

# Effects of age at photostimulation on sexual maturity and reproductive performance in rooster breeders

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**ABSTRACT** The 2 × 4 factorial experiment was designed to determine the effect of strain and photostimulation age on sexual maturity and reproductive performance of rooster breeders. A total of 96 White Leghorn (**WL**) and 120 Beijing You Chicken (**BYC**) roosters were randomly allocated to 4 treatments at 14 wk of age. The treatments represent photostimulation at 16, 18, 20, and 22 wk of age, respectively (**PS16**, **PS18**, **PS20**, and **PS22**), in both strains. Photostimulation was achieved by increasing the day length from 8L:16D to 14L:10D and by increasing lighting intensity from 10 lx to 80 lx. Three birds from each interaction were sacrificed to characterize the comb and testis weights at 4 time points: 1 d before photostimulation and 2, 4, and 6 wk after photostimulation. Semen quality and hatching performance with the semen of the experimental roosters were measured at 30 and 45 wk of age, respectively. Results showed that the testis weight of PS20 and PS22

in WL and BYC was 6.4- and 2.9-fold higher than that of PS18 before photostimulation, while testis weight of PS18 in both strains increased sharply after photostimulation. The diameter of seminiferous tubules increased in the photostimulated roosters as compared with the nonphotostimulated ones, and mature spermatozoa were produced 4 wk after photostimulation and at 20 wk of age for PS16. The WL had lower semen volume and total sperm count than BYC ( $P < 0.01$ ), but there was no difference on effective sperm count ( $P > 0.05$ ). In addition, semen quality traits were not affected by age at photostimulation ( $P > 0.05$ ) in both strains. The fertility and hatching performance were not affected by strain or photostimulation age ( $P > 0.05$ ). In summary, the sexual maturation of rooster breeders can be advanced by photostimulation at an early age, which does not lead to a difference in semen quality or hatching performance at adult stage.

**Key words:** rooster breeder, photostimulation age, sexual maturity, semen quality, fertility

2021 Poultry Science 100:101011  
<https://doi.org/10.1016/j.psj.2021.01.033>

## INTRODUCTION

Chickens are photoperiodic and may respond to long photoperiods by activation of their reproductive axis. Once the birds have reached an optimal age, BW, and frame size, their sexual maturation can be hastened by photostimulation. This process is initiated by the release of gonadotropin-releasing hormone from the hypothalamus, which stimulates gonadotropin secretion from the anterior

pituitary (Sharp, 1993), which in turn supports regulated production of gonadal steroids (Renema et al., 2007).

Previous studies have reported that the optimal age of pullets is the point at which the hypothalamo–pituitary–gonadal axis is activated by a photostimulatory cue (Lewis et al., 2008; Tyler et al., 2011; Shi et al., 2019, 2020). Moreover, many researchers suggested that the photostimulation of female breeders at 16 wk of age result in earlier sexual maturation (Renema et al., 2007; Pishnamazi et al., 2014; van der Klein et al., 2018). Such manipulation in Ross and White Leghorn (**WL**) hens results in the laying of smaller-sized and broken eggs, but a greater number of eggs (Zuidhof et al., 2007; Shi et al., 2020). Shi et al. (2019) reported that photostimulation at an early age delayed sexual organs development in Beijing You Chicken (**BYC**). Major concern with delayed photostimulation to 22 wk of age is that it shortens the length of egg-laying period (Robinson et al., 1996;

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Received August 13, 2020.

Accepted January 1, 2021.

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Pishnamazi et al., 2014) and reduces the laying peak (Shi et al., 2020). In addition, ovary weight and number of large yellow follicles decreased with delayed photostimulation in WL and thereby reducing number of potential ovulations. (Shi et al., 2020). Therefore, optimum age of photostimulation could ensure that more birds respond effectively to a photostimulatory cue (Hocking, 1996; Robinson et al., 1996). Up to date, previous studies focused mainly on effects of photostimulation age on reproductive performance of hens, but less is known about its effects in roosters. In many cases, roosters are usually housed together with breeding hens and receives the same photostimulation program. Tyler and Gous (2009) studied the response of roosters photostimulated at different ages (56, 77, 98, 119, 147, and 161 d) during the period of growth before achieving sexual maturity and found that roosters reached sexual maturity at an earlier age than hens, as measured by the start of semen production. Therefore, optimum age of photostimulation in roosters may be different from that of hens.

