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PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER CHICKENS FED LOW PROTEIN DIETS CONTAINING HYDROLYZED FEATHER MEAL, WITH OR WITHOUT PROTEASE SUPPLEMENTATION

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ABSTRACT

In a 2x2x3 factorial arrangement, two hundred and forty day-old broiler chicks were allocated randomly into eight treatment diets. There were two levels of hydrolyzed feather meal (HFM) 0 and 2%, two levels of protease 0g/kg and 5g/kg and three levels of crude protein 23%, 17.4% and 15.5%. There were ten birds in a replicate and three replicates per treatment diet. Birds were fed with treatment diets for 42 days. The feed intake (FI, g/bird), body weight gain (BWG, g/bird), carcass weights as percentage of live weight were assessed at 42 days. The feed conversion ratio was calculated from the feed intake and the body weight gain. At the end of the experiment birds on dietary treatment 4 had better BWG (1115.3g/bird) than birds fed the control dietary treatment 1, (1077.7g/bird) though not a significant improvement at p=0.05. Treatment 4 produced a lower FI (1280g/bird) compared with birds fed the control diet (1853.7g/bird). Inclusion of HFM significantly decreased BWG and FI from 1083g/bird to 896.3g/bird,and1894.7g/bird to 1403.7g/bird respectively; while protease supplementation significantly improved BWG from 872.2g/bird to 1013.8g/bird and though not significantly decreasing FI from 1553.2g/bird to 1499.8g/bird. All birds fed treatment diets with protease performed better compared with birds fed same feed without protease in terms of live weight, dressed weight dressed percent, breast, thigh and drumstick. Birds fed 15.5% CP containing HFM and protease produced breast meat comparable to birds fed the control diet.

KEYWORDS: Feather meal, Protease, Poultry, Digestibility, Feed enzyme, Performance

INTRODUCTION

All poultry birds have feathers and many poultry birds are raised on commercial scale for food all over the world. These poultry species include chickens, turkey, ducks, quails, guinea fowl etc. Poultry production on commercial scale gives rise to feathers as a by product which is increasing due to the increased production of the different poultry meats worldwide (FAO, 2009). This increased volume of feathers end up in the environment, increasing the nitrogen content of soils because most of the feathers produced are buried in landfills or piled up at dumpsites, some countries even choose the expensive alternative of incineration of feathers (Menandro, 2009).These methods lead to different types of pollution of the environment including the increase of greenhouse gases from poultry production.

Food protein quality is mainly determined by the protein content and the availability of the constituent essential amino acids (Caire-Juvera, *et al.,* 2013) which play important role in growth, reproduction and tissue maintenance. According to Darragh and Hodgkinson (2000), digestibility is the difference between the amount of nutrient intake and the amount excreted expressed as a proportion of the intake. A highly digestible nutrient will be more available for cellular usage and thus, less of the nutrient will be voided.

Feathers make up 6-10% of the weight of a mature broiler chicken (Menandro, 2009; Ajayi, 2014). Chicken feathers contain 83-87% crude protein (CP)as reported by Tiwary and Gupta (2012) and Ajayi (2014). Feathers can be hydrolyzed into feather meal by physical method (high temperature and pressure) (Kim *et al.,* 2002) or by chemical method (Kim *et al.,* 2002), but the resultant product has low digestibility leading to poor performance when fed to poultry (Shiroma and Hongo, 1974). If the protein in poultry feathers can be made more digestible, its use as animal feedstuff will be more beneficial to the animal, the environment and the farmer.

Enzymes are biological catalysts that are specific in terms of the substrate they act upon, if the substrate for an enzyme is not in the medium of reaction, the effect of the enzyme will not be evident. Exogenous feed enzymes are known to improve digestibility and nutrient availability of poorly digestible materials (Kocher *et al.,* 2002; Cowieson and Adeola, 2008), they improve the utilization of poor quality feedstuff and can be used in targeted poultry carcass part production (Ajayi, 2015). Bedford, (2001) also reported that enzyme use in broiler feeding leads to uniform body weight which results in higher profitability. Enzyme use in animal feeding can also lead to a reduction in the inclusion level of nutrients in the diets and subsequent reduction of nutrients passed out in feaces of the animals fed (Oxenboll *et al.,* 2011).

