THE EFFECT OF CHOLINE DEFICIENCY ON THE CHEMICAL COMPOSITION OF THE SKELETAL MUSCLES OF CHICKS

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "THE EFFECT OF CHOLINE DEFICIENCY ON THE CHEMICAL COMPOSITION OF THE SKELETAL MUSCLES OF CHICKS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

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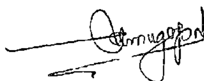

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INTRODUCTION

INTRODUCTION

Choline is essential to the animal organism as a source of methyl groups for the synthesis of amino acids, and for the formation of phospholipids and acetyl choline. The deficiency of choline in animals leads to abnormal deposition of fat and cholesterol esters in the liver, haemorrhages and muscular weakness. In chicks, ducklings and turkey poults the primary effects of choline deficiency are interference in growth and appearance of perosis. The clinical picture of perosis is well defined and is characterised by displacement of gastrocnemius tendon and paralysis of the leg. It is apparent that the thickening and twisting of the tibia and metatarsal bones, with displacement of articular cartilage is responsible for the slipping of the tendon from its condyles. It has not been elucidated with certainty whether the paralysis in perosis is a consequence of slipped tendon or due to muscular weakness. From the fact that choline supplements were effective in curing and preventing perosis in chicks, Jukes (1940, 1941) concluded that choline is linked with the metabolism of muscle and bone. The paucity of available information on the changes in chemical composition of skeletal muscle in choline deficiency in birds prompted this study. Knowledge on this aspect of study is of fundamental importance in understanding the functions of choline in the metabolism of skeletal muscles.

Present Investigation.

The study was conducted on day old chicks incorporating three different levels of choline in an otherwise normal ration.

Observations recorded:

a) During the feeding trial:

i) Weekly weight.

ii) Weekly feed consumption.

iii) Onset of perosis.

b) On gastrocnemius muscle.

i) Muscular efficiency as determined by the latent, contraction and relaxation periods and fatigue time.

ii) Total protein, lipid, phospholipid, cholesterol and creatine phosphate content.

iii) Activity of acid phosphatase, alkaline phosphatase, succinic dehydrogenase, phosphorylase, glutamate - oxaloacetate transaminase and glutamate - pyruvate transaminase.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Studies on choline and its derivatives have emphasized the biochemical importance of these compounds as structural components of tissues, as intermediates in vital metabolic reactions and as specific chemical reactants of biological potency. Ewing (1963) tentatively classified choline among B Vitamins because of its occurrence with them and its requirement for growth and nutrition of experimental animals and birds.

Choline was first isolated by Strecker (1849) from hog bile. From the seeds of white mustard Babo and Hirschbrunn (1852) obtained pure choline and called it sinkalin to indicate its origin from the alkaloid sinapin. Strecker (1862) applied the name choline to the base he obtained from hog bile. Baeyer (1866) and Dybkowsky (1867) demonstrated that neurine, a fraction separated by Liebreich (1865) from hydrolyzed crude brain extract lecithin to possess the same chemical behaviour as choline. Claus and Keese (1867) showed sinkalin to be identical with choline.

Proof of the structure of choline was furnished by Baeyer (1866) and Wurtz (1867). They established it as B hydroxy ethyl - trimethyl ammonium hydroxide and Wurtz (1867) carried out the first synthesis.

Properties of choline have been the subject of much detailed study. Griess and Harrow (1885) described the crystalline form they prepared as a colourless, odourless compound with a bitter taste, decomposing readily at elevated temperatures. Wurtz (1868) found dilute solutions to be stable to heat and concentrate solutions to decompose when boiled. Roman (1930) and

Klien and Linser (1932) studied the solubility of choline and reported that it was extremely soluble in water, formaldehyde, absolute methyl and ethyl alcohols.

Different methods were employed to extract choline from biological materials. The total content in biological fluids and foods were determined by Engel (1941, 1943), in meat cuts by McIntire *et al.* (1944), in milk by Hodson (1945) and Marquez (1942) and in brain by Sadhu (1948). Jolliffe (1957) reported egg yolk, brain and heart as the richest sources and green leafy vegetables and legumes as moderate sources of choline. According to him choline occurs in the animal tissues as lecithin and as the ester of acetyl choline.

On the basis of their observation in dogs, Hohse and Searle (1955) stated that absorption of choline took place through portal route rather than lymphatics. Flower *et al.* (1972) presented evidence for the occurrence of active transport process in addition to diffusion for the absorption of choline.

The nutritional significance of the dietary supply of choline was demonstrated by Best *et al.* (1932). They ascribed the lipotropic activity of dietary lecithin to its effective component choline. According to them choline fosters the conversion of neutral fats to phospholipids in the liver and thereby promoting the utilization and transport of fatty acids. Artom (1953) attributed the lipotropic effect of choline to the enhanced fatty acid oxidation in the liver. Jukes (1942) on the basis of his observations stated that anti-perotic and growth promoting properties of choline are

distinct. He associated the utilization of intact molecules of choline with its ability in preventing fatty livers and haemorrhagic kidneys in rats and mice and antiperosis affect in chicks and turkeys. These findings were confirmed by Welch (1936).

Jukes (1947) ascribed the growth promoting property of choline to its labile methyl group. This group is transferred to homocysteine to form methionine. Condition for creatine synthesis in muscle have been investigated by Barrenscheen and Pany (1948) who showed that creatine is formed by methylation directly from methionine and indirectly from choline.

On the basis of the results obtained by them, Lachmansoln (1945) stated that choline acetylase formed acetyl choline under aerobic condition in the presence of choline, acetate and adenosine. Chevremont (1945) claimed choline to be necessary for the formation of histocytic cells.

Only few reports are available on the participation of choline and its derivatives as co-factors in enzymatic system. Kelly and Myerhof (1950) found that a magnesium activated ATPase of muscle consisted of a lipoprotein with a choline containing phospholipid as prosthetic group. According to Sebrell and Harris (1954) the pyrophospheric acid esters of choline occur in the prosthetic groups of acid and alkaline phosphatases.

Observations on experimental animals have established fatty infiltration of the liver as the most prominent change in choline deficiency. The findings of Best and Ridout (1933) that deficiency of choline caused deposition of fat in rats were confirmed by Boxer and Stetten (1944). Similar results

were obtained in dogs by Best *et al.* (1933). In growing cats after 8 weeks of subsistence ration free of choline, daSilva *et al.* (1959) noticed fatty liver, the fatty infiltration primarily occurring in periportal spaces. Wilgram and Taylor (1959) reported that the clinical signs, jaundice and fatty liver in choline deficiency were identical in monkeys and man. The data of Marks (1968) revealed the occurrence of fatty infiltration of liver in a number of species such as rats, mice, hamsters, rabbits, dogs and ducklings in choline deficiency. Patek *et al.* (1975) reported monkeys were susceptible to cirrhosis by choline deficiency. Ostryanina (1975) observed liver involvement in rats fed choline deficient diet. Liver showed moderate fatty infiltration, marked lipohepatosis, fibrosis and nodular cirrhosis. Ewing (1963) and Fritz *et al.* (1967) studied the effects of different levels of choline in the feed on liver fat of chicks. They could not find any variation in the fat content. Swenson (1970) provided experimental evidence for the accumulation of fat in the liver of poultry when the feed was deficient in choline. Seifter *et al.* (1971) reported a case in chicks where choline deficiency caused vomiting and distension of gall bladder with high concentration of bilirubin in the bile. Surendranathan (1974) found only mild degenerative changes in the liver of choline deficient chicks. Gerlach *et al.* (1975) observed fatty liver syndrome in chicks and Wolford and Polin (1975) fatty liver haemorrhagic syndrome in laying chicken in choline deficiency.

The results obtained by Best *et al.* (1933) in dogs and Best and Ridout (1933) in rats indicated that feeding choline to deficient animals prevented

deposition of fat in liver.

Very little information is available on the effects of choline deficiency in other tissues. Griffith and Wade (1939) reported that rats showed marked enlargement and degeneration of the kidneys, a regression of thymus and an enlargement of the spleen. Griffith (1948) and Hartroft and Best (1947) found choline deficiency to produce degenerative changes in the kidneys of rats. Lack of a labile methyl group was considered responsible for haemorrhagic degeneration by Griffith and Mulford (1941). Reid (1955) reported choline deficiency in young guinea pigs produced degenerative changes in subcutaneous tissue and adrenals and paleness of kidneys. Moore (1957) described the lesions in male rats on choline deficient diets as fatty changes in the proximal tubules leading to necrosis. Necrosis later involved the cortex. He suggested vasospasm as the operative agent for necrosis. Nagler *et al.* (1968) assayed the concentration of acetylcholine and cholinesterase in the tissues of experimental and control rats to confirm their view that choline deficiency decreased acetyl choline concentration and thereby made the renal vasculature hyper-responsive to vaso pressor amines. They attributed nephropathy of acute choline deficiency to vascular disturbances brought about by the fall in acetyl choline concentration. Parke and Smith (1968) analysed the chemical composition of kidney after feeding a choline deficient diet. He found an increase in neutral lipids, lowered phospholipid concentration and cellular proliferation of kidney tubules. Neuberne *et al.* (1969) reported haemorrhagic kidneys and cardiovascular damage in choline deficient rats. Nagler *et al.* (1969)

showed that the imbalance in vasoactive mediators due to a decreased concentration of acetyl choline in choline deficiency led to vasospasm and ischemia of the kidneys of rats. Kratzing *et al.* (1973) observed a relation between kidney damage and hyper tension in choline deficient rats. According to Wilson *et al.* (1973) incidence and severity of renal lesions were greater in rats which were given choline deficient diet without cholesterol. The data of Mouserrat *et al.* (1973) revealed a high renal protein value for rats with renal necrosis. They considered alterations within to be one of the factors responsible for the necrosis in weanling rats.

Surendranathan (1974) considered the hyperplasia of the tubular epithelium of choline deficient chicks to be a milder form of kidney damage.

Wilgram *et al.* (1954) reported that young male rats fed choline deficient diet containing fat, developed cardiac lesions. The lesions were characterised by focal deposition of fat in muscle cells followed by necrosis and removal of liberated fat droplets by macrophages. Areas of pneumonia in sections of lung were the only change noticed in mice by Meader and Williams (1957).

Specific role of choline in haematopoiesis is disputed. Davis (1939) reported a depression of cobalt induced polycythemia in dogs by choline deficiency. He suggested that an increase in choline or acetyl choline and a decrease in cholinesterase caused vasodilation and increased oxygen tension in the bone marrow thereby inhibiting maturation of the cells of

the erythroid series. Augmented activity of serum phosphatase in puppies and adult dogs on choline deficient diet has been reported by Hough et al. (1943). Ebuts (1943) showed that choline deficiency caused anaemia in dogs. Similar results were obtained by Alexander and Engel (1952) in rats. Hoskins et al. (1953) revealed severe anaemia in choline deficient animals. According to them anaemia resulted from extensive damage to the liver rather than the dysfunction of bone marrow.

A fall in blood cholesterol level was noticed in puppies by Hough et al. (1943) which was in agreement with the later findings of Minoco et al. (1964) and Prasanti et al. (1968) who also reported a low blood cholesterol level in rats.

Herrmann (1946) noticed a fall in blood cholesterol level of laying hens on choline deficient ration. Surendranathan (1974) concurred with these findings and stated that both total cholesterol and phospholipid decreased in the plasma of choline deficient chicken. He further stated that the hyperplasia of the kidney tubular epithelia in experimental birds was due to the augmented blood uric acid level; the decreased growth rate and metabolic activity to the lowered plasma alkaline phosphatase, GOT and GPT activity.

