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BLOOD BIOCHEMICAL PROFILE OF BALI CATTLE FED LIGNOCELLULOLYTIC BACTERIAL PROBIOTICS AND BROILER LITTER-BASED FUNCTIONAL FEED

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ABSTRACT

This study aimed to determine the blood chemistry profile of Bali cattle given lignocellulolytic probiotics bacteria and functional feed based on broiler litter. Twelve male Bali cattle were used in a Randomized Block Design (RBD) with 3 treatments and 4 blocks. The treatments consisted of: P0 (Bali cattle fed forage and rice bran concentrate), P1 (Bali cattle fed forage and functional feed), and P2 (Bali cattle fed forage, functional feed, and lignocellulolytic bacterial probiotics). Concentrate and functional feed were given at 1% of body weight per day, while the probiotic was administered at 0.01% of body weight per day. The parameters observed included urea, glucose, total cholesterol, triglycerides, HDL, and LDL levels, measured at week 6. Analysis of variance showed that the treatments had a significant effect ($P < 0.05$) on increasing glucose, total cholesterol, and HDL levels, as well as decreasing triglyceride and LDL levels, particularly in the P2 group. However, the treatments had no significant effect ($P > 0.05$) on blood urea levels. This study concludes that the administration of lignocellulolytic bacterial probiotics combined with broiler litter-based functional feed can improve the blood chemistry profile of Bali cattle, particularly total cholesterol and especially HDL, suggesting P2 combination could be considered a potential alternative feed strategy.

Keywords: Bali cattle, Broiler litter, Lignocellulolytic bacteria, Functional feed, Blood biochemical profile

1 INTRODUCTION

Bali cattle (*Bos javanicus*) represent a superior indigenous germplasm crucial for national food security in Indonesia, owing to their high fertility rate, desirable carcass yield (~56%), and robust adaptability to tropical environments (Diwyanto and Priyanti, 2008; Tahuk *et al.*, 2018; Sudrajat *et al.*, 2022). Possessing an efficient digestive system capable of utilizing high-fiber, low-quality roughage, Bali cattle hold substantial potential for beef cattle fattening programs. Sustainable livestock production necessitates adequate nutrition; however, high-quality commercial feeds directly correlate with elevated operational costs. Consequently, developing cost-effective yet nutritious feed alternatives is

essential, which can be achieved through agricultural waste utilization integrated with biofermentation and supplementation technologies (Utama and Christiyanto, 2021).

Broiler litter represents a highly promising alternative feed resource derived from poultry waste. Composed primarily of rice hulls and chicken manure, broiler litter contains substantial nutritional value, including 13–25% crude protein, 20–25% crude fiber (Brahmana *et al.*, 2023), and rich essential minerals such as Ca, Mg, Fe, Zn, Cu, and S (Kpombrekou-A *et al.*, 2002; Utama and Christiyanto, 2021). Despite its nutrient density, the presence of antinutritional factors poses risks to animal performance, necessitating processing to degrade these limiting factors before inclusion in ruminant diets (Utama and Christiyanto, 2021).

Processing broiler litter into functional concentrates via bio-fermentation using lignocellulolytic bacterial inoculants effectively upgrades waste into functional feed. Lignocellulolytic bacteria act as efficient biocatalysts capable of cleaving crude fiber complexes in organic waste while enhancing nutrient digestibility (Mudita, 2019). Incorporating probiotics into functional feed not only satisfies basal nutrient requirements but also maintains physiological homeostasis by improving feed efficiency through the modulation of rumen microbial populations (Purwanti *et al.*, 2014). Enhanced feed efficiency and conversion driven by probiotic supplementation depend on balanced nutrient availability within the digestive tract, which positively impacts disease resistance and daily weight gain (Nugroho *et al.*, 2025). Given that this functional feed is derived from waste streams, its metabolic safety and absorption efficiency must be rigorously validated through blood biochemical profiling.

Blood biochemical parameters—such as urea, glucose, total cholesterol, triglycerides, HDL, and LDL—serve as vital physiological indicators reflecting nutrient adequacy, metabolic status, and organ function, which ultimately govern meat quality. Therefore, this study investigated the blood biochemical profile of Bali cattle supplemented with broiler litter-based functional feed and lignocellulolytic bacterial probiotics.

2 MATERIAL AND METHODS

2.1 Experimental Location and Animals

This research was conducted at the PT. Agro Sari Satwa farm (Baturiti), the Closed House Teaching Farm, and the Laboratory of the Faculty of Animal Husbandry, Udayana University. Twelve male Bali cattle with an initial body weight of 243 ± 0.44 kg were utilized. Corn stover of *Zea mays* (*tebon*) was provided as basal forage. Treatments consisted of different concentrate formulations and probiotic supplementation in drinking water. Concentrates provided were either rice bran or Microbial Protein functional feed produced from 21-day anaerobic bio-fermentation of broiler litter using bacterial inoculants.

