Immunocastration as an alternative to caponization: evaluation of its effect on body and bone development and on meat color and composition

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ABSTRACT Caponization is associated with some morbidity and mortality, which contributes to important economic losses. This practice is executed without any pain relief (neither anesthesia nor analgesia) and can be painful and without consideration of animal welfare. On the other hand, immunocastration accomplished by Improvac and Bovipriva in pigs and cattle represents a noninvasive procedure, and for that reason is regarded as an alternative with improved animal welfare. This study includes 4 experimental groups consisting of capons, slips, roosters, and birds submitted to the Improvac treatment. The administration of Improvac was associated with a considerable reduction in serum testosterone concentration (reduced by 79% compared to average serum testosterone of roosters). Regarding significant differences among experimental groups, birds from the Improvac group were intermediate between capons and slips with respect to abdominal fat pad weight and yield, breast meat water and protein contents, and femur length. Conversely, color parameters such as lightness, redness, and hue angle for Improvac birds were intermediate between roosters and capons. Thus, immunocastration with Improvac could represent an alternative solution to caponization, with considerable improvements in animal welfare.

Key words: Caponization, immunocastration, bone, meat composition, testosterone

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INTRODUCTION

Capons are male chickens whose testes have been surgically removed (testectomy) before reaching sexual maturity, a procedure named by the poultry industry as caponization. Absence of testes results in androgen deficiency and insufficient development of secondary male sexual characteristics such as comb and wattles, whereas fighting behavior and vocalization are abolished (Mast et al., 1981). The energy that is expended by roosters in territorial protection, fighting, and courting behavior is greatly reduced in capons, enabling a more efficient use of feed energy for growth and fat deposition (Rikimaru et al., 2011). Therefore, caponization contributes to an increased fat deposition and increased intramuscular fat content, enhancing meat sensorial qualities as tenderness, juiciness, and flavor (Sinanoglou et al., 2011).

Caponization requires accurate surgical skills, which are difficult to execute at farm level, and 2 major concerns are associated with caponization accuracy: 1) mortality as consequence of improper testectomy, and 2) incomplete removal of the testes. Mortality has been estimated at between 5 and 20% (Rikimaru et al., 2011), although higher mortality rates (near 50%) have been reported when caponization is performed in older birds (Gogolewski and Czerwiński, 2012). The incomplete removal of the testes leads to a bird between roosters and capons, depending upon the growth and activity of the remaining testicular tissue that was not removed (Mast et al., 1981). Such birds are called "slips" by the poultry industry.

In the poultry industry, caponization is performed in production facilities by specialized technicians, in accordance to ancient practices, but is executed without any pain relief (neither anesthesia nor analgesia control), and for that reason is a painful procedure. Despite being banned in the EU due to concerns about animal welfare, caponization is still used in traditional farming systems that use a derogation for traditional practices (Compassion in World Farming, 2013).

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Vaccination against gonadotropin-releasing hormone (**GnRH**), known as immunocastration, is regarded as an animal-friendly alternative to physical castration in male and female mammals (Bonneau and Enright, 1995). In both genders, GnRH, a hypothalamic hormone, plays a central role in the regulation of reproductive function. Therefore, immunization against GnRH, results in the neutralization of endogenous GnRH with the subsequence suppression of the gonadotropins luteinizing hormone (**LH**) and follicle-stimulating hormone (**FSH**) secretion by the anterior pituitary and consequently testicular production of testosterone and androstenone declines.

The structure and sequence of GnRH has been highly conserved throughout evolution (Dubois et al., 2002); it persists across a wide variety of vertebrates and the protein presents some regions that are highly conserved among mammal species (Gore, 2013). The assumption that Improvac, a vaccine developed for immunocastration of pigs, would provide equal effect on chicken is based on the knowledge that the amino acid sequence of mammalian GnRH differs from chicken GnRH I in just 1 amino acid and from GnRH II in 3 amino acids (Dubois et al., 2002). Therefore, the similarity in GnRH sequences between pig (*Susscrofadomesticus*) and chicken (*Gallus gallusdomesticus*) should allow Improvac to induce immunocastration in broilers, at least to some level.