Choline deficiency in animals involving the skeletal muscles has been demonstrated by a number of workers. Neumann et al. (1949) stated that

piglets in deficient diet were unthrifty and showed slow growth and incoordination of movements. Hove and Copeland (1954) observed that rabbits fed a diet deficient in choline, but adequate in vitamin E developed dystrophic lesions. Reid (1955) noted in choline deficient pigs severe retarded growth and weakening of muscles. Hove *et al.* (1957) in rabbits reported incoordination, paralysis and hyaline degeneration of skeletal muscles. The symptoms of choline deficiency in calves were described by Johnson *et al.* (1951) as weakness, inability to get up, laboured breathing and anaemia.

Deficiency of choline is rare in adult chicken. In chicks, Doyle (1931) reported slipped tendon as a sign of choline deficiency. He ascribed the bending or torsion of the bones forming the hock joint for the displacement of Achilles tendon and stated that the disease is characterised by the presence of whitish gray tumorous masses in and along the bones, in visceral organs and sometimes in the skeletal muscles. Hegsted *et al.* (1941) established the importance of choline in the diet of chicks for the growth and prevention of perosis. He could not find any difference in the bone phosphorus content between normal and choline deficient perotic chicks. Wolbach and Hegsted (1953) made comparative studies on perosis caused by manganese and choline deficiency and reported the effect on epiphyseal cartilage to be similar in both. The changes noticed by them included the abnormalities in enchondral growth of bone proliferation, granular matrix formation and maturation of cartilage cells. They concluded that both manganese and choline are essential for epiphyseal cartilage cell metabolism.

Briggs _et al_. (1953) experimentally proved that choline deficiency produced paralysis like syndrome in gosling. Creek (1959) found choline to be essential for the normal development of the hock joint in chicks and poults. Balloun and Miller (1964) reported high incidence of perosis in young turkeys and attributed it to their greater requirement of choline. Scott and Krook (1972) stated that the most prominent syndrome of choline deficiency in chicken is perosis or slipped tendon. In perosis the deformation of articular cartilage resulted in the metatarsus being thrown out of alignment with the tibia and the tendon slips from its condyles. In this condition they found that the affected leg was not able to support adequately.

Latshaw and Jensen (1972) noted a significant fall in oviposition rate, egg weight and hatchability in Japanese quail with a choline deficient diet.

The work of Gillis and Norris (1951) established the beneficial effect of added choline to a practical ration for chicks. They considered supplementation therapy as essential for differentiating perosis caused by deficiency of manganese, biotin and choline in chicks. The findings of Sutton _et al_. (1957) that the body weight of hen in production increased when they were fed choline supplemented diet were confirmed by Pattrick and Fulton (1967). Choline rich diet increased egg production and egg weight as reported by Schexnailder and Griffith (1973). Griffith and Rodriguez (1973) found no such variation in their experiments and concluded that variation in the strains were responsible for the difference in results. Kummerow

et al. (1919) studied the choline replacement value of ethanolamine in chicken kept in a high fat ration. The sparing action of Vit. B_{12} on choline requirement of chicks for and prevention of perosis was demonstrated by Schaefer *et al.* (1949). Haw *et al.* (1954) noted that the methyl groups of betaine were utilized more efficiently for tissue methionine formation than that of choline by chicks. Ketola and Young (1973) reported methyl amino-ethanol to be an effective substitute for dietary choline in Japanese quail. The data of Esh and Som (1954) did not reveal any difference in the efficiency of choline salts in promoting growth except for choline citrate. Choline in the form of chloride is generally fed to birds.

That the excess of choline in the diet has a deleterious effect was suggested by Deeb and Thornton (1959). They showed that birds on excess choline diet had lesser body weight and feed efficiency. In addition to reduced growth rate and egg production Ewing (1963) found curling of new feathers and frizzled appearance in birds that were given excess choline in their feed. According to Fritz *et al.* (1967) choline levels above 1615 mg per kg of feed retarded growth in chicken. No such change in growth or variation in feed efficiency in birds were observed by Potter and Kelly (1973) when the feed contained 5850 mg of choline per kg.

The dietary requirement of choline for chicks has been the subject of much detailed study. West *et al.* (1951) found that in the presence of ample methionine, 100 mg per cent of choline in the ration was adequate to maintain optimum growth.

For the prevention of perosis in poults Gogus and Griminger (1957) suggested 700 mg of choline per pound of feed. In chicks the level of choline required to prevent perosis was noted by Bird *et al.* (1966) as 1300 mg and Roberts and Fritz (1965) as 1450 mg per kg of feed. Fritz *et al.* (1967) reported a beneficial effect on growth of chicks when the choline content in the ration was progressively increased to a level of 1615 mg per kg of feed.

Studies of Cumming and Tribe (1956) and Ketola and Nesheim (1974) disclosed that chicks require an optimum level of 1500 mg of choline per kg of feed for maximum growth and prevention of perosis. Surendranathan (1974) concurred with the above findings.

Conflicting evidence has been recorded on the dietary requirement of choline for pullets and older hens. Abbott and Demasters (1940) reported increased egg production with dietary choline. The suggestion that choline may be synthesized by layers was made by Lucas *et al.* (1946). He found no significant difference in egg production, hatchability, fertility or body weight when birds were on a purified diet with 0.03 per cent choline. Ringrose and Davis (1946) agreed with the findings of Lucas *et al.* (1946) and concluded that synthesis of choline occurred even under conditions of low choline and methionine intake. For the best egg production and feed efficiency Holmes and Kramer (1965) observed layers to require 965.6 mg choline per kg of feed. The data of Crawford *et al.* (1969) suggested a lower value of 595 mg choline per kg to maintain egg production.

MATERIALS AND METHODS

MATERIALS AND METHODS

One day old White Leghorn chicks obtained from the University Poultry Farm, Mannuthy were used for this investigation. The chicks were individually wing banded and distributed into three groups of comparable weight, receiving the control diet, choline deficient feed 1 and choline deficient feed 2 respectively. Each treatment consisted of replicated lots of 6 chicks housed separately in a brooder with 12 hours of natural photo period. The composition of basal diet used is given in Table 1.

Table 1. Percentage composition of the feed.

Feed ingredients	Per cent
Fish meal	10
Groundnut cake	28
Rice polish	30
Tapioca	30
Mineral mixture	2
Total	100

The feed was rendered deficient in choline by repeated extraction with ethyl alcohol. The residual alcohol in the feed was got rid of by keeping it in a hot air oven maintained at 80°C. The choline and fat content of the feed so prepared were assayed by the method of Glick (1944) and Jacobs (1958) respectively. Triplicate analysis were done for each sample. The choline and fat content was restored by the addition of choline chloride and hydrogenated vegetable oil.

The methods adopted for the analysis of proximate principles in the feed were similar to those described by Jacobs (1958). Triplicate analysis were done for each sample and the mean value recorded. The chemical composition of the feed is presented in Table 2.

Table 2. Chemical composition of the feed.

Constituent	Per cent
Crude protein	21.12
Ether extract	6.00
Crude fiber	5.64
Ash	5.91
Nitrogen free extract	61.33
Total	100.00

Vitamin supplements, other than choline, were added to the feed (Table 3) in the proportion recommended by NRC (1966).

Table 3. Vitamin supplements mixed with the feed.

Vitamins	Quantity added (per kg feed)	
Vitamin A	USP[1] units	2000.00
Vitamin D	ICU[2]	200.00
Vitamin K	mg	0.53
Thiamine	mg	1.80
Riloflavin	mg	3.60

(contd.)

Vitamins	Quantity added (per kg feed)	
Pantothenic acid	mg	10.00
Niacin	mg	27.00
Pyridoxin	mg	3.00
Biotin	mg	0.09
Folacin	mg	1.20
Vitamin B_{12}	mg	0.009

1-United States Pharmacopeoia.
2-International chick units.

The ration for control birds (Group 1) was prepared by adding choline chloride to the feed mixture to maintain a level of 1500 mg per kg of feed. Rations for experimental birds were prepared similarly except that the level of choline in group 2 was adjusted to 75 mg per kg and group 3,100 mg per kg of feed.

Feeding trials

Feed and water were provided ad libitum for all the groups. The feeding trial lasted for six weeks. Initial and weekly body weight of each bird and the weekly feed intake per group were recorded. From this data, weight gain and feed efficiency ratios were calculated.

The birds in each group were closely watched for the onset of deficiency symptoms, namely the development of lethargy, lameness, slipping of the gastrocnemius tendon and the enlargement of hock joint. At the end of the experimental period, the birds were sacrificed and detailed postmortem

examination was conducted.

Biochemical study

In the biochemical studies a weighed quantity of gastrocnemius muscle (from both control and experimental birds) was taken and tissue homogenate was prepared by following the method of Colowick and Kaplan (1963). The tissue homogenate was assayed for enzymes like acid phosphatase (ACP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), succinic dehydrogenase (SDH), phosphorylase and for chemical constituents like protein, phospholipids and creatine phosphate. The method of Bodansky (1932) was adopted for assessing the acid and alkaline phosphatases. The method given by Bergmeyer (1965) was followed for the estimation of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity. Phosphorylase was estimated by the method suggested by Cahill et al. (1957). Succinic dehydrogenase was assayed by adopting the method of Kun and Abood (1949) and protein by the method of Inchiosa (1964). Phospholipid and creatine phosphate were estimated by the methods of King and Wootten (1959) and of Ennor and Rosenberg (1952) respectively, and cholesterol, as described by John and George (1967). Ether extract was estimated on dehydrated sample of the gastrocnemius muscle by adopting the soxhlet's continuous extraction (Jacobs, 1958).

The data for all the birds in each group was pooled. In the statistical analysis of the data, analysis of variance was conducted (Snedecor and Cochran, 1968). To compare the means, multiple range test was used in which

a significance level of 5 per cent was followed.

Muscular efficiency

The muscular efficiency was determined by comparing the activity of isolated gastrocnemius muscle from perotic legs, eight from each experimental group (II and III) with those isolated from normal legs (group I). After removing the muscle retaining its attachment to the bone it was rinsed in normal saline. The femur was fastened to a muscle clamp fixed on a myograph stand and the tendon of the gastrocnemius muscle was tied to an isotonic muscle lever with after load screw. A weight of 5 g was suspended from the lever and the lever was adjusted to a horizontal position. The muscle was stimulated with a single effective break induced stimuli and the movements of the lever were recorded on a smoked kymograph paper. The movements of kymograph were adjusted to a constant speed of 0.24 m/sec. The points of stimulation, contraction, peak of contraction and relaxation were marked and a time tracing was taken to determine the latent, contraction and relaxation periods. The time taken by the muscle to get fatigued was studied by applying stimuli at regular intervals and recording the first three and every subsequent tenth contraction.

R E S U L T S

RESULTS

Body weight and weight gain

The average body weight and weight gain of 24 control (group 1) and 48 experimental birds (groups 2 and 3) from the first week to sixth week of feeding trial are presented (Table 4 and 5.)

There was noticeable difference in the body weight and weight gain of experimental and control birds from the first week of feeding trial. The values for experimental birds which were lesser in the first week gradually declined and was significantly lesser than the control in the sixth week.

Choline deficient birds of group 3 (100 mg of choline per kg of feed) had significantly higher values for body weight and weight gain in the sixth week ($P < 0.05$) than the birds of group 2 (75 mg of choline per kg of feed).

Feed consumption and feed efficiency

Pairwise comparison of the different treatments showed that group 2 consumed the lowest amount of feed compared to the other two groups. The difference between the groups 1 and 3 was not significant (Table 6).

The experimental groups 2 and 3 indicated lower feed efficiency ratios compared to that of group 1 (Table 6).

Symptoms

The birds in the control group (group 1, 1500 mg of choline per kg of

Table 4. Average body weight of the birds in g.

