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Effects of ractopamine administration and castration method on the response to preslaughter stress and carcass and meat quality in pigs of two Piétrain genotypes[1]

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ABSTRACT: The objective of this study was to evaluate the effects of ractopamine supplementation, castration method, and their interaction on the behavioral and physiological response to preslaughter stress and carcass and meat quality of 2 Piétrain genotypes. A total of 1,488 male pigs (115 ± 5 kg BW) were distributed according to a 2 × 2 × 2 factorial arrangement of treatments. The first factor was ractopamine supplementation with 2 groups of pigs (376 and 380 pigs each) receiving 7.5 mg/kg of ractopamine (RAC) or not (NRAC) in their diet during the last 28 d of the finishing period. The second factor was castration method, with 744 surgical castrates (SC) and 744 immunized males (IM), and the third factor was the genotype with 2 crossbreeds containing 50% (genotype A, GA; n = 744) or 25% (genotype B, GB; n = 744) Piétrain genetics. Surgical castration took place at 2 d of age, whereas immunization against gonadotropin-releasing factor (GnRF) was performed through 2 subcutaneous injections of GnRF analog (Improvest, 2 mL) at 10 and 4 wk before slaughter. At loading more vocal stimulation was needed by the handler to drive GB pigs forward through the farm alley (P = 0.01) and RAC-fed GB pigs through the ramp (P = 0.02). Feeding RAC to IM increased the number of fights in lairage compared with SC (P = 0.03). Feeding RAC shortened fighting bouts compared with NRAC pigs (P = 0.05). The SC-GA pigs showed a greater gastrointestinal tract temperature during unloading (P = 0.05) and lairage time (P = 0.03). Blood creatine kinase (CK) concentrations were greater (P = 0.04) in SC compared with IM, and no difference was found in the concentrations of stress hormones in urine collected postmortem. Dressing yield was greater (P = 0.01) in RAC and SC-GB pigs. Carcasses from RAC pigs and IM were leaner than those from NRAC and SC pigs (P < 0.001 and P = 0.002, respectively). Feeding RAC to IM increased drip loss in the LM (P = 0.05). Warner-Bratzler shear force values were slightly greater in the LM from RAC-GB pigs and from IM compared with SC (P = 0.01 and P < 0.001, respectively) and in the semimembranosus muscle of RAC pigs (P = 0.006). In conclusion, immunization against GnRF more than the use of Piétrain genotypes appears to be a viable alternative to the use of ractopamine, as it seems to promote production of lean carcasses without compromising animal welfare and pork quality.

Key words: castration, genotype, meat quality, pigs, ractopamine, stress

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INTRODUCTION

Ractopamine (**RAC**) is a β-adrenergic agonist that, when fed to pigs, increases growth rate and carcass leanness and produces acceptable pork quality (Schinckel et al., 2001; Patience et al., 2009). However, there is evidence that RAC feeding also increases stress responsiveness in pigs, as shown by rapid heart rate and increased blood catecholamines concentrations during transport (Marchant-Forde et al., 2003), and aggressiveness in mixed groups (Poletto et al., 2010). These behavioral and physiological responses may eventually result in more animal losses and carcass condemnations (Patience et al., 2009). The use of pigs of Piétrain genetics and immunized males (**IM**) can be valid alternatives to RAC feeding. After the eradication of the Hal gene, Piétrain genotypes became more resistant to stress but maintained superior carcass quality (Fàbrega et al., 2004; Gispert et al., 2007). Immunization against gonadotropin-releasing factor (**GnRF**), through the injection of a GnRF analog (Improvest; Pfizer Animal Health, Pointe Claire, Canada) a few weeks before slaughter, proved to be a valid alternative to the painful practice of surgical castration in terms of reduced risk of boar taint in cooked pork (Font i Furnols et al., 2008) and maintained the growth performance of boars until a few weeks before slaughter (Fàbrega et al., 2010). Fàbrega et al. (2010) showed a potential for immunization against GnRF to reduce aggression and mounting behavior in the finishing pen at the farm. However, this observation was obtained in unmixed groups of pigs. No evidence of the response of IM to mixing with unfamiliar conspecifics and to other preslaughter procedures, such as handling and transportation, exists. Therefore, the objective of this study was to evaluate the effects of RAC administration, Piétrain genotype, and immunization against GnRF as single factors or in interaction on the behavioral and physiological response to preslaughter stress and carcass and meat quality in pigs.

MATERIAL AND METHODS

All experimental procedures performed in this study were approved by the institutional animal care committee on the basis of the current guidelines of the Canadian Council on Animal Care (2009).

Animals and Treatments

This study was conducted at a commercial swine growing-finishing farm and at a commercial slaughter plant in eastern Canada. A total of 1,488 pigs (115 ± 5 kg BW) were distributed according to a 2 × 2 × 2 factorial arrangement of treatments. The first factor was RAC supplementation (Paylean; Elanco Animal Health, Guelph, Canada) with 2 groups of pigs (744 pigs each) receiving 7.5 mg/kg of RAC or not (**NRAC**) in the diet during the last 28 d of the finishing period. The second factor was castration method with 744 surgical castrates (**SC**) and 744 IM, and the third factor was the genotype with 2 crossbreeds containing 25% (genotype A, **GA**; $n = 744$) or 50% (genotype B, **GB**; $n = 744$) of Piétrain genetics. Pigs were progenies from crosses of Piétrain purebred and Duroc × Piétrain crossbred sire lines with commercial hybrid sows (F-20 developed by Genetiporc Inc., St.-Bernard, Canada) resulting in crossbreeds containing 25% and 50% Piétrain genetics, respectively. Piétrain sire lines were free of the halothane gene (**Hal^N**). Surgical castration took place at 2 d of age, whereas immunization against GnRF was performed through 2 subcutaneous injections (2 mL per animal) of GnRF analog (Improvest; Pfizer Animal Health) at 10 and 4 wk before slaughter. Within each treatment combination, pigs were raised in finishing pens of 15 pigs in the first phase (February to May 2010) and 16 pigs in the second phase (May to August 2010) in terms of 6 pens per phase, each at a density of 0.79 and 0.74 m^2/pig, respectively. Pigs were shipped to slaughter over 6 wk (3 wk in May and 3 wk in August), with a slaughter day or week being the replicate (6 replicates). On the day before the shipment, 5 pigs were randomly chosen from 2 pens/treatment combination for the study of the physiological response (total of 30 pigs per treatment combination). These pigs plus 2 others randomly selected from 2 pens/treatment combination were also used for the meat quality assessment (7 pigs per treatment combination and per replicate). Feed was withdrawn for 10 h before loading and a total of 13 h before slaughter. At loading, pigs from 2 different pens within the same treatment combination were mixed in the alley to form a new group of pigs with a size ranging from 13 to 17 pigs per group depending on the size of the truck compartment. Each treatment group was then kept intact until slaughter. This procedure allowed the observation of the full expression of social behavior of mixed unfamiliar pigs in the lairage pen. Pigs were loaded onto a pot-belly trailer in groups of 5 pigs using paddles and boards. The driver of the trailer and the handler at the farm were the same throughout the 6 wk. In the trailer, pigs were distributed into 8 separate compartments in terms of 1 compartment per treatment combination, at an average loading density of 0.43 m^2/pig. A rotation of the group position in the trailer according to the treatment was done at every load to avoid the confounding effect of the truck compartment on the response of the pigs to transport.

