EFFECT OF BIOTIN SUPPLEMENTATION ON PERFORMANCE OF CHICKEN BROILERS

BY

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A THESIS SUBMITED IN PARTIAL FULFILLMENT OF THE DEGREE OF MASTER OF SCIENCE IN ANIMAL SCIENCE IN THE FACULTY OF AGRICULTURE UNIVERSITY OF NAIROBI

MAY 1991

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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ACKNOWLEDGEMENTS

I wish to express my deep gratitude and appreciation to Dr. P.N. Mbugua, for his tireless assistance, support, encouragement and counsel throughout the project work and writing of this thesis. I am grateful to Dr. B.N. Mitaru for reading, correcting and discussing this work. My thanks also go to Messrs. B.K. Chebe, Njoroge Kahiga, and Desmond Ambale for their help in carrying out the routine project duties. I also wish to thank Messrs F. Chege, R. Ragui and L. Mbugua for their help in the laboratory work.

I gratefully acknowledge the assistance provided by Hoffmann La Roche, Basle Switzerland, through Mr. Shisoka of the Nairobi Office for providing the biotin, and the vitamin/mineral premix, as well as analysing the biotin content of the raw materials and the finished diets. Thanks go to Mr. Gathungu Meta who made it possible for me to get study leave from the Ministry of Livestock Development.

To the University of Nairobi, I am grateful for awarding me a scholarship to study for this degree. I also wish to acknowledge the financial assistance received from the Norwegian Agency for International Development (NORAD), without which it would have been impossible to do the project. Special thanks go to members of the Department of Animal Production particularly Mr. J.W. Wakhungu, Dr. R.G. Wahome and Mr. B.J.O. Inyangala for their valuable assistance during the analysis of the data, and to Mr.

John Gitau who helped in the typing of this thesis.

Last but not least, I am greatly indebted to my husband Julius and daughter Beatrice for their patience and support during the long months spent in this work. To them I will forever be grateful

DEDICATION

TO MY DAUGHTER BEATRICE

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ABSTRACT

Three experiments were carried out to evaluate the effect of biotin supplementation on performance of broiler chicken. Rovimix H_2 was the source of available biotin[1]. Experiment One basal diet was formulated to provide 3152 Kcal of metabolisable energy (ME) and 230 g of crude protein (CP) per kg. The supplementary available biotin in the four experimental diets was 0, 80, 160 and 320 µg/kg of the diet. Experiment Two basal diet contained 2900 Kcal of ME and 196 g of CP per kg. which was supplemented with biotin at the rate of 0, 50, 150 and 300 µg/kg of the diet, to produce four experimental diets. In Experiment Three, a low and a high protein basal diets were formulated. To each of the basal diet, biotin was supplemented at the rate of 0, 100 and 200 µg/kg of diet, to produce six experimental diets. The low protein diets provided 2869 Kcal of ME and 216 g of CP per Kg. The corresponding values for the high protein diets were 3282 Kcal of 260 g. In the three experiments, each diet was given to a group of ten chicks, replicated four times, with equal replication for male and female chicks.

Experiment One and Two basal diets provided 142 and 355 µg/kg of total biotin while the available biotin was 108 and 166 µg/kg of the diet, respectively. In Experiment Three total biotin in the low and high protein diets were 171 and 175 µg/kg, while available biotin was 88 and 132 µg/kg, respectively. Available biotin in basal diets and supplementary biotin was summed up to obtain total available biotin in each diet.

Experiment One results showed that biotin supplementation had no effect ($P<0.01$) on growth rate, feed intake, feed efficiency, mortality or (FKLS), although it tended to improve growth rate up to 268 ug/kg. In Experiment Two, supplementation had a quadratic effect on weight gain. At 21 days of age, supplementation had a significant effect ($P<0.05$) on weight gain with the highest response obtained at a level of 266 µg/kg of the diet. At 42 days of age supplementation failed to attain statistical significance, although the best performance was obtained at an intake of 166 µg/kg of the diet. Supplementation had no effect on feed intake, feed efficiency, mortality or FKLS. In Experiment Three, response to supplementation varied with sex and dietary protein. Male chicks on high protein diet showed a linear response to supplementation. However, female chicks on the high protein diets and both male and female chicks on the low protein diets showed no response to biotin supplementation. Available biotin in fish meal and soybean meal was unusually high at 512 and 315 µg/kg of the diet respectively, while the content in wheat and white sorghum was 0 and 168 µg/kg respectively. Results from this study showed that biotin supplementation improved growth rate in sorghum based diets but this improvement became less clear with age. A level of 266 µg/kg and 166 µg/kg of diet, were found desirable in the early and late stages of growth. In wheat based diets, biotin supplementation did not elicit any significant response probably due to under-estimation of available biotin in wheat. Under field conditions, the conventional raw materials are likely to provide between 59 and 88 µg/kg of the diet, making

supplementation to 166 µg/kg, particularly in the high protein diets, desirable. A better understanding of total and available biotin in Kenya feedstuffs is desirable.

INTRODUCTION

In commercial poultry production, adequate supply of energy, proteins, inorganic elements and vitamins is essential so as to ensure good performance in weight gain, feed efficiency, production, resistance to diseases etc of the birds. Vitamins, and in particular water soluble vitamins are involved in metabolism of carbohydrates, proteins and fats. Compared to carbohydrates and proteins, the daily requirements for vitamins is low, nonetheless in inadequate supply, the process of life itself is gravely disturbed. In the last two decades, biotin has attracted a lot of interest particularly on its nutritional and metabolic roles in poultry, swine and other species. Many of the findings have become of practical importance because of the following:

(i) genetically improved and more productive animals have been bred having a higher requirement of the vitamin;

(ii) biotin rich feed ingredients such as maize have been replaced by others low in bioavailable biotin, such as wheat and wheat byproducts;

(iii) management practices particularly raising birds in cages, and drug administration have reduced the access of biotin of microbial origin;

(iv) high stocking densities induce stress, thereby increasing the requirement of biotin and other vitamins.

(v) biotin responsive conditions of commercial importance such as fatty liver and kidney syndrome have been discovered.

Biotin is required in very small quantities in the body, and it was

for a long time assumed that this requirement would be met by biotin in feedstuffs and by intestinal biosynthesis. Interest in biotin started recently when its involvement in the development of fatty liver and kidney syndrome was discovered. (Payne et al 1974, Whitehead and Blair 1974). It was also learnt that not all biotin present in feedstuffs is available for various classes of animals. This, and the spontaneous outbreaks of biotin deficiency in commercial chicken and turkey farms, has aroused interest in the study of this vitamin in poultry, and to date a clear need has been established for biotin supplementation for various classes of poultry.

In Kenya, maize is the main energy source used in poultry diets. Maize contains biotin in a form completely available to the chick, hence biotin deficiency may not be a major problem as in countries that formulate their diets based on wheat. However there is a growing need and tendency to substitute maize with other energy sources such as wheat by-products, sorghum and cassava. When this happens biotin deficiency may become a problem because of the low bioavailability in these feed ingredients. It is therefore important to determine biotin requirements in poultry under Kenyan conditions.

This study was carried with the following objectives;

(i) To assess the biotin supplementation requirement of chicken broilers

(ii) To determine the optimal level of supplementation that gives the greatest response in growth and feed efficiency.

(iii) To determine optimal level of supplementation when sorghum is used as the main source of energy.

(iv) To evaluate the effect of dietary protein on biotin requirement.

(v) To assess the biotin content of some common feedstuffs.

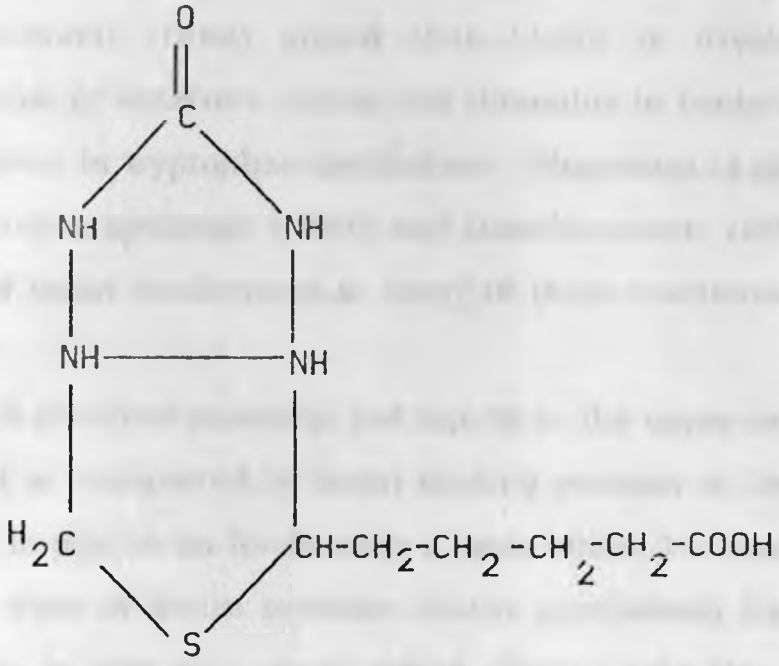
2 LITERATURE REVIEW.

2.1 Biotin in metabolism

In its pure form, biotin is a white crystalline powder, sparingly soluble in water and 95 % ethanol, but insoluble in fat and fat solvents. It is moderately heat stable, but is destroyed by nitrous acid, oxidizing agents, formaldehyde and strong acids and bases. D-biotin is a monocarboxylic acid containing a cyclic urea structure with sulphur in a thioester linkage. It crystalises as long white needles with a melting point of 232-233^{0}c. The biotin active compound is D-biotin, while the stereoisomer, L-biotin, is inactive. Homologues of biotin in which the side chain is elongated or chlorinated such as borbiotin and homobiotin, are potent antagonists of biotin. The structure of biotin is shown in Figure one:

Importance of biotin lies in it being a prosthetic group in enzymes involved in carboxylation and decarboxylation reactions in the body. The major enzymes that contain biotin are acetyl CoA carboxylase, pyruvate carboxylase, and propionyl CoA carboxylase (Bauernfeind 1969). Acetyl CoA carboxylase plays an essential role in the biosynthesis of fatty acids. In biotin deficient rats and chicks, its level in the liver is lower than in normal tissue, but is never completely absent even in severe deficiency (Arinze and Mistry 1971). Pyruvate carboxylase catalyses the carboxylation of pyruvate to oxaloacetate within the the mitochondrion, in the presence of ATP, Mg^{2+} or Mn^{2+}.

Figure one: Chemical structure of biotin



Pyruvate carboxylase which is closely related to the biotin status of the chick and the biotin content of the diet, is one of the rate limiting enzymes of gluconeogenesis via pyruvate and has been used to determine the biotin status of poultry (Bannister and Whitehead 1976, and Whitehead and Bannister 1978). Propionyl CoA carboxylase, on the other hand, is involved in propionic acid metabolism, and is important especially in propionic acid metabolism in ruminants (Mistry et al 1962). The function of the biotin moiety in these enzymes is to fix the carbon dioxide in such a way as to permit the enzyme to transfer the carbon dioxide to the appropriate substrate. The biotin molecule is attached via an amide linkage to the lysine unit of the enzyme with the aid of holocarboxylase synthatase enzymes.

These three enzymes represent the major metabolic enzymes shown to require biotin. However, effects of biotin deficiency

are noted in many reactions in the intact organism. Mistry and Dakshinamurti (1964) stated that biotin is involved in deamination of aspartate, serine and threonine in bacteria. It is also involved in tryptophan metabolism, (Shanmuga et al 1954), and in protein synthesis (Mistry and Dakshinamurti 1964). The nature of biotin involvement in many of these reactions is still obscure.

Biotin is absorbed passively, but rapidly in the upper intestinal tract and is transported by biotin binding proteins in the blood plasma. In pigs biotin levels reach a peak within 3-4 hours after a single dose of biotin injection Biotin synthesized by faecal microflora is only to a small extent absorbed by the animal (Coates et al 1968). These authors observed that pigs on biotin deficient diet showed signs of deficiency inspite of intensive biosynthesis of biotin in the intestinal tract.

2.2 Biotin binding proteins

Several proteins with specific biotin binding properties have been identified mainly in avian species. Avidin is the best known of these proteins. It is a glycoprotein with a high heat stability (upto 95^{o}C) and has the highest binding affinity for biotin (Green 1975). It is produced and secreted to the albumen in the avian oviduct in response to progestrone (O'Malley, 1969). At normal dietary biotin intakes, very little avidin in egg white has biotin bound to it (Whitehead and Bannister 1980). However albumen biotin content is related to dietary biotin and at high intake, a significant proportion of avidin can have bound biotin. The

specific role of avidin in the albumen as a specific biotin binding compound is not known. It has been suggested that avidin may protect the egg from infection by rendering biotin unavailable to invading bacteria that may require it for growth (Green 1975). Avidin is denatured by moist heat, thereby preventing it from binding biotin and hence biotin deficiency does not occur when heated egg white is fed.

