THE ACTION AND INTERACTION OF PHYSIOLOGICAL FOOD INTAKE REGULATORS IN THE LAYING HEN

By

EARL WILLIAM GLEAVES

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1953

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1961

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Thesis Approved:

Rollin H. Thayer
Thesis Adviser

John W. West
Head of the Poultry Science Department

W. S. Newcomer

Robert D. Morrison

L. J. Bush

D. R. Wilken

J. H. Boggs
Dean of the Graduate School

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INTRODUCTION

In the field of physiology, it has been recognized that certain specific physiological mechanisms in animals play a role in the maintenance of energy balance. This energy balance is kept fairly constant through adjustments of four important variables: (1) food intake, (2) stored energy, (3) work and (4) heat production. Food intake compensates for the changes in the other three variables. Therefore, the mechanisms that regulate food intake are of prime importance in the overall maintenance of energy balance.

One of the factors which led to the creation of an interest in the mechanisms that regulate food intake was obesity in man. The fact that obese people who want to lose weight often seem unable to follow diets which will allow them to achieve and maintain ideal weight has led scientists to investigate the basic mechanisms involved in the regulation of food intake. Other investigators have been motivated by an academic interest to study these mechanisms. Actually the motives which led to these investigations are of little importance here, except to emphasize the fact that a considerable amount of knowledge is now available concerning the factors that regulate food consumption in mammals and fowl.

Unfortunately, nutritionists have not utilized this knowledge to the fullest extent in nutrient requirement studies for poultry and other animals. In general, poultry nutritionists recognize that the nutrient requirements of poultry are dependent upon the action and interaction of a number of specific factors. These factors include such things as body size, level of production or growth, stress conditions, environment, the level of certain nutrients in the diet, sex, and the strain of poultry being fed. They are all related to feed intake and are, therefore, extremely important. However, a careful appraisal of the current situation will show that very little is actually being done at the present time to provide nutrient levels in a poultry ration in line with the requirements imposed by these factors.

The assumption that, when varied nutrient densities are supplied in rations being fed to poultry, varied levels of nutrients will be consumed has been relied upon too heavily. This assumption is not always true. In fact, more often than not, when dietary nutrient densities are varied there is a subsequent variation in the total feed consumed. Consequently, there is very little if any actual difference in the intake of the individual nutrients of poultry fed the various rations. This has led to conflicting reports as to the nutrient requirements for all animals including laying hens.

The experiment reported herein was designed to study the application, to laying hen nutrition, of some of the physiological mechanisms that are known to influence feed consumption. The primary objectives of the experiment were: (1) to study the main effects of dietary protein, energy, weight and volume upon feed consumption of laying hens fed under _ad libitum_ conditions, (2) to study the effects due to interactions between and among these four factors, and (3) to study the effects of these factors on egg production, egg weight and body weight change.

During the course of the analysis of the data from this experiment, it became obvious that the effect of egg production level and its influence upon nutrient expenditure was confounded with the effects of the other four factors upon feed consumption to the point that interpretation was near to impossible. For this reason a fourth objective was added. This was to estimate the effect of egg production upon feed consumption.

It is important that the correct connotation be given to some of the descriptive terms as they have been used in this introduction and as they will be used throughout the dissertation. Therefore, the following list of definitions is supplied for the benefit of the reader.

1. Physiological factors. This term pertains to those factors that are known to have some function in one or more of the physiological mechanisms that regulate food consumption. They include: dietary protein, energy, weight, volume and egg production.

2. Physical factors. This includes those factors which are normally thought of as being physical in nature, such as dietary weight and volume. These physical factors then actually become a subclass under the broader classification of physiological factors.

3. Nutrient factors. As opposed to physical factors, the term nutrient factors will be used in a broad sense to include such things as protein and energy. It is recognized that energy is not normally considered as a nutrient; however, for the purpose of brevity, and since energy is composed of carbohydrates, fat and protein it may sometimes be referred to as a nutrient.

4. Dietary factors. Any factor that is or can be incorporated as an integral part of the experimental diet will be called a dietary factor. The adjective dietary will always mean that which is included in the ration. The dietary factors of principal interest here are protein, energy, weight and volume.

5. Nutrient density. This term refers to the units of nutrient per unit weight of the experimental diets.

6. Feed intake, food intake, feed consumption and food consumption. These terms are used synonymously and mean the intake of all dietary factors considered collectively. This is opposed to statements about protein intake, for example, which mean only the intake or consumption of protein.

7. Feed intake factors. Feed intake was measured by two methods, namely, by weight and by volume. Therefore, the terms feed weight and/or feed volume intake may appear and are to be taken to mean the weight or volume of feed consumed. The intake of protein and energy will normally be spoken of as protein and/or energy intake.

Even though an attempt has been made to define terms as specifically as possible, there is one distinction that needs to be reemphasized. This is the difference between the meaning of "dietary level" and "intake level." Four of the factors (see definition of dietary factors) under study in this experiment were incorporated into the experimental diets at varied "dietary levels," but this does not mean to imply that there was, under all circumstances, varied "intake levels" of these four factors (see definition of feed intake factors). Therefore, the reader is cautioned to distinguish between "dietary level" and "intake level" in reading this dissertation. Since the experimental diets were formulated on a per hen, per day basis and because the intake of feed and feed nutrients has been reduced to a per hen, per day basis both items will be referred to in terms of units per hen, per day.

REVIEW OF LITERATURE

Historical Background and Definitions in Regulation of Food Intake

First it must be understood what it is that is being regulated in regard to food intake. Grossman (1955) submitted that it is mainly the content of nutrients in the body. Under special circumstances, regulation of other factors, such as body-heat content or body-water content, may take precedence. Although all classes of nutrients are involved in this regulatory process, it is generally concluded that the energy-yielding nutrients play the most prominent role, thus making caloric balance an important consideration (Kennedy 1952).

The store of energy in the body of a healthy adult animal remains relatively constant over long periods. It follows that the rates of energy intake and expenditure are essentially equal. The regulatory process which tends to keep them equal involves hunger and appetite. "Hunger" as defined by Grossman (1955) is the complex of sensations evoked by depletion of body nutrient stores, and "appetite" is the desire for food, as an affective state. He selected the word "fullness" to designate the complex of sensations associated with repletion of body nutrient stores. "Satiety" is the corresponding affective state in repletion signifying a lack of desire to eat or, more precisely, a desire not to eat.

In a recent review article by Anand (1961), evidence was presented to show that before the beginning of the 20th Century, the subject of "hunger" was approached largely through speculation. Three theories were advanced to explain the origin of the sensation of hunger. The theory of "peripheral" origin held that the taking of food resulted from the stimulation either of all afferent nerves by some change in the tissues or of a strictly local group of sensory nerves, mainly in the stomach. The theory of "central" origin postulated a hunger center in the brain which, being sensitive to the depletion of its energy reserves, gave a warning signal to higher centers. The third theory considered hunger a sensation of "general" origin, with all organs, including the circulating blood and brain, participating in its perception. It was suggested that the hunger center in the brain would be stimulated by a starvation state of the blood as well as by afferent impulses from all organs of the body. More recent work tends to support the suggestions contained in this theory.

Following this period of speculation hunger, appetite, satiety and fullness were regarded as problems in the domain of the physiology of digestion. However, in the last 20 years, they have been studied more and more commonly as functions of the central nervous system (Grossman, 1963). These latter studies will be summarized in the remainder of this review.

Regions of the Central Nervous System Involved in Regulation

Anand (1961) has provided a good exposition of modern concepts of hunger, viewing it as a problem in the nervous regulation of food intake. Centers in the brain facilitate or inhibit reflexes that comprise feeding behavior. The principal centers are in the hypothalamus where there is a feeding center located laterally and a satiety center located medially.

Studies with discreetly located lesions of the hypothalamus of rats provided data which were interpreted by Morgane (1961 a and b) to indicate that the lateral hypothalamic area can be fractionated into a more medial component, important in motivation to eat, and far-lateral elements, destruction of which produces not only irreversible aphagia but also a metabolic disturbance interfering with the use of food.

There is some experimental evidence that the cerebral structures of the frontal and temporal lobes included in the "limbic system" may influence food intake (Anand, et al., 1958; Morgane , 1961a). The results of these studies were used to imply that both "facilitation" and "inhibition" of feeding behavior may arise from the limbic level. They also suggest other interesting conclusions. For example, Anand, et al. (1958) noted that changes in food intake after limbic lesions were more marked in monkeys than in cats, while neither these workers nor Brobeck (1948) could find any change in food intake in rats after amygdaloid lesions. These observations suggest a process of "encephalization" in higher animals even at the limbic level. Kennedy (1952) concluded that limbic structures in the frontal and temporal lobes modify food intake through a discriminating mechanism. This he termed "appetite," while the primitive urges of "hunger" and "satiety" he attributed to the hypothalamic level.

The highest level of the brain, the neocortex, is undoubtedly involved in feeding responses. Perhaps it is responsible for the more elaborate phenomena of feeding behavior; for habits, prejudice, and other complex integrations affecting energy exchange; or for selection or preferences among the variables. Experimental data relating to this subject are limited; most of the pertinent observations have been psychological. In a recent study Anand, et al. (1961a) created bilateral lesions in some neocortical regions in cats and monkeys and studied their effects on food intake. They found no experimentally demonstrable quantitative regulation of food intake from neocortical regions. At the same time, it is recognized that feeding behavior in man can be greatly influenced in a conscious, volitional manner.

Enough evidence is available pertaining to the central nervous mechanisms regulating food intake so that they can be considered as being similar to the regulatory mechanisms for other autonomic and visceral activities, such as the regulation of blood pressure, pulmonary ventilation, gastrointestinal activity, and body temperature. Feeding behavior is probably based upon reflex mechanisms of the spinal cord and brain stem, which are facilitated or inhibited by the hypothalamic mechanisms, and further regulation comes from the higher cerebral, limbic, and neocortical regions.

Regulating System Signals

In any consideration of the individual factors which have been proposed as playing a role in regulation of food intake, it must be emphasized that no one hypothesis has proved to be entirely satisfactory. On the basis of existing evidence, it would seem unwise to designate a single specific factor as solely responsible. A multiple factor theory of regulation appears to be most reasonable.

Grossman (1960) and Anand (1961) have summarized the information available from other research workers on this subject. The following six mechanisms have been proposed as signals for the central nervous system. They are: (1) the "thermostatic" hypothesis of Strominger and Brobeck (1953), (2) the "glucostatic" hypothesis of Mayer (1955),

(3) the "lipostatic" hypothesis of Kennedy (1952), (4) the concentration of serum amino acids (Mellinkoff et al., 1956), (5) distention of the digestive tract (Janowitz and Grossman, 1949, 1951), and (6) the water concentration of the body (Adolph, 1947).

Thermostatic Regulation

Strominger and Brobeck (1953) concluded that the day to day regulation of food intake is determined by the "specific dynamic action" of the ration and not by energy expenditure. There are several circumstances in which the amounts of food eaten are not related to energy expenditure. One such circumstance is the case when animals are placed in a hot environment. Therefore, they believe that the important factor in the regulation of food intake is not its energy value, but the amount of extra heat released in its assimilation. This extra heat then signals the hypothalamic mechanism and thus adjusts the total quantity of food eaten.

These workers have tried to prove the validity of this hypothesis in a number of ways. They have tried to correlate the satiety value of food with its overall "specific dynamic action." After measuring such variables as caloric intake, protein intake, fat intake, food weight, food volume and the estimated "specific dynamic action," they found that of all the variables measured the estimated "specific dynamic action" was most highly correlated with satiety value on the first day of changed dietary composition. In support of these observations, it was found that food intake (when measured at different environmental temperatures) was higher in a cold than in a warm environment. At temperatures which produced a slight fever, the animals ate practically nothing.

Energy obtained from food is utilized by animals to do work, to increase body stores of carbohydrate, protein and fat and to maintain body temperature. Strominger and Brobeck (1953) suggest that heat production is common to all of these avenues of energy expenditure. It is, therefore, reasonable to assume that all three factors are integrated in such a way that none is allowed to vary independently of the others.

Kennedy (1952) has presented several arguments against the hypothesis of thermostatic regulation of food intake. According to this hypothesis, diets rich in calories but low in "specific dynamic action" should cause obesity. However, excessive consumption of such diets when fed for more than one day is only transient. This theory also fails to explain how the hypothalamic receptors could distinguish between the heat released from the "specific dynamic action" of a meal and the far greater amount of heat released during muscular exercise. Instead of being interpreted as a signal to eat more, the metabolism of exercise should satisfy hunger. It was also shown that in longer experiments in which heat stress and pyrexia were avoided, rats lost some weight during acclimatization, following exposure to either heat or cold. Therefore, the decreased food intake under these conditions was not considered to be related to the prevention of hyperthermia. The marked loss of weight and refusal of food in pyrexial rats exposed to high temperature is thought by Kennedy (1952) to be due to circulating metabolites, produced by tissue breakdown associated with fever.

It would appear that although the heat stress and the "specific dynamic action" of the ration may have some effect on the immediate regulation of further food intake, this cannot be the only regulating mechanism. It probably has little, if any, effect on the long-term regulation.

Glucostatic Regulation

For short-term regulation of energy exchange Mayer (1953, 1955) has proposed the "glucostatic" theory, which postulates that "glucoreceptors" in the central nervous system (probably the hypothalamus) are sensitive to the rate at which glucose is being utilized by these "glucoreceptors." Low utilization rates excite neural activity leading to hunger sensations and food-taking. High utilization rates produce the opposite effect. Mayer used arteriovenous glucose (A-V) differences as an index of utilization rate and, for the majority of his experiments, peripheral A-V differences served as the index of rate of utilization by the glucoreceptors in the central nervous system.

A variety of types of evidence have a bearing on the glucostatic hypothesis; however, only a few selected references will be presented here. Mayer argues that the existence of glucoreceptors in the central nervous system has been demonstrated in an indirect way in connection with other physiological phenomena. He demonstrated that vagotomy abolished the normal gastric response to hypoglycemia. On the other hand, hyperglycemia was produced in an isolated dog's head, which was connected to the dog's body only through the nerve supply. As a result hypoglycemia was produced in the dog's body. In addition, he showed that in normal and diabetic animals, and in animals subjected to various hormonal treatments, decreased glucose availability or utilization correlated well with increased food intake. A good correlation between decreased liver glycogen and feeding behavior was also observed. On the other hand, Janowitz and Grossman (1951) have observed in dogs that production of hyperglycemia did not decrease food intake. After glucose infusions, the slight depression of food intake that resulted was no greater than that which occurred with control injections of saline.

Other evidence in favor of the glucostatic mechanism has been provided by Marshall et al. (1955) and Debons et al. (1962) in experiments on the action of goldthioglucose. The hypothalami of goldthioglucose-injected mice show definite lesions within one to three days, including edema, pyknosis, and degeneration of nerve cells in the ventromedial area of the hypothalamus. These cells are selectively poisoned by the gold which is linked to the glucose. A single injection of this chemical into mice induces permanent overeating and obesity. The same dose of goldthioglucose in the rat produces cell degeneration in the ventromedial nucleus, but gold is so toxic to the rat that the animal does not survive long enough to show hyperphagia. The use of gold linked by a sulphur bridge to compounds other than glucose does not cause destruction of the satiety center and overeating. These compounds may be very similar to glucose, like gold thiosorbitol, or may be one of the derivatives of normal intermediaries of other pathways of metabolism, like goldthiomalate, goldthiocaproic acid, and gold thioglycerol.

The experimental evidence definitely establishes an important role of blood glucose level and glucose utilization in the regulation of the activity of hypothalamic centers. The glucoreceptor mechanism is believed to be located in the satiety centers, since alterations in their electrical activity are more pronounced than in feeding centers when blood glucose content is changed (Anand et al., 1961b). The feeding centers may be influenced indirectly by the activity of satiety centers, or there may be a direct influence on them too. The evidence presented does not exclude the possibility of the presence of other mechanisms for controlling the hypothalamic centers.

Lipostatic Regulation

Kennedy (1952) suggested that the hypothalamic satiety mechanism is concerned only in the prevention of an over-all surplus of energy intake over expenditure, which would cause the deposition of fat in depots. The simplest mechanism in which lipostasis could be achieved would be through sensitivity of hypothalamic regions to varying concentrations of circulating metabolites. The amount of fat in depots could conceivably influence the level of these blood metabolites. Kennedy points out that his findings are more compatible with the hypothesis of Mayer (1953) than that of Brobeck (1948). The fact that wide variations in the chemical composition of the diet are without effect on the caloric intake, unless palatability is altered, suggests to him that control of intake is influenced by a whole complex of metabolites in the blood stream rather than glucose alone, as Mayer has suggested.

Mayer (1955) contends that although the short-term regulation is "glucostatic" the long-term regulation of body reserves is "lipostatic." The latter idea is based on the fact that animals mobilize each day a quantity of fat proportional to the total fat content of the body. It has been observed that the amount of endogenous fat mobilized daily in *ad libitum* feeding conditions is proportional, for each type of animal, to the size of the fat depot.

Regulation by Protein and Serum Amino Acids

Mellinkoff *et al.* (1956) correlated appetite with serum amino acids and blood sugar concentrations in normal human subjects given hydrolyzed protein and glucose. They suggested that a reciprocal relationship exists between the serum amino acid concentrations and appetite. However, Anand et al. (1961b) did not find any change in the electrical activity of the hypothalamic centers in animals after intravenous transfusions of protein hydrolysates.

Additional support for the idea that protein plays some role in the regulation of food intake was reported by Gleaves (1961). In an experiment with laying hens, he found that feed consumption increased significantly as protein intake increased. When protein-depleted rats were fed balanced or imbalanced diets, Sanahwja and Harper (1962) found that feed consumption was equal for three days. After this time both growth rate and food intake of those fed the imbalanced diet dropped. When protein-depleted rats were fed the balanced or imbalanced diet, together with a protein-free diet, neither group ate the protein-free diet during the first three days. Thereafter, animals fed the imbalanced diet began to eat the protein-free diet in preference to the imbalanced diet, even though the latter would support growth and the former would not. Animals fed the balanced diet ad libitum or the imbalanced diet plus histidine did not eat the protein-free diet at all. These observations indicate that both food intake and food selection are influenced by the protein content as well as by the amino acid pattern of the diet.

Gastrointestinal Tract in Regulation of Food Intake

Janowitz and Grossman (1949) and Grossman (1955) have done a number of experiments to elucidate the role played by the upper gastrointestinal tract in producing satiety after a meal. An important factor in bringing about this state is gastric distension. In sham feeding experiments with dogs which had undergone esophogastomy so that the food passed out through esophageal fistulas, the duration of eating was repeated at short intervals. As such dogs ate after intragastric feeding, the duration and frequency of eating were inversely related to the size of the intragastric feeding. Inert bulk in the stomach was as effective as food in producing inhibition of eating. This shows that the signals for satiety mechanisms result from gastric distension. After food leaves the stomach it does not produce further inhibition.

Oropharyngeal regions meter the volume of food eaten to some extent. Dogs which had been prefed showed a greater reduction in voluntary food intake than animals into whose stomachs amounts of food equal to prefeeding had been placed directly. Thus, satiety is brought about in some degree by stimulation of oropharyngeal receptors associated with tasting, chewing and swallowing. In sham feeding experiments, however, in which food fails to reach the stomach, such animals eat far greater quantities of food than the intact ones. Thus, the oropharyngeal factor is relatively ineffective when it is not associated with the entry of food into the stomach.

Since animals with denervated gastrointestinal tracts show normal regulation of food intake, Grossman assumes that the gastric distension mechanism is dispensable. Another piece of evidence against a principal role of the stomach in the control of hunger was observed by Adolph (1947). If their food is diluted with inert material, animals quickly adjusted for the decreased caloric content per unit volume by consuming more of the diet. However, this work also demonstrated that in the presence of roughages, a compromise was effected between an excessive amount of alimentary fill and a diminished amount of nutrients.

