THE EFFECT OF PROTEIN QUALITY ON GROWTH
IN THE MINK AND RAT

ABSTRACT

Submitted to

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Lorne Charles Seier

1969

EXPERIMENT I

THE EVALUATION OF TWO DRY PROTEIN SOURCES IN RATIONS FOR YOUNG GROWING MINK

By Lorne Charles Seier

Forty-five male Sapphire kits were allotted to three treatment groups. After a two week adjustment period the kits were started on test. The control ration was a standard growing ration composed of horse meat 10%, chicken by-products 15%, raw cereal 25%, and frozen fish 50%. The protein substitutes were herring meal and soybean meal which replaced the frozen fish on an equivalent dry matter basis.

Average final weights in kilograms taken on November 7th were 1.78, 1.87, 1.92 kg. for the soybean meal, herring meal, and control treatment respectively. The differences in final weights were not statistically significant. The test period was divided into three stages; (1) growing before furring (August 1st to 29th); (2) growing and furring (August 30th to October 17th); and (3) maintenance (October 17th to November 7th). The growth rates for these three periods in grams per day were: for the control treatment 15.2 (Period I), 6.1 (Period II), 0.8 (Period III); herring treatment 18.0 (Period I), 6.7 (Period II), -2.6 (Period III); soybean treatment 11.8 (Period I), 7.8 (Period II), -0.7 (Period III).

Digestibility trials were carried out during each period. Dry matter and energy digestibility were significantly higher ($P < .05$) for the herring and control treated kits when compared to the soybean meal treated kits. There was no significant difference ($P > .05$) in nitrogen balance among treatments.

Skin biopsies were taken at pelting time to compare fur density. Average fur densities expressed as hair per pore were 19.6, 20.6, and 19.5 for the soybean, herring, and control treated kits, respectively.

EXPERIMENT II

THE EFFECT OF PROTEIN QUALITY AND QUANTITY ON HAIR GROWTH IN THE RAT

Sixty male weanling albino rats, approximately 3 weeks of age were allotted to six treatment groups. A 3 x 2 factorial experiment with three qualities of protein, egg albumen, casein supplemented with methionine and casein unsupplemented, and two levels of protein 20 per cent and 10 per cent, was initiated. The 20 per cent egg treatment was discarded due to egg white injury and the experiment was then analysed as a random design. The hair on the rats was shaved from their backs so that the hair cycles could be observed. The rats were fed _ad libitum_, and feed consumption and body weight recorded weekly.

The average length of the hair cycle in days was significant ($P < .05$) for treatments and for the 20% vs 10% orthogonal contrast, the values being SC 20% - 30.3; E 10% - 31.1; C 20% - 33.0; SC 10% - 35.0; C 10% - 40.1. Body growth, expressed as grams of gain per day was significant ($P < .05$) for each comparison and followed the same order of treatment effect as the hair cycle lengths with values of 3.61; 3.30; 2.88; 2.16; and 1.20 respectively.

Skin biopsies were taken after the second regrowth of hair and the hair densities, expressed as number of hair per follicular unit, were C 20% - 2.11; SC 20% - 2.06; SC 10% - 1.86; E 10% - 1.66; C 10% - 1.60. Treatment effects and the 20% vs 10% contrast were significant ($P < .05$). Hair diameters were measured and expressed as the ratio of the primary follicle diameters to the secondary follicle diameters. The values were E 10% - 1.44; SC 20% - 1.39; C 20% - 1.37; SC 10% - 1.33; C 10% - 1.29, and were not significantly different from each other. These data suggest that when protein is restricted body growth and hair growth are restricted proportionately.

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University of Manitoba

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1969

TABLE OF CONTENTS

A C K N O W L E D G M E N T S

My appreciation to Dr. R. J. Kirk of the Manitoba Fur and Game Station for the advice and encouragement afforded me during the course of this study.

The guidance and care offered by my advisor, Dr. T. J. Devlin is also appreciated.

My thanks for the technical assistance provided by Mr. T. Mainman, Mr. S. Kisser and Mr. E. Bazan on the staff of the Manitoba Fur and Game Station, and Miss L. Hedgecock and Mr. R. Boyla of the Department of Animal Science, University of Manitoba.

The author is indebted to the Canadian Mink Breeders Association and the Department of Animal Science, University of Manitoba for the financial assistance which made this project possible and to the Manitoba Fur and Game Station for the generous use of the animals and facilities.

Finally to my wife for her patience, help, consideration and many other attributes which have made this endeavor more worthwhile.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The mink industry, as part of the agriculture industry in general, is plagued by rising costs combined with constant or lowering returns. The response to these economic pressures has been the amalgamation of many of the small ranches into larger and more efficient units. In addition the principal ingredients of the diet for mink have changed from horse meat to scrap fish and poultry by-products.

Reduction in feed price should not sacrifice quality, but rather be those feeds which result in the most economical production of quality mink pelts. Fish and poultry by-products have in many instances replaced horse meat; however, all these products are expensive and not continually available, as well as requiring refrigeration. The trend today is away from fresh ingredients such as meat, fish, and packing house offal from livestock and poultry, and towards such dried foodstuffs as fish meal, blood meal, cereal grains, and soybean oil meal. These dry feeds represent economic savings in that no refrigeration is required, spoilage and the possibility of food poisoning is reduced, labor for ration preparation is reduced, and supplies are readily available and may be lower in price than fresh ration ingredients.

To evaluate these new diet ingredients, certain criteria must be established which relate to the performance of mink. Most research to date has used the standards established for farm animals, such as body growth or weight, body size or body length. With mink this is not necessarily adequate, as the pelt and not the carcass is the saleable product. Mink should not be pelted before the fur is prime, which is generally near the end of November, whereas, maximum body weight is reached sometime in October.

Since the pelt is the saleable product, a criterion for evaluating the dietary effect on furring would seem desirable. The basic question of what makes hair grow also warrants investigation.

The evaluation of pelt quality is very complex. However, a measurement of hair density is fairly readily obtained, and could possibly be one of the criteria used to evaluate the economic value of pelt quality, along with color and texture.

As protein sources change by the introduction of dry rations and hair is mainly protein, varying sources of protein were investigated with respect to the growth, digestibility, and effect on hair growth of growing mink. Hair density as a measure of the effect of diet on pelt quality, was the criterion used to evaluate hair growth.

An experiment, utilizing purified diets which were fed to rats, was initiated in order to establish, more clearly, the effect of protein nutrition on hair growth.

REVIEW OF LITERATURE

PROTEIN REQUIREMENTS

The National Research Council (1953) has recommended protein levels for mink of 22 to 26 per cent, on a dry matter basis, from 7 to 16 weeks of age, and 16 to 22 per cent from 16 weeks to maturity. Sinclair (1960) and Allen (1962) suggested that the protein levels recommended by N.R.C. are too low. Conventional ranch mink rations contain about 40 to 50 per cent protein on a dry matter basis (Oldfield 1968). Sinclair (1960) reported that a 27 per cent protein ration, as compared to 44, 37, or 31 per cent protein rations, was sufficient in quantity and quality for mink from 7 to 27 weeks of age. Allen (1962) suggested that a 16 per cent protein ration was not sufficient for optimum growth, a 22 per cent ration was too low for adequate disease resistance, and that a 28 per cent protein ration appeared to be nearer to the optimum for good growth. Oldfield (1968) stated that mink growth and fur production remains quite good at protein levels of 30 and even 25 per cent, but at lower protein levels both growth and fur production of the mink tend to be reduced.

DIGESTIBILITIES

Mink appear to be able to adequately digest the wide variety of products normally used in their rations. Loosli _et al_. (1940) have reported dry matter digestibilities of 70 to 78 per cent; protein digestibilities of fresh protein feeds are reported at 84 to 93 per cent; of processed protein feeds 72 to 73 per cent; of N.F.E. 65 to 85 per cent and even raw starches appear to be well digested. Leoschke (1959) using mink measured true digestibility of fats which ranged from 91.9 to 97.2 per cent.

Apparent digestible protein studies of common mink feed ingredients indicated that chicken feet had a protein digestion coeffieient of 52.4 per cent, while chicken heads had a coefficient of 78.2 per cent, and other substances registered varying percentages with horse meat having the highest coefficient 92.1 per cent. Roberts and Kirk (1964) reported that the protein digestibility of three species of fish fed to mink ranged from 87.1 to 90.6 per cent. Protein digestibility of dry cereal meal was estimated to be approximately 70 per cent (Roberts & Kirk 1964).

Loosli and Smith (1940) estimated that 1.64 grams of nitrogen were required to maintain nitrogen balance in mink fed a ration which was adequate in energy. A sparing effect of digestible energy on protein was noted by Sinclair (1960). He also reported a significant difference between age groups in nitrogen retention. Age appeared to have a variable effect upon protein digestibility of male mink. When a low protein (22 per cent) diet was fed digestibility increased as the animals became older (11 to 21 weeks). At a dietary protein level of 28 per cent the effect of age ondigestibility still could be detected although to a lesser extent. Mink fed diets containing 30 to 37 per cent protein did not demonstrate any effect of age on protein digestibility. Age and sex of mink appeared to have no effect on the digestibility of energy when protein levels were below 30 per cent. However, male and female mink digested gross energy (from 5.0 to 5.7 kcal/g) to a greater degree at 25 weeks of age than at 15 weeks of age when protein levels were between 30 and 37 per cent (Allen 1962).

ENERGY REQUIREMENTS

The National Research Council indicate the energy requirements for mink to be 124 calories/kilogram body weight, or 2,370 calories per square meter of body surface area. The National Research Council also suggests that mink can efficiently utilize raw wheat or oat cereals as a source of energy.

Allen (1962) introduced the concept of the calorie:protein ratio, the ratio of apparent digestible energy to apparent digestible protein. The ratio of gross energy to crude protein represents an estimate of the ratio calculated on an apparent digestible basis. At gross energy intakes greater than 5.0 kcal/g of dry matter, the body weight of male mink increased as the ratio of apparent digestible energy to apparent digestible protein decreased from 34 to 12. Therefore, with an energy level of 5.0 kcal/g in the ration, the optimum energy protein ratio appears to be between 12 and 13. A similar increase in growth occured for males which were fed energy levels lower than 5.0 kcal/g , but the growth increase was lower than for the mink fed the higher energy diets. It was observed, however, that liparous mink tend to produce a longer pelt, and in order to encourage fattening the energy protein ratio should be increased to 17 for males after sixteen weeks of age and this ratio maintained until pelting.

DRY RATIONS

Kifer and Schaible (1955) formulated and pelleted a dry ration consisting of soybean oil meal, fish meal, herring meal, dried meat scraps, livermeal, fish glandular hydrolysates, dried soluble blood, fish solubles, cooked cereal and yeast. Although the dry diet used in this experiment did not allow as rapid gains during growth as the control horse meat ration, the dry diet appeared to be adequate for maintainance. The weight differences between the control and experimental groups were more the result of fattening than of any other factor. Water consumption appeared to be a problem in that, in the winter with the dry diet, all the water had to be obtained from the frozen water in the watering cups. This probably restricted water intake and may have effected the performance of the mink.

