PROTEIN FEEDING METHOD AND LIPOGENESIS IN THE RAT

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PREFACE

This study is concerned with the effect of dietary protein on hyperlipogenesis with meal-feeding. Three treatment groups were used to examine the effect of dietary protein: ad lib-feeders, protein-meal-feeders, and complete-meal-feeders. The three treatment groups are compared for differences in body composition and nutrient utilization.

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CHAPTER I

INTRODUCTION

Background

The purpose of this study is to determine the effect of dietary protein on the increased fat deposition during meal feeding. When a protein load is administered as a meal feeding, the body may fail to store the protein for later use and accelerate alternate enzymatic pathways, such as lipogenesis. To test this theory, protein will be fed in three ways: (1) in the total ration with ad libitum access (nibblers), (2) in a three-hour meal with carbohydrate and fat offered the balance of the day (protein-meal-feeder), and (3) in the total ration with three hours access and fasting the balance of the day (complete-meal-feeders). Increased lipogenesis and fat deposition at the expense of protein synthesis and protein deposition would be anticipated in both the complete-meal-feeder and protein-meal-feeders if protein synthesis is rate-limiting.

Many facets of meal-eating have been studied, but the effect of periodicity of eating on protein metabolism has been studied little (1). Meal-eating causes hyperlipogenesis and increased fat deposition, but the mechanism is not understood. Some of the known contributing factors to hyperlipogenesis are animal factors (age, strain, and species) and dietary factors (carbohydrate, fat, and protein proportions in the diet;

quality of protein; type of carbohydrate; and time of day the meal is fed). Interaction of these variables in feeding studies prohibits derivation of one simple cause for the increased fat deposition in meal-feeding.

The contribution of protein metabolism or postprandial protein deficiency to hyperlipogenesis is under examination in this study. Protein-meal-feeders have been compared to nibblers, but complete-meal-feeders and protein-meal-feeders have not been compared. Complete-meal-feeding, protein-meal-feeding, and nibbling will all be compared in this study. Since protein-meal-feeders receive their protein in a meal and have ad libitum access to the calorie portion of their diet, the extent to which protein contributes to hyperlipogenesis in a meal feeding situation can be examined. By comparing fat deposition from meal-feeding protein to fat deposition from meal-feeding the entire diet, the proportion of fat deposition from dietary protein can be determined.

Controversy exists as to whether or not large protein loads increase lipogenesis. Protein ingested in an excess of immediate needs for protein synthesis is converted to lipid. But not all researchers have found increased lipogenesis with excess protein ingestion. In meal-feeding experiments, Cohn (2) found fat deposition increased with a dietary protein increase while McCracken (3) found fat deposition decreased as dietary protein increased. To further confuse the controversy some meal-feeding experiments have shown no difference in fat deposition with different levels of dietary protein (4, 5). Researchers (6) believe that protein metabolism contributes to increased fat deposition although the extent of the contribution and the mechanism of action is not known.

The experiment is designed to accentuate only meal-feeding effects. Some meal-feeding experiments in the past have fed the animal in the morning. Ad libitum fed controls, in contrast, consume the bulk of their diet at night. Recent findings indicate the time of day the meal is fed affects body composition (7). In this study feeding will be at the same time, 5 P.M., for all animals to permit the meal-feeders as well as the nibblers to be nocturnal feeders.

Theoretical Framework

The basic research question is drawn from the work on protein anabolism with meal-feeding. Questions about protein synthesis were aroused when Cohn (6) found greater fat deposition, less protein deposition, and greater urinary nitrogen output with meal-feeding. Differences in body composition from complete-meal-feeding and protein-meal-feeding may be due to a limit in the amount of dietary protein that can be utilized per unit of time for protein anabolism or stored by the liver for later mobilization. If these limits are exceeded, the absorbed amino acids cannot be used for protein synthesis and must be deaminated. The nitrogen moiety is hence extracted as urea, and the carbon fraction is catabolized or stored (5). Rogers and Harper's (8) work with meal-feeding ^{14}C-protein suggested that the carbon fraction is stored as fat. Rats were meal-fed 0, 15, 45 or 75 percent protein. Expirations of $^{14}CO_2$ was stimulated less by a high protein diet than would have been anticipated from urinary nitrogen excretion. Thus, the carbon fraction was presumed to be stored while the nitrogen moiety was excreted.

Studies with rats, sheep, cattle and man have shown that these species excrete more urinary nitrogen when meals are infrequent (9, 10, 11, 12, 13, 14) which supports the hypothesis of Cohn et al. (15). When meal-feeding, rats have an elevated arginine synthetase activity, the rate-limiting enzyme of the Kreb's urea cycle. This increased activity may account for the increased urea nitrogen excretion but could also be a result rather than a cause of the increase. In work with man, Wu and Wu (13) found a threshold for nitrogen intake above which urinary nitrogen excretion increased. These findings suggest there is a limit to the rate at which amino acids can be synthesized into protein.

If excess amino acids are deaminated, why is lipogenesis not reduced since protein is also necessary for synthesis of enzymes essential to lipogenesis? Theoretically, enzyme synthesis has priority over storage of protein. Tepperman et al. (16) found an adaptive increase in lipogenesis can occur without a supply of dietary protein or a rise in activity of fatty acid synthesis enzymes. Thus in complete-meal-feeding and protein-meal-feeding, where dietary protein will be unavailable the majority of the day, hyperlipogenesis can continue.

