Effect of Dietary Fibre and Supplementary Enzyme Levels on Nutrient Utilization and Haematological Indices of Pullet Chicks

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Abstract: One hundred and twenty 3 -week old Harco black pullet chicks were used to investigate the effects of dietary fibre and supplementary enzyme levels on nutrient utilization and haematological indices of pullet chicks. The birds were randomly divided into 8 groups of 15 birds each and assigned to 8 energetic (11.78-11.96 MJ/Kg ME) and nitrogenous (20% crude protein) diets in a 4 x 2 factorial arrangement involving four fibre levels (5.0, 6.0, 7.0 and 8.0%) and two enzyme levels (0 and 0.25%) for 8 weeks. Each treatment was replicated 3 times with 5 birds per replicate. Results showed that the intakes of crude fibre(CF) and nitrogen-free extract increased significantly (P<0.01) as the fibre level in the diet increased beyond 6%. Dry matter, nitrogen and CF retentions were significantly (P<0.01) decreased as the dietary fibre increased beyond 6% inclusion level. Increasing levels of crude fibre in the diets had no significant (P>0.01) effect on WBC and MCHC but affected the PCV, Hb, RBC, MCH and MCV significantly (P<0.01). It was concluded that pullet chicks can be fed 6% dietary fibre without supplementary enzyme and 8% dietary fibre with supplementary enzyme without adverse effects on nutrient retention and the haematology of chicks.
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1. Introduction

The poultry industry in Nigeria is expanding rapidly and egg production is an important aspect of the industry. The main factor militating against this rapid expansion of the industry is the problem of inadequate supplies of feedstuff at economic prices. The limited supply of good quality raw materials such as soybean mean, groundnut cake and cotton seed cake for the poultry feed industry has resulted in a continuous increase in the unit cost of production, causing a phenomenal rise in the unit cost of products, thus, these products have become too expensive for the majority of the population in Nigeria and elsewhere (Tewe, 2003; Esonu *et al*., 2003; Ani and Adiegwu, 2005). This, according to Tewe (2003) has further lowered productivity of stock as the qualities of cereals and protein concentrate in the mix are hardly sufficient to meet minimum nutrient requirements. Thus it has become very necessary to source for locally available, cheap and nutritionally safe alternative feedstuffs. Such feedstuffs would help resource-poor farmers to cut down their production costs and improve the efficiency of their production. A major constraint in the use of alternative feedstuffs like industrial by-products such as brewers' spent grain, wheat offal, palm kernel cake, and so on is that they contain high levels of fibre. Poultry like other monogastic animals lack the digestive frame work that can elaborately digest large amounts of fibre. There is therefore the need to incorporate exogenous enzymes in the diets of monogastric animals particularly poultry to further break down the high fibre materials not taken care of by the endogenous enzymes (ZoBell *et al*., 2000). These enzymes bind temporally to one or more of the reactants of the reaction which they catalyze and so lower the amount of activation energy needed thereby speeding up the reaction (Bennett and Frieden, 1999). The study was therefore conducted to investigate the effect of enzyme supplementation on nutrient retention and haematological indices of pullet chicks fed different levels of dietary fibre.

2. Materials and methods

The study was conducted at the Poultry Unit of the Department of Animal Science Research and Teaching Farm, University of Nigeria, Nsukka. Raw bambara nut waste and other feed ingredients used in the study were purchased at Nsukka and Enugu in Enugu State, Nigeria.

Animals and Experimental Diets

One hundred and twenty 3-week old black Harco black pullet chicks averaging 249.87 – 250.23g body weight were randomly divided into 8 groups of 15 birds each. The groups were randomly assigned to 8 energetic (11.78-11.96 MJ/Kg ME) and nitrogenous (20% crude protein) diets in a 4 x 2 factorial arrangement involving four levels (5.0, 6.0, 7.0 and 8.0%) of fibre and two enzyme levels (0 and 0.25%). The percentage composition of the diets is shown in Table1.

