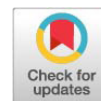


Research Article



Moringa Leaf Supplementation Improves Hematology and Immunity in Pregnant Rabbits

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Abstract | This study evaluated the effects of *Moringa oleifera* leaf meal supplementation on hematological parameters, immunoglobulin G, and mineral status in pregnant rabbits. Fifty pregnant female rabbits (8-10 months old) were allocated to five treatments using a randomized complete block design: T0 (control), T1 (2%), T2 (4%), T3 (6%), and T4 (8% *Moringa* leaf meal). Blood samples were collected during the third week of pregnancy for analysis. Results showed that *Moringa* supplementation significantly enhanced immunoglobulin G levels from 2.37 g/dL (control) to 2.67 g/dL (8% inclusion). Blood calcium increased progressively from 0.42 mM to 0.65 mM, while iron levels rose from 626.55 to 918.07 µg/dL across treatments. Hematological parameters including hematocrit, lymphocytes, and red blood cell indices remained within physiological ranges. Zinc concentrations showed stability across treatments, indicating effective homeostatic regulation. *Moringa* leaf meal supplementation effectively improved immune function and mineral bioavailability in pregnant rabbits without compromising blood health parameters.

Keywords | Moringa leaf, Blood profile, Blood minerals (Ca, Fe, Zinc), Rabbit

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INTRODUCTION

Residents in tropical regions, such as Indonesia, regularly consume *Moringa* leaves, formally referred to as *Moringa oleifera* (Adi *et al.*, 2019; Malik *et al.*, 2019; Mawardi *et al.*, 2020; Sumarni *et al.*, 2020). *Moringa* leaves have gained significant attention in animal nutrition research due to their comprehensive nutritional composition and physiologically active compounds (Fahey *et al.*, 2018; Matic *et al.*, 2018; Meireles *et al.*, 2020; Singh *et al.*, 2020; Tshabalala *et al.*, 2019), establishing their importance as a

valuable feed resource.. Based on the proximate analysis of some previous research results, *Moringa* leaves have a crude protein content that has huge functions in animal food nutrition (Chodur *et al.*, 2018; Jain *et al.*, 2019; Rébua *et al.*, 2018; Su and Chen, 2020; Sultana, 2020). Pregnancy in rabbits is a physiologically demanding period, marked by increased needs for micronutrients, immune modulation, and hematopoietic activity to support fetal development and maternal health. *Moringa* leaves, rich in iron, calcium, and antioxidants, may help fulfill these elevated nutritional demands, making them highly relevant for use during

gestation (Leone *et al.*, 2015). Specifically, Ca, Fe, and Zn were selected due to their critical roles in bone formation, oxygen transport, immune function, and enzymatic activity, all of which are essential during pregnancy.

Moringa leaves possess a higher mineral content in comparison to leaves of other plants, especially in terms of calcium (Azeez *et al.*, 2020; Borgonovo *et al.*, 2020; Kim and Kim, 2019), magnesium (Abd El-Hack *et al.*, 2018; Azeez *et al.*, 2020; Saa *et al.*, 2019), potassium (Aslam *et al.*, 2020; Fatima *et al.*, 2018; Natsir *et al.*, 2019), sodium (Ahmed *et al.*, 2020; Ali *et al.*, 2020; Arwani *et al.*, 2019; El-Hadary and Ramadan, 2019; Natsir *et al.*, 2019), copper (Das *et al.*, 2020; Galan *et al.*, 2018; Juárez-Maldonado *et al.*, 2018; Seetha *et al.*, 2020; Varkey, 2020), iron (Arora and Arora, 2021; Katata-Seru *et al.*, 2018; Khoja *et al.*, 2021; Somoza *et al.*, 2021; Tawfik *et al.*, 2021), manganese (Nawaz *et al.*, 2023), zinc (Abel *et al.*, 2021; Akintunde *et al.*, 2021; Dahran *et al.*, 2023; Irfan *et al.*, 2021; Matinise *et al.*, 2018; Ngom *et al.*, 2021) and the specified vitamins, such as ascorbic acid (Kumar *et al.*, 2021; Rodríguez *et al.*, 2020), tocopherol (Cheikhoussef *et al.*, 2018; Gharsallah *et al.*, 2021; Özcan *et al.*, 2019), beta-carotene (Abdulkadir *et al.*, 2018; Bidura *et al.*, 2020; Muteeb *et al.*, 2023; Srivastava *et al.*, 2023), vitamin A (Adi *et al.*, 2019; Singh *et al.*, 2020; Srivastava *et al.*, 2023; Sultana, 2020; Sumarni *et al.*, 2020), and B vitamins (Sumarni *et al.*, 2020) dealing with folic acid, pyridoxine, and nicotinic acid, in addition to vitamin D. *Moringa* plant's leaves also provide Polyunsaturated Fatty Acids (PUFA) in quantities ranging from 18.80 to 84.60 g/kg.

Moringa leaves have a vitamin C content (Sultana, 2020) that is seven times higher than oranges, vitamin A content that is ten times higher than carrots, a calcium content that is seventeen times higher than milk, a protein content that is nine times higher than yoghurt, a potassium content that is fifteen times higher than bananas, and an iron content that is twenty-five times higher than spinach. In addition to mineral content, moringa leaves are also confirmed to contain active plant compounds, which from various plants in Indonesia or agroindustrial waste can be integrated as livestock feed additives to support livestock production, animal health, and environmental efforts (Baihaqi *et al.*, 2019, 2020, 2022, 2023; Lisnanti *et al.*, 2023).