This study was designed to determine the effects of photostimulation age on sexual maturity and reproductive performance in WL and BYC rooster breeders. This study provides insight for a better understanding of the physiological mechanisms driving sexual maturity and reproductive traits in indigenous (BYC) and elite laying strains (WL) of chickens.

## MATERIALS AND METHODS

### Ethics Statement

The present study was approved by the Animal Care and Use Committee of Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (No. IAS2020-14) and was performed in accordance with the relevant guidelines and regulations set by Ministry of Agriculture and Rural Affairs of the People's Republic of China.

### Experimental Design and Birds

This study was designed in a  $2 \times 4$  factorial arrangement to determine the effect of 2 strains and 4 photostimulation ages on sexual maturity and reproductive performance of rooster breeders. Birds were photostimulated at 16, 18, 20, and 22 wk of age, respectively (PS16, PS18, PS20 and PS22).

A total of 96 WL and 120 BYC rooster breeders of 14 wk of age were acquired from the experimental farm station of Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. Each strain was randomly allocated to 4 treatments (room) with light-controlled facility. The chicks were housed in individual cages. Feed and water were provided as per chicken feeding standards (Ministry of Agriculture, 2004). Birds were fed commercial corn- and soybean-based diets with 16.00% CP and 11.50 MJ/kg ME. The lighting regimen was maintained at 8L:16D, and the intensity was set at 5–10 lx until photostimulation.

In the first week of the photostimulation program, the light intensity was increased to 80 lx. From the second to

the fourth week, the lighting regimen was systematically upgraded to 14L:10D by increasing light during the second week by 4 h and then by 1 h during each of the third and fourth week. Light-emitting diode lamps were suspended 2 m above the ground. Light intensity was measured at the birds' eye level with the photoreceptor sensor of a light meter (model: DT-1301; Shenzhen Everbest Machinery Industry Co. Ltd., China). Rooms had independent temperature controls and were held at 21°C during the study. The actual temperature was monitored at regular intervals to make sure that the temperatures varied in the normal range between rooms.

### BW and Sexual Organ Development

The BW was recorded weekly from 14 to 40 wk of age, and the Compertz model was used to fit the growth curve. This model was described as follows:  $W_t = A \times \exp(-B \times \exp(-K \times t))$ . The biological interpretations of the relevant variables are  $W_t = \text{BW (g)}$ ,  $t = \text{age (wk)}$ ,  $A = \text{upper asymptotic weight}$ ,  $K = \text{growth rate}$ ,  $B = \text{age at the inflection point of the growth curve}$ . In addition, 3 roosters from each interaction were sacrificed using direct cervical dislocation at 4 time points: 1 d before photostimulation and 2, 4, and 6 wk after photostimulation. The development of their comb and testes was characterized, and the weight was calculated as a percentage of BW.

### Histology of the Testis

The testis from BYC roosters of PS16 were collected at 4 time points mentioned previously. The testes from the roosters of the same age which were not stimulated were collected as control. All samples were sliced sagittally and fixed in 4% buffered formalin. Thereafter, they were embedded in paraffin and cut into 4- $\mu\text{m}$ -thick sections. These sections were then stained with Harris hematoxylin and eosin for histomorphology analysis using A Zeiss Axioskop (Carl Zeiss, Thornwood, NY) equipped with a QICam digital camera operated with NIS Elements Software (Nikon Instruments, Melville, NY).

### Reproductive Hormone

Blood samples were collected randomly from 10 BYC roosters in each treatment at the same 4 time points mentioned previously for the measurement of reproductive hormone concentration including estradiol ( $E_2$ ), follicle-stimulating hormone, and luteinizing hormone. All blood samples were centrifuged at  $3,000 \times g$  for 2 min at 4°C to collect the serum that was stored at -20°C until further analysis. The luteinizing hormone, follicle-stimulating hormone, and  $E_2$  concentrations were determined using a radioimmunoassay kit following the manufacturer's instructions (Beijing North Institute of Biological Technology, Beijing, China). Gamma radiation was measured with a radioimmunoassay counter (BFM-96; Zhongheng Electromechanical Technology Development Co., Ltd, Anhui, China).

