



**Haematology and Serum Biochemistry of Pullet Chicks Fed Diets with Low Energy and Crude Protein Contents with Enzyme as Additive**

<sup>1</sup>Agida, C. A., <sup>1</sup>Adiele, C. J., <sup>1</sup>Ogbuu, E. E., <sup>1</sup>Obioma, C. A., <sup>1</sup>Ukachukwu, S. N., <sup>1</sup>Amaefule, K.U., ·  
<sup>2</sup>E. Uchechi Ihugba

<sup>1</sup>Department of Animal Nutrition and Biochemistry, College of Animal Science and Animal Production  
Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

<sup>2</sup>Department of Agriculture, Alvan Ikoku University of Education, Owerri, Nigeria

\*[agidachrisagboje@gmail.com](mailto:agidachrisagboje@gmail.com)

**Abstract**

*This study was carried out to investigate the haematological and serum biochemical response of pullet chicks (Isa Brown) fed low energy and crude protein diets with enzyme as additive. The study was conducted from day old to 8 weeks of age with combined dietary levels of 2800, 2600 and, 2400 kcal/kg of energy and 20, 17and, 15% of crude protein (CP). The pullet chicks were assigned to 9 treatments of 60 birds per group, in four replications of 15 birds per replicate, in a 3×3 factorial in a completely randomized design (CRD). Results showed that haemoglobin concentration, packed cell volume, total white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocytes, and heterophils were significantly ( $p<0.05$ ) affected by dietary interaction (energy×protein) levels. Urea, creatinine,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and  $\text{K}^+$ , parameters for pullet chick kidney function test were found to increase significantly ( $p<0.05$ ) across interaction levels from 2800kcal/kg×20% CP to 2400kcal/kg×15% CP. Values for total protein, globulin, aspartate aminotransferase, alanine transferase, alanine phosphatase, bilirubin and glucose were found to decrease significantly ( $p<0.05$ ) across treatments from 2800kcal/kg×20% CP to 2400kcal/kg×15% CP. Lipid profile tests were found to be significantly ( $p<0.05$ ) different among dietary treatments. The results of haematological and serum biochemical parameters for pullet chicks showed no detrimental health challenge across dietary treatments for birds fed low-energy and low-crude protein diets.*

**Keywords:** Haematology, serum, biochemistry, chicks, energy, crude protein

**Introduction**

The poultry industry is under constant pressure to improve feed efficiency, minimize production costs, and reduce environmental impacts (George and George, 2023; Nawab *et al.*, 2018). Feed constitutes a significant portion of production costs, with energy and protein being the most expensive components (Pond *et al.*, 2004). Optimizing these nutrients is critical for enhancing productivity and profitability. However, challenges arise when formulating low-energy and low-crude protein (LE-LCP) diets, as they may compromise growth, egg production, and feed conversion efficiency. Supplementing these diets with enzymes has emerged as a promising strategy

to mitigate negative effects by enhancing nutrient digestibility and feed utilization (Adeola and Cowieson, 2011; Adedokun and Olojede, 2019). The humid tropics pose additional challenges for poultry production due to high temperature and humidity, which exacerbate nutrient losses and reduce feed intake (Oke *et al.*, 2024; Apalowo *et al.*, 2024; Abioja and Abiona, 2021).

Energy and protein (amino acids) are needed by pullets for maintenance, growth, egg production, immunity, and adaptation to climatic and environmental conditions (Qaid and Al-Garadi, 2021; Barszcz *et al.*, 2024). Protein is an expensive dietary component,

especially in Nigeria. It is, therefore, imperative that protein levels be manipulated in poultry diets and feeds for optimum rather than maximum bird performance at a very low financial cost (Bist *et al.*, 2024; Pest and Choct, 2023; Adedokun and Olojede, 2019). This would make eggs and other poultry products affordable to a wider segment of the populace.

In Nigeria, the dietary energy/crude protein levels recommended for pullet chicks, growers, and layer chickens have been 2800 kcal/kg/20%, 2800 kcal/kg/18%, and 2600 kcal/kg/16.50%, respectively (Amaefule *et al.*, 2004). The logic had been that dietary energy and protein levels should be increased in poultry diets to ensure constant energy and protein intake under heat stress and other adverse environmental conditions prevalent in the humid tropics. To meet these high energy and crude protein levels in pullet and layer diets meant using high levels of expensive maize, soybean meal, and fish meal (local or foreign) (Esther, 2021; Fanatico *et al.*, 2018). The diets are most times supplemented with synthetic lysine and methionine in order to meet the requirements of the pullets and layers for these two critical amino acids (Macelline *et al.*, 2021; Burley *et al.*, 2016).

Agro-industrial by-products (AIBPs) used in poultry diets are known to contain some anti-nutritional factors (ANFs) like phytate, non-starch polysaccharides (NSPs), lower metabolizable energy (ME), and, in some cases, lower protein (Chharang, 2022). Lowering the CP and ME content of pullet and layer diets has been reported to reduce feed cost and allow for greater use of alternative feedstuffs without adverse effects on growth performance (Agida *et al.*, 2019; Alagawany *et al.*, 2018). However, efficiency and rate of growth were lower (Kamran *et al.*, 2008; Belloir *et al.*, 2017; Bregendahl *et al.*, 2002). The objective of this study was to determine the

haematology and serum biochemistry of Isa brown pullet chicks fed low energy and low crude protein diets with enzyme additive.

## Materials and Methods

### Location

The study was conducted at the Poultry Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production in the Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Feedstuff and other ingredients were obtained from local Umuahia, Abia State dealers and day-old hybrid pullet chicks from a reputable hatchery in Ibadan, Nigeria. The site is located between latitudes 05°-29° North and longitudes 7°-32° East with an altitude of 123 m above sea level, with annual temperatures ranging between 22°C and 36°C and relative humidity of 50-90°C, situated in the rainforest region of South East Nigeria.

### Experimental Birds and Management

A total of 540 day-old hybrid pullet chicks were used in the study, comprising the two growth stages (pullet chick and grower) and the layer stage of table egg producing chickens. The pullet chicks, growers and layer chickens were housed and reared in a tropical-type, open-sided deep litter poultry house roofed with aluminium roofing sheets. The pens are demarcated with a concrete wall 30 cm high from the floor, while wire mesh runs from this height to 2 m high. The building has a 1.2 m high dwarf wall, with the remaining space from the dwarf wall covered with expanded metal (wire gauze). Each pen measuring 1.5 m x 1.2 m was used to house a replicate of 15 pullet chicks. Brooding was done with kerosene stoves under metal hovers while an additional four hours of light was provided at night each day (16 hours of light per day). The birds were given experimental feed and water *ad libitum*. Routine vaccination against common diseases (Newcastle, Gumboro, fowl pox and typhoid)

and medication were duly administered to the birds during the experiments.

### Experimental Diets

Three dietary energy and three dietary crude Pullet Chick Diets

protein levels for pullet chicks, giving a total of nine (9) experimental diets and four (4) replicates for each phase. The experimental diets were as follows:

Energy/CP	A (2800 kcal)	B (2600 kcal)	C (2400 kcal)
W (20%)	AW	BW	CW
X (17%)	AX	BX	CX
Y (15%)	AY	BY	CY

The control was 2800 kcal x 20% for pullet chicks.