Period	Group 1 (Control) (24)	Group 2 (Experiment 1) (24)	Group 3 (Experiment 2) (24)
Initial	34.21	33.42	34.96
First week	43.17	38.71	40.04
Second week	65.75	50.42	54.38
Third week	102.00	68.71	73.92
Fourth week	149.91	90.58	96.25
Fifth week	200.33	116.25	130.17
Sixth week	257.83	155.88	172.67

Figures in parenthesis indicate number of birds in the group.

Table 5. Average weight gain of the birds in g.

Period	Group 1 (Control) (24)	Group 2 (Experiment 1) (24)	Group 3 (Experiment 2) (24)
First week	9.17	5.29	5.08
Second week	22.11	11.71	14.33
Third week	36.25	18.71	19.96
Fourth week	47.92	21.46	22.33
Fifth week	49.17	25.67	34.75
Sixth week	57.29	39.63	42.50

Figures in parenthesis indicates number of birds in the group.

Table 6. Average weekly feed consumption per bird and feed efficiency ratio.

Parameter	Group 1 (Control)	Group 2 (Experiment 1)	Group 3 (Experiment 2)
Feed consumption - g	115.95	106.50	113.35
Feed efficiency ratio	0.31	0.18	0.19

feed) were healthy, active, alert and bright eyed throughout the period of experiment. They had normal and unruffled feathers (Plate-I a). The experimental birds (group 2, 75 mg of choline and group 3, 100 mg of choline per kg of feed)were unthrifty in appearance and showed poorly developed and ruffled feathers (Plate-II c).

Symptoms of perosis were first noticed in 2 birds(of the experimental groups 2 and 3)on the 24th day of the feeding trial. In some birds, slipping of the gastrocnemius tendon from its condyle was noticed only on the right or left leg - unilateral (Plate-I b) and in the other birds in both legs (Plate-II c).

Out of the 48 birds in group 2 and 3, 12 birds showed perosis of the right leg, 24 birds of the left leg and the rest 12 of both legs between the 24th and 42nd day of the experiment (Table 7). The time of onset of perosis was not different in the two groups. The development of lamenese synchronised with the slipping of the tendon (Plate-IIa). The perotic birds had difficulty in walking and were found sitting on their hocks. The hock joints were enlarged in the affected birds (Plate-IIb).

Table 7. Incidence of slipped tendon in choline deficient birds (group 2 and 3).

Day	Left leg	Right leg	Both legs
24th	-	1	1
25th	-	1	-
26th	3	1	2
27th	4	-	-
28th	1	1	1
29th	1	-	-
30th	1	-	-
31st	1	1	-
32nd	1	1	-
35th	1	-	-
36th	1	-	-
37th	1	1	1
38th	2	1	1
39th	2	1	1
40th	2	2	1
41st	2	1	3
42nd	1	-	1
Total	24	12	12

Biochemical studies

The results of the quantitative analysis of the chemical constituents of the gastrocnemius muscle of the control and experimental birds (perotic muscle) are given in Table 8.

Analysis of the data showed significant differences in the protein, lipid, cholesterol, creatine phosphate and phospholipid content of the gastrocnemius muscle between the control and the experimental birds.

The activities of phosphorylase, SDH, ALP, ACP, GOT and GPT were also significantly higher ($P<0.05$) in the control. Among the three groups, muscles of group 2 birds had the least cholesterol GOT, ACP and phospholipid values ($P<0.05$). Groups 2 and 3 were similar in the concentration of protein, lipid and creatine phosphate and the activities of SDH, ALP and GPT in the gastrocnemius muscles.

Muscular efficiency

The latent, contraction and relaxation periods of the gastrocnemius muscle of control birds were found to be 0.03 seconds, 0.05 seconds and 0.65 seconds respectively (Plate IIIa). The perotic muscles from group 2 birds had a latent period of 0.04 seconds, contraction period of 0.15 seconds and relaxation period of 0.28 seconds (Plate III b). The latent period for the contraction of muscle from group 3 birds was 0.035 seconds; the contraction period of the muscle was 0.10 seconds and the relaxation period, 0.24 seconds (Plate-IIIc).

The height of contraction was least for the muscle from group 2 birds.

In experimental birds the latent, contraction and relaxation periods were more than that of control. Group 2 birds had prolonged latent, contraction and relaxation periods in comparison to group 3.

Among the three groups the time required for the onset of fatigue was found to be lesser for group 2 muscles than group 1 and 3 (Plate IV a, b, and c).

Table 8. Average chemical composition of gastrocnemius muscle of chicks.

Group	Protein %/100 g tissue	Total lipids % dry wet	Cholesterol mg per 100 g wet tissue	Creatine phosphate g/100 g tissue	Phospho lipids mg/100 mg	SDH U/mg	Phosphorylase µg P/10 mg muscle tissue	GOT W.U/mg	GPT W.U/mg	ACP B.U/g	ALP B.U/g
Group 1 (Control)	20.38	1.51	231.83	0.22	6.22	0.88	300.45	41.88	7.24	2.43	3.42
Group 2 (Experiment 1)	18.51	2.36	137.21	0.17	3.32	0.46	156.46	20.03	2.83	1.69	2.61
Group 3 (Experiment 2)	18.39	2.42	148.00	0.16	3.63	0.48	187.17	25.90	3.97	2.07	2.72

D I S C U S S I O N

DISCUSSION

Feeding trial

Sebrell and Harris (1954), Houser (1955), Ewing (1963), Parkhurst (1967), Scott and Krook (1972) and Surendranathan (1974) established that deficiency of choline exerted a depressing effect on the growth of chicks. Addition of choline to a practical poultry ration increased chick growth (Gerry et al.,1948). These findings were in accordance with the reports of Gill and Norris (1951). The poor weight gain and feed efficiency ratios observed in birds of group 2 and 3 and better performance of birds in group 1 are in agreement with the above finding that choline deficiency retards growth. The similarity of feed consumption values for groups 1 and 3 suggested that choline has no influence in the palatability of feed. The difference in feed consumption between groups 2 and 3 and the lowest value for group 2 are proportional to the growth rate.

The present observation that the feed efficiency ratios for control birds (group 1) were higher than that for choline deficient birds (group 2 and 3) accorded well with the finding of Sherwood and Sloan (1954). They found that added choline improved growth rate and feed efficiency.

All the birds in the choline deficient groups 2 and 3 showed symptoms of perosis. Ewing (1963) studied the requirement of choline for prevention of perosis and for growth. He concluded that perosis in chicks developed only when the level of choline in the diet was too low, much lower than that required for growth in choline deficient ration. The experimental

birds in the present study (group 2 and 3) showed symptoms of perosis. This was consistent with the observation of Ewing (1963).

Symptoms of perosis was noticed on the 24th day of feeding trial. Though all birds developed symptoms during the experimental period, considerable variation occurred in the time of onset. This is in agreement with the finding of Surendranathan (1974) who reported that chicks have individual variation in their sensitivity to choline deficiency.

Biochemical study

There is scarcity of literature with regard to the effect of choline deficiency on the chemical composition of skeletal muscle of chicks. The low protein content of the gastrocnemius muscle of choline deficient birds (group 2 and 3) indicated that choline has influence in the total protein content of muscle. Dickerson and Widdowson (1960) reported that protein content of muscle increased during the development. The lowered protein value obtained in the present study might be due to an indirect influence of choline through its effect on growth rate. It is still not known whether perosis is another type of muscular dystrophy. Young and Dinning (1951), Weinstock and Lukacs (1964) and Peterson <u>et al.</u> (1963) reported a reduction of protein content of muscle in muscular dystrophy.

Herrmann (1946) reported that supplementing the diet of old laying hens with choline reduced the total and ester cholesterol levels in liver, heart and muscle. In the present study on the effect of choline deficiency on cholesterol content of gastrocnemius muscle, the cholesterol value was

found to be lowest in group 2 (choline deficient diet - 75 mg choline per kg of feed) and highest in group 1 (control - 1500 mg choline per kg of feed). That hypocholesterolemia occurred in choline deficiency was reported by Hough et al. (1943) in puppies, Tinoco et al. (1964) in rats and Surendranathan (1974) in chicks. Surendranathan (1974) studied the effect of raising the level of choline from 1500 mg to 3000 mg per kg of feed and stated that there was an optimum dietary level of choline in chicks for maintenance of blood cholesterol level and any additional increment of choline had no effect on the blood cholesterol level. The difference in age of the experimental birds might be responsible for the observation of Herrmann (1946) that no variation occurred in cholesterol level. The low cholesterol content of muscle was attributed to the low cholesterol level of blood in choline deficiency. The lowered cholesterol content shown in choline deficient birds in this study coincided with the results obtained by Embden and Lawaczeck (1923). They reported that the cholesterol content of muscle varied with the activity of the muscle. Perotic muscle was less active compared to that of the control. This was in agreement with the findings of John and George (1967) and Jacob (1971) that a decreased activity of the muscle reduced its cholesterol level.

In the present study fat content of the perotic muscle was found to be more than that of the control. There was no significant difference between groups 2 and 3 in this respect. The increased fat content might be attributed to lack of choline, the effect being similar to that occurring in liver.

West et al. (1966) observed that the synthesis of phospholipids in the body depends on the availability of choline, either by synthesis in the body

or through dietary sources. They further stated that choline is a dietary essential in birds, synthesis in the body being inadequate and that they have to depend, to a large measure on dietary choline for phospholipid synthesis. The low phospholipid value for skeletal muscle of perotic chicks in the present experiment accords well with the above findings.

A lowered value for creatine phosphate, in both choline deficient groups 2 and 3 indicated that creatine synthesis in muscle was disturbed as a result of choline deficiency. Barrenscheen and Pany (1948) also reported similar observations. They established that creatine is formed in muscle by methylation directly from methionine and indirectly from choline.

Compared to the control birds phosphorylase activity was lower in choline deficient birds (groups 2 and 3). Stetten and Stetten (1960) studied the role of phosphorylase in the utilisation of glycogen. They concluded that phosphorylase was essential for the phophorylative degradation and utilisation of glycogen. They also stated that the phosphorylase activity in a tissue would indicate the rate of glycolysis. Krebs and Fisher (1955) stated that phophorylase exists in the muscle in two forms, the active form 'a' and the inactive form 'b', of which the active form predominates in a contracting muscle. Rulon <u>et al</u>. (1961) showed that the active form 'a' increases during contraction of the muscle and decreases during rest. Surendranathan (1974) could not find any alterations in the glycogen content of perotic muscle of chicks. The lowered phosphorylase and reduced muscular activity in perotic muscle noticed in the present study suggested that its activity is proportional to the activity of the muscle.

Lowered GOT and GPT activities in choline deficient birds might be an indication of the role of choline in protein metabolism and its indirect influence on the growth and positive nitrogen balance.

The SDH activity of the gastrocnemius muscle was reduced in both choline deficient groups 2 and 3. This result is in agreement with the finding of Surendranathan (1974). That the level of SDH activity is an index of its capacity for oxidative metabolism and is the key enzyme in the krebs cycle have been reported by George and Talesara (1961) and George and Bergar (1966). They stated that a fall in SDH activity might point to an interference in the fat metabolism. It was concluded by them that the lowered SDH activity of perotic muscle was due to its reduced activity.

A decrease in phosphatase activity (ACP and ALP) of the muscle in choline deficient birds (groups 2 and 3) might be due to a reduction in the synthesis of these enzymes in it. Choline derivatives form part of the prosthetic group of phosphatases (Kutscher and Seig, 1950). It is interesting to note that choline deficiency reduced the muscular activity in general and therefore it directly or indirectly lowered the chemical constituents and enzyme activity.

