PHYSIOLOGY AND REPRODUCTION

Improvac immunocastration affects the development of thigh muscles but not pectoral muscles in male chickens

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ABSTRACT Improvac has been tentatively used to immune-castrate roosters. The aim of this study was to investigate whether Improvac affected skeletal muscle development in chickens. The muscle fiber type and size and the expression levels of genes related to muscle development in pectoral and thigh muscles were examined at 5, 9, and 14 wk of age in the control, early, late, and early + late Improvac-treated groups. Immunocastration with Improvac affected the development of thigh muscles and the expression of *MYH1B*, *MSTN,* and *SM.* The cross-sectional area in the early group was significantly larger than in the control group at the 14th week ($P < 0.01$). At the fifth week, the expression levels of *MYH1B*, *MYOD,* and *MSTN* in the early group were significantly higher than those in the control group ($P < 0.05$), and at the ninth week, the expression level of *SM1* in the control group was significantly lower than that in early and late groups ($P < 0.05$). Immunocastration did not affect pectoral muscle development or the expression of genes related to muscle development.

Key words: immunocastration, muscle development, Improvac, broiler chicken, fiber type

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INTRODUCTION

The caponization technique, surgical removal of the testes, has been used since ancient times as a way to produce heavier birds and more savory meat (Gesek et al., 2017). Removal of the testes results in an androgen deficiency and consequent phenotypic and behavioral changes, such as the lack of development of secondary sexual characteristics, and reduced sexual activity and aggressiveness, so that the energy formerly used for fighting was saved for lipid deposition and muscle growth. Caponization increased abdominal, subcutaneous, and intramuscular fat, and consumers showed a preference for castrated broilers (Chen et al., 2000; Tor et al., 2002; Amorim et al., 2016). The traditional surgical castration method was increasingly unsuitable for modern large-scale chicken raising because of its high technical requirements and for animal welfare issues. Vaccination against gonadotropin-releasing hormone (**GnRH**) is regarded as a mild and harmless way to castrate animals immunologically and has now supplanted surgical castration as the preferred method of capon production. A GnRH analog is injected into the animals to produce an immune response against endogenous GnRH, subsequently inhibiting the release of downstream gonadotropins (follicle-stimulating hormone and luteinizing hormone) and suppressing testosterone production as well as reproductive organ development (Brunius et al., 2011). Improvac is one of the immunocastration vaccines developed for boars, but it has also recently been used successfully on roosters (Quaresma et al., 2017; Antunes et al., 2019; Wang et al., 2019). Improvac injection decreased testosterone, inhibited spermatogenesis (Wang et al., 2019), changed fat deposition (Antunes et al., 2019), and improved carcass quality (Quaresma et al. 2017) in male chickens; but the effects of immunocastration with Improvac on muscle development have not been investigated. We hypothesized that muscle development would be hindered by a drop in testosterone levels caused by Improvac injection.

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Skeletal muscle accounts for 40 to 50% of the BW and contributes to the regulation of metabolic homeostasis. It consists of various types of myofibres with different metabolic profiles, contractile properties, as well as biochemical and biophysical characteristics, such as fibre size, color, glycogen, and lipid content (Li et al., 2007). The metabolic characteristics of pectoral and thigh muscles have a pronounced effect on the weight and quality of broilers. Poultry scientists have been studying the relationship between muscle fiber type and meat quality since the 1970s. and the types of fibers in the main muscle groups of chickens have been identified by histochemical methods (Barnard et al., 1982). There are fast-contracting muscles and slow-contracting muscles, and the contractile properties of muscle fibers depend on the type of myosin heavy-chain (**MyHC**) proteins expressed. In avian skeletal muscle, type I corresponds to slow-contraction muscles and types IIa and IIb correspond to fast-contraction muscles.

Most researchers have studied the effects of castration on the chicken carcass quality. As far as we know, there have been only 2 reports on the effect of castration on the muscle fiber types of chickens, and they used surgical castration and only looked at pectoralis major muscles. The change to chicken muscle fiber type after immunocastration has not been explored yet, and the expression levels of the genes related to muscle development, *MYOD*, *Myf5*, *MSTN* and *MYHC*, have not been measured. Our previous study with chickens demonstrated that Improvac could effectively decrease the concentration of serum testosterone and inhibit spermatogenesis. The aim of this study was to investigate if this change would affect the muscle fiber size, muscle fiber type, and expression levels of genes related to muscle development.

MATERIAL AND METHODS

Animals and Immunocastration

A total of 120 (2-week-old) male broiler chickens (*Tianfu* chicken, TF02 strain, a high-quality broiler strain) were maintained at the poultry farm of Sichuan Agricultural University, Yaan, China. The birds were randomly divided into the following 4 groups (n = 30 each): control, early, late, and early + late groups. All birds were raised in separate compartments within the same poultry shed with a density of 2 birds/m^2. All birds were subjected to a similar feeding regimen as well as environmental and sanitary conditions. Feeding methods and feed formula are shown in the Table 1 (Wang et al., 2019). The protocols for all animal experiments were approved by the Animal Welfare Committee of Sichuan Agricultural University (approval code: S20174225), and all methods strictly obeyed the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010).

The vaccination schedules and sampling for all 4 groups are shown in Figure 1. The birds were injected subcutaneously at the back of the neck with 0.6 mL of Improvac (Pfizer, Switzerland) or saline on the third and/or sixth week after hatching. The birds were euthanized under anesthesia with sodium pentobarbital as per the Guide for the Care and Use of Agricultural Animals in Research and Teaching using the most humane method possible to avoid panic and discomfort. The pectoral and thigh muscles were collected at 5, 9, and 14 wk of age.

Table 1. Composition of starter and grower feed.

Composition (%)	Starter	Grower
Total Fat ≥	2.5	3.0
Total protein ≥	19.0	15.0
Total fiber ≥	8.0	8.0
Ash ≥	9.0	9.0
Ca	0.80–1.20	0.70–1.10
P	0.55	0.50
NaCl	0.30–0.80	0.30–0.80
Methionine ≥	0.41	0.30
ME (MJ/kg) ≥	11.71	12.13

All birds were fed with the commercial starter diet (crumbs) from week 1 to 3 and were fed with the grower pellet diet from week 4 to 14.

