

Haematological profile, Henday production and effects of THI on Isa brown layers fed shrimp waste meal based rations

Victoria U. James*, Akaneren, I. Essien and Ofoninyene O. Usoro

Department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State, Nigeria.

*Corresponding author. Email: vj96171@gmail.com

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ABSTRACT: This study evaluated the replacement of fish meal with shrimp waste meal (SWM) as an animal protein source in the diets of laying hens and examined the effects of temperature and relative humidity on egg production. Ninety Point-of-Lay (POL) Isa Brown pullets were randomly assigned to five dietary treatments in a completely randomised design. Each treatment consisted of 18 birds, replicated three times with six birds per replicate. The 12-week experiment was conducted at the Akwa Ibom State University Teaching and Research Farm, Obio Akpa Campus, from November 2023 to March 2024. Five diets were formulated with fish meal replaced by SWM at 0, 25, 50, 75, and 100% levels. Treatment 1 (T1) contained 0% shrimp waste meal and 8% fish meal, T2 contained 2% SWM and 6% fish meal, T3 contained 4% SWM and 4% fish meal, T4 contained 6% SWM and 2% fish meal, and T5 contained 8% SWM and 0% fish meal. Haematological parameters were evaluated to determine the suitability of SWM as a fish meal substitute. Results showed that birds fed T5 (100% replacement) recorded significantly ($p < 0.05$) higher values of white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) than birds on other treatments. Significant ($p < 0.05$) differences were observed among treatments for most haematological parameters, except neutrophils, eosinophils, and lymphocytes. All values obtained were within normal ranges reported for laying hens. Regression and correlation analyses of Temperature Humidity Index (THI) and hen-day production (HDP) over the 12-week period revealed a weak positive correlation with morning THI values and a weak negative correlation with afternoon THI values, likely due to the mild to moderate THI conditions recorded during the study. Hen-day production generally declined with increasing duration of lay across treatments, except at the 50% SWM inclusion level, which showed a positive correlation with HDP. The study concluded that shrimp waste meal can effectively replace fish meal up to 100% in layer diets without adverse effects on haematological characteristics and may support blood production and overall bird health.

Keywords: Shrimp waste meal, layers, haematological parameters, temperature/relative humidity index, Henday production.

INTRODUCTION

The poultry industry, being a key aspect of the Agricultural sector of the Nigerian economy, plays a pivotal role in job creation, income generation, resource recycling and ultimately the provision of an affordable protein source through table eggs. Poultry production represents a significant portion of global livestock, accounting for nearly 40% of global meat production, with 74% of poultry meat

and 68% of eggs produced via intensive farming (Aladejebi *et al.*, 2014). Despite these obvious nutritional benefits and positive contributions to the economy, inadequacies in meeting standards of nutritional feed content continue to act as a barrier to the attainment of the industry's full potential. It is to this end that shrimp waste was explored in this research as a potential alternative source of animal

protein in layers' diets. Also, amongst the many factors mitigating the smooth running of the industry is the paucity of data on blood parameters. These parameters are indicative of the breeding, feeding, environmental and even genetic conditions on which the production and welfare of the animal strongly depend. The indices of blood parameters provide a reliable guide as to the health and wellness of animals, as well as the nutritional status of the individual and therefore allow for regulation in feeding. For proper assessment of the efficiency of shrimp waste as a replacement for fish meal, either partially or wholly, it is pertinent to go beyond the productivity and economic implications, which have been published as part of this work, to the blood profile, which exposes the ultimate acceptability of the test ingredients by the poultry birds. Heo *et al.* (2023) observed that protein deficient diets lead to a decline in blood production, which ultimately results in bone marrow hypoplasia, inducing structural alterations that interfere with innate and adaptive immunity alike, as well as the development of leukopenia and alterations in the bone marrow environment, resulting in haematopoietic failure. It is to this end that this study assessed the haematological characteristics of laying birds fed graded levels of the shrimp waste meal-based rations. Haematological studies on layer birds fed shrimp waste meal based diets are scarce. However, reports on analysis of blood parameters of broilers on feeding trials with shrimp waste meal have revealed variations that underscore the relationship between the test diet constitution and the state of health of the animals. Mounica *et al.* (2019) observed such variations in their studies using shrimp waste meal based diets for starter and finishing broiler birds, while James *et al.* (2024) established that shrimp waste meal has proximate fractions comparable to fish meal and that its replacement of fish meal up to 75% in layers' diet can lead to a cost saving of ₦15.48/egg produced. Henday production is a major determinant of the success of the egg production enterprise. Okon *et al.* (2009) revealed that the rate of increase in percentage egg production is highest within the first quarter (1-10 weeks) of egg production and that egg production experiences a period of rapidly increasing production, attainment of peak, and the phase of a gradual decline in production. It is noteworthy to examine the extent to which shrimp waste inclusion in the layer's ration can slow down the rate of decline in egg production, and this study also sought to address that. Also, owing to the global concern about the effects of global warming on animal production and especially during the period of the research (December 2023 to March 2024), which recorded high temperatures, the effects of temperature and relative humidity on egg production were examined. Birds are heat-stressed if they have difficulty achieving a balance between body production and body heat loss, and high ambient temperatures, coupled with high humidity, can have a major detrimental impact on the performance of commercial poultry (Zhoung *et al.*, 2019). Temperature Humidity Index (THI) has already been established to be a

