

Effect of *Indigofera sp.* Diet Towards Blood Serum Biochemistry of Indonesian Crossbred Native Chickens

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Abstract. A study was conducted to assess the effects of *Indigofera sp.* meal on the serum biochemistry of Kampung Unggul Balitbangtan (KUB) chickens, a native strain released by the Indonesian Minister of Agriculture via Decree No. 274/Kpts/SR.120/02/2014. Forty KUB chickens, aged 8-16 weeks, were raised in floor cages with ad libitum access to water. Four dietary treatments were administered: T0 (control: 25% concentrate, 35% rice bran, 40% maize); T1 (15% concentrate, 35% rice bran, 10% *Indigofera sp.* meal, 40% maize); T2 (T1 + 2cc/l Bio-B); and T3 (22% concentrate, 31.5% rice bran, 10% *Indigofera sp.* meal, 36.5% maize). Serum samples, obtained via jugular venipuncture and centrifugation, were analyzed for blood urea nitrogen (BUN), total protein, total cholesterol, and glucose. A randomized block design was employed, and data were analyzed using Duncan's post hoc test (SPSS 26). Significant differences ($p < 0.05$) in BUN and total cholesterol were observed in the T1 group compared to the control, while total protein and glucose levels remained unaffected. The findings suggest that the T1 diet is suitable for sustainable KUB chicken production.

1 Introduction

Ministerial Decree No. 274/Kpts/SR.120/02/2014, issued by the Indonesian Minister of Agriculture, officially recognized the KUB (Kampung Unggul Balitbangtan) chicken as a native breed. The KUB chicken is a native variety bred for dual-purpose use. The advantages of KUB chickens include low feed conversion ratios with good immune system, low mortality rates, and high egg productivity [1]. In line with the government's program to accelerate the fulfillment of protein consumption by poultry, the development of KUB chickens is a strategic step to be carried out through selective breeding, which is known

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through blood serum, as well as through the improvement of feed management using additional organic feed ingredients.

Improvement in feed management is being achieved through the addition of organic feed materials, such as *Indigofera sp.* meal. *Indigofera sp.*, as a leguminous plant, holds great potential for use as feed for ruminants, poultry, pets, and fish. This legume contains well-balanced amino acids enough and contain vitamins, carotenoids, and xanthophylls [2]. *Indigofera sp.* boasts a crude protein content of 27.60%-31% [3], a dry leaf yield of 4,096 kg/ha/harvest, and an in vitro dry matter digestibility of 67%-81% [4] and can be harvest in 68 days. The addition of 7.5% *Indigofera sp.* leaf meal to layer did not reduce production performance [5]. Furthermore, [6] added that up to 15% of *Indigofera sp.* meal can be used in animal diets without causing negative impacts on animal health or performance.

In general, blood serum can reflect any physiological, nutritional, or pathological changes that occur in poultry [7-8]. However, serum is not influenced by different genotypes of chickens [7], [9-10]. These biochemical indicators reflect the internal environmental homeostasis of poultry, which affects not only the health of chickens but also production parameters [11-12]. The liver and kidneys play a crucial role in removing nitrogen waste from the body of poultry. An increase in nitrogen levels in chickens may indicate liver or kidney damage [13], while the opposite condition may indicate problems with nutrition or protein metabolism in the body [14]. Additionally, glucose in blood serum is an indicator of the main energy source for poultry [15], as a precursor to steroid hormones and bile acids, cholesterol is also involved in cell proliferation and contributes to the structure of cell membranes [16-18]. Steroid hormones are crucial in poultry for regulating reproductive function, metabolic and electrolyte balance, inflammatory and immune responses, and growth regulation [16], [18-19]. The total protein value reflects the utilization of protein from feed and the level of hemoconcentration [13], [20].

Limited information is available concerning the influence of *Indigofera sp.* meal on the blood chemistry (urea, total protein, total cholesterol, and glucose) of the Ministry of Agriculture's KUB chicken breed. Therefore, this research aims to identify the missing information. This study is expected to make a significant contribution to the development of healthier and productively KUB chickens.

