NUTRITIVE VALUE OF FISH PROTEIN CONCENTRATE

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ABSTRACT

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Experiments were conducted with young Holstein calves and 21-day old weanling rats to evaluate the nutritional quality of commercially prepared fish protein concentrate. Dichloroethane or isopropanol were the extraction solvents.

Investigation with young calves revealed that supplementation of methionine to the dichloroethane-extracted fish protein concentrate (DCE-FPC) had no beneficial effect on weight gains. Growth of calves consuming 20% protein diets containing DCE-FPC or isopropanol-extracted fish protein concentrate (IP-FPC) was inferior to that on milk protein rations. Gains on the IP-FPC ration were inferior to those on the DCE-FPC ration. Calves consuming both fish protein sources developed microcytic, normochronic anemia which was typical of that found in protein-deficient animals. Calves fed DCE-FPC rations also showed a higher incidence of muscular dystrophy than those fed milk protein. Addition of chlorocholine

chloride to the milk ration tended to depress growth but dichloroethane did not effect weight gains. Extraction of DCE-FPC with ethanol improved its nutritive value for calves, but washing with water decreased weight gains. Impaired growth was also observed on IP-FPC diets and was associated with the removal of water-soluble proteins prior to extraction with isopropanol.

Improved growth in calves occurred when the DCE-FPC rations were supplemented up to twice the recommended dose of vitamin E. No alteration in weight gains was shown from vitamin E supplementation to milk protein rations. Plasma concentrations of α-tocopherol seemed related to both level of vitamin E supplemented and source of protein. A greater depression in plasma tocopherol was noted on the DCE-FPC ration compared to the milk protein ration. It is suggested that the daily requirement of vitamin E for young calves on DCE-FPC ration is about 120 mg per 100 kg body weight.

Studies with rats receiving diets containing 10% protein from various single sources showed that IP-FPC was superior for growth to casein or DCE-FPC. The nutritive value of DCE-FPC was significantly improved by extracting with methanol, ethanol or washing with water. Addition of methanolic or ethanolic extracts to casein or DCE-FPC diets significantly depressed the weight gains. Addition of chlorocholine chloride or dichloroethane to a casein diet

did not alter growth responses. In diets containing 20% or 40% protein, weight gains were the same on DCE-FPC and casein suggesting that a toxic factor was not the major reason for depressed growth of rats on DCE-FPC diet. When small amounts of casein were added to DCE-FPC diets, a significant improvement in rat growth was observed. Serum protein, serum albumin and net protein utilization of rats increased in proportion to the superiority of the protein source. Plasma free amino acid levels of rats on all fish protein diets showed lower histidine concentrations compared to those of rats fed the casein diet.

Addition of 0.075% or 0.15% L-histidine to all fish protein diets increased growth and feed intake of rats compared to unsupplemented diets. No benefit was observed by increasing hestidine level above 0.075%. Addition to fish protein diets of 0.075% L-histidine and 0.20% L-methionine, alone or in combination, resulted in improved growth. An additive effect on growth was observed with a combination of the amino acids. The protein efficiency ratio (PER) of DCE-FPC but not of IP-FPC or ethanol-extracted DCE-FPC was improved by amino acid supplementation.

When both histidine and methionine were added to FPC diets, concentrations of total essential amino acids in blood plasma of rats were lowered. When histidine and methionine were supplemented, alone or in combination, their plasma concentrations were raised. Added histidine

did not influence the methionine level in plasma, however, methionine alone depressed plasma histidine in DCE-FPC and IP-FPC diets but not on ethanol-extracted DCE-FPC. These results suggest that histidine and methionine were limiting rat growth in these fish protein concentrate diets regardless of the method of solvent extraction.

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INTRODUCTION

In recent years fish protein concentrate (FPC) has attracted attention of nutritionists as a source of protein which can help to alleviate the protein shortage of the world. Its low cost, compared to other animal protein makes it a potential source for use in underdeveloped countries.

The nutritive value of FPC has been evaluated by several investigators in various species of animals. Extreme differences in its biological value have been reported in the literature. Unsatisfactory performance of young calves when FPC was used as a sole source of protein was found by Huber and Slade (1967) and Wendlant *et al.* (1968). However, the same authors showed that satisfactory growth resulted with FPC as a partial source of protein in the diets of young calves. Morrison and Munro (1965) suggested that the organic solvent used for extracting fish greatly influences the nutritive value of the final product. Munro and Morrison (1967) later isolated chlorocholine chloride from FPC extracted with dichloroethane and proposed that it may be one of several toxic factors present. The fact that the cattle are susceptible to the

S-dichlorovinylcysteine produced in trichloroethane extracted soybean oil meal led to the speculation that young calves may be susceptible to the compounds which might result from dichloroethane extraction of FPC.

Nutritional studies (Stillings, 1967) with rats have shown that FPC is comparable to casein in biological value. More recent studies by Yanez et al. (1969) showed normal growth in infants on FPC as the only protein source.

The present study was undertaken to evaluate with young calves and rats the nutritional quality of FPC prepared by extraction with dichloroethane or is opropyl alcohol and to find out the cause of its inferior biological value. An attempt was also made to test the effect of possible contaminants such as chlorocholine chloride and dichloroethane on performance.

REVIEW OF LITERATURE

Fish protein concentrate is chiefly used as a rich protein source in human as well as animal nutrition. Conflicting reports are found in the literature on the nutritional value of fish protein concentrate (FPC). When used as a sole source of protein, FPC has generally been unsatisfactory for young calves. Calves on such diets become unthrifty and showed symptoms of vitamin E deficiency (Genskow, 1969). Dichloroethane-extracted FPC (DCE-FPC) resulted in inferior growth of rats compared to those fed casein (Morrison, 1963). Certain toxic factors in DCE-FPC have been proposed (Munro and Morrison, 1967). However, when isopropyl alcohol was used as the extraction solvent for FPC fed to rats as the sole source of protein growth was superior to that shown for milk protein (Stellaman, 1969). A discription of the processes used for preparation of fish protein concentrates and further information on chemical composition is given in Appendix Table 8, 9 and 10 respectively. A brief review of the following topics is presented:

(1) Protein sources in milk replacers fed to young calves

(2) FPC in animal nutrition

(3) Toxicity of fish protein concentrates

(4) Protein deficiency and anemia

(5) Vitamin E and muscular dystrophy

(6) Protein quality and amino acid imbalances

(7) Protein quality and plasma amino acid levels

Milk Replacer for Young Calves

Numerous attempts have been made to raise young dairy calves on milk replacers which would include animal or vegetable products as a source of protein. Though satisfactory gains have been obtained in calves fed a mixture of non-milk protein and milk protein, growth from non-milk protein alone has usually been unsatisfactory.

Shoptaw (1936) reported that calves fed a soybean milk replacer did not show a thrifty condition and that there was some difficulty in getting the calves to relish the diet. Williams and Knodt (1950) observed that when raw soybean meal constituted 40 percent of the replacer, all calves died. Studies by Noller et al. (1956) showed that the ability of the calves to utilize the soy protein increased after approximately 25 days of age. Lassiter et al. (1959) reported that the rates of growth decreased significantly as the amount of dried skim milk in milk replacer decreased and the amount of soybean meal increased. They also observed that adding proteolytic enzymes to the replacers did not improve the utilization of soybean protein.

Stein and Knodt (1954a) and Stein et al. (1954b) observed that soybean flour could effectively provide a major portion of the protein in a milk replacer containing over 30 percent protein when dried skim milk and whey provided the remainder. When dried skim milk provided less than 43 per cent of the total protein, appetite was depressed and growth was retarded. They also reported that addition of 0.05 and 0.25 percent DL-methionine did not affect growth.

Borchers (1961) reported that calves fed soybean meal supplemented with methionine, threonine and valine resulted in higher gains than unsupplemented meal. Colvin and Ramsey (1968) found that predigesting fully-cooked soy flour with various proteolytic enzyme preparations did not improve its nutritive value. However, acid digestion of the soy flour at a pH of 4.0 for five hours at 37°C markedly improved gains of calves. Later they (Colvin and Ramsey, 1969) also showed that alkali-treatment of soy flour was equally beneficial for improving calf growth as was acid treatment.

Brumbaugh and Knodt (1952) reported that the growth of calves was poor when milk replacer contained less than 35 percent dried skim milk in combination with dried blood meal, dried whey, distillers solubles and soybean meal. Archibald (1928) found that the calves fed a cereal milk replacer were unthrifty and pot-bellied with a higher incident of diarrhea than those fed milk. Williams and

Jensen (1955) reported average daily weight gains of only 0.4 lb in calves fed a milk replacer containing 50% dried skim milk, 2% dried rumen contents and 4% blood meal as the protein sources. Slade (1965) obtained extremely poor results in calves fed concentrated rumen fluid as the protein source in milk replacers. Corn distillers dried solubles were substituted for 20, 35 and 55 percent, respectively, of dried skim milk and lactose in milk replacers and weight gains of calves decreased with increased levels of the corn solubles (Bryant et al., 1963).

Brown and Varnell (1962) tested raw eggs as partial replacement for the nutrients in the whole milk. They concluded that the calves could not utilize eggs when fed only with water. Cooking of eggs did not improve the growth. Calves were in negative nitrogen balance when they received only eggs. Weight gains were higher in calves receiving whole milk compared to those on a ration of two eggs plus milk. The poor utilization of eggs by young calves is very interesting in light of the fact that egg protein is considered superior to milk for rats and other simple-stomach animals. The reason for the inferiority of eggs to milk protein in calves is not known but it may be related to the amino acid sequence or spatial configuration of the protein molecules. The inability of the calf to grow well on egg protein draws an interesting parallel to the performance

reported on fish protein, which is also considered a high quality protein source for animals other than the young calf.

Dichloroethane-extracted fish protein concentrate (DCE-FPC) has been used as an ingredient in milk replacers by several investigators and varying results have been obtained (Rupel and Wilson, 1962; Huber and Slade, 1967; Genshow et al., 1968, Wendlandt et al., 1968). In the studies at Texas A and M, Rupel and Wilson (1962) conducted feeding trials involving 100 Holstein calves. The protein in the milk replacer formulas was furnished by 0, 12, and 24 percent DCE-FPC and 55, 23 and 0 percent dried skim milk, respectively. Calves on the dried skim milk diet gained 0.63 lb per day while those on 12 percent DCE-FPC and 23 percent dried skim milk gained 0.82 lb per day. Calves receiving DCE-FPC as the only protein source in the milk replacer did not gain during the first 21-day period. Thereafter, they gained considerably less than the control group.

In a series of experiments at Virginia by Huber and Slade (1967), DCE-FPC furnished 0, 20, 33, 40, 60, 66 and 100 percent of the protein in the milk replacers. An ingredient composition of some of the replacers is listed in Table 1. Average daily gains and feed efficiencies were not significantly depressed when FPC furnished up to 40% of the dietary protein. However, when DCE-FPC furnished 60% or more of the protein a significant decrease in

TABLE 1.--Composition of milk replacer formula.

Ingredients	Percent crude protein as FPC				
	0	20	40	60	100
Dried skim milk	63.9	50.4	37.9	25.2	0
Lactose	26.0	33.6	40.3	47.0	61.0
Fish flour	-	5.9	11.7	17.7	28.9
Emulsified lard oil	10.0	10.0	10.0	10.0	10.0

growth rate occurred. In the same study when 100% of the protein came from FPC, the calves became listless, emaciated, refused the ration during the third or fourth week of the treatment and died shortly thereafter. The balance studies showed that digestibilities of dry matter, crude protein, ether extract and ash decreased as a level of DCE-FPC in milk replacers increased. The digestibility of protein in DCE-FPC was reported to be 80% compared to 90% for skim milk protein.

van Hellemond (1967) conducted a balance study with isopropanol-extracted fish protein concentrate (IP-FPC) and reported a higher apparent digestibility for fish protein (88.5%) than reported by Huber and Slade (1967). However, these studies were conducted on older animals which weighed from 53 to 139 kg. van Hellemond (1967) also observed that the IP-FPC in the reconstituted milk replacer tended to settle at the bottom of the feeding pail and necessitated stirring while being fed to the calves.

Wendlandt et al. (1968) reported that when DCE-FPC furnished 25% protein (100% of the total protein in the diet) in a milk replacer, the weight gains in calves were significantly lower than those of calves in the milk-fed control group. However, when FPC furnished 12.5% protein (50% of the total protein in the diet), weight gains were comparable to the controls. A higher death loss of calves on milk replacers where DCE-FPC furnished all the protein was also reported. In the same study a digestion trial was carried out using two calves per group at 3 weeks of age. Digestibility of crude protein decreased with increased levels of DCE-FPC in the replacer.

Williams and Rust (1968) compared milk replacers containing 10, 5, 10 and 15% DCE-FPC. Dried skim milk was added at 47.0, 64.5, 57.0 and 43.5% to the respective rations. Satisfactory gains on all the above replacers for veal calves were reported.

Preston et al. (1960, 1965) did not find a significant difference between milk replacers containing groundnut meal and groundnut meal plus fish meal. However, calves on milk replacer containing groundnut meal and fish meal gained 6 lb more in an 81 day trial. In another trial comparing groundnut meal and fish meal Whitelaw et al. (1961) observed that nitrogen retention was higher in calves on a fish meal replacer than for those on groundnut meal.

Fish Protein in Animal Nutrition

Fish protein concentrate has been investigated as a protein source for the monogastric animals including human beings.

Bender and Haizelden (1957) examined 27 samples of fish meal and deodorized fish flour for net protein utilization (NPU). The NPU's ranged from 18 to 80. The low values were thought to be due to maltreated fish meal, but the defatting and deodorizing process was not associated with poor performance. The Nutrition Division of the Food and Agricultural Organization of the United Nations (1958) reported that the proteins of fish muscle are equivalent in biological value to those of animal muscle and milk. If processed properly, fish flour was thought to retain a biological value equal to the original material. Ousterhout et al. (1959) examined several samples of fish meal for the availability of amino acids by chick assay and found that arginine, methionine, cystine, histidine and threonine were less available than indicated by their total content in fish meal.

Jaffe (1961) obtained a 2.48 protein efficiency ratio (PER) for fish meal fed to rats. The PER of wheat meal was raised from 0.23 to 1.08 by adding 2% fish meal and to 2.90 by adding 7.5% fish meal.

In a study with groups of undernourished infants, Graham et al. (1962) obtained comparable weight gains from

diets containing fish flour, a wheat-fish flour mixture, a vegetable mixture or modified cow's milk. Nitrogen retention for the vegetable mixture was slightly lower than that of diets with fish or milk protein, probably reflecting poorer digestion and absorption. In 10% protein diets fed to rats of the vegetable mixture Johnson et al. (1962) evaluated a highly-purified fish flour prepared by the VioBin Company in two ways: (1) Protein efficiency ratio and (2) Mitchell biological value. The PER values for fish flour protein was 3.24 and were superior to those observed from milk protein (2.85) or beef protein (3.15). Biological value of the fish protein concentrate was 88 percent which was equivalent to that of milk protein. When supplemented at 1 and 3 percent of the diet, fish flour significantly improved the PER of vegetable protein.

Deuel et al. (1946) reported considerably higher biological values for fish muscle protein than casein. Fish protein caused a greater recovery in weight gains of rats and a more pronounced stimulation in hemoglobin regeneration.

Fish flour was reported by Sure (1957) to be a better protein supplement to corn meal fed as the basal protein source to rats than non-fat milk solids, dried butter milk, defatted soybean flour, brewer's yeast, cultured food yeasts or peanut meal. Addition of 5% fish flour to a corn

meal diet produced a 15-fold increase in growth of rats as compared to unsupplemented corn meal.

Ferreira (1966) found the limiting amino acids in fish meal were cystine and methionine. Values were 45 to 55 percent, respectively, as high for egg protein as they were in fish meal. Smith et al. (1962) concluded that methionine was the first and arginine the second limiting amino acids in the two fish meal samples studied. In further studies with fish meal, Smith et al. (1965a, 1965b) reported that the addition of 0.25% DL-methionine increased weight gains of rats fed all the fish meal samples used, indicating an overall deficiency of sulfur amino acids in fish proteins. Heat-treated fish meal showed lack of availability of lysine and threonine.

Recently, Stillings et al. (1969) carried on a series of experiments with rats to determine the sequence of limiting amino acids in isopropanol-extracted FPC. The results of his studies have shown methionine to be the most limiting amino acid followed by the groups of: (1) histidine, tryptophan and threonine, (2) valine, isoleucine and phenylalanine and (3) leucine, lysine and arginine. In a report on nutritive value of FPC, Stillings (1967) has noted higher value of PER for isopropanol-extracted FPC as compared to casein.