### Sample collection/Laboratory analysis

At the end of the 56 days feeding trial, six birds per replicate (16 birds/treatment) were sampled and blood samples collected via wing veins using sterile 21G X 1½ (0.8 x 40mm) needles and syringes into bottles containing anticoagulant (ethylene diamine tetra acetic acid; EDTA) for haematology as described by Lamb (1981). The micro-haematocrit method determined the packed cell volume as Coles (1986) described. The haemoglobin concentration (Hb) of the blood samples were determined by the cyanomethaemoglobin method as described by Kachmar (1970). Red blood cell (RBC) and white blood cell (WBC) counts were determined by the haemocytometer method as described by Schalm *et al.* (1975). Blood samples for serum biochemistry were collected into 10ml test tubes without anticoagulant. Serum was obtained by allowing the blood sampled to clot at room temperature for 30 minutes. Then, it was centrifuged for ten minutes at 3,000 revolutions per minute using a table centrifuge. Clear serum supernatant was then carefully aspirated with a syringe and needle and stored in a clean sample bottle. Decanted sera from the coagulated blood samples were saved by freezing at -180 °c until analyzed for serum biochemistry according to Feng *et al.* (1973). Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were

determined by the Reitman-Frankel colourimetric method for the in vitro determination of ALT and AST in serum or plasma using a Quimica Clinica Aplicada (QCA) test kit according to Reitman and Frankel (1975). For the in vitro determination of alkaline phosphatase in serum or plasma using the phenolphthalein monophosphate method as described by Klein *et al.* (1960; and Babson *et al.* (1966). Total protein was determined in vitro according to Lubran (1978). The in vitro determination of albumin in serum was done using the modified Jaffe method (Blass *et al.* 1974). Serum cholesterol and glucose for in-vitro determination was analysed using the enzymatic colourimetric method (Allain *et al.* 1974). The data collected were subjected to one-way analysis of variance for haematological variables and serum biochemistry.

### Data analysis

Data obtained were subjected to analysis of variance (ANOVA) in a 3 x 3 factorial arrangement in a Completely Randomized Design (Steel *et al.*, 1997). Statistical computations were done using Mini-tab version 21. The values in percentages were subjected to arcsine transformation before ANOVA, while differences among treatment means were separated using the Tukey test.

### Results and Discussion

### **Effect of Low Energy and Protein diets with enzyme supplementation on haematological parameters of pullet chicks**

The main and interactive effects of low energy and low protein with enzyme supplementation on haematological parameters of pullet chicks are presented in Tables 2 and 3. There were significant ( $p<0.05$ ) differences on both energy and protein effects on blood haemoglobin (Hb) concentration. Low ( $p<0.05$ ) Hb levels were observed among starter chicks fed 2600kcal/kg and 17%CP dietary levels when compare with that of the control. Starter chicks fed diets with 2400kcal/kg and 15%CP were found to be statistically ( $p<0.05$ ) the same with those fed control (2800kcal/kg and 20%CP) and 2600kcal/kg and 17%CP diets. PCV showed significantly ( $p<0.05$ ) low volume for chicks fed 2600kcal/kg and 2400kcal/kg energy levels when compare with that of the control. For protein effects, the result showed significantly ( $p<0.03$ ) high PCV for chicks fed 17%CP dietary levels when compare with those fed 20%CP dietary level, chicks fed 15%CP dietary levels were found to be statistically ( $P<0.05$ ) similar with those fed 20%CP dietary levels. The results for red blood cell (RBC) and total white blood cell count (TWBC) were not significantly ( $p>0.05$ ) different across treatments for both energy and protein effects respectively.

Mean corpuscular volume (MCV) was not significantly ( $p>0.05$ ) affected by energy level, but effect of protein was significant ( $p<0.05$ ). High MCV was observed ( $p<0.05$ ) among chicks fed 17%CP when compared with those fed 20%CP dietary levels, with those fed 15%CP dietary levels observed to be statistically ( $p<0.05$ ) the same with those fed 17%CP and 20%CP dietary levels respectively. Mean corpuscular hemoglobin (MCH) was found to be significantly ( $p<0.05$ ) affected by treatments. Chicks fed diets with

2400 kcal/kg energy were found to be significantly ( $p<0.05$ ) high to those fed 2800 kcal/kg compared to the control. Low MCH was observed ( $p<0.05$ ) among starter chicks fed 2600kcal/kg dietary levels. Mean corpuscular hemoglobin concentration (MCHC) was not significant ( $p<0.05$ ) among energy and protein effects. Blood lymphocytes percentage test confirmed significant ( $p<0.05$ ) differences on both energy and protein effects. Lymphocytes percentage was found to be significantly ( $p<0.05$ ) higher among starter chicks fed 2600 kcal/kg and 2400 kcal/kg dietary energy levels when compare with those fed 2800 kcal/kg, while protein effects showed significantly ( $p<0.05$ ) higher values for chicks fed 17%CP dietary levels and lower levels for 15%CP dietary levels. Heterophils percentage showed significantly ( $p<0.05$ ) decreased levels as the dietary energy levels decreased for energy effects, while protein effects had significantly ( $p<0.05$ ) higher values for chicks fed 15%CP levels and a lower, value for chicks fed 17%CP levels when compare with those fed 20%CP levels. The results for monocytes, eosinophils, and basophils were not significant ( $p>0.05$ ) among treatment levels.

With respect to Energy x Protein interaction, haemoglobin concentration (Hb g/dl) was found to be significantly ( $p<0.05$ ) lower for starter chicks fed diets 2600kcal/kg×17CP, and 2600kcal/kg×15CP, when compare with that of control, while that of 2800kcal/kg×17CP, 2800kcal/kg×15CP, 2600kcal/kg×20CP, 2400kcal/kg×20CP, 2400kcal/kg×17CP, and 2400kcal/kg×15CP, were statistically ( $p>0.05$ ) the same with that of the control and other treatment levels. Packed cell volume (PCV) were significant ( $p<0.05$ ) across dietary treatment levels. Red blood cell (RBC) was not significant ( $p>0.05$ ) across treatments. Total white blood cell (TWBC) was significant ( $p<0.05$ ) across treatments level, a significantly ( $p<0.05$ ) low



TWBC were found among chicks fed 2400kcal/kg×17CP, 2600kcal/kg×15CP, 2600kcal/kg×20CP and 2800kcal/kg×15CP, compared with that of the control, while chicks fed 2800kcal/kg×17CP, 2600kcal/kg×17CP, 2400kcal/kg×20CP, and 2400kcal/kg×15CP, were statistically ( $p<0.05$ ) the same with the control. Mean corpuscular volume (MCV) had significantly ( $p<0.05$ ) higher volume for chicks fed 2800kcal/kg×17CP, diet, with ( $p<0.05$ ) lower values for 2600kcal/kg×15CP, and 2400kcal/kg×20CP, compared with that of the control. MCV for 2400kcal/kg×17CP, 2400kcal/kg×15CP, 2600kcal/kg×17CP, 2600kcal/kg×20CP, and 2800kcal/kg×15CP, were ( $p>0.05$ ) across treatments. Mean corpuscular haemoglobin (MCH) had significantly ( $p<0.05$ ) higher value for chicks fed 2400kcal/kg×20CP diets, with the least ( $p<0.05$ ) value found among chicks fed 2600kcal/kg×17CP, and 2600kcal/kg×15CP.. Mean corpuscular haemoglobin count (MCHC) showed significantly ( $p<0.05$ ) higher values for chicks fed 2400kcal/kg×20CP diet, and lower ( $p<0.05$ ) values for 2800kcal/kg×17CP, and 2600kcal/kg×17CP when compare with that of the control. The results also showed statistically ( $P>0.05$ ) similar values for 2400kcal/kg×17CP, and 2400kcal/kg×15CP, with 2600kcal/kg×15CP, and 2800kcal/kg×15CP, diets with that of the control. The results for lymphocytes were significantly ( $p<0.05$ ) higher for chicks fed the experimental diets when compared with that of the control which had significantly ( $p<0.05$ ) lower value. The results for heterophils showed significantly ( $p<0.05$ ) lower values across treatment levels when compared with those of the control, with significantly ( $p<0.05$ ) higher values. The results for monocytes, eosinophils, and basophils were not significant ( $p>0.05$ ) among treatment levels.

