# THE EFFECT OF CARBOHDRATE HYDROLYZING ENZYMES ADDITION ON THE NUTRITIVE VALUE OF POULTRY FEEDS

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**ABSTRACT**

The usefulness of carbohydrate hydrolyzing enzymes in animal feeds and their potential utilization in broiler diets was investigated. A 42-day of feeding trial involving 44, one week day old *Cobb 500* broiler was carried out, in a completely randomized design (CRD) to evaluate the growth characteristics using enzymes at a dietary level of 0.07% Natugrain (T1), 0.07% Digestase (T2) and 0.035% Natugrain + 0.035% Digestase (T3) meals. Daily feed intake, body weight and growth rate of feed conversion ratio was determined for each diet. Proximate analysis of starter feed was carried out. Results showed that daily feed intake was increased in T2 (0.07% Digestase) on 21 days of age until 42 days of age. T1 (0.07% Natugrain) was also showed increase in daily feed intake compared to control diet for the last two weeks of experiment. However, T3 (0.035% Natugrain + 0.035% Digestase) showed a decrease in daily feed intake on 42 days of age. There was significance difference ($p<0.05$) for body weight. Average body weight in T2 was significantly higher compared with control, T1 and T3. Feed conversion ratio (FCR) of experimental birds in T1, T2 and T3 was also within a good range in 35 to 42 days of age (1.64 – 1.97) compared with control. It is suggested that the inclusion of these hydrolytic enzymes in diets could be used in broiler commercial feed to enhance feed intake and to promote weight gain to broiler chicken.

***KESAN PENAMBAHAN ENZIM TERHADAP NUTRISI MAKANAN POLTRI***

***ABSTRAK***

*Enzim boleh ditakrifkan sebagai pemangkin yang mana adalah protin semulajadi yang dihasilkan oleh sel-sel hidup dan bertindak sebagai pemangkin dalam tindakbalas biokimia tertentu. Kajian telah dilakukan ke atas penggunaan enzim dan potensi penggunaannya dalam diet ayam pedaging. Percubaan pemberian makanan selama 42 hari yang melibatkan 44 ekor ayam pedaging Cobb 500 yang berumur satu minggu telah dijalankan dalam rekabentuk rawak untuk menilai ciri-ciri pertumbuhan pada rawatan enzim 0.07% Natugrain (T1), 0.07% Digestase (T2) dan 0.035% Natugrain + 0.035% Digestase (T3) dalam tambahan makanan. Pengambilan makanan harian, berat badan dan nisbah penukaran makanan telah ditentukan bagi setiap diet. Analisis kandungan makanan bagi makanan permulaan telah dijalankan. Keputusan menunjukkan bahawa pengambilan makanan harian meningkat dalam kumpulan T2 (0.07% Digestase) pada umur ke 21 hari dan 42 hari. Kumpulan T1 juga menunjukkan peningkatan dalam pengambilan makanan harian berbanding kumpulan kawalan untuk dua minggu terakhir kajian. Walau bagaimapun, T3 (0.035% Natugrain + 0.035% Digestase) menunjukkan penurunan dalam pengambilan makanan harian pada umur ke 42 hari. Terdapat perbezaan signifikan ($p<0.05$) antara rawatan untuk berat badan. Nisbah berat badan kumpulan T2 secara signifikan lebih tinggi berbanding kumpulan kawalan, T1 dan T3. Nisbah penukaran makanan (FCR) bagi kumpulan T1, T2 dan T3 dalam kadar yang baik pada umur ke 35 dan 42 hari (1.64 – 1.97) berbanding kumpulan kawalan. Adalah dicadangkan bahawa penambahan enzim dalam diet ayam pedaging boleh digunakan dalam makanan komersil ayam pedaging untuk meningkatkan pengambilan makanan harian dan menggalakkan pertambahan berat badan ayam pedaging.*