## Semen Quality Traits Evaluation

At 30 wk of age, 10 roosters from each interaction were trained to respond to abdominal massage technique for semen collection every other day for 1 wk before the semen quality evaluation. The ejaculate was collected manually in a weighted micropipette. Semen volume was measured by a weighing method assuming that 1 mL semen weighs 1 g (WHO, 2010). Sperm concentration and motility were estimated with a spectrophotometer (Hammerstedt, 1975) and microscopic observation ( $\times 200$  magnification; Tabatabaei and Aghaei, 2012), respectively. The motility was expressed as the percentage of motile spermatozoa with moderate-to-rapid progressive movement. At least 3 microscopic fields were examined for each sample. Total sperm count and effective sperm count were calculated for each bird using the following equations as reported previously (Liu et al., 2007):

Total sperm count = semen volume  $\times$  sperm concentration; Effective sperm count = total sperm count  $\times$  sperm motility.

## Fertility and Hatching Performance

At 45 wk of age, a total of 16 WL and 32 BYC rooster breeders were randomly selected from each strain as semen donors to inseminate hens of their respective breeds (128 WL and 256 BYC hens). Artificial insemination was accomplished on 2 consecutive day. The male-to-female ratio was 1:8. Each hen was inseminated with 20  $\mu$ L semen. Eggs were marked and collected daily from day 2 to 8 after the first insemination and stored at temperature of 18°C and RH of 75% until incubation. Abnormal shape and unclean eggs were discarded. The

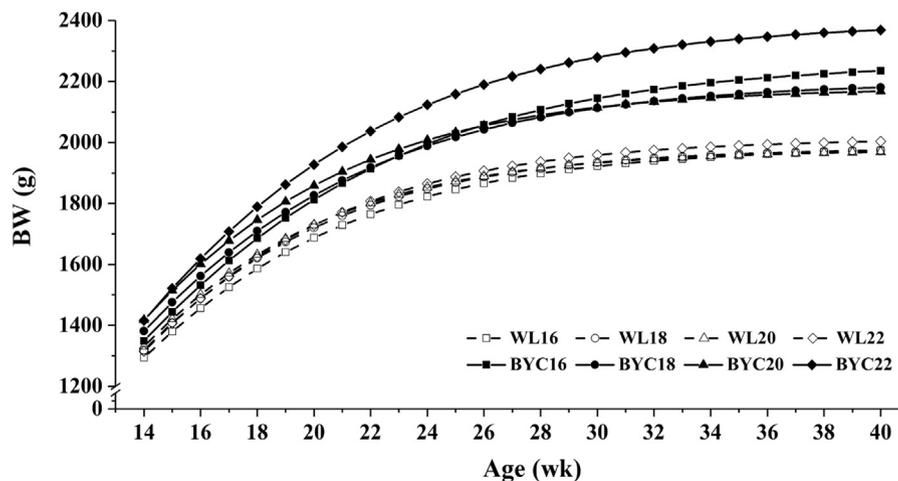
setting eggs were candled on day 11 after incubation. Those eggs without clear viable embryos were opened to determine whether they contain an early dead embryo or were unfertilized oocyte. Fertility for each rooster was determined as the percentage of fertile eggs from the total number of setting eggs. The hatchability of fertile eggs was calculated as the percentage of hatched chicks of the total number of fertile eggs. The hatchability of setting eggs was calculated as the percentage of hatched chicks of the total number of setting eggs.