major marker in dairy cattle farming. Besteiro *et al.* (2025) revealed that a THI threshold of 68 or higher generally indicates the onset of heat stress, causing reduced milk yield, lower fat and protein content, decreased feed intake, and increased risk of illness. This research work, therefore, included a check on the relationship between THI and egg production.

MATERIALS AND METHODS

The research spanned a period of 12 weeks, from 4th November 2023 to 4th February 2024. It was conducted at Akwa Ibom State University Teaching and Research Farm, Obio Akpa Campus at the Poultry Unit of the farm. Obio Akpa is in Oruk Anam Local Government Area of Akwa Ibom State and has coordinates lying between Latitude 4°9645° North and Longitude 7.75980° East of the Greenwich Meridian and belongs to the Swamp Forest ecological zone of Nigeria (Ekong and Etebong, 2018). Ninety POL Isa Brown Pullets at 19 weeks of age were used for the study in the deep litter system.

Treatments

The research treatments consisted of five diets containing five different levels of shrimp waste meal inclusion. The five treatments were designated as follows:

Treatment (T1) (control): diet containing 0% shrimp waste meal (SWM) and 8% fish meal (FM), that is, 0% replacement of fish meal by shrimp waste meal.

Treatment 2 (T2): contained 2% SWM and 6% FM, that is, 25% replacement of fishmeal by shrimp waste meal.

Treatment 3 (T3): contained 4% SWM and 4% FM, that is, 50% replacement of fishmeal by shrimp waste meal.

Treatments 4 (T4): contained 6% SWM and 2% FM, that is, 75% replacement of fishmeal by shrimp waste meal.

Treatment 5 (T5): contained 8% SWM and 0% fish meal, that is, 100% replacement of fishmeal by shrimp waste meal.

Each treatment had 3 replicates. Six (6) POL birds were randomly assigned to each of the three replicates of the five treatments.

Experimental design

The completely randomised design (CRD) was used to allocate birds to the different treatments.

Data collection and analysis

One bird per replicate (a total of 15 birds) was randomly

selected for the test, all samples being collected using a separate 2 ml syringe, via the prominent wing vein after defeathering the area. The thermal hygrometer was placed at the middle of the poultry house and was used to calculate the ambient temperature and relative humidity twice daily at 8:30 a.m. and 3:30 p.m. Eggs were collected from each replicate three times daily (9:00 am, 12:00 noon and 3:00 pm).

Full blood count was achieved using a fully automated haematology analyser (5-part differential). Elite 580 (*Ebra lachema*) using the assay method of Coulter (1953). The equipment made use of impedance and flowsychometric protocol. The temperature relative humidity index (THI) was calculated using the formula:

$$\text{THI} = 0.8T + \text{RH} (T - 14.4) 46.4$$

Where THI = temperature/relative humidity index, T = ambient temperature in degrees centigrade, and RH = relative humidity expressed as a proportion. While Henday production was calculated using the formula:

$$\text{HDP} = \frac{\text{number of eggs per day}}{\text{number of birds alive}} \times 100$$