Morrison and Munro (1965) reported that the solvent used for extraction of fish greatly influences the nutentive value of fish flour obtained. They found that fish flour extracted with

isopropanol was superior to that extracted with ethylene dichloride when tested at a critical level in rats. It was also shown that fish flour extracted with ethylene dichloride contained less available lysine and methionine than that extracted with isopropanol.

In further studies with FPC, Morrison (1963) supplemented DCE-FPC with methionine, histidine, threonine and tryptophan. When all four amino acids were added to the fish flour diets, weight gains of rats increased significantly. When histidine and methionine were omitted from the diets, weight gains were similar to unsupplemented fish flour and the combination of histidine and methionine was as effective as the combination of the four amino acids.

In further studies on the solvent used for extraction of the fish protein, Morrison and Munro. (1965) found that 1,2-dichloroethane reacts with the free sulfhydryl group of cystine to yield S,S'-ethylenebiscysteine. Munro and Morrison (1967) reported that this reaction product was not toxic to rats.

The relationship between the type of solvent used for extraction of fish flour and the amount and availability of the amino acids to enzyme hydrolysis was studied by Morrison and Munro (1965). The results showed that the solvent used markedly influenced the amount and availability of amino acids present in fish flour. (Table 2).

Total methionine values were not affected by any of the solvents. However, histidine and cystine reduced when

TABLE 2.--Effects of extracting fish protein for 16 hours with various solvents on the methionine, histidine and cystine availability and content.

Solvent	Methionine, g/16 g N		Histidine, g/16 g N		Cystine, g/16 g N	
	Acid hydrolysate	Enzyme hydrolydate	Acid hydrolysate	Enzyme hydrolysate	Acid hydrolysate	Enzyme hydrolysate
Nil	3.6	2.33	2.67	1.24	0.97	0.46
Hexane	3.1	1.76	2.30	0.88	0.92	0.42
Isopropanol	3.3	1.88	2.32	1.15	0.92	0.40
Ethylene dichloride	3.2	0.48	1.73	0.33	0.54	0.09
Ethanol	3.4	1.96	2.38	1.15	0.90	0.39

ethylene dichloride was used as extraction solvent. In another experiment it was shown that methionine, histidine and cystine on enzyme hydrolysate prepared from FPC dropped in proportion to the duration of the time of extraction (Morrison and Munro, 1965).

In a study on the effect of method of processing of fish meal on the nutritive value of herring meals, Clandinin (1948) found that meals of equal nutritive value can be produced by either the vacuum or flame methods of drying. Further results showed that meals of equivalent nutritive value were obtained at 10, 14 and 16 inches vacuum but meals dried by flame method at a temperature of 220°F were decidedly inferior to vacuum dried meals or to meals dried by the flame method at a temperature of 185°F.

Toxicity of Fish Protein Concentrate

Speculation about the toxicity in protein concentrates extracted with organic solvents probably arose from the findings that trichloroethane (TCE) extracted soybean meal was found to be toxic to cattle. This toxicity was due to S-(1,2-dichlorovinyl)-L-cysteine (DCVC) which is produced in soybean meal when TCE was used for extraction.

Trichloroethane-extracted soybean meal produced aplastic anemia when fed to cattle (Picken *et al.*, 1952, Pritchard *et al.*, 1952 and Sautter *et al.*, 1952). The solvent, TCE, by itself was not toxic because cattle are able to convert a large part of an ingested dose to

metabolites such as urochloric acid and trichloroacetic acid which are excreted through urine. Schultze et al. (1962) reported that rats are resistant to DCVC. Waibel et al. (1967) demonstrated that the turkey, in contrast to the calf, is highly resistant to toxic effects of DCVC. They further reported that the liver and kidney of turkeys have considerably higher activity of DCVC-lyase than the corresponding bovine tissues.

In 1962, Morrison et al. observed a toxic effect from a fish flour sample fed to rats to furnish a diet of 20% protein level. The fish flour was prepared by extraction with isopropyl alcohol and the bones were partially removed by flolation in chloroform. In a feeding trial using this source of FPC feed intakes at 20% protein were decreased compared to those at 10% protein diet. Weight gains of rats which normally would be superior at 20% protein were not different from those of rats fed 10% fish protein. Renal and hepatic hypertrophy were also observed in rats receiving the 20% diet.

In a later study when this fish flour was further extracted with diethyl ether, growth in rats improved. It was concluded that deleterious factor in fish flour was residual chloroform.

Recently, Munro and Morrison (1967) showed that the chlorinated solvents react with fish protein to form chlorinated compounds which can be toxic to the animals. Two

such compounds thought to be produced are S-S'-ethylenebis-cysteine and chlorocholine chloride (CCC). Very little is known about the toxicity, biochemistry or the presence of S-S'-ethylenebiscysteine in fish flour thus prepared. The other compound (CCC) results from the reaction of dichloroethane with trimethylamines (TMA) which are found in raw fish. Fish are known to contain 2 to 5% TMA (Dyre, 1952). Munro and Morrison (1967) reported that the fish flour samples extracted for 24 hrs with dichloroethane contained 400 mg/kg of CCC and when the FPC was fed as 50% of the total diet for rats, mortality was 100%. No mortality resulted when the same FPC was further extracted with methanol. Preliminary studies with rats had showed that the oral LD_{50} of CCC was 500 mg/kg body weight. Thus, a 100 g rat would need to consume 50 mg CCC daily to attain this level. A diet containing 20% crude protein, all furnished from DCE-FPC, would supply only about 1.5 mg CCC daily which is only 3% of the lethal dose.

The effect of temperature and duration of extraction on the residual DCE content in fish flour was studied by Ershoff (1967). He observed that when fish flour was extracted for 24 hrs at 87°C, the residual DCE was 30,000 ppm. However, when the fish were extracted for 45 minutes at 87°C, only 13 ppm of DCE were present. Also, when fish flour was extracted for 24 hrs, but at lower temperature (40°C), the residual DCE content was only 17 ppm.

A significant depression in growth of rats was noted with fish flours extracted at both the higher temperature for the longer period of time.

Protein Deficiency and Anemia

Anemia is defined as reduction of hemoglobin in blood. Hemoglobin is a unique protein which makes up about 1% of the body weight. It is estimated that 2.16×10^{11} red blood cells are destroyed and replaced each day in the adult human male. This would mean that 6 g hemoglobin must be produced every day. It is therefore quite logical to conclude that a shortage of protein might cause a subnormal red cell production. Whipple (1942) stated that the hemoglobin formation takes priority over the formation of other proteins and that the body protein store must be greatly reduced before the reduction in hemoglobin formation occurs. However, Platt (1956) thinks that the need for formation of new red cells represents a considerable proportion of the total body requirement for protein.

Robscheit-Robbins and Whipple (1955) have shown that a shortage of protein influences erythropoiesis. Hallgren (1953) investigated a low protein diet compared to one containing 22% egg albumin, and concluded that the animals on low protein diet developed a microcytic, normochromic anemia and that the fall in total hemoglobin on low protein was much more marked. The animals used in these studies were growing rats and competetion for protein by tissue for growth would be at a maximum.

Foy and Kondi (1957) suggested that the treatment of some anemias with iron alone is not sufficient to raise the hemoglobin level unless an adequate protein is also provided. Knowles (1957) found marked reduction in hemoglobin concentration in pigs fed a low protein diet. Anemia in pigs on a low protein diet was alleviated when they were transferred to the diets of higher protein value.

Orten and Orten (1946) observed a parallelism between the hemopoietic value of the protein and its ability to support somatic growth in rats. Various proteins fed at as low level as 2.8% resulted in a mild to moderate anemia in all cases observed.

Vitamin E and Muscular Dystrophy

It has long been known that cod liver oil added to calf diets of skim milk, produced a severe muscular disease (Blaxter et al., 1952 and Blaxter et al., 1953). Blaxter et al. (1953) also reported that the same disease could be produced in calves by giving them lard oil as a source of fat and it could be prevented by giving them very large amounts of α-tocopherol in addition to the fats. Moore et al. (1959) reported that cod liver oil contains 100 to 200 mg/kg of vitamin E. However, this amount is insufficient to protect against the highly unsaturated fatty acids it contains. Adams et al. (1959a) and Adams et al. (1959b) have reported that in addition to lard oil and cod liver oil as anti-vitamin E oils, maize oil added to the diet of

skim milk produced clinical and histological signs of muscular dystrophy in calves. In these experiments, the feeding of skim milk alone, hydrogenation of the maize oil or addition of tocopherol to the maize oil diets prevented muscular dystrophy. Certain synthetic antioxidants structurally unrelated to the tocoperhols were shown to protect vitamin E from oxidative distruction. These were N,N'-diphenyl-p-phenylenediamine, (Draper et al., 1956) methylene blue (Draper et al., 1958) and ascorbic acid (Blaxter et al., 1953).

Cawthorne et al. (1968) have suggested that since the tocopherols are biological antioxidants they also prevent auto-oxidation of vitamin A particularly in the gastro-intestinal tract. Consequently, the calves showing vitamin E deficiency, might also show vitamin A depletion despite normally sufficient dietary levels. Hardin and Hove (1951) have shown that a methionine deficiency is aggravated by rations deficient in vitamin E.

Blaxter et al. (1953) reported that the muscular dystrophy induced by diets containing high levels of poly-unsaturated fats can be prevented by giving large amounts of vitamin E, but it is unresponsive to Se. Schwartz and Foltz (1957) observed that Se. is an essential trace element for several species and that it can function as a partial alternative to tocopherol. Thomas and Okamoto (1955) have indicated that when lard oil was included in milk

replacer as a source of fat, plasma tocopherol levels were low compared to those of calves fed whole milk. They also observed that diarrhea in calves causes marked decrease in plasma tocopherol.

Maplesden and Loosli (1960) attempted to produce muscular dystrophy in calves under laboratory conditions by giving diets of very low vitamin E content and virtually free of fat. From this experiment it was implied that in the absence of unsaturated fat, a very long period on the diet is necessary to produce pathological changes.

It has been clearly established that muscular dystrophy symptoms in calves and lambs are seen in severe vitamin E deficiency. The mechanism of action of vitamin E is not understood; however, it has been hypothesized that vitamin E is a physiological antioxidant, preventing the accumulation of undesirable metabolic oxidation products in animal tissues. These products are thought to be lipid peroxides which, in absence of vitamin E or other antioxidants, can break down to give free radicals that react with sensitive cell structures and disrupt the cellular frame (Bunyan et al., 1962). Sandlers and Bird (1967) have implicated that there is an indirect relationship between tocopherol and the sulfhydryl groups on enzymes which are supposedly protected by the antioxidant action of vitamin E.

Protein Quality and Amino Imbalances

As early as 1914 Osborne and Mendle observed that certain amino acids were essential nutrients and that proteins differed in their content of amino acids. Therefore, the nutritive value of protein depends upon its amino acid composition. Later Block and Mitchell (1946) showed that by comparing the amino acid composition of one protein with that of whole egg protein, one can obtain a fairly good estimate of the biological value of that protein. They calculated which essential amino acid was in greatest deficit as compared to whole egg protein and observed that there was a high correlation between the amino acid showing the greatest deficit in that protein and its biological value. Almquist (1954) demonstrated that the efficiency with which a protein can be used for body protein synthesis depends upon the quantity and proportion of constituent amino acid. Proteins which fall far from the correct proportion are termed as unbalanced. Harper and Kumta (1959) have pointed out that the efficiency of utilization of a protein depends upon the actual quantities of essential amino acids a protein contains as well as the proportion in which they occur.

The term "amino acid imbalance" according to Harper (1964) can refer to a deficient protein source which is made more deficient by addition of an amino acid(s) other than limiting one, thus resulting in growth retardation

much greater than that caused by original deficient protein. The addition of small quantity of limiting amino acid to an imbalanced ration will prevent the growth retardation. Harper (1964) has indicated two types of amino acid imbalance. One type results from addition of a protein or a mixture of amino acids lacking in one essential amino acid to a diet containing low or moderate amounts of protein; the other type results from addition of a small amount of amino acid or amino acids to a diet that is low in protein. Such type of addition would cause substantial depression in growth rate. Yoshida et al. (1966) have put forward a hypothesis explaining the causes for retardation of growth on a diet imbalanced in amino acids. They have suggested that an imbalance leads to more efficient incorporation of the growth-limiting amino acids into tissues with the result that its concentration in blood plasma decreases within few hours after ingestion of the imbalanced meal. This phenomenon results in a signal to an appetite regulating centre. Food intake is subsequently depressed and the food intake depression results in retarded growth.

Protein Quality and Plasma Amino Acid Levels

It has long been recognized that the concentration of amino acids in the portal view is greater than those in the jugular vein after ingestion of protein. Denton and Elvehjem (1954) measured free amino acids in plasma of portal

and systemic blood of dogs fed zein, casein or beef. Plasma concentration of amino acids increased rapidly when casein or beef were fed and the increase was greater in portal than in systemic blood. Concentration of most of the amino acids decreased when zein was fed. Guggenheim et al. (1960) have also investigated the relationship between plasma amino acid levels and availability of amino acids in protein. They fed rats gluten, zein, soybean protein, casein or lactalbumin. Lysine and methionine were measured in the plasma from the portal vein of the animals. The amounts of these amino acids increased in proportion to their presence in the protein. McLaughlan (1963) concluded that the concentration of plasma amino acids (PAA) increased after a meal of good quality protein. The extent and duration of the increases were related to both the amount and composition of the protein fed. Zimmerman and Scott (1965) reported that low PAA does not necessarily indicate the deficiency of that amino acid for body functions. They demonstrated that in chicks the plasma amino acid levels did not increase significantly till the dietary intake of amino acid exceeded the amount needed for optimal growth. McLaughlan (1963) demonstrated that PAA levels were not always directly proportional to amino acid levels of the dietary proteins and that PAA levels could not be used directly to predict the nutritional quality of various proteins.

Various methods have been proposed to determine the limiting amino acid of the dietary proteins using plasma amino acid levels. Longnacker and Hause (1959) proposed the following formula to calculate the limiting amino acid.

$$\text{PAA-R} = \frac{B-A}{R} \times 100$$

where B - plasma essential amino acid (EAA) concentration after fasting 18 hrs

A - plasma EAA concentration in five consecutive blood samples drawn after feeding

R - The requirement of the EAA expressed as grams of amino acids per 16 grams of nitrogen, (NRC,1962).

For each EAA, a plasma amino acid ratio (PAA-R) is calculated. The lowest ratio indicates the limiting amino acid of the dietary protein.

McLaughlan (1964) proposed another method for calculating limiting amino acid. In this method, the EAA acid requirement of the experimental animal does not have to be known.

$$\text{PAA's} = \frac{\text{PAA level of the protein fed animals}}{\text{PAA level of the fasted animals}} \times 100$$

The lowest PAA score indicates the limiting amino acid.

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PART I

FISH PROTEIN CONCENTRATE IN MILK REPLACER FOR YOUNG CALVES

ABSTRACT

In four trials with young Holstein calves the nutritional value of fish protein concentrate (FPC) prepared by extraction with dichloroethane (DCE-FPC) or isopropanol (IP-FPC) was investigated.

In trial I the addition of methionine to DCE-FPC showed no beneficial effect on growth in young calves. In trail II calves fed DCE-FPC were inferior in weight gains to those fed a dried skim milk ration. Growth response with IP-FPC was inferior to that on DCE-FPC. Calves on FPC and low protein rations developed microcytic, normochromic anemia. This anemia was associated with poor utilitation of FPC protein. A high incidence of muscular degeneration was observed in calves on FPC rations. Addition of chlorocholine chloride 400 mg/kg protein in diets of calves on trial III tended to depress the growth; whereas an identical level of dichloroethane did not adversely affect body weight gains. Trial IV showed that extraction of DCE-FPC with ethanol improved its nutritive value for calves. Washing DCE-FPC with water depressed growth of calves compared to the original DCE-FPC. The growth depression resulting from washing DCE-FPC with water was associated with the removal of water-soluble proteins.

Introduction

Fish protein concentrate (FPC) is a very rich source of protein. A partial substitute for protein in milk replacers for young calves FPC has resulted in satisfactory growth; however, as a sole protein source poor results have been obtained (Huber and Slade, 1967). In recent studies by Genskow (1969) it was shown that deficiency of vitamin E was the principal cause of death in calves when deboned FPC which had been extracted with dichloro-ethane (DCE) was the sole source of protein in the milk replacers. Plasma amino acid levels of calves on the diet showed histidine, leucine and phenylalanine to be lower than normal.