The study presented herein was intended to evaluate the possibility of using Improvac®, a vaccine produced for immunocastration of pigs, to produce capons by immunocastration of broilers.

MATERIAL AND METHODS

Experimental Design, Animal Management, and Sample Collection

The study was conducted on a flock of 4,800 male medium-growing broilers (Redbro, Hubbard SAS, Quintin, France) reared under commercial conditions in a poultry shed, with access to a grass paddock until they were slaughtered. The production facilities and bird management were in full compliance with the specifications established by the Commission Regulation (EC) N° 543/2008, for the rearing of capons under traditional free-range conditions.

The study was designed with 3 experimental groups: Group I roosters (no intervention of any kind; n = 30); Group II capons (birds submitted to surgical caponization; n = 15); and Group III Improvac (birds submitted to the Improvac® protocol; n = 15). However, during slaughter, a new experimental group was created: the slip group, since 8 of the 15 surgical capons proved to be slips, which means that surgical caponization was performed without success in these birds. The incomplete removal of the whole testicle resulted in residual testicular tissue, which provided testosterone. The recognition that slips occurred allowed the comparison of these birds with the Improvac treatment.

In this trial, 60 male broilers (28 d old) were randomly selected among broilers belonging to a commercial flock and were randomly distributed through the 3 experimental groups. Birds assigned to the study were individually identified with a different-colored ring.

Caponization followed a commercial poultry industry protocol. All broilers allocated to this study were deprived of feed and water for 24 and 12 h, respectively, before the caponization procedure. Caponization, performed at 28 d of age, was d 0 in this study. Briefly, the feathers around the incision site, located between the last 2 ribs, were removed and the skin was swabbed with 10% solution of povidone iodine (Betadine, Mundipharma, Basel, Switzerland). A lateral incision (1.5 cm) was made between the 2 last ribs and widened by a small rib spreader, and the testectomy was performed. The incision site was then disinfected and surgical staples were used to close the surgical wound.

Broilers allocated to the Improvac treatment were injected subcutaneously at the back of the neck with 0.2 ml of Improvac (Zoetis Portugal). Improvac administration was performed 2 times, at d 28 (caponization day) and 91 (63 d before slaughter) of age.

All groups were subjected to the same feeding, environmental, and sanitary conditions. Capons and birds from the Improvac group were kept in the flock, while roosters were raised on a separate compartment within the poultry shed. The rooster group was nearby the flock but had no physical contact with the flock thanks to a net fence; such separation of roosters from the flock was done to avoid the aggressive behavior of roosters towards other birds. The flock was kept indoors at a density of 6 bird/m^2 with access to an outdoor pen in a density of four m^2/bird. To reduce the aggressiveness among roosters, their density was reduced to half, both indoors (3 birds/m^2) and outdoors (8 m^2/bird).

Feed and water were available ad libitum throughout the trial. The birds were fed a commercial starter diet presented in crumbs from d 1 to 28, a grower diet was presented as dry mash from d 29 to 55, whereas for the rest of the experimental period, they consumed a commercial finisher diet presented as pellets. The composition and analysis of the diets are presented in Table 1.

The birds, at the age of 5 months (154 d old), were placed in crates and transported to an accredited slaughterhouse, a journey time of around 2 h. Throughout the catching process, birds previously allocated to the study were weighted and placed in transport cages according to their ring color (experimental group) while roosters were the last ones to be caught and placed in transport boxes.

After arrival to the slaughterhouse, birds were hung on shackles of the slaughter line, were killed by manual exsanguination, scalded, and plucked. Subsequently,

Table 1. Composition of the free-range feed (g/kg).