Statistical analysis

Data collected throughout the period of the experiment were subjected to analysis of variance using the statistical package SPSS vs 2004. Treatment means seen to be significant were separated using the Duncan range test as prescribed by Duncan (1955). Regression and correlation analysis of egg production were carried out using the linear regression model:

$$Y = a + bX$$

RESULTS AND DISCUSSIONS

Haematological parameters of birds fed different levels of shrimp waste meal

The results of the haematological parameters of birds fed diets containing shrimp waste are presented in Table 1. The leucocyte (WBC) value of $4.67 \times 10^9/\text{L}$ of T5 was higher than those of the other treatments, while the red blood cells (RBC) or erythrocyte level of $5.70 \times 10^{12}/\text{L}$ of T5 was higher than all the other treatments and hematocrit or packed cell volume (PCV) values ranged from 23.17% to 29.00%. The mean corpuscular volume (MCV) levels ranged from 45.00fL to 51.00fL. The lowest haemoglobin (Hb) value of 7.73g/dl was found in T1-T4, while the highest value was found in T5 (10.33g/dl). MCHC values obtained from this study ranged between 33.37 and 35.63%. Birds fed 100% SWM had higher platelet (PLT) count than the other treatments, with the lowest value of $313.00 \times 10^9/\text{L}$ in birds fed 25% SWM. Significant ($p < 0.05$) differences existed

across the dietary treatments for all parameters tested except for neutrophils, eosinophils and basophils.

White blood cells (leukocytes) count

Leucocytes are part of the body's immune system and help the body to ward off invading pathogenic organisms, inflammation, allergies and leukaemia (NCI, 2024). The WBC (leukocyte) count ($4.67 \times 10^9/\text{L}$) of T5 was significantly ($p < 0.05$) higher than those (3.51 , 3.62 , 3.72 and $3.74 \times 10^9/\text{L}$) of treatments T3, T4, T1 and T2, respectively. The higher value ($4.67 \times 10^9/\text{L}$) obtained from T5 (100% SWM) agreed with the findings of Mounica *et al.* (2019), who reported higher levels ($30.22 \times 10^3/\text{L}$ and $11.07 \times 10^9/\text{L}$, respectively). The WBC (leukocyte counts) obtained in this study were within the normal range ($3.7 \times 10^9/\text{L} - 4.67 \times 10^9/\text{L}$) reported by Banerjee (2008), except the control ($3.62 \times 10^9/\text{L}$) and T3 ($3.51 \times 10^9/\text{L}$) with slightly lower values, but not low enough to allow for disease infection. This normal range of WBC shows the ability to support the immune response and defense mechanism in laying birds.

Red blood cell (RBC) count

The red blood cells (RBC) or erythrocyte level ($5.70 \times 10^{12}/\text{L}$) of T5 was significantly ($p < 0.05$) higher than all the other dietary treatments, T1, T2, T3 and T4, respectively. The values obtained in this study were higher than those ($2.52 \times 10^6 \text{ mm}^3 - 2.88 \times 10^6 \text{ mm}^3$) reported by Abun *et al.* (2023) for laying hens given fermented shrimp waste extract but similar to the range ($3.15 - 5.32 \times 10^{12}/\text{L}$) reported by Igwe (2023), for Isa Brown layers fed plantain peel meal. However, they were within the range considered normal for avian species as revealed by Bounous and Stedman (2000), except T4 ($5.03 \times 10^{12}/\text{L}$) and T5 ($5.70 \times 10^{12}/\text{L}$), which suggests a positive impact of SWM in erythropoiesis (red blood cell formation). Abun *et al.* (2023) corroborated this fact by stating that shrimp waste meal contains active ingredients in the form of astaxanthin, which has a positive impact on digestion, and Nurhalisa (2021) revealed that fermented SW extracts can increase the digestibility of nutrients, especially proteins. This increased nutrient digestibility and absorption have the potential to increase erythrocyte formation.

Haematocrit (HCT) or Packed Cell Volume (PCV)

The HCT values obtained from T5 were within the normal range (25-45%) for laying birds, while the values (23.17-24.33%) for the other treatments were slightly lower. These slightly lower values can be attributed to the higher egg production observed in those treatments (T1-T4); similarly, Shlig (2009) attributed lower HCT values to higher feed conversion efficiency. Hrabčáková *et al.* (2014)

Table 1. Haematological parameters of laying hens fed different levels of shrimp waste meal-based diets.