Muscular efficiency

There is not much information with regard to the effect of choline deficiency in muscular efficiency. The prolonged relaxation period obtained in the choline deficient muscle indicated the poor efficiency and decreased activity of the perotic muscle. The early onset of fatigue of perotic muscle might be due to a reduction in the availability of energy providing material.

S U M M A R Y

SUMMARY

The effects of choline deficiency on the body weight, feed efficiency and chemical composition of the skeletal muscles of White Leghorn chicks were studied.

Choline was incorporated to a choline extracted ration which was otherwise normal, at the rate of 1500 mg, 75 mg and 100 mg per kg and formed the three treatments. Each treatment consisted of replicated lots of 6 chicks which were housed seperately in a brooder. The birds were sacrificed when they exhibited symptoms of perosis. Chemical composition, enzyme activity and muscular efficiency of the gastrocnemius muscles were studied.

The data collected revealed the following

1. The birds fed on 75 mg and 100 mg levels of choline showed a decrease in both feed efficiency and feed utilization and rate of weight gain when the performance of these birds were compared to those which received 1500 mg of choline/kg of feed.

2. Choline deficient chicks were unthrifty, and had poorly developed and ruffled feathers. All the birds on choline deficient diets exhibited symptoms of perosis, either unilateral or bilateral starting from 24 days of age. Pronounced enlargement of the hock joint and displacement of the gastrocnemius tendon were observed.

The following observations were made on the gastrocnemius muscle of chicks

1. Lowered values for creatine phosphate, phospholipids and increased

value for fat content in the gastrocnemius muscle of perotic chicks were recorded.

2. The comparatively lower activity of GOT, GPT and lower protein values in choline deficient chicks could be attributed to the lowered protein turn over in the birds and the resultant poor growth rate.

3. Since gastrocnemius muscle is less active in choline deficient birds the phosphorylase activity is also lowered.

4. Reduction in SDH activity of the gastrocnemius muscle in choline deficiency might be the result of decreased oxidative metabolism and tonus activity of the red fibers in it.

5. Choline deficient chicks revealed significantly lower values for ALP and ACP. An interference with the synthesis of the prosthetic group concerned might be the reason for this.

6. The affected muscles showed poor muscular efficiency, as revealed by a prolonged relaxation time.

R E F E R E N C E S

REFERENCES

Abbott, O.D., and Demasters, C.U. (1940). Choline in the Diet of Chickens. J. Nutr. 19 : 47 - 55.

Alexander, H.D., and Engel, R.W. (1952). The Importance of Choline in the Prevention of Nutritional Oedema in Rats Fed Low Protein Diets. J.Nutr. 47 : 361 - 373.

Artom, C. (1960). Mechanism of Action of Choline. Am. J. Clin. Nutr. 8 : 303 - 305.

Babo, L.V., and Hirschbrunn, M. (1852). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in : "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd vol. pp. 1 - 129.

Baeyer, A. (1866). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Baeyer, A. (1867). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Balloun, S.E., and Miller, D.L. (1964). Choline Requirements of Turkey Breeder Hens. Poult. Sci. 43 : 64 - 67.

Barrenscheen, H.K., and Pany, J. (1948). Cited by Sebrell, W.H.Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Best, C.H., Ferguson, G.C., and Hershey, J.M. (1932). Choline and Liver Fat in Diabetic Dogs. J. Physiol. 79 : 94 - 102.

Best, C.H., Hershey, J.M., and Mutzman, H.H. (1933). Cited by Sebrell, W.H.Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Pres, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Best, C.H., and Ridout, J.H. (1933). The Effects of Cholesterol and Choline on Deposition of Liver Fat. J. Physiol. 78 : 415 - 448.

Bergmeyer, H.U. (1965). Methods of Enzymatic Analysis. Academic Press., New York. 1st Ed. pp. 837 - 858.

Bird, H.R., Almquist, H.J., Clandiner, D.R., Cravens, W.W., Hill, F.W., and McGinnis, J. (1966). Nutrient Requirements of Poultry. National Research Council Publication. pp. 1345.

Bodansky, (1932). Cited by Oser, B.L. (1965). in: "Hawk's Physiological Chemistry". The Blackiston Division McGraw Hill Book Company, New York. 14th Ed. pp. 1118 - 1121.

Boxer, G.L., and Stetten, D., Jr. (1944). The Effect of Dietary Choline upon the Rate of Turnover of Phosphatide Choline. J. Biol. Chem. 153 : 617 - 625.

Briggs, G.M., Hill, E.G., and Canfield, T.H. (1953). Cited by Luck, J.M., Loring, H.S., and Mackinney, G. (1955). in: "Annual Review of Biochemistry". Annual Reviews, Inc. Stanford, California, U.S.A. 16th Vol.

Cahill, G.F. Jr., Ashmore, J., Zottu, S., and Hastings, A.B. (1957). Studies on Carbohydrate Metabolism in Rat Liver Slices. IX Ionic and Hormonal Effects on Phosphorylase and Glycogen. J. Biol. Chem. 224 : 237 - 250.

Chevremont, M. (1945). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in : "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Claus, A., and Keese, C.J. (1867). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Colowick, S.P., and Kaplan, N.O. (1963). Methods in Enzymology. Academic Press, Inc. New York. Ist Ed.

Crawford, J.S., Griffith, M., Teekell, R.A., and Watts, A.B. (1969). Choline Requirement and Synthesis in Laying Hens. Poult. Sci. 48 : 620 - 626.

Creek, R.D. (1959). Perosis and Enlarged Hocks. Poult. Sci. 38 : 1196.

Cumming, R.B., and Tribe, D.E. (1956). The Effect of Floor Type and the Choline Content of the Diet upon the Incidence of Perosis in Chickens. J. Agric. Sci. 47 : 103 - 106.

Davies, J.M. (1939). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

daSilva, A., Mario, F., Guerios, M., and Monsao, S.R. (1959). The Domestic Cat as a Laboratory Animal for Experimental Nutrition Studies VI. Choline Deficiency. J. Nutr. 67 : 537 - 547.

* Dickerson, J.W.T., and Widdowson, E.M. (1960). Chemical Changes in Skeletal Muscle during Development. Biochm. J. 74 : 247.

Doyle, L.P. (1931). The Differential Diagnosis of Leg Weakness in Chicks. Poult. Sci. 10 : 393 - 395.

Dybkowsky, W. (1867). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Embden, G. and Lawaczeck, H. (1923). Cited by Jacob, E.T. (1971). in: "An Enquiry into Certain Biochemical Aspects of the Skeletal Muscles of Chicken and Ducks". M.Sc. Thesis, University of Calicut. pp. 41 - 46.

Ennor and Rosenberg. (1952). Cited by Oser (1965). Hawk's Physiological Chemistry. The Blakiston Division, McGraw - Hill Book Company, New York. 14th Ed. pp. 1118 - 1121.

Engel, R.W. (1941). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Engel, R.W. (1943). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Esh, G.C., and Som, J.M. (1954). Efficacy of Variable Choline Salts in Maintaining Normal Liver and Kidney in Rats. Indian J. Physiol. 8 : 75 - 79.

Ewing, W.R. (1963). Poultry Nutrition. The Ray Ewing Company, Pasadena, California. 5th Ed.

Flower, R.J., Pollitt, R.J., Sanford, P.A., and Smyth, D.H. (1972). Metabolism and Transfer of Choline in Hamster Small Intestine. J. Physiol. 226 : 473 - 489.

Fouts, P.J. (1943). Vitamin B Complex Studies in Dogs - Production of Cirrhosis of Liver. J. Nutr. 25 : 217 - 228.

Fritz, J.C., Roberts, T. and Boehne, J.K. (1967). The Chick's Response to Choline and its Application to an Assay for Choline in Feedstuffs. Poult. Sci. 46 : 1447 - 1454.

Gerry, R.W., Carrick, C. ., and Hauge, S.M. (1948). Methionine and Choline in a Simplified Chick Ration. Poult. Sci. 27 : 161 - 168.

George, J.C.,and Berger, A.J. (1966). Avian Myology. Academic Press, New York. Ist Ed. pp. 124 - 151.

George, J.C., and Talesara, C.L. (1961). The Succinic Dehydrogenase Levels in the Pectoral Muscles of a Few Representative Types of Birds and a Bat in Relation to the Fibre Diameter, Muscle Weight and Body Weight. Comp. Biochem. Physiol. 3 : 267 - 273.

Gerlach, H., Gylstorff, I., and Dammert, S., and Griessler, H. (1975). Lipids in Liver and Serum of White Leghorns given Different Amounts of Methionine, Choline and Vitamin B_{12}. Poult. Abstr. 1. pp. 22.

Gillis, W.D., and Norris, L.C. (1951). The Effect of Vit. B_{12} on the Response of Chicks to Betaine and Choline. J. Nutr. 43 : 295 - 302.

* Glick, D. (1944). Concerning the Reineckate Method for the Determination of Choline. J. Biol. Chem. 156 : 643 - 651.

Gogus, K., and Griminger, P. (1957). On the Vitamin Requirements of Poults. II Choline. Poult. Sci. 36 : 1121 - 1130.

Griffith, W.H. (1948). Choline Metabolism. IV. The Relation of Age, Weight and Sex of Young Rats to the Occurrence of Haemorragic Degeneration on a Low Choline Diet. J. Nutr. 19 : 437 - 448.

Griffith, W.H., and Wade, N.J. (1939). Choline Metabolism. I. The Occurrence and Prevention of Haemorrhagic Degeneration in Young Rats on a Low Choline Diet. J. Biol. Chem. 131 : 567 - 577.

Griffith, W.H., and Mulford, D.J. (1941). Choline Metabolism. IV. Haemorrhagic Degeneration and the Labile Methyl Supply. J. Am. Chem. Soc. 63 : 929 - 932.

Harris, R.S., and Thimann, K.N. (1943). Vitamins and Hormones. Academic Press. Inc. Publishers, New York. Ist Vol. pp. 1 - 51.

Hartroft, W.S., and Best, C.H. (1947). Cited by Sebrell, W.H.Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Hegsted, D.Mark., Mills, R.C., Elvehjem, C.A., and Hart, E.B. (1941). Choline in the Nutrition of Chicks. J. Biol. Chem. 138 : 459 - 466.

Herrmann, G.R. (1946). Effect of Choline on Blood and Tissues with Special Reference to Cholesterol in Old Hens. Proc. Soc. Exp. Biol. Med. 61 : 302 - 306.

Heuser, G.F. (1955). Feeding Poultry. John Wiley and Sons, Inc. New York-2. Ist Ed. pp. 39.

Hofstad, M.S., Calnek, B.W., Helmboldt, C.F., Reid, W.M. and Yoder, H.W., Jr. (1972). Diseases of Poultry. The Iowa State University Press. Ames., 6th Ed. pp. 72 - 73.

Holmes, C.E., and Kramer, C.Y. (1965). Vitamin B_{12}, Choline and Methionine in Laying Diets. Poult. Sci. 44 : 1381 - 1383.

* Hoskins, H.P., Lacroix, J.V., and Mayer, K. (1953). Canine Medicine. American Veterinary Publications, Inc. Illinois. Ist Ed. pp. 189.

Hough, V.H., Monahan, J.P., Li, T.J., and Freeman, S. (1943). The Effect of Choline and Cystine on the Serum Phosphatase and Hepatic Dye Clearance of Dogs Maintained on Deficient Diets. Am. J. Physiol. 139 : 642 - 651.

Hove, E.L., and Copeland, D.H. (1954). Progressive Muscular Dystrophy in Rabbits as a Result of Chronic Choline Deficiency. J. Nutr. 53: 391 - 405.