The work with radioactive protein, urinary nitrogen, and enzyme activity all suggest that protein metabolism may affect lipogenesis. Further, protein metabolism may have a differential effect on fat deposition when complete-meal-feeding or protein-meal-feeding. When calories are fed with protein as in nibbling and complete-meal-feeding, a protein-sparing-effect reduces the availability of protein for energy and increases protein anabolism and storage. With complete-meal-feeding, carbohydrate metabolism contributes to hyperlipogenesis. Protein-meal-feeding excludes the effects of protein-sparing and increased

hyperlipogenesis from meal-feeding carbohydrate.

With complete-meal-feeding, a rat deposits more fat and less protein but may have a similar weight gain to a nibbling animal. An inverse relation exists between body fat content and body protein plus water content; an animal with a high fat content will have a low protein content. With equal calorie deposition, the fatter animal will gain weight less rapidly since fat contains 10 percent water in contrast to 70 percent water in proteinaceous tissue.

Research Question and Hypotheses

In meal-feeding and protein-meal-feeding, does dietary protein cause hyperlipogenesis and increased fat deposition?

H_1: There is no significant difference in dry matter composition between treatments.

H_2: There is no significant difference in protein composition between treatments.

H_3: There is no significant difference in fat composition between treatments.

H_4: There is no significant difference in weight gain between treatments.

H_5: There is no significant difference in grams of body fat between treatments.

H_6: There is no significant difference in grams of body protein between treatments.

H_7: There is no significant difference in protein efficiency between treatments.

H_8: There is no significant difference in weight gain efficiency between treatments.

H_9: There is no significant difference in caloric efficiency between treatments.

Definition of Terms

Complete-meal-feeding and meal-feeding refer to a meal fed for a restricted period each day. Protein-meal-feeding refers to meal-feeding protein with ad libitum access to calories the balance of the day. Ad libitum feeding or nibbling denotes free access to the diet. Dry matter, the nonaqueous portion of the carcass is comprised of fat, protein, and ash. Protein efficiency is grams protein weight gain per gram protein consumed. Grams of weight gain per gram of food intake is weight gain efficiency. Caloric efficiency is the gain in caloric content of the carcass per calorie consumed. Protein efficiency, weight gain efficiency, and caloric efficiency are calculated in the appendix.

Assumptions

In order to test the hypotheses some assumptions must be made. It must be assumed that increased fat deposition is indicative of hyperlipogenesis rather than loss of other body components. It must be assumed that initial body composition of all animals is similar. Also, it is assumed that ad libitum fed animals consume their rations at similar rates, and that caloric absorption is equal for all treatments.

Limitations

Millard (17) found that a decrease in protein synthesis may be

countered by a decrease in protein breakdown in the tissues. A decrease in protein turnover could cloud the effect of complete-meal-feeding and protein-meal-feeding on body composition. A decrease in protein synthesis may limit growth and total caloric intake and thereby reduce deposition of fat. A final limitation to the study is inference to man. Meal-feeding affects man differently than the rat, impairing glucose tolerance in man while enhancing that of the rat. Also man synthesizes the majority of his fat in the liver while in the rat the primary area of synthesis is the adipocyte.

Significance

Little definitive work has been done on the effect of dietary protein on meal-feeding although much work has been done in the area of dietary carbohydrate and fat. If meal-feeding could be better understood in the rat, a focus would be given for studies in man. Perhaps increased fat deposition after meal-feeding is not common among all species of animals. Before inference to man can be made, meal-feeding must be better understood in the rat.

CHAPTER II

LITERATURE REVIEW

Introduction

Proportion of fat in the body is a function of the rate of biosynthesis of fat and the rate of breakdown and utilization of fat. With complete-meal-feeding, fatty acid synthesis rate is increased, but mobilization of fatty acid from adipocytes does not appear to be increased (1). In addition to complete-meal-feeding, other factors modify the rate of lipogenesis. These include dietary factors (total caloric supply, duration of energy depletion or repletion, and composition of the diet) and animal factors (strain, species, and age). The following is a discussion of the effect of meal-feeding and some of these modifying factors on the proportion of fat in the body. For discussion purposes, intermittent-starvation, intermittent-feeding, forced-feeding, and protein-meal-feeding are all types or terms for meal-feeding.

Adaptive Hyperlipogenesis

Three theories have been used to explain adaptive hyperlipogenesis, and probably all three explain different aspects of adaptation. One theory need not preclude the others. Adaptive hyperlipogenesis takes place in the adipocyte of the meal-feeding rat (18). Within nine days

of meal-feeding, the rat reaches the maximum rate of fatty acid synthesis while the adaptation remains six weeks after meal-feeding is terminated. Because of the prolonged effect of adaptive hyperlipogenesis, Leveille (1) named it "the obesity cycle". A person often diets by limiting himself to only one meal per day, a meal-eating pattern. Upon resumption of a normal eating pattern, the person rapidly regains weight. One factor that may contribute to the weight gain is continuation of adaptive hyperlipogenesis after meal-eating is abandoned.

One explanation of adaptive hyperlipogenesis is: activity of fatty acid synthesis enzymes increases in response to increased substrate. In support of this theory, Leveille (19) reported that hyperlipogenesis in the rat is accompanied by increased activity of several enzymes related to glucose metabolism and lipid synthesis (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, citrate cleavage enzyme, acetyl CoA carboxylase, and fatty acid synthetase). On the other hand, rate of lipogenesis was controlled by other unknown regulator(s) or repressor(s) in addition to enhanced enzyme activity when in vivo rates were compared to in vitro rates of lipogenesis.