Sharma _et al._ (1961) have studied the effect on hypothalamic centers of gastric distension produced by balloons. Inflation of an intragastric balloon with water or air leads to an increase in the electroencephalographically recorded activity of the satiety center. No change was observed in the activity of the feeding center or other hypothalamic areas. This emphasizes the role played by gastric distension in bringing about satiety through activation of the satiety centers.

From various experimental studies, it seems clear that sensations from the digestive tract, as well as metabolic changes occurring in the body, have a role in the short-term regulation of food intake mediated through the central nervous mechanisms. The oropharyngeal component and gastric distension contribute to bringing about satiety.

Correlation of Water and Food Intake

Adolph (1947) stated that regulation of food intake appears to be correlated with regulation of water exchange; the higher the water concentration of the diet, the greater the food intake. Animals given no water ate little or no dry food, while those given no food drank little or no water. Cizek (1961) also observed quantitative relationships between food and water intake, providing that the consumption of the diet was constant.

Anand (1961) quoting other workers stated that "it has been uniformly noted that lateral hypothalamic lesions not only lead to complete aphagia but also complete adipsia." In rats with such lesions, some animals after intubation with 10 ml. of water daily, or with a fluid diet, ultimately recover spontaneous drinking and eating behavior. At first they drink water or a special fluid diet and after a few days begin to eat solid food. Mayer (1955) thinks that adipsia is the dominant effect in these animals, and, following recovery from this adipsia, eating is resumed.

Studies in rats have been carried out by Anand (1961) to determine whether changes in food intake and water intake observed after hypothalamic lesions are interdependent or independent. Small, bilaterally symmetrical lesions spread over different regions of the hypothalamic "feeding center" resulted in complete adipsia in addition to complete aphagia. Lesions adjacent to this region regularly produced hypodipsia, regardless of increased or normal food intake, respectively. Lesions further removed from this region did not significantly change water intake, even when food intake was increased as a result of medial lesions. Based upon these experiments it would appear that the hypothalamic mechanisms controlling water intake and food intake, although physically situated in the same regions, act separately and independently.

An interesting experiment by Lepkovsky <u>et al.</u> (1960) on food intake, water intake and body water regulation of chickens, tends to support the evidence presented by Anand and to show why, in chickens, food and water intake are even more apt to be independent than in other animals. This experiment showed that feeding chickens with or without water did not greatly influence their food intake. The independence of food and water intake were attributed, at least in part, to the fact that the crop of the chicken was able to adjust its water content to water supply. Apparently the crop acts as a reservoir from which the body can withdraw water at times when water intake is low. There was more water in the crop content of chickens fed with water than in the crop content of chickens fed without water.

Indications That These or Similar Physiological Mechanisms Act in the Regulation of Food Intake in Poultry

The existence and action of the physiological mechanisms previously described, except the correlation of water and food intake, have been based on experiments with animals other than poultry. Therefore, it is desirable at this point to present evidence which indicates that the same or similar food consumption regulatory mechanisms are present and active in domestic poultry.

Evidence that the "thermostatic" mechanism is active in the regulation of feed consumption in poultry has been demonstrated by Heywang (1952), Thayer and Brooks (1956), Campos et al. (1960), Ascarelli and Bartov (1963) and others. These experiments were conducted with growing chickens and with laying hens. Even though they were not designed specifically to test the action of thermostatic regulation, the reported results were all similar in nature. It was found that as ambient temperature increased there was a subsequent decrease in feed consumption.

Indirect evidence for the presence and action of the "glucostatic" and "lipostatic" mechanisms comes from research reported by Scott et al. (1947), Hill et al. (1956), Berg and Bearse (1956), Berg et al. (1956), Anderson et al. (1957), Bolton (1958), Petersen et al. (1960), Gleaves (1961) and others. The experimental animals were either growing chickens or laying hens. In these experiments, increases in dietary energy, regardless of the source, caused a concomitant drop in feed weight consumption.

Distension of the digestive tract has been shown to be a factor which affects feed consumption in chickens. A series of experiments with normal as well as with cropectomized chicks were conducted by Fisher and Weiss

(1956) to study the effect of fiber *per se* on feed consumption. This work indicated that fiber *per se* was an important factor which influenced feed consumption independently of the energy level of the diet. Fiber, up to a given dietary level, stimulated feed consumption; but beyond that level, feed consumption remained relatively constant. Couch and Isaacks (1957) were successful in restricting the protein and energy intake in growing pullets by substituting 18.2 percent of oat hulls for an equivalent amount of milo. These workers concluded that while the fibrous bulk was restricting the total nutrient intake of the pullets, the inherent reduction of dietary energy level which accompanied the substitution of oat hulls for milo was causing an increase in feed consumption.

Gleaves *et al.* (1963a) designed an experiment to regulate the nutrient consumption of laying hens under *ad libitum* feeding conditions. The basic idea behind the experiment was to determine if dietary volume might be used to control the intake of nutrients. Although the effects of graded levels of dietary energy and protein upon feed consumption were not completely counteracted with manipulations of dietary volume, definite gradations in the intake of protein and energy were obtained. The results of this experiment indicated that dietary volume could be used to regulate protein and energy consumption, within reasonable limits, once enough information was available about the specific effects of these factors singly and in combination.

The previous experiment demonstrated that quantitative estimates of the effects of dietary protein, energy, weight and volume upon feed consumption were needed before these factors could be used successfully to regulate the nutrient consumption of laying hens. The need for this information led to the experiment to be reported in this dissertation.

In addition to the evidence which has been presented supporting the action of the hypothalamic mechanisms in chickens, there are some indications that higher centers of the central nervous system are present and active. Factors mediating food and liquid intake in chickens were studied by Jacobs and Scott (1957). It was concluded that under the conditions of their experiments the chicken could discriminate among sucrose solutions, saccharine solutions and water. The chickens preferred sucrose solution and avoided saccharine solutions and water. This preference for sucrose was not shown to be related to its caloric value. The presence of sucrose in the drinking water did not produce any measurable effect on rate of weight increase or amount of food intake. Kare *et al*. (1957) presented data that showed the chick to have a sense of taste. The response to a variety of sweet and bitter flavors suggested that the broad classifications of taste recognized by man were not applicable to the fowl, but that the sense of taste in the fowl was more than rudimentary.

EXPERIMENTAL PROCEDURE

Commercial hybrid laying hens approximately 22 weeks of age were housed in a windowless house in individual wire cages. Environmental conditions were partially controlled within the cage house throughout the experiment. Temperature varied from a low of 60 degrees Fahrenheit to a high of approximately 90 degrees Fahrenheit. Artificial light was supplied by incandescent lamps which were controlled with automatic time clocks. The hens were given 14 hours of continuous light and 10 consecutive hours of darkness per day. Each cage was equipped with a waterer, a feeder and a feed storage container. This individual hen treatment permitted each hen to be used as an experimental unit.

The hens were fed diets composed of all (81) combinations of three levels of dietary protein (13, 16 and 19 grams), three levels of dietary metabolizable energy (260, 300 and 340 Calories), three levels of dietary weight (127, 137 and 147 grams) and three levels of dietary volume (180, 230 and 280 milliliters). Hereafter, these levels will often be referred to as 1, 2 or 3 for each dietary factor, with number 1 always being the lowest dietary level and number 3 always being the highest. This factorial arrangement of treatments is presented in Table I. The nutrient composition of ingredients and the method of formulation were taken from the Poultry Nutrition Manual by Gleaves _et al_. (1963b). Ingredient volume measurements were taken from Gleaves (1961).

In order to maintain identical amino acid ratios throughout all 81

TABLE I

FACTORIAL ARRANGEMENT OF TREATMENTS

		Protein (Grams) 13			Protein (Grams) 16			Protein (Grams) 19		
		Milliliters			Milliliters			Milliliters		
Metabolizable Energy (Kilocalories)	Weight (Grams)	180	230	280	180	230	280	180	230	280
260	127	1* 1111**	4 1112	7 1113	28 2111	31 2112	34 2113	55 3111	58 3112	61 3113
	137	2 1121	5 1122	8 1123	29 2121	32 2122	35 2123	56 3121	59 3122	62 3123
	147	3 1131	6 1132	9 1133	30 2131	33 2132	36 2133	57 3131	60 3132	63 3133
300	127	10 1211	13 1212	16 1213	37 2211	40 2212	43 2213	64 3211	67 3212	70 3213
	137	11 1221	14 1222	17 1223	38 2221	41 2222	44 2223	65 3221	68 3222	71 3223
	147	12 1231	15 1232	18 1233	39 2231	42 2232	45 2233	66 3231	69 3232	72 3233
340	127	19 1311	22 1312	25 1313	46 2311	49 2312	52 2313	73 3311	76 3312	79 3313
	137	20 1321	23 1322	26 1323	47 2321	50 2322	53 2323	74 3321	77 3322	80 3323
	147	21 1331	24 1332	27 1333	48 2331	51 2332	54 2333	75 3331	78 3332	81 3333

*The number in the upper left hand corner of each square represents the diet number.

**The four numbers in the center of the square represent the dietary level combinations of protein, energy, weight and volume, respectively.

rations and to obtain the desired levels of protein and energy, it was necessary to use washed blow sand and polyethylene fluff, which are nutritionally inert, to adjust weight and volume of the experimental rations. The protein basal and the amino acid profile of the protein basal are presented in Tables II and III, respectively. The composition of the 81 experimental rations are presented in Table IV. In Table V is listed the composition of the vitamin-mineral concentrate used in these rations. Seven hens were randomly assigned to each of the rations, giving a completely randomized design with a 3^4 factorial arrangement of treatments.

The experiment began on March 21, 1963 and ended on October 31, 1963. Egg production and mortality were recorded daily. Eggs were individually weighed three days each week. The average egg weight obtained in this manner was used as an estimate of the average weight of all eggs produced during that week. Individual body weight and feed consumption data were collected and recorded every 28 days. During the course of the experiment, data were collected for eight 28-day periods; however, due to the fact that an adjustment period is necessary for the type of experimental diets fed (Gleaves <u>et al.</u>, 1963a), only the last seven periods will be reported.

The egg production, egg weight, body weight, feed consumption and mortality data were punched onto IBM cards at the end of each experimental period. IBM electronic computing equipment was utilized to make all summary and statistical computations. A summary was made of the following variables for each replicate and for each treatment:

(1) daily feed weight consumption,
(2) average daily volume of feed consumed,

TABLE II

PROTEIN BASAL

Ingredients	Grams
Ground corn	0.21888
Ground milo	0.21888
Oat mill feed	0.04858
Alfalfa meal (17% protein)	0.03337
Fish meal (herring, 70% protein)	0.09561
Soybean oil meal (50% protein)	0.21421
Blood meal (84% protein)	0.06777
Gelatin (95% protein)	0.03337
Dried whey	0.03337
Dried condensed fermented corn extractives	0.03337
dl-Methionine	0.00259
Total	1.0000

TABLE III

AMINO ACID PROFILE OF THE PROTEIN BASAL

Amino Acids	Gm. a.a./gm. prot.
Arginine	0.080
Histidine	0.037
Lysine	0.084
Tyrosine	0.037
Tryptophan	0.013
Phenylalanine	0.060
Cystine	0.017
Methionine	0.032
Serine	0.067
Threonine	0.052
Leucine	0.122
Isoleucine	0.050
Valine	0.081
Glutamic acid	0.168
Aspartic acid	0.120
Glycine	0.081
Alanine	0.073
Proline	0.084

TABLE IV

COMPOSITIONS OF THE RATIONS FOR THE 81 TREATMENTS

Treatment Number	Protein Basal Gram	Tallow Gram	Starch Gram	Sand Gram	Polyethylene Fluff Gram	Di-Ca Phos. Gram	Ca Carb. Gram	Salt Gram	VMC-60* Gram	Total Gram
1	39.60	5.00	29.85	32.44	9.91	5.30	3.50	0.60	0.80	127.00
2	39.60	5.00	29.85	44.03	8.32	5.30	3.50	0.60	0.80	137.00
3	39.60	5.00	29.85	55.61	6.74	5.30	3.50	0.60	0.80	147.00
4	39.60	5.00	29.85	19.60	22.75	5.30	3.50	0.60	0.80	127.00
5	39.60	5.00	29.85	31.19	21.16	5.30	3.50	0.60	0.80	137.00
6	39.60	5.00	29.85	42.77	19.58	5.30	3.50	0.60	0.80	147.00
7	39.60	5.00	29.85	6.76	35.59	5.30	3.50	0.60	0.80	127.00
8	39.60	5.00	29.85	18.34	34.01	5.30	3.50	0.60	0.80	137.00
9	39.60	5.00	29.85	29.93	32.42	5.30	3.50	0.60	0.80	147.00
10	39.60	10.00	30.36	27.67	9.17	5.30	3.50	0.60	0.80	127.00
11	39.60	10.00	30.36	39.25	7.59	5.30	3.50	0.60	0.80	137.00
12	39.60	10.00	30.36	50.84	6.00	5.30	3.50	0.60	0.80	147.00
13	39.60	10.00	30.36	14.83	22.01	5.30	3.50	0.60	0.80	127.00
14	39.60	10.00	30.36	26.41	20.43	5.30	3.50	0.60	0.80	137.00
15	39.60	10.00	30.36	38.00	18.84	5.30	3.50	0.60	0.80	147.00
16	39.60	10.00	30.36	1.99	34.85	5.30	3.50	0.60	0.80	127.00
17	39.60	10.00	30.36	13.57	33.27	5.30	3.50	0.60	0.80	137.00
18	39.60	10.00	30.36	25.16	31.68	5.30	3.50	0.60	0.80	147.00
19	39.60	21.55	19.85	26.17	9.63	5.30	3.50	0.60	0.80	127.00
20	39.60	21.55	19.85	37.75	8.05	5.30	3.50	0.60	0.80	137.00
21	39.60	21.55	19.58	49.33	6.47	5.30	3.50	0.60	0.80	147.00
22	39.60	21.55	19.85	13.33	22.47	5.30	3.50	0.60	0.80	127.00
23	39.60	21.55	19.85	24.91	20.89	5.30	3.50	0.60	0.80	137.00
24	39.60	21.55	19.85	36.49	19.31	5.30	3.50	0.60	0.80	147.00
25	39.60	21.55	19.85	-----	35.92	5.30	3.50	0.60	0.80	127.00
26	39.60	21.55	19.85	12.07	33.73	5.30	3.50	0.60	0.80	137.00
27	39.60	21.55	19.85	23.65	32.15	5.30	3.50	0.60	0.80	147.00

TABLE IV (CONTINUED)

Treatment Number	Protein Basal	Tallow	Starch	Sand	Polyethylene Fluff	Di-Ca Phos.	Ca Carb.	Salt	VMC-60*	Total
	Gram	Gram	Gram	Gram	Gram	Gram	Gram	Gram	Gram	Gram
28	48.72	5.00	23.10	31.19	8.93	5.15	3.51	0.60	0.80	127.00
29	48.72	5.00	23.10	42.77	7.35	5.15	3.51	0.60	0.80	137.00
30	48.72	5.00	23.10	54.35	5.77	5.15	3.51	0.60	0.80	147.00
31	48.72	5.00	23.10	18.35	21.77	5.15	3.51	0.60	0.80	127.00
32	48.72	5.00	23.10	29.93	20.19	5.15	3.51	0.60	0.80	137.00
33	48.72	5.00	23.10	41.51	18.61	5.15	3.51	0.60	0.80	147.00
34	48.72	5.00	23.10	5.51	34.61	5.15	3.51	0.60	0.80	127.00
35	48.72	5.00	23.10	17.09	33.03	5.15	3.51	0.60	0.80	137.00
36	48.72	5.00	23.10	28.67	31.45	5.15	3.51	0.60	0.80	147.00
37	48.72	11.60	21.25	26.95	8.42	5.15	3.51	0.60	0.80	127.00
38	48.72	11.60	21.25	38.53	6.84	5.15	3.51	0.60	0.80	137.00
39	48.72	11.60	21.25	50.11	5.26	5.15	3.51	0.60	0.80	147.00
40	48.72	11.60	21.25	14.11	21.26	5.15	3.51	0.60	0.80	127.00
41	48.72	11.60	21.25	25.69	19.68	5.15	3.51	0.60	0.80	137.00
42	48.72	11.60	21.25	37.27	18.10	5.15	3.51	0.60	0.80	147.00
43	48.72	11.60	21.25	1.27	34.10	5.15	3.51	0.60	0.80	127.00
44	48.72	11.60	21.25	12.85	32.52	5.15	3.51	0.60	0.80	137.00
45	48.72	11.60	21.25	24.43	30.94	5.15	3.51	0.60	0.80	147.00
46	48.72	23.60	9.25	26.27	9.10	5.15	3.51	0.60	0.80	127.00
47	48.72	23.60	9.25	37.85	7.52	5.15	3.51	0.60	0.80	137.00
48	48.72	23.60	9.25	49.43	5.94	5.15	3.51	0.60	0.80	147.00
49	48.72	23.60	9.25	13.43	21.94	5.15	3.51	0.60	0.80	127.00
50	48.72	23.60	9.25	25.01	20.36	5.15	3.51	0.60	0.80	137.00
51	48.72	23.60	9.25	36.59	18.78	5.15	3.51	0.60	0.80	147.00
52	48.72	23.60	9.25	-----	35.36	5.15	3.51	0.60	0.80	127.00
53	48.72	23.60	9.25	12.17	33.20	5.15	3.51	0.60	0.80	137.00
54	48.72	23.60	9.25	23.75	31.62	5.15	3.51	0.60	0.80	147.00

TABLE IV (CONTINUED)

Treatment Number	Protein Basal Gram	Tallow Gram	Starch Gram	Sand Gram	Polyethylene Fluff Gram	Di-Ca Phos. Gram	Ca Carb. Gram	Salt Gram	VMC-60* Gram	Total Gram
55	57.84	5.00	16.26	30.02	7.98	5.00	3.50	0.60	0.80	127.00
56	57.84	5.00	16.26	41.60	6.40	5.00	3.50	0.60	0.80	137.00
57	57.84	5.00	16.26	53.18	4.82	5.00	3.50	0.60	0.80	147.00
58	57.84	5.00	16.26	17.18	20.82	5.00	3.50	0.60	0.80	127.00
59	57.84	5.00	16.26	28.76	19.24	5.00	3.50	0.60	0.80	137.00
60	57.84	5.00	16.26	40.34	17.66	5.00	3.50	0.60	0.80	147.00
61	57.84	5.00	16.26	4.34	33.66	5.00	3.50	0.60	0.80	127.00
62	57.84	5.00	16.26	15.92	32.08	5.00	3.50	0.60	0.80	137.00
63	57.84	5.00	16.26	27.50	30.50	5.00	3.50	0.60	0.80	147.00
64	57.84	12.88	12.00	26.62	7.76	5.00	3.50	0.60	0.80	127.00
65	57.84	12.88	12.00	38.21	6.17	5.00	3.50	0.60	0.80	137.00
66	57.84	12.88	12.00	49.79	4.59	5.00	3.50	0.60	0.80	147.00
67	57.84	12.88	12.00	13.78	20.60	5.00	3.50	0.60	0.80	127.00
68	57.84	12.88	12.00	25.37	19.10	5.00	3.50	0.60	0.80	137.00
69	57.84	12.88	12.00	36.95	17.43	5.00	3.50	0.60	0.80	147.00
70	57.84	12.88	12.00	0.94	33.44	5.00	3.50	0.60	0.80	127.00
71	57.84	12.88	12.00	12.52	31.86	5.00	3.50	0.60	0.80	137.00
72	57.84	12.88	12.00	24.11	30.27	5.00	3.50	0.60	0.80	147.00
73	57.84	24.88	-----	25.94	8.44	5.00	3.50	0.60	0.80	127.00
74	57.84	24.88	-----	37.53	6.85	5.00	3.50	0.60	0.80	137.00
75	57.84	24.88	-----	49.11	5.27	5.00	3.50	0.60	0.80	147.00
76	57.84	24.88	-----	13.10	21.28	5.00	3.50	0.60	0.80	127.00
77	57.84	24.88	-----	24.68	19.70	5.00	3.50	0.60	0.80	137.00
78	57.84	24.88	-----	36.27	18.11	5.00	3.50	0.60	0.80	147.00
79	57.84	24.88	-----	-----	34.73	5.00	3.50	0.60	0.80	127.00
80	57.84	24.88	-----	11.84	32.54	5.00	3.50	0.60	0.80	137.00
81	57.84	24.88	-----	23.43	30.95	5.00	3.50	0.60	0.80	147.00

*See Table V for the composition of the vitamin-mineral concentrate (VMC-60).