Keratin (the protein of feathers) can be hydrolyzed by keratinases into the constituent amino acids but keratinases are synthesized by microorganisms but not produced by animals. Keratin under normal physiological conditions in the poultry gut is not readily digestible and does not contribute meaningfully to the protein pool in the animal. According to Odetallah *et al.* (2003), keratinase is a broad-spectrum protease that can degrade most proteins, their activities are thus carefully regulated to proceed in the intended location to avoid digestion of the animal’s tissues which could lead to inflammatory reactions in the animal (Isaksen *et al*., 2011). Feed protease has been reported to improve performance (increased amino acid availability despite low dietary protein, maintenance of weight gains from feed efficiency), healthier gut (with less undigested protein in gut, there is lower proteolytic fermentation) and a better environment because same protein retention with lower feed protein and lower feacal protein is achieved (Yan *et al*., 1990).

It was against this background that a feeding trial was conducted to investigate the effect of feeding hydrolyzed feather meal in low crude protein diets supplemented with a protease (CIBENZA DP^{100})on performance indices and carcass measures of broiler chickens.

MATERIALS AND METHODS

Two hundred and forty; one-day old unsexed broiler chickens were randomly allotted into 8 dietary treatments with 3 replicates of 10 birds each in a 2x2x3 factorial arrangement. Feathers (from 8-10 weeks old broilers) were collected, washed, chopped and boiled in a regular home pressure cooker (Masterchef pressure cooker of model MC- 11000PC, working pressure of 80Kpa± 10% Kpa and a safety pressure of 112-160Kpa) for 30 minutes. The resultant product was sundried and labelled hydrolyzed feather meal. There were 2 levels of hydrolyzed feather meal (HFM), 2 levels of protease and 3 levels of crude protein. HFM was fed at 0and 2%, while protease was at 0g and 5g/kg diet and CP levels were 23, 17.4 and 15.5% (Table 1) based on the matrix value (7.5%) of the protease used in this study. All birds were given feed and clean water ad libitum during the study period that lasted for 42 days. Medication and vaccination were administered as recommended. Body weight gain (BWG) was calculated from measured weights of birds and feed intake(FI)was assessed throughout the experimental period from measured feed in trough and remnant feed; while feed conversion ratio (FCR) was calculated from BWG and FI. On day 42, two birds representative of each experimental replicate were weighed and sacrificed by a quick severance of the jugular vein. Weight of dressed bird was taken before cutting into cut up parts to obtain carcass weights.

A two-way ANOVA of GLM procedure in SAS (2004) was employed to analyze data generated and the means were separated by Tukey's HSD; (p<0.05).

Table 1: Experimental treatment diets composition (g/kg)

Treatment/Diet No.	1	2	3	4	5	6	7	8
Protease (g/kg)	0	5	0	5	0	5	0	5
HFM (%)	0	0	2	2	2	2	2	2
CP (%)	23.0	23.0	23.0	23.0	17.4	17.4	15.5	15.5
Ingredients								
Maize	500.0	500.0	510.0	510.0	677.0	677.0	742.0	742.0
Soyabean Meal	352.5	352.5	322.5	322.5	156.0	155.5	100.5	100.5
Fishmeal	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Feather Meal	0.0	0.0	20.0	20.0	20.0	20.0	20.0	20.0
Cassava Starch	5.0	0.0	5.0	0.0	5.0	0.0	5.0	0.0
Palm oil	45.0	45.0	45.0	45.0	45.0	45.0	35.0	35.0
Limestone	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Dicalcium Phosphate	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
NaCl	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral/Vitamin premix*	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Lysine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Protease	0.0	5.0	0.0	5.0	0.0	5.0	0.0	5.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

HFM= Hydrolyzed feather meal, CP= Crude protein

* Vitamins. A 12000000iu; D3 2500000iu; E 20000mg; K3 2000mg; B1 2000mg; B2 5000mg; B6 4000mg; B12 15mg; Niacin 30000mg; Pantothenic acid 11000mg; Folic acid 1500mg; Biotin 60mg; Choline chloride 220000mg; Antioxidant 1250mg; Mn 50000mg; Zn 40000mg; Fe 20000mg; Cu 3000mg; I 1000mg; Se 200mg; Co 200mg.

RESULTS AND DISCUSSION

The results of protease supplementation and hydrolyzed feather meal levels on performance indices of broiler chickens are presented in Table 2. The inclusion of HFM significantly decreased BWG (1083g to 896.3g/bird) and FI (1894.7g to 1403.7g/bird) while protease supplementation significantly increased BWG (872.2g to 1013.8g/bird) but had no significant effect on FI of birds on the experimental diets.

