METABOLIZABLE ENERGY IN SIX FOODS AND EFFECT OF DIET ON BODY FATTY-ACIDS IN BOBWHITES

by

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INTRODUCTION

The question of the influence of dietary fatty-acid content on an animal's fatty-acid content is the topic of interest in this research. The original researchers in this area were animal breeders, concerned about the quality and flavor of meat, eggs, and milk. Early avian fat studies, such as the work of Cruickshank in 1934 on eggs and hen fat as influenced by diet, were done on poultry. Biologists later became interested in applying the question of dietary influence to migratory birds which deposit large stores of fat before migration. From there, researchers have tried to determine if, and to what extent, diets influence the fatty-acid deposits of game birds and many passerine species.

Controlled experiments on birds were first done in the laboratory with poultry, while few of the studies on wild birds were performed with factors such as temperature, photoperiod, or even diet controlled. One factor in common in these experiments has been the analysis of data by direct comparison of the fats in the diet to those of the animal fed that diet to determine if the diet influenced the fat deposits of the animal.

This study attempts to determine the influence of particular seeds on the fatty-acid composition of Bobwhites (Colinus virginanus) under controlled, simulated winter conditions by: (1) the direct comparison of the fatty-acid compositions of both the diet and the quail and (2) the use of a statistical technique called Discriminant Analysis. The metabolizable efficiency of quail on those diets will also be determined. In addition, the fatty-acid compositions of male and female Bobwhites and their eggs as influenced by a carbamate pesticide during an EPA small-pen test will be studied.

LITERATURE REVIEW

Energy Requirements

Homeothermic organisms must face particular "costs" in maintaining their body temperature. This cost of metabolism increases as the ambient temperature decreases. King and Farner (1961) have shown that a thermoneutral zone exists at which an animal can maintain its body temperature by insulatory changes alone. Body temperature is maintained below this zone by increasing the rate of metabolism (Geers and Decuypere, 1978). As an example, the existence metabolism (defined by King, 1974) of House Sparrows (Passer domesticus) has been shown to vary inversely with the mean midwinter temperature (Kendeigh and Blem, 1974). Case (1971: 51) presented equations which predict the existence energy of adult female Bobwhites (Colinus virginianus) at 1°C and 10L:14D photoperiod to be 48.14 Kcal/day; intake energy (63.22 Kcal/day) less excretory matter (15.08 Kcal/day). Results for adult male Bobwhites in the same conditions were not significantly different.

The metabolizable energy of food is important because a net synthesis and storage of fat cannot occur unless caloric input exceeds output (King, 1972). Robel (1979) and Taylor (1977) have shown that as little as 30% of the gross energy of certain foods is metabolized by various avian species. Robel et al. (1974) found no evidence that Bobwhites select high energy food but rather Bobwhites appear to base food consumption on availability. If the more abundant food is one of low metabolizable energy, caloric expenditures may exceed intake and a weight loss would ensue as some bodily reserves are metabolized. Robel (1979) states that Bobwhites in a 20% energy-deficient diet for 6 days at 2°C and 10L:14D will lose approximately 10% of their total body weight. Obtaining this energy for the maintenance

of internal body temperature becomes more critical when food is unavailable. Rubner's 1910 law of compensation held that "the heat increment of feeding contributes toward temperature regulation at low ambient temperatures. This heat is not available in the fasting bird, hence standard metabolism must increase more rapidly at low temperatures to compensate for the absence of the specific dynamic action" (Kendeigh, 1969: 444).

Robel and Linderman (1966) determined that wild quail can lose 15 to 20% of their winter weight and Robel (1969) reported Bobwhites can lose nearly 75% of their body fat during winter food stress. Errington (1939) found that some food-stressed quail die at approximately 75% of their normal body weight while others can withstand up to a 50% loss of body weight. Beardmore and Robel (1976), Bisset (1972) and Clements (1970) have shown that Bobwhites under laboratory conditions survive after being dietetically stressed to 80% of their normal body weight and 25% of their winter fat reserves. This weight and fat can be regained within 10 to 14 days if a nutritious diet is provided. Any addition of new cells such as that seen during weight gain and the refilling of fat cells is a form of growth. Growth rates after nutritional stress have been shown to be different than growth rates during regular feeding. "Growth usually proceeds at enhanced rates during realimentation following periods of restricted nutrition or malnutrition, and this phenomenon is termed <u>compensatory growth</u>." "High compensatory growth rates may result in the normal body size of the adult being exceeded, a phenomenon termed over-compensation. This is often associated with an excess of deposition of fat in the body" (Wilson and Osbourn, 1960: 354).

<u>Fat Utilization</u>

Fat reserves are particularly valuable in meeting the metabolic cost of maintaining an internal temperature during periods of fasting. The total

catabolism of the common dietary fatty-acids leads to a 41% potential energy trapping efficiency in forms of high-energy bonds (Hazelwood, 1972). King (1972: 200) lists the adaptive values of periodic fat depositions as providing fuel for migratory flight, for increased heat production in winter thermoregulation, and for extra survival time when food-finding is difficult. The mean body mass for adult American Goldfinches (Spinus tristis) increases from a July low of 11.4 g to a December/January high of 15.1 g. The seasonal variation in lipids accounted for a major portion of this observed change in mass (Carey et al., 1978).

American Goldfinches fasting in the winter for 17 h overnight at -10°C use significant amounts of body lipids (Carey et al., 1978). The question of how long a bird can actually survive with average reserves is posed often. Previous data for small passerines suggest that it is approximately 1 day (King, 1972), and 1 to 3 days for Bobwhites (Bisset, 1972).

Fat Composition

It has so far been demonstrated in the preceding discussion that homeotherms have particularly high energy needs which become increasingly greater in colder temperatures. Fat reserves can be metabolized to temporarily meet some of the energy demand. When fat reserves are replenished, the organism forms a particular fatty-acid profile. The question of what factor(s) influence or direct the formation of a bird's fatty-acid composition has been examined for many species. Most studies involving seasonal differences in the fatty-acid compositions were non-experimental. Barnett (1970) studied fatty-acids of the House Sparrow (Passer domesticus), Bower and Helms (1968) the Slate-Colored Junco (Junco hyemalis), Moss and Lough (1968) examined four species of the family Tetraonidae, and West and Meng (1968b)

the Willow Ptarmigan (Lagopus lagopus). Differences in fatty-acid compositions between seasons of capture were found and, because the diets of these birds vary with seasons, these differences were ascribed at least in part to dietary influences. In contrast, Zar (1977b) found no significant differences in the fatty-acid compositions of the House Sparrow due to either season of capture or sex. No attempts were made to determine the effect of the changing environment alone on the fatty-acid profiles.

Morton and Liebman (1974) experimented with captive White-Crowned Sparrows (Zonotrichia atricapilla) fed a constant diet and subject to natural changes in the seasons. They found no seasonal fluctations in fat compositions and concluded that most seasonal variations seen naturally were due to diet and not to the environmental changes. West and Meng (1968a) performed an experiment to test the effects of season on the fatty-acid composition of Redpolls (Acanthis flammea). They did this by varying the diets under one season. All the fatty-acid profiles were the same regardless of the diet. It was then concluded that the major factor affecting the fatty-acid composition was the environment and not the diet.

Several non-experimental field studies on fatty-acids discovered differences between species of birds collected in the same season. Tanhuanpaa and Pulliainen (1969) examined the major fatty-acid composition of organ fats in the Willow Ptarmigan and the Rock Ptarmigan (Lagopus mutus). They discovered differences in the fatty-acid profiles between the species and attributed them to dietary differences. A later study by Tanhuanpaa and Pulliainen (1975) on Crossbills (Loxia curvirostra and L. lucoptera), uncovered no differences in the compositions due to species. Both species had been feeding exclusively on spruce seeds and it was concluded that the depot fat of the Crossbills did not reflect the fatty-acids in the diet.

Walker (1964) analyzed the carcasses of 4 species of migratory birds killed by a radio tower on the birds' autumnal migration and of 1 bird killed in its spring migration. Three of the autumn species were insectivorous; the Magnolia Warbler (Dendroica magnolia), the Tennessee Warbler (Vermivora peregrina), and the Red-Eyed Vireo (Vireo olivaceus). The fourth autumn species, the Bobolink (Dolichonyx oryzivorus), is considered to be graminiverous. The species found during its spring migration was the Sora Rail (Porzana carolina), a fresh-water marsh bird. Differences were found between the fatty-acids of all these birds. Walker (1964: 64) concluded that "the similarities and differences observed suggest relationships to (a) type of food ingested, whether animal, vegetable, or mixed, (b) character of enzymatic system in lipid metabolism for the species, and (c) the stage of deposition-depletion cycle in the migratory period."

Some other experiments have been conducted to determine the effect of dietary fatty-acids on fatty-acid profiles of depot fats. Donaldson (1968) concluded in his study on Coturnix Quail (Coturnix coturnix japonica) that the mechanism(s) controlling fatty-acid desaturation and elongation is influenced by the dietary fatty-acid composition. In contrast to the previous experiment, experiments by West and Meng (1968a), Tanhuanpaa and Pulliainen (1975), and those reported by Hazelwood (1972) found that the dietary fatty-acids did not influence the fat compositions of Redpolls, Crossbills and domestic fowl, respectively. The latter three papers also suggest that some fatty-acids are preferentially retained. Three other sets of research agree with the conclusion of preferential retention. Bower and Helms (1968) calculated the level of linoleic acid ($C_{18:2}$) in the experimental food and found that it was present in the Slate-Colored Juncos at a two-fold excess over intake reflecting either synthesis in the body or selective retention. Feigenbaum and Fisher (1959) in their studies on

domestic poultry (Gallus domesticus), and the research of Morton and Liebman (1974) also suggest that certain fatty-acids, particularly linoleic acid, are selectively retained in the body. Berhard and Schoenheimer (1940) concluded from experiments with mice that animals cannot synthesize those fatty-acids containing 2 or 3 double bonds such as linoleic acid and linolenic acid ($C_{18:3}$). Even though the study by Berhard and Schoenheimer did not deal specifically with birds, the conclusion has at times been extrapolated to them, leading to the idea that these fatty-acids are selectively retained in birds and not synthesized.

Temperature has also been considered as a factor in influencing the fatty-acid composition of an organism. Fisher et al. (1962) studied the effects of three temperatures (32°C, 21°C, and 0°C) on the fatty-acid compositions of hens. The iodine values for fats from hens at 0°C were significantly greater ($P < 0.05$) than from any other hens, indicating more unsaturated fats in the 0°C hens. Yom-Tov and Tietz (1978) found that the oleic acid ($C_{18:1}$) to linoleic acid ratios found in European Starlings (Sturnus vulgaris) and Rock Ptarmigan were affected by temperature but not by photoperiod. The percentage of linoleic acid increased in birds kept in a cold (4°C) environment with short days versus those kept at high temperatures (30°C) and long days. Zar (1977a) found ($C_{20:1}$) and arachiodonic acid ($C_{20:4}$) were the major fatty-acids in the adipose tissues and foot-skins of Emperor Penguins (Aptenodytes forsteri) and concluded that the long-chain, unsaturated fats were an adaptation to cold. Zar (1977b) found that cold-stressed and starved House Sparrows had less palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), and oleic acid in their muscles and more stearic acid ($C_{18:0}$) and ($C_{22:6}$). Conversely, at high temperatures, there was a higher percentage of palmitoleic acid and less stearic acid.

Yom-Tov and Tietz (1978) present a brief discussion on the adaptive significance of unsaturated fats in cold environments. The energetic gain is considered to be of minor importance while the lowered melting point and mobility of the unsaturated fatty-acids could be useful in providing flexibility to the membranes of cells and mitochondria in legs and wings at low temperatures. Bower and Helms (1969), however, conclude that it seems unlikely to find a correlation between ambient temperature and fatty-acid composition in juncos since subcutaneous temperatures below 25°C are lethal.

Much of the research done so far on fatty-acid compositions has been nonexperimental or poorly controlled with many variables. Therefore, I designed a series of experiments using Bobwhites (Colinus virginianus) which isolate the possible factors of time and dietary fatty-acid content and examine the influence of those factors on the fatty-acid profiles of Bobwhites in controlled environmental conditions.

METHODS AND MATERIALS

The following research has been divided into three sections of study. The general purpose of the entire study is to determine the influence of dietary fatty-acids on the resultant fatty-acid composition of Bobwhites in simulated winter conditions.

The first section is designed to isolate the possible influential factors of time and dietary fatty-acid composition by altering time with one diet, or providing different diets over equal time periods. Milo (Sorghum vulgare), sunflower (Helianthus maximilliani), and sumac (Rhus glabra) were chosen as dietary items because together they make up over 50% of the quail's fall and winter diet (Robel et al., 1974) in Kansas.