After 45-min transportation, pigs were unloaded at the plant using a whip only and driven to separate lairage

Table 1. Ethogram of pig behavior during loading and unloading (Weschenfelder et al., 2012)

Pig behavior	Description
Slip/fall	Leg of pig splits away from the other legs or pig falls down (at least 2 legs buckled under)
Overlap	Pig mounts another pig, with its 2 front legs on the back of the other pig
180° turn	Pig makes a 180° turn, ending with its rear extended in the direction of intended movement
Back up	Pig moves at least 2 steps rearward, opposite the direction of intended motion
Backward	Pig moves in the intended direction with its body oriented in the opposite direction
Underlap	Head of pig goes under the body of another pig
Vocalize	Pig vocalizes
Balk	Pig refuses to walk or stops for more than 2 s
Squeeze	Pig is squeezed at the door, corridor, or the exit of the ramp (or at the trailer door, when unloading)

Table 2. Handler behavior towards pigs during loading and unloading

Handler behavior	Description
Vocal sound	Handler uses his voice to encourage forward movement of 1 or a group of pigs
Rattle noise	Handler uses the paddle to produce noise (one time)
Physical intervention	Handler uses his hands, paddle, or board (or whip at unloading) to push and encourage forward movement of 1 or a group of pigs

pens on the basis of the treatment group (no mixing). In lairage, pigs were kept at a stocking density of 0.43 m^2/pig per pen for 120 min. At the end of the lairage period, pigs were electrically stunned (head-to-chest electrical stunning) and exsanguinated in the prone position.

Behavioral Observations

Behavior at Loading. Behavior of pigs was recorded using 3 digital camcorders (DCR-HC48; Sony of Canada Ltd., Toronto, Canada) installed using 3 camera mounts overhead of the loading ramp and the alley at the loading ramp. The cameras recorded all occurrences of pig behavior (Table 1) from a predetermined start gate at the farm alley to the trailer gate. The course was divided into 2 zones: zone 1 was the alley from the start gate to the farm door, and zone 2 was between the farm door and the end of the external ramp (trailer door). With the frequency of the behaviors observed being relatively low, data were calculated as the percentage of loading groups that showed the behavior in at least 1 of the 2 zones. Video recordings were watched in real time by a trained observer using a handheld Psion Workabout (HC-110; Psion Inc., Mississauga, Canada) computer. The total time taken to move pigs from the starting gate through the door of the trailer and the occurrences of manipulator interventions needed to move the pigs (Table 2) were noted.

Behavior during Lairage. Behavior during lairage was recorded using video cameras (WV-BP50; Panasonic Canada Inc., Mississauga, Canada) installed overhead of the pens and connected to a digital encoder (Nextiva S5712e; Verint, Melville, NY). Images were captured and recorded by the Omnicast system (version 4.0; Genetec Inc., Montreal, Canada) at a frequency of 5 to 7 images/s. Scan sampling was used at 2-min intervals during the first hour of lairage to determine the number of pigs lying, sitting, and standing. Aggressive behaviors were observed continuously during the first hour of lairage and counted as number of fights and fighting duration. It was considered a fight when 2 or more animals performed a sequence of these behaviors: biting, head knocking, pushing, and shoving the other, with contacts lasting more than 3 s and no greater intervals than 10 s between attacks (Pitts et al., 2000; D'Eath, 2002).

Physiological Measurements

Gastrointestinal Tract Temperature. Approximately 8 h before loading, a total of 240 pigs (40/wk) were orally administered Thermocroni-Button data loggers (model DS1921H; Dallas Semiconductor, Maxim Integrated Products, Sunnyvale, CA) to monitor gastrointestinal tract (**GIT**) temperature using a snare, a heavy gauge metal "pig gag," and balling gun. Each data logger was programmed to begin recording from 1 h before loading until slaughter and to log temperature once per minute. The GIT temperature was measured in 0.125°C increments with ±1°C accuracy in the range of 15°C to 46°C. Data loggers of selected pigs were recovered after gross dissection of the viscera during the slaughter process. Later, data from 135 pigs (56.2% recovery rate) were downloaded onto a laptop computer. The delta GIT temperatures values within treatments were obtained by the difference between the measured GIT temperature at any determined event and the GIT temperature measured at rest (basal level).

Blood Metabolites. At exsanguination, 10 mL of blood were collected in a tube (BD Vacutainers; VWR International Ltd., Montreal, Canada) to extract the serum for creatine kinase (**CK**) analysis. Another 2 mL of blood were collected in a tube containing 3.0 mg of sodium fluoride and 6.0 mg of Na_2EDTA solution to extract plasma for lactate analysis. The 2-mL blood tubes were immediately centrifuged at 4°C for 12 min at 1,400 × *g*. The plasma was transferred into 1.5-mL Eppendorf tubes and stored at −80°C until lactate determination. Serum samples were kept at room temperature (~23°C) for 1 h

before refrigeration at 4°C. The next day, serum samples were centrifuged at 4°C for 12 min at 1,400 × *g*, transferred to Eppendorf tubes (1.5 mL), and stored at −80°C until analysis. Lactate concentrations were measured using a commercially available kit (Lactate Assay Kit, Biomedical Research Service Center, University of Buffalo, Buffalo, NY), and CK concentrations were measured with a creatine kinase-sl kit (Creatine Kinase-SL Assay of Chemicals Diagnostic Limited, Vancouver, Canada). Plasma lactate concentrations were determined with a microplate reader, and serum CK concentrations were determined with a spectrophotometer. The intra-assay CV was 8.38% and 8.68% for CK and lactate, respectively.