The pH and Color of Muscle

Meat color was estimated on the pectoralis major muscle and thigh muscles using the lightness (L*), redness (a*), and yellowness (b*) system with a CR-300 color analyzer (Minolta, Japan).This system of color identification used the coordinates, L*, a*, and b*. In each sample, color measurements were performed in triplicate on freshly cut surfaces immediately after carcass dissection. The color values were expressed as L* (lightness), a* (redness/greenness), and b* (yellowness/blueness). From these values, chroma and hue angle were calculated relative to the reference values (R. W. G. Hunt and Pointer, 2011). The pH of each sample was measured in triplicate with a PH-STAR pH meter (Matthaus, German).

Assessment of Myofiber Development

Muscle tissue samples were collected from the left pectoral and thigh of broiler chickens at 5, 9, and 14 wk of age. The tissue samples were fixed in 10% formalin in PBS at room temperature for 3 D and then dehydrated, embedded, and sectioned (thickness = 4 μm) for routine H&E staining (Wang et al., 2017). The average cross-sectional area **(CSA)** was measured using Image-Pro Plus, and a minimum of 100 fibers were counted in each muscle section (Gesek et al., 2017; Gesek et al., 2018). The densities of fibers were also measured using the formula:

$$\text{Density} = \frac{\text{Number of myofibres in one muscle bundle}}{\text{Area of this muscle bundle}}$$

Identification of Muscle Fibre Type by Immunofluorescence

Portions of the left pectoral and thigh muscle tissue samples were embedded in Tissue-Tek O.C.T.

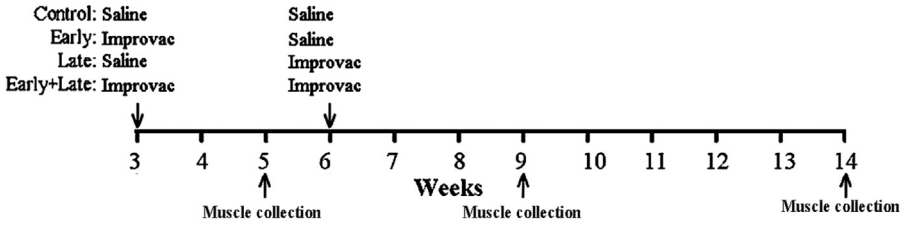

Figure 1. Time schedule for experimental design and sampling. The roosters were injected with saline and/or Improvac at the third and/or sixth week after hatching, and the thigh and pectoral muscles were collected at the fifth, ninth, and 14th week after hatching.

(Richard-Allan Scientific) and then frozen in a bath of isopentane with dry ice. The frozen tissue was sectioned at a thickness of 15 μm for immunofluorescence to measure the muscle fiber type. Refer to the previous report for detailed steps (Zhang et al., 2013; Wang et al., 2017). The sections were incubated with primary antibody (1:400 dilution) of fast MyHC (Abcam ab51263, UK) at 4°C overnight. The slides were washed 3 times with PBS for 15 min and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibody (1:1,000 dilution) (Proteintech, SA00003-1) for 1 h.

Total RNA Extraction and cDNA Synthesis

Total RNA was extracted from muscle tissue using TRIzol reagent (InvivoGen, Shanghai, China). The tissue was transfered into lysing matrix tubes (MP Biomedicals) containing 1 mL TRIzol reagent. The tubes were then ground for 30 s using a tissue-crushing apparatus MP FastPrep-24 (MP Biomedicals). Total RNA was extracted as per the manufacturer's protocol. The RNA integrity was assessed by electrophoresis on a 1% agarose gel containing formaldehyde. The RNA concentration was measured using a Beckman DU-640 spectrophotometer (Beckman). The total RNA samples were purified and subjected to reverse transcription using the Takara PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Dalian, China) and processed for cDNA synthesis as per Takara PrimeScript RT instructions (Zhang et al., 2013).

Gene Expression by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The relative expression levels of genes related to muscle MyHC type and muscle development in pectoral and thigh muscle from 5-, 9- and 14-week-old broiler chickens were analyzed by RT-PCR, which was performed in a 10 μL reaction mix containing 1 μL 2 × SYBR Premix Ex Taq II (TakaRa, Dalian, China), 3 μL dH_2O, 0.5 μL of the upstream and downstream primers, and 1 μL cDNA using a Bio-Rad CFX-96 thermocycler (Bio-Rad, CA). The reaction conditions were as follows: an initial denaturation at 95°C for 30 s and 44 cycles of amplification at 72°C for 30 s. The annealing was carried out for 40 s at temperatures specific for each target gene (Table 2). At the end of the amplification, step-wise melting curves were performed to confirm the product specificity. The cytoskeletal protein, β-actin, was used as the internal reference (Wang et al., 2017).

Data Processing and Statistical Analysis

For qRT-PCR, the control group was used as the reference group, and the relative expression levels of the genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Zhang et al., 2012). All values were expressed as the mean ± SE. Comparisons of the Improvac groups (early, late, early + late) and control group were analyzed by Student t test, and a P value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Physical Attributes

Forty minute and 5 h after euthanizing, the pectoralis major and cranial thigh muscle, respectively, was used to measure pH value and color parameters. The effect of Improvac vaccination and age on physical composition of the meat can be seen in Table 3. The pH values of pectoral and thigh muscles were not significantly ($P > 0.05$) influenced by Improvac, which agrees with the results of a previous report (Quaresma et al., 2017) and also with previous studies of capons and roosters showing that caponization had no effect on pH (Lin and Hsu, 2002; Symeon et al., 2012; Amorim et al., 2016).

Meat color and appearance are important considerations for consumers when they are choosing broilers to purchase. In this study, we compared the lightness (L*), redness (a*), yellowness (b*), chroma (C*), and hue angle (h°) of pectoral and thigh muscle of 14-week-old chickens. No differences ($P > 0.05$) were observed in any of the color parameters between the Improvac groups and controls, contrary to those reported by Quaresma et al. (Quaresma et al., 2017) who observed a higher hour° value in Improvac-treated chickens than roosters, but found no differences in the other color parameters, perhaps because they used a different breed of chicken. In general, Improvac vaccination had little influence on meat color, but surgical caponization significantly affected meat color of broilers as per other reports. A study from Miguel et al.(Miguel et al., 2008) revealed that the flesh of capons has lower redness (a*) than roosters' flesh. Several

Table 2. Primers used for qRT-PCR.

