1. Introduction

1.1 Background and justification

There is a growing disparity between the rising world population and the food producing capacity. The rate of the demand for food consumption increase is higher than the growth rate of food production. As a result, food supplies per capita are decreasing (Brown and Kane, 1994). In Cambodia where the population growth is about 2.8 %, from the year 2000 to 2002, annual supply per capita of poultry, bovine, and pig meat were reduced from 2.0, 5.0, and 8.0 to 1.9, 4.5, and 7.4 Kg respectively (FAO, 2005). On the other hand, the rising of living standards brings about a simultaneous increase in consumption of livestock products. The increasing rate of urbanisation in Cambodia burdens the requirement of increasing the urban and suburban animal production systems especially pig and broiler chicken. But the feeding systems to produce these products are based on using the same feed resources as are consumed by humans, namely cereal grains and soybean meal. It is estimated that almost 50 % of the world grain supply is consumed by livestock mainly pig and poultry (FAO, 1993). It has been argued (Preston, 1995) that if all the world's grain production were reserved for human consumption then there would be enough to feed the 10 billion inhabitants at which point the world population is expected to stabilise.

The strategy that is proposed is to find the alternatives to cereal grains as the basis of feeding systems for livestock production (Rodriguez and Preston, 1997). Reduction of poultry feed costs will be possible by optimising the local alternative feed resource into the ration. In Cambodia, about 471 thousands Mt of rice bran are annually produced from paddy rice (FAO, 2005). Research on the use of this resource in chicken diet is not yet fully done. Since little rice bran is utilized in human consumptions mainly in Far East and Southeast Asia, enormous amounts of wastage of important nutrients about 40 – 45 million tonnes are produced annually, and used largely as animal feed.

Using rice bran in broiler chicken diets is not common especially in the urban and suburban broiler chicken production system in Cambodia. Production of rice bran is seasonally – the surplus and very cheap cost during the period of paddy rice harvesting and milling and subsequently become unavailable and expensive afterwards. It is required also the transportation of rice bran from local milling into the city and storage to supply the poultry feed production in the whole year round.

Nutritionally, several factors limited its use in poultry especially broiler chicken diet. Almost half of phosphorous are in phytates form. Hull adulteration is a factor reducing the quality of rice bran (Farrell, 1994). High level of ash content indicates high level of hull (Warren and Farrell, 1990a). Other anti-nutritional factors observed in raw rice bran are trypsin inhibitors, pepsin inhibitor (Deolankar and Singh, 1979) and antithiamine (Kumar and Chaudhuri, 1976). But most disadvantageous for rice bran is its high oil content cause rapid rancidity (Shaheen et al., 1975). Because of such rapid tendency towards rancidity and because of the microbial activity generally associated with raw rice bran, it has traditionally gone into the animal feed channels, where large amount can be used in short periods of time (Babcock, 1987). Storage temperature and humidity of rice bran are important factors in determining the rate of hydrolysis of oil. Another factor is the growth of fungi produced mycotoxin (Warren and Farrell, 1990b). As a result of theses problems, diet containing more than 20% of rice bran reduces performance of broiler chicken (Farrell and Martin, 1998).

Because rice bran can be a promising feedstuff ingredient to reduce feed cost per kg weight gain of chicken (Khalil, 1997), several attempts have been done to try to overcome its instability and poor storability, both mechanically (oil-extracted, caking, roasting, extruding…) and chemically (enzymatically treated). However, the evidence of better performance of broiler chicken fed processed rice bran physically or chemically is not fully proofed (Mujahid et al., 2003, Kratzer and Payne, 1977). Concerning with the cost and applicability of different processing methods, the approach toward the use of these methods in poultry feed manufactures is not common.

