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HAEMATOLOGICAL AND SEROLOGICAL INDICES OF BROILER FINISHER CHICKENS FED DIETS WITH PALM OIL MILL EFFLUENT AS REPLACEMENT FOR MAIZE

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ABSTRACT
This study was conducted to assess the effects of substituting maize in a broiler finisher diet with energy from palm oil mill effluent (POME) using 225 marshal strain day old broiler chickens allotted to 5 experimental treatments, each replicated three times with 15 birds per replicate in a Completely Randomized Design experiment. The diets were formulated to meet the nutrients requirements of broiler finisher. Five (5) diets were formulated containing 0%, 6%, 12%, 18% and 24% level of inclusion of POME representing diet 1, 2, 3, 4 and 5 respectively. The feeding trial lasted for 8 weeks in which water and experimental diets were offered ad-libitum. Routine management practices were strictly adhered to. At the end of the 56 days feeding trial, blood samples for haematological and serum biochemistry were collected from five birds per replicate through the jugular vein. Results show that haematological indices were significantly (P<0.05) influenced by dietary treatment, and haemoglobin concentration was enhanced (P<0.05) by increased POME inclusion. The serum glucose content of the broiler chickens increased with increasing levels of POME in the diets (P<0.05), from 176.00g/dl in the control to 205.40g/dl. These results indicate that increasing level of POME in marshal strain broiler chicken diets enhanced blood haemoglobin concentration and increased glucose supply for the birds to meet the animal's metabolizable energy needs. Therefore, 12% POME replacement level for maize is considered the best in broiler finisher's diet.

Keywords: Palm oil mill effluent, broiler chickens, haematology, and serological indices

Introduction

The search for alternative feedstuffs for animal production has continued to challenge animal nutritionists in Nigeria for the past decades. The search is necessitated by high cost of the conventional feeds which are in high demand for both human and industries (Akinmutimi, 2004). Anigbogu and Ibe (2005) stated that scarcity of feed for farm animals has been a problem hindering animal husbandry in South-eastern Nigeria. There has been awareness to use agro-industrial by-products either to supplement or replace convectional grainsespecially maize in livestock rations (Ukachukwu *et al.,* 2003). In Nigeria, over dependence on crude oil with serious plan and economic will by the government to diversify its economy to invest in agriculture, has resulted in negligence and poor utilization of the enormous potential benefits inherent in the agro-industrial by-product readily available, with palm oil mill effluent (POME) as one of such.There is an enormous waste being generated from palm oil mill industry which can be converted into value-added products. POME is biodegradable, however, because of its acidic nature and very high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and heavy metals, it is necessary that it would be treated before it is discharged (Er *et al.,* 2011).

Various ways have been adopted to guarantee food security for people worldwide. This has led to the resurgence of interest in the sourcing of inexpensive alternative feed ingredients as replacement for the more expensive convectional ones in animal feed formulation. The importance of this is to increase the availability and affordability of animal products in order to alleviate global food crisis. The alternative feed ingredients also referred to as non-convectional feedstuff are mostly agro-industrial by-products. These "wastes" is best appreciated in meeting the energy and protein needs of farm animals in other to

reduce the feeding costs of farm animals (Fetuga, 1977; FAO, 2008; Onibi *et al.*, 2011). According to Kamyab et al., (2016), the raw or partially treated POME has an extremely high content of degradable organic matter.

Goh and Rajion (2007) suggested the use of indigenous feed resources as a major strategy to reduce cost of imported feed in developing countries as a panacea to develop livestock industry. In the Niger Delta and South eastern part of Nigeria, where oil palm production is common, there is a tendency of collecting a substantial quantity that are mostly discarded as waste, to meet its required demand for livestock industry. Hence meeting the consumer expectations for meat quality deserve due consideration for palm oil sludge (a solid suspension derived from the liquid effluent dietary incorporation in broiler diets). Proper accessibility of POME through broad and global acceptance research, requires due consideration because POME constitute environmental hazard. Necessary steps need to be applied for total collection during palm oil processing to reduce negative impacts and ameliorate the shortages in livestock feed supply. Thus this study was aimed at assessing the serum biochemical and haematological indices of broiler chickens fed diets containing palm oil mill effluent.