Streptavidin and stravidin are other biotin complexing agents produced by streptomyces microorganisms, which bind biotin and other related compounds in a similar manner to avidin (Chaiet and Wolf 1964). They are of practical importance if litter or feed become contaminated with such fungi (Scott 1981).

2.3 Biotin interaction with other nutrients and its toxicity

Interaction of biotin with other nutrients such as proteins, fat, and other vitamins has been studied by various researchers. In their study Whitehead et al (1976) found that in diets low in protein and fat, growth depression due to suboptimal levels of biotin was mild, but as the level of dietary protein and fat increased, biotin deficiency caused a more severe growth depression. Also the number and severity of dermal lesions was low in chicks fed low protein and fat diets, containing insufficient biotin, but dermal lesions increased in number and severity as the protein and fat level in the diet increased. However chicks on high protein and high fat diets, were free

from fatty liver and kidney syndrome even when such diets contained suboptimal levels of biotin.

Similar findings were reported by Whitehead and Bannister (1981), who observed that increasing dietary protein increased the severity of skin lesions in biotin deficient diets, although growth depression in this study was not as high as that observed previously (Whitehead et al 1976). The effect of level of protein and fat in the development of fatty liver and kidney syndrome in biotin deficient diets was also studied by Whitehead et al. (1985) who observed that biotin requirements of broiler chicken is affected by dietary protein implying that biotin requirements should be based on protein content of the diet.

Dietary fat also affects biotin requirement. Pevcek and Shull (1942) observed rapid invitro inactivation of biotin by fat and fat esters. In the presence of an antioxidant e.g. a-tocopherol, the rate of inactivation slowed down significantly. Biotin is oxidized by rancid fat to biotin sulphoxide and sulphone.

Besides protein and fat, biotin also interacts with other vitamins. It has been known to act as an antioxidant, like vitamins C and E. (Ziyad et al 1985). In studies by these researchers, biotin was observed to increase the antioxidant capacity of red blood cells by raising the level of glutathione peroxidase.They also noted a synergetic effect between biotin and vitamin E., probably due to its effect on glutathione peroxidase activity. Martin et al (1985) stated that no toxic effects of biotin are known.

2.4 Effect of biotin supplementation on animal performance.

2.4.1 Fish

Biotin is an essential nutrient for fish and signs of deficiency can be induced by feeding raw white egg in the feed. (Whitehead 1988). Casledine et al (1978) reported anaemia and erythrocyte fragmentation, while Poston and McCartney (1974) showed reduction of enzyme activities of biotin dependent enzymes in the liver, decreased lipid levels and changes in fatty acid composition, and accumulation of glycogen.

2.4.2 Minks, foxes,cats and dogs

Like in fish, experimental deficiency can be induced in these animals by feeding raw white egg. Studies by Helgebostad et al (1959) showed that in minks and foxes, biotin deficiency causes general dermatitis, characterised by hyperkeratosis, and a yellow exudate may appear on the skin. Reproductive failure and severe degenerative fatty infiltration of the liver was also reported. Biotin deficiency in cats has been studied by Carey and Morris (1977), who stated that there is accumulation of dried secretions around the mouth, nose and eyes, followed by hair loss. Food consumption and growth rate are markedly reduced. Diets low in biotin but not containing raw white egg were not found to give signs of biotin deficiency, implying biotin

biosynthesis in the gut.

Studies of Schulze and Frigg (1986) indicated that treatment with biotin is effective in preventing hair loss in dogs. Other improvements noted included hair colour, which became more shiny and less brittle, and curing of scally dermatitis and eczema. Whitehead (1988) stated that there is progressive ascending paralysis which starts in the hind leg and spreads to the forelegs and neck resulting in death, due to respiratory failure. This condition was attributed to potassium and biotin deficiencies. (Whitehead 1988)

2.4.3 Ruminants

Biotin deficiency can be induced in young ruminants by feeding diets containing avidin. In his study, Hurstel, (1982) showed that calves fed on a milk replacer diet supplemented with 4% avidin had a depressed growth rate, and a hair coat that was dull and stiff. The hooves were soft with a tendency to crack and the animals scoured. In adult animals, extensive synthesis of biotin and other water soluble vitamins occurs in the rumen, and as a result there is uncertainty as to whether and to what extent these animals experience biotin deficiency. There are times such as early lactation, when the demand for biotin dependent pathways, of carbohydrate metabolism and fat synthesis might be expected to be high. Bovine fatty liver syndrome resembles abnormalities seen in the fatty liver and kidney syndrome in the chicken. However, biochemical studies reported by Roberts and Baggot (1982), have failed to show the

dependency between biotin dependent pathways and this syndrome. Judged by pyruvate carboxylase activity, plasma biotin concentration in cows is variable with a range of 30-80 µg/100 ml which can be increased by feeding additional biotin. However, in biotin supplementation response in milk output or quality or in fertility have not been observed (Roberts and Baggot (1982). As suggested by Austic (1986), biochemical changes may be a better indicator of biotin status than milk yield. Biotin plays a role in prevention of foot lesions and lameness seen in adult ruminants (Cooke and Brumby 1982). Despite this, no evidence is available on the need to supplement ruminants with biotin. Kempton et al (1978) reported evidence of insufficient biotin for gluconeogenesis in in fasting pregnant ewes close to lambing. In this study increased rate of glucose synthesis following biotin administration was reported.

2.4.4 Swine

2.4.4.1 Reproduction

For a long time, biotin supplementation was not thought necessary in sow rations. Indeed, ARC (1967) stated that the sow had no dietary requirement for biotin, as adequate biotin was derived from normal dietary sources, and intestinal biosynthesis. Cunha (1972), reported that exact desirable level of biotin for growth in swine diets was not known, but was probably between 55 and 220 µg/kg. In 1977, it was demonstrated practically, for the first time, by Brooks and associates that

biotin supplementation in practical diets decreased incidence and severity of claw lesions in gilts and sows, and also improved reproductive performance. Sows given supplemented diets produced significantly more live piglets than the control in the second parity, and weaning to remating interval was significantly reduced. In recent years, these findings have been confirmed in several large, well controlled, and multiparity studies. Pedersen and Udesen (1980), reported more live piglets born, lower piglet mortality during suckling, and shorter intervals between furrowing to first oestrus and conception, respectively. Similar findings were reported by Misir and Blair (1983), and Bryant et al. (1985), who all found significant differences between supplemented pigs and the control, in litter size, and weaning to oestrus interval. However, Grandhi and Strain (1980) failed to get any improvement in reproductive parameters with supplementation. The studies of Bryant and Kornegay (1983), Tribble et al., (1983) and Hamilton and Veum (1984) on various reproductive parameters, showed variable response to biotin supplementation. Bryant and Kornegay (1983), showed that weaning to remating interval and conception rates were improved significantly, while number of piglets born was not affected by biotin supplementation. Tribble et al. (1983), on the other hand, reported significant differences in litter size in the parities studied, and the piglets weaned, but no significant response on weaning to remating interval was observed, while Hamilton and Veum (1984) reported significant differences in the number of piglets weaned per litter, but found no significant response in weaning to remating interval. Failure

to observe response in the work of Hamilton and Veum (1984) and Tribble et al., (1983), can be explained by the fact that the pigs had a short weaning to rebreeding interval which left no room for improvement. It is also likely that the basal diet used had enough biotin to meet the sows requirement. This could also explain the results obtained by Grandhi and Strain (1980). The breeds or strains of pigs used could also have a lower requirement for biotin.

The biochemical and physiological mechanisms through which biotin supplementation improves reproduction in female pigs is not yet understood. However, Brooks and Simmins (1980) postulated that biotin plays an essential role in gluconeogenesis and fatty acid synthesis, and hence is ultimately involved in energy yielding processes. The piglet and the sow are presumably short of readily available energy for survival and and production of milk. The energy gap could be bridged by a fully efficient biotin enzyme system. These researchers estimated the biotin requirement for reproduction to be 150 µg/kg. but the requirement could be higher under field conditions (Whitehead 1988).

2.4.4 2 **Growth and health of skin and claws.**

Growth and feed conversion are impaired when diets have insufficient biotin or contain avidin (Tagwerker 1986). An improvement in feed conversion has been observed when biotin supplementation is given to suboptimal diets (Bryant and Kornegay 1983). Besides its effect on reproduction, biotin

deficiency has profound effect on the health of the skin and claws. De Jong (1983), stated that in biotin deficient pigs, the skin develops lesions resulting in redness and dryness, while quality of the claw horn becomes affected.These symptoms can be corrected by biotin supplementation. In this study, De Jong (1983) found that supplementation of 150-200 µg/kg of biotin was adequate to restore feet, leg and skin health. Similarly Penny et al. (1981) reported that when gilts were introduced as replacements into a herd, and supplemented with biotin at 1160 µg/kg day, in pregnancy, and 2320 µg/kg day in lactation, they had significantly fewer white line lesions, heel bruises and erosions than unsupplemented controls. Severity score and number of lesions per pig were reduced by 17-18%. Brooks et al. (1977) reported improvement in hoof condition with supplements of 150-250 µg/kg, while Grandhi and Strain (1980) found that supplementation of diets based on wheat and soy-beans with 200 µg/kg, of biotin reduced severity of lesions, in Lancombe and Yorkshire sows. Despite these results, experience in the United Kingdom shows that much higher levels of 2000-3000 µg/kg are often required to resolve established lesions (Whitehead 1988).

2.5 Biotin supplementation in turkeys

The nutritional significance of biotin in the nutrition of poults was first investigated by Patrick et al., (1942), who observed that a deficiency resulted in perosis and dermatitis

that first appeared on the foot pads and extended over the feet, corners of the mouth, and sometimes on the eyelids.

Interest in the biotin needs of turkeys increased greatly because of the appearance of biotin deficiency symptoms in turkey poults on commercial farms (Richardson and Wilgus 1967). Jensen and Martison (1969) reported that biotin deficiency reduced growth rate and was associated with a high incidence of leg weakness in turkeys during the developing period. Their study showed that a level of 231-284ug/kg of diet was adequate for growth. Similar level of biotin for adequate growth was reported by Dobson (1970) who nonetheless found that deficiency symptoms were more severe in males than the females because of the higher biotin requirement by the former.

The exact biotin requirements for poults and breeders have not been fully established. However, about 280 µg of biotin per kg of diet are needed (Jensen and Martinson 1969).

In adult turkey breeder, it is possible to have some of the symptoms mentioned for growing poults (Richardson and Wilgus 1967), but more important are signs of decreased hatchability and high embryonic mortality in the first week of incubation (Fergyson et al 1961)

2.6 Effect of biotin supplementation in chickens

2.6.1 Layers and breeders.

2.6.1.1 Egg production

Evidence on whether lack of biotin can decrease egg production in chicken is not yet conclusive. Cravens et al. (1942), Brewers and Edwards (1972), and Whitehead and Bannister (1980) did not find any positive effect of biotin supplementation on egg production. However Bradley et al. (1976) got a response in Leghorn type breeders, but this observation was not supported by Whitehead and Bannister (1980) who did a much larger experiment using chicks of the same strain. Leeson, et al. (1979) found that egg production in Rhode Island Red hens declined when a biotin deficient diet was fed but this was not the case in leghorns.

Whereas several researchers have shown a requirement for biotin for layers and breeders, the optimal requirement has not been clearly stated. Cravens et al. (1942) and Brewer and Edwards (1972) used diets that were virtually devoid of biotin and egg laying continued unaffected. Bradley et al (1976) observed an increase in egg production of leghorn type hens when biotin was supplemented at the rate of 550 µg/kg, in a soy- milo diet, but Whitehead and Bannister (1980) did not get a response when a practical diet based on wheat, herring and soybean meal, with an estimated available biotin content of 30

µg/kg. was supplemented with 150 µg/kg. Other studies by Leeson et al. (1979) and Harms et al (1979) did not show any significant improvement in egg production when practical diets containing 120 µg available biotin/kg were supplemented. Under normal circumstances practical diets appear to contain sufficient biotin to sustain optimum egg production (Whitehead 1988).

