ac.kr
Secondary Email address	
Address	Executive Director, Jung P&C Institute Inc., Yongin 16951, Republic of Korea
Cell phone number	
Office phone number	
Fax number	

Abstract

Pork quality is determined by several attributes, among which odor and taste are the utmost significant. Therefore, this study was aimed to assess the effects of boar odor hormone concentration on the quality traits and sensory acceptability of pork. A total twenty-six (26) non-castrated 3-way crossbred (Landrace×Yorkshire×Duroc) pigs were selected with an average body weight (ABW) 115.6 kg before to slaughter. The three treatment groups (low, medium and high) were divided according to the androstenone concentration. In experiment 1, for meat quality traits carcass was selected based on androstenone concentration: low (LC, 0.64~0.69 μg/g, n=9), medium (MC, 0.70~0.99 μg/g, n=7) and high (HC, 1.00~1.69 μg/g, n=10). In experiment 2, for sensory evaluation carcasses were also selected based on the abovementioned conditions. Results revealed that androstenone concentration not effect on proximate components, meat quality traits and fatty acids except palmitoleic acid. Sensory evaluation data showed that boar taint and meat boar taint were significantly increased in a concentration-dependent manner from low to high, whereas, gravy and meat flavor preference were significantly increased in LC group than HC group. In addition, correlation analysis showed that boar taint and meat boar taint were positively, and gravy and meat flavor preference were negatively correlated with boar taint hormones. In essence, our findings indicate that androstenone concentration had no effect on meat qualities, but a high concentration of androstenone had a negative effect on the sensory characteristics in uncastrated pigs.

Key words: Boar taint hormone, Uncastrated pig, Meat quality traits, Sensory characteristics

Introduction

Pork is one of the most preferred meat by consumer worldwide [1]. Boar taint, a sensory off-flavor and/or offensive odor [2] connected with mainly two lipophilic compounds: androstenone and skatole (breakdown product of tryptophan) which starts to gather in the fat of sexually matured boars and creates a problem for consumers acceptability. It is a disagreeable odor or taste that is habitually apparent when cooking and/or heating of pork derived from entire male pigs [3]. This undesirable odor may be affected negatively by excess accumulation of androstenone and skatole that make obstacle for the entire male pig production [4]. In several countries, pork is only produced from barrows or gilts due to boar taint in boar [5].

To regulate boar taint, castration has conventionally applied in pig husbandry, but animal welfare concerns raised due to pain and stress during surgical castration. In past, to increase fat content pigs were castrated, because of fatter pigs were most desired to consumer as reviewed by Squires et al. [6]. However, nowadays consumer satisfactions have been shifted to lean meat to maintain their good health. Pokorná et al. [5] used a non-invasive method to examine androstenone concentration of saliva by which might improve the animal welfare in pig fattening. In welfare perspective, if castration is removed the aggressive and sexual behaviors are increases in non-castrated male pigs. Aggression may increase stress and as a result decreased immune functions, lost daily weight gain and suffered meat quality in entire males [6,7,8,9]. Moreover, in contrast entire pig production, a number of unfavorable consequences of surgical castration are higher feeding cost, reduced carcass value (due to increased fat content), more saturated fat, labor cost and reduced welfare [10].

When consumers decide the quality of pork, meat color and appearance are the most important, followed by flavor and taste. In the past, meat from uncastrated boars was known to have an unpleasant odor and taste and was treated differently from normal meat. Recently, as consumers' awareness of the importance of animal welfare has increased, especially in developed

pig farming countries, the production rate of non-castrated boar has increased, and various studies have been conducted on boar taint. In general, boars are surgically castrated within 7 days of age to improve meat quality and facilitate feeding management [11]. In pigs, boar-taint occurs mainly in non-castrated boars, and when such pork is heated, a unique flavor occurs, and in severe cases, it is so strong that it is impossible to eat. Boar taint occurs when three hormones, androstenone, skatole, and indole, accumulate excessively in the fat and lean meat [6,12,13]. On the other hand, non-castration of boars has been reported to improve feed efficiency by 9%, increase growth rate by 14%, and increase meat yield by 20% [7,14]. Since feed costs account for 60-70% of production costs in the pig farming industry, reducing feed costs is essential for reducing production costs [15].