## Statistical Analysis

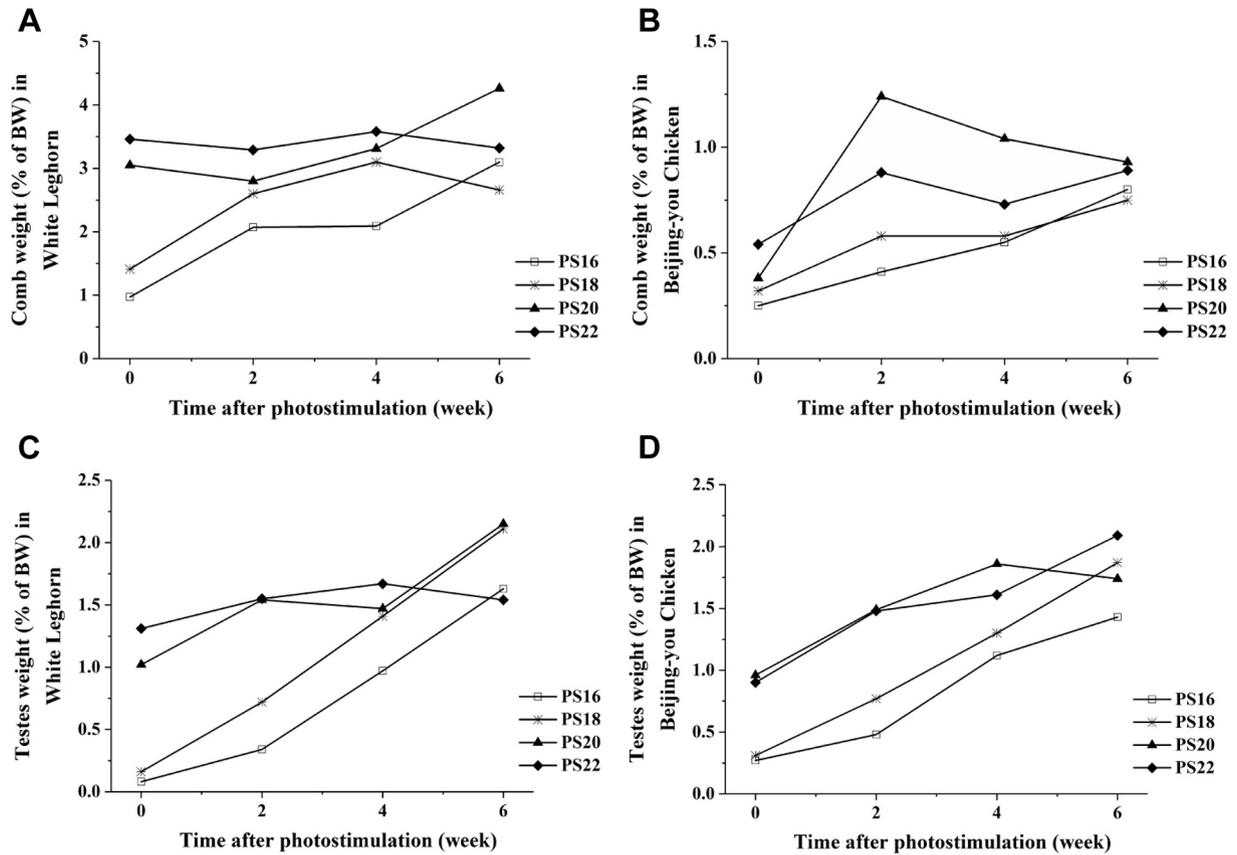
Data were analyzed using the MIXED procedure of SAS (SAS 9.1, SAS Institute Inc., Cary, NC). Strain and photostimulation age were analyzed as fixed effects. Significance was designated as  $P < 0.05$ . Means were compared by Student-Newman-Keuls multiple-range tests when a significant difference was detected.

## RESULTS AND DISCUSSION

The physiologic roles of avian's photoreceptors have been underused in male poultry management to enhance production and, consequently, economic efficiency of the birds. Although some research has been conducted in light regimen (Renden et al., 1991; Lewis et al., 2005), intensity (Lewis and Morris, 2000), and color (Chang et al., 2016) for reproductive hormone and semen quality of roosters, studies on photostimulation of roosters were rarely reported. The present study focuses on the effects of strain and photostimulation age on sexual maturity, reproductive hormone, semen quality, and reproductive performance in rooster breeders.



**Figure 1.** BW profiles for White Leghorn (WL) and Beijing You Chicken (BYC) rooster breeders photostimulated at different ages. The fitting of growth curves of each interaction are as follows: WL16:  $W_t = 1985.74703 \times \exp(-4.08526 \times \exp(-0.16108 \times t))$ ,  $R^2 = 0.993$ . WL18:  $W_t = 1982.41213 \times \exp(-4.80300 \times \exp(-0.17611 \times t))$ ,  $R^2 = 0.993$ . WL20:  $W_t = 1975.64286 \times \exp(-5.05298 \times \exp(-0.18195 \times t))$ ,  $R^2 = 0.995$ . WL22:  $W_t = 2013.41858 \times \exp(-4.71154 \times \exp(-0.99579 \times t))$ ,  $R^2 = 0.996$ . BYC16:  $W_t = 2265.61181 \times \exp(-3.70348 \times \exp(-0.14043 \times t))$ ,  $R^2 = 0.996$ . BYC18:  $W_t = 2199.80213 \times \exp(-3.96930 \times \exp(-0.15309 \times t))$ ,  $R^2 = 0.994$ . BYC20:  $W_t = 2180.82976 \times \exp(-4.35318 \times \exp(-0.16525 \times t))$ ,  $R^2 = 0.994$ . BYC22:  $W_t = 2396.48473 \times \exp(-4.13265 \times \exp(-0.14716 \times t))$ ,  $R^2 = 0.998$ . Abbreviations: BYC16, BYC rooster breeder was photostimulated at 16 wk of age; BYC18, BYC rooster breeder was photostimulated at 18 wk of age; BYC20, BYC rooster breeder was photostimulated at 20 wk of age; BYC22, BYC rooster breeder was photostimulated at 22 wk of age; WL16, WL rooster breeder was photostimulated at 16 wk of age; WL18, WL rooster breeder was photostimulated at 18 wk of age; WL20, WL rooster breeder was photostimulated at 20 wk of age; WL22, WL rooster breeder was photostimulated at 22 wk of age.