2.6.1.2 Effect of biotin supplementation on hatchability

Several studies have been done to assess the importance of biotin supplementation on layers and breeders. However, minimum requirements for maximum performance have not been established. Cravens et al., (1942) showed a marked decrease in hatchability of eggs produced by hens fed semi-purified diets of low biotin content. However, addition of 150 µg/kg of biotin reverted hatchability to normal. Similarly, Coach and Cravens (1948) using semi purified diets observed a significant increase in hatchability in hens fed 200 µg/kg compared with those fed on a biotin deficient diet. Brewer and Edwards (1972), on the other hand, showed that hatchability may decrease to zero, in biotin deficiency. However, research on the effect of biotin on hatchability has not been conclusive. For example Whitehead et al., (1985), did not observe a significant response to biotin, in hatchability and stated in their findings that total hatchability may be almost normal in diets containing suboptimal levels of biotin. However, they observed that even

though the hatchability was normal, the chicks on the deficient diets showed a high incidence of abnormalities such that hatchability in terms of commercially acceptable chicks was still depressed.

From the work of Leeson et al. (1979), it is evident that requirement for vitamins vary with strains, being higher in heavy strains, than in light strains. In broiler breeders fed ad libitum. Brewer and Edwards (1972) estimated biotin requirements for maximum hatchability to be 100 µg/kg. In contrast Bradley et al. (1976) found a significant increase in hatchability in leghorns when a diet containing about 100 µg/kg was supplemented with biotin. However, there was no significant increase in hatchability when Harms et al (1979) supplemented a diet estimated to contain 120 µg/kg. In a more recent study on broiler breeders, Whitehead et al. (1985) observed that the requirement for maximum output of commercially acceptable hatched chicks was 100 µg/kg equivalent to a daily intake of 16 µg per hen per day. However with view of need to provide chicks with a satisfactory maternal carry-over of biotin a dietary allowance of 200 µg/kg might be considered adequate (Whitehead 1988).

2.6.1.3 Chick viability

An adequate carry-over of biotin to the hatching chick is important for its subsequent growth and viability (Brewer and Edwards 1972 and Whitehead and Bannister 1980). These authors stated that chicks deficient in biotin reserves show decreased growth potential and increased mortality even when

fed diets adequate in biotin. Chicks hatching with marginal biotin reserves may look outwardly normal but may have developed minor bone defects such as chondrodystrophy which become enhanced as the chick grows (Stock and Latshaw1981). Whitehead et al (1976) also found that mortality from fatty liver and kidneys syndrome could be decreased by increasing maternal biotin supply.

2.6.2 Biotin in broiler production

In the early work done on this subject Wagstaff and Anderson (1961) estimated biotin requirement of growing chicks to be 90 µg/kg of diet. However, Anderson and Warnick (1970), in a study to find the practical diets that may require supplementation, estimated the requirement for broilers to be 150 µg/kg. This is the same amount recommended by ARC (1967) for starting chicks. Anderson and Warnick (1970) used purified diets for their experiments and this may explain why the recommendations were different from those of Whitehead and Bannister (1978) who gave the requirement of biotin as 170 µg/kg, a value which has since been increased to 180 µg/kg (Whitehead 1986).

It is apparent from the variability of results that biotin requirement of commercial broilers and factors affecting them require more study. Whitehead and Bannister (1980) looked at the effect of management on biotin requirements and observed that chicks housed on litter can supplement their biotin intake

by ingesting biotin of microbial origin from the litter. The chicks showed a higher response at the same level of supplementation than chicks housed in cages. The greatest effect was noted after 3 weeks when the proportion of faeces started increasing, and by seven weeks of age, enhancement of the biotin status was equivalent to 10 µg/kg of diet. Hence chicks housed in cages may require a higher level of supplementation than those on floor pens.

2.6.2.2 Leg lesions and fatty liver and Kidney syndrome

Besides causing depressed growth, biotin deficiency causes a high incidence of deformed legs (Al-Athari et al., 1986), and has been identified as one of the major micronutrients involved in the prevention of leg lesions in poultry. Leg abnormalities such as chondrodystrophy can be kept at low levels even in flocks fed on suboptimal levels of biotin, provided that the supply of hens is adequate. This ensures an adequate biotin content in the eggs and hence the chicks have an adequate biotin reserve (Stock and Latshaw 1981). These authors supplemented a semi-purified diet which caused a high incidence of leg weakness and impaired growth in broiler chicks with graded levels of biotin and observed that upto the highest level used, (200 µg/kg), biotin reduced the incidence of leg weakness by 25%.

Another abnormality seen in biotin deficiency is fatty liver and Kidney syndrome (FKLS) which is the most important biotin responsive condition in fast growing chicks. It is a metabolic

disorder that can be influenced by several nutritional factors. The incidence of (FLKS) can be reduced by addition of protein to the diet (Whitehead and Blair 1974), or fat (Blair and Whitehead 1975). In a practical broiler diet, fatty liver and kidney syndrome can be minimized by supplementing the diet with biotin (Payne et al.,1974, Whitehead and Blair 1974, and Whitehead et al.,1975). The characteristic changes associated with fatty liver and kidney syndrome are fatty infiltration of liver, kidney, heart, and other organs (Wight and Siller 1975), reduced plasma glucose, increased plasma free fatty acids, and triglyceride levels (Bannister, et al.,1975). Although the syndrome is associated with diets of low available biotin content, chicks dying from it do not show any signs of biotin deficiency (Whitehead, et al 1975).

Hypoglycaemia an important feature of fatty liver and kidney syndrome is thought to arise due to failure of gluconeogenesis. Biotin is cofactor for pyruvate carboxylase, a key enzyme in gluconeogenesis. Addition of biotin has been known to restore gluconeogenesis in deficient chicks (Bannister,1976). A possible explanation why chicks suddenly develop fatty liver and kidney syndrome has been advanced by Whitehead, (1980), and it revolves around the source of glucose in the chick. According to this author the principal sources of glucose are absorbed feed, gluconeogenesis via pyruvate and the hexose monophosphate shunt, via glycerol and glycogen breakdown (glycogenolysis). In biotin deficiency gluconeogenesis via pyruvate is minimal but the chick continues to derive its requirement for glucose through glycogenolysis and gluconeogenesis via glycerol.and continues to

thrive. However, a chicks glycogen reserves are small and in a chick subjected to a short period of starvation or stress, these reserves become rapidly depleted (Bannister and Cleland (1977). Furthermore, glycogenolysis releases lactate which decreases the NAD+/NADH ratio and thus inhibits gluconeogenesis via glycerol (Bannister and Cleland (1977). Hence the chick has now lost three important sources of glucose, and in the short term, digestion of food does not seem to provide an adequate energy supply. The chick develops hypoglycaemia, which becomes progressively severe over a period of a few hours, until death occurs. Elevation of lactate levels may cause acidosis which contributes to death (Balnave et al., 1977). Fatty liver and kidney syndrome is thus a condition where dietary factors, mainly lack of biotin, leave the bird in a metabolically susceptible condition, but where the actual initiation of the syndrome is triggered by environmental factors such as stress or starvation.

Biotin in broiler diets is thus required for optimal growth, for prevention of leg abnormalities, and fatty liver and kidney syndrome. Different researchers have stated different levels of biotin requirement. The requirements have been changing over time. Wagstaff and Anderson (1961) gave the requirement as 90 µg/kg,which was considerably lower than that of 150 µg/kg, given by Anderson and Warnick (1970). This figure is similar to that of NRC (1984) but is lower than 170 µg/kg recommended by Whitehead and Bannister (1978) and which was later increased to 180 µg/kg (Whitehead (1986). There are many reasons that may account for these different observations. Firstly

the basal diets used have shown great variation in biotin content. Total biotin content in grains as an example varies depending on plant varieties, harvesting and processing methods and storage conditions (Tagweker (1983). Secondly, the method of assessing requirement may cause variation. The three main methods used in assaying for biotin namely microbial assays, chick bioassay and use of pyruvate carboxylase seem to give a range depending on which one is used. Thirdly, the year the study was carried out seems to affect the requirements. Over the years, it seems like biotin requirement for the broilers has been increasing.This is to be expected since breeding has produced more productive animals having high requirements per unit weight of feed. (Tagwerker 1986)

2.6.3 Assessment of biotin status of poultry

Tissue or plasma biotin concentrations of chicken are related to dietary intake and can be used as a criteria of the biotin status of the chicks or diets (Whitehead, 1988). In young chicks, liver biotin content has been used to give information on their biotin status in relation to the occurrence of fatty liver and kidney syndrome (Hood et al., 1976). Plasma concentrations have also been used Frigg et al. (1973), as an example, associates concentrations of 2-3 ng/ml with biotin deficiency in chicks 4-5 weeks of age. Concentrations of 1 ng/ml or less were found in chicks showing severe foot lesions and growth depression. Whitehead (1988), stated that there is a linear relationship between dietary biotin and plasma concentrations upto a dietary

level of 300 µg/kg. Plasma concentrations reach a plateau when the diet contains about 500 µg/kg, but rises again slowly in response to very high intakes

Biotin deposition in the egg has also been used to assess the biotin status in layers and broiler breeders (Buenrostro and Kratzer 1984). These researchers stated that the overall shape of the response curve resembles that of plasma biotin but is linear over a meaningful range of dietary intake. With a dietary biotin content of 150 µg/kg, yolk concentrations range from 500 µg/g of yolk wet weight in leghorn type hens (Buenrostro and Kratzer 1984.,) to 700 µg/g in IsA Brown (White and Whitehead 1987). The data given in all these studies refer to hens at peak of egg production

Biotin dependent enzymes have also been used to assess biotin status in poultry. Pyruvate carboxylase, a mitochondrion enzyme is commonly used .It is a more sensitive indicator of the biotin status in chicks than growth response (Bannister and Whitehead 1976). Acetyl CoA carboxylase has also been used by Glatzle and Frigg (1975). In the tissues, activity of pyruvate carboxylase in liver and kidney is the most sensitive to dietary biotin (Shen and Mistry 1977). In the liver it is sensitive over a wide range of dietary levels, but unfortunately pyruvate carboxylase activity changes with age (Atwal et al. 972) and is affected by other aspects of diet such as protein or fat level (Whitehead et al 1978).

2.5 Biotin content and availability in feedstuffs.

Since 1975, Hoffmann La Roche, Basle, has analysed microbiologically more than 1600 samples for total biotin content. Biotin content in cereals and other feed ingredients show great variation. Maize, for instance, has an average content of 79 µg/kg with a range of 79 to 115 µg/kg. Tagwerker (1983) stated that in cold countries grains are of low biotin content, while in hot climates, grains tend to have a higher biotin content. Oil seed meals are good sources of biotin. Soybean meal, for instance, has an average content of 270 µg/kg with comparatively small variation. Animal proteins on the other hand, are unreliable sources of biotin. Highly variable contents are found in fish meal which has an average of 135 µg/kg, ranging between 11 to 421 µg/kg.

studies by various researchers indicate that poultry and pigs cannot fully utilize biotin as present in many feed ingredients. Frigg (1987) stated that biotin from wheat is completely unavailable to the chick, while that from other grains such as. sorghum, millet, and barley is only partially available. In oil seed meals biotin is fully available in soybean, but only partially available in ground nut, rape seed, and sunflower seed meals. Biotin from animal proteins such as fish meal, meat meal, and meat and bone meal, is fully available to the chick.

It is apparent that calculation of total biotin content in feeds will give variability. Measurement of available biotin may be more reliable than the total biotin content.

3 STUDIES ON BIOTIN NUTRITION IN BROILER CHICKEN

3.1 EXPERIMENT ONE:

Broiler performance on a wheat based diet supplemented with biotin

3.1.1 Introduction

Biotin is an essential nutrient, required for growth and prevention of fatty liver and kidney syndrome, and other biotin related deficiency conditions. Biotin requirements in poultry under Kenyan conditions has not been elucidated. As stated in the Literature Review bioavailability of biotin in wheat is low. There has been an increased use of wheat particularly its by products in poultry feeds in Kenya. It is therefore necessary to determine biotin requirements when such feeds are used.

3.1.2 Objectives.

The objectives of this experiment were;

(i) To assess biotin requirements for broilers

(ii) To determine the optimal level of supplementation that gives the greatest response in growth.

3.1.3 Materials and methods.

3.1.3.1 Management of the chicks and experimental diets.

One hundred and sixty day old 'starbro' broiler chicks were obtained from a commercial hatchery, and sexed on arrival.Males and females were raised separately. The chicks were fed on a commercial diet for the first two days and on the third day, they

were weighed in groups of ten picked at random and allocated to experimental floor pens, in a completely randomised design. Each pen measured 1.2Mx1.2M providing a floor space of $0.144M^2$ per chick. The floor was covered with wood shavings to a depth of ten centimetres. Infra red bulbs suspended above the pens were used for brooding. A temperature of 32^oc was maintained in the first week, and was reduced by 2^oc every week by raising the height of the bulbs, until the end of four weeks, when the pen temperature was maintained at 26^oc. Feed and water were provided ad libitum.