Microbial treatment can be an alternative approach to optimise the use of rice bran in poultry feeding. Yeast using in the feed industry has been sporadic over last 40 years. Onifade et al. (1999) found that inclusion of yeast culture of *Saccharomyces cerevisiae* increase the bioavailability of minerals and nutrients and nutrient retention. Although less is known about the mode of action of yeast culture in chicken, a stimulatory effect on hindgut fermentation is a possible contribution to the digestibility response (Stewart, 1995). However, Brake (1991) found lack of effect of a live yeast culture on broiler breeder and progeny performance while depression of growth was reported from broiler chicken fed dry yeast (Oguntona et al., 1985). Application of probiotics either live bacteria or yeast to chicken in dry form or as premix supplement is still questionable while the short life span of chicken supports a very limited opportunity for the desired microbe to counteract with the indigenous flora as a first step to colonize and dominate in the intestine –time consuming until the benefit can be yielded. Anatomically, the length of gastrointestinal tract of broiler chicken is short, therefore, providing fewer sites for the implanted microbe.

Another approach to increase the involvement of yeast as inoculums and feed is fermentation of feed before feeding to animal. The studies with probiotics added to dry feed directly is incomparable with fermented diet fed to animal. In fermented diets, both yeast and their fermented products are presented, whereas in dry diets, animal consume only the bacteria and the results of their metabolism appear in the gastrointestinal tract of the animal. There is also a huge difference in the number and the proliferation of microorganism inoculants and their activities. In fermented diets the inoculants grow rapidly on substrate whereas in dry feed it is in spore form and require more time to accelerate (Scholten et al., 1999).

1.2 The problem statements

The present research attempts to evaluate the linkage between maximising the use of rice bran in broiler chicken diet and optimising the level of yeast as inoculums. To reach this approach, it is required to know what would be the level of unprocessed rice bran which reduce the performance and feed digestibility of broiler chicken when feed is formulated isonitrogenously and isocalorically.

On the other hand, if the use of yeast as fermentation inoculums could be an approach to improve the quality of rice bran, what is the appropriate means to conduct. Because feeding lived yeast cell may change the intestinal ecology of broiler chicken, what would be the optimum level to improve the performance and digestibility of chicken. Moreover, yeast used in the fermentation process requires also nutrients for its proliferation. The further research question, therefore, could be raised whether fermented rice bran could substitute totally unprocessed rice bran in poultry feed and what is its impact on chicken performance and feed digestibility.

Finally, what is the maximum level of rice bran that can be incorporated into poultry feed after it is fermented with yeast? And is there any different performance response between male and female chicken? Large-scale broiler chicken industry try to dominate the small-scale farmer in ordering only male chick from hatchery.

1.3 Research hypothesis

Fermentation process will increase the possibility to use rice bran in broiler chicken feed. Fermented rice bran will improve the performance and digestibility of broiler chicken.

1.4 Objective

The main objective of this research is to increase the possibility of using rice bran in broiler chicken feed by fermenting with yeast.

The specific objectives of the research are to:

- determine the effect of different levels of raw rice bran in diets on apparent digestibility and growth performance of chicken
- assess the optimum methodology to inoculate rice bran with yeast *Saccharomyces cerevisiae*
- evaluate the effect of graded levels of yeast inoculated into dietary rice bran on apparent digestibility and growth performance of chicken
- compare the effect of substituting unprocessed rice bran with fermented rice bran on apparent digestibility and growth performance of chicken
- and determine the optimum level of fermented rice bran that can be used in broiler chicken diets.

2. Literature review

2.1. The implication of dietary fibre in poultry feed

Feed ingredients used in poultry diets are mostly of vegetable origin. These feedstuffs include a variety of by-products of the food and feed industries. Some are high in starch and protein and others are rich in fibre such as hull or bran fractions of cereal and legume seeds obtained as by-products from the feed milling industry. The positive contribution of fibre to the feeding value of poultry feedstuffs is of negligible importance as its fraction is indigestible.

Consumption of fibre-rich material has nutritional, physiological and also microbiological implications. The effect of dietary fibre on performance of broiler chicken is not only simply related to the physical properties of feedstuffs but also involved with a complex change of micro-ecology of the gastrointestinal tract of the host animals.