The fish were administered *Moringa* leaf seed extract at a concentration of 2.4 mg/L for a duration of 7, 14, 21, 28, and 35 days. The study examined the potential impact on the quantity of red blood cells, the average size of red blood cells by Mean Corpuscular Volume (MCV), the average amount of haemoglobin in red blood cells by Mean Corpuscular Hemoglobin (MCH), and the levels of glucose in the blood. In a study conducted by Awodele *et al.* (2012), it was found that orally providing *Moringa* leaf

extract to mice at doses of 250, 500, and 1500 mg/kg over a period of 60 days did not result in any notable changes in the biochemical and haematological parameters of the mice's blood. Therefore, the aim of this study is to determine the effects of giving *Moringa* leaves on the haematological and mineral composition of the blood of pregnant rabbits.

MATERIALS AND METHODS

EXPERIMENTAL LOCATION AND ANIMALS

The experiment was conducted at the Animal Science Department research facility, Universitas Islam Kadiri, Kediri, East Java, Indonesia, from March to June 2023. Fifty pregnant female rabbits (8-10 months old) and twelve male rabbits were utilized. All animals were sourced from the same local breeder to minimize genetic variation and underwent comprehensive health screening two weeks prior to experimentation. Initial screening included physical examination, body weight measurement, and assessment of physiological parameters including body temperature (38.5-39.5°C), respiratory rate (30-60 breaths/minute), and heart rate, which were within normal ranges for adult rabbits. Only clinically healthy females showing regular estrus cycles and males with proven fertility were selected. Exclusion criteria included signs of respiratory infection, digestive disorders, external parasites, or abnormal behavior. Selected rabbits were dewormed using ivermectin (0.4 mg/kg BW) and vaccinated against common rabbit pathogens.

EXPERIMENTAL DESIGN AND TREATMENTS

A randomized complete block design with five blocks and five treatments was implemented. Each block contained ten rabbits (two rabbits per treatment), with blocks differentiated by initial body weight ranges: Block 1 (2.8-3.0 kg), Block 2 (3.1-3.3 kg), Block 3 (3.4-3.6 kg), Block 4 (3.7-3.9 kg), and Block 5 (4.0-4.2 kg). This blocking strategy minimized potential confounding effects of body weight on treatment responses.

Treatments consisted of: T0 (control diet with 0% *Moringa*), T1 (2% *Moringa* leaf meal), T2 (4% *Moringa* leaf meal), T3 (6% *Moringa* leaf meal), and T4 (8% *Moringa* leaf meal). All diets were formulated as isocaloric and isonitrogenous biscuits, with *Moringa* leaf meal replacing equivalent amounts of base ingredients to maintain nutritional balance. In order to accomplish this objective, we conducted a study on the blood cell profile, immunoglobulin G levels, and blood mineral profiles (Ca, Fe, and Zn) of pregnant rabbits. The study involved 50 pregnant female rabbits and twelve male rabbits, all of which were raised in cages under identical upbringing and environmental conditions. The rabbits were treated with *Moringa* leaf treatment. The feed therapy regimen consisted of biscuits containing *Moringa* leaves, with a recommended daily intake of 6 biscuits.

We monitor the levels of immunoglobulin G and blood mineral profiles (Ca, Fe, Zn) in pregnant rabbits for our research study because *Moringa* leaf has a higher mineral content, particularly Ca, compared to other plant leaves. We facilitate the rabbits' access to potable water. The decision to select pregnant rabbits was driven by a lack of research on this subject and limited access to relevant literature. This study focuses on the effects of giving *Moringa* leaves in the form of biscuits on hematology, IgG, and blood minerals in pregnant rabbits.

FEED PREPARATION AND HOUSING

Fresh *Moringa* leaves were sourced from Blora Regency, Central Java, and processed through controlled drying at 60°C for 24 hours followed by grinding to achieve uniform particle size (mesh 40). The resulting *Moringa* leaf meal was incorporated into biscuit formulations at specified concentrations. Each biscuit weighed 30 grams, with daily feed allocation consisting of six biscuits per rabbit (180 grams total). Fresh *Moringa* leaves were dried at 60°C for 24 hours and ground using a 40-mesh sieve to ensure uniform particle size. Feeding began after mating and continued throughout the pregnancy period. Blood samples were collected in the early morning (06:00–08:00) to minimize diurnal variation, and the nutrient values in Table 1 are expressed as percentages of dry matter (% DM).

Individual battery cages elevated 80 cm above ground level provided adequate ventilation and standardized housing conditions. Environmental parameters were monitored daily, maintaining temperature (24–28°C) and humidity (65–75%) within optimal ranges for tropical rabbit production. Feed and water containers were positioned to allow unrestricted access throughout the study period.

FEED COMPOSITION AND NUTRITIONAL ANALYSIS

Table 1 presents the ingredient composition and nutritional content of experimental diets. All diets were analyzed for dry matter, crude protein, ether extract, crude fiber, and ash content using standard AOAC procedures.

FEED INTAKE AND PERFORMANCE MONITORING

Daily feed intake was recorded throughout the 21-day experimental period by weighing feed offered and refused. Individual rabbit body weights were measured weekly using a digital scale (accuracy ±1 gram). Feed conversion efficiency was calculated as the ratio of feed consumed to body weight gain.

BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples (5 mL) were collected from the marginal ear vein during the third week of pregnancy using sterile procedures. Morning collections (06:00–08:00 hours) were standardized to minimize diurnal variations in blood

parameters. Samples were immediately divided into two portions: EDTA tubes for hematological analysis and plain tubes for serum separation.

Table 1: Ingredient composition and nutritional content of experimental diets.