Table 1: Percentage composition of experimental diets

Crude fiber level (%)	5.00		6.00		7.00		8.00	
Enzyme levels (%)	0	0.25	0	0.25	0	0.25	0	0.25
Ingredients /Diets	1	2	3	4	5	6	7	8
Maize	46.11	46.11	34.26	34.26	38.56	38.56	37.94	9.37
Wheat offal	8.53	8.28	12.72	12.47	19.40	19.40	9.62	9.66
Ground nut cake	15.74	15.74	16.04	16.04	10.26	10.26	13.92	13.92
Soybean meal	11.81	11.81	12.58	12.58	8.26	8.26	9.30	9.30
Palm kernel cake	7.87	7.87	9.70	9.70	4.31	4.06	9.30	9.30
Bambara nut offal	3.94	3.94	8.70	8.70	13.21	13.21	13.92	13.92
Fish meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral-vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme	0.00	0.25	0.00	0.25	0.00	0.25	0.00	0.25
Total	100	100	100	100	100	100	100	100
Calculated composition:								
Crude protein	20	20	20	20	20	20	20	20
Crude fiber	5.02	5.02	6.02	6.02	7.09	7.09	8.02	8.02
Energy (MJ/kgME)	11.96	11.96	11.91	11.91	11.80	11.80	11.78	11.78

Each treatment was replicated 3 times with 5 birds per replicate placed in 2m x 3m deep litter pens of fresh wood shavings. Kerosene stoves placed under metal hovers for 28 days provided heat. Feed and water were supplied *ad libitum* to the birds. The birds were properly vaccinated against New Castle disease in the first, third and sixth weeks. They were also vaccinated against Gumboro disease in the second and fourth weeks and against fowl pox in the fifth week. Prophylactic treatment against coccidiosis with Embazin forte at two weeks of age was also given to the birds. The experiment lasted for a period of 8 weeks.

Apparent Nutrient Retention by Pullet Chicks

During week 8 of the experiment, a seven-day excreta collection from three birds per treatment (one per replicate) was carried out to determine the apparent retention of the proximate components. During this period birds were housed individually in metabolism cages and weighed quantity of feed (90% of the daily feed intake) was offered to each bird daily. The birds were allowed two days to adjust to the cage environment before droppings were collected. Daily feed consumption was recorded as the difference between the quantity offered and the quantity left after 24 hours. Faecal droppings were collected from separate cages in detachable trays placed beneath the wire mesh floor of the cages, oven-dried and weighed over a seven day period. At the end of the collection period, all faecal samples from each bird were bulked and preserved for analysis.

Blood collection and evaluation

During the 8^{th} week of the experiment, three birds were randomly selected from each treatment group (one bird per replicate) and blood samples were collected from their jugular veins with sterile needles. The blood samples were collected into properly labeled sterilized bottles containing EDTA (Ethylene diamine tetra-acetic acid) for haematological analysis. Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined by the methods described by Lamb (1991). Red blood cell (RBC) and total white blood cell (WBC) counts were estimated using the haemocytometer, while mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were calculated according to Mitruka and Rawnsley(1977).

PROXIMATE AND STATISTICAL ANALYSES

Feed and excreta samples were assayed for proximate composition by the methods of AOAC (1990). Gross energy of feed and faecal samples was determined in a Parr oxygen adiabatic bomb calorimeter. Data collected were subjected to analysis of variance (ANOVA) in a completely randomized design (Steel and Torrie, 1980). Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955).

3. Results and discussion

Table 2 shows the proximate composition of the experimental diets while data on nutrient intake by pullet chicks fed varying dietary fibre levels and supplementary enzyme are presented in Table 3. While there were no significant ($P>0.01$) differences among treatments in the intake of dry matter (DM), nitrogen and ether extract (EE), significant ($P<0.01$) differences existed among treatments in the intake of crude fibre (CF) and nitrogen-free extract (NFE). Chicks on treatments 5 to 8(7 and 8 % fibre diets with and without enzyme supplementation) had similar ($P>0.01$) CF intake values and these were significantly ($P<0.01$)

higher than the comparable (P>0.01) CF intake values of chicks on other treatments. The NFE intake values of chicks on treatments 1, 2, 3, 4, 5, 7 and 8 were comparable (P>0.01) and these were significantly (P<0.01) higher than the NFE intake value of chicks on treatments 6. While there was no significant (P>0.01) interaction between fibre and enzyme levels on CF intake, significant (P<0.01) interaction existed between fibre and enzyme levels on NFE intake. Enzyme supplementation reduced (P<0.01) NFE intake at the 7% fibre inclusion level.