To reduce off-odor, immunocastration or surgical castration is usually applied to male piglets in many countries. But to ensure animal welfare issue most European countries have imposed a voluntary ban on this method [16]. Ban of surgical castration without anaesthesia became a frequent topic [17]. Environmental condition and diet manipulation could be associated with genetic selection [18] and genomic selection seems to be beneficial to reduce boar taint [19]. Previous literatures also showed that a number of long-term (genetics) and short-term strategies (management practices) have been executed to control boar taint, but these can have inconsistent results and much variability. Therefore, in addition to the animal welfare aspect, a non-castrated boar production method is very necessary from an economic point of view. This study was conducted to present basic data for the production of non-castrated boars by analyzing the meat quality and sensory characteristics according to androstenone concentrations in non-castrated boars.

Materials and Methods

Ethics statement

This study was approved by the Animal Ethics Committee of Jeonbuk National University (approval number: CBNU2018-097). All the experiments were conducted following the guidelines and regulations of Jeonbuk National University.

Animals

A fat biopsy was collected by the method of Baes et al. [20] from 26 non-castrated 3-way crossbred (Landrace×Yorkshire×Duroc) pigs with an average body weight of 115.6 kg prior to slaughter. The biopsy instrument was used to extract the fat of a living pig. Boston butts (*M. subscapularis*) were collected from the slaughterhouses. All pigs were raised under uniform housing conditions and fed ad libitum commercial diet.

Experimental design

In experiment 1, to analysis the meat quality traits three treatment groups were divided according to the androstenone concentration based on the research of Meier-Dinkel et al. [21]: low concentration (LC, 0.64~0.69 μg/g, n=9), medium concentration (MC, 0.70~0.99 μg/g, n=7) and high concentration (HC, 1.00~1.69 μg/g, n=10). In experiment 2, for sensory evaluation carcasses were also selected based on the abovementioned conditions.

Analysis of boar taint compounds

Concentration of boar taint compounds were measured by GC-MS from fat samples. Pure fat sample in the vial without muscle and skin was melted in microwave oven (700 W) for 1 min. Liquid fat of 0.2 g was taken, transferred at centrifuge tube (2 mL) and mixed with 0.8 mL of reagent (methanol:n-hexane, 9:1, v/v). The centrifuge tubes were vortex mixed for 1 min, and incubated at ultrasonic wave incubation at 50℃ for 40 min with vortex mix every 5 min. After incubation, samples were cooled at room temperature and centrifuged at 15,000 rpm for 30 min and supernatant was collected into injection vial for boar taint compound analysis.

The GC-MS system from Shimadzu (GC-MS, QP-2010, Kyoto, Japan) and column was Rtc-5Sil MS column (30 m x 0.25 mm, 0.25 μm, film thickness, Restex, USA). During whole

analyze times, inject was split mode (split ratio, 10:1) at 300℃ and inject volume was 1 μL. The oven temperature was programed at 40℃ for 1 min, increased the temperature of 10℃/min to 250℃ (5 min hold), and then again increased 15℃/min to 300℃ and hold 10 min. The ionization performed using electron impact ionization (EI) method at voltage 70 eV and the ion source temperature was 260℃. A scan range of m/z 40-550 was chosen and MS spectrum was referred to the Wiley library. In order to analyze the correct BTCs, qualification was performed using selective ion monitoring (SIM) mode with m/z 257 (androstenone), 130 (skatole) and 117 (indole).

Proximate component

Proximate component analysis was performed according to AOAC [22], moisture content was determined by oven drying method, fat was partially modified from Folch et al. [23] was used, and the raw material was incinerated at 550° C for 5 hours in an incinerator (FPX-14, HANIL, Korea), then the content was measured and the results were expressed as the percentage (%).

Meat quality

The meat color (*L**, *a**, and *b**) per sample were taken at three different locations on the bloomed cut surfaces of the sample blocks was measured using a Minolta chromameter (CR-410, Minolta Co., Ltd., Japan) calibrated by a black-and-white calibration plate. The color results were reported as CIE *L**, *a**, and *b** (Lightness, Redness and Yellowness), analyzed by Spectra Magic Software (Minolta Co., Ltd., Japan).