Ingredients (%)	Starter	Grower	Finisher
Corn	63.40	66.00	73.00
Soybean meal 47%	22.04	20.41	18.50
Sunflower meal 28%	4.00	—	—
Rape meal	3.00	4.50	4.30
Wheat		5.00	—
Wheat bran	2.90	—	—
Calcium carbonate	1.33	1.02	1.00
Calcium phosphate	1.10	0.53	0.47
Vitamin-mineral premix	0.20	0.20	0.20
Phytase	0.05	0.05	0.05
Enzymes	0.05	0.05	0.05
Threonine	0.09	0.07	0.06
Salt	0.18	0.22	0.22
Sodium bicarbonate	0.18	0.17	0.14
Broiler fat	0.72	1.17	1.44
Methionine	0.30	0.24	0.22
Lysine	0.46	0.37	0.35
Proximate composition (%)			
Dry matter	88.11	87.86	87.83
Crude protein	18.85	17.72	16.70
Total fat	3.50	3.99	4.40
Crude fiber	4.10	3.13	3.07
Total ash	5.80	4.61	4.37
Starch	45.46	44.31	45.47
Carbohydrates	2.92	3.03	2.92
Vitamins	7.46	11.07	10.91
ME (kcal/kg)	2915	2915	3130

the feet, head, and neck were removed and evisceration was performed manually. Edible viscera (giblets) were individually packed in numbered plastic bags for subsequent weighting. Carcass weight was measured immediately after evisceration (warm carcass weight), then they were placed in a refrigeration tunnel until they reach a temperature of 4°C for cooling and dripping. Afterwards, carcasses were weighted (cold carcass weight) and cut into parts (leg, wing, breast, and carcass remnant; EC regulation 543/2008, European Union, 2008). The breast was separated from the back at the shoulder and along the junction of the vertebral and sternal ribs. Afterwards, in the day after slaughter, in the laboratory, the *pectoralis major* was used to measure pH and color parameters. The wings were separated from the carcass at the shoulder. The legs were disarticulated at the hip (iliofemoral) joint, whereas the right leg was divided into drumstick and thigh at the tibiofemoral joint.

The left leg and left breast portions were individually packed, stored under refrigeration (< 4°C) and transported to the laboratory, where both meat portions were trimmed of skin, bones, and major visible fat and connective tissues. The muscle portion was then homogenized in a domestic food processor (Moulinex, France) vacuum packed and stored in the freezer (–20°C) until analysis.

Right humerus and the larger right leg bones (femur and tibia) were separated from their muscles. The bones were then macerated in water for sufficient time to facilitate the removal of the remaining tissues. After drying for 5 d at 40°C, the bones were weighed and measured: length and width of diaphysis.

Proximate Analysis

Homogenized meat samples from boneless and skinless breast and leg portions from each individual bird were used to estimate their proximate composition, according to the AOAC (2000) methods. Moisture (950.46), total protein (981.10), ether extract (991.36), and ash (920.153) contents of meat homogenates were analyzed in duplicate.

Testosterone Serum Concentration

Regarding blood collection for serum separation, blood was collected immediately after manual exsanguination, by placing a collection tube (7.5 mL for serum collection SARSTEDT, Monovette®) against the jugular vein until it was filled. Afterwards, the collection tubes were identified and closed, keeping them at room temperature for 60 min (to allow clot formation), then being refrigerated for 24 h (2 to 5°C.), before serum collection.

Total testosterone measurements were performed in serum, using a solid phase competitive chemiluminescent enzyme immunoassay (Immulite 2000, Siemens).

Color Measurements

The color measurements were carried out with a Minolta CR 300 colorimeter (Konica Minolta Holdings Inc., Tokyo, Japan) with a C illuminant and a 2° standard observer in the CIELAB space, after 1 h of blooming to allow oxygenation. In each sample, color measurements were performed in triplicate, recording lightness ($\mathbf{L}^*$), redness ($\mathbf{a}^*$) and yellowness ($\mathbf{b}^*$). The chroma ($\mathbf{C}^*$) was calculated as $\sqrt{(a^*2 + b^*2)}$, and the hue angle (h°) as $\tan^{-1}$ (b^*/a^*). Breast meat color measurements were taken on the medial surface of the fillet (bone side) in an area free of obvious color defects (bruises, discolorations, hemorrhages, full blood vessels, or any other condition that may have effect on a uniform color reading).