Previous research has already determined the metabolizable energy in most of the seeds used in this study. The actual metabolizable energy of this collection of seeds could, however, vary from the previously reported values, and the metabolizable energy of false sunflower has not been reported. The second section is designed to determine the metabolizable energy of the experimental foods used in section I.

The third section reports the analyses of Bobwhites' fatty-acid compositions and the fatty-acid composition and calorie value of their eggs from an EPA small-pen test conducted in the summer of 1979 to test the effects of a carbamate pesticide on wildlife (Bobwhites). The fatty-acid compositions of Bobwhites and the fatty-acid composition and energy in their eggs are studied.

SECTION I

This section of the research isolates the factors of time and different diets in order to assess the influence of dietary fatty-acid composition on the fatty-acid composition of Bobwhites consuming those diets in winter

conditions. The changes in fatty-acid composition and total grams of fat in dietary items after exposure to winter weathering is also studied.

General Experimental Procedure

Only adult male Bobwhites were used in this study to reduce differences due to age and/or sex. All quail were leg-banded, individually confined in 48 x 25 x 13-cm polypropylene cages with 1.3-cm hardware cloth tops, and housed in an environmental chamber which simulated Kansas winter conditions: 9L:15D photoperiod and 5°C temperature. Relative humidity ranged from 81 to 88%. The maintenance diet was P-18, a balanced mash consisting of 20.5% protein, 2.7% fat, and 3.6% crude fiber prepared and pelleted by the Kansas State University Department of Grain Science and Industry (Appendix 1). Food and water were provided ad libitum except during weight reduction periods which simulated times of winter food unavailability to ground feeders. Only water was provided during those periods. After a 7-day acclimation period in which birds were not handled, Bobwhites were weighed daily to 0.1 g during the last 2 h of the dark period when the birds were inactive, non-feeding, and had defecated. This was done to insure minimal variation in body weight data (West, 1960). The weight of each bird was considered to be "stable" when daily variations were less than $\pm$2% of the average weight over the most recent 15 days. When the weights of the birds were stable, the birds were ranked from light to heavy, divided into 5 equal-sized blocks, and an even number of 5-bird, weight-stratified experimental groups were formed by randomly selecting 1 bird from each of the 5 weight blocks. Extra quail were kept as replacements in case of losses. The even number of experimental groups was divided at random into 2 major subdivisions; I and II. Experimental groups in subdivision I were sampled for fatty-acid analysis by killing the birds, and groups in subdivision II were sampled for fatty-acid analysis by removing not more than 3 cc of blood from the wing veins of the birds. Subdivision II

birds were kept an additional 15 days after sampling to determine if the procedure was harmful to them. All samples were collected during the last hour of the dark period and stored at -20°C to inhibit deterioration by oxidation (Kendeigh and West, 1965). Body fat reserves were purged from Bobwhites by denying them food until a 20% or greater loss of total stable body weight was achieved.

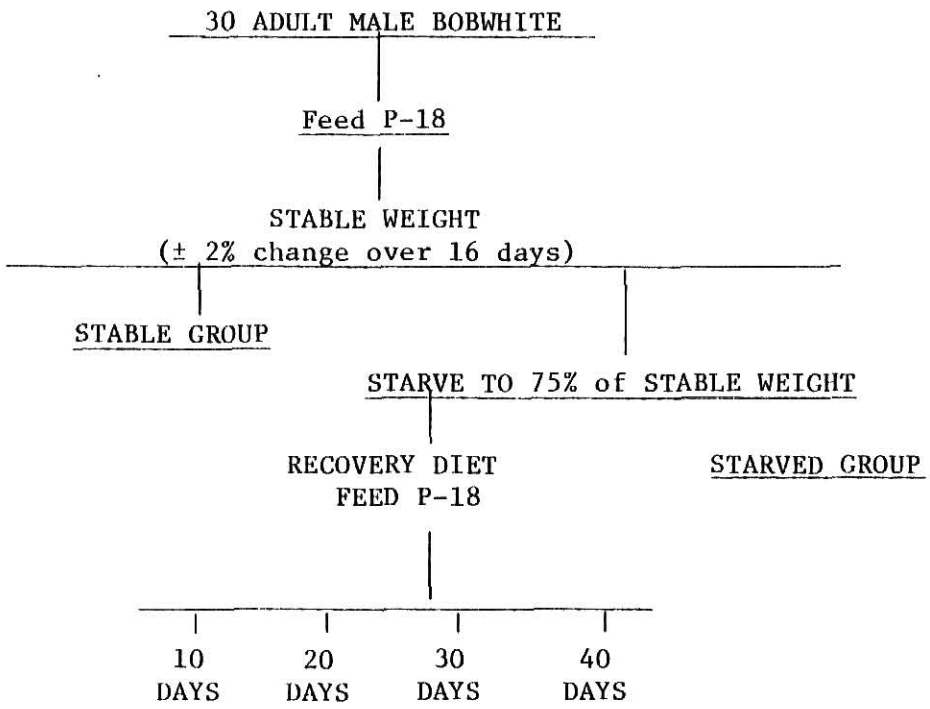
Experiments

The first experiment was designed for assessing the changes over time in the fatty-acid composition of Bobwhites fed a constant diet of P-18. Seventy-five adult male Bobwhites were obtained January 12, 1979 from the Kansas Fish and Game Commission Quail Farm at Pittsburg, Kansas. When the birds' weights were stable, 60 quail from 5 weight blocks were randomly assigned to twelve 5-bird groups equally divided into subdivision I and II. No less than 31 days passed before quail were considered to be weight stable. One stable group of randomly chosen birds was sampled from each subdivision, the remaining 10 groups were starved to 80% or less of their stabilized weights, and one group of starved birds was sampled from each subdivision. The final 8 groups were fed P-18 as a recovery diet. At 10-day intervals the 5-bird group was sampled from each subdivision (Flow Diagram 1).

The second experiment studied the differences between the fatty-acid compositions of groups of Bobwhites fed different diets over the same length of time. Seventy-five adult male Bobwhites were acquired April 6, 1979 from the Kansas Fish and Game Commission Quail Farm at Pittsburg, Kansas. When the birds were of stable weight, 50 quail from 5 weight blocks were randomly assigned to ten 5-bird experimental groups equally divided into subdivision I and II. No less than 32 days passed before quail were considered to be

FLOW DIAGRAM 1

EXPERIMENTAL DESIGN FOR THE DETERMINATION OF THE FATTY-ACID COMPOSTIONS OF BOBWHITES FED P-18 BALANCED MASH OVER DIFFERENT LENGTHS OF TIME

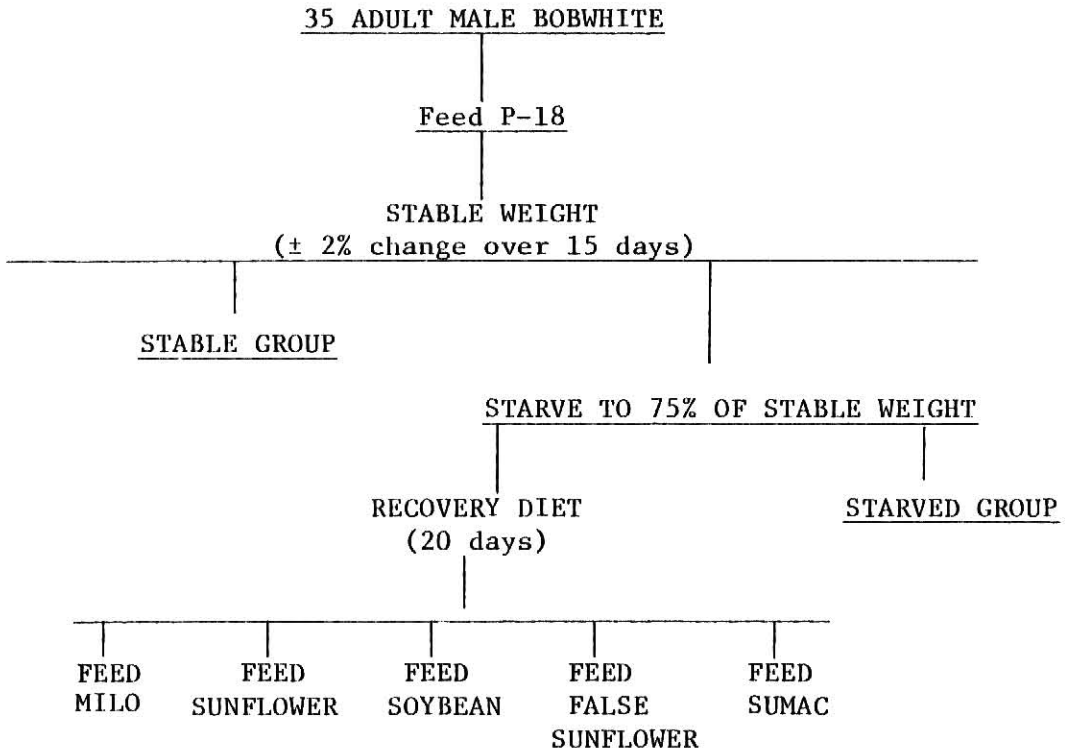


stable in weight. One group of randomly selected birds was sampled from each subdivision, the other 4 groups in each subdivision were starved to 80% or less of their original stable weights, and one group of starved birds was sampled from each subdivision. Of the final 6 groups; 2 were fed sunflower (Helianthus maximiliani), 2 were fed milo (Sorghum vulgare), and 2 were fed sumac (Rhus glabra) for 20 days. Ten of the extra quail were randomized from the weight blocks into 2 groups, each provided false sunflower (Heliopsis helianthoides) as a recovery diet, and the last 3 replacement quail were used as a partial group in subdivision I feeding on soybean (Soja max) as a recovery diet (Flow Diagram 2). All seeds were fed in their natural form except for milo which was pelleted. All seeds with the exception of sumac were acquired from the Kansas State University Department of Agronomy and were pesticide free. The sumac was collected in November 1978 from a site 15 km west of Manhattan, Kansas on the Fort Riley Military Reservation. Seeds were stored at -20°C until needed for feeding trials. Samples for fatty-acid analysis were taken from the frozen reserves.

In the third experiment, a study was made on the changes in fatty-acid composition and grams of total fat in seeds through the winter season. Samples of sumac, milo, and sunflower (Helianthus annuus) were collected on November 1, 1978; each from one location to reduce differences in the energy content of each seed type due to different locations (Johnson and Robel, 1968). Two 1-g subsamples of each seed were stored on November 1 at -20°C for subsequent fatty-acid analysis. The remaining seeds were placed on top of a piece of muslin cloth over a 1.3-cm mesh hardware cloth floor in a 76.0 x 36.0 x 30.5-cm box with wooden sides and a top of 1.3-cm mesh hardware cloth. The box with the seeds was stored on the south roof of Ackert Hall, Kansas State University where the seeds were exposed to maximum weathering

FLOW DIAGRAM 2

EXPERIMENTAL DESIGN FOR THE DETERMINATION OF THE FATTY-ACID COMPOSITION OF BOBWHITES FED DIFFERENT RATIONS



process but protected from consumption by birds or mice and from being blown away. Two 1-g subsamples of each seed were taken every 30 days from the box and stored at -20°C in glass vials with cork stoppers. The first sample was taken November 1, 1978 and the last on March 1, 1979.

Chemical Analysis of Fatty-Acids

Wings, feet, skin, and crop contents were removed from frozen experimental Bobwhites and carcasses were individually weighed to 0.1 g and ground in a Universal No. 3 Food Chopper (fine cutter). Subcutaneous fat remained on the frozen carcasses after skin removal. Lipids were extracted individually from ground quail in a Model 5BA60VL22 Waring laboratory blender using a technique from Folch et al. (1957) in which a 2:1 (volume:volume) extraction solution of Chloroform ($CHCl_3$) and Methanol (CH_3OH) was used. The final volume of extraction solution per quail was modified from the recommended 20 ml per 1 g of sample to 500 ml per quail. Ground quail were first blended in 300 ml of extraction solution and vacuum filtered through a buchner funnel using a No. 2 Whatmann filter paper. Debris from the filter paper was collected, re-extracted in 200 ml of extraction solution, and refiltered with fresh filter paper. Both liquid portions were transferred to a 500 ml separatory funnel, washed with 250 ml (half of the final extraction volume) of deionized, distilled water using gentle rocking motions, and left standing for not longer than 2 h to separate the top proteinaceous waste/water layer from the heavier lipid/chloroform layer. The lipid layer was drained from the funnel, the waste layer discarded, and the washing, separation, and lipid collection procedure was repeated. The final collected lipids were concentrated to 1 to 2 ml in a round bottom flash over warm non-boiling water using a rotary evaporator, poured into individual 8-dram glass vials with screw caps, flushed gently for at least 15 seconds with nitrogen gas to reduce oxidation, and stored at -16°C until further treatment (see esterification procedure).

