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Abstract

The experiment was carried out to determine if inclusion of cocoa pod husks (CPH) in layer diets will affect laying performance and egg characteristics. Two hundred and sixteen (216) Bovan Brown {BB} layers (92 weeks old) were randomly assigned to twelve experimental diets for 12 weeks in a completely randomized design. There were three levels of CPH inclusion; 0%, 10% and 15%. For each level of CPH, diets were further sub-divided into four and each portion treated with, i) no enzyme, ii) phytase only, iii) a commercial enzyme cocktail only and iv) a combination of both phytase and cocktail. The enzyme cocktail was added at a rate of 200g per tonne of complete feed. The phytase was added at the rate of 250g per ton of complete feed to give a phytase activity of 500 FTU (Phytase Units)/kg of complete feed.

Overall, adding CPH did not affect average daily feed intake (ADFI). Hen day egg production for layers on diets with 0%, 10% and 15% CPH, with a combination of phytase plus an enzyme cocktail (76.19, 73.81 and 66.34 respectively), was better than that of hens on diets without enzymes. Adding either phytase, a cocktail enzyme, or a combination of the two improved egg weight. There were no effects of CPH or enzyme addition on egg quality characteristics. Cocoa pod husk (up to 15%) plus exogenous enzymes can effectively be used in layer diets without adversely affecting production performance or egg quality characteristics.

Key words: *egg production, egg quality, enzymes*

Introduction

Agriculture is the most dominant sector of the economies of many developing countries. In Ghana for example, it contributes about 33.6% of the Gross Domestic Product (GDP) and about 75% of export earnings (GSS 2008). The livestock and poultry sub-sector is estimated to contribute about 6% of Agricultural GDP. It provides employment and helps to meet the basic meat and animal product requirements for the growing Ghanaian population (MoFA 1990). Commercial poultry production, in particular, provides easily accessible and affordable meat and eggs. About 80% of the world's population get most of their basic nutrients like proteins, fats and vitamins from meat and eggs (FAO 2009).

Feeds and feeding constitute a large proportion of the total expenditure in the poultry industry especially when ingredients like grains are used (Teguia and Beynen 2004). Cereals such as maize, rice, wheat and millet constitute the major part of poultry feed and makes up to 60-70% (Ravindran and Blair 1993). In many developing countries, most of the cereal that is grown is for human consumption, and hopefully, surpluses are used for animal feeding. However, the reality is that whatever is available is competed for by humans and livestock for food and feed, respectively. This competition raises the cost of poultry production. A high cost of animal production, translates into a high cost of animal protein. Reducing this cost component in animal (particularly poultry) production therefore is one way of reducing the cost of poultry products. It is, therefore, imperative to find local agricultural residues and by-products to use as alternative feed ingredients in poultry production. These by-products should be cheap, readily available and not be detrimental to the birds' health and productivity (Teguia and Beynen 2004).

Various by-products from the beer and milling industries, and from the cocoa processing industry like defatted cocoa cake (a by-product of the cocoa fermentation process) and cocoa pod husk (CPH), have been used to varying degrees of success in monogastric feeds (Obikaonu and Udedibie, 2006; Adama et al 2007; Husaini et al 2010; Nortey et al 2013a; Manu-Barfo et. al 2013). Cocoa pod husk is a major by-product from commercial cocoa farming, (Alemawor et al., 2009) and forms over 70% (w/w) of the whole matured fruit of cocoa. Ghana grows some of the best cocoa in the world and its economy depends heavily on this crop. However, once the beans have been obtained from the pod, the latter is of little value. Some of this by-product is used for products such as some local soaps, but most of it is not utilized and left to rot on the land. It can however be potentially incorporated into layer diets to reduce the maize (main energy source) requirement. It has high levels of protein, energy and other nutrients. It is however, high in fibre and has high levels of lignin (14%), non-starch polysaccharides (NSP) like hemicellulose (11%), cellulose (35%) and pectin (6%) (Alemawor et al 2009). These nutrients are not readily available to monogastrics (poultry and pigs) because this class of animals lack fibre-degrading enzymes needed to hydrolyze NSP (Barrera et al 2004). Undigested NSP can influence intestinal transit time and increase digesta viscosity. Both result in inefficient nutrient absorption which ultimately affects growth and egg laying performance of animals and birds. In addition, phosphorous (P) in plants and plant products such as CPH is available as phytate-phosphorous (Humer et al 2014) and is not readily available to monogastrics because they lack the enzyme phytase which is responsible for phytate hydrolysis. Even if they do, the quantities are insufficient (Golovan et al 2001). Hence for efficient use of CPH in monogastric diets, it is important to include exogenous fibre-degrading and phytase enzymes in such diets. These enzymes are able to hydrolyse fibre and phytate, improve nutrient utilization and improve performance (Mroz et al 1994; Liao et al 2005; Nortey et al 2013b). Phytase has also been shown to improve amino acid and energy utilization (Nortey et al 2007).