Materials and Methods

Experimental Site and Materials Collection

The experiment was carried out at the Poultry unit, Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The farm is located within latitude 05^0 29^1 North and longitude 07^0 32^1 East. The farm lies in the altitude of 122m and within the rainforest zone of south-east Nigeria, which is bimodal rainfall pattern and total annual rainfall of 2177mm, maximum ambient temperature range of 22 to 36^0 C during the hot dry season of the year (November-March) and minimum ambient temperature range of 20to 26^0 C during the cold rainy season (April-October). The relative humidity ranges from 50-90% and is located in warm humid tropics (NRCRI, 2017).

Procurement/processing of POME

The POME used for the experiment was collected from Ishiborr Community in Ogoja Local Government Council Area of Cross River State, Nigeria. It was locally processed based on traditional method of oil palm processing. The POME was packed in a sack bag and allowed to stay for 24hours to enable the water to drain out, leaving the sludge which was later air dried for 21 days. This was then stored for chemical analysis and feed formulation. The dried palm oil effluent was used in formulating the experimental diets.

Processing of Palm Oil and Collection of Palm Oil Mill Effluent (POME)

Collection of POME was from a local/indigenous processing method in Ogoja.

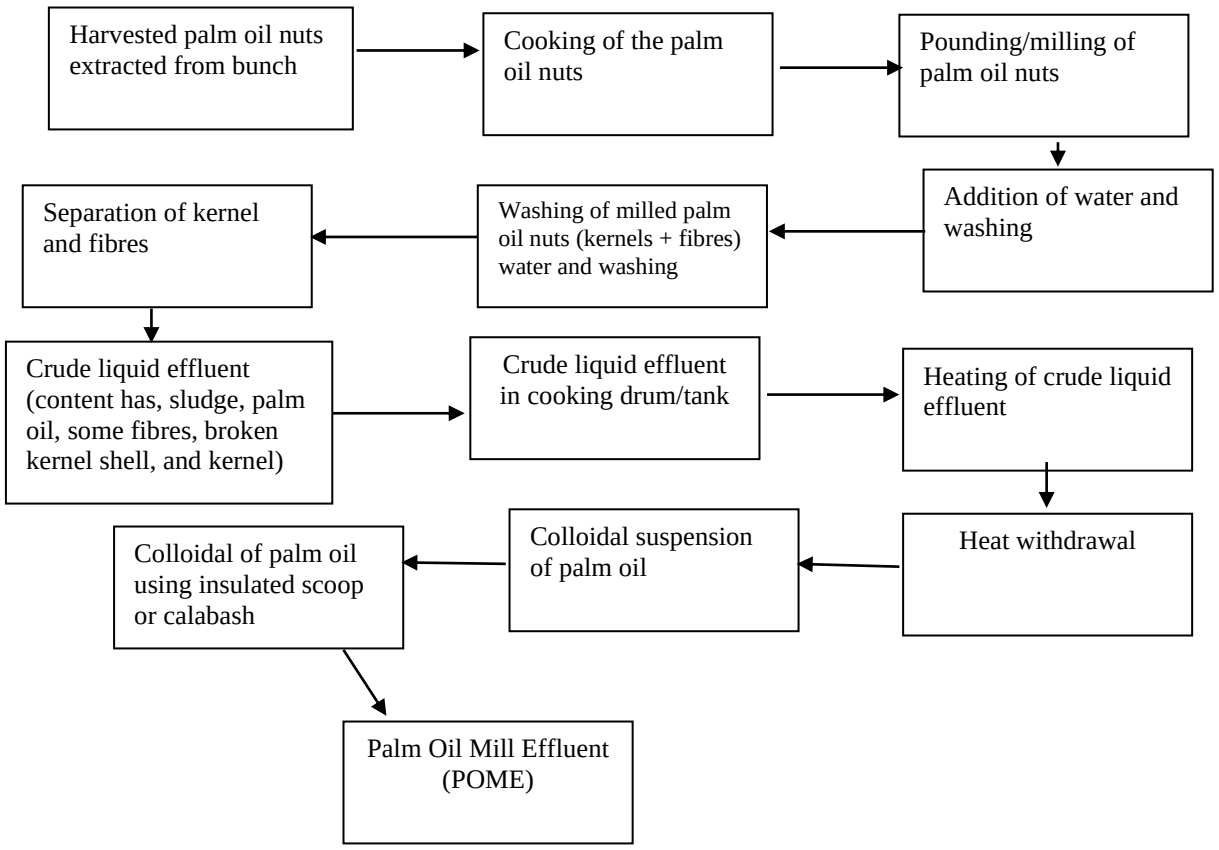

Figure 1: Flow Chart for POME Collection

The palm oil mill effluent was then packed in a bag to drain out the water leaving behind the slurry/sludge.

Experiment Diets

Five experimental diets (D1, D2, D3, D4 and D5) were formulated with POME included at 0%, 6%, 12%, 18% and 24% respectively to meet the nutrient requirements of the broiler finisher. The nutrient compositions of the experimental diets are shown in Table 1.

Experimental Layout/Experimental animals and Management

The experimental design was completely randomized and the birds were reared on deep litter using wood shavings. Two hundred and twenty five day old chicks (Marshal breed/strain) were allotted randomly to five experimental diets and replicated 3 times with 15 birds per replicate. The experiment lasted for 8weeks. The birds were placed on the starter diets for four weeks and thereafter on finisher diets for four weeks. Feed and water were given *ad-libitum*, electric bulb, stoves and lantern were used in providing illumination and heat for the brooding stage. Routine drugs and vaccine administration and hygienic conditions, were followed and maintained.