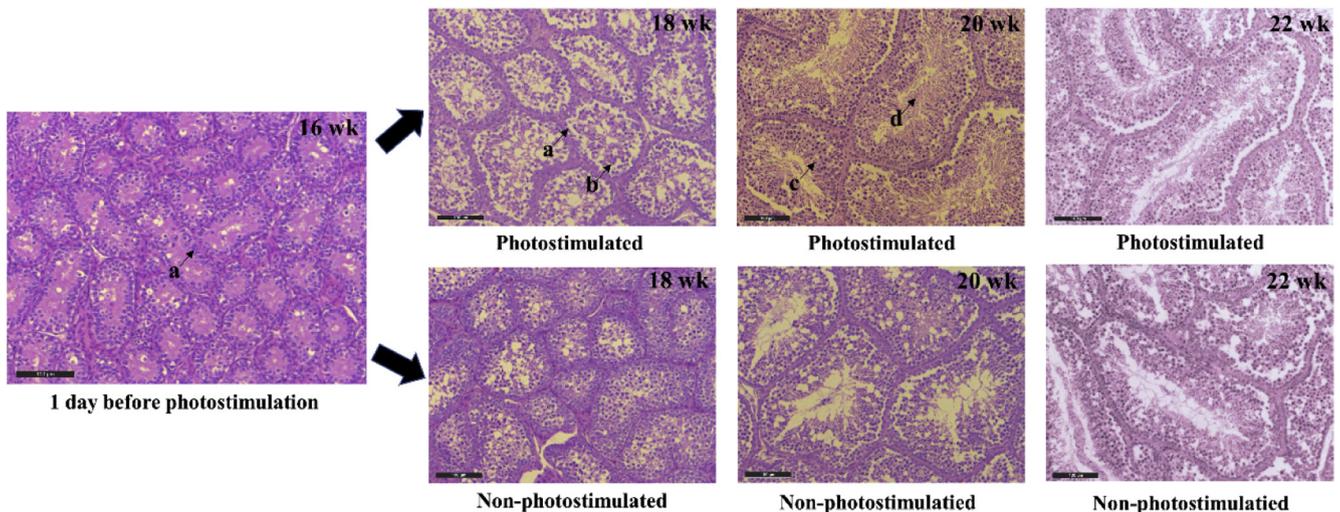


**Figure 2.** Change in comb weight and testes weight of White leghorn (WL) and Beijing-You Chicken (BYC) rooster breeders photostimulated at different ages. “0,” “2,” “4,” and “6” on the x axis means 1 d before photostimulation, 2, 4, and 6 wk after photostimulation, respectively. Abbreviations: PS16, rooster breeders were photostimulated at 16 wk of age; PS18, rooster breeders were photostimulated at 18 wk of age; PS20, rooster breeders were photostimulated at 20 wk of age; PS22, rooster breeders were photostimulated at 22 wk of age.

