ASSESSMENT OF THE NUTRITIVE VALUE OF SELECTED POULTRY FEED SUPPLEMENTS AND TOXICOLOGICAL EVALUATION OF METABISULPHITE-TREATED 5EEDS .

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D E D I C A T I O N

Dedicated to all those who are continually searching to understand nature's language and to tame the wild world.

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A B S T R A C T

The nutritive value of brewers' spent grains (BSG) and two commercial poultry feeds produced try Arewa and Pfizer companies and the effect of feeding sodium metabisulphite-treated (1%) feed to day-old pullets have been investigated.

The feed mashes were found to contain inadequate levels of a number of amino acids some of which are essential for birds.

The BSG was found to be fairly rich in essential amino acids but it alone could not support the growth of starter pullets while 25% BSG added to Pfizer chick mash when fed to starter pullets, resulted in significant ($P < 0.05$) growth retardation and increased mortality rate. However, a similar addition to Pfizer growers mash had no detrimental effect on the chicks.

Addition of sodium metabisulphite to the wet BSG (3,000 ppm) enhanced the keeping quality of the BSG.

Feeding day-old pullets with Arewa diet containing 1% sodium metabisulphite for 8 weeks did not have any significant effect on digestibility and feed consumption but resulted in gradual growth retardation without abnormal histological changes in the liver, kidneys and spleen.

Blending the grain size of Arewa feed to fine particles resulted in a marked retardation in growth of and the amount of the feed consumed by the day-old chicks.

INTRODUCTION

Protein-calorie malnutrition is a global nutritional problem, especially in the developing countries of the world, where a majority of the people are poor and cannot afford to feed on adequate amounts of protein-rich diets due to the high cost placed on them (19, 58, 83). This problem of malnutrition is further aggravated by the ever-rising population in these areas of the world without a corresponding increase in food production.

If this trend continues unchecked for the next decade, the problem might assume an alarming proportion. There are, however, two alternative solutions to this problem. The first alternative could involve a deliberate inhibition of population growth so as to match the available food resources. This may be achieved through proper birth control system. With the improvement in medical care and general hygiene, mortality figures are fast falling. The present public attitude to family planning in these areas of the world and the falling mortality rate are likely to make the success of this method very remote or near impossible. The second alternative is to increase food production to match the increasing demand for food. This alternative is more likely to succeed but needs careful planning.

It is therefore not an overstatement to assert that food, an item which man unconditionally needs to maintain life, determines his survival. Human civilization therefore demands the production of a wide variety of food in large quantities to go round the entire population.

This necessitates the immediate amplification of the food production machinery.

Although starvation is still plaguing some parts of the world today, inadequate intake of protein in the diet is no doubt more widespread. This is because the protein-rich foods are usually expensive (73). The people within the low-income group are forced to feed more on foods rich in carbohydrates but deficient in proteins and vitamins (6). This implies that this group of people, although not starving, are feeding on unbalanced diets. In the light of this, a bold step towards the production of high quality protein foods at reasonably cheap rates that are compatible with the purchasing power of the people would be a well placed priority.

Although animals ultimately depend on the food manufactured by green plants (48, 88), animal proteins are superior to plant proteins (24, 36, 73). The productivity of the animals (chicken, cattle etc.) which serve as the sources of high quality protein for man is directly affected by the quality and quantity of the feeds given to them (22). Of these animals, poultry animals are the fastest and possibly the most efficient converter of the plant proteins to animal proteins (43). Well-fed chickens start laying after about 22 weeks of age while well fed broilers are usually ready for consumption after 12 weeks. Poultry requires a top quality well balanced diet - an easily digestible feed which contains the required protein, energy, vitamins, mineral elements and all the other ingredients necessary for the full expression of the genetic potential of the birds.

In Nigeria, one of the greatest limitations to the expansion of poultry industry is the high cost of the standard commercial feeding stuffs (3). This problem has either driven away many people from poultry business or prevented them from starting at all. The few successful poultry farmers are forced by the high cost of production to sell their products at prices which many people cannot afford, thereby reserving these products for only the rich. Social justice demands that this state of affair be eliminated within the shortest possible period. A need therefore arises for the immediate search into the possible ways of obtaining maximum production in poultry with minimum expenditure, so that the products can be sold at relatively low prices compatible with what most people can afford. This objective may be achieved by producing and feeding the birds with relatively cheaper feeds of comparable nutritional quality with the existing expensive commercial poultry feeds whose prices are perpetually sky-rocketting. The introduction of such a cheap but efficient feed into poultry industry will go a long way to encourage many more farmers to enter into the business.

This forms the basis for the decision to embark on this project. In this study, the possibility of including brewers spent grains in poultry feeds as well as the level tolerated by chicken have been investigated. Brewers spent grain is the insoluble residue obtained after filtering the wort in the brewing process. This by-product of the beer industry is presently being wasted or not properly utilised in Nigeria.

If by possible fortification or supplementation, this by-product can be converted into a poultry feed, we would then have taken a giant step towards reducing the cost of production which will ultimately lead to a fall in the selling price of the products. The nutritive value of two standard commercial feeds made by Pfizer and Arewa companies have also been assessed in this study. While Pfizer Feeds Company is a subsidiary of the international parent Pfizer Company, the Arewa Feed Company is a local feed processing company located in Zaria.

Another factor which can limit the expansion of poultry industry may be the losses resulting from microbial contamination of the feeds and poultry products with viable pathogenic bacteria or toxic products of microorganisms. Contamination may result in losses in the following ways.

(i) It may result in diseases leading to increased mortality for the chicks.

(ii) Growth and development of chicks may be retarded.

(iii) It may cause a decline in production.

(iv) Food poisoning may result if contaminated products are consumed by man and this will subsequently lead to the rejection or black-listing of the products and may also attract the fury of the Food and Drug Administration authority or the food legislating bodies.

In the light of the problems enumerated above, the search for an effective method of preventing microbial growth in the feed could not be a misplaced priority. Various agents such as irradiation, application of heat and chemicals have been employed in the preservation of foods.

The use of irradiation is not promising here as the feed bags presently being used do not prevent recontamination after the feed might have been rendered sterile by irradiation. The use of heat is unsuitable as certain components of the feeds may be destroyed by heat. Secondly, the problem of recontamination is also there.

A chemical antimicrobial agent which has been classified as a safe food additive by the FAO and WHO may well turn out to be the most suitable weapon against the microorganisms and other harmful organisms. The possibility of employing sodium metabisulphite, one of the safe food additives (55, 56) and its toxic effect on the chicks if any have also been investigated in this study.

LITERATURE REVIEW

The disparity between the needs of an increasing global population and decreasing food resources poses one of the most intractable problems mankind has ever faced. Infact, nutritional problems appear to be the heaviest socio-economic problems facing the third world today. Finding solutions to these problems is a task of staggering proportions and is indeed one of the most difficult assignments which nature has given to man (11, 64, 87).

Of the nutritional problems, protein-calorie malnutrition (95) and possibly, disorders resulting from vitamin deficiency are the most prominent and most complex. Clinical consequences of malnutrion such as marasmus, kwashiorkor and various vitamin deficiency problems among children in the tropics have been reported by Mcfie (62). The acute shortage of protein in the world, especially in developing countries, including Nigeria, has even stimulated researches directed at increasing food production by recovering protein from plant materials which are not normally considered for food (30). Oke (69) also reported on the need for a new source of proteins to meet the increasing demand. It has been reported by Oke (70, 71) that protein extract of some leafy vegetables contain good quality proteins which may be of value in preventing protein-calorie malnutrition. Chibnall, Ress and Lugg (15) have also reported on the nutritive value of protein extract from the leaves of several plants. The fact that sorghum, a staple food in Northern Nigeria (38), cassava and cassava products widely eaten among the Southern Nigerians (68) are

poor sources of protein has been of major concern to the government. The intake of animal protein which would have supplemented this deficiency has been very low among Nigerian peasants (5).

However, it is not likely anything positive can be achieved unless definate steps are taken to increase the production of essential food items at such a rate that would cause a significant fall in the market prices of these items. The problem is further exacerbated by the population that is growing rapidly at a rate of about three percent per annum (50, 71). Seing the impending danger, the former Federal Military Government of Nigeria launched the 'Operation Feed the Nation' in 1976 followed by the 'Green Revolution Programme' of the present Presidential Government launched in 1979, both of which aim at increasing food production.

Poultry, apart from being an efficient converter of feed to valuable animal products, such as meat and eggs, has the advantage of being reared in a small space in the back-yard of a building. Poultry products can play a very important role in human nutrition. Like other meats, poultry is valuable for its protein and it is also a good source of iron and phosphorus. The eggs contain mineral elements such as calcium, phosphorus and iron and a number of vitamins (22).

However, the quality of the feed fed to the animals is the keynote to successful poultry keeping (22). Because of the importance of feeds, any attempt aimed at boosting poultry production must start with the feeds. The feed cost accounts for about two-thirds of the total cost of production on the average commercial poultry farm (22).

In Nigeria various poultry feeds (mashes) are sold commercially in various grain sizes. It is not yet clear whether the grain size of the mash has any significant influence on the nutrition of the chicks. Most of these feeds contain the following ingredients in varying proportions: corn, wheat middlings, fish meal, groundnut cake, palm kernel cake, brewers yeast, bone meal, oyster shell, salt, vitamin premix and mineral premix. Sometimes small doses of antibiotic and antioxidant are added. Data on the nutritive quality of these feeds are scanty, if not non-existent. For any meaningful progress to be made in improving poultry industry in Nigeria such data must be obtained by subjecting these products to nutritive assessment tests. On the basis of such data, the companies producing the feeds could be advised to improve on nutrients that are inadequate and reduce those that are in excess, thereby making their products balanced for the poultry.

In assessing whether or not an essential nutrient is present in adequate amounts, its level is usually compared with the level recommended by the National Research Council (NRC) of the National Academy of Sciences, Washington D.C. (66) given in tables 18 and 19 (see appendix). These values have been accepted almost internationally.

Chicks fed with diets which are grossly deficient in these essential nutrients have always developed deficiency problems which, in acute cases, have resulted in the death of many chicks. The common problems associated with the deficiency or overdose of the major classes of nutrients are discussed below.

Protein.

Protein is required for the formation of new protoplasm. If deficient in the diet, growth is significantly retarded or may even stop. In severe cases there will be loss of weight due to withdrawal of protein from certain less vital tissues to maintain the functions of the more vital ones (24). A minimum of 20 percent protein is required in the diet of starter chicks (45, 46). However, it is just not enough to have 20 percent protein in the diet but it must be able to meet the amino acid requirements of the chicks, which inturn depends on the amino acid composition of the protein, its digestibility and the availability of the amino acids to the animals (39). Ideally, only eleven amino acids are essential for poultry, these include arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (24, 65). However, chicks can synthesize glycine but this does not proceed at a rate sufficient to meet the requirements for maximum growth (65).

Miles and Featherson (63) have reported that chicks fed with a diet supplemented with the limiting amino acid of the diet grew more rapidly and efficiently and excreted less uric acid than the chicks fed with unsupplemented diet even though the levels of crude proteins were indentical in both cases. Poultry starter feeds based on cereals such as corn and wheat require lysine supplementation with a component that is rich in lysine (16).

Nitrogen-free extract and crude fibre.

Carbohydrate is used by the body as the source of energy. Any excess may be stored in the body or incorporated into egg yolk as fat (25, 44). It has been reported by Jensen, Chang and Wyatt (52) that fat accumulation in the liver of hens is influenced by the carbohydrate source. Carbohydrate is not usually lacking in most feeds especially when the major component of the feeds is derived from cereal grains, and as such special attention needs not be devoted to the total carbohydrate content of the feed. However, the level of crude fibre in the diet is very important as crude fibre is poorly digested by poultry. Moreover, a high crude fibre content in the diet makes other nutrients less available (27). A high crude fibre level has been reported to interfere with availability of amino acids in man and monogastric animals by Ranhotra, Hepburn and Bradley (79).

Although chicks can tolerate diets with crude fibre content between 8 and 9 percent without harmful effects on mortality, rate of growth, feed consumption, age of maturity and production, the general opinion amongst poultry experts and feed manufacturers is that fibre content of the ration should be kept below 7 percent.

Current views in the medical field hold that the depletion of fibre in modern western diets may be responsible for some metabolic diseases that are common in the western world but rarely found in Africa (12, 21, 80). Such diseases include diverticular diseases of the colon (74), constipation, adenomatous polyps of the colon, cancer of the colon and rectum (12) and hiatus hernia (13). However, it is not known whether these apply to poultry.

Lipid

This serves as the source of energy and heat in the body and of fat in the body and egg yolk. Fat constitutes about 20 percent of the entire weight of an average size hen, about 10 percent of the whole egg and about 32 percent of the egg yolk (26). It has been reported that large proportions of fat in the feed may retard digestion and upset the normal metabolism of the other nutrients (26). Moreover, feeds which contain large amounts of fat, especially unsaturated fatty acids, are apt to become rancid in hot wheather (26, 44), a condition which might be injurious to poultry. Consequently, large amounts of fat are always avoided in animal feeding. The desired goal is to have just that quantity that meets the requirements for essential fatty acids and the requirements for the transportation of the fat-soluble vitamins. A fat content of 8 percent and above is not recommended in poultry feeds (26).

Mineral elements

Minerals constitute about 3 - 4 percent of the life weight of the fowl and about 10 percent of whole egg (28). The functions of mineral elements include maintenance of osmotic pressure and surface tension in various fluids of the body, regulation of PH of blood and tissue fluids and control of irritability. Minerals are also integral parts of living protoplasm and form a greater part of bones. Iron and copper are essential components of haemoglobin, the oxygen - carrying pigment of blood.

The following elements are required in poultry nutrition at the

levels specified by NRC (66): sodium, potassium, calcium, phosphorus, magnesium, manganese, iodine, copper, iron, zinc and selenium. However, a good ration made up of natural feeding stuffs is likely to be deficient only in sodium chloride, calcium, phosphorus (44) and possibly in potassium and magnesium to the extent that will require special addition.

Provision of excess amounts of mineral elements is both uneconomical and detrimental to the normal development and production of chickens. The deficiency and toxicity problems associated with the mineral elements have been well documented (44).

Vitamins and other components.

About 13 vitamins are required in poultry nutrition (66). Vitamins are not usually present in adequate amounts in rations formulated from natural feedstuffs. As such, vitamin fortification is usually carried out by adding adequate amounts of commercial vitamin premix. It is important that a margin of safety to compensate for possible losses of vitamins during feed processing, transportation and storage be added above the normal requirement. The vitamin deficiency problems frequently encountered in poultry have been well documented (44, 66).

Some other components that were not normally included in poultry rations are now currenty added, following the discovery that they could play beneficial roles. The addition of antioxidants is known to prevent oxidative rancidity (26) while the addition of a small amount of antibiotic is known to stimulate growth (44, 92) and to favour more economic feed-conversion (54). Water is essential for poultry nutrition and should

normally be fed *ad libitum*.

Inclusion of brewers' spent grains in poultry feeds.

Although high crude protein values of between 20 and 28 percent have been reported for dried brewers' spent grains (18, 29), sufficient information is not available as regards the level of spent grains that can be tolerated by poultry in their diets. On the basis of the high crude protein content, one would expect it to serve as a good protein supplement for poultry if the protein is of good quality, well digested and the amino acids so produced are readily available to the animals. Ademosun (3) has reported that not more than 10 percent of spent grains obtained from the 'West African Breweries Limited, Abeokuta' should be incorporated in starter chicks diet. However, since the composition of the spent grains depends on the starting raw materials and the adjuncts present during the brewing operations, a value established for the product of one brewery may not be applicable to the products of other breweries. It therefore follows that meaningful recommendations can only be made to feed manufacturers with respect to the particular brewery whose products is to be used.

Toxicological evaluation of metabisulphite - treated feed.

It is just not enough to produce abundant amounts of the feed but the feed must be safeguarded against microbial contamination and subsequent biodeterioration. Microbial contamination of the feed or poultry products is of public health significance since the causative agents of diseases such as anthrax, salmonellosis, brucellosis tuberculosis and many others are easily transmitted between man and animal (47).

Sulphur dioxide is widely used in foods and drinks as an antioxidant, antimicrobial and antibrowning agents (33, 51, 55, 67, 89, 90). The sulphur dioxide is usually introduced into foods in the form of sodium or potassium metabisulphite, bisulphite and sulphite (33). Metabisulphite has the advantage of being a cheap but efficient antimicrobial agent and its use in the preservation of fruits, vegetables, wine, beer, vinegar, sugars, minced and fresh sausages and pectin solutions has long been recognised. Metabisulphite levels equivalent to 100mg of sulphur dioxide per kg to 2000mg per kg of the food are being used in practice (55). The antimicrobial action of sulphite or metabisulphite is both pH (60) and moisture (2) dependent.

In view of the promising future for metabisulphite in the field of food preservation, it is pertinent that its interaction with vital organs and processes in the body of the organism consuming the food be well understood. Few researches have been carried out with different organisms in this regard. Lockett and Natoff (59) have reported that feeding of sodium metabisulphite, 750 ppm as SO_2 in the drinking water of rats through three generations had no effect on growth, food intake and fluid output in faeces, fertility and weight of newborn. It also did not increase the frequency with which tumours developed.

Til, Feron and DeGroot (89); Til, Feron, DeGroot and Van der Wal (90) have respectively reported on rats fed with diets containing 0 to 2 percent sodium metabisulphite for a period of 2 years over three generations and on pigs fed similar levels of metabisulphite for 15 to 48

weeks. Pigs fed with 0.83 percent level of sodium metabisulphite and above showed progressive growth retardation. At the 1.72 percent level, there was loss of appetite and marked reduction in food consumption and growth. There were however, no distinct histological changes in the liver and kidneys but inflammatory and hyperplastic changes were reported to have occurred in the stomach.

A seventeen kg body weight dog fed with 3g of sodium sulphite daily by stomach intubation for 23 days showed no abnormalities on autopsy (55). A rabbit given 3g of sodium sulphite daily by stomach intubation for 185 days showed loss of weight but all organs were normal at post mortem (55). Thiamin destruction has been reported (59, 89, 90). As such, addition of metabisulphite to foods not rich in thiamin is undesirable except allowances are made for the anticipated losses.

Although available data seem to suggest that reasonable levels of metabisulphite are tolerated by these animals that have been tested, nothing has been reported on the metabisulphite tolerance of poultry. Sulphite is actively oxidized to sulphate in the liver and eliminated from the body in the urine and faeces (7, 32, 33, 86).

It has been reported that sulphite ions react with a variety of pyrimidine bases (40, 41, 42, 84). Shapiro, Cohen and servis (85) have reported on the possible genetic hazard of sulphur dioxide and bisulphite to living organisms. However, researches into the genetic effect of sulphur dioxide and related compouds on living organisms are still in their rudimentary stages and therefore opinions held on their possible mutagenic hazard may well be termed speculative at the moment.

MATERIALS AND METHODS

Sample collection and initial processing.

Samples of the brewers' spent grains, the malt sweeping and the brewers' yeast were all obtained from the North Brewery, Kano, Nigeria. The samples of spent grains which were collected hot and wet are usually discharge with steam into the waste tank. As a result of the high moisture content (about 75% by weight), after cooling, the spent grains are usually susceptible to contermination with microorganisms. This has been one of the factors limiting the wide usage of spent grains in poultry feed supplements.

All the samples collected were first dried at 60°C for 24 hours and then at 80°C to constant weight or the desired moisture content (about 10% for the samples of spent grains used for animal feeding). As it was noticed that the spent grains could not keep its quality for more than two days unless the moisture content was significantly reduced or/and an antimicrobial agent was added, the drying was started the very day the samples were collected from the brewery.

