A Comparative Study of Hematological Profiles And *Mycoplasma gallisepticum* Prevalence in Philippine Native Chickens Raised Using CPU Technology and Backyard Free-Range Methods

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Abstract. Philippine Native chickens are typically raised using a free-range system in backyards. The management style is characterized by its low-input, low-output nature. To address the growing demand for native chicken, production is intensified through the strategic use of available feed resources, occasionally supplemented with commercial feed additives. Native chickens exhibit notable disease resistance and adaptability to local climatic conditions, making them more favored by local communities. However, these free-ranging habits expose them to diseases and nutritional deficiencies. In response to the identified challenges, Central Philippine University developed a technology package for raising native chickens. This innovative approach features confined housing and employs antibiotic-free feeding management and phytomedicines. This technology was evaluated, focusing on the hematological profiles and the prevalence of *Mycoplasma gallisepticum* in native chickens. The findings revealed that 73% of the sample population of backyard chickens tested positive for *M. gallisepticum* infection, whereas chickens raised under CPU technology exhibited only a 6% positive rate. Hematological and antibody test results corroborated these findings, allowing for practical disease evaluation. Notably, the free-range chickens displayed elevated mean corpuscular volume (MCV) values compared to standard reference ranges. Hence, this technological application significantly reduced the incidence of *M. gallisepticum* in native chickens, addressing a respiratory disease that leads to an alteration in erythrocyte size.

Keywords: Philippine native chicken, hematological profile, *Mycoplasma gallisepticum*, poultry production

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1 Introduction

Native chickens are essential to the poultry sector in the Philippines, particularly in backyard farming. As of April 1, 2025, the Western Visayas region reported a total poultry inventory of 12.9 million birds, with native chickens comprising 63.9%, approximately 8.3 million birds, surpassing broilers and layers. Iloilo is the leading contributor, representing 58.4% of the native chicken population [1]. This significant presence highlights their resilience and adaptability, underscoring their vital role in the country's poultry production [2]. Backyard-raised native chickens, characterized by free-ranging habits, require a dependable nutritional source. It is also characterized by a system with minimal input and output [3].

Increased consumer demand for native chickens necessitates a rise in production among farmers. This often involves the use of commercial feed supplements, resulting in higher costs. Unfortunately, as the popularity of raising native chickens continues to rise, there is also a growing incidence of disease among these flocks [4]. Respiratory infections are the leading cause of morbidity among poultry farms, particularly among native chickens. Avian mycoplasmosis is an economically important and frequently reported avian respiratory disease caused by a mollicute, *Mycoplasma gallisepticum*. This is often characterized by a 10–20% reduction in weight gain, 10–20% decrease in feed conversion efficiency, 5–10% mortality rate, and 10–20% of carcasses condemned and a decrease in egg hatchability, causing high economic losses in chicken farms [5]. Furthermore, free-range chickens have a high prevalence of the disease, with a 56.4% prevalence among free-range native chickens in Tarlac, Philippines [6].

Native chickens are known for their resilience, adaptability to local climate and conditions, and low requirements for maintenance from external resources. However, in free-range systems, poultry are primarily sustained by scratch feed, resulting in insufficient nutrition, inadequate monitoring, suboptimal housing, and limited protection from inclement weather and predation. Furthermore, deficiencies in management and support systems negatively impact productivity and increase disease susceptibility [7]. Because of these identified problems, Central Philippine University (CPU) developed a technology package for growing Philippine Native Chickens. This technology package includes housing design, feed and phytomedicines, feed and water management, and marketing support.

CPU technology is characterized by a confined housing design from the brooding stage to the breeder stage. Each stage incorporates a designated feed for each growing period, developed by the research center using locally sourced materials, an integral component of the technology. Phytomedicines are also an essential part of this technology, considering they serve as natural substitutes for commercial antibiotics. Emulsified concentrates of phytomedicines, such as garlic and lemongrass, have been developed and tested for their efficacy in normalizing hematological and biochemical parameters in native chickens. Demonstrating efficacy against *M. gallisepticum* and the internal parasite of domestic fowl, specifically *Ascaridia galli* [8]. Hence, this study further validates the effectiveness of the technology package by comparing the native chickens reared under the CPU technology with those raised using backyard free-range methods in terms of hematological analysis and Antibody determination using enzyme-linked immunosorbent assay.

