

THE UNIVERSITY OF MANITOBA

EFFECTS OF ENZYME SUPPLEMENTATION ON THE
NUTRITIONAL VALUE OF FABABEANS IN CHICKEN DIETS
AS ASSESSED BY GROWTH AND AVAILABLE ENERGY STUDIES

BY

© JOSE-IGNACIO RODRIGUEZ-CASTANON

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A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

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ABSTRACT

Fababeans (Vicia faba L.) contain antinutritional factors which may decrease the productivity of monogastric animals. Experiments were made with chicks to determine if the effects of the antinutritional factors in fababeans could be reduced by adding of enzymes to the diet, by autoclaving, and by dehulling. An additional objective was to identify factors that might affect the reliability of metabolizable energy values of different fababean fractions as determined by the true metabolizable energy (TME) system.

In study one, which comprised three experiments, the effects of adding various crude enzyme preparations to diets containing raw, autoclaved and fermented fababeans were measured. Weight gain, feed efficiency and dry matter retention responses were assessed using Leghorn chicks that were 7 to 21 days of age. The addition of enzymes at levels that ranged from 0.25 to 0.50% of the raw fababean-based diet did not affect ($p>0.10$) the performance of chicks in the first week of the experiment, but increased ($p<0.05$) weight gains by more than 10% with protease, 6-7% with cellulase, and 8% with the combination of cellulase plus protease during the second week of the experiment. The supplemental enzymes had no effect on feed consumption and dry matter

retention but did improve the efficiency of feed utilization by 5% ($p < 0.05$). The addition of enzymes to autoclaved fababeans did not alter chick responses. The combined addition of cellulase plus protease to fermented fababeans improved weight gain in the second week and feed efficiency ($p < 0.01$). Other enzyme treatments of fermented fababeans did not produce any beneficial response ($p > 0.10$). Autoclaving improved ($p < 0.01$) weight gain, feed efficiency, and dry matter retention by more than 15%. Fermentation of fababeans improved ($p < 0.05$) efficiency of feed utilization and dry matter retention by 10%, but had no effect ($p > 0.10$) on weight gain. The responses of chicks fed fermented fababeans were inferior to those fed autoclaved fababeans, mainly due to a decrease in feed intake.

Study two comprised two experiments. The first experiment determined the effect of dietary enzyme treatments on the true digestibilities of nitrogen (TDN) and energy (TDE), true metabolizable energy corrected to zero nitrogen balance (TMEn) and the true amino acid availability (TAAA) of soybean meal, rapeseed meal, and different fractions of raw and autoclaved fababeans (whole, dehulled and hulls). In the second experiment the effects on TMEn values of autoclaving, amount ingested and duration of excreta collection were determined using fababeans and fababean hulls. Dehulling increased ($p < 0.01$) TDE and TMEn of fababeans. The addition of enzymes to fababeans and soybean meal reduced or tended to reduce ($p < 0.10$) TDN and TDE, but in rapeseed tended to increase TDN. The addition of enzymes to soybean meal decreased ($p < 0.05$) TMEn. TAAA were not affected ($p > 0.10$) by the

addition of enzymes. The apparent stress situation of birds influenced the results obtained when the TME system was applied to fababeans by affecting the rate of passage. However, the endogenous excretions of gastrointestinal origin appeared to be independent of the condition of birds. Autoclaving dramatically increased the rate of passage of fababean hulls. The high TMEn value and low recovery of the indigestible raw fababean hulls was attributable to a collection period (96 h) that was too short for complete voidance of undigestible residues. Such an effect would increase the apparent nutritive value of raw feedstuffs and may explain the difference between the TMEn value of fababeans in the first and second experiment of study two. Increasing the amount of fababeans ingested from 30 to 80 g decreased the TMEn value of raw fababeans by 13% ($p < 0.01$) but not that of autoclaved fababeans ($p > 0.10$). Autoclaving increased the TAAA and TMEn of fababeans by 10 ($p < 0.05$) and 22.4% ($p < 0.01$) respectively when fed at 80 g and by 17 ($p < 0.01$) and 13.5% ($p < 0.01$) respectively when fed at 30 g. The TMEn values (MJ/kg DM) of dehulled fababeans, soybean meal and rapeseed meal in experiment 4 were 14.0, 11.4 and 9.0 respectively. The TMEn values of raw fababeans, autoclaved fababeans and autoclaved hulls in experiment 5 were 10.7, 12.2 and 0.004 respectively. The mean TAAA of raw and autoclaved fababeans in experiment 5 was 76 and 89%, respectively.

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LIST OF ABBREVIATIONS

DM	dry matter
min	minutes
h	hours
d	days
kcal	kilocalories
kJ	kilojoules
MJ	megajoules
kg	kilograms
g	grams
ppm	parts per million
IU	international units
ml	millilitres
l	litres
cm	centimetres
mm	millimetres
C	Celsius degrees

1. INTRODUCTION

There has been extensive research to determine the relationships between dietary inclusion of fababeans (Vicia faba L.) and animal productivity. Recent work has focussed on methods to improve the nutritional value of fababeans (heat treatment, dehulling, addition of methionine).

Enzyme supplementation of cereals, particularly the addition of beta-glucanases and cellulases to barley, rye and oats is another active area of research. However, the effects of enzyme supplements on protein feeds have received little attention. Potential effects of enzymes on fababeans are: cellulases and polysaccharidases may reduce the viscosities of carbohydrates which can interfere with digestion and absorption of proteins and other nutrients; amylases may assist in the digestion of starch; and, proteases may neutralize the effects of some of the antinutritive factors and assist in the digestion of proteins.

The true metabolizable energy (TME) system and chick growth trials are commonly used to assess the nutritive values of feedstuffs for poultry. However, little has been done to determinate if the TME system

is applicable to feeds containing antinutritional factors. Use of the system to study the effects of different processing methods, including enzyme treatments, on energy and amino acid availabilities of different fababean fractions has not been attempted, previously.

The objectives of this Thesis are:

Study one:

1) To identify a blend of enzyme preparations that will enhance the utilization of fababeans by growing chickens.

2) To measure the responses of chicks when enzymes are added to diets containing raw, autoclaved and fermented fababeans.

Study two:

3) To estimate the effects of enzyme supplements on the TMEn and TAAA values of soybean meal, rapeseed meal and various fractions of fababeans.

4) To compare autoclaving and enzyme supplementation in terms of the TMEn and TAAA values of various fractions of fababeans.

5) To identify factors affecting the applicability of the TME method when used to evaluate feedstuffs that contain antinutritional factors and indigestible fibrous material, such as fababeans.

2. LITERATURE REVIEW

2.1.- Introduction.

The most extensively used diet for monogastric animals on a world wide basis is a corn-soybean meal diet supplemented with methionine, fish meal, vitamins and minerals. Soybean meal has become the protein source of choice in many areas because it is plentiful, usually inexpensive, and has a good balance of amino acids with a low concentration in antinutritional factors. However, in some parts of the world other protein sources are more economically produced. For example, fababeans is an important crop due to its relatively high protein content and high yield potential.

2.2.- Use of fababeans in poultry nutrition.

2.2.1.- Chemical composition.

The chemical composition of fababeans is similar to that of other seed legumes. Hull represents 13-17% of the total seed weight (Hill et al,1977) and contains almost all of the fiber, lignin, calcium and condensed tannins. Most of the protein, phosphorus, fat, vicine plus convicine, and hemagglutinins are present in the endosperm fraction (Marquardt et al,1975). The content of calcium in fababean seeds is approximately 0.08% and that of phosphorus is from 0.6 to 0.9%. About 40-60% of phosphorus is in form of phytates (Griffiths and Jones,1977). The fat content is low (1.0-4.0%) (Newton and Hill,1983) and most fatty

acids are unsaturated, with more than half being linoleic acid (Clarke,1970).

The crude fiber content depends on the cultivar and growing conditions, and varies between 6-15% (Newton and Hill,1983). Cellulose represents 5% and lignin 1.1% of the dry matter of fababeans (Beroard-Cerning and Filliatre,1977). There is a high negative correlation between neutral detergent fiber and protein (De Haro et al,1979).

The carbohydrate concentration of fababeans is approximately 50 to 60%, with 30 to 45% being starch (Newton and Hill,1983). The availability of carbohydrates seems to depend on the cultivar, being 70% in fall cultivars and 45 to 65% in spring cultivars (Pritchard et al,1973). The starch of legumes is more resistant to enzyme hydrolysis than that from non-leguminous seeds and as a result is slowly digested and absorbed (Leeds,1982). Pritchard et al (1973) reported that the unavailable fraction consists of non-water soluble sugars, hemicellulose, cellulose and lignin.

The mean crude protein content of fababeans is 26.5% in fall cultivars, and 31.5% in spring cultivars (Bond and Toynbee-Clarke,1968). However, Ilian et al (1985) reported that approximately 35% of fababean nitrogen is non-protein. Fababean protein has a low concentration of the sulfur amino acids, cystine and methionine, and there is a negative correlation between the content of protein and that of lysine, methionine and cystine (Bond,1970;Frolich et al,1974;Mosse

and Baudet, 1977). Protein digestibility for monogastric animals is 80-85% which is 10% lower than that of soybean meal (Waring and Shannon, 1969; Palmer and Thompson, 1975; Henry and Bourdon, 1977; Petersen and Schulz, 1979).

The Institute National de la Recherche Agronomique (I.N.R.A., 1985) reported that the gross energy of fababeans is 3900 kcal/kg, the digestible energy for swine is 3300 kcal/kg, and the metabolizable energy for poultry is 2700 kcal/kg. Similarly, the National Research Council (N.R.C., 1984) reported that the metabolizable energy of fababeans, corrected to zero nitrogen balance, is 2400 kcal/kg. The metabolizable energy in spring cultivars tends to be higher than that of fall cultivars (Carpenter and Johnson, 1968; Waring and Shannon, 1969). The Food and Agriculture Organization (F.A.O., 1973) reported a low biological value (55%) of protein of fababeans, which is explained by the low sulfur amino acid content, particularly that of methionine (Palmer and Thompson, 1975). This value can be greatly improved by supplementation with a relatively small amount of methionine (Marquardt and Campbell, 1974).

2.2.2.- Antinutritive factors.

Fababeans contain compounds such as tannins (condensed proanthocyanidins), protease inhibitors, hemagglutinins, phytates, favism factors (vicine and convicine), saponins, and unavailable carbohydrates (alpha-galactosides and fiber). These factors are not very toxic, but do affect the performance of animals. Some of them are thermolabile

(tannins, protease inhibitors, and lectins), being readily inactivated by heat treatments; this is discussed subsequently.

Fababean hulls contain between 0 and 8% of condensed tannins depending on cultivar (Griffiths and Jones,1977), storage and growing conditions (Marquardt et al,1978b). The mean tannin content in the testa is 4.3%, with the concentration being related to cultivar (Marquardt et al,1978b). The tannin content is 0.8% in dehulled fababeans and this value is not influenced by cultivar (Griffiths and Jones,1977). The tannin content is related to seed and flower colour: white seeds and flowers are usually tannin-free (Marquardt et al, 1978b). Tannins form indigestible and insoluble complexes with proteins, and inhibit enzyme systems (Swain,1965;Martin-Tanguy et al, 1977a,1977b;Griffiths,1979;Aw and Swanson,1985;Sievwright and Shipe,1986). Griffiths and Moseley (1980) suggested that fababean tannins increase pancreatic secretion of digestive enzymes and that in the gut, tannins are bound to alpha-amylase and trypsin, but not to lipase. The net effect is that the retention of nutrients is greater for birds fed tannin-free, as compared to those fed tannin-containing, cultivars (Marquardt and Ward,1979). Dry matter and protein digestibilities and nitrogen retention values were inversely proportional to tannin content (Carrera et al,1973;Lindgren,1975; Marquardt et al,1977;Guillaume and Bellec, 1977;Poulsen and Peterson, 1982). Antitriptic activity, mainly concentrated in the hull (Marquardt et al,1975), has an insignificant effect on animal performance compared to the effect of the condensed tannins (Wilson and McNab,1972;Marquardt

et al,1975;Ward et al,1977; Griffiths,1979;Liener and Kakade,1980;Ilian et al,1985).

Hemagglutinins or lectins specifically agglutinate erythrocytes. Their effect is minimum in chicks (Marquardt et al,1975) and rats (Grant et al,1983). These substances are destroyed by heat (Rodriguez and Bayley,1987), but resist digestive proteolysis to some extent (Levy-Benshimol,1986).

The phytate content of fababeans is lower than that of cereals (Su et al, 1982) and does not affect seriously the nutritive value of seeds, especially if the diets are formulated to correct for their effect. Heat treatment has no effect on phytate acid content.

Vicine and convicine are thermostable glucosides that reduce the concentration of glutathione in erythrocytes, causing favism (hemolytic anemia). However, favism effects are not important in domestic animals, except for the laying hens which are affected by dietary vicine and convicine, being the net effect the reduction of egg size (Muduuli et al,1981;Marquardt,1982).

Dietary saponins reduce the concentration of blood cholesterol and increase bile acid secretion. Their effect on the animal performance is relatively insignificant (Whitehead et al,1981;Sim et al,1984;Birk,1986).

The mean alpha-galactoside content of fababeans is 2.16% (Lattanzio et al,1986). Alpha-galactosides are not digested in the small intestine but are subjected to fermentation in the large intestine, yielding gases (CO_2 , H_2 and CH_4) which are responsible for the flatulence phenomena. These fermentations seem to decrease chick growth (Guillaume,1977).

Fiber and tannin concentrations appear to be related. Tannin-free cultivars have, in addition to a lower percent testa, proportionally lower concentrations of lignin but higher concentrations of ADF and cellulose (Marquardt et al,1978a). This fiber may inhibit the utilization of other nutrients.