Table 2: Effect of protease supplementation and HFM level on performance indices of broilers (0-42d)

	HFM		Protease		*p-values*	
Parameter	0	2	0	5	HFM*Protease	SEM
BWG (g/bird)	1083.0^{a}	896.3^{b}	872.2^{b}	1013.8^{a}	0.9685	76.6
FI (g/bird)	1894.7^{a}	1403^{b}	1553.2^{a}	1499.8^{a}	0.7178	147.4
FCR	1.8	1.6	1.7	1.6	0.8691	0.2

^{a,b,c} Figures along the row with same superscript are not significantly different statistically.
HFM= Hydrolysed Feather Meal, BWG= Body weight gain, FI= Feed Intake, SEM= Standard Error of Mean, (P= 0.05)

The results of performance indices of birds fed protease, different CP and HFM levels are as presented in Table 3, birds fed diets containing HFM with adequate CP (23%) supplemented with protease had the highest weight gain (1115g/bird) which is comparable to the weight gain of birds on the control diet (1077.7g/bird). The least FI was recorded in birds fed treatment diet 4 (2% HFM, 23% CP and 5g/kg protease) which was similar statistically to diet 8 (2% HFM, 15.5% CP and 5g/kg protease). Birds fed 23% CP containing 2% HFM without protease were among the birds with the lowest weight gains among the treatments. Birds fed the lowest CP level (15.5%) with HFM and protease supplementation (diet 8) performed better than birds fed dietary treatment 3 (containing HFM, 23% CP) in terms of weight gain (Table 3). All birds had FCR in the range of 1.3 to 1.9 with birds fed treatment 4 diets (2% HFM, 23% CP and protease) having the lowest value (Table3).

Table 3: Performance indices of broiler chickens fed protease, different CP and HFM levels (0-42days)

	Treatment No.								
Parameter	1	2	3	4	5	6	7	8	SEM
BWG	1077.7^{a}	1088.3^{a}	848.7^{c}	1115.3^{a}	733.7^{d}	862.3^{c}	828.7^{c}	989.3^{b}	76.6
FI	1853.7^{c}	1935.8^{c}	1484.3^{b}	1280.0^{a}	1429.1^{b}	1450.9^{b}	1445.6^{b}	1332.4^{ab}	147.4
FCR	1.8^{cd}	1.8^{cd}	1.6^{bc}	1.3^{a}	1.9^{d}	1.7^{c}	1.6^{bc}	1.5^{b}	0.2

^{a,b,c} Figures along the row with same superscript are not significantly different statistically. HFM= Hydrolyzed Feather Meal, CP= Crude Protein, BWG= Body weight Gain, FI= Feed Intake, FCR= Feed Conversion Ratio, SEM= Standard Error of Mean, (P= 0.05)
1= 23%CP, 0%HFM and 0g/kg protease; 2=23%CP, 0%HFM and 5g/kg protease; 3=23%CP, 2%HFM and 0g/kg protease; 4=23%CP, 2%HFM and 5g/kg protease; 5=17.4%CP, 2%HFM and 0g/kg protease; 6=17.4%CP, 2%HFM and 5g/kg protease; 7=15.5%CP, 2%HFM and 0g/kg protease; 8=15.5%CP, 2%HFM and 5g/kg protease

Inclusion of HFM had a significant decreasing effect on the live weight (1017.4g to 870.3g) and dressed weight (749.7g to 637.4g), both were improved with protease supplementation. Dressed percent was significantly improved from 72.4 to 74.2% with protease supplementation but remained statistically the same with HFM inclusion. A 2% HFM inclusion in the diets reduced the weight of drumstick significantly from 101.3g to 84.7g while protease effect on drumstick was not significant at $p<0.05$ (Table 4). HFM inclusion decreased weight of breast and thigh while protease supplementation improved them but these effects were not significant at $p<0.05$ (Table 4).

Table 4: Effect of protease supplementation and HFM level on carcass weights of broilers (0-42d)

	HFM		Protease		*p-value*	
Parameter	0	2	0	5	HFM*Protease	SEM
LW(g)	1017.4^{a}	870.3^{b}	880.5^{b}	933.5^{ab}	0.7142	117.9
D.wt (g)	749.7^{a}	637.4^{b}	638.9^{b}	692.1^{ab}	0.7952	88.5
DP (%)	73.7^{ab}	73.2^{ab}	72.4^{b}	74.2^{a}	0.6056	1.8
Breast (g)	182.0	152.7	151.7	168.3	0.1931	32.7
Thigh (g)	104.8	87.8	88.4	95.8	0.4633	17.2
Drumstick (g)	101.3^{a}	84.7^{b}	84.5^{b}	93.2^{ab}	0.3027	14.0

^{a,b,c} Figures along a row with same superscript are not significantly different statistically. HFM= Hydrolyzed Feather Meal, LW= Live weight, D.wt= Dressed weight, DP= Dressed Percent, SEM= Standard Error of Mean, (P= 0.05

Table 5 shows the effect of HFM inclusion, protease supplementation and CP levels on the carcass weights of broiler chickens. Birds fed dietary treatment 8 (15.5% CP, 2% HFM plus protease) had breast comparable to birds on treatment diets with adequate CP (23%) (diets 1, 2 & 4) except for birds fed treatment diet 3.