The pH of each sample was measured in triplicate with a HI 99,163 portable pH-meter (Hanna Instruments, USA).

Statistical Analysis

The statistical analysis was accomplished using the MIXED procedure of SAS (SAS Institute, Cary, NC), version 9.3. The model considered a single effect (experimental group). Least squares means (**LSMeans**) were presented and compared, using the LSD test, when differences between experimental groups were statistical significant ($P < 0.05$). LSMeans and Residual Standard Deviation (**RSD**) are presented in the results.

Table 2. Data on serum testosterone levels (ng/100 dL) observed within experimental groups.

Testosterone	Capon	Improvac	Slip	Rooster
n	7	15	8	14
Average	ND	28.3[b]	51.9[b]	134.7[a]
Range[1]	ND	20.1–32.5	25.9–81.8	56.5–342

ND, not determinate; testosterone concentration below the lower limit of detection (<20 ng/100 ng/dL).

[1]range of values between minimum and maximum serum testosterone levels within experimental groups.

Different superscripts in the same row diverge significantly ($P < 0.05$).

RESULTS AND DISCUSSION

The study was performed within the production facilities and with minimum interference at the production level. Surgical caponization was performed by the production technicians, following practices used in traditional capon production, without any intervention of the research team.

The unexpected occurrence of slips among birds from the capon group(8 out of 15) reveal the difficulties associated with capon production at commercial level, that is: 1) at farm level and with the intensity required to accomplish the early caponization of a full commercial flock (4,800 birds) in a relatively short period of time (1 wk), it is difficult to achieve the required accuracy necessary to perform caponization; 2) at farm level and at slaughter there is no commercial differentiation between capons and slips, therefore, capons reaching the market enclose slips, which confers the product an undesirable variability. Such a situation appears to be a reality among industrial capon production, but does not seem to occur among traditional capon production (as observed on Capão de Freamunde-PGI), where caponization is performed at a lower scale and birds are submitted to a physical appraisal before their sale, since commercially they are sold alive.

Testosterone, primary male sexual hormone, is produced by Leydig cells in testes, under hormonal control of the hypothalamic-pituitary axis. Therefore immunization against gonadotropin-releasing hormone (**GnRH**) in male broilers should reduce or nullify testosterone production in testes, as previously confirmed on pigs (Brunius et al., 2011) and cattle (D'Occhio et al., 2001). The assumption that Improvac, a vaccine developed for immunocastration of pigs would provide equal effect on broilers is based on the knowledge that GnRH has been highly conserved throughout evolution (Dubois et al., 2002).

Serum testosterone concentration

The serum testosterone concentration (expressed as ng/dL) for birds belonging to different experimental groups is presented in Table 2. Detectable testosterone was measured in all birds with the exception of the capons (n = 7) in which the serum testosterone concentration was below the lower limit of detection (20 ng/dL).

The averaged serum concentration of roosters (135 ng/dL) was significantly higher ($P < 0.01$) than was obtained on slips (52 ng/dL) and in birds belonging to the Improvac group (28 ng/100 ng/dL). Nevertheless, no significant differences ($P > 0.05$) were observed on serum testosterone concentration between birds injected with Improvac and slips.