Urinary Hormones. Urine samples were collected directly on the slaughter line from the bladders of the 239 sentinel pigs. After the addition of HCl 6 *M* (1% of urine volume) as a preservative, samples were immediately frozen (−45°C) pending analysis of urinary free corticoids (cortisol and cortisone) and catecholamines (**CA**), such as norepinephrine, epinephrine, and dopamine. Cortisol and cortisone were assayed using a solid-phase extraction procedure on C18 cartridges followed by HPLC (Agilent Technologies, Massy, France) with UV absorbance detection (254 nm) as described by Hay and Mormède (1997a). Limits of detection, defined by the capacity of the computing integrator to discriminate between the baseline noise and peaks, were approximately 2 ng present in the urine sample for glucocorticoids. Catecholamines (norepinephrine, epinephrine, and dopamine) were assayed using an ion-exchange purification procedure followed by HPLC (Agilent Technologies, Massy, France) with electrochemical detection, as described previously (Hay and Mormède, 1997b). Limits of detection for catecholamines were approximately 12.8 ng present in the urine sample. Concentrations of hormones in urine were expressed as their ratio to creatinine content (ng:mg creatinine) to correct for the variable dilution of urine. Creatinine concentration was measured by a colorimetric quantitative method (procedure 500, Sigma Diagnostics, Saint-Quentin-Fallavier, France) to correct for urine dilution.

Carcass Quality Measurements

After slaughter, carcasses were eviscerated, split, and chilled according to standard commercial practices. Hot carcass weight and carcass lean percentage (by Destron probe) were recorded, and HCW was used to calculate dressing percentage.

Skin damage was assessed on the day of slaughter in the cooler using the 5-point photographic scale (1 = none to 5 = severe; MLC, 1985), whereas bruises were classified as fighting-type bruises (1 = fewer than 10 bruises, 2 = 11 to 20 bruises, and 3 = more than 20 bruises) or mounting-type bruises (1 = fewer than 5 bruises, 2 = 6 to 10 bruises, and 3 = more than 10 bruises) by visual assessment of shape and size according to the photographic standards of the Institut Technique du Porc (**ITP**, 1996) as described by Faucitano (2001). According to the ITP scale, bruises due to biting during fighting are recognized as being 5 to 10 cm in length, comma shaped, and concentrated in high number in the anterior (head and shoulders) and posterior (ham) regions of the carcass. Long (10 to 15 cm), thin (0.5 to 1 cm wide), comma-shaped bruises densely concentrated on the back of pigs caused by the fore claws were classified as mounting-type bruises. Lacerations and scratches normally produced when pigs are handled aggressively and run in closed and tight spaces were also noted and classified as "other-type bruises."

To distinguish between bruises inflicted at the farm and in the antemortem period, bruises were also classified by the age of infliction based on the color change, in terms of "red," corresponding to a fresh bruise (occurred within 10 h), and "dark" (occurred more than 24 h before) for an old bruise as described by Gracey and Collins (1992) and Strappini et al. (2012). As there is evidence that the typical boar behavior is maintained until the second vaccine injection (Fàbrega et al., 2010), this measurement particularly provides indirect information on the difference in social behavior of boars before and after mixing before transport.

Meat Quality Measurements

Meat quality was assessed on 336 carcasses (7/group) by measuring pH at 24 h postmortem (**pHu**) in the LM (at the third/fourth last rib level) and in the semimembranosus (**SM**) and adductor (**AD**) muscles using a pH meter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted with a Cole Parmer spear-type electrode (Cole Palmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation (**ATC**) probe. At 24 h postmortem, these measurements were also taken in the LM and SM muscles: light reflectance by a Minolta Chromameter CR 300 (D65 light source with 0° viewing angle geometry; Minolta Canada, Inc., Mississauga, Canada) according to the reflectance coordinates [Commission international de l'éclairage (**CIE**) L*, a*, b*] and subjective color score with the Japanese color standards (from 1 = pale to 6 = dark color; Nakai et al., 1975) after exposing the muscle surface to 30 min blooming time. Drip loss was measured in the LM and SM muscle by a modified EZ-Driploss procedure (Correa et al., 2007). Briefly, 3 25-mm-diam. cores were removed from the center of 2.5-cm-thick LM (removed at third/fourth last rib level) and SM chops by a cork borer, weighed, and placed into plastic drip loss con-

tainers (Christensen Aps Industrivaengetand, Hilleroed, Denmark) before being stored for 48 h at 4°C. At the end of the 48-h storage period, muscle cores were removed from their container, surface moisture was carefully dabbed, cores were reweighed, and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight.

Muscle chops (10 to 20 cm in length) were taken from the LM at the third/fourth last rib level and from the middle region of the SM muscle. Both muscle chops were aged for 5 d at 4°C and frozen at −20°C pending analyses of the Warner-Bratzler shear force (**WBSF**) and myofibrillar fragmentation index (**MFI**). The WBSF analysis was conducted by a Warner-Bratzler device attached to a TAXT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) according to a modified procedure described by Van Oeckel et al. (1999). In brief, the meat chops (approximately 300 g) were heated in a sealed plastic bag in a water bath at 75°C for approximately 50 min, followed by cooling in cold tap water for 40 min. For each meat chop, 12 (for LM) and 9 (for SM muscle) rectangular cores (1 cm^2), parallel to the longitudinal orientation of the muscle fibers, were taken and analyzed. The greatest and least values for each sample were disregarded, and then the mean of the remaining values was used to get the final result for WBSF.

The analysis of the MFI was performed according to the method described by Hopkins et al. (2000), with adaptations for pork meat. In brief, meat was blended with a Polytron (System POLYTRON PT 3100; Kinematica AG, Luzern, Switzerland) placed in ice-cold vessels for 10 s. Myofibril suspensions were filtered into 50-mL centrifuge tubes (1.0-mm mesh strainers) to remove connective tissue. The samples were centrifuged at 1,400 × *g* for 10 min at 4°C. Finally, 1 mL of homogenate was transferred for borosilicate glass tubes (16 × 100 mm) with 4 mL of cold buffer. The protein concentration of the suspensions was determined using a Bio-Rad kit (Bio-Rad DC Protein Assay; Bio-Rad Laboratories, Hercules, CA) with BSA as a reference and was measured at 750 nm with a spectrophotometer (Ultrospec model 300 UV/Visible; Pharmacia Biotech Ltd., Cambridge, UK). Aliquots of the suspensions were diluted in cold buffer to a final protein concentration of 0.5 mg/mL in triplicate. The absorbance was measured immediately at 540 nm with a spectrophotometer (Ultrospec model 300 UV/Visible; Pharmacia Biotech Ltd.). The average of the triplicate absorbance readings was multiplied by 150 to give MFI values according to Hopkins et al. (2000).