TABLE V

COMPOSITION OF VMC-60

Vitamins and Minerals	Units per Gm of Concentrate
Vitamin A	353.00 U.S.P.
Vitamin D_3	5.30 I.C.U.
Vitamin E	0.26 I.U.
Vitamin K	0.13 mg.
Vitamin B_{12}	0.35 mg.
Riboflavin	0.18 mcg.
Niacin	1.41 mg.
Pantothenic Acid	0.35 mg.
Choline Chloride	22.00 mg.
Manganese	1.22 mg.
Iodine	0.96 mg.
Cobalt	0.03 mg.
Iron	0.96 mg.
Copper	0.07 mg.
Zinc	1.00 mg.

(3) daily protein consumption,
(4) daily energy consumption,
(5) average number of eggs produced,
(6) average egg weight,
(7) total body weight gain or loss, and
(8) number of periods that any one hen was dead.

Analyses of variance for the factorial arrangement of treatments were performed on each of the eight responses listed above. A publication by Yates (1937) was used to aid in the interpretation of the results.

After the experiment was completed, an estimate of the effect of egg production and its interacting effects upon feed consumption were obtained by selecting two groups of hens with different levels of egg production. Two hens were selected from each treatment with a "high" egg production and two hens were selected from each treatment with a "low" egg production. At attempt was made to select hens that had laid 25 eggs per 28-day period for the "highs" and 15 eggs per hen, per 28-day period for the "lows." Selection of the "high" egg producing hens resulted in a mean egg production of 24.4 eggs per hen, per 28-day period with a standard error of only 0.082. However, selecting hens with a "low" egg production was more difficult due to the fact that in some treatments egg production never dropped, while in others it was extremely low. The mean for the "low" egg producers was 15.20 eggs per hen per 28-day period with a standard error of 0.225.

Once these birds had been selected, the same type of summaries and statistical analyses described previously were made on the "highs," "lows" and "combined" factorial arrangements. The "high" and "low" factorial arrangements were exactly the same as that shown in Table I, with the exception that in these analyses there were only two hens per treatment as compared to seven in the original factorial arrangement. The "combined" design also had only two hens per treatment, but a fifth factor was added (egg production), which made a 3^4x2 factorial. This arrangement of treatments is presented in Table VI.

TABLE VI

FACTORIAL ARRANGEMENT OF TREATMENTS WITH EGG PRODUCTION AS A FIFTH FACTOR

Metabolizable Energy (Kilocalories)	Weight (Grams)	Eggs	Protein (Grams)								
			13			16			19		
			Milliliters			Milliliters			Milliliters		
			180	230	280	180	230	280	180	230	280
260	127	Eggs* 1	11111**	11121	11131	21111	21121	21131	31111	31121	31131
260	127	2	11112	11122	11132	21112	21122	21132	31112	31122	31132
260	137	Eggs 1	11211	11221	11231	21211	21221	21231	31211	31221	31231
260	137	2	11212	11222	11232	21212	21222	21232	31212	31222	31232
260	147	Eggs 1	11311	11321	11331	21311	21321	21331	31311	31321	31331
260	147	2	11312	11322	11332	21312	21322	21332	31312	31322	31332
300	127	Eggs 1	12111	12121	12131	22111	22121	22131	32111	32121	32131
300	127	2	12112	12122	12132	22112	22122	22132	32112	32122	32132
300	137	Eggs 1	12211	12221	12231	22211	22221	22231	32211	32221	32231
300	137	2	12212	12222	12232	22212	22222	22232	32212	32222	32232
300	147	Eggs 1	12311	12321	12331	22311	22321	22331	32311	32321	32331
300	147	2	12312	12322	12332	22312	22322	22332	32312	32322	32332
340	127	Eggs 1	13111	13121	13131	23111	23121	23131	33111	33121	33131
340	127	2	13112	13122	13132	23112	23122	23132	33112	33122	33132
340	137	Eggs 1	13211	13221	13231	23211	23221	23231	33211	33221	33231
340	137	2	13212	13222	13232	23212	23222	23232	33212	33222	33232
340	147	Eggs 1	13311	13321	13331	23311	23321	23331	33311	33321	33331
340	147	2	13312	13322	13332	23312	23322	23332	33312	33322	33332

*Number 1 represents the average "low" egg production level of 15.2 eggs per hen, per 28-day period. Number 2 represents the average "high" egg production level of 24.4 eggs per hen, per 28-day period.

**The five digits in the number within the rectangle represent the dietary level combinations of protein, energy, weight, volume and the level of egg production, respectively.

RESULTS AND DISCUSSION OF ORIGINAL FACTORIAL ARRANGEMENT

Data for this experiment were collected for seven 28-day periods. However, the period-to-period trends were consistent with the accumulated averages for the entire seven periods; therefore, only the accumulated treatment means will be presented. These accumulated treatment means were computed on a hen-housed basis. Normally in an experiment of this type, a hen-day basis might be more desirable. This is especially true when mortality is a random consequence of the normal death loss that usually accompanies any experiment with laying hens. There were several factors that led to the decision to report the experiment on a hen-housed basis. These factors were: (1) the experiment was conducted over a relatively short span of time, seven 28-day periods as compared to a full year, (2) there were no disease outbreaks, (3) mortality was high, and (4) there was, during the course of the experiment and in the accumulated averages, a correlation of death loss with certain experimental diets. Evidence to support the fourth factor is presented in Table VII and will be discussed in more detail later.

Mortality

Mortality means are presented (Table VII) as the average number of periods dead per hen, per treatment. This was done in order to allow full credit to each treatment for all periods that the hens on that treatment were able to survive. A brief explanation of what these means represent is necessary for a complete understanding of their meaning.

TABLE VII

MORTALITY MEANS (AVE. NO. OF PERIODS DEAD PER HEN) FOR EACH TREATMENT

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13, Milliliters 180	13, 230	13, 280	16, Milliliters 180	16, 230	16, 280	19, Milliliters 180	19, 230	19, 280
260	127	0	0	1.43	0	0.43	0.71	0	0	0
	137	0	0	0.43	0	0.43	0.43	0.71	0	0
	147	0.57	0.29	0	0	0	0.57	0	0.57	0
300	127	1.43	0.86	0	0.43	0	0	0.29	0	1.00
	137	1.86	0.86	0.29	0	0.57	0	0	0.43	0.86
	147	0.71	0.86	1.00	0.71	0	0.43	0	0	0
340	127	0.86	2.57	2.29	2.14	1.71	0	0	1.00	0.43
	137	2.29	1.00	1.71	0	0	1.43	0	0.43	0
	147	1.57	1.43	1.29	0	0	0	0	0	0

An example is perhaps the easiest way to accomplish this. A zero means that all seven hens survived for all seven periods, while a one means that the original seven hens survived for an average of six out of the seven periods. Thus, the numbers are relative and a zero indicates better livability than a one, and a one indicates better livability than a two.

An analysis of variance (Table VIII) was performed on the original data from which the means in Table VII were computed. This analysis gave the final evidence needed to make the decision to report all treatment means on a hen-housed basis. It verifies the fact that mortality was due, at least for the most part, to the treatment that the hens received. The main effects of protein, energy, and weight upon mortality were all statistically significant. The linear protein and energy effects were significant at the one percent level of probability, and the linear effect due to weight was significant at the five percent level. The only interaction effect that was significant was protein x energy ($P<0.01$).

In a consideration of the analyses of variance computed from the data of this experiment, since a fixed model was employed, the relative sizes of the mean squares are meaningful. Even though the mean squares contain a component of variance due to the error term, it is a constant in every case. Thus, in a relative sense, the mean squares represent an estimate of variance for each variable tested. The estimate of variance due to the effect of protein was much larger than that due either to energy, weight or protein x energy interaction. This is the first indication that protein is the most important single factor involved in mortality rate.

The significant main effect means are presented in Table IX. An inspection of these means reveals that as dietary protein was increased

TABLE VIII

ANALYSIS OF VARIANCE OF MORTALITY DATA
(NO. OF PERIODS DEAD PER HEN)

Source of Variation	df	SS	MS	F
Total (Corrected)	566	1,059.70	1.87	
Treatment	[80]			
Protein (P)	(2)			
P_{Linear} (L)	1	51.75	51.75	30.99**
$P_{Quadratic}$ (Q)	1	0.87	0.87	0.52
Energy (E)	(2)			
E_L	1	31.79	31.79	19.04**
E_Q	1	0.19	0.19	0.11
Weight (W)	(2)			
W_L	1	7.41	7.41	4.44*
W_Q	1	0.02	0.02	0.01
Volume (V)	(2)			
V_L	1	0.09	0.09	0.05
V_Q	1	0.02	0.02	0.01
Interactions	(72)			
P x E	4	32.40	8.10	4.85*
P x W	4	1.46	0.37	0.22
P x V	4	1.73	0.43	0.26
E x W	4	11.14	2.79	1.67
E x V	4	4.68	1.17	0.70
W x V	4	1.64	0.41	0.24
P x E x W	8	8.67	1.08	0.65
P x E x V	8	17.13	2.14	1.28
P x W x V	8	17.94	2.24	1.34
E x W x V	8	16.32	2.04	1.22
P x E x W x V	16	36.73	2.30	1.38
Error	486	813.71	1.67	

*Significant at the five percent level of probability

**Significant at the one percent level of probability

there was a linear drop in mortality rate. Just the reverse is true in the case of dietary energy. As dietary energy increased there was a linear increase in mortality. Surprisingly, the trend due to the effect of dietary weight is the reverse of what might be expected. As dietary weight was increased there was a linear decrease in mortality. With the data that are presently available, the effect due to dietary weight is unexplainable and it might be considered as one of those "one in one hundred" occurrences that has no real meaning. However, as will be shown, the effect of dietary weight upon some of the other dependent variables was unexpected. Therefore, it is the opinion of the author that this effect is real even though an explanation cannot be presented.

TABLE IX

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY AND WEIGHT UPON MORTALITY

Factor	Level*		
	1	2	3
Protein	0.95	0.37	0.21
Energy	0.24	0.47	0.82
Weight	0.65	0.51	0.37

*Protein levels 1, 2 and 3 represent 13, 16, and 19 grams of protein, respectively. Energy levels 1, 2 and 3 represent 260, 300 and 340 Calories of metabolizable energy, respectively. Weight levels 1, 2 and 3 represent 127, 137 and 147 grams of feed, respectively.

Since the protein x energy interaction effect upon mortality was significant, care must be taken in the interpretation of the significant main effects due to these two factors. From the means for the protein x energy interaction effect (Table X), it can be seen that at each of the three levels of dietary energy (260, 300 and 340 Calories) there was, in general, a linear decrease in mortality as dietary protein was increased. However, the effects of these dietary energy levels when fed in combination with each of the three levels of protein (13, 16 and 19 grams) did not follow a pattern that is as consistent. At the low level of protein there was a linear increase in mortality as dietary energy was increased. The effects of dietary energy upon mortality of hens fed the second level of protein were more nearly quadratic in nature, with the least mortality occurring among those hens fed 300 Calories of metabolizable energy. Again the effects of the three levels of energy fed in combination with the third level of protein were quadratic, but this time the greatest mortality was with the 300 Calorie diets.

TABLE X

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY UPON MORTALITY

Energy Level	Protein Level*		
	1	2	3
1	0.30	0.29	0.14
2	0.87	0.24	0.29
3	1.67	0.59	0.21

*See Table IX footnote for the dietary equivalents of levels 1, 2 and 3 for protein and energy.

Thus, the inconsistency of the effects of dietary energy upon mortality, when fed in combination with each of the three levels of dietary protein, and the relatively consistent effects of protein on mortality regardless of energy level, lead to the conclusion that the linear main effects of protein are more interpretable than the linear main effects of energy. The failure of dietary energy to act the same in combination with each of the three levels of protein is the reason that the protein x energy interaction effect was significant.

These same data are presented graphically in Figure 1, which more clearly depicts the combined effects of protein and energy upon mortality. The predominant effect of protein level upon mortality is again evident. As dietary protein level was increased, the effect of energy level on mortality became less. Livability was relatively high among hens fed the third level of protein, irrespective of the energy level that was fed in combination with the protein.

With the above facts in mind, and knowing that dietary energy is a major factor controlling feed intake, it is logical to assume that the linear main effect of energy on mortality was indirect even though it was statistically significant. As dietary energy was increased there was a reduction in feed consumption and consequently a reduction in protein intake. Therefore, the primary action of dietary energy was that of restricting protein consumption, which in turn resulted in increased mortality as dietary energy was increased. Protein consumption levels below the level that is required to maintain life appear to be the principal reason for a high mortality rate among those hens fed certain experimental diets. This is true whether low protein consumption was caused by a low dietary level of this nutrient or a high level of dietary energy which resulted in a restricted intake of protein regardless of the dietary protein level. Further evidence to support this reasoning will be presented when feed and nutrient intake data are discussed.

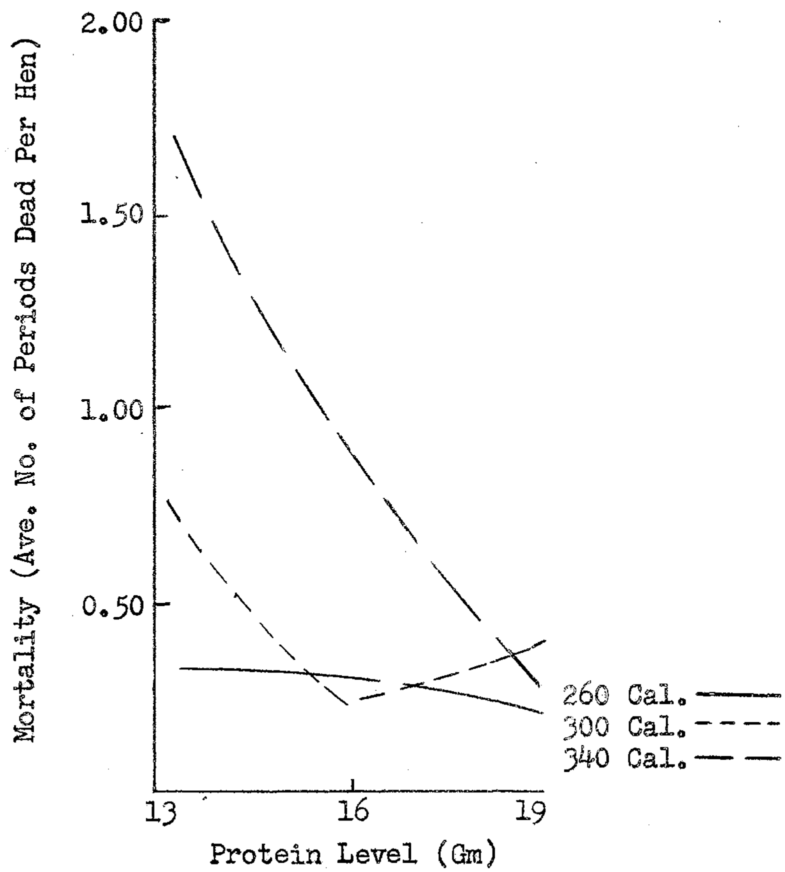

Figure 1

Protein x Energy Interaction Effect Upon Mortality

Feed Weight Consumption

Feed weight consumption means (grams per hen, per day) for each treatment are presented in Table XI. The analysis of variance computed from the original individual hen summaries is given in Table XII. The linear main effects of protein, energy, weight and volume upon feed weight consumption were all highly significant ($P<0.01$). In addition the interaction effects of protein x energy ($P<0.01$) and protein x energy x weight ($P<0.05$) were significant.

Again the relative size of the mean squares for the significant main effects is of interest. The order of size, with the largest first, is energy, weight, protein and volume, respectively. This can be taken as an indication of the relative degree with which these factors regulate feed weight consumption. However, it must be pointed out that two interaction effects were significant and a third one (protein x energy x weight x volume) was approaching significance at the five percent level of probability. Thus, the effects of protein, energy, weight and volume are not independent. From a statistical standpoint this means that estimating a quantitative effect for each one of the four factors separately is meaningless. Each one of the four should be considered in combination with the other three, which makes it necessary to study individual treatments in this particular situation (Table XI). In addition to these complications there is an effect due to egg production level upon feed consumption, and this is confounded with the effects of the other four factors.

The author recognizes that these interactions exist, that they disrupt the additive effect of the four dietary factors, and that egg

TABLE XI

AVERAGE GRAMS OF FEED CONSUMED PER HEN, PER DAY

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13, Milliliters 180	13, 230	13, 280	16, Milliliters 180	16, 230	16, 280	19, Milliliters 180	19, 230	19, 280
260	127	117.4	125.4	116.7	125.9	120.8	122.0	129.4	122.3	122.2
260	137	132.3	113.5	127.6	131.0	126.0	127.4	125.2	135.8	125.8
260	147	142.6	138.9	131.4	151.5	140.1	135.6	133.2	137.4	139.2
300	127	103.1	100.5	100.1	116.2	100.8	101.3	107.4	108.2	113.6
300	137	118.7	105.4	105.6	121.7	117.7	113.1	125.7	116.0	111.1
300	147	127.6	124.5	104.5	115.1	121.0	117.2	125.0	127.1	122.9
340	127	80.0	73.1	75.5	80.3	92.0	86.8	101.8	88.9	96.4
340	137	84.7	90.1	79.2	87.0	94.5	83.6	102.5	101.8	107.0
340	147	84.6	87.0	79.6	106.0	114.7	111.8	121.5	119.2	103.2

TABLE XII

ANALYSIS OF VARIANCE OF FEED WEIGHT CONSUMPTION DATA

Source of Variation	df	SS	MS	F
Total (Corrected)	566	276,114.63	487.83	
Treatments	80			
Protein (P)	(2)			
P_{Linear} (L)	1	11,666.43	11,666.43	61.12**
$P_{Quadratic}$ (Q)	1	290.78	290.78	1.52
Energy (E)	(2)			
E_L	1	120,378.58	120,378.58	630.65**
E_Q	1	545.67	545.67	2.86
Weight (W)	(2)			
W_L	1	24,446.65	24,446.65	128.07**
W_Q	1	217.88	217.88	1.14
Volume (V)	(2)			
V_L	1	2,440.63	2,440.63	12.79**
V_Q	1	32.41	32.41	0.17
Interactions	(72)			
P x E	4	7,353.39	1,838.35	9.63**
P x W	4	523.46	130.87	0.68
P x V	4	411.59	102.90	0.54
E x W	4	745.86	186.46	0.98
E x V	4	770.15	192.54	1.01
W x V	4	933.09	233.27	1.22
P x E x W	8	3,488.13	436.02	2.28*
P x E x V	8	1,090.69	238.71	1.25
P x V x W	8	1,141.10	142.64	0.75
E x W x V	8	1,044.19	130.52	0.68
P x E x W x V	16	5,005.30	312.83	1.64
Error	486	92,769.64	190.88	

*Significant at the five percent level of probability

**Significant at the one percent level of probability

production level is confounded with the dietary factors. In spite of these facts, the size of the mean squares for the main effects justifies further study of their actions. The means for the main effects of dietary protein, energy, weight and volume are presented in Table XIII.