In Ghana, except for a few of the major feed mills, the use of phytases and carbohydrases routinely in feed formulation is not widespread despite the fact that a large proportion of monogastric feeds are based on plant material. This can result in the under-utilization of available feed nutrients and large amounts of undigested nutrients being excreted into the environment. Ultimately there is a drop in production and an increase in production costs. However, routine use of fibre-degrading and carbohydrase enzymes will enable feedmills to take advantage of the vast and diverse feed resources (including non-conventional and sometimes high fibre feed ingredients) available in the West African sub-region. Thus the hypothesis of this study was that CPH together with exogenous enzymes can effectively be used in laying hen diets without adversely affecting performance. The objectives of this study were therefore to determine the effect of exogenous enzyme supplementation in diets with added CPH on ADFI, hen day egg production, feed conversion efficiency (FCE) and egg quality characteristics.

Materials and Methods

The trial was carried out at the Livestock and Poultry Research Centre (LIPREC), College of Basic and Applied Sciences (CBAS) of the University of Ghana (UG).

Processing of cocoa pod husk

Fresh cocoa (*Theobroma cacao*) pods were harvested from the cocoa plantations of the Cocoa Research Institution of Ghana, New-Tafo in the Eastern Region of Ghana. They were cracked open to remove the cocoa beans together with the placenta. The husks were then chopped into slices (average sized 2cm) at the Product Development Unit of the Research Institution. They were dried in the sun for about 24 hours to reduce the moisture content to about 80%. The pre-dried slices were then passed through a combination mincer and pelleting machine to produce pellets (about10-12 mm). The pellets were again dried for about 48-72 hours to further reduce moisture content to about 10% and stored until use.

Experimental diets

Twelve (12) experimental diets (T1 to T12) were used in the trial. Three main diets were initially formulated to contain 0%, 10% and 15% CPH respectively. Each main diet was further sub-divided into four parts. Part one was not treated any further. Parts two, three and four were treated with phytase alone (300g/tonne of complete feed), a commercial enzyme cocktail alone (250g/tonne of complete feed), and a combination of both phytase and a cocktail enzyme at the stated inclusion levels. Thus T1 (CHO), T2 (CHO-PHY), T3(CHO-EC) and T4 (CHO-PHY-EC) represented diets with a) 0% CPH with no exogenous enzyme, b) 0% CPH plus phytase alone, c) 0% CPH plus enzyme cocktail alone, and d) 0% CPH plus a combination of phytase and an enzyme cocktail. Treatments five to eight (CH10, CH10-PHY, CH10-EC and CH10-PHY-EC), and nine to twelve (CH15, CH15-PHY, CH15-EC and CH15-PHY-EC) represented diets with 10% and 15% CPH respectively, and with the same combination of enzymes as was in T1 to T4. The enzyme cocktail contained phytase, amylase, protease, cellulase, xylanase, β-glucanase and pectinase and was supplied by Zoetis under the brand name Enziver.