Oregon State University Experimental Station has conducted growth trials for several years using dry diets mixed into a paste. The dry ration consisted of 20.2 per cent herring meal; 14.8 per cent blood meal; 16.2 per cent lard; 29.6 per cent oat groats and 19.3 per cent of supplemental ingredients such as soybean meal, molasses, brewers yeast, beet pulp, and wheat bran. The growth curves of mink on the paste diets were generally lower than the control diets, but fur color was superior for the mink on the paste diets. Fur quality of the paste diet fed mink was equivalent or lower then the controls. The incidence of wet belly, an unprime condition on the belly, was reduced about a third for the mink on the paste diet compared to the control diet, but feed wastage due to the lack of binding in the paste diet resulted in increased feed costs.

HAIR CLASSIFICATION, GROWTH, and REPLACEMENT

Three general classifications of hair cycles have been suggested by Ryder (1964) which consist of: first, seasonal moults e.g. mink; second, waves of growth which regularly pass across the body, e.g. rats; third, hairs are replaced irregularly with each follicle having its own cycle apparently independent of that of its neighbour, e.g. man.

Mink have two moults annually; a spring and autumn moult, which consist of the old hairs being replaced by new hairs while in contrast, the fox only moults once a year.

In the rat, the formation and elongation of the hair ends at about 17 days of age, and constitutes the growing stage of the hair.

The transformation into a quiescent condition occurs within an interval of three or four days and usually by 21 days of age the root of the hair is securely lodged in a resting follicle, which remains inactive until 31 or 32 days of life. This resting period and growth period constitutes a hair cycle, which occurs approximately every 30 to 34 days. This cyclic hair growth in rats occurs as a wave over the body. Activity in follicles of the venter may be observed 2 to 3 days before it occurs in the dorsum. Thus, the wave begins in the venter, spreads dorsally, then anteriorly and posteriorly (Butcher 1951).

Ebling (1964) has broken the hair cycle down into three phases, instead of the two of Butcher: first, anagen, or the period of activity of growth; second, catogen, a short transition phase during which the hair forms a club; third, telogen, the resting stage.

Noback (1951) has written a review on the morphology and phylogeny of mammalian hair from which the following classification of hair types was taken.

TYPES OF MAMMALIAN HAIR

1. Hair with specialized follicles containing erectile tissue. Large, stiff hairs that are preeminently sensory have been variously designated as feelers, whiskers, sensory hairs, tactile hairs, vibrissal, etc. These occur in all mammals except man, and are grouped essentially as follows:

I. Active tactile hairs - under voluntary control.

II. Passive tactile hairs - not under voluntary control.

a) Follicles characterized by a circular sinus.

b) Follicles without a circular sinus.

2. Hairs with follicles not containing erectile tissue. Most of the remaining types of hair are more or less defensive or protective in function.

In many cases, the follicles have a good nerve supply, endowing the hair with a passive sensory function. These hairs are grouped according to their size and rigidity.

I. Coarser, more or less stiffened "overhair", guard hair, top hair.

a) Spines: Greatly enlarged and often modified defensive hairs, quills.

b) Bristles: Firm, usually subulate, deeply pigmented and generally scattered hairs. "Transition hairs", Leithaore, "protective hair", "primary hair", "overhair". This group also includes mane hairs.

c) Awns: Hairs with a firm, generally mucronate tip but weaker and softer near the base. "Grannenhaare", "overhair", "Protective hair".

II. Fine uniformly soft "underhair", ground hair", "underwool".

a) Wool: Long, soft usually curly hair.

b) Fur: Thick, fine, relatively short hair, "underhair", "wool hair".

c) Vellus: Finest and shortest hair. "down", "wool", "fuzz", "languo".

The following comments, again from Noback (1951), supplement the above classification. The guard hairs are listed in a series from greater to lesser rigidity; (in order, spines, bristles, and awns). There are many interguard hairs between the typical bristle and the typical awn and these hairs are also interspersed between the typical fur hair.

Noback (1951) also presents a brief statement on the relation of the fiber generations. The first follicles to differentiate, the primary follicles, form a trio follicular group. The essential point is that each of these primary follicles will be the central follicle of different hair groups. Later in development, other follicles of the hair group, the lateral trio follicles, differentiate in relation to these central trio follicles, forming a trio of trio's. The ontogenetic studies of follicle arrangement have added confirmatory evidence to DeMeijere's basic concept that in mammals there is a universal and regular grouping of hair follicles.

In general, the early differentiating follicles (central trio follicles) form the coarse overhair, while the late differentiating follicles (secondary follicles) form the fine underhair. Lateral trio follicles produce overhair like that of the central follicles or intermediate types such as awns, which are classified as overhair.

Carter (1964) describes the arrangement of hair by stating that from the pre-natal events of skin ontogeny there emerges, in each species, the tiny complex of structures with a pattern of its own that may reasonably in most cases, be termed a "hair follicle group". Each of these hair follicle groups in turn become arranged into a pattern of their own. These hair follicle groups in turn become arranged into a pattern of patterns over the available extent of the common integument. These patterns form the individual and specific variations in the pelage so evident among mammals.

In the fetus of mink, during the early period of hair follicle formation within the skin, the distribution of hair is uniform. In this early period of formation only the guard hair follicles are present and these are equidistant from each other. As development proceeds, each guard hair follicle is flanked on either side by a follicle that will give rise to an intermediate guard hair, thus forming a trio group to form three distinct bundles of follicles (Dolnick et al. 1960). It is this "hair follicle unit" or bundle which Dolnick et al. (1960) use as a criterion of hair density.

Tanaka et al. (1965) have verified the existance of these bundles in the rat. They found that hair on the rat forms a bristle which ordinarily contains three hairs in a hair pore, with one of the hairs being large and possessing a medulla. From the point of view of development, the pilo-sebaceous system, the sebaceous gland and the hair bristle have to be emphasized and considered as a unit.

Robbins et al. (1965) studying urinary calculi in rats found that hair density as measured by the criterion of the "hair follicle group" varied in that the number of hairs per group decreased when a low potassium diet was fed.

Hardy (1951) suggested that the potential size of the follicle groups (i.e. the number of secondary follicles is dominantly inherited), but the actual group size (number of active follicles) in the mature animal (sheep) seems to vary according to the diet fed in the first year of life. Thus it seems possible to alter the group size by nutrition. Therefore, many properties of the coat of the sheep seem to depend on the inherited follicle group pattern and the modification of this by the environment. Perhaps the same principles apply to other mammals.

Dolnick (1959) also suggested that hair density may be succeptible to environment. The hair follicles are formed from buds which arise from the basal cell layer of the epidermis. Once the full complement of hair follicles is obtained, subsequent hair generations arise at the base of the follicles already established and, as far as is known, no new follicles develop. It is in the growing stage that this can be influenced by outside factors. When the hair is in the resting stage, it is almost invulnerable to change.

NUTRITIONAL EFFECTS ON HAIR GROWTH

Many varied factors affect hair growth and were summed up by Houssay et al. (1964) when they stated that varied nutritive, hormonal, or traumatic factors can modify the normal rhythm of hair growth waves. However, it appears that, while hair loss in animals is an early and frequent sign of a deficiency syndrome, severe nutritional deprivations are necessary in man before hair growth is impaired (Flesh 1961). Slee (1964) suggested that poor nutrition impeded species moulting and good nutrition merely allowed it to proceed under the influence of genetic and other environmental factors. Ryder (1964) in sheep, Hutchinson (1964) also in sheep, and Raddi (1968) in deer have all observed that a low plane of nutrition affected the appearnace and texture of the coat, due to impairment of the normal cycle.

Chase and Eaton (1959) mention that sick animals, and pregnant females generally initiate no new hair growth or, at best, have occasional islands or limited waves. Here a lack of requisite nutrition for the follicles is indicated. However, "recovery" from these states results in extensive hair growth, usually of such an explosive nature that little or no wave phenomena can be detected. In a sense, the follicles were over ready to go into anagen.

From his studies, Butcher (1959) also consluded that the epiderm and its derivatives are easily and quickly affected by the toxic substance or, in reality, by a lack of nutrition. Butcher (1937) using rats, studied hair growth in relationship to body size and quantity of food intake, and found that underfeeding greatly retarded hair growth in that it slowed down the cycle. The evidence that nutrition affects hair growth is convincing, but the question arises as to what aspect of nutrition in mink is concerned with hair growth.

Sims (1968) proposed from his studies of the protein deficiency disease, kwashiorkor, that the measurement of the diameter of scalp hair was a useful index of protein synthesis in the management of cases of malnutrition. The increase in hair diameter in response to one month of adequate protein indicates that hair is sensitive to dietary protein.

In the rat, the finding that a deficiency of sulfur containing amino acids leads to defective hair growth is to be expected, in view of the important role of the sulfur containing amino acids in keratin formation (Lorincz 1961).

Reis and Schinckel (1962) found that two grams daily of L-cystine, or an equivalent amount of DL-methionine, or sixty grams of casein, when given abomasally to sheep increased rate of total wool growth per sheep from 35 to 130%. There was also an increase in the sulfur content of the wool. DL-methionine was found to be able to replace an equivalent amount of L-cystine. This was not unexpected as it is known that both D and L-methionine can be converted to cystine, via homocystine and cystathionine. It thus appears that protein, apart from its energy value, can specifically stimulate wool growth and that the sulfur containing amino acids may be especially important. However, the specific function of cystine in stimulating wool growth is not known at present. The greater increase in wool growth obtained with casein, was possibly a consequence of the supply of an additional factor or factors limiting wool growth.

There is no evidence at present to indicate whether the variations in the sulfur content of wool are associated with any change in the internal structure of the fibers. Wool fibers can be degraded into several components of which the largest two are α - keratose and β - keratose. The α - keratose contains 2.5% sulfur, while the β - keratose contains 6.0% sulfur. The altered sulfur content of the whole fibers observed was probably associated with a relatively greater synthesis of a high sulfur component of wool protein, presumably β - keratose. The alternative would be a uniform change in the sulfur content of these protein fractions (Reis and Schinckel 1962).

Evidence available from studies with mink fed purified diets has indicated that mink require three unidentified factors, in addition to the known cystalline vitamins for growth and survival (Schaefer et al. 1948). One of the unknown factors which is present in liver has been designated the residual factor as it is present in the insoluble residue from a 60% mathanol extract of liver. Mink kits fed purified rations exhibit characteristic signs of the residue factor deficiency by September or October. It should be pointed out that the time of occurence of the residue factor deficiency in the mink is concurrent with the fur production phases of the animals' life (Leoschke and Elvehjem 1959). Leoschke and Elvehjem (1959) present results which indicate that arginine and methionine may replace the residual factor required by the mink for growth and quality fur production. This indicates the need for protein, in particular a balanced protein for fur production of mink.

