Clamshell and Fishbone Can Improve Growth Performance and Metabolism in Bangkok Rooster

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Abstract. A lot of zinc in clamshell produces an aromatase blocker attribute that enhances testosterone production. Protein-rich traits enable fishbone as a protein source for animals. Testosterone amplifies muscle protein synthesis and proliferation. Furthermore, T3 and T4 regulate fat and protein conversion into energy. The fluctuation of body fat and protein influences body weight. Bangkok roosters (*Gallus gallus*) develop massive muscle and aggressive physical activity that portray testosterone manifestations. But, the effect of natural aromatase blockers on T3 and T4 and body weight needs further study, especially in Bangkok roosters with clamshell and fishbone treatment. This research aimed to assess the clamshell and fishbone effects on T3, T4, and body weight. Three-month-old Bangkok roosters underwent an experiment for 35 days with weekly weighing. The weekly T3 and T4 measurements employed an enzyme immunosorbent assay. All rooters consisted of three groups: P0 ($n = 6$) did not receive any treatment, P1 ($n = 6$) received clamshell and fishbone treatment, and P2 ($n = 6$) consumed fishbone treatment only. The significant results were the data that showed $p < 0.05$ in the ANOVA test. Roosters in P1 showed a drop in the T3/T4 ratio ($p < 0.05$) at the end of the experiment. No significant changes appeared in body-weight ($p > 0.05$). The fall of T3/T4 represents that the roosters are free from the energy deficit. The increase of T3 proceeds fat and protein breakdown to fulfill energy needs. T4 has a similar function to T3 but also presents as a T3 prohormone. The intact body weight indicates that the treatment is desirable for energy balance. Clamshell and fishbone treatment can be a supportive feed supplement to improve metabolism in Bangkok roosters. Current results proved that clamshell and fishbone supplementations reduced T3/T4 ratio but did not influence body weight in Bangkok roosters. This study suggested that clamshell and fishbone were effective natural substances for modifying growth and metabolism in chickens.

Keywords: Aromatase blocker · bangkok rooster · body weight · T3/T4 ratio

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1 Introduction

Modern poultry raises chicken breeds that produce optimum growth rates [1–3]. The rapid growth of chicken is not only genetic but also the outcome of husbandry. Feed advancement is part of precision husbandry that can increase chicken productivity. An alternative feed supplement can improve chicken meat production and sustain feed availability. Various feedstuffs studies have been done in recent years for feed advancement in poultry [4–6].

Clamshell and fishbone are natural substances that emerge as the byproduct of aquaculture and marine industries. A high zinc level in clamshell expresses an aromatase blocker effect that enhances testosterone levels [7, 8]. Fishbone possesses a high protein amount which is desirable for muscle improvement [9, 10]. The muscular androgen receptor will interact with testosterone which provokes myofiber proliferation and protein synthesis [11, 12]. The androgenic improvement in muscle can boost chicken productivity. The clamshell action is safer than the exogenous androgen. Exogenous testosterone treatment directly impairs the reproductive system in humans and animals [13]. Because extra testosterone consumption heavily suppresses gonadotropin secretion. Clamshell aromatase blocker increases endogenous testosterone, which decreases gonadotropin physiologically. Therefore, clamshell and fishbone are desirable to increase chicken productivity by testosterone elevation and muscle performance improvement.

The thyroid is a gland that secretes triiodothyronine (T3) and thyroxine (T4). The secretion of T3 and T4 are under the regulation of thyroid-stimulating hormone (TSH) from the anterior pituitary. The low T3/T4 ratio increases TSH secretion by stimulating the hypothalamus to secrete TSH-releasing hormone (TRH) [14]. Otherwise, T3 escalation suppresses hypothalamic TSH production [14]. The T3 and T4 activities governing body fat and protein affect body weight. T3 and T4 modulate energy extraction from fat and protein mainly for physical activity and thermoregulation [15, 16]. Exogenous T3 and T4 treatments minimize body weight gains in broilers [15]. Chronic thyroxine treatment reduces body weight and enhances blood glucose levels in broilers [16]. The aromatase blocker effect of clamshell on T3 and T4 needs further research because of the influences on muscle protein.