Experimental birds and design

Two hundred and sixteen (216) Bovan Brown {BB} layers at 92 weeks old were randomly assigned in battery cages to twelve experimental diets in a Completely Randomized Design for 12 weeks. The experiment was set up in a 3 x 4 factorial arrangement of treatments (3 levels of CPH x 4 levels of enzyme treatment). There were 18 birds in each treatment and 6 birds per replicate. The laying hens were allowed seven (7) days on the experimental diets for conditioning. The experiment consisted of three periods of 28 days each. Birds were fed the same treatment diet during the experimental period. Water was provided *ad libitum.* A known amount of feed was provided every morning, and feed left over after 24 hours was collected and weighed to determine feed disappearance. This amount represented the average daily feed intake (ADFI). Feed conversion efficiency (FCE) was calculated as the ratio of weight of eggs to feed intake. Eggs were collected twice a day at 08:00 and 16:00 and grouped according to treatment and replicate. Records of egg weight, hen-day and hen-housed egg production performance, were kept daily and summarized on a weekly basis.

Chemical Analysis

The proximate chemical composition of all the major ingredients used was analyzed using methods outlined in AOAC (1995). Calcium and phosphorus were determined according to the methods outlined by James (1996) and AOAC (1995).

Egg quality analysis

External and internal egg quality measurements were determined on days 28, 56 and 84. Two eggs laid within a two hour period were selected from each replicate group. Parameters measured included shell thickness, egg length and width and albumen height. These measurements were taken within 24 hours after collection and at room temperature. A digital caliper was used to measure egg width (at the broadest end) and length. Egg weight was measured with an electronic balance (OHAUS-Pioneer ™, Ohaus Corp., USA) with a sensitivity of 0.01g. The egg was then cracked and shell thickness and albumin height determined using a digital caliper (A & D Company Ltd). The height of the thick albumen was measured between the yolk and the edge of thick albumen. Three points were measured and the average taken. The Haugh Unit was calculated as has been described by Haugh (1937):

Where:

- HU = Haugh unit
- H= observed height of the albumen in millimeters
- W = weight of egg in grams
- G = the gravitational constant, 32.2

Statistical Analysis

All data gathered were subjected to statistical analysis using the Generalised Linear Model procedure of the Statistical Analysis Systems Institute (SAS 1999). Significant differences among means were separated using the Student Newman-Kuels (SNK) Test. The results from the different breeds were handled separately.

A priori, it was decided to compare the following treatments which were of particular interest, using contrasts:

- All diets without enzymes versus all diets with both phytase plus cocktail
- All diets without enzymes versus all diets with only a cocktail
- All diets without enzymes versus all diets with only phytase
- All diets with only phytase versus all diets with only a cocktail
- All diets with phytase alone versus all diets with both phytase plus a cocktail
- All diets with only a cocktail versus all diets with both phytase and a cocktail

Results

Table 1a shows the chemical composition of CPH. The diets used in the experiment, showing CPH levels and combination of enzymes (T1 – T12) are shown schematically in Table 1b. The composition and calculated values of the layer diets are shown in Table 2. Protein levels dropped from 16.24 to 15.34 as the level of wheat bran reduced and CPH increased. This was due to the relatively lower protein content of CPH compared to wheat bran. A higher fibre content of CPH also meant that the levels of total dietary fibre increased as the level of CPH also increased.

Table 1a: Chemical composition of cocoa pod husk

Parameter	Concentration (%)
Dry matter	85.7
Crude protein	7.04
Crude fibre	31.1
Total ash	9.6
Ether extract	5.93
Calcium	0.81
Phosphorous	0.44

Production parameters

Average Daily Feed Intake

For birds on 0% CPH, those on CH0 and CH0-PHY recorded the minimum and maximum ADFI respectively. For diets with a cocktail enzyme alone (CH0-Phy), ADFI was not different from what was obtained for birds fed a diet with only phytase. At the 10% CPH inclusion, a similar trend was observed, where birds on diets with no enzyme ate less than those on diets with enzymes. However for birds on CH10-PHY, ADFI was lower than for birds on CH10-PHY-EC. Similarly, at the 15% of level of CPH inclusion, the trend in ADFI as was observed at lower inclusion levels, was observed here as well. Birds on CH15 had an ADFI which was lower than the ADFI of birds on CH15-PHY-EC but similar to that of birds on the remaining two diets.