Gene symbol	Sequence (5′-3′)	Ta(°C)	Product size (bp)	Accession number
SM	F: CCA AGA AGG CCA TCA CTG R: GAT GGT CTG CTC CAT GTT	60	107	XM_424041.4
FRM(MYH1B)	F: AAC CTC ACC AAG TTC CGC AAG R: CCA TGA AAC TCC CGG CTC TT	61	113	NM_204228
FWM(MYH1F)	F: TGC CTC AGG TCA CAC TTT AGC R: AGC TGT CCA ATG TCA ACC TTT CC	62	180	NM_001319015.1
MYoD	F: ATC ACC AAA TGA CCC AAA GC R: GGG AAC AGG GAC TCC CTT CA	60	149	FJ977569.1
Myf5	F: ACT CCC CAA AGT GGA GAT CCT G R: CAG TCC GCC ATC ACA TCG	60	159	NM 001030363.1
MSTN	F: AGC GGG TAG CGA CAA CAT C R: GCT TTT GAT GAG ACT GGA CGA G	60	173	GU181328.1
β- actin	F: GGC TGT GCT GTC CCT GTA TGC R: CTC TCG GCT GTG GTG GTG AAG	60	207	NM_205518.1

Abbreviations: F, sense primers; qRT-PCR, quantitative real-time polymerase chain reaction; R, antisense primers; Ta, annealing temperature.

studies showed similar color changes in flesh from roosters after surgical castration, with an increase in L*, b* and h° and a decrease in a* (Lin and Hsu, 2003; Symeon et al., 2010; Symeon et al., 2012; Quaresma et al., 2017). These data indicate that Improvac immunization has less effect on meat color than surgical caponization.

Myofiber Development

The light photomicrographs of H&E staining of pectoral and thigh muscles can be seen in Figures 2 and 3. In general, the pectoral and thigh muscles at 5 and 9 wk of age showed no differences in CSA between controls and Improvac-treated animals (Figures 4A, 4C) ($P > 0.05$). At 14 wk of age, however, the CSA of thigh muscles from the early Improvac group were significantly larger than those of the control group (Figure 4C) ($P < 0.01$). No difference was observed in the density of pectoral and thigh muscles (Figures 4B, 4D) ($P > 0.05$). Although several studies confirmed that caponization decreased the diameter of chicken skeletal muscle compared with noncastrated roosters (Lin and Hsu, 2002; Gesek et al., 2017; Gesek et al., 2018), these changes mainly occurred from 20 to 28 wk of age. In our previous study, we found that Improvac could inhibit the secretion of testosterone. And, at the age of 14 wk, the concentration of serum testosterone in the early group was significantly lower than in the control group (Wang et al., 2019), whereas the CSA in the early group was significantly higher than in the control group. Some reports indicated that androgen suppressed the muscle growth of chickens (Chen et al., 2010). Fennell et al. (Fennell et al., 1996) found that testosterone reduced the skeletal muscle growth of young chickens, suggesting that immunocastration could result in a larger volume of thigh muscle.

To understand the effects of Improvac immunocastration on the fiber types in pectoral and thigh muscle, sections were stained for immunofluorescence with the antifast myosin skeletal heavy-chain antibody. For better observation and calculation, we used pectoral muscle, which is composed of a single type of fiber. We also analyzed the thigh muscle that is composed of multiple fiber types. All the fibers in the pectoralis major belong to the fast-twitch type, and Improvac treatment did not change the percentage of fast-twitch muscle of pectoralis or thigh (Figure 5). Gesek et al. analyzed the effect of caponization on pectoral muscle fiber type in different breeds of chickens (Gesek et al., 2017; Gesek et al., 2018) and found that all fibers were type IIB, which is one kind of fast-twitch muscle. Our results also agree with those of Smith et al. (Smith and Fletcher, 1988) and Chiang et al. (Chiang et al., 1995).

Table 3. The pH values and colorimetric parameters of skeletal muscle from 14-week-old chickens.

Sampling part	N = 10	pH (40 m)	pH (5h)	L*	*a**	*b**	C*	h°
Pectoral muscles	C	6.59 ± 0.18	6.36 ± 0.11	49.26 ± 0.56	5.07 ± 0.92	-1.28 ± 0.51	5.60 ± 0.83	-2.82 ± 1.39
	E	6.57 ± 0.09	6.08 ± 0.09	49.82 ± 0.28	3.81 ± 0.26	-2.91 ± 0.95	5.35 ± 0.64	0.37 ± 0.82
	L	6.31 ± 0.08	5.97 ± 0.11	51.11 ± 0.45	3.63 ± 0.33	-1.36 ± 0.74	4.58 ± 0.27	-1.58 ± 0.39
	E + L	6.51 ± 0.09	6.16 ± 0.06	49.79 ± 0.72	4.36 ± 0.36	-2.93 ± 0.28	5.26 ± 0.45	-1.3 ± 0.07
Thigh muscles	C	6.72 ± 0.04	6.63 ± 0.03	54.43 ± 0.83	7.76 ± 0.61	-1.99 ± 0.94	8.55 ± 0.60	-1.10 ± 0.69
	E	6.78 ± 0.04	6.62 ± 0.05	53.14 ± 0.97	6.45 ± 0.22	-1.84 ± 0.56	7.17 ± 0.30	-1.89 ± 0.51
	L	6.77 ± 0.08	6.67 ± 0.02	57.05 ± 0.85	7.13 ± 0.54	-2.30 ± 0.20	7.53 ± 0.54	-3.24 ± 0.36
	E + L	6.70 ± 0.03	6.54 ± 0.02	51.27 ± 1.21	6.84 ± 0.52	-3.64 ± 0.77	7.97 ± 0.71	-0.13 ± 0.91

Abbreviations: a*, redness; b*, yellowness; C, control group; C*, chroma; E, early group; h°, hue angle; L, late group; E + L, early + late group; L*, lightness.

Values were expressed as the mean ± SD.