The addition of 3,000 ppm sodium metabisulphite (equivalent to about 8,520 ppm when dried to the moisture content of 10.21%) prevented spoilage even when the sample was maintained wet for 3 - 5 days. However, this does not imply a total destruction of microorganisms but that it simply enhanced the stability and keeping quality of the spent grains. In the case of the samples of spent grains used for animal feeding experiment, the moisture content was only reduced to about 10% instead of complete drying and no metabisulphite was added. At the end of the drying period, the

samples were blended in a Waring blender (type 211.2.00), and stored in clean feed bags.

The malt sweeping and the brewers' yeast were obtained dry from the brewery and were not subjected to further drying and grinding process.

The commercial Arewa feeds were bought from the Arewa Agricultural Enterprices Limited Zaria, a local feed milling factory while the Pfizer feeds were obtained from Komson Agro-Services (Nig) Agencies Zaria, Nigeria, dealers in Pfizer feeds, drugs and equipments. Pfizer Feeds Limited, which processes and markets Pfizer feeds is a subsidiary of Pfizer Products International.

Sampling procedure.

The representative sample for a particular bag of the commercial feeds was obtained by taking numerous small (15 - 20g) samples from different points in the bag into a large crucible. These were then mixed thoroughly, ground into very fine powder, and stored in clean storage bottles.

The representative sample for a particular commercial feed group, for example Arewa feed, was obtained by taking small samples from three different bags of the feed into a clean container. The mixture was then ground into fine powder after thorough mixing. This was stored in clean storage bottles for use in subsequent analysis.

In the case of the brewers' spent grains, the representative sample was obtained by selecting numerous small samples from the bulk and mixing thoroughly.

Determination of moisture content (4c).

Principle. This is an indirect distillation and involves the measurement of the weight loss due to the evaporation of water from the food material at a temperature of 80°C. The method, which measures the 'free' water and to a less extent, the 'bound' water, gives accurate results when considered on a comparative basis. Loss of volatile oils and decomposition of some sugars that were likely to occur at high temperatures would not occur to any significant extent at the temperature of 80°C that was applied in this investigation.

Procedure. A clean porcelain crucible was ignited at 100°C for 30 minutes, cooled to room temperature in a desiccator and weighed. A known weight (3 - 5g) of the sample was taken into the crucible, thinly spread, and dried to a constant weight in an oven maintained at a temperature of 80°C. The crucible with its contents was removed into a desiccator, cooled to room temperature, and the weight was recorded. This procedure was repeated with five more samples.

In the case of the brewers' spent grains, the samples were moistened with toluene to inhibit fermentation during drying and first dried for 24 hours in an oven maintained at 60°C. After this, the temperature of the oven was increased to 80°C, and the samples were dried further to constant weights which were then recorded.

The weight loss in each case represented the weight of the moisture present in the sample. The percentage moisture was computed from the following expression.

$$\text{Percentage moisture} = \frac{\text{Loss in weight on drying} \times 100}{\text{Initial weight of sample}}$$

Determination of ash content (75)

Principle. The ash of a feedstuff is the inorganic residue remaining after the organic matter has been burnt away. In the determination, a known weight of the dry feed was ignited in a muffle furnace at 600°C. The organic matter was then burnt off leaving behind the ash which was cooled in a desiccator and weighed. The percentage ash was then calculated from the following expression.

$$\text{Percentage ash} = \frac{\text{Wt. of the resulting ash} \times 100}{\text{Wt. of sample taken}}$$

Procedure. A clean porcelain crucible was ignited at 100°C for 30 minutes, cooled in a desiccator and weighed.

About 2.5 to 3.5g of the feed were accurately weighed into the crucible and ignited in a muffle furnace at 600°C for 24 hours. At the end of the 24 hours when the organic matter was burnt up, the crucible containing the resulting ash was taken into a desiccator, cooled to room temperature and weighed. The weight of the ash was then calculated. Five other trials were treated in the same way as above.

Determination of crude protein content.

The semi-micro kjeldahl method was used (75).

Principle. The crude protein content of a particular sample is the figure obtained usually by multiplying the nitrogen content by the factor of 6.25. Sometimes the factor could vary depending on the percentage of nitrogen that is known to be present in the protein of the sample.

The method involves the conversion of the nitrogenous components of the

feed to ammonium sulphate by digesting with concentrated sulphuric acid. Ammonia gas is distilled from the ammonium sulphate by heating with 40% sodium hydroxide solution, trapped into 2% boric acid solution and titrated with standard acid solution. The nitrogen content, hence the crude protein content is then estimated.

It must be noted that this method assumes that all the nitrogen comes from protein and that all proteins contain 16% nitrogen except where a conversion factor other than 6.25 is used.

Calculation

$$NH4OH + HCl \longrightarrow NH4Cl + H_2O$$

$$1 \text{ mole of } HCl \equiv 1 \text{ mole } NH_3$$

$$\therefore 1 cm^3 \text{ of } 0.01M\ HCl \equiv 0.14 mg\ N.$$

$$\text{Total Protein} = \text{Nitrogen} \times 6.25$$

Reagents.

1. Catalyst mixture (96% anhydrous sodium sulphate, 3.5% cupric sulphate and 0.5% selenium dioxide).
2. Sodium hydroxide solution, 40% (w/v).
3. Standard hydrochloric acid solution, 0.01M.
4. Concentrated sulphuric acid
5. Boric acid solution, 2% (w/v).
6. Screned methyl red indicator, containing 0.016% (w/v) methyl red plus 0.083% (w/v) bromocresol green in ethanol.

Procedure. About 0.5g of the well ground powder of the sample was accurately weighed into a digestion flask containing 4g of the catalyst

mixture and a few grains of anti-bumping granules. Ten ml of concentrated sulphuric acid was added gently to the flask and set up in the digestion rack in the fume cupboard. The flask was heated gently for one hour to avoid charring and then it was heated strongly until a clear solution was obtained. The heating was continued for 4 hours after obtaining a clear solution. At the end of the digestion, the flask with its contents was cooled to room temperature, 5 ml of distilled water was added and then the contents were poured gently into a 100 ml volumetric flask. The digestion flask was rinsed three times with 2 ml portions of distilled water into the volumetric flask and the volume was made up to the mark with distilled water.

Ten ml of the digest was pipetted into the Markham distillation apparatus, 10 ml of the 40% sodium hydroxide solution was added, and the mixture was distilled into 10 ml of the boric acid solution containing three drops of the screned methyl red indicator. After 50 ml of the distillate had been collected, it was titrated with the 0.01N hydrochloric acid. Five other determinations and the blank were carried out as above, obtaining three titre values for each digest.

Determination of crude petroleum ether extract or lipid (4d).

Principle. The lipid is extracted exhaustively with a suitable lipid extractive solvent. The solvent is then distilled off and the weight of the resulting lipid estimated.

$$\text{Percentage lipid} = \frac{\text{Wt. of lipid extract}}{\text{Wt. of the sample}} \times 100.$$

Reagent

Petroleum ether (b.p. 60 - 80°C.).

Procedure. About 5g of the dry powdered feed was weighed accurately into dry fat-free thimbles.

To each of six 500 ml round-bottomed distillation flasks which had been previously weighed with a few grains of anti-bumping granules was added 300 ml of petroleum ether. The soxhlet extractors containing the thimbles with the feed were then fitted unto the round-bottomed flasks. Heating was increased gradually until the solvent started boiling. The extraction was continued for 8 hours.

At the end of the extraction, the petroleum ether was distilled off on the Flash rotary evaporator attached to a Liebig condenser. The flasks with the extracted lipid were then dried for 2½ hours in an oven maintained at 50°C to remove the last traces of petroleum ether. The flasks were then cooled in a desiccator and the weights were recorded. The increase in the weight of each flask represented the weight of the lipid extracted in the respective cases.

Determination of crude fibre content (4b).

Principle. The crude fibre represents the organic components that remain after the removal of crude protein, crude lipid and nitrogen-free extract. It is made up mainly of cellulose and lignin and is resistant to hot 1.25% (w/v) sulphuric acid and hot 1.25% (w/v) sodium hydroxide (27), hence the loss in weight on ignition after successive hydrolysis with these two solutions represents the crude fibre content.

$$\text{Percentage crude fibre} = \frac{\text{Wt. loss on ignition after acid and alkali hydrolysis}}{\text{Wt. of sample taken}} \times 100$$

Reagents.

1. Petroleum ether (b.p. 60 - 80°C).
2. Sulphuric acid, 1.25% (w/v).
3. Sodium hydroxide solution, 1.25% (w/v).
4. 95% ethanol.
5. Pretreated asbestos:- asbestos was heated at 600°C for 16 hours in a muffle furnace, then boiled with 1.25% sulphuric acid for 30 minutes, filtered and washed thoroughly with distilled water. The asbestos was then boiled with 1.25% sodium hydroxide solution for 30 minutes, filtered, washed once with 1.25% sulphuric acid and then washed thoroughly with distilled water. It was then dried and ignited at 600°C for 2 hours.

Procedure. About 2 - 4.5g of the dried powdered sample was extracted with petroleum ether for 8 hours. The resulting sample was then transferred quantitatively into a 500 ml round-bottomed flask fitted with a condenser. To this were added a few grains of anti-bumping granules, 1g of the pretreated asbestos, and 200 ml of boiling 1.25% sulphuric acid. The flask was then boiled for exactly 30 minutes with an electric heating mantle, and the contents were then filtered hot to isolate the residue. The residue was washed with four 50 ml portions of boiling distilled water and then boiled with 200 ml of 1.25% sodium hydroxide solution as above. The content of the flask was then filtered hot, washed with the

sulphuric acid, then three 50 ml portions of distilled water and finally with ethanol.

The anti-bumping granule grains were removed, the residue was dried at 130°C for 2 hours and then cooled in a desiccator and later weighed. The residue was then ignited for 2 hours at 600°C, cooled and reweighed.

The blank determination was made by heating 1g of the pretreated asbestos with acid and alkali as in the determination. Any loss in weight was subtracted from the loss in weight of the sample on ignition.

Determination of carbohydrates (excluding crude fibre) or nitrogen-free extract (81).

The nitrogen-free extract was estimated by difference obtained after the subtraction of total crude protein, lipid, ash and crude fibre from the total dry matter.

If, for example, the percentage:

dry matter	=	a
crude protein	=	b
ash	=	c
lipid	=	d
crude fibre	=	e

Then the percentage nitrogen-free extract = a - (b + c + d + e).

Amino acid analysis

Principle. The working principle of an amino acid analyser is based on an elution chromatography from buffered columns of ion - exchange resin. The separation of the amino acids largely depends on their pka values and to some extent on the affinity of the side chains for the resin (94). The separated amino acids are automatically reacted with ninhydrin reagent and a purple or yellow colour is developed by passage through a coil heated in a water bath. The absorbance of the resulting effluent is measured with a colorimeter at 570 nm for all the amino acids except proline and hydroxyproline which are measured at 440 nm and plotted on a strip of chart recorder. The area under each peak gives an estimate of the quantity of each amino acid present.

The identification of each amino acid is possible, since, under controlled conditions the position of emergence of each amino acid is constant (the elution time is characteristic of each amino acid).

There are usually two ion-exchange columns - a short column for the analysis of basic amino acids and a long column for the acidic and neutral amino acids.

Preparation of acid hydrolysate.

Reagents.

1. Standard hydrochloric acid, (6M), saturated with nitrogen gas.
2. Sample buffer (citrate buffer, pH 2.2).

Procedure. To 100 mg of the finely ground feed in the hydrolysis ampoule was added 10 ml of the 6M hydrochloric acid.

The contents of the ampoule were solidified in liquid nitrogen and the ampoule was rendered air-tight and sealed. A duplicate preparation was made as above, then the ampoules were kept for 24 hours in an oven, thermostatically controlled at $110^{\circ}C \pm 1^{\circ}$.

The ampoules were then removed from the oven, cooled to room temparature and carefully opened with a glass cutter. The hydrochloric acid was removed in vacuo from the hydrolysate mixture using the Flash rotary evaporator at $50^{\circ}C$. Two 3 ml portions of distilled water were added to the mixture and subsequently evaporated to rinse excess of the acid. The hydrolysate was then redissolved in 10 ml of the sample buffer.

Calibration of the analyser and the estimation of amino acids.

The Technicon automatic amino acid analyser (Technicon Instruments Co. Ltd.) was used for the analysis.

The analyser was calibrated by applying a standard mixture of amino acids (supplied by the Technicon Instruments Co. Ltd) containing a known amount (0.25 micromoles) of each amino acid to the column.

The concentration of each amino acid present was indicated by the integrated area under the peak representing it on the chromatogram. The area of each peak was integrated in terms of its absorption values and multiplied by its width in terms of the plotted points at the half peak height.

The concentration of each amino acid present in the hydrolysate was determined by comparing the integrated area under its peak with that of the corresponding standard with known concentration.

Determination of tryptophan

Although the hydrolysis with constant boiling 6M hydrochloric acid has the advantage of giving the best recoveries for most amino acids as well as the advantage of the acid being easily removed at the end of the hydrolysis, it is not without problems. The most serious disadvantage of hydrolysing a protein with 6M constant boiling hydrochloric acid is the destruction of tryptophan (20, 61, 78).

Several other methods such as the spectrophotometric procedure of Goodwin and Morton (37), the dye binding colorimetric method of Barman and Koshland (8) and more recently, the alkaline hydrolytic method of Blackburn (10) using 3M barium hydroxide and the enzymic (pronase) hydrolytic method of Oyeleke (72) have been used for the determination of tryptophan.

The alkaline hydrolytic method of Blackburn (10) was adapted in this study and tryptophan in the hydrolysate was determined by the ferric chloride reagent method.

Preparation of the alkaline hydrolysate (10).

Reagents.

1. Barium hydroxide, $Ba(OH)_2 . 8H_2O$.
2. Hydrochloric acid, 6M.
3. Sodium sulphate solution, 1M.

Procedure. About 250 mg portions of the finely ground feed samples were accurately weighed into dry hydrolysis ampoules and 4.73 g of finely ground barium hydroxide powder was added to each ampoule which was then treated with 5 ml of hot distilled water. This quantity of barium

hydroxide is equivalent to 5 ml of 3M $Ba(OH)_2 . 8H_2O$ which could not be prepared as barium hydroxide is not very soluble in water.

The ampoules were carefully sealed under nitrogen and kept for 24 hours in an oven thermostatically maintained at $110^{\circ}C \pm 1^{\circ}$, occationally shaking the ampoules to distribute the contents uniformly. At the end of the 24 hours, the ampoules were allowed to cool, carefully opened with a glass cutter and the contents of each were neutralised with 5 ml of 6M HCL. This was then transferred into a clean centrifuge tube and treated with sodium sulphate solution which was added dropwise until all the barium ions were precipitated. The suspension was then centrifuged at 3,000 r.p.m. for 15 minuts on a Gallenkamp bench centrifuge.

The supernatant was further treated with a drop of sodium sulphate solution to test-proof the absence of barium ions in solution and the solution then carefully poured into a 25 ml volumetric flask. The residue was washed three times with 2 ml portions of distilled water, centrifuged and it was added to the flask. The volume was finally made up to the mark. Duplicate hydrolyses were carried out for each sample. The amount of tryptophan in each flask was determined spectrophotometrically at 420 nm on Sp 6 - 400 uv spectrophotometer.

Estimation of tryptophan by the ferric chloride reagent method.

The method of Horacio and Lynn (49) as described by Oyekele (72) was adapted.

Principle. The principle is based on the reaction of the indole ring of tryptophan with the ferric chloride to produce a colour, the intensity of

which is measured spectrophotometrically at 420 nm.

Reagents.

1. Ferric chloride reagents. Exactly 270 mg of ferric chloride hexahydrate was dissolved in 0.5 ml of distilled water then transferred into a 1 litre volumetric flask and made to volume with glacial acetic acid. Equal volumes of this solution and concentrated sulphuric acid were mixed one to two hours before use.
2. Stock solution of tryptophan and working standard samples: stock solution contained 1 mg/ml.

Determination: Working standard tryptophan solutions of concentrations 2.5 mg, 5.0 mg, 7.5 mg, 10.0 mg and 15.0 mg per 100 ml were prepared from the stock tryptophan solution and used for preparing the calibration curve.

To 1 ml of each standard tryptophan solution mentioned above, 4 ml of ferric chloride reagent was added. The mixture was shaken vigorously and the colour was developed by incubating for 15 minutes in a water bath at 65°C. The test tubes were then cooled to room temperature and the absorbances were measured at 420 nm with sp 6 - 400 u.v. spectrophotometer.

In the case of the experimental samples, 1.0 ml of the hydrolysate, was treated with 4 ml of the ferric chloride reagent, mixed vigorously and the mixture was in cubated for 15 minutes in a water bath at 65°C for colour development. After cooling to room temperature, the absorbance was measured at 420 nm. The concentration of tryptophan in the hydrolysates was estimated from the calibration curve.

Determination of some mineral elements.

Wet ashing. Although the ashing of feed samples at a relatively low ignition temperature such as 550°C, minimises the decomposition of ash constituents or loss by volatilization, when the absolute values of alkali and alkaline earth metals are required it is not the method of choice as the chlorides of these elements are slowly volatilized (57). Wet ashing method which has the advantage of abviating difficulties resulting from the loss of volatile constituents and the slow solution of the residue after ashing is therefore preferred (57, 76).

Reagents.

Perchloric acid (70 - 72%, w/v).

Concentrated nitric acid.

Concentrated sulphuric acid.

Procedure. A known weight (2g) of the feed was accurately weighed into each Kjeldahl digestion flask. To this were added 15ml of concentrated nitric acid, 5ml of concentrated sulphuric acid and 10ml of perchloric acid. The flasks were set up in duplicate for each feed sample. The flasks were heated gently to boiling in a fume cupboard. The heating was continued until all the organic materials had been oxidized and a colourless solution was obtained.

Each flask was then allowed to cool and the digest was filtered into a 100ml volumetric flask. The digestion flask and the filter paper were thoroughly washed into the filtrate with deionized water and the volume was made up to 100 ml.

Suitable dilutions of the perchloric acid digest were made for the determination of sodium and potassium with the flame photometer and also for the determination of calcium and magnesium with the atomic absorption spectrophotometer. The solutions meant for the determination of calcium and magnesium were treated with 0.2% and 0.1% (w/v) lanthanum chloride respectively. Lanthanum chloride which is a releasing agent depresses interference from other elements (96).

All the solutions were stored in polythene bottles.

Determination of sodium and potassium by flame photometry.

The method of Vogel (91) was adapted.

Principle. The principle is based on the fact that excitation of atoms of a metal in a flame gives rise to emission of a characteristic colour. The excitation is obtained by spraying a solution of the substance into a flame and the concentration of the element present is determined from the intensity of the radiation emitted. The region of the spectrum appropriate to the element being determined is selected by means of a suitable optical filter.

Reagents.

1. Stock sodium solution (1,000 ppm Na^+): This was prepared by dissolving 2.542g of dry sodium chloride in deionized water and diluting to 1 litre.
2. Stock potassium solution (1,000 ppm K^+): It was prepared by dissolving 1.909g of potassium chloride in deionized water and diluting to 1 litre.

The stock solutions were stored in polythene bottles.

Procedure. The following working standard solutions of sodium and potassium (2, 4, 6, 8 and 10 ppm) were prepared from their respective stock solutions.

The standard and the sample solutions were ran on the Eel flame photometer with the correct filter in position and the percentage emission recorded for each solution. The concentration of sodium and potassium in each sample was estimated from the calibration curve constructed from the results of the standard solutions. The amount of sodium or potassium in the sample was calculated in the following way. Suppose the concentration read from the calibration curve and converted to mg per ml of digest was 'x' and the dilution factor and the weight of sample taken were 'D' and 'y' g respectively, then the amount of sodium or potassium in mg/100g of sample.

$$= \frac{100\ Dx}{y}$$

Determination of calcium and magnesium by atomic absorption spectrophotometry

The standard method of the Association of Official Analytical Chemists, A.O.A.C. (4a) was followed.