Lipids were extracted from seeds according to Folch et al. (1957) with no modifications. A 1-g sample of each seed type was individually pulverized using a mortar and pestle. Fatty-acids in each 1-g sample of pulverized seed material were extracted by shaking the seed sample vigorously in a 20 ml solution of 2:1 $CHCl_3$:CH_3OH in an 8-dram glass, screw-cap vial for 1 min, and vacuum filtering the mixture through a buchner funnel using a No. 2 Whatmann filter paper. The debris was collected from the paper, reextracted in an additional 20 ml of extraction solution per g of sample, and refiltered using fresh paper. The liquid portions were transferred to a 100 ml separatory funnel, washed with 20 ml of deionized, distilled water per 1-g of sample seed using gentle rocking motions, and left to stand for not longer than 1 h to separate the top proteinacous waste/water phase from the heavier lipid/chloroform phase. The remainder of the procedure (from the second wash on) follows that described previously for the extraction of fatty-acids from quail.

Methyl esters of the fatty-acids of seeds and quail for analysis by gas-liquid chromatography (GLC) were formed from the frozen, concentrated extracted lipids. Frozen lipids were thawed in their individual vials at room temperature, 5 ml of Boron Trichloride in Methanol (Sigma Grade, Sigma Chemical Company) was added to each vial which was recapped, shook, and left at room temperature for 1 h. To this mixture was added 2 to 3 ml of hexane (reagent grade) and approximately 10 ml of deionized, distilled water as a wash. Three separate layers formed in each vial; a lower water/waste layer, a thin middle emulsion waste layer, and an upper organic layer. More hexane was added if the organic layer did not rise from the bottom. The organic layer was transferred by pasteur pipette to a clean, dry 8-dram vial; washed gently with 5 to 10 ml of deionized, distilled water, then transferred into a clean, dry 2-dram vial containing a small layer of anhydrous Na_2SO_4.

All vials were flushed with a gentle stream of nitrogen gas for at least 15 seconds to prevent oxidation of the fatty-acid esters and were stored in a refrigerator for analysis by GLC.

During chromatography, a 5-lambda aliquot of each sample was analyzed on a Barber-Colman Selecta Systems Model 5002 Gas-Liquid Chromatograph using columns of diethylene glycol succinate (DEGS) with nitrogen as the carrier gas (Barber-Colman Company Manual). Column temperatures ranged 170°C to 180°C. A standard containing known fatty-acids was analyzed on the chromatograph at the onset of each series of samples.

Saturated fatty-acids of 14, 16 and 18 carbon lengths and the $C_{16:1}$ and $C_{18:1}$ unsaturated fatty-acids were identified by direct comparison of the sample's chromatograph peak retention times to the peak retention times of the standard fatty-acids. To identify the remaining fatty-acids in the sample, the log retention times of the sample's identifiable saturated fatty-acids were plotted on the y-axis against their respective carbon numbers on the x-axis, the retention times of the unknown fatty-acids were located on the plot, and the corresponding x-axis value was compared with the table II of Hofstetter and Holman (1969:539) to determine the most probable fatty-acid in that location on a DEGS column. Replicate analysis of 5 quail samples were conducted to measure the repeatability of the methodology. The percent composition of the individual fatty-acids in the replicate analysis were very similar between runs done on subsequent days (See appendix, Table 2).

The percent fatty-acid composition was measured by quantifying the areas under the chromatographic peaks using the peak-height times the width at one-half the height method (Hfetman, 1967). Individual fatty-acids were expressed as percentages of the total fatty-acids in the sample.

Chemical Analysis of Total Fats

The amount of total ether soluble material (fatty-acids plus complex lipids) in quail and seeds was determined using a Goldfisch fat extractor. Samples of seeds and quail were individually ground or pulverized as described previously for fatty-acid extraction, weighed to 0.0001 g, vacuum dried at 95°C and 26 pounds of pressure for 5 h, and reweighed to 0.0001 g to determine water content. Dried samples weighed to 0.0001 g were fluxed for 12 h on the Goldfisch extractor using ethyl ether as the extracting solution. Extraction beakers were vacuum dried and weighed to 0.0001 g before and after the extraction; the difference was the amount of ether soluble material present in the dried sample.

An internal standard fatty-acid was used to estimate the grams of fatty-acids present in a quail using GLC. One gram of behenic acid ($C_{22:0}$) was added prior to the first $CHCl_3$:CH_3OH extraction where any losses of fats during the process would be consistent for the unknown fats and the standard. The retention time of the behenic acid was determined by dividing a ground quail into two equal portions, adding behenic acid to one half only, then extracting the fatty-acids from both halves and analyzing using GLC. The additional chromatographic chart peak of the sample half containing behenic acid marked the retention time of the internal standard. This sample half was used as a standard at the onset of each series of GLC analyses. The area under a sample's behenic acid peak was said to represent 1 gram of fatty-acid and the total grams of fatty-acid in the sample was calculated by dividing the total area under the sample fatty-acid peaks by the area under that sample's behenic acid peak.

The gross amount of fatty-acids determined by using the behenic acid internal standard was adjusted to an estimate of the grams of total ether extractable fats in each sample. Eleven quail with prepared carcass weights

ranging from 83.3 to 209.0 g were individually processed through the Universal No. 3 food chopper (fine cutter), mixed so as to be homogenous, and divided into two portions of unequal size; the smaller of which weighed approximately 10 g. The ether extractable fats in the smaller sample were determined using the Goldfisch extraction and the grams of fatty-acids in the larger portion were estimated using GLC and the behenic acid internal standard (1.0 g per sample).

Statistical Analyses

Total ether extractable fat estimates derived from the Goldfisch extraction from the 11 quail were plotted against the estimate of grams of fatty-acids derived from the behenic acid standard. Three regression models were tested to describe the plot:

Simple linear model: $Y = \beta_0 + \beta_1 X$ where Y = the total ether extractable fat of the Goldfisch data and X = the unadjusted estimate of grams fatty acid.

Partial quadratic model: $Y = \beta_0 + \beta_2 X^2$

Full quadratic model: $Y = \beta_0 + \beta_1 X + \beta_2 X^2$ where β_0 is the Y intercept and β_1 and β_2 are constant coefficients.

Arcsine transformations of raw data (Snedecor and Cochran, 1967: 327-329) were performed on percentage data where values were less than 25% or greater than 70%. Variances are greatest in these regions resulting in a skewdness of error contrary to assumptions in Analysis of Variance (ANOVA). Using transformed variables reduces the chances of producing too many significant F-test results.

The fatty-acid compositions, prepared carcass weights, percent saturated and unsaturated fatty-acids, and the total ether extractable fats of the quail fed a diet of P-18 during lengthening time periods were analyzed for differences with respect to the experimental group using Analysis of Variance (ANOVA) and Least Squares Separation of Means.

Percent composition of fatty-acids, percent saturated and unsaturated fatty-acids, carcass weights, and the grams of ether extractable fats in the quail fed different diets over the same time were analyzed with respect to the treatment groups using ANOVA and Least Squares Separation of Means. The percent composition of fatty-acids, percent saturated and unsaturated fatty-acids, and the percent ether extractable fats in the dietary foods were also analyzed using ANOVA and Least Squares Separation of Means. These variables were then analyzed for quail and diets combined using ANOVA and Least Squares Separation of Means. Allowable comparisons were made on the fatty-acids in birds and their respective diets based on the variances found in the separate analyses of quail and diets. The percent composition of fatty-acids were analyzed for quail only using Discriminant Analysis.

The percent composition of individual fatty-acids and the percent saturated and unsaturated fatty-acids of the sunflower, milo, and sumac collected at 30-day intervals from November 1 through March 1 were analyzed with respect to the seed type and month of collection using ANOVA and Least Squares Separation of Means. The grams of total ether extractable fat and the percent fat using dry seed weight of the monthly sunflower, milo, and sumac collections were analyzed for differences with respect to seed type, month of collection and a seed type by month interaction using ANOVA and Least Squares Separation of Means. The probability level chosen for use in analyzing the statistical significance of all results was $P = 0.05$.

RESULTS

Section I

The equations and respective R^2 values of the three regression models using the internal standard derived weight of fat as the independent variable and the Goldfisch derived weight of total fat as the dependent variable are:

Linear
$Y = 0.813 + 1.815\ X$ $\qquad R^2 = 0.774$

Simple Quadratic
$Y = 2.465 + 0.252\ X^2$ $\qquad R^2 = 0.580$

Full Quadratic
$Y = 1.226 + 4.460\ X - 0.439\ X^2$ $\qquad R^2 = 0.892$

A graph (Figure 1) shows the predicted total fat values (Y) plotted against their respective internal standard derived fat values (X) with the plot of the actual Goldfisch fat values against their respective internal standard fat values.

The acquisition of blood from the wing veins of the quail was unsuccessful. One quail died from the blood sampling process after 2 cc of blood was removed from its jugular vein. From only two of the quail were 3 cc of blood acquired, most samples consisted of less than 1 cc of blood. These volumes were thought to be too small for fatty-acid analysis using GLC. The largest (3 cc) sample of blood was analyzed using GLC and no fatty-acid peaks were found. No other blood samples were analyzed.

Two to 6 days ($\bar{x}$ = 3.3 days) were required in the P-18 diet fed over varying lengths of time experiment to starve the quail to a mean of 76.4% of their original stable weights. Two quail died during the food deprivation time, one on the 2nd day of food denial at 80% stable weight and one at 5 days after an overnight drop from 80% to 65% stable weight. The mean carcass weight (Table 1) and the weight of total fat using the full

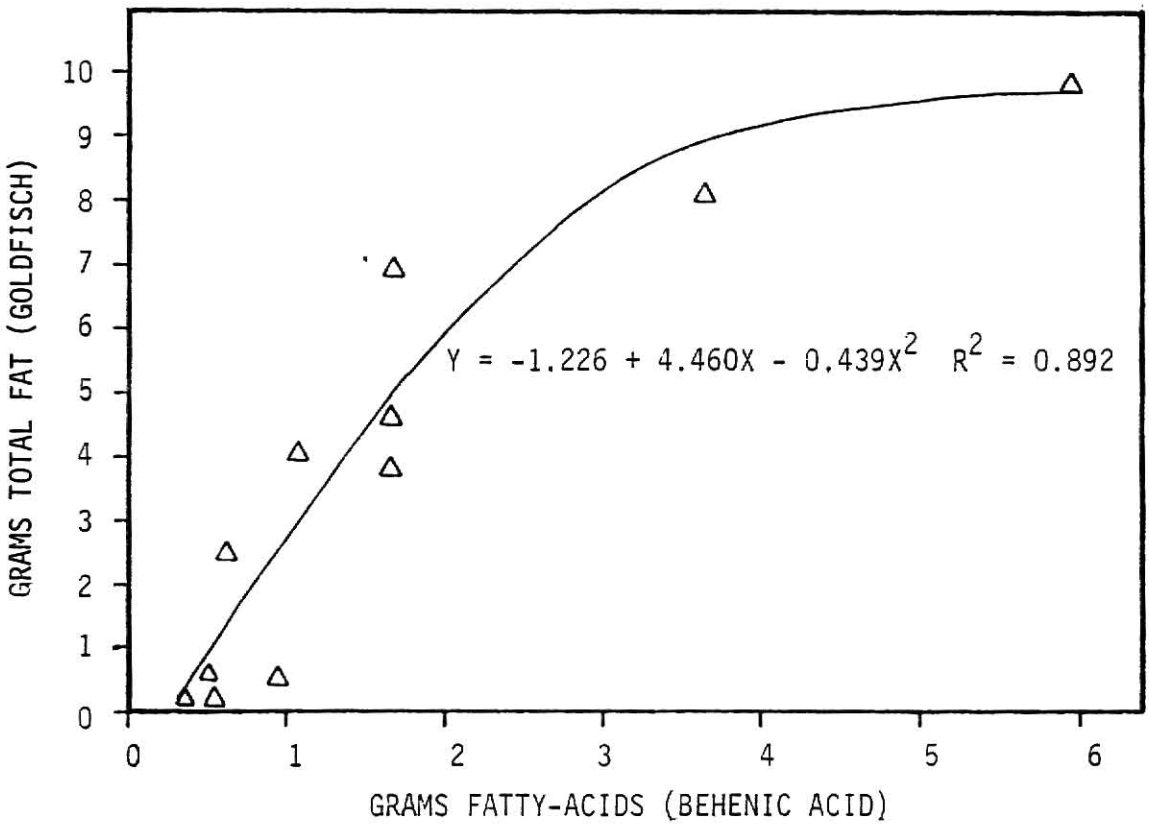


Figure 1. Ether extractable fat values using a Goldfisch extraction unit versus those estimated from GLC Analysis with a behenic acid standard.

Table 1. Mean weights and fat data from 5-bird groups of Bobwhites fed a balanced mash (P-18) for various lengths of time.