Later, Leiville (19) suggested that enzyme activity is not responsible for increased fatty acid synthesis since lipogenesis increased after five days of complete-meal-feeding and enzyme activity increased after nine days. Thus enzyme activity is not responsible for the initial increase in fatty acid synthesis, but the increased flux through the pathway increased fat synthesis.

The third theory of adaptive hyperlipogenesis is that the ingestion and absorption of nutrients alters the activities of rate limiting enzymes through some regulator, such as cyclic-AMP (21). A low

cyclic-AMP is associated with lipogenesis while a rise in cyclic-AMP results in increased glucose production due to increased glycogenolysis, increased gluconeogenesis, and decreased glycogen synthesis. Cyclic-AMP is one possible regulator substance involved; hormonal control is also indicated. Insulin and prostaglandin E_1 are associated with lipogenesis while epinephrine, norepinephrine, glucagon, ACTH, and several other hormones are associated with lipolysis (22).

Absorption

The gastrointestinal tract of the complete-meal-feeding animals adapts by increasing in size thus increasing the physical capacity and absorptive area (19). The small intestine and glucose absorption are increased by approximately 40 percent. Enzyme activity in the intestinal mucosa increases in response to complete-meal-feeding (23, 24). Increased in vivo absorption of fat emulsion has also been found (24). Hypertrophy of the gastrointestinal tract enables the animal to accommodate the stress of meal-eating. However, amino acid absorption, motility of the intestine, and the intestinal microflora are not affected by meal-eating (24). Friend (4) found that fecal nitrogen loss was similar in meal-eaters and nibblers.

Sex and Age

Although many contradictions appear in the literature on the effect of sex and age on lipogenesis, it is generally assumed that sex has no effect and age has a definite effect. The findings of Sullivan et al. (20) indicate that the age of the rat influences the rate of in vivo

lipogenesis with meal feeding. They reported that lipogenesis is increased in immature female Sprague-Dawley rats. Other investigators (4, 25) found increased lipogenesis and fat deposition in the immature male rat. Increased fat deposition with complete-meal-feeding is found more often in the immature animals and may in part be explained by hyperplasia of the adipocyte in the young animal (22). It is now fairly well established that adipocytes do not increase in number after about 15 weeks of age in the rat. Decreased enzyme activity has been found to accompany aging and may explain decreased lipogenesis with increased age (20).

Dietary Composition

Dietary composition has been reported to affect the extent of fat deposition with complete-meal-feeding, but experimental results vary. Many experiments have been conducted where protein level is held constant, and fat and carbohydrate are varied. Cohn et al. (5) found less fat deposition with complete-meal-feeding on a high carbohydrate diet than high fat. Fabry (24) found that a high fat diet did not increase fat deposition with complete-meal-feeding whereas a high carbohydrate diet increased fat deposition. The lipogenic capacity of adipose tissue was inversely related to fat content of the diet in work done by Leveille (19). In the same study the differences between fat deposition in complete-meal-feeders and nibblers disappeared as the fat content of the diet increased. More investigators have found complete-meal-feeding a high fat diet does not lead to increased fat deposition, but the findings of Cohn et al. (5) cannot be ignored as they force pair-fed meal-eaters to ad lib controls. Forced-feeding insures that meal-eaters

consumed the same amount as nibblers whereas "trained" animals consume 75 percent as much as nibblers (19).

Different types of carbohydrate have also been reported to affect fat deposition. When sucrose or cornstarch are the carbohydrate portion of the ration, sucrose causes greater fat deposition with complete-meal-feeding (26). Sullivan et al. (20) complete-meal-fed rats a 70 percent carbohydrate diet and varied the carbohydrate source. A 70 percent fructose diet gave the highest rate of lipogenesis followed in descending order by sucrose, glucose, and starch. The rates of lipogenesis may correspond to variances in either rates of carbohydrate absorption or some other unknown factor. Cohn et al. (5) found no difference between sucrose and cornstarch on fat deposition with force-fed complete-meal-feeders.

The changes in fat composition of the rat may be secondary to the inability of the rat to handle large protein loads in meal-feedings. The meal-fed animal deposits less protein and excretes more urinary nitrogen, indicative that protein synthesis may be limited in meal-feeding. The protein load has been increased in complete-meal-feeding by increasing the protein content of the diet, and as the protein was increased there was a relatively greater fat deposition in complete-meal-feeders than nibbling controls (5). Also low quality protein or a low level of protein can increase fat deposition (26). Increased fat deposition can occur on a low-protein diet as well as a high-protein diet, in the latter case excess amino acids can be deaminated and used for formation of body fat (24).

With rats consuming similar amounts, protein-meal-feeders had a lower body protein, greater body fat and lower weight gain than ad lib

fed controls (7). Protein-meal-feeders responded similarly to complete-meal-feeders in body composition. The lower weight gain is not as easily understood. A protein-meal-feeding experiment involving children also found lowered weight gains with protein-meal-feeding when compared to a nibbling regime (27).

With complete-meal-feeding or protein-meal feeding, protein is available for a limited period each day subjecting the animal to possible protein deficiency the balance of the day. This theory is supported by Krebs' (28) finding that the degradation of excess amino acids (not needed for growth and replacement) takes precedence over carbohydrate or fat. It is possible that meal-fed protein is in exess of the immediate needs of the animal and is thus degraded creating a protein deficiency later in the day. Krebs (28) also reported that there is a high degree of variability in the regulation of amino acid degradation and some adaptation could occur to preserve the essential amino acids when the supply of substrate is low. A full adaptive increase in lipogenesis can occur whether there is a continuing supply of protein or not (29). Evidence of this was shown when the rate of lipogenesis was just as high after refeeding a zero-protein diet as a high-carbohydrate-high-protein diet.