Hove, E.L., Copeland, D.H., Herndon, J.F., and Salimon, W.D. (1957). Further Studies on Choline Deficiency and Muscular Dystrophy in Rabbits. J. Nutr. 63 : 289 - 299.

Hew, B., Aderson, E.G., Weiss, S., and Stekol, J.A. (1955). Cited by Luck, J.M., Loring, H.S., and Mackinney, G. (1955). in: "Annual Review of Biochemistry". Annual Reviews. Inc. Stanford, California, U.S.A. 16th Vol.

Inchiosa, M.A. (1964). Biuret Assay for Tissue Homogenate. J. Lab. Clin. Med. 63 : 319.

Jacob, M.B. (1958). The Chemical Analysis of Foods and Food Products. D.Van. Nostrand Company. Inc. New York. 3rd Ed.

Jacob, E.T. (1971). An Enquiry into Certain Biochemical Aspects of the Skeletal Muscles of Chicken and Ducks. M.Sc. Thesis. University of Calicut. pp. 41 - 46.

Jolliffe, N.M.D. (1957). Vitamin Manual. The Up John Company. Kalamazoo. Michigan. pp. 57 - 59.

* John, T.M., and George, J.C. (1967). Seasonal Variation in Cholesterol Level in the Migratory Starting. Sturnus roseus. Ravo. 5 : 29.

Johnson, B.C., Mitchell, H.H., Pinkos, J.G., and Morril, C.C. (1951). Choline Deficiency in the Calf. J. Nutr. 43 : 37 - 48.

Jukes, T.H. (1940). Prevention of Perosis by Choline. J. Biol. Chem. 134 : 789 - 790.

Jukes, T.H. (1941). Studies of Perosis in Turkeys. I. Experiments Related to Choline. Poult. Sci. 70 : 751.

Jukes, T.H. (1942). Cited by Harris, R.S., and Thimann, K.N. (1943). in: "Vitamins and Hormones". Academic Press, Inc. Publishers, New York. Ist Vol. pp. 1 - 51.

Jukes, T.H. (1947). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Kelly, W.W., and Myerhof, O. (1950). Studies on Adenosine Triphosphatase of Muscle. III. The Lipoprotein Nature of the Magnesium Activated Adenosine Triphosphatase. J. Biol. Chem. 183 : 391 - 401.

Ketola, H.G., and Young, R.J. (1973). The Need for Dietary Choline by Young Japanese quail. Poult. Sci. 52 : 2362 - 2367.

Ketola, H.G., and Nesheim, M.C. (1974). Influence of Dietary Protein and Methionine Levels on the Requirements for Choline by Chicken. J. Nutr. 104 : 1484 - 1489.

* King, E.J., and Wootton, I.P.D. (1959). Microanalysis in Medical Biochemistry. J & A. Churchill Ltd., 104 Gloucester Place. W.1. 3rd Ed.

Klien, G., and Linser, H. (1932). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Krebs, E.G., and Fisher, E.H. (1955). Phosphorylase Activity of Skeletal Muscle Extracts. J. Biol. Chem. 216 : 113 - 120.

Kummerow, F.A., Weaver, R. and Boustead, H. (1949). Cited by Sutton, J.B., Pasvogel, M.W., Kemmerr, A.R., and Varich, M.G. (1957). in: "The Influence of Choline and Methionine on the Deposition of Fat in the Liver of the Mature Laying Hen. Poult. Sci. 36 : 1161 - 1168.

* Kun, E. and Abood, L.G. (1949). Colorimetric Estimation of SDH by Triphenyl Tetrazolium Chloride. Science. 109 : 144.

Kutscher, W., and Sieg, H. (1950). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Latshaw, J.D., and Jensen, L.S. (1972). Choline Deficiency and Synthesis of Choline from Precursors in Mature Japanese quail. J. Nutr. 102 : 749 - 755.

Liebreich, O. (1865). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Lucas, H.L., Norris, I.C., and Heuser, C.F. (1946). Observations on the Choline Requirements of Hens. Poult. Sci. 25 : 373.

Luck, J.M., Loring, H.S., and Mackinney, G. (1955). Annual Review of Biochemistry. Annual Reviews, Inc. Stanford, California, U.S.A. 24th Vol.

Marks, J. (1968). The Vitamin in Health and Diseases - a Modern Reappraival J and A Churchil Ltd., 104, Gloucester Place, London, W-1.

Marquez, V.M. (1942). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

McIntire, J.M., Schiveigert, B.S., and Elvehjem, C.A. (1944). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1-129.

Meader, R.D., and Williams, W.L. (1957). Choline Deficiency in the Mouse. Am. J. Anat. 100 : 167 - 203.

Mouserrat, A.J., Hamilton, F., Ghoshal, A.K., Porta, E.A., and Hartroft, W.S. (1973). Lysosomes in the Pathogenesis of the Renal Necrosis of Choline Deficient Rats. Am. J. Path. 8 : 113 - 146.

Moore, H.C. (1957). The Acute Renal Lesions Produced by Choline Deficiency in the Male Weanling Rat. J. Path. Bact. 74 : 171 - 184.

Nachmansoln, D., Machado, A.L. (1943). Cited by Sebrell, W.H. Jr., and Harris, R.S.(1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Nagler, A.L., Dettabrn, W.D., Seifter, E., and Levenson, S.M. (1968). Tissue Levels of Acetyl Choline and Acetyl Cholinesterase in Weanling Rats Subjected to Acute Choline Deficiency. J. Nutr. 94 : 13 - 15.

Nagler, A.L., Baez, S., and Levenson, S.M. (1969). Status of Microcirculation During Acute Choline Deficiency. J. Nutr. 97 : 232 - 236.

Neuberne, P.M., Bresnahan, M.R., and Kula, N. (1969). Effects of Two Synthetic Antioxidants Vit. E. and Ascorbic Acid on the Choline Deficient Rats. J. Nutr. 97 : 219 - 231.

Neumann, A.L., Krider, J.I., James, M.P., and Jonson, B.C. (1949). The Choline Requirement of the Baby Pig. J. Nutr. 38 : 195 - 203.

Oser, B.L. (1965). Hawk's Physiological Chemistry. The Blakiston Division McGraw-Hill Book Company. New York. 14th Ed. pp. 1118 - 1121.

Ostryanina, A.D. (1975). Content of Total and Esterified Cholesterol in Liver Tissue and Blood During Prolonged Choline - Protein Deficiency. Chem. Abstr. 83 : 7783.

Parkhurst, R.T. (1967). Commercial Broiler Production. U.S. Department of Agricultural Research Service, Washington. pp. 35.

Passanti, G.T., Guerrant, N.B., and Thompson, R.C. (1958). Effect of Supplementary Methionine and Choline on Tissue Lipids and on the Vascular Structure of Cholesterol Fed Growing Rats. J. Nutr. 66 : 55 - 74.

Patek, A.J.Jr., Subhash, D., and Hayes, K.C. (1975). Cirrhosis of Choline Deficiency in the Rhesus Monkey. Possible Role of Dietary Cholesterol. Proc. Soc. Exp. Biol. Med. 148 : 370 - 374.

Pattrick, K.H.R., and Felton, S.R. (1967). Methionine and Choline Supplementation on Diets for Laying Hens. Rec. Agric. Res. 16 : 69 - 74.

Peterson, J.M., Keevil, N.B., and McHenry, B.W. (1944). Choline and Prevention of Haemorrhagic Kidneys in the Rats. II Phospholipid Turnover Determined with Radio Active Phosphorus. J. Biol. Chem. 153 : 489 - 493.

Potter, L.M. and Kelly, K. (1973). Added Fat, Methionine, Choline and other Vitamins on Variables in Diets of Young Turkeys. Poult. Sci. 52 : 2075 - 2079.

Reid, M.M. (1955). Nutritional Studies with the Guinea Pig. III Choline. J. Nutr. 56 : 215 - 229.

Ringrose, R.C., and Davis, H.A. (1946). Choline in the Nutrition of Laying Hens. Poult. Sci. 25 : 646 - 649.

Roberts, T., and Frits, J.C. (1968). Specificity of the Chicks Requirement for Choline. Poult. Sci. 47 : 1711 - 1714.

Rohse, W.G., and Searle, G.W. (1955). Absorption of Choline from Intestinal Loops in Dogs. Am. J. Physiol. 18 : 207 - 209.

Roman, W. (1930). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Rulon, R.R., Schottelius, D.D., and Schottelius, B.A. (1961). Effect of Stimulation on Phosphorylase Levels of Excised Anterior Tibial Muscles of the Mouse. Am. J. Physiol. 200 : 1236 - 1238.

Sadhu, D.P. (1948). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Schafer, A.F., Salmon, W.D., and Strength, D.R. (1949). Interrelationship of Vitamin B_{12} and Choline. II Effect on Growth of the Chick. Proc. Soc. Exp. Biol. Med. 71 : 202 - 204.

Schexnailder, R., and Griffith, M. (1973). Liver Fat and Egg Production of Laying Hens as Influenced by Choline and Other Nutrients. Poult. Sci. 52 : 1188 - 1194.

Scott, M.L., and Krook, L. (1972). Cited by Hofstad, M.S., Calnek, B.W., Helmboldt, C.F., Reid, W.N., Yoder, H.W. Jr. (1972). in : "Diseases of Poultry". The Iowa State University Press. Ames. 6th Ed. pp. 72 - 73.

Sebrell, W.H. Jr., and Harris, R.S. (1954). The Vitamins. Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Seifter, E., Rettura, G., Seifter, J., Megler, A., and Levenson, S.M. (1971). Gall Bladder Distension in Choline Deficient Chicks. J. Nutr. 101 : 1233 - 1240.

Sherwood, D.H., and Sloan, H.J. (1954). Vitamin B_{12} and Choline in Corn - Soy rations for Starting Poults. Poult. Sci. 33 : 1015 - 1021.

Snedecor, G.W., and Cochran, W.G. (1968). Statistical Methods. Oxford and IBH Publishing Co., Calcutta. 6th Ed. pp. 258 - 280.

Stetten, D. Jr., and Stetten, M.R. (1960). Glycogen Metabolism. Physiol. Rev. 40 : 505 - 537.

Strecker, A. (1849). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Strecker, A. (1862). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamins". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Surendranathan, K.P. (1974). Studies on Certain Biochemical Aspects of Thiamine, Riboflavin and Choline Deficiency in Poultry. Ph.D. Thesis University of Calicut. pp. 82 - 122.

Swenson, M.J. (1970). Duke's Physiology of Domestic Animals. Comstock Publishing Associates, Ithaca. 8th Ed. pp. 634 - 663.

Sutton, J.B., Pasvogel, M.W., Kemmer, A.R., and Varich, M.G. (1957). The Influence of Choline and Methionine on the Deposition of Fat in the Liver of the Mature Laying Hen. Poult. Sci. 36 : 1161 - 1168.

Tinoco, J., Shannon, A., and Lyman, R.L. (1964). Serum Lipids in Choline Deficient Male and Female Rats. J. Lipid. Res. 5 : 57 - 62.

Weinstock, I.M., and Lukacs, M. (1964). Enzyme Studies in Muscular Dystrophy IV. Acid Deoxyribonuclease in Nutritional and Heriditory Muscular Dystrophy. Proc. Soc. Exp. Biol. Med. 145 : 716 - 718.

Welch, A.D. (1936). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). inL "The Vitamin". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

West, J.W., Carrick, C.W., Hauge, S.M., and Mertz, E.T. (1951). The Relationship of Choline and Cystine to the Methionine Requirement of Young Chickens. Poult. Sci. 30 : 880 - 885.

West, E.S. Todd, W.B., Mason, H.S., and VanBruggen, J.T. (1966). Text

Book of Biochemistry. The MacMillian Company, New York. 4th Ed. pp. 959 - 1198.