In conclusion, the main antinutritive factors in faba beans are the tannins and the unavailable carbohydrates (fiber and alpha-galactosides), and vicine and convicine in laying hens diets. Most of the tannins and crude fiber can be removed by dehulling. Tannins can be eliminated by heating or by using tannin-free cultivars. Hydrolysis of some of the antinutritional compounds with various enzyme preparations may also improve the digestive utilization and the nutritive value of fababeans.

2.2.3.- Use of raw fababeans in monogastric diets.

Chicks responded to increasing levels of raw fababeans in the diet by increasing feed consumption, but the increase in consumption was not sufficient to maintain a body weight similar to that obtained

with soybean meal based diets (Campbell and Marquardt,1977). There was a reduction in the intestinal disaccharidase activity (Santidrian et al,1981) and in the intestinal absorption of glucose and galactose (Santidrian,1981) in rats fed fababeans. There was also a considerable increase in protein metabolism and degradation of amino acids (Bello et al,1975;Cenarruzabeitia et al,1979) and an increase in the urinary nitrogen losses (Grant et al,1987) in chickens or rats fed legumes. Levels as high as 20% of fababeans can be used in chick diets (Sanz-Arias,1964;Lanza et al,1971a;Bhargava,1976;Bhargava and O'Neil,1979;Mateos and Puchal,1981a;Classen et al,1984;Jeroch et al,1986). Levels can be increased up to 30% if methionine at 0.20-0.30% (Wilson and McNab,1972;Marquardt and Campbell,1974;Marquardt and Campbell,1975) and fat are added to the diet. The additions correct energy and methionine deficiencies in diets that contain a high concentration of fababeans (Sanz-Arias,1964;Wilson,1977;Gardiner et al,1980). Carcass quality was not affected in broilers when high levels of fababeans were present in the diets (Lanza et al,1971a;Liebert et al,1983).

Savage et al (1986) reported that raw fababeans could be fed to day-old turkey poults at levels up to 60% with dietary supplementation of energy, methionine and choline.

When fababeans were fed to laying hens, egg production always tended to be lower than that obtained with hens fed soybean meal control diets (Robblee et al, 1977;Davidson,1980;Campbell et al,1980). It appears that more than 10-15% of fababeans in layer diets decreases egg weight (Lanza et al, 1971b;Wilson,1977;Guillaume,1977;Mateova et

al,1984), and more than 20-25%, even with addition of methionine, decreases the laying rate (Sanz-Arias,1964;Davidson,1973;Vogt,1973;Dun and Beer,1974;Robblee et al,1977;Wilson,1977;Campbell et al,1980). Supplementation with tryptophan increased the rate of lay but did not affect the egg weight reduction (Larbier,1980). Some authors have reported that condensed tannins are responsible for the decreased rate of egg production and egg size (Guillaume and Bellec,1977;Martin-Tanguy et al,1977a), while others reported that the two factors were not affected by condensed tannins (Davidson,1980). Marquardt (1982) demonstrated that egg size depression and associated effects were caused by vicine and convicine. It can be concluded that 20% of fababeans, using standard dietary formulations, can be safely given in rearing feeds but not more than 10-15% should be given in laying feeds (Wilson and Teague,1974;Mateos and Puchal,1980,1985).

In swine, the suggested maximum level of fababeans in the diet is 20-25% (Lightfoot et al, 1974;Levillet and Castaing,1974;Castell,1976; King and Green,1976;Onaguise et al,1976;Onaguise and Bowland,1977; Aherne et al, 1977;Petersen and Schulz,1979;Mateos and Puchal,1981b). This level can be raised to 30-35% if methionine and tryptophan are added to the diet (Balboa et al,1966;Henry et al,1976;Henry and Bourdon,1977;Davidson,1977). Total replacement of soybean meal by fababeans in the diets of pigs had no adverse effect during the finishing phase (Onaguise and Bowland,1977;Haider,1979), for lactating sows (Etienne et al,1975) or for pregnant sows (Etienne,1977). However levels higher than 10% decreased growth and feed efficiency (Fekete et

al,1985) and nitrogen retention (Myer et al,1982) of weaned piglets. Henry and Bourdon (1977) concluded that when only half of the soybean meal was replaced, or when diets were supplemented with tryptophan, or when older pigs were used, responses were not different from those of the controls.

2.2.4.- Treatments to improve the nutritive value of fababeans.

Growth and feed efficiency of chickens was improved when dietary fababeans were heat treated, by microwave irradiation (Marquardt et al,1976), micronization (McNab and Wilson,1974;McNab,1977b), steam pelleting (Blair et al,1970;Wilson,1977;Marquardt et al,1976;McNab, 1977b;Liebert et al,1983), extrusion (Marquardt et al,1976;Cerning-Beroard,1977;Melcion and Valdebouze,1977;Moscicki et al,1984), and autoclaving (Wilson and McNab,1972;Bello et al,1972;Marquardt and Campbell,1973;Marquardt and Campbell,1974;Marquardt et al,1974; Marquardt et al,1976;Campbell and Marquardt,1977). The effect of autoclaving was more pronounced at high dietary levels of fababeans (Marquardt and Campbell,1973;Campbell and Marquardt,1977;Bezares et al,1980). The effect of heat treatment has been attributed to an inactivation of the condensed tannins (Rys and Zima,1977;Marquardt and Ward,1979;Ilian et al,1985;Rodriguez and Bayley,1987) which in turn improved digestibility of starch and protein (McNab,1977a;Guillaume, 1978) and possibly the compounds associated with the testa of the seed (Marquardt et al,1976). Better results were obtained when the moisture content of the beans was increased before heat-treatment (Marquardt et al,1976). Autoclaving increased the apparent metabolizable energy by

10-15% (McNab,1977a;Shannon and Clandinin,1977;Bhargava and O'Neil, 1979;Rys and Fras,1982; Nwokolo and Oji,1985). As expected, the response to autoclaving was greater for tannin-containing than for tannin-free fababean cultivars (Marquardt and Ward,1979), and for whole as compared to dehulled seeds (Cerning-Beroard, 1977). Although autoclaving increases nitrogen digestibility (Guillaume,1978) by inactivation of the tannins, it decreases the biological availability of some amino acids (Shannon and Clandinin,1977;Ilian et al,1985;Mitaru et al,1985;Kadam et al,1987). Levels as high as 35-40% of heat-treated fababeans can be used in chick diets (Campbell and Marquardt,1977; Bezares et al,1980;Ilian et al,1985), replacing at least 60% of the soybean meal (Bhargava and O'Neil,1979;Moscicki et al,1984). Diets with autoclaved beans supplemented with methionine allowed young chickens to grow as well as when soybean meal is the main protein source (Bhargava, 1976).

Egg production in laying hens fed a diet containing 35% of autoclaved fababeans supplemented with methionine was the same as that obtained when standard corn-soybean meal diet were fed (Davidson, 1977). The decrease in egg size however was not prevented by heat treatments or removal of the testa (Guillaume and Bellec,1977;Davidson, 1977;Olaboro et al,1977;Campbell et al,1980;Olaboro et al,1980; Davidson,1980). Autoclaving did not prevent this effect as egg size depression is caused by vicine and convicine, which are not inactivated by heat treatment (Marquardt, 1982).

Heat treatments of fababeans did not affect nutrient digestion in the small intestine of the pig (Ivan and Bowland,1976) and did not

alter productivity (Aherne et al,1977).

Treatments with formaldehyde inactivated the tannins of the testa (Ford and Hewitt,1979a), but when the amount of formaldehyde was increased there was a reduction in the availabilities of some amino acids and a decrease in the digestibility of the protein when fababeans were fed to poultry (Pisulewski and Krasnodebska,1978). Treatment of high-tannin sorghum grain with ammonia inactivated the tannins and improved its nutritional value when fed rats and chickens (Ford and Hewitt,1979b). Mitaru et al (1983,1985) reported that high moisture storage of high tannin sorghums decreased their tannin content and improved poultry performance.

Marquardt et al (1975) reported that in vitro digestibility by rumen microorganisms was 34.4% for hulls and 87.4% for dehulled fababeans. Although dehulling increased the trypsin inhibitor activity by 20% (Melcion and Valdebouze,1977), it reduced the fiber and the tannins (Marquardt et al,1975) and as a result increased nitrogen digestibility (Meier et al, 1977) thereby improving chick performance.

Fermentation (water soaking) of fababeans decreased the concentrations of NDF, ADF and crude fiber (Centers et al,1985;Bednarski and Tomasik,1985), and increased the content of total and essential amino acids by about 10%, with the largest increase being that of methionine (Laurent et al,1980; Centers et al,1985). The most dramatic improvement occurred in fababeans with the lowest content of

protein (Centers et al,1985). Fermentation also decreased slightly the activities of proteolytic enzyme inhibitors (Bednarski and Tomasik, 1985). Pak and Barja (1973) reported that water soaking was more effective than autoclaving for improving protein utilization.

2.3.- Use of supplementary enzymes in monogastric nutrition.

Enzyme supplementation of monogastric diets is the subject of numerous research reports. Jensen et al (1957) reported that the nutritive value of barley for chicks was improved by enzyme supplementation. The effects of enzymes on fababeans, however, have not been studied.

Rexen (1981) reported that enzymes (amylases, proteases or cellulases) only improved feed utilization (5-10%) when added to diets containing poorly digested ingredients. Collier and Harly (1986) reported that enzyme supplements enhanced utilization of ingredients with low biological value. The greatest response to enzyme supplementation appears to be in the youngest animals (Reese et al, 1983;Kojal et al,1983;Nurtdinov,1986;Nurtdinov and Korshum,1986).

Crude enzyme preparations have been obtained by controlled fermentation of selected strains of fungi, bacteria or yeasts. The enzymes most commonly used as feed supplements are the cellulases which hydrolyze the beta-D-1,4-glucosidic bonds of the cellulose, its oligomers, and derivatives, converting cellulose to glucose. Polysaccharidases hydrolyze the glucosidic bonds of cellulose, hemicellulose and branched pectin substances, degrading the plant cell

walls and producing mainly low molecular weight carbohydrates. Hemicellulases, xylanases and arabanases can partially hydrolyze the pentosans of rye and other cereals, and as a result reduce the viscosity of the highly viscous, water-soluble pentosans. Beta-glucanases hydrolyze the beta-glucans of barleys and oats, and thereby reduce the viscosity. Alpha-amylases, which hydrolyze 1,4-alpha-glucosidic linkages in amylose and amylopectin at random, yield substantial quantities of maltose and glucose. Proteases hydrolyse proteins to lower molecular weight peptides and amino acids.

The supplemental enzymes used in animal nutrition must be able to withstand pelleting, other proteolytic enzymes and pH conditions in the small intestine. It is unlikely that available commercial enzymes preparation formulated for purposes other than for animal feeds will be adequate (Lyons,1987).

Most enzyme preparations used to supplement feed are crude mixtures containing many of the enzymes discussed in this section. Consequently, it is not feasible to conclude that an effect is attributable to a specific enzyme; this can only be done if a pure enzyme preparation is used. An example of misinterpretation is that of Grammer et al (1982) who stated that a pectinase improved the utilization of rye. Rye contains essentially no pectin but is rich in the highly viscous water-soluble pentosans, which have been identified as the principal antinutritional factor (Ward and Marquardt,1987). The hemicellulase activity in the "pectinase" preparation was probably

responsible for its beneficial effects.

2.3.1.- Beta-glucanases.

The inferior feeding value of barley for chickens, as compared to other cereals, seems to be caused mainly by the presence of beta-glucans, because their hydrolysis does not occur in the normal digestive processes of the chick (Burnett,1966;White et al,1981). The beta-glucans dilute the energy value of barley and interfere with digestion and absorption of nutrients; as a result barley is generally limited to 20-25% in broiler-diets and 25-35% in layer-diets (Gohl et al,1978). In addition, birds fed barley may have an increased level of water consumption and produce sticky droppings. This fact have been attributed to the water-soluble beta-glucans (Gohl et al,1978).

Beta-glucanases hydrolyze polysaccharides consisting of beta-1,3- and beta-1,4-glucopyranose units and reduce their viscosity in water (Rotter et al,1987). Weight gains of chicks fed barley based diets supplemented with beta-glucanase varied from 0 to 20% with the degree of response being influenced by cultivar (Rotter et al,1987). The net effects of enzymes are to increase metabolizable energy and apparent digestibility of barley (Leong et al,1961), and to improve weight gains and efficiency of feed conversion (Campbell et al, 1984;Newman and Newman,1987). Also dry matter content of feces was increased (Rotter et al,1987) and their viscosity was reduced allowing for easier management and cleaning of litter, cages, birds, etc. White et al (1981) reported that the performance of chicks fed barley based diets supplemented with cellulase was saturable and that increasing amounts of enzyme above

0.005% produced no further improvement. Beta-glucanases have also been added with success to the drinking water (Hesselman et al,1981).

White et al (1983) suggested that factors other than glucose release are important in the growth response of chicks fed beta-glucanase supplemented barley diets because, if the total amount of beta-glucans were hydrolyzed, only about 3% of the diet would be converted to glucose; however a 10% increase in chick growth on enzyme supplemented feed is typical. Burnett (1966) suggested that the response to beta-glucanase may be attributed not only to glucose release from the hydrolysis of beta-glucans, but also to higher feed consumption as a consequence of improving intestinal passage (reduced viscosity) as well as to increasing availabilities of nutrients. Beta-glucanase inclusion also favoured utilization of starch in the more anterior portion in the small intestine (Hesselman and Aman,1985). Addition of beta-glucanases to low-energy diets such as those containing oats also improved growth rate in chicks (Campbell et al,1987). However, when added to maize diets, beta-glucanases did not affect chick performance (Broz and Frigg,1986).

2.3.2.- Hemicellulases, cellulases and pectinases.