Parameters	Treatment					SEM	P-Value	LOS
	1	2	3	4	5			
WBC ($10^9/L$)	3.72 ^b	3.74 ^b	3.51 ^b	3.62 ^b	4.67 ^a	0.14	0.016	*
RBC ($10^{12}/L$)	4.87 ^b	4.87 ^b	4.63 ^b	5.03 ^b	5.70 ^a	0.12	0.029	*
HGB (g/dl)	8.17 ^b	8.17 ^b	7.73 ^b	7.97 ^b	10.33 ^a	0.31	0.014	*
HCT (%)	24.33 ^b	24.33 ^b	23.17 ^b	23.83 ^b	29.00 ^a	0.73	0.052	*
MCV (fl)	50.00 ^a	50.00 ^a	50.00 ^a	45.00 ^b	51.00 ^a	0.76	0.058	*
MCH (pg)	16.83 ^{ab}	16.83 ^{ab}	16.70 ^{ab}	15.80 ^b	18.13 ^a	0.29	0.127	*
MCHC (%)	33.57 ^b	33.57 ^b	33.37 ^b	33.43 ^b	35.63 ^a	0.29	0.028	*
PLT ($10^9/L$)	319.00 ^{bc}	313.00 ^c	332.00 ^{abc}	338.67 ^{ab}	344.00 ^a	4.16	0.060	*
NEU (%)	30.00	30.67	30.00	26.67	27.33	0.79	0.426	NS
EOS (%)	5.93	6.80	6.37	6.30	5.47	0.29	0.714	NS
MON (%)	2.73 ^{ab}	5.20 ^a	4.30 ^{ab}	1.37 ^b	2.53 ^{ab}	0.50	0.090	*
BAS (%)	0.00	0.00	0.00	0.00	0.00	0.00	-	NS
LYM (%)	61.33 ^{ab}	57.33 ^b	59.33 ^{ab}	65.67 ^a	64.67 ^a	1.12	0.061	*

SEM = standard error of mean, LOS = level of significant, * = significant at $p < 0.05$, ** = significant at $P < 0.01$, NS = not significant, ^{a,b,c} = means with different superscript along the row are significantly different.

reported a drop in the total number of erythrocytes at the peak of egg production for laying Pheasants, a period which is accompanied by significant amounts of metabolic stress that is reflected in a reduced number of red blood cells.

Mean Corpuscular Volume (MCV)

The lowest MCV content was produced in the T4 treatment with an average value of (45.00fl), while the others were significantly higher ($p < 0.05$). The highest value of MCV content was produced in the T5 treatment with a total of 51.00 fl. MCV is a parameter used to determine the average volume of an individual red blood cell in the circulatory system (Onyishi *et al.*, 2017) or the average volume of one red blood cell (Hong *et al.*, 2021). Therefore, this increased value of MCV at 100% replacement of fishmeal by shrimp waste meal is a testimony to the ability of the test ingredient to contribute meaningfully to the overall welfare of layers.

Mean Corpuscular Haemoglobin (MCH)

The lowest Haemoglobin (Hb) value (7.73 g/dl) was found in T1-T4, while T5 had the highest value (10.33 g/dl). These values were well within the range (7-13 g/dl) for the normal haemoglobin count of chickens. Dietary treatments containing 100% replacement of fish meal by shrimp waste meal (T5) had the highest value (18.13pg) of mean corpuscular Haemoglobin (MCH) in this study, although not different from T1-T3, while that of 75% replacement (T4) had the lowest value (14.8 pg). There were significant ($p < 0.05$) differences between the various treatments.

Mean Corpuscular Haemoglobin Concentration (MCHC)

Mean Corpuscular Haemoglobin Concentration (MCHC) is a measure of the average amount of haemoglobin in a single red blood cell as it relates to the volume of the cell; it is used to determine whether the RBC is carrying an appropriate amount of haemoglobin (Kamfrath, 2022). Values of MCHC obtained from this study were significantly ($p < 0.05$) different across dietary treatments but fell well within the range considered normal as put forward by Guard and Hawley (1990).

Platelets (PLT), Lymphocytes (LYM), and Monocytes (MON)

Values of platelet counts observed in this study were well within the range ($150-450 \times 10^9/L$) revealed by Adeyemo *et al.* (2018). Lymphocyte values recorded in this study were well within the normal range (47.20-85%) reported by Mitruka and Rawnsley (1977), Riddell (2011). Birds fed up to 75%, and 100% replacement of FM by SWM had significantly ($p < 0.05$) higher (65.67%) lymphocyte values, while birds on 25% replacement had the lowest (57.33%). But the exact reverse was the case, for monocytes, where there were significant ($p < 0.05$) differences across the treatments. Birds fed 25% replacement of FM with SWM had the highest (5.20%) value compared to that of 75% replacement, which had the lowest value (1.37).