A wheat based basal diet was formulated to contain 230 g. crude protein and 3152 kcal of metabolizable energy per kg. Graded levels of biotin, in the form of Rovimix H-2[1], were added to the basal diet (Table One) to produce four experimental diets. The four diets, i.e diets I, II, III, and IV, were obtained by adding and thoroughly mixing 0, 400, 800, and 1600 mg of Rovimix H-2, per 100 kg of the basal diet, respectively. Supplemented available biotin was therefore 0, 80, 160 and 320 µg/kg. To ensure that the basal diet did not receive any biotin from the vitamin/mineral premix, Zoodry vm 115[2] free of biotin was specially prepared and sent for this study by Hoffmann La Roche. Each diet was given to four groups of ten chicks each, with two male and two female groups per diet. The chicks were on the experimental diet for thirty five days.

1: Rovimix H-2 was provided by Hoffmann La Roche, Basle, Switzerland. It contains 2 percent biotin, which is all availabe to the chick. Each gram contains 20000000 particles, each of which contains biotin to ensure homogenous distribution in feeds.

2. See footnote Table One.

Table 1: Composition of the basal diet used in Experiment One.

Ingredient	%
Wheat	70.20
Soya bean meal	9.60
Fishmeal (Danish)	15.00
Corn oil	2.85
Dicalcium phosphate	0.40
Limestone	1.20
Iodised salt	0.50
Vit/mineral premix[1]	0.25
Total	100.00

[1]Vitamin mineral premix was zoodry vm 115. Composition of premix per kilogram: vitamin A, 5,500,000 IU; vitamin D_3, 1,000,000 IU ; vitamin E., 16,000 mg.; vitamin K, 2,500 mg; vitamin B_1, 1200mg; vitamin B_2, 3000mg; vitamin B6, 2000mg; Niacin, 18,000 mg; D pantothenic acid, 6000mg; vitamin B_{12}, 14 mg; folic acid, 400 mg; choline chloride, 180,000 mg; avoporcine, 4,000 mg; manganese, 60,000 mg; iron, 16,000mg; zinc,18,000mg; copper, 2,000 mg; iodine, 560 mg; cobalt, 80 mg; selenium, 60mg; dl methionine, 40,000 mg; l lysine, 40,000 mg; betaine, 40,000 mg; ethoxyquin, 40,000 mg.

Proximate composition of the raw materials and the basal diet was done according to the standard procedures of Association of Analytical chemists (AOAC 1984).

Mineral composition of the diet was done using an Atomic absorption spectrophotometer (Perkin Elmer Model 2380) Biotin content in the ingredients and the basal diet was determined microbiologically using Lactobacilus plantarum (ATCC 8014). This analysis was done by Hoffmann La Roche, Basle Switzerland. Available biotin was calculated using the analysed biotin content in the raw materials and the availability of the biotin (Hoffmann La Roche 1987).

3.1.3.2 Data collection

Body weight gain and feed intake were determined weekly. Body weight gain was calculated as the difference in weight between two consecutive weighings. At the beginning of the experiment the estimated feed required for each replicate was weighed into a bucket of known weight. At the end of each week, the feed in the troughs was emptied back into the respective bucket and weighed. Feed intake was calculated as the deference in feed offered and feed left over at the end of every week. Feed efficiency was determined as the ratio between feed intake and body weight gain. During the weighing the chicks were checked for lesions and other abnormalities. The chicks that died were taken for post mortem.

3.1.3.3 Statistical analyses.

Data on body weight, gain, feed intake and feed conversion at thirty five days of age were subjected to statistical analysis. The sum of squares due to biotin were partitioned into the linear, quadratic and cubic effects, using a least squares programme (Harvey 1987)

Model: $Y_{ijk} = \mu + Ai + Bi + Cj + (A B)_{ij} + eij$

Yijk=observation e.g weight gain, feed intake, feed efficiency etc per chick of the ith sex and the jth biotin level

μ= underlying population mean.

Ai=effect of the ith sex.

Bj=effect of the jth biotin level

(AB)ij= effect of the interaction.

eijk= the error term.

3.1.4 Results and discussion.

3.1.4.1 Raw materials and experimental diets

Table 2 shows the chemical composition of the raw materials and the basal diet used. The energy, protein, and crude fibre in the basal diet were within NRC (1984) and Scott et al (1982) specifications. However, lysine and methionine levels were slightly higher than stipulated by NRC, which was attributed to the high levels of fish meal used. The basal diet provided 108 μg/kg of available biotin, which was higher than expected.

Table 2: Chemical composition of raw materials and the basal diets used in Experiment One

	Wheat	Soybean meal	Fish meal	Basal diet
Analysed (As fed basis)				
Dry matter(%)	88.30	90.88	89.60	90.15
Crude protein(%)	12.00	45.16	68.65	23.21
Crude fibre(%)	3.50	1.24	0.86	3.60
Fat(%)	2.49	3.91	12.53	7.94
Ash (%)	2.45	5.98	14.65	6.39
Total phosphorous(%)	-	-	-	0.59
Calcium(%)	-	-	-	0.92
Total biotin(µg/kg)	106.00	315.00	521.00	142.00
Calculated analysis (DM Basis)				
M.E,. (Kcal/kg).	-	-	-	3152.00
Available phosphorous(%)	-	-	-	0.46
Methionine, (% protein)	-	-	-	2.20
Lysine, (% protein)		-	-	5.77
Available biotin (µg/kg)	0.00	315.00	521.00	108.00

This high level arose from the inclusion of fish meal at 15%, and the fact that the fish meal used in this experiment had an unusually high level of biotin, Hoffmann La Roche (1987) have determined the average biotin content in fish meal to be 135 µg/kg, but the one used in this study had 521 µg/kg, a fact that was not known before the start of the experiment, as it was not possible to assay for biotin then. Similarly, soybean meal had a biotin content of 315 µg/kg, while the average determined by Hoffmann La Roche over the years is 270 µg/kg. Despite this, available biotin in the basal diet was below that required for optimal growth in chicken broilers (NRC 1984, Whitehead and Bannister 1978, and Whitehead 1986). Total available dietary biotin was 108, 188, 268, and 428 µg/kg for diets I, II,III, and IV, respectively.

3.1.4.2 Broiler performance.

The results of body weight gain, feed intake, feed efficiency, and mortality at thirty five days of age are shown in Table 3. Biotin supplementation did not have significant linear or quadratic effect ($P>0.05$) on body weight gain. The mean body weight per broiler was 1204 g. Males were significantly ($P<0.05$) heavier than the females whose respective weights were 1251.8 g. and 1157.4 g. These weights were higher than those reported by Scott et al 1982, but were similar to those quoted in NRC (1984). The protein and energy levels in this experiment were higher than those quoted by Scott et al (1982) at the fourth and fifth weeks of age, which explains the higher body weights.

Table 3: Effect of biotin supplementation on body weight, weight gain, feed intake, feed efficiency and mortality.at thirty eight days of age.

Item	Diets Sex	Biotin supplementation (µg/kg) 0 I	80 II	160 III	320 IV	Mean	SE
Total available biotin (µg/kg)		108.39	189.39	268.39	428.3	-	-
Body weight (g/chick)	M	1253.4	1257.30	1299.00	1197.00	1251.8*	19.46
	F	1087.1	1159.50	1207.00	1176.00	1157.4	19.46
	Mean	1170.3[a]	1208.4 [a]	1253.1[a]	1186.4 [a]	1204.6	55.10
Body weight.gain (g/broiler)	M	1186.8	1193.5	1233.50	1131.10	1186.2*	16.02
	F	1026.0	1096.0	1144.60	1114.10	1095.4	6.02
	Mean	1102.7[a]	1144.8[a]	1189.1[a]	1122.6[a]	1140.8	5.30
Feed intake (g/chick)	M	2113.6	2124.7	2178.80	1988.15	2102.0	39.05
	F	1901.3	1982.4	2124.30	1978.00	1995.0	49.05
	Mean	2007.5[a]	2053.6[a]	2151.6[a]	1983.1[a]	2048.8	14.04
Feed conversion (gain/feed)							
	M	1.77	1.77	1.77	1.76	1.77	0.025
	F	1.85	1.81	1.86	1.78	1.80	0.025
	Mean	1.82 [a]	1.80[a]	1.82[a]	1.77[a]	1.79	0.070
Mortality (%)	-	7.5	7.50	7.50	2.50	6.30	

S.E. standard error of mean.

*Significant differences between males and females ($P<0.05$). Means in a row with different superscripts are significantly different. ($P<0.05$)

Biotin supplementation had no significant (P>0.05) effect on feed intake and feed efficiency. The mean feed intake per broiler was 2031 g, which was close to the breeders specification of 1935 g (Shaver 1987). Males had a significantly higher feed intake than females.($p<0.05$). The mean feed efficiency was 1.80. The mean for males was 1.78, while that of females was 1.8. These two were not significantly different (P>0.05). There was no significant interaction between sex and biotin ($p> 0.05$).

A total of ten chicks died during the experimental period. Post mortem results showed that the chicks died from aflatoxicosis. None of the deaths noted was associated with fatty liver and kidney syndrome .This was probably due to the fact that the basal diet used in this experiment was high in protein and fat. Whitehead and Blair (1974) stated that low protein and low fat diets have a higher propensity to cause fatty liver and kidney syndrome than high protein and high fat diet. Besides, presence of aflatoxins in the basal diet, had a confounding effect on fatty liver and kidney syndrome.

Whitehead (1986) estimated biotin requirement to be 180 µg/kg. The basal diet used in this study therefore provided about 60% of the biotin required. As shown in table 3 supplementing the basal diet with biotin had no significant effect on weight gain. However, broilers that received the basal diet only, had the least weight gain of 1102.7 g. per broiler, while those on 80 µg/kg gained 1144.85 g representing an improvement of 3.8 % over the control group. The available biotin intake for this group was 188 µg/kg, which is the level recommended by Whitehead (1986). It was however noted in this study that a higher level of

biotin (268 µg/kg), gave the highest response in growth. The group fed on this level of biotin had a 7.8 % increase over the control. For the chicks fed on 320 µg/kg, the available biotin per kilogram was 428.39 µg/kg and the corresponding weight gain was 1122.6 g. which represented 1.8% improvement over the control.

Results of these study do not agree with those of Frigg and Brubacher (1976), and Whitehead, et al (1976), who found that biotin supplementation gave a significant increase in weight. In their studies, Frigg and Brubacher (1976) fed a basal diet that contained 52 µg/kg of biotin, and supplemented it with either 50 µg/kg or 500 µg/kg of biotin, and obtained significant response in growth for the two supplementary levels. Similarly, Whitehead et al (1976) using a basal diet containing 15 µg/kg got a significant increase in growth by supplementing the basal diet with 500 µg/kg of biotin. Al-Athari et al. (1986) in a study to investigate the effect of dietary lipid and biotin on chick growth and other parameters observed significant increase in growth,when he supplemented a purified biotin free basal diet with 70,130, and 200 µg/kg. In all the studies refered to, it is worth noting that the basal diets had lower biotin than the one used in this experiment

The results of this experiment are however consistent with those of Anderson and Warnick (1970), who found that in corn and wheat based diets, response to biotin supplementation did not attain statistical significance for weight gain. Similarly Balnave, (1975) also failed to attain significant differences in weight gain . Harms,et al (1979) in a study to investigate foot

pad lesions in broilers, supplemented chicks with 200µg/kg of biotin and failed to note any significant differences in weight gain over the control group.

The lack of a significantly different response to supplementation could be due to a number of factors. Response depends on the biotin content in the basal diet, and its availability to the chick. The basal diet used in this study was similar to the one used by Whitehead and Bannister (1978). which contained 75, 12, and 6% wheat, herring meal, and isolated soya protein respectively, providing 22.7 % , crude protein and 5.4 % ether extract. Differences between this study and that of Whitehead and Bannister could therefore be due to the levels of available biotin in the basal diet which were 108 and 33 µg/kg, respectively.

Besides the level of available biotin in the basal diets, the raw materials used could also help in explaining the differences observed. In place of isolated soya protein, soybean meal was used in this study. Also, as explained earlier, fish meal used in this study was unusually high in total biotin. Since the basal diet used in this study provided 60 % of available biotin recommended by Whitehead (1986), the response to supplementation was not great. Management could also be another factor that influenced the results. Whitehead and Bannister (1980) suggested that birds kept on litter are able to meet 10 % of their requirements from pecking on the litter. Since these chicks were managed on deep litter, which is the system under which broilers are reared in this country, coupled with the high level of biotin in the basal diet, the response to

supplementation was not high. A fact worth noting in this experiment is that the chicks fed on diet containing 160 µg/kg, where the total available biotin in the diet was 268 µg/kg, had the highest weight gain.Their weight was higher than that of the chicks fed on 188 µg/kg, which is the most recent biotin requirement recommendation. The chicks fed on a diet containing 428 µg/kg, had the least weight gain among the supplemented birds.