	Experimental Groups					
	Weight		Fed			
	Stable	Starved	10 days	20 days	30 days	40 days
Weight (g)						
Carcass	143.5[a]	100.7[b]	127.2[a]	141.6[a]	142.5[a]	145.1[a]
Fat	8.1[a]	2.4[b]	6.8[a]	8.6[a]	7.8[a]	8.8[a]
Percent of Stable Weight	100.0[a]	76.4[b]	90.9[c]	99.7[a]	101.8[a]	102.4[a]
Fat (Percent of)						
Carcass weight	5.6[a]	2.5[a]	5.3[a]	6.1[a]	5.5[a]	6.1[a]
Saturated	32.5[a]	26.9[b]	27.2[ab]	29.7[a]	32.1[a]	35.0[a]
Unsaturated	62.9[a]	73.1[ab]	72.7[bc]	70.4[bc]	69.6[c]	65.1[c]

a,b,c = Least squares means with common superscripts within a row are not significantly different (P = 0.05).

quadratic equation of the starved birds were significantly lower than those of the other experimental groups. Bobwhites fed P-18 until stable, starved to 76% of their stable weight, and placed on a P-18 recovery diet, recovered to within 90% of their pre-starved weight after 10 days, and regained their pre-starved stable weight within 20 days. The weight of total fat, ranging from 2.4 g to 8.6 g, and the mean carcass weights were not significantly different between any of the other experimental groups fed P-18 over varying lengths of time (Table 1). The percent fat ranged from a low of 2.5% for the starved birds to 6.1% for the quail fed P-18 as a recovery diet for 20 and 40 days. The original amount of total fat was also regained in 10 to 20 days. These values were not significantly different between groups of birds.

The following fatty-acids were found in all groups of quail fed P-18 over varying lengths of time: $C_{12:0}$, 14:0, 14:1, 16:0, 16:1, 16:2, 18:0, 18:1, 18:2, 20:0 and 20:4 (Table 2, Figure 2). A fatty-acid that appeared to be $C_{20:5}$ (using Hofstetter and Holman, 1969) was found in 1 of the 5 birds in the 30-day feeding group and in 2 of the 5 birds from the 40-day feeding group. Fatty acid $C_{16:2}$ was found in the smallest amount (0.0 to 0.8%) and $C_{18:1}$, 18:2, and 20:4 were found in the greatest amounts. Unsaturated fatty-acids were found in greater quantity (62.9 to 73.1%) than saturated fatty-acids. A period of 40 days was required to return the level of unsaturated fats in quail fed P-18 as a recovery diet to the original pre-starved level (Table 1). Starved quail had a significantly greater percentage of the fatty-acid $C_{20:4}$ than the stable or recovering quail, and a lower or equal percentage of all other fatty-acids. The percentage of $C_{20:4}$ in the body returned to the original level in 10 to 20 days. The levels of all of the fatty-acids of starved quail fed P-18 for 10 days were not significantly different from the pre-starved levels though

Table 2. Mean percent composition of individual fatty-acids of 5-bird groups of quail fed one diet over varying lengths of time.

Fatty-acid (Carbon:Unsaturated)	Experimental Groups					
	Weight		Recovery Time (days)			
	Stable	Starved	10	20	30	40
12:0	4.7^{ab}	2.0^{a}	3.2^{ab}	4.9^{b}	5.3^{b}	4.0^{ab}
14:0	4.3^{a}	1.3^{b}	2.2^{ab}	3.7^{ab}	4.6^{ab}	3.2^{ab}
14:1	6.1^{ab}	2.5^{c}	3.4^{bc}	5.0^{abc}	7.8^{a}	7.0^{a}
16:0	14.9^{a}	10.7^{a}	13.8^{a}	13.6^{a}	15.0^{a}	15.5^{a}
16:1	0.9^{ab}	0.2^{a}	2.2^{bc}	2.4^{cd}	3.4^{d}	1.1^{bc}
16:2	0.3^{a}	0.3^{a}	0.0^{a}	0.1^{a}	0.8^{a}	0.7^{a}
18:0	9.0^{a}	12.9^{a}	8.0^{a}	7.7^{a}	7.4^{a}	12.4^{a}
18:1[1]	17.1^{ac}	13.0^{c}	22.1^{ab}	23.7^{b}	20.9^{ab}	17.4^{ac}
18:2[1]	22.4^{ab}	18.0^{b}	26.8^{a}	25.7^{a}	22.7^{ab}	21.6^{ab}
20:0	0.5^{ab}	0.1^{b}	0.9^{ab}	0.8^{ab}	1.0^{a}	0.5^{ab}
20:4[1]	15.9^{a}	38.4^{b}	18.1^{a}	13.2^{a}	11.7^{a}	13.0^{a}
20:5	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{a}	2.0^{a}

a,b,c = Least square mean values with common superscripts within a row are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data were arcsine transformed.

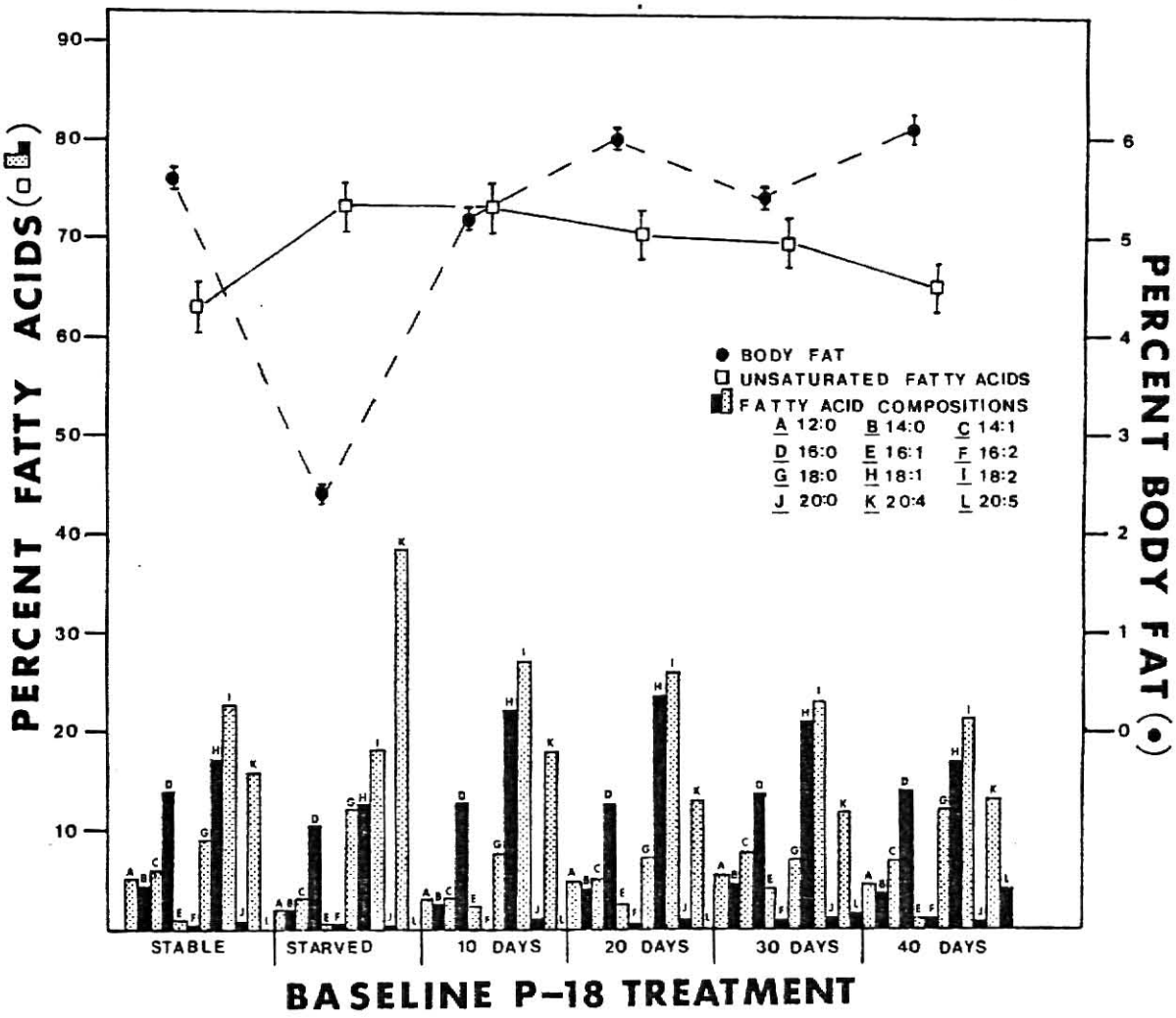


Figure 2. Percent composition of fatty-acids and percent body fat for quail fed P-18 for different lengths of time.

the levels of $C_{16:1}$, 18:1, and 18:2 in quail fed P-18 for 10 days after starvation were higher (140, 30, and 20%, respectively) than the pre-starved levels. The levels of $C_{16:1}$ and 18:1 continued to rise significantly then declined to the pre-starved level. The level of $C_{18:2}$ declined directly from the 10 day level to reach that of the pre-starved level. The level of $C_{14:1}$ after 10 days of P-18 recovery diet was 45% lower than the pre-starved level which was recovered in 30 days.

In the experiment in which quail were fed different diets for 20 days; 2 to 6 days ($\bar{x}$ = 2.6 days) were required to starve experimental, weight stable, P-18-fed quail to a mean of 74.4% of their original stable weight. Nine quail out of 45 died during the food denial period with a weight range of 75 to 90% of their original stable weight ($\bar{x}$ = 83% stable weight). Two quail died within 1 day, 4 died in 2 days, and 3 died in 3 days. All false sunflower- and sumac-fed quail died within 1 to 4 days at means of 65.3% and 68.6% of their original stable weight, respectively, which were lower than but not significantly different from the mean 74.4% stable weight of the surviving starved (food deprived) birds (Table 3). Preparation of carcasses revealed food in the crop of all experimental quail (both those which died before the completion of the experiment and those killed by the experimenter). Two soybean-fed quail died after 15 and 19 days of weight gains, and carcass preparation revealed their crops impacted with soybeans. One sunflower-fed quail died after a 17-day weight gain. No cause for the death could be found during necropsy. Only 2 quail of the starved (food deprived) group were alive when their weight reduction to 75% stable weight was achieved. The mean carcass weight (Table 3) ranged from 79.9 g for the false sunflower-fed quail to the high of 119.9 g for the milo-fed birds which was significantly higher than the soybean-, false sunflower-, and sumac-fed groups. The mean percent original stable weights of the

Table 3. Mean number days alive on treatment, carcass weight, and percent saturated and unsaturated fatty-acids of quail fed different foods.

	Experimental Group						
	Weight		Recovery Group				
	Stable	Starved	Milo	Sunflower	Soybean	False Sunflower	Sumac
N	5	2	5	4	3	7	6
Weight (g)							
Carcass	136.8^{a}	101.3^{bcd}	119.9^{b}	107.1^{bc}	91.4^{cd}	79.9^{d}	81.8^{d}
Fat	8.3^{a}	7.9^{ab}	8.3^{a}	4.7^{b}	8.4^{a}	6.2^{ab}	6.0^{ab}
Percent of Stable Weight	100.0^{a}	74.4^{bd}	89.0^{c}	75.5^{be}	81.7^{bc}	65.3^{d}	68.6^{de}
Fat (Percent of)							
Carcass Weight	6.0^{a}	7.8^{a}	7.0^{a}	4.4^{a}	9.2^{a}	8.5^{a}	7.7^{a}
Saturated	40.0^{a}	31.0^{b}	29.0^{b}	29.3^{b}	32.0^{b}	26.3^{b}	26.8^{b}
Unsaturated	60.1^{b}	69.0^{b}	68.6^{b}	71.3^{b}	68.0^{b}	73.6^{b}	73.2^{b}
Number of Days Alive	----	----	23.0^{a}	23.7^{a}	18.0^{a}	2.1^{b}	1.3^{b}

a,b,c,d,e = Least Square mean values with common superscripts within a row are not significantly different (P = 0.05).

experimental groups (Table 3) was highest (89% stable weight) for the milo-fed quail and all were significantly less than 100% of the original stable weight as represented by the P-18-fed, weight stable quail.