Table 2: Proximate composition of experimental diets

Dietary fibre Levels (%)	5.00		6.00		7.00		8.00	
Enzyme levels	0	0.25	0	0.25	0	0.25	0	0.25
Components/Diets	1	2	3	4	5	6	7	8
Dry matter (%)	91.00	90.40	90.25	90.95	91.10	88.90	90.85	90.80
Crude protein (%)	20.01	20.02	20.04	20.07	20.08	20.02	20.05	20.03
Ether extract (%)	3.45	3.33	3.70	3.60	3.20	3.05	3.75	3.30
Crude fibre(%)	5.03	5.02	6.03	6.02	7.07	7.09	8.02	8.03
Ash (%)	5.2	5.6	4.15	4.2	4.8	7.7	3.75	4.45
Nitrogen-free extract (%)	57.31	56.43	56.33	57.06	55.95	51.04	55.28	54.99

Table 3: Nutrient intake of pullet chicks fed varying dietary fibre levels and supplementary enzyme

Dietary fibre Level (%)	5.00		6.00		7.00		8.00		
Enzyme level(%)	0	0.25	0	0.25	0	0.25	0	0.25	SEM
Parameters/Treatments	1	2	3	4	5	6	7	8	
Dry matter intake (g)	54.04	53.98	53.82	54.68	54.72	53.50	54.34	54.56	0.35
Crude protein intake (g)	12.19	11.62	12.35	12.04	12.16	11.57	12.08	12.18	0.19
Crude fibre intake (g)	13.73^{b}	13.70^{b}	15.60^{b}	16.45^{b}	20.60^{a}	20.60^{a}	22.90^{a}	21.59^{a}	0.79
Ether extract intake (g)	10.48	10.93	11.15	10.69	9.54	9.09	11.52	9.46	0.30
Nitrogen-free extract intake (g)	34.58^{a}	33.55^{a}	34.35^{a}	34.22^{a}	33.46^{a}	26.44^{b}	33.40^{a}	33.11^{a}	0.55

a,b, means on the same row with different superscripts are significant (P<0.01) different.
SEM= Standard error of mean.

The apparent retention of nutrients by pullet chicks fed varying dietary fibre levels and supplementary enzyme is presented in Table 4.

Table 4: Apparent retention of nutrients by pullet chicks fed varying dietary fibre levels and supplementary enzyme

Dietary fibre Level (%)	5.00		6.00		7.00		8.00		
Enzyme level(%)	0	0.25	0	0.25	0	0.25	0	0.25	SEM
Parameters/Treatments	1	2	3	4	5	6	7	8	
Dry matter (%)	70.77^{cd}	77.78^{a}	73.22^{bc}	74.41^{b}	60.03^{e}	68.71^{d}	63.18^{e}	62.08^{e}	1.29
Nitrogen (%)	54.47^{c}	59.34^{b}	56.83^{c}	69.51^{a}	56.53^{c}	56.73^{c}	39.01^{e}	49.08^{d}	1.71
Crude fibre (%)	72.13^{a}	73.59^{a}	66.42^{b}	73.29^{a}	58.37^{d}	61.62^{c}	50.95^{e}	57.35^{d}	1.67
Ether extract (%)	62.01^{cd}	59.44^{d}	80.65^{b}	82.06^{b}	62.61^{c}	89.09^{a}	43.59^{e}	80.49^{b}	3.02
Nitrogen-free extract (%)	45.25^{d}	75.21^{a}	40.56^{e}	45.79^{cd}	42.27^{e}	50.38^{b}	47.85^{c}	51.21^{b}	2.14

a,b,c…e means on the same row with different superscripts are significant (P<0.01) different.
SEM= Standard error of mean.