Principle. The vapour of an element which contains free atoms absorbs light having wavelengths as that which the atoms of this element are capable of emitting. When light of this wavelength passes through a flame containing a cloud of free atoms of the respective element, the intensity of the light is diminished due to absorption. The measured decrease in intensity of the light is proportional to the concentration of the atoms in the flame having the corresponding wavelength which in

turn is proportional to the concentration of the atoms in the solution.

Reagents.

1. Stock calcium solution (1,000 ppm Ca^{2+}). This was prepared by dissolving 2.497g of oven-dried calcium carbonate in 25 ml of 1M hydrochloric acid before diluting to 1 litre with deionized water.
2. Stock magnesium solution (1,000 ppm Mg^{2+}). It was prepared by dissolving 1.000g of magnesium metal in 50 ml of 5M hydrochloric acid. Excess acid was removed from the solution by evaporating it in a water bath maintained at 80°C. The container was washed several times with deionized water and the wash poured into a 1 litre volumetric flask then finally diluted to the mark with deionized water.

The stock solutions were stored in polythene bottles.

Procedure. Six working standard calcium solutions of concentrations 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ppm Ca^{2+}, each containing 0.2% (w/v) lanthanum chloride were prepared from the stock calcium solutions. Six working magnesium standards of concentrations 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm Mg^{2+}, each containing 0.1% (w/v) lanthanum chloride were also prepared from the stock magnesium solution.

The absorbance values of the standard calcium solutions and samples were measured at a wavelength of 423nm on the Pye unican Sp 1900 atomic absorption spectrophotometer while magnesium was determined at 285 nm. The calibration curve for each set of determination was plotted and used for determining the amount of calcium and magnesium present in the samples.

Determination of phosphorus (4C).

Principle. The determination is a colorimetric method based on the reaction of molybdovanadate with inorganic phosphorus to form a pink-coloured complex, phosphomolybdovanadate which is measured at 400 nm.

Reagents.

1. Molybdovanadate reagent: 40g of ammonium molybdate tetrahydrate was dissolved in 400 ml of hot distilled water and then cooled. Two g of ammonium metavanadate was dissolved in 250 ml of hot distilled water and then cooled. To the metavanadate solution, 313 ml of 60% (w/v) perchloric acid was added. The molybdate solution was gradually added to the metavanadate solution with constant stirring and the solution was diluted to 2 litres.
2. Stock standard phosphorus solution (2 mg P/ml): This was prepared by dissolving 8.788 g of potassium dihydrogen phosphate (KH_2PO_4) in distilled water and diluting to 1 litre.

Procedure. A known weight (2g) of the sample was ashed in a muffle furnace at 600°C until the sample became carbon-free. The ash was cooled and washed into a 250 ml beaker with 40 ml of concentrated hydrochloric acid in several small portions. To this was added 1.5 ml of concentrated nitric acid and heated to boiling, then cooled and filtered into a 250 ml volumetric flask. The volume was made up with distilled water. Ten ml of this solution was treated with 20 ml of the molybdovanadate reagent, and the mixture was diluted to 100 ml with distilled water. The absorbance of this solution was measured at 400 nm against the blank.

Phosphorus working standard solution (0.1 mg P/ml) was prepared by diluting 50 ml of the stock solution to 1 litre.

Aliquots of the working standard solution containing 0.5, 0.8, 1.0 and 1.2 mg of phosphorus were transferred into 100 ml volumetric flasks and each was treated with 20 ml of molybdovanadate reagent and the mixture was then diluted to volume. A blank solution containing 20 ml of molybdovanadate reagent but no phosphorus was also prepared as above. The absorbance at 400 nm was read after 10 minutes on sp 6 - 400 u.v. spectrophotometer. A calibration curve was then plotted and the amount of phosphorus in the samples determined.

Determination of phytic acid content.

The method of Wheeler and Ferrel (93) was adapted.

Principle. The phytate is extracted from a known quantity of finely ground dry sample with 3% (w/v) trichloroacetic acid (TCA) solution and precipitated as the iron salt (iron phytin). The precipitated iron is then determined colorimetrically and the phytate content calculated using the constant $4Fe^{3+}$: 6P molecular ratio in the precipitate.

Reagents.

1. Trichloroacetric acid (TCA), 30% (w/v) and 3% (w/v) solutions.
2. Iron (III) chloride solution, 2 mg Fe^{3+} per ml of 3% TCA.
3. Sodium hydroxide solution, (1.5M).
4. Nitric acid solution, (3.2M).
5. Potassium thiocyanate solution, (1.5M)
6. Iron (III) nitrate hexahydrate solution, (250 mg/litre).

Procedure. A known weight (2g) of finely ground sample was extracted with 50 ml of 30% TCA for 45 minutes with constant swirling on the Gallenkamp Orbital Incubator. The sample was then centrifuged at 3,000 r.p.m. for 10 minutes. Exactly 20 ml portion of the supernatant was transferred into a 40 ml centrifuge tube and treated with 4 ml of Ferric Chloride solution. The tube was then heated in boiling water for 45 minutes, cooled to room temperature, centrifuged for 15 minutes at 3,000 r.p.m. and the supernatant was decanted. The precipitate was washed twice by dispersing well in 25 ml of 3% TCA and heating it in boiling water bath for 10 minutes. It was then cooled and centrifuged. The precipitate was washed in distilled water, dispersed in 2 ml of distilled water and 3 ml of the sodium hydroxide solution, and then the volume was made up to 30 ml with distilled water. This was boiled for 30 minutes and filtered hot quantitatively. The resulting precipitate was then washed with 60 - 70 ml of hot distilled water and dissolved in 40 ml of hot 3.2M nitric acid. The volume was made up to 100 ml in a 100 ml volumetric flask. Five ml portions of this solution were transferred into 100 ml volumetric flasks and the volume was made up to approximately, 70 ml with distilled water (ie. by adding 65ml of water). The contents of the flasks were then diluted to 100 ml with the 1.5M potassium thiocyanate solution and the absorbance was read at 480 nm within one minute on the sp 6 - 400 u.v. spectrophotometer.

Suitable quantities of the 25 mg% Iron (111) nitrate solution contaning 0.04 to 0.32 mg of iron were dispensed into eight 100 ml volumetric flasks. To each flask were added, 2 ml of the 3.2M nitric

acid and 65 ml of distilled water and the volume was made up to the mark with 1.5M potassium thiocyanate. The absorbance was read within one minute at a wavelength of 480 nm. A calibration curve was plotted and the amount of iron precipitated was calculated for each sample.

Calculations.

Phytic acid content in sample ('a' mg)

is given by the expression

$$a = \frac{660.}{4 \times 56} \text{ mg of iron} \times 2.5 \times 20$$

$$\therefore \text{Percentage phytic acid} = \frac{a \times 100.}{2000}$$

Determination of the pH of the feeds (1).

Procedure. Finely ground dried samples (10g) were suspended in 90 ml of distilled water. This was subjected to thorough blending in a Waring blender and the pH of the decanted supernatant was measured immediately with Corning pH-meter which had been calibrated with standard buffers of pH 4, 7 and 9. Triplicate determinations were carried out for each sample.

Microbial count of the feed.

Procedure. To 90 ml of sterile 1.0% (w/v) peptone water (Difco) was added 10g of the feed and shaken constantly for 45 minutes at 100 r.p.m. on the Gallenkamp orbital incubator, then allowed to settle and the supernatant was decanted. Three successive decimal serial dilutions (1/10) of the supernatant were made with sterile 1.0% peptone water and

plated out in triplicate on sterile plate count agar (PCA, Difco) by the pour plate method.

In this procedure, 0.1 ml of the dilutions was transferred into the sterile PCA agar that had been cooled to about 40°C) by means of a sterile pipette. Two sterile blank agar plates, which were not inoculated were also included to acertain absence of contamination from the incubator during the incubation period.

The plates were then incubated at 30°C for 3 days. At the end of the incubation period, the number of colony-forming units (CFU) on each plate was recorded and the total microbial count was calculated as colony-forming units per g of the feed (CFU/g of feed). No attempt was made to indentify the fungi or bacteria present on the plates. Where growth was observed in the sterile blank agar plates (indicating contamination) the whole set of result was regarded as unreliable and discarded.

Osmophilic moulds (moulds capable of growing even in high osmotic environment) count was determined by plating the serial dilutions prepared as above on MY - 40 agar (2% malt extract, 0.5% yeast extract, 40% sucrose, and 2% bacto-agar, Difco). All the other steps were the same as above.

Biological assay.

Although the nutrient composition as well as the amino acid composition of a feed gives useful information about the quality of the feed, the extent to which these may be utilised or are available to animals when fed in the diets is not shown by such data (31).

Biological assessment of the nutritive value of a feed through aminal feeding experiment is therefore essential for obtaining full information on the biological quality of the feed.

Management of the chicks.

One hundred and thirty day-old Warren breed of Leghorn layer chicks with mean body weight of 35.68 $\pm$ 1.33 g were obtained from Arewa Agricultural Enterprices Limited, Zaria.

The chicks were randomly assigned to six groups. The first four groups contained 25 chicks per group while groups five and six contained 15 chicks each. Each group was assigned a large wooden cage covered on the top with a wire mesh. The cages were kept in the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The design of the animal house was such that temperature fluctuation was reduced to a minimum if the door was constantly kept closed.

Warmth was supplied to the cages by means of a 100 watts electric lamp installed in such locations as to give a cage temperature of $31^{o}C \pm 2^{o}$ for the first three weeks of life. After this, the 100 watts lamps were replaced with 60 watts electric lamps giving cage temperatures of $28^{o}C \pm 2^{o}$. This was done up to the sixth week of life and the chicks were left at the temperature of the animal house ($25^{o}C \pm 3^{o}$) throughout the subsequent period.

The chicks in group 1 were fed with the commercial Arewa poultry feed

while those in group 2 were fed with the commercial Pfizer poultry feed. Those in group 3 were started on the brewers' spent grains to which 1% (by weight) of brewers' dried yeast had been added. However, this diet was replaced with a diet consisting of 25% brewers' spent grains and 75% (by weight) of Pfizer feed on the fifth day as it was observed that the chicks did not like or could not make use of the spent grains. This became evident from the finding that only a small amount of the feed was consumed and some parts of the little eaten were found intact in the faeces. A high mortality rate was also recorded within the short period.

The chicks in group 4 were initially fed with spent grains to which 2% malt sweeping and 1% (by weight) of brewers' dried yeast had been added but also this diet had to be replaced on the fifth day with a diet consisting of 23% spent grains, 2% malt sweeping and 75% (by weight) of Pfizer feed. This was because the spent grain-based diet was found to be nutritionally inadequate for and unacceptable to the chicks.

The chicks in group 5 were fed with Arewa feed to which 1% (by weight) of sodium metabisulphite had been added. The metabisulphite was ground into very fine powder before mixing with the feed.

Group 6 chicks were fed with Arewa feed whose grain sizes had been reduced by blending. This was done only during the period the chick mash was fed.

In all the groups, the chick mash was only fed either wholly or incorporated into the diet during the first 8 weeks of life, after which it was replaced with growers mash.

Food and water were fed ad libitum to all the groups. No antibiotic was added to the drinking water. The feeders were thoroughly cleaned and the drinking containers were thoroughly washed every morning to minimise contamination.

Determination of food consumption, Protein efficiency ratio, Feed efficiency ratio and apparent digestibility.

To determine the food consumption, a known weight of excess food was supplied to the chicks and the food left over was collected every morning between 8.00 a.m. and 9.00 a.m. Contaminants such as feathers were removed and the food was then dried and weighed. This weight was then subtracted from the weight of food supplied to obtain the weight of food consumed.

The faeces were collected every morning, dried in an oven at 50°C, cooled to room temperature and then weighed and stored. These measurements were made for 8 weeks and the mean was calculated for each week. The total nitrogen voided in the faeces between the second week and the sixth week of life of the chicks was determined by the Kjeldahl's method. The first two weeks of life was left out as this was the acclimitization period.

Calculations of the protein efficiency ratio (PER), the Feed efficiency ratio (FER) and apparent digestibility were done as follows:

The protein efficiency ratio,

$$PER = \frac{\text{Gain in live weight}}{\text{Total protein consumed (N x 6.25)}}.$$

The feed efficiency ratio,

FER = Weight of feed consumed
Gain in life weight of the chicks.

The apparent digestibility using crude nitrogen as the index,

= Total nitrogen consumed - Total nitrogen voided in faeces x 100
Total nitrogen consumed by the chick

Determination of the effect of grain size of the feeds on the feed Consumption and utilization by the chicks.

The Arewa chick mash samples was found to have more coarse grain sizes than the Pfizer chick mash. The Arewa chick mash was further ground to reduce the grain size such that when 100g portion of the feed was sieved on a number 18 mesh size sieve, 25 - 30g of the fall out was obtained. This grain size was found to be close to the grain size of Pfizer chick mash. This was the feed that was fed to group 6 chicks. The influence of grain size of the feed on the chicks was then determined by comparing the feed consumption, gain in life weight, apparent digestibility, PER, and FER of this group with group one chicks (the control group).

Gross pathological and histopathological studies.

Procedure. Two birds each from the groups of pullets fed with metabisulphite-treated Arewa feed and the group fed with non-metabisulphite treated Arewa feed were weighed and sacrificed under aseptic conditons at the ages of six weeks and eight weeks. Internal organs were examined superficially for gross pathological changes if any. The liver and the

kidneys, the respective sites of metabolism and excretion of metabisulphite, were removed immediately and weighed. They were then subjected to further processing for histopathological examination.

Tissue Processing. Blocks of the liver, kidney and spleen were fixed in 10 percent buffered neutral formalin for about three days, trimmed and processed in a technicon, then sectioned at 6 microns thickness, and stained with haematoxylin and eosine (H & E). The slides were then observed under light microscope and photographed.

TABLE 1: Proximate composition of the feed trials.

Feed	Moisture (% of wet weight)	Dry matter (% of wet weight)	Ash (% of dry weight)	Crude Protein (% of dry weight)	Petroleum-ether extract (% of dry weight)	Crude fibre (% of dry weight)	Nitrogen-fr extract (% of dry w
Arewa Chick Mash	9.35 ± 0.06	90.65 ± 0.06	6.52 ± 0.06	21.52 ± 0.21	3.88 ± 0.13	3.47 ± 0.14	64.61
Arewa growers mash	4.82 ± 0.09	95.18 ± 0.09	9.27 ± 0.04	19.84 ± 0.34	4.28 ± 0.09	3.27 ± 0.10	63.34
Arewa Layers mash	7.02 ± 0.07	92.98 ± 0.07	13.66 ± 0.02	21.77 ± 0.04	3.92 ± 0.20	2.32 ± 0.02	58.33
Pfizer chick mash	7.56 ± 0.16	92.44 ± 0.16	8.73 ± 0.20	24.40 ± 0.21	3.58 ± 0.37	3.10 ± 0.10	60.19
Pfizer growers mash	5.87 ± 0.22	94.13 ± 0.22	9.69 ± 0.04	17.40 ± 0.20	5.32 ± 0.05	3.16 ± 0.10	64.43
Pfizer layers mash	8.25 ± 0.07	91.75 ± 0.07	17.00 ± 0.77	18.98 ± 0.04	2.95 ± 0.04	3.34 ± 0.05	57.73
Brewers spent grains	75.71 ± 0.15	24.29 ± 0.15	3.65 ± 0.02	21.07 ± 0.62	7.66 ± 0.09	18.79 ± 0.07	48.83

Each value represents the mean of six determinations and the standard errors.

TABLE 2: Proximate composition of the feed trials as fed to the chicks

Feed	Moisture (%)	Ash (%)	Crude Protein (%)	Petroleum-ether extract (%)	Crude Fibre (%)	Nitrogen-free extract (%)
Arewa Chick Mash	9.35	5.91	19.51	3.52	3.15	58.56
Arewa growers mash	4.82	8.82	18.88	4.07	3.11	60.30
Arewa layers mash	7.02	12.70	20.24	3.64	2.16	54.24
Pfizer Chick Mash	7.56	8.07	22.56	3.31	2.87	55.63
Pfizer growers mash	5.87	9.12	16.38	5.01	2.97	60.65
Pfizer layers mash	8.25	15.60	17.41	2.71	3.06	52.97
Brewers' Spent grains	10.21	3.28	18.92	6.88	16.86	43.85

The values reported here were calculated from mean values in table 1, taking into account the moisture content of the feeds, except in the case of the brewers spent grains for which moisture content was dired down to 10.21%.

TABLE 3: Physiological fuel value (PFV) of the feed trials calculated from proximate composition.

Feed.	PFV (Kcal/Kg dry matter)
Arewa chick mash	3,794.4
Pfizer chick mash	3,705.8
Arewa growers mash	3,712.4
Pfizer growers mash	3,752.0
Arewa layers mash	3,556.8
Pfizer layers mash	3,333.9
Brewers' spent grains	3,485.4

The PFV values were obtained by multiplying the mean weight (g) of the ether extract or fat, crude protein and nitrogen-free extract or Carbohydrate per Kg of the feed by 9, 4, 4 respectively and taking the sum of the products (77).

TABLE 4: Variation in the proximate composition of Arewa feeds.

Nutrients	Chick mash			Growers mash			Layers Mash		
	Sample 1	Sample 2	Representative Sample	Sample 1	Sample 2	Representative Sample	Sample 1	Sample 2	Representative Sample
Moisture % of wet weight	8.89	9.77	9.35	5.39	6.13	4.82	7.52	7.26	7.02
Ash % of dry matter	6.69	7.18	6.52	10.06	8.89	9.27	14.12	12.63	13.66
Crude protein % of dry matter	21.15	20.52	21.52	19.49	20.12	19.84	21.42	21.52	21.77
Petroleum-ether extract % of dry matter	3.57	3.48	3.88	4.05	3.76	4.28	2.36	2.57	3.92
Crude fibre % of dry matter	3.41	3.49	3.47	2.87	3.35	3.27	2.93	2.46	2.32
Nitrogen-free extract % of dry matter	65.18	65.33	64.61	63.53	63.88	63.34	59.17	60.82	58.33

Each value represents the mean of three determinations except in the case of the representative samples where values represent the mean of six determinations. The representative samples are mixtures of random samples from three different bags. Samples 1 and 2 are samples from each of two feed bags respectively.

TABLE 5: Variation in the proximate composition of Pfizer feeds.

Nutrients	Chick mash			Growers mash			Layers mash		
	Sample 1	Sample 2	Representative Sample	Sample 1	Sample 2	Representative Sample	Sample 1	Sample 2	Representative Sample
Moisture % of wet weight	7.83	7.51	7.56	6.41	6.06	5.87	8.42	8.76	8.25
Ash % of dry matter	8.72	8.68	8.73	10.12	9.10	9.69	15.99	16.49	17.00
Crude protein % of dry matter	23.69	23.73	24.40	16.87	17.30	17.40	18.85	19.16	18.98
Petroleum-ether extract % of dry matter	3.42	3.42	3.58	5.28	5.26	5.32	2.90	2.92	2.95
Crude fibre % of dry matter	3.10	3.25	3.10	3.11	3.31	3.16	3.32	3.25	3.34
Nitrogen-free extract % of dry matter	61.07	60.91	60.19	64.62	65.03	64.43	58.94	58.18	57.73

Each value represents the mean of three determinations except in the case of the representative samples where values represent the mean of six determinations. The representative samples are mixtures of random samples from three different bags. Samples 1 and 2 are samples from each of two feed bags respectively.

Proximate Composition.

Tables 4 & 5 show the variation of proximate composition of different batches of Arewa and Pfizer feeds respectively. Generally, the composition of the Arewa feeds appear to vary more widely from one bag to another than the Pfizer feeds. Infact relatively large lumps of groundnut cakes were found in the Arewa feeds.