2.2 Functional Feed Preparation

Functional feed processing involved drying and sterilizing broiler litter, followed by inoculation with a lignocellulolytic bacterial solution calibrated to reach a 50% moisture content. Anaerobic fermentation was carried out for 21 days in plastic drum silos. Subsequently, the fermented mass underwent pelleting and a 5-day tiered drying regimen (initial drying at 40°C for 2 days, followed by final finishing and packing).

2.3 Experimental Design and Feeding Regimen

A Randomized Block Design (RBD) was implemented with 3 treatments and 4 blocks (grouped by body weight). The experimental treatments were:

- **P0 (Control):** Bali cattle fed forage and rice bran concentrate at 1% of body weight/day
- **P1:** Bali cattle fed forage and broiler litter-based functional feed fermented with lignocellulolytic bacteria at 1% of body weight/day.
- **P2:** Bali cattle fed forage, lignocellulolytic bacterial probiotics at 0.01% of body weight/day, and broiler litter-based functional feed fermented with lignocellulolytic bacteria at 1% of body weight/day.

Diets were offered twice daily (morning and evening) following a 2-week dietary adaptation period.

2.4 Blood Sampling and Biochemical Analysis

Blood samples were collected at week 6 of the trial, exactly 4 hours post-morning feeding. A 10 mL blood sample was drawn from the jugular vein of each animal using plain vacuum syringes. Serum was separated via centrifugation. Blood biochemical parameters were analysed using the Colorimetric End-Point method (Glory® Diagnostics kit), with absorbance measured on a spectrophotometer at specific wavelengths (340 nm for urea; 500 nm for glucose, total cholesterol, triglycerides, HDL, and LDL).

2.5 Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA). Differences among treatment means were evaluated using Tukey's Honestly Significant Difference (HSD) test at a significance level of $P < 0.05$.

3 RESULTS AND DISCUSSION

The experimental results regarding the blood biochemical parameters—including blood urea, glucose, total cholesterol, triglycerides, High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL)—in male Bali cattle subjected to treatments P0, P1, and P2 are summarized in table 1.

Table 1: Blood biochemical profile of Bali cattle.

Parameter	Experimental treatments ¹			
	P0	P1	P2	SEM ²
Urea (mg/dl)	21.08 ^a	23.11 ^a	22.98 ^a	0.54 ^(ns)
Glucose (mg/dl)	28.49 ^c	58.18 ^a	41.34 ^b	1.93
Triglycerides (mg/dl)	24.34 ^a	27.06 ^a	20.88 ^c	0.79
Total Cholesterol (mg/dl)	113.62 ^b	159.25 ^a	123.33 ^b	4.86
HDL (mg/dl)	93.96 ^b	129.08 ^a	109.96 ^b	3.98
LDL (mg/dl)	14.80 ^b	24.77 ^a	9.20 ^c	0.81

Note: 1. Experimental treatments: P0= Bali cattle fed forage and rice bran concentrate at 1% of body weight/da. P1= Bali cattle fed forage and broiler litter-based functional feed fermented with lignocellulolytic bacteria at 1% of body weight/day.. P2= Bali cattle fed forage. lignocellulolytic bacterial probiotics at 0.01% of body weight/day. and broiler litter-based. functional feed fermented with lignocellulolytic bacteria at 1% of body weight/day. ²SEM= Standard Error of The Treatment Means. ³Means with different superscript letters within the same row differ significantly ($P < 0.05$)

3.1 Blood Urea Concentrations

Based on the analysis of variance (ANOVA) presented in table 1, blood urea concentrations in male Bali cattle for treatment groups P0, P1, and P2 were recorded at 21.08 (mg/dL), 23.11 (mg/dL), and 22.98 (mg/dL), respectively. Statistical evaluation demonstrated no significant difference ($P > 0.05$) among the experimental diets.

When compared with the findings of Mudita (2019), who reported blood urea levels ranging between 22.82(mg/dL) and 25.60(mg/dL) in Bali cattle fed rations fermented with lignocellulolytic bacteria, the urea values obtained across all groups in this trial fell within a highly consistent physiological range. Although the urea values in P0, P1, and P2 were slightly lower than the upper range reported in earlier literature, the overall consistency confirms that dietary protein degradation and hepatic urea synthesis operated under stable physiological conditions typical for cattle receiving lignocellulolytic inoculants.

