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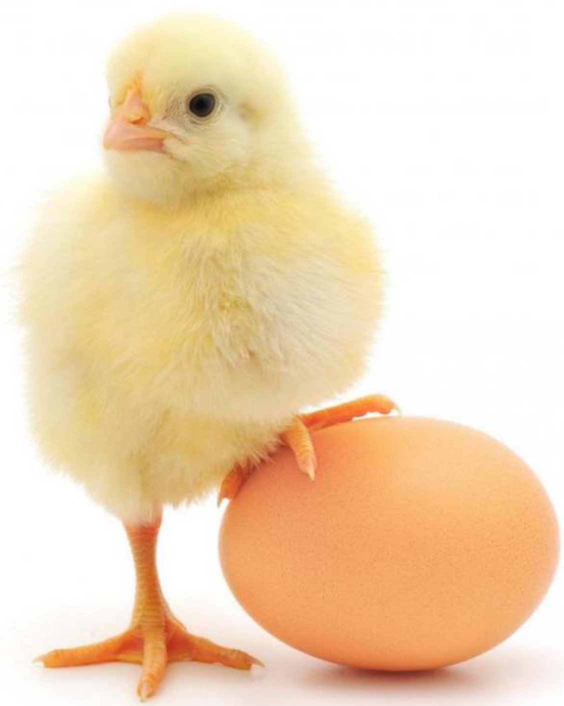

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Response of Broiler Chicks Fed Increasing Levels of Copra Meal and Enzymes

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Abstract: A study was performed to test the efficacy of three types of commercial enzymes, to determine how much copra meal can be used in chick diets and to investigate whether there is an interaction between type of enzyme and level of copra meal in the diets. Sixty four male Ross chicks were used in a two way factorial design experiment with four levels of copra meal (0, 10, 30 and 50 percent) and four enzyme treatments (no enzymes, "Hemicell", "Allzyme SSF" and a combination of "Gamanase", "Hemicell" and "Allzyme SSF") with four replicates. All levels of inclusion of copra meal decreased weight gain, feed consumption, feed conversion efficiency, DM digestibility, nutrient digestibility, AME and jejunal content viscosity significantly. In general, the amount of decrease was proportional to the amount of copra meal in the diet. The inclusion of each enzyme significantly increased weight gain, feed conversion efficiency, DM digestibility, nutrient digestibility and decreased jejunal content viscosity. However, none of the enzyme treatments was able to completely overcome the growth depression of chicks caused by the inclusion of copra meal in the diet.

Key words: Copra meal, enzymes, young chicks, broiler performance

Introduction

Copra meal, the by-product of coconut oil extraction, has been reported to be qualitatively poor due to low concentrations of several limiting amino acids (Creswell and Brooks, 1971; NRC, 1994), heat damage (Butterworth and Fox, 1962) and high dietary fiber (Rama *et al.*, 1965; Knudsen, 1997). These problems have attempted to be addressed by either carefully formulating a diet to meet poultry requirements, particularly amino acids (Panigrahi *et al.,* 1987), or by the inclusion of enzymes (Pluske *et al.*, 1997). Panigrahi *et al.* (1987) obtained satisfactory growth of broilers by 25% copra meal diet with 0.5 % lysine.

Nutritionally, copra dry matter contains 48% carbohydrate, 5% lignin, 21% protein and 5.7% lipid (Sundu and Dingle, 2003). On a dry matter basis, copra meal contains 25-30% mannan as both pure mannan and galactomannan. Balasubramaniam (1976) found that the NSP of copra meal is in the form of mannan (26%), galactomannan (61%) and cellulose (13%). Since the galactomannan in legumes such as guar bean, locust bean and soybean, has been found to be anti-nutritional, it was likely that copra galactomannans would be anti-nutritional as well. Some mannans and galactomannans have also been found to be linked to cellulose (Balasubramaniam, 1976; Whitney *et al.*, 1998). From the composition of carbohydrates of copra meal, it seemed that three enzymes; mannanase, alpha galactosidase and cellulase may be needed to break down the main polysaccharide components. The use of mannanase alone may not be effective in digesting mannan when it is linked to galactose and cellulose. Balasubramaniam (1976) used cellulase, galactosidase and mannanase with some success in the laboratory to break down these substances. In this trial, "Hemicell" (β-mannanase), "Gamanase" (containing β-mannanase and α-galactosidase) and a mixed enzyme preparation, "Allzyme SSF" (containing 7 enzymes, namely cellulase, pentosanase, protease, phytase, β-glucanase, amylase and pectinase) were used singly and in combination to test their efficacy in improving the value of copra meal for broiler chicks.