Feeds from both Arewa and Pfizer, especially the layers mash contain much sand. This was evident from the fact that grains of sand were found in the ash.
It is likely that the sand made a significant contribution to the total ash content obtained with the layers mash.

TABLE 6: Proxinate composition of brewer's spent grains from the old and new brew houses.

Nutrients	Spent grains from the new brew house.	Spent grains from the old brew house
Moisture % of wet weight	75.71 ± 0.15	79.61 ± 0.08
Ash % of dry matter	3.65 ± 0.02	3.44 ± 0.02
Crude protein % of dry matter	21.07 ± 0.62	18.78 ± 0.41
Petroleum-ether extract % of dry matter	7.66 ± 0.09	5.59 ± 0.11
Crude fibre % of dry matter	18.79 ± 0.07	16.08 ± 0.15
Nitrogen-free extract % of dry matter	48.83	55.11

Values for each iten represent the mean of six determinations and standard errors. The new brew house is used more frequently than the old brew house. So the samples used for the feeding experiment were obtained from the former.

TABLE 7: Total amino acid composition of the feed trials expressed as percentage of dry matter.

Amino acid	Level (%) in feed						
	Chick Mash		Growers Mash		Layers Mash		Brewers' Spent grains
	Arewa	Pfizer	Arewa	Pfizer	Arewa	Pfizer	
Tryptophan	0.31	0.26	0.21	0.30	0.24	0.24	0.33
Lysine	0.67	1.48	0.99	0.87	1.08	1.52	0.80
Histidine	0.43	0.62	0.57	0.50	0.85	0.74	0.51
Arginine	1.10	2.05	1.76	1.54	1.65	1.33	1.25
Aspartic acid	2.22	1.77	1.23	1.08	2.11	1.31	1.63
Threonine	0.76	0.73	0.50	0.44	0.76	0.66	0.81
Serine	1.06	0.94	0.65	0.57	0.72	0.78	0.95
Glutamic acid	4.46	4.33	3.27	2.86	4.46	2.92	4.02
Proline	1.39	1.20	0.98	0.86	1.31	1.06	2.15
Glycine	1.13	0.99	0.77	0.67	1.15	0.91	0.80
Alanine	1.16	1.06	0.90	0.79	1.35	0.93	1.02
Cysteine	-a	-	-	-	-	-	-
Valine	0.86	0.85	0.83	0.73	1.35	1.04	1.15
Methionine	0.17	0.39	0.33	0.29	0.46	0.59	0.35
Isoleucine	0.83	0.75	0.58	0.51	0.96	0.63	1.01
Leucine	1.66	1.55	1.16	1.02	1.68	1.38	1.87
Tyrosine	0.42	0.67	0.46	0.41	0.54	0.44	0.62
Phenylalanine	0.98	0.85	0.73	0.64	0.87	0.79	1.36

[a] Not detected.

TABLE 8: Essential* amino acid composition of the feed trials expressed as percentage of dry matter.

Amino acid	Level (%) in feed.						
	Chick mash		Growers mash		Layers mash		Brewers
	Arewa	Pfizer	Arewa	Pfizer	Arewa	Pfizer	Spent grains
Tryptophan	0.31	0.26	0.21	0.30	0.24	0.24	0.33
Lysine	0.67	1.48	0.99	0.87	1.08	1.52	0.80
Histidine	0.43	0.62	0.57	0.50	0.85	0.74	0.51
Arginine	1.10	2.05	1.76	1.54	1.65	1.33	1.25
Threonine	0.76	0.73	0.50	0.44	0.76	0.66	0.81
Glycine	1.13	0.99	0.77	0.67	1.15	0.91	0.80
Valine	0.86	0.85	0.83	0.73	1.35	1.04	1.15
Methionine	0.17	0.39	0.33	0.29	0.46	0.59	0.35
Isoleucine	0.83	0.75	0.58	0.51	0.96	0.63	1.01
Leucine	1.66	1.55	1.16	1.02	1.68	1.38	1.87
Phenylalanine	0.98	0.85	0.73	0.64	0.87	0.79	1.36

* Essential for poultry.

Note Chick mash is recommended for the first 8 weeks of life, growers mash between 8 - 18 weeks and layers mash after 18 weeks.

Amino acid composition.

Tables 7 and 8 respectively show the total amino acid composition and the essential (essential for poultry) amino acid composition of the feed trials. From table 7, tryptophan and methionine are the two limiting amino acids in all the feed trials. Glutamic acid was found to be the most abundant in all the feed trials including the pure brewers' spent grains. Aspartic acid level was also found to be fairly high in all the feed trials.

The brewers' spent grain has a high level of proline, and infact its proline content is much higher than any of the levels recorded for all the other feed trials. Cystein was not detected in any of the feed trials.

Comparing the levels of each essential amino acid in table 8 with the values recommended by NRC (Table 18), the Arewa chick mash was found to be grossly deficient in lysine and methionine, and the level of arginine was marginal

The Arewa growers mash had marginal levels of threonine, methionine, glycine and isoleucine. However, the Arewa layers mash was found to adequately meet the demand for all the essential amino acids except methionine.

The Pfizer chick mash and the layers mash were found to contain the recommended levels of the essential amino acids except methione whose level was low in the chick mash. The Pfizer growers mash was however, found to contain a low level of methionine and marginal levels of threonine, glycine and isoleucine.

Except the levels of lysine and methionine which were found to be marginal when compared with the level recommended by NRC (66) for starter chicks' diet, brewers spent grains contained adequate amounts of amino acids that are essential for poultry.

TABLE 9: The level of some mineral elements in the feeds

(as percentage of dry matter)

Mineral Element	Level (%) in feed						
	Chick mash		Growers mash		Layers mash		Brewers' Spent grains
	Arewa	Pfizer	Arewa	Pfizer	Arewa	Pfizer	
Sodium	0.222	0.212	0.263	0.257	0.392	0.365	0.042
Potassium	0.267	0.232	0.342	0.252	0.512	0.425	0.062
Magnesium	0.035	0.048	0.034	0.048	0.036	0.049	0.055
Calcium	1.52	2.10	2.00	2.86	2.55	3.72	0.19
Phosphorus	0.628	0.645	0.620	0.633	0.655	0.667	0.725

TABLE 10: The pH of, phytic acid and phytic acid phosphorus content of the feeds.

Feed	pH Value	Phytic acid (% of dry matter)	Phytic acid phosphorus (% of dry matter)
Arewa Chick Mash	6.0	0.447 ± 0.014	0.126
Pfizer Chick Mash	6.3	0.514 ± 0.010	0.145
Arewa Growers Mash	5.8	0.539 ± 0.010	0.152
Pfizer growers mash	6.2	0.593 ± 0.010	0.167
Arewa Layers Mash	5.8	0.729 ± 0.074	0.205
Pfizer Layers Mash	6.2	0.832 ± 0.015	0.234
Brewers' Spent grains	4.9 - 5.2	0.448 ± 0.003	0.126

TABLE I1: Total microbial counts of the feeds.

Feed	Mould count (CFU/g)		Bacterial Count/g
	Moulds[a]	Osmophilic Moulds	
Arewa Chick Mash	7.0×10^4	4.17×10^3	9.0×10^4
Pfizer Chick Mash	2.52×10^5	-b	-c
Arewa growers Mash	1.0×10^4	2.17×10^3	3.0×10^4
Pfizer growers mash	4.50×10^4	-	-
Arewa Layers Mash	9.5×10^4	2.33×10^3	7.5×10^3
Pfizer Layers Mash	2.55×10^5	9.5×10^3	-
Brewers Spent grains	4.25×10^4	2.0×10^3	-

[a]These are total mould counts irrespective of physiological species characteristics.

[b]No mould was detected.

[c]No bacteria were detected.

TABLE 12: Nutritive Value of the feed trials.

Feed type	Total feed consumed (g) (in 4 weeks)	Total protein consumed (g)	Total nitrogen consumed (g)	Total faeces voided (g.dry weight)	Total nitrogen voided in faeces (g).	weight gain (g)	Feed efficiency ratio (FER)	Protein efficiency ratio (P.E.R.)	Apparent digestibil
A.F.	774.08	151.02	24.16	119.45	6.14	159.54	4.85	1.06	74.59
P.F.	631.29	140.52	22.48	76.67	3.22	179.98	3.51	1.28	85.68
B.S.G.	798.87	178.99	28.64	141.75	7.98	194.53	4.11	1.09	72.14
M.S.	765.24	145.84	23.32	98.41	5.26	188.73	4.05	1.29	77.44
M.T.F.	751.66	146.64	23.46	151.69	5.85	157.17	4.78	1.08	75.10
G.A.F.	679.73	132.61	21.21	127.19	6.69	129.82	5.24	0.98	68.46

A.F. Arewa feed.
P.F. Pfizer feed.
B.S.G. Brewers spent grains diet (25% of spent grains and 75% Pfizer feed).
M.S. Malt sweeping blended diet (23% spent grains, 2% malt sweeping and 75% Pfizer feed).
M.T.F. Metabisulphite-treated Arewa feed.
G.A.F. Ground Arewa feed.

Nutritive value of the feed trials.

Table 12 shows the nutritive value of the feed trials. The Pfizer feed showed the highest apparent digestibility.

Addition of 25 percent spent grains to commercial Pfizer feed led to a reduction in the apparent digestibility of the Pfizer product.

The metabisulphite treatement of Arewa feed has had no significant effect on the digestibility of the Arewa feed.

The grinding of the Arewa feed led to a reduction in the apparent digestibility of the feed by the chicks.

TABLE 13: Mean weights of day-old pullets fed the different feed trials (weights recorded in grammes).

WEEK	*GROUP 1	*GROUP 2	*GROUP 3	*GROUP 4	*GROUP 5	*GROUP 6
1/7	37.08 ± 1.50	36.56 ± 1.36	35.08 ± 1.04	36.15 ± 1.36	34.07 ± 1.42	35.13 ± 1.32
1	41.10 ± 1.90	53.62 ± 2.34	29.17 ± 1.92	35.33 ± 3.58	41.03 ± 3.08	35.46 ± 2.12
2	58.15 ± 5.58	77.22 ± 6.38	44.83 ± 2.78	55.94 ± 11.50	57.43 ± 6.52	41.75 ± 6.04
3	73.23 ± 6.68	100.28 ± 8.58	67.39 ± 7.50	79.56 ± 14.54	80.64 ± 9.14	59.56 ± 9.80
4	108.43 ± 13.92	143.38 ± 11.62	102.28 ± 14.10	111.72 ± 16.06	116.86 ± 13.28	87.71 ± 14.50
5	158.00 ± 21.56	213.80 ± 18.14	163.44 ± 20.94	171.67 ± 18.22	165.86 ± 21.64	123.00 ± 17.86
6	217.69 ± 28.96	257.20 ± 23.94	239.31 ± 22.76	244.67 ± 24.02	215.71 ± 30.16	166.57 ± 26.66
7	276.31 ± 32.42	317.21 ± 22.60	323.25 ± 32.18	311.44 ± 23.42	273.08 ± 43.70	240.40 ± 34.90
8	323.22 ± 36.68	368.83 ± 24.86	389.38 ± 37.84	376.25 ± 27.94	338.33 ± 51.18	288.20 ± 38.32
9	422.25 ± 44.95	472.09 ± 27.00	445.88 ± 42.26	451.50 ± 21.76	389.40 ± 50.50	310.00 ± 25.52
10	520.97 ± 53.04	575.65 ± 31.16	542.69 ± 48.74	554.44 ± 25.40	459.80 ± 61.00	354.67 ± 22.68
11	613.34 ± 60.70	642.74 ± 31.12	580.50 ± 55.32	606.69 ± 22.32	596.20 ± 60.66	483.67 ± 5.84
12	687.62 ± 66.00	691.17 ± 34.12	626.13 ± 58.10	649.38 ± 25.84	651.60 ± 55.82	540.33 ± 4.24
13	787.62 ± 71.62	799.78 ± 38.86	735.00 ± 70.30	757.00 ± 32.06	681.80 ± 55.56	607.33 ± 23.62
14	861.87 ± 77.66	890.61 ± 41.96	830.71 ± 79.82	847.75 ± 35.18	695.60 ± 59.10	650.00 ± 36.50
15	968.87 ± 80.46	968.61 ± 44.42	932.57 ± 82.86	938.13 ± 35.72	736.50 ± 62.00	687.00 ± 39.46
16	1041.38 ± 84.82	1042.48 ± 4356	1009.43 ± 81.00	1006.38 ± 42.84	782.90 ± 64.42	740.67 ± 52.66

*see legend.

Legend to Tables 13 and 14.

Group 1. Chicks fed with Commercial Arewa poultry feed.

Group 2. Chicks fed with Commercial Pfizer poultry feed.

Group 3. Chicks started on spent grains with 1% (w/w) brewers dried yeast diet but the diet was replaced on the fifth day with a diet consisting of 25% spent grains and 75% (by weight) of Pfizer feed.

Group 4. Chicks started on spent grains with 2% malt sweeping and 1% (by weight) of brewers dried yeast but the diet was replaced on the fifth day with a diet consisting of 23% spent grains, 2% malt sweeping and 75% (by weight) of Pfizer feed.

Group 5. Chicks were fed with Arewa feed to which sodium metabisulphite (1%) had been added.

Group 6. Chicks fed with ground Arewa feed.

Note Values of weight gain used in constructing Fig. 1 were derived from Table 13.

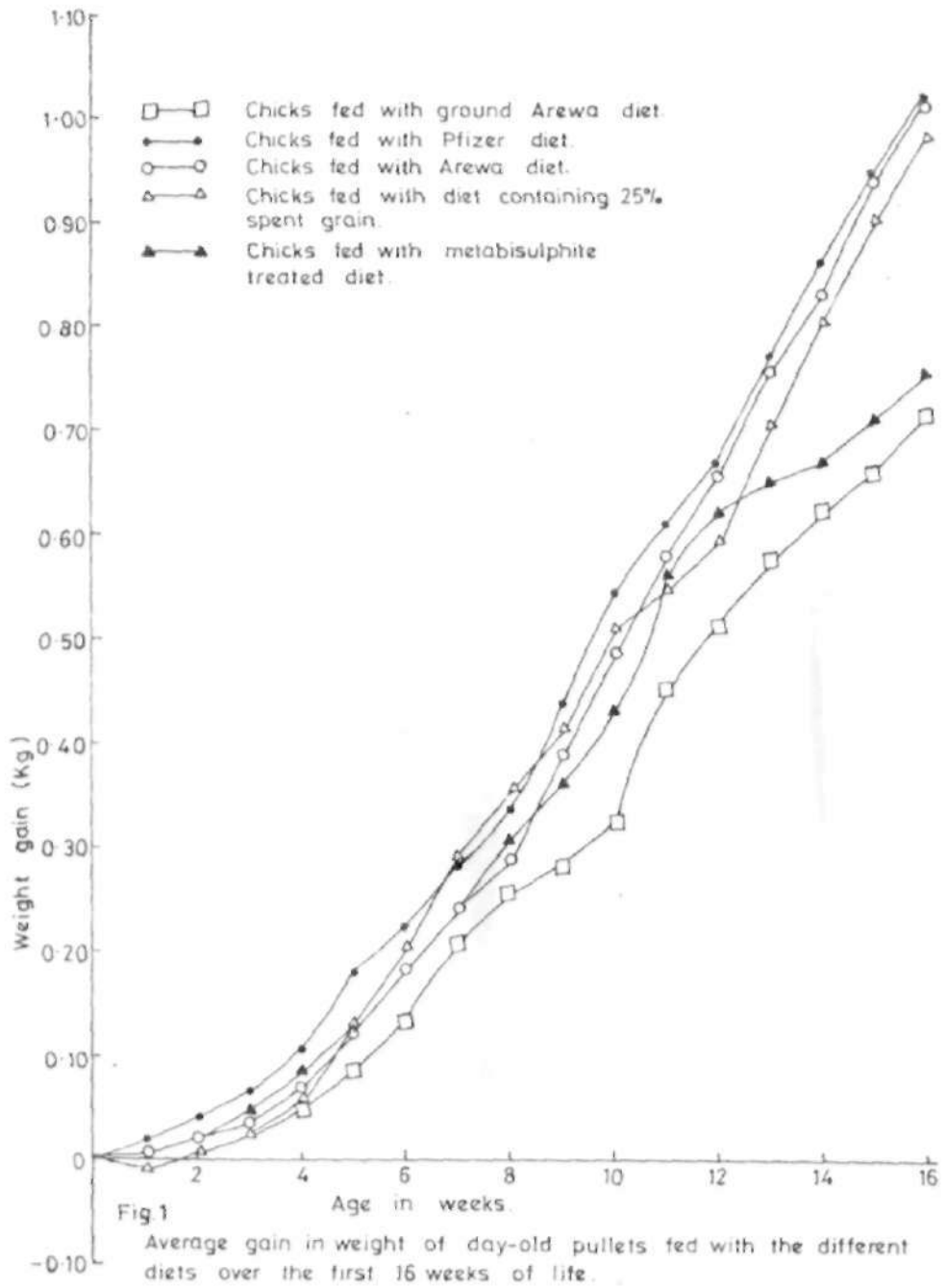


Fig.1 Average gain in weight of day-old pullets fed with the different diets over the first 16 weeks of life.

Growth of the chicks in relation to diet.

Figure 1 shows how the average gain in life weight of the chicks varies with the type of diet. The mean gain in life weight of chicks fed with Commercial Pfizer chick mash was significantly ($P < 0.05$) higher than that for chicks fed with Commercial Arewa chick mash. The chick mash from the two sources (Pfizer and Arewa Companies) were fed to the chicks during the first 8 weeks of life.

There was no significant ($P > 0.05$) difference between the gain in weight of chicks fed with growers mash from the two sources (Pfizer and Arewa Companies). Although the gain in weight of the chicks fed with Pfizer growers mash was slightly higher, the Pfizer growers mash cannot be said to be outstandingly superior to Arewa growers mash since the difference was not significant ($P > 0.05$).

The inclusion of the brewers spent grains at a level of 25% (w/w) in the Pfizer chick mash resulted in a significant ($P < 0.05$) loss in weight gain when the mixture was fed to chicks within the first 5 weeks of life. After this age, there was no significant ($P > 0.05$) difference between the gain in life wight of the control (chicks fed with pure Pfizer mash) and the experimental (chicks fed on Pfizer diet containing 25% spent grains) chicks. The inclusion of spent grains at 25% (w/w) level in the diet appears not to be tolerated by starter chicks.

The mean gain in life weights of chicks fed with metabisulphite-treated Arewa feed were similar to mean gain in life weights of the control (chicks fed with metabisulphite-free Arewa diet) chicks for the first 9 weeks of life.

However, it became apparent in the tenth week that growth of the chicks fed with the metabisulphite-treated feed was gradually being retarded but the retardation did not become significant ($P > 0.05$) until the thirteenth week.

There was a significant ($P \leq 0.05$) decrease in the weight gain when the day-old chicks were fed with Arewa feed whose particle size was reduced to fine particles. This was investigated by comparing the mean weekly weight gain of the group fed with unground feed (control group) with the group fed with the ground feed (experimental group). The chicks fed with the ground feed also showed haemorrhage of the feet during the first two weeks of life.

