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Original article

Effects of crude cowpea (*Vigna unculata*) meal supplemented with enzyme on nutrient digestibility and growth performance of broiler chickens

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ABSTRACT

This study was designed to assess the digestibility of feed components and the growth performances of broilers fed on raw Cowpea based diet supplemented with fibrolytic enzyme (Naturzyme®). A control ration without Cowpea and enzyme was compared to six others rations in which raw cowpea was incorporated at 15 and 25% and supplemented with 350 and 500g enzyme/ton of feed. The experimental rations were randomly assigned to 252 twenty-one-day old Cobb500 broiler chickens in a 2x2 factorial design (two level of enzyme and two level of cowpea). At 49 days old, faeces were collected over a period of 3 days to assess the digestibility of feed components. The main results showed that the increasing rate of raw cowpea in the rations induced a linear decrease ($p<0.05$) in feed intake. The inclusion of 25% of cowpea in the ration irrespective of the rate of incorporation of the enzyme tends to increase the digestibility of feed component as compared to the control. Growth parameters of broilers inversely decrease with the incorporation rate of raw cowpea in the ration. The highest carcass yield ($p<0.05$) was recorded with 15% raw cowpea

supplemented with 500g of enzyme per ton of feed. The relative weight of the gizzard increased significantly ($p<0.05$) with the increasing incorporation rate of raw cowpea in the diet with or without enzyme supplements. In conclusion, raw cowpea supplemented with enzyme did not substantially improve the growth performance of broilers. Other processing methods like boiled or fermented should be considered for better utilization of cowpea in broilers diet.

1. Introduction

In most African countries, high animal feed cost is in general associated with the high cost of imported protein feedstuff such as soybean meal which is the major source of plant protein in poultry diet. This has resulted to the search for locally available and cheap feedstuffs (Teguia and Beynen, 2005; Mube, 2011). The leguminous seed which has been widely studied appear to be a good substitute to soybean since the amino acid profile are comparable (Kana et al., 2012; Defang et al., 2014). One of such ingredients is cowpea (*Vigna unguiculata* WAL). Cowpea called "white cold" is cultivated in the five agro-ecological zones of Cameroon and is well integrated into local population feeding habits (Djilé et al., 2016). Recent works revealed its promising potential as a feedstuff for poultry. In fact, Cowpea contains 63% of carbohydrates, 14-24% crude protein (Chinma et al., 2008; Chathuni et al., 2018) while the metabolizable energy value varies from 3375.7 to 3606.7kcal/kg, methionine (0.28-0.32g/kg) and lysine (1.52-1.67g/kg). However, the utilization of raw cowpeas is limited in the monogastric diet due to its high antinutritional factors content that negatively affect broilers' performances (Teguia and Fru, 2007; Defang et al., 2008; Kana et al., 2012). This seed contains protease inhibitors, tannins, lectins, and alkaloids which impair broiler growth (Frota et al., 2017). The successful use of legumes grains as a source of protein in diets of broilers thus depends on the type of treatment used to eliminate the antinutritional factors they contain (Kana et al., 2012; Abdou et al., 2019). Among the proposed chemical methods, the utilization of exogenous fibrolytic enzymes appears to be promising effective ways (Saad, 2011). Fibrolytic enzyme is able to improve diet digestibility, reduce the production cost, improve meat quality and maintain animal health (Saad, 2011). Previous works revealed that 0,025% of enzyme in a ration containing 30% of wheat bran leads to a rise of 1,77% of the live weight of broiler as compared to the rations containing the same rate of wheat without enzyme (Seifi, 2013). The main objective of this study is to investigate the effects of cowpea supplemented with enzyme on production performance of broiler chickens.

2. Materials and methods

This study was conducted at the poultry unit of the Teaching and Research Farm of the University of Dschang, Cameroon for field work and in the Laboratory of Animal Nutrition for laboratory analysis. The experimental zone is located at altitude 1420m and the annual temperature varies from 10°C to 25°C. The rainfall ranges from 1500 to 2000mm per annum. The rainy season last for a period of over 9-month (March-November) (Tankou et al., 2013).