The grams of total fat (Table 3) ranged from 4.7 g in sunflower-fed birds to a high of 8.3 g in the milo-fed birds. With the exception of the sunflower-fed quail, none of these fat amounts were significantly different from the 8.3 g of fat found in pre-starved quail. The mean percent fat (Table 3) in the body was not significantly different for any groups. The following fatty-acids were found in all groups of quail: $C_{14:0}$, 16:0, 18:0, 18:1, 18:2, and 20:4 (Table 4). Fatty-acid $C_{16:1}$ was not present in soybean-, sumac- or false sunflower-fed birds; $C_{16:2}$ was not present in P-18-, milo-fed, or starved (food deprived) birds; and $C_{20:0}$ was not present in starved, P-18-, false sunflower-, or sumac-fed birds. Fatty-acids $C_{20:4}$ and 18:2 were present in the largest amounts (13.8 to 48.8% and 11.8 to 45.5%, respectively) in the recovery groups. Fatty-acids $C_{16:1}$, 16:2 and 20:0 were present in the least amount (0 to 5.6%, 0 to 0.5%, and 0 to 0.4% respectively). There was no significant difference between recovery groups in the levels of $C_{12:0}$, 14:0, 14:1, 16:0, 16:2, and 18:0. Milo-fed quail had significantly more $C_{16:1}$ than the other recovery groups, and sunflower- and sumac-fed quail had significantly less $C_{18:1}$ than the other recovery groups. The largest differences in the fatty-acid compositions between quail fed different diets is seen in the fatty-acids $C_{18:2}$ and $C_{20:4}$. The list of highest to lowest composition of $C_{18:2}$ in quail according to their diets is: sunflower, soybean, milo, false-sunflower, starved, and sumac. A similar ranking by diet of high to low composition of $C_{20:4}$ in quail is nearly the reverse, i.e., sumac, starved, false sunflower, milo, soybean, and sunflower. Starved quail and quail fed false sunflower and sumac had

Table 4. Mean percent composition of individual fatty-acids of quail fed different foods.

Fatty-Acid (Carbon:Unsaturated)	Experimental Group						
	Weight		Recovery Diet				
	Stable	Starved	Milo	Sunflower	Soybean	False Sunflower	Sumac
12:0	10.0^{a}	1.6^{b}	2.1^{b}	1.6^{b}	2.4^{b}	2.3^{b}	2.1^{b}
14:0	9.4^{a}	1.4^{b}	2.3^{b}	2.2^{b}	1.9^{b}	1.4^{b}	1.4^{b}
14:1	5.5^{a}	1.4^{b}	1.4^{b}	2.4^{b}	2.4^{ab}	2.9^{ab}	2.4^{b}
16:0	15.7^{a}	15.2^{a}	11.4^{a}	10.9^{a}	14.9^{a}	14.7^{a}	12.1^{a}
16:1	0.1^{ab}	0.1^{ab}	5.6^{b}	0.1^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
16:2	0.0^{a}	0.0^{a}	0.0^{a}	0.5^{a}	0.1^{a}	0.1^{a}	0.0^{a}
18:0	7.5^{a}	14.5^{ab}	12.3^{ab}	15.0^{b}	12.1^{ab}	11.6^{ab}	11.8^{al}
18:1	17.2^{ab}	14.1^{abc}	18.9^{a}	9.3^{c}	19.1^{ab}	12.9^{bc}	10.3^{c}
18:2[1]	22.6^{ab}	14.7^{bc}	23.4^{ab}	45.5^{d}	25.1^{a}	18.5^{ab}	11.8^{c}
20:0	0.0^{a}	0.0^{a}	0.2^{a}	0.1^{a}	0.4^{a}	0.0^{a}	0.0^{a}
20:4[1]	15.9^{ab}	39.6^{cd}	25.6^{a}	13.8^{b}	21.7^{ab}	39.3^{c}	48.8^{d}
N	5	2	5	4	3	7	6

a,b,c,d = Least square mean values with common superscripts within a row are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data were arcsine transformed.

the greatest amount of fatty-acid $C_{20:4}$. Level of unsaturated fatty-acids (Table 3) were found to make up the greater percentage of fats (68.0 to 73.6%) in the recovery groups rather than saturated fatty-acids (26.3 to 32.0%).

The analysis of the dietary items showed the percent fat values of dry seeds determined by the Goldfisch extraction (Table 6) were significantly different between seeds with a range of 3.3% in P-18 to 28.2% in sunflower. Pulverized sumac contained 12.9% fat as opposed to whole sumac which yielded only 5.6% fat. The two mean sumac values were not significantly different from each other based on Least Significant Differences. The following fatty-acids were found in all experimental seeds: $C_{16:0}$, 18:0, 18:1, and 18:2. Fatty-acid $C_{14:1}$ was present in P-18 only (0.2%), $C_{16:1}$ was present in P-18, milo, and sumac (0.4, 0.4, and 0.4%, respectively) and $C_{20:0}$ was found only in P-18 (0.7%) and sumac (8.8%). Fatty-acid $C_{18:2}$ was present in the greatest amount and $C_{14:1}$ was present in the least amount (Table 5). The analysis of the fatty-acid composition of the dietary items showed the largest differences in percent composition (Table 6) to be in fatty-acids $C_{18:2}$ and $C_{16:0}$. A list of seeds containing fatty-acids $C_{18:2}$ from highest to lowest percentage is: sunflower (73.9%), false sunflower, P-18, milo, soybean, and sumac (25.6%). A similar ranking of high to low percentages of fatty-acid $C_{16:0}$ is nearly a reversal of the preceeding list; i.e., soybean (24.1%), sumac, milo, P-18, sunflower, and false sunflower (10.0%). The seeds vary in the percent composition of other fatty-acids, but not in an inverse relationship. Soybean and sumac had significantly greater percentages (14.4 and 16.9%, respectively) of $C_{18:0}$ than the other seeds, and sunflower had a significantly lower percentage (13.0%) of $C_{18:1}$.

Because of differences in variances, comparisons between a diet's fatty-acid composition and the comparable fatty-acids in quail fed that diet

Table 5. Mean percent composition of fatty-acids of foods used in this study.

Fatty-Acid (Carbon:Unsaturated)	Seed Type					
	P-18	Milo	Sunflower	Soybean	False Sunflower	Sumac
N	2	2	2	2	2	2
14:1	0.2^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
16:0	16.5^{ab}	16.7^{ab}	10.6^{b}	24.1^{a}	10.0^{b}	23.5^{a}
16:1	0.4^{a}	0.4^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.4^{a}
18:0	2.4^{a}	2.7^{a}	4.4^{a}	14.4^{b}	5.5^{a}	16.9^{b}
18:1	31.7^{a}	39.0^{a}	13.0^{b}	28.1^{a}	35.0^{a}	36.0^{a}
18:2[1]	54.3^{a}	51.2^{a}	73.9^{b}	44.5^{a}	56.8^{a}	25.6^{c}
20:0	0.7^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	8.8^{b}
Unsaturated[1]	81.4^{a}	82.7^{a}	85.8^{ab}	68.8^{bc}	85.2^{a}	55.4^{c}
Saturated[1]	18.6^{ab}	17.8^{b}	14.2^{b}	34.7^{a}	14.8^{b}	44.6^{c}

a,b,c,d = Least Square mean values with common superscripts within a row are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data arcsine transformed.

Table 6. Mean percent fat by dry weight of foods used in this study.

Seed Type	N	% Fat
P-18	3	3.3^{a}
Milo	3	3.6^{a}
Sunflower	3	28.2^{b}
Soybean	3	22.4^{bc}
False Sunflower	3	18.8^{bcd}
Whole Sumac	3	5.6^{ad}
Pulverized Sumac	3	12.9^{acd}

a,b,c,d = Mean values with common superscripts are not significantly different (P = 0.05) using Least Significant Differences.

could be made only for fatty-acids $C_{16:0}$, 18:0, 18:1, 18:2, percent unsaturated, and saturated fatty-acids (Table 7, Figures 3 and 4). There was significantly more $C_{16:0}$ in the soybean (24.1%) and sumac (23.5%) than in the quail fed those foods (14.9 and 12.1%, respectively); less $C_{18:0}$ in sunflower (4.4%), milo (2.7%), and P-18 (2.4%) than in the quail (15.0, 12.3, and 7.5%, respectively); and more $C_{18:1}$ in P-18 (31.7%), milo (39.0%), false sunflower (35.0%), and sumac (36.0%) than in the corresponding quail; P-18-fed (17.2%), milo-fed (18.9%), false sunflower-fed (12.9%), and sumac-fed (10.3%). There was significantly more $C_{18:2}$ in all seeds than in the quail and significantly more unsaturated fatty-acids in all seeds with the exception of P-18 and P-18-fed quail (Table 7).

Discriminant analyses (Table 8) of the fatty-acids in the bodies of quail were used to predict the diet of the quail. Fatty-acids in body tissues allowed the correct classification of all P-18-fed, milo-fed, false sunflower-fed, and sumac-fed quail into their proper experimental feeding groups. One starved (food deprived) quail was misclassified into the milo-fed group with a probability of 0.9950 of belonging in that group. The other starved quail was misclassified with a probability of 0.9330 into the sumac-fed group. One of the three soybean-fed quail was correctly classified into the soybean-fed group (Probability of 0.9984) but the other two were misclassified into the milo-fed group (Probabilities of 0.9908 and 0.9949 of belonging to the milo-fed group).

The fatty-acid compositions of sumac, milo, and sunflower which were exposed to natural winter weathering and collected at the 1st of each month from November 1978 through March 1979 were not analyzed statistically with respect to an interactive effect between seed type and month of collection because there were no replications. The fatty-acids present in all 3 seeds

Table 7. Mean percent fatty-acid composition of dietary items and quail fed those diets.

Dietary Treatment	N	Percent Fatty-Acid (Carbon:Unsaturated)					
		16:0	18:0	18:1	18:2[1]	Saturated[1]	Unsaturated[1]
P-18	2	16.5[a]	2.4[a]	31.7[a]	54.3[a]	18.6[a]	81.4[a]
Quail	5	15.7[a]	7.5[b]	17.2[b]	22.6[b]	40.0[b]	60.0[b]
Milo	2	16.7[a]	2.7[a]	39.0[a]	51.2[a]	17.8[a]	82.7[a]
Quail	5	11.4[a]	12.3[b]	18.9[b]	23.4[b]	29.0[b]	68.6[b]
Sunflower	2	10.6[a]	4.4[a]	13.0[a]	73.9[a]	14.2[a]	85.8[a]
Quail	4	10.9[a]	15.0[b]	9.3[a]	45.5[b]	29.3[b]	71.3[b]
Soybean	2	24.1[a]	14.4[a]	28.1[a]	44.5[a]	34.7[a]	68.2[a]
Quail	3	14.9[b]	12.1[a]	19.1[a]	25.1[b]	32.0[a]	68.0[a]
False Sunflower	2	10.0[a]	5.5[a]	35.0[a]	56.8[a]	14.8[a]	85.2[a]
Quail	7	14.7[a]	11.6[a]	12.9[b]	18.5[b]	26.3[b]	73.6[b]
Sumac	2	23.5[a]	16.9[a]	36.0[a]	25.6[a]	44.6[a]	55.4[a]
Quail	6	12.1[b]	11.8[a]	10.3[b]	11.8[b]	26.8[b]	73.2[b]

a,b = Least Square mean values with common superscripts within a column in a section are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data arcsine transformed.

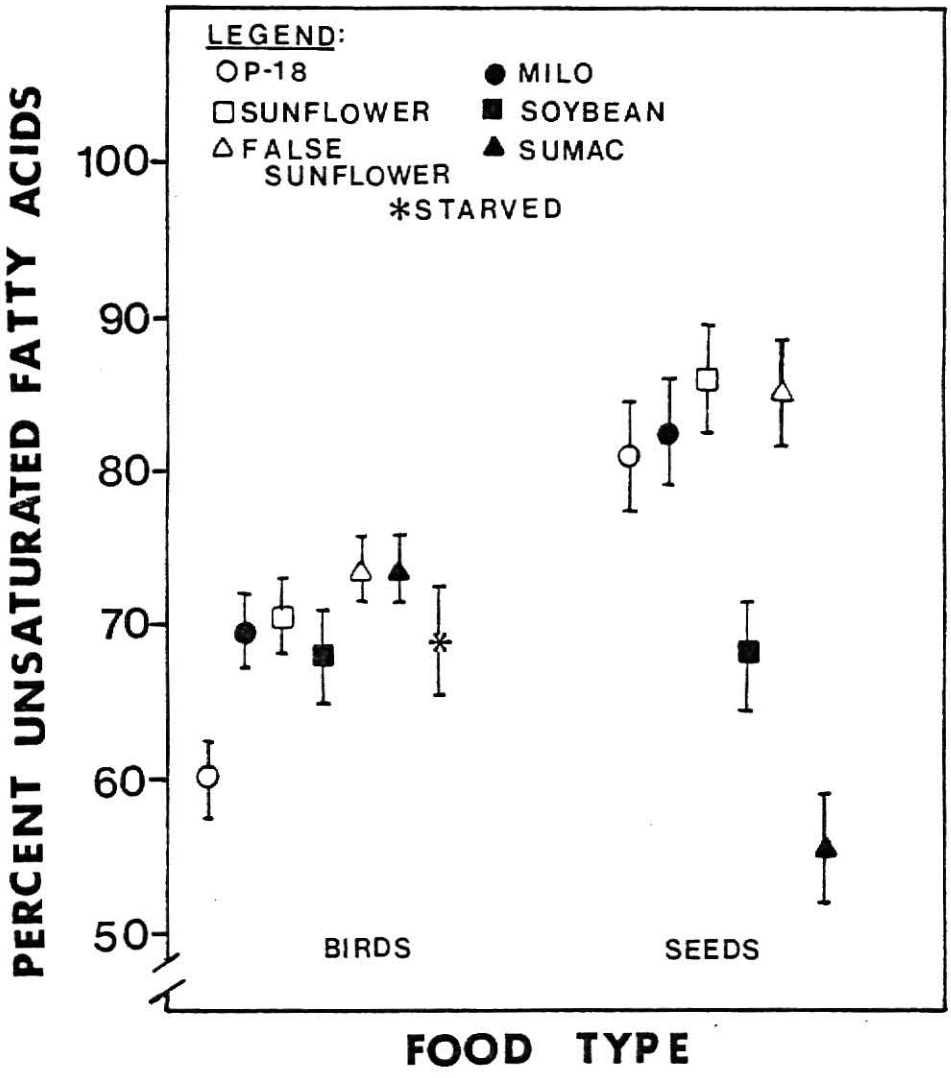


Figure 3. Percent composition of unsaturated fatty-acids in experimental diets and in quail fed those diets.