The muscle is an organ that plays a significant role in protein metabolism. An additional protein intake can improve the muscle structure in chickens. An extra protein ration in seven-day-old Kampung chicken produces sounder pectoralis muscle development [17]. The protein-rich properties of the fishbone are a potential protein source to increase muscle performance. The T3 and T4 activities modulate muscle performance because of the protein breakdown effect to fulfill energy demand. A level up of T3 and T4 treatments increase pectoral muscle weight but decreases abdominal fat in the broiler [6, 15]. The protein breakdown rate is quicker when T3 and T4 escalate [15]. The study of T3 and T4 is crucial to understand muscle protein regulation.

Bangkok rooster bears a superior muscle performance due to its original cockfighting use in Thailand [3]. The Indonesian people hybridize the Bangkok chicken for various utilization, involving meat production [18, 19]. Broilers have a vigorous genetic selection to achieve a rapid growth rate, which affects muscle characteristics [1, 20, 21]. Bangkok rooster potentially becomes an excellent meat-producing chicken with a unique meat characteristic. Muscle and fat composition influence body weight in chicken [3, 20,

21]. The robust muscle trait in Bangkok roosters affects the body weight gain pattern. The investigation of clamshell and fishbone effects on body weight is necessary for the Bangkok rooster.

The demand for protein sources encourages the poultry industry to develop precision husbandry. Therefore, the potency of clamshell and fishbone to boost productivity can be essential in precision husbandry. T3/T4 ratio and body weight are pivotal to comprehending chicken productivity. This research aimed to evaluate the effect of clamshell and fishbone treatments on T3/T4 profiles and body weight in Bangkok roosters.

2 Materials and Methods

2.1 Animal

This research subjected 18 three-month-old Bangkok roosters (*Gallus gallus*), which originated from an ornamental chicken breeder in Bantul Regency, Yogyakarta Special Region, Indonesia. The rearing and experiments took place in Bantul Regency, Yogyakarta Special Region, Indonesia, from February until March 2022. The rooster husbandry method was semi-intensive, with individual platform wooden chicken houses and slit bases. All procedures in this study fulfilled the standard from the Ethics Committee of the Integrated Testing and Research Laboratory Universitas Gadjah Mada (LPPT UGM) with certificate number 00009/04/LPPT/III/2021.

The study design in this research was the Randomized Control Trial (RCT) method. Three rooster groups underwent the treatment for 35 days. Each treatment group comprised six roosters that consumed treatment material. The treatment groups were P0: without treatment; P1: fishbone 3.3 g/day + clamshell 6.6 g/day, and P2: fishbone 3.3 g/day. The fishbone powder was the brackish water milkfish (*Chanos chanos*) bones that underwent a water extraction. Atomic absorption spectrometry (AAS) revealed 35.75% protein in the milkfish thorns. The clamshell carried 61.55 mg/kg of Zn, according to the previous study [7]. Zinc is also available in the fishbone, particularly 1.40 mg/100 g in sea bream and 0.59 mg/100 g in tuna [22, 23]. All animals received 300 g/bird/day standard feed and ad libitum water. The roosters had a weekly weighing to collect body weight data.

2.2 Serum T3 and T4

The serum T3 and T4 analysis employed an Enzyme-linked immunosorbent assay (ELISA) according to the manual (Calbiotech T4 ELISA T4224T and T3 ELISA T3379T, USA). The sera from days 0, 7, 14, 21, 28, and 35 of treatment were collected and saved at −20 °C for analysis. The T3/T4 ratio was the result of the division of T3 levels by T4 levels.

2.3 Statistical Analysis

The statistical analysis employed one-way ANOVA at a 95% confidence level ($\alpha = 0.05$) with SPSS 16.0 software. Then, the Duncan test complemented the ANOVA to identify the different values.

3 Results

The results of ELISA showed a decrease of the T3/T4 ratio in the P1. Table 1 presented the profile of T3/T4 in the Bangkok rooster during 35 days of the experiment. These results revealed that clamshell and fishbone might suppress the T3/T4 ratio in the Bangkok rooster.