The factors that are responsible for the poor utilization of rye by chickens appear to be the water-soluble and highly viscous pentosans. These compounds are not only undigestible but also interfere with the digestion of other nutrients including minerals, carbohydrates, amino acids, vitamins and particularly fats (Marquardt et al,1987). In addition pentosans produce wet sticky excreta which

also causes management problems. Birds, particularly young chicks, may be more sensitive to the effects of these compounds than other monogastric animals because of their more elementary digestive system (Marquardt and Fengler,1985). The pentosans of rye were rapidly hydrolyzed to non-viscous short chain polysaccharide units (Pawlik et al,1986) and chick performance were improved (Marquardt et al,1987) by adding fungal crude enzymes (presumably xylanases) to their diets.

Nahm and Carlson (1985) reported that cellulase supplementation improved the digestibility of cell wall components of wheat fed broilers. Wheat bran supplemented with cellulase gave improved weight gains in rats, and dry matter digestibility increased by 20% (Saunders et al,1972). Kuzmicky et al (1978) reported that wheat bran supplemented with a cellulolytic enzyme system dramatically increased the apparent metabolizable energy for broilers and the in vitro digestibility of protein. Cellulase supplementation of diets based on barley, oats or rye markedly improved both the growth rate and feed conversion of chickens, but did not improve broiler performance when added to commercial diets (White et al,1980;Reese et al,1983;Broz and Frigg,1986). Cellulase, when added to hen diets, increased cellulolytic activity in the digestive tract, intensified hydrolysis of non-readily hydrolysable polysaccharides, increased utilization of nitrogen, fat, fibre and N-free extract, increased egg yield and improved efficiency of feed utilization (Kvitkin and Tishenkova,1982). Utilization of nutrients was increased in poultry when hemicellulase was added to commercial diets (Tishenkova et al,1986). On the other hand, Halverson

(1985) reported that addition of a fungal cellulase did not affect the performance of rats fed barley.

Addition of cellulase to pig diets increased total volatile fatty acids in the caecum by 10-15% (Tang et al,1983), improved weight gains by 5% and feed efficiency by 9% (Kanev et al,1977). On the other hand, Jelic et al (1982) reported than there were no significant differences in average daily gain or efficiency of feed utilization in growing pigs given cellulase. Bodart (1979), in agreement with the results of Leong et al (1961), reported that average daily gain and feed intake were independent of the crude fiber content of the diet when a cellulase was used in fattening pig diets.

Pectinase when added to the diet increased nitrogen, fat and crude protein utilization in broiler chicks, ducks, geese and laying hens (Tishenkova et al,1986) and stimulated the rate of passage of digesta through the alimentary tract (Issiki and Nakahiro,1984). Pectinesterase when added to a barley and wheat based diet (low in metabolizable energy) had effects on carbohydrate intake in broilers that were similar to those produced by the supplementation of 2% edible fat (Mikhailov et al, 1981).

2.3.3.- Alpha-amylases.

Alpha-amylase when added to commercial diets improved both weight gain and feed utilization of turkeys (Parkany-Gyarfas,1975), chicks (Reese et al,1983;Petkov and Kuceroval,1986), and pullets

(Berezhnova,1981), and increased egg yield by 20% (Dogan et al,1973).

Amylase supplementation of diets for pigs, from 15 to 100 days of age, improved weight gains by up to 25% and efficiency of feed utilization by 5% (Popehina and Gajstruk,1971). Newman et al (1980) reported that the addition of diastase to barley-based diets tended to improve weight gains and efficiency of feed utilization in pigs. Goryachev et al (1974) reported that there was 5% more meat in the carcasses of pigs fed supplemental amylase, but there was no differences in average daily gain or feed intake.

On the other hand, alpha-amylase supplementation of commercial diets had no effects on gain or feed efficiency in pigs or broilers (Cheshmedzhiev et al,1976;Kanev et al,1977;Jelic et al,1982). Supplementation of barley-based diets with alpha-amylase did not affect rate of growth or the efficiency of feed utilization of chickens (Willingham et al,1959; Broz and Frigg,1986).

2.3.4.- Proteases.

Vogt and Harnisch (1978) reported that the addition of a crude protease to broiler diets containing a reduced content of protein (80% of normal) yielded performance values that were almost the same as those obtained with control soybean meal diets. The treatment also increased nitrogen retention and metabolizable energy values when laying hens were fed protein deficient diets.

Other studies have demonstrated that weight gain and feed efficiency were also improved when proteases were added to diets for broilers (Cibulka et al,1981;Heger et al,1984), pullets (Berezhnova,

1981) and pigs (Popehina and Gajstruk, 1971). In contrast, other studies have indicated that proteolytic enzymes had no effect on gain or feed efficiency in broilers (Stoyanov, 1972; Lewis and Hiller, 1980; Reese et al, 1983), piglets (Casteels and Buysee, 1977) or pigs (Cheshmedzhiev et al, 1976).

2.3.5.- Mixed enzymes.

Mixed enzyme preparations containing amylase, protease and cellulase activity when added to the diet improved weight gains in fattening pigs (Podhorsky et al, 1985) and increased egg yield but not egg weight in layers (Zarirov et al, 1977).

Berezhnova (1981) reported that growth of pullets was increased when amylolytic and proteolytic enzymes were given together. With an enzyme preparation containing protease and starch-splitting enzymes, broiler chicks grew faster and ate 10% more than the controls (Nierodzik et al, 1986). However, enzyme preparations with amylolytic and proteolytic activity did not affect the digestibilities of nutrients, weight gain or feed intake of pigs (Stibic, 1971; Polivoda and Yuditseva, 1974) or growing cocks (Rosochacki et al, 1981). Jelic et al (1982) also reported that an alpha-amylase and cellulase mixture did not improve average daily gain or feed efficiency in growing pigs. Addition of a mixture of cellulase, pectinase and protease only had beneficial effects if the feed mixture was made up of ingredients of low digestibility (Rexen, 1981).

2.3.6.- Enzyme supplements to plant protein feedstuffs.

Endogenous proteases and amylases play an important role in breaking down the macromolecules in the leaf, pod and seed coat. However, the activities of these enzymes decrease with maturation (Rauf,1980).

Fermentation of soybeans with 10 species of *Aspergillus* improved weight gain and feed efficiency in broilers due to a greater supply of the essential amino acids, with the response being more pronounced in chicks fed the low dietary levels of protein (Chah et al,1975).

Beta-glucosidase hydrolyzed vicine and convicine of fababeans with the degree of hydrolyses being dependent on the duration of treatment (Arbid and Marquardt,1985).

In vitro hydrolysis of canola meal with protease increased its soluble protein content (Ma and Ooraikul,1986). Enzyme supplementation of rapeseed meal, however, did not affect body weight gains and feed conversion efficiency in broilers (Goh et al,1982). Longstaff and McNab (1987) reported that cellulase when added to a diet containing ground peas, improved fiber digestion but that it did not improve the digestibility of starch or metabolizable energy values.

Hemicellulase addition increased growth and feed efficiency of chickens (Verma and McNab,1982;Patel and McGinnis,1985), but had no effect on egg yield when given with raw or autoclaved guar meal (Patel and McGinnins,1985).

3. STUDY ONE: EFFECTS OF ENZYME ADDITIONS ON THE NUTRITIVE VALUES OF RAW, AUTOCLAVED AND FERMENTED FABABEANS AS ESTIMATED FROM CHICK GROWTH TRIALS.

3.1.- Introduction.

Three experiments assessed the effects of several enzymes at concentrations of 1.5, 2.5 and 5.0 g/kg of diet on the nutritive values of raw, autoclaved and naturally fermented fababeans. Weight gain, dry matter retention, feed consumption and feed efficiency were measured using male White Leghorn chicks. The birds were fed the experimental diets from 7 to 21 days of age.

3.2.- Materials and Methods.

3.2.1.- Materials and Diet composition.

The fababean cultivar used in this and subsequent studies was Diana and was grown at the University of Manitoba research farm. The mean weight of fababeans was 0.30 g/seed. The testa was 14% of the seed and had a mean tannin content of 4.56%. The composition of the beans is given in Table 1 (page 29). Prior to use the fababeans were ground in a hammer mill having a sieve size of 2 mm. The crude enzyme preparations were liquid in form with a density of 1.25 g/ml and were from Novo Industrias (Copenhagen, Denmark). Chemicals were from Fisher Scientific

Company (New Jersey, USA). Single Comb White Leghorn chicks were obtained from a commercial hatchery. The composition of the diets is given in Table 2 (page 30). Chromic oxide was used as a non-absorbed indicator in the feed.

3.2.2.- Experimental design, and chemical and statistical analyses.

The first experiment involved two levels (2.5 and 5.0 g/kg of air-dry diet) of four different enzymes (Celluclast, a cellulase from Trichoderma reesei; SP249, a polysaccharidase from Aspergillus niger; Alcalase 0.6L, a protease from Bacillus licheniformis, and BAN, an alpha-amylase from B. subtilis), and two combinations of these enzymes (polysaccharidase + protease, and polysaccharidase + cellulase) at a concentration of 0.125% of each. The control diet contained fababeans with no enzyme additions. The enzymes, prior to their addition to the diets, were diluted in 200 ml of water, added to 2.5 kg of fababeans and then mixed with the rest of the diet. The total number of diets which contained raw fababeans was 11.

In the second experiment the effects on chicks of autoclaved fababeans with and without enzyme supplements were measured. Fababeans were autoclaved for 20 min at 121 C at a depth of 2 cm. After autoclaving, the beans were dried at room temperature during one day, so their dry matter content was 90.7%, similar to that of raw beans. Three different enzymes (cellulase at 2.5, protease at 2.5, and alpha-amylase at 1.5 g/kg diet) and three combinations of them (protease at 2.5 + alpha-amylase at 1.5, cellulase at 2.5 + alpha-amylase at 1.5,

and cellulase at 2.5 + protease at 2.5 g/kg diet) were tested. The last combination was used with both raw and autoclaved fababeans. The two control diets contained raw or autoclaved fababeans with no enzyme supplementation. The total number of dietary treatments was 9.

In the third experiment the effects of enzymes on the nutritive value of fermented ground fababeans was studied. Fababeans were soaked in the proportion 1 kg beans:1 l of water, with the different enzymes being added to the water. The beans were anaerobically fermented for 24 h at 21-25 C and were then dried at 30-34 C for five days. The dry matter content of fababeans before the treatment was 88-89% and after drying was 92-93%. After drying the beans were reground and the diets were prepared. Two enzymes (cellulase at 2.5 and protease at 2.5 g/kg diet) and one combination of them, each at 2.5 g/kg diet, were tested. Other treatments included diets containing raw, autoclaved or fermented fababeans with no enzyme additions. The total number of diets was 6.

Analyses of dry matter, ash, ether extract, calcium, phosphorus, protein and NDF in diet and fecal samples were carried out according to procedures outlined in A.O.A.C. (1984). Amino acids were determined according Moore and Stein (1963). ADF was analyzed according Goering and Van Soest (1975). Chromic oxide was determined according Williams et al (1962). Gross energy was determined using a PARR adiabatic oxygen bomb calorimetry. Condensed tannins were analysed according to the method outlined by Price et al (1978), which is a modification of the original procedure of Burns (1971).

All experimental trials were set up as completely randomized designs. There were 8 replicates of each diet with 6 birds per replicate in each of the three experiments. Linear contrasts among treatments were conducted for all data using the general linear model procedure (GLM) of the Statistical Analysis System (SAS Institute, Inc., 1985). Standard errors were also calculated using SAS and are based on eight observations per treatment. The use of the 10% level of probability is very unusual, however it was utilized in this thesis because the high standard errors associated to diets containing enzymes. Therefore, the significant differences at 10% level indicate only a tendency since these differences will occur 10% of the time by chance alone.

3.2.3.- Birds and Management.

One-day-old male leghorns were housed in electrically heated, thermostatically controlled Jamesway brooders with raised wire floors and provided with continuous lighting for 7 d prior to the start of an experimental trial. All chicks were fed commercial chick starter crumbles containing a minimum of 21% protein during the pre-experimental period.

When the chicks were 7 day old, they were starved for 4 h to decrease gut fill. The birds were then individually weighed and placed into appropriate groups having weight ranges of 4 g. The heavy and light birds were discarded and the remainder were randomly assigned to

the experimental groups (replicates) so the mean weight of birds in each replicate was similar. The replicates contained six birds and there were eight replicates per treatment.

Birds on test were housed in Petersime battery brooders with continuous light and heat, and raised wire floors. Feed and water were provided ad libitum. The room and battery temperatures during the experiments were 21 and 32 C, respectively. At the end of the 7 and 14 day experimental periods, the birds were starved again for a 4 h period before weighting. Weight gain was measured by weighing each cage of birds individually. The difference between the initial and final pen weights of the chicks was divided by the number of birds in the pen and this was taken as the change in body weight. Weight gain, feed consumption and the feed to gain ratio (feed efficiency) were calculated.

Dry matter retention values were determined on excreta collected from four replicates per treatment during the last three days of the experiment. The excreta were dried at room temperature for two days and stored at -20 C until analyzed. The equation used to calculate dry matter retention (Marquardt et al, 1979) was:

$$\% \text{ DM retention} = \left(1 - \frac{\% \text{ DM feces}}{\% \text{ DM feed}} \times \frac{\% \text{ Cr feed}}{\% \text{ Cr feces}} \right) \times 100$$

TABLE 1.- Composition of fababeans used in this thesis.

Composition ¹	(g/kg)
Dry matter	90.18
Ash	3.54
Crude protein (N x 6.25)	31.35
Ether extract	0.90
ADF	10.38
NDF	26.14
Calcium	0.06
Phosphorus	0.65
Gross energy (MJ/kg DM)	18.47
Amino acids:	
Aspartic acid	2.97
Threonine	1.04
Serine	1.49
Glutamic acid	4.61
Proline	1.20
Glycine	1.18
Alanine	1.11
Cystine	0.27
Valine	0.94
Methionine	0.21
Isoleucine	0.88
Leucine	1.97
Tyrosine	0.75
Phenylalanine	1.14
Histidine	0.69
Lysine	1.72
Arginine	2.86

1: Values, except for dry matter content, are expressed on a dry matter basis.