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## LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

| | |
|---|---|
| Sdn. Bhd | *Sendirian Berhad* (Private Limited) |
| $H_0$ | Null Hypothesis |
| $H_1$ | Alternative Hypothesis |
| NSP | Non-starch Polysaccharide |
| ANF | Anti-Nutritional Factor |
| Wt. | Weight |
| p | probability |
| CRD | Completely Randomized Design |
| HCl | Hydrochloric acid |
| NaOH | Sodium Hydroxide |
| $H_2SO_4$ | Sulphuric acid |
| DFI | Daily Feed Intake |
| BW | Body Weight |
| FCR | Feed Conversion Ratio |
| ANOVA | Analysis of Variance |
| SPSS | Statistical Package for the Social Science |
| T1 | 0.07% Natugrain |
| T2 | 0.07% Digestase |
| T3 | 0.035% Natugrain + 0.035% Digestase |
| SSA | School of Sustainable Agriculture |
| UMS | Universiti Malaysia Sabah |
| mm | Milimeter |
| °C | Celcius |
| % | percent |
| g | gram |
| kg | kilogram |
| Sd | Standard deviation |
| CF | crude fibre |

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

All animals use enzymes to digest feeds. Not only animals, but human also needs enzymes in the process of digestion of feed. Enzymes are produced by animals itself or by the microbes naturally present in the gut. Monogastric lacks specific enzymes that break down certain components in the feeds. The poultry industry has come to rely on the use of exogenous enzymes in feeds to improve the nutrient digestibility of raw materials, increase yield from cereals, improve litter quality and reduce waste excretion.

The main problem in animal production system is the feed cost. If feeds are not digested by the animal as efficiently as they could be, there is a cost to both the producer and the environment. The nutritional values of feeds ingredients can be improved by supplementing the feed with specific enzymes thus increase the efficiency of digestion. Feed enzymes can help in break down anti-nutritional factors that present in many feed ingredients. Anti-nutritional factors interfere with normal digestion which reduce meat and egg production. Feed efficiency will be low and can also trigger indigestion.

There are many benefits of feeds enzymes for an efficient digestion of animal feed. First and foremost, to improve efficiency and to reduce cost. Feeds enzymes are very useful for a better digestion environment. Consistency can be improved by reducing the nutritional variation in feed ingredients. Enzymes can also helps in maintaining gut health.

Enzymes are categorized according to the substrates they act upon. According to the current market and expected developments, there are some enzymes used in animal feed. Carbohydrases divide into two separate degrading enzymes which are fibre-degrading enzymes and starch-degrading enzymes. Another enzyme which can be used in feed additive is proteases. Proteases are protein-digesting enzymes which are used in pigs and poultry nutrition. Phytases is also used as feed additive in animal feed. Most of the phosphorus in plant-derived ingredients is in the form of phytate, which is the main storage form of phosphorus in plant seeds.

## 1.2 Justification

In Malaysia, livestock industry is one of the most important industries for food supply. Malaysians prefer meats from ruminants and poultry as daily protein source. Demand for meats has lead to the increasing of ruminants and poultry production. Therefore, this study is important to evaluate the effect of enzymes on the nutritive value of poultry feeds.

Even though we have reached self sufficiency in poultry production, it is still not efficient if we cannot produce our own feed for poultry. Production cost is very high especially for commercial feed production. We still import most of the ingredients in formulated feed such as corns, soy beans and others.

If the animal cannot digest feed as efficiently as they could, this will increase the cost of production. Therefore, with the supplementation of enzymes in feed, it can help in breaking down feed ingredients and to increase efficiency of digestion, absorption and availability of nutrients.

## 1.3 Objective

I. To evaluate the nutritive value of poultry feed supplemented with feed enzymes.

II. To evaluate the effect of feed enzymes addition on growth performance of broiler chicken.

## 1.4 Hypotheses

$H_0$: There is no significant difference in the average growth performance of broiler chicken between control and three treatment diets.

$H_1$: There is significant difference in the average growth performance of broiler chicken between control and three treatment diets.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Since late 1980s, feed enzymes have played a major part in helping radically to improve the efficiency of meat and egg production by changing the nutritional profile of feed ingredients. Broiler starter and finisher may contain Aflatoxin, one of the antinutritional factors in broiler feed (Martin Weidenbörner, 2007). Positive effect on the bird's performance can be explained by the presence of additional activities of hemicellulase, cellulose, xylanase and the main activities of protease and amylase could facilitate the digestion of nutrients and improve the digestion of fibre (Viveros *et al.,* 1993). Feeds enzymes allows pig and poultry to extract more nutrient from the feed because it target specific anti-nutrients in certain feed ingredients (Barletta, 2001). By this, feed efficiency can be improved.