Table 2 displayed the body weight of the Bangkok rooster through 35 days of treatment. All roosters underwent body weighing every seven days. The results showed that giving fishbone and a fishbone-clamshell mixture supplement could increase body weight, although statistically did not show a significant difference among others ($p > 0.05$).

Table 1. Serum T3/T4 ratio of Bangkok rooster under 35 days of treatment

P	Mean ± SD of T3/T4 ratio, days -					
	0	**7**	**14**	**21**	**28**	**35**
P0	1.27 ± 0.71^{a}	1.02 ± 0.41^{a}	1.61 ± 0.41^{a}	1.52 ± 0.91^{a}	2.20 ± 1.45^{a}	1.83 ± 0.44^{a}
P1	1.45 ± 0.17^{a}	1.47 ± 0.87^{a}	1.60 ± 0.83^{a}	1.50 ± 0.79^{a}	1.80 ± 0.47^{a}	1.14 ± 0.39^{b}
P2	1.92 ± 0.64^{a}	1.64 ± 0.42^{a}	1.93 ± 0.63^{a}	2.02 ± 0.60^{a}	2.12 ± 1.14^{a}	2.49 ± 0.74^{a}

[a,b] Different letters in the same column show significant differences ($p < 0.05$). P0: control, P1: milkfish thorn 3.3 g + clam shell 6.6 g, P2: milkfish thorn 3.3 g. P: treatment, SD: standard deviation.

Table 2. Bodyweight of Bangkok rooster under 35 days of treatment

P	Mean ± SD of bodyweight (kg), days -					
	0	**7**	**14**	**21**	**28**	**35**
P0	1.36 ± 0.15^{a}	1.53 ± 0.12^{a}	1.67 ± 0.12^{a}	1.91 ± 0.08^{a}	1.96 ± 0.11^{a}	2.15 ± 0.08^{a}
P1	1.50 ± 0.15^{a}	1.68 ± 0.15^{a}	1.82 ± 0.12^{a}	1.98 ± 0.13^{a}	2.06 ± 0.12^{a}	2.35 ± 0.17^{a}
P2	1.31 ± 0.34^{a}	1.51 ± 0.33^{a}	1.64 ± 0.31^{a}	1.82 ± 0.30^{a}	1.92 ± 0.35^{a}	2.27 ± 0.25^{a}

[a] Different letters in the same column show significant differences ($p < 0.05$). P0: control, P1: milkfish thorn 3.3 g + clam shell 6.6 g, P2: milkfish thorn 3.3 g. P: treatment, SD: standard deviation.

4 Discussion

Clamshell has an aromatase blocker property due to its high zinc amount. The blood zinc enters the Zn transporter into the intracellular zone [24]. Intracellular zinc becomes a second messenger that phosphorylates cellular proteins [24, 25]. The rise of intracellular phosphorylation blocks the aromatase activity [26]. Aromatase blockers elevate testosterone activity by hindering the conversion of testosterone into estradiol. Testosterone can cause muscle hypertrophy in humans and animals. The androgenized female rats show enlargement of the gastrocnemius and tibialis anterior muscles [27]. Furthermore, testosterone therapy has been widely effective as a therapeutic agent for muscle-wasting disorders [28–30].