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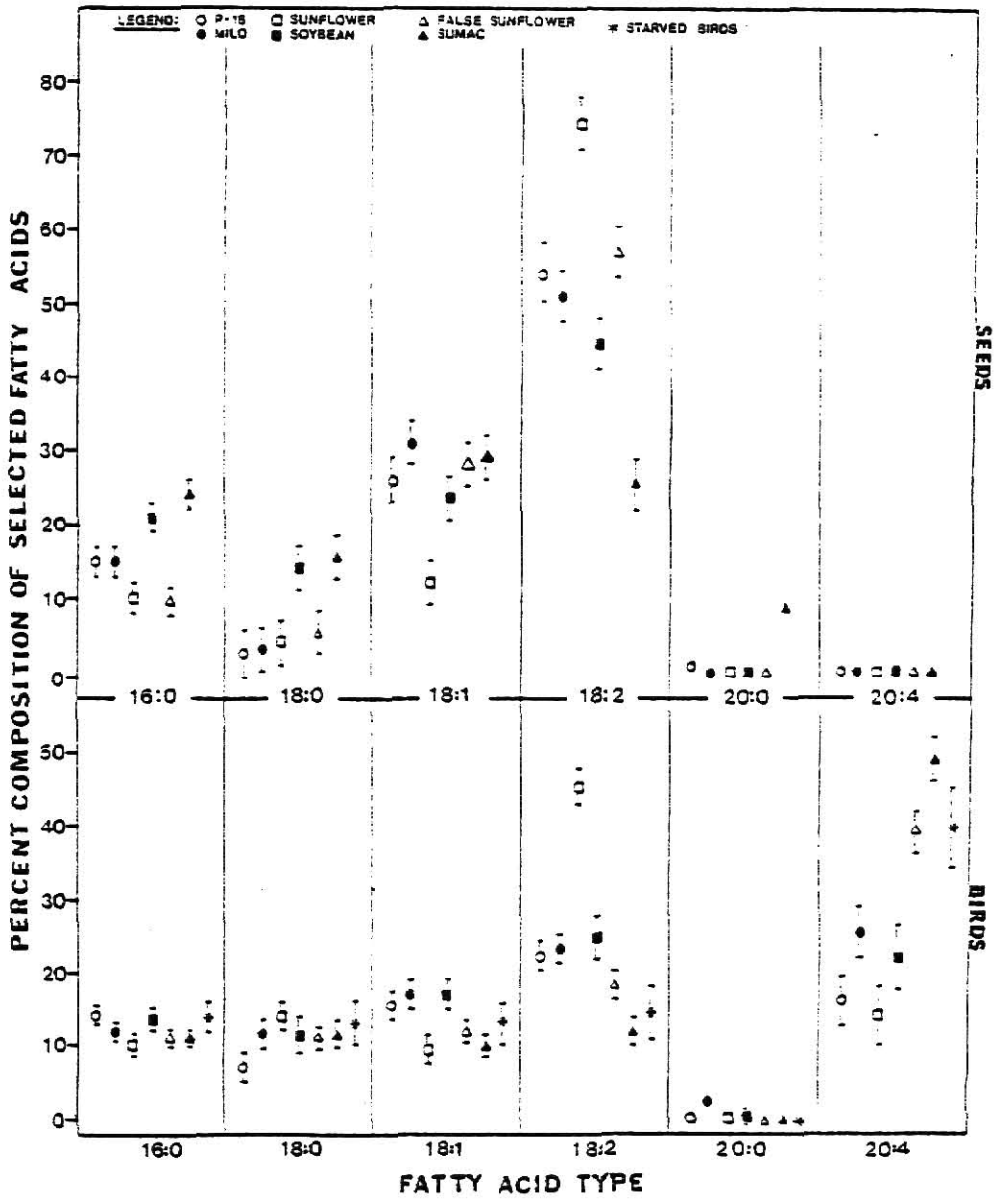

Figure 4. Percent composition of individual fatty-acids in experimental diets and in quail fed those diets.

Table 8. Discriminant analysis: Classification probabilities of experimental treatments.

ACTUAL EXPERIMENTAL TREATMENT		CLASSIFIED INTO TREATMENT	POSTERIOR PROBABILITY OF MEMBERSHIP IN: ACTUAL TREATMENT	MISCLASSIFIED TREATMENT
BIRDS FED:	SAMPLE			
P-18	A	P-18	1.0000	------
	B	P-18	0.9995	------
	C	P-18	1.0000	------
	D	P-18	1.0000	------
	E	P-18	1.0000	------
Starved	A	Milo	0.0000	0.9950
	B	Sumac	0.0000	0.9330
Milo	A	Milo	0.9999	------
	B	Milo	0.9602	------
	C	Milo	0.9983	------
	D	Milo	0.8087	------
	E	Milo	0.9999	------
Sunflower	A	Sunflower	1.0000	------
	B	Sunflower	1.0000	------
	C	Sumac	0.2000	0.7990
	D	Sumac	0.0022	0.9978
Soybean	A	Milo	0.0091	0.9908
	B	Milo	0.0050	0.9949
	C	Soybean	0.9984	------
False Sunflower	A	False Sunflower	1.0000	------
	B	False Sunflower	1.0000	------
	C	False Sunflower	1.0000	------
	D	False Sunflower	1.0000	------
	E	False Sunflower	1.0000	------
	F	False Sunflower	1.0000	------
	G	False Sunflower	1.0000	------
Sumac	A	Sumac	0.9997	------
	B	Sumac	1.0000	------
	C	Sumac	1.0000	------
	D	Sumac	0.9997	------
	E	Sumac	1.0000	------
	F	Sumac	0.9999	------

were $C_{16:0}$, 18:0, 18:1, and 18:2 (Table 9). Fatty-acid $C_{16:1}$ was not found in milo or sumac and was found only in small amounts in sunflower (0.7%). There was no significant difference among the 3 seed types in amount of $C_{16:1}$. Fatty-acid $C_{20:0}$ was found only in sumac (6.2%) which was significantly more than the 0.0% found in sunflower or milo. There was a significantly smaller amount of $C_{16:0}$ in sunflower (9.0%) than in the sumac or milo seeds and significantly less $C_{18:1}$ in sunflower than in sumac or milo. Sumac and milo did not differ significantly between them in amounts of $C_{16:0}$ and $C_{18:1}$. Sumac had a significantly larger percent of $C_{18:0}$ (12.6%) than milo (4.6%) and more (but not significantly) than sunflower (8.0%). Sunflower had almost 50% more $C_{18:2}$ than milo and 150% more than sumac. No fatty-acids were significantly different with respect to month of collection.

The mean percent fat values of dried seeds which had been exposed to natural winter weathering and collected montly (Table 10, Figure 5) were found to be significantly different with respect to an interaction between the seed type and the month of collection. In sunflower and sumac seeds, there was a decrease in the percent fat from November (24.6 and 14.6%, respectively) to significantly lower January levels of 15.5 and 9.0%, respectively. There was a subsequent rise into March with levels of 21.5% for sunflower and 15.2% for sumac which were not significantly different from the original November levels. The percent fat of dry milo seeds also declined from 2.4% in November to a December low of 1.5% with a subsequent increase to the 2.6% level in March. None of the milo percent fat levels were significantly different from each other. The February percent fat levels in sunflower was identical to that of the February sumac seeds (19.0%). In all other months, the percent fat levels in sunflower were greater than that of sumac collected in the same month. The percent fat in milo was significantly less than both sunflower and sumac in all months.

Table 9. Mean percent composition of individual fatty-acids of seeds collected monthly and analyzed with respect to seed type. (Pooled over the months)

Fatty-Acid (Carbon:Unsaturated)	Seed Type		
	Sunflower	Sumac	Milo
N	5	5	5
16:0	9.0[a]	23.3[b]	21.7[b]
16:1	0.1[a]	0.0[a]	0.0[a]
18:0	8.0[ab]	12.6[a]	4.6[b]
18:1[1]	22.7[a]	37.7[b]	34.0[b]
18:2[1]	60.4[a]	24.0[b]	40.1[c]
20:0	0.0[a]	6.2[b]	0.0[a]

a,b,c = Least square mean values with common superscripts within a row are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data arcsine transformed.

Table 10. Mean percent total fat from winter exposed seeds collected monthly and analyzed with respect to seed type by month interaction.

Month Collected[1]	Seed Type		
	Sunflower	Sumac	Milo
November	24.6^{a}	14.6^{b}	2.4^{d}
December	24.3^{a}	12.1^{f}	1.5^{d}
January	15.5^{b}	9.0^{g}	1.7^{d}
February	19.0^{c}	19.0^{c}	2.2^{d}
March	21.5^{e}	15.2^{b}	2.6^{d}

a,b,c,d,e,f,g = Least Square mean values with common superscripts are not significantly different (P = 0.05).

[1]Seeds collected on or about the first of each month.

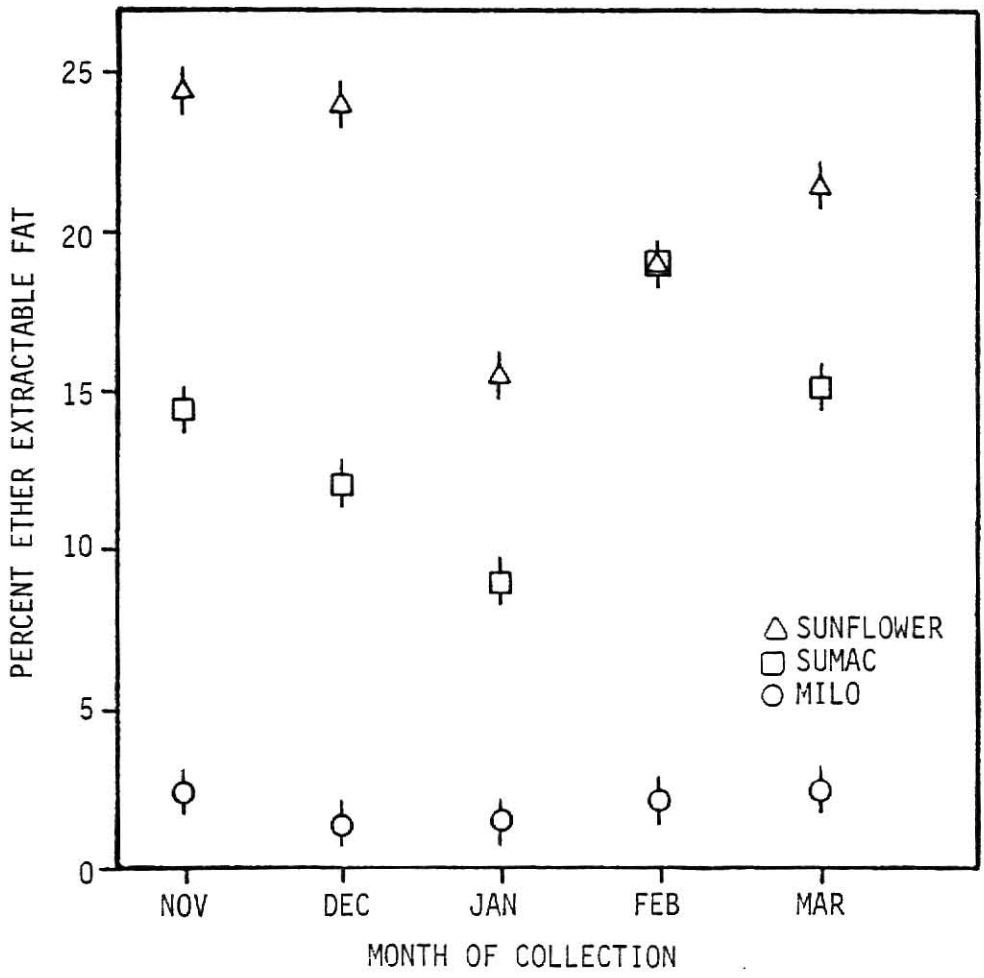

Figure 5. Mean percent total ether extractable fat from winter-exposed seeds by month of collection.