Table 1: Composition of experimental diets

Diets	1	2	3	4	5
Ingredient (%)	**0**	**6.0**	**12.0**	**18.0**	**24.0**
Maize	57.9	51.9	45.9	39.9	33.9
Soya bean meal	35.6	35.6	35.6	35.6	3.56
POME	-	6.0	12.0	18.0	24.0
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	1.00	1.00	1.00	1.00	1.00
Vitamin/mineral premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total (kg)	**100**	**100**	**100**	**100**	**100**
Crude Protein (%)	19.518	19.639	19.758	19.878	19.999
ME (kcal/kg)	2955.6	3142.8	3330.0	3517.2	3704.4
Ether Extract (%)	3.46	3.84	4.55	4.58	4.93
Crude Fibre (%)	2.92	4.02	5.18	6.20	7.23

Vitamin-mineral premix supplied (per kg of diet); Vitamin A, 1500Iu, Vitamin D3 1600Iu, Riboflavin 9.0g, Biotin 0.25mg, Partothenix acid 6.0mg, Vitamin B2 2.5mg, Vitamin B12 8.0mg, Nicotinic acid 8.0mg Iron 5.0mg, Manganese 10mg, Zinc 4.5mg, Cobalt 0.02mg, Selenium 0.01mg.

Sample collection/Laboratory analysis

At the end of the 56 days feeding trial, five birds per replicate (15 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 aceticacid; EDTA) for haematology as described by Lamb (1981). Pack cell volume was determined by the micro-haematocrit method as described by Coles (1986). The haemoglobin concentration (Hb) of the blood samples were determined by the cyano-methaemoglobin method as described by Kachmar (1970). Red blood cell (RBC) and white blood cell (WBC) counts weredetermined by the haemo-cytometer 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, after which it was centrifuged for ten minutes at 3, 000 revolutions per minute using a table centrifuge.The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle. Decanted sera from the coagulated blood samples were saved by freezing at -180cuntil analyzed for serum biochemistry according to Feng *et al.*, (1973). AlanineAminotransferase (ALT) and Aspartate Amino Transferase (AST) were determinedby the Reintman-Frankel colorimedric 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 phosphate in serum or plasma using phenolphthalein monophosphate method as described by (Klein *et al.*, 1960; 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 modified Jaffe method (Blass *et al.* 1974). Serum cholesterol and glucose for in-vitro determination was analysed using enzymatic colorimetric method (Allain *et al.* 1974). The data collected were subjected to one-way analysis of variance for haematological variables serum biochemistry.