Haematological analysis revealed significant differences in haemoglobin concentration, PCV, and TWBC across treatments. Haemoglobin levels at both energy and protein varying composition were lower than the normal range (7.0-13.0g/dl). Except for the control, haemoglobin concentration was below the normal range (7.0-13.0g/dl) as reported by Ross *et al.*(1978) and Mitruka and Rawnsley (1977). The values for PCV were within normal range (25-45%) except for 2600kcal/kg×20CP, and 2600kcal/kg×15CP, interaction (energy×protein), which were below the normal range (Ross *et al.*, 1978 and Mitruka and Rawnsley, 1977). Also, RBC and TWBC at both main effects and interaction (energy×protein), all values recorded were within normal range ( $2.0-4.0\times10^6\text{ mm}^3$  and  $9.0-31.0\times10^3\text{ mm}^3$ ), as reported by Ross *et al.* (1978) and Mitruka and Rawnsley (1977). Levels for MCV for chicks fed 20%CP diets were within normal range, while those from interaction (energy×protein) were below normal range (90.0-140.0fl). MCH and MCHC for both main effects and interaction (energy×protein) were also below normal range (33.0-47.0pg; 26-35g/dl) except for 2400kcal/kg×20CP, as reported by Ross *et al.* (1978) and Mitruka and Rawnsley (1977). For lymphocytes, both main effects and interaction (energy×protein) were all within normal range (45-80%) as reported by Ross *et al.* (1978) and Mitruka and Rawnsley (1977). Birds on high-energy diets had better blood profiles, indicative of improved physiological and immune function. Higher energy and protein diets generally support better blood profiles. Lower protein diets (15% CP) showed reduced values. This is consistent with Boshou *et al.* (2017), who reported that dietary protein influences haematological parameters by supporting erythropoiesis and immune system function. The addition of exogenous enzymes further enhances these effects by improving nutrient bioavailability. Haematological profile of starter pullets

reflect the nutritional adequacy of their diet, influencing growth and overall health. Both energy and protein levels in the diets significantly influenced Hb and PCV values. Starter chicks fed 2800 kcal/kg with 20% CP (control) exhibited higher Hb and PCV values compared to lower energy and protein diets. This finding aligns with studies that highlight adequate energy and protein as essential for optimal erythropoiesis and oxygen transport in poultry (Leeson and Summers, 2009). Neither RBC nor TWBC counts showed significant differences across treatments. This suggests that moderate reductions in energy and protein do not critically impair erythrocyte production or immune cell abundance. This stability could be attributed to the dietary enzyme supplementation mitigating potential deficits (Odetola *et al.*, 2012). Protein levels significantly influenced MCV and MCH, with higher MCV observed at 17% CP. The MCH was highest in chicks fed 2400 kcal/kg energy, suggesting that low energy diets may still support sufficient haemoglobin synthesis when paired with enzyme supplementation (Abudabos *et al.*, 2017). Lymphocyte percentages were higher in lower energy diets, while heterophil percentages declined. This may indicate a stress-related response, as energy deficits can affect immune modulation, increasing lymphocyte production as a compensatory mechanism (Adeyemi *et al.*, 2020).

#### Effects of Low Energy and Low Protein diets with enzyme supplementation on the kidney function test of pullet chicks

The effects of low energy and protein diets with enzyme supplementation on the kidney function test of pullet chickens are presented in Tables 4.0 and 5.0. The effects of energy and protein levels on urea, creatinine, sodium ion, chlorine ion, and hydrogen carbonate showed significantly ( $p<0.05$ ) higher values for chicks fed 2400kcal/kg and 15%cp dietary levels, followed by chicks fed 2600kcal/kg

and 17%cp dietary levels. potassium ion ( $K^+$ ) had significantly ( $p<0.05$ ) low value for chicks fed 2600kcal/kg dietary levels when compared with that of the control, while chicks fed 2400kcal/kg had significantly ( $p<0.05$ ) higher and the same value when compare with that of the control. Protein main effects had significantly ( $p<0.05$ ) higher values for chicks fed 15% CP dietary levels, followed by 17% CP, when compared with those of the control diet.

Urea interaction (energy $\times$ protein) showed significant ( $p<0.05$ ) increased across treatments, with higher ( $p<0.05$ ) urea values found with chicks fed 2400kcal/kg $\times$ 15CP, 2400kcal/kg $\times$ 17CP, 2400kcal/kg $\times$ 20CP, 2600kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 17CP, 2600kcal/kg $\times$ 20CP, 2800kcal/kg $\times$ 15CP, and 2800kcal/kg $\times$ 17CP, when compare with those fed control diets. Creatinine and Sodium ion ( $Na^+$ ) interaction (energy $\times$ protein) had significantly ( $p<0.05$ ) higher values for pullet chicks fed 2400kcal/kg $\times$ 15CP diets, followed by 2400kcal/kg $\times$ 17CP, 2400kcal/kg $\times$ 20CP, 2600kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 17CP, 2600kcal/kg $\times$ 20CP, 2800kcal/kg $\times$ 15CP, and 2800kcal/kg $\times$ 17CP, when compare with that of the control. Chlorine ion ( $Cl^-$ ), hydrogen carbonate ( $HCO_3^-$ ), and potassium ion ( $K^+$ ) interaction (energy $\times$ protein) were observed to show significantly ( $p<0.05$ ) higher values for all experimental treatments when compared with those of the control.

Main effects values and those of the interaction for urea were all below the normal range (4.40-6.38mg/dl), which might have been affected by the quality of the diets consumed (Subayo *et al.*, 2013). Except for 2800kcal/kg ME main effects levels and T1 interaction (energy $\times$ protein), creatinine levels were all above normal range (0.10-0.40mg/dl), Ritchie *et al.* (2013). Main effects and interaction (energy $\times$ protein) for  $Na^+$  were all within normal range (130-150mEq/l), that of  $Cl^-$  were below normal range (114-120mEq/l),