Lesions were not noted in this study. Three cases of chondrodystrophy were observed in treatment one.

Lesions characteristic of biotin deficiency were observed in studies of Frigg and Brubacher (1976), Whitehead and Bannister (1978) and Whitehead and Bannister (1981), in the control rather than in test diets.

3.1.5 Conclusions

The average daily biotin intake was 6, 11, 16 and 24 µg/day/chick and the corresponding weight gain was 31, 33, 34, and 32 g/day/chick for diets I, II, III, and IV respectively.

Biotin supplementation had no significant effect on weight gain, feed intake, feed efficiency or fatty liver and kidney syndrome. The basal diet used in this study provided 108µg/kg of available biotin, which was high. However even with this high level of biotin in the basal diet, there was some response. The best performance was obtained when supplementation was at 160 µg/kg, implying available biotin of 268 µg/kg, or a daily intake of 16 µg/chick/day. Chicks fed on the diet with the the highest

biotin level in this trial of 428 µg/kg, had the least weight gain amang the birds on biotin supplemented diets. However, the quadratic response was not significant. The biotin content of soybean meal and fish meal used in this experiment was higher than that reported by various researchers, but the content in wheat was similar to the findings of Hoffmann La Roche 1987.

Lesions characteristic of biotin deficiency were not found in this study, neither was there mortality due to fatty liver and kidney syndrome.

3.2 EXPERIMENT TWO:

Evaluation of chicken broiler performance given sorghum based diets supplemented with biotin.

3.2.1 Introduction

There is a growing need in Kenya to reduce the use of maize in poultry feeds. Sorghum is one of the grains that has aroused interest as an alternative source of energy, for animal feeds. No information is available on biotin content of local varieties of sorghum. This experiment was therefore carried out to evaluate the effect of substituting sorghum for maize in poultry feeds, on biotin requirements in broilers, and the effect this would have on weight gain, feed intake, and feed efficiency.

3.2.2 Objectives.

(i) To asses biotin levels and availability in sorghum.

(ii) To assess the importance of biotin supplementation in in sorghum based diets.

3.2.3 Materials and methods.

3.2.3.1 Management of the chicks and experimental diets.

One hundred and sixty shaver 'Starbro' broiler chicks were obtained from a commercial hatchery and sexed on arrival. Males and females were raised separately in electrically heated floor pens. The chicks were fed on the control diet (no biotin) for the first three days and on the fourth day, they were weighed in groups of ten picked at random and allocated to experimental pens, in a randomised design. Each pen measured 1.2M x1.2M providing a floor space of $0.144M^2$ per chick. The chicks were managed as described in section 3.1.3.1

White sorghum was used to formulate 560 kg of a basal diet to provide 196g crude protein and 2900 kcal of metabolizable energy per kg as shown in Table 4. The basal diet was then divided into four equal volumes of 140 kg each. To the first, second, third and fourth volumes 0, 350, 1050, and 2100 mg, of Rovimix H-2 were added respectively and thoroughly mixed, to form four experimental diets i.e. diets I, II, III and IV. Supplemental biotin levels in the diets were thus 0, 50, 150 and 300 µg/kg, for diets I, II, III and IV, respectively. Each diet was given to groups of ten chicks with two male and two female groups per diet.

Table 4: Composition of the basal diet used in Experiment Two.

Ingredients	%
White sorghum	62.90
Fish meal (Danish)	10.38
Sunflower seed cake	8.00
Pollard	15.61
Dicalcium Phosphate	0.89
Iodised salt.	0.50
L-lysine	0.39
DL-methionine	0.07
Vit/mineral premix[1]	0.25
Limestone	1.02
Total	100.00

1: See footnote Table1

3.2.3 2 Data collection

The biotin content of the raw materials used, and the basal diet was determined in the laboratories of Hoffmann La Roche, Basle, Switzerland as explained in Experiment One. Similarly the available biotin was calculated as described in section 3.1.3.1. Data on body weight, weight gain and feed intake was taken once every week as described in section 3.1.3.2

3.2.3.3 Statistical analysis

Body weight, gain, feed intake, and feed conversion data were collected at 21 and 42 days of age and were subjected to statistical analysis as explained in section 3.1.3.3

3.2.4 Results and discussion.

3.2.4.1 Raw materials and experimental diets

Proximate composition of the raw materials used are shown in Table 5. Diets in this experiment were formulated to be low in energy and protein so as to mimick the commercial broiler starter diets available in Kenya. The Kenya bureau of Standards specifies that the minimum metabolizable energy content in a starter diet should be 3,000 kcal/kg, (KBS 1978). This was however not achieved because there was an attempt to maintain a proper energy:protein ratio in the diet.

Table 5: Chemical composition of raw materials and the basal diet used in Experiment Two:

	White sorghum	Pollard	Sunflower seed cake	Fishmeal	Basal diet.
analysed composition					
Dry matter(%)	90.80	88.50	93.50	88.02	89.02
Protein(%)	11.84	17.75	24.50	68.65	19.60
Ether extract(%)	3.57	4.74	11.04	12.53	5.12
Total biotin (µg/kg)	344.00	328.00	582.00	521.00	355.00
Total phosphorous(%)	-	-	-	-	0.72
Calcium(%)	-	-	-	-	0.90
Crude fibre(%)	5.90	0.25	36.90	0.86	6.74
Calculated composition					
Available phosphorous(%)	-	-	-	-	0.50
Methionine,(% protein)	-	-	-	-	2.55
Lysine,(% protein)	-	-	-	-	6.63
Available biotin(µg/kg)	168.80	16.40	203.70	521.00	116.26
ME (kcal/kg)	-	-	-	-	2960.00

The total biotin in the basal diet was 344 µg/kg, of which 116µg/kg was estimated to be available. The basal diet therefore did not provide enough bioavailable biotin to meet the requirements of chick (Whitehead 1986). Calculated lysine and methionine levels were slightly higher than expected, and this was attributed to the high levels of fish meal in the diets. Crude fibre, calcium, and available phosphorous were all within the NRC (1984) recommendations.

3.2.4 2 Broiler performance at 21 and 42 days of age

Evaluation of effect of biotin supplementation was done at twenty one and forty two days.This was found necessary because response varied with age. In contrast to the response observed with a wheat based basal diet in Experiment one, the response to supplementation was observed to be high at the beginning of the experiment and decreased as the chicks grew older.

The weekly response in growth, to biotin supplementation is shown in Figure Two, while the broiler performance at twenty one and forty two days of age is shown in Tables 4 and 5. At twenty one days of age there were significant differences in body weight, and weight gain, between the supplemented and the control groups ($P<0.05$). Biotin supplementation had significant linear and quardratic effects on body weight and weight gain as described by Morris (1989). This is shown in Figure 3. There was no mortality at 21 days of age. The response to biotin supplementation decreased with age, and at 42 days, there were no significant differences between the control and the

supplemented groups (Table 5).

Similar findings have been reported by Anderson and Warnick (1970) using sorghum based diets fed for 25 days. These researchers found that effect of supplementation was highest in the first eleven days of age, and thereafter the percentage increase in weight decreased gradually. They concluded that there is an unrecognizable factor that affected the performance of these chicks. They attempted to explain this performance by assuming that biotin in sorghum is unavailable to the chick in the early stages of growth, but becomes more available as the chicks grow older. It is likely that a similar situation occurred in this experiment. The calculated available biotin in the basal diet was 116 mg/kg, practically it is possible that the actual availability was less than this. The availability of biotin in sorghum to chicken and factors affecting it need more study.

During the early growth phase, biotin supplementation at the rate of 150µg/kg gave the best response. However, when the entire experimental period is considered, the best performance is obtained at a supplementation level of 50 µg/kg, where available biotin is 166 µg/kg. This level was lower than that recommended by Whitehead (1986), but was close to that of NRC (1984) of 150 µg/kg. Feed intake and feed efficiency were not significantly affected by biotin supplementation ($P>0.05$).

Figure 2: Weekly response in weight gain to biotin supplementation.

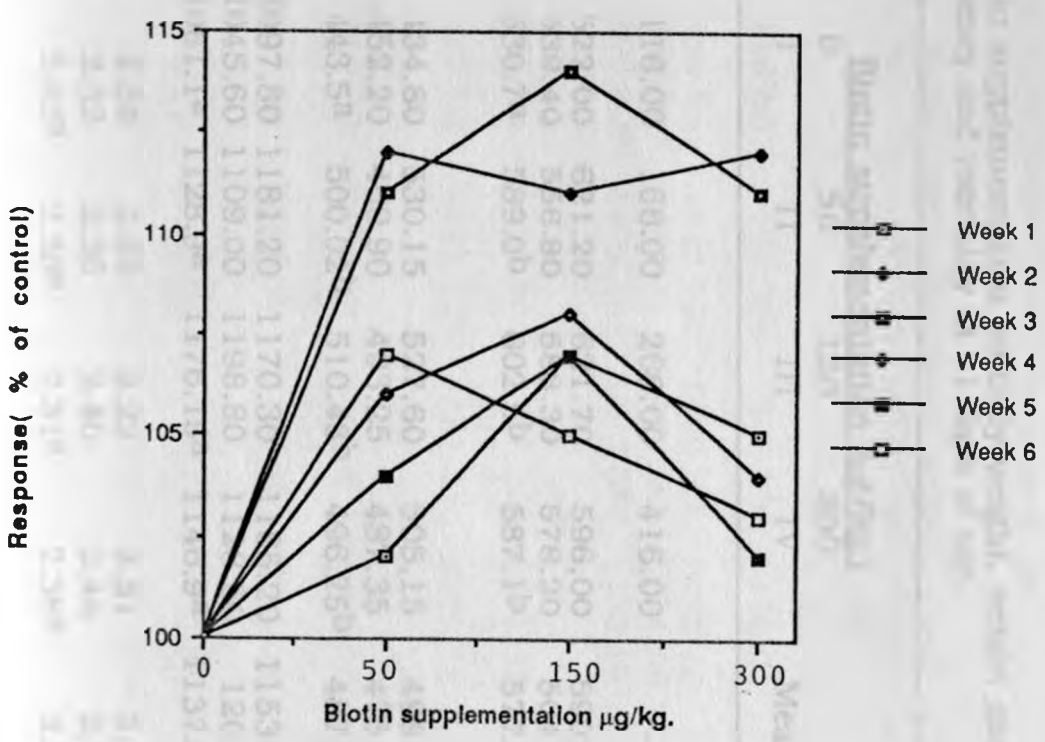


Table 6: Effect of biotin supplementation on body weight, weight gain, feed intake, feed efficiency and mortality at 21 days of age.

		Biotin supplementation (µg/kg.)					
		0	50	150	300		
	Diets	I	II	III	IV	Mean	SE[1]
Item	Sex						
Available biotin (µg/kg)		116.00	166.00	266.00	416.00	-	-
Body weight, (g/chick)	M	522.00	621.20	621.70	596.00	590.20 *	9.51
	F	539.40	556.80	583.30	578.20	564.43	9.51
	Mean	530.7[a]	589.0[b]	602.5[b]	587.1[b]	577.31	26.90
Weight gain g/chick	M	434.80	530.15	527.60	505.15	499.42*	9.74
	F	452.20	469.90	493.25	487.35	475.69	9.74
	Mean	443.5[a]	500.02[b]	510.43[b]	496.25[b]	487.54	27.55
Feed intake, (g/broiler)	M	1097.80	1181.20	1170.30	1165.20	1153.61*	4.50
	F	1045.60	1109.00	1198.80	1128.60	120.50	24.50
	Mean	1081.1[a]	1128.9[a]	1176.15[a]	1146.9[a]	1137.03	69.21
Feed efficiency, (feed/gain)	M	2.54	2.23	2.22	2.31	2.32*	0.059
	F	2.32	2.36	2.40	2.44	2.36	0.059
	Mean	2.43[a]	2.30[a]	2.31[a]	2.38[a]	2.34	0.17
mortality,(%)	Mean	0.00	0.00	0.00	0.00	0.00	-

*Means in a row with differing superscripts are significantly different (P<0.05).
SE is standard error of mean. * No significant differences between males and females

Table 7 Effect of biotin supplementation on body weight, weight gain, feed intake feed efficiency and mortality at forty two days of age

		Biotin supplementation µg/kg.					
		0	50	150	300		
	Diets	I	II	III	IV	Mean	SE.
	Sex						
Available biotin (µg/kg)		116.00	166.00	266.00	416.00	-	-
Final body weight (g/chick)	M	1454.55	1544.15	1486.30	1462.15	1487.30*	25.57
	F	1365.05	1474.45	1461.00	1404.05	1426.10	125.57
	Mean	1409.80[a]	1509.3[a]	1473.6[a]	1433.1[a]	1456.70	72.30
Weight gain (g/chick)	M	1367.00	1456.00	1392.20	1371.35	1396.87*	26.00
	F	1277.90	1387.60	1370.90	1313.20	1337.30	26.00
	Mean	1322.50	1422.10	1381.55	1342.28	1367.13	73.50
Feed intake (g/chick)	M	3241.00	3460.80	3492.8	3301.00	3374.07*	54.80
	F	3082.00	3189.00	3454.3	3171.00	3224.31	54.80
	Mean	3161.9[a]	3325.0[a]	3473.6[a]	3236.4[a]	3299.19	55.00
Feed conversion (feed/gain)	M	2.37	2.38	2.51	2.41	2.42*	0.03
	F	2.41	2.28	2.53	2.30	2.38	0.04
	Mean	2.40[a]	2.33[a]	2.52[a]	2.36[a]	2.40	0.11
Mortality (%)	Mean	0.00	3.00	0.00	2.00	2.50	-

Means in a row with different superscripts are significantly different ($P<0.05$)

SE: standard error of mean. *Males were not significantly different from females.