Statistical Analysis

Behavior data were analyzed by the FREQ procedure (SAS Inst. Inc., Cary, NC) using the treatment group as the experimental unit. Categorical variables relative to the animals had low frequencies, and were transformed to binary variables, and were analyzed by the LOGISTIC procedure of SAS. Analyses of handler intervention data, load time, fighting events, and fighting duration were made through an adjustment to nonnormal distributions using the GLIMMIX procedure of SAS, except for vocal sound and posture during lairage, which presented normal distribution. In this case, the data were analyzed by the MIXED procedure of SAS. Severity of skin bruises was analyzed with a Cochran-Mantel-Haenszel (**CMH**) test and the LOGISTIC procedures of SAS to determinate interactions between factors. The MIXED procedure of SAS was applied to analyze the physiological data using the treatment combination group as the experimental unit and carcass and meat quality data using the experimental individual animal as the experimental unit. Urinary hormone data were transformed into their logarithmic score ($\log_{10}$) for data normalization before analysis. The model included treatment, week, and their interaction as fixed effects. Comparisons between treatment means were adjusted for multiple comparisons with a Tukey-Kramer correction. A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests, whereas probability levels of $P \leq 0.10$ were considered to be a tendency.

RESULTS AND DISCUSSION

Behavioral Response

Loading. Loading pigs onto the truck is considered the most critical stage of the transport period as shown by the increased heart rate and greater concentrations of stress indicators (cortisol and lactate) in the blood, with these effects lasting until slaughter (Bradshaw et al., 1996; Correa et al., 2010). In this study, behavioral data concerning handler interventions and pig behavior during loading, which were significantly affected by treatments, are presented in Tables 3 and 4, respectively. Of the behaviors observed during loading (Table 1), many had infrequent occurrence, slip/fall (1.6% of loading groups in the alley, 0.5% in the ramp), overlap (4.2% and 2.6%, respectively), 180° turn (7.3% in the ramp), backward (15.6% in the alley), back up (alley: 14.6%; ramp: 4.2%), underlap (alley: 3.6%; ramp: 0.5%), and balk (13.0% in the alley), and were not affected by treatments ($P > 0.10$). In the ramp, 35.4% of loading groups vocalized, but this behavior was not affected by treatments. However, in this study it was observed that GB pigs needed more vocal interventions from the handler

Table 3. Effects of ractopamine supplementation, castration method, and Piétrain genetic types on the number of handler interventions during loading[1]

		RAC				NRAC					*P*-values[2]				
		SC		IM		SC		IM							
Variable	Zone	GA	GB	GA	GB	GA	GB	GA	GB	SEM	RAC	CAS	GEN	RAC × CAS	CAS × GEN
Vocal sound	Alley	5.93	6.69	5.83	7.75	6.14	7.19	7.23	7.83	0.95	NS	NS	0.01	NS	NS
Vocal sound	Ramp	4.31	5.52	3.90	6.17	4.98	4.52	5.35	5.35	0.56	NS	NS	0.08	NS	NS
Physical contact	Alley	8.89	9.23	7.36	8.44	6.73	5.71	8.16	6.93	—	0.02	NS	NS	0.07	NS
Physical contact	Ramp	7.54	8.60	6.81	10.63	10.69	6.17	8.67	9.35	—	NS	NS	NS	NS	0.02
Rattle noise	Alley	5.12	4.79	6.32	7.22	6.18	4.73	5.55	6.76	—	NS	0.002	NS	NS	0.02

[1]RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain, CAS = castration method, GEN = genotype.

[2]NS = $P > 0.10$.

in the alley ($P = 0.01$; Table 3) and tended to get more squeezed in the ramp compared with GA pigs ($P = 0.07$; Table 4) at loading. Differences between GA and GB pigs are especially significant when they were RAC fed, with more vocal and physical interventions in the ramp for RAC-fed GB pigs compared with RAC-fed GA pigs ($P = 0.02$ and $P = 0.01$, respectively; Fig. 1). The lower easiness of handling was reflected by a trend for more balking in the ramp ($P = 0.06$) and longer load duration ($P = 0.004$) in these pigs (Table 4).

The RAC feeding alone influenced only the easiness of handling, with RAC-fed pigs needing more physical contacts to be driven in the alley compared with NRAC pigs (8.45 vs. 6.83; $P = 0.02$; Table 3). Finally, when compared with SC pigs, more handler interventions, in terms of use of rattle noise in the alley and physical contact in the ramp, were necessary to drive IM-GB pigs at loading ($P = 0.02$ for both). Overall, these observations show that RAC-fed pigs and leaner pigs (GB pigs and IM based on carcass leanness in this study; see later in the carcass quality results) were harder to handle, as previously reported by Marchant-Forde et al. (2003) and Busse and Shea-Moore (1999), respectively. The greater level of interventions needed to handle IM at loading may be explained by their increased excitability compared with SC, which has been reported in a number of previous studies (Cronin et al., 2003; Zamaratskaia et al., 2008; Fàbrega et al., 2010).

Lairage. A greater proportion of GB pigs tended to lie during lairage compared with GA pigs (52.3% ± 3.2% vs. 45.5% ± 3.4%; $P = 0.09$; Fig. 2). The tendency for a greater proportion of GB pigs to lie and to fight less in lairage may be associated with a greater need for recovery from a greater loading stress due to their lower easiness of handling (Tables 2 and 3). On the other hand, a greater ($P = 0.04$) proportion of IM were standing compared with SC pigs (31.6 ± 2.1 vs. 36.8 ± 2.1). Increased activity in IM compared with SC was already reported by a number of previous studies (Cronin et al., 2003; Zamaratskaia et al., 2008; Fàbrega et al., 2010). Similar to these studies, where IM were kept in the same group in the finishing pen, in this study the increased activity did not result in increased agonistic acts or fighting between IM, even when they were mixed with unfamiliar conspecifics. To our knowledge, this is the first evidence of social behavior of IM in a mixed-group situation. These

Table 4. Effects of ractopamine supplementation, castration method, and Piétrain genetic types on animal behavior during loading[1]

		RAC				NRAC				*P*-values[2]					
		SC		IM		SC		IM							
Variable	Zone	GA	GB	GA	GB	GA	GB	GA	GB	RAC	CAS	GEN	RAC × CAS	RAC × GEN	CAS × GEN × RAC
180° turn, %	Alley	41.67	37.50	37.50	45.83	33.33	50.00	45.83	25.00	NS	NS	NS	NS	NS	0.07
Vocalize, %	Alley	62.5	58.33	50.0	79.17	50.0	62.5	62.5	45.83	NS	NS	NS	NS	NS	0.02
Squeeze, %	Alley	29.17	25.00	16.67	25.00	20.83	12.50	37.50	33.33	NS	NS	NS	0.03	NS	NS
Squeeze, %	Ramp	8.33	25.00	12.50	16.67	4.17	16.67	12.50	29.17	NS	NS	0.07	NS	NS	NS
Backward, %	Ramp	16.67	16.67	8.33	29.17	41.67	12.50	33.33	29.17	NS	NS	NS	NS	0.02	NS
Balk, %	Ramp	12.50	16.67	0.00	8.33	4.17	0.00	8.33	4.17	NS	NS	NS	NS	0.06	NS
Load duration, s	Both	17.15	21.98	17.41	20.86	22.59	18.51	21.39	21.47	NS	NS	NS	NS	0.004	NS

[1]RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain, CAS = castration method, GEN = genotype.