$C^* = \sqrt{a^{*2} + b^{*2}}$, $h^\circ = \arctan\frac{b^*}{a^{*\circ}}$

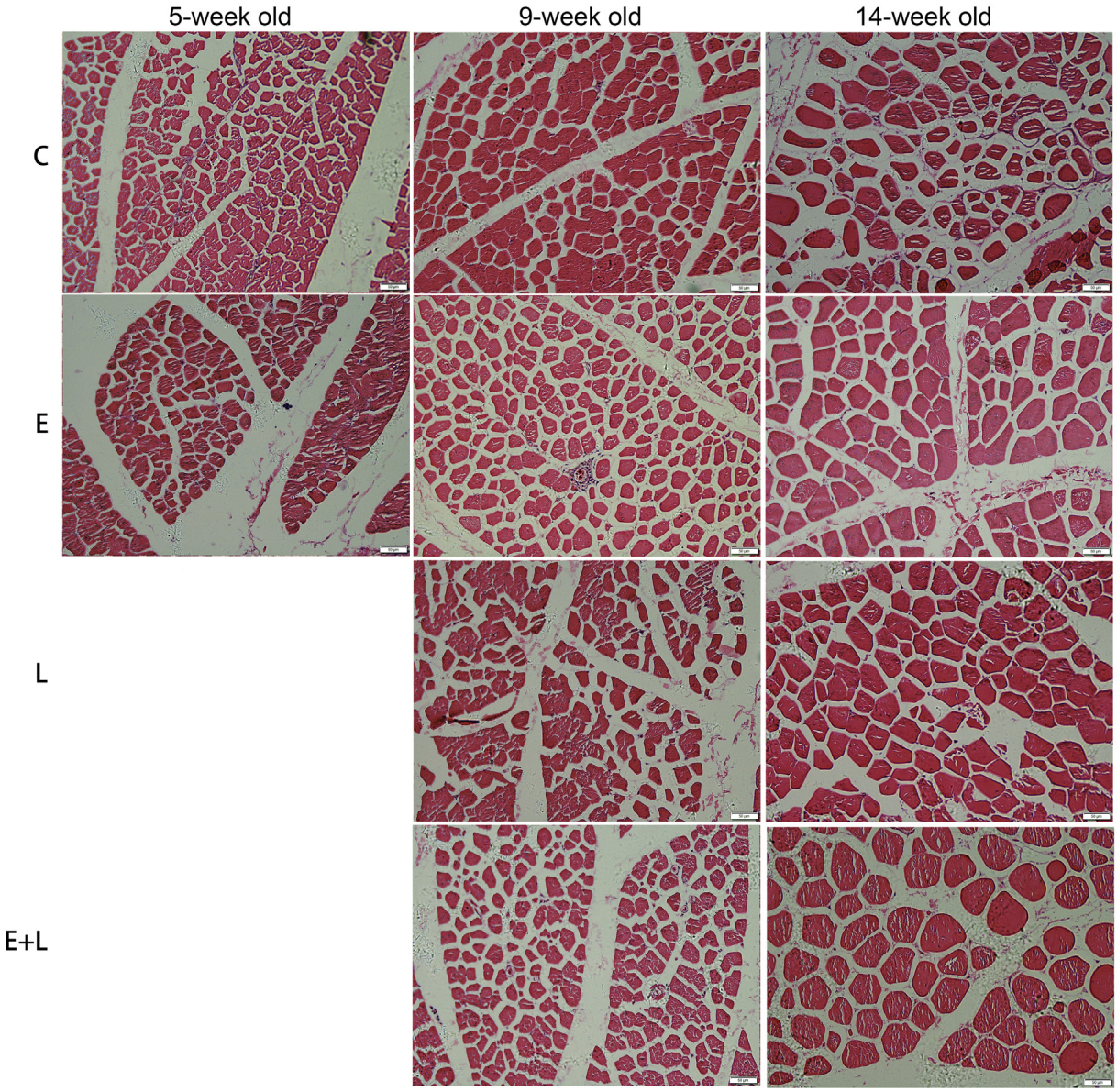

Figure 2. The light photomicrographs of H&E staining of pectoral muscles. Abbreviations: C, control group; E, early group; L, late group; E + L, early + late group. Scale bar = 50 μm.

These reports all indicated that the sex of chickens had no influence on the proportion of muscle fiber types.

Expression of Genes Related to Muscle Development and Fibre Type

The relative expression of genes involved in muscle development, *MYOD, Myf5,* and *MSTN,* is shown in Figure 4. There were no significant differences in the expression levels in pectoral muscle (Figures 6A–6C; $P > 0.05$). In thigh muscles, the expression level of *MSTN* and *MYOD* in the early group was significantly higher than in the controls ($P < 0.05$) at 5 wk (Figure 6D). However, no difference was observed in the expression level of *Myf5*. This result indicated Improvac has an effect on early thigh muscle development. The relative expression of MyHC isoform genes, *SM, FWM,* and *FRM,* is shown in Figure 5. Similar to the muscle developmental genes, no differences were observed in MYHC genes in pectoral muscle at the fifth and ninth week (Figures 7A, 7B; $P > 0.05$). In thigh muscles at week 5, the *MYH1B* expression in the control group was significantly lower than that in the early group (Figure 7D; $P < 0.05$). At week 9, the expression level of *SM1* was significantly higher in the control group than that in the late groups (Figure 7E; $P < 0.05$).

The data for the mRNA levels and the results of muscle fiber sections did not correlate well. As a negative regulator gene of muscle growth, MSTN had a negative effect on muscle fiber diameter. Although in the thigh muscle, the expression level of MSTN was higher in the early Improvac-immunized group than