Dolnick et al. (1960) mixed a high meat diet with uncooked ground wheat (40%) and choice white grease (12%). The number of underfur fibers growing in each follicular bundle of this treatment was reduced, compared to the high meat diet alone. In mid-December, mink fed the high meat diet produced a ratio of underfur to guard hair of 29:1 and the mink fed the low meat diet a ratio of 19:1.

Lightbody and Lewis (1929a), using rats, indicated that by limiting the protein in the diet, both body growth and hair production are limited compared to adequately fed animals. The demands for protein for growth of hair appeared to be secondary in importance to the demands for growth of what is considered the more essential tissue of the body.

In a second experiment, Lightbody and Lewis (1929b) suggested that in the period of rapid hair growth, hair of a cystine content similar to that of adults may be produced, if excess cystine is present in the diet. If an adequate but not excessive amount of cystine is furnished in this early growth period, the hair may contain a higher percentage of cystine than that produced by younger animals. Under these circumstances, however, the cystine content of the hair is not as great as in the adult or in the younger animals which have received an excess of cystine in the diet. Retardation of growth alone did not result in a reduction in the cystine content of hair. A diet deficient in lysine, when fed to rats retarded growth, but did not result in production of hair with an abnormally low content of cystine or sulfur.

It appears that any limiting amino acid may stimulate hair growth when the general growth-promoting power of that diet is increased by the addition of the limiting amino acid. A lowering of the cystine content of hair results only from a cystine-deficient diet and the deficiency effect is removed by adding cystine to the diet. Hair with a normal cystine content is thus produced. It seems clear, therefore, that on a diet deficient in cystine, the rat will produce hair containing a medulla differing in optical appearance from that of a normal hair.

This hair from a cystine-deficient rat will have proportionally broader cells, be regularly disposed, and to all appearance occupy a greater proportion of the fiber. The microscopical findings suggest that the lower cystine content of hair produced by the feeding of a cystine-deficient diet is the result of defective keratinization of the hair fiber, which in consequence contains more of the sulfur-poor medullary substance and less of the sulfur-rich cortex (Smuts *et al.* 1932).

E X P E R I M E N T I

THE EVALUATION OF TWO DRY PROTEIN SOURCES IN RATIONS FOR YOUNG GROWING MINK

Mink rations in commercial operations are often composed primarily of frozen whole fish. In this study frozen fish was replaced by dry protein sources on an equivalent dry matter basis to formulate test rations. Kirk (1968) presents data which suggests that fish meals may be used to replace 50 per cent whole fish in the diet. Thus, by changing the protein source, some information might be gained as to the usefulness of soybean meal and herring meal in dry rations, as well as to compare the utilization of plant and animal protein by mink. Soybean meal, a protein source relatively low in methionine, was of special interest for its possible effect on hair growth.

MATERIALS and METHODS

Forty-five male Sapphire kits, born and raised at the Manitoba Fur and Game Station were randomly allotted to three treatment groups. Mink were penned individually in a conventional breeding pen, with a nest box, and inside feed boards. They were fed a growing ration from weaning to the start of the experimental period. The experiment started on July 18th or when the kits were approximately 60 days of age. The experimental rations were introduced to the mink over a two-week period by substituting five or ten per cent increments of the test diets to the standard ration, so that the animals were being fed their respective test diets by August 1st. The mink were tested for Aleutian disease at the start of the trial and only negative mink were used.

Composition of rations are presented in Table I and analyses of rations are presented in Table II. Each ration analysis represents an average of the analyses from three diet mixes. Two analyses are presented for the herring meal diet, one containing the added fat, and one the average of two analyses of lots with no added fat. The high protein herring meal increased the ration protein percentage and reduced the ration calorie/protein ratio to a level considered to be less than optimum for mink. To more nearly equalize the calorie/protein ratio of the three rations at approximately thirteen, stabilized animal tallow was added to the herring meal ration. The mink fed the herring meal ration, subsequently appeared to be depositing considerable fat during the early part of the trial, which could possibly have an effect on furring. The fat was therefore removed from the ration on September 7th in an attempt to overcome this difficulty and equalization of the calorie/protein ratio was sacrificed.

TABLE I

COMPOSITION OF RATIONS

Ingredient (kg. per mix)	RATIONS Control	Soybean MEAL	Herring MEAL
Horse meat	4.55	4.55	4.55
Hypro*	6.82	6.82	6.82
Raw Cereal (A meal)^	11.36	11.36	11.36
Frozen Fish	22.73	-	-
Soybean Meal	-	6.91	-
Herring Meal	-	-	6.55
Beet Pulp	-	-	.29
Tallow**	-	-	2.27
Water	11.36	22.73	20.45

* A commercial chicken waste product manufactured by Maytex Corp., New York.

** Removed on September 7th.

^ Formula of Manitoba Fur and Game Station:

41.0% Fine Ground Flaked Wheat
41.0% Fine Ground Rolled Oats
9.0% Brewers Yeast
5.0% Whey
3.0% Iodized Salt
1.0% Mineral and Vitamin Mix containing:

10g.	Iron Sulphate	203,125 IU Vit. A
6g.	Manganese	2,500 IU Vit. E
2g.	Cobalt	46,875 IU Vit. D_3
		1.1 mg. Vita B_{12}

TABLE II

CHEMICAL ANALYSIS OF MINK RATIONS*

	Control	Soybean Meal	Herring Meal With fat	Herring Meal Without fat
Dry Matter %	36.1	40.5	47.9	42.2
Fat %	12.6	7.2	19.3	9.0
Energy Cal/g	4.97	4.72	5.35	4.90
Ash %	7.7	6.0	6.9	7.5
Protein %	38.5	34.5	38.2	41.5
Cal/protein ratio	12.9	13.7	14.0	11.8

* An average of three analyses for each ration except herring meal ration which was one analysis with the added fat and an average of two analyses without the added fat.

The feces of the mink fed the herring meal ration were very loose, and this condition was corrected by the addition of approximately .29 kg. of beet pulp to each ration mix. The horse meat, hypro, and fish, were ground in a half inch grinder, and each ration was mixed in approximately 50 kg. lots in a paddle type mixer. Water was added to make a hamburger like consistency. The average quantities of water added were: control 11.36 kg; herring 20.45 kg; soybean 22.73 kg; and the rations were then stored at 0°F until fed. Feed was removed from the freezer daily and allowed to thaw until feeding time. The mink were fed each afternoon at 4:00 p.m. and a small feeding each morning at 8:00 a.m.

Nitrogen balance trials were carried out during each of the three periods of the test. The balance trials were conducted beginning on August 14th, corresponding to the late growth period; October 1st, corresponding to the furring period; and November 7th, corresponding to priming. Three mink were selected from each treatment group and were used for all balance trials, except; one mink on the soybean meal ration which was replaced after the first trial. A five day collection period was preceded by a four day adjustment period in each of the balance trials. The same feeding schedule was followed in the balance trial as for the growth study, as described earlier. Total feces and spilled or refused feed were collected before each feeding and stored at 0°F. Urine collected in two ml. conc. HCl was measured daily, before the afternoon feeding, and then placed in the total collection of urine which contained fifty ml. of toluene. The funnels were washed with 500 ml. of water daily which was also added to the urine collection.

Following completion of the balance trial a respresentative sample of the total urine from each mink was analysed for nitrogen. Feed and feces were oven-dried at seventy degrees Centigrade to a constant weight, and finely ground in a Wiley mill. Feed and feces were analysed for dry matter, protein, fat, ash, and energy.

Nitrogen analysis on feed, feces, and urine was carried out using a macro-Kjeldahl procedure (A.O.A.C.) and gross energy was determined using a Parr adiabatic oxygen bomb calorimeter. Ether extract was determined by the A.O.A.C. method.

Mink were anaesthetized with ether and skin sections were obtained from a random sample of nine mink on the first of August or prior to being fed the test rations, and from fifteen mink, five from each treatment group, in late November or December. A biopsy tool, similar to that of Carter and Clark (1957) was used and the samples were obtained just off the central dorsal line over the last rib. The skin tissues, fixed in a ten per cent formalin solution, were cut horizontally at a thickness of eight microns, using routine histological techniques. Sections obtained at the depth of the sebaceous gland were stained with haematoxylin and Van Gieson's counter-stain. The number of hair follicles per pore or the ratio of underfur to guard hair, was the criterion used to evaluate density. Ten pores per animal were counted using a microscope at a magnification of 280X.

Statistical analyses were performed following the methods outlined in Snedecor and Cochran 1967.

R E S U L T S

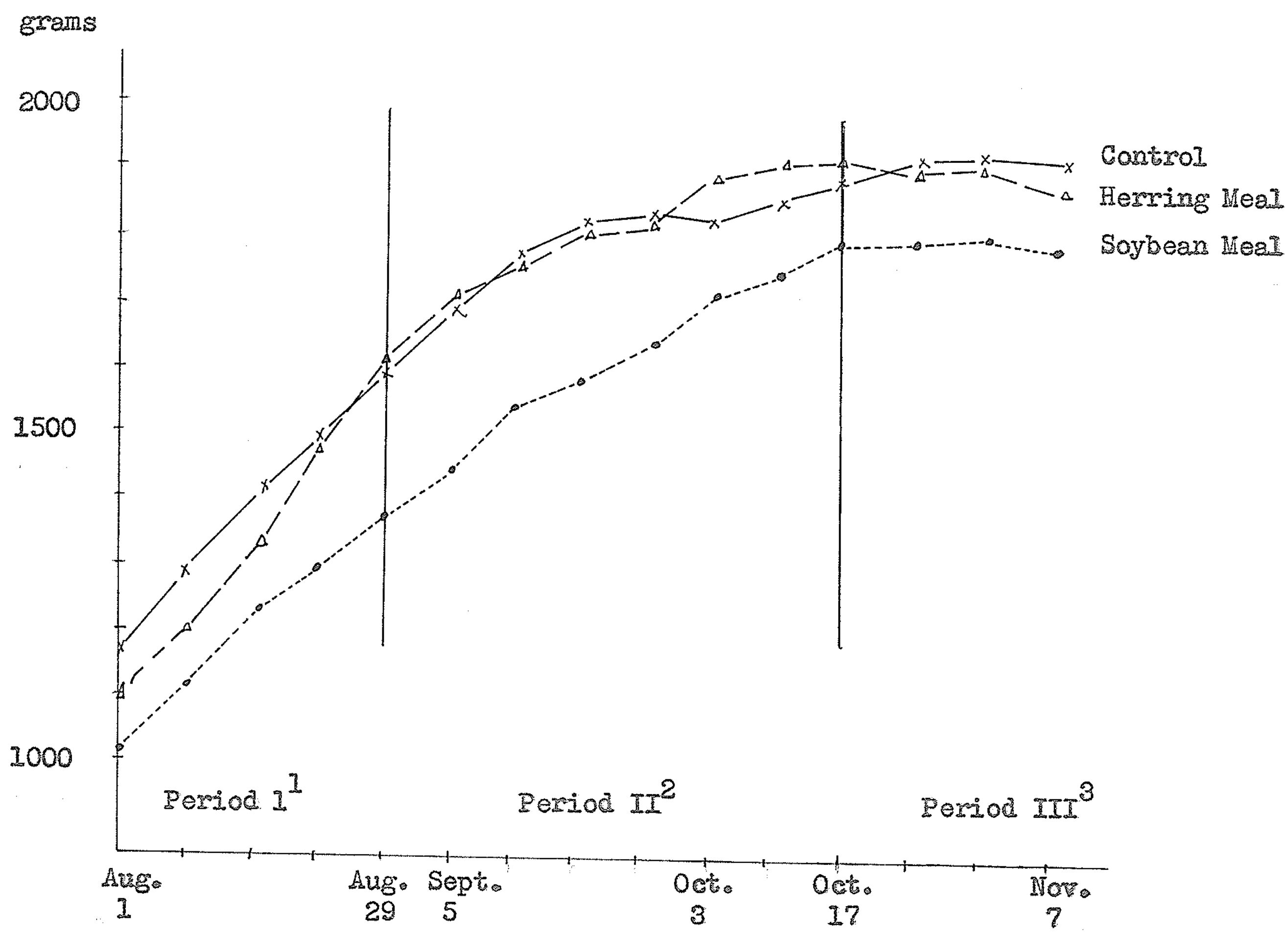
Average weekly weights of the mink on each treatment are separated into the three physiological periods, late growth, furring, and priming, and are presented in Figure I. Weight gains for treatments for each period are shown in Table III. At the conclusion of the trial, on November 7th the average weights of the mink were: 1.92 kg; 1.87 kg; 1.78 kg; for the control, herring and soybean diets, respectively. There was no significant difference ($P > .05$) in final weight. In average daily gain among the treatments, there was, however, a significant ($P < .01$) interaction between months and treatments, in that the mink on soybean treatment exhibited a different growth rate pattern than the mink on the other two dietary treatments.