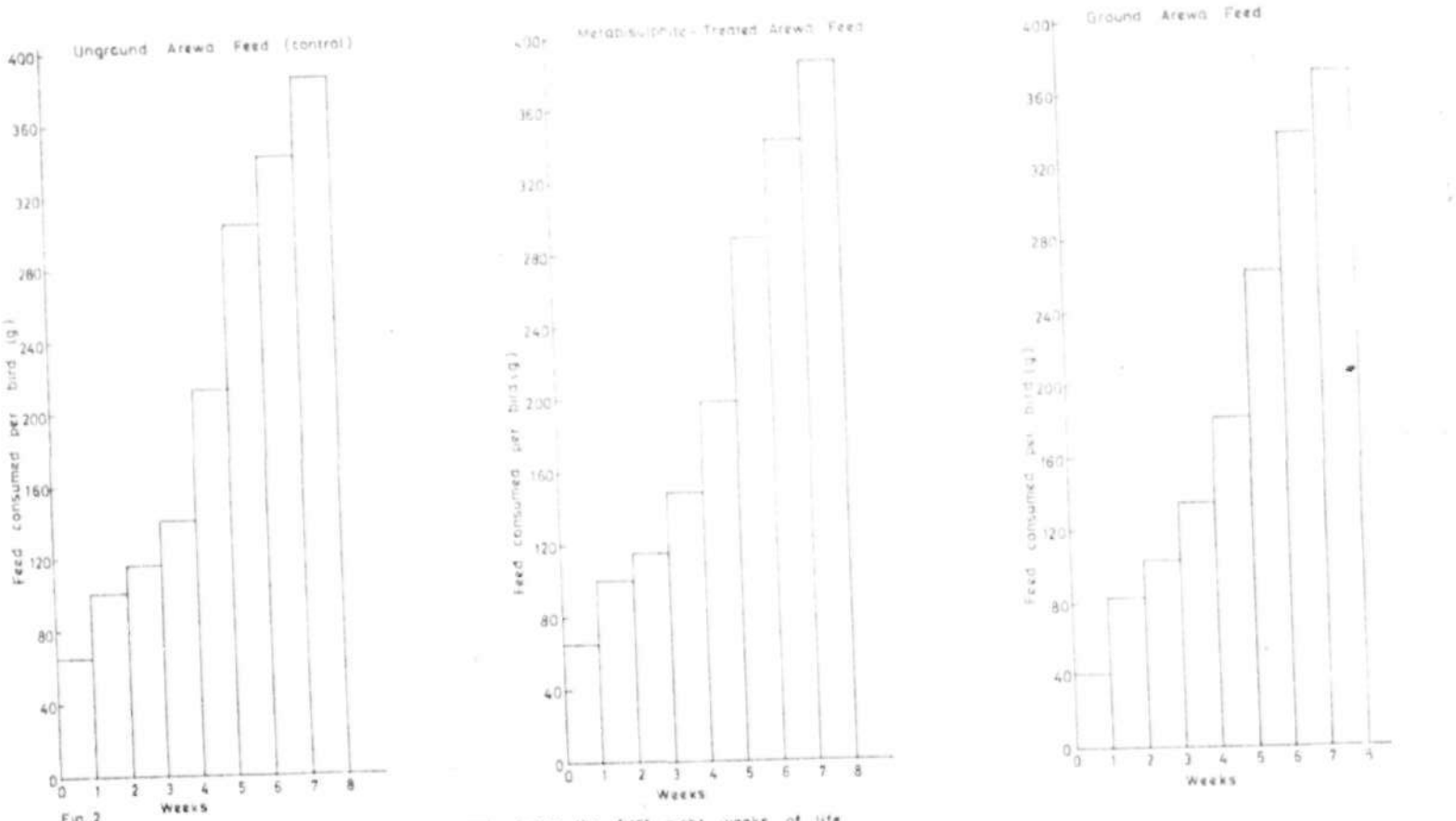


Fig. 2 Average feed consumption per bird per week during the first eight weeks of life

Feed Consumption: There was no pronounced difference between the consumption of the metabisulphite-treated and that of the non-metabisulphite treated Arewa feeds by the chicks (Fig. 2).

The grinding of the Arewa feed to reduce the particle size led to a pronounced reduction in the amount of the feed consumed by the chicks. Generally, the chicks were found to eat more on the days that there was electric power failure and warmth could not be supplied. This was true for all the feed trials.

TABLE 14: Mortality rate expressed as the percentage of the Original number of day-old chicks that die weekly.

Week	Percentage mortality.					
	Group.1	Group 2.	Group 3.	Group.4	Group 5	Group 6.
1	-a	-	40.0	64.0	-	6.6
2	4.0	-	24.0	-	6.6	40.0
3	-	-	-	-	-	-
4	12.0	-	-	-	-	-
5	16.0	-	-	-	-	-
6	4.0	-	4.0	-	-	-
7	-	4.0	-	4.0	-	-
8	-	4.0	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	-	-	4.0[b]	-	-	-
14	-	-	-	-	-	-
15	-	-	-	-	-	-
16	-	-	-	-	-	-

[a] No death.

[b] Accidental death.

Mortality: Table 14 shows the mortality rate of chicks fed with different feed trials. On the whole, 36 percent of the day-old chicks fed with the Commercial Arewa feed died and all the deaths occurred during the first 6 weeks of life. Only 8 percent of the chicks fed with Commercial Pfizer feed died. More than half of the chicks fed on the diet containing 25% of spent grain died during the first two weeks of life while over 60 percent of the chicks fed with the diet containing the malt sweeping died during the first week of life.

The inclusion of metabisulphite in the diet appears to be accompanied with a reduction in mortality rate of the chicks (Table 14).

The chicks fed with the ground Arewa feed showed higher mortality than those fed with unground Arewa feed (the control chicks).

TABLE 15: Internal organ weights of pullets fed with metabisulphite-treated feed at the sixth week of life.

Treatment	Body wt. (g)	Liver wt. (g)	Kidney wt. (g)	Liver wt. per 100g body wt.	kidney wt. per 100g body wt.
M1.	196	7.820	2.587	3.99	1.32
M2.	240	9.540	3.403	3.97	1.42
C1.	161	6.517	2.190	4.05	1.36
C2.	220	8.954	2.354	4.07	1.07

M1. and M2 were chicks fed with metabisulphite-treated Arewa feed.

C1. and C2 were (controls) chicks fed with Arewa feed with no metabisulphite.

TABLE 16: Internal organ weights of pullets fed with metabisulphite-treated feed at the eighth week of life.

Treatment	Body wt.(g)	Liver wt. (g)	Kidney wt. (g)	Liver wt.per 100g body wt.	Kidney wt. per 100g body wt.
M3	339	9.763	3.729	2.88	1.10
M4	307	9.271	3.530	3.02	1.15
C3	302	8.879	3.450	2.94	1.14
C4	379	11.565	4.570	3.05	1.21

M3 and M4 were chicks fed with metabisulphite-treated Arewa feed (experimental chicks). C3 and C4 were (controls) chicks fed metabisulphite-fee Arewa feed.

Organ weight at autopsy. The organ weights per 100g body weight for the liver and the kidneys of the chicks fed with metabisulphite-treated feed were almost identical with the values obtained for the controls (Tables 15 and 16). However, the organ weights per 100g body weight appear to decrease with increasing age, the decrease being more pronounced with the liver. This observation was noticed in both the control and the experimental chicks.

Gross pathological examination of internal organs: There were no significant findings other than slight intramuscular haemorrhage of the chest and thigh muscles. However, similar findings were noticed in both the chicks fed with metabisulphite-treated feed (the experimental chicks) and the controls (chicks fed with metabisulphite-free diet). The bursa of Fabricius was found to be enlarged, Oedematous and involuted along its longitudinal folds both in the experimental and the control chicks.

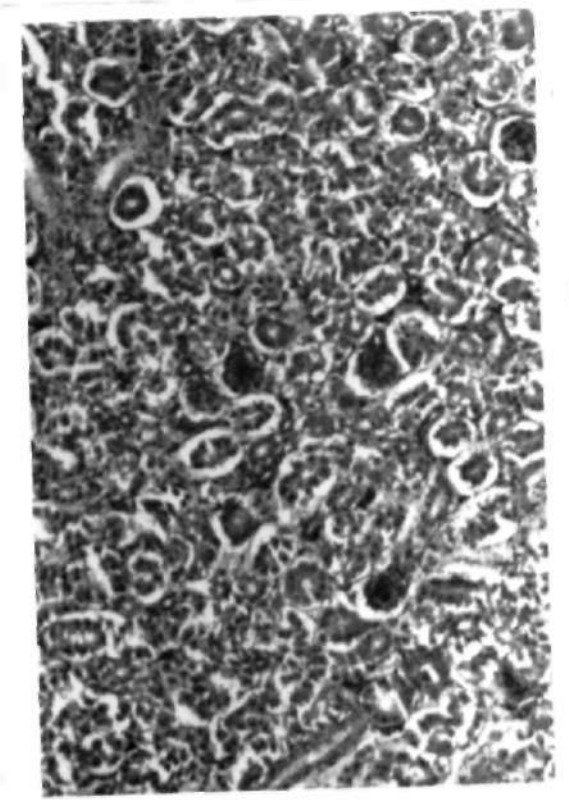


PLATE 1: Photomicrograph of the kidney section (x 100) of chick fed with metabisulphite-free Arewa feed (control) at the age of six weeks. There were no signficant findings.

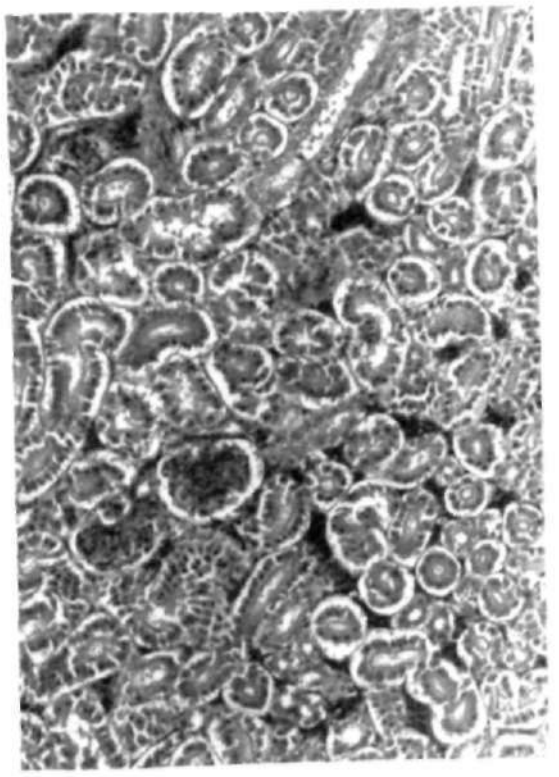


PLATE 2: Photomicrograph of the kidney section (x 100) of chick fed with metAbisulphite-treated Arewa feed at the age of six weeks. There were no significant findings.



PLATE 3: Photomicrograph of the liver section (x 100) of chick fed with metabisulphite-free Arewa feed (control) at the age of six weeks. Shows focal accumulation of leucocytes around the bile ducts and some of the blood vessels.

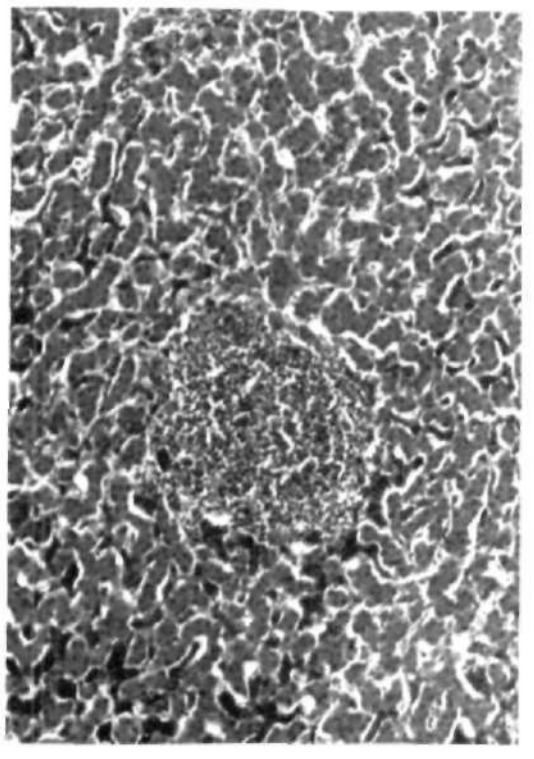


PLATE 4: Photomicrograph of the liver section (x 100) of chick fed with metabisulphite-treated Arewa feed at the age of six weeks. Shows focal accumulation of leucocytes around the bile ducts and some of the blood vessels.

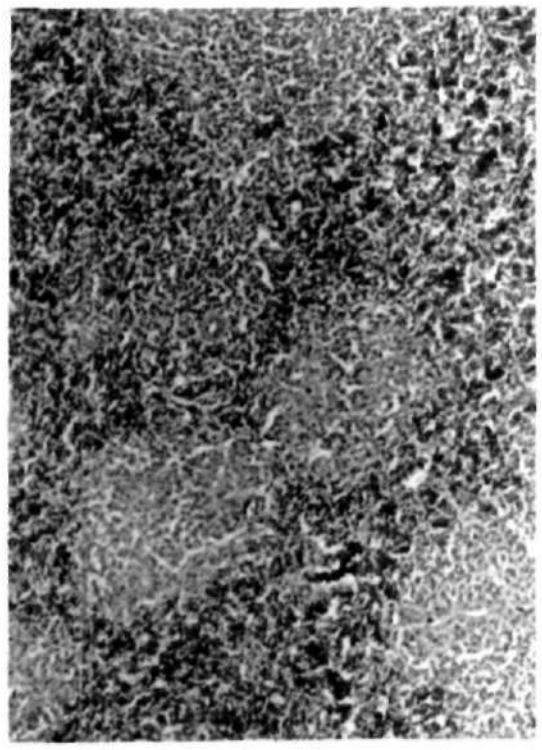


PLATE 5: Photomicrograph of the spleen section (x 100) of chick fed with metabisulphite-free Arewa feed (control) at the age of six weeks. There were no significant findings.

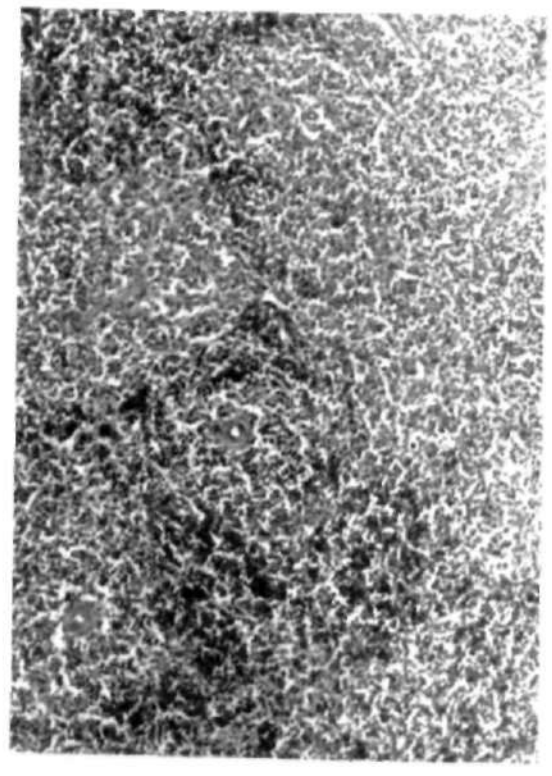


PLATE 6: Photomicrograph of the spleen section (x 100) of chick fed with metabisulphite-treated Arewa feed at the age of six weeks. There were no significant findings.

Results obtained with chicks sacrificed at the age of eight weeks were identical with those reported above, in the respective cases.

D I S C U S S I O N

Proximate Composition. Although it is possible to make many more determinations, the ordinary report of the chemist on the routine feed analysis usually gives values for moisture, crude protein, fat or ether extract, crude fibre, mineral matter or ash and nitrogen-free extract (44). The proximate analysis is probably the most generally used chemical scheme for describing foodstuffs, despite the fact that the information it gives may often be of uncertain nutritional significance. Theoretically, it is possible to formulate a feed which is complete in all nutritional essentials but fails to be appetizing to the animals or birds (23). However, each of the components mentioned above plays an important role in achieving the desired goal for which the feed was formulated.

Moisture. The moisture level of a feed affects the stability (82) and the acceptablility of the feed to the animals. Crampton and Harris (17) reported that foods containing more than 14 percent moisture cannot be stored in bulk as they are readily attacked by moulds. However, animals, especially poultry, universally deslike dusty or hygroscopic feeds (17, 23). The moisture content of the representative samples of all the commercial feeds fell between 4.73 and 9.41% while the moisture content of the brewers' spent grains (from the new brew house) fell between 75.56 and 75.86% (Table 1). The moisture content of the growers mash from both Arewa and Pfizer companies were fairly low (4.73 to 6.09, see Table 1). The other mashes (chick and layers mashes) satisfy the conditions needed for bulk storage, but they were not too dry as to approach dustiness.

The moisture content of the brewers' spent grains was found to be too high and could not therefore be stored in bulk. The spent grain was therefore found to be extreemly difficult to preserve. Apart from the considerably high cost of drying the spent grains by artificial heat, the heat method alone is not likely to prevent attack by moulds and other micro-organisms if drying is to be carried out slowly at relatively low temperatures to prevent the cooking of the grains. It is most likely that the attack by moulds and other micro-organisms and the subsequent spoilage are among the most serious limiting factors militating against the use of brewers' spent grains in poultry feeds. It has however been found out in this study that the addition of 3,000 ppm of sodium metabisulphite (1010.5 ppm SO_2 equivalent) to the wet spent grains enhanced the stability, making it possible for the grains to be dried slowly or even in the sun without spoilage taking place.

Crude Protein.

The crude protein contents of all the commercial feed mashes analysed (Table 1) were found to be adequate when compared with the levels recommended by NRC (66). The protein content of the brewers' spent grains was found to be fairly high. However, this does not necessarily mean that all the feed mashes or the spent grains are capable of fully meeting the protein requirements of the chicks. This is because the quality of a protein depends on its amino acid composition, digestibility and availability of the amino acids.

Methionine and tryptophan were found to be the limiting amino acids in all the feeds (Table 7). However, the levels of tryptophan recommended by NRC for the different categories of poultry feed were found to be lower than the levels obtained in all the feeds including spent grains. Cystein was not detected in any of the feeds. Byers (14) has reported that methionine and cystein are usually present in foods in small amounts relative to other amino acids. The low level of cystein coupled with its extensive destruction in acid hydrolysis (20) is possibly responsible for the failure to detect it in the feeds. Where the absolute level of cystein is required, the samples are first subjected to performic acid oxidation before the acid hydrolysis (97). Under this condition, cystein and methionine are oxidized to cystoic acid and sulphone respectively (14). In this study, such steps were not taken to determine cystein as cystein is not essential for chicken. The high levels of glutamic acid and aspartic acid obtained in the feeds (Table 7), were partly due to the decomposition of glutamine and asparagine respectively (20),

The Arewa chick mash (with low levels of lysine, methionine and arginine) and the Arewa growers mash (with marginal levels of threonine, methionine, glycine and isoleucine) can be improved by increasing the amount of fish meal and or soyabean in the feed. The Pfizer growers mash also appear to require similar fortification or supplementation.

Ideally, there are three ways of supplementing the essential amino acids that are either deficient or present at marginal levels in feeds.

(i) Increasing the level of protein in the diet, but this is wasteful and is of little application.

(ii) Fortification by the direct addition of the deficient amino acid. However, this is too expensive and the method is seldomly used.

(iii) Feeding a combination of proteins from different sources such that the deficiency of one protein is supplemented by abundance of others. This is the method that is most widely used.

Carbohydrate.

All the feeds analysed were found to be rich in carbohydrates both on dry weight basis and as fed to the chicks (Tables 1 and 2 respectively). This is not unexpected, judging from the fact that cereal grains form a major component of all the feeds. The physiological fuel value of all the feeds including the brewers' spent grains (Table 3) were found to be above the value recommended for all categories of chicks by the NRC (Table 18 in the appendix). As far as the supply of energy is concerned therefore, all the feeds appeared to be nutritionally adequate for the chicks.