Testosterone concentration quantified in roosters is in the range of values previously presented for other strains and within the variability observed among birds from the same group (Sexton et al., 1989; Lin et al., 2012). Data on serum testosterone was consistent with the observations at slaughter, at 2 levels: 1) absence of visible testicular masses was associated with a serum testosterone concentration below the lower limit of detection (20 ng/dL); 2) different testicular masses with variable size observed on slips was in agreement with the fluctuation observed on serum testosterone levels (25.9 to 81.8 ng/dL). In other studies, caponization was associated with a significant decline in serum testosterone concentration (Lin et al., 2012), but quantification was still possible, which was not the case in our study. Such apparent discrepancy could be a consequence of different genetic background between broilers used in these studies. We found 2 possible explanations for such discrepancy: 1) it may rely on the phenotypic variability of slips, as some of these birds resembled capons and their differentiation was only achieved due to the full monitoring of the slaughter process, which could have not been executed in other studies; 2) different testosterone quantification assays used in different studies may be associated with different accuracy and detection threshold, which may have contributed to the differences observed between studies.

The administration of Improvac was associated with a considerable reduction on serum testosterone concentration (reduced by 79%) compared to the average serum testosterone concentration of roosters, still, such reduction in the testosterone serum concentration occurred without testicular mass reduction. Nevertheless, it demonstrates that immunization against GnRH can be achieved using Improvac, at least partially, in roosters as it is accomplished in pigs and cattle. To the best of our knowledge, this is the first study performed to test the immunocastration of male broilers with Improvac. Therefore, the protocol of administration and dosage tested in this study could be improved in subsequent studies.

Additional important data recorded in this study was the degree of aggressiveness that diverged between groups. The aggressive behavior of roosters was responsible for mortality throughout the growing period (16 of 30 roosters died in this period). Among survivors, the aggressive behavior was also evident at slaughter, since the absence of feathers in most of the neck revealed clear signs of fighting, as did edema, bruises, and scars at the neck and also in the comb, which were not observed in

Table 3. Live body weight, warm and cold carcass weights, body portions, and giblets weight (g), and yield (%) for different experimental groups.

					Statistics	
	Capon	Improvac	Slip	Rooster	RSD	*P*
n	7	15	8	14		
Live body weight[1]	4,865.1	5,011.3	4,747.0	4,605.6	573.6	0.30
Warm carcass weight	3,961.9	4,085.5	3,881.5	3,818.4	468.8	0.48
Cold carcass weight	3,942.6	4,077.5	3,865.4	3,808.1	464.9	0.46
Meat portions and giblets weight						
Wing	370.1	382.0	384.6	371.2	42.6	0.82
Leg	1,195.6	1,255.1	1,139.6	1,162.4	141.8	0.22
Breast	761.3	760.0	748.3	688.9	122.9	0.40
Abdominal fat pad	183.3[a]	137.9[a, b]	118.8[b, c]	92.92[c]	43.1	<0.001
Heart	25.9	28.6	24.1	27.6	3.77	0.05
Liver	53.0	52.1	48.1	53.9	7.70	0.40
Gizzard	44.7	52.5	45.8	50.6	7.51	0.08
Yield (%)[2]						
Wing	9.47	9.38	10.1	9.82	0.99	0.37
Leg	30.3	30.8	29.5	30.6	1.15	0.07
Breast	19.3	18.6	19.3	17.9	1.45	0.12
Abdominal fat pad	4.66[a]	3.35[b]	3.16[b, c]	2.32[c]	1.03	<0.001
Heart	0.66	0.70	0.63	0.73	0.01	0.18
Liver	1.34	1.27	1.25	1.44	0.24	0.19
Gizzard	1.13	1.30	1.21	1.33	0.21	0.18

RSD = Relative standard deviation.
Different superscripts in the same row diverge significantly ($P < 0.05$).
[1]Live body weight obtained immediately before slaughter.
[2]expressed as percentage of cold carcass weight.

other birds from the flock. It is therefore important to highlight that in roosters, aggressive behavior was responsible by the death of 53% of the roosters, which occurred primarily within the last month of the study.