Insulin

Fabry (24) suggested that increased fat deposition is secondary to increased insulin secretion. The large load of carbohydrate provided by meal-feeding increases insulin secretion. Insulin, in turn, increases the rate of glucose transport across the cellular membrane, the

rate of glucose metabolism, the storage of glycogen, the synthesis of fatty acids, and the entry of fatty acids into the adipocyte (21). This hypothesis is in agreement with Fabry's (24) finding that in contrast to high carbohydrate diets, a high fat meal feeding does not increase fat deposition.

Glycogen

Glycogen, in addition to lipid, serves as significant energy storage in the complete-meal-feeding rat. The complete-meal-fed rat has a higher fasting level of glycogen than the nibbler indicating that glycogen metabolism differs with feeding regime (18). The differences are not attributed to liver glycogen as the rate of accumulation is similar for complete-meal-feeders and nibblers. In contrast, the rates of glycogen accumulation are higher in diaphragm and adipose tissue of the complete-meal-feeder. The differences are greatest in adipose tissue since glycogen is found in the complete-meal-feeder and is almost nonexistent in the nibbler. The pattern of glycogenesis in the complete-meal-feeder is characterized by glycogen and fat storage in the first eight hours following a meal; 30 percent of ingested energy is utilized, 48 percent is stored as lipid and 22 percent is stored as glycogen. From 8 to 14 hours after a meal, glycogen is utilized. Lipid is then oxidized until the initiation of the next meal (18).

Species Differences

Chickens, receiving all of their ration in a daily two hour period, have increased fatty acid synthesis both in vivo and in vitro (30). Since the chickens consumed less energy than nibbling controls, the body

weight gain and body fat percentage was lower. A hyperlipogenic state was found in the meal-eating pig also, although the state was not accompanied by increased fat deposition (31). The work on other species than rats suggests that hyperlipogenesis is a common raction to complete-meal-feeding, but increased fat deposition resulting from hyperlipogenesis is unique to the rat.

Man

Metabolic studies on meal-feeding in man, to date, have not shown that meal-feeding causes increased fat deposition (32). Epidemiological studies indicate a trend towards obesity as meals are less frequent. Fabry (33, 34, 35) conducted three different epidemiological studies on men, women, and children and found that in general, incidence of overweight increases as meal frequency decreases. But much more work needs to be done before any conclusions can be drawn from meal-feeding of man.

CHAPTER III

METHODS AND PROCEDURES

Introduction

The effect of protein metabolism with meal-feeding on body composition was examined. Thirty rats were followed over a three-week observation period, a 10-day training period, and a 21-day experimental period. The three treatments were ad libitum feeding, protein-meal-feeding, and complete-meal-feeding. Following the experimental period, the rats were sacrificed and body composition was determined.

Experimental Subjects

Thirty-five, Sprague-Dawley, male rats weighing between 60 and 75 grams were purchased for the experiment. Highly inbred strains, such as Sprague-Dawley rats, have less variability in body composition than other strains with less inbreeding (36). Since body composition was a focus of the study, it was important to minimize any external source of variability. The animals were maintained for three weeks on a rat chow to adjust to laboratory conditions before the experiment was begun. At the beginning of the experiment the animals weighed between 150 and 178 grams.

Experimental Diet

The diet of Peret et al. (7) was used as a guideline for the diet in this study. Since 80 percent protein-casein was used, the calculated level of protein in the diet was 10.6 percent and was verified by determining Kjeldahl nitrogen of the complete and protein rations (37). The previous work on protein-meal-feeding suggested keeping the dietary protein level at 8 percent as this was sufficient for protein synthesis without providing any excess for gluconeogenesis (7). In preliminary work to this study, such a low level of dietary protein was found to be unacceptable to the weanling rat. The protein requirement declines with age from 28 percent dietary protein at 30 days of age to 10 percent at 50 days (38). To avoid the problems of protein-deficiency the experiment did not start until the animals were 50 days old.

The Peret et al. (7) formula was also altered by adding cystine to the diet. The diet is slightly deficient in sulphur-containing amino acids, methionine and cystine. Using lower quality dietary protein can increase fat deposition independent of the meal-feeding effect and is thus to be avoided (26). Cystine was used as it was found to be more palatable to the rat than methionine.

For the ad libitum and complete-meal-feeding animals the diet was completely mixed while for the protein-meal-feeders the protein ration was kept separate from the calorie ration. All of the rations were prepared at the beginning of the experiment and kept under refrigeration for the duration of the study.

TABLE I

COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredients	Percent in Complete Dry Mix	Percent in Protein Mix	Percent in Calorie Mix
Casein	13.00	13.00	
Cystine	0.20	0.20	
Sucrose	20.00	2.80	17.20
Cornstarch[a]	46.05		46.05
Corn oil	10.00		10.00
Cellulose	5.00		5.00
Salt mix	4.00		4.00
Vitamin mix	1.00		1.00
$CaCO_3$	0.40		0.40
NaCl	0.25		0.25
Choline	0.10		0.10

[a]Vitamin E was added to provide 68 I.U./kg feed.