for  $K^+$ , except for 2400kcal/kg, they are all slightly below normal range (4.00-6.00mEq/l), Sturkie (1965). The main effects and interactions (energy $\times$ protein) kidney function tests are critical for assessing the impact of dietary interventions on renal health. Parameters such as urea, creatinine, and electrolyte balance are very important in assessing the health of the animals. Significant increases in urea and creatinine levels were observed with diets containing reduced energy and protein. Chicks fed 2400 kcal/kg and 15% CP exhibited the highest values, suggesting increased protein catabolism or impaired renal clearance under nutrient-limited conditions (Musa *et al.*, 2021). This finding explains the importance of balanced diets to minimize metabolic stress on the kidneys. Electrolyte levels, including  $Na^+$ ,  $Cl^-$ , and  $HCO_3^-$ , were significantly higher in chicks fed diets with lower energy and protein. These changes may reflect compensatory mechanisms to maintain acid-base balance under stress (Karcher *et al.*, 2015). Potassium levels varied significantly, with the highest values in chicks fed 2400 kcal/kg and 15% CP. Elevated  $K^+$  may indicate shifts in cellular ion balance due to energy deficits (Ajakaiye *et al.*, 2011). Highlighting the significant main effects of reduced energy and protein on renal health indicators. Higher levels of urea and creatinine in diets with 2400 kcal/kg and 15% CP suggest heightened nitrogen excretion and potential renal strain. Dietary enzymes might partially mitigate these effects by enhancing nutrient digestibility (Zhao *et al.*, 2020). Electrolyte imbalances were evident, with higher  $Na^+$ ,  $Cl^-$ , and  $HCO_3^-$  levels in chicks fed lower energy and protein diets. Potassium levels, however, followed a similar trend, reflecting cellular adjustments to dietary stress (Ajakaiye *et al.*, 2011). Optimal kidney function requires sufficient dietary energy and protein, with enzyme supplementation playing a supportive role.

### **Effects of Low Energy and Low Protein diets with enzyme supplementation on liver function test of pullet chicks**

The effects of low-energy and low-protein diets with enzyme supplementation on the liver function test of pullet chicks are presented in Tables 6 and 7. Total proteins was found to be significantly ( $p<0.05$ ) lower among pullet chicks fed 2400kcal/kg and 15%cp, followed by 2600kcal/kg and 17%cp dietary levels when compared with those fed 2800kcal/kg and 20%cp dietary levels, respectively. Albumin had a significantly ( $p<0.05$ ) higher value for chicks fed 2400kcal/kg dietary levels, followed by those fed 2600kcal/kg dietary levels when compared with those fed 2800kcal/kg dietary levels. Also, albumin protein main effects had significantly ( $p<0.05$ ) higher values for chicks fed 17% CP, and lower ( $p<0.05$ ) levels for chicks fed 15% dietary levels when compared with those fed 20% CP levels. Main levels effects energy and protein on globulin, AST, ALT, ALP, bilirubin and glucose were found to be significantly ( $p<0.05$ ) different across treatment levels, which were observed to decrease significantly ( $p<0.05$ ) as the dietary energy and crude protein levels decreased.

With respect to energy $\times$ protein interaction total protein was observed to be significantly ( $p<0.05$ ) lower for chicks fed 2400kcal/kg $\times$ 15CP diet, followed by 2400kcal/kg $\times$ 17CP, 2400kcal/kg $\times$ 20CP, 2600kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 20CP, 2600kcal/kg $\times$ 17CP, 2800kcal/kg $\times$ 15CP, and 2800kcal/kg $\times$ 17CP, when compared with that of the control diet. Meanwhile, Albumin had significantly ( $p<0.05$ ) higher value for chicks fed 2400kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 17CP, 2600kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 20CP, 2400kcal/kg $\times$ 20CP, 2400kcal/kg $\times$ 17CP, and 2800kcal/kg $\times$ 17CP, whereas 2800kcal/kg $\times$ 15CP was significantly ( $p<0.05$ ) lower when compare with that of the control. Albumin value interaction (energy $\times$ protein)

also had statistically ( $p < 0.05$ ) the same results for 2400kcal/kg $\times$ 17CP, 2400kcal/kg $\times$ 20CP, and 2600kcal/kg $\times$ 20CP, similar results were also recorded for 2600kcal/kg $\times$ 17CP, and 2600kcal/kg $\times$ 15CP. The results for globulin interaction (energy $\times$ protein) showed a significant ( $p < 0.05$ ) decrease in values across treatments, with the least value observed ( $p < 0.05$ ) among chicks fed 2400kcal/kg $\times$ 15CP diets. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were also found to decrease significantly ( $p < 0.05$ ) across treatments, with the least values found with chicks fed 2400kcal/kg $\times$ 15CP diets respectively. Alanine phosphatase (ALP) was found to significantly ( $p < 0.05$ ) different among chicks fed diets, with lower ( $p < 0.05$ ) ALP observed among chicks fed 2400kcal/kg $\times$ 15CP, 2400kcal/kg $\times$ 17CP, 2400kcal/kg $\times$ 20CP, 2600kcal/kg $\times$ 17CP, 2600kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 20CP, 2800kcal/kg $\times$ 15CP, and 2800kcal/kg $\times$ 17CP, when compared with that of the control diets. The results for bilirubin and glucose were found to decrease significantly ( $p < 0.05$ ) across treatments, with the least value found among pullet chicks fed 2400kcal/kg $\times$ 15CP diets.

The liver's role in metabolism makes its function a key indicator of dietary adequacy. Values of main effects and interaction (energy $\times$ protein) for albumin, total protein and globulin levels were slightly above normal range (2.5-4.5g/dl; 1.15-1.53g/dl), reported by Monika *et al.* (2012) and Murray *et al.* (2009). Glucose range for 2800kcal/kg $\times$ 20CP, 2800kcal/kg $\times$ 17CP, 2800kcal/kg $\times$ 15CP, 2600kcal/kg $\times$ 20CP, and 2600kcal/kg $\times$ 17CP, were within normal range (180-250mg/dl), while those fed 2600 kcal/kg $\times$ 15CP, 2400kcal/kg $\times$ 20CP, 2400kcal/kg $\times$ 17CP, and 2400kcal/kg $\times$ 15CP, were below normal range (180-250mg/dl) (Hazelwood, 2000; Sturkie (1965). This explores total protein, albumin,

and globulin as enzymatic markers for liver function. Total protein and globulin levels declined significantly in chicks fed diets with reduced energy and protein. Conversely, albumin levels were higher in these groups, potentially indicating shifts in protein allocation to maintain plasma oncotic pressure (Ebeid *et al.*, 2012). Enzyme activities (AST, ALT, ALP) decreased significantly with lower energy and protein diets, suggesting reduced hepatic metabolic activity. This may reflect the sparing effect of enzyme supplementation in mitigating liver workload (Omede *et al.*, 2017). Lower bilirubin and glucose levels in nutrient-deficient diets further highlight reduced hepatic function and energy availability. Adequate energy and protein intake are crucial for maintaining liver health and metabolic efficiency. Total protein and globulin levels declined under low energy and protein diets emphasised the role of dietary adequacy in maintaining protein synthesis. Albumin levels increased in lower energy diets, possibly due to compensatory mechanisms (Ebeid *et al.*, 2012). Reduced enzyme activity and glucose levels in low-energy and protein diets highlight the metabolic strain imposed by nutrient deficits. Enzyme supplementation may alleviate some of these effects by improving nutrient bioavailability (Zhao *et al.*, 2020).