Morrison and Munro (1965) indicated from their studies with rats that the nutritive value of FPC is greatly influenced by the solvent used for extraction. They suggested that the sulfur amino acids and histidine became less available to the animal because of DCE extraction. Studies have shown that cattle fed trichloroethane-extracted soybean meal develops aplastic anemia. (Picken *et al.*, 1952; Pritchard *et al.*, 1952; Sautter *et al.*, 1952).

The present study was undertaken to investigate the causes of poor performance of calves fed FPC as the sole source of protein. Blood parameters and the changes in certain tissues were also observed. Because rat data showed a marked improvement in performance from methanol, ethanol or water washing of DCE-extracted FPC, similar treatment was applied to material fed to calves.

Experimental Procedure

Four trials were conducted with Holstein bull calves to evaluate the nutritional value of FPC as a sole protein source in milk replacers for young calves.

Morrison and Munro, (1965) reported a poor availability of methionine in dichloroethane extractracted FPC (DCE-FPC). Trial I was therefore designed to study the effect of supplementation of methionine to a milk replacer diet in which DCE-FPC furnished all of the protein. The ingredient composition of the milk replacer formulas used in this trial is listed in Table 3. To the control ration 0.40% urea was added in order to make it isonitrogenous with other rations. Rations 2 and 3 were supplemented with 1.24% DL-methionine and 1.24% DL-glutamic acid. Six calves were allotted to three treatments. The calves received colostrum for the first three days after birth and one of the milk replacers as the only source of food from 3 to 56 days. Calves were fed solids at 1.2% of the body weight during the first week of treatment and at 1.3% for the

TABLE 3.--Ingredient composition of rations fed in Trial I.

Ingredient	Rations[a]		
	1	2	3
	----------(%)----------		
Fish flour[b]	27.20	27.20	27.20
Lactose	62.15	61.30	61.30
Emulsified lard oil	10.00	10.00	10.00
Urea	0.40	-	-
DL-methionine	-	1.24	-
Glutamic acid	-	-	1.24
Aurofac-10	0.25	0.25	0.25

[a]Vitamin A and D were added to all rations at 3230 IU and 300 IU, respectively.

[b]Dichloroethane-extracted fish protein concentrate obtained through courtesy of Viobin Corp., Montecello, Ill.

remainder of the treatment period. The milk replacer formulas were diluted to 15% solids and fed by open pail. Calf weights were recorded weekly. Blood samples were drawn at weekly intervals from the jugular vein and analyzed for hematocrit by the micro method and for hemoglobin by the cyanmethemoglobin method, Wintrobe (1953). At the end of the experimental period the calves were sacrificed by electrocution and autopsies were performed.[1]

[1]Autopsy of the calves was performed by Dr. R. L. Michel, Department of Pathology, Michigan State University, East Lansing, Michigan.

Trial II

The results of Trial I showed poor growth and a large decrease in hemoglobin irrespective of methionine supplementation. Trial II was therefore designed to further investigate into growth, hematological changes and changes in the various tissues of calves receiving FDC as the only source of dietary protein. Two levels of protein, 20% and 10% from dried skim milk and FPC were incorporated into the treatment rations. In addition to the regular DCE-FPC used in Trial I, a low ash DCE-FPC (from which bones were partially removed through mechanical screening) and also fish extracted with isopropanol (IP-FPC) were tested. Ingredient composition of the six treatment rations is shown in Table 4. Thirty Holstein bull calves were allotted to the six treatments (five per group) and were fed and weighed as described in Trial I. Blood samples were drawn from jugular vein at weekly intervals and were analyzed for hemoglobin and hematocrit as shown previously and for red blood cells and white blood cells by using the electronic Coulter Counter. Data were statistically analyzed using analysis of variance and differences between treatments were tested by Duncuns new multiple range test. On completion of the eight week treatment period, all calves were sacrificed by electrocution

TABLE 4.--Ingredient composition of rations used in Trial II.[ab]

Ingredient	Rations					
	I	II	III	IV	V	VI
	(%)					
Dried skim milk	57.1	28.6	-	-	-	-
Fish protein concentrate	-	-	29.0[d]	14.5[d]	24.0[e]	24.3[f]
Lactose[c]	-	23.0	30.4	36.6	33.0	32.7
Cerelose	32.6	36.3	30.4	36.7	32.8	32.8
Emulsified lard oil[g]	10.0	10.0	10.0	10.0	10.0	10.0
Dicalcium phosphate	-	2.0	-	2.0	-	-
Aurofac-10	0.25	0.25	0.25	0.25	0.25	0.25

[a]All diets contained (per kg) 3230 I.U. vitamin A, 300 I.U. vitamin D, 46 mg vitamin E, 5.0 mg thiamine, 3.5 mg riboflavin, 20 mg niacin, 5.0 mg pyridoxine, 0.15 mg biotin, 15.0 mg pantothenic acid, 2.0 g choline and 68 mg vitamin B_{12}.

[b]All diets (per kg) were supplemented with Fe 22.5 mg, Zn 30.0 mg, Cu 4.5 mg, Co, 1.10, I, 0.76 and Mn, 20 mg.

[c]Furnished through courtesy of Foremost Dairies, Inc., San Francisco, Calif.

[d]Viobin Corp., Monticello, Ill. Whole fish extracted by the azeotropic method using dichloroethane as the organic solvent.

[e]Viobin Corp., Monticello, Ill. Extracted by azeotropic method - bones partially removed.

[f]Furnished through courtesy of Astra Pharmaceutical Products, Inc., Sweden. Fish extracted using isopropanol as the organic solvent.

[g]Milk Specialties, Inc., Dundee, Ill.

and an autopsy was performed.[1] In addition, certain tissues were subjected to histopathological examination.

Trial III

Chlorocholine chloride (CCC) is the reaction product of trimethylamine (a component usually found in raw fish) and the DCE used as the extraction solvent. The CCC content of DCE-FPC prepared by Munro and Morrison (1967) was reported to be 400 mg/kg air dried weight. Chlorocholine chloride is toxic to rats at the very high level of 500 mg per kg body weight. A high residual content of DCE in DCE-FPC extracted for 24 hrs was reported to cause a depression in growth of rats (Ershoff, 1967).

The samples used in the present studies were analyzed for CCC content by the method described by Munro and Morrison (1967) as adopted from the procedure for choline analysis by Ackerman and Salmon (1960). Dichloroethane content of FPC was determined by gas chromatography. Chlorocholine chloride and DCE concentration in the DCE-FPC used in this study are shown in Table 5. The two samples were quite different in their CCC content (average 376 mg/kg whereas DCE level was less variable and averaged 330 mg/kg).

A trial was therefore designed to asses the effect of the presence of CCC and DCE in calf diets containing DCE-FPC. Four Holstein bull calves were allotted to two diets

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[1]Autopsy of the calves was performed by Dr. R. L. Michel, Department of Pathology, Michigan State University, East Lansing, Michigan.

TABLE 5.--Chlorocholine chloride and dichloroethane contents in fish protein concentrate used in Trial I and Trial II.

Sample	Chlorocholine chloride	Dichloroethane
	mg/kg	(mg/kg)
1	524	360
2	228	300

containing 400 mg/kg of CCC or DCE per kg of protein. The other ingredients in the rations were similar to those in ration I of Trial II. Calves were fed and weighed as described in Trial I. Hemoglobin and hematocrit determinations were made weekly by the methods shown previously. Autopsy of all calves were performed at the end of the eight week experimental period.

Trial IV

The DCE-FPC used in one of the diets was further extracted with ethanol. Extraction was performed in batches of 4.5 kg using a 1:3 proportion of DCE-FPC to ethanol. The DCE-FPC was held in ethanol suspension at 65°C for 1 hr and then filtered through a nylon cloth of fine mesh. Residual ethanol was removed by air drying overnight at room temperature and further drying in a forced draft oven for 2 hrs at 60°C. The DCE-FPC thus prepared contained 77% protein and 0.29% ether extract, which was considerably

lower than ether extract values (approximately 1.5%) determined for the original DCE-FPC.

A second treatment included DCE-FPC washed with hot water. The washing procedure was similar to that used for extraction with ethanol except that the temperature employed while DCE-FPC was in water was 55°C. Fish protein concentrate thus prepared contained 76.25% protein and 1.40% ether extract. Other ingredients of the treatment diets were the same as listed for ration III of Trial II (Table 4). Six bull calves were used in this trial, and it ran concurrently with the trial dealing with vitamin E levels, calves on the dried skim milk diet served as control for both trials. Methods for feeding, weighing and care of animals were the same as employed in Trial I.

Results and Discussion

Trial I

Supplementation of methionine to DCE-FPC did not improve the growth of calves over unsupplemented DCE-FPC diets. Supplementation of 1.24% DL-methionine increased the ration content of L-methionine 0.62% which was approximately equal to the methionine supplied by the original DCE-FPC (Morrison and Munro 1965). Since the growth response with methionine supplementation was not different from other FPC rations, the data might suggest that methionine is not a limiting amino acid for calves fed

TABLE 6.--Growth and hemoglobin levels in calves fed fish protein concentrate supplemented with methionine and glutamic acid - Trial 1.

Ration	Weight changes		Hemoglobin changes	
	30 days	56 days	30 days	56 days
	(lb)		(g%)	
FPC	-1.0	+7.0	-3.60	-5.78
FPC + methionine	-5.5	-	-3.00	-
FPC + glutamic acid	-2.0	-6.5	-3.13	-5.70
S.E. of treatment mean	3.25	5.93	1.07	1.62

DCE-FPC as the only protein source. However, it is well known that excessive methionine can be detrimental to growth and it is possible that lower level of methionine addition might have been beneficial.

A significant finding of this experiment was the marked anemia in the calves on all treatments. During the 8-week feeding period, the hemoglobin values dropped to about one-half their original level. These results differ from those obtained by Genskow (1969) who observed no change in hemoglobin of calves fed milk replacers in which all the protein came from DCE-FPC.

Blood smears from the calves were examined by a competent pathologist[1] and showed no immature elements.

[1]Dr. R. L. Michel, Department of Pathology, Michigan State University, East Lansing, Michigan.

However, autopsy of the calves revealed hypoplastic bone marrow. The blood changes observed and the atrophy of the thymus shown in most of the calves on the study might be expected to result from a general malnutrition and not from any specific dietary factor.

Trial II

Growth of calves fed 20% DCE-FPC diet in Trial II (Table 7) was superior to that on DCE-FPC treatment in Trial I. Although the DCE-FPC used in Trials I and II was prepared by a similar process but the rations in Trial II were supplemented with additional vitamins and trace minerals, while those of Trial I were not. These data are in general agreement with those of Genskow (1969) who obtained better growth and less mortality in calves on DCE-FPC diets which were supplemented with vitamin E than on unsupplemented diets.

Weight gains during the 8-week treatment period were lower on DCE-FPC diets than on dried skim milk (DSM) at corresponding protein levels. Weight gains on screened DCE-FPC ration were inferior to those obtained on DCE-FPC containing 20% protein, but differences were not significant ($P<0.05$). A marked growth depression on the IP-FPC ration compared to other protein sources were observed. This was partially due to poor acceptance by the calves. Calves started refusing the IP-FPC ration after about 3 weeks on trial. Average refusals amounted to about 25% of the total dry matter offered.

TABLE 7.--Effect of different sources of protein in milk replacers on body weight gains, feed consumption and protein efficiency ratio (PER) in calves, Trial II.

Treatment Diet	Body weight			Total feed cons.	PER
	Initial	Final	Weekly change		
	----------(lb)----------			(lb)	
Dried skim milk-20% P	81.0	116.2	4.40^{d}	64.69^{a}	2.68
Dried skim milk-10% P	98.4	101.2	1.12^{ab}	59.31^{a}	0.58^{a}
DCE-FPC-20% P	93.6	107.8	1.92^{a}	67.15^{a}	0.96^{a}
DCE-FPC-10% P	104.4	90.8	-1.65^{c}	56.28^{ac}	-2.64
DCE-FPC screened-20% P	98.0	103.4	0.80^{ab}	65.85^{a}	0.41^{a}
IP-FPC-20% P	91.8	81.4	-1.61^{c}	47.35^{bc}	-1.21
S.E. of treatment mean	-	-	0.62	4.10	0.13

^{abc}Values not sharing a common superscript are significantly different ($P<0.05$).

During the preparation of IP-FPC, raw fish is pressed to remove the water. Preparation of DCE-FPC does not involve pressing of fish before extraction with DCE. It can therefore be assumed that water-soluble proteins are removed from IP-FPC, while they are conserved in DCE-FPC. It has been shown that during early life, calves do not grow satisfactorily when water-soluble proteins are low or absent in milk or milk replacers (Shillam et al., 1960; Shillam and Roy, 1963; McCoy et al., 1967). Superior growth of calves on DCE-FPC compared to IP-FPC may be related of the presence of water soluble proteins in DCE-FPC. The favorable results obtained by European workers (Stellaman, 1969) with milk replacers in which 75% of the protein came from IP-FPC and the other 25% from whey suggests a highly favorable effect of the whey which might be attributable to the whey protein being highly digestible and completely water soluble.

Feed consumption did not decrease on FPC milk replacers except for the IP-FPC and the 10% DCE-FPC diets (Table 7). The lower weight gains and PER ($P<0.05$) on FPC than on corresponding DSM replacers shows that the nutritive value of FPC for calves is markedly lower than that of protein from dried skim milk. Efficiency of protein utilization was markedly decreased when milk replacers contained only 10% protein whether from DSM or DCE-FPC. The possibility that the growth retardation was due to deficiency of certain

vitamin or trace elements is not ruled out; but all the rations were supplemented with recommended levels of micro-elements. However, a later study indicated that the quantity of vitamin E added to DCE-FPC rations may have been too low to support optimal gains. Another contributing factor to the depressed growth was a lower protein digestibility of FPC than for milk protein. (Huber and Slade, 1967 and van Hallemond, 1967).

Studies with rats have shown increased weight gains when FPC diets were supplemented with one or more of the following amino acids: methionine, histidine, tryptophan, threonine and arginine (Ousterhout et al., 1959, Smith et al., 1962, Morrison and Sabry, 1963; Shillings, et al., 1969). Blood plasma of calves fed a milk replacer in which DCE-FPC was the protein source had low concentrations of histidine (Genskow, 1969). The author suggested a low availability of this amino acid to calves fed DCE-FPC. Fish protein concentrate is not markedly low in histidine and it is not fully understood why histidine in FPC might be limiting in its availability to the animal.

Hematological data (Tables 8 and 9) reveal that calves on all FPC milk replacers as well as those on 10% DSM milk replacer developed mild to severe anemia. The severity of the anemia appeared to be inversely proportional to body weight gains during the 8 week feeding period (Fig. 1 and 2). It is well known that protein deficiency

TABLE 8.--Hematological data on calves fed different sources of protein in the milk replacer, Trail II.

Treatment diet	Hemoglobin			Hematocrit		
	Initial	Final	Weekly change	Initial	Final	Weekly change
	---------(g/%)----------			----------(%)----------		
Dried skim milk-20% P	10.0	9.8	-0.018[a]	32.6	31.0	-0.198[a]
Dried skim milk-10% P	11.9	9.0	-0.360[b]	34.6	27.2	-0.896[ab]
DCE-FPC-20% P	9.9	7.8	-0.282[b]	32.4	23.6	-1.134[bc]
DCE-FPC-10% P	12.1	8.8	-0.470[b]	38.6	26.6	-1.712[c]
DCE-FPC screened-20% P	11.9	8.6	-0.416[b]	36.8	25.0	-1.472[bc]
IP-FPC-20% P	10.0	8.2	-0.296[b]	31.0	24.8	-1.026[bc]
S.E. of treatment mean			0.073			0.249

[abc]Values not sharing common superscript are significantly different ($P<0.05$).

TABLE 9.--Changes in hematological values in calves fed different sources of protein in milk replacers, Trial II.

Treatment ration	Mean corpuscular hemoglobin (MCH)	Mean corpuscular volume (MCV)	Mean corpuscular hemoglobin concentration (MCHC)
	(μμg)	(μ^3)	(%)
Dried skim milk-20% P	-0.12[a]	+0.10[a]	-0.28[bd]
Dried skim milk-10% P	0.00[a]	+1.20[a]	-1.00[cd]
DCE-FPC-20% P	-0.74[a]	-5.30[c]	+2.00[ab]
DCE-FPC-10%	-3.00[b]	-11.40[d]	+3.70[a]
DCE-FPC screened-20% P	-1.50[a]	-6.20[bc]	+2.00[ab]
IP-FPC-20% Protein	-0.50[a]	-2.70[ab]	+0.90[bc]
S.E. of treatment mean	0.49	1.60	0.77

[abcd]Values not sharing common superscript are significantly different ($P<0.05$).

is one cause of anemia. Since the milk replacers were adequately supplied with Fe, Cu, Co and vitamin B_{12}, anemia observed in these calves is not attributed due to lack of those nutrients.