In poultry science, the carbohydrate component whose level should be critically watched in poultry feed is the crude fibre. The inability of hens to effectively digest crude fibre and the interference of fibre with availability of amino acids have already been discussed (27). The crude fibre contents of all the commercial feeds were all found to be below 7 percent (Table 1). This is what most poultry feed manufacturers strive to achieve when formulating rations for hens, especially starter chicks (27). The crude fibre content of the spent grain was found to be

quite high (about 18.7%). This possibly contributed to the inability of the starter chicks to make good use of the spent grains. Ademosun (3) has implicated the high crude fibre content of brewers grains as being responsible for its limited use by poultry.

Although the role of dietary fibre in preventing some diseases of the alimentary canal in man has been reported (12), there are yet no data to show that such findings apply to poultry.

Petroleum ether extract (fat).

The levels (2.91 - 7.75%) of petroleum ether extract in the representative samples of all the feeds (Table 1) were found to fall within the recommended range of 2 - 8% (26). However, oxidative rancidity, a problem commonly associated with the fat component of the feed is a function of the level of unsaturated fatty acids rather than the total fat content of the feed.

Total ash and mineral elements.

The representative samples of the layers mashes showed higher total ash (13.64 - 17.77%) than all the other feeds analysed (Table 1). The reason for this seems to be that more bone meal and oyster shell are usually added to the layers mash than the chick or growers mash.

The level of calcium in the Arewa layers mash (2.55%) was found to be marginal when compared to the level (2.75%) recommended by NRC (Tables 9 and 19). There is therefore a need to improve this situation by the addition of oyster shell or calcium carbonate, as restricted calcium intake has been reported to cause cessation of laying in sexually mature hens (34, 35).

The phosphorus content of the chick mashes of both Arewa and Pfizer feeds were also found to be marginal. A more disturbing observation was the fact that a significant proportion of the phosphorus was present in phytic acid (Table 10). This is disturbing because phytic acid phosphorus is less readily utilised than inorganic phosphorus. However, since no phosphorus deficiency symptoms were observed in the chicks fed with these diets, it may be fair to infer that the level present was able to meet the phosphorus requirements of the chicks.

Variation in proximate Composition.

The wide variation of the proximate composition of the Arewa feeds from one bag to another (Table 4) possibly resulted from improper mixing of the bulk of the feed before packaging. This wide variation in composition is undesirable as customers buying feeds from the same source are likely to get diverse feeding results. The variation in the case of Pfizer feeds (Table 5) was found, on the average to be less pronounced than in Arewa feeds. The difference could be attributed to proper mixing of the Pfizer feeds. It is indeed necessary that individual ingredients should be thoroughly mixed to ensure that the proportion of the mash consumed by one bird will have the same composition as that prescribed by the formular. The finding that the nitrogen-free extract of the spent grains from the new brew house was lower than the value obtained for spent grains from the old brew house (Table 6) suggestes that the procedure adopted in the former was more efficient at extracting the soluble carbohydrates.

pH

All the feeds analysed had pH values in the acidic region. However, both the Arewa and Pfizer feeds were found to be only weakly acidic (Table 10). The brewers' spent grain was found to be more acidic than any of the commercial feeds. Although the effect of this on the palatability of the grains feed to the chicks was not known, it apparently had the advangage of suppressing bacterial growth. The freshly dried brewers' spent grain was found to be devoid of bacteria but fungi were present (Table 11). Moreover, the antimicrobial action of metabisulphite which was being tested for possible use in preventing spoilage of the spent grains is usually pH dependent, being more active at low pH values (60). This is therefore an added advantage.

Microbial Count.

The microbial count of a feed may also be considered as one of the indices for assessing the quality as some of the microorganisms are either pathogenic or could cause the spoilage of the feeds. Although some of the feeds analysed were found to contain viable micro-organisms (Table 11), it is difficult to tell whether they were present in the raw materials or whether they entered the products during the post-processing storage period. However, the incorporation of metabisulphite into the feed seemed to militate against the survival of the micro-organisms irrespective of their point of entry into the feed. This has an advantage over the use of heat and/or irradiation which may be less

effective in controlling post-processing contamination when in the hands of the customers.

Nutrive Value of the feed trials.

The addition of 25% spent grains to Pfizer diet led to a reduction in the apparent digestibility of the Pfizer diet (Table 12). This possibly was responsible for the finding that despite the fairly high level of essential amino acids in spent grain (Table 8), poor reseults were obtained when attempts were made to feed it as the only protein source. The high crude fibre content might have been responsible for the poor digestibility (3). The significant ($P < 0.05$) loss in the weight gain (Fig. 1) and the increased mortality rate (Table 14) of starter chicks when fed with the diet containing 25% spent grain showed that spent grain was not tolerated by the starter chicks at this level (25%) and so it is not recommended as sole chick feed. However, 25% addition of spent grains to growers diet had no significant ($P > 0.05$) effect on weight gain and mortality rate and such supplementation may be recommended (Fig 1 and Table 14). The addition of malt sweeping in the diet did not result in any distinct benefit and so no further attempt was made to include this component in the feed trials.

The treatment of the Arewa feeds with sodium metabisulphite (1% w/w) did not have any pronounced effect on the digestibility and the consumption of the feed (Table 12 and Fig. 2). Although the growth of the pullets fed with metabisulphite-treated feed was gradually retarded

without any distinct toxic symptoms on consumption of feeds containing 1% metabisulphite for 8 weeks, whether or not this retardation has any effect on the onset of egg laying and subsequently on egg production by the chicks require further investigation. Til, Feron, DeGroot and Van der Wal (90) have reported similar growth retardation in pigs fed with a diet containing 0.83% sodium metabisulphite for 15 to 48 weeks.

The fall in the digestibility of Arewa chick mash on grinding it to fine particulate size was due to the short time spent in the gizzard by fine food particles (23) thereby preventing any reasonable digestion. The haemorrhage of the feet might be due to vitamin K deficiency which in turn might have resulted from insufficient intake of this vitamin as the feed consumption was reduced (Fig.2). It therefore appeared that the reduction in the particle size adversely affected the appetite of the chicks. Infact, Ewing (23) had reported that fine ground mash was not as palatable as one that was of medium coarse in granulation.

Toxicological Studies.

The Joint FAO/WHO Expert committee on food additives (53) has recommended the use of the following indices in toxicological evaluation: growth rate, hepatic and renal functions and where indicated, the functions of other organs, organ weight at autopsy and histopathological investigation.

Neither the control chicks nor the chicks fed with metabisulphite-treated feed showed gross pathological changes in the liver or the kidneys. The weights of these two organs relative to the body weight in the experimental chicks were identical to values obtained with the controls (Tables 15 and 16).

Both the control and the experimental chicks showed slight intramuscular haemorrhage of the chest and thigh muscles. These findings could therefore, not be attributed to the metabisulphite treatment. Haemorrhage could result from vitamin K deficiency but since the vitamin levels of the feeds were not determined in this study, vitamin K can neither be categorically implicated nor ruled out.

The microscopic examination of the kidney of the chick fed for 6 weeks with the metabisulphite-treated feed (Plate 2) was identical to that of the control (Plate 1). The metablisulphite therefore had no adverse effect on the kidneys. No significant pathological changes occurred in the spleen as a result of the metabisulphite treatment (Compare Plates 5 and 6).

The fact that focal accumulation of leucocytes around the bile ducts and some blood vessels was found in liver sections of both the experimental and the control chicks, (Tables 3 and 4), implied that the metabisulphite could not be implicated as being responsible for the observation. Mononuclear lymphoid cell accumulation as discrete foci or as diffuse collections, particularly in periportal areas are sometimes found in normal avian livers (9). Til, Feron, DeGroot and Van der Wal (90) have reported that pigs fed with a diet containing a level of sodium metabisulphite as high as 1.72% for 15 weeks showed no distinct histological changes in the liver and the kidneys although the relative weights of these organs were increased. The metabisulphite tolerance of

an animal definitely depends on the ability of the liver to metabolise the metabisulphite into non-toxic products. It can therefore be inferred that since there were no significant histological abnormalities in the livers of the experimental chicks, the level of sodium metabisulphite employed, 1% or 10,000 ppm (equivalent to about 3,368 ppm of sulphur dioxide) falls within the range the liver could deal with physiologically. Sodium matabisulphite may therefore be of value in preventing spoilage of the spent grains even when dried slowly at low temperature and in controlling spoilage of poultry feeds during the post-processing storage period. Metabisulphite concentration of 8,000 ppm was found to render 10% rehydrated bone meal free of osmophilic moulds in simulated contamination (1).

It is felt that the level of metabisulphite that is capable of eliminating osmophilic moulds will eliminate most micro-organisms, since osmophilic moulds are among the most resistant groups of micro-organisms.

C O N C L U S I O N S.

The moisture content of the brewers' spent grains (BSG) from North Brewery Limited, Kano, Nigeria was found to be quite high (up to 75%). As a result of this, it was difficult to ordinarily sun-dry the BSG without mycotic contamination and subsequent spoilage. The addition of sodium metabisulphite to wet BSG (3,000 ppm metabisulphite, equivalent to 8,520 ppm metabisulphite when dried to a moisture content of 10.21%) enhanced the stability and longevity thereby permitting the grains to be dried at convenience provided that this was done within 3 - 5 days after the discharge.

Inspite of the findings that BSG was fairly rich in crude protein and essential amino acids, it cannot serve as the only protein source in poultry diets as it was poorly digested by starter chicks in particular. The high crude fibre content was possibly responsible for this poor digestibility. Addition of 25% BSG to Pfizer chick mash fed to starter chicks resulted in a significant growth retardation and increased mortality rate and therefore BSG alone could not be recommended as chick feed. However, a similar additon to the growers diet had no significant effect on growth and mortality rate. It is hoped that with such additon, the price of the diet will fall significantly.

Methionine was deficient in all the commercial feeds except Pfizer layer mash where the level was found to be adequate. The level of this amino acid and other amino acids present at sub-optimal level can be

improved by increasing the proportion of fish meal in the ration.

Feeding day-old pullets with a diet containing sodium metabisulphite (10,000 ppm equivalent to 3,368 ppm of sulphur dioxide) for 6 - 8 weeks does no significant damage to the liver, the kidneys and the spleen of the pullets. As day-old chicks are more vulnerable to toxic agents than older chicks, it is felt that older chicks can tolerate this level of metabisulphite too, even though this conclusion is without prejudice to the cumulative effect of the metabisulphite.

SUGGESTIONS FOR FURTHER STUDIES

It may be necessary to carry out further studies in order to resolve the following unanswered questions which may becloud the use of brewers' spent grains and metabisulphite in poultry feeds.

1. The effect of feeding layer chicks with a diet containing 25% BSG on the onset of lay and the laying performance of the birds.
2. The effect of feeding pullets with metabisulphite-treated feed on the onset of laying and laying performance.
3. The relationship between storage time and temperature of the feed and the residual concentration of metabisulphite in the feed with a view to finding the best storage conditions.
4. The interaction between the metabisulphite and the components of the feeds.
5. The effect of metabisulphite-treatment on the normal flora of the alimentary canal of the chicks.

R E F E R E N C E S.

1. Abalaka, J.A. (1977). Combined effect of Moisture and sodium metabisulphite on the destruction of salmonella and other bacteria in bone meal and gelatin. Ph.D. thesis, University of Wisconsin, Madison, U.S.A.

2. Abalaka, J.A. and Deibel, R.H. (1980). Viability of Salmonella typhimurium in metabisulphite-treated bone meal: effect of inocula and moisture variation on the lethal potency of metabisulphite. Microbios 27, 167 - 174.

3. Ademosun, A.A. (1973). Evaluation of brewers dried grains in the diets of growing chickens. Brit. Poultry Sci. 14 (5), 463 - 468.

4. A.O.A.C. Association of Official Analytical Chemists (1975) 'Official methods of analysis', 12th Ed.

   4a. A.O.A.C. P. 22.

   4b. A.O.A.C. P. 136.

   4c. A.O.A.C. P. 143.

   4d. A.O.A.C. P. 185.

   4e. A.O.A.C. P. 192.

5. Bassir, O. (1953). Nutrition in Nigeria. W. Afr. Med. J. 2, 30 - 41

6. Bassir, O. (1964). Improving the level of nutrition. W. Afr. J. Biol. Appl. chem. 7 (3), 32 - 40.

7. Bhaghat, B. and Lockett, M.F.(1960). The absorption and elimination of metabisulphite and thiosulphate by rats. J. Pharm. Pharmcol. 12, 690 - 694.

8. Barman, T.E. and Koshland, D.E. Jr. (1967). The colorimetric procedure for the quantitative determination of tryptophan residues in Proteins. J. Biol. Chem. 242 (24), 5771 - 5776.

9. Benirschke, K., Garner, F.M. and Jones, J.C. (1978). Pathology of laboratory animals vol. 1, Springer - Verlag N. York, P. 252.

10. Blackburn, S. (1968). Amino acid determination methods and techniques. N.Y. Marcel Dekker Inc., pp. 95 - 97.

11. Borgstron, G. (1975). Neglected aspects of world food issue. In 'The Man/Food Equation' (Proceedings of a symposium held at the Royal Institution London 1973). Steel, F. and Bourne, A. Eds., 1975. Academic Press London, pp. 43 - 51.

12. Burkitt, D. P (1969). Related desease - Related cause? Lancet 2, 1229 - 1231.

13. Burkitt, D.P. and James, P.A. (1973). Low-residue diets and hiatus hernia. Lancet 2, 128 - 130.

14. Byers, M. (1971). Amino acid composition and in vitro digestibility of some protein fractions from three species of leaves of various ages. J. Sci. Fd. Agric. 22, 243 - 251.

15. Chibnall, A.C., Ress, M.W. and Lugg, J.W. (1963). The amino-acid composition of leaf proteins. J. Sci. Fd. Agric. 14, 234 - 239.

16. Cough, J.R. and Rayton J.K. (1974). Amino acids and protein in broiler nutrition. Poultry Sci. 53, 750 - 755.

17. Crampton, E.W. and Harris, L.E. (1969). Applied Animal Nutrition. 2nd Ed. (ch.3. Proximate analysis of feeds). W.H. Freeman and Co. San Francisco, pp. 30 - 55

18. Crampton, E.W. and Harris, L.E. (1969). Applied Animal Nutrition. 2nd Ed. W.H. Freeman and Co. San Francisco, p. 605

19. Davidson, S., Passmore, P., Brock, J.F. and Truswell, A.S. (1975) Human Nutrition and Dietetics. 6th Ed. English Language Book Society Churchill Livingstone Edinburgh pp. 302 - 304.

20. Davies, M.G. and Thomas, A.J. (1973). An investigation of hydrolytic techniques for the amino acid analysis of foodstuffs. J. Sci. Fd. Agric. 24, 1525 - 1540.

21. Eastwood, M.A. (1974). Dietary fibre in human nutrition. J. Sci. Fd. Agric. 25, 1523 - 1527.

22. Ewing, W.R. (1951). Poultry Nutrition 4th Ed. (Ch. 1. Importance of quality feeds). W. Ray Ewing pub., South Pasadena, California, pp. 3 - 19.

23. Ewing, W.R. (1951). Poultry Nutrition 4th Ed. (ch. 3. Digestibility and palatability). W. Ray Ewing Pub., South Pasadena, California, pp. 33 - 50.

24. Ewing, W.R. (1951). Poultry Nutrition. 4th Ed. (Ch. 6. Proteins and amino acids). W. Ray Ewing Pub., South Pasadena, California, pp. 148 - 405.

25. Ewing, W.R. (1951). Poultry Nutrition. 4th Ed. (Ch. 7. Carbohydrates in the poultry ration). W. Ray Ewing Pub., South Pasadena, California, pp. 406 - 468.

26. Ewing, W.R. (1951). Poultry Nutrition. 4th Ed. (Ch. 8 Fats in the poultry ration). W. Ray Ewing Pub., South Pasadena, California, pp. 469 - 483.

27. Ewing, W.R. (1951). Poultry Nutrition. 4th Ed. (Ch. 9. Fibre in the poultry ration). W. Ray Ewing Pub., South Pasadena, California pp. 484 - 507.

28. Ewing, W.R. (1951). Poultry Nutrition 4th Ed. W.Ray Ewing Pub., South Pasadena, California p. 508.

29. Ewing, W.R. (1951). Poultry Nutrition. 4th Ed. W. Ray Ewing Pub., South Pasadena, California P. 1052.

30. Federal Institute of Industrial Research, Nigeria Annual Report, (1960). W. Afr. J. Biol. Appl. Chem. 4 (1), 14 - 19.

31. Fetuga, B.L., Babatunde, G.M. and Oyenuga, V.A. (1973). Protein quality of some feedstuffs II. Biological evaluation of protein quality. J. Sci. Fd. Agric. 24, 1515 - 1523.

32. Fridovich, I. and Handler, P. (1956). The initial step in enzymic sulphite oxidation. J. Biol. Chem. 223, 321 - 325.

33. Gibson, W.B. and Strong, F.M. (1973). Metabolism and elimination of sulphite by rats, mice and monkeys. Fd. Cosmet. Toxicol. 11, 185 - 198.

34. Gilbert, A.B. and Blair, R. (1975). Comparison of the effects of two low-calcium diets designed to regulate egg production in the fowl. Brit. Poultry Sci. 16, 547 - 552.

35. Gilbert, A.B., Peddie, J., Teague, P.W. and Mitchell, G.G. (1978). The effect of delaying the onset of laying in pullets with a low calcium diet on subsequent egg production. Brit. Poultry Sci. 19, 21 - 34.

36. Girdwood, R.H. (1974). Nutritional disorders. In Textbook of medical Treatment. Alstead, S. and Girdwood, R.H. Eds., Churchill Livingstone Edinburgh., pp. 396 - 421.

37. Goodwin, T.W. and Morton, R.A. (1946). The spectrophotometric determination of tyrosine and tryptophan in proteins. Biochem. J. 40, 628 - 632.

38. Haikerwal, H. and Mathieson, A.R. (1971). The protein content and amino acid composition of sorghum grains. Cereal Chemistry 48, 690 - 698.

39. Hammond J.C. (1943). Incompartibilities in mixed feeds. Official Publication of the Association of American Feed Control Officials, 1943. pp. 55 - 61.

40. Hayatsu, H. (1969). The oxygen-catalysed reaction between 4 - Thiouridine and sodium sulphite. J. Amer. Chem. Soc. 91 (20), 5693 - 5694.

41. Hayatsu, H. and Miura, a. (1970). The mutagenic action of sodium bisulphite. Biochem. Biophys. Res. Comm. 39 (1), 156 - 160.

42. Hayatsu, H., Watuya, Y. and Kai, K. (1970). The addition of sodium bisulphite to uracil and cytosine. J. Amer. Chem. Soc. 92 (3), 724 - 726.

43. Heuser, G.F. (1955). Poultry Feeding. 2nd Ed. (ch. 2. Objects and principles in feeding poultry). John Wiley and sons Inc. N. York, pp. 13 - 19.

44. Heuser, G. F. (1955). Poultry Feeding 2nd Ed. (ch. 3. Composition of plants, animals and rations). John Wiley and sons Inc. N. York, pp. 20 - 79.

45. Heuser, G.F. and Norris, L.C. (1930). The influence of the protein level on the rate of growth in chickens. Proc. iv World's poultry Congress London pp. 328 - 342.

46. Heuser G.F. and Norris, L.C. (1933). The influence of the protein level on the growth of chickens and its relation to subsequent behaviour. Proc. v World's Poultry Congress Rome II. pp. 551 - 558.

47. Hobbs, B.C. (1976). Microbiological hazards of international trade. In Microbiogy in Agriculture, Fisheries and Food. The society for Applied Bacteriology Symposium series no. 4. Skinner, F.A. and Carr, J.G. Eds., 1976. Academic Press London, pp. 161 - 180.