### **Effects of Low Energy and Low Protein diets with enzyme supplementation on Lipid profile of pullet chicks**

The effects of Low Energy and Protein diets and their interaction with enzyme supplementation on liver function test of pullet chicks are presented in Tables 8 and 9. Total cholesterol had significantly ( $p < 0.05$ ) lower values for chicks fed 2400kcal/kg and 15%CP, followed by, 2600kcal/kg and 17%CP when compared with those fed 2800kcal/kg and 20%CP. triglyceride also had significantly ( $p < 0.05$ ) low values for chicks fed 2400kcal/kg and 2600kcal/kg dietary energy,



when compared with those fed 2800kcal/kg dietary energy. Protein main levels effects for triglyceride had significantly ( $p<0.05$ ) higher value for chicks fed diets containing 17%CP level, and significantly ( $p<0.05$ ) lower value for chicks fed 15%CP dietary levels when compare with those fed 20%CP dietary levels. Energy and protein effects for high-density-lipo-protein (HDLP) had significantly ( $p<0.05$ ) low values for 2400kcal/kg and 15%CP, followed by 2600kcal/kg and 17%CP dietary levels when compare with chicks fed 2800kcal/kg and 20%CP dietary levels. Low-density-lipo-protein (LDLP) main levels effects had significantly ( $p<0.05$ ) higher values for chicks fed diets containing 2400kcal/kg and 15%CP dietary levels, followed by 2600kcal/kg and 17%CP dietary levels when compare with those fed 2800kcal/kg and 20%CP dietary levels. Main levels effects for energy and protein effects for very-low-density-lipo-protein (VLDLP) had significantly ( $p<0.05$ ) low values for 2400kcal/kg and 15%CP, followed by 2600kcal/kg and 17%CP dietary levels when compare with chicks fed 2800kcal/kg and 20%CP dietary levels.

With regards to energy×protein interaction, total cholesterol had significantly ( $p<0.05$ ) lower value for starter chicks fed 2400 kcal/kg×15CP, followed by 2400 kcal/kg×17CP, 2400 kcal/kg×20CP, 2600 kcal/kg×15CP, 2600 kcal/kg×17CP, 2600 kcal/kg×20CP, 2800 kcal/kg×17CP, and 2800 kcal/kg×15CP, hens compared with the control. Triglyceride was found to be significantly ( $p<0.05$ ) higher for starter pullet chicks fed 2600kcal/kg×17CP diets, while other treatment diets were significantly ( $p<0.05$ ) lower, with the least value found among chicks fed 2400 kcal/kg×15CP diet when compared with that of the control. High density lipo-protein (HDLP) had significantly ( $p<0.05$ ) lower value for chicks fed 2400kcal/kg×15CP, followed by those fed

2400kcal/kg×17CP, 2400kcal/kg×20CP, 2600kcal/kg×15CP, 2600kcal/kg×17CP, 2600kcal/kg×20CP, 2800kcal/kg×17CP, and 2800kcal/kg×15CP respectively, while the highest value was recorded among the control. Low-density lipo-protein was found to be significantly ( $p<0.05$ ) higher among starter chicks fed 2400kcal/kg×15CP diets, followed by 2400kcal/kg×20CP, 2600kcal/kg×15CP, 2600kcal/kg×17CP, 2400kcal/kg×17CP, 2800kcal/kg×15CP, 2600kcal/kg×20CP, and 2800kcal/kg×17CP when compare with those fed control diet.

Total cholesterol values for chicks fed diets with 2800kcal/kg, 2800kcal/kg×20CP, 2800kcal/kg×17CP, and 2800kcal/kg×15CP within normal range and other treatments below (129-297mg/dl). triglycerides normal range is 150mg/dl of which all parameters were found to be below and which it is considered normal (Simaraks *et al.*, 2004). High density lipoprotein (HDLP), which is considered good cholesterol with higher levels being preferable, was found to be below the normal range (77.00-87.10mg/dl) for Energy and protein levels and their interactions as reported by Ross (1978). Low-density lipoprotein (LDLP) is considered bad cholesterol, with lower levels considered better, and was found to be within normal range (50-82mg/dl) across treatments (Hariyanto, 2017). All parameters for both main effects and interaction (energy×protein) for very low-density lipoprotein (VLDLP) were found to be below the normal range (23.77-32.47mg/dl), (Simaraks *et al.*, 2004). The interactions (energy×protein) and main effects for lipid profile reflect the energy status and metabolic health of poultry. Chicks fed 2400 kcal/kg and 15% CP exhibited the lowest cholesterol levels, while triglyceride levels varied, peaking in the 17% CP group. These findings align with the role of dietary energy and protein in modulating lipid metabolism (Panda *et al.*, 2014). HDL levels decreased,

and LDL levels increased with lower energy and protein diets, indicating a shift towards less favourable lipid profiles. Enzyme supplementation may partially offset these changes by enhancing lipid utilisation (Abudabos *et al.*, 2017). Reduced energy and protein diets for main effects led to lower HDL and higher LDL levels, consistent with impaired lipid metabolism. Triglyceride levels were lowest in the 15% CP group, reflecting reduced energy availability (Agida, *et al.*, 2019; Panda *et al.*, 2014). Thus, optimizing dietary energy and protein is critical for maintaining favourable lipid profiles and overall metabolic health in starter pullets.

## Conclusion

Blood haematological and serum biochemical indice were affected by dietary levels of Energy and Protein and their interaction. Values for haemtological parameters observed in the study showed that low-energy and low-crude protein did not have any adverse effects on the pullet chicks. Kidney function tests howed the pullet chicks to have increased urea, and creatinine levels. Also, the value for electrolytes levels were higher for pullet chicks fed low-energy and low-crude protein. The value for liver function test and lipid profile levels, except for albumin and low-density lipoprotein were found to decrease across treatments. However, the results did not show any serious health challenges among pullet chicks.

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Table 1: Percentage composition of ingredients and nutrients of the experimental diets.

Ingredients	2800 kcal/20%Cp	2800 kcal/17%Cp	2800 kcal/15%Cp	2600 kcal/20%Cp	2600 kcal/17%Cp	2600 kcal/15%Cp	2400 kcal/20%Cp	2400 kcal/17%Cp	2400 kcal/15%Cp
Maize	49.02	52.07	59.00	35.67	43.54	47.31	30.32	29.39	33.44
Soybean meal	29.28	19.27	14.33	28.17	19.36	14.11	28.10	18.59	12.98
Wheat offal	6.00	9.19	11.24	19.20	20.49	25.47	25.83	35.93	39.26
Palm kernel cake	12.00	15.77	11.73	13.26	12.91	9.41	12.05	12.39	10.62
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Bonemeal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin/Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Enzyme (20g)</b>	+	+	+	+	+	+	+	+	+
<b>Calculated</b>									
ME (kcal/kg)	2800.036	2800.300	2800.001	2603.660	2600.010	2601.17	2400.780	2400.75	2404.275
Crude Protein (%)	20.031	17.040	15.002	20.019	17.012	15.001	20.029	17.112	15.195
Ether extract (%)	4.763	4.851	4.889	5.701	5.907	5.956	6.793	6.845	6.896
Crude fibre (%)	5.400	5.857	6.157	8.973	9.667	9.914	12.824	13.121	13.362
Calcium (%)	1.079	1.074	1.071	1.126	1.125	1.123	1.177	1.283	1.307
Phosphorus (%)	0.372	0.363	0.361	0.358	0.361	0.330	0.383	0.357	0.361
Lysine (%)	0.817	0.731	0.639	0.878	0.771	0.647	0.877	0.752	0.681
Methionine (%)	0.299	0.280	0.287	0.354	0.334	0.328	0.406	0.384	0.370

\*Vitamin mineral premix contains per 1kg: Vitamin A 15,000 IU, Vitamin D3 13,000 IU, Thiamine 2mg, Biotin 0.08mg, Riboflavin 6mg, Pyridoxine 4mg, Niacin 40mg, Cobalamine 0.05g, Choline chloride 0.05g, Manganese 0.09g, Zinc 0.06g, Iron 0.024g, Copper 0.006g, Iodine 0.014g, Selenium 0.24mg, Cobalt 0.024mg and Antioxidant 0.125g.