Young animals are usually more aggravated by a low protein ration than older ones since protein is also demanded for body growth. The greater anemia observed in calves on FPC than DSM rations at equal protein intake was attributed primarily to the poor protein quality of the FPC.

Hematological indices (Table 9) showed that mean compuscular volumes (MCV) decreased more in the calves on FPC than DSM milk replacers. However, the mean corpuscular hemoglobin concentration (MCHC) increased in calves fed the DCE-FPC milk replacers, compared to DSM or IP-FPC. It has been reported by Plat (1956), Knowles (1957) and Schalm (1965) that protein deficiency causes normochronic, microcytic anemia. The anemia in the present study has been characterized as normochromic, microcytic type.

The observation that the reduction in hemoglobin concentration was almost parallel to the depression in growth (Fig. 1 and 2) suggests that there is competition for protein between demands for body growth and for hemoglobin synthesis.

A summary of the autopsy reports on the calves performed at the end of the 8-week experimental period is

TABLE 10.--Weekly weight gain changes in calves fed rations with different protein sources (expressed as % of initial weight).

Ration	Weeks								
	Initial	1	2	3	4	5	6	7	8
20% DSM	100.0	100.0	102.2	107.9	115.0	123.9	129.6	137.2	143.4
10% DSM	100.0	98.6	98.4	97.8	97.6	99.1	103.0	105.4	109.1
20% DCE-FPC	100.0	95.7	96.5	100.4	104.0	105.7	110.0	113.0	121.0
10% DCE-FPC	100.0	93.5	91.2	88.7	89.3	87.3	85.3	85.2	85.0
20% DCE-FPC screened	100.0	98.1	97.9	98.5	100.4	102.0	102.8	104.2	107.5
20% IP-FPC	100.0	95.2	94.3	93.2	91.7	91.1	93.3	98.6	-

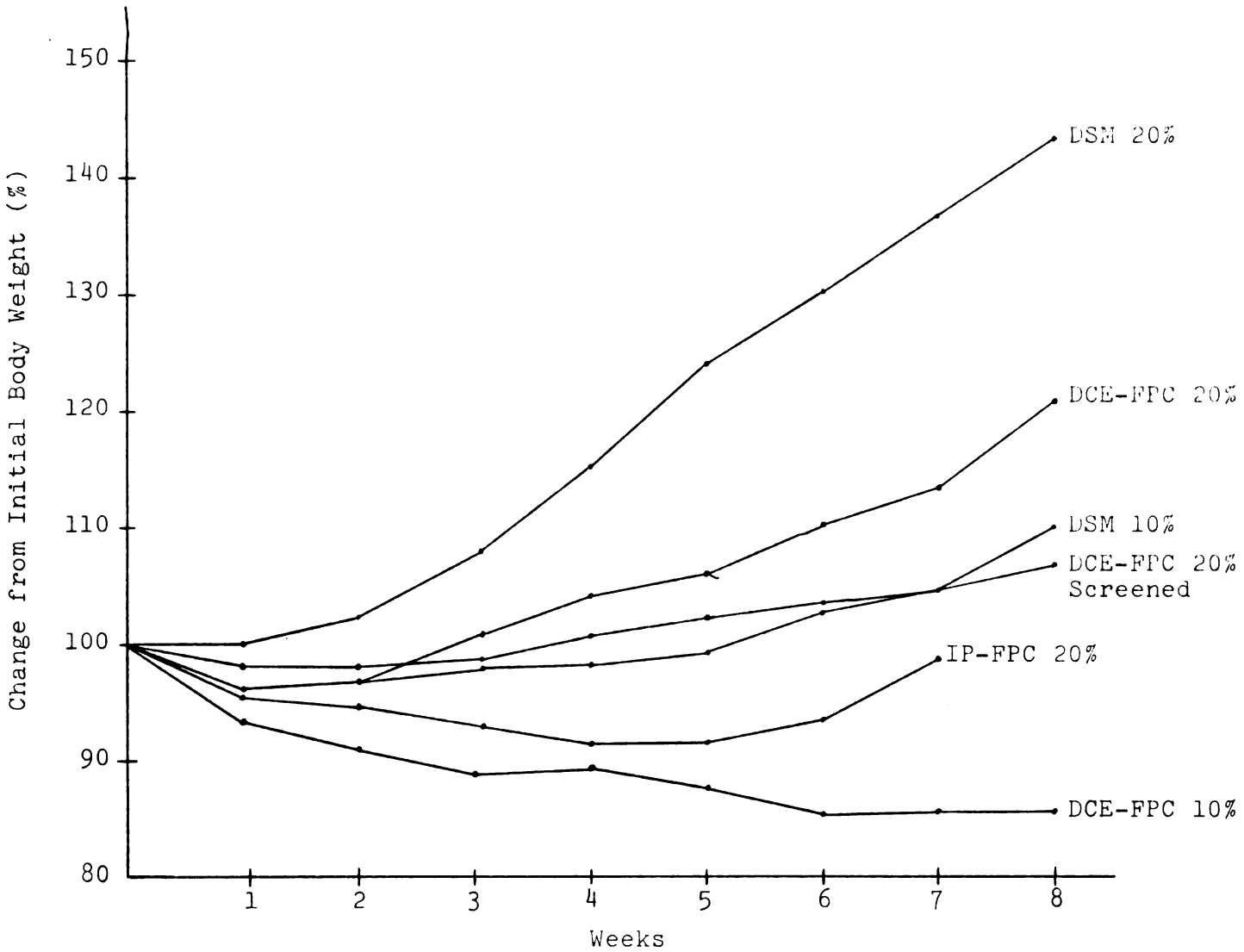

Figure 1.--Changes in Body Weight of Calves Fed Different Protein Sources.

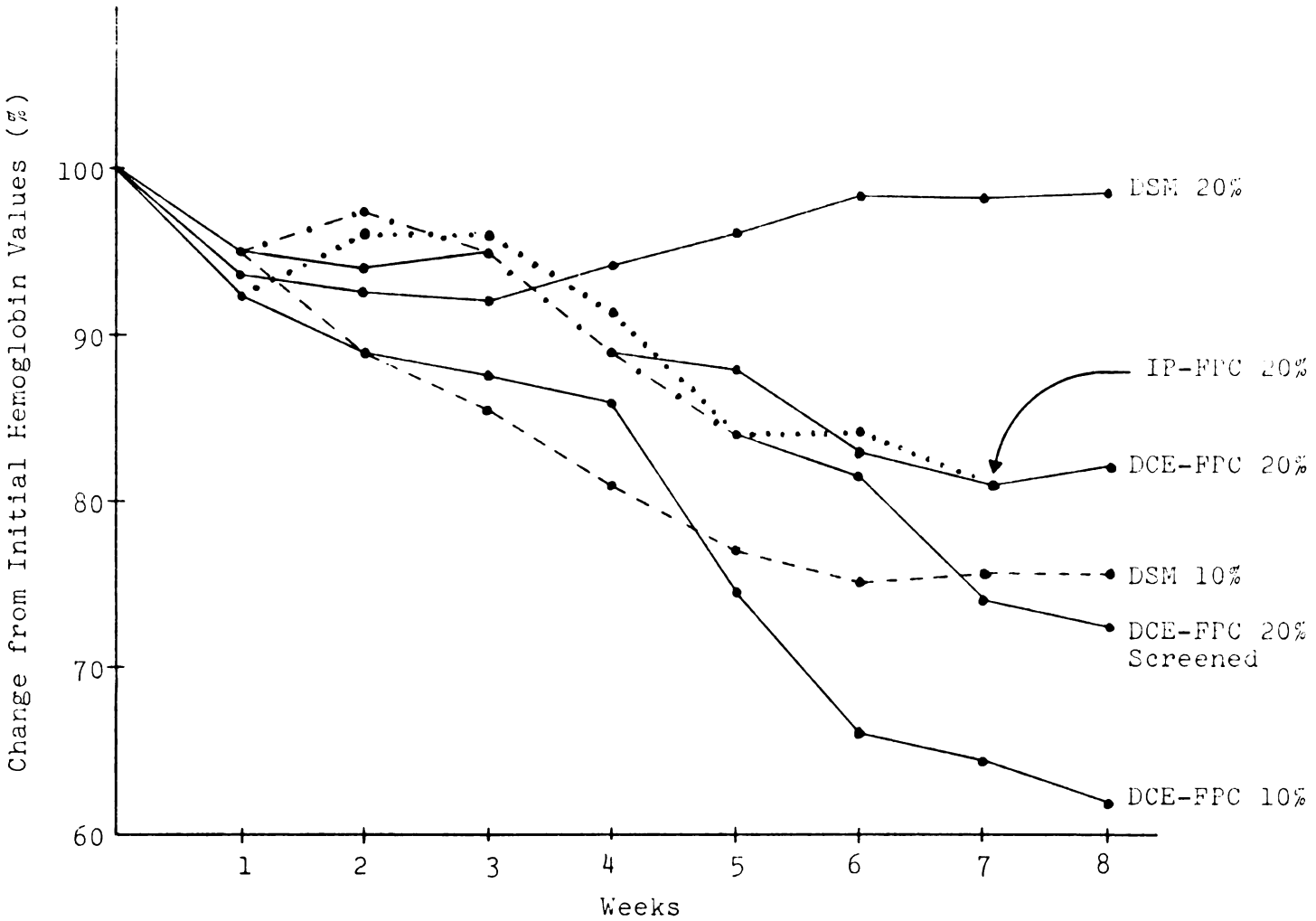

Figure 2.--Changes in Hemoglobin Values in Calves Fed Different Protein Sources.

given in Appendix Table I. The main autopsy finding showed that the calves on FPC-milk replacers had a higher incidence of muscular degeneration than those on milk protein, although the recommended levels of vitamin E (Huber and Thomas 1967) was supplemented to all the rations. Because it appeared that DCE-FPC increased vitamin E requirement of the calves, further study on the relationship of protein source and response to supplemental vitamin E was undertaken and is reported later in this thesis.

Trial III

As indicated in studies by Munro and Morrison (1967) and Ershoff (1967), CCC and residual DCE exert depressing effect on growth in rats. Effect of these two compounds on the growth of calves was studied in Trial III. Results of this trial (Table 11) showed that when CCC was added to the diet at the level of 400 mg per kg milk protein, a slight but nonsignificant depression in growth was obtained. The calves consumed 1.53 mg CCC per kg body weight. The LD_{50} of CCC for calves is not known. Munro and Morrison (1967) reported that the LD_{50} of CCC for rats was 500 mg/kg body weight. Calf numbers in this trial were not sufficient to unequivacally conclude a depressing effect of CCC on calf growth. However, it appears that the calves are more sensitive to this compound than rats who showed no adverse effect on the growth at levels comparable to those fed to calves as reported elsewhere in the thesis.

TABLE 11.--Effect of chlorocholine chloride (CCC) and dichloroethane (DCE) on growth in calves (Trial III)[a].

Ration	Weekly body weight change (lb)	
Dried skim milk replacer	4.40±0.50[a]	(5)[b]
Dried skim milk replacer + CCC	3.69±0.78	(2)
Dried skim milk replacer + DCE	4.56±0.78	(2)

[a]S. E. treatment mean.

[b]Figure in parentheses indicate the number of calves used per treatment.

Residual DCE in DCE-FPC used in this study was 360 ppm. When 400 mg DCE per kg protein were added to dried skim milk replacer, growth was not altered compared to control diet. In a study by Ershoff (1967) with rats, a diet containing FPC extracted with DCE for 24 hrs (which contained 30,000 mg/kg of DCE) exerted marked depression in growth of rats. This level of residual DCE is one hundred-fold higher than found in commercially prepared FPC where the period of extraction is only 1 to 2 hrs.

Trial IV

Superior weight gains were obtained when DCE-FPC was further extracted with ethanol compared to unextracted DCE-FPC (Table 12). However, weight gains were still lower than those of calves fed dried skim milk replacer. The DCE-FPC used in this study contained 1.43% ether extract.

TABLE 12.--Body weight changes in calves fed FPC extracted with ethanol or washed with water.

Ration	Weekly body weight change (lb)	
DSM (20% Protein)	4.56[a]	0.61*
DCE-FPC (20% Protein)	2.04[a]	0.70
DCE-FPC extracted with ethanol (20% protein)	3.35[a]	0.70
DCE-FPC washed with water (20% Protein)	-1.55[b]	0.70

*S. E. of treatment mean.

[ab]Values not sharing a common superscript are significantly different ($P<0.05$).

After extraction with ethanol, FPC contained only 0.29% ether extract. Thus, ethanol extraction removed most of the lipids. It is well established that diets high in polyunsaturated fatty acids increase the vitamin E requirement of calves (Adams et al., 1959a). As shown by Genskow (1969) and also later in this study, the addition of vitamin E to a DCE-FPC milk replacers improved weight gains and decreased mortality of calves. The muscular degeneration observed in the calves in Trial III also suggest a vitamin E deficiency on DCE-FPC milk replacers. Studies by Munro and Morrison (1957) showed that further washing with methanol of FPC, originally prepared by extracting 24 hrs in DCE, resulted in a decrease in mortality of rats.

In light of the findings of Ershoff (1967), it seems likely that the primary deleterious factor removed by the methanol in Canadian study (Munro and Morrison 1967) was the residual DCE. However, in our studies with rats, as explained in a later section, a marked improvement in growth also resulted from methanol, ethanol, or water washing of DCE-FPC. This improvement did not appear related to a decrease in residual DCE or CCC, but more specifically to an increase in efficiency of protein utilization. Removal of polyunsaturated fatty acids and of CCC are the probable factors responsible for improved growth of calves observed after ethanol-washing of DCE-FPC.

When DCE-FPC was washed with water and included in calf diets as the only protein source, a loss of 1.55 lb body weight per week was observed. This weight loss was comparable to that obtained with the IP-FPC milk replacer in Trial II. During the preparation of IP-FPC, water soluble proteins from fish are removed; but water-soluble constituents of DCE-FPC are conserved during its original preparation. These data suggest that removal of water soluble fractions from FPC milk replacers decreases its nutritive value for calves. These calf data are in general agreement with the findings of Shillam et al. (1960) and Shillam and Roy (1963) who observed unsatisfactory growth in calves fed milk replacers in which 72% of the whey protein was denatured by high heat treatment. However, in our

rat studies, removal of the water-soluble constituents markedly improved performance. These data also support the observation of McCoy et al. (1967) who showed an improvement in calf performance when the whey protein non-fat dried milk fed to calves was at least 4.7 mg/g. The reason for need of water-soluble protein in the young calf has not been clarified but one possibility is that the rapid release of certain amino acids are important for efficient protein digestion in the calf and a source comprised totally of water insoluble protein would not meet this necessity.

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PART II

VITAMIN E ADDITION TO DCE-FPC MILK REPLACERS

ABSTRACT

Nineteen Holstein calves were placed on five treatments. Two rations contained dried skim milk with (46 mg/kg dry matter) or without vitamin E. The other three rations contained dichloroethane extracted fish protein concentrate (DCE-FPC) with 0, 46 and 92 mg/kg vitamin E. Growth response improved as the level of vitamin E in DCE-FPC ration increased. However, no alteration in weight gains was observed with different levels of vitamin E supplemented to milk protein rations. Plasma tocopherol concentrations were related to both level of supplemental vitamin E and protein source. More depression in plasma tocopherol concentration was noted in calves on the DCE-FPC ration compared to the milk protein ration. It is suggested that the vitamin E requirement for the young calf receiving DCE-FPC as the only protein source is approximately 120 mg per 100 kg body weight.

Introduction

In the preceeding studies with calves fed milk replacers containing dichloroethane extracted fish protein concentrate (DCE-FPC) as a sole source of protein, symptoms of vitamin E deficiency, particularly muscular dystrophy, were observed, although the rations were supplemented with recommended levels (Huber and Thomas, 1967) of vitamin E. It has been well documented that muscular dystrophy can be produced in several species of animals by feeding a vitamin E deficient diet (Stafford et al., 1954). Moore et al. (1961) have reported that cod liver oil contains a considerable amount of vitamin E, the range being 100-200 mg/kg. However, this level of vitamin E is insufficient to protect against the highly unsaturated fatty acids also present. Thus, animals fed diets containing cod liver oil have an increased requirement for vitamin E.