TABLE 2.- Ingredients and composition of diets used in study one.

Ingredients ¹	(g/kg)
Fababeans	90.65
Tallow	5.00
Calcium phosphate	2.00
Calcium carbonate	0.50
Vitamins ²	1.00
Minerals ³	0.35
Methionine	0.20
Chromic oxide	0.30
Enzymes ⁴	variable
Composition ⁵	
Dry matter	88.00
Ash	6.00
Ether extract	5.75
Crude protein (N x 6.25)	23.75
ADF	10.25
NDF	24.75
Calcium	0.65
Phosphorus	0.85
Gross energy	17.35 MJ/kg DM

1: Values are expressed on a air dry basis.

2: Mineral mix composition (g/kg premix): manganese oxide (60% Mn), 26.20; zinc oxide (72% Zn), 19.85; ferrous sulphate (35% Fe), 73.75; copper sulphate (25% Cu), 5.65; sodium selenite (0.02% Se), 142.50; iodized salt, 732.05.

3: Vitamin mix composition (g/kg premix): vitamin A (500,000 UI/g), 1.65; vitamin D3 (40,900 UI/g), 0.50; vitamin E (40 UI/g), 27.25; vitamin B12 (1 mg/g), 1.15; vitamin B58 (per kg of vitamin B58: riboflavin, 5.5 g; Ca-Pantothenate, 8.8 g; niacin, 13.2 g; choline chloride, 220.0 g), 125.0; niacin 98%, 3.75; menadione Na-bisulfate (50%), 0.22; choline chloride (60%), 150.0; folic acid (pure), 0.075; biotin (20 mg/g), 1.25; delaquin, 18.75; methionine DL (99%), 50.0; Strep-Pen, 10.0; amprol, 100.5; vegetable oil, 10.0; wheat middlings, 550.405.

4: Enzymes were added at concentrations ranged from 0 to 0.50 % of the diet.

5: Values, except for dry matter content, are expressed on a dry matter basis.

3.3.1.- Experiment 1

The addition of enzymes to raw fababeans (Tables 3 and 4) did not affect significantly ($p>0.10$) weight gain in the first week of the experiment. In the second week the addition of protease at 0.25% improved weight gain by 11% ($p<0.01$) while the addition of cellulase at 0.25% and at 0.50% improved it by 6-7% ($p<0.05$). Over the entire course of the experiment, the addition of protease to the diet at 0.25% improved weight gain by 6% ($p<0.05$), and addition of cellulase at 0.50% and alpha-amylase at 0.25% tended to increase it by 5% ($p<0.10$). The addition to the diet of both levels of polysaccharidase reduced feed consumption by 6% ($p<0.05$) while the addition of protease at 0.50% and the combinations cellulase+polysaccharidase and protease + polysaccharidase tended to reduce it by 4-5% ($p<0.10$). Feed efficiency was improved by 6% ($p<0.01$) when protease at 0.25% was added, and by 4-5% ($p<0.05$) with cellulase at 0.50% and with protease at 0.50%. Dry matter retention was dramatically reduced by addition of polysaccharidase at 0.25% ($p<0.01$), but was not affected ($p>0.10$) by any of the other enzymes. Both levels of polysaccharidase and its combinations with protease and cellulase yielded feed consumption and dry matter retention values that were lower ($p<0.01$) than those obtained with the other enzymes.

In general, the best performance values were obtained when the diets were supplemented with the low concentration of enzyme. Protease at 0.25% yielded better weight gains in the second week ($p<0.01$) and for the entire period ($p<0.10$) than when at a concentration of 0.50%.

TABLE 3.- Weight gains of chicks fed enzyme supplemented raw fababeans (experiment 1).

Enzyme	Weight gain ¹ (g)		
	7-14 days	14-21 days	7-21 days
No enzymes	49.7	61.0	111
Cellulase at 0.25%	50.3	65.4	116
Cellulase at 0.50%	51.7	65.5	116
Polysaccharidase at 0.25%	48.7	58.0	107
Polysaccharidase at 0.50%	50.7	58.1	109
Protease at 0.25%	49.9	67.9	118
Protease at 0.50%	50.4	60.9	111
Alpha-amylase at 0.25%	52.8	63.5	116
Alpha-amylase at 0.50%	51.2	59.2	111
Cellulase 0.125% + Polysacch. 0.125%	46.8	62.7	110
Protease 0.125% + Polysacch. 0.125%	49.9	60.2	110
Standard error	1.7	1.6	2.5
Linear contrasts	7-14 days	14-21 days	7-21 days
Effect of enzyme addition to raw beans			
No enzymes vs Cellulase at 0.25%	NS	**	NS
No enzymes vs Cellulase at 0.50%	NS	**	*
No enzymes vs Polysacch. at 0.25%	NS	NS	NS
No enzymes vs Polysacch. at 0.50%	NS	NS	NS
No enzymes vs Protease at 0.25%	NS	***	**
No enzymes vs Protease at 0.50%	NS	NS	NS
No enzymes vs Alpha-amylase at 0.25%	NS	NS	*
No enzymes vs Alpha-amylase at 0.50%	NS	NS	NS
No enzymes vs Cellulase + Polysacch.	NS	NS	NS
No enzymes vs Protease + Polysacch.	NS	NS	NS
Effect of enzyme level			
Cellulase, 0.25% vs 0.50%	NS	NS	NS
Polysaccharidase, 0.25% vs 0.50%	NS	NS	NS
Protease, 0.25% vs 0.50%	NS	***	*
Alpha-amylase, 0.25% vs 0.50%	NS	*	*

1: Weight gains were significantly different ($p < 0.01$) between week 1 and 2.

NS: no significant difference ($p > 0.10$)

*: significant difference ($p < 0.10$)

**: significant difference ($p < 0.05$)

***: significant difference ($p < 0.01$)

TABLE 4.- Feed consumption, feed efficiency and dry matter retention of chicks fed enzyme supplemented raw fababeans (experiment 1).

Enzyme	Feed consumption (7-21 days) (g)	Feed efficiency ¹ (7-21 days) (g/g)	Dry matter retention (18-21 d) (%)
No enzymes	290	2.63	48.5
Cellulase at 0.25%	296	2.56	48.8
Cellulase at 0.50%	293	2.52	51.2
Polysaccharidase at 0.25%	273	2.56	37.5
Polysaccharidase at 0.50%	275	2.53	46.1
Protease at 0.25%	293	2.49	48.3
Protease at 0.50%	278	2.50	49.2
Alpha-amylase at 0.25%	297	2.55	50.0
Alpha-amylase at 0.50%	295	2.67	49.2
Cellulase 0.125% + Polysacch. 0.125%	279	2.55	43.2
Protease 0.125% + Polysacch. 0.125%	279	2.53	46.4
Standard error	5.8	0.04	2.7

Linear contrasts

	Consumption	Efficiency	Retention
Effect of enzyme addition to raw beans			
No enzymes vs cellulase at 0.25%	NS	NS	NS
No enzymes vs cellulase at 0.50%	NS	**	NS
No enzymes vs polysacch. at 0.25%	**	NS	***
No enzymes vs polysacch. at 0.50%	**	*	NS
No enzymes vs protease at 0.25%	NS	***	NS
No enzymes vs protease at 0.50%	*	**	NS
No enzymes vs alpha-amylase at 0.25%	NS	NS	NS
No enzymes vs alpha-amylase at 0.50%	NS	NS	NS
No enzymes vs cellulase + polysacch.	*	NS	NS
No enzymes vs protease + polysacch.	*	*	NS
Effect of enzyme level:			
Cellulase, 0.25% vs 0.50%	NS	NS	NS
Polysaccharidase, 0.25% vs 0.50%	NS	NS	*
Protease, 0.25% vs 0.50%	*	NS	NS
Alpha-amylase, 0.25% vs 0.50%	NS	**	NS

1: feed consumption/weight gain

NS: no significant difference ($p > 0.10$)

*: significant difference ($p < 0.10$)

**: significant difference ($p < 0.05$)

***: significant difference ($p < 0.01$).

Also feed consumption tended to be higher ($p < 0.10$) at 0.25% than at 0.50%. Chicks fed diets containing alpha-amylase at 0.25% also tended to have higher weight gains in the second week ($p < 0.10$) and over the two week experimental period ($p < 0.10$), and better overall feed efficiency ($p < 0.05$) compared to those obtained at the 0.50% concentration level. The response to cellulase treatment, however, was not affected ($p > 0.10$) by the concentration of cellulase (0.25 or 0.50%).

3.3.2.- Experiment 2

Autoclaving fababeans (Tables 5 and 6) improved weight gain by 10% ($p < 0.01$) in the first week, by 17% ($p < 0.01$) in the second week, and by 14% ($p < 0.01$) over the two week period. It reduced feed consumption by 4% ($p < 0.01$) and improved the feed to gain ratio by 16% ($p < 0.01$). In general, addition of enzymes to autoclaved fababeans did not improve chick performance, however, addition of both protease+alpha-amylase and cellulase+alpha-amylase improved dry matter retention by 11% ($p < 0.01$), but reduced feed efficiency by 3% ($p < 0.05$).

When a combination of cellulase at 0.25% + protease at 0.25% was added to raw fababeans, weight gain in the second week was improved by 8% ($p < 0.01$) and by 5% ($p < 0.05$) over the two week period. Feed efficiency was also improved by 5% ($p < 0.01$). Weight gain in the first week and feed consumption over the two week period were not significantly affected ($p > 0.10$). Performance of chicks fed the enzyme supplemented raw fababeans were inferior ($p < 0.01$) to those fed autoclaved fababeans.

TABLE 5.- Weight gains of chicks fed enzyme supplemented autoclaved fababeans (experiment 2).

Treatment and enzyme	Weight gain ¹ (g)		
	7-14 days	14-21 days	7-21 days
Raw fababeans			
No enzymes	49.3	65.4	115
Cellulase 0.25% + Protease 0.25%	49.5	70.5	120
Autoclaved fababeans			
No enzymes	54.2	76.5	131
Cellulase at 0.25%	53.6	76.4	130
Protease at 0.25%	54.6	76.6	131
Alpha-amylase at 0.15%	53.4	75.2	129
Cellulase 0.25% + Protease 0.25%	54.5	77.7	132
Cellulase 0.25% + Alpha-amylase 0.15%	52.6	74.5	127
Protease 0.25% + Alpha-amylase 0.15%	52.9	75.0	128
Standard error	0.9	1.5	2.0
Linear contrasts	7-14 days	14-21 days	7-21 days
Effect of autoclaving			
Raw (no enz) vs autoclaved (no enz)	***	***	***
Effect of enzyme addition to raw beans			
No enzymes vs cellulase + protease	NS	***	**
Effect of enzyme addition to autoclaved beans			
No enzymes vs cellulase	NS	NS	NS
No enzymes vs protease	NS	NS	NS
No enzymes vs alpha-amylase	NS	NS	NS
No enzymes vs cellulase + protease	NS	NS	NS
No enzymes vs cellulase + alpha-amylase	NS	NS	NS
No enzymes vs protease + alpha-amylase	NS	NS	NS

1: Weight gains were significantly different ($p < 0.01$) between week 1 and 2.

NS: no significant difference ($p > 0.10$)

** : significant difference ($p < 0.05$)

***: significant difference ($p < 0.01$)

TABLE 6.-Feed consumption, feed efficiency and dry matter retention of chicks fed enzyme supplemented autoclaved fababeans (experiment 2).

Treatment and enzyme	Feed consumption (7-21 days) (g)	Feed efficiency ¹ (7-21 day) (g/g)	Dry matter retention (18-21 d) (%)
Raw fababeans			
No enzymes	295	2.58	-
Cellulase 0.25% + Protease 0.25%	293	2.44	50.3
Autoclaved fababeans			
No enzymes	283	2.16	48.7
Cellulase at 0.25%	285	2.20	50.5
Protease at 0.25%	282	2.15	47.7
Alpha-amylase at 0.15%	282	2.19	47.2
Cellulase 0.25% + Protease 0.25%	283	2.14	51.5
Cellulase 0.25% + Alpha-amylase 0.15%	283	2.23	54.5
Protease 0.25% + Alpha-amylase 0.15%	284	2.22	54.4
Standard error	3.4	0.02	1.4
Linear contrasts			
	Consumption	Efficiency	Retention
Effect of autoclaving			
Raw (no enz) vs autoclaved (no enz)	***	***	-
Effect of enzyme addition to raw beans			
No enzymes vs cellulase + protease	NS	***	-
Effect of enzyme addition to autoclaved beans			
No enzymes vs cellulase	NS	NS	NS
No enzymes vs protease	NS	NS	NS
No enzymes vs alpha-amylase	NS	NS	NS
No enzymes vs cellulase + protease	NS	NS	NS
No enzymes vs cellulase + alpha-amylase	NS	**	***
No enzymes vs protease + alpha-amylase	NS	**	***

1: feed consumption/weight gain

NS: no significant difference (p>0.10)

** : significant difference (p<0.05)

***: significant difference (p<0.01)

3.3.3.- Experiment 3

In the third experiment (Tables 7 and 8), autoclaving of fababeans improved weight gain by 9% ($p < 0.01$) in the first week, by 17% ($p < 0.01$) in the second week, and by 13% ($p < 0.01$) over the two week experimental period. Dry matter consumption was reduced by 4% ($p < 0.05$) and feed efficiency was improved by 15% ($p < 0.01$). Autoclaving also improved dry matter retention (13%, $p < 0.01$).

Fermentation of fababeans improved weight gain by 5% ($p < 0.05$) in the first week, but not in the second week nor over the two week period. Dry matter consumption was reduced by 9% ($p < 0.01$), efficiency of feed utilization was improved by 10.5% ($p < 0.01$) and dry matter retention was increased by 9.5% ($p < 0.01$).