48. Holum, J.R. (1975). Elements of General and Biological Chemistry. 4th Ed. John Viley and Sons Inc., N. York, P. 322.

49. Horacio, H.H. and Lynn, S.B. (1969). Res. Bull. 13, 1. Cited from Oyeleke (see 72).

50. Idusogie, E.O. (1975). The nutritional requirements of the Nigerian population. Afr. J. Med. Sci. 3, 53 - 66.

51. Jay, J.M. (1978). Modern Food Microbiology 2nd Ed. D. Van Nostrand Co., N. York, pp. 166 - 167.

52. Jensen, L.S. Chang, C.H. and Wyatt, R.D. (1976) Influence of carbohydrates source on liver fat accumulation in hens. Poultry Sci. 55, 700 - 709.

53. Joint FAO/WHO (1965). Expert Committee on Food Additives. 8th Rept. Specification for the identity and purity of food additives, and their toxicological evaluation: Food colours and some antimicrobials and antioxidants. FAO Nutr. meeting Rept. Ser. No. 38, WHO Techn. Rept. ser. No. 309, p. 12.

54. Joint FAO/WHO (1969). Expert Committee on Food Additives. 12th Rept. Specification for the identity and purity of food additives and their toxicological evaluation: Some antibiotics. FAO Nutr. Meetings Rept. ser. No. 45, WHO Techn. Rept. Ser. No. 430 p. 6.

55. Joint FAO/WHO (1971). Expert Committee on Food Additives, 14th Rept. A Review of the technological efficacy of some antimicrobial agents. FAO Nutr. Meetings Rept. Ser. 48c., WHO/FAO Food Add/70/41. pp. 33 - 40.

56. Joint FAO/WHO (1974). Expert Committee on Food Additives. 17th Rept. Toxicological evaluation of some food additives including anti-craking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. FAO Nutr. Meetings Rept. Ser. No. 53A., WHO Techn. Rept. Ser. No. 539. pp. 130 - 142.

57. Joslyn, M.A. (1970). Methods in Food Analysis 2nd Ed. Academic Press N. York and London., pp. 109 - 140.

58. Jul, M. (1974). Proteins in world nutrition. J. Trop. Paed. Envir. child Health 20 (2), 60 - 67.

59. Lockett, M.F. and Natoff, I.L. (1960). A Study of the toxicity of sulphite 1. J. Pharm. Pharmcol. 12, 488 - 496.

60. Lucker, C., Mosser, J and Hanson, R.S. (1975). Effect of sulphite on microorganisms. In Fd. Res. Ann. Rept., 1975 (University of Wisconsin, Madison). pp. 378 - 379.

61. Mahler, H.R. and Cordes, E.H. (1971). Biological Chemistry. 2nd Ed., Harper and Row Pub. Inc. N. York, p. 72.

62. Mcfie, J. (1964). The clinical consequences of malnutrition. W. Afr. J. Biol. Appl. Chem. 8 (1), 20.

63. Miles, R.D. and Featherson, W.R. (1976). Uric acid excretion by the chicks as an indicator of dietary protein quality. Poultry Sci. 55, 98 - 102.

64. Narain, R.D. (1975). Population and food supply. In 'The Man/Food Equation'. (Proceedings of a symposium held at the Royal Institution, London 1973). Steel, F. and Bourne, A. Eds. Academic Press London, pp. 3 - 22.

65. National Research Council, NRC (1962) Nutrient requirements of domestic animals. No. 1 Nutrient requirements of poultry:- A report of the committee on animal nutrition. National Academy of Sciences, Washington D.C.

66. National Research Council, NRC (1971). Nutrient requirements of domestic animals, No. 1. Nutrient requirements of poultry. National Academy of Sciences, Washington D.C.

67. Nickerson, J.T. and Sinskey, A.J. (1977). Microbilogy of Foods and Food Processing. Elsevier North-Holland Inc. P. 126.

68. Oke, O.L. (1965) Chemical Studies on some Nigerian foodstuffs - 'Lafun'. W. Afr. J. Biol. Appl. Chem. 8 (3), 53 - 56.

69. Oke, O.L. (1966). The need for a new source of protein. W. African Pharmacist 8 (6), 112 - 116.

70. Oke, O.L. (1972). Leaf protein for better nutrition. Appropriate Technology 1 (4), 11 - 12.

71. Oke, O.L. (1973). Leaf protein research in Nigeria: a review. Tropical Science 15 (2), 139 - 155.

72. Oyeleke, O.A. (1977). Assessment of the nutritive value of two varieties of sorghum grain, Sorghum bicolor (L) moench. MSc. thesis, department of biochemistry, Ahmadu Bello University Zaria, Nigeria.

73. Oyenuga, V.A. (1960). Problems of nutrition in agricultural production. W. Afr. J. Biol. Appl. Chem. 4 (2), 23 - 28.

74. Painter, N.S., Almeida, A.Z. and Colebourne, K.W. (1972) Unprocessed bran in treatment of diverticular deseases of the colon. Brit. Med. J. 2, 137 - 140.

75. Pearson, D. (1976). The Chemical Analysis of Foods. 7th Ed. Churchill Livingstone Edinburgh, London, pp. 7 - 11.

76. Perrin C.H. (1949). Determination of potash in fertilizers. Anal. Chem. 21 984 - 986.

77. Pike, R.L. and Brown, M.L. (1975). Nutrition an Integrated Approach. 2nd Ed. John Wiley and Sons, Inc. N. York p. 853.

78. Plummer, D.T. (1971). An Introduction to Practical Biochemistry. McGraw - Hill Book Co Limited Maidenhead. Berkshire. England. p. 169.

79. Ranhotra, G.S., Hepburn, F.N. and Bradley, W.B. (1971). Effect of fibre on availability of protein from wheat shorts. Cereal Chemistry 48, 9 - 13.

80. Reilly, R.W. and Kirsner, J.B. (1975). Fibre deficiency and colonic disorders. Amer. J. Cli Nutr. 28 (3), 293 - 294.

81. Schneider, B.H. and Flatt, W.P. (1975), The evaluation of feeds through digestibility experiments. The Univ. of Georgia Press Athens p. 96.

82. Scott, W.J. (1956). Water relations of food spoilage microorganisms. Advan. Food Res. 7, 84 - 127.

83. Shakir, A.. (1975)• The Surveillance of Protein-calorie malnutrition by simple economical means. J. Trop. Paed. Envir. child **Health* 21** (2), 69 - 74.

84. Shapiro, it. Servis, R.E. and Welcher, M. (1970). Reactions of uracil and cytosine derivatives witli sodium bisulphite. *A* specific deamination method J. Aner. Chem. Joe. _22, 422 - 424.

85. Shapiro, It., Cohen, B.I. and oervis, **EJ2.** (1970). Specific deamination of RNA by sodium bisulphite. Nature, London, 227. 1047 - 1048.

86. Strong, F.M. and Giteon, W.B. (1973). Safety of sulphited foods. In Fd. Res. Ann. Rept., 1973- (University of Wisconsin, Madison), pp. 256 - 258.

87. Sukhatmo, 1.V. (1975). Human protein needs and the relative role of energy and protein in meeting them. *In* 'The ^an/F Equation[1]. (Proceedings of a sy: lposiun at the Royal Institution, London 1973). Steel, F. and Bourne, ... Eds. Academic Press, London pp. 53 - 75.

88. Sutie, J.W, (1972). Introduction to Biochemistry. Holt, itinehart and Winston Inc., N. York. p. 273.

89. Til, ii.P., Feron, V.J. and DeGroot, A.P. (1972). The toxicity of sulphite 1. Long-term feeding and **multi.** eneration studies in rats. Fd. Cosmet. Toxicol. JO, 291 - 310.

90. Til, H.P., Feron, V.J., DeGroot, A.P. and Van der Wal, F. (1972). The toxicity of sulphite II. Short - and long-term feeding studies in pigs. Fd. Cosmet. Toxicol. 10, 463 - 473.

91. Vogel, A.I. (1962). A Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis. Richard Clay and Co. Ltd. Bungay, pp. 884 - 887.

92. Welch, H. (1957). Antibiotics in food preservation. Science 126 (3284), 1159 - 1161.

93. Wheeler, E.C. and Ferrel, R.E. (1971). A method for phytic acid determination in wheat and wheat fractions. Cereal chemistry 48, 312 - 320.

94. White, A., Handler, P. and Smith, E.L. (1964). Principles of Biochemistry. 3rd Ed. McGraw-Hill Book Co. N. York. P. 118.

95. Whitehead R.G. and Alleyne, C.O.A. (1972) Pathophysiological factors of importance in protein-calorie malnutrition. Brit. Med. Bull. 28 (1), 72 - 78.

96. Whiteside P.J. (1975). Pye Unican Atomic Absorption Data Book. Pye Unican Ltd. England pp. 16 and 36.

97. Wilson, R.F. and Tilley, J.M.A. (1965). Amino acid composition of lucerne and grass protein preparation. J. Sci. Fd. Agric. 16, 173 - 178.

A P P E N D I X

TABLE 17: Mean weekly feed consumption per bird. (weights recorded in grammes).

Week	Arewa feed	Pfizer feed	Spent grain diet	Malt sweeping blended diet	Metabisulphite-treated feed	Ground Arewa feed
1	65.32	72.54	19.12	51.50	63.89	41.16
2	101.21	102.08	70.82	76.89	99.10	83.46
3	117.17	118.48	161.03	124.95	115.70	103.05
4	140.79	133.88	176.10	139.45	148.64	134.32
5	212.35	170.26	205.39	178.12	198.10	181.04
6	303.77	209.07	256.35	222.81	288.22	261.32
7	341.51	250.79	346.57	286.35	341.93	337.47
8	384.74	310.05	401.32	349.51	385.10	370.75

TABLE 18: Energy, Protein and Amino Acid Requirements of chicks (66)

Nutrient	Broilers		Pullets			Laying & Breeding Hens
	0 - 6 WEEKS	6 - 9 WEEKS	0 - 6 WEEKS	6 - 14 WEEKS	14 - 20 WEEKS	
Metabolizable Energy (Kcal/kg)	3,200	3,200	2,900	2,900	2,900	2,850
Protein %	23	20	20	16	12	15
Arginine. %	1.40	1.20	1.20	0.95	0.72	0.80
Glycine and/or Serine. %	1.15	1.00	1.00	0.80	0.60	?a
Histidine %	0.46	0.40	0.40	0.32	0.24	?
Isoleucine %	0.86	0.75	0.75	0.60	0.45	0.50
Leucine %	1.60	1.40	1.40	1.10	0.84	1.20
Lysine %	1.25	1.10	1.10	0.90	0.66	0.50
Methionine %	0.86	0.75	0.75	0.60	0.45	0.53
OR						
Methionine %)	0.46	0.40	0.40	0.32	0.24	0.28
*Cystine %)	0.40	0.35	0.35	0.28	0.21	0.25
Phenylalanine %	1.50	1.30	1.30	1.05	0.78	?
OR						
Phenylalanine %)	0.80	0.70	0.70	0.55	0.42	?
*Tyrosine %)	0.70	0.60	0.60	0.50	0.36	?
Threonine %	0.80	0.70	0.70	0.55	0.42	0.40
Tryptophan %	0.23	0.20	0.20	0.16	0.12	0.11
Valine %	1.00	0.85	0.85	0.70	0.50	?

[a] value not yet established.

* cystine and Tyrosine are not essential for poultry but when present at the level specified can respectively supplement low levels of methionine and phenylalanine.

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TABLE 19: Vitamin and Mineral Requirements of Chicks (as percentage or amount per Kg. of feed) (66).

Nutrient	Starter Chicks 0 - 8 WKS.	Growing Chicks 8 - 18 WKs.	Laying Hens	Breeding Hens.
Vitamin A Activity (IU)	1,500	1,500	4,000	4,000
Vitamin D (ICU)	200	200	500	500
Vitamin E (IU)	10	?a	?	?
Vitamin K (mg)	0.53	?	?	?
Thiamin (mg)	1.80	?	?	?
Riboflavin (mg)	3.60	1.80	2.20	3.80
Pantothenic Acid (mg)	10	10	2.20	10
Niacin (mg)	27	11	10	10
Pyridoxine (mg)	3	?	3	4.5
Biotin (mg)	0.09	?	?	0.15
Folacin (Starch diet) (mg)	0.55	?	0.25	0.35
Vitamin B12 (mg)	0.009	?	?	0.003
Choline (mg)	1,300	?	?	?
Calcium %	1.00	0.80	2.75	2.75
Phosphorus %	0.70	0.40	0.60	0.60
Sodium %	0.15	0.15	0.15	0.15
Potassium %	0.20	0.16	?	?
Manganese (mg)	55	?	?	33
Iodine (mg)	0.35	0.35	0.30	0.30
Magnesium (mg)	500	?	?	?
Iron (mg)	80	?	?	?
Copper (mg)	4	?	?	?
Zinc (mg)	50	?	?	65
Selenium (mg)	0.10	?	?	?

[a]Value not established.

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TABLE 20: Amino acid composition of brewers grains as reported by NRC (66)

Amino acid	Amounts as percentage of dry feed.
Arginine	1.3
Cystine	-[a]
Glycine	-
Histidine	0.5
Isoleucine	1.5
Leucine	2.3
Lysine	0.9
Methionine	0.4
Phenylalanine	1.3
Serine	-
Threonine	0.9
Tryptophan	0.4
Tyrosine	1.2
Valine	1.60

[a] Not detected.

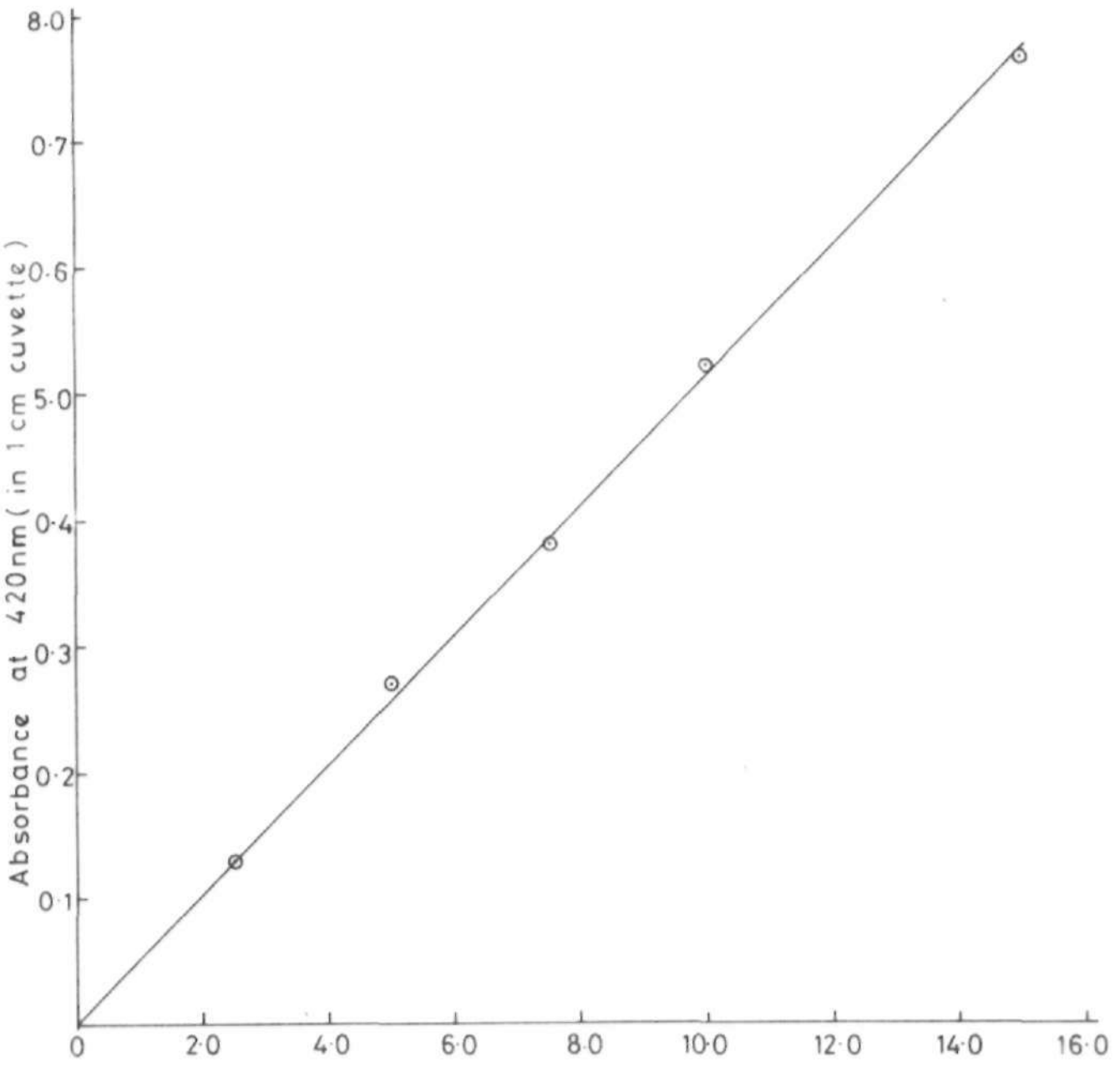


Fig. 3 Calibration curve for the determination of tryptophan.

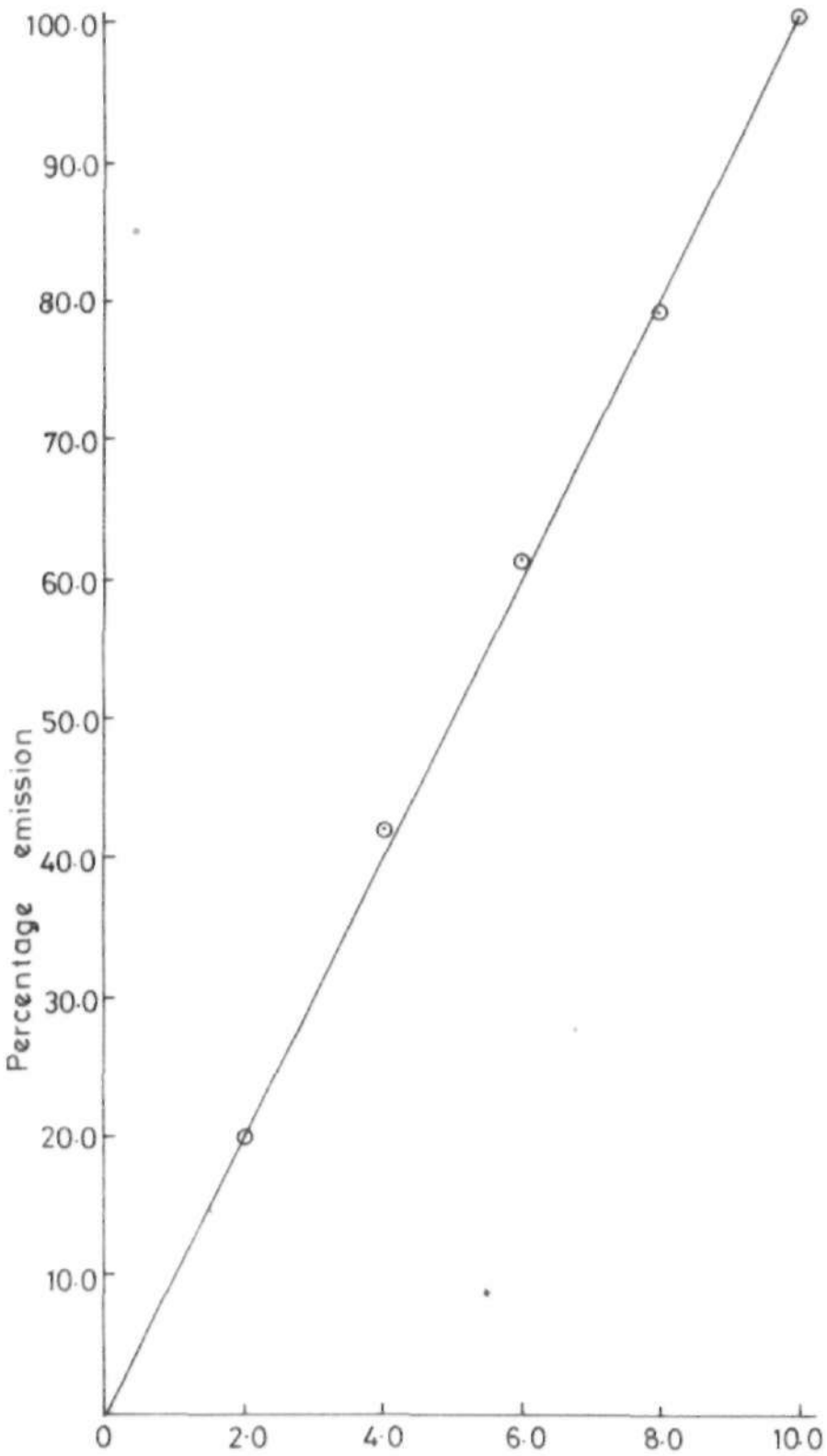


Fig. 4 Calibration curve for the determination of sodium by Flame Photometry.