The purpose of this experiment was to determine the level of vitamin E in a DCE-FPC milk replacer ration which will prevent muscular dystrophy and bring about optimum growth in calves. The interaction of protein source and response to vitamin E was also studied.

Experimental Procedures

Nineteen Holstein calves (10 bulls and 9 heifers) were placed on five treatments. Ingredient composition of the rations is presented in Table 13. All the rations were supplemented with the vitamins and trace minerals suggested for semi-purified diets for calves (Huber and Thomas, 1967) except vitamin E. The calves were fed and weighed as described in previous experiments. At weekly intervals, blood samples were taken from jugular vein for α-tocopherol determinations. The α-tocopherol concentrations were determined according to the method described by Duggan (1959). At the end of the 8-week treatment period, an autopsy and histopathological examination was performed on two bull calves from each diet.

Results and Discussion

Vitamin E exerted marked effect on weight gains of calves receiving DCE-FPC but did not alter growth on milk protein (Table 14). When 60 mg vitamin E per 100 kg body weight were supplied to calves fed DCE-FPC milk replacer, weight gains improved over the unsupplemented fish protein diet. Growth was further improved by doubling the supplemental vitamin E. However, none of the DCE-FPC diets were equal to milk protein diet which contained no added vitamin E.

TABLE 13.--Ingredient composition of rations used in Trial V.[a,b]

Ingredient	Rations				
	I	II	III	IV	V
	(%)				
Dried skim milk	56.50	56.50	-	-	-
Fish protein concentrate	-	-	26.50	26.50	26.50
Lactose[d]	17.00	17.00	31.70	31.70	31.70
Cerelose	16.20	16.20	31.50	31.50	31.50
Emulsified lard oil	10.00	10.00	10.00	10.00	10.00
Aurofac-10	0.25	0.25	0.25	0.25	0.25
Vitamin E[c]	+	-	+	-	++

[a]All rations contained (per kg) 3230 I.U. vitamin A, 300 I.U. vitamin D, 5.0 mg thiamine, 3.5 mg riboflavin, 20 mg niacin, 5.0 mg pyridoxine, 0.15 mg biotin, 15.0 mg pantothenic acid, 2.0 g choline and 68.0 mg vitamin B_{12}.

[b]All rations contained (per kg) Fe 22.5 mg, Zn 30.0 mg, Cu 4.5 mg, Co 1.10 mg and I 0.76 mg.

[c]Rations I and III contained 46 mg α-tocopherol acetate per kg. Ration V contained 96 mg α-tocopherol acetate per kg.

[d]Furnished through courtesy of Foremost Dairies, Inc., San Francisco, California.

Decreases in plasma α-tocopherol were noted for all treatments, but seemed related both to level of vitamin E supplementation and source of protein (Table 15). At the normal level of E supplementation plasma tocopherols were more greatly depressed on fish protein than milk protein.

TABLE 14.--Growth of calves fed milk or fish protein with or without supplemented vitamin E.

Ration	Weight gains per week
	(lb)
I Dried skim milk + E[d]	4.56[a]±0.62*
II Dried skim milk - E	4.28[a]±0.62
III Fish protein concentrate + E[d]	2.04[ab]±0.71
IV Fish protein concentrate - E	1.06[b]±0.62
V Fish protein concentrate + 2E[e]	3.66[a]±0.62

*S. E. of treatment mean.

[abc]Values not sharing common superscript are significantly different. ($P<0.05$)

[d]Supplemented at 46 mg/kg diet with α-tocopherol acetate.

[e]Supplemented at 96 mg/kg diet with α-tocopherol acetate.

However, when twice the normal vitamin E dose was added to DCE-FPC replacer, plasma tocopherol concentrations of calves fed this diet were comparable to those found in calves receiving normal dried skim milk ration at recommended level of vitamin E. The very low concentrations of plasma α-tocopherol shown in calves on both protein sources receiving no supplemented vitamin E raises the question as to the minimum levels of plasma α-tocopherol that can be reached and the calf still live. The concentrations observed in this study were lower than the author could find elsewhere in the

TABLE 15.--Vitamin E concentrations in plasma of calves fed supplemented and unsupplemented rations with different protein sources (ug/100 ml).

Time of Sampling	Ration				
	I	II	III	IV	V
Initial	152.59	192.53	146.70	151.97	181.69
4th Week	103.66(68)[a]	56.03(29)	77.90(53)	64.64(43)	124.99(69)
8th Week	89.22(58)	3.60(2)	47.60(33)	12.97(9)	88.88(49)

[a]Value in parenthesis represents the percent of the initial concentration.

literature. It is also interesting to note that the unsupplemented calves on dried skim milk grew as well as the supplemental group despite the tremendously low plasma α-tocopherol concentrations. These data strongly suggest that DCE-FPC affords less protection to vitamin E than does dried skim milk.

The lipid content of the DCE-FPC used in this study was 1.43%. A 100 lb calf on DCE-FPC replacer would consume 2.3 g lipid per day. Since fish lipids contain highly unsaturated fatty acids, it is suggested that higher levels of vitamin E would be needed in a DCE-FPC than a dried skim milk diet to afford adequate protection against unsaturated fatty acids.

A higher incidence of diarrhea was observed in calves on the DCE-FPC replacers. It was found that the diarrhea in calves causes marked decrease in plasma tocopherol (Thomas and Okamoto, 1955). The diarrhea may have further aggravated the vitamin E stress of calves on DCE-FPC diets.

Calves on the unsupplemented DCE-FPC replacers were lethargic, muscularly weak and some were unable to stand without assistance towards the end of the treatment period. Histological examination (Appendix Table II) revealed extensive muscular lesions in calves on milk replacers not supplemented with vitamin E regardless of protein source; whereas muscular lesions were prevented at all levels of E supplementation.

Since α-tocopherol is a biological antioxidant, it prevents the auto-oxidation of vitamin A (Cawthorne et al., 1968). This implies that the calves showing vitamin E deficiency symptoms would need higher levels of vitamin A in the diet. Vitamin A concentrations in the blood of calves were not determined, but no visible symptoms of vitamin A deficiency were evident. These data support those of Genskow (1969) who showed 50 and 100 mg/day of vitamin E supplemented to calves fed milk replacers in which deboned DCE-FPC was the sole source of protein decreased death losses, improved performance and increased plasma α-tocopherol concentrations. No difference was observed between diets furnishing 50 and 100 mg/day of vitamin E. The data also show that amount of vitamin E needed for supplementation to DCE-FPC is over 30 mg/day (the approximate amount the calves on ration IV received).

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PART III

EVALUATION OF FISH PROTEIN CONCENTRATE AS A SOURCE OF PROTEIN

ABSTRACT

Fish protein concentrates extracted with dichloroethane (DCE-FPC) or isopropanol (IP-FPC) were evaluated for their nutritional quality. The IP-FPC produced superior growth response and PER to casein or DCE-FPC. Biological value significantly improved ($P<0.05$) when DCE-FPC was further extracted with methanol or ethanol or washed with water. Heating of DCE-FPC tended to improve the weight gains in rats. Addition of methanolic and ethanolic extracts of DCE-FPC to casein or DCE-FPC diets depressed rat gains but addition of chlorocholine chloride (CCC) or dichloroethane (DCE) to casein diet did not alter growth response. At 20% and 40% protein, weight gains were same on DCE-FPC and casein suggesting that toxicity of DCE-FPC was not a major factor depressing growth in rats. When small amounts of casein were added to DCE-FPC diets, a significant improvement ($P<0.05$) in weight gains were observed. Serum protein, serum albumin and net protein utilization of rats increased in proportion to the superiority of the protein source. Plasma of rats on all FPC diets was lower in histidine than plasma of rats on the casein diet.

Introduction

The solvent used to remove oil and water from fish to produce fish protein concentrate (FPC) greatly influences the nutritive value of the final product (morrison and Munro, 1965). Chlorocholine chloride (CCC), a reaction product of trimethylamine in fish and the dichloroethane (DCE), was toxic to rats at high levels (Munro and Morrison, 1967). It was also found that a high residual content of DCE in FPC markedly depressed growth in rats (Ershoff, 1967). Favorable growth responses were reported when dichloroethane extracted FPC (DCE-FPC) was supplemented with milk protein (Ershoff, 1967) or when FPC was prepared using isopropanol as the extraction solvent (Stillings, 1967).

The aim of the present study was to determine the importance of certain toxic compounds reportedly present in FPC and whether further extraction or heating of DCE-FPC would alter its nutritive value. An effort was also made to determine the response of rats to varying levels of casein added to DCE-FPC diets.

Experimental Procedures

Experiment I

This experiment was designed to gain further information on the toxicity of DCE-FPC. Three levels of protein were fed and response of rats to methanol extraction of DCE-FPC was also investigated.

Fifty, 21-day old weanling male rats of the Sprague-Dawly strain were alloted to the ten diets shown in Table 16. Diets 1, 2 and 3 contained 10, 20, and 40% protein from casein. Diets 4, 5 and 6 contained same levels of protein from DCE-FPC. Identical protein levels from methanol-extracted DCE-FPC were replicated in diets 7, 8 and 9. Diet 10 contained 40% protein from casein plus the methanolic extract from DCE-FPC. The amount of methanolic extract added was equal to that obtained from extraction of DCE-FPC used in diet 9. Rats were housed in individual cages with wire bottom floors. Food and water were given ad libitum. Rats were weighed every week and food intake was measured for each rat. The PER for each rat was calculated from weight gain and food consumption data. Treatment was for 28 days. At the end of treatment rats were sacrificed and bled. Hemoglobin and hematocrit were determined as described previously for calves. Histopathological examination of tissues were also made as mentioned earlier from selected animals. Because no differences between treatments were observed for hemoglobin and hematocrit and all

TABLE 16.--Ingredient composition of diets used in Experiment I.

Diet	Diets									
	1	2	3	4	5	6	7	8	9	10
					%					
Casein[a]	11.0	22.0	44.0	-	-	-	-	-	-	44.0
DCE-FPC[b]	-	-	-	13.8	27.6	55.2	-	-	-	-
DCE-FPC methanol extracted	-	-	-	-	-	-	12.1	24.2	48.4	-
Corn starch	39.5	34.0	23.0	39.1	33.2	19.4	40.0	34.9	22.8	23.0
Cerelose	39.5	34.0	23.0	39.1	33.2	19.4	39.9	34.9	22.8	23.0
Vegetable oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt mix[c]	4.0	4.0	4.0	2.0	-	-	2.0	-	-	4.0
Vitamin mix[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[a]Nutritional Biochemicals Corp., Cleveland.

[b]Viobin Corp., Monticello, Ill.

[c]General Biochemicals Corp., Chagrin Falls, Ohio. Salt mix containing (%): $CaCO_3$, 21; $Ca_3(PO_4)_2$, 14.9; $CuSO_4.5H_2O$, 0.039; $ScPO_4.4H_2O$, 1.47; $mgSO_4$, 9.0; $MnSO_4$, 0.02; $K_2Al(SO_4)_2.24H_2O$, 0.009; KCl, 12.0; KI, 0.005; KH_2PO_4, 31.0;NaCl, 10.5; NaF, 0.057.

[d]Nutritional Biochemicals Corp., Cleveland. Vitamin diet fortification mixture containing (g/100 lb diet): vitamin A (200,000 IY/g), 4.5; vitamin D (400,000 IU/g), 0.25; α-tocopherol, 5.0; ascorbic acid, 45; inositol, 5.0; choline chloride, 76.0; menadione, 2.25; p-amino benzoic acid, 5.0; niacin, 4.5, riboflavin, 1.0; pyridoxine-HCL, 1.0; calcium pantothenate, 3.0 (mg/100 lb diet): biotin, 20, folic, 90; and vitamin B_{12}, 1.35.

tissues examined were essentially normal, the data are not presented in greater detail.

Experiment II

Fish protein concentrate prepared by extraction with dichloroethane or isopropanol were compared. Moreover, the addition to casein diet of CCC and DCE, separately and in combination to casein diets, was tested. Eighty weanling male rats were alloted to the eight diets shown in Table 17. All the diets in this experiment as well as in subsequent experiments contained 10% protein. Ethanol-extracted DCE-FPC was prepared in the same as described in calf Trial IV. The amount of ethanolic extract added to casein diet was equivalent to that obtained from extracting sufficient DCE-FPC to make 10% protein diet. Chlorocholine chloride and DCE were added to casein diets at the rate of 400 mg per kg of protein. Data on weekly weight gains and total feed consumption were obtained for each rat. At the end of the experiment, the liver and pancreas were removed from all rats and the weights of these organis were recorded.

Experiment III

This experiment was designed to study the effect of different extraction treatments on the nutritive value the original DCE-FPC, and also the effect of adding varying levels of casein to DCE-FPC diets. There were fourteen treatments with 10 rats per diet (Table 21). In diets 1, 2 and 3 casein, IP-FPC and DCE-FPC respectively furnished

TABLE 17.--Ingredient composition of diets used in Experiment II.

Ingredient	Casein	IP-FPC	DCE-FPC		Casein			
			Untreated	Ethanol extracted	+ CCC	+ DCE	+ CCC+DCE	+Ethanol extracted
	(%)							
Casein[a]	11.0	-	-	-	11.0	11.0	11.0	11.0
IP-FPC[b]	-	12.0	-	-	-	-	-	-
DCE-FPC[c]	-	-	13.5	12.8	-	-	-	-
Corn starch	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
Cerelose	39.5	39.7	39.0	39.7	39.5	39.5	39.5	39.5
Vegetable oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt mix[d]	4.0	2.8	2.0	2.0	4.0	4.0	4.0	4.0
Vitamin mix[e]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[a]Nutritional Biochemicals Corp., Cleveland.

[b]Furnished through courtesy of Astra Pharmaceutical Co., Sweden.

[c]Viobin Corp., Monticello, Ill.

[d]General Biochemicals Corporation, Chagrin Falls, Ohio.

[e]Nutritional Biochemicals Corp., Cleveland.

the sole source of protein. In diet 4 the DCE-FPC was further extracted with ethanol as previously described. Diets 5 and 6 corresponded to diets 3 and 4, respectively, but differed in that ethanolic extracts obtained from extraction of DCE-FPC were added at same level described in Experiment II. The DCE-FPC in diet 7 was moistened with small quantity of ethanol and heated at 65°C for 2 hours. Water-washed DCE-FPC (as described in calf Trial IV) was the protein source in diet 8. Casein was added to DCE-FPC in diets 9, 10 and 11 to furnish 30%, 20% and 10% of the total protein. Diets 12, 13 and 14 contained identical amounts of casein added to ethanol extracted DCE-FPC. Except for protein source, all the diets were of the same basic composition as shown in Table 17. The rats were weighed each week and total feed intake was measured for each rat.

At the end of 4 weeks of treatment, blood from the abdominal aorta was drawn from five rats each on diets 1, 2, 3, 4 and 8 for determination of plasma free amino acids as well as total serum protein and its components. Samples for plasma free amino acid determination were prepared according to Purser et al. (1966) using norleucine as an internal standard. Amino acid analysis was performed on a Technicon TSM Amino Acid Analyzer using a lithium citrate buffer. Total serum protein was determined by modified Lowry method (Miller 1959). Electrophoretic separation

of serum protein components was performed as described by Cowley and Eberhardt (1962).

Net protein utilization (NPU) of rats on treatments 1, 2, 3, 4 and 8 was determined according to method described by Bender (1958) with following modification: An intial group of five rats was sacrificed at the beginning of the experiment. Rats on the five treatment diets were sacrificed after the four-week treatment period. The contents of the gastrointestinal tract were removed from each rat. The rats were autoclaved and homogenized as described by Mickelsen and Anderson (1959) and the carcass nitrogen determined by Kjeldahl (AOAC, 1965). Net protein utilization was calculated by the following formula:

$$NPU = \frac{\text{Nitrogen retained in carcass}}{\text{Nitrogen intake}} \times 100$$

Results and Discussion

Experiment I

Growth of rats on the DCE-FPC diet was significantly ($P<0.05$) lower than on the 10% casein diet, (Table 18). At the 10% protein, methanol extraction of DCE-FPC significantly improved growth and PER compared to the original DCE-FPC, but they still did not equal those observed for casein, (Table 19). No significant difference was noted in the 20% or 40% protein levels between casein, DCE-FPC or methanol extracted DCE-FPC. This may have been due to

TABLE 18.--Effect of fish flour on weight gains of rats

Diet	Level of protein in the ration		
	10%	20%	40%
Casein	$128.4^{c*} \pm 8.09$	$186.3^{ab} \pm 8.09$	$187.2^{a} \pm 8.09$
DCE-FPC	$53.0^{d} \pm 8.09$	$178.0^{ab} \pm 8.09$	$175.9^{ab} \pm 8.09$
Methanol-extracted DCE-FPC	$86.9^{e} \pm 8.09$	$176.2^{ab} \pm 8.09$	$179.6^{ab} \pm 8.09$
Casein+methanolic extract	----	----	$162.7^{b} \pm 8.09$

^{abcd}Values not sharing common superscript are significantly different ($P<0.05$).