Data analysis

Data collected were subjected to one-way analysis of variance (ANOVA) as described by Steel and Torrie (1980). Where significant differences were found, the means were separated and compared using Duncan's

New Multiple Range Test (DNMRT) (Duncan, 1980). Statistical computations were done using IBM SPSS (20th Edition).

Results and Discussion

The results of haematological indices/variables of the broiler chickens are shown in Table 2. Mean corpuscular haemoglogin and mean corpuscular haemoglogin concentration were not significantly ($p>0.05$) affected by dietary treatment levels. All other haematological parameters measured were significantly ($P<0.05$) influenced by dietary treatments with, consistent trend established in the haemoglobin concentration. Birds fed diets 5 had significant ($P<0.05$) and highest pack cell volume (PCV) contents, compared to the control. These were not similar with the haematological variables reported by Onibi *et al*,(2011). The PCV contents were compared between the normal ranges of PCV reported by Mitruka and Rawnsley (1977) and Ross *et al.*, (1978). Goodwin et al., (1992) reported PCV for healthy broiler chickens at age 3 to 49 days old between 26 to 33%. The PCV is an indication of the proportion of blood that is made up of cells, which is expressed as a percentage of cells in the blood. The determination of PCV is to identify if the fed diets has detrimental health implications on the broiler chickens as a result of possible nutritional disorder from the tested diets. The results showed that broiler chicken fed diet containing POME were not anemic or polycythemic. RBC were significantly ($p<0.05$) higher in birds fed diets 3. RBC for all the treatments were between the range for broilers chickens reported by Mitruka and Rawnsley, (1977), and Ross *et al.,* (1978). Onyishi *et al*., (2017) also reported RBC for a healthy broiler chicken week at 7 to be 2.19 $\times 10^{12}$/l. Red blood cell is produced in the bone marrows of long bones of the body, and adequate production is dependent on the amount of iron absorbed from the digested food. White blood cells (WBC) were significantly ($P<0.05$) higher in broilers chicken fed diet 3. WBC is essential component of the blood necessary for defence against infectious disease, and for immune system. High amount above normal range may indicate pathogenic disease.

Table 2: Haematological indices of broiler chickens fed diets containing air dried palm oil mill effluent

Parameters	1	2	3	4	5	SEM
Pack cell volume (%)	28.50^{c}	30.25^{b}	30.25^{b}	28.75^{c}	33.75^{a}	0.52
Red blood cell (x 10^6/µl)	2.06^{ab}	2.08^{ab}	2.49^{a}	2.31^{ab}	2.03^{b}	0.07
White blood cell x (10^3µl)	11.15^{ab}	11.63^{ab}	15.55^{a}	10.13^{b}	13.35^{ab}	0.75
Haemoglobin concentration (g/dl)	16.58^{b}	17.77^{ab}	18.56^{a}	18.56^{a}	18.56^{a}	0.28
Mean corpuscular haemoglobin (p_g)	78.33	89.49	75.55	79.26	92.35	2.83
Mean corpuscular (g/dl)	139.30^{bc}	145.90^{b}	123.35^{c}	127.40^{bc}	166.95^{a}	4.83
Mean corpuscular haemoglobin concentration (g/dl)	58.15	61.35	61.33	61.83	55.66	1.00

^{abc} indicates the same letters superscripts within the same row are not significantly different ($P<0.05$).