Wolbach, S.B., and Hegsted, D.M. (1953). Perosis. Epiphyseal Cartilage in Choline and Manganese Deficiences in the Chick. Arch. Path. 56 : 437 - 453.

Wolford, J.H., and Polin, D. (1975). Effect of Inositol, Lecithin, Vitamins (B_{12} with Choline and E) and Iodinated Casein on Induced Fatty Liver - Haemorrhagic Syndrome in Laying Chickens. Poult. Sci. 54 : 981 - 997.

Wurtz, A. (1867). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamin". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Wurtz, A. (1868). Cited by Sebrell, W.H. Jr., and Harris, R.S. (1954). in: "The Vitamin". Academic Press, Inc. Publishers, New York. 2nd Vol. pp. 1 - 129.

Young, J.M., and Dinning, J.S. (1951). A Relationship of Vitamin E to Nucleic Acid Metabolism. J. Biol. Chem. 193 : 743 - 745.

* Original not consulted.

I L L U S T R A T I O N S

Plate - Ia

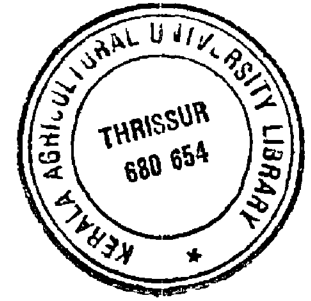
Control chick - Normal in health vigour and alertness.

Plate - I b

Choline deficient chick - exhibiting unilateral perosis.

170021

Plate - I



a

b

Plate - II a

Choline deficient chick - showing slipping of the tendon of gastrocnemius muscle.

Plate - II b

Choline deficient chick with enlarged hock joints.

Plate - II c

Choline deficient chick - showing bilateral perosis and ruffled feathers.

Plate - II

170021

a

b

c

Plate - III a

Simple muscle twitch - control
(group 1)

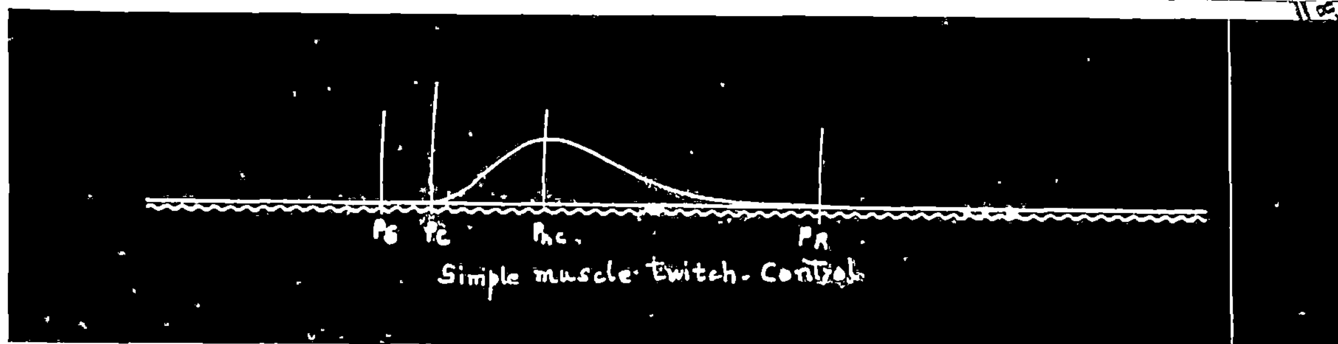
Plate - III b

Simple muscle twitch - Experiment 1
(group 2)

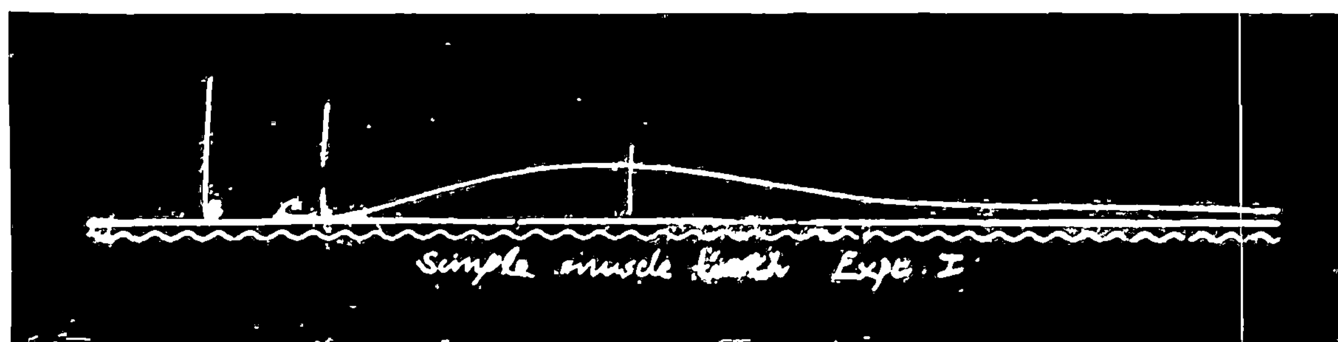
Plate - III c

Simple muscle twitch - Experiment II
(group 3)

Plate - III



a



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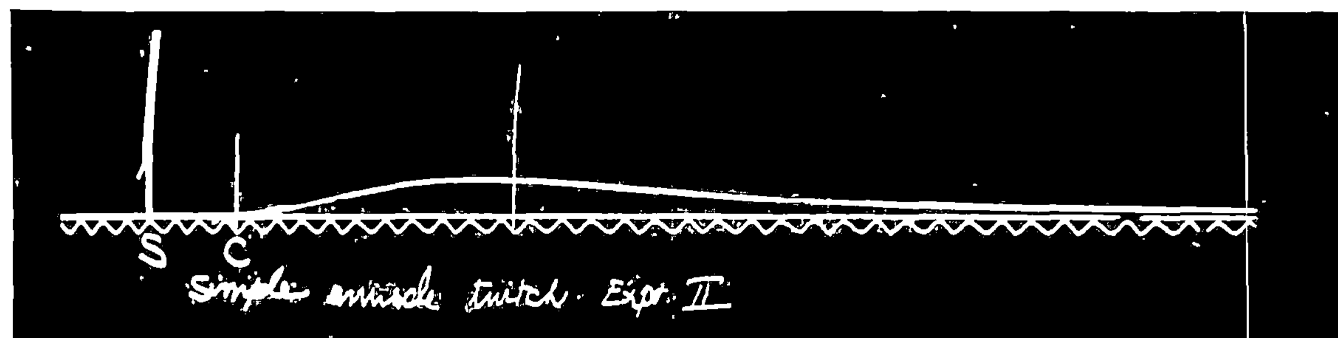


c

Plate - IV a

Fatigue - Control (group 1)

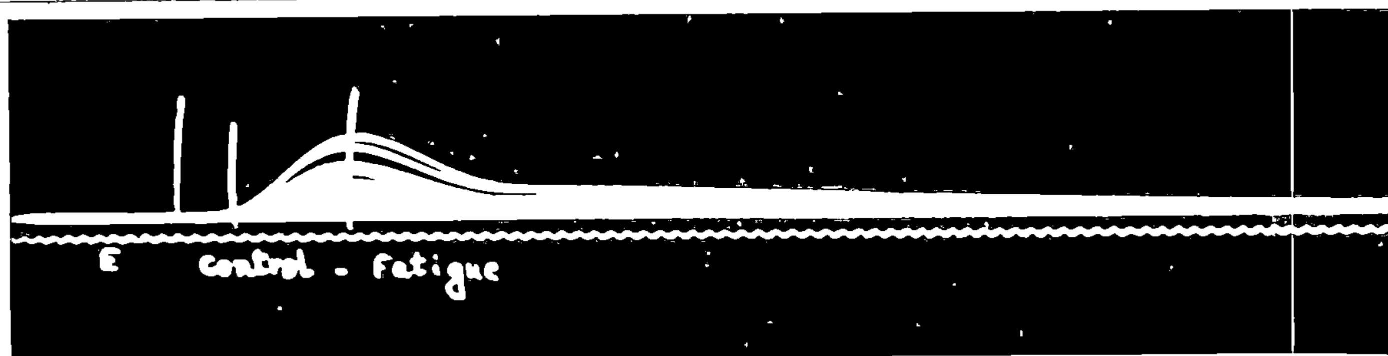
Plate - IV b

Fatigue - Experiment I (group 2)

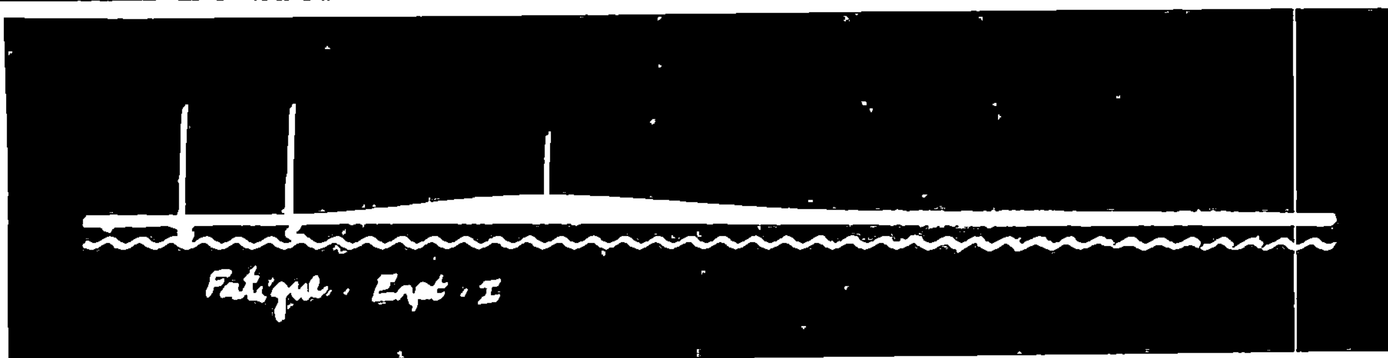
Plate - IV c

Fatigue - Experiment II (group 3)

Plate - IV



a



b

c

A P P E N D I X

APPENDIX

Table 1. Initial weight, weekly weight and weight gains (g) of the chicks in group I (control)

		Weight						Weight gain					
Sl. No.	Initial	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	First week	Second week	Third week	Fourth week	Fifth week	Sixth week
1	36	38	55	95	160	220	300	2	17	40	65	60	80
2	35	50	90	143	205	270	320	15	40	53	62	65	50
3	35	38	70	108	155	230	280	3	32	38	47	75	50
4	34	38	60	90	125	205	280	4	22	30	35	80	75
5	35	40	70	110	150	205	250	5	30	40	40	55	45
6	36	41	60	105	148	192	260	5	19	45	43	44	68
7	29	38	55	95	152	200	245	9	17	40	57	48	45
8	34	40	55	86	150	215	280	6	15	31	64	65	65
9	34	42	60	95	140	180	240	8	18	35	45	40	60
10	40	50	70	116	190	240	300	10	10	46	74	50	60
11	35	45	65	105	170	235	275	10	20	40	65	65	40
12	35	39	60	105	175	200	280	4	21	45	70	25	80
13	32	40	60	90	140	180	250	8	20	30	50	40	70
14	35	40	58	93	148	195	265	10	18	35	55	47	45
15	30	42	58	90	135	175	240	12	16	32	45	40	65
16	35	52	70	105	158	188	245	17	18	35	53	30	57
17	40	48	62	100	138	168	205	8	14	38	38	30	37
18	34	45	75	100	140	180	230	11	30	25	40	40	50
19	32	45	60	110	158	190	240	13	15	50	48	32	50
20	32	45	80	100	130	185	238	13	35	20	30	45	53
21	35	50	80	120	141	190	245	15	30	40	21	49	45
22	33	45	60	90	115	170	248	12	15	30	25	55	78
23	35	45	78	92	130	187	232	10	33	14	38	57	55
24	30	40	67	105	145	188	240	10	27	38	40	43	52
Mean	34.21	43.17	65.75	102.00	149.91	200.33	257.83	9.17	22.17	36.25	47.92	49.17	57.29