[2]NS = $P > 0.10$.

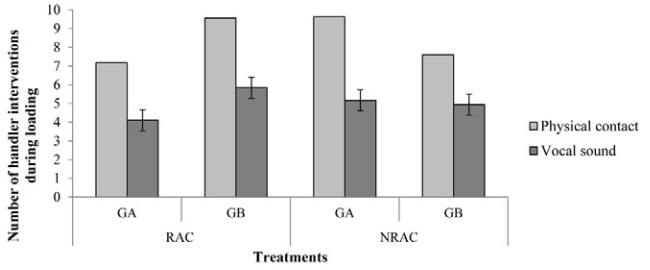

Figure 1. Number of handler interventions during loading. A greater proportion of RAC-fed GB required more vocal and physical interventions in the ramp compared with RAC-fed GA pigs (P = 0.02 and P = 0.01, respectively). RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; GA = 25% Piétrain, GB = 50% Piétrain.

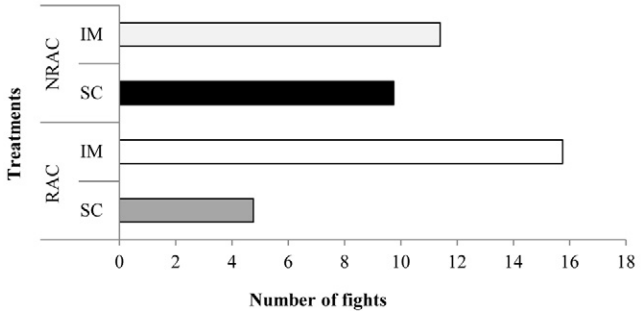

Figure 3. Effects of ractopamine supplementation and castration method on number of fights in lairage. The RAC-fed immunized males presented a greater (P = 0.03) number of fights in the lairage pen compared with RAC-fed surgical castrates. NRAC = without ractopamine, RAC = with ractopamine; IM = immunized males, SC = surgical castrates.

results indicate that immunization against GnRF is as efficient as surgical castration in removing aggressiveness in pigs. Nevertheless, when IM were fed RAC, the number of fights significantly increased (P = 0.03), showing the additive effect of RAC feeding on IM social behavior (Fig. 3). A more than 2-fold greater (P = 0.008) number of fights were observed in GA pigs compared with GB pigs (14.8 vs. 6.17; data not shown). In this study, fighting activity of RAC-fed pigs was of shorter duration compared with NRAC pigs (P = 0.05; Fig. 4), which may indicate that these pigs become easily fatigued during fighting or were more tired after transport and needed some rest between the multiple aggressive bouts, although no difference in the postural time budget during lairage was observed in these pigs (P > 0.10; Fig. 2).

Physiological Measurements

The GIT temperature can increase as a result of the increased adrenal cortex activity after disturbing events, such as handling and transport (Broom and Johnson, 1993). In this study, the delta GIT temperature tended to be lower in IM-GA pigs during traveling (P = 0.06) and was lower at unloading (P = 0.05) and in lairage (P = 0.03) compared with SC-GA pigs (Table 5). These results may indicate a reduced response to transport stress and handling at unloading and faster recovery rate in lairage in IM-GA pigs.

As shown in Table 6, serum CK concentrations increased in RAC-fed GB compared with RAC-fed GA pigs (P = 0.05) and were greater (P = 0.04) in SC compared with IM pigs (3.74 ± 0.05 vs. 3.69 ± 0.05). As CK is an indicator of long-term physical stress because it achieves the maximum concentration peak until 6 h after the stress and does not return to basal concentrations until 48 h (Anderson, 2010), the increased exsanguination CK concentrations in RAC-fed pigs are indicative of their long-term response to overall handling stress before slaughter. The greater fighting rate (Fig. 3) may have prevented RAC-fed pigs from resting in lairage, resulting in greater physical fatigue at slaughter. A relationship between RAC administration and muscle fatigue induced by physical exercise has been associated with the more glycolytic muscle metabolic type of these

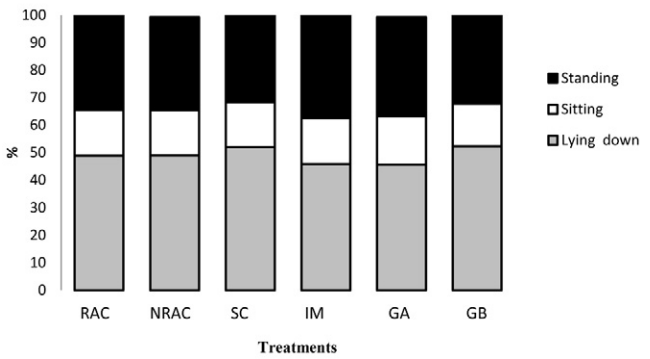

Figure 2. Effects of ractopamine supplementation, castration method, and Piétrain genetics on animal behavior in lairage. A greater proportion of GB pigs tended to lie down compared with GA pigs (P = 0.09), whereas a greater (P = 0.04) proportion of IM stood compared with SC. RAC = with ractopamine, NRAC = without ractopamine; SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain.

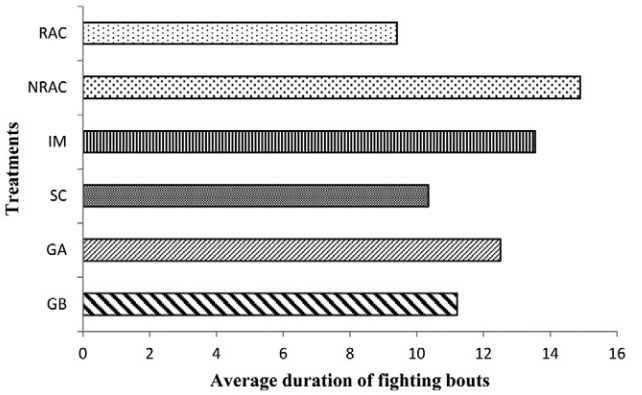

Figure 4. Effects of ractopamine supplementation and castration method and Piétrain genetics on average fighting bout duration during lairage. The NRAC pigs fought longer than RAC pigs (P = 0.05). RAC = with ractopamine, NRAC = without ractopamine; IM = immunized males, SC = surgically castrates; GA = 25% Piétrain, GB = 50% Piétrain.