Dried Cowpea was bought at the Dschang local market, ground in a Harmed mill before processing. Six experimental diets were formulated from a control ration (T0) with soybean as main protein sources by substituting 15% (T15) and 25% (T25) of soybean with Cowpea (Table 1) supplemented with 0.035 and 0.5% fibriolytic enzyme (Nutuzyme®).

The experimental birds consisted of 252 chicks of 21 days old of Cobb 500 strain with an average weight of 738.08±47.91g. The chickens were reared on litter floor with 10 birds/m^2 density. Vaccination and other routine poultry management practices were maintained. Chicks were randomly assigned to the experimental rations in a completely randomized design with 36 birds per treatment. Each group was further subdivided into 3 replicates of 12 birds each (06 males and 06 females).

Data on feed intake and body weight gain were collected on a weekly basis and used to calculate feed conversion ratio. At the end of the feeding trial (49 days), 8 birds from each treatment group were randomly selected, fasted for 24 hours and slaughtered for carcass evaluation.

For digestibility evaluation, 3 birds per treatment were randomly selected, their feaces were collected each morning for 3 consecutive days. These faeces was dried in an oven at 60°C until constant weight for the proximate analysis according to the procedure described by A.O.A.C (1990) and Neutral Detergent Fiber (NDF) by the method of Van Soest et al. (1991). The apparent digestive coefficient of DM, OM, CB, CF, NDF were calculated according to the formula: Apparent digestibilty= (intake-excreted)/intake×100

Table 1
Composition of experimental diets.

	Rations		
Ingredients %	**T0**	**T15**	**T25**
Maize	63.5	55	50
Wheat bran	0	4.5	2
Cotton cake	6	8	25
Soybean cake	20	5	3
Cowpea	0	15	25
Fish meal	5	5	5
Blood meal	0	4	3.5
Oyster shell	1	1	1
Bone meal	1	1	1
Premix 0,5%	0.5	0.5	0.5
Palm oil	1	1	1
Total	100	100	100
Chemical composition of the experimental diet			
Crude protein %	20.24	2094	21.25
Metabolizable energy (kcal/kg)	3029.5	3134	3174
Energy/protein ratio	149.65	149.69	149.37
Calcium (%)	1.13	1.12	1.11
Phosphorous (%)	0.49	0.49	0.46
Calcium/phosphorous(%)	2.28	2.28	2.43
Lysine (%)	1.26	1.72	1.98
Methionine (%)	0.42	0.49	0.55
Magnesium	3.69	6.70	3.54

Premix 5%: crude proteins=40%, Lys=3.3%, Meth=2.40%, Ca=8%, P=2.05%, Metabolizable energy=2078kcal/kg

2.1. Data analysis

The data were analyzed statistically using two ways (Cowpea level and Enzyme rate) ANOVA test by General Linear Model's procedure. The Statistical Package for Social Science (SPSS 21.0) software was used. Significant differences between treatment means were separated using Duncan's multiple range tests at 5% probability (Steel and Terrie, 1980).

3. Results and discussion

3.1. Digestibility of experimental diets

The digestibility of the diets components varied with increasing levels of cowpea and enzyme in the rations (Table 2). Thus, the incorporation of 15% of crude cowpea supplemented by 350g of enzyme tends to reduce the digestibility of DM, OM, CF, and NDF. Meanwhile the reverse trend was observed when 25% of Cowpea was supplemented by 500g of the enzyme. In addition, the digestibility of crude fiber dropped with the increasing rate of Cowpea in the ration irrespective of the rate of enzyme considered.

Table 2
Apparent digestibility of feed components of broiler fed on diets containing graded levels cowpea supplemented with enzyme.