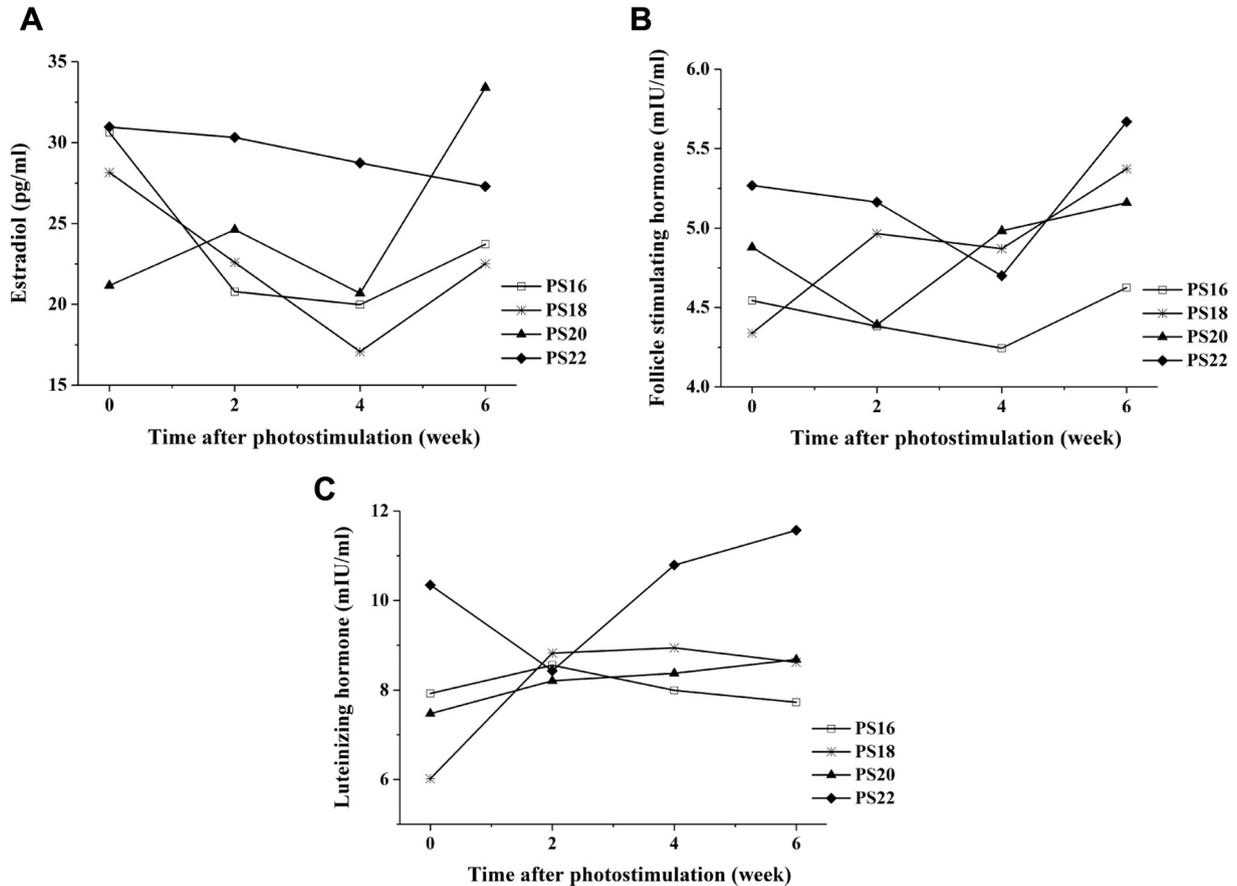
### BW and Sexual Organ Development

In the present study, the BW of WL and BYC roosters in PS16 increased slowly after stimulation comparing with other treatments; however, this situation disappeared with aging (Figure 1). Therefore, early photostimulation in roosters such as 16 wk of age may slow

down the growth during sexual maturity. Previous study reported that photostimulation have a stimulatory effect on the proliferation and differentiation of satellite cells and a promoting effect on the uniformity of the muscle fibers in the early posthatch period (Rozenboim et al., 2013). A more likely explanation is that photostimulation indirectly affects myoblast proliferation by activating the



**Figure 3.** Histology analysis of hematoxylin and eosin stained sections of the testis in Beijing You chickens from 16 to 22 wk of age. Bar = 500  $\mu$ m. a, spermatogonia; b, spermatocytes; c, spermatids; d, mature sperms.



**Figure 4.** Change in estradiol, follicle stimulating hormone, and luteinizing hormone concentration of Beijing-You Chicken roosters photostimulated at different ages. “0,” “2,” “4,” and “6” on the x axis means 1 d before photostimulation, 2, 4, and 6 wk after photostimulation, respectively. Abbreviations: PS16, rooster breeders were photostimulated at 16 wk of age; PS18, rooster breeders were photostimulated at 18 wk of age; PS20, rooster breeders were photostimulated at 20 wk of age; PS22, rooster breeders were photostimulated at 22 wk of age.

endocrine system; the latter receives photic cues from the retinal or extraretinal photoreceptors (Dishon et al., 2017). However, whether this mechanism works in rooster needs further study. The process of sexual maturation in birds represents a major shift in its physiological status (Johnson et al., 2009), and its surplus nutrients demand to guide the sexual organ development during sexual maturation. In addition, bright light used in the present study could cause more activity of rooster breeders. This may explain the reduced BW of PS16. Similar result was reported by Olanrewaju et al. (2014), who found that bright light as 100 lx have stimulated the activity of chickens to the extent that more energy was used toward maintenance instead of growth.