2 Materials and methods

2.1 Study area

The sampling area was selected purposefully based on data regarding technology adopters and non-adopters from the CPU Research Center for Philippine Native Chicken. The

technology's adopters generally maintain local chicken flocks, especially the Jolo and Bisaya native chicken crossbreeds, which number between 150 and 1,000. Implement the research center feed formulated from locally sourced natural feedstuffs, incorporating phytobiotics such as lemongrass and garlic essential oils, while adhering to the mandated confined housing and prohibiting synthetic substances in feeds or growth stimulants. Conversely, non-adopters tend to maintain indigenous Jolo or Bisaya chicken flocks, typically numbering under 150 birds, frequently employing free-range practices, and occasionally utilizing commercial feeds and antibiotics.

2.2 Pre-collection preparation

Before collecting blood and serum samples, sterile serum separating tubes for serum collection, dipotassium ethylenediaminetetraacetate acid (K2 EDTA) tubes for whole blood samples, one cubic centimeter (cc) syringes, an ice box, and alcohol were prepared and utilized. The site and the representative chickens were identified. The symptoms, such as a runny nose and fatigue, were noted. Sample tubes were appropriately labeled.

2.3 Collection of blood and serum samples

Blood samples were collected from randomly selected representative chickens from the flock with an age range of 1-3 months. The blood was extracted from the birds' brachial veins using a one cc sterile syringe. 0.3 ml of the blood was transferred to the K2 EDTA tubes, and the remaining blood sample was transferred to the serum-separating tubes. K2 EDTA tubes were gently inverted to mix the anticoagulant and blood properly before storing both tubes inside the ice box to transport samples.

2.4 Blood hematology on whole blood samples

Blood samples from K2 EDTA tubes were utilized in the hematological analysis of chickens. Blood parameters were determined using the PKL PPC 610H Auto Hematology Analyzer. The data collected were red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), hemoglobin (HGB), and red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

2.5 Enzyme-linked immunosorbent assay (ELISA) on serum samples

Samples from serum-separating tubes were used to determine *Mycoplasma gallisepticum* (MG) infection in chickens. The study employs an indirect ELISA protocol to measure the relative level of *M. gallisepticum* antibodies in chicken serum. Following the IDEXX MG Ab test kit protocol, the antigen is coated on 96-well plates, forming a complex with the antibodies in the serum after incubation. After washing away the unbound antibodies, a conjugate is added. Unbound conjugates are then washed away, and an enzyme substrate is introduced. This is where color development begins, which is directly related to the amount of *M. gallisepticum* antibody present in the test samples [9]. The absorbance of each sample in 96-well plates was determined using the RT-2100C Micro-plate Reader at 630 nm.

2.6 Data collection and analysis

Data collected from the Hematology and ELISA utilized the actual values and means presented in a tabular form to aid the presentation and discussion of results. Hematological

data were compared via an independent sample t-test; Antibody Titers of the serum were determined by absorbance at 630 nm. The end-point titer is calculated using the formula provided in the IDEXX MG AB TEST KIT.

$$Log_{10}Titer = 1.09\ (\log_{10}S/P) + 3.36 \text{ whereas;} \quad \{1\}$$

$$S/P = \frac{Sample\ Mean - NC\bar{x}}{PC\bar{x} - NC\bar{x}} \quad \{2\}$$

$$NC\bar{x} = Negative\ Control\ 1 + Negative\ Control\ 2 \quad \{3\}$$

$$PC\bar{x} = Positive\ Control\ 1 + Positive\ Control\ 2 \quad \{4\}$$

Serum samples exhibiting S/P ratios ≤ 0.50 were classified as negative; conversely, samples with S/P ratios > 0.50 and titers> 1076 were classified as positive, indicative of *Mycoplasma gallisepticum* exposure.