Apparent digestion coefficients and percentage of absorbed nitrogen retained are presented in Table IV. The digestibility of protein and energy were the criteria used to measure the ability of the mink to utilize the diets during each period.

Digestibility of dry matter (Fig. II) was significantly ($P < .01$) less for the mink fed the soybean diet than for the mink fed the herring diet. The dry matter digestibility of the control diet was significantly greater ($P < .01$) than the dry matter digestibility of the herring and soybean diets. Periods were not significantly different ($P > .05$), but the interaction between period and treatments was highly significant ($P < .01$), which was due to the removal of the fat from the herring ration. Digestibility of energy followed the same pattern as dry matter digestibility except that periods were significantly different ($P < .05$) for energy.

FIGURE I

AVERAGE WEEKLY WEIGHTS OF MINK (GROWTH CURVES)



1. Period I - growing
2. Period II - furring and growing
3. Period III - priming and maintenance

TABLE III

AVERAGE DAILY GAIN (GRAMS) OF THE MINK FOR EACH PERIOD

	Soybean	Herring	Control
Period I Aug. 1 to Aug. 29 (growing)	11.8	18.0	15.2
Period II Aug. 30 to Oct. 17 (furring and growing)	7.8	6.7	6.1
Period III Oct. 18 to Nov. 7 (priming and maintenance)	-0.7	-2.6	0.8
Standard errors	± 0.94	± 0.87	± 0.84

TABLE IV

APPARENT DIGESTIBILITY COEFFICIENTS**

Treatment Period		Dry Matter	Organic Matter	Protein	Retained N % absorb	Energy	Fat
SOYBEAN DIET	Aug. 14	66.6	67.8	79.6	4.6	70.2	86.0
	Oct. 1	69.5	70.7	82.1	10.8	74.1	91.0
	Nov. 6	66.6	67.6	80.5	14.6	70.8	88.5
HERRING DIET	Aug.14*	79.9	81.9	83.7	14.8	83.5	93.1
	Oct. 1	74.5	76.8	82.8	16.7	79.1	93.7
	Nov. 6	73.5	75.5	81.4	10.4	77.5	91.4
CONTROL DIET	Aug. 14	76.5	79.3	84.7	11.6	81.5	93.1
	Oct. 6	76.4	78.9	85.9	15.5	82.0	94.8
	Nov. 6	75.4	78.2	84.8	6.7	80.7	92.8

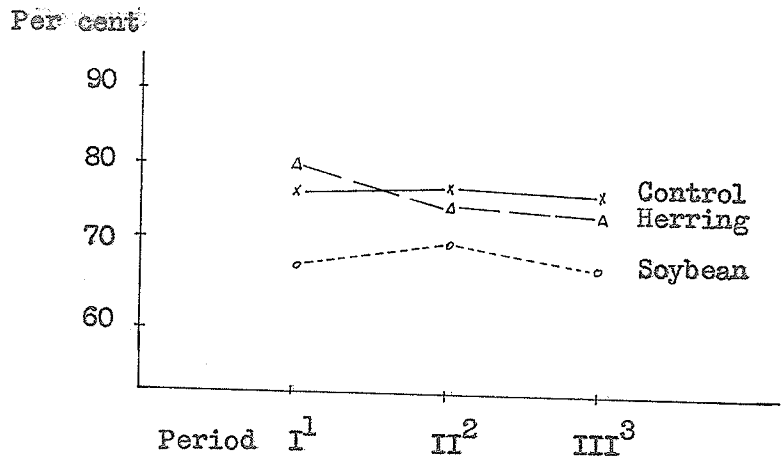
* - Aug. 14 Herring ration had added fat.

** - Average of 3 mink per observation.

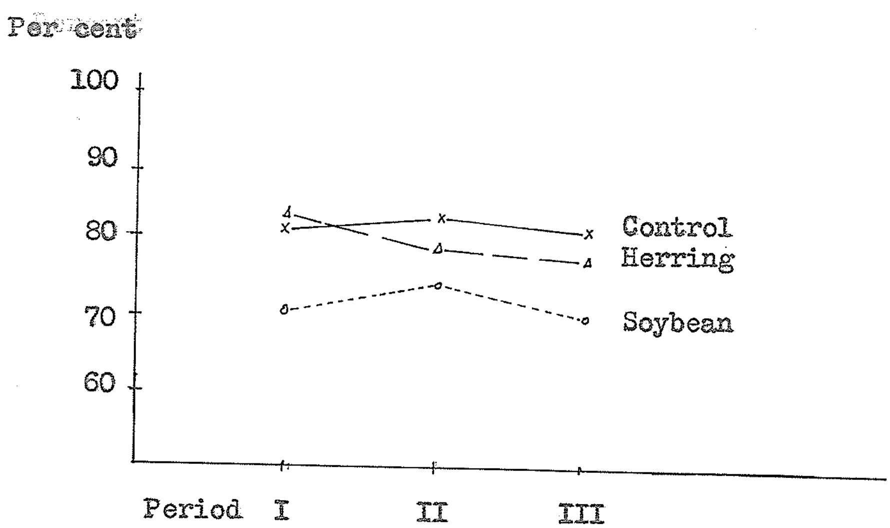
F I G U R E I I

APPARENT DIGESTIBLE COEFFICIENTS FOR DRY MATTER AND ENERGY

A. Dry Matter



B. Energy

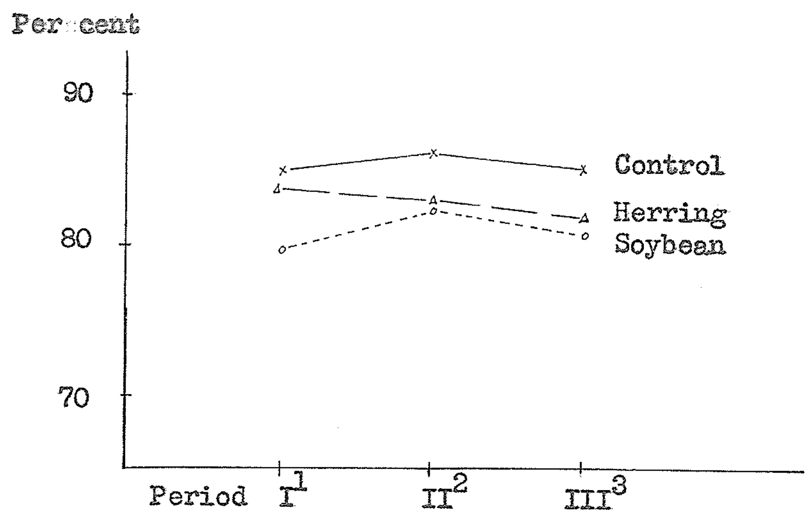


1. growing
2. furring and growing
3. priming and maintenance

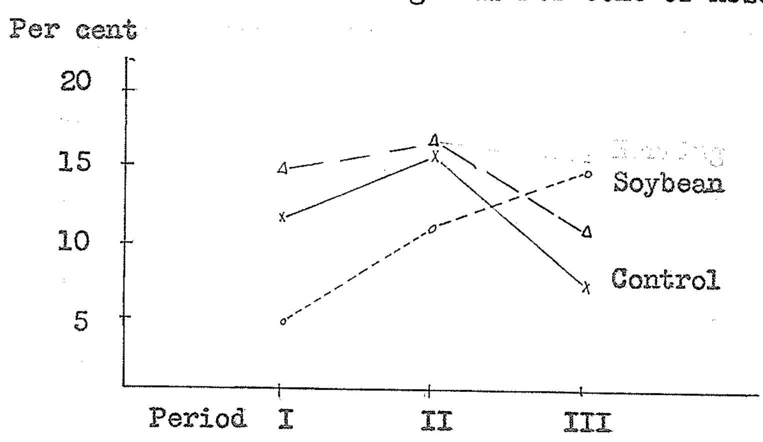
FIGURE III

NITROGEN UTILIZATION

A. Per Cent Digestible Protein



B. Retained Nitrogen as Per Cent of Absorbed Nitrogen



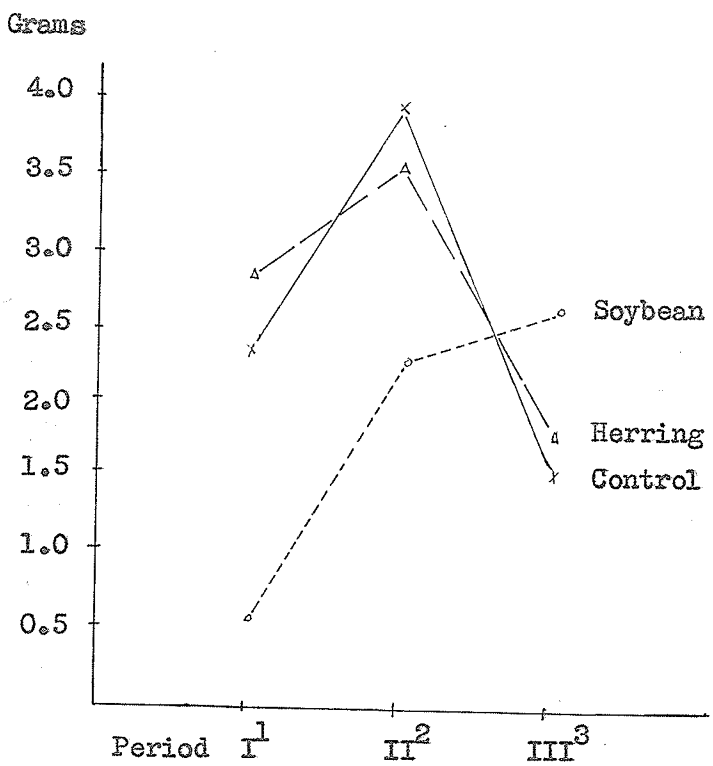
1. growing
2. furring and growing
3. priming and maintenance