Blood haemoglobin concentration (Hb) were significantly ($P<0.05$) higher in birds fed diets 3, 4 and 5. Onyishi et al., (2017) analyzed Hb profile for healthy broilers at 7 weeks of age at 18.58g/dl. The results showed that palm oil mill effluent has significant influence ($p<0.05$) on the blood haemoglobin. This indicates high concentration of nutritional iron in the test ingredients "palm oil mill effluent" (POME). Mean corpuscular volume were significantly ($P<0.05$) higher in birds fed diet 5. The results also showed birds fed diets 2 and 3 had MCV within the range reported by Onyishi et al., (2017) as 92.42 to 136.80g/dl. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were not significantly ($P>0.05$) affected by POME. Parameters that are mostly affected by dietary treatments include Red blood cell (RBC), pack cell volume (PCV) and haemoglobin concentration (Hb) (Aletor and Egberongbe, 1992). Onibi *et al.* (2011) reported no influence by dietary treatments of POS on haematological variables of the broiler chickens but increase in the lipid contents of the muscles with increasing levels of POS inclusion in the diets. Haematological indices served as indicators of the animal's health status (Awotwi, 1990; Aletor and Egberongbe, 1992; Babatunde *et al.,* 1992) and there exists positive relationship between organo-lipids and coronary heart diseases in humans (Your total health, 2008).

The serum biochemistry parameters of the broiler chickens in (Table 3.) were significantly ($P<0.05$) influenced by dietary treatments, except the enzymes (AST, ALT and ALP). Aspartate amino-transferase (AST) is a non-specific, very sensitive biomarker of liver disease in birds. Alanine amino-transferase (ALT) is more specific in detecting liver injury. Alkaline Phosphatase (ALP) is influenced by the phosphorus levels in the diets. High ALP activity is in response to decreasing phosphorus levels in the diet of birds (Schmidt et al., 2007) and this effect could be induced by osteoblast where growth or remodelling of bone is taking place.

Serum total protein had statistically similar values in broilers chicken fed diets 1, 2 and 5 and decreased as the level of POME increased. Blood plasma proteins plays important role in homeostasis maintenance in the animal system, in forming enzymes, transport of minerals and hormones and building the immune system in the animal. Birds fed diet 2 had significantly (P<0.05) higher value of albumin. The values for albumin content within normal range for broilers chicken as reported by Ross *et al.,* (1978) as 2g/dl to 3.5g/dl.

Table 3: serum biochemistry, enzymes and organo-lipids profile of broiler chickens fed diets containing air dried palm oil mill effluent

Parameters	1	2	3	4	5	SEM
Glucose (g/dl)	176.00^{b}	184.30^{ab}	186.60^{ab}	203.63^{a}	205.40^{a}	4.14
Serum total protein (g/dl)	3.56^{a}	3.67^{a}	3.23^{b}	3.14^{b}	3.70^{a}	0.06
Albumin (g/dl)	1.43^{ab}	1.65^{a}	1.47^{ab}	1.55^{ab}	1.39^{b}	0.04
Globulin (g/dl)	2.13^{ab}	2.02^{b}	1.76^{c}	1.60^{c}	2.31^{a}	0.07
Plasma-protein (g/dl)	4.19^{a}	4.13^{ab}	3.54^{c}	3.30^{c}	4.12^{ab}	0.17
Urea (mg/dl)	3.71^{a}	2.67^{bc}	2.02^{c}	3.23^{ab}	3.78^{a}	0.20
Creatinine (mg/dl)	0.63^{a}	0.46^{ab}	0.31^{ab}	0.15^{c}	0.42^{ab}	0.05
Organo-lipids profiles						
Cholesterol (mg/dl)	50.00^{ab}	53.85^{ab}	57.70^{a}	51.93^{ab}	48.08^{b}	1.30
Triglyceride (mg/dl)	28.44^{bc}	28.91^{bc}	49.16^{ab}	13.70^{c}	56.88^{a}	5.10
High density Lipo protein (mg/dl)	41.19^{c}	47.08^{bc}	85.23^{a}	64.72^{b}	62.76^{b}	4.69
Low density lipo-protein (mg/dl)	53.13^{ab}	54.84^{a}	36.39^{c}	20.33^{c}	22.02^{c}	4.45
Enzymes						
Aspartate Amino Transterase (µ/l)	68.22	63.04	62.00	63.39	64.78	1.62
Alanine Amino Transferase (µ/l)	4.70	4.84	5.55	5.98	7.26	0.38
Alkaline Phosphates (µ/l)	175.00	177.90	185.85	185.85	187.50	1.94

^{abc} indicates the same letters superscripts within the same row are not significantly different (P>0.05)
SEM: standard error of mean, P-protein: plasma protein.