TABLE XIII

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY, WEIGHT AND VOLUME UPON GRAMS OF FEED WEIGHT CONSUMPTION

Factor	Level*		
	1	2	3
Protein	106.29	113.37	117.40
Energy	129.51	113.74	93.81
Weight	104.75	111.48	120.83
Volume	114.73	112.69	109.64

*See Table XI for the dietary equivalents of levels 1, 2 and 3 for each of the four factors.

As dietary protein level was increased there was a linear increase in feed weight consumption. However, it will be shown later that the protein effect is the primary one that is confounded with egg production level. Therefore, this effect may not be real in the sense that protein has a direct bearing on feed weight consumption. In fact, it will be shown eventually that as dietary protein was increased there was a concomitant increase in egg production which resulted in increased feed weight consumption. The indications are that protein acts indirectly through egg production to increase feed weight consumption.

On the basis of the references cited in the review of literature concerning the effects of graded levels of dietary energy on feed consumption of laying hens, and on the basis of the "glucostatic" and "lipostatic" hypotheses, the effect of dietary energy upon feed weight consumption was as expected. As dietary energy was increased there was a subsequent reduction in feed weight consumption (Table XIII). The 300 Calorie diets reduced feed weight intake 15.77 grams below that of the 260 Calorie diets. This is a reduction of 0.394 grams of feed for each extra Calorie of metabolizable energy in the diet. The 340 Calorie diets reduced feed weight intake by 19.93 grams below the 300 Calorie diets or a reduction of 0.497 grams per Calorie. The fact that these differences were not equal indicates the existence of a quadratic effect. Even though this quadratic effect due to energy was not statistically significant there appeared to be a trend in the quadratic direction. Evidence, that the quadratic effect due to energy was greater than that due to the other factors, is supported by the fact that the mean square for the quadratic effect of energy was larger than for the quadratic effect of the other factors. A quadratic effect of dietary energy upon feed consumption was reported by Gleaves (1961) and Gleaves et al. (1963a). The presence of this quadratic effect, the presence of interaction and the confounding due to egg production make it impossible to assign an absolute value to the reduction in feed consumption caused by each Calorie that is added to a diet.

However, in those experiments conducted earlier as well as the present one, the mean squares due to the linear effect of dietary energy on feed weight consumption were much larger than for the quadratic effect. For this reason, an estimate of the reduction in feed intake caused by one Calorie being added to the diet can be based on the linear effect of dietary energy. From the data in Table XIII, this estimate would be 0.446 grams of feed reduction for each Calorie added above 260 Calories to the daily diet of laying hens. This estimate should not be construed as being applicable in every feeding situation with laying hens, but rather as an estimate determined under these particular experimental conditions.

The effect of dietary weight upon feed weight consumption (Table XIII) was that as dietary weight was increased there was a linear increase in feed weight consumption. This amounted to an increase in feed weight consumption of 0.804 grams for each increase of one extra gram in dietary weight above 127 grams. This could account for the fact that hen livability increased as dietary weight was increased, especially if this increase in feed weight consumption resulted in a greater intake of nutrients. However, in an analysis of these feed weight and feed consumption data, the complications presented previously must be kept in mind. The 0.804 grams increase in feed consumption per one gram of extra dietary weight is not an absolute value but merely an estimate with many possibilities for error.

Again on the basis of the evidence presented in the review of literature, the effect of dietary volume upon feed weight consumption was as to be expected. As dietary volume was increased there was a corresponding decrease in feed weight consumption. An estimate of this effect based upon the means in Table XIII is a 0.05 grams reduction in the feed consumption per milliliter of dietary volume above 180 milliliters.

Based on these estimates of the effects on feed weight consumption per unit variable, the order of magnitude of importance of the four factors has changed from that observed as indicated by the size of the mean squares. The order of relative importance from the per unit factor estimates is: weight (0.804 grams/gram), energy (0.446 grams/Calorie), volume (0.05 grams/ml), and protein (indirect). It is assumed that the action of protein is indirectly through egg production and, therefore, impossible to estimate. The reason for this change is obvious when it is considered that there is a spread of 80 Calories between treatments as compared to a spread of only 20 grams in dietary weight. Therefore, caution must be used in looking only at the relative sizes of the mean squares. Regardless of how these factors are considered, there is little room to doubt that all four are important considerations in a study of the factors affecting the feed weight consumption of laying hens.

Means for the interaction effect of protein x energy upon feed weight consumption are shown in Table XIV. The presence of this interaction is not at all unexpected. In fact it would be surprising if it were not present. An interrelationship between dietary protein and energy has been reported by many research workers, not only from animal nutrition studies but from biochemical studies as well. It is a well established fact that excess protein intake, due either to a high dietary level of protein or an amino acid imbalance, can be utilized by the animal body as an energy source. Consequently, it would be expected that excess protein should have the same effect upon feed consumption (perhaps slower in action) as dietary energy. Therefore, with varied levels of dietary protein, and since a perfect amino acid profile is not yet known, it would be surprising if there was not a protein x energy interaction effect on feed weight consumption.

TABLE XIV

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY UPON GRAMS OF FEED WEIGHT CONSUMPTION

Energy Level	Protein Level*		
	1	2	3
1	127.33	131.14	130.05
2	110.00	113.77	117.45
3	81.54	95.20	104.70

*See Table XI for the dietary equivalents of levels 1, 2 and 3 for protein and energy.

It can be seen from Table XIV and Figure 2 that the three levels of dietary protein acted differently when fed in combination with each of the three levels of dietary energy. The slope of the line (Figure 2) due to the action of the three protein levels was always in a positive direction (increased feed consumption with increases in dietary protein) at each of the three energy levels, but the rate of change in the slope was different. A possible explanation for this action could be that at the lower energy levels (260 and 300 Calories) feed consumption was enough greater than that at 340 Calories to result in an excess protein intake on the diets containing 16 and 19 grams of protein. This protein was used as energy and acted to reduce the rate of increase in feed consumption.

The interaction effect of protein x energy x weight upon feed weight consumption (Table XV) is more difficult to interpret. However, the interaction with the 300 and 340 Calorie diets appears to be due primarily to the protein x energy effect. On the other hand, with the 260 Calorie diets it was obvious that each level of weight acted differently

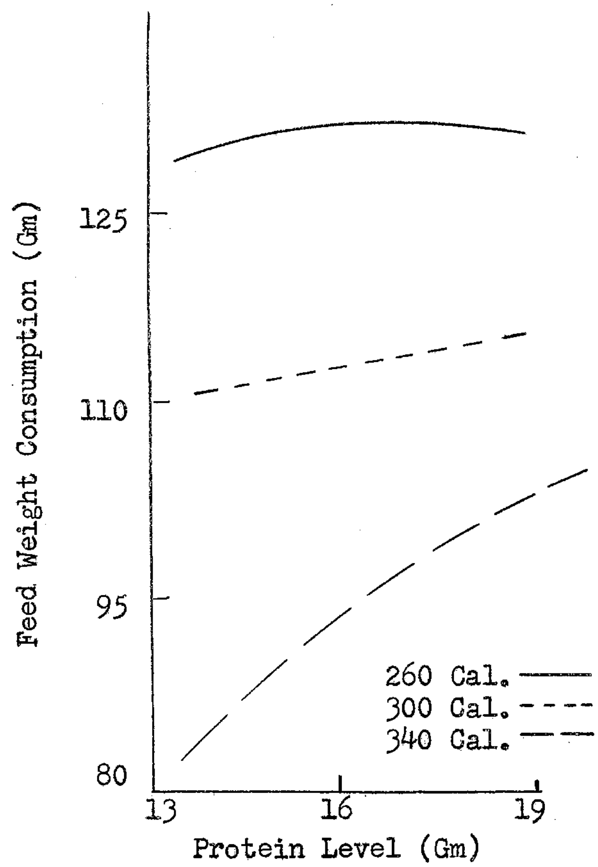

Figure 2

Protein x Energy Interaction Effect Upon Feed Weight Consumption

TABLE XV

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY X WEIGHT UPON GRAMS OF FEED WEIGHT CONSUMPTION

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13	16	19
260	127	119.85	122.89	124.63
	137	124.49	128.14	128.92
	147	137.65	142.39	136.60
300	127	101.23	106.08	109.76
	137	109.91	117.47	117.60
	147	118.87	117.76	124.99
340	127	76.20	86.38	95.70
	137	84.67	88.36	103.78
	147	83.75	110.85	114.63

at each level of protein. An explanation for this behavior is beyond the scope of the data available to the author at this time.

Protein, Energy and Volume Consumption

The summaries and analyses for protein, energy and volume consumption are included to help clarify and explain further the results obtained from the feed weight consumption data. In an experiment of this type, where consumption levels (protein, energy, weight and volume intakes) of the independent factors (dietary protein, energy, weight and volume) are considered as dependent variables, it is necessary to look at an analysis for each factor to complete the picture. This is true because the effect of each independent factor (dietary level) upon its respective dependent counterpart (intake level) must be estimated. For example, an estimate of the effect of dietary weight upon feed weight consumption comes from an analysis of variance for feed weight consumption. An estimate of the effect of dietary protein upon protein consumption comes from an analysis of variance for protein consumption, and so on for the other factors.

The average grams of protein, Calories of energy and milliliters of feed consumed per hen, per day, per treatment are given in Tables XVI, XVII and XVIII, respectively. The analyses of variance relative to these data are presented in Table XIX. Due to the fact that the mean squares for the protein x energy x weight x volume interactions are significant ($P<0.05$) for protein and volume consumption, and are approaching significance ($P>0.05$) for energy consumption, Tables XVI, XVII and XVIII should be studied on an individual treatment basis. However, the author feels again that the relatively large size of the mean squares caused by the significant main effects and other interactions justify further breakdown

TABLE XVI

AVERAGE GRAMS OF PROTEIN CONSUMED PER HEN, PER DAY

		Protein (Grams)								
		13			16			19		
		Milliliters			Milliliters			Milliliters		
Metabolizable Energy (Kilocalories)	Weight (Grams)	180	230	280	180	230	280	180	230	280
260	127	12.05	12.88	11.99	15.92	15.29	15.43	19.36	18.30	18.28
	137	12.60	10.81	12.15	15.37	14.78	14.94	17.37	18.83	17.44
	147	12.65	12.32	11.65	16.56	15.31	14.81	17.22	17.77	18.00
300	127	10.59	10.32	10.28	14.69	12.75	12.82	16.07	16.19	17.00
	137	11.30	10.04	10.05	14.27	13.80	13.27	17.44	16.09	15.41
	147	11.32	11.04	9.27	12.58	13.22	12.81	16.17	16.43	15.89
340	127	8.22	7.51	7.74	10.15	11.64	10.98	15.22	13.30	14.39
	137	8.07	8.57	7.54	10.20	11.08	9.81	14.21	14.12	14.84
	147	7.51	7.72	7.06	11.59	12.54	12.22	15.71	15.41	13.35

TABLE XVII

AVERAGE CALORIES OF METABOLIZABLE ENERGY
CONSUMED PER HEN, PER DAY

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13, Milliliters 180	13, 230	13, 280	16, Milliliters 180	16, 230	16, 280	19, Milliliters 180	19, 230	19, 280
260	127	242.43	259.14	241.00	260.57	250.43	252.57	268.57	253.57	253.29
	137	253.43	217.57	244.43	251.86	242.14	244.43	241.00	260.86	241.86
	147	254.57	248.14	234.57	271.29	250.57	242.71	238.86	246.29	249.29
300	127	243.57	237.43	236.14	277.29	240.43	241.71	256.57	258.71	271.43
	137	259.86	230.86	231.29	269.29	260.29	250.14	278.43	256.71	246.14
	147	260.43	254.14	213.43	237.00	249.57	241.86	258.00	262.29	253.71
340	127	215.86	197.29	203.43	216.86	248.57	234.71	275.29	240.29	260.14
	137	211.71	225.14	198.14	217.86	236.71	209.29	256.71	255.29	268.14
	147	197.14	203.00	185.43	247.43	267.57	261.14	283.86	278.43	241.14

TABLE XVIII

AVERAGE MILLILITERS OF FEED CONSUMED PER HEN, PER DAY

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13, Milliliters 180	13, 230	13, 280	16, Milliliters 180	16, 230	16, 280	19, Milliliters 180	19, 230	19, 280
260	127	166.33	227.16	257.39	178.56	218.84	268.93	183.39	221.49	269.43
	137	173.87	190.67	260.86	172.21	211.54	260.33	164.53	227.94	257.09
	147	174.53	217.41	250.27	185.61	219.20	258.20	163.20	215.01	265.20
300	127	146.14	181.99	220.66	164.60	182.49	223.40	152.19	196.03	250.59
	137	155.99	177.04	215.81	159.86	197.59	231.16	165.23	194.71	227.10
	147	156.14	194.84	199.10	140.99	189.39	239.60	153.06	198.89	234.09
340	127	113.43	132.37	167.67	113.71	166.67	193.01	144.19	160.97	214.03
	137	111.31	151.20	161.94	114.30	158.60	170.96	134.69	171.00	218.71
	147	103.67	136.24	151.56	129.87	179.50	213.06	148.69	186.49	196.71

and analyses of these effects.

Main Effects Upon Protein Consumption: The analysis of variance for the protein consumption data (Table XIX) shows the linear main effects of protein, energy and volume to be significant at the one percent level of probability. This is essentially the same pattern that was obtained with the feed weight consumption analysis (Table XII). However, there was one important difference. Dietary weight had no significant effect upon protein consumption. In addition the largest mean square due to the effect of dietary weight was quadratic and not linear, as was the situation with the feed weight consumption analysis.

It is apparent from both the analysis of variance for protein consumption (Table XIX) and the main effect means (Table XX) that the effect of dietary protein upon protein consumption appears to be essentially the same as the effect of dietary protein upon feed weight consumption. As dietary protein was increased there was an increase in protein intake. The two analyses of variance for feed weight consumption and protein consumption appear to show the same effect due to dietary protein. However, it is proposed that the effect of dietary protein upon protein intake is a real and direct action which results in higher egg production from those hens consuming the higher levels of protein. In turn, the higher egg production levels ultimately lead to the indirect effect of a linear increase in feed weight consumption as dietary protein is increased.

As would be expected, linear main effects of dietary energy and volume (Table XX) upon protein consumption were identical to their effects upon feed weight consumption. As dietary energy was increased and as dietary volume was increased there was in each case a concurrent decrease in protein consumption.

TABLE XIX

ANALYSES OF VARIANCE OF PROTEIN, ENERGY AND VOLUME CONSUMPTION DATA

Source of Variation	df	MS Protein	MS Energy	MS Volume
Total (Corrected)	566	11.63	1,254.37	2,162.05
Treatment	[80]			
Protein (P)	(2)			
P_{Linear} (L)	1	3,596.69**	73,982.20**	34,919.99**
$P_{Quadratic}$ (Q)	1	1.00	1,594.51	1,303.24
Energy (E)	(2)			
E_L	1	1,550.65**	2,390.94	337,856.67**
E_Q	1	5.65	27,079.44**	1,629.29
Weight (W)	(2)			
W_L	1	0.20	4.17	28.79
W_Q	1	3.24	971.87	889.30
Volume (V)	(2)			
V_L	1	29.08**	11,206.95**	552,037.43**
V_Q	1	0.23	194.39	173.08
Interaction	(72)			
P x E	4	4.48	11,394.70**	4,665.51**
P x W	4	1.56	684.19	589.24
P x V	4	0.50	521.06	1,101.46
E x W	4	5.13	1,553.04	613.93
E x V	4	2.00	951.65	2,445.13**
W x V	4	2.78	1,072.06	604.74
P x E x W	8	4.75*	2,116.55**	822.93
P x E x V	8	3.50	1,261.86	523.18
P x V x W	8	1.74	602.71	598.98
E x W x V	8	1.78	674.57	319.84
P x E x W x V	16	4.57*	1,520.13	911.84*
Error	486	2.40	959.41	518.71

*Significant at the five percent level of probability

**Significant at the one percent level of probability

TABLE XX

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY AND VOLUME UPON GRAMS OF PROTEIN CONSUMPTION

	Level*		
Factor	1	2	3
Protein	10.12	13.29	16.29
Energy	15.19	13.37	11.14
Volume	13.50	13.26	12.94

*See Table XVII for the dietary equivalents of levels 1, 2 and 3 for each of the three factors.

Main Effects Upon Energy Consumption: The main effects of dietary protein, energy and volume upon energy consumption were all highly significant (Table XIX), but dietary weight had no significant effect. The quadratic mean square due to the effect of dietary weight was larger than the linear mean square. Also, it is important to note that the significant main effects of dietary protein and volume upon energy consumption were linear as was observed in the cases of feed weight and protein intake. However, the significant effect of dietary energy upon energy consumption was quadratic.

The 300 Calorie diets resulted in an average energy intake of 250.99 Calories per hen, per day (Table XXI). The lowest energy consumption of 234.69 Calories occurred among hens fed the 340 Calorie diets, while the lowest energy diets (260 Calories) resulted in an intermediate consumption of 248.72 Calories per hen, per day. Undoubtedly, this quadratic effect of dietary energy upon energy consumption accounts for the quadratic effect of dietary energy (although not significant) observed upon feed weight consumption (Table XII).

TABLE XXI

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY AND VOLUME UPON CALORIES OF METABOLIZABLE ENERGY CONSUMPTION

Factor	Level*		
	1	2	3
Protein	229.61	247.20	257.59
Energy	248.72	250.99	234.69
Volume	249.84	245.61	238.95

*See Table XVII for the dietary equivalents of levels 1, 2 and 3 for each of the factors.

The effects of dietary protein and volume upon energy consumption are the same as they were upon feed weight consumption. Therefore, they support the previous discussion, but add nothing new.

Main Effects Upon Volume Consumption: An absence of a significant effect of dietary weight upon volume consumption is again noticeable (Table XIX). The familiar significant linear effects of dietary protein and energy that were observed upon feed weight consumption are apparent from the feed volume consumption data and analysis (Tables XXII and XIX, respectively). However, the significant effect of dietary volume upon feed volume consumed was the reverse of that observed previously upon feed weight consumption (Table XII). As dietary volume was increased there was a corresponding increase in feed volume consumption.

Protein x Energy Interaction Effects: The protein x energy interaction effects upon both energy and volume consumption were highly significant ($P<0.01$) (Table XIX). The respective interaction means for these analyses are presented in Tables XXIII and XXIV. The pattern of this interaction in each case is the same as that observed for feed weight

(Table XII). Consequently, the explanation of the interaction is the same.

TABLE XXII

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY AND VOLUME UPON MILLILITERS OF FEED CONSUMPTION

Factor	Level*		
	1	2	3
Protein	177.61	190.44	196.84
Energy	217.00	190.69	157.21
Volume	150.74	189.08	225.07

*See Table XVII for the dietary equivalents of levels 1, 2 and 3 for each of the three factors.

TABLE XXIII

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY UPON CALORIES OF METABOLIZABLE ENERGY CONSUMPTION

Energy Level	Protein Level*		
	1	2	3
1	243.92	251.84	250.40
2	240.79	251.95	260.22
3	204.13	237.79	262.14

*See Table XVII for the dietary equivalents of levels 1, 2 and 3 for protein and energy.