Table 2. Effect of low Energy and Protein diets with enzyme supplementation on Haematological parameters of pullet chicks

	Energy			SEM	P value	Protein			
	2800	2600	2400			20	17	15	P value
Hb (g/dl)	6.57 <sup>a</sup>	5.57 <sup>b</sup>	6.08 <sup>ab</sup>	0.236	0.020	6.78 <sup>a</sup>	5.47 <sup>b</sup>	5.97 <sup>ab</sup>	0.002
PCV (%)	31.33 <sup>a</sup>	26.23 <sup>b</sup>	27.13 <sup>b</sup>	0.722	<0.0001	26.63 <sup>b</sup>	30.33 <sup>a</sup>	27.73 <sup>b</sup>	0.004
RBC (×10 <sup>6</sup> mm <sup>3</sup> )	3.00	3.61	3.02	0.192	0.042	3.13	3.30	3.16	0.787
TWBC (×10 <sup>3</sup> mm <sup>3</sup> )	27.11	16.75	20.73	3.47	0.122	27.53	17.05	20.01	0.107
MCV (fl)	51.98	46.19	45.38	2.38	0.120	43.93 <sup>b</sup>	53.60 <sup>a</sup>	46.02 <sup>ab</sup>	0.019
MCH pg	22.02 <sup>a</sup>	15.45 <sup>b</sup>	22.92 <sup>a</sup>	1.53	0.003	22.91 <sup>a</sup>	16.73 <sup>b</sup>	20.76 <sup>ab</sup>	0.026
MCHC (g/dl)	20.43	22.01	22.78	0.966	0.233	25.37 <sup>a</sup>	18.17 <sup>c</sup>	21.69 <sup>b</sup>	<0.0001
Lymphocytes (%)	71.67 <sup>b</sup>	84.00 <sup>a</sup>	87.00 <sup>a</sup>	1.60	<0.0001	79.50 <sup>ab</sup>	84.67 <sup>a</sup>	78.50 <sup>b</sup>	0.024
Heterophils (%)	28.33 <sup>a</sup>	15.83 <sup>b</sup>	13.00 <sup>c</sup>	1.61	<0.0001	20.33 <sup>ab</sup>	15.33 <sup>b</sup>	21.50 <sup>a</sup>	0.027
Monocytes (%)	0.00	0.00	0.00	*	*	0.00	0.00	0.00	*
Eosinophils (%)	0.00	0.00	0.00	*	*	0.00	0.00	0.00	*
Basophils (%)	0.00	0.00	0.00	*	*	0.00	0.00	0.00	*

<sup>abcde</sup> Means in a subset in a row with different superscripts are significantly ( $p < 0.05$ ) different. Hb=Hemaglobin; PCV=Pack cell volume; RBC=Red blood cell; TWBC=Total white blood cell count, MCV=Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin count



**Table 3. Effect of Energy and Protein interaction diets with enzyme supplementation on haematological parameters of pullet chicks**

Energy (kcal/kg)	2800			2600			2400			SEM	P-Value
Protein (%)	20	17	15	20	17	15	20	17	15		
Hb (g/dl)	7.40 <sup>a</sup>	5.60 <sup>ab</sup>	6.70 <sup>ab</sup>	6.10 <sup>ab</sup>	5.30 <sup>b</sup>	5.30 <sup>b</sup>	6.85 <sup>ab</sup>	5.50 <sup>ab</sup>	5.90 <sup>ab</sup>	0.408	0.681
PCV (%)	31.00 <sup>a</sup>	33.00 <sup>a</sup>	30.00 <sup>ab</sup>	24.50 <sup>bc</sup>	30.50 <sup>a</sup>	23.70 <sup>c</sup>	24.40 <sup>bc</sup>	27.50 <sup>abc</sup>	29.50 <sup>abc</sup>	1.25	0.014
RBC (×10 <sup>6</sup> mm <sup>3</sup> )	3.44	3.00	2.45	3.56	3.66	3.63	2.38	3.28	3.41	0.333	0.074
TWBC (×10 <sup>3</sup> mm <sup>3</sup> )	28.32 <sup>a</sup>	18.32 <sup>ab</sup>	17.20 <sup>b</sup>	14.00 <sup>b</sup>	19.28 <sup>ab</sup>	16.96 <sup>b</sup>	22.76 <sup>ab</sup>	13.56 <sup>b</sup>	25.88 <sup>ab</sup>	6.00	0.032
MCV (fl)	47.92 <sup>ab</sup>	60.42 <sup>a</sup>	47.61 <sup>ab</sup>	48.13 <sup>ab</sup>	50.04 <sup>ab</sup>	40.42 <sup>b</sup>	35.76 <sup>b</sup>	50.35 <sup>ab</sup>	50.03 <sup>ab</sup>	4.12	0.108
MCH pg	19.35 <sup>bc</sup>	18.80 <sup>bc</sup>	27.92 <sup>ab</sup>	17.39 <sup>bc</sup>	14.48 <sup>c</sup>	14.49 <sup>c</sup>	31.99 <sup>a</sup>	16.90 <sup>bc</sup>	19.88 <sup>abc</sup>	2.65	0.006
MCHC (g/dl)	21.91 <sup>abc</sup>	17.06 <sup>c</sup>	22.33 <sup>abc</sup>	25.85 <sup>ab</sup>	17.47 <sup>c</sup>	22.73 <sup>abc</sup>	28.37 <sup>a</sup>	19.97 <sup>bc</sup>	20.01 <sup>bc</sup>	1.67	0.131
Lymphocytes (%)	65.00 <sup>c</sup>	82.00 <sup>a</sup>	68.00 <sup>bc</sup>	89.50 <sup>a</sup>	80.00 <sup>ab</sup>	82.50 <sup>a</sup>	84.00 <sup>a</sup>	92.00 <sup>a</sup>	85.00 <sup>a</sup>	2.77	0.001
Heterophils (%)	35.00 <sup>a</sup>	18.00 <sup>c</sup>	32.00 <sup>ab</sup>	10.00 <sup>c</sup>	20.00 <sup>bc</sup>	17.50 <sup>c</sup>	16.00 <sup>c</sup>	8.00 <sup>c</sup>	15.00 <sup>c</sup>	2.79	0.001
Monocytes (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*	*
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*	*
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*	*

<sup>abcdef</sup> Means in a subset on a row with different superscripts are significantly different. Hb=Hemoglobin; PCV=Pack cell volume; RBC=Red blood cell; TWBC=Total white blood cell count, MCV=Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin count