Figure 3. Weight gain at 21 days of age.

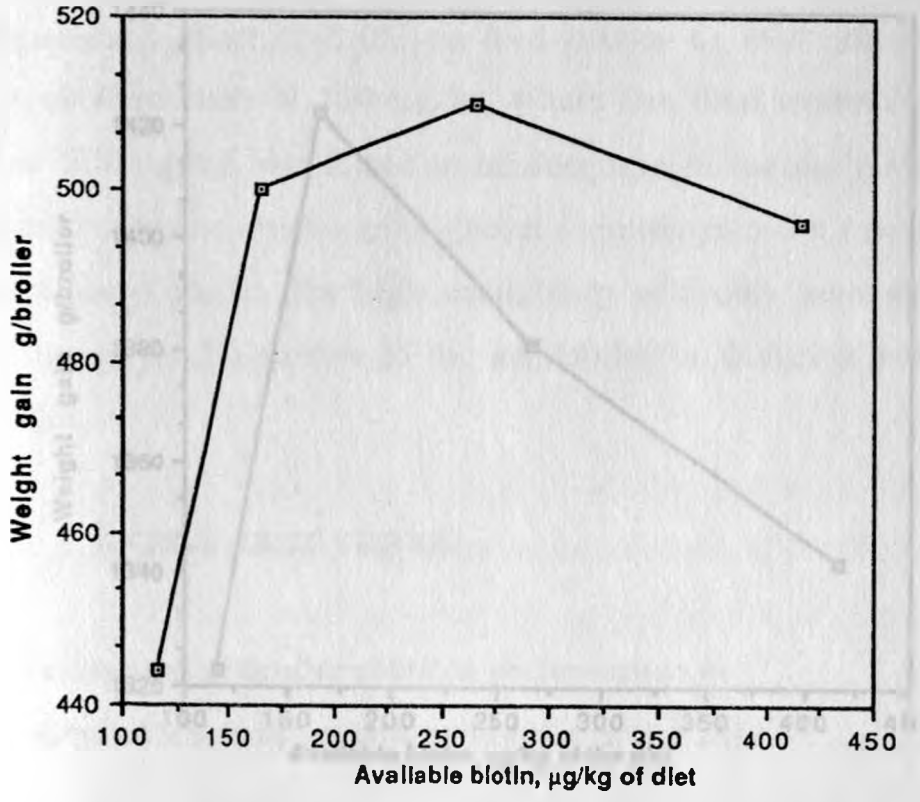
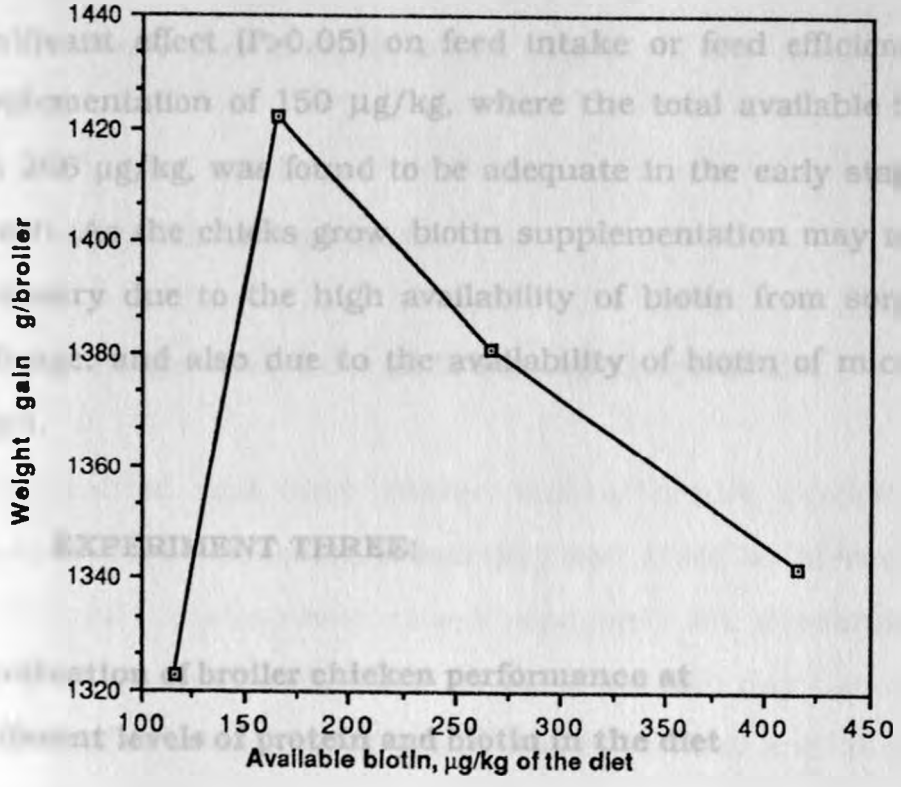


Figure 4. Weight gain at 42 days of age.



3.2.5 Conclusions

The total biotin content in white sorghum based diets was found to be 344 µg/kg, while available biotin was estimated to be 116 µg/kg. Biotin supplementation to these diets, improved growth rates significantly in the early stages of growth. but it had no significant effect (P>0.05) on feed intake or feed efficiency. A supplementation of 150 µg/kg, where the total available biotin was 266 µg/kg, was found to be adequate in the early stages of growth. As the chicks grow, biotin supplementation may not be necessary due to the high availability of biotin from sorghum with age, and also due to the availability of biotin of microbial origin.

3.3 EXPERIMENT THREE:

Evaluation of broiler chicken performance at different levels of protein and biotin in the diet

3.3.1 Introduction.

The requirement of biotin by broiler chicken is affected by several factors among which are the levels of protein and fat in the diet. In this experiment the effect of protein level on the performance of broiler chicken at three biotin levels was investigated.

3.3.2 Objectives

The objectives of this experiment were;

(i) To determine the effect of protein level on biotin requirement.

(ii) To assess the effect of biotin intake on the hepatic lipid content.

3.3.3 Materials and methods

3.3.3.1 Management of the birds and experimental diet.

Two hundred and forty shaver 'stabro' broiler chicks were obtained from a commercial hatchery and sexed on arrival. The males and females were raised separately on a commercial starter feed, for the first four days.On the fifth day the chicks were weighed in groups of ten picked at random and allocated to 24 experimental pens located in two separate houses. Each house constituted a block and contained twelve pens.The treatments were replicated equally within the two blocks. Each pen measured 1.2Mx1.2M providing a total floor space of $0.144m^2$ per chick, and was covered with wood shavings to a depth of ten cm. The chicks were managed as in experiments One and Two.

Two basal diets each weighing 240 kg were formulated. The first batch was formulated to provide 216 g of crude protein and 2869 kcal of metabolizable energy per kg, while the corresponding values for the second batch were 260 and 3282.

Each of the batches was then divided into three equal volumes of 80 kg. To the first second and third volume of each of the basal diets 0 400 and 800 mg of Rovimix H-2 were added so as to supply 0, 100 and 200 µg of supplemental biotin per kg. Thus, a total of six diets were obtained, the first three being low protein diets and the next three being high protein diets. The composition of the basal diets is shown in Table 8.,

In the first two experiments, fatty liver and kidney syndrome was not observed. In this experiment, a hypothesis that "a low intake of biotin is likely to lead to a high hepatic lipid content." was postulated. To test this hypothesis hepatic lipid content was determined at thirty three days of age. Two chicks from each replicate were picked at random, killed by cervical dislocation, and the liver excised. The gall bladder was also removed and the liver wrapped in an aluminium foil and frozen to await analysis. The lipid content was determined according to the standard procedures (AOAC 1984). The whole liver was minced using a Moulinex shredder. 3-4 g of the minced liver were weighed into a thimble containing small amounts of sand and mixed with a glass rod. The thimble and glass rod were removed from the oven and cooled. The thimble containing the liver was then put in a soxhlet extractor and fat was collected in a beaker of known weight for 18 hours. The fat collected was divided by the weight of the sample taken, and then expressed as a percentage. For each treatment, a total of eight livers were analysed and the mean for each treatment obtained.

Table 8: Composition of the basal diet used in Experiment Three. (%)

Ingredients	Dietary protein level low	high
Wheat	71.70	59.73
Soy bean Meal	18.60	17.01
Fish meal (Danish)	5.60	15.01
Corn oil	1.10	5.90
Dicalcium Phosphate	1.50	0.50
Limestone	1.60	1.10
Iodized salt	0.50	0.50
premix[1]	0.25	0.25
DL methionine	0.10	0.00
Total	100.00	100.00

[1]Composition of the mineral/vitamin premix is as shown in footnote Table 1.

3.3.3.2 Data collection

Data on body weight, weight gain, and feed intake was taken weekly upto the end of the experimental period, as described in Experiment One.

3.3.3.3 Statistical analysis.

This was done as in Experiment One. The model used for this experiment was:

yijk=m+Ai +Bj+Ck+ Dl + (AB)ij+ (AD)il + (BD)jl where;

Yijk = observation e.g weight gain , feed intake, feed efficiency etc per chick of the ith protein level, fed on the jth biotin level and in the lth block.

μ = population mean.

Ai = the effect of the ith protein level.

Bj = the effect of the jth biotin level.

Ck = the effect of the kth block.

Dl = the effect of the lth sex

(AB)ij= the interaction of the ith protein level with the Jth biotin level.

(AD)il = the interaction of the ith protein level with the lth sex

(BD)jl = the interaction of the jth biotin level with the lth sex.

eijk = is an error term.

3.3.4 Results and Discussion.

3.3.4.1 Raw materials and experimental diets

Proximate composition of fish meal, soybean meal and wheat is shown in Table 2, while that of the basal diet is shown in Table 9. The low protein diet was formulated to mimick commercial diets available in Kenya. Both diets were adequate in calcium and phosphorous, and were reasonably low in crude fibre, but the high protein diet was high in lysine and methionine, due to the high levels of fish meal used.

3.3.4.2 Broiler performance

Results of Body weight, weight gain, feed intake, feed efficiency, hepatic lipid content and mortality data obtained at 32 days of age, are presented in Table 10. Chicks on the high protein diet had a better growth rate and feed efficiency than those on low protein diets ($P < 0.05$).

The average weight of the broilers in the low protein diet was 988.2 g. with means for males and females being 1003.5 and 932.8 g, respectively. These were significantly different ($P < 0.01$). The mean body weights of birds on the low protein diets were below NRC (1984) specifications.of 1250 g for the males and 1035 g for the females.

Table 9: Chemical composition of the basal diet used in Experiment Three.

	Dietary protein level	
	Low	High
Analysed composition (As fed basis)		
Dry matter(%)	91.41	89.39
Crude protein(%)	21.63	25.96
Ash(%)	6.25	6.31
Ether extract(%)	2.74	9.25
Crude fibre(%)	5.74	5.05
Calcium(%)	1.20	1.04
Total phosp.(%)	0.75	0.68
Sodium(%)	0.13	0.17
Total biotin (µg/kg)	171.00	175.00
Calculated analyses (DM basis)		
M.E. kcal/kg.	2869.00	3282.00
Lysine, % of protein	5.50	5.93
Methionine, % of protein	2.27	2.04
Available biotin(µg/kg)	88.00	132.00

Table 10: Effect of biotin supplementation on weight gain, feed intake, feed efficiency, mortality and hepatic lipid content 32 days of age.