3 Results and discussion

3.1 Blood hematology

Hematology is a commonly used clinical tool for disease diagnosis. During stress or an infection caused by a pathogen, a noticeable difference is observed in the blood profile, particularly in leukocytes, also known as white blood cells (WBCs). WBCs play a crucial role in the body's immune system in combating various diseases through phagocytic mechanisms and antibody production [10]. It is classified into granulocytes and agranulocytes. Granulocytes release enzymes when a foreign substance attacks the immune system, while the agranulocytes produce specific antibodies for a specific antigen. It is further categorized into two types: monocytes and lymphocytes (LYM). Lymphocytes have major subsets like T lymphocytes, responsible for cell-mediated immunity, B lymphocytes for immunoglobulin production, and the killer cells [10]. Consequently, lymphocyte and white blood cell count assessment is crucial for diagnosing infectious and other diseases.

Table 1 shows that the WBC of the chickens reared under CPU technology has an average WBC of 124.57 and 135.31 ($1x10^9$/L), respectively. The %LYM of the chickens reared with or without the CPU technology is 90.40% and 88.48%, respectively. The WBC and %Lym values of the two groups are not significantly different. In comparison, both values fall within the standard WBC and %LYM values among healthy native chickens.

On the other hand, erythrocytes or red blood cells (RBCs) contain hemoglobin (HGB), which transports oxygen from the lungs to cells and carbon dioxide from cells to the lungs. HGB and Hematocrit (HCT) are essential in determining blood viscosity and arterial oxygen content. Furthermore, RBCs alone do not accurately calculate the blood oxygen-carrying capacity and are not used to diagnose anemia directly. That is why the red cell indices must be evaluated [11,12].

Red cell indices are calculated using HGB, HCT, and RBC. These include, but are not limited to, mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH), and mean corpuscular Hemoglobin concentration (MCHC). The MCV is used to calculate the average RBC size, MCH to measure the amount of HGB per blood cell, and MCHC to determine the amount of HGB relative to the size of the cell per RBC [11].

Statistical analysis shows a significant difference in RBC, HGB, and HCT values between chickens raised using CPU technology and those raised free-range. Average values were 2.97 and 3.39 $1x10^{12}$/L for RBC, 12.58 and 15.31 (grams per deciliter) g/dL for HGB, and 28.50% and 33.61% for HCT, respectively. Analysis reveals that chickens raised using backyard free-range technology exhibit superior RBC values compared to those raised under CPU technology; this difference is correlated with elevated HGB and HCT levels in the free-range group. This finding corroborates the linear correlation between hemoglobin and hematocrit,

mediated by red blood cell volume [12]. While elevated, the RBC, HGB, and HCT values of free-range chickens remain within the established normal range for healthy chickens.

Table 1. Hematological analysis of Philippine native chicken reared under CPU technology and backyard free-range technology.

Samples	WBC $1x10^9$/L	LYM %	RBC $1x10^{12}$/L	HGB g/dL	HCT%	MCV fL	MCH pg	MCHC g/dL
CPU	124.57^{ns}	90.40^{ns}	2.97^{*}	12.85^{*}	28.50^{*}	96.30^{*}	43.26^{*}	45.01^{*}
Backyard Free-range	135.31	88.48	3.39	15.31	33.61	99.29	45.10	45.51
STD	123.89± 23.56	88.20± 2.43	2.87±0. 59	13.14±2 .74	27.48±5 .74	95.62±2 .34	45.34±1 .34	47.50±0 .69

Note: STD–standard hematological levels among healthy native chickens (Cabarles, 2025)
^{ns}–no significant difference
* –with significant difference at $p<0.05$ level

Additionally, a statistically significant difference exists between the red cell indices of both groups. The values for the MCH of CPU technology and backyard free-ranged are 43.26 picograms (pg) and 45.10 pg, respectively, which are within a normal range for both groups. Conversely, free-range chickens exhibited a markedly higher mean corpuscular volume (MCV) with 99.29 femtoliters (fL) than their counterparts raised using CPU technology with 96.30 (fL) and standard levels of MCV among healthy chickens. This elevated MCV may indicate macrocytic anemia, potentially stemming from vitamin deficiencies, pathogenic conditions, or infectious agents [11,13,14]. Analysis revealed a significant difference ($p<0.05$) in MCHC between groups, with values of 45.01 g/dL and 45.51 g/dL for the CPU and free-range backyard groups, respectively; however, neither value falls within the established values for regular chickens. This may suggest the early stages of iron deficiency anemia, given that both RBC and HGB levels currently fall within the reference range [11].