2 Material and methods

2.1 Animal and experimental design

The research took place in the Tembuku area of Bangli Regency, Bali, Indonesia, during the months of July and August 2022. A total of 40 KUB chickens were raised from the age of 8-16 weeks and were used in the study. Ad-Libitum drinking was given to the chickens, and the cage system used was a floor system. The KUB chickens were divided into 4 treatments with 5 replications each. Four distinct treatments, identified as T0 through T3, were included in the experimental design, as illustrated in Table 1.

Table 1. The dietary treatments

Ingredients	T0	T1	T2	T3
Concentrate (%)	25	15	15	22
Rice bran (%)	35	35	35	31.5
<i>Indigofera sp.</i> Meal (%)	0	10	10	10
Corn (%)	40	40	40	36.5
Bio B (ml/liter)	0	0	2	0
Nutrient*	T0	T1	T2	T3
Dry Matter (%)	86.35	86.65	86.65	86.71

Ash (%)	5.14	4.51	4.51	4.63
Crude Protein (%)	13.94	12.71	12.71	13.74
Ether Extract (%)	7.32	7.82	7.82	7.60
Crude Fiber (%)	5.93	5.96	5.96	6.00
Gross Energy (cal/g)	3005	3028.75	3028.75	3009.88
Calcium	0.26	0.19	0.19	0.25
Phosphor	0.83	0.75	0.75	0.75

2.2 Blood collection and analytical determination

Serum blood parameters were measured in five randomly selected chickens from each of the treatment groups. Blood samples were collected from the jugular vein and subsequently processed using centrifugation techniques. Urea, total protein, total cholesterol, and glucose levels were measured using biochemical analysis. The statistical analysis was designed using ANOVA and analyzed through the SPSS 26 program. Statistical significance of treatment differences was determined using Duncan's test at a p-value of less than 0.05.

3 Result and discussion

Research on Balitbangtan's native chicken (KUB) examined urea, total protein, cholesterol, and glucose levels across different treatments (Table 2). While the T2 treatment exhibited a statistically significant difference in urea levels ($p < 0.05$), and the T1 treatment showed a significant difference in cholesterol levels ($p < 0.05$), no significant differences were found for total protein and glucose levels ($p > 0.05$).

Table 2. Serum blood parameters of KUB chickens

Variable	T0	T1	T2	T3
Urea (g/dL)	1.45±0.26a	1.70±0.14ab	3.29±3.55b	1.51±0.16a
Total protein (g/dL)	4.61±0.43	4.93±0.33	4.97±0.44	5.03±0.50
Total cholesterol (g/dL)	1.16±0.32ab	1.39±0.14c	1.05±0.14a	1.32±0.14bc
Glucose (g/dL)	2.33±0.18	2.42±0.20	2.48±0.13	2.36±0.32

*significance 0.05

The inclusion of 10% *Indigofera sp.* meal in treatments T1, T2, and T3 led to elevations in urea, total protein, and glucose levels compared to the control group (T0). Total cholesterol also increased in T1 and T3, but not in T2. The urea levels in the T2 treatment showed the worst values, which could be due to the stress of feed or the environment on KUB chickens, rather than low protein digestibility. It was confirmed by the higher total protein in T2 than T1, even not significantly different. Furthermore, the protein in T3 showed the greater result, which was due to well protein concentrate digestibility than substitution with *Indigofera sp.* meal. [21] explained that probiotics contribute to fiber degradation, immunity, and regulation of gut microflora homeostasis. Increased in urea levels is influenced by the protein metabolism whose excretion is carried out by the kidneys. If there is damage to the glomerulus cells in the kidney, it could cause a decrease in the glomerulus filtration rate [22-23]. Protein and purine metabolism in birds culminates in the production of uric acid, which is then excreted through the feces, a process distinct from that in mammals, where urea and creatinine are the primary excretory products. It is toxic less than ammonia and urea [24]. Dietary protein levels above requirements result in elevated uric acid excretion in birds. In addition to its function in eliminating nitrogenous waste, uric acid is a key component of the blood's antioxidant system and may be a factor in increased lifespan [25].