Components	T0	T1	T2	T3	T4
Feed ingredients (%)					
<i>Moringa</i> leaf meal	0.0	2.0	4.0	6.0	8.0
Dried water spinach	10.0	10.0	10.0	10.0	10.0
Indigofera leaves	10.0	10.0	10.0	10.0	10.0
Teak leaves	5.0	5.0	5.0	5.0	5.0
Bamboo leaves	3.0	3.0	3.0	3.0	3.0
Banana leaves	5.0	5.0	5.0	5.0	5.0
Guava leaves	3.0	3.0	3.0	3.0	3.0
Soy sauce dregs	7.0	7.0	7.0	7.0	7.0
Bread waste	8.0	8.0	8.0	8.0	8.0
Soybean meal	15.0	15.0	15.0	15.0	15.0
Pollard	8.0	8.0	8.0	8.0	8.0
Rice bran	5.0	5.0	5.0	5.0	5.0
DDGS	5.0	5.0	5.0	5.0	5.0
Molasses	5.0	5.0	5.0	5.0	5.0
Mill corn	10.0	8.0	6.0	4.0	2.0
Premix	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100
Nutritional content (% DM)					
Dry matter	87.31	88.09	89.88	90.66	91.45
Crude protein	17.17	17.60	18.03	18.46	18.89
Ether extract	5.28	5.37	5.46	5.55	5.64
Crude fiber	11.36	11.58	11.80	12.02	12.24
Ash	10.37	10.56	10.75	10.95	11.14

Analysis conducted at NMT Laboratory, Faculty of Animal Husbandry, Brawijaya University, 2023

For serum collection, samples were centrifuged at 3,000 rpm for 10 minutes at room temperature. Separated serum was transferred into Eppendorf tubes and stored at -20°C until analysis for immunoglobulin G and mineral determinations.

LABORATORY ANALYSES
HEMATOLOGICAL PARAMETERS

Complete blood counts were performed using an automated hematology analyzer (Mindray BC-5000 Vet) calibrated for rabbit blood. Parameters measured included hematocrit (%), lymphocyte count (%), platelet count (×10³/μL), red blood cell distribution width-standard deviation (RDW-SD, fL), red blood cell distribution width-coefficient of variation (RDW-CV, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean

corpuscular hemoglobin concentration (MCHC, g/dL).

IMMUNOGLOBULIN G DETERMINATION

IgG concentrations were quantified using sandwich ELISA technique with a commercial rabbit IgG ELISA kit (Cloud-Clone Corp., SEA803Rb, USA). Serum samples were diluted 1:10,000 in sample diluent buffer and analyzed against a seven-point standard curve (range: 0.312–20 ng/mL). Absorbance was measured at 450 nm with wavelength correction at 630 nm using a microplate reader (Multiskan™ FC, Thermo Scientific™). All samples were analyzed in duplicate with intra-assay coefficient of variation maintained below 8%.

MINERAL ANALYSIS

Serum calcium, iron, and zinc concentrations were determined using atomic absorption spectrophotometry (AAS) with a PerkinElmer AAnalyst 400 instrument. Calcium was measured at 422.7 nm wavelength, iron at 248.3 nm, and zinc at 213.9 nm. Standard curves were prepared using certified reference materials, and all samples were analyzed in triplicate.

STATISTICAL ANALYSIS

Data were analyzed using analysis of variance (ANOVA) appropriate for randomized complete block design using SAS software version 9.4. Treatment means were compared using Duncan's Multiple Range Test when ANOVA indicated significant differences ($P < 0.05$). Effect sizes were calculated using Cohen's d , and practical significance was assessed using eta-squared (η^2) values.

RESULTS AND DISCUSSION

THE TREATMENT EFFECT ON BLOOD HEMATOCRIT OF PREGNANT MOTHER RABBITS

The *hematocrit* examination is a highly comprehensive and straightforward procedure for determining the severity of anaemia or *Polycythemia*. The *Hematocrit* measurement is also used to compute the mean erythrocyte value. Typically, this measurement is obtained using either venous blood or capillary blood. *Hematocrit* is a haematological statistic used to quantify the proportion of red blood cell volume in relation to the overall blood volume.

The analysis of hematocrit levels serves as a fundamental tool in evaluating blood parameters, offering insights into anemia and polycythemia, with normal values in rabbits ranging from 33–48% (Willems *et al.*, 2016). In our study, hematocrit values ranged from 35.70% to 43.68% across treatment groups, indicating that Moringa leaf supplementation maintained blood parameters within physiological ranges while supporting maternal health during pregnancy (Gordeuk *et al.*, 2019; Folsom *et al.*,

2020). A decreased *hematocrit* level may suggest the presence of health conditions such as anaemia, nutritional deficiencies, bleeding, *hemolysis*, malnutrition, dengue fever, right heart failure, hypoxemia, or bone marrow disorders leading to abnormal red blood cell levels (Folsom *et al.*, 2020; Gordeuk *et al.*, 2019; Xu *et al.*, 2020). The potential health complications include *Hemoptysis*, fibrosis, renal neoplasms, and *Polycythemia vera*. Elevated *Hematocrit* levels may be indicative of health conditions such as dehydration, pulmonary disease, cardiovascular disease, renal malignancy, and genetic disorders. Elevated *Hematocrit* levels pose a risk to the body and are indicative of *Polycythemia*.