Materials and Methods

One hundred day old male Ross broiler chicks were placed in a floor pen from day 1 to 4 and given a control diet. After the initial 4-day period, 64 chicks, weighing between 51 and 76 g (65.5±0.77; mean±SE), were selected to minimize animal variation and transferred into individual cages in a controlled temperature room and exposed to 23 h light and 1 h dark and fed the experimental diets ad libitum for 10 days, from day 4 to 14. Four chicks were allocated randomly to each treatment. After data collection for production parameters, three representative chicks from each treatment were kept in the metabolism cages for 6 more days, from day 14 to 20, to measure digestibility.

Copra meal obtained from Papua New Guinea was used in this experiment (Table 1). The diets contained 0, 10, 30 and 50% copra meal and were formulated to

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Table 1: Fractions of copra meal used in this experiment

Fractions	Concentrations (mg/g)
DM	903
Crude protein	217
Arginine	30.5
Lysine	5.5
Methionine	3.3
Gross energy (k Cal/kg)	4,247
Crude fibre	141
Neutral detergent fibre	617
Lipid	69
Ash	56
Bulk density	0.49
Water holding capacity	4.14

meet the nutrient requirements of starter broilers as recommended by NRC (1994) (see Table 2). Each of these basal diets had one of four enzyme mixtures added; 0, Hemicell®, Allzyme SSF® or a mixture of Hemicell, Gamanase® and Allzyme SSF.

Twenty ml of a solution of "Gamanase" was dissolved in 250 ml tap water and sprayed onto 10 kg feed (to produce a dose rate of 0.2%) using a small pressure sprayer while the diets were being mixed in a cement mixer. The diets were then air-dried for 3 days prior to feeding. A multi-enzyme supplement "Allzyme SSF" and "Hemicell" were added as powders to the diets as recommended by the manufacturers (0.02% and 0.05% of the diet respectively). Feed and water were given ad libitum. Feed intake and weight gain were measured four times, at age 4, 11 and 14 days of age or day 0, 7 and 10 days of feeding the test diets.

A method of Kyriazakis and Emmans (1995) was used to measure water holding capacity (WHC). A 0.5 gram oven-dried sample was placed in a 15 ml tube and soaked for 24 hours in distilled water. It is then centrifuged at 6000 g for 15 min. The supernatant fraction was decanted and the fresh weight of feed was measured. After freeze-drying, the samples were weighed and the WHC was calculated as g water/ gram feed. Amino acid was analyzed by a method based on Siriwan *et al.* (1993) and Ravindran *et al.* (1999). Bulk density of the material was measured by weighing the amount of material in a known volume container.

Faeces were collected from three birds per treatment for three days (from day 18 to 20), weighed daily and each day's production was placed in marked plastic bags and stored frozen. Representative samples of the mixed faeces of all birds on a diet for the 3 days were then dried at 65°C for 48 hours for analysis of dry matter, lipid, protein, NDF and energy according to the methods of the AOAC (1970). The feed intake of each chicken was also recorded daily. Dry matter digestibility and apparent metabolizable energy (AME) were calculated using the marker " celite" (acid insoluble ash). Analysis of acid insoluble ash was based on the method of Siriwan *et al.* (1993).