Hen day egg production

Birds on CH0-PHY-EC and CH10-PHY-EC had hen day egg production values which were similar, but higher than egg production values of hens on the remaining treatments. For the rest of the treatments with 0% CPH, birds on the first three treatments had similar egg production values. On the average birds on diets with 15% CPH recorded lower egg production values compared to those on either 0 or 10% CPH.

Table 1b: Set-up of layer diet showing CPH levels and combination of enzymes used

Treatment	Diet	% CPH inclusion	Phytase inclusion	Enzyme cocktail inclusion
T1	CH0	0	yes	no
T2	CH0-PHY	0	no	no
T3	CH0-EC	0	yes	yes
T4	CH0-PHY-EC	0	no	yes
T5	CH10	10	yes	no
T6	CH10-PHY	10	no	no
T7	CH10-EC	10	yes	yes
T8	CH10-PHY-EC	10	no	yes
T9	CH15	15	yes	no
T10	CH15-PHY	15	no	no
T11	CH15-EC	15	yes	yes
T12	CH15-PHY-EC	15	no	yes

Egg weight

For each level of CPH inclusion (0, 10 and 15%) egg weights seemed to increase with enzyme supplementation. At the 0% CPH inclusion, egg weights for birds on CH0-PHY-EC was similar to those on CH0-PHY, but heavier than those on the remaining two. At 10% CPH inclusion, birds on CH10-PHY-EC had the heaviest eggs which was heavier than that for birds on 10CP-Cont and similar to the remaining two.

Average daily feed intake of, birds on treatments without any added enzyme was lower than that of birds with a combination of both enzymes. Birds fed on diets with either phytase, or a cocktail alone irrespective of CPH inclusion level, ate less than birds that were fed diets with a combination of phytase and a cocktail.

Enzyme addition

Average hen day egg production of birds on diets without any enzyme irrespective of CPH inclusion was 60.6% and was lower than egg production of birds on both phytase plus an enzyme cocktail (72.11%). Adding a cocktail enzyme resulted in an egg production of 65.1%, which was better than adding only phytase to the diet (64.32%). The trends noted for hen day production were observed in egg weights as well. Egg weight of birds on diets without added enzyme irrespective of CPH inclusion (61.15g) was lower than egg weight of birds that had been fed diets containing both phytase plus an enzyme cocktail (63.72g). Compared to eggs from diets that had phytase alone (63.05g), birds fed diets with a cocktail enzyme laid eggs that averaged 63.24g in weight, and these were different from one another.

Table 2: Composition of the layer diets

Ingredients (%)	0%CPH*	10%CPH	15%CPH
Corn	51.6	51.6	51.6
Soybean meal	18.0	18.0	18.0
Wheat bran	20	10	5
Cocoa pod husk	0	10	15
L-Lysine HCl	0.15	0.15	0.15
DL-Methionine	0.15	0.15	0.15
Limestone	8.0	8.0	8.0
Dicalcium phosphate	0.70	0.70	0.70
Salt	0.50	0.50	0.50
Layer premix	0.25	0.25	0.25
BE3[1]	0.50	0.50	0.50
Toxin binder[2]	0.20	0.20	0.20
Total	100	100	100
Calculated Analysis			
ME, MJ/kg	9.75	9.55	9.45
CP%	16.2	15.6	15.3
CF%	3.57	4.38	4.79
Lys %	0.98	0.98	0.98
Met%	0.40	0.38	0.36
Ca%	3.12	3.14	3.14
P%	0.65	0.57	0.53

*Cocoa pod husk

1: *Lactobacillus spp, Bacillus spp, Saccharomyces spp* and Fermentation products.

2: Mycofix® Select 3.0 by Biomin

A. priori contrasts were performed to determine which of the enzymes and/or combination of enzymes resulted in improved production characteristics irrespective of CPH inclusion. A comparison between diets with added cocktail enzymes or those with only phytase, versus diets with no enzymes at all indicated that the birds did not benefit significantly (Table 5). However, comparing the efficacy of the two classes of enzymes showed that improvements occurred only when both enzymes were added to the diet and the results compared to diets without any added enzyme.A similar trend was observed in hen day egg production and egg weight, where the birds reacted favourably only when a combination of the two enzymes was added to the diet. Comparing the two enzymes separately however showed that the cocktail enzyme performed better than phytase.