Table 2 indicates that the *Hematocrit* level of the rabbits that received treatment ranged from 35.70% to 43.68%. The statistical analysis revealed that there were no significant changes between the treatments ($P \geq 0.05$). Additionally, the values obtained for the rabbits fell within the normal range of 33–48% (Abdulkadir *et al.*, 2018). The animal's overall health has a big impact on its *hematocrit* level. The data's location falls within the expected range and does not exhibit any major deviations, suggesting that the administration of *Moringa* biscuits does not adversely affect the livestock's health, thereby not disturbing the red blood cell count.

The maintenance of hematocrit values within the normal physiological range (35.70–43.68%) in this study suggests that Moringa leaf supplementation may contribute to hematopoietic stability during pregnancy. This stability is potentially supported by the high antioxidant content, iron bioavailability, and essential micronutrients present in Moringa, which are known to enhance erythropoiesis and prevent anemia (Sánchez-Machado *et al.*, 2010; Leone *et al.*, 2015). The absence of hematocrit decline in pregnant rabbits indicates that Moringa supplementation effectively supports maternal blood health and prevents the onset of nutrition-related hematological disorders.

Although the overall ANOVA did not show statistically significant differences across all treatment groups ($P > 0.05$), post-hoc analysis using Duncan's Multiple Range Test revealed that the T8 group exhibited a significantly higher hematocrit value compared to the control. This suggests that a higher inclusion level of Moringa leaf meal (8%) may enhance erythropoiesis, potentially due to improved iron availability and antioxidant support

Although MCV, MCH, and MCHC values did not show significant changes in the short term, the bioactive compounds in Moringa such as flavonoids, polyphenols, and essential trace minerals may contribute to long-term improvements in erythropoiesis and overall hematological stability (Leone *et al.*, 2015). These compounds have been

shown to support liver function, antioxidant defense, and iron metabolism, which indirectly affect red blood cell formation and quality over time (Sánchez-Machado *et al.*, 2010; Gopalakrishnan *et al.*, 2016). Therefore, while no immediate effect was detected, longer-term or stress-challenged conditions could potentially reveal indirect benefits of Moringa supplementation on these hematological indices.

Table 2: Effects of moringa leaf treatment on blood parameters in pregnant rabbits.

Variable	Treatment	Mean±SD	95% CI	Cohen's d	η ²	Significance
Hematocrit (%)	T0	35.78±2.27	34.21-37.35	-	0.47	*
	T2	35.18±1.97	33.83-36.53	0.29		
	T4	37.30±1.75	36.11-38.49	0.75		
	T6	35.70±1.34	34.80-36.60	0.04		
	T8	43.68±1.37 ^a	42.76-44.60	4.12		
Lymphocytes (mg/dL)	T0	55.44±18.32	42.54-68.34	-	0.31	ns
	T2	56.06±16.09	44.74-67.38	0.04		
	T4	57.58±11.21	49.67-65.49	0.14		
	T6	57.12±8.78	50.91-63.33	0.12		
	T8	59.32±14.94	48.78-69.86	0.23		
Platelets (mg/dL)	T0	394.2±21.9	378.6-409.8	-	0.35	ns
	T2	345.6±8.9	339.2-352.0	2.92		
	T4	382.6±12.6	373.6-391.6	0.63		
	T6	437.6±13.0	428.3-446.9	2.33		
	T8	364.8±14.9	354.1-375.5	1.54		
RDW-SD (g/dL)	T0	37.26±5.23	33.61-40.91	-	0.28	ns
	T2	36.58±5.04	33.06-40.10	0.13		
	T4	36.08±2.59	34.24-37.92	0.29		
	T6	37.42±4.20	34.47-40.37	0.03		
	T8	38.50±5.38	34.75-42.25	0.24		
IgG (g/dL)	T0	2.37±0.77 ^b	1.82-2.92	-	0.38	**
	T2	2.27±0.23 ^b	2.10-2.44	0.17		
	T4	2.49±0.43 ^b	2.18-2.80	0.19		
	T6	2.52±0.47 ^b	2.18-2.86	0.24		
	T8	2.67±0.59 ^a	2.24-3.10	0.44		

Notes: CI = Confidence Interval, Cohen's d represents effect size compared to control (T0), η² (Eta squared) represents proportion of variance explained by treatment, Different superscripts (^a,^b) within columns indicate significant differences (p<0.05), ns = not significant, p<0.05; ** p<0.01, Effect size interpretations: Cohen's d: Small (0.2), Medium (0.5), Large (0.8), η²: Small (0.01), Medium (0.06), Large (0.14).

THE TREATMENT EFFECT ON MATERNAL RABBIT BLOOD LYMPHOCYTES

Lymphocytes are a subset of leukocytes that have a crucial function in the immunological response (Folsom

et al., 2020). Lymphocytes serve to enhance the efficiency of the immune system in safeguarding the body against a multitude of diseases (Khan and Ghazanfar, 2018; Krishnamurty and Turley, 2020; Ruffo *et al.*, 2019; Sia *et al.*, 2022). Lymphocytes play a crucial role in the body's defence against harmful bacteria, viruses, and poisons, effectively combating illnesses. Abnormally low or high lymphocyte numbers can serve as an indication of underlying health issues. Reduced lymphocyte levels can be indicative of various health issues, including infections, cancer, autoimmune illnesses, blood abnormalities, and kidney problems. Elevated lymphocyte levels may suggest an ongoing immune response against a specific disease or infection.

The research results indicated that the lymphocyte levels of the rabbits that received treatment ranged from 55.32 to 59.58 mg/dL. The statistical analysis showed that there was no significant difference in lymphocyte levels between the treatments (P≥0.05). This lack of difference could be attributed to the absence of infection or the administration of illness therapy to the rabbits, which led to a substantial increase in the lymphocyte counts. Multiple studies conducted on different animal species have demonstrated that lymphocyte numbers can be affected by numerous factors, including dietary composition, vitamin supplementation, livestock health, and environmental conditions. While the observed difference may not have been statistically significant, there was a clear trend of increasing lymphocyte count with higher levels of Moringa leaf flour supplementation in the rabbit biscuit meal. This can suggest that the rabbit's overall health can be improved by augmenting the feed with a higher quantity of Moringa leaves.