Table 5: Effect of protease supplementation, HFM inclusion and different CP levels on carcass weights of broilers (0-42d)

Treatment	1	2	3	4	5	6	7	8	SEM
LW(g)	995.1^{ab}	1039.6^{a}	916.9^{bc}	1001.3^{ab}	739.1^{d}	811.1^{cd}	870.9^{c}	882.2^{c}	117.9
D.wt (g)	727.7^{ab}	771.7^{a}	669^{bc}	743.1^{ab}	527.7^{d}	594.3^{cd}	631.6^{c}	659.2^{bc}	88.5
DP (%)	73.1^{a}	74.3^{a}	72.8^{b}	74.2^{a}	71.6^{b}	73.4^{a}	72.6^{b}	74.7^{a}	1.8
Breast (g)	171.6^{a}	192.4^{a}	155.9^{b}	176.3^{a}	136.2^{b}	141.6^{b}	143.3^{b}	163.0^{a}	32.7
Thigh (g)	100.4^{ab}	109.3^{a}	90.4^{ab}	101.8^{a}	70.9^{c}	83.8^{bc}	88.2^{b}	91.8^{ab}	17.2
Drumstick (g)	97.8^{ab}	104.9^{a}	90.5^{bc}	106.8^{a}	70.7^{d}	72.1^{d}	79.2^{cd}	88.9^{bc}	14

^{a,b,c} Figures along a row with same superscript are not significantly different statistically. HFM= Hydrolysed Feather Meal, LW= Live weight, D.wt= Dressed weight, DP= Dressed Percent, SEM= Standard Error of Mean, (P= 0.05) 1= 23%CP, 0%HFM and 0g/kg protease; 2=23%CP, 0%HFM and 5g/kg protease; 3=23%CP, 2%HFM and 0g/kg protease; 4=23%CP, 2%HFM and 5g/kg protease; 5=17.4%CP, 2%HFM and 0g/kg protease; 6=17.4%CP, 2%HFM and 5g/kg protease; 7=15.5%CP, 2%HFM and 0g/kg protease; 8=15.5%CP, 2%HFM and 5g/kg protease

The main interactive effect of the fixed variables on the carcass weight of broiler chickens are as presented in Table 6. There was no significant interaction effect between the variables on the carcass weights when p=0.05.

Table 6: Main interaction effect on carcass weights of broilers fed HFM with protease supplementation at different CP levels (0-42d)

	P-Anova			
PARAMETERS	CP*HFM	CP*Pro	HFM*Pro	CP*HFM*Pro
Liveweight (g)	0.7572	0.9083	0.7142	0.9046
Dressed weight (g)	0.7844	0.8717	0.7954	0.9298
Dressed percent	0.8507	0.8450	0.6056	0.7477
Breast(g)	0.7957	0.4937	0.1931	0.3712
Thigh(g)	0.7536	0.2448	0.4633	0.9565
Drumstick(g)	0.6109	0.0670	0.3027	0.4862

CP= Crude protein, HFM= Hydrolyzed feather meal, Pro= Protease

Many researchers have reported different effects of feeding exogenous enzymes to poultry, in terms of performance characteristics. Some used similar feed enzymes but got different results while some used different enzymes with similar results. The specificity of the enzymes from different sources, the effect of other ingredients in the feed (for instance enzyme cocktails), the physiological conditions of the animal and the dynamics of how these enzymes work within the animals (whether they are same species or different) used is still not well understood. For instance, Ghazi *et al.* (2003) and Freitas *et al.* (2011) reported improvements in BWG of broiler chickens fed diets with protease, though they used proteases from different sources and with different reactivities. Walk *et al.* (2011) however reported a no effect on the BWG of broilers fed a protease. Both BWG and FI were significantly increased at the starter, grower and finisher phases when two enzymes, Kemin and Rovabio were fed to broiler chickens by Goli and Shahryah, (2015). Dersjant-Li *et al.* (2016) used an enzyme blend containing a serine protease in combination with a direct fed microbial (DFM) to reduce the effect of a coccidial challenge in cobb 500 broiler chicks. Oliaei *et al.* (2016) also fed a multi enzyme containing a protease and after feeding broiler chicks for 21 days they recorded a significant improvement in the BWG but the improvement did not reflect in the carcass weights of same birds at slaughter. Yadav and Sah, (2005) fed different levels of a protease and birds fed the recommended level of protease had similar BWG to birds fed the basal diet though there was no significant effect of the protease on the dressing percent. When turkey poults were fed a mono-component protease by Vieira *et al.* (2013) there was no improvement on the BWG of the poults due to protease supplementation.