Experimental Conditions

The lighting was controlled from 5:00 A.M. to 5:00 P.M. with temperature 25 $\pm$ 1° C and controlled relative humidity. The animals had free access to water. All meals were initiated at 5:00 P.M. since the rat normally consumed the bulk of his diet in the evening and night. Feeding a meal during the day would introduce another variable not under study in this experiment. Peret et al. (7) found that protein-meal-feeders receiving their protein meal in the day consumed less food and gained less body protein and fat than animals receiving their protein meal at night. When protein-meal-feeders received their meal at night, their eating behavior was similar to nibbling rats. Since the animals were pair-fed, it was essential to elicit a similar response to the diet between treatments.

Experimental Procedure

After receiving the animals, they were placed in separate, labelled cages and observed for health and vigor. Unhealthy rats were removed. During this period the animals had free access to rat chow. At the end of the observation period the animals were weighed and stratified by weight into ten groups of three animals each. From each group or block, one animal was randomly assigned to each of the three treatments. Each block was randomly assigned to a different rack in the rat cages. Within a rack, the treatments were randomly assigned to cages.

The training period lasted ten days and began with a 24-hour fast for all animals. The three treatment groups consisted of nibbler, protein-meal-feeder, and complete-meal-feeder. The nibblers had free

access to food for the duration of the study. The protein-meal-feeders had access to their protein ration for three hours (from 5:00 to 8:00 P.M.) and their calorie ration the balance of the 24-hour period. The complete-meal-feeders had access to the complete ration for three hours only per day. On the last day of the training period the 24-hour consumption of the nibblers was determined.

The experimental period was a triplicate feeding situation where the triplet was based on similarity in weight of the animals. In each of the ten weight categories, the complete-meal-feeder and protein-meal-feeder received the amount of food the nibbler consumed on the previous day. Food wastage was kept to a minimum by using nonspilling food pots. The food pots as well as the cages were color coded to minimize laboratory errors when feeding.

The experimental period lasted for 21 days with the weight of the animals recorded at the beginning of the period and once weekly thereafter. The weight was followed to detect any abnormalities that might have occurred. A 21-day feeding period was used since increased lipogenesis can be detected any time after 14 days (6).

The method used for animal weighing gave good reproducible results and was adapted from a technique designed to facilitate giving intravenous injections into the tail vein of the rat (39). The rat was placed on a turkish towel with the body perpendicular to the centerfold of the towel and the nose touching the centerfold. Half the towel is folded along the centerfold over the rat and is then rapidly rolled up. The rat is kept still without any harm and can be weighed on a top-loading balance.

Response Criteria

At the end of the study all animals were fasted for 15 hours to minimize differences in weight due to "digestive-tract fill" (4). The rats were killed by carbon dioxide inhalation, weighed, wrapped individually in plastic bags, labelled, and stored at -14° C. Each frozen carcass was chopped into one inch cubes and returned to frozen storage. To obtain homogeneous samples for carcass analysis, each carcass was frozen in liquid nitrogen and blended for three minutes in a Waring five liter blender. All the contents of the blender were carefully removed and further mixed using a stirring rod. For each carcass two-10 grams samples were dried for 24 hours in a vacuum oven at 60° C and 20 pounds pressure to determine carcass dry matter (37). A macro-Kjeldahl nitrogen procedure was used to determine carcass protein in two-5 grams samples (37). Ether extractions were done on the dried samples to determine body fat composition (37).

In analysis of the feed, a Parr adiabetic bomb calorimeter was used to determine the caloric content (37). Protein content of the ration was found from Kjeldahl nitrogen.

Statistical Analysis

The least significant difference, two-way analysis of covariance statistic was used to examine the difference between treatments, in body protein, fat, water, and weight. Covariance removed the effect of any differences in food intake. The randomized complete-block design increased precision, reduced experimental error, and aided calculation of missing data when an experimental subject died (40). Because the effect

of intake is calculated into protein efficiency, weight gain efficiency, and caloric efficiency, two-way analysis of variance was used to determine the differences in treatments on these values. The data was collected, coded, key-punched, and analyzed using a statistical software computer package located in the University Computer Center, Oklahoma State University.

CHAPTER IV

RESULTS

Introduction

All of the treatments consumed a lower level of ration than was expected. A consumption of 17.3 grams per day would have ensured adequate growth, but the controls consumed 15.9 grams daily (39). Accordingly, the controls reached an average weight of 218 grams by the end of the experiment in contrast to an expected weight of 293 grams. Respiratory infections, low dietary protein, and possible unknown factors explained the low dietary intake. One animal died of a respiratory infection during the course of the experiment; calculation of missing values by analysis of covariance supplied the missing data. As was expected, the complete-meal-feeders consumed less than ad lib controls. Leveille (19) reported that complete-meal-feeders consumed 75 to 80 percent as much as nibblers; this study, in support of his work, found complete-meal-feeders consumed 82 percent as much ration.

To test the accuracy of the laboratory procedure, correlation coefficients were calculated on body composition to determine if experimental data corresponded to known relationships about body composition.

Dry matter and dry protein were negatively related ($r = -.70$). This indicates that as body protein increases, body water increases (or dry matter decreases). This relationship is expected since protein binds

the majority of body water. As body protein and water decrease, fat and dry matter increase which is supported by the high positive correlation of percent dry matter and dry fat (r = .91). Fat and protein on a dry basis have a high negative correlation (r = -.76) as expected. The data for fat and protein on a wet basis does not have as high a degree of significance as the dry figures suggesting that some error exists in the laboratory analysis of moisture. The ground carcasses were wrapped in plastic bags, and some moisture was lost through the plastic. An unequal moisture loss may account for the lower negative correlation between wet fat and protein. The dry figures show a high degree of accuracy.