For pH measurement, 2 g of meat sample was homogenized with 18 mL of distilled water at 11,000 rpm for 1 minute using a homogenizer (Polytron PT 10-35 GT, Kinematica AG, Luzern, Switzerland). The filtrate of each sample was filtered with filter paper (followed by Whatman No. 4) and measured with a pH meter (Seven Excellence™, METTLER TOLEDO, Switzerland) at room temperature.

To measure the water holding capacity (WHC), 5 g of the pulverized sample is placed in a 50 mL tube with a filter with pores, and the weight of the sample is measured after centrifugation by centrifugation (Combi-514R, HANIL, Korea) at 1,000 rpm at 5°C for 10 minutes. Thus, the value was calculated by the formula: [(weight of total sample (g) - weight of free water (g))/weight of total sample (g) × 100].

Cooking loss was measured by the method of Honikel [24]. Samples were weighed and put in a plastic bag, which was placed in an 80°C water bath until the internal temperature reached 75°C. When this temperature was reached, the samples were cooled and weighed again. The difference in weight before and after boiling was expressed as percentage cooking loss.

The Warner-Bratzler shear force (WBSF) was determined by taking meat sample blocks using an Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, MA). Meat samples were cooked and cooled before measurement of WBSF parallel to the muscle fiber direction. Samples were cut into a size of 30×50×10 mm, heat it to a core temperature of 75°C (LLOYD instruments, UK) was equipped with a Warner-Bratzler blade and the shear force (unit: kgf) was measured while the muscle texture of the sample was perpendicular to the blade. The speed was 2.0 mm/s and the post-test speed were 5.0 mm/s.

Fatty acid composition analysis

The fatty acids composition of porcine M. subscapularis muscle was estimated by the method of [25], with a minor modification. The assay was performed using a Gas Chromatograph-Flame Ionization Detector (7890 series, Agilent, Santa Clara, CA, USA) under the following conditions: injector split mode with split ratio of 25:1, temperature 250℃. High purity air, high purity H2, and high purity He were used as carrier gases. The flow rate was maintained at 40 mL/min for H2 and 400 mL/min for air. An HP-88 column (60 m×250 μm×0.2 mm) was used for the analysis. Fatty acid composition is expressed as a percentage.

Sensory attributes evaluation of pork by trained sensory panelists

The use of trained persons as instruments for the evaluation of sensory properties of pork is a common practice. To conduct sensory evaluation, three specific areas have to be considered: 1) panel conditions used for sensory evaluation; 2) training and selection of sensory panelists; and 3) the step and structure of evaluation that will be used to address sensory characteristics in pork. For the sensory test, the longest abdominal muscle containing back fat was heated to a core temperature of 75 ℃ under the same cooking conditions, and a certain amount of the sample was presented to the sensory evaluator. For the evaluation of the sample, a 9-point scale was used based on the 5-point scale of uncastrated pigs, and broth boar odor (1 point = very weak, 9 points = very strong), broth flavor preference (1 point = very bad, 9 points = very good), meat odor (1 point = very weak, 9 points = very strong), and meat flavor preference (1 point = very bad, 9 points very good).

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) followed by the Duncan's multiple range test procedure by SAS software (SAS version 9.3, USA). All data were presented as mean ± standard error (SE). Statistical significance was set at $p < 0.01$ and $p < 0.05$.

Results

Boar taint compounds

Table 1 showed the concentrations of boat taint compounds based on treatment groups. The androstenone, skatole and indole concentrations ranged from 0.610 to 1.685 μg/g, from 0.081 to 0.092 μg/g, and from 0.043 to 0.074 μg/g, respectively. Various studies have been conducted to determine the minimum detectable concentrations of androstenone and skatole in the boat taint compounds. Bonneau and Chevillon [26] reported that the minimum androstenone concentration detectable by humans is around 2-3 μg/g in fat. In another study, Xue et al. [27] suggested that androstenedione and skatole concentrations should be at least 1.5 μg/g and 0.25 μg/g or higher

for detection. Bañón et al. [28] also stated that people can detect androstenone and skatole concentrations of at least 0.5 μg/g and 0.1 μg/g, respectively, in heat-treated loin meat.