Table 3: Effect of cocoa pod husk on egg laying performance

Diet /Parameter	Inclusion level of Cocoa pod husk													
	0%				10%				15%					
	Enzyme				Enzyme				Enzyme					
	#CH0	CH0-PHY	CH0-EC	CH0-PHY-EC	CH10-	CH10-PHY	CH10--EC	CH10-PHY-EC	CH15	CH15-PHY	CH15-EC	CH15-PHY-EC	SEM	P-Value
ADFI (g)	105^{cd}	110^{a}	109^{ab}	108^{4abc}	102^{e}	105^{d}	106^{cd}	108^{abc}	106^{d}	107^{bcd}	107^{bcd}	108^{abc}	0.77	<0.001
HDE (%)	67.3^{c}	67.7^{c}	68.1^{c}	76.2^{a}	61.8^{d}	66.1^{c}	70.6^{bc}	73.8^{ab}	52.7^{f}	59.2^{de}	56.6^{e}	66.3^{c}	1.19	<0.001
Egg weight (g)	62.8^{de}	64.3^{ab}	63.9^{bc}	64.7^{a}	59.5^{g}	62.7^{de}	63.1^{cde}	63.4^{cd}	61.1^{f}	62.1^{e}	62.8^{de}	63.1^{cde}	0.25	<0.001
FCE	0.60	0.59	0.59	0.61	0.59	0.61	0.61	0.59	0.59	0.59	0.59	0.59	0.01	0.198

*CHO= No enzyme added: PHY = Phytase added; EC = Enzyme cocktail added; SEM = Standard Error of Means; HDE =Hen Day Egg Production; CH = Cocoa Pod Husk; #0, 10, 15 = Level of inclusion of CH

Table 4: Effect of cocoa pod husk on egg quality characteristics

Diet /Parameter	Inclusion level of cocoa pod husk													
	0%				10%				15%					
	Enzyme				Enzyme				Enzyme					
	#CH0	CH0-PHY	CH0-EC	CH0-PHY-EC	CH10	CH10-PHY	CH10--EC	CH10-PHY-EC	CH15	CH15-PHY	CH15-EC	CH15-PHY-EC	SEM	P-Value
Egg Length (mm)	55.9	61.2	60.2	60.5	59.0	58.8	59.8	62.5	60.2	61.7	59.2	60.8	1.35	0.735
Egg width (mm)	44.0	43.0	44.7	42.7	42.7	42.3	41.7	44.0	41.8	42.5	42.3	43.2	0.75	0.167
Shell thickness (mm)	0.38	0.45	0.45	0.45	0.41	0.40	0.37	0.42	0.43	0.34	0.39	0.43	0.03	0.598
Albumin height (mm)	7.50	7.11	7.27	7.27	7.18	7.31	7.56	7.22	7.31	7.11	7.12	7.18	0.21	0.45
Haugh Unit	70.0	66.7	68.2	68.1	67.2	68.4	70.7	67.5	68.4	66.6	64.4	67.3	1.78	0.63

**CHO= No enzyme added: PHY = Phytase added; EC = Enzyme cocktail added; SEM = Standard Error of Means; HDE =Hen Day Egg Production; CH = Cocoa Pod Husk; #0, 10, 15 = Level of inclusion of CH*

Discussion

Production parameters measured in this trial included ADFI, hen-housed egg production, egg weight and FCE. External and internal egg parameters measured included shell thickness, egg length and width, albumin height and Haugh unit.

Effect of CPH

There was similarity in ADFI in spite of increasing fibre levels and slightly reducing energy densities as the level of CPH increased in the diet. Generally where gut fill is not a factor, monogastrics like birds will eat more of a diet that is low in nutrient density, in an attempt to meet their daily nutrient requirements, particularly energy (Leeson and Summers 1997). This phenomenon has been observed in broilers by Alemawor et al (2010) and in layers by Umar Faruk et al (2010). However since it was not observed in the current study, it may be concluded that dietary energy dilution was not enough to elicit an increased feed intake. The layers used in this trial were 92 weeks old and thus probably had well developed caeca with a great number and diversity of microbes which were capable of breaking down fibre and releasing nutrients in the form of volatile fatty acids (VFA). This will contribute to the maintenance energy requirements of the birds and prevent any expected increase in ADFI.