In the northern Europe, wheat and Barley are the main cereals grains used in poultry diets. These increase the viscosity of bird's gut because of their high levels of soluble carbohydrates (Barletta, 2001). High levels of gut viscosity reduce bird weight gain and feed efficiency due to a reduced rate of digestion and impaired nutrient absorption (Barletta, 2001). Apart from that, wet litter is also one of the problems which lead to high incidences of hock burns and breast blisters that reduce carcass quality. In addition, market value might also reduce due to the low carcass quality.

By addition of enzyme, the nutritive value may improve when soluble fibres break down by specific enzymes and lead to the increase of digestion efficiency. Feed cost will be reduced when the digestion efficiency increase. Furthermore, animal growth performance can be improved by increasing the efficiency of nutrients used in the feed given.

## 2.2 Characteristics of Enzymes

### 2.2.1 Unaffected By the Reactions That They Speed Up

This characteristic increases the efficiency of enzymes because they can be reused. Enzymes will remain same as the previous shape after the reaction with the substrate (Saul, 2002). This concept is same as a wrench remains unaltered after it has unscrewed thousands of bolts, so does enzyme keep working after completes a chemical reaction.

### 2.2.2 Highly Specific

The action of enzymes is highly specific because of the specification of enzymes and their substrate. For example, protein will react with protease and for carbohydrate substrate, the enzyme is carbohydrase. Each enzyme only works with specific molecules called the substrate (Saul, 2002). Another example to show that enzyme is highly specific, the rates and degree of cellulose hydrolysis also affected by substrate characteristics. Different molecular weights of cellulose substrate affect the action of cellulose enzyme (Mansfield *et al.,* 2008). The lock and key theory can illustrate the enzyme and substrate reaction (Bennett *et al.,* 1972).

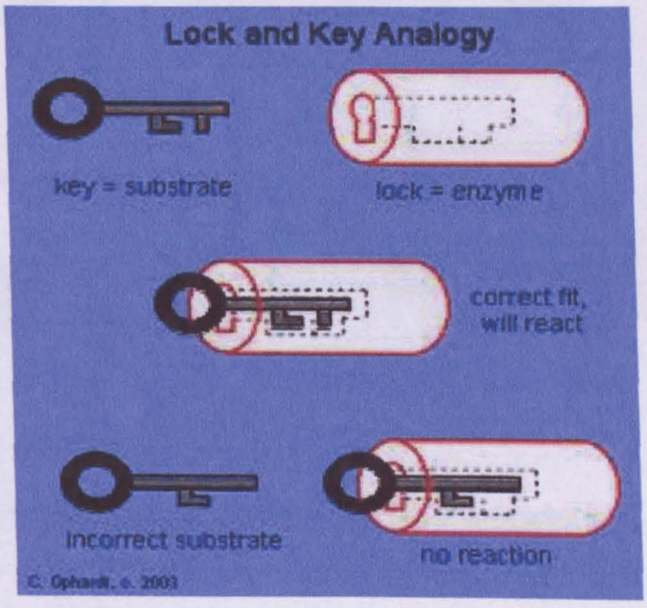


Figure 2.1 Model of lock and key theory of enzyme and substrate reaction

### 2.2.3 Can Speed Up the Same Chemical Reaction Going In Opposite Direction

Enzymes can break a molecule (substrate) into two pieces, but if only it is provided with the pieces, it will put it back together (Saul, 2002). In this case, it might not only break down molecules but can put it back together. The chemical reaction of enzyme and substrate can also going in opposite direction. Some theory that can illustrate this reaction is a wrench that can either take things apart or put them together but, depend on the condition of the things whether it is already assembled or not.

### 2.2.4 Enzymes Are Proteins

Enzyme is made up of chains of amino acids joined by peptide bonds. All known enzymes are proteins (Holum *et al.,* 1972). But not all proteins are enzymes. The chemical reaction involves the process of dehydration of water. When the carboxyl group and amino group react, water will be eliminated and a peptide bond (covalent bond) is formed.