The mink fed the control diet digested significantly ($P < .01$) more protein than the mink fed the herring and soybean meal diets (Fig. III). The mink fed the soybean meal diet also had lower ($P < .01$) digestible protein coefficients than mink fed the herring meal diet. The within treatment variation was very small for the analysis of apparent per cent digestible protein. The nitrogen retention data (Fig. lV), however, showed such a large variation that F tests were not statistically significant ($P > .05$). In fact all the F values were less than one. The variation remained regardless of how nitrogen retention was expressed: as grams nitrogen retained; as a per cent of absorbed nitrogen retained; or as a per cent of consumed nitrogen retained. Values for Nitrogen retained as a per cent of absorbed nitrogen and digestibility of protein during each time period is shown in Figure III, and illustrates the trend of the treatment by period interaction, even though it was not statistically significant ($P > .05$).

Due to the large animal variation in nitrogen balance, it was decided to investigate the influence of age on the nitrogen balance data (Fig. V). For the balance trials three groups of three littermates were originally to be used to reduce animal variation. A third set of three was not available, so three mink of approximately the same age were used. One mink on the soybean meal treatment was replaced, which broke up a littermate set, but an animal of the same approximate age was selected as a replacement. The average birth date for these three groups was: group 1, May 8th; group 2, May 11th; group 3, May 12th. The effect of the three groups (Fig. V) of mink was removed statistically and was highly significant ($P < .01$). Once this source of variation was removed, the analysis of variance table was more reasonable in that the values for F were greater than one.

F I G U R E   I V

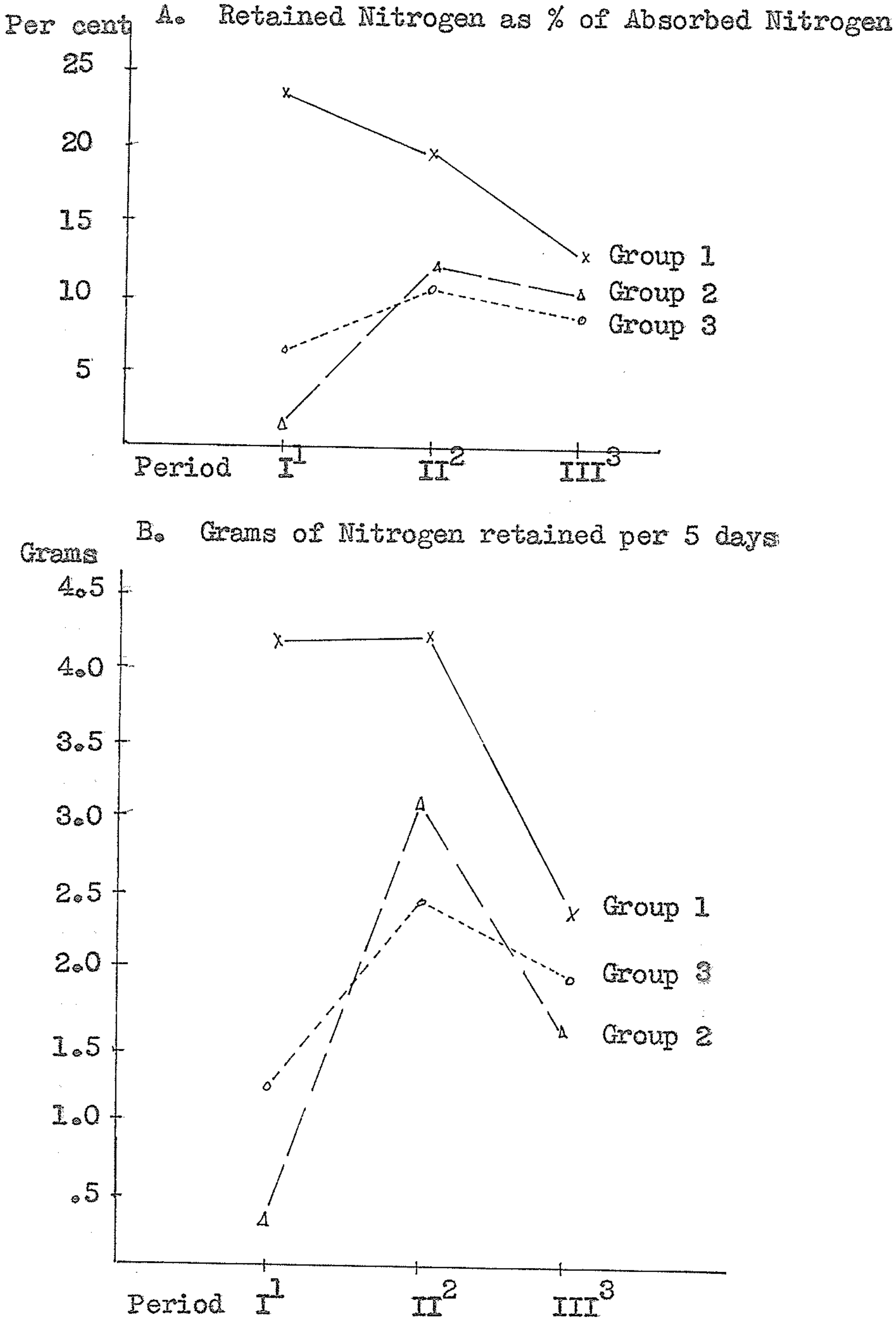
GRAMS OF NITROGEN RETAINED PER 5 DAYS



1. growing
2. furring and growing
3. priming and maintenance

F I G U R E V

NITROGEN RETENTION BY AGE GROUPS



1. growing
2. furring and growing
3. priming and maintenance

Accurate feed consumption data was difficult to obtain with mink due to wastage and the change in the moisture content of the feed over time. Feed consumption was analysed using feed consumption data from the three, five day digestibility trials as representative samples of feed consumption during each of the three periods, and consumption was analysed. The consumption figures shown in Table V represent the average of three mink for five days.

The kilocalories of energy digested was significantly greater ($P < .01$) for the mink on the control treatment than for the mink on the herring and soybean treatments. There was no statistically significant difference ($P > .05$) in energy digestibility between the mink fed the herring and control diet treatments. Mink appeared to digest more kcal. of energy in October than in either August or November, and this period effect was significant ($P < .01$). There was no significant interaction between treatment and period ($P > .05$). An attempt was made, at the beginning of the experiment, to equate the gross calorie/protein ratio of the three diets. The apparent digestible energy/apparent digestible protein ratio (Table V) is, therefore, also fairly constant among the treatments. The grams of protein digested closely parallels the pattern of the kcal. of energy digested. The control diet treated mink digested significantly ($P < .01$) more protein as compared to mink on the other two treatments. In the month of October a significantly ($P < .01$) greater amount of protein was digested by mink than in either of the months of August or November. There was no statistically significant ($P > .05$) effect for treatment by period interaction.

TABLE V

FEED CONSUMPTION* OVER 5 DAYS FOR MINK

		Consumed Dry Matter (g.)	Digested Dry Matter (g.)	Digested Energy (kcal)	Digested Protein (g.)	ADE/ADP Cal/prot. Ratio	Retained N (g.)
	Aug.	400.3	266.7	1,302.43	110.8	11.8	0.58
SOYBEAN	Oct.	480.5	334.1	1,707.75	138.0	12.4	2.29
	Nov.	402.8	267.4	1,343.27	109.9	12.2	2.48
	Aug. **	385.4	307.7	1,720.69	123.2	14.0	2.83
HERRING	Oct.	403.2	266.5	1,568.66	135.5	11.6	3.60
	Nov.	309.6	227.8	1,268.88	104.1	12.2	1.77
	Aug.	380.2	316.9	1,669.41	131.9	12.7	2.33
CONTROL	Oct.	473.4	361.6	1,973.20	158.2	12.5	3.97
	Nov.	437.0	329.0	1,723.91	144.3	11.9	1.52

* - Average of 3 mink per observation.

** - Extra fat in ration.

The variation in the grams of nitrogen retained resulted in no statistically significant differences in treatments, periods, or treatment by period interaction. Grams of nitrogen retained over time (Fig. IV) illustrated the trends among the treatments even though they are not statistically significant. Removing age effects by blocking, from the analysis of variance indicates age was highly significant ($P < .01$) and periods was significant at the $P < .10$ level.

To examine the effect of ration on hair growth, skin sections were taken at the start of the test in August. The number of hairs per pore was 13.6 $\pm$ 2.06 for a sample of nine mink. Skin sections were taken from five mink per treatment in the months of November and December to estimate hair density of mink as affected by dietary treatment. The hair densities for the three treatment groups of mink were: control, 19.5; herring, 20.6; soybean, 19.6. Statistical analysis did not show any significant difference among the three treatments.

Several mink were retained for breeding stock from the three groups of experimental animals. The pelt lengths of those pelted were; soybean meal diet (average of 7) 26.3 inches; herring meal diet (average of 9) 26,7 inches; control diet (average of 6) 25.8 inches, which was not statistically analysed as pelt lengths from the mink kept for breeders were not available.

DISCUSSION

In order to evaluate the response of mink to various proteins during the three periods, growing; furring and growing; priming and maintenance, digestibilities and nitrogen balance trials were conducted. These data were intended to aid in the explanation of any observed differences in body growth and hair density due to treatment. Differences in weight gains and hair density were, however, not statistically significant ($P > .05$). A significant ($P < .01$) treatment by period interaction was observed in the analysis of daily weight gains. The interaction suggests that the mink fed the soybean meal diet followed a different growth curve as compared to the herring meal and the control fed mink. In the second period (furring) the weight gain of the mink fed the soybean treatment decreased approximately thirty per cent from that observed in Period 1, as compared to a sixty per cent decrease by the herring and the control fed mink.