2.1.1. Structure of plant fibre

Since the early 1950s, various studies paid attention to the characterisation of plant cell walls and their effect on health and disease. In 1953, Hipsley defined dietary fibre as "non-digestible constituents that make up the plant cell wall, encompassing the so-called unavailable carbohydrate". Trowell et al. (1976) revised the definition of dietary fibre as "the plant polysaccharides and lignin which are resistant to hydrolysis by digestive enzymes of man". Recently, Fuller (2004) defined dietary fibre as plant polysaccharides and lignin that cannot be hydrolysed by the endogenous digestive enzyme of higher animals. The term dietary fibre differs from crude fibre, the fat-free insoluble residues of samples after treatment with a certain concentration of acid and alkali in Proximate Analysis. Depending on the nature of diet or sample analysed, a variable proportion of fibre can be underestimated.

Because it is difficult to determine the dietary fibre, a new alternative term "Non-Starch Polysaccharide" (NSP) was defined (Englyst et al., 1982). The term NSP is widely adopted particularly in non-ruminant animal feeding to cover all polysaccharide molecules excluding starch. Jassen and Carré (1989) classified NSP into two categories, namely water-soluble (SNSP) and water-insoluble NSP (INSP), the so-called unstructured and structured fibre by Englyst (1989). The water-soluble NSP comprises chemically the non-starch water-soluble polysaccharides, including β (1 $\rightarrow$ 3) glucan from barley, arabinoxylan from rye, highly methylated pectin from fruits, galactomannan from leguminous plants such as guar gum, and other polysaccharides from algae. Water-insoluble NSP consists of insoluble cell wall material, it includes cellulose, hemicellulose, pectin substances, and lignin. Fig. 1 below summarises the analytical terminologies used in relation to dietary fibre.

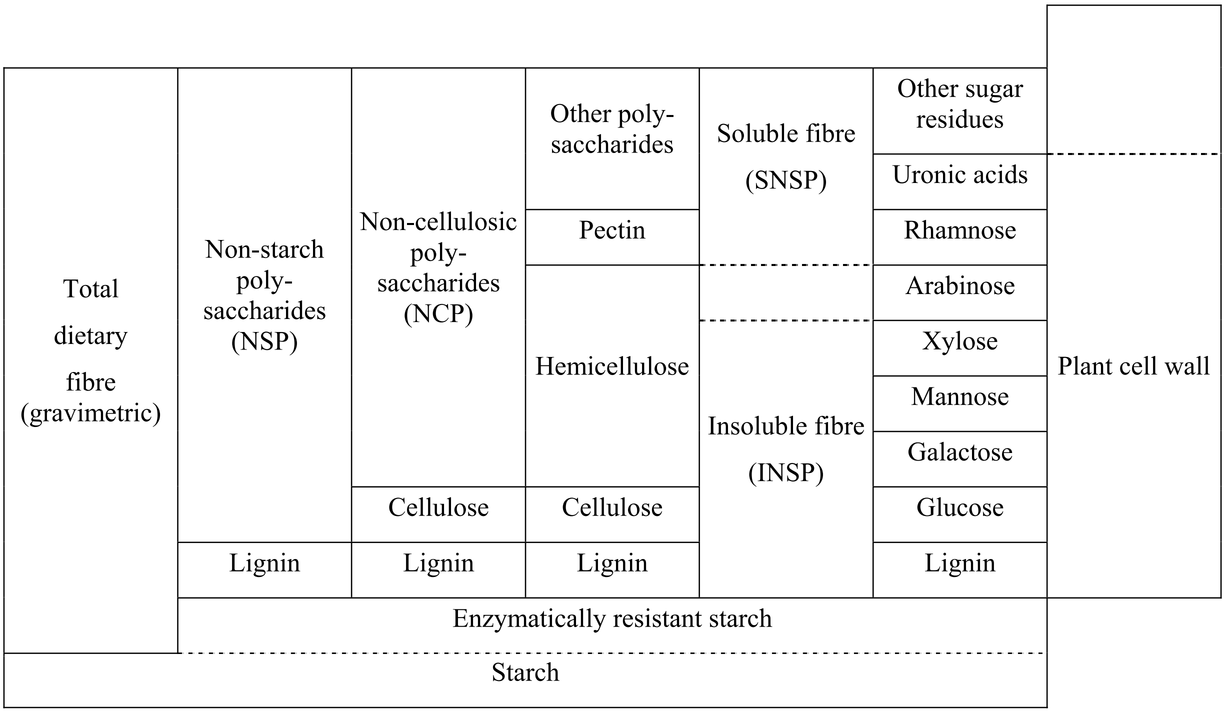

Figure 1. The relationship between the different components of "dietary fibre" fraction. Broken lines indicate boundaries that are not absolutely divided (modified from Asp and Johansson, 1984)