**Table 4. Effect of low Energy and Protein diets with enzyme supplementation on kidney function test of Pullet chicks**

	Energy					Protein				
	2800	2600	2400	SEM	P value	20	17	15	SEM	P value
Urea (mg/dl)	3.63 <sup>c</sup>	3.97 <sup>b</sup>	4.22 <sup>a</sup>	0.00282	<0.0001	3.83 <sup>c</sup>	3.94 <sup>b</sup>	4.05 <sup>a</sup>	0.00282	<0.0001
Creatinine (mg/dl)	0.39 <sup>c</sup>	0.50 <sup>b</sup>	0.57 <sup>a</sup>	0.00245	<0.0001	0.45 <sup>c</sup>	0.50 <sup>b</sup>	0.52 <sup>a</sup>	0.00245	<0.0001
Na <sup>+</sup> (mEq/l)	135.28 <sup>c</sup>	135.99 <sup>b</sup>	137.50 <sup>a</sup>	0.019	<0.0001	135.70 <sup>c</sup>	136.29 <sup>b</sup>	136.79 <sup>a</sup>	0.019	<0.0001
Cl <sup>-</sup> (mEq/l)	99.46 <sup>c</sup>	100.73 <sup>b</sup>	102.56 <sup>a</sup>	0.017	<0.0001	100.13 <sup>c</sup>	101.12 <sup>b</sup>	101.50 <sup>a</sup>	0.017	<0.0001
HCO <sub>3</sub> <sup>-</sup> (mmol/l)	19.90 <sup>c</sup>	20.50 <sup>b</sup>	21.33 <sup>a</sup>	0.0137	<0.0001	20.37 <sup>c</sup>	20.46 <sup>b</sup>	20.90 <sup>a</sup>	0.0137	<0.0001
K <sup>+</sup> (mEq/l)	3.43 <sup>a</sup>	3.85 <sup>b</sup>	4.18 <sup>a</sup>	0.00222	<0.0001	3.68 <sup>c</sup>	3.86 <sup>b</sup>	3.92 <sup>a</sup>	0.00222	<0.0001

<sup>abcdef</sup> Means in a subset on a row with different superscripts are significantly ( $p < 0.05$ ) different. Na<sup>+</sup>=Sodium; Cl<sup>-</sup>=Chlorine; HCO<sub>3</sub><sup>-</sup>=Hydrogen carbonate; K=Potassium

**Table 5. Interaction between low Energy and low Protein diets with enzyme supplementation on kidney function test of Pullet chicks**

Energy (kcal/kg)	2800			2600			2400			SEM	P-Value
Protein (%)	20	17	15	20	17	15	20	17	15		
Urea (mg/dl)	3.42 <sup>i</sup>	3.61 <sup>h</sup>	3.85 <sup>g</sup>	3.92 <sup>f</sup>	3.98 <sup>e</sup>	4.01 <sup>d</sup>	4.15 <sup>c</sup>	4.22 <sup>b</sup>	4.30 <sup>a</sup>	0.00488	<0.0001
Creatinine (mg/dl)	0.32 <sup>g</sup>	0.42 <sup>f</sup>	0.43 <sup>f</sup>	0.47 <sup>e</sup>	0.50 <sup>d</sup>	0.53 <sup>c</sup>	0.55 <sup>b</sup>	0.57 <sup>ab</sup>	0.59 <sup>a</sup>	0.00425	<0.0001
Na <sup>+</sup> (mEq/l)	135.00 <sup>g</sup>	135.35 <sup>f</sup>	135.50 <sup>f</sup>	135.70 <sup>e</sup>	136.01 <sup>d</sup>	136.28 <sup>c</sup>	136.40 <sup>c</sup>	137.50 <sup>b</sup>	138.00 <sup>a</sup>	0.033	<0.0001
Cl <sup>-</sup> (mEq/l)	99.20 <sup>h</sup>	99.49 <sup>g</sup>	99.70 <sup>f</sup>	99.90 <sup>e</sup>	101.20 <sup>cd</sup>	101.10 <sup>d</sup>	101.30 <sup>c</sup>	102.68 <sup>b</sup>	103.70 <sup>a</sup>	0.029	<0.0001
HCO <sub>3</sub> <sup>-</sup> (mmol/l)	19.70 <sup>i</sup>	19.89 <sup>h</sup>	20.10 <sup>g</sup>	20.50 <sup>e</sup>	20.30 <sup>f</sup>	20.70 <sup>d</sup>	20.90 <sup>c</sup>	21.20 <sup>b</sup>	21.90 <sup>a</sup>	0.0238	<0.0001
K <sup>+</sup> (mEq/l)	3.39 <sup>i</sup>	3.43 <sup>h</sup>	3.47 <sup>g</sup>	3.52 <sup>f</sup>	3.97 <sup>e</sup>	4.07 <sup>d</sup>	4.13 <sup>c</sup>	4.18 <sup>b</sup>	4.23 <sup>a</sup>	0.00222	<0.0001

<sup>abcdeghij</sup> Means in a subset on a row with different are significantly different. Na<sup>+</sup>=Sodium; Cl<sup>-</sup>=Chlorine; HCO<sub>3</sub><sup>-</sup>=Hydrogen carbonate; K=Potassium

**Table 6. effects of Low Energy and Protein diets with enzyme supplementation on liver function test of pullet chicks**

	Energy					Protein				
	2800	2600	2400	SEM	P value	20	17	15	SEM	P value
Total protein (g/dl)	5.65 <sup>a</sup>	5.55 <sup>b</sup>	5.41 <sup>c</sup>	0.00591	<0.0001	5.58 <sup>a</sup>	5.54 <sup>b</sup>	5.50 <sup>c</sup>	0.00591	<0.0001
Albumin (g/dl)	2.91 <sup>c</sup>	3.30 <sup>b</sup>	3.31 <sup>a</sup>	0.00176	<0.0001	3.25 <sup>b</sup>	3.28 <sup>a</sup>	3.00 <sup>c</sup>	0.00176	<0.0001
Globulin (g/dl)	2.41 <sup>a</sup>	2.25 <sup>b</sup>	2.10 <sup>c</sup>	0.00123	<0.0001	2.32 <sup>a</sup>	2.26 <sup>b</sup>	2.18 <sup>c</sup>	0.00123	<0.0001
AST (u/l)	76.60 <sup>a</sup>	66.07 <sup>b</sup>	58.61 <sup>c</sup>	0.0287	<0.0001	70.30 <sup>a</sup>	67.11 <sup>b</sup>	63.87 <sup>c</sup>	0.0287	<0.0001
ALT (u/l)	46.67 <sup>a</sup>	39.30 <sup>b</sup>	34.36 <sup>c</sup>	0.0193	<0.0001	42.52 <sup>a</sup>	40.08 <sup>b</sup>	37.73 <sup>c</sup>	0.0193	<0.0001
ALP (u/l)	35.43 <sup>a</sup>	29.43 <sup>b</sup>	25.37 <sup>c</sup>	0.0255	<0.0001	31.90 <sup>a</sup>	29.60 <sup>b</sup>	28.73 <sup>c</sup>	0.0255	<0.0001
Bilirubin (mg/dl)	4.61 <sup>a</sup>	4.22 <sup>b</sup>	4.12 <sup>c</sup>	0.00232	<0.0001	4.47 <sup>a</sup>	4.28 <sup>b</sup>	4.20 <sup>c</sup>	0.00232	<0.0001
Glucose (mg/dl)	189.67 <sup>a</sup>	181.00 <sup>b</sup>	175.67 <sup>c</sup>	0.192	<0.0001	184.67 <sup>a</sup>	181.67 <sup>b</sup>	179.00 <sup>c</sup>	0.192	<0.0001

<sup>abcde</sup> Means in a subset on a row with different superscripts are significantly different. AST=Aspartate Amino Transferase; ALT=Alanine amino transferase; ALP= Alkaline phosphatase

**Table 7. Interaction between Low Energy and Low Protein diets with enzyme supplementation on liver function test of pullet chicks**