TABLE 19.--Effect of fish flour on protein efficiency ratios (PER) of rats.

Diet	Level of protein in the ration		
	10%	20%	40%
Casein	$3.19^{a*} \pm .088$	$2.15^{b} \pm .088$	$1.15^{d} \pm .088$
DCE-FPC	$1.17^{d} \pm .088$	$2.05^{b} \pm .088$	$0.92^{d} \pm .088$
Methanol-extracted DCE-FPC	$2.22^{b} \pm .088$	$1.75^{c} \pm .088$	$0.97^{d} \pm .088$
Casein+methanolic extract	----	----	$0.90^{d} \pm .088$

^{abcd}Values not sharing common superscript are significantly different ($P<0.05$).

a masking of adverse effects of the DCE-FPC by higher levels of protein. When the methanolic extract of DCE-FPC was added to the 40% casein diet a significant decrease ($P<0.05$) in growth was noted.

Average PER values for the DCE-FPC diet at 10% protein were significantly lower than that for casein. When DCE-FPC was extracted with methanol, an improved PER was noted at 10% protein level. Similar to growth observations, source of protein did not significantly affect PER at 20% and 40% protein.

The results obtained in this study are not in complete agreement with those of Munro and Morrison (1967) who observed complete mortality of rats fed DCE-FPC. The FPC used in their study was prepared by extraction of cod fillets for 24 hours with DCE, while that used in this study was commercially prepared FPC extracted with DCE for a much shorter period of time. Ershoff (1967) has observed that when FPC was extracted with DCE for 24 hours, the residual DCE content was 30,000 ppm. Poor growth was obtained when this FPC was fed to the rats. Residual DCE in FPC used in the present study was 360 ppm. Smaller accumulation of residual DCE and other deleterious compounds may explain the superior results obtained in these studies compared to those of Munro and Morrison (1967). Huber and Slade (1967) and also the present study have shown that DCE-FPC diets caused depressed growth and/or death of calves. It was also shown that CCC, a compound

formed by the reaction between trimethyleamine in raw fish and DCE, slightly depresses growth of calves when added to milk protein diets.

Unavailability of certain amino acids reported by Morrison and Munro (1965) and a low level toxicity may be responsible for the depression in growth observed in rats on DCE-FPC diets containing 10% protein but these problems were overcome at the higher protein intake. If the toxicity described by Munro and Morrison (1967) was of significance in commercially prepared DCE-FPC, the higher levels of DCE-FPC (in the 20% and 40% protein diets) should have caused poor growth than 10% protein diet. Since the growth improved at the higher level of DCE-FPC, it seems that a low biological value of the protein rather than any toxic substance present is the main contributing factor to the inferior growth on the DCE-FPC diets.

Experiment II

The IP-FPC was superior to all other protein sources, (Table 20). Growth on the DCE-FPC (untreated or ethanol extracted) diets showed a trend similar to that previously observed. When CCC and DCE were added separately at 400 mg per kg casein, no adverse effects on growth or PER were noted. However, when added in combination, a small decrease in growth was observed, though not significantly different from values obtained for casein. When ethanolic extract of DCE-FPC were added to casein, growth was improved. The

TABLE 20.--Data on growth, PER and weight of organs from rats fed different sources of protein.

Diet	Weight gain	PER	Liver weight	Pancreas weight
	(g)		(% BW)	(% BW)
1. Casein	138.1^{a}	3.19^{a}	4.38^{b}	0.25
2. IP-FPC	153.3^{d}	3.11^{a}	5.03^{a}	0.26
3. DCE-FPC	96.8^{c}	2.78^{b}	4.83^{a}	0.22
4. DCE-FPC Ex.	130.1^{ab}	3.13^{a}	4.70^{a}	0.24
5. Casein+CCC	135.7^{a}	3.24^{a}	4.35^{b}	0.25
6. Casein+DCE	139.9^{a}	3.29^{a}	4.74^{a}	0.24
7. Casein+CCC+DCE	130.7^{ab}	3.09^{a}	4.88^{a}	0.25
8. Casein+Ethanol ex.	120.3^{b}	3.14^{a}	4.77^{a}	0.24
S. E. treatment mean	4.47	0.068	0.10	0.019

^{abc}Values not sharing common superscript are significantly different. ($P<0.05$)

DCE-FPC used in this study contained 228 mg/kg CCC and 300 mg/kg DCE. A 100 g rat on the DCE-FPC diet would receive 0.34 and 0.45 mg of CCC and DCE per day, respectively. This level of CCC is about a hundred times lower than the LD_{50} reported for rats. (Munro and Morrison, 1967) So it is not surprising that no adverse results were noted in our studies. A growth-depressing effect of DCE-FPC was observed by Ershoff (1967) when the residual DCE content was as high as 30,000 ppm. The DCE content in the FPC used in this study was 300 ppm which was also a hundred times lower

than that which adversely affected growth. However, when the ethanolic extract of DCE-FPC was added to the casein diet, weight gains were significantly decreased. This coupled with the observation that the ethanol-extracted DCE-FPC gave superior growth to the original DCE-FPC suggests the possibility that there might be compounds in the ethanolic extract of FPC which may cause a depression in growth of rats other than CCC and DCE.

Since the PER values on diets with added CCC plus DCE or ethanolic extract of DCE-FPC were not different from those for casein diets, it appears that a decreased feed intake was responsible for the slightly poorer growth observed when compared to casein.

Liver weights (as a % of body weight) were higher for the casein and casein plus CCC diets than for all others. This effect on liver weights is not explainable on the basis of growth response obtained on the different diets. Treatment had no significant effect on pancreas weights.

One can conclude from these results that neither CCC nor DCE is responsible for the depression of growth noted on DCE-FPC diets. Even though a combination of these compounds slightly decreased gains, the effect was not significant.

The superior gains of rats on IP-FPC diets to those on DCE-FPC are not readily explainable. In addition to different extraction solvents, fish used for IP-FPC were caught in the North Sea and were predominantly Herring,

while DCE-FPC was prepared from Red Hake caught off New England Coast. However, Munro and Morrison (1967) using the same type of fish (cod fillets) demonstrated a superiority of IP-FPC over DCE-FPC. Another difference between the processes was that the pressed fish cake from which water-soluble materials had been removed was used in the IP-FPC process; whereas, water-solubles were conserved in DCE-FPC. Studies in Sweden have shown a beneficial effect from removal of water-soluble components from FPC (Stellaman 1969). The importance of water-soluble components was further studied in Experiment III.

Experiment III

The average weight gains, total feed consumption and PER values for different diets are summarized in Table 21. Similar trends for the casein, IP-FPC, DCE-FPC and ethanol extracted DCE-FPC diets were obtained as in the previous Experiments I and II. Heating of DCE-FPC slightly improved the weight gains and PER, though increases were not statistically significant for the original DCE-FPC. It is possible that mild heating at 65°C for 2 hrs improved the biological value of the protein in DCE-FPC by increasing the availability of methionine. Morrison (1963) observed that steaming of DCE-FPC did not alter the organic chloride content but the methionine became more available.

TABLE 21.--Effect of different protein sources on weight gains, feed consumption and protein efficiency ratio in rats (10 per treatment).

Diet	Weight gains	Feed consumption	PER
	(g)	(g)	
1. Casein	105.1^{bcd}	316.2^{bcd}	3.31^{ab}
2. IP-FPC	151.4^{h}	387.4^{a}	3.91^{f}
3. DCE-FPC	81.3^{fg}	276.3^{ef}	2.94^{e}
4. DCE-FPC Ex.[i]	98.4^{cde}	302.9^{bcde}	3.24^{abc}
5. DCE-FPC+extract	76.1^{g}	266.8^{f}	2.85^{e}
6. DCE-FPC Ex.+extract	92.4^{def}	283.8^{cdef}	3.24^{abc}
7. DCE-FPC heated	85.1^{efg}	283.1^{def}	3.03^{de}
8. DCE-FPC washed	106.6^{bc}	314.8^{bcd}	3.38^{a}
9. DCE-FPC+30% casein protein	98.1^{cde}	318.1^{bcd}	3.06^{cde}
10. DCE-FPC+20% casein protein	97.2^{cde}	308.1^{bcde}	3.15^{bcd}
11. DCE-FPC+10% casein protein	91.4^{def}	300.9^{bcdef}	3.02^{de}
12. DCE-FPC+Ex. 30% casein protein	121.4^{a}	366.5^{a}	3.32^{ab}
13. DCE-FPC+Ex. 10% casein protein	112.4^{ab}	331.9^{b}	3.37^{a}
14. DCE-FPC+Ex. 10% casein protein	107.7^{bc}	318.0^{bcd}	3.39^{a}
S.E. of treatment mean	4.48	11.20	0.071

^{abcdefgh}Values not sharing common superscript are significantly different ($P<0.05$).

[i]Ethanol extracted DCE-FPC.

When the DCE-FPC was washed with water, weight gains were significantly higher ($P<0.05$) than for the original DCE-FPC. After washing the FPC with water, DCE-FPC was put in the oven at 65°C until completely dry. It is presumed that during washing of FPC, certain water soluble substances which might cause growth depression were removed. It therefore appears that beneficial effect of water washing of DCE-FPC might be due to combined effect of mild heating and the removal of deleterious substances.

The ethanolic extract of DCE-FPC resulted in a slight but nonsignificant growth depression when added back to original DCE-FPC or ethanol-extracted DCE-FPC diets. Extraction with ethanol, heating or washing of DCE-FPC with water also improved PERs of the original DCE-FPC.

The addition of casein to the original DCE-FPC diets or to the ethanol-extracted DCE-FPC diets at all levels (10, 20 or 30% of the protein) resulted in a beneficial effect on growth of rats, (Table 21). The increased gains were proportional to the amount of casein added. Ethanol-extracted DCE-FPC was superior to the original DCE-FPC at all levels of added casein. Moreover, it is interesting to note that when ethanol extracted DCE-FPC furnished 70% and casein 30% of the total dietary protein (diet 12) for rats, growth was superior ($P<0.05$) to the casein diet. This probably demonstrates the complementary effect of the two protein sources.

Amino acid analyses reveal that fish protein is slightly lower than milk protein in tryptophan, isoleucine, leucine, phenylalanine and histidine, (Table 22). It was further shown that availability of histidine, cystine and methionine was reduced when FPC was extracted for a prolonged period with DCE (Morrison and Munro (1965). If we assume that essential amino acids from casein are fully available to the growing rat, then the addition of casein to the DCE-FPC diet should tend to compensate for the amino acids which may be limiting. Improved growth when casein furnished 10, 20 or 30% of the protein in DCE-FPC diets supports this assumption. The increased growth from addition of casein was associated with higher feed intakes and no change in PER was observed. Previous studies with FPC diets fed to rats established that improvement of the protein source usually resulted in a greater consumption of food (Morrison, 1963).

Plasma free amino acid (PFAA) levels in rats on casein, IP-FPC, DCE-FPC and ethanol extracted DCE-FPC are shown in Table 23. Compared to PFAA levels on the casein diet, it was observed that histidine was markedly decreased on all FPC diets. Small differences were noted for some of the other amino acids, with threonine, valine, cystine, leucine, tyrosine, and lysine slightly lower on FPC; whereas, methionine and isoleucine were slightly higher. Because histidine appeared so greatly depressed in the blood of rats on FPC diets, this amino acid was selected for further study.

TABLE 22.--Essential amino acid composition of milk protein and fish protein concentrate.*

Amino acid	Milk protein	DCE-FPC
	----------g/16 g N----------	
Tryptophan	1.47	1.05
Threonine	4.82	4.54
Isoleucine	6.68	4.63
Leucine	10.27	7.90
Lysine	8.14	8.54
Methionine	2.56	3.35
Cystine	0.93	0.78
Phenylalanine	5.07	4.30
Valine	5.33	5.34
Histidine	2.76	2.11

*Alpine Marine Protein Industries, Inc., Bedford, Mass.

It has been previously suggested that PFAA levels are somewhat dependent on the amino acid composition of the protein ingested (Richardson et al., 1953; Longenecker and Hause, 1959). Comparison of the relative concentrations of amino acids in casein and FPC with their PFAA concentrations in rats fed different protein sources tends to support this observation.

Total serum protein and serum albumin were closely related to weight gains and PERs, (Table 24). Values for the IP-FPC diet were significantly higher than for all

TABLE 23.--Plasma free amino acid levels in the plasma of rats fed different protein sources.

Amino acid	Casein	IP-FPC	DCE-FPC	DCE-FPC Ex.
	----------uM/100 mℓ----------			
Histidine	5.02	1.43	1.64	0.95
Methionine	2.09	2.95	2.42	2.44
Threonine	26.10	14.09	21.41	24.52
Valine	9.04	9.25	6.77	6.66
Isoleucine	3.74	6.02	4.30	4.45
Cystine	0.47	0.91	0.29	0.37
Leucine	6.38	6.15	5.83	5.60
Tyrosine	5.40	4.62	3.09	3.79
Phenylalanine	2.77	2.80	2.50	2.76
Lysine	26.82	26.82	23.18	23.37
Arginine	4.46	7.05	4.07	4.09

other treatment. These serum components were not significantly different on the casein and DCE-FPC diets. Diets had no significant incluence on the golbulin levels. It has been generally observed that when animals were kept on a diet low in protein or deficient in a single amino acid, low serum protein and serum albumin levels resulted (Albanese, 1963).

The net protein utilization on IP-FPC diet was significantly higher ($P<0.05$) than on all other treatments. This observation corresponds well with the superior weight gains and PER values on this diet. Values for NPU on DCE-FPC

TABLE 24.--Net protein utilization, (NPU), serum protein and its components in rats fed different sources of protein.

Diet	NPU	Serum protein	Serum albumin	Serum globulin
	%	%	%	%
Casein	77.15[a*]	5.83[b]	3.74[b]	2.09
IP-FPC	81.55[b]	6.96[a]	5.06[a]	1.90
DCE-FPC	72.15[d]	4.91[b]	3.24[b]	1.67
DCE-FPC Ex.	75.52[ac]	5.86[b]	3.98[b]	1.88
DCE-FPC washed	74.93[c]	5.49[b]	3.76[b]	1.73
S.E. of treatment mean	0.59	0.37	0.26	0.15

[abcd]Values not sharing common superscript are significantly different ($P<0.05$).

diet was significantly lower than for all other diets. Extraction with ethanol or washing with water significantly improved NPU values over the original DCE-FPC. The correlations between PER and NPU, serum protein and serum albumin were 0.95, 0.95 and 0.96, respectively. In view of the high correlation between these parameters (Fig. 3) the following equation using serum protein or serum albumin as the independent (X) variable can be used for prediction of PER (Y):

$$Y_{(PER)} = 0.446X_{(Serum\ Protein)} + 0.767 \text{----------} (1)$$

$$Y_{(PER)} = 0.506X_{(Serum\ Albumin)} + 1.371 \text{----------} (2)$$

The results of this experiment showed that the nutritive value of DCE-FPC can be significantly improved by further extraction with ethanol, or washing with water or by adding a small amount of casein to it.