Body Weight, Carcass Weight, Carcass Parts and Edible Viscera Weights

Data on live body weight at slaughter, warm and cold carcass weights, and the weight and yields of meat portions and giblets for all experimental groups is depicted on Table 3. No significant differences ($P > 0.05$) were observed among experimental groups on live body weight (averaging 4,807 g), warm and cold carcass weights (averaging 3,937 and 3,923 g, respectively). Warm carcass and cold carcass weights represent 81.9% and 81.6% of live body weight, wipe loss represented 1.6% of overall weight loss. The carcass yield obtained in this study was higher than was previously reported in roosters and capons of Castellana Negra ((72 to 74%) and Penedesenca Negra (73 to 78%) breeds, Spanish autochthonous breeds (Tor et al., 2002; Miguel et al., 2008), and even higher than was previously observed on Redbro capons and roosters (73 and 75% respectively) (Symeon et al., 2010).The higher carcass yield observed in this study could be associated with the slaughter age; birds in our study were slaughtered at 22 wk of age, while birds from other studies were slaughtered between 24 and 29 wk old. Older birds tend to accumulate higher fat depots; however, abdominal fat pad contribute to an increased live weight but are not included in carcass weight, thus reducing the carcass yield.

No significant differences were observed among experimental groups ($P > 0.05$) on weights and yields of prime meat portions (wing, breast, and leg) and giblets (heart, liver, and gizzard), but significant differences were observed on abdominal fat pad weight and yield ($P < 0.001$). Capons displayed significantly ($P < 0.001$) higher abdominal fat pad weight and yield (183 g and 4.6% of carcass weight) than slips (119 g and 3.2% of carcass weight) and roosters (93 g and 2.3% of carcass weight). Complete caponization has contributed to the accumulation of almost twice the amount of abdominal fat pad stored by roosters, while incomplete caponization resulted in 28% increase in the amount of abdominal fat accumulated, comparatively to roosters. On the other hand, Improvac administration resulted in 48% increase in the amount of abdominal fat pad stored, compared to roosters. Therefore, birds from the Improvac group displayed the second heaviest fat pad, presenting an amount of abdominal fat midway between capons and roosters, not differing significantly from capons and slips ($P > 0.05$), but diverging significantly from roosters ($P < 0.05$).

Data previously presented revealed that growth occurred evenly and in a similar pattern in all experimental groups, while differences observed on abdominal fat pad weight, revealed that lipid deposition in the abdominal cavity occurred in a different rate. It is possible to observe an inverse relation between abdominal fat weight and serum testosterone concentration, which is in agreement with results previously described among roosters, slips, and capons (Chen et al., 2006b; Sirri et al., 2009). Such results may be explained by the negative correlation observed between plasma

Table 4. Bone parameters (weight, length and width) for major long bones (Humerus, Femur and Tibia) for different experimental groups.

						Statistics	
		Capon	Improvac	Slip	Rooster	RSD	*P*
Humerus	Weight (g)	8.319	9.046	9.221	9.511	1.47	0.36
	Length (mm)	94.36	94.51	97.39	96.94	4.04	0.21
	Width (mm)	8.717	8.596	8.710	8.763	0.62	0.93
	Weight/Length	0.086	0.095	0.094	0.096	0.014	0.41
Femur	Weight (g)	3.080	3.088	3.041	3.250	0.39	0.70
	Length (mm)	96.42^{c}	$97.15^{b, c}$	$99.83^{a, b}$	100.86^{a}	4.02	<0.001
	Width (mm)	12.61	14.04	14.76	16.26	1.65	0.06
	Weight/Length	0.032	0.031	0.031	0.031	0.003	0.85
Tibia	Weight (g)	4.48	4.32	4.16	4.33	0.90	0.71
	Length (mm)	155.55	145.25	161.03	162.87	23.2	0.77
	Width (mm)	18.34	19.69	20.14	20.01	4.24	0.29
	Weight/Length	0.030	0.048	0.025	0.026	0.004	0.47

RSD = Relative standard deviation.
Different superscripts in the same row diverge significantly ($P < 0.05$).

Table 5. Proximate composition (g/100 g edible portion) of breast and leg meat portions for different experimental groups.