The three different dietary protein sources used; one a dried animal protein (herring meal); another a plant protein (soybean meal); and the third fresh fish, resulted in no diffeeence in overall performance of mink. The reason for the lack of an effect may be clarified by an investigation of the digestibility and balance data. Three possible hypotheses lend themselves to the explanation of the data with regard to the similar observations obtained in hair density and body growth among treatments.

1 - Energy or some ingredient other than protein was the limiting factor in the diet.

2 - Protein quality does not effect hair growth.

3 - All three proteins tested were of equal biological value (either good or poor) resulting in similar body growth and hair density.

ENERGY:

The fact that the soybean meal diet was least well utilized was indicated by reduced apparent digestibility of dry matter which is probably a reflection of the fiber content of the soybean meal diet. The short alimentary canal and rapid passage of food in mink would not allow maximum utilization of fiber. Mink fed on the control diet digested the most dry matter. The treatment by period interaction in the analysis of variance probably arose from the change in digestible dry matter of the herring meal diet from period I to period II due to the removal of the fat after period I. Periods or months were not significantly different ($P > .05$).

Apparent digestible energy, a reflection of the digestible dry matter, indicates the control diet was superior to the soybean and herring diets. Similarily the herring diet was superior as compared to the soybean diet. The significance of the period by treatment interaction again reflects the withdrawal of the fat from the herring diet.

The analysis of the kilocarlories of energy apparently absorbed or digested revealed statistical significance ($P < .01$). The mink fed the control diet digested more energy than the mink fed the herring diet but there was no difference between mink fed the herring and soybean diets. Periods were highly significant ($P < .01$), more energy being digested in the month of October than in either August or November. Whether this increased consumption of energy during October was a result of fat deposition or whether the increased demands of protein synthesis for hair growth precipitated the response was not readily apparent.

The digestibility of energy data appears to indicate that the control diet was superior to both the herring and soybean diets, with the herring diet being superior to the soybean diet. This order or ranking of the treatments, 1 - control, 2 - herring, 3 - soybean, parallels the rank of the treatments considering final weight, even though the difference among the final weights of the treatments was not statistically significant.

Other dry diet formulae (Oregon Quart. Bul. 1967 and VanLimborgh et al. 1969) use approximately 20% fat in the diets as compared to this experiment in which the control diet contained 12% fat; soybean 7.2%; herring without added fat, 9.0% fat. As fat is efficiently utilized by mink, increasing the apparent digestible energy level by adding fat may slightly improve the body weight gain of mink fed the herring and soybean diets and perhaps even the mink on the control diet. Energy, however, could not have limited body growth very much as the mink appeared to grow well and produce an adequate pelt length. The pelt lengths were:

soybean meal diet	(average of 7)	26.3 inches
herring meal diet	(average of 9)	26.7 inches
control diet	(average of 6)	25.8 inches

The Canadian Mink Breeders Association has classified pelt lengths into five categories in which mink pelts are sold:

1 - 28 inches and over

2 - 26 to 28 inches

3 - 24 to 26 inches

4 - 22 to 24 inches

5 - under 22 inches

The averages of the pelted soybean, herring and control treated mink are near the bottom of the second category which while not exceptional is a satisfactory size for a mink pelt, particularly when in this case the superior mink were selected and retained for breeders.

PROTEIN:

Apparent digestibility of protein by mink fed the control diet was significantly greater ($P < .01$) as compared with the mink fed the herring and soybean diets. Apparent digestibility of protein for the mink fed the herring diet was also significantly greater ($P < .01$) as compared with mink fed the soybean diet. When food intake, as well as percentage digestible protein, is taken into account it was found that the grams of protein digested by mink on the control treatment was greater than for mink on the herring or soybean treatments. There was no difference in grams of protein digested between mink on the herring and soybean treatments. Periods were significant ($P < .01$); and in the month of October, more protein was digested by mink on all treatments than in either of the months of August or November. The increased consumption as stated above, may be due to increased demands for energy, protein, or a combination of both. Nitrogen retention by mink also tends to increase in October supporting the hypothesis that the increased demands for protein may result in the increased feed consumption.

The nitrogen retention data revealed such large variations that the usual analysis reveals all "F" tests to be less than one. The use of three groups of littermates for the balance trials, resulted in a statistically significant ($P < .01$) age effect. This would account for some of the abnormally high variation in nitrogen balance. Nitrogen retention vs period is plotted in Figure V and it appears that age of mink in group 1 in period 1 did not follow a similar pattern to the two other age groups.

An explanation may be that, the balance trial conducted on August 14th was close to the initiation of furring, and perhaps group 1 mink, the oldest group, had indeed started furring, resulting in the higher nitrogen retention, as compared to the other two age groups.

When the age effect was removed no significant difference ($P > .05$) was found among treatments or periods in nitrogen retention. Age, however, was highly significant ($P < .01$) in that group 1 retained more nitrogen than the other two groups. The lack of significance due to the treatment effect would tend to indicate that there was no apparent difference in utilization of all three proteins by mink. The grams of nitrogen retained was not statistically differant among the treatments, but periods were significant ($P < .10$). Thus the grams of nitrogen retained appears to follow the same pattern as grams of protein digested and kilocalories of energy digested, in that the retention was higher in October than in either August or November.

As the pelt length of the mink seemed to be adequate, protein quality and quantity would appear to have been sufficient for body growth. The amount of nitrogen retained was the lowest by the mink on the soybean diet but the fact that nitrogen retention and growth of these mink continued to increase after period II (October) could possibly indicate a delay in the maturity of the mink on the soybean diet as compared with mink on the control and the herring diets. The same pattern seems to be present in the growth curve of mink on the soybean diet. In period I growth rate of the mink on the soybean diet was well below the mink receiving the control and herring diets, but in period II, when the mink on the control and herring diets showed a decreased growth rate of approximately sixty per cent, the mink on the soybean diet only decreased their growth rate thirty per cent.

This may have been a result of a retardation in maturity of the mink on the soybean diet, in that in period II the herring and control treated mink had reduced growth rate but the soybean treated mink, not attaining equal maturity continued to grow into period II.

Lightbody and Lewis (1929a) suggested that body growth seemed to have a certain degree of priority over hair growth for protein. This would then mean that the additional requirements for protein for fur or hair growth would have an additive effect on the nitrogen retention, i.e. nitrogen retention would have to increase during furring, due to both growth and furring requirements. The nitrogen retention data of the mink tend to follow this pattern, increasing during furring. However, no difference in hair density was observed among the three treatments. The question which now arises is whether the increase in nitrogen retention in the furring period was adequate for optimum hair growth. As stated previously, the nitrogen retention of the mink on the soybean treatment appeared to be marginal, but the hair density of mink on the soybean treatment was equivalent to the other treatments.

Dolnick et al. (1960) found that, by mixing 40% of a high meat ration (85% meat) with 48% cereal grains and 12% fat, hair density of mink was decreased as compared to the mink on the 85% meat diet. In early August the high meat ration (group 1) had a hair density of 16:1 per follicular bundle and the diluted ration (group 2) a hair density of 8:1. In mid-December group 1 and 2 averaged 29:1 and 19:1 respectively. In early August a representative sample of nine mink from this experiment were biopsied having an average density of 12.6:1 per follicular bundle.

As there did not appear to be any difference among treatments in December all three treatments were averaged resulting in a December average of 18.8:1. In comparison to Dolnick's mink in early August the test mink were half way between her good group and poor group. In December, however, the test mink were only just equal to the poor group. From this comparison, the inference may be drawn that mink on all three test treatments, control, herring, and soybean, had low hair densities or poor hair growth, and, therefore, that the additional nitrogen retention observed was not adequate for optimum hair growth.

SUMMARY OF EXPERIMENT I

A feeding experiment was conducted using three protein sources, frozen fish, herring meal and soybean meal in rations for mink. There was no statistical difference in either body weight gain or hair growth among treatments. Digestibility studies indicated the frozen fish diet was utilized more efficiently than the herring meal and soybean meal diets and the herring meal diet was utilized more efficiently than the soybean meal diet. During the physiological period of furring in October, feed consumption and nitrogen retention increased. The significance of this phenomenon, whether it was related to hair growth or associated with the deposition of fat for winter, was not established.

EXPERIMENT II

THE EFFECT OF PROTEIN QUALITY AND QUANTITY ON HAIR GROWTH IN THE RAT

The previous experiment using mink did not demonstrate any difference in hair density although Dolnick *et al.* (1960) had shown that hair growth in mink was affected by nutrition. In the Dolnick *et al.* (1960) experiment a high meat diet (85% meat) was diluted with 48% cereal grains and 12% fat, which must have lowered the crude protein content and also the biological value. In the mink experiment proteins of supposedly different biological values were fed, but whether in fact the biological values were different as judged from the nitrogen balance data of the mink, is questionable. Therefore, an experiment with rats was initiated in order to investigate the effect of quality and quantity of protein on hair growth. The rat was chosen as the experimental animal because it initiates a new hair cycle at approximately thirty day intervals.

MATERIALS and METHODS

Sixty male weanling white rats were individually penned and randomly allotted to six treatments. A 3 x 2 factorial experiment was initially set up using three protein sources; dried egg albumen, casein supplemented with methionine and unsupplemented casein. Two levels of protein, 20 per cent and 10 per cent, were used for each protein source. The rats on the 20 per cent egg treatment, however, had to be discarded, due to egg white injury. The remaining five treatments were then analysed as a completely random design. The composition and analysis of the diets are presented in Tables VI and VII.

The rats were weighed weekly and feed comsumption data were recorded. On the first day of the experiment, the rats were shaved along a strip of the back. When the hair appeared to have completely regrown, the rats were shaved again and subsequently shaved every two days until hair growth ceased in the shaved area. After the second regrowth of hair, this procedure was repeated. The length of one complete hair cycle was measured from the time the hair ceased growing initially until it ceased growing the second time. Skin biopsies were taken after the third shaving. The biopsy was obtained to the right of the mid dorsal line, over the last rib. The skin sections were processed through routine histological procedures and stained with haematoxylin and Van Gieson's counter stain. Hair density was determined as the number of hairs per pore, in a manner similar to that used previously for mink.

The average time interval of the hair cycle for each treatment was calculated and the portion of the growth curve, corresponding to a hair cycle, was analysed with respect to average daily weight gain and average daily feed consumption of the rats.

TABLE VI

COMPOSITION OF RATIONS AS A PER CENT

	Unsupplemented Casein 10%	Unsupplemented Casein 20%	Supplemented Casein 10%	Supplemented Casein 20%	Egg 10%	Egg 20%
Protein Source	10.7	23.5	10.7	23.5	11.5	25.0
Soybean Oil	6.0	5.0	6.0	5.0	6.0	5.0
Cellulose*	3.0	3.0	3.0	3.0	3.0	3.0
Mineral Mix**	3.7	3.7	3.7	3.7	3.7	3.7
Vitamin Mix***	1.0	1.0	1.0	1.0	1.0	1.0
Dextrose	72.2	60.4	72.2	60.4	71.4	58.9
KH_2PO_4	3.4	3.4	3.4	3.4	3.4	3.4
DL-Methionine	--	--	0.2	0.2	--	--

* - Alphacel (Nutritional Biochemical Co.)