The birds were killed by cervical dislocation on day 21 and jejunal digesta (from the distal part of duodenal loop to Meckel's diverticulum of the small intestine) was collected and then stored frozen. Jejunal viscosity was analyzed based on the procedure of Perez-Maldonado *et al.* (1999). The samples were thawed and centrifuged at 1459 X g for 15 minutes at a temperature between 22°C-25°C. A sample of 0.5 ml was taken from the supernatant fraction and the viscosity was measured using a Brookfield LVTCP model viscometer with a CP-40 cone and expressed in centipoise (cP) units.

The experimental design was a two way factorial with four basal diets, four enzyme treatments and four replicate chickens in single bird cages in which birds were randomly allocated to each cage. Data was analyzed by analysis of variance using the SAS 6.2 statistical program (SAS Institute, 1990). The significance of difference between pairs of treatment means within any overall treatment effects, found significant by analysis of variance, was tested by Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results

The means of feed intake, weight gain, FCR and DM digestibility, nutrient digestibility, AME of the diets and jejunal viscosity of chicks fed the different levels of CM from d 4 to 14 are shown Table 3. Chicks fed the control diet consumed significantly more feed and had higher weight gain and lower FCR than those fed the copra based diets. There were significant linear ($P<0.05$) decreases in feed intake, weight gain, feed efficiency and DM digestibility with increasing levels of copra meal in the diets. Protein and NDF digestibility, AME and viscosity decreased significantly at the higher levels of CM in the diet. Lipid digestibility increased significantly at the higher levels of CM in the diet (Table 3).

The addition of each enzyme treatment improved the weight gain, FCR, DM digestibility and nutrient digestibility and AME of the diets (see Table 4) but no significant change occurred in feed intake. Viscosity, on the other hand, decreased significantly when mannanase was added to the diets. There was no significant interaction between basal diet and enzyme addition for any parameters except for DM and nutrient digestibility, where the addition of the mixed enzymes produced a significantly greater improvement in the DM and nutrient digestibilities of the 50 % CM diet than in the other basal diets. The interactions between feed and enzyme were found in DM digestibility, protein digestibility, lipid digestibility and AME of the diet (Table 5).

Discussion

Early findings by Teves *et al.* (1989) and Pluske *et al.* (1997) indicated that the inclusion of mannanase in a

Table 2: Diet composition

Dietary components (g/kg)	Diet 1	Diet 2	Diet 3	Diet 4
Copra meal	0.00	100.0	300.0	500.0
Maize	569.4	481.5	316.4	175.0
Soybean	253.3	213.5	135.0	30.0
Fish meal	100.0	105.0	115.0	134.3
Sunflower oil	44.6	65.0	105.0	138.0
Limestone	5.0	4.9	3.8	0.2
Dicalcium phosphate	6.2	4.9	1.8	0.5
Salt	1.9	5.2	2.5	3.8
Celite	15.0	15.0	15.0	15.0
Vitamin mix	1.0	1.0	1.0	1.0
Mineral mix	1.0	1.0	1.0	1.0
DL-Methionine	2.0	1.5	1.5	1.5
L-Lysine	1.5	1.5	2.0	3.5
Calculated analysis				
ME (MJ/kg)	13.39	13.39	13.39	13.39
Protein	230.1	230.1	230.4	230.3
Meth + Cys	9.0	9.0	10.0	10.0
Avail lysine	11.0	11.0	11.0	11.0
Arginine	14.3	16.2	20.1	23.6
Ca	10.0	10.5	11.2	11.9
Avail phosphorus	0.54	0.59	0.68	0.83
Analyzed composition				
Gross energy (MJ/kg)	17.79	18.11	18.51	19.14
Protein	249	230	235	232
Lipid	73	89	114	150
NDF	116	177	253	354
Bulk density (g/cm^3)	0.68	0.63	0.59	0.56
WHC (g water/ g feed)	2.6	2.8	3.2	3.6

copra diet improved feed intake. A very small (but non-significant) improvement of feed intake of chicks fed the experimental diets from d 4 to 14 occurred when the diets were supplemented with enzymes in this experiment (see Table 4). This may indicate that there was an increased digesta flow rate due to the greater hydrolysis of copra by the added enzymes in the diets. Although the feed intake did not increase significantly, the feed conversion ratio and weight gain did increase significantly with enzyme addition and they appear to correlate well with the increases in DM digestibility.