The fact that the protein x energy interaction effect upon protein consumption was not significant is obvious (Table XIX). Although definite proof cannot be presented at this time, it is the opinion of the author that this is due to the overall low consumption of protein (Table XVI). This is especially true for the two lower levels (13 and 16 grams) of dietary protein. At these relatively low levels of protein consumption, excess protein would not be available to act as an energy source. Consequently, dietary protein would not exert a restricting influence on protein consumption. It is reasoned that the 19-gram-protein diets were responsible for the fairly large mean square for the protein x energy interaction effect on protein intake. The protein intakes of the hens fed 260 and 300 Calories in combination with 19 grams of protein were high enough that some of the protein could have been used as energy.

TABLE XXIV

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY UPON MILLILITERS OF FEED CONSUMPTION

Energy Level	Protein Level*		
	1	2	3
1	213.16	219.25	218.59
2	183.08	192.12	196.87
3	136.60	159.96	175.05

*See Table XVII for the dietary equivalents of levels 1, 2 and 3 for protein and energy.

<u>Energy x Volume Interaction Effect</u>: The energy x volume interaction effect upon volume of feed consumption was significant at the one percent level of probability (Table XIX). This is the first time this interaction has been statistically significant. The means for this effect are presented in Table XXV. A graphic representation of these means is shown in Figure 3.

TABLE XXV

MEANS FOR INTERACTION EFFECT OF ENERGY X VOLUME UPON MILLILITERS OF FEED CONSUMPTION

Volume Level	Energy Level* 1	Energy Level* 2	Energy Level* 3
1	173.56	154.91	123.76
2	216.59	190.33	160.39
3	260.85	226.83	187.52

*See Table XVII for the dietary equivalents of levels 1, 2 and 3 for energy and volume.

An inspection of these data reveals that this interaction was caused by an increased rate of restriction of volume consumption by dietary energy as dietary volume was increased from 180 to 230 to 280 milliliters, respectively. Conversely, volume of feed consumption increased at a faster rate as dietary volume was increased in combination with the low level of dietary energy than with the two higher levels of dietary energy. This is evidence that dietary energy is exerting more influence over feed consumption than is dietary volume. Perhaps an explanation of the mechanism for this interaction can be delineated from the data in Table XXI. It can be seen from the means in this table that dietary volume does restrict energy consumption, which in turn would tend to increase feed consumption. Thus, the interaction of energy x volume may be a result of these interrelationships.

<u>Interaction Effects of Protein x Energy x Weight</u>: The protein x energy x weight interaction effects upon protein and energy consumption were both statistically significant (Table XIX). However, this interaction

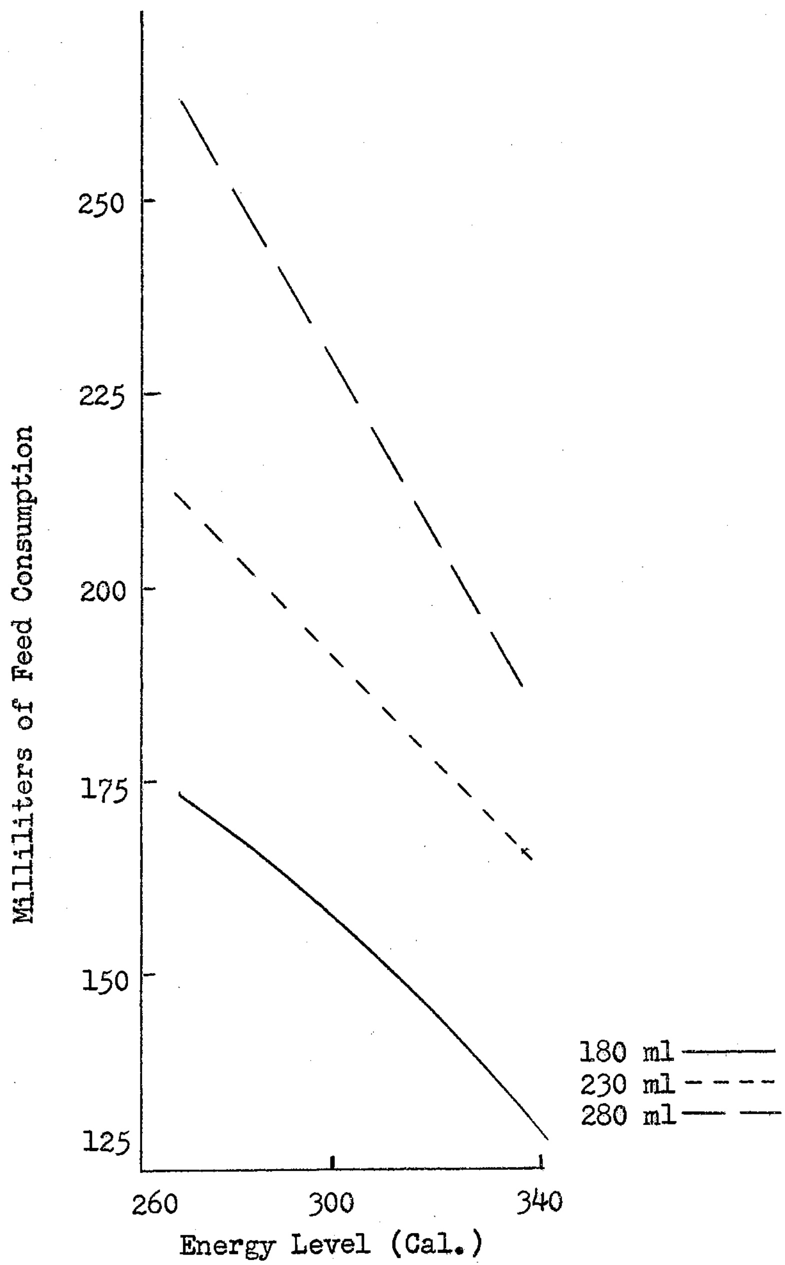

Figure 3

Energy x Volume Interaction Effect Upon Feed Volume Consumption had no significant effect upon volume of feed consumed. This is understandable from the standpoint that the predominant reason for this interaction was the protein x energy effect and that there was little evidence of a weight x volume or a protein x volume interaction.

The means for these three-way interactions are listed in Tables XXVI and XXVII for protein and energy consumption, respectively. Although the mechanism of these interactions cannot be delineated, the reason that weight is involved can be seen more clearly from these tables than from the table for feed weight consumption. The reason for the quadratic mean squares being larger than the linear mean squares, for the main effects of dietary weight on protein and energy consumption (Table XIX), can be seen also in these tables. These means are discussed in the following paragraphs.

The interaction effect of protein x energy x weight could be caused by a reversal of this quadratic effect from energy level to energy level. For example, an observation of the means for the 13-gram-protein-260-Calorie diets shows a low point in a quadratic nutrient (protein and energy) consumption to be at the middle level (137 grams) of dietary weight, while on the 13-gram-protein-340-Calorie diets just the reverse is true. The high point in nutrient consumption occurs with 137 grams of dietary weight. The quadratic effect of weight was less prominent on the 16-gram-protein diets. However, it was present and reversals were obvious, but it did not follow the same pattern as that observed with the 13-gram-protein diets. The quadratic effects of dietary weight upon nutrient consumption were not evident at all in the 19-gram-protein diets. In fact, at the low level of dietary energy, there was a linear decrease in nutrient consumption as dietary weight was increased.

TABLE XXVI

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY X WEIGHT UPON GRAMS OF PROTEIN CONSUMPTION

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13	16	19
260	127	12.31	15.55	18.65
	137	11.85	15.03	17.88
	147	12.21	15.56	17.66
300	127	10.40	13.42	16.42
	137	10.46	13.78	16.31
	147	10.54	12.87	16.16
340	127	7.82	10.93	14.30
	137	8.06	10.37	14.39
	147	7.43	12.12	14.82

TABLE XXVII

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY X WEIGHT UPON CALORIES OF METABOLIZABLE ENERGY CONSUMPTION

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13	16	19
260	127	247.52	254.52	258.48
	137	238.48	246.14	247.90
	147	245.76	254.86	244.81
300	127	239.05	253.14	262.24
	137	240.67	259.90	260.43
	147	242.67	242.81	258.00
340	127	205.52	233.38	258.57
	137	211.67	221.29	260.05
	147	195.19	258.71	267.81

A similar pattern exists at the middle level of dietary energy, but at the high energy level this trend was reversed. Although no significant energy x weight interactions have been observed, it seems almost certain that some interrelationship does exist between dietary energy and dietary weight.

The prevalent occurrence of a protein x energy x weight interaction, that is unexplainable, suggests the need for an experiment designed with a wider range of dietary weight levels and perhaps a narrower range of dietary energy levels than were used in this experiment. Such an experiment might reveal some main effect due to dietary weight that would help explain this persistent interaction.

Dietary Factor Effects Upon Production Traits From Data of Original Factorial Arrangement

The average total body weight change per hen, average total number of eggs produced per hen and the average egg weight are listed in Tables XXVIII, XXIX and XXX, respectively. There were no significant four-way interaction effects on these production traits. However, in order to complete the record of this experiment, it was deemed necessary to include these three tables. The analyses of variance related to these means are given in Table XXXI.

The main effects of dietary protein and energy were significant upon all three production traits that were measured. The effect of dietary volume upon body weight change (significant at the one percent level) was the only evidence that the physical factors exerted any influence upon the production traits of laying hens. It is interesting to note also from the analysis of variance for body weight change that the effect of

TABLE XXVIII

AVERAGE BODY WEIGHT CHANGE IN GRAMS PER HEN FOR ENTIRE EXPERIMENT

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13 Milliliters 180	13 230	13 280	16 Milliliters 180	16 230	16 280	19 Milliliters 180	19 230	19 280
260	127	+72.86	+145.71	+77.57	+111.43	+25.71	+100.00	+305.71	+110.00	+177.14
	137	+84.29	-34.29	-25.71	+211.43	+30.00	-98.43	+47.14	+211.43	+57.14
	147	+52.86	-30.00	-41.43	+241.43	+237.14	+127.14	+72.86	+121.43	+75.71
300	127	-172.86	-110.00	+105.71	+144.29	+54.29	0	+262.86	+275.71	+198.29
	137	+20.43	-140.00	-141.43	+284.29	100.00	+18.57	+307.14	+117.14	+195.71
	147	+82.86	+60.00	-117.14	-81.43	+205.71	-87.14	+130.00	+324.29	+130.00
340	127	-171.43	-447.17	-119.43	-212.86	-171.43	-31.43	+234.29	+12.86	+5.71
	137	-355.71	-173.29	-191.43	-138.57	-30.00	-181.43	+252.86	+152.86	+178.57
	147	-178.57	-141.43	-207.14	+2.86	+44.29	+90.00	+280.00	+257.14	+60.00

TABLE XXIX

AVERAGE NUMBER OF EGGS PRODUCED PER HEN FOR ENTIRE EXPERIMENT

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13, Milliliters 180	13, 230	13, 280	16, 180	16, 230	16, 280	19, 180	19, 230	19, 280
260	127	159.57	153.29	117.14	152.14	141.86	146.86	160.00	149.00	151.71
260	137	153.14	120.00	141.00	152.00	144.43	146.57	139.43	163.43	160.00
260	147	142.14	143.14	139.00	155.14	148.86	126.86	140.86	146.86	162.57
300	127	105.14	127.00	112.29	140.71	146.29	147.43	137.86	143.14	128.71
300	137	89.86	114.57	112.43	132.14	147.43	150.14	166.71	146.71	122.43
300	147	116.14	119.29	101.57	128.86	143.71	128.57	150.00	167.00	141.00
340	127	93.57	60.57	45.14	85.71	105.57	142.00	160.14	117.14	140.71
340	137	59.14	81.29	64.71	120.86	127.29	99.00	152.71	146.00	147.29
340	147	57.14	80.00	69.29	128.71	150.43	146.86	151.86	152.57	144.57

TABLE XXX

AVERAGE EGG WEIGHT IN GRAMS

Metabolizable Energy (Kilocalories)	Weight (Grams)	Protein (Grams) 13			16			19		
		Milliliters 180	230	280	Milliliters 180	230	280	Milliliters 180	230	280
260	127	51.64	54.54	52.46	54.10	54.77	55.16	54.03	55.39	55.90
	137	52.89	51.99	54.39	55.39	54.13	54.33	53.97	54.90	53.04
	147	53.46	54.06	55.19	56.20	54.97	53.79	55.83	53.31	54.76
300	127	51.54	51.36	54.33	53.16	53.03	54.49	54.07	53.94	54.74
	137	52.84	52.21	52.57	56.27	53.06	53.99	55.09	54.49	52.63
	147	53.06	53.50	53.30	53.60	53.94	56.20	54.03	53.64	54.51
340	127	52.61	50.06	51.86	54.57	53.73	50.90	54.36	53.46	54.37
	137	50.90	50.76	50.20	52.77	52.41	52.24	54.23	54.03	55.50
	147	51.43	49.70	50.49	54.99	52.94	52.50	55.19	56.13	55.50

TABLE XXXI

ANALYSES OF VARIANCE FOR BODY WEIGHT LOSS OR GAIN, EGG PRODUCTION AND EGG WEIGHT DATA FROM THE ORIGINAL FACTORIAL ARRANGEMENT

Source of Variation	df	MS Body Wt. Change	MS Egg Production	MS Egg Weight
Total (Corrected)	566	75,090.43	1,804.75	8.94
Treatment	80			
Protein (P)	(2)			
P_{Linear} (L)	1	5,715,507.88**	160,485.95**	431.96**
$P_{Quadratic}$ (Q)	1	9,852.77	11,067.36**	41.04*
Energy (E)	(2)			
E_L	1	1,723,739.54**	111,372.87**	175.04**
E_Q	1	398,909.70**	886.70	1.96
Weight (W)	(2)			
W_L	1	70,584.78	1,635.38	17.55
W_Q	1	60,929.90	117.13	14.58
Volume (V)	(2)			
V_L	1	301,667.63**	1,190.93	1.08
V_Q	1	1,031.32	1,836.38	18.38
Interaction	(72)			
P x E	4	342,994.67**	19,591.66**	37.64**
P x W	4	51,492.24	504.10	0.58
P x V	4	45,572.44	942.21	7.69
E x W	4	131,368.64	1,352.44	1.99
E x V	4	34,167.52	1,013.92	8.63
W x V	4	163,420.72*	873.20	5.07
P x E x W	8	80,534.15	1,227.87	8.33
P x E x V	8	61,936.35	2,041.56	7.95
P x W x V	8	59,479.59	1,554.79	2.06
E x W x V	8	80,292.16	1,098.15	13.00
P x E x W x V	16	54,656.32	1,344.63	7.89
Error	486	57,616.16	1,166.44	7.69

*Significant at the five percent level of probability

**Significant at the one percent level of probability

weight x volume upon body weight change was significant ($P<0.05$). In addition to the significant effects that have been mentioned, the effect of protein x energy upon all three production traits was highly significant. Table XXXII includes the means for the significant main effects due to dietary protein, energy and volume upon body weight change, number of eggs and average egg weight.

TABLE XXXII

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY AND VOLUME UPON BODY WEIGHT CHANGE, NUMBER OF EGGS PER HEN AND AVERAGE EGG WEIGHT

	Dietary Factors						
	Protein Effects			Energy Effects			Volume Effects
Level*	Body Wt. Change (Gm)	No. Eggs Per Hen	Ave. Egg Wt. (Gm)	Body Wt. Change (Gm)	No. Eggs Per Hen	Ave. Egg Wt. (Gm)	Body Wt. Change (Gm)
1	-77.26	106.58	52.34	+91.71	146.56	54.24	+70.02
2	+36.88	136.53	53.99	+80.27	132.12	53.69	+44.75
3	+168.67	147.79	54.48	-43.70	112.23	52.88	+13.52

*See Table XXX for the dietary equivalents of levels 1, 2 and 3 for each of the three factors.

Protein Effects: The linear main effects of dietary protein upon body weight change, egg production and egg weight were all highly significant ($P<0.01$). The directions of these effects were all the same (Table XXXII). As dietary protein was increased there was an increase in body weight gain, egg production and egg weight.

Similar results for the effect of dietary protein upon the performance of laying hens have been reported by Quisenberry and Bradley

(1962). They found that hen-day production, egg weight and feed efficiency were significantly improved as dietary protein was increased from 13 to 17 percent. Harms and Waldroup (1963) found that feeding low levels of protein significantly reduced the length of a laying cycle, which resulted in a significantly lower rate of egg production. In addition, Biely and March (1964) have recently reported that hens receiving 16 percent of protein in the ration consistently laid larger eggs than did those receiving 14 percent of protein.

It was shown earlier in this discussion that protein intake increased as dietary protein was increased. Therefore, there is little doubt that protein consumption is an important consideration in obtaining the best performance from laying hens. In making this statement, the assumption is made that the dietary protein will be composed of the best possible amino acid profile.

In addition to the significant linear effects of protein, the quadratic effects of protein upon egg production and egg weight were also significant (Table XXXI). This effect was caused by the relatively large increase in egg production and egg weight between the first and second level of dietary protein as compared to a small increase between the second and third levels (Table XXXII). It is reasoned that this results from the hens approaching an over consumption of protein between the second (16 grams) and third levels (19 grams) of dietary protein. There may not be an excessive protein consumption occurring between the 16 and 19-gram-protein diets, but it would appear that the point of diminishing returns is being approached. The average intake of protein on the 19-gram-protein diets was 16.29 grams (Table XX), which was found by Gleaves (1961) to be near the optimal intake of protein for laying hens. At protein intakes greater than 17 grams there was actually a decrease in egg production, egg size and body weight gain. Essentially the same results were reported by Touchburn and Naber (1962). They found the minimum protein intake for maintenance of 72 percent egg production in a four-pound hen to be 17 grams per day.

Energy Effects: The linear effects of dietary energy upon body weight change, egg production and egg weight were all statistically significant. However, the trends were reversed from those due to the effects of dietary protein (Table XXXII). As dietary energy was increased there was a concurrent decrease in body weight gain, egg production and egg weight. A similar effect of dietary energy upon egg weight has been reported by March and Biely (1963). Donaldson (1962) reported that the feeding of a balanced diet containing 30.4 percent of added fat to Leghorn pullets reduced egg production. However, he reported that body weight gain of the pullets fed this diet was greater than for those pullets fed the control diet. Although these results for the effect of dietary energy upon body weight change appear to contradict the results of the experiment reported here, they do not. Donaldson's diets (both control and experimental) had the same Calorie-protein ratio. Therefore, protein intake with the high-fat diet was high enough to maintain body weight but not egg production.

These facts support the hypothesis that balanced protein intake is the key to high performance from laying hens. The results of this experiment, in which dietary protein, energy, weight and volume were all controlled, indicate that the effect of energy on the three measured production traits is indirect. When dietary energy was high enough to restrict the intake of protein and other nutrients, there was a drop in production performance of the hens. This should not be taken to mean that energy is not a necessary component in laying hen diets. However, it is much less critical than protein, for if it becomes necessary the hens can utilize protein for energy. There is no doubt that hens must consume a certain minimum level of energy in order to utilize the protein that is available, but beyond the minimum level, energy functions mainly as a regulator of feed consumption.

Another possible reason that the hens in Donaldson's experiment did not lose weight is that total dietary energy may not have been high enough to begin to restrict energy intake as such. This was shown to be possible in the discussion of the data in Table XXI of this report. The quadratic effect of dietary energy upon body weight change was also significant at the one percent level (Table XXXI). From Table XXXII it can be seen that, at the highest dietary energy level fed, there was an average of 43.7 grams of weight loss per hen. This sharp decrease in body weight gain from that obtained at the second level of dietary energy accounts for the significant quadratic effect of dietary energy upon body weight change.