		Low protein				High protein				
Biotin µg/kg		0	100	200	Mean	0	100	200	Mean	SE
Diets		I	II	III		IV	V	VI		
	Sex									
Item										
Available biotin (µg/kg)		88.0	188.0	288.0	-	132.0	232.0	332.0	-	-
Body wt	M	963.8	1023.6	1023.2	1003.5**	1175.3	1229.9	1296.3	1233.8**	20.5
(g/broiler)	F	908.3	932.8	953.2	931.4	1149.7	1111.8	1117.9	1126.4	20.5
	Mean	936.0[a]	978.2[a]	988.2[a]	967.5	1162.5[b]	1170.8[b]	1207b	1180.1	14.5
Weight.gain	M	887.7	948.5	940.3	925.5**	1101.9	1153.5	1221.8	1159.1**	18.5
(g/broiler)	F	834.5	860.4	879.3	858.0	1056.5	1038.9	1040.5	1045.3	18.5
	Mean	861.1[a]	904.5[a]	909.8[a]	891.8	1079.2[b]	1096[b]	1131.15[b]	1102.2	13.1
Feed intake.	M	1961.3	1969.6	2032.5	1987.7*	1975.9	2073.4	1988.3	2012.5*	20.7
(g/broiler)	F	1871.9	1850.8	1894.3	1872.3	1954.6	1891.6	1828.8	1891.6	20.7
	Mean	1916.6[a]	1910.2[a]	963.4[a]	1930.0	1921.1[b]	1982.5[b]	1908.5	1952.1	14.6
	M	2.26	2.09	2.16	2.17	1.80	1.81	1.62	1.74	0.04
Feed	F	2.28	2.15	2.16	2.20	1.85	1.83	1.70	1.81	0.04
efficiency	Mean	2.27[a]	2.12[a]	2.16[a]	2.185	1.83[b]	1.82[b]	1.69[b]	1.77	0.03
(Feed/gain)										
Mortality (%)		0	5	7.5	6.25	5	5	22.5	10.8	
Liver fat, (%)		3.5[a]	3.6[a]	3.9[a]	3.66	3.58[a]	3.79[a]	4.14[a]	3.88	0.12

Means in a row with different superscripts are significantly different. * Significant differences between males and females
SE standard error of mean

The weights were also below the breeders specifications (Shaver 'Starbro' broiler management guide 1987), whose expected mean weights were 1250 g for males and 1110 g for females. This is expected because NRC specifications are based on weights of chicks fed on a diet containing metabolizable energy at 3200 kcal/kg and a protein level of 23% in the first three weeks of life. The mean weight of the chicks on the high protein diet was 1179.3 g. Males weighed 1233.8 g, while the females weighed 1126.4 g. These weights were within NRC (1984) and the Breeders specifications.

Feed intake was significantly affected by sex ($p<0.01$), while FCE was affected by dietary protein ($p<0.01$). Within the high protein diet, FCE had a significant linear response to biotin supplementation ($p<0.05$), while there was no response in the low protein diets. No death occured among the chicks on control diet while the highest mortality was recorded in the chicks on a high protein diet and receiving 200 µg/kg of biotin supplementation. This was also the group that had the highest growth rate. Post mortem results indicated that the major cause of death was ascites. Ascites has been recorded in Kenya in chicken broilers on high plane of nutrition (Maxwell and Mbugua 1989).

For the low protein diets the available biotin was 88, 188 and 288 µg/kg, for diets I, II, and III. respectively, while corresponding values for the high protein diets were 132 232 and 432 µg/kg for diets IV, V, and VI, respectively. The biotin level in the two basal diets did not meet the optimal requirement for growth. (NRC 1984 and Whitehead et al 1986)

Generally supplementation gave a growth response over the two levels of protein studied, and over all levels of biotin included. However, this improvement did not attain statistical significance either for the low or high protein diets ($P>0.05$). In the high protein diet, maximum daily gain was obtained at a supplementation level of 200 µg/kg. Within the low protein diet, there was no improvement in gain between the chicks receiving a supplementation of 100 and 200 µg/kg. The biotin intakes for these two groups were 188 and 288 µg/kg of feed. This could imply that a biotin intake of 188 µg/kg was adequate for growth at this protein level. This is the same value suggested by Whitehead (1986), but is higher than the values recommended by Anderson and Warnick (1970) and NRC (1984). These researchers have given different levels of biotin requirement. All along, the values quoted are expressed in µg/kg of diet. Ultimately these values should be related to feed intake and the available biotin in the basal diet. From this experiment, a daily intake of 13.2 µg/chick/day was adequate for the low protein diets. This level is comparable to 180 µg/kg recommended by (Whitehead 1986), and is lower than the level of 21.7 µg/chick/day or 232 µg/kg, which gave the maximal response in the high protein diets.

Response to biotin supplementation therefore varied with dietary protein level. This agrees with the findings of Whitehead et al (1975), and Whitehead and Bannister (1981), who showed that extra protein increased requirement for biotin. With low dietary protein, biotin deficiency results in moderate depression, while in high protein there is a more severe

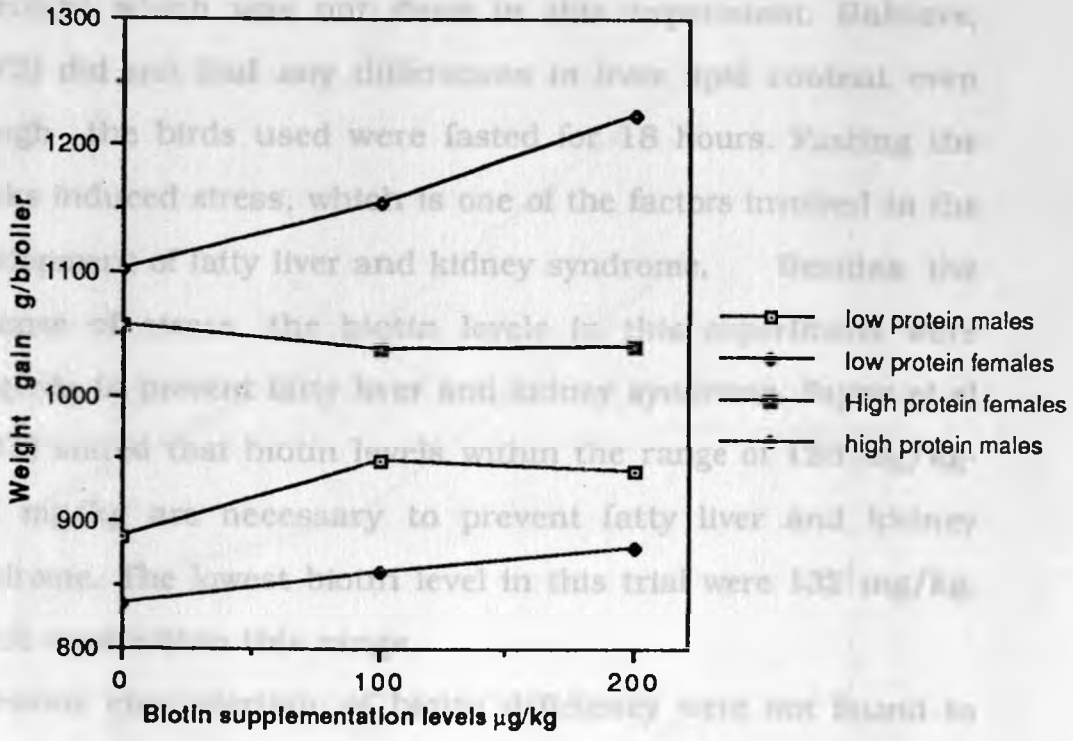
depression of growth. Although the argument put forward by Whitehead and Associates (Whitehead et al 1975, Whitehead and Bannister 1981) regarding the interaction of biotin and protein is plausable, it is also true that a high plane of nutrition increases biotin requirements. From this experiment, it appears that protein rather than biotin was the nutrient limiting growth.

Although protein*biotin interaction was not significant it was observed that the rate of gain was highest in males on a high protein diet. Within the range of biotin supplementation, male chicks showed a linear response to biotin intake. However, the females on the high protein diet had minimal response to biotin supplementation. Also males and females on the low protein diet did not respond to biotin supplementation. Response in weight gain to biotin supplementation is shown in figure 5.

Generally, requirements for nutrients varies between individuals of the same breed in a population. Also sex differences are likely to occur. Generally males grow faster than females and their requirements for various nutrients including biotin are bound to differ. Optimal biotin requirement for males in the high protein diets was estimated to be 332 µg/kg, while that for females was 132 µg/kg.

Effect of biotin supplementation on hepatic lipid content is shown is shown in figure 6. Biotin supplementation had a linear though not significant effect on hepatic lipid content ($P>0.05$). However, chicks on the high protein diets had a relatively higher hepatic lipid content than those on the low

Figure 5: Effect of biotin at different protein levels on body weight gain.at 32 days of age.

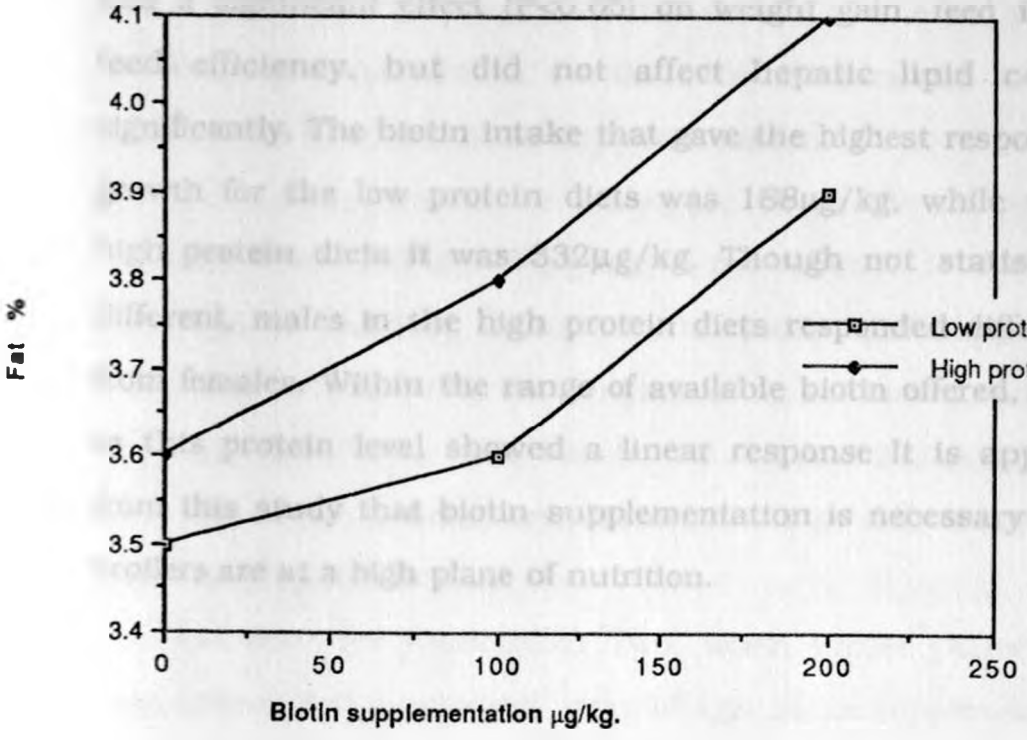


protein diets. This is expected since lipogenesis is influenced by the plane of nutrition.

Balnave and Brown (1967), found fatty livers in biotin deficient chicks. However the chicks in their experiment were fasted overnight which was not done in this experiment. Balnave, (1975) did not find any differences in liver lipid content even though the birds used were fasted for 18 hours. Fasting the chicks induced stress, which is one of the factors involved in the development of fatty liver and kidney syndrome. Besides the absense of stress, the biotin levels in this experiment were adequate to prevent fatty liver and kidney syndrome. Payne et al (1973) stated that biotin levels within the range of 120 mg/kg-145 mg/kg are necessary to prevent fatty liver and kidney syndrome. The lowest biotin level in this trial were 132 mg/kg, which were within this range.

Lesions characteristic of biotin deficiency were not found in this experiment. This is consistent with the findings of Whitehead and Bannister (1978) who found no signs of dermal lesions in chicks housed on litter, but which appeared on chicks housed on wire floor from 4-6 weeks of age.

Figure 6: Effect of protein and biotin levels on hepatic lipid content. at 31 days of age.



3.3.4 Conclusions

Biotin supplementation had no effect on weight gain, feed intake, feed efficiency, and hepatic lipid content. Dietary protein had a significant effect ($P<0.05$) on weight gain, feed intake, feed efficiency, but did not affect hepatic lipid content significantly. The biotin intake that gave the highest response to growth for the low protein diets was 188µg/kg, while in the high protein diets it was 332µg/kg. Though not statistically different, males in the high protein diets responded differently from females. Within the range of available biotin offered, males in this protein level showed a linear response It is apparent from this study that biotin supplementation is necessary when broilers are at a high plane of nutrition.

