The Effect of Zinc, Shell, and Fishbone Supplementations on Comb Width and Length of Bangkok Rooster

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Abstract. The Bangkok rooster is a prestigious ornamental bird in Indonesia. Zinc and shell have an aromatase blocker effect that results in testosterone elevations. Additionally, fishbone contains rich protein for optimum biological functions. Serum testosterone regulates comb size in the chicken. This study aimed to investigate the effect of natural aromatase blockers and protein in the comb of Bangkok roosters. This experiment divided thirteen Bangkok roosters into control and treatment groups with zinc, fishbone-shell, and fishbone supplementations. The weekly comb lengthening and widening were intervals between week 0 and the measurement week in this study. The results suggested that fishbone and fishbone-shell supplementations escalated the comb lengthening. Fishbone–shell treatment significantly increased the comb lengthening in the second and fourth weeks ($p < 0.05$). The significant elongation change in the fishbone group only appeared in the second week ($p < 0.05$). Otherwise, the treatments produced lower comb widening in the roosters. Fishbone and fishbone-shell supplementation significantly caused a lower widening in every measurement week ($p < 0.05$). This study uncovered the effects of zinc, shell, and fishbone supplementations on reproductive phenotype in Bangkok roosters.

1 Introduction

The transboundary effects of COVID 19 have weakened the global economy, including the poultry industry [1]. Bangkok chicken breeders must also suffer this pandemic crisis. Bangkok roosters have commonly bred as fighting and ornamental chicken, which is prestigious in Indonesia. The weakened economy of the consumer reduces chicken demand. Besides, chicken performance and production efficiency are critical for the poultry industry. An adaptive strategy to cope with this pandemic is necessary to maintain Bangkok chicken breeding.

Fish and oysters are popular food materials in the world. Fishbone and shell is the leftover of fish and oyster consumption. An accumulation of this waste becomes an environmental problem in several places [2,3]. Fishbone and shell may be useful as a feed supplement to enhance chicken performance. Furthermore, the utilization of leftover material may reduce production costs. This application can also solve the environmental problems due to fishbone and shell accumulation.

Testosterone is a class of gonadosteroid that spreads in several tissues in the animal. Aromatase converts testosterone into estradiol to regulate testosterone levels [4–6]. Testosterone levels regulate spermatogenesis and other reproductive traits of a male animal. Therefore, testosterone aromatization is a significant process that modulates animal reproduction. Inhibition of aromatase consequently modifies animal reproductive phenotypes.

Zinc is an essential nutrient for an animal. Studies show that zinc has several roles in male reproduction. Zinc deficiency causes several disorders, including reproductive diseases and infertility [7–9]. Furthermore, zinc has caused aromatase suppression and testosterone elevation in animals [2,10,11]. Aromatase inhibition results in testosterone elevation by blocking testosterone conversion into estrogen. The testosterone increase in animals causes reproductive phenotype modifications. Aromatase suppression has appeared in the shell of an oyster. An oyster shell contains zinc and aromatase blocker activity in vertebrates [2,12]. Shell consumption causes testosterone elevations and aromatase inactivation in the brain and Leydig cells of rats [2]. Besides testosterone activity, proper protein intake also significantly influences reproductive performance. Fishbone possesses a high protein content beneficial as a protein source for animals [3]. Further study is crucial for the advanced application of shell and fishbone to boost reproductive performance.

A comb is one of the secondary sexual characteristics of chicken. In practice, farmers have used comb size as a selection indicator for performance in commercial chickens [13]. Moreover, gonadal hormones such as testosterone levels govern comb size in roosters [13,14]. The correlation between testosterone and comb size underlies the usefulness of comb as a reproductive indicator. This study used a comb of Bangkok rooster to

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investigate the effect of aromatase inhibitor and additional protein.