** - Brigg's Salt Mixture (Nutritional Biochemical Co.)

*** - The composition which is expressed as mg/14.4 kg of ration mix.

Tocopherol 25mg; Menadione 50 mg; Choline 135,000 mg; Thiamine 150 mg; Riboflavin 200 mg; Niacin 150 mg; Pyridoxine 50 mg; Pantothenic Acid 450 mg; Folic Acid 1,250 mg; Biotin 5 mg; Vitamin A 100 mg; Vitamin B_{12} 6,000 mg; Vitamin D 400 mg; Inositol 50 mg; Paraminobenzoic Acid 600 mg.

TABLE VII

CHEMICAL ANALYSIS OF RATIONS, EXPERIMENT II

	Unsupplemented Casein		Supplemented Casein		EGG	
	10%	20%	10%	20%	10%	20%
Energy cal/gm.	3931	4087	3939	4083	3846	3996
Protein %	9.4	19.9	9.2	20.3	9.4	20.0
Per cent Amino Acid* (expressed as a % of the sample)						
Lysine	0.79	1.95	0.66	1.78	0.68	1.45
Histidine	0.27	0.68	0.28	0.61	0.22	0.47
Ammonia	0.07	0.15	0.06	0.15	0.06	0.12
Arginine	0.33	0.86	0.31	0.50	0.50	1.05
Aspartic Acid	0.72	1.60	0.69	1.52	0.94	2.21
Threonine	0.38	0.85	0.38	0.82	0.40	0.91
Serine	0.51	1.15	0.51	1.17	0.61	1.39
Glutamic Acid	2.66	5.99	2.62	6.09	1.55	3.50
Proline	0.96	2.17	0.98	2.29	0.32	0.76
Glysine	0.22	0.48	0.21	0.47	0.34	0.86
Alanine	0.25	0.55	0.24	0.56	0.48	1.05
Cystine	0.07	0.13	0.00**	0.00**	0.04	0.20
Valine	0.57	1.27	0.58	1.31	0.58	1.27
Methionine	0.13	0.37	0.36	0.73	0.32	0.69
Isoleucine	0.45	1.08	0.47	1.07	0.46	1.02
Leucine	0.87	1.96	0.87	2.01	0.77	1.70
Tyrosine	0.48	1.13	0.46	1.16	0.32	0.75
Phenylalanine	0.48	1.08	0.46	1.12	0.56	1.24

* - The samples were hydrolysed by a modification of the procedure according to Bragg *et al.* (1966). Hydrolysing time was 15 hours and amino acids eluted with a pH 2.2 sodium citrate buffer. Amino acids were determined by the method of Benson and Patterson (1965).

** - Negative values were left at zero.

Hair diameters were traced on paper using a camera leucida, and the diameter of each hair was measured. The diameter of the largest hair per group (primary follicle) was divided by the average diameter of the smaller hairs (secondary follicles) to obtain a ratio of the size of the primary follicle to the secondary follicles.

Statistical analyses were performed following the methods outlined in Snedecor and Cochran (1967).

RESULTS

The average weekly weights of the rats on the respective treatments are presented in Fig. VI. Animal performance was not analysed over the whole time of the test but only for the interval of one hair cycle, which was indicated on each treatment growth curve. Rat weight gains per day, (Table VIII), for the treatments were highly significant ($P < .01$) which when broken into orthogonal contrasts showed the 20% superior to the 10% rations, Table VIII. The supplemented casein 20% and casein 20% were not significantly different ($P > .05$), but the egg 10% was superior to the supplemented casein 10% and casein 10% while the supplemented casein 10% was superior to the casein 10%.

The 20% protein rations were less efficient for protein utilization than the 10% protein rations, Table VIII. There was no significant difference ($P > .05$) in protein efficiency ratios between the supplemented casein 20% and casein 20% rations. The rats fed the egg 10% diet demonstrated a significantly greater ($P < .05$) protein efficiency ratio than rats fed either the supplemented and unsupplemented 10% casein diets. The supplemented casein 10% treatment was significantly better ($P < .05$) than the casein 10% treatment in protein efficiency ratio.

The hair cycle length responded to treatment. The rats fed the 20% level of protein had shorter hair cycles than the rats fed the 10% protein rations. The rats fed the 10% egg diet tended to have a shorter hair cycle than the rats fed the casein and supplemented casein 10% diets ($P < .10$). The supplemented casein vs casein contrasts with regard to hair cycle length for both the 10% and 20% level of protein were not significantly different ($P > .05$).

FIGURE VI

AVERAGE WEEKLY WEIGHTS OF RATS (GROWTH CURVES)

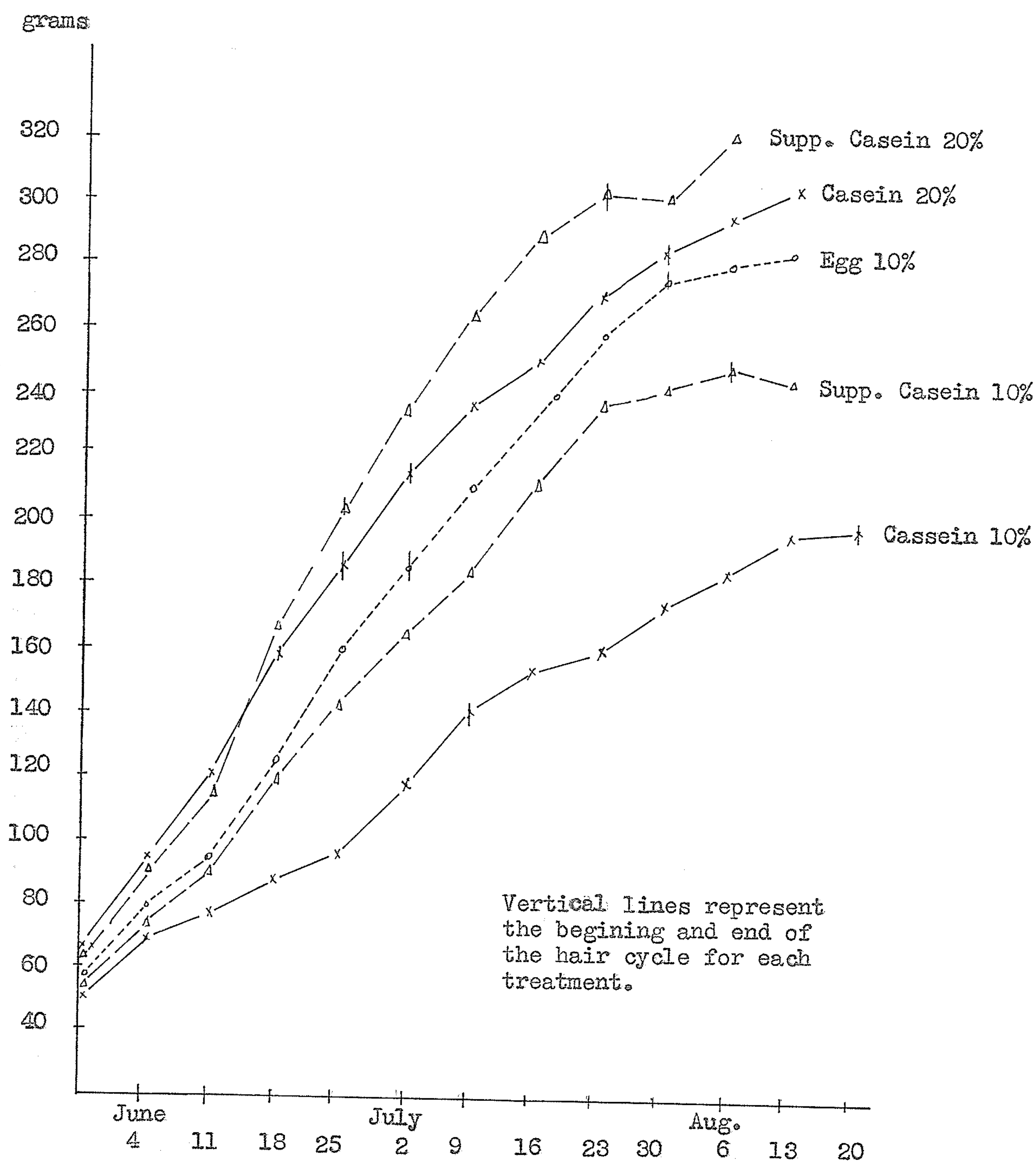


TABLE VIII

COMPARISON OF BODY GROWTH AND HAIR GROWTH CHARACTERISTICS†

1. Average Daily Gain (grams).

SC 20%$_{a}$†	E 10%$_{a/b}$	C 20%$_{b}$	SC 10%$_{c}$	C 10%$_{d}$
3.61	3.30	2.88	2.16	1.20
± 0.25††	± 0.25	± 0.25	± 0.26	± 0.29

2. Length of Hair Cycle (days).

SC 20%$_{a}$	E. 10%$_{a}$	C 20%$_{a}$	SC 10%$_{a}$	C 10%$_{b}$
30.3	31.1	33.0	35.0	40.1
±1.69	±1.51	±1.81	±1.61	±1.81

3. Hair Density (hair per follicular bundle).

C 20%$_{a}$	SC 20%$_{a}$	SC 10%$_{a}$	E 10%$_{b}$	C 10%$_{b}$
2.11	2.06	1.86	1.66	1.60
±0.12	±0.12	±0.12	±0.14	±0.15

4. Hair Diameter (ratio of primary to secondary diameters).

E 10%$_{a}$	SC 20%$_{a}$	C 20%$_{a}$	SC 10%$_{a}$	C 10%$_{a}$
1.44	1.39	1.37	1.33	1.29
±0.04	±0.04	±0.04	±0.04	±0.04

†abcd - Treatments with differing sub-scripts are significantly different ($P < .05$).

†† - Standard errors.

ORTHOGONAL CONTRASTS	Weight Gains	Protein Effic. Ratios	Hair Cycle Length	Hair Density	Hair Diameter Ratios
Treatments	* *	* *	* *	*	n.s.
20% vs 10%	* *	* *	* *	* *	n.s.
SC 20% vs C 20%	*	n.s.	n.s.	n.s.	n.s.
SC 10% & C 10% vs E 10%	* *	* *	n.s.	n.s.	n.s.
SC 10% vs C 10%	*	* *	n.s.	n.s.	n.s.

Hair density measurements taken at the completion of the third hair cycle revealed a significant difference between the 20% and 10% levels of protein but other orthogonal comparisons were not significantly different, Table VIII.

The analysis of hair diameter on a representative sample of six rats per treatment showed no significant difference ($P > .05$) due to treatment, Table VIII.