					Statistics	
	Capon	Improvac	Slip	Rooster	RSD	*P*
Breast						
Water	70.83^{c}	72.07^{a}	$71.06^{b, c}$	$71.87^{a, b}$	0.93	0.03
Protein	25.93^{a}	24.79^{c}	$25.71^{a, b}$	$25.05^{b, c}$	0.61	<0.001
Intramuscular fat	2.14	1.97	2.09	1.93	0.96	0.52
Ash	1.09	1.17	1.14	1.15	0.10	0.44
Leg						
Water	70.41	71.42	71.18	71.93	1.90	0.39
Protein	19.06	19.41	19.81	19.70	0.99	0.41
Intramuscular fat	9.53	8.18	8.05	7.38	2.49	0.31
Ash	0.99	0.99	0.96	0.98	0.06	0.94

RSD = Relative standard deviation.
Different superscripts in the same row diverge significantly ($P < 0.05$).

testosterone concentration and the activity of major hepatic lipogenic enzymes (Chen et al., 2006b).

Bone Characteristics

Capons are normally raised throughout a long period, during which they achieve a considerable live weight. Therefore, they require a strong skeleton to support the increasing body mass. It is known that androgens play an important role in bone development (Chen et al., 2006a); therefore, caponization is associated with a significant loss of bone weight and bone mineral content (Lin et al., 2012).

The weight, length, and width of humerus, femur, and tibia are shown on Table 4. The results revealed that femur length was the only parameter presenting significant differences between experimental groups. Roosters presented a longer femur than capons ($P < 0.05$), while slips displayed an intermediate length, not diverging significantly from them ($P > 0.05$). On the other hand, the femur from birds belonging to the Improvac group revealed an intermediate length in between capons and slips not differing significantly from them ($P > 0.05$). Despite the absence of significant differences on femur weight and width ($P > 0.05$), femur width revealed a statistical tendency for difference ($P = 0.06$). The results presented herein show a direct relation between serum testosterone concentration and femur length, which is in agreement with the results previously presented by others, since caponization has been associated with decreased breaking strength, reduced cortical thickness, lower bone ash content, and lower calcium, phosphorus, and magnesium contents (Lin et al., 2012).

Proximate Composition

The proximate composition of breast and leg meat portions is depicted in Table 5. On breast meat significant differences between experimental groups were limited to water and protein content of breast meat. On the other hand, no significant differences were observed on leg meat portion ($P > 0.05$). The comparison of breast meat portion from capons, slips, and roosters shows that capons displayed the highest protein content (25.9 g/100 g of edible portion) and lowest water content (70.8 g/100 g of edible portion), diverging significantly from roosters (25.1 and 71.9 g/100 g of edible portion for protein and water content, respectively), while slips presented protein and water content

Table 6. pH values and colorimetric parametersfor different experimental groups.

					Statistics	
	Capon	Improvac	Slip	Rooster	RSD	*P*
pH_{24}	5.67	5.65	5.67	5.67	0.09	0.82
Lightness (L*)	56.06[a]	53.71[b]	55.66[a, b]	53.53[b]	2.17	0.003
Redness (a*)	1.80[b]	2.75[a]	1.66[b]	3.08[a]	0.91	<0.001
Yellowness (b*)	3.28	2.33	2.39	1.54	1.64	0.057
Chroma (C*)	4.04	3.82	3.09	3.63	1.33	0.364
Hue (h°)	60.69[a]	36.91[b, c]	51.78[a, b]	26.34[c]	19.7	<0.001

RSD = Relative standard deviation.
Different superscripts in the same row diverge significantly ($P < 0.05$).

between capons and roosters, not differing significantly from them ($P > 0.05$). No significant differences were observed among experimental groups on breast lipid and ash contents ($P > 0.05$); still, it was observed an inverse association between serum testosterone concentration and lipid content. Breast meat from the Improvac group displayed, in all the parameters, intermediate values between capons and slips.