The use of Moringa leaf-based biscuits as a functional feed additive in rabbit farming presents promising practical implications. Beyond improving hematological and mineral profiles in pregnant does, Moringa leaves are widely available in tropical regions and can be processed into biscuits using cost-effective, small-scale production methods, enhancing their scalability for rural and commercial farms (Leone *et al.*, 2016). While this study did not directly assess litter size or offspring health, improved maternal immunity and mineral status particularly calcium, iron, and zinc are known to influence fetal development, neonatal survival, and milk quality in rabbits (Lebas *et al.*, 2019). Thus, future studies should investigate the intergenerational effects of Moringa supplementation to validate its long-term benefits and optimize its integration into reproductive management strategies.

The primary limitation of this study lies in its focus on hematological and mineral parameters solely during the third week of pregnancy, which may not capture the full

physiological changes occurring throughout gestation and lactation. Different pregnancy stages early gestation, late gestation, and post-partum are characterized by distinct metabolic demands, hormonal fluctuations, and nutrient partitioning, potentially leading to varied responses to dietary interventions such as Moringa supplementation.

THE TREATMENT EFFECT ON BLOOD PLATELETS OF MOTHER RABBITS

Table 2 indicates that there is no statistically significant difference ($P > 0.05$) in the platelet levels of baby rabbits throughout the study period. This lack of difference can be attributed to various factors that can influence the platelet levels of rabbit offspring, particularly during the pre-weaning phase. These factors include the genetic makeup of the mother, the mother's condition (age, weight, litter size, interval, lymphocyte count, and the age and weight of the weaned offspring), the management practices employed during rearing, and the prevailing environmental conditions (such as climate, temperature, and wind) (Suwignyo *et al.*, 2017; Meireles *et al.*, 2020; Budisatria *et al.*, 2021).

Baby rabbits with a low lymphocyte count and poor quality experience persistent hunger and heightened susceptibility to illness, leading to an elevation in platelet levels. Baby rabbits rely exclusively on milk as their sole source of nourishment until they reach the age of 21 days. When the juvenile rabbit reaches 21 days old, it leaves its enclosure and starts eating the food that its mother has provided. Maternal milk serves as the initial means of introducing immune protection into the digestive system of new-born rabbits, enhancing their resistance to diseases. These results indicate a strong correlation between the mortality of new-born rabbits within the first 21 days of life and the secretion of maternal milk, as the latter serves as both a nourishing food supply and the primary provider of immunity. Rabbits supplemented with extra Moringa leaf flour exhibit elevated lymphocyte levels compared to rabbits without the supplementation, ensuring adequate fulfilment of the treated rabbits' requirement for maternal milk.

THE TREATMENT EFFECT ON RDW-SD AND RDW-CV OF MATERNAL RABBIT BLOOD

RDW-SD and RDW-CV are two parameters used in haematological examination to quantify the heterogeneity in the dimensions of erythrocytes (Gordeuk *et al.*, 2019; Willems *et al.*, 2016). These two factors, namely the size distribution of red blood cells in a blood sample, can be utilised to offer a comprehensive assessment of animal health.

RDW-SD quantifies the heterogeneity of red blood cell

size by assessing the standard deviation of the distribution of red blood cell sizes. A high RDW-SD value signifies significant heterogeneity in the size of red blood cells within the sample, with the presence of both very small and extremely large red blood cells. RDW-SD is mostly employed in specialised blood analyses, such as reticulocyte counts, to assess the size distribution of red blood cells with greater precision.

RDW-CV quantifies the variability in the size of red blood cells by assessing the coefficient of variation of the distribution of red blood cell sizes. The RDW-CV value is determined by dividing the standard deviation of the distribution of red blood cell sizes by the average red blood cell size. A high RDW-CV score signifies substantial fluctuations in the size of the red blood cells present in the sample. The term RDW-CV is frequently employed in standard blood tests and is typically included in a comprehensive blood panel known as a CBC. Both of these parameters can offer indications regarding the existence of specific health issues (Gordeuk *et al.*, 2019). For instance, an elevated RDW number may suggest the presence of anaemia or a dysfunction in the generation of red blood cells.

The RDW-SD research results presented in Table 2 above fall within the range of 36.08–38.50 g/dL. Statistical calculations indicate that there were no notable disparities between treatments ($P > 0.05$) in rabbits who did not get Moringa leaf flour or those that were given Moringa leaf flour up to 8% in biscuit feed. This may be attributed to the treatment's lack of a direct impact capable of inducing substantial alterations in RDW levels. Multiple studies indicate that the RDW can be affected by a range of factors, including anaemia, renal or liver impairment, thyroid disorders, blood transfusions, physical exertion, and dietary choices. The inclusion of Moringa leaf flour at a concentration of 8% did not have any impact on the RDW value.