From a physiological perspective, blood urea concentration directly reflects the dynamic equilibrium between nitrogen degradation in the rumen and microbial protein synthesis. The subtle, non-significant numerical increase in urea observed in P1 and P2 relative to P0 aligns with the presence of non-protein nitrogen (NPN) compounds—primarily uric acid—inherent to chicken manure within broiler litter (Utama and Christiyanto, 2021). Rumen microorganisms rapidly hydrolyze uric acid into free ammonia (NH₃), which is subsequently absorbed across the rumen epithelium into the portal circulation and synthesized into urea by hepatic tissues (Tahuk *et al.*, 2018). However, because this elevation was statistically non-significant ($P > 0.05$), it indicates that the excess ammonia generated from broiler litter NPN was effectively synchronized with fermentable energy liberated by the probiotics. Consequently, rumen microbes efficiently utilized the available ammonia for microbial protein synthesis rather than allowing it to accumulate as excess blood urea (Mudita, 2019).

3.2 Blood Glucose Dynamics

Analysis of variance revealed that dietary treatments exerted a highly significant effect ($P < 0.05$) on blood glucose levels in Bali cattle. Mean blood glucose values were 28.49(mg/dL) for P0, 58.18(mg/dL) for P1, and 41.34(mg/dL) for P2. Post-hoc analysis using Tukey's HSD test established that the P1 group achieved the significantly highest glucose concentration, followed by P2, while the control group (P0) exhibited the lowest values.

According to reference values established by Mitruka *et al.* (1977) in Tahuk *et al.* (2018), physiological blood glucose concentrations in healthy cattle typically range between 43(mg/dL) and 100(mg/dL). The glucose level observed in P1 (58.18(mg/dL)) fell squarely within this normal reference range, whereas values for P0 (28.49(mg/dL)) and P2 (41.34(mg/dL)) were positioned slightly below the standard threshold. By comparison, Mudita (2019) reported higher blood glucose ranges (72.7-81.0(mg/dL)) in Bali cattle fed lignocellulolytic fermented diets.

The marked increase in blood glucose observed in P1 and P2 compared to P0 is primarily attributed to the enzymatic activity of the lignocellulolytic bacterial consortium incorporated into the functional feed. These bacteria degrade recalcitrant crude fiber complexes within broiler litter into fermentable carbohydrates, yielding volatile fatty acids (VFAs)—particularly propionate, which serves as the principal substrate for gluconeogenesis in the ruminant liver. Interestingly, P2 yielded lower glucose concentrations than P1. This phenomenon suggests that direct probiotic supplementation in drinking water modulates hepatic gluconeogenesis rates, optimizing intracellular energy utilization and preventing excessive glycemic spikes. Overall, the significant difference ($P < 0.05$) confirms that broiler litter-based functional feed delivers superior energy availability compared to traditional rice bran feed.

3.3 Total Cholesterol Profile

The treatment diets produced a statistically significant impact ($P < 0.05$) on total blood cholesterol levels, with measured means of 113.62(mg/dL) (P0), 159.25(mg/dL) (P1), and 123.33(mg/dL) (P2). Tukey's HSD test showed that P1 was significantly higher than P0 and P2, whereas P0 and P2 exhibited no statistically significant difference from one another.

In previous research by Mudita (2019), total cholesterol levels in Bali cattle fed lignocellulolytic fermented rations ranged between 111.59(mg/dL) and 116.90(mg/dL). The total cholesterol values for P0 (113.62(mg/dL)) and P2 (123.33(mg/dL)) in the present study closely mirror this historical baseline. In contrast, the substantial cholesterol spike in P1 (159.25(mg/dL)) significantly exceeded earlier benchmarks, demonstrating a distinct metabolic response to functional feed feeding without direct probiotic co-administration.

Total blood cholesterol comprises various circulating lipid fractions, primarily High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and triglycerides. The notable elevation in P1 aligns with enhanced fiber fermentation by lignocellulolytic microbes, which generates elevated amounts of acetate VFA. In hepatocytes, acetate is converted into cytosolic Acetyl-CoA—the primary precursor required for endogenous cholesterol biosynthesis (Mansilla *et al.*, 2024). The higher total cholesterol in P1 indicates active anabolic processes, wherein energy liberated from the functional feed is directed toward synthesizing structural cell membranes and steroid hormones essential for muscle growth.

Conversely, the return of total cholesterol levels in P2 toward control levels (123.33(mg/dL)) underscores the regulatory hypocholesterolemic action of added probiotics. Probiotic microorganisms suppress excessive systemic cholesterol through several physiological mechanisms, including bile salt deconjugation, direct cholesterol assimilation into microbial cell walls, and short-chain fatty acid (SCFA) signaling that inhibits hepatic cholesterol synthesis. Consequently, probiotic inclusion in P2 successfully maintained cholesterol homeostasis, preventing hypercholesterolemia while preserving the nutritional benefits of the broiler litter feed.

3.4 Blood Triglyceride Levels

Blood triglyceride levels differed significantly ($P < 0.05$) among treatments, recorded at 24.34(mg/dL) for P0, 27.06(mg/dL) for P1, and 20.88(mg/dL) for P2. Tukey's HSD test indicated that P1 and P0 were statistically comparable, whereas P2 was significantly lower than both groups.