TABLE II

CORRELATION COEFFICIENTS FOR BODY COMPOSITION

x	y	r	Significance
% Dry Matter	% Protein (Dry)	-0.7013	0.001
% Dry Matter	% Fat (Dry)	0.9149	0.001
% Fat (Dry)	% Protein (Dry)	-0.7567	0.001
% Fat (Wet)	% Protein (Wet)	-0.2454	0.104

Dry Matter and Water

Tables III, IV, V and VI show the analysis by treatment and block of body composition: dry matter, protein, and fat. Table III indicates

no significant difference at $P \leq .05$ for percent dry matter. On review of Table VI, the percent body water means (100 minus percent dry matter) do not significantly differ at $P \leq .10$. Theoretically, the nibbler has more body protein which binds more water thus increasing the water content of the body and decreasing the dry matter. All of the animals consumed the ration at a suboptimal level which may have interfered with regular protein deposition and water composition.

Protein

Table IV indicates no significant difference in percent body protein at $P \leq .05$, and Table VI indicates no significant difference $P \leq .10$. Previous research findings indicate there should have been a significant difference in protein composition between the complete-meal-feeder and the nibbler with the nibbler depositing more protein (6). Less is known about the protein-meal-feeder, but Peret *et al*. (7) found protein deposition to be similar in the nibbler and the protein-meal-feeder. The lack of differences in protein composition are attributed to the suboptimal food intake which may have interfered with regular nutrient metabolism in each treatment.

Fat

Tables V and VI show a significant difference in percent of body fat at $P \leq .10$. Although a low level of significance is found, a trend toward increased fat deposition in the complete-meal-feeder and protein-meal-feeder is indicated. Possibly, the trend would be more clearly defined if food intake was optimal. Previous research indicates that complete-meal-feeders and protein-meal-feeders are fatter than nibbling

TABLE III

ANALYSIS OF VARIANCE TABLE--PERCENT DRY MATTER

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Main Effects	44.971	11	4.088	2.137	0.074
Treatment	6.347	2	3.173	1.659	0.217
Block	38.624	9	4.292	2.243	0.069
Explained	44.971	11	4.088	2.137	0.074
Error	34.439	18	1.913		
Total	79.410	29	2.738		

TABLE IV

ANALYSIS OF VARIANCE TABLE--PERCENT PROTEIN

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Main Effects	14.108	11	1.283	0.678	0.999
Treatment	1.411	2	0.705	0.373	0.999
Block	12.697	9	1.411	0.746	0.999
Explained	14.108	11	1.283	0.687	0.999
Error	34.039	18	1.891		
Total	48.146	29	1.660		

TABLE V

ANALYSIS OF VARIANCE TABLE--PERCENT FAT

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Main Effects	118.725	11	10.793	2.016	0.090
Treatment	26.043	2	13.022	2.432	0.115
Block	92.682	9	10.298	1.924	0.113
Explained	118.725	11	10.793	2.016	0.090
Error	96.360	18	5.353		
Total	215.086	29	7.417		

TABLE VI

MULTIPLE CLASSIFICATION ANALYSIS OF BODY COMPOSITION ADJUSTED FOR TREATMENT AND BLOCK

	Nibblers	Protein-Meal-Feeders	Complete-Meal-Feeders
Water, %	65.08[1,a]	64.90[a]	64.63[a]
Protein, %	19.95[a]	19.64[a]	19.42[a]
Fat, %	7.33[a]	9.28[b]	9.33[b]
Total	92.36	93.82	93.38

[1]Adjusted means not followed by the same letter are significantly different at $P \leq 0.10$ using Least Significance Difference procedure.

controls (6, 7).

Weight Gain

Tables VII, VIII, IX and X show the analysis by treatment and block with intake of body gains. Table VII indicates a significant difference in weight gain at $P \leq .05$ when the effect of differences in food intake is removed. Further examination of weight gain in Table X shows the complete-meal-feeders to have a significantly greater weight gain at $P \leq .05$ than protein-meal-feeders. As previous research has not compared protein-meal-feeders and complete-meal-feeders, a new finding is indicated. The lower weight gain in protein-meal-feeders has been shown in comparison to nibblers (28). The difference is not clearly understood except for the explanation that protein may not be utilized as efficiently in the absence of calories thus impairing growth. Greater weight gains in complete-meal-feeders as compared to nibblers have been reported (19), however the weight gain of complete-meal-feeders and nibblers was not significantly different at $P \leq .05$. With more cases per treatment a significant difference between complete-meal-feeders and nibblers would probably be found. In actuality, adjustment for intake removes part of the treatment effect, and each treatment may be significantly different from the other.

Total Body Fat

Tables VIII and X indicate total body fat to be significantly different at $P \leq .05$. Body fat is found to be significantly higher in complete-meal-feeders than nibblers with protein-meal-feeders midway between the two treatments. The significant difference between

complete-meal-feeders and nibblers is supported by research findings (6), but little is known about protein-meal-feeding. Although protein-meal-feeding does not differ significantly from the other treatments, it does represent a mean weight halfway between the complete-meal-feeder and nibbler which may significantly differ if more cases were added per treatment.

Total Body Protein

Table IX indicates body protein to be significantly different at $P \leq .05$. Further review of body protein in Table X shows that protein is significantly higher (at $P \leq .05$) in complete-meal-feeders than nibblers and protein-meal-feeders. The protein-meal-feeder does not appear to metabolize protein in the same way as the complete-meal-feeder.