Table 2. Initial weight, weekly weight and weight gain (g) of the chicks in group 2 (Experiment 1)

	Weight							Weight gain					
Sl.No.	Initial	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	First week	Second week	Third week	Fourth week	Fifth week	Sixth week
1	36	45	60	90	125	162	205	9	15	30	35	37	43
2	34	38	46	65	79	95	125	4	8	19	14	16	30
3	35	38	58	80	100	126	160	3	20	22	20	26	34
4	35	39	52	75	100	130	170	4	13	23	25	30	40
5	35	38	50	70	95	125	160	3	12	20	25	30	35
6	35	39	55	80	96	115	150	4	16	25	16	19	35
7	32	41	50	65	92	115	170	9	9	15	27	23	55
8	36	48	52	68	92	125	190	12	4	16	24	33	65
9	35	43	59	80	105	135	210	8	16	31	25	30	75
10	33	40	50	65	85	110	170	7	10	15	20	25	60
11	30	34	45	60	80	100	160	4	11	15	10	20	60
12	30	35	45	50	70	92	145	5	10	5	20	22	53
13	30	35	45	65	90	120	155	5	10	20	25	30	35
14	35	40	50	78	108	143	183	5	10	28	30	35	40
15	30	33	42	60	80	108	146	3	9	18	20	28	38
16	35	39	52	65	83	105	135	4	13	13	18	22	30
17	35	40	50	68	84	102	122	5	10	18	16	18	20
18	32	36	45	65	80	100	120	4	9	20	15	20	20
19	35	40	50	68	93	123	158	5	10	18	25	30	35
20	35	40	52	65	85	111	143	5	12	13	20	26	32
21	30	35	48	60	81	110	138	5	13	12	21	29	28
22	35	40	51	68	90	115	145	5	11	17	22	25	30
23	30	36	50	69	92	112	142	6	14	19	23	20	30
24	34	37	53	70	89	111	139	3	16	17	19	22	28
Mean	33.42	38.71	50.42	68.71	90.58	116.25	155.88	5.29	11.71	18.71	21.46	25.67	39.63

Table 3. Initial weight, weekly weight and weight gain (*g*) of the chicks in group 3 (Experiment 2)

	Weight							Weight gain					
Sl.No.	Initial	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	First week	Second week	Third week	Fourth week	Fifth week	Sixth week
1	36	38	60	80	100	132	170	2	22	20	20	32	38
2	37	40	65	95	111	152	195	3	25	30	16	41	43
3	35	38	52	71	92	122	155	3	14	19	21	30	33
4	36	38	56	90	105	142	205	2	18	34	15	37	63
5	40	46	70	95	105	150	210	6	24	25	10	45	60
6	36	40	55	72	100	140	195	4	15	17	28	40	55
7	36	37	50	60	75	110	155	1	13	10	15	35	45
8	36	45	65	90	115	175	235	9	20	25	25	60	60
9	35	46	65	80	100	135	180	11	19	15	20	35	45
10	34	44	70	86	122	155	210	10	26	16	36	33	55
11	36	42	56	72	98	115	165	6	14	16	26	17	50
12	33	38	45	65	75	136	172	5	7	20	10	61	36
13	40	46	56	78	106	136	171	6	10	22	28	30	35
14	35	40	52	77	102	132	168	5	12	25	25	30	36
15	34	38	47	67	92	117	155	4	9	20	25	35	38
16	30	34	44	62	87	107	142	4	10	18	25	30	35
17	30	36	48	68	96	131	171	6	12	20	28	35	40
18	36	41	52	71	93	121	153	5	11	19	22	28	32
19	32	37	47	72	101	135	175	5	10	25	29	34	40
20	35	40	48	56	80	110	148	5	8	18	24	30	38
21	35	41	51	67	87	115	147	6	10	16	20	28	32
22	32	36	45	64	89	119	154	4	9	19	25	30	35
23	35	40	50	67	90	118	156	5	10	17	23	28	38
24	35	40	56	69	89	119	157	5	16	13	20	30	38
Mean	34.96	40.04	54.38	73.92	96.25	130.17	172.67	5.08	14.33	19.96	22.33	34.75	42.50

17003

Table 4 (a). Analysis of variance - body weight - first week.

Source	df	SS	MSS	F
Due to diet	2	251.36	125.68	8.86 *
Error	69	979.25	14.19	
Total	71	1230.61		

* Critical difference at 5% level : 2.14

Table 4 (b). Analysis of variance - body weight - second week.

Source	df	SS	MSS	F
Due to diet	2	3041.36	1520.68	26.89 *
Error	69	3901.96	56.55	
Total	71	6943.32		

* Critical difference at 5% level : 4.25

Table 4 (c). Analysis of variance - body weight - third week.

Source	df	SS	MSS	F
Due to diet	2	15393.08	7696.54	66.88 *
Error	69	7940.79	115.08	
Total	71	23333.87		

* Critical difference at 5% level : 6.07

Table 4 (d). Analysis of variance - body weight - fourth week.

Source	df	SS	MSS	F
Due to diet	2	51461.33	25730.66	114.28 *
Error	69	15536.17	225.16	
Total	71	66997.50		

* Critical difference at 5% level : 8.49

Table 4 (e). Analysis of variance - body weight - fifth week.

Source	df	SS	MSS	F
Due to diet	2	97496.33	48748.16	75.99 *
Error	69	44267.17	641.55	
Total	71	141763.50		

* Critical difference at 5% level : 14.33

Table 4 (f). Analysis of variance - body weight - sixth week.

Source	df	SS	MSS	F
Due to diet	2	143446.58	71723.29	34.68 *
Error	69	142673.29	2067.73	
Total	71	286119.87		

* Critical difference at 5% level : 25.73

Table 5 (a). Analysis of variance - weight gain first week.

Source	df	SS	MSS	F
Due to diet	2	253.87	126.94	14.22 *
Error	69	616.12	8.93	
Total	71	869.99		

Critical difference at 5% level : 1.69

Table 5 (b). Analysis of variance - weight gain second week.

Source	df	SS	MSS	F
Due to diet	2	1421.03	710.52	20.09 *
Error	69	2439.63	35.36	
Total	71	3860.66		

* Critical difference at 5% level : 3.36

Table 5 (c). Analysis of variance table - weight gain - third week.

Source	df	SS	MSS	F
Due to diet	2	4597.53	2298.77	48.14 *
Error	69	3294.42	47.75	
Total	71	7891.95		

* Critical difference at 5% level : 3.91

Table 5 (d). Analysis of variance table - weight gain - fourth week.

Source	df	SS	MSS	F
Due to diet	2	10842.53	5421.27	61.65 *
Error	69	6067.13	87.93	
Total	71	16909.66		

* Critical difference at 5% level : 5.31

Table 5 (e). Analysis of variance table - weight gain - fifth week.

Source	df	SS	MSS	F
Due to diet	2	6740.78	3370.39	31.00 *
Error	69	7501.16	108.71	
Total	71	14241.94		

* Critical difference at 5% level : 5.90

Table 5 (f). Analysis of variance table - weight gain - sixth week.

Source	df	SS	MSS	F
Due to diet	2	4313.35	2156.68	14.04 *
Error	69	10596.59	153.57	
Total	71	14909.94		

*Critical difference at 5% level : 702

Table 6(a). Weekly feed consumption data (total feed consumption per group in g).

Week	Group 1 (24)	Group 2 (24)	Group 3 (24)
First	1131.12	1017.84	1063.68
Second	2021.52	1835.76	1980.00
Third	2296.08	2264.64	2450.40
Fourth	3067.92	2720.88	2838.72
Fifth	3592.32	3437.76	3630.00
Sixth	4588.32	4060.32	4359.84

Figures in parenthesis indicate numbers of birds.

Table 6(b). Weekly feed consumption data (average feed consumption per bird in g).

Week	Group 1	Group 2	Group 3
First	47.13	42.41	44.32
Second	84.23	76.49	82.50
Third	95.67	94.36	102.10
Fourth	127.83	113.37	118.28
Fifth	149.68	143.24	151.25
Sixth	191.18	169.18	181.66
Mean	115.95	106.50	113.35

Table 7. Analysis of variance - average weekly feed consumption.

Source	df	SS	MSS	F
Due to diet	2	285.62	142.81	7.91 *
Due to week	5	35363.13	7072.63	
Error	10	180.60	18.06	
Total	17	35829.35		

* Critical difference at 5% level : 5.47

Table 8. Feed efficiency ratio.

Week	Group 1	Group 2	Group 3
First	0.19	0.12	0.11
Second	0.26	0.15	0.17
Third	00.38	0.20	0.20
Fourth	0.37	0.19	0.19
Fifth	0.33	0.18	0.23
Sixth	0.30	0.23	0.23
Mean	0.31	0.18	0.19

Table 9. Analysis of variance - feed efficiency ratio.

Source	df	SS	MSS	F
Due to diet	2	0.06	0.03	30 *
Due to week	5	0.04	0.008	8
Error	10	0.01	0.001	
Total	17	0.11		

* Critical difference at 5% level : 0.04

Table 10. Chemical composition of gastrocnemius muscle of chicks group 1.

Sl. No.	Protein %/100 g tissue	Total lipids % dry weight	Cholesterol mg per 100 g wet tissue	Creatine Phosphate g/100 g tissue	Phospho lipids mg per 100 mg	SDH U/mg	Phosphorylase μg P/10 mg muscle/15 mts.	GOT W.U/mg	GPT W.U/mg	ACP B.U/g	ALP B.U/g
1	21.01	1.38	264.55	0.16	6.92	0.83	342.30	54.54	6.23	2.67	3.33
2	20.17	1.27	263.16	0.15	5.50	1.95	282.35	65.60	15.00	2.40	3.20
3	20.04	1.87	257.89	0.24	6.66	0.90	327.27	34.50	4.13	2.57	3.21
4	20.40	1.90	264.55	0.19	5.33	0.75	266.60	59.09	11.36	2.34	3.13
5	20.70	1.41	200.00	0.22	6.55	0.84	313.04	31.71	4.36	2.95	3.55
6	20.10	1.42	190.48	0.21	6.55	1.32	327.27	37.04	12.22	3.14	3.79
7	19.18	1.53	172.41	0.20	6.31	0.95	320.00	24.64	2.41	3.23	4.15
8	20.47	1.96	281.35	0.16	6,00	0.99	307.68	34.05	4.66	2.08	2.76
9	18.18	1.45	216.22	0.22	6.10	1.29	313.04	53.57	5.52	1.78	2.37
10	19.17	1.36	247.52	0.17	6.25	0.89	315.78	21.41	5.71	1.81	2.17
11	20.14	1.62	238.09	0.23	6.32	0.45	327.27	50.00	12.40	2.44	3.05
12	20.18	1.45	218.86	0.16	5.71	0.37	282.35	68.03	8.19	1.67	2.52
13	18.20	1.20	242.44	0.31	6.90	0.85	318.40	34.15	6.78	2.51	2.95
14	20.18	1.54	218.71	0.19	5.95	0.79	310.50	59.09	4.75	1.97	3.07
15	22.41	1.42	198,35	0.18	5.48	0.95	248.15	53.57	6.75	2.48	3.13
16	19.78	1.32	142.34	0.24	5.97	0.92	298.40	40.18	7.17	2.58	4.25
17	22.10	1.48	198.42	0.28	6.12	0.86	287.18	31.78	5.18	2.75	3.95
18	21.10	1.28	242.74	0.25	6.35	0.87	288.70	34.05	7.15	3.15	3.21
19	20.78	1.37	239.14	0.25	6.48	0.88	276.18	28.41	4.58	1.75	5.51
20	20.08	1.91	247.15	0.27	6.46	0.88	298.75	34.05	4.36	1.97	2.95
21	21.00	1.78	208.45	0.22	6.33	0.79	310.10	37.56	12.10	2.48	2.78
22	22.41	1.09	288.45	0.29	6.18	0.83	279.18	39.08	11.35	2.75	3.07
23	19.70	1.45	264.75	0.23	6.15	0.87	277.14	38.75	7.17	2.40	4.75
24	21.71	1.70	257.89	0.24	6.78	0.81	293.15	40.17	4.17	2.35	5.18
Mean	20.38	1.51	231.83	0.22	6.22	0.88	300.45	41.88	7.24	2.43	3.42

Table 11. Chemical composition of gastrocnemius muscle of chicks group 2.