Table 5. Effects of castration method and genetic types on delta gastrointestinal tract (GIT) temperature at the time periods of loading, transport, unloading and lairage[1,2]

Variable	SC		IM			*P*-values[3]			
(n = 135)	GA	GB	GA	GB	SEM	RAC	CAS	GEN	CAS × GEN
Loading	0.43	0.13	0.04	0.18	0.18	NS	NS	NS	NS
Traveling	0.63	0.23	0.13	0.24	0.13	NS	0.06	NS	0.06
Unloading	0.21	−0.14	−0.18	−0.01	0.14	NS	NS	NS	0.05
Lairage	−0.01	−0.31	−0.37	−0.09	0.11	NS	NS	NS	0.03

[1]Delta GIT is the difference in GIT temperatures values within treatments (GIT temperature at each determined event minus GIT temperature at the rest level).

[2]SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain, RAC = ractopamine (Elanco Animal Health, Guelph, Canada), CAS = castration method, GEN = genotype.

[3]NS = $P > 0.10$.

pigs (Depreux et al., 2002). The decreased exsanguination blood CK concentrations in IM reflects their faster recovery from preslaughter stress, which may have contributed to the aforementioned GIT results. Plasma lactate concentrations were greater ($P = 0.04$) in GB compared with GA (24.1 ± 2.3 vs. 21.9 ± 2.3 mmol). On the basis of the relatively rapid speed of plasma lactate rise (4 min) and return to basal levels (2 h) after physical exercise (Anderson, 2010), the greater lactate concentration in blood of GB pigs at slaughter may reflect their greater responsiveness to handling just before slaughter, which may have resulted from the memory of the previous negative handling experience at loading (Table 2). This result confirms what was previously reported by Edwards et al. (2010) on the basis of the significant correlation ($P < 0.001$) between blood lactate measured at loading and at exsanguination.

Variability in hypothalamic-pituitary-adrenal (**HPA**) axis activity has been shown to be influenced by genetic factors (Hazard et al., 2008; Mormède et al., 2011) and to be related to great metabolic differences between genotypes (Foury et al., 2007). Except for marginal variations in urinary norepinephrine and dopamine, no difference was found for urinary cortisol, cortisone, and epinephrine between treatments. Postslaughter urinary norepinephrine concentrations tended to increase in RAC-fed IM compared with RAC-fed SC ($P = 0.08$). When GA pigs were fed with RAC, the concentrations of dopamine tended to increase compared to the other treatments ($P = 0.08$). To our knowledge, this is the first study documenting the effects of immunization against GnRF and RAC feeding on the variation of urinary hormones in pigs. Overall, the variation of these urinary hormones may indicate no effects of the treatments on the preslaughter HPA axis activity of the pigs assessed in this study.

Carcass Quality Traits

In agreement with previous studies (Aalhus et al., 1992; See et al., 2004; Hinson et al., 2011), compared with NRAC pigs, RAC dietary supplementation increased HCW (93.1 ± 0.4 vs. 91.6 ± 0.4; $P = 0.001$), dressing yield (79.7 ± 0.2 vs. 79.2 ± 0.2; $P = 0.01$), and carcass leanness (63.1 ± 0.2 vs. 62.5 ± 0.2; $P < 0.001$; Table 7). According to Crome et al. (1996), the greater

Table 6. Effects of ractopamine supplementation, castration method, and genetic types on blood metabolites and urinary hormones[1]

	RAC				NRAC					*P* values[2]				
	SC		IM		SC		IM							
Variable	GA	GB	GA	GB	GA	GB	GA	GB	SEM	RAC	CAS	GEN	RAC × CAS	RAC × GEN
Blood (n = 238)														
Serum CK, $\log_{10}$	3.723	3.827	3.753	3.733	3.762	3.666	3.649	3.532	0.07	0.005	0.04	NS	NS	0.05
Plasma lactate, mmol	24.12	24.93	20.62	24.30	21.84	24.42	21.19	22.87	2.65	NS	NS	0.04	NS	NS
Urine (n = 239)														
Cortisol, $\log_{10}$	1.55	1.51	1.59	1.42	1.57	1.55	1.48	1.49	0.07	NS	NS	NS	NS	NS
Cortisone, $\log_{10}$	1.52	1.50	1.52	1.44	1.56	1.54	1.50	1.47	0.06	NS	NS	NS	NS	NS
Epinephrine, $\log_{10}$	1.21	1.11	1.25	1.16	1.10	1.19	1.18	1.09	0.07	NS	NS	NS	NS	NS
Norepinephrine, $\log_{10}$	1.27	1.23	1.40	1.32	1.27	1.35	1.32	1.28	0.07	NS	NS	NS	0.08	NS
Dopamine, $\log_{10}$	1.27	1.23	1.29	1.24	1.19	1.25	1.20	1.23	0.06	NS	NS	NS	NS	0.08

[1]RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain; CAS = castration method, GEN = genotype, CK = creatine kinase.

[2]NS = $P > 0.10$.

Table 7. Effects of ractopamine supplementation, castration method and Piétrain genetics on carcass characteristics[1]

	RAC				NRAC					*P*-values[2]			
	SC		IM		SC		IM						
Variable	GA	GB	GA	GB	GA	GB	GA	GB	SEM	RAC	CAS	GEN	CAS × GEN
HCW, kg	92.6	94.4	92.6	92.6	91.5	94.0	91.0	90.1	0.66	0.001	0.0008	0.06	0.005
Dressing yield,%	80.1	81.2	78.8	78.9	79.2	81.1	78.0	78.5	0.34	0.01	<0 .0001	0.0001	0.01
Lean yield,%	62.9	62.6	63.3	63.7	62.5	62.1	62.9	62.4	0.28	0.0004	0.002	NS	NS

[1]RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain; CAS = castration method, GEN = genotype.