METHODS AND MATERIALS

SECTION II

This section of the thesis describes methods used to determine the metabolizable energy in the 6 experimental foods under simulated winter conditions of Section I.

Metabolizable Energy in Six Experimental Foods

Twenty-five adult male Bobwhites were obtained June 4, 1979 from Van Arsdales Hatchery, Towanda, Kansas, and were leg banded, individually confined in 48 x 25 x 13-cm polypropylene cages with 1.3-cm mesh hardware cloth tops and removable floors, and housed in an environmental chamber which simulated Kansas winter conditions; 9L-15D photoperiod and 5°C temperature. The maintenance diet, method and time of weighing, and the definition of stabilized weight are the same as that described in Section I. When weights of the birds were stable, the birds were ranked from light to heavy and divided into 5 equal sized blocks. Fifteen quail were assigned to three 5-bird experimental groups by randomly selecting 1 bird from each of the 5 weight blocks. A minimum of 32 days passed before any birds were considered weight stable.

Each of the 3 stable, 5-bird experimental groups were provided ad libitum rations of either P-18, milo, or soybean. Body weight and food intake were monitered daily over two 3-day periods. All spilled food and excreta were collected at the end of each 3-day period, carefully separated by hand with forceps and a hand lens, and oven dried in petri dishes at 65°C for a minimum of 72 h. All birds were provided P-18 ad libitum at the end of the two 3-day feeding trials until the weights stabilized (average time 29 days), reweight stratified, and assigned to 5 equal-sized groups. The 15 birds were assigned to three 5-bird experimental groups by

randomly selecting 1 bird from each of the weight groups and were used to determine the metabolizable energy in sunflower, sumac, and false sunflower using the previoulsy described procedure. In this second series of trials, the first feeding period was 2 days instead of 3 because of the rapid weight loss of birds feeding on false sunflower and sumac. Excreta and spilled food were collected daily during the second feeding period. Extra quail from the appropriate weight block were randomly chosen to replace any quail that died during the first 3-day (acclimation) trial or before the second day of the second 3-day trial. Substitute quail underwent both of the 3-day feeding trials. Data from the replaced quail were discarded.

Analysis

Dried feed and excreta were weighed to 0.0001 g, pulverized separately in a mortar and pestle, reweighed and analyzed for caloric content in a Parr Series 1200 adiabatic calorimeter using a Parr 1101 oxygen bomb under 30 atm of oxygen. Sample sizes in the bomb ranged from 0.2 g to 0.8 g ($\bar{x}$ = 0.4125). Each sample was analyzed 3 times, the mean of which was the energy value used for statistical analyses. If replications differed by more than +5% of the mean, an additional replication was conducted and the value differing by more than 5% was discarded.

The percent dry weight of the feeding trial seeds was determined by oven drying 3 samples each of the six seeds in petri dishes for 4 days at 65°C and dividing the dry weight by the original wet weight. The dry weight of food consumed by any bird was determined by subtracting the oven dry weight of spilled food from the calculated dry weight of the food supplied. The gross energy consumed (Kcal) was determined by multiplying the dry weight of food ingested (g) by its energy content (Kcal/g). Excreted energy (Kcal) was calculated by multiplying the dry weight of excreta

produced (g) by the energy content (Kcal/g) of feces from that bird and was corrected for energy from the breakdown of body tissue during weight loss. The corrections were made using the formula from Bisset (1972:55): Corrected Kcal = Excreted energy - [(grams weight lost) (.335 Kcal/g)]. Metabolized energy is the gross energy consumed minus the gross energy excreted. Metabolizable efficiency is the metabolized energy divided by the gross energy consumed.

Statistical Analysis

Arcsine transformations of raw data (Snedecor and Cochran, 1967: 327-329) were performed on percentage data where values were less than 25% or greater than 70%. Variances are greatest in these regions resulting in a skewdness of error contrary to assumptions in Analysis of Variance (ANOVA). Using transformed variables reduces the chances of producing too many significant F-test results.

The metabolic efficiency, weight change of the quail over the second 3 days of the feeding trials, energy (Kcal) ingested per day, and energy assimilated per day [(ingested energy (Kcal) minus excreted energy (Kcal)) divided by days fed] were analyzed for differences due to the food types using Analysis of Variance (ANOVA) and Least Squares Separation of Means at $P = 0.05$ significance level.

RESULTS

SECTION II

The mean gross energy (expressed as Kcal/g) of dry foodstuff (Table 11) was significantly lower for milo and P-18 (4.3 Kcal/g each) and highest for soybean and sunflower (5.8 and 5.9 Kcal/g). False sunflower contained 5.6 Kcal/g and sumac 5.1 Kcal/g. The inner seed of the sumac contained 4.5 Kcal/g which was not significantly different from the outer hull's 5.0 Kcal/g. The hull averaged 49% of the dry weight of the entire weight of the entire sumac seed.

All 6 sumac-fed quail were dead by the 3rd to 5th days of the feeding trial, 2 of the 5 false sunflower-fed quail lived only 3 days, the other 3 quail surviving 5 days. Metabolic efficiencies adjusted for weight losses were significantly different among the groups (Table 12, Figure 6). Milo-fed quail had the highest efficiency (84.6%) which was not significantly higher than the 72.7% for P-18-fed quail or the 64.7% of sunflower-fed quail. The efficiencies of the latter two groups were not significantly different from the 48.2% efficiency of false sunflower-fed quail. The efficiency of soybean-fed quail (45.0%) and sumac-fed quail (31.2%) were significantly lower than all other feeding groups with the exception of the false sunflower-fed group. The mean energy (Kcal) assimilated per day, also adjusted for weight loss, were significantly higher for the quail fed milo (70.3 Kcal) and P-18 (69.1 Kcal) and lowest for the quail fed sumac (6.6 Kcal) and false sunflower (23.6) (Table 12, Figure 6). P-18-fed birds ingested the most energy (95.0 Kcal), though they assimilated less than milo-fed birds. The amount of energy ingested by P-18-, milo- and sunflower-fed quail were not significantly different though sunflower-fed quail ingested 19% less energy than P-18-fed quail. The amount of energy ingested by soybean-fed quail was not significantly different than that of the milo-and sunflower-fed

Table 11. Mean gross energy (Kcal/g) and metabolizable energy of dry dietary items.

Seed Type	N	Gross Energy (Kcal/g)	Metabolic Efficiency (%)	Metabolizable Energy (Gross Energy x Metabolic Efficiency) (Kcal/g)
P-18	3	4.3^{a}	72.7^{ab}	3.1
Milo	3	4.3^{a}	84.6^{a}	3.6
Sunflower	3	5.9^{b}	64.7^{ab}	3.8
Soybean	3	5.8^{bc}	45.0^{c}	2.6
False Sunflower	3	5.6^{cd}	48.2^{bc}	2.7
Sumac				
Whole	3	5.1^{ad}	31.2^{c}	1.6
Inner Seed	3	4.5^{ad}	----	---
Outer Hull	3	5.0^{ad}	----	---

a,b,c,d = Values with common superscripts within a column are not significantly different (P = 0.05).

Table 12. Mean ingested and assimilated energy per day, metabolic efficiency, and the weight change over the test period of quail fed different foods, corrected for weight loss.

Ration of Experimental Group	N	Energy		Metabolic Efficiency (%)	Weight Change (g/3 days)[1]
		Ingested (kcal/day)	Assimilated (kcal/day)		
P-18	5	95.0^{a}	69.1^{ab}	72.7^{ab}	$+2.5^{a}$
Milo	5	83.2^{ab}	70.3^{a}	84.6^{a}	$+1.0^{a}$
Sunflower	5	76.8^{ab}	49.6^{bc}	64.7^{ab}	-6.2^{a}
Soybean	5	66.6^{bc}	34.8^{cd}	45.0^{c}	-8.6^{a}
False Sunflower	3	48.9^{cd}	23.6^{de}	48.2^{bc}	-31.0^{b}
Sumac	4	25.3^{d}	6.6^{e}	31.2^{c}	-41.9^{b}

a,b,c,d,e = Least square mean values with common superscripts within a column are not significantly different (P = 0.05).

[1]Weight loss for birds on a sumac diet occurred over a 2-day period.

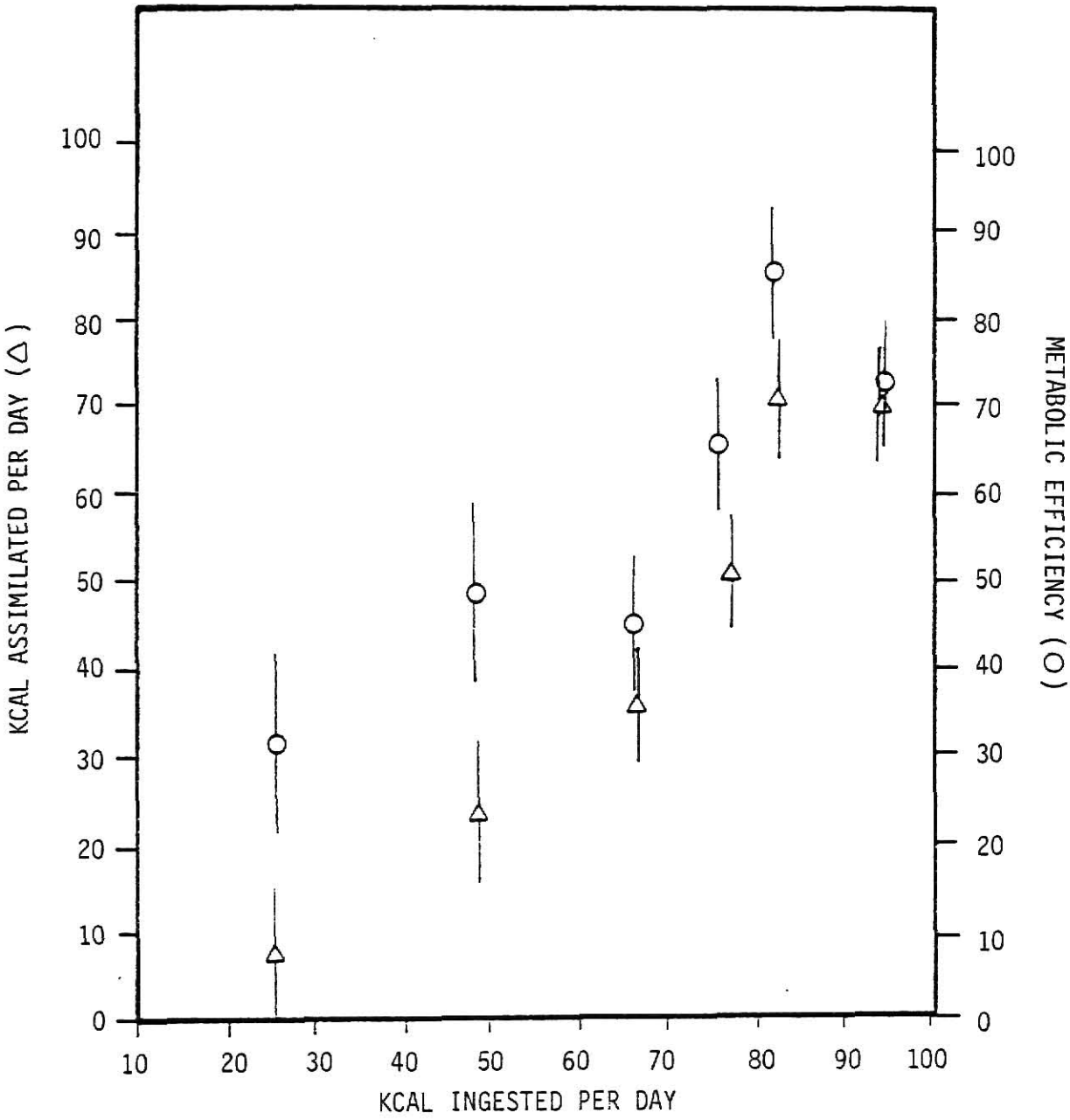

Figure 6. Mean energy (Kcal) assimilated per day and metabolic efficiency versus mean ingested energy (Kcal) per day.

quail or the false sunflower-fed quail. Sumac-fed quail ingested 73.4% less energy than P-18-fed quail and 48% less energy than the false sunflower-fed quail. The amount of energy ingested by the false sunflower- and sumac-fed quail were not significantly different from one another. False sunflower- and sumac-fed quail had significantly greater weight changes (-31.0 g and -41.9 g, respectively) than any other group. The weight changes of P-18- and milo-fed quail (+2.5 g and +1.0 g, respectively) were not significantly different from those of sunflower-and soybean-fed quail (-6.2 g and -8.6 g, respectively), the former two were biologically different in that they were the only groups which had a mean weight gain over the 2nd 3-day feeding trial (Table 12).