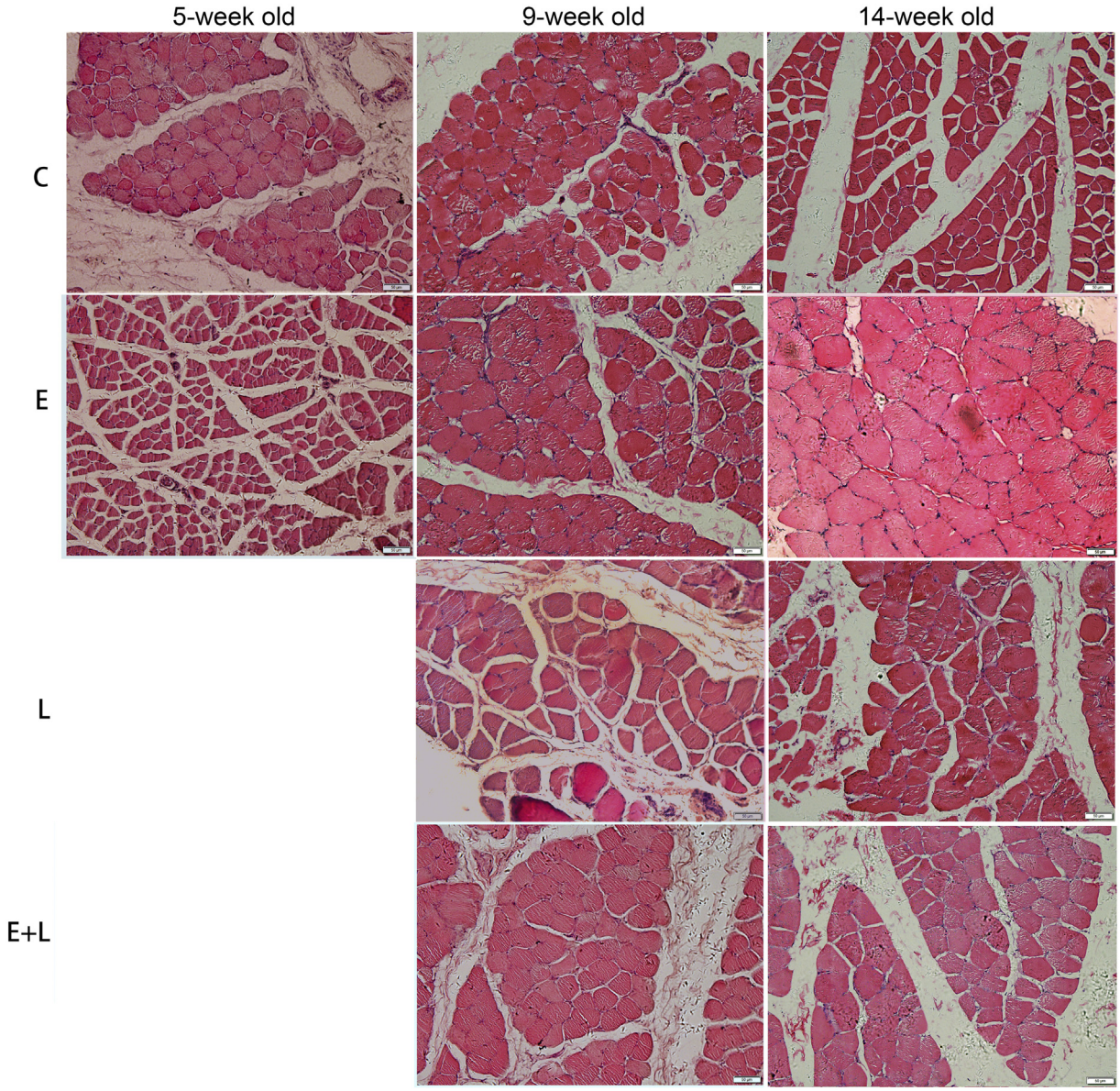

Figure 3. The light photomicrographs of H&E staining of thigh muscles. Abbreviations: C, control group; E, early group; L, late group; E + L, early + late group. Scale bar = 50 μm.

in controls, we did not observe a difference in muscle CSA and density between these 2 groups. *MYOD* and *Myf5* belong to myogenic regulatory factor family and play an important role in the differentiation of progenitor cells and the proliferation of myoblasts in the primary stage of muscle development (Arnold and Winter, 1998). Myofiber formation is regulated by MYOD and Myf5, and the change in expression of *MYOD* indicates that the early Improvac immunocastration affected the normal development of myofibers from thigh muscles at a young age. Slow-twitch myofibres are small, oxidative fibres with red color, high lipid content, and many mitochondria, whereas white fast-twitch fibres are the largest glycolytic fibres possessing high glycogen content and few mitochondria. Red fast-twitch myofibers are intermediate oxidative–glycolytic fibres that are similar to slow-twitch myofibres in color but resemble white fast-twitch fibres in their contractile property, possessing both aerobic and anaerobic metabolic capabilities (Li et al., 2007). Although the results of qRT-PCR showed changes of expression level of genes related to myofiber type after Improvac immunization, the immunofluorescence observations did not show any clear effect of Improvac on the proportion of myofibers in the pectoral and thigh muscles. This discrepancy may be due to the fact that the classification of muscle fiber types in immunofluorescence experiments and qRT-PCR do not correspond well. Gene transcription and translation are complex processes involving protein modification and processing, which may cause a divergence between mRNA levels and protein expression. In general, Improvac immunocastration of male chickens had little effect on the myofiber type.