[2]NS = $P > 0.10$.

carcass yield can be explained by the increased lean tissue deposition in the carcass of RAC-supplemented animals compared with the growth of organs and viscera. The increased lean yield may be explained by the effect of RAC on repartitioning of nutrients to lean and away from the adipose tissue (Xiong et al., 2006). Carcasses from IM are generally reported to be heavier than those from SC as a result of the increased growth rate due to the decreased aggressiveness and sexual activity after the second vaccination (Dunshea et al., 2001; Cronin et al., 2003; Fàbrega et al., 2010). However, in this study SC carcasses were heavier than IM carcasses, especially in GB pigs ($P = 0.005$). Greater ($P = 0.01$) dressing yield in GB pigs were also found in SC compared with IM carcasses (80.4% ± 0.2% vs.78.5% ± 0.2%). This effect of immunological vs. surgical castration has been reported elsewhere (e.g., Dunshea et al., 2001; Pauly et al., 2009; Gispert et al., 2010) and was considered to be mostly due to gastrointestinal tract and to the presence of testes and associated tissue, bulbourethral glands, seminal vesicles, and a thicker skin. Greater HCW and dressing yield have already been reported in the carcass of Piétrain genotypes, regardless of the presence of the Hal gene in the crossing (Gispert et al., 2007). Thus, the additive effect of the Piétrain genotype on SC carcass traits is not surprising.

Consistent with previous reports (D'Souza and Mullan, 2003; Jaros et al., 2005; Fàbrega et al., 2010), carcasses from IM were leaner than SC pigs (62.9% ± 0.2% vs. 62.5% ± 0.2%; $P = 0.002$), indicating that immunization against GnRF can still deliver the carcass quality attributes of boars to producers.

No differences were found in the overall skin damage score on the carcass between treatments (data not shown). However, NRAC pigs showed a greater ($P = 0.007$) percentage of severe (score 3) dark bites compared with RAC pigs (Fig. 5). As the age of a dark brownish bruise can be dated to 24 h or more before the skin assessment (Gracey and Collins, 1992), these bruises in NRAC pigs were likely inflicted in the finishing pen at the farm. A greater ($P = 0.01$) proportion of dark scratches due to mounting were observed on IM carcasses compared with SC carcasses (Fig. 6). These bruises may likely be related to sexual behavior of boars before the full immunization occurred (Cronin et al., 2003; Fàbrega et al., 2010). The low proportion of fresh scratches on the IM carcass skin and the lack of difference with SC carcasses confirm the aforementioned lack of difference in social behavior between the 2 genders during the antemortem period.

Meat Quality

Meat quality variation was mostly influenced by RAC feeding alone or in combination with castration method and Piétrain genotype in this study (Table 8). Compared with RAC pigs, the LM muscle of NRAC pigs was slightly paler (greater L* value; 51.6 ± 0.5 vs. 50.7 ± 0.5; $P = 0.007$) and more red and yellow for LM [greater a* (7.2 ± 0.1 vs. 6.0 ± 0.1) and b* values (4.7 ± 0.1 vs. 3.8 ± 0.14)] and SM muscle [greater a* (8.5 ± 0.1 vs. 7.5 ± 0.1) and b* values (4.6 ± 0.1 vs. 4.1 ± 0.1); $P <$

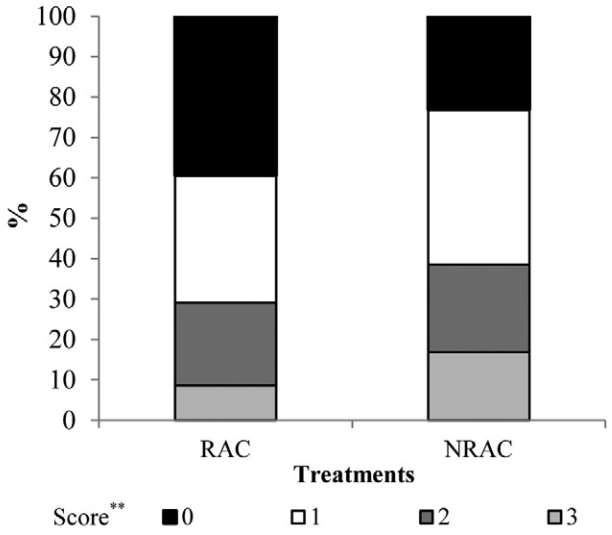

Figure 5. Effects of ractopamine dietary supplementation on the distribution of fighting-type dark bruises on the carcass. The NRAC pigs showed a greater ($P = 0.007$) percentage of severe (score 3) dark bites compared with RAC pigs. RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; fighting-type bruise score: 1 = less than 10 bruises, 2 = 11 to 20 bruises, and 3 = greater than 20 bruises.

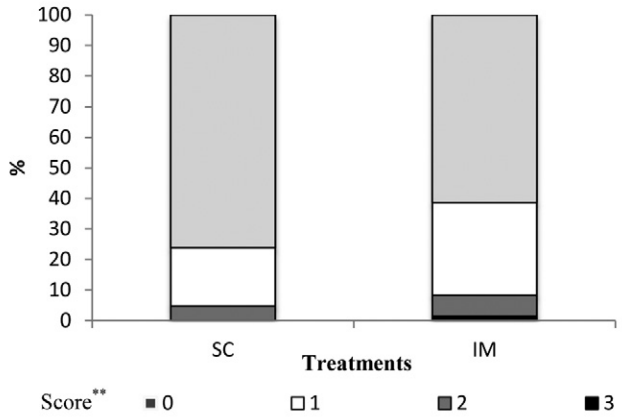

Figure 6. Effects of castration method on the distribution of mounting-type dark scratches on the carcass. A greater ($P = 0.01$) proportion of dark scratches were observed on IM carcasses compared with SC ones. SC = surgically castrates, IM = immunized males; mounting-type bruise score: 1 = less than 5 bruises, 2 = 6 to 10 bruises, and 3 = greater than 10 bruises.