van Soest (1977) developed alternative procedures to determine and thereby classify the components of fibre: Neutral-Detergent Fibre (NDF), the residue after extraction with boiling neutral solution of sodium lauryl sulphate and ethyenediaminetetraacetic acid (EDTA), consists mainly of lignin, lignin-N compounds, cellulose and hemicellulose and is regarded as a measurement of plant cell wall material. Acid-Detergent Fibre (ADF) is the residue after refluxing with 0.5 M sulphuric acid and cetyltrimethylammonium bromide, and represents crude lignin, silica and cellulose fractions (McDonald et al., 1995).

2.1.2 Biological effects of dietary fibre in small intestine

Prolonged feeding of fibre to animals cause morphological changes and adaptations in the gastrointestinal tract. The effects of dietary fibre throughout the gastrointestinal tract vary from more simple mechanic interferences to complex hormonal interactions according to the different fractions of fibre. Water-soluble and insoluble NSP actively cause different physiological responses.

2.1.2.1 The effect of SNSP on gastrointestinal physiology of chicken

In the gastrointestinal tract, the viscosity-inducing ability of SNSP primarily provokes effects on the digesta in the foregut (Chesson, 1990). It modifies the gastrointestinal physiology and the ecosystem (Choct, 2002). The antimotility properties of viscous SNSP delay the gastric emptying rate, impair convection or mixing in the upper small intestine, alter the absorptive site (Read and Eastwood, 1992) and delay small intestine transit time (Read and Eastwood, 1992, and Chesson, 1990). This leads to increasing endogenous losses of nutrients (Chesson, 1990).

2.1.2.1.1 The ability of SNSP to induce digesta viscosity and transit time

The SNSP is able to increase digesta viscosity along the gut and this in turn reduces the efficiency of nutrient utilisation in chicken (Choct et al., 1999). Viscosity of digesta is defined as the frictional resistance of its fluid, which reduces its ability to flow freely (Fuller, 2004), and therefore impair the propulsive and mixing effects of gastrointestinal tract contraction (Read and Eastwood, 1992). Regardless to the source of NSP, there is no difference in the physical effect of viscosity on nutrient digestion and absorption (Read and Eastwood, 1992).

High gut viscosity caused by SNSP can also delay the rate of digesta passage in the stomach (Fuller, 2004) and small intestine (Gohl and Gohl, 1977). However, because it increases the transit time in the large intestine, SNSP results in a general increase throughout the whole digestive tract (Fuller, 2004). The interest in transit time is based on the view that if transit time is increased in large intestine, then less time is available for the microbial fermentation of undigested dietary residues which result in poorer use of the diet (Latymer et al., 1990).

2.1.2.1.2 Fibre in gastric emptying

Under normal physiological conditions, solid contents of feed are retained in the fundus[1] of the stomach until the majority of liquid has been released. This content, then enter the antrum where it is disrupted by abrupt acceleration and deceleration forces of antral propulsion and

[1] Stomach of non-ruminant animal is subdivided into the Cardia (entrance area), located nearest the esophagus, continued by the Fundus (the dome-shaped part of stomach) that is adjacent to the Corpus (the rounded base or bottom) and together they form the middle portion subjected to enlargement. The Antrum is the constricted part of the stomach that joins the duodenum.

pyloric closure until it is light enough to enter the axial stream and be propelled through the pylorus[2] ahead of the advancing antral contraction (Meyer et al., 1986). Increasing the viscosity of gastric contents prevent the separation into discrete solid and liquid components. Solid compounds are no longer retained in the fundus, and gravity does not cause them to settle out. The accelerated emptying of solid contents reduce the absorption of nutrients because when gastric contents are viscous, solids are no longer disrupted by antropyloric contraction, rather they enter the duodenum in larger bulk, and the digestion is impaired by the reduction in surface area (Read and Eastwood, 1992).