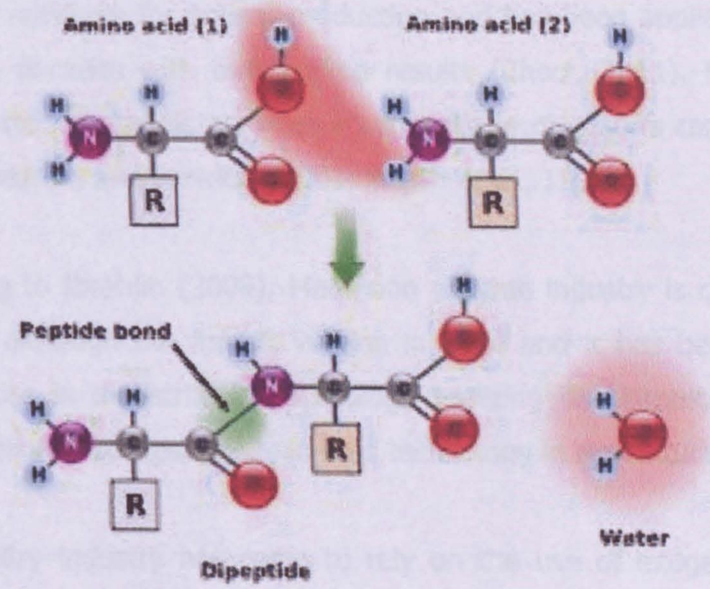


Figure 2.2 The formation of peptide bond and elimination of water

## 2.3 Current Status of the Uses of Enzyme in Industry

The current market size for global feed enzyme is estimated to be about US$550-600 million (Paloheimo et al., 2001). In the 1980s, the introduction of feed enzyme technology in Europe revolutionized the poultry industry (Barletta, 2001). The uses of enzyme as one of feed supplement are not new. If poultry is given the opportunity to satisfy its nutrition requirement, it would consume a range of seeds, roots, inorganic materials and insects (Isaksen *et al.,* 2001). However, to satisfy the consumers demand for 'vegetarian' animal production of farm animals, the feed given rarely sufficient for the animal's digestive system (Cowieson *et al.,* 2001). If the feed is not sufficient, animal does not have the sufficient amount of nutrition for growth and also for maintenance.

Europe, Canada, Australia and New Zealend are markets where wheat and barley feature prominently in pig and poultry diets (Barletta, 2001). Today, for barley-and-wheat-based poultry and piglets feeds contain xynalase and β-glucanase feed enzymes (Barletta, 2001). Various biotechnologies such as feed enzymes will continually play a significant role in food production through improving the use of poorly digested nutrients for animal production and has been applied in practice for more than two decades with outstanding results (Choct, 2011). Furthermore, the addition of selected hydrolytic enzymes to animal's feedingstuffs can improve animal performance (Bedford and Partridge 2001; Walsh *et al.,* 1994).

According to Ibrahim (2008), Malaysian enzyme industry is considered almost non-existence, although the import volume is large and it has become one of the national priorities in industrial biotechnology realizing the importance of enzymes applications in bioindustry especially in feed technology in agricultural sector.

The poultry industry has come to rely on the use of exogenous enzymes in feeds to improve the nutrient digestibility of raw materials, increase yield from cereals, improve litter quality and reduce waste excretion (Pattison et al., 2008). According to Pattison et al. (2008), the benefit of enzyme addition either as powders added to the feed mix or liquids applied to pelleted feeds have been considerable. In addition, enzymes are commonly used to improve the nutritive value of feeds for non-ruminants.

## 2.4 Types of Enzymes Used In Animal Feed

### 2.4.1 Phytase

Out of US$550-600 million of global feed enzyme market size, phytase having the greatest share of about 50% of this and the non starch polysaccharide (NSP) enzymes (Laughton, 2008). Since 1980s, phytases (myo-inositol(1,2,3,4,5,6) hexakisphosphate phosphohydrolases) have attracted considerable attention from both scientists and entrepreneurs in the areas of nutrition, environmental protection and biotechnology (Greiner, 2002).