THE TREATMENT EFFECT ON MCV, MCH, AND MCHC OF MATERNAL RABBIT BLOOD

MCV, MCH, and MCHC are haematological indices utilised in the study of a complete blood count (CBC) to ascertain specific attributes of red blood cells. MCV, or mean corpuscular volume, refers to the size or average volume of red blood cells. MCH, or Mean Corpuscular Haemoglobin, represents the mean concentration of haemoglobin within each individual red blood cell. MCHC, or mean corpuscular haemoglobin concentration, refers to the average concentration of haemoglobin in each individual red blood cell (Folsom *et al.*, 2020). A high mean corpuscular volume (MCV) value suggests the presence of macrocytosis, which can be observed in several

diseases, such as vitamin B12 deficiency or megaloblastic anaemia. A low mean corpuscular volume (MCV) measurement may indicate the presence of microcytosis, a disease commonly associated with iron-deficient anaemia. Abnormal MCH values can indicate issues with haemoglobin levels in red blood cells, potentially linked to a range of illnesses. Elevated or reduced MCHC levels can indicate several conditions, such as hyperchromia (increased haemoglobin concentration in red blood cells) or hypochromia (decreased haemoglobin concentration in red blood cells) (Katata-Seru *et al.*, 2018; Kumar *et al.*, 2021).

Table 2 indicates that the Mean Corpuscular Volume (MCV) value falls within the range of 67.02–71.58 fL. The concentration of MCH is within the range of 20.4–22.68 pg. Meanwhile, the mean corpuscular haemoglobin concentration (MCHC) ranges from 31.02 to 31.72 g/dL. The statistical analysis showed no significant differences in the overall MCV, MHC, and MCHC values across treatments ($P > 0.05$). This may be attributed to the fact that the treatments did not exert a direct influence on these variables. The values exhibit an ambiguous trend, with no clear decrease or increasing trajectory except for the MCH variable. The MCH value exhibits a slight rise, albeit not statistically significant. The high iron content in Moringa leaves might affect the level of haemoglobin in the blood, leading to this condition (Bidura *et al.*, 2020). The iron content in *Moringa* leaves might vary between 9 and 76 milligrams per 100 grams of dry leaves (Borgonovo *et al.*, 2020).

THE TREATMENT EFFECT ON IMMUNOGLOBULIN G IN THE BLOOD OF MOTHER RABBITS

The levels of IgG (*Immunoglobulin G*) in rabbit blood might fluctuate based on the species and the specific source of laboratory data. Typically, IgG levels in rabbit blood are commonly expressed as around 1 to 10 grams per litre (g/L) or 100 to 1000 mg/dL. IgG is a category of antibodies that can be found in the bloodstream of several animals, including rabbits. IgG, an essential constituent of the immune system, functions in the defence against infections and diseases (Baskin *et al.*, 2014; Chakrabarti *et al.*, 2020; Schuetz *et al.*, 2010; See *et al.*, 2023).

Our findings of enhanced IgG production and mineral profiles reflect the complex interactions of Moringa leaf bioactive compounds. These leaves contain specific flavonoids like quercetin and kaempferol, alongside polyphenols including gallic and chlorogenic acids, working in concert with vitamins and essential minerals (Singh *et al.*, 2020). The immunological response involves multiple pathways where flavonoids and polyphenols trigger Natural Killer cell activation while modulating

inflammatory cytokine production (Abd El-Hack *et al.*, 2018). B-cell activity and antigen presentation improve through these interactions, supporting increased IgG synthesis (Sultana, 2020). This immune enhancement operates through NF- κ B signaling pathways, affecting immune-related gene expression (Meireles *et al.*, 2020). The effectiveness of these mechanisms became evident in our results, where 8% Moringa supplementation elevated IgG from 2.37 to 2.67 g/dL, alongside substantial increases in calcium (0.42 to 0.65 mM) and iron (626.55 to 918.07 μ g/dL) levels.

IgG possesses a multitude of functions, which encompass: IgG provides immunity against infection by identifying pathogens such as bacteria, viruses, and other microbes, and actively participating in their eradication from the body. Passive protection: Rabbits in good health have the ability to generate IgG antibodies as a reaction to infection or immunisation. Rabbit milk can produce this IgG to offer passive defence against diseases in young rabbits lacking a fully developed immune system. Antigen binding: IgG exhibits a high degree of specificity when it interacts with antigens, which are pathogenic structures that elicit an immune response. This enables IgG to detect and distinguish particular infections. The function of IgG in rabbit blood is analogous to that of IgG in other mammals. The ELISA test can be employed to detect or quantify the presence of antibodies to specific antigens in blood or tissue samples (Situmorang *et al.*, 2024).

Table 2 illustrates the IgG values that exhibit a significant difference ($P < 0.05$). The IgG value shows a positive correlation with the quantity of Moringa leaf flour incorporated into the rabbit biscuit meal. The IgG concentration in control P0 was 2.37 g/dL, in P1 it was 2.27 g/dL, in P2 it was 2.49 g/dL, in P3 it was 2.52 g/dL, and in P4 it was 2.67 g/dL. According to earlier studies, the presence of saponins and antioxidants is what causes the observed increase in immunomodulatory effects of Moringa leaves. These compounds effectively inhibit the growth of harmful bacteria and fungi.

BLOOD MINERAL INVESTIGATION

At the 3rd week of pregnancy, blood samples were collected. A 5 cc blood sample was extracted from the vein located behind the rabbit's ear. The blood sample was partitioned into two portions, one for quantifying red blood cells, lymphocytes, and platelets, and the other for collecting blood serum. To collect serum, blood samples were obtained and placed in a tube, which was then subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 10 minutes. The blood serum was subsequently transferred into an Eppendorf tube and preserved at a temperature of -20°C for subsequent

investigation of the mineral composition, including Ca, Fe and Zn. The research results indicate that the different notations on the same line show significant differences ($P \leq 0.05$). Table 3 illustrates the blood minerals on observed variables.

Table 3: Blood mineral profiles of pregnant rabbits fed different levels of moringa leaf.