The reduction in weight gain due to the inclusion of even 10 % CM indicates that copra meal impaired the quality of the diets even though the nutrients contents were similar. The dry matter (DM) digestibilities of the diets containing CM were lower than that of the non-supplemented diet (see Table 3) and they had a strong negative correlation (R^2=0.98; Y= 0.35x + 79.3) with the percentage of CM in the diet. A similar pattern was also shown in the protein digestibility and in the AME of the diet. Low digestibility of protein and lipid due to increase level of dietary fibre may be due to the dietary fibre blocking the access of enzymes to cell contents (Knudsen, 1997).

The main factor that impaired the digestibility of the diet was not related to viscosity but it may have been related to the increase in dietary fibre in the diet (see Table 2). Young chicks have a limited ability to digest dietary fibre (Bolton, 1955). Thus, it is not difficult to postulate that an increased intake of dietary fibre decreases total digestibility of the diet. The consequence is less energy absorbed and that may be the reason for lower AME of the CM diets in this present study. The high viscosity of corn/soy diet found in this study was possibly contributed to by the water soluble NSPs in soybean (Jackson, 2001). Accordingly, when soybean inclusion in the diet decreased as a consequence of an increased level of CM, jejunal viscosity decreased. However, it is of interest that the galactomannan of CM did not increase digesta viscosity, unlike the galactomannan of guar meal (Dingle and McNab, 1979; Rayment *et al*., 1995).

Over-heating of copra meal during processing, either in the drying or oil extraction processes may also be a possible factor in causing low digestibility in copra-based diets. The very dark brown colour of the copra meal used in this experiment may be an indicator that over-heating took place. According to Guarte *et al*. (1996), during drying, copra becomes totally brown at a temperature of 100^{o}C. The Maillard products generated during processing could have been responsible for

Table 3: The effect of level of CM on the response of broiler chicks, day 4-14

Parameters	0 % CM	10 % CM	30 % CM	50 % CM
Weight gain (g)	300.1[a]	207.2[b]	210.7[b]	148.5[c]
Feed intake (g)	339.0[a]	291.8[b]	294.3[b]	250.4[c]
FCR	1.13[a]	1.42[b]	1.41[b]	1.72[c]
DM digestibility (%)	80.1[a]	75.3[b]	70.3[c]	64.0[d]
Protein digestibility (%)	73.3[a]	69.4[b]	70.0[ab]	66.7[b]
Lipid digestibility (%)	93.4[c]	93.2[c]	94.5[b]	95.4[a]
AME diet MJ/kg	13.33[a]	12.95[ab]	12.78[b]	12.21[c]
Viscosity (cP)	2.34[a]	2.21[b]	1.84[c]	1.61[d]

Values with the same superscript within a row are not significantly different (P<0.05)

Table 4: The effect of enzyme supplements on the response of broiler chicks, day 4-14

Parameters	Nil	Mannanase	SSF	Mannanase+ Gamanase + SSF
Weight gain (g)	200.5[b]	221.3[a]	220.1[a]	224.9[a]
Feed intake (g)	290.8	295.4	300.5	298.0
FCR	1.56[a]	1.39[b]	1.42[b]	1.37[b]
DM digestibility (%)	67.2[b]	75.1[a]	73.9[a]	73.5[a]
Protein digestibility (%)	63.7[b]	72.2[a]	70.4[a]	73.1[a]
Lipid digestibility (%)	92.8[b]	94.8[a]	94.8[a]	94.1[a]
AME diet MJ/kg (%)	12.13[b]	13.03[a]	13.10[a]	13.02[a]
Viscosity (cP)	2.09[a]	1.90[c]	2.06[a]	1.98[b]