The results from this study is similar to those reported by Odetallah *et al.*, (2003) where they fed broiler chicks with a keratinase enzyme and their results showed that all birds had improved body weight gain though birds fed low protein diets with the enzyme did not perform as good as those fed the control diet with enzyme; in this study birds on treatment 5, 6, 7 and 8 (diets containing HFM and with inadequate CP) had significantly lower BWG compared to birds on treatment 3 and 4, the control diets (containing HFM) with and without protease respectively. Treatment 4 (23% CP, 2% HFM plus protease) produced similar BWG to treatment 1 (23% CP, 0%HFM minus protease) and treatment 2 (23% CP, 0% HFM plus protease) which is significantly different from the BWG of treatment 3 (23% CP, 2% HFM minus protease), this shows that the inclusion of HFM is likely responsible for the decreased BWG but the supplementation with the serine protease with keratinase activity improved it. The required CP level in treatment 1, 2, 3 and 4 provides enough substrates for the enzymes to allow for maximum availability of the protein components which should result in better body weight gain, however, birds fed diet 3 had lower BWG; showing that the CP though adequate was not available for use by the birds. A factor to consider is the constituent amino acids of any protein source, a digested protein will only make available the amino acids that makes it up; the amino acid requirement of the animal determines to an extent the utilization of the amino acids from the amino acid pool.If the amino acids required for muscle protein synthesis are available, muscle mass will increase which will result in increased body mass and weight, though fat deposition can also increase body weight.

All birds fed diets containing HFM (treatment 3, 4, 5, 6, 7 and 8) had lower FI which were similar except for treatment 4 which produced a significantly lower FI ($p<0.05$).This same trend (lower) was seen in the BWG of birds fed HFM diets (3, 5, 6, 7 and 8) except in birds on diet 4, which produced the best BWG among all treatments. The decreased feed intake may be as a result of post ingestion effects of HFM as suggested by Ferket and Gernat, (2006) but not as a result of energy content (all feeds had adequate energy content of 3000kcal/kg to 3200kcal/kg) or texture since all feeds were pelletized and crumbled to allow birds pick the feed in a form similar to seeds. Since the amino acid profile of feather meal shows a deficiency of essential amino acids for poultry, this could also reduce feed intake (Ferket and Garnet, 2006) and the supplementation of feather meal with these amino acids may improve feed intake. A warm ambient temperature can cause a reduction in feed intake, the warm temperatures during the experimental period (March, 25 - 34°C) could have negatively influenced feed intake in this study because the environmental temperature recommended for Ross 304 broiler chickens (used in this study) after 27 days of age is 20°C (Aviagen bulletin, 2010 and2014).

Though these results are comparable to those of Odetallah *et al*., (2003) since diets used in both studies had adequate energy contents, Fru-Nji *et al.,* (2011) reported a no significant effect of feeding a similar protease to male broiler chicks, this is not at variance per se with the results here when the substrate is considered. In the study by Fru-Nji *et al.,* (2011), the diets did not contain sources of keratin which the protease used would act on but the substrate (HFM) was included in the treatment diets in this study to provide the substrate. This study has raised the issues of amino acid requirements and the formulation of feeds based on the amino acid content of the protein source;

which probably is a better way of assessing any protein source and its utilization for animal feeding. This is more so as alternative, nonconventional and cheaper protein feed sources are increasingly being used in animal feeds and feeding.

CONCLUSION

It can be concluded from these results that supplementation of diets (containing Hydrolyzed feather meal) with a serine protease fed to broiler chickens significantly improved weight gain and dressed percent. Also, that birds fed adequate CP diets containing HFM and protease will perform comparably to birds fed a control diet without HFM (with or without protease supplementation).

In addition, birds fed 15.5% CP containing HFM and protease produced breast meat comparable to birds fed the control diet; though the interactive effect of HFM, CP levels and protease supplementation was not statistically significant in any way.

However, the reduced feed intake observed with HFM inclusion is still a challenge to its effective use in broiler chicken feeding which the protease used in this study did not address.

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