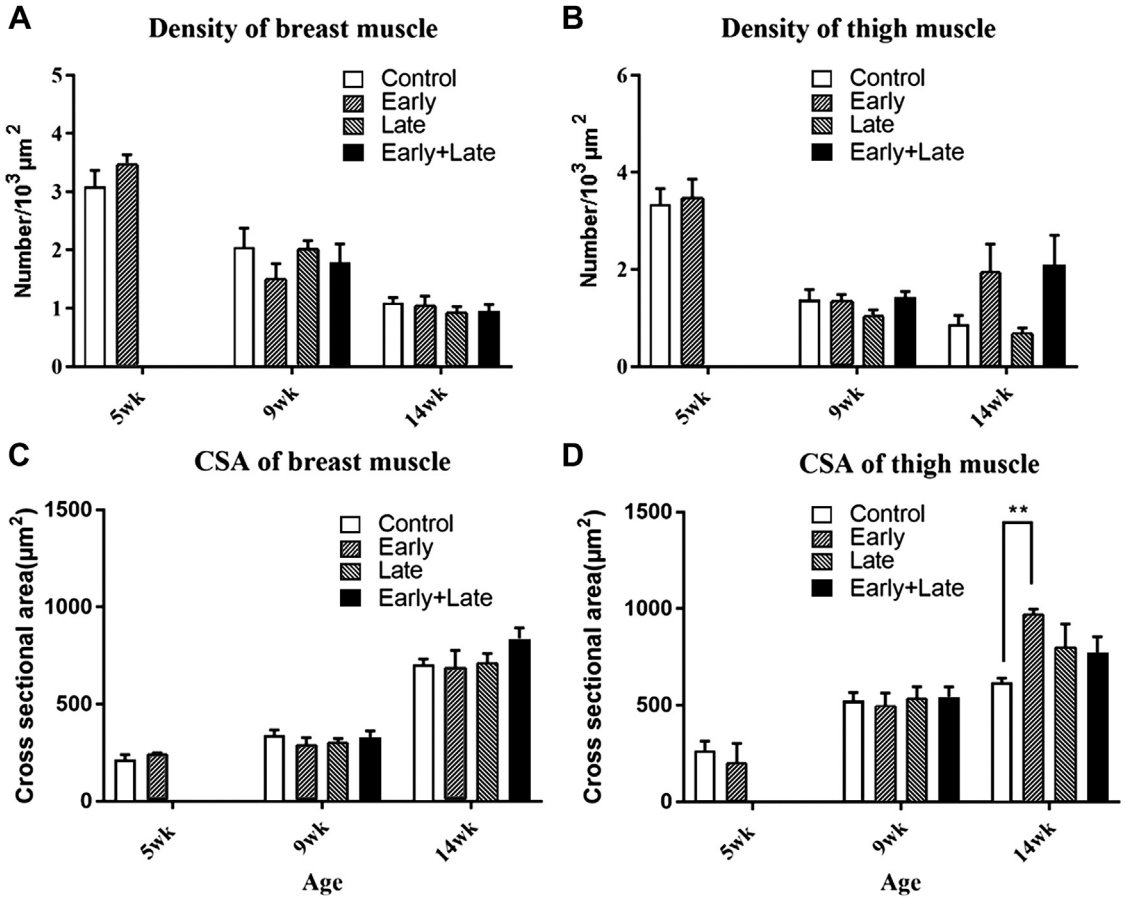

Figure 4. The cross-sectional area (CSA), density of pectoral and thigh muscles. (A) CSA of pectoral muscle; (B) density of pectoral muscle; (C) CSA of thigh muscle; (D) density of thigh muscle; Values are expressed as mean ± SE (n = 10),$^{**}P < 0.01$.

CONCLUSIONS

The results of our study revealed differences in CSA and expression levels of MyHC genes in thigh muscles from chickens after Improvac immunization. Improvac injection had some effects on muscle development and thigh muscles appeared to be more sensitive to Improvac treatments than pectoral muscles. Because our study was the

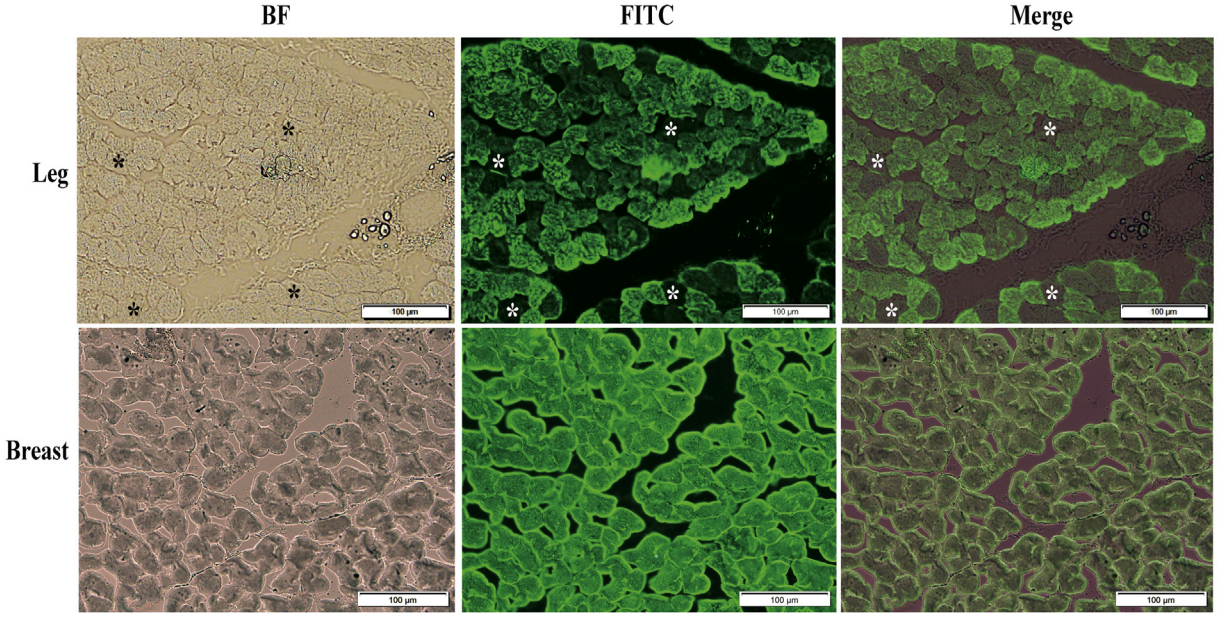

Figure 5. Fiber types of pectoral and thigh muscles. Abbreviations: BF, bright-field; FITC, Fast muscle labeled with mouse anti-rabbit Fast MyHC (myosin skeletal heavy chain)–conjugated FITC; Merge, the merged images of bright-field and FITC staining of the same visual field; Pec, pectoral muscles; Th, thigh muscles. Scale bar = 100 μm. * indicates slow-twitch muscle in the thigh.