3.2 ELISA for *Mycoplasma gallisepticum* antibody

Indirect ELISA, an enzyme immunoassay leveraging enzymatic catalysis, facilitates the detection and quantification of immunological reactions. It is frequently applied in clinical analyses, particularly antibody detection [9]. In this study, the detection of *Mycoplasma gallisepticum* antibodies was employed.

Table 2 shows that out of 35 chickens raised under CPU technology, three (3) have symptoms of a runny nose and fatigue. However, when tested for *M. gallisepticum* infection, only two (2) were positive, showing a 6% positive rate in the sample population. On the contrary, out of 59 backyard free-range chickens, only nine (9) had symptoms of a runny nose and fatigue; however, 43 were identified as positive, making 73% of the sample population.

Moreover, one chicken per group displayed symptoms, yet tested negative for *M. gallisepticum* antibodies. This suggests that the observed symptoms might reflect different pathogenic etiologies. In consequence, symptomatic evaluation alone may prove insufficient for the definitive identification of a specific infection.

These results show a correlation with the MCV hematological analysis data of the free-range chickens. *M. gallisepticum* infection impacted MCV elevation because of red blood cell invasion, resulting in altered surface morphology, decreased cell size, and perforation. [15].

Table 2. Enzyme-Linked Immunosorbent Assay for *Mycoplasma gallisepticum* Antibody.

Samples	n	Chickens with symptoms	With symptoms but negative	Positive	Negative	% Positive	% Negative
CPU technology	35	3	1	2	33	6	94
Backyard Free-range	59	9	1	43	16	73	27

Note: The positive and negative results were interpreted using the formula given by the IDEXX MG AB Test Kit

The hematological data show superior health in chickens raised using CPU technology compared to free-range chickens, exhibiting elevated MCV values indicative of erythrocyte size abnormalities. This was further substantiated by the ELISA results for *M. gallisepticum* antibodies, with 73% of the sample population identified as positive for infection. Backyard-raised chickens were susceptible to the disease, as shown by ELISA and hematological analysis of MCV.

Previous research into the developed phytomedicines: garlic tea, lemongrass emulsified concentrate, and garlic emulsified concentrate, indicated enhanced levels of several blood biochemical parameters, including cholesterol, triglycerides, uric acid, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and creatinine. The hematological profile was normalized, with a reduction in *M. gallisepticum* colonies [8].

4 Conclusion

This comparative study highlights the significant advantages of the CPU technology package for raising Philippine native chickens over traditional backyard free-range methods. The hematological analysis revealed that chickens raised using CPU technology exhibited healthier blood profiles, particularly regarding red blood cell indices. Free-range chickens showed elevated mean corpuscular volume (MCV) values, indicating potential macrocytic anemia or other health issues. The enzyme-linked immunosorbent assay (ELISA) showed results for *Mycoplasma gallisepticum* antibodies, with only 6% of chickens raised under CPU technology testing positive, compared to 73% of backyard free-range chickens. This substantial difference in *M. gallisepticum* prevalence shows the effectiveness of the CPU's confined housing design, specialized feeding protocols, and use of phytomedicines in preventing respiratory infections. The study also underscores the limitations of relying solely on symptomatic evaluation for disease diagnosis, as some chickens tested positive without showing clinical signs. Overall, these findings validate the CPU technology package as a superior approach for native chicken production, offering improved health outcomes and reduced disease susceptibility compared to traditional free-range methods.