The total cholesterol in T1 showed the highest result compared to other treatments. In poultry, cholesterol plays a vital role in both egg formation and the bird's metabolic functions. While typical cholesterol levels in poultry pose little risk, a significant elevation can negatively impact both the birds' well-being and egg quality [26-27]. The results of this study align with the findings reported by [28] in their research, that *Indigofera sp.* treatment was able to produce a negative correlation between cholesterol levels and protein content. Xanthophyll and γ -carotene found in *Indigofera sp.* at a concentration of 507.6 mg/kg could reduce total cholesterol in poultry blood [28-29]. However, the cholesterolemic effect of feed components in poultry could be influenced by factors such as breed, sex, age, and feed composition [28] [30]. The glucose levels in the T2 treatment showed the great values in the study. [28] explained that there was a trend for glucose levels to increase in native chickens supplemented with *Indigofera sp.* compared to supplementation with *Brachiria sp.* and *Axonopus sp.* Blood glucose levels in native chickens indicate the amount of energy that could be utilized. According to [10], a lack of energy would stimulate the process of glycogenolysis to produce glycogen, while if the body lacks glucose, the process of lipolysis would produce energy reserves in the fat form. The values of this study indicate that the blood glucose levels in entire treatments were constantly within normal limits and sufficient to cover energy requirements.

Blood parameters are capable of providing information on metabolism, stress response, and their impact on poultry production performance. Stress can elevate blood levels of corticosteroids, glucose, cholesterol, and high-density lipoprotein, potentially impacting production [14]. Previous research has suggested that the levels of uric acid, total protein, and total cholesterol could be influenced by age, pathologies, housing models, and feed diet [8] [31-34]. A comparison of average urea levels revealed that KUB chickens had higher values than Hisex Brown chickens [14], but worse than Noi Vietnam chickens [10]. The total protein content was greater in KUB chickens than in Ross 38 chickens [35] and Saudi native chickens [9], but it worse than five natives chickens in Nigeria [36]. A study [14] comparing Hisex Brown laying hens (30-45 weeks old) in cage and floor systems found differences in several blood parameters. Hens in cages exhibited total protein, urea, and total cholesterol levels of 4.51 g/dL, 3.33 mg/dL, and 1.13 g/dL, respectively. Corresponding values for floor-housed hens were 4.67 g/dL, 3.99 mg/dL, and 1.19 g/dL. Meanwhile, [12] found that the average total protein was 5.56 g/dL and the total cholesterol was 1.51 g/dL in 25-week-old Hyline chickens. A study by [28] on total cholesterol levels in native chickens fed *Indigofera sp.* meal showed a value of 1.49 g/dL. A study by [10] on Noi Vietnam chickens revealed the following average levels: glucose (2.51 g/dL), total protein (7.34 g/dL), albumin (3.40 g/dL), globulin (3.94 g/dL), albumin/globulin ratio (1.75), triglyceride (1.48 g/dL), cholesterol (1.90 g/dL), creatinine (0.44 mg/dL), and uric acid (1.44 mg/dL), although none of these parameters were statistically significant. The total protein levels in Nigerian and Saudi local chickens were 7.18 g/dL [36] and 3.35 g/dL [9], respectively.

4 Conclusion

Research revealed that a 10% *Indigofera sp.* meal inclusion in KUB chicken diets altered serum urea and total cholesterol concentrations, while protein and glucose levels remained unchanged. The T1 treatment is considered highly suitable for promoting the sustainable growth of KUB chicken production.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement

Rachmad Dharmawan: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. Ida Ayu Parwati: Data curation, Writing- Reviewing and Editing. I Nyoman Suyasa: Investigation, Writing- Reviewing and Editing. Anastasia Sischa Jati Utami Investigation, Writing- Reviewing and Editing.

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