Chicks on treatment 2 (5% fibre diet with enzyme supplementation) had significantly (P<0.01) higher DM retention value than those chicks on other treatments. Chicks on treatments 5, 7 and 8 had the least DM retention values and these were comparable (P>0.01). Chicks on treatment 4(6% fibre diet with enzyme supplementation) had the highest nitrogen retention value while chicks on treatment 7(8% fibre diet without enzyme supplementation) had the least nitrogen retention value (P<0.01). The CF retention values of chicks on treatments 1, 2 and 4 were comparable and these were significantly (P<0.01) higher than the CF retention values of chicks on other treatments. Chicks on treatment 7 had the least CF retention value. The EE retention value of chicks on treatment 6 was significantly (P<0.01) higher than the EE retention values of chicks on other treatments. Chicks on treatment 7 had the least EE retention value. The NFE retention value of chicks on treatment 2 was significantly (P<0.01) higher than the NFE retention values of chicks on other treatments. Chicks on treatments 3 and 5 similar NFE retention values and these were the least. There were significant (P<0.01) interactions between fibre and enzyme levels on the retention of DM , nitrogen, CF, EE and NFE. Enzyme supplementation increased(P<0.01) the retention of

DM at the 5% and 7 %, fibre inclusion levels; increased (P<0.01) nitrogen retention at the 5%, 6% and8% fibre inclusion levels; increased (P<0.01) CF retention at the 6%,7% and 8% fibre inclusion levels and increased(P<0.01) EE and NFE retentions at the 7% and 8% fibre inclusion levels.

As shown in Table 3, as the fibre level in the diet increased beyond 6%, there was a significant increase in the intake of nutrients such as CF and NFE. This was as a result of increase in feed intake by birds fed the 7and 8% CF diets which had no supplementary enzyme (Table3). Pond and Maner (1974) had shown that as the level of crude fibre increased in the diet, there was a concomitant increase in feed intake vis-à-vis nutrient intake. This was attributed to the bulky nature and low total digestible nutrient content of such highly fibrous feed. It is well known that in chickens feed intake is inversely related to dietary energy concentration. Increase in dietary fibre level is known to result in decrease in dietary energy level of the diets (Kung and Grueling, 2000; Macdonald *et al*., 2002). Since birds eat to satisfy their nutrient (energy) requirement (Jurgens, 2007), those birds fed the 7and 8% CF diets had to consume more feed than other birds in order to meet their nutrient requirements. Beside decrease in energy level, dietary fibre has a laxative effect. It acts by changing the nature of the contents of the gastrointestinal tract and by changing how other nutrients and chemicals are absorbed (Eastwood and Kritchevsky, 2005). Soluble fibre absorbs water to become a gelatinous, viscous substance and is fermented by bacteria in the digestive tract. Insoluble fibre has bulking action and is not fermented (Anderson *et al*., 2009). The laxative nature of dietary fibre might have therefore increased the rate of gastric evacuation in the birds. A high rate of gastric evacuation is usually compensated by increased feed intake vis-à-vis nutrient intake (Payne and Wilson, 1999; Williamson and Payne, 2000).

Tables 4 shows that increasing dietary fibre levels had adverse effect on nutrient absorption as evidenced by the significant reduction in the retention of DM, nitrogen, CF, EE and NFE . Abdelsamie *et al*., (1983) and Jokthan *et al*. (2006) had reported similar depression in nutrient digestibility in broiler chicks and rabbits, respectively. Kass *et al*. (1980) and Fielding (1991) attributed such depression in apparent nutrient digestibility to higher rate of passage of digester in animals fed on high fibre diets. Fibre tends to limit the amount of intake and the retention of the available energy by birds and contributes to excessive nutrient excretion (Kung and Grueling, 2000). According to Macdonald *et al*. (2002), the percentage of crude fibre affects the digestibility of feeds as the higher the percentage of crude fibre in the diet, the lower the digestibility of other nutrients. As a matter of fact, the content of crude protein and digestibility of dry matter decreased.