Chicks fed autoclaved fababeans gained more weight than those fed fermented fababeans during the second week (11%, $p < 0.01$) and during the complete experiment (18%, $p < 0.01$). Autoclaving as compared to fermentation of fababeans resulted in 5% ($p < 0.01$) greater feed consumption and a 5% ($p < 0.01$) improvement in feed efficiency. Autoclaved and fermentated fababeans yielded similar ($p > 0.10$) dry matter retention values.

The addition of cellulase or protease each at 0.25% to fermented fababeans did not affect weight gain, feed consumption or dry matter retention values. The growth and feed efficiencies of chicks fed enzyme supplemented fababeans that had been fermented were inferior ($p < 0.01$) to those fed autoclaved fababeans. However, the addition to fermented

TABLE 7.- Weight gains of chicks fed enzyme supplemented fermented fababeans (experiment 3).

Treatment and enzyme	Weight gain ¹ (g)		
	7-14 days	14-20 days	7-20 days
Raw fababeans			
No enzymes	51.0	56.9	108
Autoclaved fababeans			
No enzymes	55.5	66.5	122
Fermented fababeans			
No enzymes	53.4	56.4	110
Cellulase at 0.25%	53.3	57.4	111
Protease at 0.25%	54.2	55.3	109
Cellulase 0.25% + Protease 0.25%	55.3	61.0	116
Standard error	1.1	1.6	2.1
Linear contrasts	7-14 days	14-20 days	7-20 days
Effect of the treatment			
Raw (no enz) vs autoclaved (no enz)	***	***	***
Raw (no enz) vs fermented (no enz)	**	NS	NS
Autoclaved (no enz) vs fermented (no enz)	NS	***	***
Effect of enzyme addition to fermented beans			
No enzymes vs cellulase	NS	NS	NS
No enzymes vs protease	NS	NS	NS
No enzymes vs cellulase + protease	NS	**	**

1: Weight gains were significantly different ($p < 0.01$) between week 1 and 2.

NS: no significant difference ($p > 0.10$)

** : significant difference ($p < 0.05$)

***: significant difference ($p < 0.01$)

TABLE 8.- Dry matter consumption, dry matter efficiency and dry matter retention of chicks fed enzyme supplemented fermented fababeans (experiment 3).

Treatment and enzyme	Feed consumption (7-20 days) (g)	Dry matter efficiency ¹ (7-20 day) (g/g)	Dry matter retention (18-20 d) (%)
Raw fababeans			
No enzymes	246	2.28	53.9
Autoclaved fababeans			
No enzymes	235	1.93	60.9
Fermented fababeans			
No enzymes	223	2.04	59.0
Cellulase at 0.25%	220	1.99	59.7
Protease at 0.25%	222	2.03	59.2
Cellulase 0.25% + Protease 0.25%	221	1.90	57.4
Standard error	3.6	0.03	1.5

Linear contrasts

	Consumption	Efficiency	Retention
Effect of the treatment			
Raw (no enz) vs autoclaved (no enz)	**	***	***
Raw (no enz) vs fermented (no enz)	***	***	***
Autoclaved (no enz) vs fermented (no enz)	***	***	NS
Effect of enzyme addition to fermented beans			
No enzymes vs cellulase	NS	NS	NS
No enzymes vs protease	NS	NS	NS
No enzymes vs cellulase + protease	NS	***	NS

1: dry matter consumption/weight gain
 NS: no significant difference ($p > 0.10$)
 **: significant difference ($p < 0.05$)
 ***: significant difference ($p < 0.01$)

fababeans of a combination of cellulase at 0.25% + protease at 0.25% improved weight gains by 8% ($p < 0.05$) in the second week and by 6% ($p < 0.05$) during the two week period, and overall feed efficiency by 7% ($p < 0.01$). This enzymatic combination did not affect ($p > 0.10$) weight gain in the first week, dry matter consumption nor dry matter retention. Feed efficiency of fermented beans that had been supplemented with cellulase or with the cellulase plus protease combination was not significantly different ($p > 0.10$) from that obtained with autoclaved fababeans.

3.3.4.- General results.

Autoclaving of fababeans improved weight gains by 9-10% ($p < 0.01$) in the first week of the experiments, by 17% ($p < 0.01$) in the second week, and by 13-14% ($p < 0.01$) over the two week period. Autoclaving reduced feed consumption by 4% ($p < 0.01$) and improved feed efficiency by 15-16% ($p < 0.01$). Also it improved dry matter retention by 13% ($p < 0.01$) (experiment 3, table 8).

Fermentation of fababeans improved weight gains by 5% ($p < 0.05$) in the first week of the experiment, but not in the second week nor over the two week period. Fermentation of fababeans decreased dry matter consumption by 9% ($p < 0.01$), improved feed efficiency by 10.5% ($p < 0.01$) and increased dry matter retention by 9.5% ($p < 0.01$). Chicks fed fermented fababeans as compared to those fed autoclaved fababeans had lower weight gains, feed consumption and feed efficiency ($p < 0.01$). Dry matter retention, however, was not significantly different ($p > 0.10$).

between chicks fed autoclaved and fermented fababeans.

Enzyme addition to raw fababeans did not affect chick performance during the first week, but they improved it during the second week by increasing weight gain by more than 10% with protease, 6-7% with cellulase, and 8% with the combination protease+cellulase. In general enzymes did not affect feed consumption nor dry matter retention. Cellulase, protease and their combination, however, improved feed efficiency by more than 5%. An increased concentration of cellulase did not affect chick performance, whereas an increased concentration of protease or alpha-amylase tended to reduce chick performance. The addition of polysaccharidase to raw fababeans reduced feed consumption and dry matter retention. In general the addition of enzymes to autoclaved or fermented fababeans did not affect chick performance.

3.4.- Discussion.

The fababeans used in this study had, on dry matter basis, 31.35% protein and 18.47 MJ/kg gross energy. Tannin content of the testa was 4.56%, a little higher than the mean value reported by Marquardt et al (1978b).

As expected, autoclaving increased the nutritive value of fababeans. The weight gain was increased about 15%. This, as referenced in the literature review section, may be attributed to the inactivation of antinutritional factors which decrease the digestibility of nutrients. The greater weight gain improvement during the second week

in chicks fed autoclaved as compared to those fed raw fababeans, maybe due to the accumulated negative effects of the tannins in the gastrointestinal tract of chicks fed raw fababeans, or perhaps to a better utilization in the older birds of nutrients made available by autoclaving. The net effect of improved nutrient digestion when fababeans were autoclaved appeared to be a reduced level of feed consumption and therefore an improved efficiency of feed utilization.

Water soaking has been shown to improve the nutritive value of cereals (Marquardt et al,1987) and could become a more common treatment with the implementation of liquid feeding systems. In the current study both weight gains and efficiency of feed utilization were increased by fermentation. Some authors reported that there was a loss of tannins when beans were soaked, and that the reductions were greater when beans were soaked in salt solutions (Deshpande and Cheryan,1983; Sievwright and Shipe,1986). Other authors (Centers et al,1985; Moslehuddin and Hang,1987) reported that total and essential amino acids were increased about 10% in fermented legumes as compared with unfermented ones. Fermentation appears to induce a decrease in the palatability and a concomitant decrease in feed consumption, which prevented an increase in the weight gain. The improvement in dry matter retention is difficult to explain. Grant et al (1987) reported that consumption of kidney beans induced a depletion of lipid and glycogen reserves, and there was a compensatory increase in water retention. As reported by Shariff et al (1981), fermented fababeans were inferior to autoclaved beans, mainly due to a decrease in intake.

There was some response to addition of enzymes to fababeans, but as reported by others (Chah et al,1975;Reese et al,1983), the results were not always consistent. Thus, the standard deviation of performance when enzymes were used increased considerably, maybe due to variable individual predisposition to use the enzymes. As observed with autoclaving, the addition of enzymes to raw fababeans produced a more dramatic response in the second week. When levels of enzymes were increased from 0.25 to 0.50%, no effect or negative responses were observed, perhaps because high quantities of exogenous enzymes can adversely affect the digestive system. White et al (1981) used five concentrations of crude enzyme from T. viride and observed no further improvements in the rate of growth of broiler beyond the initial enzyme level (0.005%).

Although alpha-amylase had no effect on the nutritive value of fababeans, cellulase, protease and the combination cellulase+protease did. Several authors also reported no improvements in chick responses when barley or sorghum-based diets were supplemented with alpha-amylase (Rexen,1981;Broz and Frigg,1986;Madacsi et al,1988). Cellulase could hydrolyze part of the fiber of beans, yielding glucose available to the chicks. As referenced in the literature review section, other authors also reported improvements in chick responses when added cellulase to feedstuffs with a great content of fiber. Addition of protease to the beans also increased the productivity of chicks presumably due to hydrolyze of proteins. The results of this study demonstrated that the addition of some types of commercial diets can improve the utilization

of fababeans by chicks in a similar proportion as they do when supplement cereals. It is expected that the use of enzymes in animal nutrition increase in the next years, when the mode of action of the exogenous enzymes be better understood, and new and more specific products be available. Therefore, further studies are required to establish optimal doses and types of enzymes. On forages, the degradation of cell walls by chemical pretreatments such as sodium hydroxide or SO_2 , facilitated the activity of the enzymatic preparations (Hartley, 1983; Miron and Ben-Guedalia, 1987). Chemical pretreatments of fababeans could also have a synergistic effect with enzymatic treatments. In general, addition of enzymes to autoclaved or fermented fababeans did not further improve chick performance. However, when the enzymatic combination of cellulase+protease was added to fermented fababeans, weight gain in the second week and feed efficiency were improved.

4. STUDY TWO: DETERMINATION OF THE DIGESTIBILITY OF NITROGEN AND ENERGY AND THE METABOLIZABLE ENERGY OF RAW, AUTOCLAVED AND ENZYME SUPPLEMENTED FABABEAN FRACTIONS.

4.1.- Introduction.

This study comprised two experiments (experiments 4 and 5 of this thesis). In experiment 4 the effects of enzyme addition to soybean meal, rapeseed meal and different fababean fractions (whole fababeans, dehulled fababeans and fababean hulls) were measured in terms of nitrogen and energy digestibilities and metabolizable energy values. The effects of enzyme addition and autoclaving on the metabolizable energy of fababean fractions were also compared. In experiment 5 the applicability of the true metabolizable energy system to raw and autoclaved fababeans and fababean hulls was determined for birds given two amounts of feed (30 or 80 g) and when excreta were collected after 96 h. Experiment 4 was conducted at the Animal Research Centre in Ottawa and experiment 5 at the University of Manitoba in Winnipeg.

4.2.- Materials and Methods.

4.2.1.- Materials.

The fababean cultivar and enzymes used in this study were the same as those in study one. Dehulled fababeans and the hulls were prepared

by coarse grinding of whole fababeans followed by separation of the two fractions by air classification. The feedstuffs were ground in a hammer mill having a sieve size of 1 mm. The roosters, two-year-old Leghorns, used in experiment 5 were different of those used in experiment 4.

4.2.2.- Experimental design, and chemical and statistical analyses.

Thirteen samples were tested in experiment 4. They were fababeans, dehulled fababeans, fababean hulls, soybean meal, rapeseed meal, autoclaved fababeans, autoclaved dehulled fababeans, autoclaved fababean hulls, fababeans + 0.25% cellulase + 0.25% protease, dehulled fababeans + 0.25% alpha-amylase + 0.25% protease, fababean hulls + 0.25% cellulase, soybean meal + 0.25% cellulase + 0.25% protease, and rapeseed meal + 0.25% cellulase + 0.25% protease.

Six samples at two different concentrations were tested in experiment 5; raw fababeans at 30 and 80 g, autoclaved fababeans at 30 and 80 g, raw fababean hulls at 30 g and autoclaved fababean hulls at 30 g.

The feedstuffs were tested according to the TME system (Sibbald, 1986). A 48 h of fast was used to clear feed residues from alimentary canals. Each rooster was then force-fed, by intubation, 30 g of feed in experiment four, and 30 or 80 g in experiment five. The total number of roosters per diet was 6 or 7, and 12 birds in the control (fasted) group. The feeds were assayed for energy, nitrogen, amino acids and dry matter. Excreta were collected on trays after intubation, over a 48 h

period in experiment 4 and over 96 h in experiment 5. Collections in each experiment were made, at 24 h intervals, into plastic containers, frozen, lyophilized, equilibrated with atmospheric moisture and weighed. The collections from each bird were then combined into a single sample, ground, mixed and assayed for energy, nitrogen, uric acid and amino acids. Analyses of diets and fecal samples were made according to procedures outlined in study one. Uric acid was analyzed according to Marquardt (1983).

The enzymes (2 ml) were diluted in 10 ml of water and added to 10 g of feed. The mixture was then mixed with 100 g of feed and this was mixed with 890 g of feed. Autoclaving was at 121 C for 20 min at a depth of 2 cm. The chemical compositions of fababean fractions, rapeseed meal and soybean meal are presented in Table 9 (page 51).

Linear contrasts among treatments were conducted for all data using the general linear model procedure (GLM) of the Statistical Analysis System (SAS Institute, Inc., 1985).

4.2.3.- Theoretical calculations.

True metabolizable energy (TME) was calculated according to Sibbald (1986). In experiment 4, 48 h collection data were used and in experiment 5, 96 h collection data and 48 h estimated collection data were used. The theoretical method to estimate for 48 h excreta collection period in experiment 5 is given subsequently.