2.1.2.1.3 Reduction of intestinal mixing

Mixing of intestinal contents by contraction is to release material trapped in the polysaccharide matrix, increase the interaction between digestive enzymes and the complex macromolecules in food, and facilitate the access of the digestive products to the intestinal epithelium (Read and Eastwood, 1992). Viscous SNSP act as antimotility agents that reduce the diffusion of nutrients, and as intestinal surface coating agents that increase the thickness of the unstirred water layer (Gerencser et al., 1984). When the gastrointestinal contents are rendered viscously by the incorporation of SNSP, INSP trap complex food molecules within the matrix hindering their access to digestive enzymes and to the intestinal epithelium (Read and Eastwood, 1992). Chemo-physiologically, SNSP interact with the glycocalyx[3] of the intestinal brush border and thicken the rate-limiting unstirred water layer of the mucosa, which reduces the efficiency of nutrient absorption through the intestinal wall (Johnson and Gee, 1981).

2.1.2.1.4 The endogenous losses of nutrients

The SNSP change the function of gut by modifying endogenous secretion of water, protein, electrolytes and lipids (Johnson and Gee, 1981). In the case of protein losses, the study of Angkanaporn et al., (1994) demonstrated that endogenous losses of amino acids in chickens are significant even with a low level (1.5 %) of SNSP in the diet. Moreover, SNSP enhances bile acid secretion and subsequently result in significant loss of these acids in the faeces

[2] The inner aspect of stomach has specific regions according to cell type: the esophageal, cardiac gland, fundus gland, and pyloric gland regions altogether so-called gastric glands secrete gastrin, mucus, HCl, and pepsinogen.

[3] Glycocalyx, a biological term, which refers to the carbohydrate layer on the outer surface of animal cells which is composed of the oligosaccharide termini of the membrane glycoproteins and glycolipids.

(Ikegami et al., 1990). The net effect is to alter the lipid metabolism in the intestine, resulting in increased hepatic synthesis of bile acids from cholesterol to re-establish the composite pool of these metabolites in the enterohepatic circulation. The continued secretion of bile acids and lipids sequestration are eliminated as faecal acidic and neutral sterols.

2.1.2.2 The effect of INSP on gastrointestinal physiology of chicken

Insoluble NSP play a role in increasing the bulkiness of digesta and on excreta consistency, digesta transit time, and has some effect on animal behaviour (Choct, 2002). It provides little or no effect on nutrient utilization in non-ruminant animals but contributes to the bulkiness of the total fibre in the diets (Bedford et al., 1990). Stephen and Cummings (1979) reported that dietary fibre accumulate the flow of substrate into the colon and, therefore, increase faecal weight by promoting microbial growth. Moreover, through the water-holding capacity of their large hydrophilic molecules, INSP causes wet and sticky excreta and litter, which increase the incidence of illness, dirty animals, and dirty products.

2.1.3. Fibre induced morphological changes of the digestive tract

Morphological changes of digestive tract as results of feeding fibre-rich diets are another characteristic effect of NSP. Feeding fibrous feed to non-ruminant animals results in a change of the digestive tract length, weight and thereby functions of the organs.

Changes of mass of small and large intestine and their villus are observed. Dietary fibre cause atrophic changes to the major absorptive sites in the proximal small intestine. As a result, villi are stunned, disaccharidase production is reduced, and there are compensatory increases in crypt cell production rate and cell turn over (Johnson and Gee, 1986). An increase in small intestine length in rats given diets enriched with fibre is accompanied by an increase in mucosal mass (Jacobs, 1983), indicating an increased rate of cell proliferation. Johnson et al., (1988) reported this phenomenon as an effect of viscous SNSP such as pectin and guar gum.

Kass et al. (1980) reported an increase in the weight of the colon of pigs fed on diets containing increasing amounts of lucerne meal. Stanogias and Pearce (1985) found that increasing intake of fibre from different sources caused an increase in weight of different intestinal segments, particularly that of colon.