Volume Effects: The linear main effect of dietary volume upon body weight change was significant at the one percent level of probability (Table XXXI). As dietary volume was increased there was a concomitant decrease in body weight gain (Table XXXII). It was established earlier that as dietary volume was increased there was a corresponding decrease in protein intake (Table XX). Therefore, the effect of dietary volume upon body weight change may be an indirect one, as was the case with dietary energy. The ability of dietary volume to restrict feed consumption is much less than that of dietary energy. Therefore, dietary volume never restricted feed consumption to the point that hens actually lost weight; instead, the higher levels of dietary volume did not permit the hens to gain as much weight (Table XXXII). This would explain why egg production was not affected by dietary volume. If the experiment had been conducted for a longer period of time, egg production could have been reduced also by the action of the high levels of dietary volume.

The discussion on the actions of dietary protein, energy and volume has been centered around protein, mainly because protein was one of the controlled variables in this experiment. However, it is recognized also that other nutrients such as the vitamins and minerals may become limiting under the feed intake restriction influence exerted by dietary energy and volume. All of these nutrients including protein are extremely important in enzyme formation and enzyme function in metabolism. Very little discussion has been devoted to these factors because the experiment was not designed to study such variables.

Protein x Energy Interaction Effects: The protein x energy interaction effects upon body weight change, egg production and egg weight were all statistically significant ($P<0.01$). At the low level of dietary energy (260 Calories) the increase in body weight gain, egg production and egg weight was less (Table XXXIII and Figure 4) at each increase in dietary protein than at the second level of dietary energy (300 Calories). At the third level of dietary energy (340 Calories) the increase in body weight gain, egg production and egg size was much greater with each increase in dietary protein than at the other two levels of dietary energy.

Experimental results to support the existence of an interrelationship between protein and energy have been reported by many research workers, but only one of the more recent reports will be cited here. Touchburn and Naber (1962) found that a Calorie-protein ratio of 80 (productive

TABLE XXXIII

MEANS FOR INTERACTION EFFECTS OF PROTEIN X ENERGY UPON BODY WEIGHT CHANGE, NUMBER OF EGGS PER HEN AND AVERAGE EGG WEIGHT

Effects on	Energy Level	Protein Level* 1	Protein Level* 2	Protein Level* 3
Body Wt. Change (Grams)	1	+34.65	+109.54	+130.95
	2	-45.83	+70.95	+215.68
	3	-220.62	-69.84	+159.37
No. Eggs Per Hen	1	140.94	146.08	152.65
	2	110.92	140.59	144.84
	3	67.87	122.94	145.89
Ave. Egg Wt.	1	53.40	54.76	54.57
	2	52.75	54.19	54.13
	3	50.89	53.00	54.75

*See Table XXX for the dietary equivalents of levels 1, 2 and 3 for protein and energy.

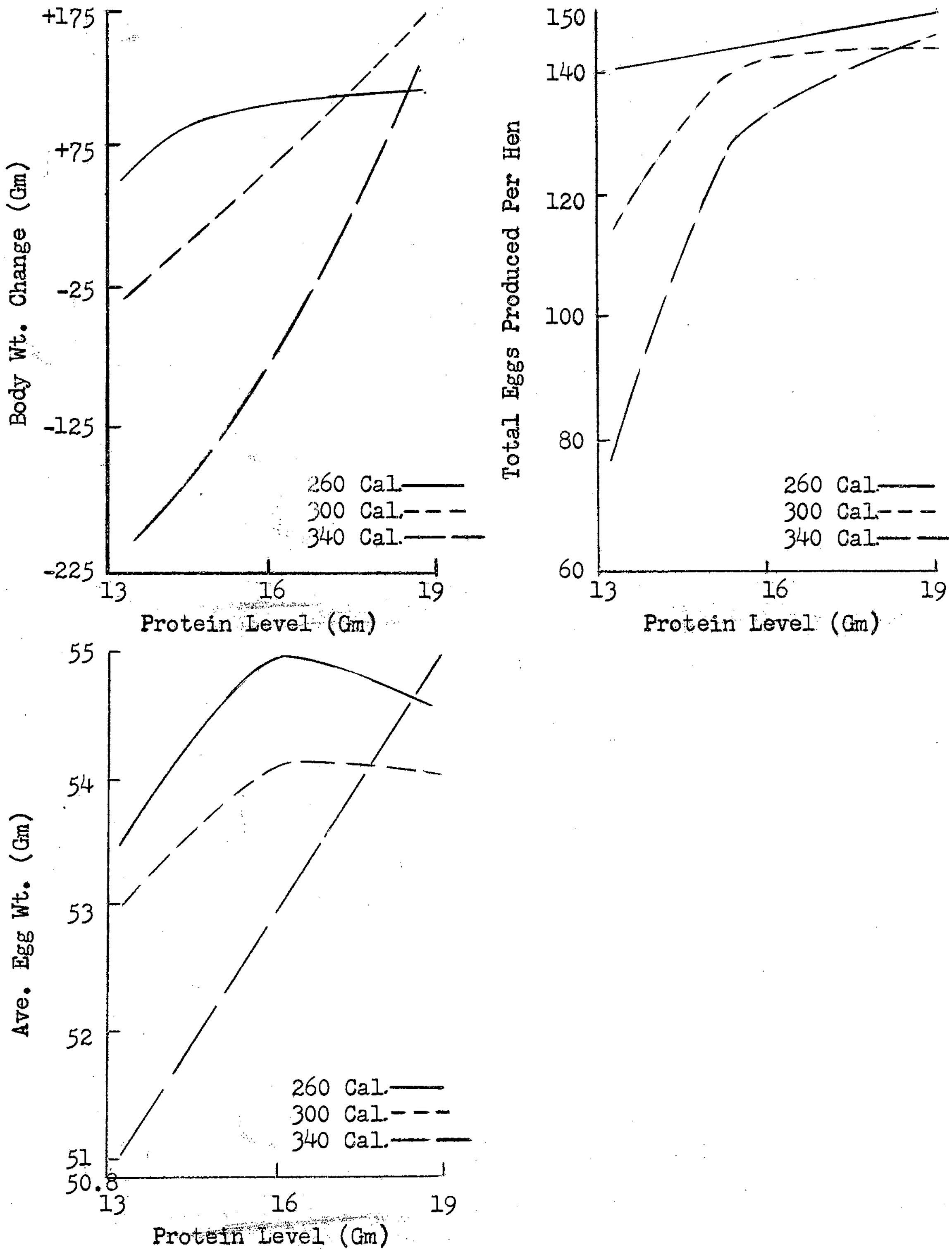

Figure 4

Protein x Energy Interaction Effects Upon Body Weight Change, Number of Eggs Produced and Average Egg Weight energy values were used) in a 12 percent protein ration caused a decrease in egg production. A Calorie-protein ratio of 75 in a 16 percent protein ration did not depress egg production. Because of the difference in units, it is rather difficult to correlate these findings with those of this experiment. However, the important point is that both experiments demonstrate that dietary protein and energy are interrelated in their effects upon the reproductive performance of the hen.

Weight x Volume Interaction Effect: The weight x volume interaction effect upon body weight change was significant ($P<0.05$). It is apparent from the means presented in Table XXXIV and depicted graphically in Figure 5 that the effect of increases in dietary weight acted differently at each level of dietary volume. It is apparent also that there was no consistent pattern; this makes interpretation very difficult. At the first level of dietary weight (127 grams) the hens gained less at the second level (230 milliliters) of dietary volume than at either 180 or 280 milliliters. This effect was definitely quadratic. At the second level of dietary weight (137 grams) the effect of dietary volume upon body weight gain was almost linear. Body weight gain decreased with each increase in dietary volume. At the third level of dietary weight (147 grams) the effect of dietary volume upon body weight gain was again quadratic, but in the opposite direction to that observed at the first level of dietary weight. The hens fed the second level of dietary volume gained more than those fed either of the other two levels of dietary volume.

This interaction effect is just as confusing as that observed for the effect of protein x energy x weight upon feed weight consumption. Even though there were no obvious main effects due to dietary weight, it definitely exerts an influence in the overall scheme of poultry nutrition.

There is enough evidence from this experiment to state that dietary weight should be controlled, either by being held constant or varied at definite intervals, in future nutritional experiments with laying hens.

TABLE XXXIV

MEANS FOR INTERACTION EFFECT OF WEIGHT X VOLUME UPON BODY WEIGHT CHANGE IN GRAMS

Volume Level	Weight Level*		
	1	2	3
1	+63.81	+79.25	+66.98
2	-11.59	+25.98	+119.84
3	+57.06	-20.94	+4.44

*See Table XXX for the dietary equivalents of levels 1, 2 and 3 for weight and volume.

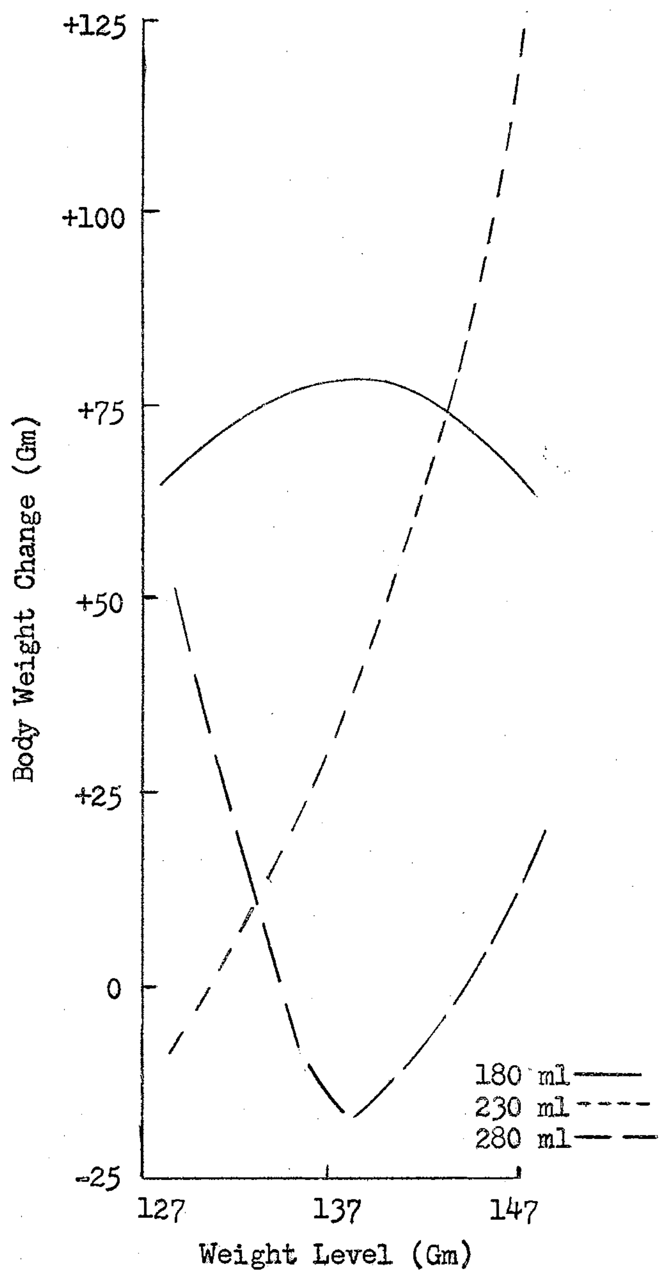

Figure 5

Weight x Volume Interaction Effect Upon Body Weight Change

RESULTS AND DISCUSSION WITH EGG PRODUCTION AS A FIFTH FACTOR

Egg production is undoubtedly one of the primary pathways of nutrient expenditure in laying hens, but quantitative estimates of the effect of egg production level upon feed consumption are very difficult to obtain. At the present time it is impossible to design an experiment with controlled egg production levels, especially where several dietary factors are involved. The interrelationship between nutrient intake and utilization and nutrient expenditure is so great that present techniques permit only crude quantitative estimates to be made of the factors affecting feed consumption in laying hens. However, as more knowledge becomes available from experiments of the type reported herein, better estimates of the effect of such factors as individual nutrients, physical dietary factors, egg production and body maintenance upon feed consumption can be made.

In this experiment an attempt was made to overcome the tremendous variation in egg production (Table XXIX) by selecting four hens from each treatment on the basis of egg production. Two hens were selected as "low" producers and two as "high" producers. The results of this selection upon egg production are shown in Table XXXV. The mean egg production for the "lows" was 15.2 eggs per hen, per 28-day period with a standard error of 0.225. The mean egg production for the "highs" was 24.4 eggs per hen, per 28-day period with a standard error of 0.082. It is evident from Table XXXV and the standard errors for these selections that egg

TABLE XXXV

AVERAGE EGG PRODUCTION PER TREATMENT FOR THOSE HENS SELECTED AS "LOW" AND "HIGH" PRODUCERS, RESPECTIVELY

Metabolizable Energy (Kilocalories)	Weight (Grams)	Eggs	Protein (Grams) 13, Milliliters 180	13, 230	13, 280	16, Milliliters 180	16, 230	16, 280	19, Milliliters 180	19, 230	19, 280
260	127	1*	17.2	18.0	18.7	18.0	17.8	19.5	18.3	16.8	18.3
		2	25.2	24.2	24.2	25.3	25.5	25.0	25.3	24.5	24.3
	137	1	19.0	14.7	18.7	14.3	15.0	18.8	16.3	17.8	16.3
		2	24.5	23.5	24.5	24.3	24.8	25.5	25.5	25.3	25.5
	147	1	17.5	18.5	16.0	17.8	15.8	12.5	14.5	17.8	20.5
		2	25.2	24.2	25.0	24.3	25.5	24.8	24.3	25.3	25.0
300	127	1	15.2	15.0	11.2	15.0	14.5	14.3	12.5	18.3	17.0
		2	24.7	24.7	23.7	24.8	24.3	24.3	26.0	24.8	25.5
	137	1	15.7	13.7	12.0	13.5	19.0	16.5	20.5	18.8	18.8
		2	23.2	24.7	22.2	25.0	25.0	25.3	25.0	25.3	25.5
	147	1	13.0	14.0	12.7	16.0	17.0	16.5	13.8	17.3	13.0
		2	24.7	24.0	21.5	24.8	24.0	24.8	24.5	25.3	24.5
340	127	1	10.5	8.0	7.0	11.3	15.5	15.8	19.8	13.8	18.5
		2	22.5	21.5	20.2	24.0	25.0	23.5	25.0	24.8	23.8
	137	1	11.0	8.5	8.7	12.0	10.3	13.0	16.8	15.0	18.5
		2	23.2	21.7	24.0	24.3	24.3	23.8	25.8	25.5	25.3
	147	1	8.7	8.7	7.0	13.8	12.8	15.5	18.0	17.3	17.3
		2	21.5	22.0	24.0	23.5	23.8	24.0	25.0	24.0	25.0

*Number 1 represents the average "low" egg production level of 15.2 eggs per hen, per 28-day period. Number 2 represents the average "high" egg production level of 24.4 eggs per hen, per 28-day period.

production is much more constant in the "highs" than in the "lows." It would have taken many more hens on each original treatment to have improved selection by reducing the standard error within each production group.

The average egg weight for the "lows" was 56 grams and for the "highs" 52 grams. During the course of the experiment the "lows" gained an average of 34 grams in body weight per hen and the "highs" lost an average of five grams per hen. These data are presented to show that even when variation in egg production level is reduced, egg weight and body weight are at least two other pathways of nutrient expenditure remaining as uncontrolled factors. However, these two pathways are probably minor as compared to egg production.

Feed Weight, Protein, Energy and Volume Consumption With Egg Production as a Fifth Factor

The average feed weight, protein, energy and feed volume consumption data per hen, per day, per treatment are presented in Tables XXXVI, XXXVII, XXXVIII and XXXIX, respectively. The analyses of variance related to the data presented in these four tables are given in Table XL. A glance at these tables reveals that egg production level is a tremendous factor in controlling feed consumption in laying hens. Conversely, feed consumption level has a tremendous effect upon egg production. Be that as it may, the important point is that feed consumption and egg production cannot be studied independently of each other.

Main Effects: The significant main effects due to dietary weight, protein, energy and volume are identical to those discussed earlier under the original factorial arrangement. In addition, the interaction effects

TABLE XXXVI

AVERAGE GRAMS OF FEED CONSUMED PER HEN, PER DAY
WITH EGG PRODUCTION AS A FIFTH FACTOR

			Protein (Grams)								
			13			16			19		
			Milliliters			Milliliters			Milliliters		
Metabolizable Energy (Kilocalories)	Weight (Grams)	Eggs*	180	230	280	180	230	280	180	230	280
260	127	1	113.4	122.1	112.9	153.4	114.4	123.4	136.3	119.7	125.5
		2	117.9	134.1	115.0	122.2	134.9	123.9	117.9	129.7	122.8
	137	1	127.2	101.9	122.3	114.6	121.9	138.3	118.8	139.6	129.8
		2	142.8	136.1	127.4	155.5	129.5	129.6	143.0	134.7	127.2
	147	1	137.9	141.8	139.4	144.2	146.4	127.5	100.6	130.0	146.4
		2	155.9	138.8	147.5	152.1	149.9	153.9	151.0	142.9	143.5
300	127	1	83.2	95.1	87.9	100.1	88.1	84.2	109.6	98.8	110.3
		2	144.9	110.3	115.0	110.4	109.5	101.7	110.9	102.8	114.5
	137	1	113.7	85.4	95.8	120.0	118.3	104.9	121.2	111.1	114.1
		2	132.5	135.2	117.3	119.7	115.9	128.9	124.9	120.0	114.2
	147	1	104.5	111.4	107.1	134.1	123.8	107.7	110.7	130.9	124.7
		2	161.6	147.5	123.0	122.1	119.9	135.6	136.2	118.1	126.7
340	127	1	73.7	73.3	75.4	73.8	84.1	81.0	94.6	93.3	95.1
		2	90.9	89.2	94.6	99.6	106.5	90.0	103.1	100.8	107.0
	137	1	92.0	73.9	66.2	75.1	90.2	73.6	96.5	90.9	113.2
		2	113.6	116.1	104.9	105.8	102.0	103.9	110.3	101.0	114.8
	147	1	81.1	68.9	67.9	92.0	86.9	110.2	120.6	89.0	94.6
		2	128.3	111.0	114.9	120.5	110.9	117.6	107.2	122.6	93.5

*Number 1 represents the average "low" egg production level of 15.2 eggs per hen, per 28-day period. Number 2 represents the average "high" egg production level of 24.4 eggs per hen, per 28-day period.