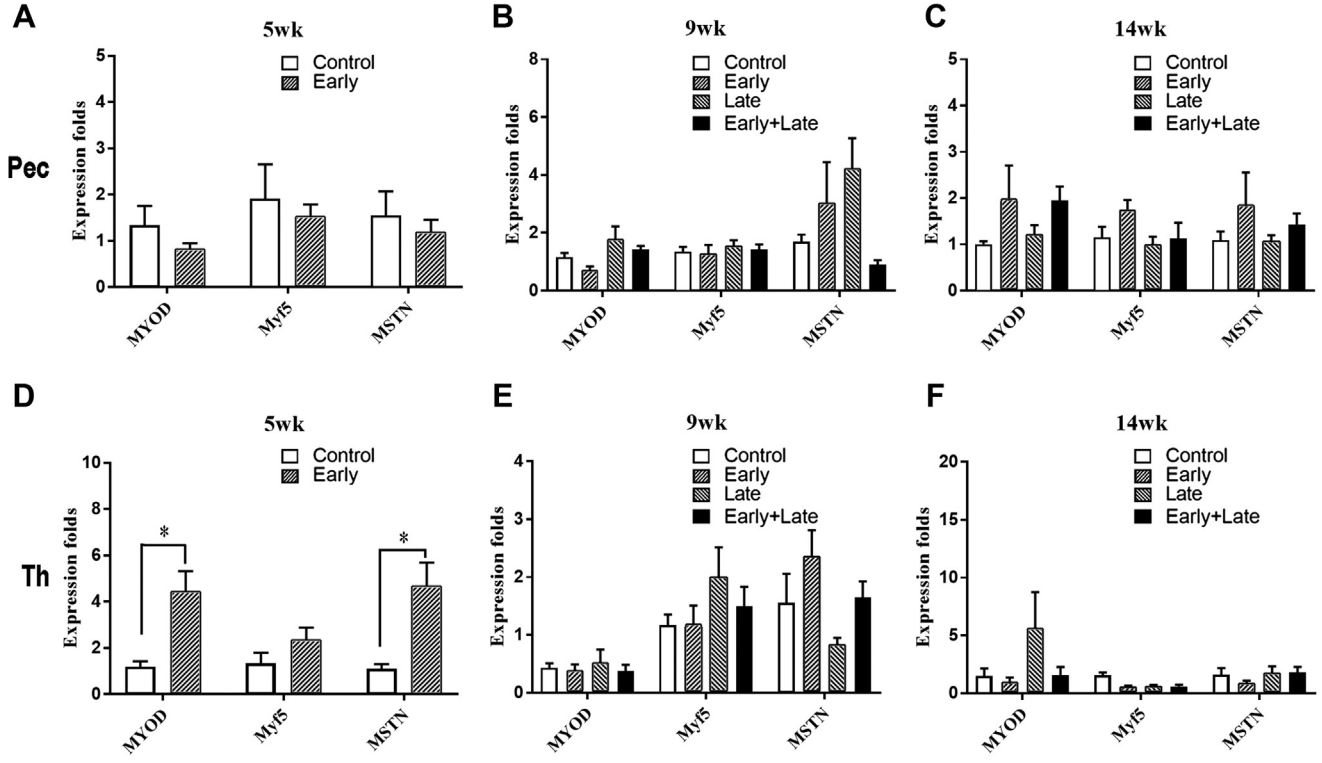

Figure 6. The relative expression levels of muscle development genes. Abbreviations: Pec, pectoral muscles; Th, thigh muscles. Values are expressed as mean ± SE (n = 10); $^*P < 0.05$.

first to measure the effects of different Improvac treatments on CSA, density, and fiber type of muscles as well as expression levels of muscle development genes in male chickens, more trials are needed to confirm our results and to determine the effects of different doses and injection times of Improvac on chicken muscle characteristics.

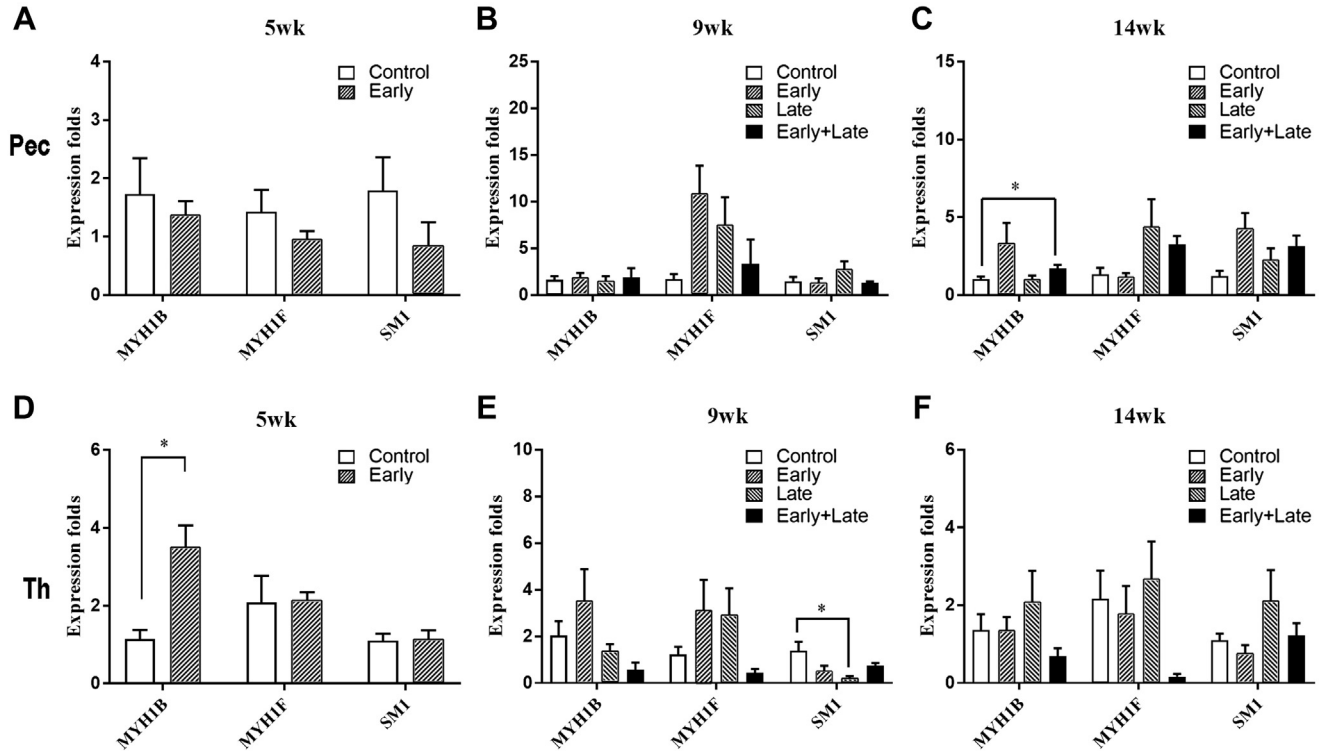

Figure 7. The relative expression levels of muscle MyHC isoform genes. Abbreviations: MyHC, myosin skeletal heavy chain; Pec, pectoral muscles; Th, thigh muscles. Values are expressed as mean ± SE (n = 10), $^*P < 0.05$.

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Conflict of Interest Statement: The authors declare no conflicts of interest.