TABLE VII

ANALYSIS OF COVARIANCE TABLE--WEIGHT GAIN WITH COVARIATE-INTAKE

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Covariate	3675.385	1	3675.385	22.683	0.001
Main Effects	2989.225	11	271.748	1.677	0.163
Treatment	1536.558	2	768.279	4.741	0.023
Block	1508.089	9	167.565	1.034	0.454
Explained	6664.609	12	555.384	3.428	0.011
Error	2754.566	17	162.033		
Total	9419.176	29	324.799		

TABLE VIII

ANALYSIS OF COVARIANCE TABLE--TOTAL BODY FAT
WITH COVARIATE-INTAKE

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Covariate	338.351	1	338.351	11.330	0.004
Main Effects	755.370	11	68.670	2.299	0.060
Treatment	243.080	2	121.540	4.070	0.035
Block	442.374	9	49.153	1.646	0.180
Explained	1093.720	12	91.143	3.052	0.018
Error	507.697	17	29.865		
Total	1601.417	29	55.221		

TABLE IX

ANALYSIS OF COVARIANCE TABLE--TOTAL BODY PROTEIN
WITH COVARIATE-INTAKE

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Covariate	1341.377	1	1341.377	106.320	0.001
Main Effects	458.693	11	41.699	3.305	0.014
Treatment	338.704	2	169.352	13.423	0.001
Block	143.532	9	15.948	1.264	0.323
Explained	1800.071	12	150.006	11.890	0.001
Error	214.479	17	12.616		
Total	2014.550	29	69.467		

TABLE X

MULTIPLE CLASSIFICATION ANALYSIS OF BODY GAINS ADJUSTED FOR TREATMENT AND BLOCK

	Nibblers	Protein-Meal-Feeders	Complete-Meal-Feeders
Weight gain, gm.	53.97[1,ab]	45.48[a]	63.42[b]
Body fat, gm.	14.67[a]	18.14[ab]	22.51[b]
Body protein, gm.	40.39[a]	37.96[a]	46.33[b]

[1]Adjusted means not followed by the same letter are significantly different at $P \leq 0.05$ using Least Significant Difference procedure.

Protein Efficiency

The efficiency scores of protein, weight gain, and calories serve as a verification of the analysis of covariance of body gains. The efficiency scores are divided by a factor of intake thus the problem of removing differences in intake by covariance is eliminated. In this study, the use of covariance to adjust for differences in intake removes part of the treatment effect and makes interpretation of the results difficult (41).

Tables XI, XII, XIII and XIV show the analysis by treatment and block of efficiency in gains. Table XI indicates a significant difference in protein efficiency at $P \leq .05$. Further investigation of protein efficiency in Table XIV indicates that efficiency is significantly lower in the protein-meal-feeder than the complete-meal-feeder at $P \leq .05$. It can be deduced that the protein-meal-feeder used significantly more dietary protein for energy purposes than the complete-meal-feeder. From

Table X one could deduce that protein deposition significantly differs in the complete-meal-feeder and nibbler, but closer analysis in Table XIV indicates no significant difference. The false assumption drawn from Table X is due to using analysis of covariation to remove differences in intake.

Weight Gain Efficiency

Table XII indicates no significant difference in weight gain efficiency at $P \leq .05$. Closer inspection of weight gain efficiency in Table XIV indicates a significant difference between protein-meal-feeders and complete-meal-feeders at $P \leq .05$. The findings in Tables XII and XIV confirm the findings in Tables VII and X.

Caloric Efficiency

Tables XIII and XIV indicate a significant difference in caloric efficiency at $P \leq .05$. Caloric efficiency is significantly higher at $P \leq .05$ in the complete-meal-feeder than the nibbler while protein efficiency is not significantly different at $P \leq .05$. Therefore the higher caloric efficiency in the complete-meal-feeder is attributed to increased fat gains. Previous findings show complete-meal-feeders to deposit more fat than nibblers (6). Because protein efficiency is lower in the protein-meal-feeder than the nibbler and caloric efficiency is the same, more fat may be deposited in the protein-meal-feeder.

TABLE XI

ANALYSIS OF VARIANCE TABLE--PROTEIN EFFICIENCY

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Main Effects	0.126	11	0.011	3.126	0.016
Treatment	0.047	2	0.023	6.392	0.008
Block	0.079	9	0.009	2.401	0.054
Explained	0.126	11	0.011	3.126	0.016
Error	0.066	18	0.004		
Total	0.192	29	0.007		

TABLE XII

ANALYSIS OF VARIANCE TABLE--WEIGHT GAIN EFFICIENCY

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Main Effects	0.015	11	0.001	1.875	0.114
Treatment	0.007	2	0.004	4.841	0.021
Block	0.008	9	0.001	1.216	0.345
Explained	0.015	11	0.001	1.875	0.114
Error	0.013	18	0.001		
Total	0.028	29	0.001		

TABLE XIII

ANALYSIS OF VARIANCE TABLE--CALORIC EFFICIENCY

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance of F
Main Effects	0.036	11	0.003	5.335	0.001
Treatment	0.021	2	0.011	17.464	0.001
Block	0.015	9	0.002	2.639	0.038
Explained	0.036	11	0.003	5.335	0.001
Error	0.011	18	0.001		
Total	0.047	29	0.002		

TABLE XIV

MULTIPLE CLASSIFICATION ANALYSIS OF EFFICIENCY SCORES ADJUSTED FOR TREATMENT AND BLOCK

	Nibblers	Protein-Meal-Feeders	Complete-Meal-Feeders
Protein Efficiency	$0.39^{1,ab}$	0.34^{a}	0.44^{b}
Weight Gain Efficiency	0.13^{ab}	0.11^{a}	0.15^{b}
Caloric Efficiency	0.21^{a}	0.21^{a}	0.27^{b}

[1]Adjusted means not followed by the same letter are significantly different at $P \leq 0.05$ using Least Significance Difference procedure.