This is probably due to high rate of passage of digesta through the alimentary tract and that is why highly fibrous feeds are laxative. Besides fibre, the anti-nutritional factors as protease inhibitors, tannins and haemaglutinins found in raw bambara nut(Liener, 1986; Ensminger *et al*., 1996), one of the major sources of fibre in the diets may have contributed to the observed reduction in nutrient digestibility and retention. This agrees with the reported findings of Osho (1989) and Marquardt (1997) that ANFs in raw beans cause depression in nutrient digestibility, absorption and retention. Poor nutrient, digestibility, absorption and retention could lead to depressed rate of growth in animals. However, enzyme supplementation of some of the diets improved nutrient retention. This corroborates earlier reports (Ikegami, 1990; Francesch *et al*., 1994) that enzymes improved the digestion and absorption of nutrients. Bedford (1997) reported that exogenous enzyme supplementation on diets improves production efficiency of poultry by increasing the digestion of low quality products and reducing nutrients loss through excreta, disallowing the reduction of diets nutritional levels with likely economic advantages. Toibipont and Kermanshahi (2004) had also shown that apparent metabolizable energy, apparent lipid digestibility and apparent protein digestibility were all significantly improved when arabinoxylanase and beta-glucanase enzymes were added to wheat-soybean meal based diets. Perhaps enzymes might have helped to reduce the viscosity of the encapsulated nutrients to enhance nutrient uptake and absorption. Slominoski *et al*. (2006) had shown that inclusion of enzyme in the diet helps to reduce such non-digestive residues that have negative impacts on digesta viscosity.

Table 5 shows the effect of varying dietary fibre and supplementary enzyme on the haematological indices of pullet chicks. While there were no significant (P>0.05) differences among treatments in mean cell haemoglobin concentration(MCHC) and white blood cell count(WBC), significant (P<0.05) differences existed among treatments in haemoglobin concentration(Hb), packed cell volume(PCV), red blood cell count(RBC), mean cell haemoglobin (MCH) and mean cell volume(MCV).

Effect of Dietary Fibre and Supplementary Enzyme Levels on Nutrient Utilization and Haematological Indices

of Pullet Chicks

Table 5: Effect of varying dietary fibre levels and supplementary enzyme on haematological parameters of pullet chicks

Dietary fibre Level (%)	5.00		6.00		7.00		8.00		
Enzyme level (%)	0	0.25	0	0.25	0	0.25	0	0.25	SEM
Parameters/Treatments	1	2	3	4	5	6	7	8	
Packed cell volume (%)	16.00^{cd}	17.50^{bcd}	16.50^{bcd}	30.00^{a}	22.50^{d}	23.50^{abc}	22.50^{abcd}	26.50^{ab}	1.61
Hemoglobin Con.(g/100ml)	4.50^{cd}	5.15^{bcd}	4.95^{cd}	9.65^{a}	2.80^{d}	7.40^{abc}	7.00^{abc}	8.40^{ab}	0.66
Red blood cell count (10^6/mm3)	2.50^{bc}	2.74^{bc}	2.49^{cd}	4.91^{a}	1.99^{d}	3.79^{abc}	3.37^{abc}	4.30^{ab}	0.28
White blood cell count ($10^6/mm^3$)	11.40	14.85	14.70	22.75	13.55	14.90	12.95	16.00	1.18
Mean Cell Hem. Con. (%)	64.11	64.06	66.55	61.13	63.76	62.09	61.62	68.28	1.18
Mean Cell hemo. (pg)	18.01^{a}	18.72^{a}	19.76^{a}	19.65^{a}	14.28^{b}	19.58^{a}	19.53^{a}	21.24^{a}	0.56
Mean cell Volume (um3)	28.13^{b}	29.25^{ab}	29.83^{ab}	32.14^{a}	22.41^{c}	31.53^{ab}	31.70^{ab}	31.10^{ab}	0.81

$^{a,b,c.....g}$ Means on the same row with different superscripts are significant (P<0.01) different.
SEM= Standard error of mean.