Most of the phosphorus in plant derived ingredients is in the form of phytate, which is the main storage form of phosphorus in plant seeds. In the plant, phytate forms complexes with minerals, protein and starch, making them unavailable for absorption (Barletta, 2001). When this is used as feed ingredients, the composition of phytate is still present in the feed. The feed eaten by animal with no phytase enzyme production in their digestive tract will take this as an anti-nutritional factor. According to Greiner and Konietzny (2001), the ability of phytase to hydrolise phytate in the digestive tract is determined by its enzymatic properties. However, the negative effect of of phytate on the nutritive value of protein is not clearly confirmed in studies with monogastric animals (Sebastian *et al.,* 1998). Peter and Baker (2001), suggested that phytate does not affect protein digestibility. In addition, Cowieson (2001) reported that amino acid availability improved with the decreasing levels of phytate and supplemental phytase was reported to improve utilization of minerals by animals (Lei *et al.,* 1993; Adeola *et al.,* 1995; Lei and Stahl, 2001; Debnath *et al.,* 2005b). Furthermore, phytase supplementation results in increased energy utilization in monogastric animals (Selle and Ravindran, 2007).

The first commercial phytase products were launched on to the market in 1991 (Haefner *et al.,* 2005). Phytase is also apply in food processing or the production of pharmaceuticals (Greiner and Konietzny, 2006) and used as feed additives in diets largely for swine (Selle and Ravindran, 2008) and poultry (Selle and Ravindran, 2007), and to some extent for fish (Debnath *et al.,* 2005a).

The upper part of digestive tract in small intestine of monogastric has lack of significant endogenous phytase activity and low microbial activity (Iqbal *et al.,* 1994) causing poor availability of phytate phosphorus in monogastric animals (Walz and Pallauf, 2002). Substrate phytate was reported to act as an inhibitor of many histidine acid phytase activities and the lowest phytate concentration necessary to inhibit phytase activity ranges from 300 µM for the maize root enzyme (Gibson and Ullah, 1988). This inhibitor disturbs the phytase activity and the local environment of the catalytic domain of the enzyme. In addition, when the concentration of substrate phytate is higher, the inhibition of histidine acid phytase activity will increase thus inhibit the conversion of enzyme-substrate complex to enzyme and product.

Based on Greiner and Konietzny (2001), the ability of phytase to hydrolyse phytate in the digestive tract is determined by its enzymatic properties. Regarding to phytate dephosphorylation in the gastrointestinal tract of animals, consideration of low pH in the crop of poultry between pH 4.0 to 5.0 and in the proventriculus and gizzard of poultry and stomach of pigs and fish range between pH 2.0 to 5.0 (Simon and Igbasan, 2002). On the other hand, the pH in small intestine of animals ranges from 6.5 to 7.5 which presents a neutral pH environment. Two main types of phytases that have been identified is acid phytases showed maximal phytate dephosphorylation around pH 5.0 and alkaline phytases with a pH optimum around pH 8.0 (Konietzny and Greiner 2002).

With regard in phytate hydrolysis in the digestive tract, all phytases used as animal feed from the class of histidine acid phytases. Therefore, their reaction in animals is more efficient under acidic condition present in the forestomach or the stomach. Although the phytase action site in the gastrointestinal tract of poultry has received little attention, the primary site of phytate dephosphorylation by supplementary phytase was reported very probably in the crop or forestomach (Selle and Ravindran,
2007). A phytase requires a sufficiently high stability under the pH condition in the stomach and intestine and high resistance to proteolytic activities, mainly the activities of pepsin in the stomach and the pancreatic proteases in the small intestine (Greiner and Konietzny, 2001). As mentioned, there are many activities of enzymes in the digestive tract including the stomach and the small intestines. These enzymes activities might disturb phytase reaction for the phytate dephosphorylation.

As reported by Eeckhout and De Paepe (1994), all of the agronomic species of cereals, legumes, and oilseeds possess some phytase activity, but only cereals such as barley, wheat, rye, and triticale possess appreciable amounts of phytase activity. The efficacy of phytase in dephosphorylating phytin in plant-derived ingredients and thereby improving its availability for pigs and poultry is established (Adeola *et al.,* 2003).