	Cowpea incorporation rate (%)	
Enzyme (g/t)	**15**	**25**
Dry Matter (%)		
0	92.43	94.47
350	93.06	95.71
500	93.40	95.46
Organic matter (%)		
0	81.68	79.11
350	87.84	92.93
500	86.05	94.57
Nitrogen (%)		
0	67.77	19.2
350	62.92	31.39
500	67.39	38.42
Crude Fiber (%)		
0	81.81	79.11
350	87.84	92.93
500	86.05	94.57
NDF (%)		
0	87.48	95.86
350	85.23	98.08
500	91.12	97.32

3.2. Growth performances

The growth performances of broiler as affected by raw cowpea supplemented with enzyme are summarized in Table 3. Incorporation of Cowpea supplemented by enzyme in broiler diet induced a significant reduction ($p<0.05$) in feed intake as compared to the control ration. With 25% of cowpea in the ration, growth parameters were lower ($p<0.05$) when the ration was supplemented by 350g of enzyme per ton of feed as compared to ration supplemented with 500g per ton of feed.

The incorporation of crude cowpea in the ration whatever the rate of enzyme considered induced a significant reduction ($p<0.05$) in the live body weight and body weight gain of broiler as compared to the control ration containing soybean as main protein sources (Table 3).

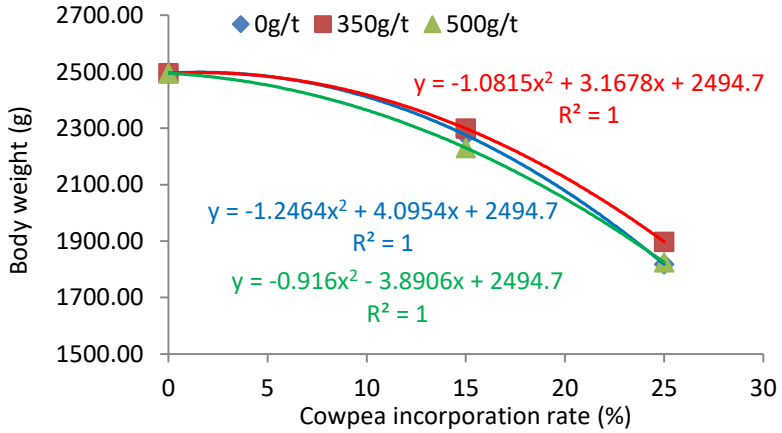

Fig. 1. Regression of body weight on cowpea incorporation rate in the rations supplemented by enzyme.

This reduction is materialized by Figure 1 which shows the relation between body weight and incorporation rate of cowpea in the ration supplemented by enzyme at different rates. It appears that, when cowpea is included in the rations at 15 and 25% without enzyme, body weight decreases from 9 to 37% respectively as compared to control diet. Whereas, this reduction is about 8.51 and 31% with a supplementation of 350g/t and 11 and 36% with 500g of enzyme per ton of feed containing 15 and 25% of cowpea.

Table 3
Effects of cowpea supplemented with enzyme on growth performances of broiler chickens.