Proximate compositions

Table 2 presents the results of proximate compositions. Results revealed that boar taint hormone concentration not effect on proximate components. Moisture content refers to the content of fixed and free water present in meat, and these moisture contents have a close relationship with the WHC of meat and juiciness when chewing meat, depending on the degree of electrical bonding between proteins and water molecules in the meat. Meat have fat, along with carbohydrates and proteins, is one of the main nutritional components of food, and gives food a unique rich flavor and texture.

Meat quality

Table 3 showed meat quality parameters such as color, pH, WHC, cooking loss and shear force. It was observed that no significant differences in the meat qualities according to the concentration of androstenone. However, pH and WHC were increased in the LC group than other two groups without any significant.

Fatty acid composition

The results of fatty acid compositions are presented in Table 4. Palmitoleic acid (C16:1), which is one of the major fatty acid of monounsaturated fatty acids (MUFA) was significantly higher in LC and MC groups than HC group. In addition, polyunsaturated fatty acid (PUFA) is lower in LC group than HC group without any significant differences.

Sensory evaluation

Comparison on sensory properties of pork between LC, MC and HC groups is shown in Table 5. Data showed that boar taint and meat boar taint were significantly increased in a concentration-dependent manner from low to high, whereas, gravy and meat flavor preference were significantly increased in LC group than HC group.

Correlation index between boar taint hormones and sensory characteristics

The analysis of the correlation index between androstenone, indole, and skatole, which are boar odor hormones, and the sensory characteristics of pork are presented in Table 6. In the primary sensory evaluation, boar odor felt when eating broth and meat, and androsterone, indole, and skatole in pork showed a positive (+) correlation. A negative (-) correlation was shown in the preference for gravy and meat flavor preference.

Discussion

In this study, androstenone concentrations not affect the general compositions and meat quality traits of pork. It is known that pH has a high correlation with meat qualities such as meat color and WHC. Generally, the meat pH is related to WHC, and the higher pH, the higher WHC. Some previous studies showed that better pH values [29], higher WHC [30] and lower PSE [31] in entire male pigs when compared to the castrated pig. Cooking loss refers to the degree to which meat drips are released during the heating stage. The age of meat can be changed by complex effects such as the amount of moisture and fat, the amount and chemical state of connective tissue, and whether or not actomyosin is formed. Our results indicate that a high concentration of androstenone slightly effects on quality traits of meat in uncastrated pigs because it accumulates in the fat and when heated up, androstenone becomes volatile and can be detected in the cooked pork.

Individual fatty acid plays a significant role not only in the taste, but also in the formation of flavor by decomposing fat during cooking or creating volatile substances in meat. In addition, fatty acids affect the color, hardness, and storability of meat, and excessive intake of saturated fatty acids is known to cause heart disease. The foremost MUFA of palmitoleic acid (C18:1) showed a significantly higher content at low and medium concentrations than at high concentrations. In addition, PUFA is lower in LC group than HC group without showing any

228 significant. The previous study Mörlein & Tholen [16] explored that extremely low androstenone
229 containing boars had lower SFA and higher PUFA in subcutaneous adipose tissue. However,
230 these results are totally opposite from our study. As the most abundant MUFA in meat, oleic acid,
231 which is known to differentially affect the flavor of meat, showed an average content of about 37
232 to 39%, and have no significant differences between the treatment groups. Cameron & Enser
233 [32] reported that a higher MUFA concentration and a lower PUFA concentration had a positive
234 effect on the taste of meat. Also, among PUFA, there was a study result that linoleic acid (18:2)
235 was affected by the type of feed rather than the variety, and that the higher concentration,
236 negatively affected the flavor of meat [33]. In this study, the MUFA were low, the PUFA were
237 high, and the concentration of linoleic acid (18:2) was high in the HC group when compared to
238 the LC and MC groups. It is known that the fatty acid content differs slightly by breed and that
239 there is a difference in the content due to feed ingredients during breeding rather than breeding.
240 During oxidative processes, the flavor is affected by fatty acids, which is well documented.
241 Literature also showed that higher concentrations of n-3 PUFA are accountable for the
242 production of some lipid degradation products, mainly aroma active aldehydes in meat [34].
243 Specifically, the aldehyde is responsible for off-flavors in food [35].