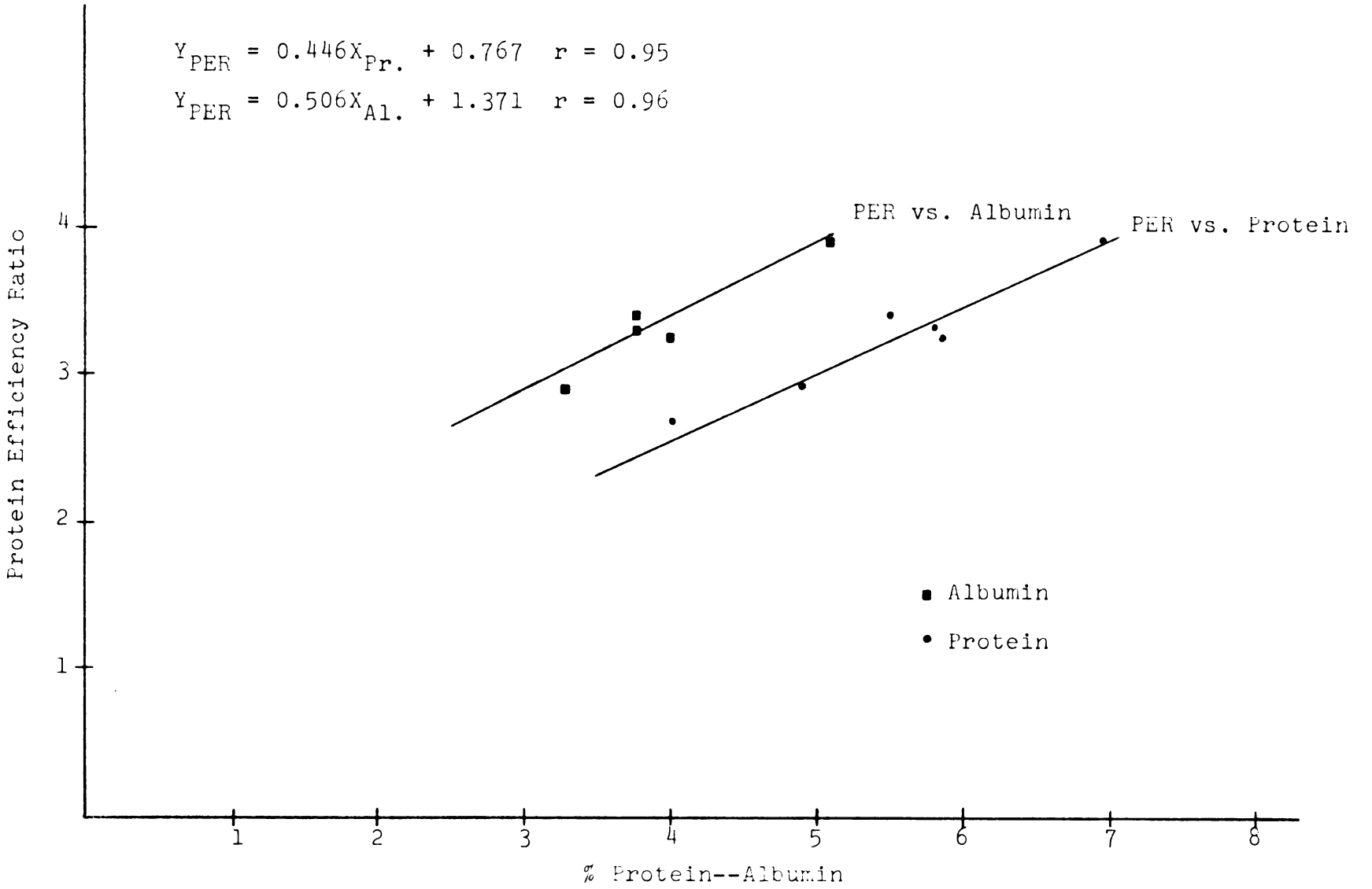

Figure 3.--Relationship between PER and blood protein fractions.

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PART IV

SUPPLEMENTATION OF HISTIDINE AND METHIONINE TO FISH PROTEIN CONCENTRATE DIETS FOR RATS

ABSTRACT

The nutritive value of fish protein concentrate extracted with isoproanol (IP-FPC), dichloroethane (DCE-FPC) or ethanol extracted DCE-FPC (DCE-FPC-Ex) was studied with male weanling rats. Earlier investigations showed IP-FPC superior for rat growth to casein or DCE-FPC. In Experiment I two levels of L-histidine, (0.075% and 0.15%) were added to FPC diets containing 10% protein. Both histidine levels increased growth and feed intake on all the FPC diets ($P<0.05$). No benefit was observed by increasing histidine above 0.075%. In Experiment II 0.075% L-histidine and 0.20% L-methionine, alone or in combination were added to FPC diets. Improved growth resulted with added histidine or methionine. Histidine plus methionine produced additive effects on growth. In both trials protein efficiency ratios were improved ($P<0.05$) by amino acid supplementation to DCE-FPC.

Plasma free amino acids in rat blood showed lower levels of total essential amino acids when both histidine and methionine were added to fish protein diets. When histidine and methionine were supplemented, alone or in combination, their plasma concentrations were raised.

Added histidine did not influence the methionine level in plasma; however, methionine alone depressed plasma histidine in rats on DCE-FPC and IP-FPC but not for those receiving ethanol-extracted DCE-FPC. This study suggests that histidine and methionine were limiting in these FPC diets regardless of method of solvent extraction.

Introduction

Previous experiments with rats on FPC diets showed low biological values of protein in DCE-FPC diets fed to rats compared to casein diets. Improved response obtained by addition of varying amounts of casein to DCE-FPC and the low plasma histidine levels on FPC diets suggested that histidine might be limiting rat growth on FPC diets. Studies with young calves (Genskow, 1969) have indicated low plasma histidine, leucine and phenylalanine on DCE-FPC rations. Rat studies (Morrison and Munro, 1965; Stillings, 1969) proposed that FPC prepared either by extracting with dichloroethane or isopropanol might be limiting in one or more of the following amino acids: methionine, histidine, tryptophan, threonine, valine, isoleucine, phenylalanine, leucine, lysine and arginine. Smith and Scott (1965) compared unheated and heated fish meal by chick biological assay and noted a decrease in availability of lysine and threonine due to heat treatment.

In reviewing the amino acid composition of fish protein, it appears that the magnitude of a deficit of amino acids in FPC would not be very great. Since the plasma histidine levels for rats on FPC diets were very low in a previous experiment, this amino acid was selected for

further study. Addition of methionine alone or in combination with histidine was also tested.

Experimental Procedures

Experiment I

Sixty weanling male rats of the Sprague-Dawley strain were alloted to 10 different diets. The basic composition of the diets is shown in Table 25. The three FPC diets were supplemented with 0, 0.075 or 0.15% L-histidine. All diets were made isonitrogenous by adding glutamic acid. The rats were fed the diets ad libitum for 14 days and weekly weight gains as well as feed intakes were recorded. Protein efficiency ratios were calculated for each rat.

TABLE 25.--Basic composition of diets used in Experiment I and Experiment II.

Ingredients	Source of protein			
	Casein	IP-FPC	DCE-FPC	DCE-FPC Ex.
	(%)			
Casein	11.0	-	-	-
IP-FPC	-	12.0	-	-
DCE-FPC	-	-	13.5	12.8
Corn starch	39.5	39.5	39.5	39.5
Cerelose	39.5	39.7	39.0	39.5
Vegetable oil	5.0	5.0	5.0	5.0
Salt mix	4.0	2.8	2.0	2.0
Vitamin mix	1.0	1.0	1.0	1.0

Experiment II

In Experiment II L-methionine and L-histidine when added alone or in combination to FPC diets. Casein, IP-FPC, DCE-FPC and ethanol-extracted DCE-FPC were again used as the protein sources. Ninety-six 21-day-old male rats were alloted to 16 treatment diets. The basic composition of all the diets was the same as shown in Table 25. Diets 2, 6, 10 and 14 were supplemented with 0.75% L-histidine; diets 3, 7, 11 and 15 were supplemented with 0.20% L-methionine and diets 4, 8, 12 and 16 were supplemented with 0.075% L-histidine plus 0.20% L-methionine. Diets were again made isonitrogenous by adding glutamic acid. The rats were housed and fed as described in Experiment I. At the end of the 14-day treatment period, blood samples from abdominal aorta from three rats on each treatment diet were collected. Plasma was harvested from each blood sample and samples were pooled from each treatment and prepared for amino acid determination as described earlier.

Results and Discussion

Experiment I

Addition of L-histidine at the rate of 0.075% or 0.15% of diet significantly improved growth rates in all of the FPC diets, (Table 26). Since plasma on all the FPC diets in previous experiment showed very low concentrations of histidine, the improved growth response with histidine

TABLE 26.--Effect of adding histidine at two different levels on weight gains and protein efficiency ratio.

Diet	Weight gains	PER
	(g)	
1. Casein	64.6[f]	3.15[e]
2. IP-FPC	74.5[e]	3.69[a]
3. IP-FPC+H[g]	83.2[a]	3.72[a]
4. IP-FPC+2H[h]	84.5[a]	3.67[a]
5. DCE-FPC	42.2[d]	2.74[f]
6. DCE-FPC+H	51.2[c]	2.95[c]
7. DCE-FPC+2H	51.0[c]	2.92[c]
8. DCE-FPC Ex.	49.3[c]	3.24[b]
9. DCE-FPC Ex.+H	58.8[b]	3.26[b]
10. DCE-FPC Ex.+2H	57.8[b]	3.38[d]
S.E. treatment means	1.57	0.038

[abcdef]Means not sharing common superscript are significantly different ($P<0.05$).

[g]Represents 0.075% supplemental L-histidine.

[h]Represents 0.15% supplemental L-histidine.

supplementation is not too surprising. There is considerable evidence (Richardson et al.,1953) that an increase or decrease in the dietary level of an essential amino acid results in corresponding change in concentration of that amino acid in plasma. Histidine content of FPC is not markedly low compared to casein but these data suggest that

the availability of the histidine in FPC is reduced. Morrison and Munro (1965) found a marked reduction in total histidine released during in vitro digestion of FPC. Results of the present study are substantiated by those of Morrison and Munro (1965) and Stillings (1969) who obtained improved growth of rats by addition of histidine to FPC diets. Addition of 0.15% histidine had no beneficial effect over that observed for 0.075%.

Increased PER ($P<0.05$) was obtained when histidine was supplemented to DCE-FPC, however no change in PER resulted on other FPC sources due to addition of histidine. The addition of histidine apparently resulted in a more balanced protein and thereby increased feed intake of the rats.

Experiment II

Growth responses and PER values from Experiment II when histidine or methionine or both were supplemented to the different protein sources are shown in Table 27. Addition of histidine to all FPC diets again resulted in superior growth compared to the unsupplemented FPC. Addition of histidine to casein did not affect growth suggesting that casein is already adequate in its available histidine content. Growth response of the same magnitude as with supplemental histidine was obtained when methionine was added to FPC diets. Addition of methionine to casein produced better growth than unsupplemented casein.

Since casein is low in methionine this response was expected. When both histidine and methionine were added to FPC diets, an additive effect of both the amino acids was demonstrated in growth response on all supplemented FPC diets over the unsupplemented. Addition of histidine or methionine or both improved ($P<0.05$) the PER values on DCE-FPC compared to unsupplemented DCE-FPC, while no improvement was noted for the other FPC diets.

Plasma free amino acid (PFAA) levels of rats in Experiment II are shown in Table 28. When both histidine and methionine were added to the diet, a marked lowering in concentrations of total essential amino acid (TEAA) in plasma occurred. It was also on these diets that the best growth was noted. The data suggests that at maximum rates of protein anabolism, a lowering of total PFAA results. These observations are at variance with Hill and Olsen (1963) who correlated low PFAA levels with poor nutrition and poor protein quality. It is also known that feeding a N-free diet adequate in calories results in very low PFAA levels (Bergen and Purser, 1968). Levels of TEAA generally correspond with the growth response obtained with the unsupplemented FPC diets.

When histidine and methionine were supplemented alone or in combination, the plasma levels of these amino acids were raised. Histidine supplementation did not influence the methionine levels in plasma; however, methionine

TABLE 27.--Effect of adding combination of histidine and methionine on weight gains and protein efficiency ratio.

Diet	Weight gains	PER
	(g)	
1. Casein	65.8[c*]	3.09[de]
2. Casein+H[j]	63.0[d]	3.02[e]
3. Casein+M[k]	69.0[b]	3.15[cd]
4. Casein+H+M	68.0[bc]	3.15[cd]
5. IP-FPC	73.5[i]	3.73[a]
6. IP-FPC+H	81.3[a]	3.74[a]
7. IP-FPC+M	81.0[a]	3.82[a]
8. IP-FPC+H+M	86.0[f]	3.77[a]
9. DCE-FPC	42.5[h]	2.72[g]
10. DCE-FPC+H	51.5[fg]	2.87[f]
11. DCE-FPC+M	50.0[g]	2.88[f]
12. DCE-FPC+H+M	53.0[f]	2.91[f]
13. DCE-FPC Ex.	50.0[g]	3.20[bcd]
14. DCE-FPC Ex.+H	58.0[e]	3.21[bc]
15. DCE-FPC Ex.+M	57.0[e]	3.25[b]
16. DCE-FPC Ex.+H+M	63.0[d]	3.29[b]
S.E. treatment means	0.88	0.038

[abcdefghi]Means not sharing common superscript are significantly different ($P<0.05$).

[j]Represents 0.075% supplemental L-histidine.

[k]Represents 0.20% supplemental L-methionine

TABLE 28.--Amino acid levels in plasma of rats fed different protein sources (uM/100mℓ).

Amino acid	Casein				IP-FPC				DCE-FPC				DCE-FPC-Ex.			
	-	H[a]	M[b]	H+M[c]	-	H[a]	M[b]	H+M[c]	-	H[a]	M[b]	H+M[c]	-	H[a]	M[b]	H+M[c]
Threonine	55.14	56.45	9.93	12.10	35.66	40.09	18.57	8.92	34.41	44.69	32.08	12.64	43.73	49.33	43.74	14.89
Cystine	00.17	00.19	1.00	1.26	00.28	00.33	02.14	00.80	00.32	00.87	1.62	00.71	00.63	00.63	1.89	1.80
Valine	16.01	16.72	9.83	11.42	13.48	13.87	17.11	7.80	8.47	12.28	6.43	4.32	10.63	13.66	9.31	6.57
Methionine	5.09	5.36	6.02	6.08	3.74	4.93	7.95	4.68	4.44	5.85	6.48	3.77	7.12	8.77	6.31	4.51
Isoleusine	8.99	10.14	6.27	7.75	7.58	7.84	11.70	5.18	4.64	6.73	3.99	3.31	5.26	5.77	5.60	4.26
Leusine	11.00	15.50	6.37	8.96	10.88	11.84	11.94	7.79	7.50	11.17	6.37	4.13	9.07	11.97	8.23	6.12
Tyrosine	16.67	16.92	12.88	10.90	6.55	7.90	28.18	4.04	4.88	5.38	5.51	2.50	5.26	6.77	6.07	3.31
Phenylalanine	4.39	7.41	3.96	4.61	6.04	6.36	7.70	3.94	4.68	5.38	4.27	3.09	4.87	6.27	5.87	3.37
Lysine	162.80	126.08	114.06	109.04	116.23	112.68	114.40	85.85	85.58	99.23	90.66	81.07	83.20	105.38	112.61	78.03
Histidine	13.77	22.44	16.76	12.46	9.03	12.72	3.04	12.28	6.78	13.57	5.47	12.22	4.95	14.69	8.47	12.56
Arginine	13.00	24.97	27.11	17.39	16.86	19.71	21.10	23.78	22.89	21.90	29.92	27.78	35.85	23.97	33.74	25.16
Total essential amino acids	307.03	302.18	214.19	201.97	226.33	238.27	243.53	165.06	184.59	217.05	192.80	155.54	210.61	247.21	241.84	160.58

[a]These diets were supplemented with 0.075% L-histidine.

[b]These diets were supplemented with 0.20% L-methionine.

[c]0.075% L-histidine and 0.20% L-methionine both added to these diets.

supplementation alone depressed plasma histidine levels in DCE-FPC and IP-FPC, but not in ethanol extracted DCE-FPC.

The general increase in PFAA for all the FPC diets when histidine was supplemented may indicate a catabolic state, despite increased growth response. This suggests that the level of histidine supplementation was in excess of the requirement of the animal. Harper (1964) has indicated that in an imbalance situation only a very small amount of supplemental limiting amino acid is needed to correct the imbalance situation.

If histidine and methionine was co-limiting amino acids, plasma methionine level should have decreased when histidine alone was added to the diet and plasma histidine level should have decreased when methionine alone was added. As was mentioned, methionine supplemented to FPC diets did depress plasma histidine but supplemented histidine did not depress plasma methionine, even though growth and PER data strongly suggest that both amino acids were co-limiting. These results indicate that PFAA levels can be used as a guide rather than conclusive evidence for a specific limiting amino acid in a particular protein source.

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SUMMARY AND CONCLUSIONS

Experiments were conducted with young calves and weanling rats to evaluate the nutritive value of fish protein concentrate (FPC) as a sole source of protein. The FPC used in the present studies was prepared by extraction with dichloroethane (DCE-FPC) or isopropanol (IP-FPC).