Sl. No.	Protein %/100 g tissue	Total lipids % dry weight	Cholesterol mg per 100 g wet tissue	Creatine Phosphate g/100 g tissue	Phospho lipids mg per 100 mg	SDH U/mg	Phosphorylase µg P/10 mg muscle/ 15 mts.	GOT W.U/ mg	GPT W.U/ mg	ACP B.U/g	ALP B.U/g
1	18.67	2.31	142.80	0.16	3.10	.49	160.00	20.74	1.88	1.67	2.51
2	17.16	2.18	113.64	0.16	3.58	.54	184.61	27.86	3.93	2.18	2.73
3	18.69	2.67	152.84	0.20	2.92	.61	150.00	11.80	1.64	1.69	2.55
4	18.91	2.42	117.65	0.18	2.72	.79	133.33	15.80	2.90	1.67	2.09
5	19.17	2.56	100.00	0.15	4.80	.26	133.33	20.00	1.80	2.33	3.09
6	18.71	2.42	92.31	0.17	2.92	.45	266.66	20.82	1.71	1.83	2.44
7	18.43	2.38	157.89	0.19	4.62	.58	150.00	21.88	4.07	1.87	2.30
8	17.19	2.47	155.00	0.18	4.07	.32	114.23	17.89	6.57	1.68	2.25
9	18.10	2.09	166.66	0.17	3.13	.43	208.69	17.29	2.03	2.48	3.72
10	17.10	2.18	181.03	0.16	2.50	.34	156.52	14.75	2.45	1.60	2.57
11	18.48	2.07	103.84	0.16	3.80	.31	114.29	20.89	3.99	1.07	2.57
12	18.10	2.48	118.64	0.14	2.92	.34	171.17	21.42	1.38	1.45	2.36
13	19.18	2.45	107.50	0.17	2.95	.35	151.18	19.15	1.75	1.15	3.75
14	18.75	2.75	157.84	0.19	3.15	.47	141.17	18.75	1.41	1.40	2.95
15	18.76	2.18	147.54	0.15	3.18	.61	108.75	14.51	2.98	2.17	2.91
16	19.15	2.71	107.56	0.17	3.75	.58	152.75	22.75	1.75	2.18	3.51
17	17.17	2.18	149.58	0.15	3.15	.49	148.75	25.17	4.57	1.75	1.91
18	18.76	2.45	152.84	0.19	3.15	.51	175.18	21.48	4.08	1.56	2.48
19	18.18	2.18	142.56	0.18	3.91	.39	191.18	20.85	3.95	1.41	2.15
20	19.12	2.15	155.50	0.25	4.18	.41	180.71	25.14	3.15	1.48	2.19
21	20.17	2.18	171.18	0.15	2.75	.41	156.52	27.18	2.78	1.57	2.09
22	19.42	2.75	118.45	0.17	2.18	.48	141.15	15.19	2.95	1.58	3.07
23	18.75	2.15	138.45	0.19	3.17	.41	135.75	19.41	2.15	1.45	2.54
24	18.17	2.18	141.80	0.17	3.08	.37	129.15	20.11	1.99	1.39	1.95
Mean	18.51	2.36	137.21	0.17	3.32	.46	156.46	20.03	2.83	1.69	2.61

Table 12. Chemical composition of gastrocnemius muscle of chicks - group 3.

Sl. No.	Protein %/100 g tissue	Total lipids % dry weight	Cholesterol mg per 100 g wet tissue	Creatine Phosphate g/100 g tissue	Phospho lipids mg per 100 mg	SDH U/mg	Phosphorylase µg P/10 mg muscle/ 15 mts.	GOT W.U/ mg	GPT W.U/ mg	AGP B.U/g	ALP B.U/g
1	18.42	2.18	198.58	0.13	3.00	.59	150.00	28.17	1.69	1.84	2.76
2	18.72	2.16	147.36	0.15	1.91	.46	192.00	32.28	2.44	1.05	2.50
3	18.31	2.08	137.43	0.16	2.92	.45	150.00	26.09	8.80	1.71	2.57
4	18.91	2.18	143.46	0.14	4.31	.42	212.12	29.86	3.67	2.29	3.06
5	18.48	2.50	136.36	0.15	5.51	.92	206.66	23.04	3.26	2.35	3.13
6	18.71	2.47	126.31	0.13	4.13	.21	208.68	19.98	3.99	1.38	2.76
7	18.31	2.80	142.80	0.16	4.80	.36	208.20	21.05	7.89	2.57	3.42
8	17.12	2.41	105.26	0.16	3.87	.32	191.70	29.06	2.79	1.36	3.30
9	18.48	2.82	134.85	0.17	4.39	.45	218.18	27.06	3.53	2.36	2.95
10	18.24	2.51	195.70	0.13	5.39	.33	205.71	27.94	9.91	1.51	1.82
11	18.24	2.94	145.83	0.10	5.80	.65	180.00	25.68	2.58	2.54	4.20
12	17.14	2.10	142.11	0.18	4.13	.32	218.18	29.03	2.58	2.78	3.50
13	19.18	2.18	175.18	0.15	2.17	.50	171.51	25.18	3.75	1.98	2.18
14	17.15	2.75	123.19	0.19	2.75	.58	155.11	24.17	2.91	1.75	2.17
15	18.35	2.17	138.17	0.21	3.14	.45	165.11	23.17	3.98	1.78	2.51
16	19.18	2.46	145.18	0.19	3.41	.41	191.11	19.48	3.15	2.15	2.71
17	18.75	2.75	147.18	0.18	5.18	.47	170.19	20.17	4.17	2.75	3.18
18	18.18	2.51	151.75	0.17	4.10	.58	192.18	26.09	3.75	1.97	2.10
19	18.75	2.17	138.75	0.17	3.75	.61	188.75	29.18	2.98	1.98	2.17
20	18.09	2.18	141.18	0.16	3.10	.71	175.75	30.15	3.67	2.75	2.18
21	18.17	2.75	195.18	0.18	3.18	.41	190.18	28.17	3.15	1.98	2.05
22	19.09	2.75	137.18	0.15	2.17	.43	200.10	27.18	3.75	1.77	3.08
23	18.10	2.15	109.75	0.19	2.18	.25	191.75	24.17	3.14	1.84	2.75
24	19.18	2.08	192.83	0.17	2.00	.39	159.10	25.18	3.74	1.75	2.18
Mean	18.39	2.42	148.00	0.16	3.65	0.48	187.17	25.90	3.97	2.07	2.72

Table 13. Analysis of variance - protein.

Source	df	SS	MSS	F
Due to diet	2	60.03	30.01	41.69 *
Error	69	49.40	0.716	
Total	71	109.43		

*Critical difference at 5% level : 0.48

Table 14. Analysis of variance - total lipids.

Source	df	SS	MSS	F
Due to diet	2	12.46	6.23	100.48 *
Error	69	4.3	.062	
Total	71	16.76		

*Critical difference at 5% level : 0.14

Table 15. Analysis of variance - cholesterol.

Source	df	SS	MSS	F
Due to diet	2	128767.20	64383.6	178.17 *
Error	69	24934.14	361.36	
Total	71	153701.34		

* Critical difference at 5% level : 10.76

Table 16. Analysis of variance - creatine phosphate.

Source	df	SS	MSS	F
Due to diet	2	.04	.02	28.57 *
Error	69	.05	.0007	
Total	71	.09		

* Critical difference at 5% level : 0.02

Table 17. Analysis of variance - phospholipids.

Source	df	SS	MSS	F
Due to diet	2	121.85	60.93	338.47 *
Error	69	12.64	0.183	
Total	71	134.49		

* Critical difference at 5% level : 0.24

Table 18. Analysis of variance - SDH

Source	df	SS	MSS	F
Due to diet	2	2.74	1.37	51.12 *
Error	69	1.85	0.27	
Total	71	4.59		

* Critical difference at 5% level : 0.09

Table 19. Analysis of variance - phosphorylase.

Source	df	SS	MSS	F
Due to diet	2	275995.47	137997.74	194.37 *
Error	69	48989.19	709.99	
Total	71	324984.66		

* Critical difference at 5% level : 15.08

Table 20. Analysis of variance - GOT

Source	df	SS	MSS	F
Due to diet	2	6133.84	3066.92	50.29 *
Error	69	4207.37	60.98	
Total	71	10341.21		

* Critical difference at 5% level : 4.43

Table 21. Analysis of variance - GPT.

Source	df	SS	MSS	F
Due to diet	2	251.48	125.74	22.29 *
Error	69	388.89	5.64	
Total	71	640.37		

* Critical difference at 5% level : 1.34

Table 22. Analysis of variance - ACP.

Source	df	SS	MSS	F
Due to diet	2	6.55	3.28	18.22 *
Error	69	12.66	.18	
Total	71	19.21		

* Critical difference at 5% level : 0.24

Table 23. Analysis of variance - ALP.

Source	df	SS	MSS	F
Due to diet	2	9.21	4.61	9.04 *
Error	69	35.22	0.51	
Total	71	44.43		

* Critical difference at 5% level : 0.41

A B S T R A C T

THE EFFECT OF CHOLINE DEFICIENCY ON THE CHEMICAL COMPOSITION OF THE SKELETAL MUSCLES OF CHICKS.

BY

P. A. PHILOMINA

ABSTRACT OF A THESIS
submitted in partial fulfilment of
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MASTER OF VETERINARY SCIENCE

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ABSTRACT

The results and conclusions drawn from a study carried out to determine the effects of choline deficiency on the chemical composition of the skeletal muscles of chicks are presented. The levels of choline viz., 1500 mg, 75 mg and 100 mg per kg were added to a choline extracted but an otherwise normal ration and were fed to three groups. Twentyfour, one-day old chicks were allotted to each treatment. Data on growth rate, weight gains, and feed efficiency were significantly lower for choline deficient chicks, indicating the importance of choline in poultry. All the chicks on deficient diets exhibited typical symptoms of perosis, either unilateral or bilateral from 24 days of age. Creatine phosphate, phospholipids and cholesterol levels of gastrocnemius muscles of chicks affected by perosis were lowered either due to interference or impairment in their formation. Reduced SDH activity, observed might be due to the impaired oxidative metabolism. Slight increase in lipid content might be due to the lowered SDH level. The reduced activity of GOT and GPT and total protein content probably attributed to the lowered growth rate in choline deficient chicks. ALP and ACP values were lowered which can be attributed to the interference in the synthesis of their prosthetic groups. Poor muscular efficiency was revealed by the affected muscles.