Energy (kcal/kg)	2800			2600			2400			SEM	P-Value
Protein (%)	20	17	15	20	17	15	20	17	15		
Total protein (g/dl)	5.71 <sup>a</sup>	5.65 <sup>b</sup>	5.60 <sup>c</sup>	5.56 <sup>cd</sup>	5.57 <sup>cd</sup>	5.52 <sup>d</sup>	5.46 <sup>e</sup>	5.40 <sup>f</sup>	5.38 <sup>f</sup>	0.0102	0.002
Albumin (g/dl)	3.20 <sup>e</sup>	3.25 <sup>d</sup>	2.27 <sup>f</sup>	3.28 <sup>c</sup>	3.30 <sup>b</sup>	3.31 <sup>b</sup>	3.28 <sup>c</sup>	3.28 <sup>c</sup>	3.37 <sup>a</sup>	0.00304	<0.0001
Globulin (g/dl)	2.51 <sup>a</sup>	2.40 <sup>b</sup>	2.33 <sup>bc</sup>	2.28 <sup>cd</sup>	2.27 <sup>cd</sup>	2.21 <sup>de</sup>	2.18 <sup>de</sup>	2.12 <sup>e</sup>	2.01 <sup>f</sup>	0.0214	0.050
AST (u/l)	82.10 <sup>a</sup>	75.50 <sup>b</sup>	72.20 <sup>c</sup>	68.70 <sup>d</sup>	66.30 <sup>e</sup>	63.20 <sup>f</sup>	60.10 <sup>g</sup>	59.53 <sup>h</sup>	56.20 <sup>i</sup>	0.0498	<0.0001
ALT (u/l)	50.53 <sup>a</sup>	45.90 <sup>b</sup>	43.60 <sup>c</sup>	40.70 <sup>d</sup>	39.50 <sup>e</sup>	37.70 <sup>f</sup>	36.33 <sup>g</sup>	34.85 <sup>h</sup>	31.90 <sup>i</sup>	0.0085	<0.0001
ALP (u/l)	38.20 <sup>a</sup>	35.60 <sup>b</sup>	32.50 <sup>c</sup>	30.78 <sup>d</sup>	27.90 <sup>f</sup>	29.60 <sup>e</sup>	26.70 <sup>g</sup>	25.30 <sup>h</sup>	24.10 <sup>i</sup>	0.0441	<0.0001
Bilirubin (mg/dl)	4.98 <sup>a</sup>	4.52 <sup>b</sup>	4.33 <sup>c</sup>	4.28 <sup>d</sup>	4.23 <sup>e</sup>	4.15 <sup>f</sup>	4.14 <sup>f</sup>	4.12 <sup>g</sup>	4.104 <sup>h</sup>	0.00403	<0.0001
Glucose (mg/dl)	195.00 <sup>a</sup>	189.00 <sup>b</sup>	185.00 <sup>c</sup>	183.00 <sup>d</sup>	181.00 <sup>e</sup>	179.00 <sup>f</sup>	176.00 <sup>g</sup>	175.00 <sup>g</sup>	173.00 <sup>h</sup>	0.333	<0.0001

<sup>abcde</sup> Means in a subset on a row with different superscripts are significantly different. AST=Aspartate Amino Transferase; ALT=Alanine amino transferase; ALP= Alkaline phosphatase

**Table 8 Effects of Low Energy and Protein diets with enzyme supplementation on lipid profile of pullet chicks**

	Energy			SEM	P value	Protein			SEM	P value
	2800	2600	2400			20	17	15		
Total Cholesterol (mg/dl)	133.00 <sup>a</sup>	126.22 <sup>b</sup>	117.57 <sup>c</sup>	0.040	<0.0001	127.81 <sup>a</sup>	125.11 <sup>b</sup>	123.87 <sup>c</sup>	0.040	<0.0001
Triglyceride (mg/dl)	55.50 <sup>a</sup>	51.33 <sup>b</sup>	33.23 <sup>c</sup>	0.0266	0.377	49.07 <sup>b</sup>	52.32 <sup>a</sup>	38.68 <sup>c</sup>	0.0266	<0.0001
HDLP (mg/dl)	61.10 <sup>a</sup>	48.10 <sup>b</sup>	37.56 <sup>c</sup>	0.381	<0.0001	54.95 <sup>a</sup>	46.94 <sup>b</sup>	44.87 <sup>c</sup>	0.381	<0.0001
LDLP (mg/dl)	60.45 <sup>c</sup>	69.10 <sup>b</sup>	73.32 <sup>a</sup>	0.0104	<0.0001	62.93 <sup>c</sup>	69.00 <sup>b</sup>	70.96 <sup>a</sup>	0.0104	<0.0001
VLDLP (mg/dl)	11.10 <sup>a</sup>	8.29 <sup>b</sup>	6.64 <sup>c</sup>	0.0104	<0.0001	9.79 <sup>a</sup>	8.50 <sup>b</sup>	7.73 <sup>c</sup>	0.0104	<0.0001

<sup>abcdef</sup> Means in a subset on a row with different superscripts are significantly different. HDLP= High density lipoprotein; LDLP=Low density lipoprotein; VLDLP=Very low-density lipoprotein

**Table 9: Interaction between Low Energy and Protein diets with enzyme supplementation on lipid profile of pullet chicks**

Energy (kcal/kg)	2800			2600			2400			SEM	P-Value
Protein (%)	20	17	15	20	17	15	20	17	15		
Total Cholesterol (mg/dl)	135.18 <sup>a</sup>	130.33 <sup>c</sup>	133.50 <sup>b</sup>	128.75 <sup>d</sup>	127.60 <sup>e</sup>	122.30 <sup>f</sup>	119.50 <sup>g</sup>	117.41 <sup>h</sup>	115.80 <sup>i</sup>	0.069	<0.0001
Triglyceride (mg/dl)	65.73 <sup>b</sup>	52.55 <sup>c</sup>	48.23 <sup>d</sup>	45.80 <sup>e</sup>	70.60 <sup>a</sup>	37.60 <sup>f</sup>	35.70 <sup>g</sup>	33.80 <sup>h</sup>	30.20 <sup>i</sup>	0.0460	<0.0001
HDLP (mg/dl)	70.17 <sup>a</sup>	56.50 <sup>b</sup>	56.63 <sup>b</sup>	54.39 <sup>b</sup>	46.82 <sup>c</sup>	43.10 <sup>d</sup>	40.28 <sup>de</sup>	37.50 <sup>f</sup>	34.89 <sup>f</sup>	0.660	<0.0001
LDLP (mg/dl)	51.80 <sup>i</sup>	63.26 <sup>h</sup>	66.29 <sup>f</sup>	65.00 <sup>g</sup>	70.60 <sup>d</sup>	71.70 <sup>c</sup>	71.99 <sup>b</sup>	62.71 <sup>e</sup>	73.07 <sup>a</sup>	0.0512	<0.0001
VLDLP (mg/dl)	13.09 <sup>a</sup>	10.57 <sup>b</sup>	9.63 <sup>c</sup>	9.16 <sup>d</sup>	8.18 <sup>e</sup>	7.52 <sup>f</sup>	7.14 <sup>g</sup>	6.76 <sup>h</sup>	6.03 <sup>i</sup>	0.0180	<0.0001

<sup>abcdefghi</sup> Means in a subset on a row with different are significantly different. HDLP= High density lipoprotein; LDLP=Low density lipoprotein; VLDLP=Very low-density lipoprotein