TABLE XXXVII

AVERAGE GRAMS OF PROTEIN CONSUMED PER HEN, PER DAY
WITH EGG PRODUCTION AS A FIFTH FACTOR

Metabolizable Energy (Kilocalories)	Weight (Grams)	Eggs*	Protein (Grams) 13: Milliliters 180	13: 230	13: 280	16: Milliliters 180	16: 230	16: 280	19: Milliliters 180	19: 230	19: 280
260	127	1	11.65	12.54	11.59	19.41	14.47	15.61	20.39	17.90	18.77
		2	12.11	13.77	11.82	15.45	17.07	15.68	17.63	19.40	18.37
	137	1	12.11	9.70	11.64	13.44	14.30	16.22	16.47	19.36	18.00
		2	13.59	12.96	12.13	18.23	15.19	15.20	19.84	18.68	17.64
	147	1	12.23	12.58	12.37	15.75	16.00	13.93	13.00	16.81	18.93
		2	13.83	12.31	13.09	16.62	16.39	16.83	19.53	18.47	18.55
300	127	1	8.55	9.77	9.03	12.66	11.14	10.65	16.39	14.78	16.50
		2	14.88	11.33	11.81	13.96	13.85	12.85	16.59	15.38	17.13
	137	1	10.82	8.13	9.12	14.08	13.88	12.31	16.81	15.41	15.83
		2	12.62	12.87	11.17	14.04	13.60	15.12	17.33	16.65	15.84
	147	1	9.27	9.89	9.50	14.65	13.53	11.77	14.32	16.93	16.12
		2	14.33	13.08	10.91	13.35	13.10	14.82	17.61	15.27	16.37
340	127	1	7.57	7.53	7.74	9.33	10.64	10.25	14.15	13.96	14.19
		2	9.33	9.16	9.70	12.59	13.46	11.39	15.42	15.08	15.96
	137	1	8.76	7.03	6.30	8.81	10.58	8.63	13.38	12.61	15.69
		2	10.81	11.06	9.98	12.40	11.96	12.18	15.30	14.01	15.92
	147	1	7.19	6.12	6.02	10.06	9.50	12.04	15.59	11.51	12.23
		2	11.38	9.85	10.19	13.17	12.12	12.85	13.87	15.85	12.09

*Number 1 represents the average "low" egg production level of 15.2 eggs per hen, per 28-day period. Number 2 represents the average "high" egg Production level of 24.4 eggs per hen, per 28-day period.

TABLE XXXVIII

AVERAGE CALORIES OF METABOLIZABLE ENERGY CONSUMED PER HEN, PER DAY WITH EGG PRODUCTION AS A FIFTH FACTOR

			Protein (Grams)								
			13			16			19		
			Milliliters			Milliliters			Milliliters		
Metabolizable Energy (Kilocalories)	Weight (Grams)	Eggs*	180	230	280	180	230	280	180	230	280
260	127	1	234.5	252.5	233.5	317.5	237.0	255.5	282.5	248.5	260.5
		2	243.5	277.0	237.5	253.0	279.5	256.5	244.5	269.0	255.0
	137	1	243.5	195.5	234.0	220.0	234.0	265.5	228.5	268.5	249.5
		2	273.5	261.0	244.0	298.5	248.5	248.5	275.0	259.0	244.5
	147	1	246.5	253.0	249.0	258.0	261.5	228.0	180.0	233.0	262.5
		2	278.0	248.0	263.0	272.0	268.5	275.5	270.5	256.0	257.0
300	127	1	196.5	224.5	207.5	239.0	210.0	201.0	261.5	236.0	263.5
		2	342.5	260.5	272.0	263.5	261.5	242.5	264.5	245.5	273.5
	137	1	248.5	187.0	210.0	265.5	261.5	232.0	268.5	246.0	252.5
		2	290.5	296.0	257.0	264.5	256.5	285.0	276.5	265.5	253.0
	147	1	213.0	227.5	218.5	276.0	255.5	222.0	228.5	270.0	257.0
		2	330.0	301.0	251.0	252.0	247.0	279.5	281.0	244.0	261.0
340	127	1	198.5	197.5	203.0	199.5	227.0	218.5	256.0	252.5	256.5
		2	245.0	240.5	254.5	269.0	287.0	243.0	278.5	272.5	288.0
	137	1	230.0	185.0	165.5	188.0	226.0	184.0	241.5	227.5	283.5
		2	284.0	290.0	262.0	265.0	255.5	260.0	276.5	253.0	287.5
	147	1	189.0	160.5	158.0	215.0	203.0	257.5	281.5	208.0	221.0
		2	299.0	258.5	267.5	281.0	259.0	274.5	250.5	286.5	218.0

*Number 1 represents the average "low" egg production level of 15.2 eggs per hen, per 28-day period. Number 2 represents the average "high" egg production level of 24.4 eggs per hen, per 28-day period.

TABLE XXXIX

AVERAGE MILLILITERS OF FEED CONSUMED PER HEN, PER DAY WITH EGG PRODUCTION AS A FIFTH FACTOR

Metabolizable Energy (Kilocalories)	Weight (Grams)	Eggs*	Protein (Grams) 13			16			19		
			Milliliters			Milliliters			Milliliters		
			180	230	280	180	230	280	180	230	280
260	127	1	160.7	221.0	248.9	217.4	207.2	272.1	193.1	216.7	276.7
		2	167.0	242.7	253.6	173.1	244.3	273.3	167.0	234.8	270.7
	137	1	167.1	171.1	249.8	150.5	204.7	282.7	156.1	234.4	265.2
		2	187.6	228.5	260.3	204.3	217.4	264.8	188.0	226.1	259.9
	147	1	168.8	221.9	265.5	176.6	229.0	242.7	123.2	203.5	279.0
		2	190.8	217.2	231.0	186.3	234.7	293.2	185.0	223.6	273.4
300	127	1	118.0	172.2	193.7	141.9	159.5	185.7	155.3	178.9	243.2
		2	205.4	199.7	253.6	156.4	198.2	224.0	157.2	186.2	252.4
	137	1	149.4	143.3	195.9	157.7	198.7	214.5	159.2	186.5	233.3
		2	174.2	227.0	239.8	157.2	194.6	263.4	164.2	201.5	233.4
	147	1	127.9	174.4	204.0	164.2	193.7	220.1	135.5	204.9	237.5
		2	197.7	230.9	234.2	149.7	187.5	277.0	166.7	184.8	241.2
340	127	1	104.4	132.8	167.5	104.6	152.3	180.0	134.0	168.9	211.0
		2	128.7	161.5	210.1	141.1	192.8	200.1	146.0	182.5	237.4
	137	1	120.9	123.9	135.3	98.7	151.4	150.4	126.7	152.6	231.3
		2	149.3	194.9	214.3	139.0	171.2	212.3	144.9	170.0	234.5
	147	1	99.3	107.9	129.3	112.7	136.0	209.9	147.6	139.3	180.2
		2	157.2	173.7	218.8	147.6	173.4	224.1	131.3	191.9	178.1

*Number 1 represents the average "low" egg production level of 15.2 eggs per hen, per 28-day period. Number 2 represents the average "high" egg production level of 24.4 eggs per hen, per 28-day period.

TABLE XL

ANALYSES OF VARIANCE OF FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION DATA WITH EGG PRODUCTION AS A FIFTH FACTOR

Source of Variation	df	MS Weight	MS Protein	MS Energy	MS Volume
Total (Corrected)	323	527.16	11.33	1,391.46	2,314.40
Treatments	161				
Protein (P)	(2)				
P_{Linear} (l)	1	1,303.95**	1,677.57**	10,194.53**	5,225.39**
$P_{Quadratic}$ (Q)	1	22.44	0.08	49.99	140.97
Energy (E)	(2)				
E_L	1	68,375.94**	904.73**	7,814.93**	191,881.01**
E_Q	1	53.97	0.15	2,714.59	97.41
Weight (W)	(2)				
W_L	1	14,671.22**	0.40	13.50	1.62
W_Q	1	2.87	0.01	0.95	56.60
Volume (V)	(2)				
V_L	1	1,207.63**	12.62*	5,987.57**	986.22**
V_Q	1	222.26	3.04	1,083.77	416.87
Eggs (G)	(1)	17,956.00**	205.22**	92,314.69**	47,912.35**
Interactions	(152)				
P x E	4	496.60*	3.90	2,621.01*	1,175.37*
P x W	4	252.57	4.31	1,310.00	895.92
P x V	4	241.73	2.25	1,146.16	1,026.07
P x G	2	2,567.42**	16.28**	13,400.40**	7,084.79**
E x W	4	249.32	2.74	857.75	672.40
E x V	4	91.21	1.19	462.18	1,618.23**
E x G	2	978.77**	11.04*	8,349.62**	3,128.33**
W x V	4	21.25	0.19	87.16	73.62
W x G	2	317.15	2.24	734.03	362.98
V x G	2	160.78	2.07	655.09	126.77
P x E x W	8	167.79	2.19	889.31	489.46
P x E x V	8	109.93	1.62	525.07	205.42
P x E x G	4	523.97**	4.96	2,713.71*	1,105.50
P x V x W	8	112.56	1.43	596.87	416.67
P x W x G	4	60.29	0.68	276.20	123.13
P x V x G	4	134.73	1.78	501.27	454.13
E x W x V	8	125.72	1.80	619.29	338.92
E x W x G	4	222.86	3.61	1,097.70	508.24
E x V x G	4	98.89	1.52	402.55	467.16
W x V x G	4	124.22	2.11	368.03	222.59
P x E x W x V	16	207.22	3.07	1,016.23	691.61
P x E x W x G	8	182.52	2.14	828.60	396.34
P x W x V x G	8	381.20**	4.78	1,758.94*	944.22*
P x E x V x G	8	288.76	3.66	1,415.92	621.29
E x W x V x G	8	412.07**	6.13*	1,781.52*	903.62
P x E x W x V x G	16	164.23	2.43	781.22	391.65
Error	162	173.76	2.44	867.52	470.35

*Significant at the 5 percent level of probability

**Significant at the 1 percent level of probability of protein x energy and energy x volume upon feed consumption were identical. The recurrence of these significant effects adds strength to the validity of the observations discussed earlier. However, a further discussion of these effects would be redundant. In light of this, it was deemed unnecessary to include the tables of means related to these effects.

The main effects of egg production level upon feed weight, protein, energy and feed volume consumption were all significant at the one percent level of probability (Table XL). In every case consumption was greater at the "high" egg production than at the "low" (Table XLI). From the means in Table XLI, it can be estimated that for each additional egg produced in a 28-day period, it takes an additional 1.52 grams of feed weight, 0.174 grams of protein and 3.66 Calories of energy. In a study of these estimates it must be remembered that egg production was not independent of the other factors. Even though the mean square for the effect of egg production level was much larger than those for the interactions which were present (Table XL), the fact remains that the interactions were present and thus limited the interpretation.

TABLE XLI

MEANS FOR MAIN EFFECT OF EGG PRODUCTION LEVEL UPON FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION

Egg Prod. Level	Feed Weight Cons. (Gm)	Protein Cons. (Gm)	Energy Cons. (Cal)	Feed Volume Cons. (ml)
15.2	107.1	12.64	232.7	179.8
24.4	121.1	14.24	266.4	204.1

<u>Protein x Egg Effects</u>: The protein x egg interaction effect upon

consumption of feed weight, protein, energy and feed volume was significant ($P<0.01$) in each case. At the "low" egg production level the consumption of each factor increased as dietary protein was increased (Table XLII). The reverse was true at the "high" level of egg production. As dietary protein was increased there was a concomitant decrease in the consumption of each factor with the exception of protein. The increase in consumption was much greater with each increase in dietary protein at the "low" egg production than was the corresponding decrease in consumption at the "high" level of production.

TABLE XLII

MEANS FOR INTERACTION EFFECT OF PROTEIN X EGG UPON FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION

Dietary Protein Level*	Feed Wt. Cons. (Gm) at Egg Prod. Level		Protein Cons. (Gm) at Egg Prod. Level		Energy Cons. (Cal) at Egg Prod. Level		Vol. Cons. (Ml) at Egg Prod. Level	
	1	2	1	2	1	2	1	2
1	99.1	124.7	9.43	11.85	213.4	271.4	165.7	207.4
2	108.6	121.2	12.72	14.20	235.5	264.7	182.0	203.7
3	113.5	120.0	15.78	16.66	249.1	263.2	191.6	201.2

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein.

This interaction is thought to be due to at least two factors, for which reasonable explanations can be given. First, protein intake was high enough at the "high" egg production level, which made some protein available to be used as energy, thus slightly decreasing feed consumption at each increase in dietary protein. The second fact is that even though an attempt was made to select two constant levels of egg production, there was an increase in egg production at the "low" level (Table XXXV) with each increase in dietary protein. Therefore, the effect of protein intake upon egg production was not completely eliminated. Consequently, the increase in egg production at each increase in dietary protein could be a factor in causing feed consumption to increase as dietary protein was increased at the "low" level of egg production.

Energy x Egg Effects: The energy x egg interaction was statistically significant for the consumption of feed weight, protein, energy and feed volume (Table XL). From the means in Table XLIII it can be delineated that as dietary energy was increased there was a corresponding decrease in feed consumption at both egg production levels. However, the decrease was much greater at the "low" level than at the "high" level.

TABLE XLIII

MEANS FOR INTERACTION EFFECT OF ENERGY X EGG UPON FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION

Dietary Energy Level*	Feed Wt. Cons. (Gm) at Egg Prod. Level		Protein Cons. (Gm) at Egg Prod. Level		Energy Cons. (Cal) at Egg Prod. Level		Vol. Cons. (Ml) at Egg Prod. Level	
	1	2	1	2	1	2	1	2
1	127.7	136.3	15.00	15.94	245.7	261.4	215.0	227.7
2	107.3	122.9	12.66	14.29	236.3	271.0	179.6	205.8
3	86.2	106.7	10.27	12.48	216.1	266.9	144.8	178.7

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein.

These results indicate that, under conditions where large quantities of energy are spent in the formation of eggs, the influence of dietary energy upon feed consumption is reduced. In the situation where less energy is used for egg production, energy exerts more control over feed consumption.

The quadratic effect of dietary energy upon energy intake, which is again apparent from the energy consumption data in Table XLIII, appears to be greater at the "low" level of egg production than at the "high." This would add support to the assumption that, when less energy is needed for egg production, dietary energy exerts more influence upon feed consumption.

Protein x Energy x Egg Effects: This interaction effect was only significant for feed weight and energy consumption. However, the mean squares for the protein x energy x egg effect upon protein and volume consumption approached significance at the five percent level of probability. Therefore, the table of means (Table XLIV) for the interaction effect of protein x energy x egg includes all four dietary factors. The existence of this three-way interaction is not surprising in light of the fact that the interaction effects of protein x energy, protein x egg and energy x egg were all significant. The effect of each one of these two-way interactions can be delineated from the means in Table XLIV. However, the delineation of these effects provides no additional information for discussion.

Protein x Weight x Volume x Egg Effects: The interaction effects of protein x weight x volume x egg upon feed weight, energy and feed volume consumption were all statistically significant (Table XL). The mean square for this interaction effect upon protein consumption was approaching significance. The means for the effect of protein x weight x volume x egg upon feed weight, protein, energy and feed volume consumption

TABLE XLIV

MEANS FOR INTERACTION EFFECT OF PROTEIN X ENERGY X EGG UPON FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION

Feed Wt. Cons. (Gm) at Egg Prod. Level*	Protein Level* 1			2			3		
	Energy Level			Energy Level			Energy Level		
	1	2	3	1	2	3	1	2	3
1	124	98	75	132	109	85	127	115	99
2	135	132	107	139	118	106	135	119	107
Protein Cons. (Gm) at Egg Prod. Level									
1	11.8	9.3	7.1	15.5	12.7	10.0	17.7	15.9	13.7
2	12.8	12.6	10.2	16.3	13.9	12.5	18.7	16.5	14.8
Energy Cons. (Cal) at Egg Prod. Level									
1	238	215	187	253	240	213	246	254	248
2	258	289	267	267	261	266	259	263	268
Feed Vol. Cons. (Ml) at Egg Prod. Level									
1	208	164	125	220	182	144	216	193	166
2	225	218	179	232	201	178	225	199	180

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein and energy.

are presented in Tables XLV, XLVI, XLVII and XLVIII, respectively.

At the "low" level of egg production the effects upon feed weight consumption due to changes in dietary weight and volume were very erratic. The only consistent pattern observed was with the high level of dietary volume (280 ml), where each increase in dietary weight resulted in an increase in feed weight consumption (Table XLV). However, this increase in feed weight consumption was not great enough to result in an increase in nutrient consumption (Tables XLVI and XLVII).

It was postulated that if evidence were available to indicate why livability was better as dietary weight was increased (Table IX), it would appear in the nutrient consumption data of those hens that were laying at a relatively "low" rate. From this standpoint, it is interesting to note that at the "low" level of egg production, increases in dietary volume caused very little decrease in nutrient consumption (Tables XLVI and XLVII). In fact those hens fed the third level of dietary protein (19 grams) in combination with the high level of volume (280 ml) and the two higher levels of weight (137 and 147 grams) actually consumed more protein than those fed the lower level of weight (127 grams) and the two lower levels of volume (180 and 230 ml). Although this evidence is very meager, it is at least an indication that, at certain dietary factor combinations, protein consumption and therefore livability were higher at the higher levels of dietary weight.

At the second level of egg production, dietary volume appeared to be exerting its normal physiological influence upon feed consumption. As dietary volume was increased there was, for the most part, a concurrent decrease in feed consumption (Table XLV). However, there is evidence (Table XLVI) that increases in dietary volume do not always result in

TABLE XLV

MEANS FOR INTERACTION EFFECT OF PROTEIN X WEIGHT X VOLUME X EGG UPON FEED WEIGHT CONSUMPTION (GRAMS)

		Egg Production Level 1			Egg Production Level 2		
		Volume Level			Volume Level		
Protein Level	Weight Level	1	2	3	1	2	3
1	1	90.1	96.8	92.0	117.9	111.2	108.2
	2	110.9	87.0	94.7	129.6	129.1	116.5
	3	107.8	107.3	104.8	148.6	132.4	128.4
2	1	109.1	95.5	96.2	110.7	116.9	105.2
	2	103.2	110.1	105.6	127.0	115.8	120.8
	3	123.4	119.0	115.1	131.5	126.9	135.7
3	1	113.5	103.9	110.3	110.6	111.1	114.7
	2	112.1	113.8	119.0	126.1	118.5	118.7
	3	110.6	116.6	121.9	131.5	127.8	121.2

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein, weight and volume.

TABLE XLVI

MEANS FOR INTERACTION EFFECT OF PROTEIN X WEIGHT X VOLUME X EGG UPON PROTEIN CONSUMPTION (GRAMS)

		Egg Production Level 1			Egg Production Level 2		
		Volume Level			Volume Level		
Protein Level	Weight Level	1	2	3	1	2	3
1	1	9.25	9.94	9.45	12.10	11.42	11.11
	2	10.56	8.29	9.02	12.34	12.29	11.09
	3	9.56	9.53	9.29	13.18	11.74	11.39
2	1	13.80	12.08	12.17	14.00	14.79	13.30
	2	12.11	12.92	12.39	14.89	13.58	14.16
	3	13.49	13.01	12.58	14.38	13.87	14.83
3	1	16.97	15.54	16.48	16.55	16.62	17.15
	2	15.55	15.79	16.50	17.49	16.44	16.46
	3	14.30	15.08	15.76	17.00	16.53	15.67

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein, weight and volume.

TABLE XLVII

MEANS FOR INTERACTION EFFECT OF PROTEIN X WEIGHT X VOLUME X EGG UPON ENERGY CONSUMPTION (CALORIES)

		Egg Production Level 1			Egg Production Level 2		
		Volume Level			Volume Level		
Protein Level	Weight Level	1	2	3	1	2	3
1	1	209.8	224.8	214.7	277.0	259.3	254.7
	2	240.7	189.2	203.2	282.7	282.3	254.3
	3	216.2	213.7	208.5	302.3	269.2	260.5
2	1	252.0	224.7	225.0	261.8	276.0	247.3
	2	224.5	240.5	227.2	276.0	253.5	264.5
	3	249.7	240.0	235.8	268.3	258.2	276.5
3	1	266.7	245.7	260.2	262.5	262.3	272.2
	2	246.2	247.3	261.8	276.0	259.2	261.7
	3	230.0	237.0	246.8	267.3	262.2	245.3

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein, weight and volume.