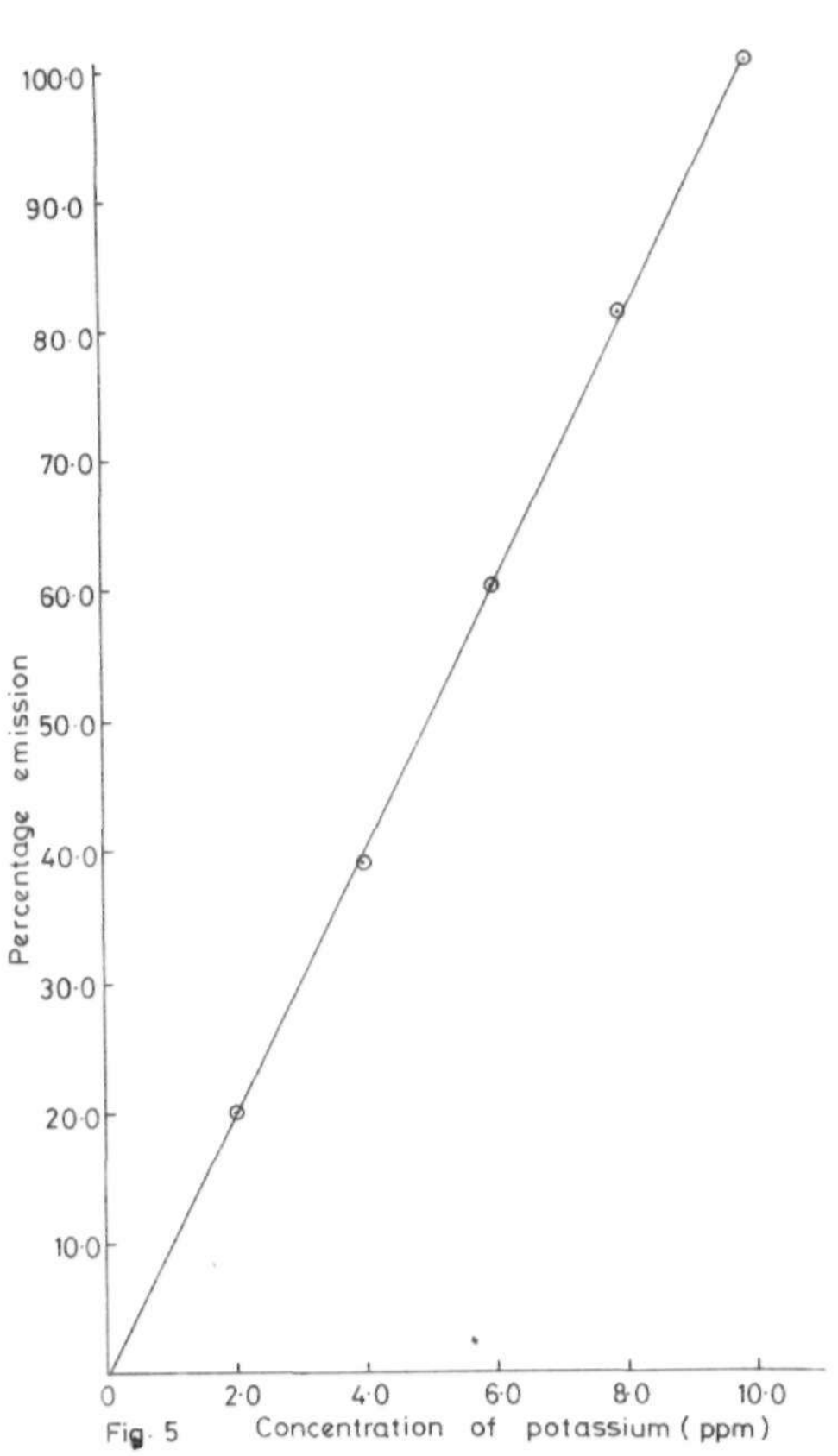


Fig. 5 Calibration curve for the determination of potassium by Flame Photometry.

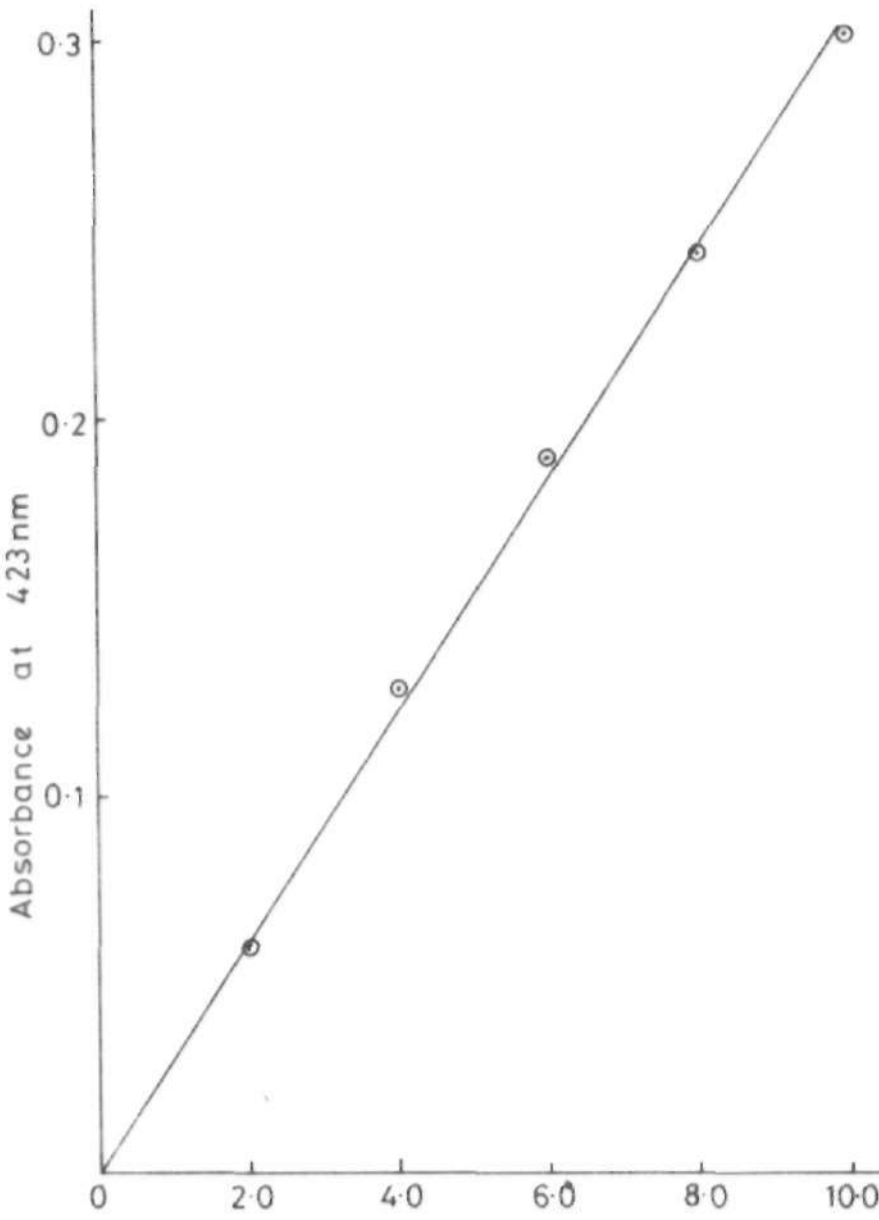


Fig. 6 Calibration curve for the determination of calcium by Atomic Absorption Spectrophotometry

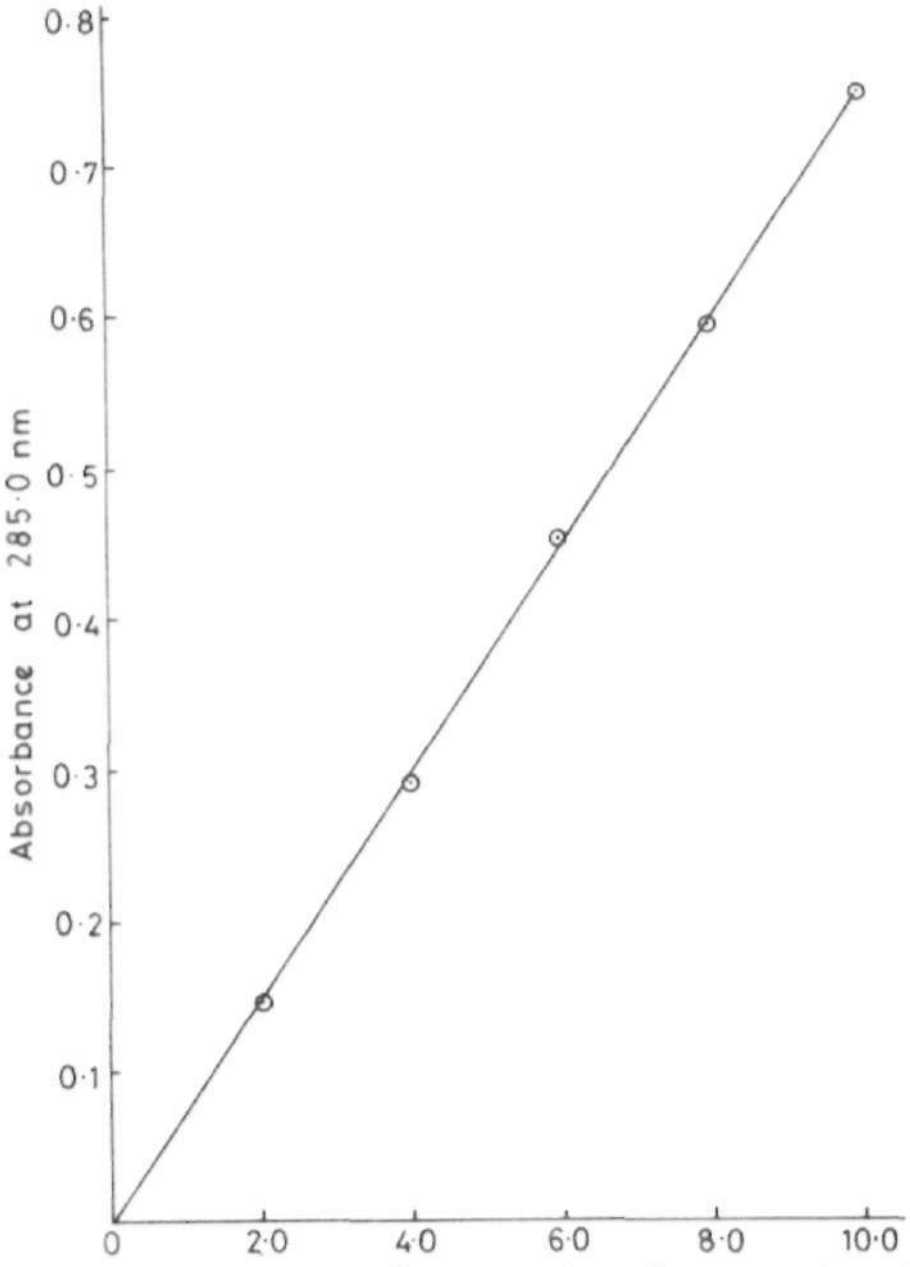


Fig. 7
Calibration curve for the determination of magnesium by Atomic Absorption Spectrophotometry

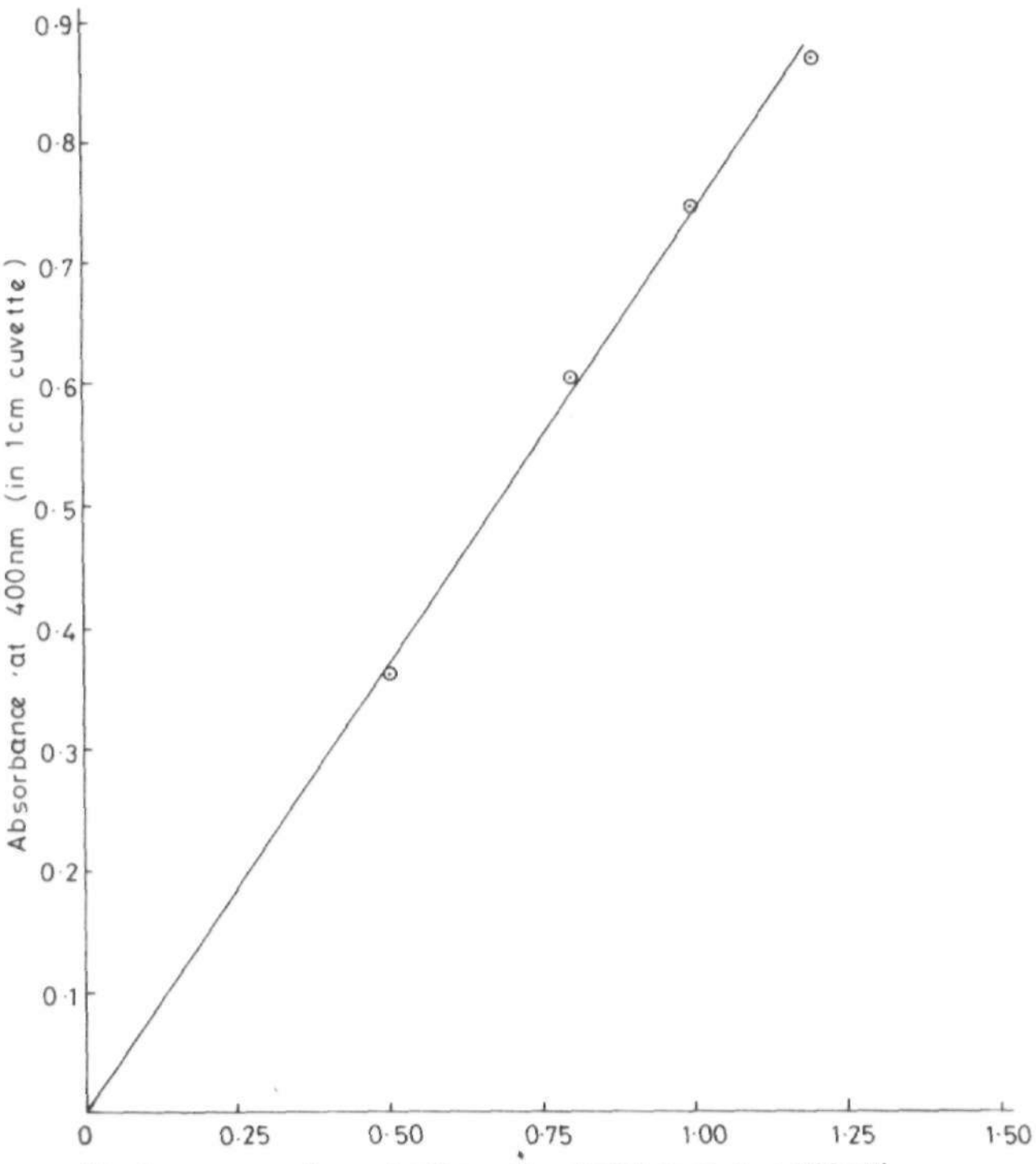


Fig. 8 Calibration curve for the colorimetric determination of phosphorus.

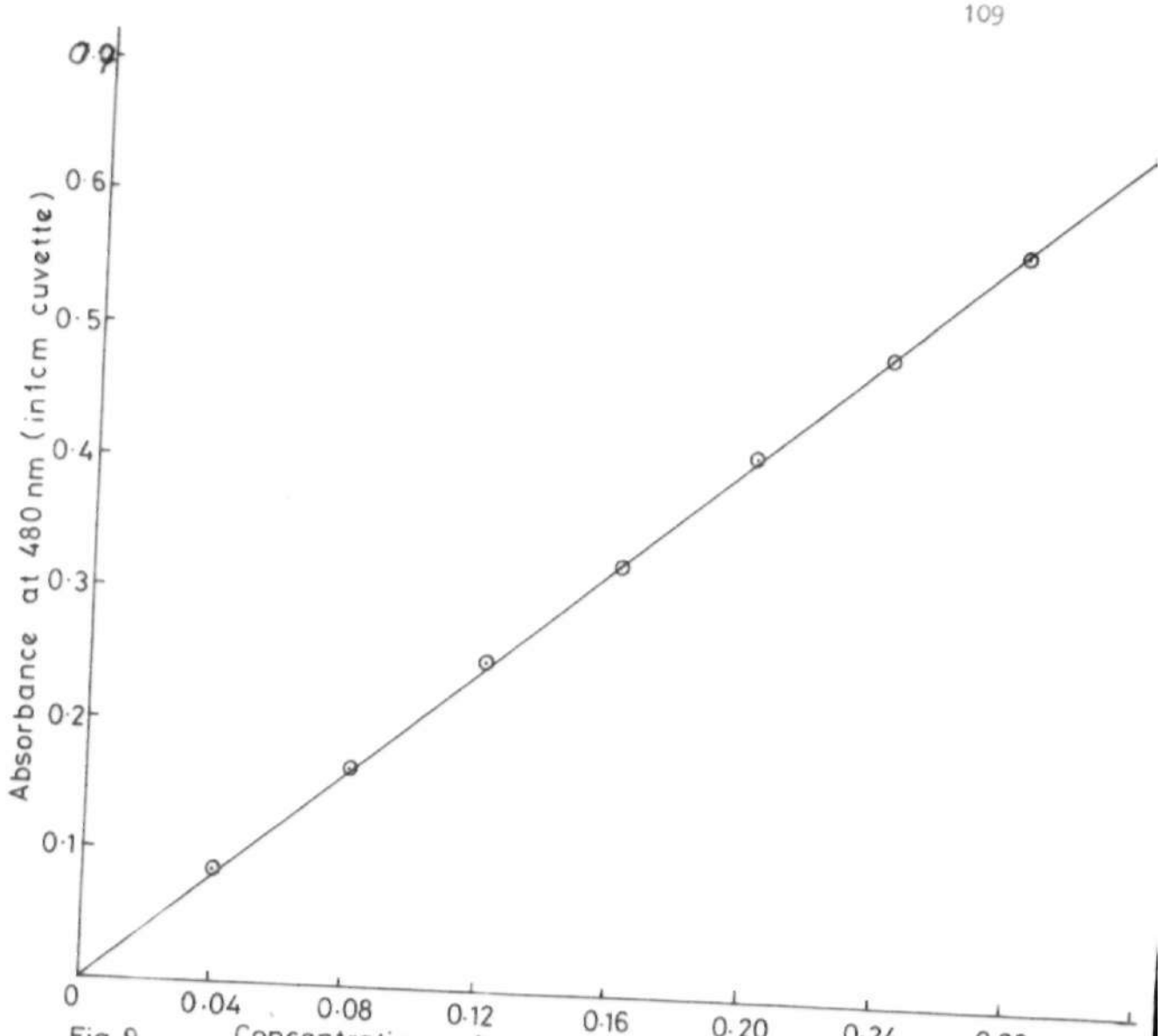


Fig. 9 Calibration curve for the determination of phytic acid.