CHAPTER V

CONCLUSIONS

Purpose

The effects of dietary fat and carbohydrate on hyperlipogenesis with complete-meal-feeding have been reported in the literature (24). But effects of dietary protein on hyperlipogenesis and consequent fat deposition have escaped attention. Comparison of protein-meal-feeders to nibblers and complete-meal-feeders examined the effect of dietary protein on fat deposition. Protein-meal-feeders have not previously been compared to complete-meal-feeders.

Findings

The findings are summarized in Table XV. The protein-meal-feeder and complete-meal-feeder had 26 percent and 27 percent more fat in the carcass than the nibbler ($P < .09$), therefore protein-meal-feeding alters body composition in a similar manner as complete-meal-feeding. It does not appear, though, that protein-meal-feeding increases the efficiency of nutrient utilization above nibblers (0 percent) as is the case with complete-meal-feeding (29 percent). The protein-meal-feeder had lower efficiency ($P < .05$) than the complete-meal-feeder in use of protein (23 percent) and weight gain (17 percent).

TABLE XV

SUMMARY TABLE OF PROTEIN FEEDING METHOD AND RAT CARCASS COMPOSITION

	Nibblers	Protein-Meal-Feeders	Complete-Meal-Feeders	Significance of F
Water, %	65.08[1,a]	64.90[a]	64.63[a]	0.074
Protein, %	19.95[a]	19.64[a]	19.42[a]	0.999
Fat, %	7.33[2,c]	9.28[d]	9.33[b]	0.090
Weight gain, gm.	53.97[ab]	45.48[a]	63.42[b]	0.011
Body fat, gm.	14.67[a]	18.14[ab]	22.51[b]	0.018
Body protein, gm.	40.39[a]	37.96[a]	46.33[b]	0.001
Protein efficiency	0.39[ab]	0.34[a]	0.44[b]	0.016
Weight gain efficiency	0.13[ab]	0.11[a]	0.15[b]	0.114
Caloric efficiency	0.21[a]	0.21[a]	0.27[b]	0.001
Intake, gm./day	15.90	15.60	13.10	

[1]Adjusted means not followed by the same letter are significantly different at $P < .05$ using Least Significant Difference procedure.

[2]Adjusted means not followed by same letter are significantly different at $P < .10$.

This suggests that the protein-meal-feeder, like the complete-meal-feeder, deposits more fat than the nibbler. Increased body fat content may be due to a low protein efficiency. Protein was used for energy storage instead of protein deposition. In comparison, the complete-meal-feeder has a high protein efficiency, indicating protein was not used extensively for fat deposition. Hence, the protein-meal-feeder and

complete-meal-feeder have a similar fat composition but for differing reasons: the protein-meal-feeder deposits more fat because of low protein efficiency, and the complete-meal-feeder deposits more fat because of high caloric efficiency.

Recommendations

One important factor to alter in future study is increasing food consumption. To improve the acceptability of the ration, the protein content could be increased. Intake decreased most after the fourteenth day of the experimental period. A ten-day training period with a 14-day experimental period would probably be sufficient to indicate hyperlipogenesis without decreasing food intake.

The protein-sparing-effect of carbohydrate was lost in the protein-meal-feeder because of the three-hour protein meal before carbohydrate was introduced. A one-hour meal for the protein-meal-feeder could decrease the amount of protein used for energy and change results. One-hour meal-feedings have been used successfully for complete-meal-feeding. To insure the most accuracy tube-feeding equal calories could be used.

To improve the laboratory analysis, an electric sausage grinder could be used to homogenize rat carcasses. Animals could be prepared by chopping into one-inch cubes and freezing in liquid nitrogen before grinding.

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APPENDIXES

CALCULATION OF EFFICIENCY TERMS

Protein Efficiency

A simplified form of calculating protein efficiency is:

$$\frac{\text{final body protein - initial body protein}}{\text{protein consumed}}.$$

The above formula served as a model for calculation of protein efficiency and was actually calculated as follows:

$$\frac{\text{(gm. final body protein) - (gm. initial body weight x .15)}^{a}}{\text{gm. intake x .106}^{b}}$$

Weight Gain Efficiency

Weight gain efficiency was computed as follows:

$$\frac{\text{gm. weight gain}}{\text{gm. intake}}.$$

Caloric Efficiency

The simplifed form of caloric efficiency is:

$$\frac{\text{final carcass caloric content - initial carcass caloric content}}{\text{caloric intake}}.$$

The technical calculations follow:

[(gm. final body protein x 5.65^{c}) + (gm. body fat x 9.40^{d})] - [(initial weight x $.15^{e}$ x 5.65^{c}) + (initial body weight x $.107^{f}$ x 9.40^{d})]/gm. intake x 4.5^{g}.

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[a]Fraction of weanling carcass that is protein (6).

[b]Fraction of ration that is protein determined from Kjeldahl nitrogen.

[c]Atwater fuel value for kilocalories per gram of body protein.

[d]Atwater fuel value for kilocalories per gram of body fat.

[e]Fraction of weanling carcass that is protein (6).

[f]Fraction of weanling carcass that is fat (6).

[g]Kilocalories per gram of feed as determined by bomb calorimetry.

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