TME (kJ) is defined as the retention of energy corrected with the endogenous losses:

$$TME = IE - EE + FEE$$

IE: Ingested energy (kJ)

EE: total energy excreted by the fed bird (kJ)

FEE: total energy excreted by the fasted bird (kJ)

The TME was corrected to zero nitrogen balance (TMEn, kJ):

$$TMEn = TME + k \times (EN - IN - FEN)$$

k: energy content of the excretory products resulting from the catabolism of a gram of tissue nitrogen:

$$k = 36.53 \text{ kJ/g nitrogen}$$

EN: total nitrogen in the fed bird excreta (g)

IN: ingested nitrogen (g)

FEN: total nitrogen in the fasted bird excreta (g)

The TMEn value is given per kilogram of dry matter:

$$TMEn \text{ (MJ/kg DM)} = \frac{TMEn \text{ (MJ)}}{\text{Ingestion (kg)} \times \text{dry matter (\%)}} \times 100$$

Although the composition (Richardson et al, 1968) and energy (Waring and Shannon, 1969) of urine varies slightly with diets, to calculate the digestibility of nitrogen (TDN) and energy (TDE) it was assumed that all urinary nitrogen comes from uric acid. To calculate the TDE it was also assumed that all urinary nitrogen had the same energy value as uric acid nitrogen (34.39 kJ/g UAN).

The digestibility of nitrogen (TDN, %) is:

$$\text{TDN} = 100(\text{IN} - \text{FN} + \text{FFN})/\text{IN} = 100 (\text{IN} - \text{EN} + \text{FEN} + \text{UAN} - \text{FUAN})/\text{IN}$$

FN: nitrogen in the feces of fed birds (g)

FFN: nitrogen in the feces of fasted birds (g)

UAN: uric acid nitrogen in the fed bird excreta (g)

FUAN: uric acid nitrogen in the fasted bird excreta (g)

The digestibility of energy (TDE, %) is:

$$\text{TDE} = 100 (\text{IE} - \text{FE} + \text{FFE})/\text{IE} = 100 (\text{TME} + \text{UE} - \text{FUE})/\text{IE}$$

FE: energy in feces of fed birds (kJ)

FFE: energy in feces of fasted birds (kJ)

UE: energy in urine of fed birds (kJ)

$$\text{UE} = 34.39 \times \text{UAN}$$

FUE: energy in urine of fasted birds (kJ)

$$\text{FUE} = 34.39 \times \text{FUAN}$$

$$\text{TDE (\%)} = 100 (\text{TME} + 34.39 (\text{UAN} - \text{FUAN}))/\text{IE}$$

The true amino acid availability (TAAA, %) for each amino acid is:

$$\text{TAAA} = 100 (\text{IAA} - \text{EAA} + \text{FEAA})/\text{IAA}$$

IAA: amount of amino acid ingested (mg)

EAA: amount of amino acid excreted by fed birds (mg)

FEAA: amount of amino acid excreted by fasted birds (mg)

The effect of excreta collection time would most satisfactorily be established by analysis of samples that were collected daily. This, however, is extremely labor intensive. Therefore the total amount of excreta voided during the four day period was pooled. The amounts voided each day, however, were determined (Table 14). It was assumed

that the excretions in days 3 and 4 of the experiment were endogenous excretions.

The second assumption was that the concentration of energy, nitrogen and uric acid of the excreta of fasted birds was constant throughout the four days of the experiment. Thus, the amount of energy, nitrogen and uric acid excreted in days 3 and 4 for each fasted bird was calculated using the following equation:

$$X_L = (X_T \times E_L) / E_T$$

X_L = amount of energy, N or UAN excreted during days 3 and 4

X_T = amount of energy, N or UAN excreted during the four days

E_L = amount of excreta during days 3 and 4

E_T = amount of excreta during the four days

Sibbald and Wolynetz (1984) reported that the excreted energy was correlated with the nitrogen content of excreta voided by fasted birds. In experiment 5 there was also an excellent correlation (in all cases $R^2 > 0.99$) between energy (kJ), nitrogen (g) and uric acid nitrogen (g) voided by fasted birds during days 3 and 4 and the amount of excreta (g) voided also during days 3 and 4:

Energy of excreta = $16.07 + 9.34 \times \text{excreta during days 3 and 4}$

Nitrogen of excreta = $-0.457 + 0.281 \times \text{excreta during day 3 and 4}$

Uric acid nitrogen = $-0.396 + 0.252 \times \text{excreta during days 3 and 4}$

The excretion of energy, nitrogen and uric acid nitrogen by fed birds during days 3 and 4 were estimated from the above regression equations. The excretions of energy, nitrogen and uric acid nitrogen during days 1 and 2 were estimated by difference between total

excretions during the four day collection period and those for days three and four. It was assumed that the excretions during days one and two of birds fed fababean hulls were one-half of the total four day excretions since the amount of excreta voided were constant throughout the four days of excreta collection.

TABLE 9.- Composition of the feedstuffs used in study two (experiments 4 and 5)¹.

	Fababeans (g/kg)	Dehulled (g/kg)	Hulls (g/kg)	Soybean meal (g/kg)	Rapeseed meal (g/kg)
Dry matter	90.18	90.70	89.76	90.25	91.56
Ash	3.54	3.56	2.64	7.49	7.46
Crude protein (N x 6.25)	31.35	34.27	5.48	55.58	46.40
Ether extract	0.90	1.28	0.16	0.72	3.37
ADF	10.38	3.03	66.43	5.40	20.11
NDF	26.14	14.81	70.71	8.68	28.04
Calcium	0.06	0.05	0.33	0.48	0.60
Phosphorus	0.65	0.66	0.05	0.79	1.19
Energy (MJ/kg DM)	18.47	18.28	17.59	19.15	20.03
Amino acids:					
Aspartic acid	2.97	3.32	0.50	6.77	3.25
Threonine	1.04	0.98	0.18	1.95	1.75
Serine	1.49	1.65	0.28	2.81	1.91
Glutamic acid	4.61	5.17	0.41	13.28	7.95
Proline	1.20	1.31	0.32	2.30	2.59
Glycine	1.18	1.28	0.50	2.20	2.20
Alanine	1.11	1.23	0.20	2.24	1.87
Cystine ²	0.14	0.20	0.09	0.43	0.39
Valine	0.94	0.97	0.23	1.96	1.67
Methionine ²	0.13	0.17	0.11	0.59	0.76
Isoleucine	0.88	0.86	0.21	1.93	1.32
Leucine	1.97	2.13	0.33	3.93	2.86
Tyrosine	0.75	0.83	0.27	1.61	1.06
Phenylalanine	1.14	1.28	0.22	2.60	1.78
Histidine	0.69	0.73	0.17	1.34	1.10
Lysine	1.72	1.84	0.30	3.31	2.23
Arginine	2.86	3.18	0.46	4.19	2.89

1: Values, except for dry matter content, are expressed on a dry matter basis.

2: Non oxydized values.

4.3.- Results.

4.3.1.- Experiment 4

Analytical data describing the excreta of fasted and fed birds are given in Table 10. It was assumed, for the calculations of TDN and TDE that the composition of urine was uric acid and water. The TDN (Table 11) of rapeseed (70%) was significantly lower ($p < 0.05$) than that of soybean meal (87%) and whole and dehulled fababeans (81% in both). The TDE (Table 11) and the TMEn (Table 11) were significantly different ($p < 0.01$) among soybean meal, rapeseed meal, whole fababeans and dehulled fababeans. The TDE was high in dehulled fababeans (85%), intermediate in whole fababeans and soybean meal (76-74%), and low in rapeseed meal (54%). Dehulled and whole fababeans had the highest content of TMEn (14.0 and 12.6 MJ/kg DM, respectively). The TMEn content of soybean meal was 11.4 MJ/kg DM and that of rapeseed meal was 9.0 MJ/kg DM.

The TDN of fababean hulls was not calculated because the amount of ingested nitrogen was negligible and higher than the standard error of the voided nitrogen. The TDE and TMEn values of raw hulls were 69% and 12.1 MJ/kg DM respectively (Table 11). There was twice as much excreta obtained from birds fed autoclaved hulls as compared to those fed raw or enzyme supplemented hulls (Table 10). The reason for the low excretion of the highly indigestible hulls is discussed subsequently. The data describing the hulls were omitted from the statistical analyses because of low excretion.

Dehulling increased TDE (12%, $p < 0.01$) and TMEn (11%, $p < 0.01$), but

not the digestibility of nitrogen. Autoclaving whole and dehulled fababeans did not have a significant detectable effect ($p>0.10$) on their nutritive values.

The addition of enzymes to the diets also did not affect ($p>0.10$) the nutritive value of dehulled fababeans. However, when added to whole fababeans, they decreased ($p<0.05$) TDN by 14%. The addition of enzymes to soybean meal also tended to reduce TDE by 4% ($p<0.10$) and TMEn by 13% ($p<0.05$).

Some of the amino acids of fababeans (serine, aspartic acid, glutamic acid, glycine and tyrosine) had lower bioavailability than those of soybean meal ($p<0.05$, Table 12). In contrast, the bioavailabilities of aspartic acid, threonine, serine, lysine and arginine were greater in fababeans than in rapeseed meal ($p<0.05$). The mean bioavailability of amino acids (TAAA) of soybean meal and dehulled fababeans was greater ($p<0.01$) than that of rapeseed meal. The mean TAAA of raw whole fababeans also tended to be greater ($p<0.10$) than that of rapeseed meal. The mean TAAA of soybean meal and whole and dehulled fababeans were not different ($p>0.10$). Dehulling increased the bioavailability ($p<0.05$) of aspartic acid, serine, glycine, alanine and leucine. The TAAA of dehulled fababeans was not different ($p>0.10$) to that of soybean meal. In general, autoclaving did not affect ($p>0.10$) the TAAA of whole or dehulled fababeans.

The addition of enzymes to fababeans decreased the bioavailability ($p<0.05$) of threonine and lysine but did not have an effect on the bioavailabilities of the remaining amino acids. Addition of enzymes to dehulled fababeans, soybean meal or rapeseed meal did not affect

($p > 0.10$) the TAAA.

4.3.2.- Experiment 5

The fasted roosters in this experiment produced a larger amount of excreta than those on comparable treatments in experiment 4 (Table 13). The fasted birds produced 6.0 ± 1.0 g of excreta during two days in experiment 4, while those in experiment 5 yielded 31.2 ± 12.8 g in four days. The estimated amount of excreta for 48 h in experiment 5 was 13.1 ± 6.5 g.

There was a significant increase ($p < 0.05$) in the amount of excreta (Table 14) voided by fasted birds in days 3 and 4 compared to that excreted on day 1 of the experiment. The excretion on day 1 by roosters fed 30 g of raw or autoclaved fababeans was greater ($p < 0.05$) than the excretion on the other three days. Among days 2, 3 and 4, however, the excretions were not different ($p > 0.10$). The birds fed 80 g of raw or autoclaved fababeans excreted significantly ($p < 0.01$) more on days 1 and 2 than on days 3 and 4, for which the amounts of excreta were not different ($p > 0.10$). The birds fed autoclaved fababean hulls voided more excreta ($p < 0.05$) on the first two days than on the two last days of the experiment, with the amount being the same in the latter two days ($p > 0.10$). There was no difference ($p > 0.10$) in the amount of excreta voided in each day of the experiment by birds fed raw fababean hulls.

The effect of the level of ingestion on the daily excretion was analyzed. Birds fed 80 g of raw fababeans excreted significantly more ($p < 0.01$) during the first two days than birds fed 30 g, but there was

no difference ($p>0.10$) between input in the amount excreted in the last two days. Autoclaved as compared to raw fababeans did not affect ($p>0.10$) the amount of excreta voided in birds fed 30 g of fababeans but caused a reduction in this value ($p<0.05$) during the second day in birds fed 80 g of fababeans (Table 14). The amount of excreta voided by birds fed raw hulls as compared to those fed autoclaved hulls was lower in day 1 ($p<0.01$), similar in day 2 ($p>0.10$) and higher in days 3 ($p<0.01$) and 4 ($p<0.12$).

The TDN was not calculated in this experiment because the individual variation in uric acid excretion was greater than the amount of ingested nitrogen. To calculate the TDE (Table 15) it was assumed that all urinary nitrogen was uric acid nitrogen. The amounts of either raw or autoclaved fababeans that were ingested did not affect ($p>0.10$) the TDE. Autoclaving increased the TDE at both levels of fababeans (from 60 to 70%, $p<0.01$). The calculated energy digestibility of raw and autoclaved hulls was 9.5 and 1.0% respectively.

The TMEn value of raw fababeans (Table 15) decreased by 13% ($p<0.01$) when the level of ingestion was increased from 30 to 80 g. The TMEn value of autoclaved fababeans tended to decrease by 6% when the level of ingestion increased, but this was not significant ($p>0.10$). Autoclaving dramatically increased the TMEn value of fababeans by 22.4% ($p<0.01$) when fed at 80 g, and by 13.5% ($p<0.01$) when fed at 30 g. The TMEn values of raw and autoclaved fababean hulls were 1.46 and 0.004 MJ/kg DM, respectively.

The TAAA values (Table 16) of raw or autoclaved fababeans were not affected ($p>0.10$) by the level of ingestion except for the

bioavailabilities of lysine and arginine which increased ($p < 0.01$) with the ingestion of raw fababeans. The TAAA values of raw and autoclaved hulls were not valid, as discussed subsequently, and therefore are not reported. Autoclaving increased the mean TAAA of fababeans by 17% ($p < 0.01$) when fed at 30 g and by 10% ($p < 0.05$) when fed at 80 g.

When data were corrected to a 48 h of collection period (Tables 10 and 15, experiment 5c), the TDE and TMEn values of raw and autoclaved fababeans at both levels of ingestion were the same as for the 96 h collection period (Table 15, experiment 5c). However, the estimated energy digestibility and the TMEn values of raw hulls dramatically increased when the 48 rather than the 96 h collection period was used; those of autoclaved hulls remained essentially constant.

4.3.3.- General results.

The amount of excreta voided by the birds in experiment 5 was much higher than that voided by the birds in experiment 4 (Table 10). In experiment 5, most of excreta of the birds fed 30 g of raw or autoclaved fababeans (Table 14) were voided in the first day after feeding, and that of the birds fed 80 g of raw or autoclaved fababeans or 30 g of autoclaved hulls were voided in the first two days after feeding. The amount of excreta voided by the birds fed raw hulls were constant during the four days of excreta collection and considerably lower than the amounts voided by birds fed autoclaved hulls during day 1, but higher than that excreted during days 3 and 4. Autoclaving apparently increased the rate of passage of fababean and fababean hull residues (Table 14).