Growth response of young calves on FPC milk replacers was significantly inferior to dried skim milk. Growth response on IP-FPC (from which most of the water-soluble substances had been removed before extraction with the solvent) was inferior to DCE-FPC. The data suggest that water-soluble proteins are essential in the diets of young calves but more research on this problem is needed.

Calves on FPC milk replacers developed microcytic, normochronic anemia suggesting a poor utilization of protein from FPC diets.

Muscular degeneration occurred in calves on DCE-FPC rations supplemented to provide 60 mg/100 kg body weight of vitamin E. However, raising the level of vitamin E to twice this level prevented muscular degeneration, resulted

in normal tocopherol concentrations in blood, and improved growth compared to that shown for DCE-FPC diets containing lower levels of vitamin E.

The DCE-FPC contained about 370 mg/kg of chlorocholine chloride (CCC) and 360 mg/kg of dichloroethane (DCE). Adding 400 mg CCC per kg protein to a dried skim milk ration tended to depress the growth in young calves. Identical levels of DCE did not exert an adverse effect upon growth.

Extraction of DCE-FPC with ethanol produced superior growth in calves to the original DCE-FPC, but washing with water resulted in depressed growth similar to what was observed on IP-FPC diets.

In rat studies using diets containing 10% protein, the IP-FPC resulted in superior growth, protein efficiency ratio and net protein utilization when compared to casein or DCE-FPC. Casein was better than DCE-FPC. No difference between casein and DCE-FPC was noted at 20 and 40% protein which discounted earlier suggestions that the main reason for depressed rat growth on commercially prepared DCE-FPC was the presence of toxic substances. Addition of CCC or DCE alone did not decrease weight gains of casein fed rats. However, the combination of both contaminants slightly depressed growth.

An improvement in the nutritive value was noted when DCE-FPC was further extracted with ethanol or methanol or when it was washed with water. Addition of ethanolic and

methanolic extracts of DCE-FPC to casein or FPC diets decreased weight gains and reduced feed intakes.

The addition of casein at 10%, 20% or 30% of the total protein in DCE-FPC or ethanol-extracted DCE-FPC diets resulted in rat gains superior to those for diets containing only fish protein.

Plasma free amino acid levels in rats on all FPC diets showed low histidine concentrations. Supplementing FPC diets with 0.075% L-histidine or 0.20% L-methionine, alone or in combination, produced superior weight gains to unsupplemented FPC diets.

APPENDIX

TABLE 1.--Summary of the description of muscular changes in calves fed various protein sources in milk replacer.

Calf	
	Ration I. Dried skim milk--20% protein
888	Normal
380	Normal
709	The overall appearance of the section examined gave an impression of greatly increased number of nuclei. In addition, there were scattered fibres which had undergone various degenerative changes, and some were frankly necrotic.
935	There were no distinct changes of myopathy, but the overall impression was one of increased numbers of nuclei.
716C	Normal
	Ration II. Dried skim milk--10% protein
857	There were a few very small areas of degeneration
437	There were occasional eosinophilic fibres with pyknotic nuclei.
436	Normal
970	A few isolated groups of fibres in cross sections examined exhibited degenerative changes, including central nuclei, increased eosinophilia and swelling.
895	Normal
	Ration III. DCE-FPC--20% protein
400	There was very limited evidence of nutritional myopathy.
335	Normal
868	There was scattered necrosis and degeneration of fibers with some mineralization.
376	There was a moderate number of swollen, brightly eosinophilic fibers with pyknotic nuclei. Some fibers were frankly necrotic. There were increased numbers of nuclei around such fibers. In a few places, these changes were accompanied by early mineralization.
374	There was one small focus of fiber necrosis.
	Ration IV. DCE-FPC--10% protein
402	There was a moderate degree of hyaline change with increased eosinophilia, hypercellularity and necrosis of some fibers.
389	There was swelling and degeneration of scattered fibers with some increase in the number of nuclei present. The changes were limited but unmistakable.
394	There were diffuse, scattered, necrotic, or degenerative fibers, some of which contained fine mineral granules. The overall appearance of the section was of one containing an excessive number of nuclei.
873	There were numerous, scattered, small foci of necrosis, most of which had undergone advanced mineralization.
969	There were scattered individual fibers which had undergone degenerative changes and necrosis. Changes noted included swelling, increased affinity for the eosin stain, fragmentation and pyknosis. The overall appearance gave an impression of abnormally large number of nuclei.
	Ration V. DCE-FPC screened--20% protein
392	There were scattered foci of necrosis and the overall picture was one of hypercellularity.
858	Normal
384	There were changes of nutritional myopathy. These included an increased number of nuclei, necrosis, swelling, pyknosis and increased affinity for the eosin stain.
772	There were a few focal lesions of fiber degeneration and necrosis. These changes were limited but unmistakable.
716D	There were very limited changes of nutritional myopathy. Changes noted included fragmentation, intense eosinophilia and mineralization.
	Ration VI. IP-FPC--20% protein
401	There was widespread degeneration of muscle fibers.
843	No degenerative changes were observed.
386	There were a limited number of small foci (probably representing individual fibers) of degeneration, necrosis, mineralization and infiltration by mesenchymal cells.
391	There were scattered, focal degenerative areas of mineralization and necrosis. There was a distinct increase in cellularity.
826	There were necrosis of some of the fibers and infiltration of mesenchymal cells. Other changes noted in some fibers included loss of cross striations, fragmentation, mineralization and the arrangement of nuclei in rows in centers of fibers.

TABLE 2.--Summary of description of muscular changes in calves fed FPC with vitamin E supplementation.

Calf	Ration: Dried skimmilk supplemented with vitamin E (60 mg/100 kg body wt)
908	Norml
462	Normal
	Ration: Dried skim milk unsupplemented with vitamin E
455	Normal
463	There was fairly extensive but scattered fragmentation and swelling of fibres with infiltration by mesenchymal cells in one section in which the fibres were cut longitudinally. In another section in which the fibres were viewed in cross section, there were a few scattered, swollen fibres with pyknotic nuclei. Several other sections of skeletal muscle appeared essentially normal.
	Ration: DCE-FPC supplemented with vitamin E (60 mg/100 kg body wt)
880	Normal
460	Normal
	Ration: DCE-FPC unsupplemented with vitamin E
811	In several sections occasional fibres exhibited an increased affinity for the eosin stain, were swollen and their nuclei were pyknotic. There was also fragmentation of fibres and some increase in mesenchymal cell nuclei. There was loss of cross straition and some fibres cut in cross section contained central nuclei.
456	There were a few scattered fibres which were fragmented, swollen and had pyknotic nuclei. In one section some of the degenerative fibres appeared to have fine granules of dystrophic mineralization.
	Ration: DCE-FPC supplemented with vitamin E (120 mg/100 kg)
458	Normal
461	Normal

TABLE 3.--Data on individual calf on trial I.

Calf	Milk Replacer	Weight Changes lb.		Hemoglobin Changes g/100 ml.	
		30 days	56 days	30 days	56 days
7	DCE-FPC	-6.0	- 1.0	-4.52	-7.55
8	DCE-FPC	+4.0	+15.0	-2.68	-4.02
9	DCE-FPC + Met.	-8.0	-	-4.13	-
10	DCE-FPC + Met.	-3.0	-	-1.87	-
11	DCE-FPC + Glu.	-1.0	- 4	-2.71	-4.86
12	DCE-FPC + Glu.	-3.0	- 9	-3.55	-6.84

TABLE 4.--Changes in growth and hematological values on individual calves fed milk replacers for eight weeks (Trial II)

Calf No.	Milk Replacer	Weight	Hemoglobin	Hematocrit	RBC
		(lb)	(g/100 ml)	(%)	(million/mm3)
709	DSM 20% P	31.0	- 0.4	- 5.0	- 1.0
395	DSM 20% P	23.0	+ 1.2	+ 4.0	+ 0.8
716C	DSM 20% P	42.0	- 0.4	- 3.0	- 0.3
888	DSM 20% P	31.0	- 1.6	- 5.0	- 1.1
380	DSM 20% P	49.0	+ 0.5	+ 1.0	+ 0.3
Avg.		35.2	- 0.14	- 1.60	- 0.26
857	DSM 10% P	11.0	- 0.9	- 3.0	- 0.9
970	DSM 10% P	18.0	- 3.1	- 8.0	- 2.4
437	DSM 10% P	9.0	- 2.5	- 7.0	- 1.8
895	DSM 10% P	1.0	- 1.9	- 3.5	- 1.6
436	DSM 10% P	6.0	- 6.0	-16.0	- 4.7
AVG.		9.0	- 2.88	- 7.50	- 2.28
400	DCE-FPC 20% P	27.0	- 1.2	- 3.0	- 0.3
335	DCE-FPC 20% P	0.0	- 2.8	-10.0	- 1.3
868	DCE-FPC 20% P	9.0	- 2.1	- 9.0	- 2.1
374	DCE-FPC 20% P	21.0	- 1.9	-10.0	- 1.3
376	DCE-FPC 20% P	20.0	- 2.8	-12.0	- 1.0
Avg.		15.4	- 2.16	- 8.80	- 1.20
873	DCE-FPC 10% P	1.0	- 4.3	-14.0	- 0.6
389	DCE-FPC 10% P	-20.0	- 3.6	-13.0	- 0.8
969	DCE-FPC 10% P	-20.0	- 3.6	-15.0	- 2.5
402	DCE-FPC 10% P	- 8.0	- 3.1	-11.0	- 0.4
394[a]	DCE-FPC 10% P	- 9.0	- 1.9	- 7.0	- 0.9
Avg.		-11.2	- 3.30	-12.00	- 1.04
858	DCE-FPC screened 20% P	- 2.0	- 1.5	- 6.0	- 1.0
392	DCE-FPC screened 20% P	+ 1.0	- 2.6	- 5.0	- 0.7
716D	DCE-FPC screened 20% P	4.0	- 4.5	-16.0	- 2.0
384	DCE-FPC screened 20%	18.0	- 3.4	-13.0	- 2.1
772	DCE-FPC screened 20% P	11.0	- 4.7	-19.0	- 2.5
Avg.		6.4	- 3.34	-11.8	- 1.66
391[b]	IP-FPC 20% P	-17.0	- 1.0	- 3.5	+ 0.1
401[b]	LP-FPC 20% P	-14.0	- 3.6	-11.0	- 2.2
386[c]	IP-FPC 20% P	- 9.0	- 1.4	- 7.0	- 1.7
843[b]	IP-FPC 20% P	- 4.0	- 0.8	- 3.0	- 0.8
387[d]	IP-FPC 20% P	-14.0	- 2.1	- 6.0	- 1.1
Avg.		-11.6	- 1.78	- 6.10	- 1.18

[a] 4 weeks data

[b] 7 weeks data

[c] 6 weeks data

[d] 5 weeks data

TABLE 5.--Data on individual calves on trial III.[a]

Calf No.	Milk Replacer	Weight Changes (8 weeks)
		(lb)
953	DSM + CCC	26.0
438	DSM + CCC	33.0
Avg.		29.5
767	DSM + DCE	41.0
854	DSM + DCE	32.0
Avg.		36.5

[a]Data for calves on DSM ration shown in appendix table 4.

TABLE 6.--Data on individual calves on trial IV.[a]

Calf No.	Milk Replacer	Weight Changes (6 weeks)
		(lb)
446	DCE-FPC Ex.	23.0
472	DCE-FPC Ex.	15.0
991	DCE-FPC Ex.	22.0
Avg.		20.0
888*	DCE-FPC washed	-11.0
468	DCE-FPC washed	- 1.0
473	DCE-FPC washed	-14.0
Avg.		- 8.7

[a]Data on calves on DSM and FPC rations shown in table 7.

*5 weeks data.

TABLE 7.--Data on individual calves fed milk replacer supplemented or unsupplemented with vitamin E.

Calf No.	Milk Replacer	Weight change (8 weeks)	Plasma tocopherol conc.		
			Initial	4th week	8th week
		(lb)	(μg/100 ml)		
908	DSM + E	53.0	100.6	65.7	79.1
462	DSM + E	40.0	116.8	86.3	73.1
453	DSM + E	20.0	179.0	102.5	91.4
1099	DSM + E	33.0	213.9	160.1	113.3
Avg.		38.5	152.6	103.6	89.2
455	DSM - E	31.0	179.5	62.4	3.9
463	DSM - E	24.0	258.0	116.1	3.8
1100	DSM - E	39.0	179.3	17.9	3.4
1106	DSM - E	43.0	153.3	27.6	3.2
Avg.		34.2	192.5	56.0	3.6
880	DCE-FPC + E	15.0	117.0	70.1	58.9
460	DCE-FPC + E	20.0	137.6	73.1	60.6
1105	DCE-FPC + E	14.0	185.5	90.2	23.4
Avg.		16.3	146.7	77.9	47.6
811	DCE-FPC - E	-5.0	99.3	34.0	17.1
456	DCE-FPC - E	15.0	97.6	50.9	7.9
1115	DCE-FPC - E	18.0	193.1	79.7	21.1
1110	DCE-FPC - E	6.0	217.8	91.6	5.7
Avg.		8.5	151.9	64.6	12.9
458	DCE-FPC + 2E	32.0	166.4	123.4	79.1
461	DCE-FPC + 2E	19.0	172.8	100.0	80.1
1112	DCE-FPC + 2E	37.0	197.2	119.9	97.4
1101	DCE-FPC + 2E	29.0	190.3	156.9	98.9
Avg.		29.3	181.7	125.0	88.9

TABLE 8.--Brief description of the processes used in preparation of fish protein concentrate.

Process		Description
(1) Guttman and Vandenheuval	(i)	The heads of fish are removed and discarded. The backbone and adhering flesh are ground in a meat chopper to 1/4 inch size.
	(ii)	Equal weights of water and ground material are well mixed, acidified to pH 5.4-5.5 with polyphosphoric acid and the acidified slurry is heated and stirred for 1/2 hour at 150° F.
	(iii)	The slurry is centrifuged and washed with hot water until the effluent is clear. The material is then mixed with twice its weight of isopropanol, heated to 180° F for 15 minutes and vacuum filtered.
	(iv)	The extracted material is dried in an oven drier at 100% F for 24 hours.
	(v)	Dried material is screened through 16 and 20-mesh screens to separate bones and skins from protein. Protein fraction is ground in a grinder using 1/32 inch screens.
(2) Astra	(i)	The fish is first cooked with constant heating and stirring. The fat in the fish is then extracted with various solvents. Chlorinated solvent like dichloroethane are avoided. The solvent is introduced into a mixing tank where the extraction is carried out. After the solvent has been removed from the solid, the extraction operation is repeated until defatting is complete. Isopropanol was the solvent employed for material used in the present study.

(3)	Viobin	(i)	Whole fish is washed with water and ground with meat grinder. This material is washed with five washes of boiling solvent to remove the water and fat. This product is again washed with boiling solvent to remove the odor and non-protein nitrogen, the remaining oil and more of water. Dichloro-ethane was the solvent employed for material used in the present study.
(4)	Lever Brothers	(i)	This process consists of grinding fresh fish, mixing it with 1 per cent of its weight of sodium sulfite and sufficient aqueous sodium hydroxide to raise the pH to 10. The mass so obtained is drum-dried and the dried material extracted with acidified 95% ethanol. At the completion of the extraction, residual alcohol is evaporated. The extracted material suspended in water and the pH adjusted to 7. The material is filtered, washed with water and finally tray-dried at 50° C.
(5)	Vacuum dehydration	(i)	Fresh fish is vacuum dehydrated at 40-50° C with the help of heat transfer medium. The cominuted material is slurried with a vegetable or other edible oil and subjected, with agitation, to vacuum dehydration. The material is thus rapidly dried, and oil and other lipids removed from the meal by a suitable means.

TABLE 9.--Proximate composition of fish protein concentrate.

	VioBin (DCE FP)		Alpine	Astra
	With Bones	Without Bones	(DCE-FPC)	(IP-FPC)
Crude protein	71.3	77.5	75.0	82.3
Ether extract	2.0	2.0	1.5	0.3
Ash	18.9	13.8	16.5	11.4
Moisture	7.2	7.5	7.0	6.0

TABLE 10. Mineral content of fish protein concentrate.[a]

Element	mg 1100 g.
Calcium	3,780
Phosphorus	2,930
Sodium	353
Potassium	593
Chloride	1,000
Bromide	1.5
Iodide	0.1
Flouride	13
Iron	18.9
Selenium	0.2
Copper	0.9
Manganese	0.9

[a]United States Department of the Interior. Fish and Wildlife Service. Bureau of Commercial Fisheries. Fishery leaflet 584.