This study aimed to evaluate the effect of aromatase blockers and additional protein in the comb size of Bangkok roosters. The Bangkok roosters grouping consisted of control, zinc, fishbone-shell, and fishbone treatments. The roosters underwent measurement of comb length and width during the treatment period. Shell and fishbone have an advantageous potency as a supplement for poultry during this pandemic.

2 Methodology

2.1 Animal Treatment

The study was conducted from October to November 2021. Thirteen 8-12 months old Bangkok roosters (*Gallus gallus*) participated in this study. All animals underwent acclimation for seven days before the treatments started. The experiments took place at Bantul regency, Special Region of Yogyakarta, Indonesia. All roosters were divided into control (n = 1); Zn (n = 2); fishbone-shell (FS) (n = 5); and fishbone (F) (n = 5) groups. The zinc group consumed zinc powder of about 37 mg daily. Zinc powder originated from food-grade zinc tablets. FS group ingested a 6.6 g shell powder and a 3.3 g fishbone powder daily. F group consumed only a 3.3 g fishbone powder daily. The treatment groups received a daily oral treatment and standard feed simultaneously. All treatments had performed for 28 days.

2.2 Fishbone and Shell Powder Processing

Fishbone of freshwater milkfish (*Chanos chanos*) originated from local markets in Daerah Istimewa Yogyakarta, Indonesia. This research adopted a fishbone processing method from the previous study [3]. Afterward, shell powder originated from the blood clamp (*Anadara granosa*) shell. The shell processing procedure agreed with the previous work [2].

Fig. 1. Comb length and width measurement in Bangkok rooster. The yellow line represents width measurement, while the blue line represents length measurement.

2.3 Comb Size and Statistical Analysis

Comb length was the longest distance between the rostral and caudal end. The longest distance between the dorsal and ventral end was comb width. **Fig. 1.** depicted comb measurement in a Bangkok rooster. Comb lengthening was the sum of length in the measurement week (week 2-4) minus pre-treatment (week 0). Meanwhile, comb widening was the sum of width in the measurement week (week 2-4) minus pre-treatment (week 0). This study used One-Sample T and Binomial tests following the normality test. The test value in One-Sample T-test for each week was the control value. This study used the Binomial test for the abnormally distributed data to confirm the significant changes. The control values became the cut point in the Binomial test with a 0.50 test proportion. The significant results had a p-value under 0.05.

3 Results and discussions

Table 1. Means ± Standard of Error Means of the comb lengthening and comb widening in Bangkok roosters. The asterix (*) indicates $p < 0.05$ compared to the control values.

Comb lengthening	**Week 2**	**Week 3**	**Week 4**
Control (cm)	- 1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Zn (cm)	- 0.25 ± 0.25	0.00 ± 0.00	0.75 ± 0.25
FS (cm)	0.00 ± 0.22*	0.10 ± 0.14	0.60 ± 0.15*
F (cm)	0.00 ± 0.16*	0.00 ± 0.16	0.20 ± 0.12
Comb widening	Week 2	Week 3	Week 4
Control (cm)	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
Zn (cm)	0.00 ± 0.50	0.25 ± 0.75	0.00 ± 0.50
FS (cm)	0.06 ± 0.17*	0.10 ± 0.10*	0.20 ± 0.12*
F (cm)	0.06 ± 0.12*	- 0.10 ± 0.10*	0.10 ± 0.10*

Comb lengthening of Bangkok roosters had shown in Table 1. The control rooster showed no alteration of comb lengthening but a 1 cm reduction in the second week. The zinc group showed comb lengthening by – 0.25 ± 0.25; 0.00 ± 0.00; and 0.75 ± 0.25 cm in the second, third, and fourth weeks, respectively. Fishbone-shell supplementation yielded comb lengthening by 0.00 ± 0.22; 0.10 ± 0.14; and 0.60 ± 0.15 cm at the second, third, and fourth weeks, respectively. Fishbone treatment only induced a comb lengthening by 0.2 ± 0.12 cm in the week four. The comb lengthening data showed a normal distribution. Fishbone–shell treatment increased the comb lengthening in the second and fourth weeks ($p < 0.05$). The elongation change in the fishbone group only appeared in the second week ($p < 0.05$). Zinc did not significantly change comb lengthening.