The results on meat proximate composition (breast and leg meat portions) is in agreement with other studies previously presented in Portugal for the traditional capon of Freamunde (Brito et al., 2009) and elsewhere (Sirri et al., 2009; Volk et al., 2011). Differences observed on proximate composition between breast and leg meat portions revealed differences in accordance with data published on this subject (Brito et al., 2009; Sirri et al., 2009b; Volk et al., 2011). However, there is a considerable heterogeneity of results; some studies observed no significant differences in meat proximate composition between roosters and capons in both breast and leg meat portions (Volk et al., 2011), while in others, significant differences in both breast and leg meat portions were found on total lipid and ash content (Sirri et al., 2009). Different studies enclose different breeds, different diets and feeding management, different caponization and slaughter ages, which may be responsible for differences observed between studies.

Physico-Chemical Attributes and Colorimetry

No significant differences on breast meat pH values were observed between groups (Table 6), revealing a quite constant pH value (5.65 to 5.67) that was below the pH values previously reported on breast meat from both capons and roosters (5.71 to 5.88) (Sirri et al., 2009; Volk et al., 2011). Differences on breast meat pH value between studies suggest different muscular glycogen content at slaughter time, which may be consequence of several variables that were not within the scope of this study.

Color is the prime characteristic of meat that is evaluated by consumers at the point of sale. Defined by Commission Internationale de l'Eclairage (**CIE**), the CIELAB color coordinates is an objective and quantitative method available to measure color. In the CIELAB color space, L* indicates lightness value, a* is the red/green coordinate, and b* is the yellow/blue coordinate. The chromaticity coordinates can also be used to calculate the hue angle (h°) and chroma (C*). The hue angle corresponds to what is commonly called color, being function of the wavelength of reflected light, while chroma indicates how pure the color is, i.e., the degree of deviation for gray (Serpil Sahin, 2006). The hue angle depends mainly on pigment content and chemistry (Renerre, 2000), while chroma indicates the perceived intensity of a determined color and depends mostly on the myofibrillar structure and on the ultimate meat pH (Renerre, 2000).

Breast meat lightness (L*), redness (a*) and the hue angle (h°) diverged significantly between groups ($P < 0.05$), while yellowness (b*) revealed a statistical tendency ($0.05 < P < 0.10$). Rooster and capons revealed themselves as the most different groups among all in comparison, diverging significantly on L*, a* and h°, while slips presented intermediate values of L* and h°. On the other hand, birds submitted to the Improvac protocol presented the L*, a* and h° values similar to those presented by roosters, not diverging significantly from them ($P > 0.05$). Therefore, according to CIELAB color evaluation, meat from birds belonging to the Improvac group was closer to rooster than capon meat.

The comparison of our results with others revealed higher values of L*, similar values of a* and lower values of b* (Sirri et al., 2009; Volk et al., 2011). Despite differences between studies they share a similar pattern, that is, when compared with roosters, capons displayed higher values of L*, b* and h° and lower values of a*.

In chickens, meat color is significantly influenced by heme pigments; a negative correlation has been established between total heme pigments concentration and L*, while there is a positive correlation between total heme pigments concentration and a* (Sirri et al., 2009), suggesting that testosterone has a positive influence on meat heme pigments, which is in agreement with the results presented here.

CONCLUSION

Improvac administration was associated with a considerable reduction on serum testosterone concentration (reduced by 79% comparatively to the average serum

testosterone concentration of roosters), revealing that immunization against GnRH can be achieved, at least partially, in male broilers.

Improvac administration was associated with increased homogeneity and higher live weight comparatively with roosters and capons, and it was also associated with a nearly 50% increase in the amount of abdominal fat pad stored, compared to roosters.

Regarding the parameters associated with significant differences between groups, birds from the Improvac group displayed results between capons and slips concerning abdominal fat pad weight and yield, breast meat content of water and protein, and femur length. On the other hand, with respect to color parameters, data on lightness, redness, and hue angle fell between those of roosters and capons.

The study presented herein shows, for the first time, that immunocastration with Improvac could represent an alternative solution to caponization, with considerable improvements regarding animal welfare.

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