TABLE XLVIII

MEANS FOR INTERACTION EFFECT OF PROTEIN X WEIGHT X VOLUME X EGG UPON FEED VOLUME CONSUMPTION (ML)

			Egg Production Level 1			Egg Production Level 2		
			Volume Level			Volume Level		
Protein Level	Weight Level		1	2	3	1	2	3
1		1	127.7	175.3	203.3	167.0	201.3	239.1
		2	145.8	146.1	193.6	170.3	216.8	238.1
		3	132.0	168.1	199.6	181.9	207.2	244.7
2		1	154.6	173.0	212.6	156.8	211.8	232.4
		2	135.6	184.9	215.9	166.8	194.4	246.8
		3	151.1	186.2	224.2	161.2	198.6	264.8
3		1	160.8	188.2	243.6	156.7	201.2	253.5
		2	147.3	191.2	243.2	165.7	199.1	242.6
		3	135.4	182.5	232.2	161.0	200.1	230.9

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary protein, weight and volume.

decreases in protein consumption. This was especially true at the highest level of dietary weight.

Although it is apparent that protein, weight, volume and egg production are not independent of each other, it is extremely difficult to interpret the full meaning of any four-way interaction. Therefore, the author submits that many possible explanations of this interaction may have been overlooked.

Energy x Weight x Volume x Egg Effects: The effects of this interaction upon feed weight, protein and energy consumption were statistically significant and its effect upon volume consumption approached significance (Table XL). The means for feed weight, protein, energy and volume consumption are listed in Tables XLIX, L, LI, and LII, respectively.

The energy x egg interaction that was discussed earlier is discernible from these means, as are many of the other effects that have already been described. For example, feed and nutrient consumption were generally higher at the "high" than at the "low" level of egg production (Tables XLIX, L and LII). The quadratic effect of dietary energy upon energy intake (Table LI) was evident at both egg production levels, but appeared to be greater at the "low" level of production.

These facts again make it clear that dietary energy, weight, volume and egg production level are not independent of each other. They should all be considered in any attempt to estimate the feed consumption of laying hens. These two latter interaction effects (P x W x V x G and E x W x V x G) reemphasize the need at least to hold dietary weight and volume constant in nutritional experiments designed to determine the dietary nutrient requirements of laying hens. The existence of these interactions also points out that nutrient requirements should be studied on the basis of a particular egg production level.

TABLE XLIX

MEANS FOR INTERACTION EFFECT OF ENERGY X WEIGHT X VOLUME X EGG UPON FEED WEIGHT CONSUMPTION (GRAMS)

			Egg Production Level 1			Egg Production Level 2		
			Volume Level			Volume Level		
			1	2	3	1	2	3
Energy Level 1	Weight Level	1	134.4	118.7	120.6	119.3	132.9	120.6
		2	120.2	121.1	130.1	147.1	133.4	128.0
		3	127.5	139.4	137.7	153.0	143.8	148.3
Energy Level 2	Weight Level	1	97.6	94.0	94.1	122.1	107.5	110.4
		2	118.3	104.9	104.9	125.7	123.7	120.1
		3	116.4	122.0	113.1	139.9	128.5	128.4
Energy Level 3	Weight Level	1	80.7	83.6	83.8	97.8	98.8	97.2
		2	87.8	85.0	84.3	109.9	106.3	107.8
		3	97.9	81.6	90.9	118.7	114.8	108.6

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary energy, weight and volume.

TABLE L

MEANS FOR INTERACTION EFFECT OF ENERGY X WEIGHT X VOLUME X EGG UPON PROTEIN CONSUMPTION (GRAMS)

Energy Level	Weight Level	Egg Production Level 1, Volume Level 1	Egg 1, Volume 2	Egg 1, Volume 3	Egg Production Level 2, Volume Level 1	Egg 2, Volume 2	Egg 2, Volume 3
1	1	17.15	14.97	15.32	15.06	16.74	15.29
	2	14.00	14.45	15.28	17.22	15.61	14.99
	3	13.66	15.13	15.07	16.66	15.72	16.15
2	1	12.53	11.89	12.06	15.14	13.52	13.93
	2	13.90	12.47	12.42	14.66	14.37	14.04
	3	12.74	13.45	12.46	15.10	13.81	14.03
3	1	10.35	10.71	10.72	12.44	12.57	12.35
	2	10.31	10.07	10.21	12.84	12.34	12.69
	3	10.94	9.04	10.10	12.80	12.61	11.71

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary energy, weight and volume.

TABLE LI

MEANS FOR INTERACTION EFFECT OF ENERGY X WEIGHT X VOLUME X EGG UPON ENERGY CONSUMPTION (CALORIES)

			Egg Production Level 1			Egg Production Level 2		
			Volume Level			Volume Level		
Energy Level		Weight Level	1	2	3	1	2	3
1		1	278.2	246.0	249.8	247.0	275.2	249.7
		2	230.7	232.7	249.7	282.3	256.2	245.7
		3	228.2	273.5	249.2	257.5	246.5	265.2
2		1	232.3	223.5	224.0	290.2	255.8	262.7
		2	260.8	231.5	231.5	277.2	272.7	265.0
		3	239.2	251.0	232.5	287.7	264.0	263.8
3		1	218.0	225.7	226.0	264.2	266.7	261.8
		2	219.8	212.8	211.0	275.2	266.2	269.8
		3	228.5	190.5	212.2	276.8	268.0	253.3

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary energy, weight and volume.

TABLE LII

MEANS FOR INTERACTION EFFECT OF ENERGY X WEIGHT X VOLUME X EGG UPON FEED VOLUME CONSUMPTION (ML)

		Egg Production Level 1			Egg Production Level 2		
		Volume Level			Volume Level		
Energy Level	Weight Level	1	2	3	1	2	3
1	1	190.4	214.9	265.9	169.0	240.6	265.8
	2	157.9	203.4	265.9	193.3	224.0	261.7
	3	156.2	218.1	262.4	187.4	225.1	282.5
2	1	138.4	170.2	207.5	173.0	194.7	243.3
	2	155.4	176.2	214.5	165.2	207.7	245.5
	3	142.5	191.0	220.5	171.4	201.0	250.8
3	1	114.3	151.3	186.2	138.6	178.9	215.8
	2	115.4	142.7	172.3	144.4	178.6	220.3
	3	119.8	127.7	173.1	145.3	179.7	206.9

*See Table XXXIX for the quantitative equivalents of levels 1 and 2 for egg production and levels 1, 2 and 3 for dietary energy, weight and volume.

Analyses of Variance at "Low" Egg Production Level

Analyses of variance were performed on the data from which the "low" egg production level (No. 1) means in Tables XXXVI, XXXVII, XXXVIII and XXXIX were calculated. These tables contain the means for feed weight, protein, energy and feed volume consumption per hen, per day, per treatment, respectively.

The analyses of variance (Table LIII) for the low egg production level reveal at least one interesting fact. Dietary volume had no significant effect upon feed weight, protein or energy consumption. In all other analyses dietary volume did have a significant effect upon feed weight, protein and energy consumption. However, it was noted from the data in Tables XLVI and XLVII that, at the "low" level of egg production, increases in dietary volume did not alter nutrient consumption in any consistent manner. Therefore, these analyses substantiate the earlier observation.

The failure of the gastrointestinal physiological mechanism to be triggered may have been a result of the low feed consumption associated with "low" egg production. The hens' "stomachs" were never filled to the point where dietary volume could exert its main effect.

Another noticeable difference between the analyses in Table LIII and those presented earlier (Tables XII, XIX, XXXI and XL) is that the only significant interaction effect was protein x energy. This may be a result of the small number of hens (2) per treatment, which permitted only 81 degrees of freedom in the error term. In this situation, the error mean square was large relative to that from the original factorial where there were seven hens per treatment and 486 degrees of freedom for

TABLE LIII

ANALYSES OF VARIANCE OF FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION DATA AT "LOW" EGG PRODUCTION

Source of Variation	df	MS Weight	MS Protein	MS Energy	MS Volume
Total (Corrected)	161	585.10	13.69	1,468.38	2,545.17
Treatments	[80]				
Protein (P)	(2)				
P_{Linear} (L)	1	5,644.69**	1,086.55**	34,334.16**	18,081.11**
$P_{Quadratic}$ (Q)	1	187.89	0.53	660.26	404.59
Energy (E)	(2)				
E_L	1	46,655.51**	604.48**	23,640.33**	133,212.55**
E_Q	1	2.59	0.03	1,056.79	2.42
Weight (W)	(2)				
W_L	1	4,730.89**	3.22	699.51	255.47
W_Q	1	48.10	0.46	179.79	207.19
Volume (V)	(2)				
V_L	1	148.85	1.27	920.85	155,242.92**
V_Q	1	176.91	2.64	962.35	508.40
Interaction	(72)				
P x E	4	578.19*	2.49	3,466.11*	1,375.11
P x W	4	208.29	3.87	1,054.18	667.57
P x V	4	194.58	2.73	810.98	1,016.66
E x W	4	390.01	5.92	1,853.73	1,076.92
E x V	4	108.94	1.48	458.05	1,645.82
W x V	4	56.64	1.12	159.13	117.42
P x E x W	8	247.84	2.89	1,059.61	615.04
P x E x V	8	258.56	3.75	1,281.63	483.96
P x V x W	8	312.88	3.75	1,403.76	686.73
E x W x V	8	328.87	4.99	1,485.82	671.44
P x E x W x V	16	253.54	3.94	1,123.20	722.90
Error	81	212.56	3.07	1,023.80	605.37

*Significant at the five percent level of probability

**Significant at the one percent level of probability

error. The point is that the interactions that were significant in the original factorial may exist at the "low" egg production level, but there were not enough hens on each treatment to show the effect to be significant. On the other hand, it is quite possible that at a constant egg production level these 3- and 4-way interactions do not exist. If this is the case, it will be possible to design experiments for the determination of the quantitative effects of these four factors at different egg production levels. Even though protein and energy are interrelated, this would be much less frustrating and confusing to interpret than if all four dietary factors are interrelated.

Analyses of Variance at "High" Egg Production Level

The analyses of variance performed on the data from the "high" egg producers are presented in Table LIV. The means related to these analyses are given in Tables XXXVI, XXXVII, XXXVIII and XXXIX under the number 2 level of egg production.

An inspection of these analyses reveals that neither dietary protein nor energy exerted any significant influence upon energy consumption. It is reasoned that this was probably due to the small number of hens (2) involved, and to the high level of energy needed to support an average egg production of 87 percent.

As was the case with the "low" egg production level, it was encouraging to find protein x energy to be the only significant interaction effect. In fact, the mean squares due to protein x energy x weight x volume were less, in most cases, than were the error mean squares. This is a stronger indication than was noted in the discussion of the "low" egg production analyses that at a constant egg production level the four-way interactions

TABLE LIV

ANALYSES OF VARIANCE OF FEED WEIGHT, PROTEIN, ENERGY AND FEED VOLUME CONSUMPTION DATA AT "HIGH" EGG PRODUCTION

Source of Variation	df	MS			
		Weight	Protein	Energy	Volume
Total (Corrected)	161	360.96	7.75	749.80	1,800.41
Treatments	[80]				
Protein (P)	(2)				
P_{Linear} (L)	1	578.55*	623.04**	1,806.63	1,039.22
$P_{Quadratic}$ (Q)	1	50.10	0.10	244.27	11.01
Energy (E)	(2)				
E_L	1	23,654.72**	322.24**	828.67	64,771.45**
E_Q	1	74.62	0.21	1,702.97	248.68
Weight (W)	(2)				
W_L	1	10,508.24**	0.80	447.25	307.73
W_Q	1	20.16	0.39	155.76	13.80
Volume (V)	(2)				
V_L	1	1,365.29**	15.19**	6,254.50*	165,863.99**
V_Q	1	60.36	0.71	253.81	41.31
Interaction	(72)				
P x E	4	442.38*	6.36*	1,868.62*	905.76*
P x W	4	104.57	1.12	523.03	351.48
P x V	4	181.88	1.30	836.45	463.54
E x W	4	82.17	0.43	101.73	103.72
E x V	4	81.17	1.23	406.68	439.57
W x V	4	88.83	1.18	296.06	178.74
P x E x W	8	102.47	1.44	658.30	270.76
P x E x V	8	140.13	1.53	659.37	342.74
P x V x W	8	180.88	2.47	952.05	674.18
E x W x V	8	208.92	2.94	914.99	571.10
P x E x W x V	16	117.92	1.56	611.25	360.36
Error	81	134.97	1.82	711.23	335.33

*Significant at the five percent level of probability

**Significant at the one percent level of probability

may not exist.

The linear main effects of dietary protein, energy, weight and volume upon feed weight consumption were all statistically significant. The effects of the latter three factors were significant at the one percent level of probability, while the effect due to dietary protein was significant at the five percent level (Table LIV). From the means in Table LV it can be seen that the directions of these linear main effects were the same as those observed under the original factorial (Table XIII) except for protein. A possible reason for the opposite effect of dietary protein upon feed weight consumption was given in the discussion of protein x egg effects (Table XLII).

TABLE LV

MEANS FOR MAIN EFFECTS OF DIETARY PROTEIN, ENERGY, WEIGHT AND VOLUME UPON GRAMS OF FEED WEIGHT CONSUMPTION AT A "HIGH" EGG PRODUCTION

	Level*		
Factor	1	2	3
Protein	124.65	121.16	120.02
Energy	136.26	122.91	106.66
Weight	111.83	122.44	131.56
Volume	125.93	121.08	118.82

*See Table XXXIX for the dietary equivalents of levels 1, 2 and 3 for each of the four factors.

Discounting the effect of dietary protein as being indirect, it is interesting to look at the quantitative <u>estimates</u> of the effects of the other three factors under conditions of a relatively high and relatively constant egg production level. For each extra dietary Calorie added beyond 260 Calories there was a concomitant reduction in feed consumption of 0.37 grams. For each extra gram of dietary weight above 127 grams there was a concurrent increase in feed weight consumption of 0.986 grams. There was a reduction in feed weight consumption of 0.0711 grams for each extra milliliter of dietary volume above 180 milliliters. Thus, two sets of quantitative estimates for the effects of dietary energy, weight and volume upon feed weight consumption are available from this experiment, one from this "high" constant egg production level and the other from the feed weight consumption data under the original factorial. These two sets of estimates are quite different. In the case of "high" egg production, the effect due to dietary energy was less than in the original factorial, while the effects due to dietary volume and weight were greater. This leaves little doubt that such estimates will have to be made under many sets of conditions (particularly egg production levels) before absolute values for the prediction of future feed consumption of laying hens can be calculated.

SUMMARY AND CONCLUSIONS

An experiment with a 3^4 factorial arrangement of treatments was designed to study the effects of dietary protein, energy, weight and volume upon feed consumption and the reproductive performance of laying hens. Mortality was relatively high among the hens fed certain experimental diets. This mortality was found to be due to low protein consumption. Since mortality was due to treatment effect, all means reported herein were on a hen-housed basis.

Dietary protein, energy, weight and volume were all found to exert a significant linear effect upon feed weight consumption. As dietary protein was increased there was a concurrent increase in protein consumption and in feed weight consumption. However, it was concluded that the effect of dietary protein upon feed weight consumption was an indirect one resulting from an increase in egg production as protein intake increased. The increase in egg production brought about an increase in feed weight consumption. At a "high" constant level of egg production, the significant linear effect upon feed weight consumption due to dietary protein was the reverse of the previous situation. In this case protein consumption was high enough so that some of the protein was available to be used as energy. Consequently, dietary protein had the same effect upon feed weight consumption as did dietary energy. This is additional evidence that the effect of dietary protein upon feed consumption is an indirect one which is dependent upon the circumstances under which it is fed.

As dietary energy was increased a quadratic effect was exerted upon energy consumption. The highest level (340 Calories) of dietary energy actually caused the intake of energy to be reduced below that of the second level (300 Calories). This effect of dietary energy upon the consumption of feed weight was not significant. However, the mean square for the quadratic effect of dietary energy upon feed weight consumption was large and this quadratic trend could be delineated from the feed weight consumption means.

Under all sets of conditions studied, the most prominent effect of increasing levels of dietary energy was to decrease feed weight consumption. This effect was statistically significant at the one percent level of probability in all cases.

The only discernible main effects of dietary weight were upon feed weight consumption and mortality. As dietary weight was increased there was a significant linear increase in feed weight consumption and a significant linear decrease in mortality. Dietary weight appeared in several of the significant interactions, which indicates that it does have an important influence upon the consumption of dietary nutrients. It can be concluded that further experimentation needs to be conducted using a wider range of dietary weight levels.

Generally, the effect of increases in dietary volume was to decrease the consumption of all factors except volume consumption, which increased. As dietary volume was increased there was a corresponding significant linear decrease in feed weight, protein and energy consumption. There was one major exception. At a "low" level of egg production, the effect of dietary volume upon protein and energy intake was not significant.

Under these conditions feed volume consumption was low and it was postulated that a certain minimum volume consumption is necessary before the physiological mechanism triggered by dietary volume is brought into action.

Quantitative estimates of the effects of dietary energy, weight and volume upon feed weight consumption were determined and discussed under two sets of conditions. Quantitative estimates for the effect of egg production level upon feed weight consumption were determined. The primary purpose of these estimates was to demonstrate the need for additional experiments in which these same five factors are studied singly and in combination at different levels than were employed in this experiment.

The existence of several significant interaction effects demonstrated that the factors under study were not independent of each other. These interactions were presented and discussed to the best of the author's ability. The most promising aspect of this discussion was that at constant egg production levels the only significant interaction effect was protein x energy. Fortunately more basic knowledge is available concerning this interaction than for any of the others, thus making it possible to interpret its meaning. Another encouraging aspect of this type of experimentation was that the mean squares for the main effects were much larger than those for the interaction effects. This indicates that, even though the main effects of the factors studied are not independent, much knowledge can be gained by studying their action.

Dietary protein and energy exerted the most influence upon the reproductive performance of laying hens. Under the conditions of this experiment, the hens produced the best (74 percent egg production with eggs that weighed an average of 54 grams) when diets were fed that contained 19 grams of protein in combination with 260 Calories of energy.

The consumption of protein and energy under these conditions was approximately 16.5 grams and 249 Calories, respectively. However, in this experiment, both dietary volume and weight were controlled. Therefore, care must be taken in the application of these findings where these two factors are not controlled.

The only main effect of dietary volume upon the reproductive performance of laying hens was upon body weight change. As dietary volume was increased there was a resultant decrease in body weight gain. Hens fed the third level (280 ml) of dietary volume gained an average of 13.5 grams during the course of the experiment. This might be considered the best level of dietary volume to feed, but there was a significant weight x volume interaction effect upon body weight change. Therefore, the level of dietary volume must be considered at some particular level of dietary weight. The best combination used in this experiment, as far as body weight gain was concerned, was the 280-milliliter diet with 137 grams of weight.

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VITA

Earl William Gleaves

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE ACTION AND INTERACTION OF PHYSIOLOGICAL FOOD INTAKE REGULATORS IN THE LAYING HEN

Major Field: Animal Nutrition

Biographical:

Personal Data: Born: Miami, Oklahoma, April 3, 1930.

Education: Attended grade school at Spring River Grade School located in Ottawa County, Oklahoma; graduated from Wyandotte High School, Wyandotte, Oklahoma in 1947; received the Bachelor of Science degree from the Oklahoma State University, with a major in Poultry Science in May of 1953; received the Master of Science degree from the Oklahoma State University, with a major in Poultry Nutrition in August, 1961; completed requirements for the Doctor of Philosophy degree in August, 1964.

Professional experience: Manager of a laying hen farm in Santa Fe, New Mexico, May, 1953-April, 1956; Manager of a farm store in Parsons, Kansas, April, 1956-September, 1957; Research Assistant and Instructor, Poultry Science Department, Oklahoma State University, September, 1957 to present.

Organizations: Alpha Zeta, Phi Sigma, Sigma Xi and Poultry Science Association.

Date of Final Examination: August, 1964.