In this study, comb weight for PS20 and PS22 in WL was at least 2.2-fold higher than that in PS16 and PS18 1 d before photostimulation, and the comb weight of PS16 and PS18 increased sharply in 2 wk after photostimulation (Figure 2A). Meanwhile, testis weight for PS20 and PS22 in WL was at least 6.4-fold higher than that in PS16 and PS18 1 d before photostimulation, and the testis weight of PS16 and PS18 increased sharply from 0 to 6 wk after photostimulation (Figure 2C). This result indicates that sexual organs can develop without photostimulation, which is similar to the reports in hens (Shi et al., 2020). However, photostimulation triggers the development of

the testis. The testis weight increased tremendously and continuously in BYC roosters that were photostimulated at early age more than those stimulated after 20 wk of age (Figure 2D). In contrast, Shi et al. (2019) reported delayed development of the ovary and oviduct in BYC hens until after 4 wk after photostimulation. Taken together, photostimulation of roosters at 16 wk of age in both WL and BYC strains facilitates the development of the comb and testis, which is in agreement with a previous study showing that roosters respond to earlier photostimulation than hens (Tyler and Gous, 2009).

The histologic structure of the testis of photostimulated and nonphotostimulated BYC roosters at 16, 18, 20, and 22 wk of age was shown in Figure 3. For the PS16 roosters, the diameter of seminiferous tubules of the roosters photostimulated for 2 wk increased as compared with the nonphotostimulated roosters of the same age, and mature spermatozoa were produced 4 wk after photostimulation and at 20 wk of age.

### Reproductive Hormone

An immediate decrease of serum  $E_2$  was observed in roosters of PS16, PS18, and PS22 after photostimulation (Figure 4A). This result is in consist with a previous study in roosters showing that  $E_2$  level was the highest

**Table 1.** Effect of genetic strain and age at photostimulation (PS) on semen quality traits of White Leghorn (WL) and Beijing You Chicken (BYC) rooster breeders at 30 wk of age.

	Strain	PS (wk)	Semen volume	SEM	Sperm concentration	SEM	Sperm motility	SEM	Total sperm count	SEM	Effective sperm count	SEM
			( $\mu\text{L}$ )		( $10^8/\text{mL}$ )	(%)	( $\times 10^8$ )	( $\times 10^8$ )				
Strain	WL		247.30 <sup>b</sup>	25.23	11.04	0.69	57.64	3.79	3.30 <sup>b</sup>	0.39	1.94	0.27
	BYC		438.59 <sup>a</sup>	23.35	10.83	0.52	48.26	3.01	4.83 <sup>a</sup>	0.34	2.41	0.25
Age at PS		16	349.47 <sup>a,b</sup>	29.78	10.93	0.91	54.47	5.14	3.93	0.43	1.98	0.26
		18	342.22 <sup>a,b</sup>	45.67	10.68	0.85	55.11	4.98	4.08	0.64	2.57	0.55
		20	275.53 <sup>b</sup>	47.68	10.42	0.97	48.06	5.43	3.49	0.63	1.61	0.29
		22	411.00 <sup>a</sup>	34.53	11.51	0.70	53.72	4.27	4.84	0.46	2.61	0.35
Strain $\times$ Age at PS	WL	16	243.33	27.23	10.92	1.66	64.91	7.31	2.82	0.56	1.74	0.39
		18	243.33	55.95	11.29	1.09	50.07	7.20	3.43	0.81	1.81	0.57
		20	133.33	42.88	10.52	1.58	54.74	8.80	2.15	0.77	1.13	0.37
		22	357.00	47.07	11.32	1.36	60.50	7.32	4.38	0.85	2.74	0.66
	BYC	16	445.00	25.22	10.95	1.02	45.08	6.07	4.82	0.49	2.17	0.36
		18	441.11	57.34	10.08	1.35	60.15	6.88	4.73	1.02	3.32	0.91
		20	403.50	57.66	10.36	1.30	42.05	6.45	4.43	0.87	1.94	0.40
		22	465.00	46.58	11.70	0.45	46.94	3.61	5.30	0.36	2.49	0.27
Source of variation	<i>P</i> -value											
Strain			<0.01		0.06		0.80		0.01		0.11	
Age at PS			0.03		0.73		0.85		0.22		0.15	
Strain $\times$ Age at PS			0.38		0.15		0.95		0.80		0.44	

<sup>a,b</sup>Values within a row and treatment group lacking a common superscript differ ( $P < 0.05$ ).

before sexual maturity and decreased steadily from sexual maturation (Weil et al., 1999). However, there was no consistent or stable trend for follicle-stimulating hormone or luteinizing hormone of BYC roosters photostimulated at different ages (Figures 4B and 4C).