0.001 for both muscles]. A RAC × CAS × GEN interaction was found for the L* value (lightness) in the SM muscle, with RAC-IM-GB hams being slightly paler ($P = 0.04$) than in RAC-SC-GB pigs. Results for the effects of RAC on pork lightness (L* value) are conflicting, with some studies either showing paler loins in RAC pigs (Leick et al., 2010) or showing no effect (Stoller et al., 2003; Patience et al., 2009; Kutzler et al., 2011). Reduced a* and b* values in pork with low dietary dose of RAC (5 to 10 mg/kg) were already reported in previous studies (Carr et al., 2005; Patience et al., 2009). The RAC feeding decreased drip loss in LM (3.3 ± 0.3 vs. 3.8 ± 0.3; $P = 0.02$) and increased WBSF values in GB loins ($P = 0.01$) and in the SM muscle ($P = 0.006$). The reduced drip loss in RAC loins may be related to the effects of RAC feeding on the reduced fat deposition and increased protein deposition, resulting in greater water retention (Crome et al., 1996). On the basis of the relationship between WBSF and MFI (LM: $r = -0.53$; SM: $r = -0.49$; $P < 0.001$ for both muscles) in this study, the greater toughness of pork from RAC pigs may be explained by the effects of RAC dietary supplementation on the reduced postmortem ageing process (lower MFI; $P < 0.001$). The effects of RAC feeding on pork toughness (greater WBSF values) confirm those reported by Patience et al. (2009) using low doses of RAC (5 mg/kg) in the finishing diet and may be explained by the increased amount of glycolytic (IIB) fibers and larger fiber size in the muscle of RAC pigs (Depreux et al., 2002). To our knowledge, this study provides the first evidence of the negative effect of RAC feeding on pork tenderness in pigs of Piétrain genetics. This result disagrees with previous reports showing no effect of the interaction between RAC and genotype on pork quality (Schinckel et al., 2001).

Minor effects of immunization against GnRF on pork quality were found in this study. Compared with SC, WBSF values were slightly greater ($P = 0.01$) in the LM (3.0 ± 0.05 vs. 3.2 ± 0.05) and tended to be greater ($P = 0.06$) in the SM muscle of IM (3.9 ± 0.1 vs. 4.1 ± 0.1). These results disagree with Moore et al. (2009), who reported a decrease in WBSF values with immunization against GnRF. Both the increased water exudation and toughness in pork evaluated in this study may be explained by the greater proportion of glycolytic fibers and larger fiber size in the muscles of leaner pigs (Rehfeldt et al., 2000; Ryu and Kim, 2005), such as IM in this study. Apart from the aforementioned slight increase in drip loss, similar to the results of Moore et al. (2009), RAC dietary supplementation had no major additive effect on pork quality variation in IM in this study.

Greater ($P = 0.04$) pHu values were found in the LM muscle of GA than in GB pigs (5.75 ± 0.03 vs. 5.71 ± 0.03), which is in disagreement with Werner et al. (2010), who reported lower values of pHu for Piétrain crossbred compared with Duroc purebred pigs. A trend for greater ($P = 0.07$) drip loss value was recorded in GB pigs. Affentranger et al. (1996) and Edwards et al. (2003) also reported greater drip loss values in pork from crossbreeds containing a greater proportion of Piétrain genetics.

Conclusions

The results of this study show that the RAC administration in the pig diet over the last weeks before slaughter may have a negative impact as both a single and an additive factor on the behavioral and physiological response of pigs to preslaughter handling. Ractopamine administration, although it increased carcass leanness, increased drip loss and toughness of pork. However, given the small magnitude of the difference between treatments, water exudation found in pork meat in this study cannot be considered of economic importance for the processing industry, and the between-treatment variation in WBSF values is less than 1 kg, which is the threshold value for the average consumer detection of meat toughness (Aalhus et al., 1990). The additive effects between treatments, mostly found on animal welfare measurements, were mostly negative in this study, indicating that their combination may not be favorable to pork producers and processors. Contrary to Moore et al. (2009), no additive effect between RAC and immunization against GnRF on carcass quality traits was found in this study.

Besides reducing dressing yield, immunization against GnRF as a single factor did not have a negative influence on response of the pigs to preslaughter handling and meat quality. Thus, on the basis of the results

Table 8. Effects of ractopamine supplementation (RAC), castration method (CAS), and Piétrain genotype (GEN) on meat quality characteristics[1]

	RAC				NRAC					*P*-values[2]					
	SC		IM		SC		IM								
Variable	GA	GB	GA	GB	GA	GB	GA	GB	SEM	RAC	CAS	GEN	RAC × CAS	RAC × GEN	CAS × GEN × RAC
LM															
pHu[3]	5.8	5.7	5.8	5.7	5.7	5.7	5.7	5.7	0.03	NS	NS	0.04	NS	NS	NS
L*	50.7	50.7	50.7	50.9	51.3	52.3	51.4	51.6	0.63	0.007	NS	NS	NS	NS	NS
a*	6.1	5.9	6.1	5.9	7.1	7.3	6.9	7.3	0.19	<0.0001	NS	NS	NS	NS	NS
b*	3.9	3.7	3.8	3.7	4.8	5.0	4.5	4.8	0.2	<0.0001	NS	NS	NS	NS	NS
Drip loss, %	2.9	2.9	3.9	3.5	3.5	4.0	3.7	4.0	0.40	0.02	0.03	NS	NS	NS	NS
WBSF,[4] kg	2.9	3.4	2.9	3.6	2.7	2.9	3.0	3.2	0.11	0.001	0.01	<0.0001	NS	0.01	NS
MFI[5]	64.0	64.3	63.5	58.8	78.2	74.1	69.9	66.7	4.68	<0.0001	0.02	NS	NS	NS	NS
SM muscle															
pHu	5.8	5.9	5.9	5.8	5.8	5.7	5.8	5.8	0.03	NS	NS	NS	NS	NS	NS
L*	48.2	47.2	47.3	48.4	48.0	48.3	47.7	47.3	0.48	NS	NS	NS	NS	NS	0.04
a*	7.4	7.5	7.5	7.5	8.4	8.5	8.5	8.7	0.17	<0.0001	NS	NS	NS	NS	NS
b*	4.2	4.0	4.0	4.2	4.6	4.6	4.5	4.6	0.16	0.0001	NS	NS	NS	NS	NS
Drip loss, %	2.6	2.7	2.8	3.5	2.4	3.3	3.1	3.0	0.3	NS	NS	0.07	NS	NS	NS
WBSF, kg	4.1	4.2	4.2	4.3	3.5	3.7	3.9	3.9	0.017	0.006	0.06	NS	NS	NS	NS
MFI	47.9	43.2	52.8	45.4	62.1	64.1	53.7	51.0	7.74	0.002	NS	NS	0.02	NS	NS

[1]RAC = with ractopamine (Elanco Animal Health, Guelph, Canada), NRAC = without ractopamine; SC = surgically castrates, IM = immunized males; GA = 25% Piétrain, GB = 50% Piétrain, CAS = castration method, GEN = genotype.

[2]NS = $P > 0.10$.

[3]pHu = pH at 24 h postmortem.

[4]WBSF = Warner-Bratzler shear force.

[5]MFI = myofibrillar fragmentation index.

arising from the present study, immunization against GnRF more than the use of Piétrain genetics appears to be a viable alternative to the use of RAC as it ensures the production of lean carcasses without any major effect on animal welfare and pork quality.

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