	Cowpea incorporation rate (%)			
Enzyme (g/t)	**0**	**15**	**25**	**p**
Feed intake (g)				
0	4594.72±359.12^{A}	4274.61±205.94^{AB}	3951.22± 91.70^{aC}	0.049
350	4594.72±359.12^{A}	4286.24±163.42^{AB}	3793.22±136.30^{bC}	0.052
500	4594.72±359.12^{A}	4267.75±104.26^{AB}	4047.20±137.12^{aC}	0.069
p		0.990	0.044	
Body weight (g)				
0	2494.73±62.20^{A}	2275.72±36.89^{B}	1818.11±56.00^{C}	0.000
350	2494.73±62.20^{A}	2298.90±42.14^{B}	1897.97±95.08^{C}	0.000
500	2494.73±62.20^{A}	2230.28±28.50^{B}	1824.97±101.99^{C}	0.000
p		0.140	0.500	
Body weight gain (g)				
0	1838.73±62.20^{A}	1619.72±36.89^{B}	1162.11±56.00^{C}	0.000
350	1838.73±62.20^{A}	1642.91±42.14^{B}	1241.96±95.08^{C}	0.000
500	1838.73±62.20^{A}	1574.28±28.50^{B}	1168.97±101.99^{C}	0.000
p		0.140	0.500	
Feed conversion ratio				
0	2.5±0.11^{B}	2.64±0.08^{B}	3.40±0.15^{aA}	0.000
350	2.5±0.11^{B}	2.61±0.03^{A}	3.08±0.54^{aA}	0.141
500	2.5±0.11^{B}	2.71±0.10^{B}	3.48±0.39^{bA}	0.005
p		0.307	0.036	

a.b.c: Means with the same superscript on the same column are not significantly different (P>0.05). SEM=standard error of mean, P=probability. A,B:Means with the same superscript on the same line are not significantly different (P>0.05).

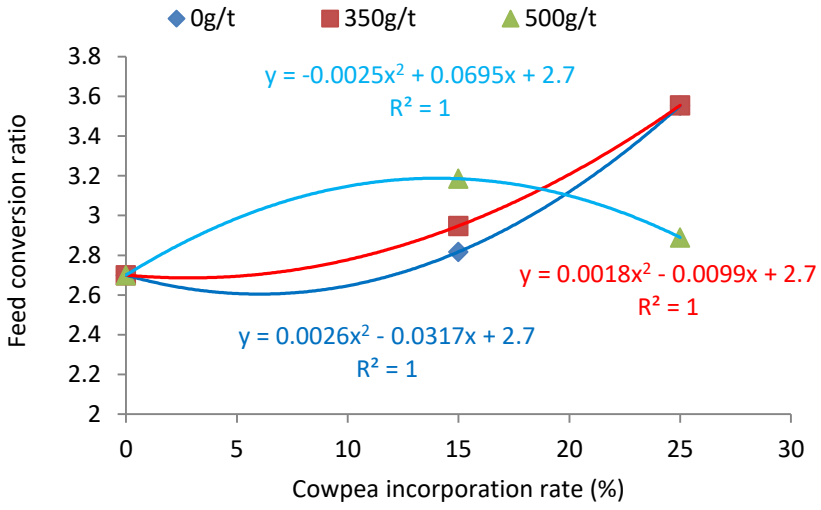

Fig. 2. Regression of feed conversion ratio on incorporation level of cowpea in the diet supplemented with enzyme.

Feed conversion ration tend to decrease with the incorporation rate of the crude cowpea in the rations. However, without enzyme and with the incorporation of 500g of enzyme per ton of feed, FCR of the diet containing 15% of cowpea were comparable ($p<0.05$) with that of the control groups (Table 2). While at 25% of cowpea, FCR was higher ($p<0.05$) as compared to other diet irrespective of the rate of enzyme used. This parameter increases from 5 to 26% when crude cowpea was incorporated at 15 and 25% respectively in the ration without enzyme (Figure 2). But when the cowpea was supplemented with 350g/t the reduction rate was 4 to 18% and 7 to 28% with 500g/t for 15% and 25% crude cowpea incorporation respectively.

Data on carcass yield and the proportion of different carcass components as % of live weight are summarized in Table 4. Globally, incorporation of cowpea in the rations tends to increase carcass characteristics whatever the enzyme level. Hence, diet containing 15% of crude cowpea supplemented with 500g/t of enzyme recorded the highest ($p<0.05$) carcass yield as compared to other rations.

Table 4
Carcass characteristics of broilers fed on diets containing crude cowpea supplemented with enzyme.