244 In sensory evaluation, flavor (scent) is evaluated as a sense that is felt by combining the
245 stimuli of taste felt by the tongue and smell felt by the nose. As a result of comparing the sensory
246 evaluation (which revealed that it was the subjective evaluation of the sensory evaluation
247 personnel who participated in this experiment) conducted twice on pork, the difference between
248 high and medium concentrations was not significant, but at low concentrations it was clearly
249 found that there was a difference. At high concentration, the boar taint of the broth was stronger
250 and the flavor preference of the broth was lower than that of the low concentration treatment
251 group. Since these results are the result of limited evaluation personnel, additional consumer

252 investigation is necessary, and it is judged that more specific experimental studies are needed on
253 the direct causes (flavor substances such as free amino acids, nucleic acids, etc.).

254 Correlation index between sensory attributes (trained panel) and boar taint compounds
255 (analytical values) were significantly correlated in pork (Table 6). Boar taint and meat boar taint
256 assessed by the trained panel were significantly correlated along with androstenone, indole and
257 skatole levels. The boar taint attribute and analytically evaluated androstenone, indole and
258 skatole levels were significantly correlated (r = 0.356, 0.574 and 0.583, respectively). On the
259 other hand, gravy flavor and meat flavor attributes were negatively correlated with androstenone
260 (r = -0.521 and -0.528, respectively), indole (r = -0.633 and -0.740, respectively) and skatole (r =
261 -0.642 and -0.748, respectively) levels. Other previous studies also exposed that boar taint and
262 both androstenone (ranges 0.42 to 0.60) and skatole (ranges 0.46 to 0.83) contents were
263 significantly correlated [36,37,38,39]. These findings are supported our hypothesis. Several
264 researchers also acknowledged that the excess accumulation of boar taint hormones
265 (androstenone and skatole) are the potential causes for decreased consumer preference of boar
266 meat. Besides, some short chain fatty acids and other compounds (16-androstenone steroids, 4-
267 ethyphenol, p-cresol) are also intricated with sensory boar taint.

268 In conclusion, androstenone concentration had no effect on proximate components and meat
269 quality traits but sensory properties like boar taint and meat boar taint were significantly
270 increased in HC, whereas, gravy and meat flavor preference were significantly increased in LC
271 group. Our data indicate that a high concentration of androstenone has a negative effect on the
272 sensory characteristics in uncastrated pigs and could create complications for consumer
273 preferences and commercial pig production. However further research will warrant this
274 association.

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Table 1. Least-square means and standard error of boar taint compounds concentration of porcine *M. subscapulari*s muscle for the three treatments

Boar taint Compound (μg/g)	LC (n=9)	MC (n=7)	HC (n=10)	Total (n=26)
Androstenone	0.651^{c} ±0.046	0.844^{b} ±0.114	1.426^{a} ±0.263	1.035±0.469
Skatole	0.086±0.046	0.086±0.046	0.089±0.026	0.087±0.036
Indole	0.046±0.027	0.053±0.024	0.058±0.019	0.055±0.014

LC: low concentration; MC: medium concentration; HC: high concentration

Means in the same row with different letter are statistically significant at 10% of significance

Table 2. Effects of androstenone concentration on proximate composition of porcine *M. subscapularis* muscle

Proximate component (%)	LC (n=9)	MC (n=7)	HC (n=10)	SEM[1]
Moisture	75.38	74.88	75.22	0.16
Crude protein	23.65	24.44	23.8	0.19
Fat	1.64	2.02	1.81	0.14
Crude Ash	1.14	1.13	1.11	0.01

LC: low concentration; MC: medium concentration; HC: high concentration

[1]SEM: standard error of the means.