The baseline study by Mudita (2019) documented blood triglyceride values between 21.93(mg/dL) and 25.37(mg/dL). Values for P0 (24.34(mg/dL)) and P2 (20.88(mg/dL)) fell within this established range, whereas P1 (27.06(mg/dL)) slightly surpassed the upper limit. The alignment of these figures confirms that overall lipid metabolism in this trial remained consistent with ruminants fed lignocellulolytic-treated roughage.

Triglycerides function as the primary form of energy storage deposited in adipose tissues, liver, and skeletal muscle. The lack of significant difference between P1 and P0 indicates that substituting rice bran with broiler litter-based functional feed alone is insufficient to alter baseline lipid deposition. However, the significant drop in triglycerides observed in P2 demonstrates that probiotic supplementation actively inhibits excessive lipogenesis and enhances the oxidation efficiency of VFAs. By supplying a steady stream of VFA-derived energy, probiotics reduce the necessity for body fat mobilization, ultimately dampening triglyceride synthesis and secretion into systemic blood circulation.

3.5 High-Density Lipoprotein (HDL) Concentrations

Dietary treatments significantly increased ($P < 0.05$) HDL blood concentrations in Bali cattle. Recorded values were 93.96 mg/dL (P0), 129.08 mg/dL (P1), and 109.96 mg/dL (P2). Tukey's HSD test revealed that P1 and P2 were statistically equivalent to each other, and both were significantly higher than the P0 control group.

Comparatively, Mudita (2019) reported HDL levels ranging from 70.79(mg/dL) to 76.93(mg/dL). All treatment groups in the present study exhibited higher baseline HDL concentrations than those reported in earlier literature. Nevertheless, the overall trajectory remains highly consistent: cattle receiving broiler litter-based functional feeds exhibited markedly superior HDL concentrations compared to control animals fed standard rations.

HDL plays a vital physiological role in reverse cholesterol transport, shuttling excess cholesterol from peripheral tissues back to the liver for degradation or excretion into bile acids. Higher HDL levels thus signify an improved lipid metabolic state and enhanced cardiovascular health in cattle. The significant HDL rise in P1 and P2 highlights that VFA energy generated from lignocellulolytic fibre degradation fully satisfied hepatic energy demands for synthesizing high-density apolipoproteins. The non-significant difference between P1 and P2 confirms that probiotic addition does not impair HDL biosynthesis, but rather maintains high protective HDL levels while selectively suppressing atherogenic lipids.

3.6 Low-Density Lipoprotein (LDL) Profiles

Low-Density Lipoprotein (LDL) levels exhibited significant variation ($P < 0.05$) across all three treatments, measuring 14.80 mg/dL in P0, 24.77 mg/dL in P1, and 9.20 mg/dL in P2. Post-hoc testing confirmed that all three treatment groups differed significantly from one another.

In contrast to Mudita (2019), who reported LDL ranges of 33.88 -36.23 mg/dL, the LDL values in this trial were substantially lower across all groups. Most notably, the P2 group (9.20 mg/dL) demonstrated an exceptionally potent suppression of LDL, reaching levels far below those observed in previous studies using lignocellulolytic fermented diets.

LDL serves as the primary transport vehicle delivering cholesterol from the liver to peripheral tissues; however, elevated LDL levels increase the risk of lipid accumulation along vascular walls. The higher LDL in P1 (24.77 mg/dL) reflects its higher total cholesterol baseline, resulting from active hepatic lipogenesis driven by increased energy intake. Conversely, the dramatic reduction of LDL in P2 (9.20 mg/dL) is driven by probiotic-induced Bile Salt Hydrolase (BSH) enzymatic activity. Probiotic bacteria produce BSH, which hydrolyses conjugated bile salts in the intestinal lumen, rendering them insoluble and preventing their reabsorption via enterohepatic circulation. To replace excreted bile salts, hepatocytes upregulate LDL receptor expression and extract circulating LDL cholesterol from the blood as raw material for de novo bile acid synthesis. This mechanism drastically depresses systemic LDL concentrations, producing an optimal HDL-to-LDL ratio that supports superior metabolic health in Bali cattle.

4 CONCLUSION

Dietary supplementation with lignocellulolytic bacterial probiotics combined with broiler litter-based functional feed improves the blood biochemical profile of Bali cattle, particularly by elevating protective HDL cholesterol. The treatment combination P2 effectively lowers circulating LDL and triglycerides while maintaining metabolic homeostasis, demonstrating strong potential as a safe and efficient feeding strategy for beef cattle fattening.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that we have no conflict of interest.

Statement of ethical approval

The research activities underlying this publication were conducted in accordance with the ethical clearance guidelines issued by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University (Certificate No.: B/191/UN14.2.9/PT.01.04/2025).

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