### Semen Quality Traits, Fertility, and Hatching Performance

As shown in Table 1, semen volume and total sperm count in WL was lower than those of BYC ( $P < 0.05$ ); however, there was no difference on effective sperm count ( $P > 0.05$ ). Roosters of PS16, PS18, and PS22 had higher semen volume than those of PS22

( $P = 0.03$ ), but there was no difference in other semen quality traits ( $P > 0.05$ ). Although photostimulation at an early age could accelerate sexual organ development, it is not accompanied by improved or impaired semen quality of roosters at adult age. As shown in Table 2, fertility and hatching performance were not affected by strain or photostimulation age ( $P > 0.05$ ). Based on the aforementioned information, the photostimulation age does not affect semen quality and fertility characteristics of adult rooster breeders.

In fact, photostimulation age has an important role on sexual maturity and egg production performance in hens. Optimal age of stimulation was strain-specific, for example Shi et al. (2019, 2020) suggested that the indigenous breed BYC should be photostimulated at 20 wk,

**Table 2.** Effect of strain and age at photostimulation (PS) on fertility and hatch performance of White Leghorn (WL) and Beijing You Chicken (BYC) rooster breeders at 45 wk of age.

	Strain	PS (wk)	Fertility (%)	SEM	Hatchability of fertile eggs (%)	SEM	Hatchability of setting eggs (%)	SEM
Strain	WL		94.57	1.76	77.15	3.21	72.89	3.24
	BYC		92.72	1.16	81.60	1.73	75.60	1.82
Age at PS		16	93.23	1.54	78.10	3.64	72.90	3.86
		18	92.96	1.80	79.92	2.61	74.11	2.38
		20	91.50	2.70	83.06	3.58	75.73	3.59
		22	95.67	1.55	79.39	2.96	76.04	3.27
Strain $\times$ Age at PS	WL	16	96.87	1.05	74.35	9.96	72.19	10.11
		18	95.26	1.84	75.57	3.99	72.06	4.41
		20	90.07	5.75	79.19	7.97	70.61	6.01
		22	96.09	3.91	79.47	4.61	76.72	6.74
	BYC	16	91.41	1.99	79.98	2.90	73.26	3.62
		18	91.82	2.52	82.09	3.26	75.13	2.95
		20	92.21	3.14	85.00	3.87	78.30	4.45
		22	95.46	1.54	79.35	4.01	75.70	3.92
Source of variation	<i>P</i> -value							
Strain			0.48		0.79		0.92	
Age at PS			0.38		0.21		0.46	
Strain $\times$ Age at PS			0.61		0.90		0.85	

which was 2 wk later than that of the elite layer strain WL. For rooster breeders, sexual maturation can be advanced by photostimulation at 16 wk of age for both strains. The practice of early photostimulation in laying hens may arise the concern that the laying performance could be induced. This situation in rooster breeders seems different in the way that the early photostimulation may accelerate the testis development and sperm production but did not lead to impaired semen quality, fertility, or hatching performance at the adult stage. It is normally accepted that earlier sexual maturity of roosters than the laying hens is beneficial for the timely production of fertilized eggs. As per the results of this study, it is suggested that the rooster breeders could be photostimulated earlier than the females if the facility permits or the males and females housed separately.

## CONCLUSIONS

This study showed that accelerated development of primary and secondary sexual organs of roosters can be activated by photostimulation at an early age, which does not however lead to an improved or impaired semen quality traits, fertility, or hatching performance. Early photostimulation of rooster breeders is therefore possible to increase the production of fertilized eggs especially at the early laying stage.

## ACKNOWLEDGMENTS

The authors thank Adamu Mani Isa (Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China) for careful proofreading of this article and thank Nodark Biolight Technology Co., Ltd., Wuxi, China, for providing lighting instrument of this experiment. Financial support of this study was provided by the National Key Research and Development Program of China (grant number 2016YFD0500502, 2017YFD0502004), Chinese Agricultural Research System (grant number CARS-40), and the Agricultural Science and Technology Innovation Program (grant number ASTIP-IAS04).

## DISCLOSURES

The authors declare no conflicts of interest.

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