Table 3. Effects of androstenone concentration on meat quality traits of porcine *M. subscapularis* muscle

Color	LC (n=9)	MC (n=7)	HC (n=10)	SEM[1]
CIE L* (Lightness)	56.43	56.10	53.10	1.22
CIE a* (Redness)	15.81	16.81	16.83	0.36
CIE b* (Yellowness)	6.10	6.70	5.78	0.47
Other quality trait				
pH (24-hour)	5.56	5.57	5.75	0.11
WHC (%)	69.67	69.5	73.73	2.04
Cooking loss (%)	24.08	22.3	20.83	1.03
Shearing force (Kgf)	4.92	5.79	4.93	0.55

LC: low concentration; MC: medium concentration; HC: high concentration

[1]SEM: standard error of the means.

480 **Table 4.** Effects of androstenone concentration on fatty acid compositions (% of total fatty acids)

481 of porcine *M. subscapularis* muscle

Fatty acid (%)	LC (n=9)	MC (n=7)	HC (n=10)	SEM[1]
C10:0 (Decanoic)	0.11	0.11	0.09	0.00
C12:0 (myristic)	0.10	0.10	0.10	0.00
C14:0 (myristic)	1.29	1.36	1.26	0.06
C16:0 (palmitic)	21.37	21.74	21.15	0.40
C16:1 (palmitoleic)	2.75[a]	2.84[a]	2.35[b]	0.13
C18:0 (stearic)	11.15	10.78	11.43	0.26
C18:1n9c (oleic)	38.81	39.94	37.93	0.87
C18:2n6c (linoleic)	14.90	14.21	15.85	0.66
C18:3n6 (gamma-linolenic)	0.58	0.63	0.62	0.02
C20:2 (eicosadienoic)	0.36	0.37	0.41	0.02
C20:3 (Dihomo-γ-linolenic)	0.47	0.43	0.47	0.03
C20:4 (arachidonic)	3.45	3.26	3.58	0.30
C20:5n3 (eicosapentaenoic)	0.11	0.10	0.11	0.01
C22:6 (Cervonic)	0.10	0.11	0.10	0.02
C24:1 (Nervonic)	0.60	0.52	0.59	0.05
ΣSFA	34.01	34.08	34.02	0.64
ΣUFA	62.12	62.39	62.00	0.50
ΣMUFA	42.15	43.30	40.86	0.95
ΣPUFA	19.97	19.09	21.14	1.01
ΣUFA/SFA	1.83	1.84	1.83	0.04
Σn-6/n-3	14.84	14.11	15.21	0.45

482 SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty

483 acid; USFA, unsaturated fatty acid; ω6, omega-6 fatty acid; ω3, omega-3 fatty acid.

484 Values in a row with different superscript letters are significantly different (p <0.05). Lack of

485 superscript letters indicates no significant difference.

486 LC: low concentration; MC: medium concentration; HC: high concentration

487 [1]SEM: standard error of the means.

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Table 5. Comparison of sensory evaluation of pork at different androstenone concentrations of porcine *M. subscapulari*s muscle

	LC (n=9)	MC (n=7)	HC (n=10)
Boar taint[1]	5.29^{b}±0.25	5.47^{ab}±0.13	5.65^{a}±0.14
Gravy flavor preference[2]	4.55^{a}±0.10	4.29^{b}±0.08	4.16^{b}±0.03
Meat boar taint[1]	5.41^{b}±0.05	5.57^{ab}±0.08	5.69^{a}±0.04
Meat flavor preference[2]	4.59^{a}±0.03	4.47^{ab}±0.01	4.20^{b}±0.01

LC: low concentration; MC: medium concentration; HC: high concentration

The results are represented as the mean±SE. ^{a,b} means with different superscripts differ (p <0.05).

[1)] Boar odor attributes are scored on a 9-point scale where1 point very weak ~ 5 points (control) ~ 9 points very strong.

[2)] Flavor preference attributes are scored on a 9-point scale where 1 point very bad - 5 points (control) - 9 points very good.

Table 6. Correlation index between boar taint compounds and sensory attributes of porcine *M. subscapularis* muscle

	Androstenone	**Indole**	**Skatole**
Boar taint	0.356*	0.574*	0.583**
Gravy flavor preference	-0.521*	-0.633*	-0.642**
Meat boar taint	0.447*	0.595**	0.603**
Meat flavor preference	-0.528**	-0.740**	-0.748**

$^{**}p$ <0.01, $^{*}p$ <0.05 significant