	Cowpea incorporation rate (%)			
Natuzyme (g/t)	**0**	**15**	**25**	**p**
Carcass yield (% BW)				
0	60.30 ± 2.20^{B}	71.92 ± 0.80^{A}	70.54 ± 1.87^{A}	0.000
350	60.30 ± 2.20^{C}	72.55 ± 1.27^{A}	68.19 ± 6.03^{B}	0.000
500	60.30 ± 2.20^{C}	73.08 ± 0.98^{A}	68.42 ± 1.74^{B}	0.000
p		0.102	0.405	
Gizzard (% PV)				
0	1.15 ± 0.15^{B}	1.64 ± 0.13^{A}	1.62 ± 0.13^{abA}	0.000
350	1.15 ± 0.15^{B}	1.69 ± 0.19^{A}	1.57 ± 0.11^{bA}	0.000
500	1.15 ± 0.15^{B}	1.63 ± 0.09^{A}	1.79 ± 0.20^{aA}	0.000
p		0.718	0.046	
Intestine density (g/cm)				
0	0.468 ± 0.050^{A}	0.469 ± 0.059^{A}	0.446 ± 0.049^{aA}	0.614
350	0.468 ± 0.050^{AB}	0.440 ± 0.052^{B}	0.516 ± 0.093^{abA}	0.010
500	0.468 ± 0.050^{AB}	0.387 ± 0.145^{B}	$0.560\pm0\ .062^{bA}$	0.006
p		0.243	0.014	
Liver(% PV)				
0	1.26 ± 0.09^{C}	1.89 ± 0.16^{B}	2.17 ± 0.33^{A}	0.000
350	1.26 ± 0.09^{C}	1.92 ± 0.20^{B}	2.23 ± 0.31^{A}	0.000
500	1.26 ± 0.09^{C}	1.89 ± 0.20^{B}	2.30 ± 0.14^{A}	0.000
p		0.944	0.626	
Heart (% PV)				
0	0.39 ± 0.07^{B}	0.47 ± 0.13^{AB}	0.51 ± 0.10^{A}	0.103
350	0.39 ± 0.07^{B}	0.55 ± 0.13^{A}	0.53 ± 0.07^{A}	0.007
500	0.39 ± 0.07^{B}	0.50 ± 0.11^{A}	0.47 ± 0.08^{AB}	0.083
p		0.321	0.391	
Abdominal fat (% PV)				
0	1.10 ± 0.42^{A}	1.60 ± 0.73^{A}	1.42 ± 0.55^{A}	0.247
350	1.10 ± 0.42^{A}	1.65 ± 0.66^{A}	1.32 ± 0.44^{A}	0.131
500	1.10 ± 0.42^{A}	1.37 ± 0.45^{A}	0.98 ± 0.74^{A}	0.375
p		0.652	0.320	

a.b.c: Means with the same superscript on the same column are not significantly different (P>0.05). SEM=standard error of mean, P=probability. A,B:Means with the same superscript on the same line are not significantly different (P>0.05).

The relative weight of the liver increased linearly and significantly ($p<0.05$) with the rate of cowpea in the rations. The variation of the rate of enzyme did not have any significant effect ($p>0.05$) on this organ weight.

The incorporation of 25% of cowpea supplemented with 350 and 500g of enzyme /t of feed tend to increase gizzard weight as compared to other rations. The highest weight of the gizzard and intestine density was recorded with 25% of cowpea supplemented with 500g of enzyme per ton in feed. Intestinal density decreases with 15% inclusion level of cowpea in broilers ration with increasing level of enzyme.

The present result reveals that feed intake decrease with the increasing rates of crude cowpea in the diet regardless of the enzyme supplemented rate considered. This result is in close agreement with that of Kana et al. (2012) who incorporated 20% of crude cowpea supplemented by 0.2% of *Canarium schweinfurthii Engl* charcoal in broiler diet. This could be attributed to the effect of the anti-nutritional factors present in raw cowpea. We can therefore suggest that enzyme used was unable to destroy these anti-nutritional factors. In fact, it was reported by Gai et al. (2010) that high tannin contents induce reduction in feed consumption due to the capacity of tannin to combine with saliva proteins and membranous mucous leading to a deterioration of the rations palatability. Furthermore, anti-nutritionals factors such as hemagglutinins and lectins present in crude cowpea possessed depressive effect on feed intake by moderating the endocrinal activity leading to appetite reduction (Akande, 2010; Balaiel, 2014).