Hen housed egg production generally decreased with increasing levels of CPH. There was a 2 percentage point drop in egg production when the level of CPH in the diet went from zero to 10%, whilst a 10% dip in production occurred when the CPH level was further increased to 15%. It can be assumed from this observation that any ANF present in CPH exerted a negative effect on egg production at levels beyond 10%.

There were similar egg weights for diets with 0, 10, and 15% CPH respectively. Among the factors affecting the weight of an egg ADFI plays a key role. Thus similarities in ADFI over the three levels of CPH inclusion, could explain the lack of differences in egg weight that were observed. Feed conversion efficiency in egg production is the efficiency of converting feed into eggs. With the observed trend in ADFI and egg weights the lack of effect of CPH level in the diet on FCE was expected. The lack of an effect of CPH on shell thickness may be due to the fact that the levels of both calcium and P in the diet, which are the main minerals needed for egg shell formation were adequate and not out of balance. Internal egg parameters were not affected by the level of CPH in the diet.

Effect of enzyme addition

Enzymes are biological catalysts that speed up chemical reactions in biological systems. Although addition of phytase or a cocktail enzyme by themselves tended to increase ADFI in this trial, a combination of both phytase and a cocktail, caused a more drastic effect. This trend was the reverse of what was observed with pigs (Nortey et al 2013b; Kies et al 2005; Madrid et al 2013) and in poultry (Osei and Oduro 2000) where enzyme addition tended to reduce ADFI.

Table 5: p-values of A priori treatment comparisons of interest: production parameters

Parameter	*None vs PHY +EC	None vs EC	None vs PHY	PHY vs EC	PHY vs PHY+EC	EC vs PHY+EC
ADFI	0.007	0.089	0.805	0.147	0.003	<0.001
Egg production	<0.001	0.058	0.284	0.003	<0.001	<0.001
FCE	0.082	0.482	0.815	0.636	0.131	0.299
Egg weight	<0.001	0.100	0.249	0.005	<0.001	<0.001

**None= No enzyme added; PHY = Phytase added; EC = Enzyme cocktail added; SEM = Standard Error of Means*

The theory behind a drop in ADFI upon enzyme supplementation is the phenomena of nutrient uplift. It is generally assumed that exogenous enzyme addition causes a release of nutrients. This will cause the daily nutrient requirement of animals to be met faster. In a situation like this, some mechanisms that control feed intake including the glucostatic, thermostatic, lipostatic, aminostatic and ionostatic theories will come into play to stop further feed ingestion. However, the glucostatic theory of feed intake regulation as observed in some mammals is said to be either not present in poultry or is not well understood (Richardson 1970; Smith and Bright-Taylor 1974). In the absence of such a theory occurring in this trial, one can speculate that inclusion of enzymes, in addition to releasing nutrients, may have reduced (or prevented) any possible fibre-inducing intestinal viscosity. This will result in slightly faster intestinal transit times leading to a feeling of emptiness and hence the need to increase ADFI. The fact that a combination of both enzymes in the diet resulted in greater ADFI compared to either enzyme alone indicates that there is synergy between both enzymes. Hen day average egg production and egg weights also increased with added enzymes at each level of CPH inclusion. This could be a direct result of the increased ADFI. Some factors that influence egg production and weight are level of protein and balance of amino acids, fat, and water intake. For diets that contained CPH, levels of these nutrients tended to reduce slightly as a result of the dilution effect of increased fibre from the CPH. However since hen day production and egg weights tended to improve, it can be speculated that enzyme addition improved nutrient availability.

The observed results in the *a priori* comparisons were expected since in addition to phytase, the cocktail enzymes contained other enzymes which together would act synergistically to improve nutrient digestibility (Nortey et al 2008).

Conclusions

- Cocoa pod husk was included in diets for layers up to a level of 15% and in the presence of enzymes without affecting production.

- There was a synergistic effect between phytase and a cocktail enzyme in improving egg laying and production performance.

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