References

1. Rama, T.K.E. & Tongol, M.A.S. (2024). Digestibility of different carbohydrate sources in the diet of native chicken (*Gallus gallus domesticus*). *International Research Journal*

of Science, Technology, Education, and Management, *4*(2), 158-166. https://doi.org/10.5281/zenodo.12731074
2. Tangalin, P. J. B., & Medalla, J. B. (2025). Value Chain Analysis of ZamPen Native Chicken for Sustainable Agribusiness Development in Zamboanga Peninsula Region. *Cognizance Journal of Multidisciplinary Studies*, *5*(3), 222–241. https://doi.org/10.47760/cognizance.2025.v05i03.018
3. Haunshi, S., & Rajkumar, U. (2020). *Native chicken production in India: present status and challenges*. Cipav.org.co. http://www.lrrd.cipav.org.co/lrrd32/11/ullen32181.html
4. Cadmus, K. J., Mete, A., Harris, M., Anderson, D., Davison, S., Sato, Y., Helm, J., Boger, L., Odani, J., Ficken, M. D., & Pabilonia, K. L. (2019). Causes of mortality in backyard poultry in eight states in the United States. *Journal of Veterinary Diagnostic Investigation*, *31*(3), 318–326. https://doi.org/10.1177/1040638719848718
5. Galluzzo, P., Migliore, S., Galuppo, L., Condorelli, L., Hussein, H. A., Licitra, F., Miriana Coltraro, Sallemi, S., Antoci, F., Cascone, G., Puleio, R., & Guido Ruggero Loria. (2022). First Molecular Survey to Detect Mycoplasma gallisepticum and Mycoplasma synoviae in Poultry Farms in a Strategic Production District of Sicily (South-Italy). *Animals*, *12*(8), 962–962. https://doi.org/10.3390/ani12080962
6. Paragas, A., & Beltran, M. A. (2023). Prevalence of Mycoplasma gallisepticum and Mycoplasma synoviae Antibodies in Free Range Chickens as Detected by ImmunoComb Assay. *Philippine Journal of Arts, Sciences and Technology*. http://tau.edu.ph:8085/index.php/pjast/article/view/38
7. Fiorilla, E., Birolo, M., Ala, U., Xiccato, G., Trocino, A., Schiavone, A., & Mugnai, C. (2023). Productive Performances of Slow-Growing Chicken Breeds and Their Crosses with a Commercial Strain in Conventional and Free-Range Farming Systems. *Animals*, *13*(15), 2540. https://doi.org/10.3390/ani13152540
8. Cabarles, J. (2025). Verification and Standardization of Processing and Concentration of Garlic, Betel nut, and Asiatic Bitter Yam Against *Mycoplasma gallisepticum* and Internal parasites (*Ascaridia galli*) of Philippine Native Chicken [Unpublished Report]. DA-BAR Completion Report
9. Alhajj, M., Farhana, A., & Zubair, M. (2023). Enzyme Linked Immunosorbent Assay (ELISA). PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK555922/
10. Tigner, A., Ibrahim, S. A., & Murray, I. (2022). Histology, white blood cell. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK563148/
11. Brihi, J. E., & Pathak, S. (2024). Normal and Abnormal Complete Blood Count with Differential. Www.ncbi.nlm.nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK604207/
12. van der Veen, P. H., Muller, M., Vincken, K. L., Westerink, J., Mali, W. P. T. M., van der Graaf, Y., & Geerlings, M. I. (2015). Hemoglobin, hematocrit, and changes in cerebral blood flow: the Second Manifestations of Arterial Disease-Magnetic Resonance study. *Neurobiology of Aging*, *36*(3), 1417–1423. https://doi.org/10.1016/j.neurobiolaging.2014.12.019
13. Moore, C. A., & Adil, A. (2022). Macrocytic Anemia. Nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK459295/
14. Korver, D. (2023). Vitamin Deficiencies in Poultry - Poultry. Veterinary Manual. https://www.msdvetmanual.com/poultry/nutrition-and-management-poultry/vitamin-deficiencies-in-poultry
15. Vogl, G., Plaickner, A., Szathmary, S., Stipkovits, L., Rosengarten, R., & Szostak, M. P. (2008). Mycoplasma gallisepticum Invades Chicken Erythrocytes during Infection. *Infection and Immunity*, *76*(1), 71–77. https://doi.org/10.1128/IAI.00871-07