The incorporation of 15% crude cowpea in the ration supplemented with 350g of enzyme tends to reduce the apparent digestibility of the dry matter, organic matter and nitrogen. These results are similar to those of González-Alvarado et al. (2010); Berrocoso et al. (2014, 2015) who reported a reduction in nutrient digestibility of the diet when fibrous ingredients were incorporated in broiler diet. Furthermore, it is well known that the digestibility of a ration is inversely proportional to its fiber content (Zhang et al., 2013). On the other hand, digestibility of NDF in the rations containing crude cowpea supplemented by enzyme was higher than that of the ration without cowpea and enzyme. This result is in agreement with Abdou et al. (2019) who recorded an increase in the digestibility of NDF from 12 to 13% with the addition of fibrolytic enzymes in the ration of broiler containing 40% of palm kernel. The improvement of the digestibility of the NDF by the addition of enzyme can be explained by the reduction of the viscosity of the rations in the digestive tract and the degradation of the plant cell wall by this enzyme (Natalie, 2016).

Body weight and body weight gain of broiler were reduced with the incorporation of crude cowpea in the diet with or without enzyme supplement. According to Defang et al. (2008); Kana et al. (2012) this depressive effect of cowpea on the weight could be attributed to the anti-nutritional factors such as protein inhibitors present in the cowpea seed that reduced protein digestibility and therefore depress growth. This result suggested that Naturzyme used was unable to completely detoxified cowpea seed. Moreover, the low feed consumption recorded might have led to an insufficient level of essential amino acids which affected protein synthesis and other metabolic processes (Defang et al., 2014).

Birds fed on control diet recorded lower feed conversion ratio as compared to those fed on rations containing crude cowpea independently of the rate of enzyme used. This observation is in agreement with that of Abdelgani et al. (2013) and Kana et al. (2012) which reported that FCR is depressed by addition of Cowpea in broiler diet.

Except relative weight of abdominal fat, the ration containing 15% crude cowpea supplemented by 500g/t of enzyme recorded the highest carcass yield ($P<0.05$) as compared to the rest of the rations. This result is contrary with that obtained by Leng (2015) who reported a reduction in broiler carcass yield by incorporation of industrial palm kernel in the diet. Crude cowpea supplemented by the enzyme significantly increases the relative weights of the gizzard, liver and heart as compared to the control ration and rations containing cowpea without enzyme. The increase in the relative weight of the liver and the gizzard according to Defang et al. (2008), is related to the intense activity undertaken by these organs due to the toxic effect of the anti-nutritionals factors. This result is close to those of Kana et al. (2012) and Defang et al. (2014) who reported a heavier liver and gizzard of broiler subjected to diet containing crude cowpea and commons bean respectively.

The relative weight of the pancreas increases significantly with the increasing rates of cowpea supplemented or not with enzyme. This result is in agreement with that of Nasara (2009). According to this author, the increase in the volume of the pancreas is caused by the release of the cholecystokinin when the chickens consume feed containing trypsin inhibitors as it is the case with cowpea. In fact, Kapas (2010) reported that the cholecystokinin stimulates the production of the enzymes in the pancreas (trypsin and chymotrypsin), which contributes to hypertrophy this organ.

4. Conclusion

The results of this research indicates that supplementation of crude cowpea by fibrolytic enzyme had no beneficial effect on growth performance and carcass yield of broilers. Other processing methods like boiled or fermented should be considered for better utilization of cowpea in broilers diet.

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