The fishbone treatment boosts dietary protein in the Bangkok rooster. A high-protein diet promotes protein deposition in muscle. Besides, an elevation of testosterone amplifies muscle protein absorption and production. Protein deposition contradicts T3 and T4 functions which induces protein breakdown [15]. Thus, the reduction in T3/T4 ratio supported muscle growth in this study. The low T3/T4 ratio is the outcome of low T3 and high T4 in the individuals. The hypothalamic TRH secretion occurs under a low circulating T3/T4 ratio [14]. The TRH activates the anterior pituitary to stimulate the thyroid gland by producing TSH. The thyroid gland secretes T4 and a small amount of T3 in the blood. Meanwhile, peripheral tissues mainly contribute to serum T3 by transforming serum T4 into T3 [31]. A serum T3 increase gives negative feedback to the hypothalamus and pituitary to maintain the T3/T4 ratio [14]. The fall of circulating T3 occurs in an individual under heat stress, food restriction, and negative energy balance [15, 32, 33]. The metabolism and excretion of thyroid hormones are under deiodinase regulations. Deiodinase enzymes comprise deiodinase 1 (Dio1), Dio2, and Dio3. Dio1 modulates the conversion of T4 into the inactive T3 metabolite or reverse T3 (rT3). Dio2 promotes T3 activity by T4 deiodination. Dio3 converts T4 to rT3 and T3 to diiodothyronine. The administration of testosterone depresses T3 activity in chickens [8, 34]. The aromatase blockage might result in a low T3/T4 ratio due to a high testosterone level in the P1 roosters. The high testosterone level can excite the hypothalamus to increase TRH release [33]. The blood testosterone elevation activates the thyroid gland to produce predominantly T4 and a few T3 into circulation [35]. Besides, testosterone also induces hepatic deiodinase 1 (dio1) to metabolize serum T4 and T3 [35, 36]. Dio1 scarcely catalyzes T3 but intensively metabolizes sulfated-T3 (T3S) to recollect iodothyronines [37]. Testosterone likely induced thyroidal T4 production and hepatic T3 breakdown in Bangkok roosters.

The low T3/T4 ratio in P1 animals suggested that clamshell and fishbone induced energy deposition. Androgen and additional proteins reduce energy usage, which improves protein synthesis. Physiological energy demand such as exercise and thermoregulation escalates T3 secretion [33, 37]. But, negative energy balance status depletes T3 activity to prevent energy loss [33]. The androgen receptor inactivation disrupts glucose metabolism, which triggers late obesity and excessive insulin [38]. Treatment in P1 seemed to prevent energy loss and enhance protein synthesis in Bangkok roosters. High levels of T3 and T4 can coexist with heavier pectoral muscle but less abdominal fat in the broiler [6, 15]. Broiler has a considerably higher intramuscular fat than indigenous Thai chickens [20, 39]. T3 and T4 promote fat and protein degradation to

increase blood glucose and heat production [16]. High body fat in broiler may substitute the protein degradation hence protein synthesis and muscle growth remain unimpaired. The β-hydroxy-β-methyl butyrate calcium (HMB-Ca) can drive muscle growth and protein synthesis in the broiler with high thyroid hormones [6]. Dietary HMB-Ca is a leucine metabolite that potentially increases muscle mass in animals and humans [6]. The Bangkok rooster is originally a cockfighting breed in Thailand [18, 19]. Cockfighting roosters typically develop massive muscles and aggressive physical activity [3]. The low T3/T4 ratio may be suitable for the Bangkok rooster to develop muscle performance with efficient energetics.

The T3 and T4 activity also regulate chicken body weight. T3 and T4 elevation coexist with smaller body weight gain in broilers [15]. Chronic hyperthyroidism also causes body weight loss in broiler [1]. Consumption of HMB-Ca results in elevation of T3 and T4 with heavier body weight in broiler [6]. The P1 roosters showed the heaviest body weight among the others at the end of the treatment. However, no Bangkok rooster showed a significant body weight difference in the current research. The insignificant weight differences indicate that the low blood T3 does not directly regulate body weight. The high level of circulating T4 probably can maintain the body weight of the P1 rooster. The intracellular Dio2 activity can transform T4 into T3 in the Bangkok rooster. Thus, the intracellular T3 induces fat degradation in the peripheral tissues to prevent uncontrollable weight gain. Although, severe T3 and T4 depletions cause metabolic disorders that lead to obesity, hypothermia, and developmental impairments in individuals [33]. The low serum T3 activity in Bangkok rooster may still be able to prevent fat formation that influences body weight. This finding suggested that the decrease of T3/T4 slightly increased the body weight of the Bangkok rooster. This study also provided an information about the physiological activity of T3 and T4 on body weight in Bangkok roosters.

5 Conclusion

The supplementation of fishbone and clamshell mix can increase body weight by lowering the activity of the thyroid hormone in the Bangkok rooster. This study proved that clamshells and fishbone could improve metabolism and growth performance in the chicken.

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