Comb widening of Bangkok roosters appeared in Table 1. The control rooster showed a stagnant comb widening by 0.5 cm along the measurement weeks. Zinc treatment only yielded a 0.25 ± 0.75 cm comb widening in the third week. Fishbone-shell supplementation produced a comb widening by 0.06 ± 0.17; 0.1 ± 0.1; and 0.2 ± 0.12 cm in the second, third, and fourth weeks, respectively. Fishbone supplementation yielded comb widening by 0.06 ± 0.12; - 0.1 ± 0.1; and 0.1 ± 0.1 cm in the second, third, and fourth weeks, respectively. The comb widening data did not distribute normally. Fishbone and fishbone-shell treatment caused lower widening than the control group in every measurement week ($p < 0.05$). Otherwise, zinc did not cause any significant changes.

Gene, health, nutritional status, social interaction, and ambient temperature affect comb shape in chicken [13,15]. Comb size is a good indicator of performance in commercial chicken. Naturally, a big and attractive comb is useful for males in sexual and social aspects. A high serum testosterone level proves the superior reproductive performance in the roosters with a big comb [16,17]. A large comb also represents the dominancy of a rooster in a flock. The subdominant males tend to shrink their comb to minimize confrontations [16]. A large comb is also sexually attractive to females [17]. Bangkok rooster have a small comb and wattles. This ornamental characteristic originates from selective breeding to cope with aggressive behavior [18]. Most aggressive roosters have small comb and wattle that is adaptive for aggression. Comb and wattle anatomically possess a high vascularization for thermoregulation [19]. The large comb and wattle risk the rooster bleeding in a fight due to the vascularization [19–21].

Testosterone influence ornamental organs, including comb size, in a bird. Testosterone implantation successfully enlarge the comb in red grouse and influence social interaction between males [16]. As testosterone determines comb size, the testicular size also affects the ornamental organs. For example, Italian roosters show a correlation between testicular and comb size [22]. Testicular size affects the sexual phenotypes of birds because of testosterone production. However, the testosterone-dependent effect on comb size may involve other factors. Wattle size alteration do not appear in Bangkok roosters with a continuous testosterone treatment for 35 days [12]. Moreover, red grouse with smaller comb shows relatively similar testosterone levels to the others, indicating a modulation of testosterone-dependent effect in comb size [16].

3.1 Aromatase Inhibition Affects Comb Size in the Roosters

Shell powder supplementation increased the lengthening but not widening of the comb in the Bangkok rooster. The results showed that shell powder in fishbone-shell supplementation progressively lengthened the comb from the second to the fourth week. Androgen plays a role in the comb size regulation of the birds. The androgenic effect on the comb originates from testosterone metabolism. The hormonal synthesis and removal regulate serum testosterone levels. Aromatase converts testosterone into estradiol, which results in a serum testosterone reduction. Inhibition of aromatase activity will slow down testosterone conversion, which increases serum testosterone. The aromatase inhibition effect of the shell may elevate testosterone levels and elongate the comb. Low aromatase activity in Leydig cells of rats coexists with serum testosterone elevation [2]. Besides various chemicals, zinc shows aromatase blocker effect in several organs. Natural materials such as oyster shells possess high zinc content and aromatase blocker effect. Oyster shell treatment suppresses aromatase activity in the brain and Leydig cells of the rats [2]. Thus, the result of this experiment supports the idea that aromatase inhibitors influence ornamental organs in chickens.