The globulin values of the POME diet groups were significantly (P<0.05) different from that of the control diet, but broilers chicken fed diet 5 had significantly (P<0.05) highest value. Globulins are immune molecules that are produced by the immune system in response to invasion of the body of agents that are perceived by the system as being foreign. The glucose values of the POME diet fed birds were significantly (P<0.05) different from .that of the control diet (1), but broiler chickens on diet (4) and (5) had a significantly higher value than that of the other treatment groups. The values for glucose content fall between the normal ranges of biochemical glucose level as reported by Ross *et al,* (1978). The high glucose values observed on diets (4) and (5) which increased as POME levels inclusion increased indicates that "POME" contains high quality of digestible carbohydrate which served as a good source of energy in broiler feeds replacement for maize.

Low density Lipo-protein (LDL) values were significant (p<0.05). Birds fed diet 2 were statistically higher. Low density lipo-protein (LDL) decreased as POME increased beyond 6% inclusion level. The results for high density lipo-protein contents showed significant (P<0.05) differences among treatments with diet 3 as the highest. Ozdogan and Aksit (2003) reported high HDL in broilers fed diet containing corn oil, sunflower oil and soybean oil, and low LDL in the same diets. The results showed that broilers fed diets containing POME has no effects on the HDL and LDL. Serum cholesterol content are significantly (P<0.05) different on diet 3 and 5, while diet 1, 2 and 4 are statistically similar. Diet 3 had the highest cholesterol content. The cholesterol serum level is in contrast with the study reported by Onibi *et al,* (2011) that the serum cholesterol level increased with increasinglevels of palm oil sludge inclusion in broiler chickens diets. Onibi *et al.* (2011), further examined no influence by dietary treatments of POS on haematological variables of the broiler chickens and increase in the lipid contents of the muscles with increasing levels of POS inclusion in the diets.
Broiler chickens fed diet 5 had significant and highest (p<0.05) values of triglycerides. Broiler chickens fed diets 2 were not significant (p>0.05) from the control. Both cholesterol and triglyceride serum levels are genetically dependent as one of the reasons for their great variability revealed by different researchers in experiments on growing chickens (Krasnodebska-Depta and Koncicki 2000).

Plasma protein content of broiler chickens fed diets containing POME were significantly (P<0.05) different. The control diets had significant higher values compared to other treatment diets, but broiler chickens fed diet 2 and 5 were not significantly (p>0.05) different with the control. Blood urea levels of broiler chickens fed diets containing POME were significant (P<0.05), broiler chicken fed diets 4 and 5

were not significantly (p>0.05) different from the control. But broiler chicken on diet 5 and control were higher compared with other treatments. Creatinine content levels of broiler chickens fed diet containing POME were significant (P<0.05). Broiler chickens fed control diet were significantly higher (p<0.05) than other treatments, broiler chickens fed diets 2, 3 and 5 were not significantly (p<0.05) different from the control. The results showed that increased levels of POME inclusion in the diets of broiler chickens did not increase (p>0.05) as POME levels inclusion increased. The values for urea are indications of protein quality in the diet. WebMD, 2014 and Wikipedia, (2014) noted an increase in blood urea in animals have been caused by high protein diet. The values for Aspardate Amino Treansferase (AST), Alanine Amino Transferase (ALT) and Alkaline Phosphatase (ALP) were not significant (P<0.05).

Conclusion

The results obtained showed that the replacement of maize with dried POME in diets of broiler chickens at between 6%-24% in starter and finisher diets can be achieve with no adverse effects on the haematological indices, and serum biochemical profiles in the broiler chickens. The values for glucose and haemoglobin concentration content were seen to have increased with increasing levels of POME inclusion in the broilers diets. The broiler chickens glucose and haemoglobin concentration content showed that palm oil mill effluent is a good source of digestible carbohydrate readily available for the animal's energy requirement.

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