Values with the same superscript within a row are not significantly different (P<0.05)

Table 5: The effect of enzyme supplements in the different levels of CM on the response of broiler chicks, day 4-14

Diet	Enzyme	Digestibility (%)			AME (Kcal/kg)
		Dry Matter	Protein	Lipid	
Corn-soy	Nil	79.8[ab]	72.3[a]	93.4[abcde]	3190[a]
	M)	81.1[ab]	75.1[a]	95.3[abcd]	3205[a]
	SSF	81.7[a]	75.1[a]	93.0[cde]	3220[a]
	M+G+SSF	78.4[ab]	69.4[a]	92.0e	3112[a]
10 % CM	Nil	72.9[ab]	65.0[ab]	91.9e	2989[a]
	M)	77.8[ab]	73.6[a]	93.8[abcde]	3142[a]
	SSF	75.1[ab]	66.0[ab]	94.2[abcde]	3139[a]
	M+G+SSF	76.0[ab]	72.5a	92.8[de]	3098[a]
30 % CM	Nil	68.8[ab]	68.3[a]	92.8[de]	2981[a]
	M	68.4[ab]	66.2[ab]	93..2[bcde]	3003[a]
	SSF	72.2[ab]	71.7[a]	96.2[ab]	3115[a]
	M+G+SSF	70.9[ab]	74.0[a]	95.6[abcd]	3023[a]
50 % CM	Nil	47.1[c]	51.2[b]	93.0[cde]	2422[b]
	M	72.3[ab]	71.4[a]	96.6[a]	3165[a]
	SSF	66.5[b]	69.2[a]	96.0[abc]	3057[a]
	M+G+SSF	69.6[ab]	74.9[a]	96.1[abc]	3080[a]

M: Mannanase; SSF: multi enzyme Allzyme SSF; G: Gamanase

depressing feed digestibility (Van Soest and Mason, 1991).

The use of enzymes to improve the quality of feedstuffs has been shown for a number of feedstuffs (Choct, 1996; Kumar *et al.,* 1997). The significant improvement of weight gain, FCR and DM, protein and lipid digestibility and AME of the diet found in chickens fed the enzyme supplements indicates that all the enzymes used in this current study were effective. The improvement in nutrient digestibility indicates that protein and lipid may have been linked to the mannan backbone. Knudsen (1997) found that the endosperm cell wall encloses intra-cellular protein and lipid and thus prevents their digestion and absorption. Accordingly, breaking down the mannan backbone through the addition of mannan-degrading enzyme may have been the reason why the digestibility of all nutrients, particularly lipid and protein, increased. The interaction between diet and enzyme was found in the 50 % CM diet. Enzyme inclusion in this diet increased DM

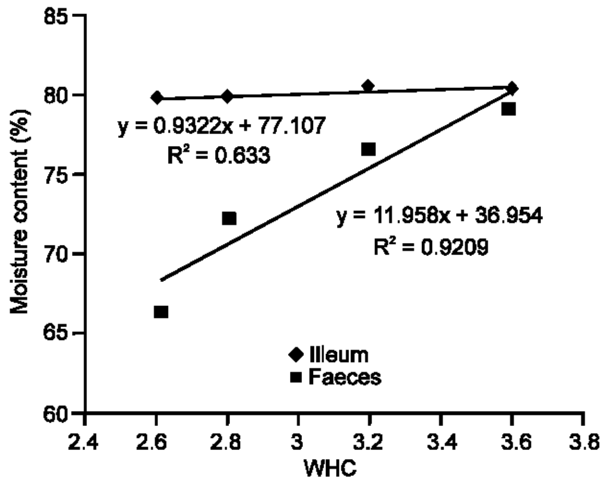

Fig. 1: Correlation between water holding capacity (WHC) of the diets and moisture content of ileal digesta and faeces

digestibility, protein digestibility and AME of the diet (See Table 5). The efficacy of Allzyme SSF could not be maintained in the 50 % CM diet, compared with the corn-soy diet.