3.2 Zinc as an Aromatase Inhibitor

Aromatase governs androgen and estrogen concentration in serum and tissues by transforming testosterone into estradiol [4,12,23]. Intracellular zinc modifies aromatase activity as a second messenger [24]. An elevation of zinc increases the phosphorylation of aromatase. The phosphorylation inhibits aromatase activity to convert testosterone [4,25]. Besides aromatase inhibition, zinc-metalloproteins activate gonadal hormone receptors [10]. The interaction between Zn-metalloprotein and gonadal hormone receptors leads to the path of the androgenic effect in the target cells. Zinc also mediates dihydrotestosterone synthesis that induces stronger androgenic effects [10]. Zinc deficiency causes structural alteration of Leydig cells that leads to androgen depletion. Aged male animals show testosterone elevation after zinc supplementation [26]. The alteration of comb lengthening and widening suggests the influence of zinc as an aromatase inhibitor in the oyster shell.

The results did not demonstrate a significant effect of zinc on the lengthening and widening of the comb. Conversely, the fishbone-shell treatment showed a reduction in widening and an increase in lengthening. Mineral analysis reveals an abundant amount of zinc in an oyster shell [2]. This phenomenon indicates that oyster shells depress aromatase activity in the roosters. The aromatase inhibition elevates testosterone levels and mediates the secondary sexual phenotype alteration. Comb size alterations suggest that zinc acts as an aromatase blocker in the shell. The lower effect in zinc treatment than the fishbone-shell is unclear. The combination of several minerals inside the oyster shell may modulate aromatase inhibition. For instance, the Fe supplementation causes higher Zn absorption in animals [27,28]. Oyster shells contain an abundant amount of Fe along with Zn. Nevertheless, these results prove an excellent effect of oyster shells as natural aromatase blockers.

The fishbone-shell supplementation induced superior comb lengthening to the fishbone and zinc group. This trend suggests that the combination of protein and zinc in the shell yields a better effect on comb size. Aromatase inhibition and supplemental protein improve the comb size in the Bangkok rooster.

3.3 Effect of Protein in Comb Size

Fishbone and fishbone-shell supplementation cause alterations in the comb size of Bangkok roosters. These results reveal an essential nutrient inside the fishbone. Biochemical analysis shows a high protein content in the frame of milkfish [3]. Protein deficiency disrupts reproductive performance, even fertility, in an animal. Protein is a fundamental molecule in the hormonal synthesis and receptors and the modulation of the hormones themselves [29]. Thus, the results of this study support the idea that combs lengthening indicates the protein status of chicken [13].

3.4 Width Reduction in the Treated Roosters Indicated a Conservation of Small Comb

Gene determines the morphology of the comb [13,30]. Environmental factors, such as ambient temperature, also influence the size of the comb [19]. Large combs are prone to frostbite in the cold climate. A high vascularization of the comb allows the chicken to regulate thermal movements. However, a high vascularization risks the chicken bleeding due to physical aggression [18,19]. The other factors are physical aggression and intra-sexual competition. Physical aggression and competitions of chickens were normal behavior for territorial defense and hierarchical status. Bangkok roosters have a relatively high aggressiveness. Human selective breeding results in small comb formation in aggressive roosters [18,19]. A small comb is favorable because of its minimal risk of bleeding during aggression. Long selective breeding might pool the undersized comb genotype in the Bangkok rooster population. The minimum comb widening in Bangkok roosters might be the expression of the small comb genes. Birds can suppress their comb size to adapt to the environment. Subdominant male red grouse shrinks their comb to prevent competition against the dominant rooster [16]. The roosters impede an extensive comb widening to preserve small size.

4 Conclusion

Fishbone and oyster enhance lengthening and reduce the widening of comb in Bangkok rooster. Aromatase inhibitory effect from oyster shell and protein content from fishbone shape the comb of Bangkok rooster. This study suggests the benefit of fishbone and oyster shells as a feed supplement to boost reproductive performance. Fishbone and oyster shells may be a solution for efficient poultry production during the pandemic.

Ethical Clearance Statement

Ethics Committee of the Integrated Testing and Research Laboratory Universitas Gadjah Mada (LPPT UGM) has approved all procedures with certificate number 00030/04/LPPT/VI/2020.

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