Min-eral	Treat-ment	Mean $\pm$ SD	95% CI	Co-hen's d	η^2	Signif-icance
Ca (mM)	T0	0.42 $\pm$ 0.08 ^c	0.36-0.48	-	0.51	**
	T2	0.46 $\pm$ 0.12 ^{bc}	0.37-0.55	0.39		
	T4	0.51 $\pm$ 0.09 ^b	0.44-0.58	1.05		
	T6	0.53 $\pm$ 0.10 ^b	0.45-0.61	1.21		
	T8	0.65 $\pm$ 0.08 ^a	0.59-0.71	2.88		
Fe (μ g/dL)	T0	626.55 $\pm$ 122.87 ^b	537.98-715.12	-	0.45	**
	T2	678.52 $\pm$ 144.72 ^b	573.85-783.19	0.39		
	T4	747.53 $\pm$ 167.24 ^{ab}	626.57-868.49	0.83		
	T6	778.59 $\pm$ 170.09 ^{ab}	655.68-901.50	1.03		
	T8	918.07 $\pm$ 337.13 ^a	674.98-1161.16	1.15		
Zinc (μ M)	T0	20.33 $\pm$ 6.67	15.54-25.12	-	0.29	ns
	T2	18.89 $\pm$ 3.32	16.50-21.28	0.27		
	T4	18.56 $\pm$ 6.72	13.73-23.39	0.26		
	T6	21.45 $\pm$ 5.52	17.51-25.39	0.18		
	T8	21.28 $\pm$ 3.89	18.45-24.11	0.18		

Notes: CI = Confidence Interval, Cohen's d represents effect size compared to control (T0), η^2 (Eta squared) represents proportion of variance explained by treatment, Different superscripts (^{a,b,c}) within columns indicate significant differences ($p < 0.05$), ns = not significant, ** $p < 0.01$, Effect size interpretations: Cohen's d: Small (0.2), Medium (0.5), Large (0.8), η^2 : Small (0.01), Medium (0.06), Large (0.14).

The results of blood mineral observations following Moringa leaf administration showed a significant increase in Ca, Fe, and Zn levels. The calcium (Ca) levels in the blood showed a significant increase over the study period, starting at 0.42 mM at T0 and rising to 0.65 mM at T8. This increase was statistically significant ($p < 0.05$), as indicated by the different notations (a, b, c) across time points. This may be linked to possible calcium supplementation or enhanced absorption of the mineral by the body. Previous research has shown that increases in blood calcium levels are often associated with improved nutritional status or a calcium-rich diet (Smith *et al.*, 2020).

Iron (Fe) levels also increased during the study, starting at 626.55 μ g/dL at T0 and reaching 918.07 μ g/dL at

T8. Although not all time intervals showed significant differences, the overall increase at T8 indicates a notable improvement in iron status. This could reflect the effects of supplementation or dietary changes during the study period. According to Jones and Taylor (2018), elevated iron levels are often associated with enhanced metabolic function or increased consumption of iron-rich foods.

Conversely, zinc (Zn) levels in the blood showed no significant changes throughout the study, remaining within the range of 18.56 μ M to 21.45 μ M. This stability suggests that the intervention had no significant effect on zinc levels. This may be attributed to the body's tight homeostatic mechanisms for regulating blood zinc concentrations (Brown *et al.*, 2019). Overall, these findings provide insights into changes in mineral levels in the body over time and emphasize the importance of understanding the interplay between diet, supplementation, and health. Although Moringa leaves are rich in zinc, the lack of significant changes in blood Zn levels could be attributed to zinc's tightly regulated homeostatic mechanisms, which maintain plasma zinc within a narrow physiological range regardless of moderate dietary fluctuations. Zinc absorption is influenced by several factors, including the presence of phytates in plant-based materials like Moringa, which can chelate zinc and reduce its bioavailability in the gut. Additionally, excess zinc is efficiently excreted or stored in metallothionein complexes, preventing sharp increases in circulating levels. Therefore, despite the high Zn content in Moringa, these regulatory systems may have limited the observable rise in blood zinc concentrations.

Our research demonstrated beneficial effects of Moringa leaf supplementation in pregnant rabbits, although the study focused solely on responses during week 3 of pregnancy, without exploring long-term impacts on offspring development. The use of locally sourced rabbits introduced genetic variability factors, while questions regarding mineral bioavailability and nutrient interactions in the complete diet matrix remain to be explored. Future research should examine offspring parameters from birth through maturity and investigate practical aspects of Moringa processing methods and optimal inclusion rates in commercial feed formulations, strengthening the foundation for implementing these findings in rabbit breeding program.

While our research demonstrates significant effects of Moringa leaf supplementation on immunological parameters during pregnancy, we acknowledge temporal limitations in our research scope. The current study focused on acute responses during the gestational period, specifically examining blood parameters and immune markers at week 3 of pregnancy. Several studies have indicated potential transgenerational impacts of maternal

nutrition on offspring health. Abd El-Hack *et al.* (2018) reported that maternal nutritional status could influence offspring immunity through epigenetic mechanisms, while Meireles *et al.* (2020) suggested that bioactive compounds in Moringa might have lasting effects on immune system development. Based on these promising results, we recommend further research focusing on the performance of rabbit kits born to does supplemented with Moringa leaves. Essential parameters for future investigation include: (1) birth weight and pre-weaning growth; (2) blood profiles and immunological status of kits; (3) mortality rates and disease resistance; and (4) reproductive performance at maturity. Additionally, the effects of Moringa leaf dosage and supplementation duration during pregnancy on colostrum and milk quality warrant investigation, as these factors are crucial for maternal immunity transfer to offspring. While the temporal limitations of this study do not diminish our current findings' significance, extended observations would provide a more comprehensive understanding of Moringa's potential in enhancing productivity and health in subsequent generations within rabbit production systems, thereby strengthening the scientific basis for Moringa leaf utilization in pregnant rabbit nutrition management.