Neutrophils (NEUT), Eosinophils (EOS), and Basophils (BAS)

These granulocytes were not significantly ($p > 0.05$) different

Table 2. Regression and correlation equations relating hen day production (Y) to temperature / humidity index (X) for 12 weeks (1-12) of egg production.

Treatment	Equation	r	r ²	r ² change	SEE	Significant
Morning	Y = 80.473 + 0.042X	0.029	0.001	-0.012	5.31	0.798
Afternoon	Y = 91.354 - 0.100X	0.035	0.001	-0.011	5.31	0.755

Table 3. Regression and correlation equations of hen day Production (Y), and duration of lay in weeks (X), of layers fed different dietary levels of replacement of fish meal by shrimp waste meal (n = 84).

Treatment	Equation	r	r ²	r ² change	SEE	Significant
1	Y = 97.472 - 0.270X	0.601	0.362	0.354	8.70	P<0.000
2	Y = 89.529 - 0.088X	0.171	0.029	0.017	12.34	P<0.122
3	Y = 67.821 + 0.203X	0.530	0.280	0.089	15.02	P<0.001
4	Y = 92.638 - 0.107X	0.295	0.087	0.076	8.41	P<0.007
5	Y = 89.529 - 0.246X	0.426	0.182	0.172	12.67	P<0.000

different across the five treatment groups; however, the values obtained for basophils were well within the range (0-15%) observed by Riddell (2011). Values of neutrophils obtained in the study also fell within the range obtained by Branson *et al.* (1994), while those of eosinophils (5.47-6.80%) were lower than those (0.67-2.00%) observed by Egbewande (2019), who also stated that these fractions of white blood cells support birds against parasitic infections and inflammatory responses.

Monocytes

Values (1.37-5.20%) of this study corresponded to the reference value (2-5%) of monocytes for avian species as put forward by Nanbol *et al.* (2016) and varied across treatments at P<0.05, with T3 (50% SWM and 50% FM) having the highest value of 4.30%.

Regression and correlation equations of temperature and relative humidity (THI) for 12 weeks of egg production

The results obtained from this study are presented in Table 2. A weak positive correlation between Henday production and Temperature Humidity Index was observed in the morning values, while a weak negative correlation existed in the afternoon values. This could be due to the THI values calculated and used in the regression analysis being within the mild and moderate range. No significant (p>0.05) relationship existed between THI and Henday production. This finding agrees with Tůmová and Gous (2012), who observed no significant (p>0.05) effect of temperature on the rate of lay. The susceptibility of today's chickens to heat stress owing to their high productivity (Lin *et al.*, 2006) makes these results of noninterference of THI (72-89THI) during the test period a welcome one.

Regression and correlation analysis between Henday production and duration (weeks) in lay for the five treatments

The relationship between Henday production and duration in lay for the laying birds in each of the five treatments was investigated using the simple linear regression and correlation model. These results are presented in Table 3. The general trend indicated for the five treatments was the characteristic decreasing Henday production with increasing duration of lay within the 12 weeks period, except in treatment 3, which showed a positive correlation with HDP. A significant (p<0.05) relationship existed between Henday production and duration in lay of this study for the five treatments. The trend of characteristic decrease in HDP with increasing duration in lay, which agrees with earlier studies of Essien (1988) and Williams *et al.* (2009), for different strains of laying birds. Also, the regression equations developed from the study for each of the Treatments were reliable predictions of Henday production within the period of study, as confirmed by McHugh (2008), that the standard error of estimate and the level of significance (p<0.01), indicate the strength of predictability of the equation derived for all treatment groups containing various levels of shrimp waste meal replacement of fishmeal.

Conclusion and Recommendation

The research revealed that including shrimp waste meal in layers' diets supported the production of blood parameters at levels considered optimum for the birds' well-being, while temperature and humidity during the experiment had no adverse effects on hen-day production. A 100% replacement of fishmeal by shrimp waste meal resulted in a higher boost in the production of the majority of the blood parameters tested. Based on these findings, it was

recommended that shrimp waste meal be used to replace fishmeal in layers' diets, even up to 100%, to maintain optimum haematological status and sustain HDP throughout the production period.

CONFLICT OF INTEREST

The author declared that they have no conflict of Interest.

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