DISCUSSION

In this experiment the 20 per cent egg ration was discarded because weekly weight gains decreased until they became negative and also hair cycles could not be measured as only half of the rats regrew normal hair. The possibility that the avidin in the egg white was not destroyed by the drying process was investigated by dividing the rats on the egg 20 per cent treatment group into two groups. Additional biotin was supplemented to one of the groups and within a week, weight gain of the supplemented group of rats had increased and new hair growth was evident. Thus vitamins particularly biotin apparently affects hair growth. It would seem reasonable to assume that, because body growth and hair growth of the rats receiving the 10 per cent egg ration appeared more normal than for the rats receiving the 20 per cent egg diet, some biotin was available in the diet.

The weight gains in grams per day of the rats in treatment groups arranged in order of magnitude from high to low (Table VIII) are: SC 20%; E 10%; C 20%; SC 10%; C 10%. The rats growth rates were significantly different ($P < .05$) for each orthogonal contrast performed (Table VIII). Feed consumption in grams per day for the treatments averaged: SC 20% - 18.99; E 10% - 18.11; C 20% - 17.97; SC 10% - 14.88; C 10% - 13.77. As all five diets were isocaloric, protein, the only variable among the diets, must have been related to the differences in feed consumption which resulted in different rat performance. An imbalance of amino acid in proteins appears to affect feed consumption which in turn is reflected in body weight gain.

A 20 per cent protein diet is the National Research Council recommended level for rats. This protein level was probably more than adequate and corrected any possible imbalance. Feed consumption or body growth were not affected to any great extent when the 20 per cent protein diet was fed.

Munro (1964) suggests that at low intakes virtually 100 per cent of the absorbed amino acids from whole egg are utilized for tissue replenishment. The biological value for egg albumin and casein have been cited at 97 and 69 respectively (Munro 1964). The 10 per cent egg ration, therefore, should not have an imbalance, and consumption should be similar to the 20 per cent protein level diets as was the case. The rats fed the 10 per cent supplemented casein and 10 per cent casein diets ate less feed and had lower weight gains suggesting an imbalance of amino acids in the diet.

The question arising now is whether hair growth follows the same trend as body growth. If the effect due to ration on hair growth follows the same trend as the effect of ration on body growth this would indicate that body growth has priority for protein over hair growth. If no ration effect on hair growth is shown or if a trend differing from that shown with body growth is exhibited, then hair growth would seem to be independent of body growth.

The length of the hair cycles (Table VIII), when arranged in order of magnitude from high to low, seems to follow the same trend as the order of the weight gains. The means of weight gain, hair cycle, and hair density, were tested by a Duncans test, which is shown in Table VIII.

The hair density measurement was difficult to obtain due to the problem in defining the follicular unit. In the rat the follicular unit is not as clearly defined as in mink. The order or rank of the hair density measurements from high to low is: C 20%; SC 20%; SC 10%; E 10%; C 10%. The trend is similar to that of weight gains, except for the egg 10 per cent ration.

The ratio of the diameters of the primary to secondary hairs was, at best, a crude measurement as the diameters were traced on a page with a camera leucida.

No significant difference was shown among treatments, but the rank was: E 10%; SC 20%; C 20%; SC 10%; C 10%.

The treatment which deviated or varied most in the three measurements of hair was the egg 10 per cent. The supplemented casein 20 per cent and 10 per cent, the casein 20 per cent and 10 per cent all followed a pattern similar to the pattern of the weight gains, for all three criteria of hair growth. This variation in the ranking of the egg 10 per cent ration may or may not be a result of the avidin present even though the acute symptoms of biotin deficiency were not observed. Presumably, the low level of dried egg albumen, 11.5 per cent, did not contain enough avidin to combine with or complex with all of the biotin in the ration. Whether enough avidin was present to effect hair growth by limiting the biotin available is not known, however, this possibility exists.

If a score of one for first position, two for second position, etc. is attached to each treatment for its ranking with respect to the three hair growth criteria, then an average rank for the hair growth criteria can be obtained which is: SC 20% - 1.67; E 10% - 2.33; C 20% - 2.33; SC 10% - 3.33; C 10% - 5.00. The general trend of hair growth in response to protein seems to parallel the response of body growth to the protein source, thereby indicating a priority of protein for growth over hair. The analysis of variance, using orthogonal contrasts (Table VIII) demonstrates a significant superiority ($P < .01$) of the 20 per cent level to the 10 per cent level of protein. The proximity of the egg 10 per cent treatment to the casein 20 per cent treatment in body growth and hair growth is interesting in that it suggests that quality as well as quantity of the protein affects hair growth and body growth.

Methionine supplementation of casein did not appear to improve the performance of casein to equal egg indicating that hair growth does not seem to be limited by total nitrogen or sulphur containing amino acid but seems to respond to protein quality in general.

SUMMARY OF EXPERIMENT II

A feeding trial was conducted to examine the effect of three protein sources fed at two levels in the diet of rats. Body growth was significantly different ($P < .05$) for each dietary treatment. The effect of the various protein levels on hair growth was approximately proportional to the effect on body growth.

Hair growth in the rat appears to be limited when dietary protein is restricted either in quality or quantity. Protein is apparently conserved for more vital body functions by diverting the protein needed for hair growth. The diversion is not complete in that hair growth does not cease completely but is decreased with the effect of:

1 - Increasing the length of time required to complete one full hair cycle.

2 - Fewer hairs per pore.

3 - The diameter of the primary hair may be reduced.

GENERAL DISCUSSION

The three protein sources, frozen fish, herring meal and soybean meal, compared in experiment I appeared to be sufficiently utilized by the mink to provide adequate mink growth. The data indicated that either or both of the dry protein sources, herring meal and soybean meal, could be formulated into a dry mink diet in order to contribute a protein source to the diet.

The level of dietary protein used in the experiment was approximately 35% which is higher than the 28% level that Sinclair (1960)and Allen (1962) have suggested as being adequate. The cost of dry ration formulae may be reduced by lowering the quantity of the protein in the diet. The high level of protein in the experimental diets may have masked any differences in hair growth and body growth. Feeding excessive amounts of protein to supply the limiting amino acid in adequate amounts also supplies excessive amounts of other amino acids which is wasteful and opens the possibility that this "imbalance" may actually be restrictive to growth (Balloun 1967).

To reduce protein quantity and maintain animal performance, protein quality must be maintained or improved. For example in experiment II the 10% egg diet fed to the rats produced body weight gains equivalent to the supplemented casein 20% and casein 20% rations. Balloun (1967) states that, "It is commonly accepted that feed (or food) protein must be balanced to the needs of the animal in its content of essential amino acids in order to insure a maximal state of nutrition". Three general methods which have been employed to insure this balance of amino acids are:

1. Increasing the total quantity of protein ingested daily.
2. Providing protein from a combination of sources.
3. Supplementation with a specific amino acid which is apt to be lacking.

Mink Producers generally, according to Oldfield (1968) are formulating and feeding mink diets containing 40 to 50% protein. This high concentration of protein has been thought to be necessary due to the poor quality proteins and the high proportion of by-products used in the rations. Sinclair (1960) and Allen (1962) suggest that the protein requirements appear to be met with a 28% protein diet, which possibly can be reduced even further provided a high quality protein is fed.

Protein quality may be increased by combining two protein sources which mutually complement one another. Both herring meal and soybean meal appear to be adequately utilized as protein sources for mink rations. Good quality fish products do blend well with soybean meal into a combination in which the protein sources mutually complement each other for chicks since fish products tend to be a good source of sulfur containing amino acids (Balloun 1967). A mixture of soybean meal and herring meal may well provide an effective protein source for mink.

As defined above, a balanced protein or high quality protein must meet the needs of the animal with respect to essential amino acids. A problem that arises with mink, however, is that the essential amino acids necessary for growth and/or fur production have not been clearly defined. Hair has a different amino acid composition than body tissue and, therefore, mink may require a different amino acid ratio during the furring period than during nonfurring for optimum fur production.

The apparent increase in nitrogen retention for mink observed in October suggests two possibilities. Either the required pattern of amino acids changes during furring as compared to growth only or nitrogen is used more efficiently thereby allowing the increased retention. The additional nitrogen retention observed during the furring period could represent an additional nitrogen requirement for hair growth. If this extra nitrogen is for hair growth, the question arises as to how much extra nitrogen is required for optimum hair growth, and what amino acid pattern would give optimum nitrogen retention.

Dolnick et al. (1960) have shown that mink hair growth was affected by nutrition, in that the number of hair per follicular unit was changed. In Experiment I none of the three protein sources, soybean meal, herring meal, or fish, produced an effect on hair growth, in mink. The high level of protein and the contribution of protein from the other ingredients relative to these three sources in the ration may have masked any effect of the individual protein source on hair growth.

In the rat, protein has been shown to affect hair growth (Heard & Lewis 1938, Lightbody & Lewis 1929 a, b, and Smutts et al. 1932). In Experiment II, involving rats, a priority of body growth over hair growth for protein was suggested. When protein is marginal, the rat seems to conserve protein by diverting it from hair growth. The three criteria of hair growth measured; hair cycle length, hair density, and hair diameter all seemed to be affected by protein. Therefore, protein seems to be conserved by:

1. Growing less hair per day, i.e. the length of time required for one hair cycle varies disectly with the supply of protein.
2. The hair coat is less dense or fewer hairs are grown per pore.
3. The ratio of the diameters of the primary to secondary follicles decreases with poorer nutrition.

Butcher (1937) found that by restricting the daily feed of the rats to four grams, the hair cycle was retarded. At this level of feeding both protein and energy were limiting. A similar effect was found in Experiment II by limiting proteins but not energy.

A retardation in the appearance of hair after plucking the hair from underfed deer compared to adequately fed deer was observed by Raddi (1968). Raddi (1968) found that the size of the medulla was also affected by nutrition in deer. Smuts et al. (1932) noted that a cystine deficient diet fed to rats produced hair containing a medulla differing in optical appearance from that of normal hair of rats. In the rat, the primary hair possess a medulla but the other hair in the bristle are fine and without medulla, (Tanaka et al. 1965). Thus the diameter of the primary hair should vary more relative to the secondary hair resulting in a lower ratio with poorer nutrition. Sims (1968) proposed the measurement of scalp hair diameter in humans as a useful index of protein synthesis in the management of cases of malnutrition (kwashiorkor). Lightbody and Lewis (1929b) found that the cystine content of the hair varied if cystine was limiting in the diet of rats, suggesting some change in the make-up of the hair. Sheep have a continuous rather than cyclic pattern of hair growth. However, Reis and Schinckel (1962) have demonstrated that supplements of L-cystine, DL-Methionine, or casein, given to sheep per abomasum increased wool growth from 35 to 130%. Thus showing that protein does affect wool growth in sheep.

Thus hair growth seems to respond to protein nutrition in rats, and sheep. Dolnick et al. (1960) also suggests hair growth in mink responds to protein nutrition. It appears advantageous to further investigate the response of hair growth in mink to protein in order to produce quality pelts with maximum efficiency.

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