REFERENCES

Amorim, A., S. Rodrigues, E. Pereira, and A. Teixeira. 2016. Physicochemical composition and sensory quality evaluation of capon and rooster meat. Poult. Sci. 95:1211–1219.

Antunes, I. C., M. A. G. Quaresma, M. F. Ribeiro, S. P. Alves, P. Martins da Costa, and R. J. B. Bessa. 2019. Effect of immunocastration and caponization on fatty acid composition of male chicken meat. Poult. Sci. 98:2823–2829.

Arnold, H.-H., and B. Winter. 1998. Muscle differentiation: more complexity to the network of myogenic regulators. Curr. Opin. Genet. Dev. 8:539–544.

Barnard, E., J. M. Lyles, and J. Pizzey. 1982. Fibre types in chicken skeletal muscles and their changes in muscular dystrophy. J. Physiol. 331:333–354.

Brunius, C., G. Zamaratskaia, K. Andersson, G. Chen, M. Norrby, A. Madej, and K. Lundström. 2011. Early immunocastration of male pigs with Improvac®–effect on boar taint, hormones and reproductive organs. Vaccine 29:9514–9520.

Chen, T. T., C. C. Huang, T. Y. Lee, K. J. Lin, C. C. Chang, and K. L. Chen. 2010. Effect of caponization and exogenous androgen implantation on muscle characteristics of male chickens. Poult. Sci. 89:558–563.

Chen, K., C. Wu, and Y. Hong. 2000. Meat quality and carcass traits of capon in comparison with intact male and female Taiwan country chickens. J. Chin. Soc. Anim. Sci. 29:77–88.

Chiang, W., M. Solomon, and K. Kotula. 1995. Muscle fiber types of selected muscles from broiler chickens in relation to age and sex 1. J. Muscle Foods 6:197–210.

Fennell, M. J., S. V. Radecki, J. A. Proudman, and C. G. Scanes. 1996. The suppressive effects of testosterone on growth in young chickens appears to be mediated via a peripheral androgen receptor; studies of the anti-androgen ICI 176,334. Poult. Sci. 75:763–766.

Gesek, M., D. Murawska, I. Otrocka-Domagała, K. Michalska, and M. Zawacka. 2018. Effects of caponization and age on the histology, lipid localization, and fiber diameter in muscles from Leghorn cockerels. Poult. Sci. 98:1354–1362.

Gesek, M., M. Zawacka, and D. Murawska. 2017. Effects of caponization and age on the histology, lipid localization, and fiber diameter in muscles from Greenleg Partridge cockerels. Poult. Sci. 96:1759–1766.

Li, Y., L. Yuan, X. Yang, Y. Ni, D. Xia, S. Barth, R. Grossmann, and R.-Q. Zhao. 2007. Effect of early feed restriction on myofibre types and expression of growth-related genes in the gastrocnemius muscle of crossbred broiler chickens. Br. J. Nutr. 98:310–319.

Lin, C. Y., and J. C. Hsu. 2002. Effects of surgical caponization on growth performance, fiber diameter and some physical properties of muscles in Taiwan country chicken cockerels. Asian-Australasian J. Animal Sci. 15:401–405.

Lin, C.-Y., and J.-C. Hsu. 2003. Influence of caponization on the carcass characteristics in Taiwan country chicken cockerels. Asian-Australasian J. Animal Sci. 16:575–580.

Federation of Animal Science Societies. 2010. Poultry. Pages 102–105 in Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. Federation of Animal Science Societies, Champaign, IL.

Miguel, J. A., J. Ciria, B. Asenjo, and J. L. Calvo. 2008. Effect of caponisation on growth and on carcass and meat characteristics in Castellana Negra native Spanish chickens. Animal 2:305–311.

Quaresma, M. A. G., I. C. Antunes, M. F. Ribeiro, S. Prazeres, R. J. B. Bessa, and P. Martins da Costa. 2017. Immunocastration as an alternative to caponization: evaluation of its effect on body and bone development and on meat color and composition. Poult. Sci. 96:3608–3615.

Hunt, R. W. G., and M. R. Pointer. 2011. Relations between colour stimuli. Pages 41–72 in Measuring Colour. 4th ed. John Wiley & Sons, Hoboken, NJ.

Smith, D., and D. Fletcher. 1988. Chicken breast muscle fiber type and diameter as influenced by age and intramuscular location. Poult. Sci. 67:908–913.

Symeon, G. K., F. Mantis, I. Bizelis, A. Kominakis, and E. Rogdakis. 2010. Effects of caponization on growth performance, carcass composition, and meat quality of medium growth broilers. Poult. Sci. 89:1481–1489.

Symeon, G. K., F. Mantis, I. Bizelis, A. Kominakis, and E. Rogdakis. 2012. Effects of caponization on growth performance, carcass composition and meat quality of males of a layer line. Animal 6:2023–2030.

Tor, M., J. Estany, D. Villalba, E. Molina, and D. Cubiló. 2002. Comparison of carcass composition by parts and tissues between cocks and capons. Anim. Res. 51:421–431.

Wang, C., F. Yue, and S. Kuang. 2017. Muscle histology characterization using H&E staining and muscle fiber type classification using immunofluorescence staining. Bio-Protoc. 7:e2279.

Wang, C., Y. T. Zeng, X. Y. Chen, Q. Y. Wu, L. Q. Yang, L. Xu, Y. Zhang, I. H. Qazi, G. B. Zhou, C. J. Zeng, Z. Z. Zuo, T. Z. Song, Q. Zhu, and M. Zhang. 2019. Improvac induces immunocastration by affecting testosterone levels and disrupting spermatogenesis in male broiler chickens. Poult. Sci. 98:6034–6045.

Zhang, M., Y.-L. Liu, C.-Y. Fu, J. Wang, S.-Y. Chen, J. Yao, and S.-J. Lai. 2013. Expression of *MyHC* genes, composition of muscle fiber type and their association with intramuscular fat, tenderness in skeletal muscle of *Simmental* hybrids. Mol. Biol. Rep. 41: 833–840.

Zhang, M., T. Nii, N. Isobe, and Y. Yoshimura. 2012. Expression of Toll-like receptors and effects of lipopolysaccharide on the expression of proinflammatory cytokines and chemokine in the testis and epididymis of roosters. Poult. Sci. 91:1997–2003.