The TDN of fababeans was lower than that of soybean meal, but higher than that of rapeseed meal. The TDN of fababeans was not increased by dehulling or autoclaving (experiment 4, Table 11). The TDE of fababeans decreased 20% in experiment 5 compared to experiment 4 (Tables 11 and 15). The level of ingestion did not affect the TDE of raw or autoclaved fababeans (Table 15). Dehulling increased the TDE of fababeans by 12% (Table 11). When compared the TDE of dehulled fababeans in experiment 4 with that of whole fababeans in experiment 5, dehulling increased the TDE by 41% (Tables 11 and 15). Autoclaving fababeans increased TDE by 17% in experiment 5, but not in experiment 4.

The TMEn value of fababeans as determined in experiment 5 was 15% lower than the value obtained in experiment 4 (Tables 11 and 15). Increasing the amount of feed ingested from 30 to 80 g decreased the TMEn value of raw fababeans by 13% ($p < 0.01$) and that of autoclaved fababeans by 6% ($p > 0.10$). Dehulling increased the TMEn value of fababeans by 11% (Table 11). The TMEn value of dehulled fababeans in experiment 4 compared with that of whole fababeans in experiment 5 increased in value by 31% (Tables 11 and 15). The TMEn value of autoclaved fababeans fed at 30 g was similar in experiments 4 and 5. Autoclaving did not affect ($p > 0.10$) the TMEn value of whole or dehulled fababeans in experiment 4, but autoclaving increased ($p < 0.01$) the TMEn value of whole fababeans by 22.4% when fed at 80 g and by 13.5% when fed at 30 g in experiment 5.

The TAAA values (Table 12) of dehulled fababeans and soybean meal were similar. Autoclaving did not affect the TAAA of fababeans in

experiment 4 (Table 12). In experiment 5 autoclaving increased the mean TAAA (Table 16) by 17% ($p < 0.01$) when fed at 30 g and by 10% ($p < 0.05$) when fed at 80 g. The mean TAAA of autoclaved fababeans was similar in experiments 4 and 5, but it was lower in experiment 5 compared to experiment 4 for raw fababeans.

In general, the addition of enzymes to fababeans, soybean meal and rapeseed meal did not affect, or tended to reduce, the TDN and TDE when the period of excreta collection was 48 h (experiment 4).

TABLE 10.- Analysis of excreta of roosters (experiments 4 and 5).

No. birds	Ingested			Excreta			
	Amount (g)	Energy (kJ)	Nitrogen (g)	Amount (g)	Energy (kJ)	Nitrogen (g)	UAN (g)
Experiment 4							
Fasted 12	0	0	0	6.0+1.0	71+13	1.2+0.3	0.8+0.2
FB 7	30	500	1.4	15.3+0.8	208+13	1.9+0.2	1.3+0.1
FB+E 7	30	494	1.3	16.0+1.2	218+16	2.2+0.2	1.4+0.2
AFB 7	30	496	1.3	15.7+1.0	206+18	2.0+0.3	1.4+0.2
DFB 7	30	497	1.5	13.9+1.9	171+24	2.2+0.3	1.5+0.2
DFB+E 7	30	498	1.5	14.1+1.2	174+15	2.1+0.2	1.5+0.1
ADFB 6	30	498	1.5	14.1+1.1	178+13	2.2+0.3	1.5+0.2
SBM 6	30	518	2.4	19.4+0.8	247+15	2.7+0.3	2.0+0.2
SBM+E 6	30	518	2.4	19.6+0.6	259+12	2.7+0.2	1.9+0.1
RSM 6	30	550	2.0	25.1+1.1	343+17	2.7+0.3	1.7+0.2
RSM+E 6	30	545	2.0	24.8+1.3	356+19	2.5+0.3	1.7+0.2
H 6	30	474	0.2	15.4+3.8	223+63	1.5+0.4	1.0+0.2
H+E 7	30	476	0.2	18.3+5.6	267+93	1.8+0.5	1.2+0.3
AH 6	30	481	0.2	33.2+7.7	511+118	2.2+0.7	1.3+0.5
Experiment 5							
Fasted 11	0	0	0	31.1+12.8	319+119	8.0+3.4	7.2+3.2
FB 6	30	491	1.2	44.4+14.8	538+137	9.6+3.9	7.9+3.5
FB 5	80	1308	3.3	62.2+11.8	862+120	8.7+2.8	7.6+2.9
AFB 5	30	484	1.2	38.8+10.2	458+101	8.4+2.9	7.2+2.5
AFB 6	80	1290	3.2	52.3+4.8	694+72	8.1+1.3	6.6+1.3
H 6	30	478	0.4	54.2+12.8	747+161	8.1+3.1	7.0+3.1
AH 6	30	474	0.4	51.8+3.2	717+58	6.3+1.2	5.1+1.3
Experiment 5c ²							
Fasted 11	0	0	0	13.1+6.5	135+53	3.4+1.6	3.0+1.4
FB 6	30	491	1.2	25.9+7.1	330+64	4.3+1.9	3.1+1.7
FB 5	80	1308	3.3	45.8+4.8	693+61	4.5+0.8	3.9+0.8
AFB 5	30	484	1.2	20.5+3.7	271+40	3.7+1.1	2.9+1.0
AFB 6	80	1290	3.2	34.7+6.6	530+77	4.0+0.5	3.0+0.8
H 6	30	478	0.4	26.5+9.9	374+80	4.0+1.6	3.5+1.5
AH 6	30	474	0.4	35.2+1.4	555+22	2.6+0.5	1.6+0.7

1: FB:fababeans, AFB:autoclaved fababeans, DFB:dehulled fababeans, ADFB:autoclaved dehulled fababeans, SBM:soybean meal, RSM:rapeseed meal, H:fababean hulls, AH:autoclaved fababean hulls, +E:plus enzymes.

2: Estimated data for a 48 h excreta collection period.

TABLE 11.- Digestibility of nitrogen (TDN) and energy (TDE) and TMEn values (experiment 4).

Treatment ¹	TDN (%)	TDE (%)	TMEn (MJ/kg DM)
FB	81.1	75.9	12.57
FB + enzymes	69.6	74.5	12.26
FB, autoclaved	76.2	76.6	12.63
DFB	81.1	84.9	13.95
DFB + enzymes	80.7	84.0	13.80
DFB, autoclaved	80.6	83.7	13.74
SBM	87.0	74.0	11.43
SBM+ enzymes	83.6	71.0	10.91
RSM	70.0	54.1	9.00
RSM + enzymes	74.0	53.2	8.71
H	-	69.4	12.11
H + enzymes	-	61.8	11.03
H, autoclaved	-	12.1	2.64
Standard error ²	3.24	1.08	0.17
Linear contrasts			
	TDN	TDE	TMEn
Effect of type of feedstuff			
FB vs SBM	NS	NS	***
FB vs RSM	**	***	***
FB vs DFB	NS	***	***
DFB vs SBM	NS	***	***
DFB vs RSM	**	***	***
SBM vs RSM	***	***	***
Effect of autoclaving and dehulling			
FB vs AFB	NS	NS	NS
FB vs DFB	NS	***	***
AFB vs DFB	NS	***	***
DFB vs ADFB	NS	NS	NS
Effect of enzyme addition			
FB vs FB + enzymes	**	NS	NS
DFB vs DFB + enzymes	NS	NS	NS
SBM vs SBM + enzymes	NS	*	**
RSM vs RSM + enzymes	NS	NS	NS

1: FB:fababeans, DFB:dehulled fababeans, SBM:soybean meal, RSM:rapeseed meal, H:fababean hulls, AFB:autoclaved fababeans, ADFB:autoclaved dehulled fababeans.

2: Hulls were not considered in the statistical analyses.

NS: no significant difference ($p > 0.10$)

*: significant difference ($p < 0.10$)

**: significant difference ($p < 0.05$)

***: significant difference ($p < 0.01$)

TABLE 12.- Bioavailabilities of amino acids (experiment 4).

Treatment ¹	ASP (%)	THR (%)	SER (%)	GLU (%)	PRO (%)	GLY (%)	ALA (%)	ISO (%)	VAL (%)	LEU (%)	TYR (%)	PHE (%)	HIS (%)	LYS (%)	ARG (%)	Mean (%)
FB	90	88	87	92	86	84	87	88	84	88	85	87	88	88	94	88
FB + enzymes	88	82	86	91	83	82	85	83	82	87	84	81	85	80	89	85
FB, autoclaved	90	86	90	93	88	84	89	88	86	91	89	88	87	87	91	88
DFB	93	89	93	94	89	89	90	89	87	92	90	92	92	88	93	91
DFB + enzymes	93	89	92	95	94	89	91	92	91	93	90	93	93	87	93	91
DFB, autoclaved	94	92	95	96	94	90	92	92	91	95	93	94	93	88	92	93
SBM	93	90	93	96	90	90	90	92	89	92	91	90	91	85	92	91
SBM + enzymes	94	88	93	96	90	88	91	92	89	92	93	93	92	90	94	92
RSM	84	81	81	92	82	82	86	83	82	88	82	85	87	79	88	84
RSM + enzymes	83	88	93	96	92	80	84	83	80	87	80	87	83	78	88	83
Standard error	0.9	1.6	1.1	0.8	1.6	1.8	1.2	2.1	2.1	1.2	1.8	2.4	1.8	2.2	1.6	1.4

Linear contrasts

Type of feedstuff

FB vs SBM	**	NS	***	**	*	**	NS	NS	NS	*	**	NS	NS	NS	NS	NS
FB vs RSM	***	***	***	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	**	**	*
FB vs DFB	***	NS	***	*	NS	**	**	NS	NS	**	*	NS	NS	NS	NS	NS
DFB vs SBM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DFB vs RSM	***	***	***	**	***	***	**	**	NS	**	***	**	**	**	**	***
SBM vs RSM	***	***	***	***	***	***	*	**	**	*	***	NS	NS	*	NS	***

Effect of autoclaving and dehulling

FB vs AFB	NS	NS	*	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
FB vs DFB	***	NS	***	*	NS	**	**	NS	NS	**	*	NS	NS	NS	NS	NS
AFB vs DFB	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
DFB vs ADFB	NS	NS	NS	NS	**	NS	NS	NS	NS	*	NS	NS	**	NS	NS	NS

Effect of enzyme addition

FB vs FB+enzymes	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	*	NS
DFB vs DFB+enzymes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SBM vs SBM+enzymes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RSM vs RSM+enzymes	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

1: FB:fababeans, DFB:dehulled fababeans, SBM:soybean meal, RSM:rapeseed meal, AFB:autoclaved fababeans, ADFB:autoclaved dehulled fababeans.

NS:no significant difference ($p>0.10$)

*: significant difference ($p<0.10$)

** : significant difference ($p<0.05$)

***: significant difference ($p<0.01$)

TABLE 13.- Comparison of the composition of excreta from fasted birds in experiments 4 and 5.

	Experiment 4 (mean±SD)	Experiment 5 (mean±SD)
Duration of excreta collection (h)	48	96
Amount of excreta (g)	6±1	31±13
Energy of excreta (kJ)	71±13	319±119
Nitrogen of excreta (g)	1.2±0.3	8.0±3.6
Uric acid nitrogen of excreta (g)	0.8±0.2	7.2±3.2
Uric acid of excreta (g)	2.5±0.7	21.8±9.6
Uric acid energy of excreta (kJ)	28±8	247±109
Excreta of fecal origin (g)	3.6±0.5	9.8±3.3
Nitrogen of fecal origin (g)	0.35±0.10	0.82±0.45
Energy of fecal origin (kJ)	44±8	72±17
Daily nitrogen of fecal origin (g)	0.18±0.05	0.21±0.11
Daily energy of fecal origin (kJ)	21.9±3.8	18.1±7.7
Daily excretion of amino acids (mg):		
Aspartic acid	28	32
Threonine	18	16
Serine	22	24
Glutamic acid	40	45
Proline	21	23
Glycine	23	26
Alanine	15	15
Valine	13	18
Isoleucine	12	20
Leucine	18	29
Tyrosine	12	10
Phenylalanine	14	14
Histidine	10	12
Lysine	21	24
Arginine	19	21
Total	286±87	329±136

TABLE 14.- Daily amount of excreta voided (experiment 5).

Treatment ¹	Day 1 (g)	Day 2 (g)	Day 3 (g)	Day 4 (g)
Fasted	5.9	7.2	9.0	9.0
FB, 30 g	16.5	9.1	10.1	10.5
FB, 80 g	26.7	19.1	7.7	8.7
AFB, 30 g	13.6	6.8	8.7	9.6
AFB, 80 g	26.3	11.7	7.0	8.9
H, 30 g	11.8	14.7	15.4	12.4
AH, 30 g	20.5	14.7	7.0	8.6
Standard error	2.10	2.14	1.63	1.60

Linear contrast

	Fasted	FB,30	FB,80	AFB,30	AFB,80	H	AH
Daily excretion:							
Day 1 vs day 2	NS	**	**	***	***	NS	NS
Day 1 vs day 3	**	**	***	**	***	NS	***
Day 1 vs day 4	**	**	***	**	***	NS	***
Day 2 vs day 3	NS	NS	***	NS	***	NS	**
Day 2 vs day 4	NS	NS	***	NS	*	NS	NS
Day 3 vs day 4	NS	NS	NS	NS	NS	NS	NS

	Day 1	Day 2	Day 3	Day 4
Effect of level of ingestion				
FB, 30 vs 80 g	***	***	NS	NS
AFB, 30 vs 80 g	***	NS	NS	NS

Effect of autoclaving

	Day 1	Day 2	Day 3	Day 4
FB vs AFB, 30 g	NS	NS	NS	NS
FB vs AFB, 80 g	NS	**	NS	NS
H vs AH, 30 g	***	NS	***	NS

1: FB:fababeans, AFB:autoclaved fababeans, H:hulls, AH: autoclaved hulls.