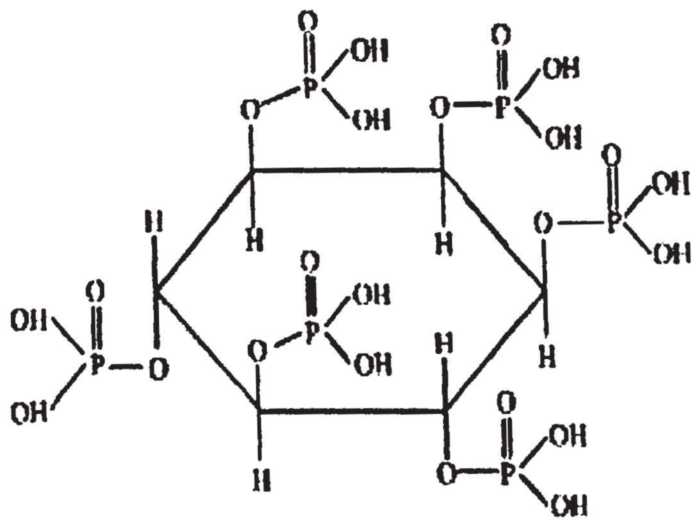

Figure 2.3 The molecular structure of phytic acid

**2.4.2 Proteases**

Proteases are protein-digesting enzymes used in pig and poultry nutrition to break down storage proteins in various plant materials and proteinaceous anti-nutrients in vegetable proteins (Barletta, 2001) whose catalytic function to hydrolyse peptide bonds of protein. Protein is made up of polymers of amino acids connected by peptide bonds. Peptide bond is produced from the reaction of carboxyl group and amino group of amino acids chain and results in elimination of water. Each amino acid has in addition a side group, which has different chemical properties and is the basis for grouping the amino acids into hydrophobic, hydrophilic or aromatic groups (Isaksen *et al.,* 2001).

According to Whitcomb and Lowe (2007), proteases are characterized by their ability to hydrolyse bonds before or after specific amino acids. The proteases degrading protein in the digestive system have been reviewed extensively. There are two major proteinaceous anti-nutrients, trypsin inhibitors and lectins. Barletta (2001), has found trypsin inhibitor in raw vegetables protein such as soybeans. Trypsin inhibitor can block trypsin and inhibit digestion of protein in the small intestine. Lectins are sugar binding protein which reduce digestibility (Barletta, 2001). Trypsin inhibitor and lectins in soy product can be reduced by heating process. However, excess heating might reduce particular lysine.

Pancreas is the major source of proteases in the gastrointestinal tract (Isaksen, 2001). Pancreas secreted proteolytic enzymes initially the gastric enzyme pepsin which break down protein materials in the small intestine. The precursors are called trypsinogen, chymotrypsinogen, proelastase and procarboxypeptidase. An enzyme enterokinase secreted from the walls of small intestine which transformed trypsinogen to trypsin (Setia, 2013).

Pepsinogen, the precursor for pepsin is produced in the proventriculus of poultry. With regard to the acidic environment, pepsinogen is excreted into the digestive tract and activated by pepsin. Pepsin is an endoprotease that hydrolyse peptide bonds containing phenylalanine, tyrosine and leucine at a pH range of 1.8 to 3.5 (Piper and Fenton, 1965). Chicken pepsin is irreversibly inactivated at slightly alkaline pH and active at less acidic conditions than pepsin in human and pigs (Bohak, 1969).



On the other hand, low protein diet supplemented with amino acids help to reduce nitrogen excretion from pigs' facilities; the exogenous enzymes improve the feedstuffs utilization (Reyna *et al.,* 2006). The crude protein in diets for pigs can be reduced by replaced soybean meal to sorghum grain and supplementation of exogenous enzyme (proteases) can improve the nutritive value of feedstuffs. Proteases are also added to feed with the purpose of increasing dietry protein hydrolysis and improved nitrogen fixation (Oxenboll *et al.,* 2011) thus enabling better nitrogen utilization. This will result in reducing the nitrogen content in the manure and reduce diet protein content.

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