The PCV value of birds on treatment 4 was comparable (P>0.05) to the PCV values of birds on treatments 6, 7 and 8 and this was significantly (P<0.05) higher than the PCV values of birds on other treatments. Birds on treatment 4 had significantly (P<0.05) higher Hb value than birds on treatments 1, 2, 3 and 5 which have similar Hb value. Birds on treatments 1,2, 3, 6 and 7 had comparable Hb values (P>0.05). Birds on treatment 4 had significantly (P<0.05) higher RBC values than birds on treatments 1, 2, 3 and 5. Birds on treatments 1, 2, 3, 6 and 7 had similar RBC values. Birds on treatments 3 and 5 had comparable RBC values (P>0.05). Birds on treatment 5 had significantly (P<0.05) lower MCH value than birds on other treatments which had comparable MCH values (P>0.05). The MCV value of birds on treatment 4 was comparable to the MCV values of birds on treatments 2,3, 6, 7 and 8 and this was significantly (P<0.05) higher than the MCV values of birds on treatments 1 and 5. Birds on treatment 5 (7% CF diet without supplementary enzyme) had the least MCV value. There were significant (P<0.01) interactions between fibre and enzyme levels on PCV, Hb, RBC, MCH and MCV. Enzyme supplementation increased (P<0.01) PCV, Hb and RBC at the 6% and 7 %, fibre inclusion levels and increased (P<0.01) MCH and MCV at the 7% fibre inclusion level.

Increasing levels of crude fibre in the diets had no significant effect on the WBC and MCHC. However, the PCV, Hb, RBC, MCH and MCV were significantly affected by treatments (Table 5). Considering the importance of the PCV as an indicator of blood dilution, the Hb as a measure of the ability of an animal to withstand some levels of respiratory stress, and the prominent role of white blood cells in disease resistance especially with respect to the generation of antibodies and the process of phagocytosis, it could be suggested that the dietary levels of fibre in the present study were not detrimental to these blood indices. Interestingly, there was a remarkable increase in PCV, Hb, RBC, MCH and MCV at the 6% fibre inclusion level when compared to other CF inclusion levels. This tends to indicate that although the blood indices examined were not adversely affected at all the levels of fibre inclusion in the diets, the quality of the 6% fibre diet was optimal. Clarke and Myra (1975) had shown that red blood cells count is an indication of feed quality. Swenson and Reece (1993) also indicated that nutritional status is an important factor that can affect blood composition. It is well known that animals' blood serves as a medium that transports nutrients from the gastro intestinal tract to the tissue. It also carries metabolic waste from the cells to the organs of excretion. The fact that PCV in particular was not adversely affected by the dietary treatments tends to suggest that the temperature and relative humidity of the environment (Nsukka) where the experiment was conducted were conducive. According to Yahar *et al* .(1997), higher temperatures of rearing chickens is associated with significant hypervolemia that results in low PCV as this provides the fluid needed for heat dissipation by panting in contrast to low temperatures. Similar effects of temperature on PCV had also been reported in chickens (Oyewale, 1987; Yahar *et al*. 1997). As a matter of fact, the values of packed cell volume (30%) and haemoglobin concentration(9.65 mg/100ml) obtained at the 6% fibre inclusion level in the present study compare well with the PCV values (30-33%) and the Hb values (6.5-9.00mg/ml) reported by Schalm (1975) and Swenson and Reece(1993) for birds. The supplementation of some of the diets with exogenous enzyme (Roxazyme G®) resulted in significant (P<0.01) increases in PCV, Hb and RBC at the 6% and 7 %, fibre inclusion levels. Enzyme supplementation also increased (P<0.01) MCH and MCV at the 7% fibre inclusion level. The enzyme included in some of the diets might have helped to boost the immune system of the birds. This view is strongly supported by the report of Joshi *et al*. (2002) that blood is a good indicator of the health of an organism and acts as pathological reflector of the whole body; hence haematological parameters are important in diagnosing the functional status of animals exposed to certain dietary treatments. The results obtained in the present study tend to show that inclusion of exogenous enzyme in some of the chicks' diets improved the utilization of nutrients by the birds as well as their haematological indices.

CONCLUSION

The results obtained in this study show that pullet chicks can be fed 6% dietary fibre without supplementary enzyme and 8% dietary fibre with supplementary enzyme without adverse effects on nutrient retention and the haematology of chicks.

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