GENERAL DISCUSSION

The major objective of this study was to determine the effect of biotin supplementation in practical diets on broiler performance. The study was also aimed at assessing biotin content in some of the common raw materials used in poultry feeds in Kenya. The purpose of this section is to bring together the findings obtained from the three experiments with a view of defining the need for biotin supplementation.

The three experiments were designed to provide a wide range of biotin intake (88 to 428 µg/kg), a pre-requisite in describing response to nutrient input (Morris 1989). The experiments were also run for different lengths of time. Thus Experiment One, Two and Three were run for 35, 42 and 28, days of age. This was done to determine requirements at different growing periods (Whitehead 1986).

Other than for Experiment Two, when broiler performance was assessed at twenty-one days of age, biotin supplementation had no significant effect on weight gain. Failure to obtain significant differences was partly associated to the high available biotin in the basal diet and partly due to the criteria used in assessing biotin status. Available biotin in the basal diets ranged between 88 µ/kg, and 132 µg/kg. Response to biotin supplementation has been documented in experiments where the basal diets provided a lot less biotin than that used in this study (Frigg and Brubacher 1976, and Whitehead et al 1976). Therefore, at the level of available biotin in the basal diet, supplementation was unlikely to produce dramatic results. In his review on requirements of vitamins, Whitehead (1986) stated

that the composition of the basal diet must be well defined before the requirement can be adequately stated. This however was not the case in this study, as biotin content in the feedstuffs could not be determined before the start of the experiments. Biotin responsive conditions such as fatty liver and kidney syndrome (FLKS) and leg lesions are also used to assess the adequacy of dietary biotin. Neither FLKS nor leg lesions were observed in this study indicating that the basal diet provided adequate amounts of biotin to prevent these conditions. In Experiment Three, hepatic lipid content was found to increase with increase in dietary biotin. Contrary to the hypothesis posed, low levels of biotin did not cause fat infiltration in the liver. Biochemical changes such as pyruvate carboxylase activity has been stated to be a better criteria for assessing biotin status than weight gain (Austic 1986). This was not done in this experiment.

Although biotin supplementation did not result in significant weight gain, for most of the situations, it did elicit some response. An attempt was therefore made to determine the level of biotin intake leading to highest weight gain. Initially, a curvelinear equation as shown below was used.

$Y = a + b_1x + b_2 X^2$

Where Y = weight gain (g); X = biotin intake (µg/kg)

This was done for all experiments, individually or combined. In all cases, the regression was not significant, with a coefficient of determination of 0.25 or less. It was however pertinent to determine the level of biotin intake that resulted in highest

weight gain. When determined at the end of the experiment, maximum response was obtained with an intake of 268 and 166 µg/kg for experiments One and Two, and 188 and 332 µg/kg for experiment Three high and low protein diets respectively. The intake of 166 µg/kg was comparable to that reported by Anderson and Warnik (1970) and Whitehead and Bannister (1978) but higher than that reported by Scott et al. (1969). Experiment Two data analysed at 21 days of age, showed that biotin had a significant effect ($P<0.05$) on weight gain, with maximum response obtained at an intake of 266 µg/kg, which is higher than the theoretical value of 147 µg/kg calculated by Whitehead (1986), at this stage of growth. The higher requirement seen in the early stage of growth, could be explained by the fast growth rate of the chick which needs a high nutrient intake commensurate to the rate of growth.

When the data from the three experiments was analysed at twenty-one days of age, no significant differences were observed for both experiments One and Three. Available biotin in the low protein diet (Experiment Three) was lower than that of Experiment Two, and yet response to supplementation was obtained in the latter. It appears that there was underestimation of a biotin in the wheat based diets, and that the chicks were receiving close to their optimal requirement. Indeed, this is best seen in experiment Three. For the low protein diets biotin supplementation did not elicit any response. If biotin required is between 90 and 180µg/kg (Scott et al. 1969 and Whitehead (1986), then the basal diet was providing adequate or nearly

adequate available biotin. Available biotin in wheat is variable (Frigg 1976), which could explain the underestimation. Another possible explanation for the differences in response between the Two experiments could be the raw materials used. In Experiment Three, soybean meal was used, which has been documented to be a good source of biotin (Hoffmann La Roche 1987), while in Experiment Two, it was substituted with sunflower seed cake and pollard where biotin is only partly available.

There was no clear cut trend in response to biotin supplementation between male and female chicks. In Experiment One, female chicks showed a better response than male chicks. The reverse was the case in Experiment Two, although the difference in response was not large. In Experiment Three, a better performance was seen in male than female chicks. In the high protein diets, male chicks showed a linear response to biotin intake, while female chicks showed no response at all. On average males have a a higher nutrient requirement than females due to their faster growth. This could explain the observation made in Experiment Three. The opposite response seen between male and female chicks in Experiment One and Three (high protein) is not easy to explain. The strain of the chicks used was the same in all the experiments. and hence it was expected that response would be the same.

Other than for males in Experiment Three, there was a quadratic response in weight gain to biotin supplementation. Biotin toxicity has not been reported. However, interaction

between biotin and other B vitamins has been reported (Whitehead and Randall, 1982). Probably, a negative interaction between biotin and other vitamins took place in this study, a proposition which can be furthomed, since metabolic balance between vitamins must be considered in animal nutrition. Indeed, (Hulan et al 1979) showed that a high biotin intake must be coupled with a high intake of folic acid, pyridoxine, and thiamine.

The raw materials used in this study were:- wheat, white sorghum, pollard, sunflower seed cake, soya bean meal and fish meal. Other than for fish meal, biotin content in the feedstuffs was within the expected range (Hoffmann La Roche 1987). Danish fish meal was used in this study and was found to be unusually high in biotin.

it is not easy to explain the reason for the high biotin but it is plausible to argue that this fishmeal was not handled or stored property thereby encouraging bacterial growth, which could have synthesized biotin. The mean biotin content in fish meal has been estimated at 135 µg/kg with a range of 11 to 421 µg/kg (Roche 1987). The value of 521 µg/kg obtained in this study was 24 % above the upper limit reported.

5 CONCLUSIONS

1. With the exception of the sorghum based diets in the early stages of growth, the raw materials used in these experiments provided adequate biotin for growth and hence there was no significant response to supplementation

2. Biotin supplementation had a significant effect on weight gain in sorghum based diets but not in wheat based diets. During the first 21 days, an intake of 266 µg/kg resulted in the highest gain but this decreases to 166 µg/kg.

3. Biotin supplementation did not elicit any response in wheat based diet. This was probably due to an underestimation of biotin in wheat.

4. Biotin requirement is higher in high plane of nutrition than in low plane of nutrition.

5. It is estimated that the practical feeding situation, the local feeds will provide 59 to 88µg/kg of available biotin.

6. A better understanding of available biotin content of the feedstuffs used in poultry feeds in Kenya is needed.

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APPENDICES
Experiment One.
Appendix 1.1

Analysis of variance for body weight, feed intake, and feed efficiency at 35 days of age.

Source of variation	df.	Mean sum of squares. Body weight	Feed intake	Feed efficiency $x10^{-3}$
Biotin	3	5172	2003.0	7.650
Linear	1	1696	1.6	6.00
Quardratic	1	11046	9346.0	11.00
Cubic	1	2775	2655.0	5.00
Sex	1	26947*	70662.0*	0.025
Block	1	1092	883.0	0.100
Biotin*sex	3	4183	6180.0	3.000
Remainder	7	3031	12190.0	5.140
R squared		0.726	0.545	0.479

* $P<0.05$

Appendix 1.2. Experiment One. Body Weights (grammes per bird) 0-5 weeks of age

Age in weeks	Sex	Biotin supplementation µg/kg 0	80	160	320
1	M	192	185	189	196
	F	178	191	188	188
	Mean	185	188	187	192
2	M	400	402	406	405
	F	371	391	395	399
	Mean	386	397	401	402
3	M	716	731	733	731
	F	639	678	705	697
	Mean	678	705	719	714
4	M	1013	1035	1048	1040
	F	905	955	1001	973
	Mean	959	995	1025	1007
5	M	1253	1257	1209	1197
	F	1089	1160	1232	1175
	Mean	1171	1209	1221	1186

Appendix 1.3

Experiment One. Feed intake (grammes per bird) 0-5 weeks of age.

Age in weeks	Sex	Biotin supplementation µg/kg 0	80	160	320
1	M	184	182	199	194
	F	173	177	175	179
	Mean	179	180	188	187
2	M	364	367	389	359
	F	313	335	333	370
	Mean	339	351	361	365
3	M	531	536	518	543
	F	455	505	502	493
	Mean	493	521	510	518
4	M	603	650	632	681
	F	548	548	581	576
	Mean	576	599	607	629
5	M	628	650	632	641
	F	548	548	581	576
	Mean	588	599	607	609

Experiment Two.

Appendix 2

Analysis of variance for body weight, feed intake, and feed efficiency.at 42 days of age.

Source of variation.	df	Mean squares		
		Body weight	Feed intake	Feed efficiency
Biotin	3	7888	71818	0.0270
Linear	1	221	27665	0.0010
Quadratic	1	19888	160220	0.0090
Cubic	1	3555	27569	0.0720
Sex	1	14963	89715	0.0056
Biotin*sex	3	735	9223	0.0066
Remainder	8	5232	23999	0.0124
R Squared		0.49	0.630	0.5200

No significant differences observed (P>0.05)

Appendix 2.1 Analysis of variance for body weight, feed intake and feed efficiency at 21 days of age.

Source of variation	df	Mean sum of squares		
		Body weight	Feed intake	Feed efficiency
Biotin	3	4050*	8915	0.013
Linear	1	6669*	14047	0.012
quadratic	1	5432*	12327	0.013
cubic	1	51	371	0.009
Sex	1	2657	4399	0.005
Biotin x sex	1	1193	1902	0.036
Remainder	8	723	4790	0.027
R Squared		0.76	0.49	0.410

* Significant (P<0.05)

Appendix 2.2

Experiment Two. Feed intake (grammes per bird) 1-6 weeks of age

Age in weeks	Sex	Biotin supplementation µg/kg 0	50	150	300
1	M	231	257	253	257
	F	249	225	269	254
	Mean	240	241	261	256
2	M	342	398	383	391
	F	351	336	383	370
	Mean	347	367	383	381
3	M	525	527	534	517
	F	446	493	547	504
	Mean	486	460	541	511
4	M	675	694	662	631
	F	706	739	776	703
	Mean	691	717	719	667
5	M	733	853	821	758
	F	706	739	776	703
	Mean	720	796	799	731
6	M	733	808	840	750
	F	709	713	810	693
	Mean	721	761	825	722

Appendix 2.3

Experiment Two Weight gain (grammes per bird) 1-6 weeks of age

Age in wks	Sex	Biotin supplementation µg/kg 0	50	150	300
1	M	102	115	114	114
	F	126	119	130	117
	Mean	114	117	127	116
2	M	246	283	298	297
	F	268	285	298	293
	Mean	257	284	298	295
3	M	435	530	528	505
	F	452	470	493	487
	Mean	444	500	511	496
4	M	675	694	662	631
	F	722	729	776	721
	Mean	699	712	719	676
5	M	1038	1123	1129	1097
	F	1038	1039	1084	1027
	Mean	1038	1081	1107	1067
6	M	1367	1457	1392	1371
	F	1278	1382	1371	1371
	Mean	1323	1420	1382	1371

Experiment Three.

Appendix 3.

Analysis of variance for Body weight, feed intake and feed efficiency.

Source variation	df	Mean squares: Body weight	Feed intake	Feed efficiency*10^2
Biotin	2	4683	214	3.00
linear	1	9360	97	5.70
quadratic	1	6	331	0.30
Protein	1	271383**	17136*	136.000**
Sex	1	48339	172771**	0.007
Protein*sex	1	1874	17631	4.000
protein*biotin	2	632	1001	1.780
Sex*biotin	2	3819	2472	5.900
Remainder	13	2511	2561	9.700
R squared		0.913	0.876	0.921

** P<0.01

* P<0.05

Appendix 3.1

Experiment 3. Body weight (grammes per bird) 0-4 weeks of age

Age in weeks	Sex	Low protein diets Biotin supplementation µg/kg 0	100	200	High protein diets 0	100	200
1	M	183	201	218	234	248	245
	F	204	203	201	248	231	236
Mean		193	202	210	241	240	241
2	M	36	386	404	516	518	507
	F	388	391	386	493	446	479
Mean		378	389	395	505	482	493
3	M	649	698	727	878	878	887
	F	651	666	665	803	795	782
Mean		650	682	696	841	837	834
4	M	964	1024	1023	1175	1230	1296
	F	908	933	953	1150	1112	1118
Mean		936	978	988	1163	1171	1207