NS: no significant difference ($p > 0.10$)

*: significant difference ($p < 0.10$)

**: significant difference ($p < 0.05$)

***: significant difference ($p < 0.01$)

TABLE 15.-Digestibility of energy (TDE) and TMEn values (experiment 5).

Treatment ¹	Experiment 5		Experiment 5c ²	
	TDE (%)	TMEn (MJ/kg DM)	TDE (%)	TMEn (MJ/kg DM)
FB, 30 g	60.4	10.73	60.7	10.64
FB, 80 g	59.1	9.36	59.0	9.38
AFB, 30 g	70.6	12.18	70.6	12.09
AFB, 80 g	69.3	11.46	69.1	11.49
H, 30 g	9.5	1.46	53.5	9.20
AH, 30 g	1.0	0.004	1.1	1.02
Standard error ³	2.41	0.29	2.3	0.29
Linear contrasts				
	(%)	(MJ/kg DM)	(%)	(MJ/kg DM)
Effect of level of ingestion				
FB, 30 vs 80 g	NS	***	NS	***
AFB, 30 vs 80 g	NS	NS	NS	NS
Effect of autoclaving				
FB vs AFB, 30 g	***	***	***	***
FB vs AFB, 80 g	***	***	***	***

1: FB:fababeans, AFB:autoclaved fababeans, H:hulls, AH:autoclaved hulls.

2: Estimated values for a 48 h excreta collection period.

3: Raw hulls were not considered in the statistical analysis.

NS: no significant difference ($p > 0.10$)

***: significant difference ($p < 0.01$)

TABLE 16.- Bioavailabilities of amino acids (experiment 5).

Treatment ¹	ASP (%)	THR (%)	SER (%)	GLU (%)	PRO (%)	GLY (%)	ALA (%)	ISO (%)	VAL (%)	LEU (%)	TYR (%)	PHE (%)	HIS (%)	LYS (%)	ARG (%)	Mean (%)
FB, 30 g	89	81	88	90	76	78	87	57	78	74	85	86	75	44	60	76
FB, 80 g	90	84	87	89	73	74	82	70	69	81	75	78	78	79	86	80
AFB, 30 g	96	92	96	96	89	83	92	81	83	85	90	95	94	81	87	89
AFB, 80 g	93	88	93	95	85	85	90	81	85	87	90	94	88	79	84	88
Standard error	2.5	2.7	3.1	1.7	4.6	3.8	4.4	5.4	5.4	3.1	4.4	4.5	4.3	4.8	5.9	3.0

Linear contrasts

Effect of level of ingestion

FB, 30 vs 80 g	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	***	NS
AFB, 30 vs 80 g	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Effect of autoclaving

FB vs AFB, 30 g	*	**	*	**	**	NS	NS	***	NS	**	NS	NS	***	***	***	***	***
FB vs AFB, 80 g	NS	NS	NS	**	*	*	NS	NS	*	NS	**	**	NS	NS	NS	**	**

¹: FB:fababeans, AFB:autoclaved fababeans.

NS:no significant difference ($p>0.10$)

*: significant difference ($p<0.10$)

**: significant difference ($p<0.05$)

***: significant difference ($p<0.01$)

4.4.- Discussion.

The amounts of excreta, energy, nitrogen, uric acid and amino acids voided by roosters in experiment 5 were much higher than those voided by roosters in experiment 4. However, the composition of endogenous excretions of gastrointestinal origin of fasted birds in experiments 4 and 5 (Table 13), assuming that all urine is uric acid, was not different ($p>0.10$). In experiment 5 the TME trial was conducted in a room different from that the roosters were between TME experiments. In addition, new designed cages, unknown for the roosters, were used in this experiment. As a result, the stress of these birds was considerable and the net effect was an apparently greater excretion of the nitrogen reserves of the body in the form of uric acid in the latter as compared to the former experiment. There was also a high individual variation in the excretion of energy, nitrogen and uric acid, which reflects the individual variation in the endogenous losses of metabolic origin which are characteristic of each bird (Sibbald and Price, 1980; Sibbald and Wolynetz, 1985). This variation seems to be larger in birds having a stress situation, such as those used in experiment 5.

The duration of the excreta collection period can have a considerable influence on the results. Long collection periods may produce low values because the endogenous excretions seem to increase in the latter stages of the fasting period. This effect may influence the TME value when the bird is used to estimate its own endogenous excretions. The use of fasted birds, however, should correct for the

increase in the endogenous excretions. On the other hand, short collection periods can produce large TME values because of incomplete clearance of the gastrointestinal tract. Forty eight hours of excreta collection is of sufficient duration to allow for the clearance of most feedstuffs from gastrointestinal tract (Sibbald,1979). However, when the feedstuff has a high concentration on antinutritional factors or undigestible compounds, a longer period is required. Thus, at least 52 h are required to collect the excreta from chickens fed oats (Sibbald,1980), and 72 h to collect the excreta from chickens fed alfalfa meal (Sibbald and Morse,1982;Sibbald and Wolynetz,1987).

The period of excreta collection in the first experiment was 48 h, but this period was inadequate to void all residues of hulls because the amount of feces excreted by chickens fed raw hulls was 15 g, versus 33 g excreted by chickens fed autoclaved hulls (Table 10). Even after 96 h of collection (experiment 5) birds fed raw hulls voided excreta of feed origin. Autoclaving appeared to dramatically increase the rate of passage of hulls. The difference in the voided amount of excreta would explain the differences in the TME_n value of raw and autoclaved hulls (Tables 12 and 15) as the TME_n value of the raw hulls in experiment 4 is high; i.e., the portion of the undigested hulls that was not excreted was assumed to have been digested. As indicated, a longer collection period would have solved this problem. Thus, when the collection period was prolonged to 96 h (experiment 5, Table 15), the TME_n value of raw hulls dramatically decreased. The TME_n value of raw hulls also increased dramatically when estimated for a period collection of 48 h in experiment 5. The differences between the TME_n

values of fababeans obtained in experiment 4 and 5 are attributed to the difference in collection time. In experiment 5 the longer collection period would permit the more complete clearance of fababean residues from the gastrointestinal tract compared to the 48 h standard collection time used in experiment 4. However, the TMEn value of fababeans in experiment 5 was the same for an estimated collection period of 48 h (experiment 5c) as for the 96 h collection period (Table 15). This was attributed to a higher rate of passage of fababeans in birds in experiment 5 than in those used in experiment 4. Forty eight hours would have been sufficiently long to collect all of the excreta of birds fed 30 g of raw or autoclaved fababeans in experiment 5, and maybe of sufficient time to collect the excreta of birds fed autoclaved fababeans in experiment 4 as the TMEn and TAAA values of autoclaved fababeans were similar in experiments 4 and 5. However, 48 h were not enough to collect the excreta of less stressed birds fed raw fababeans in experiment 4.

The rate of passage seems to depend, in addition to the type and amount of feedstuff, on the stress of the bird. It would be, therefore, useful to determinate the optimal collection time, as indicated by Sibbald (1986), before starting the TME trial, particularly when non-common feedstuffs are studied. Otherwise, erroneously high values may be obtained. The optimal period of excreta collection can be determinated by using non-absorbed markers, such as chromic oxide or acid insoluble ash, in the feed and by studing the recovery in the excreta.

The effect on the rate of passage of fababeans may be primarily

attributed to the effect of raw hulls. This effect, however, may not have occurred with autoclaved fababeans for which the TMEn value is similar in experiments 4 and 5, and maybe not with dehulled fababeans, and therefore the values obtained with dehulled beans in experiment 4 may be close to the true values.

The TDN of fababeans was lower than that of soybean meal (81 and 87%, respectively) but higher than that of rapeseed meal (70%). These values are approximately the same as the mean values for TAAA. The respective values of TAAA for fababeans, soybean meal and rapeseed meal are 88, 91 and 84%. On the basis of these results it would appear that the correction of excreta nitrogen for its uric acid content could produce a basis for estimating total protein digestibility. The lower values for TDN than for TAAA could be due to undigestible nitrogenous compounds in the feeds. Although the low TDN of fababeans may be partially attributed to the presence of tannins in the testa (Martin-Tanguy et al, 1977a; Marquardt et al, 1977; and others), no increase in TDN was obtained by dehulling. This may be attributed to an insufficient collection period (48 h, experiment 4). The TDE of fababeans was similar to that of soybean meal (76%) and higher than that of rapeseed meal (55%). The TMEn value of fababeans was higher than that of soybean meal and rapeseed meal (12.6, 11.4 and 9.0 MJ/kg DM, respectively). However, the TDE and the TMEn values of fababeans were 20 and 15% lower when the period of excreta collection was prolonged to 96 h. The TMEn values of fababeans, soybean meal and rapeseed meal obtained in experiment 4 are in agreement with the data

compiled by Sibbald (1986). The TMEn value of raw fababeans obtained in experiment 5 is similar to that reported by N.R.C. (1984).

Although autoclaving reduced the mean tannin content of fababean hulls from 4.56 to 0.54%, it did not affect the nutritive value of fababeans in experiment 4, maybe because the short duration of excreta collection. The period of excreta collection presumably did not affect the autoclaved fababean values but yielded erroneously high values for the raw fababeans, and as a result the two values were similar. However, when the period of excreta collection was prolonged (experiment 5), autoclaving increased the TMEn value by 13% when 30 g were fed, and by 22.5% when 80 g were fed. The more dramatic decrease in the TMEn value obtained when feed intake was increased in birds fed raw fababeans as compared to those fed autoclaved fababeans (Table 15) would suggest that the total amount of antinutritive factors may modify results in a non-proportional manner. This may be attributed to the ability of the rooster to partially adapt to small amounts of antinutritive factors (mainly condensed tannins) but not larger amounts. That is, condensed tannins in birds fed 30 g of fababeans neutralize digestive enzymes less severely than in birds fed 80 g. The latter response may better represent the situation in the ad libitum-fed chicken. This is in accordance with other authors (Marquardt and Campbell, 1973; Campbell and Marquardt, 1977; Bezares et al, 1980) who reported that the beneficial effects of autoclaving were most pronounced in chickens fed high levels of fababeans. Edwards (1977) and Shannon and Clandinin (1977) reported increases in the metabolizable

energy content of about 15% when fababeans were autoclaved. Boldaji et al (1986), in contrast, reported that the TMEn of fababeans, white lupin or yellow peas when tested according to the TME system were not always improved when they were autoclaved. This may also be attributed to an insufficiently long collection period or to the low levels of fababean intake.

Edwards and Duthie (1973) reported that dehulling increased the metabolizable energy value by over 30% by removing of the testa and the antinutritional factors associated with it. In this study (experiment 4) the increase in the TMEn value was 12% probably because of the inadequate duration of excreta collection. However, the TMEn of dehulled fababeans was 31% higher than that of whole fababeans obtained in experiment 5.

The TAAA was increased 17% ($p < 0.01$) by autoclaving. The TAAA values of hulls were not consistent. This was attributed to the negligible amount of protein ingested by birds fed hulls as compared to individual variation in the excretion of endogenous protein. To correct this effect in the calculation of TAAA it is suggested that birds must be fed with a larger intake of those feeds low in protein content, which would decrease the effect of the endogenous excretions.

On the basis of the results of this study the estimated TMEn values determined according the TME system of whole fababeans, dehulled fababeans, autoclaved fababeans, autoclaved fababean hulls, soybean

meal and rapeseed meal are 10.73, 13.95, 12.18, 0.04, 11.43 and 9.00 MJ/kg DM, respectively. However, the TME_n value of raw fababeans decreased when the amount ingested increased. Values which were confounded by an inadequate collection time have been excluded.

In general, the addition of enzymes did not affect the TAAA (Table 13) of the feedstuffs. However, the addition of enzymes to fababeans or soybean meal reduced or tended to reduce the digestibilities of nitrogen and energy, while in rapeseed meal tended to increase the digestibility of nitrogen. In soybean meal it also decreased the metabolizable energy. Enzymes had no effect on dehulled fababeans. High concentrations of enzymes may depress nitrogen and energy digestibilities. Further dose response studies are required to establish optimal concentrations of each enzyme.

SUMMARY AND CONCLUSIONS

- 1.- The TMEn value of raw but not of autoclaved fababeans decreased with the amount of feed intake . This effect may be attributed to the condensed tannin content of raw fababeans, which are inactivated by autoclaving.
- 2.- The addition of exogenous enzymes, such as cellulases or proteases, to fababeans improved weight gains, efficiency of feed utilization and nutrient retention by 5%. This effect, however, was not detected using the TME system, perhaps because of an insufficient excreta collection period.
- 3.- Dehulling dramatically increased the TDE and the TMEn value of fababeans. This may be attributed to the elimination the testa which contains tannins and is, itself, highly undigestible.
- 4.- Fermentation of fababeans improved feed efficiency by 10%, but not weight gains because of a decrease in the feed intake. In some instances, the addition of enzymes to fermented fababeans improved performance by 6%.

5.- Autoclaving of fababeans improved weight gain, feed efficiency and dry matter retention by more than 15%. Autoclaving improved the TMEn value of fababeans, particularly when high levels were fed. The mean TAAA of fababeans was also improved by autoclaving. The addition of enzymes to autoclaved fababeans did not produce further improvements.

6.- The duration of the period for the complete clearance of the alimentary canal seems to depend on the stress situation of birds and the type and amount of feedstuff ingested. Fibrous feeds that contain antinutritive factors, such as fababean hulls, have a greatly reduced rate of transit through the gastrointestinal tract. These effects erroneously increase the TMEn and TAAA values of the feedstuff. Under these conditions a longer collection period must be used. The use of a marker, such as chromic oxide or acid insoluble ash, would probably assist in identifying these feedstuffs.

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