The coefficient of variation of the weight gain also improved when enzymes were included, decreasing from 36 % (no enzymes) to 24 % (for the three enzyme treatment). Although the use of enzymes successfully improved weight gain, uniformity, DM digestibility and decreased FCR of chicks at d 14, the enzymes used did not increase the weight gain to the same level as chicks fed the maize-soy based diet. Due to the increased digestibility of nutrients of the enzyme supplemented diets in the short period of this trial, it is possible that if birds are fed enzyme supplemented diets for a longer period of time, there may be a better result from feeding enzyme supplemented CM.

Since many previous findings indicated that copra meal could be used at the level of 20% and even 40% in an amino acid-supplemented diet, the negative effect of using copra meal in this current study may be due to the quality of copra meal used. The dark brown colour of the copra meal indicated that over-heating and nutrient destruction might have taken place during the drying and oil extraction processes.

A correlation of 0.92 was found between level of CM in the diet and moisture content of the faeces whereas there was a low correlation of 0.63 between the level of CM in the diet and moisture content in the ileum. It appears that the moisture content in the ileum was fairly constant (about 80 %) but that about a quarter of this moisture is reabsorbed by the chicken fed a corn-soy diet. However, very little of this moisture is able to be reabsorbed by the colon of a chicken fed a 50 % copra diet (Fig. 1)

The substitution of 10-50% copra meal in a maize-soybean diet depressed feed intake by between 13% (30% CM) and 26% (50% CM). This marked reduction in feed intake may have been due to the bulkiness and NSPs of copra meal. The current finding is different from the finding of Wignjosoesastro *et al*. (1972), who found that the inclusion of increased concentrations of copra meal in a Leghorn pullet diet fed for 168 days increased feed intake linearly. A possible reason for the difference between the two studies is the age of the birds, because older birds have a greater intake, a greater ability to digest NSPs (Bolton, 1955; Panigrahi *et al*., 1987; Carre *et al*., 1995) and possibly a greater ability to increase gut capacity than young chicks. Panigrahi *et al*. (1987) also noted that feed intake was lower in the first two weeks of life for chicks fed 12.5% copra meal and in the first four weeks of life for chicks fed 25% copra meal. It appears that young chicks take some time to adapt to consuming copra meal.

The weight gain of chicks from 4-14 days was negatively related to the amount of CM in the diet. This finding is consistent with the previous finding of Thomas and Scott (1962). The pattern of weight gain of chickens fed copra diet has been studied by Panigrahi *et al*. (1987), who found that birds fed 25% CM had lower body weight in the first 5 weeks and accelerated their growth in the following weeks. This indicates that chickens gain a better ability to handle CM as they get older.

There was a significant linear correlation between feed intake and weight gain (R^2 = 0.99). Calculation of daily intakes of crude protein, lysine and methionine + cystine showed that the inclusion of CM decreased the intake of protein by 13-26%, lysine by 18-30% and methionine by 3-17%, so that daily amino acid intakes were below those required for growth of broilers (NRC, 1994).

It is possible that the decrease in bulk density with each increase in the levels of CM in the diet (Table 2) contributes to the decrease in feed intake. The volume of the mean feed intake for each of the diets was [339 X1/0.68] = 498.5, [207.2 X 1/0.63] = 328.8, [210.7 X 1/0.59] = 357.1 and 148.5 X 1/0.56] = 265.2 for 0, 10, 30 and 50% CM respectively. The ratio between intake volume and chicken weight was fairly constant, being 1.7, 1.6 1.7 and 1.8 for 0, 10, 30 and 50% CM diets respectively.

In conclusion, increased levels of CM in the diet impaired feed digestibility, AME of the diet and broiler production. Jejunal digesta viscosity decreased due to CM addition. Inclusion of enzymes improved weight gain, feed conversion ratio, and feed digestibility and decreased jejunal digesta viscosity.

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