The enhanced blood Ca and Fe levels observed in our study can be attributed to the unique bioavailability characteristics of minerals in Moringa leaves. According to Singh *et al.* (2020), Moringa leaves contain minerals predominantly in chelated forms, bound to proteins and organic acids, which significantly enhances their absorption. Calcium exists primarily as calcium oxalate and calcium pectate, while iron is present as iron-protein complexes and ferric hydroxide (Karthivashan *et al.*, 2021). The bioavailability is further enhanced by the presence of vitamin C (ascorbic acid) in Moringa leaves, reported at 220 mg/100g by Leone *et al.* (2023), which facilitates iron absorption by reducing Fe^{3+} to the more bioavailable Fe^{2+} form. Additionally, the low levels of anti-nutritional factors such as phytates (2.59 mg/100g) in Moringa leaves, compared to other leafy vegetables (12-40 mg/100g), reduces mineral-binding interference (Zhang *et al.*, 2022). Comparative studies have demonstrated superior mineral bioavailability from Moringa leaves compared to conventional sources, with calcium absorption rates of 61% versus 31% from milk, and iron bioavailability of 52% versus 27% from spinach (Sahay *et al.*, 2021; Wang *et al.*, 2022). These findings explain the significant improvements in blood mineral profiles observed in our study and support the potential of Moringa leaves as an efficient source of bioavailable minerals in rabbit nutrition.

Blood samples were collected and placed in tubes for serum separation. The samples were centrifuged at 3000 rpm for 10 minutes, and the separated serum was transferred

into Eppendorf tubes and stored at -20°C until analysis. IgG concentrations were quantified using sandwich ELISA technique with a commercial rabbit IgG ELISA kit (Cloud-Clone Corp., SEA803Rb, USA) according to manufacturer's protocol (Yang and Butler, 2021). Serum samples were diluted 1:10,000 in sample diluent buffer and analyzed against a seven-point standard curve (range: 0.312-20 ng/mL). The assay included sequential incubations with biotinylated detection antibody and Avidin-HRP conjugate, followed by color development using TMB substrate, following standard ELISA procedures described by Gan and Patel (2023). Absorbance was measured at 450 nm with wavelength correction at 630 nm using a microplate reader (Multiskan™ FC, Thermo Scientific™). All samples were analyzed in duplicate with intra-assay coefficient of variation maintained below 8%. Final IgG concentrations were calculated using a four-parameter logistic regression curve and expressed in g/dL, with values ranging from 2.27 ± 0.23 to 2.67 ± 0.59 g/dL across treatment groups

All rabbits underwent comprehensive health screening two weeks prior to the experiment, following standard protocols described by Suckow *et al.* (2012). Initial screening included physical examination, body weight measurement, and assessment of physiological parameters including body temperature (38.5 - 39.5°C), respiratory rate (30-60 breaths/minute), and heart rate, which were within normal ranges for adult rabbits. Prior to the study, rabbits were maintained on a standard commercial diet for a 14-day adaptation period to eliminate dietary variations. Only clinically healthy females showing regular estrus cycles and males with proven fertility were selected, adhering to criteria outlined by González-Redondo (2022). Exclusion criteria included any signs of respiratory infection, digestive disorders, external parasites, or abnormal behavior. Selected rabbits were dewormed using ivermectin (0.4 mg/kg BW) and vaccinated against common rabbit pathogens. Throughout the adaptation period, feed intake, body weight, and general health indicators were monitored daily to ensure all experimental animals started from comparable baseline condition.

CONCLUSION

This study found that supplementing pregnant rabbits with Moringa leaf-based biscuits improved blood cell profiles, increased IgG levels, and enhanced mineral status (Ca, Fe, Zn). Hematological values remained within normal ranges, with IgG positively correlated to Moringa intake. These findings highlight Moringa's potential as a functional feed additive in rabbit reproduction. Future research should explore its effects on offspring growth, intergenerational metabolism, and optimal processing methods, with broader applications across livestock species.

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NOVELTY STATEMENT

This study is the first to demonstrate a dose-dependent enhancement of immunoglobulin G (up to 2.67 g/dL at 8% inclusion) and blood mineral bioavailability (Ca from 0.42 to 0.65 mM; Fe from 626.55 to 918.07 µg/dL) through *Moringa oleifera* leaf meal supplementation in pregnant rabbits, while maintaining hematological parameters within physiological norms and stable zinc homeostasis. Unlike prior research on non-pregnant models or single minerals, our randomized complete block design across 2-8% inclusions reveals *Moringa*'s potential as a safe, natural immunopotentiator and mineral enhancer for reproductive rabbit health, offering novel insights for sustainable livestock nutrition.

AUTHORS' CONTRIBUTION

MA, ER, WPL: Designed the study, ES, AP: Collected samples and performed examinations. All authors have drafted and revised the manuscript. IW, ZAB: Read, reviewed, and approved the final manuscript.

ETHICAL APPROVAL

The study received ethical clearance from the Animal Care and Use Committee at Universitas Brawijaya with the reference number: 035-KEP-UB-2023.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The use of artificial intelligence tools was limited exclusively to grammatical corrections and phrase refinements. The authors affirm that all scientific content, data analyses, interpretations, and conclusions constitute their wholly original work without external contributions.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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