METHODS AND MATERIALS

SECTION III

This section reports the procedures used to assess the effects of a carbamate pesticide on the fatty-acid composition of Bobwhites and their eggs during an EPA small-pen test in the summer of 1979.

Fatty-Acid Compositions of Quail Exposed to a Carbamate Pesticide

Adult male and female Bobwhites were obtained June 4, 1979 from Van Arsdales Hatchery at Towanda, Kansas and leg banded. Thirty male and 30 female quail were randomly selected for a weight stratified pool of 225 birds and placed on cultivated soil outdoors in 4 x 5 x 1-ft (1.2 x 1.5 x 0.3 m) wood frame pens; one male/female pair per pen. The sides and top of the pens were covered with 1.3-cm hardware cloth. Each pen was equipped with a 12 x 12 x 8-in (30 x 30 x 20-cm) wooden shelter. Water was provided ad libitum in 1-ℓ watering fonts. P-18 mash (20.5% protein, 2.7% fat, and 3.6% crude fiber) prepared and pelleted by the Kansas State University Department of Grain Science and Industry was provided each morning and was equivalent to 140% of the quails' existence energy as estimated from Case and Robel (1974). The experimental area for the small-pen test was an acre of bottomland on the Konza Prairie Research Area 16 km southwest of Manhattan, Kansas.

Pens of quail were equally assigned randomly to one of the three treatments: no pesticide (control), pesticide applied 5-cm below soil surface (in-furrow), and pesticide applied on the surface (surface banded) and the order in which they were placed in a treatment row. A 16-day experimental period began after a 16-day acclimation period. Pens of quail were placed on the appropriate area of treated, corn-planted soil the evening the area was treated. Every 4th day, quail were captured and weighed to

1-g on a spring balance, eggs were collected, labeled, and weighed, and the pen was shifted to a new position on the appropriate treatment. All quail were killed on day 16, and birds from alternate pens of each treatment were stored at -16°C for later fatty-acid analysis. Eggs were refrigerated for fatty-acid analysis after which the remainder were frozen at -20°C for later calorimetric analysis.

Analysis

Five eggs per treatment per weighing date were randomly selected and analyzed for fatty-acids. Whole eggs and egg shells were first weighed to 0.1g. Content weight was determined as the difference between the whole weight divided by the whole egg weight, and multiplying this answer by 100. The fatty-acid analysis follows that described previously (Section I) using the recommended Folch et al. (1956) dilution of 20 ml of 2:1 $CHCL_3$:CH_3OH per gram of sample. Both the yolk and the albumin underwent extraction. Analysis of the fatty-acid composition of the 30 quail followed that described previously in Section I. Replicate analyses of 5 of the samples were run on the day following their initial analysis to check repeatability (Appendix Table 2). The other 25 quail were analyzed only once.

Three eggs per treatment per weighing date were randomly chosen from the remaining pool of eggs. They were punctured, oven dried in petri dishes at 65°C for 3 days, ground individually with mortar and pestle, and returned to the drying oven for another day. Three energy determinations were conducted on each egg following procedure described previously (Section II).

Statistical Analysis

Arcsine transformations of raw data (Snedecor and Cochran, 1967: 327-329) were performed on percentage data where values were less than 25%

or greater than 70%. Variances are greatest in these regions resulting in a skewdness of error contrary to assumptions in Analysis of Variance (ANOVA). Using transformed variables reduces the chances of producing too many significant F-test results.

The percent composition of individual fatty-acids, prepared carcass weight, and percent unsaturated fatty-acids of the EPA small-pen test quail were analyzed for differences with respect to the experimental treatment, sex, and treatment by sex interaction using Analysis of Variance (ANOVA) and Least Squares Separation of Means. The cage was considered to be the experimental unit as two birds were housed in each cage.

Whole egg weight, wet weight of contents, percent content weight, percent composition of individual fatty acids, percent saturated and unsaturated fatty-acids, whole egg dry weight, and energy (Kcal/g) of dry eggs from the small pen test were analyzed for differences with respect to the experimental treatment, date of collection, and a treatment by date interaction using ANOVA and Least Squares Separation of Means. The probability level chosen for use in analyzing the statistical significance of all results was $P = 0.05$.

RESULTS

SECTION III

Treatment type alone had no significant effect on the eggs laid by the EPA small pen test Bobwhites. The mean wetweight, dryweight, and mean energy (Kcal) per gram of the eggs which were randomly selected for calorimetric analysis were found to have no significant differences with respect to the pecticide treatment, the date of collection, or the interaction of pesticide treatment with the collection date. The mean energy per dry gram of egg ranged from 5.8 Kcal to 6.8 Kcal ($\bar{x}$ = 6.4 Kcal/g), the mean dry egg weights ranged from 2.8 g to 3.8 g ($\bar{x}$ = 3.4 g), and the mean egg wetweights ranged from 7.9 g to 9.5 g ($\bar{x}$ = 9.0 g) (Table 13).

The use of or manner of pesticide treatment did have an effect on the eggs selected for fatty-acid analysis when it was considered with the date of collection. Egg content weight (Table 14) of eggs laid on areas where pesticide was applied by surface banding increased significantly from 7.9 g on day 4 of the experiment to 9.2 g on day 8, decreasing back to 7.3 g on day 12. The whole egg weights did not change significantly but the percent content weights decreased significantly from the 82.5 and 85.0% of days 4 and 8 of the experiment to 77.9% on day 12. Neither the egg content weights nor the whole egg weights of the eggs laid on areas where pesticide was applied in-furrow changed significantly over the experiment, but the percent content weight declined from 84.3% on day 4 to 79.5% on day 8 and increased again to 85.7% on day 12. Eggs laid by quail on the areas where pesticide was banded on the surface had no $C_{14:0}$ on the 4th or 8th day of the experiment, but was present as 1.2% of the fatty-acids on day 12 (Table 14). Eggs laid by quail in areas where pesticide was applied in the furrow exhibited a significant decline in $C_{14:0}$ from 1.1% on day 4 of the experiment to 0.0%

Table 13. Mean wet and dry weight and energy content based on dry weight of EPA small pen test eggs; 3 eggs per sample.

Treatment Type	Collection Day	Wet Weight (g)	Dry Weight (g)	Energy Content (kcal/g)
Control: (no pesticide)	4	9.2[a]	3.8[a]	6.5[a]
	8	8.9[a]	3.3[a]	6.1[a]
	12	7.9[a]	2.8[a]	6.6[a]
Pesticide applied:				
Surface banded	4	9.4[a]	3.3[a]	6.5[a]
	8	8.0[a]	3.3[a]	6.8[a]
	12	8.9[a]	3.3[a]	5.8[a]
In furrow	4	9.1[a]	3.5[a]	6.3[a]
	8	9.5[a]	3.4[a]	6.4[a]
	12	9.3[a]	3.5[a]	6.2[a]

a = Mean values with common superscripts within a column are not significantly different (P = 0.05).

on day 8 and 0.2% on day 12. Eggs laid in the control areas where no pesticide was applied showed no significant change in the amount of $C_{14:0}$ present.

The amount of fatty-acid $C_{20:0}$ fluctuated over the 12 days in the eggs laid on all three treatment types (Table 14). While the fluctuations were not statistically significant, the amount of $C_{20:0}$ in eggs laid by birds on control areas increased from 1.5 and 0.8% of days 4 and 8 to 2.7% on day 12. The amount of $C_{20:0}$ in eggs laid on the surface-banded sites (2.0% on day 4, 9.6%-day 8, and 0.0%-day 12) and the in-furrow pesticide sites (7.6%-day 4, 11.5%-day 8, and 1.0%-day 12) increased on day 8 but decreased on day 12 (Figure 7). The raw data making up these means is highly variable making the means non-significant statistically.

The day of the experiment on which the eggs selected for fatty-acid analysis were collected influenced the whole egg weight (Table 15) and percent $C_{16:1}$ (Table 15). The eggs pooled from all three experimental treatments increased in weight from 9.7 g on day 4 to 10.3 g on day 8 and back to 9.6 g on day 12. The percent $C_{16:1}$ in all the eggs increased non-significantly from 0.1% on day 4 to 0.4% on day 8 then increased significantly to 3.5% on day 12. No other fatty-acids were altered significantly in percentage either statistically or biologically. The following fatty-acids were found in all eggs: $C_{14:0}$, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, and 20:0 (Table 15). Fatty-acid $C_{12:0}$ was present (0.1%) in the eggs laid by hens on the areas where pesticide was applied as bands on the surface (Table 14). Fatty-acid $C_{16:0}$ was present in the largest amount (35.4 to 47.7%) and $C_{18:1}$ was present in the second largest amount (21.2 to 29.1%) (Table 14).

There were no deaths of any EPA small pen test quail. The type of pesticide treatment and the sex of the Bobwhites interacted to significantly effect the prepared carcass weights of females (Table 16). The mean carcass weight was highest for female Bobwhites on areas with pesticide

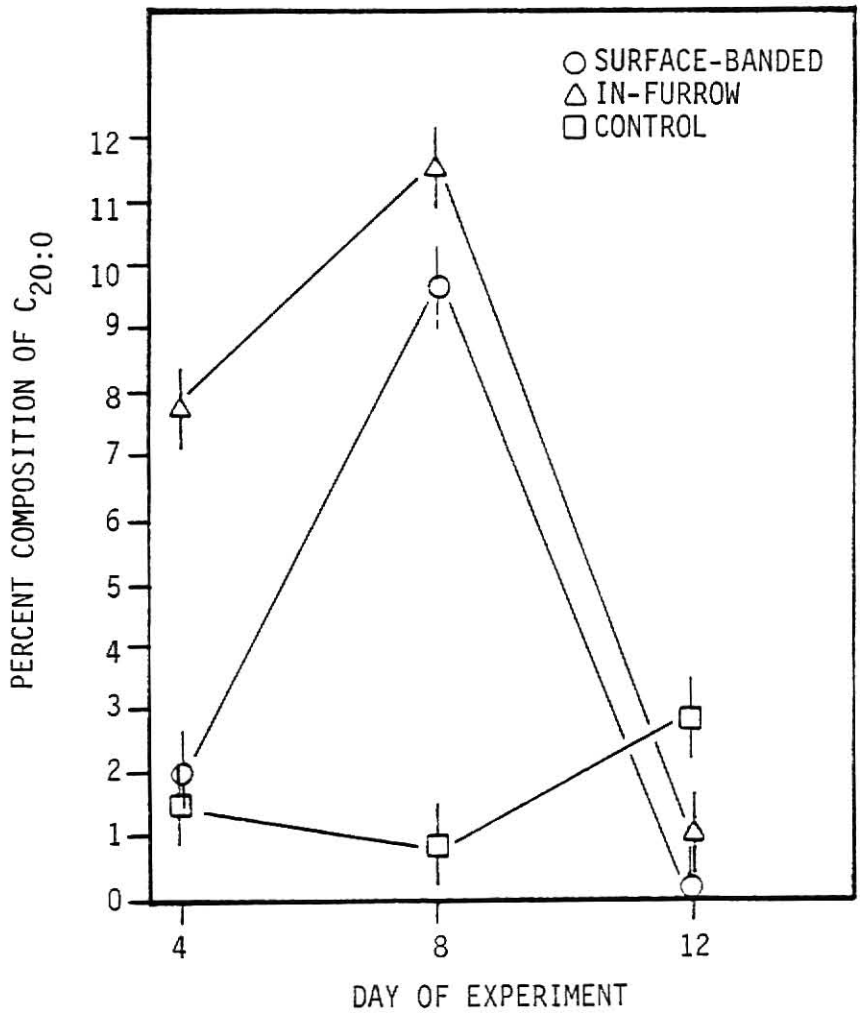

Figure 7. Changes in the percent composition of fatty-acid $C_{20:0}$ in eggs laid by EPA Small-Pen Test Bobwhites during the first 12 days of the experiment.

Table 14. Mean egg weight, percent egg content weight, and percent composition of individual fatty-acids of eggs laid by quail during an EPA small pen test and analyzed with respect to the treatment type in conjunction with the collection day.

	Treatment								
				Pesticide Applied					
	Control: (No Pesticide)			Surface-Banded			In-Furrow		
Date Collected:	4	8	12	4	8	12	4	8	12
N	5	5	5	5	5	5	5	5	5
Weight (g)									
Whole Egg	10.1^{a}	9.7^{a}	9.3^{a}	9.5^{a}	10.8^{a}	9.4^{a}	9.4^{a}	10.5^{a}	10.0^{a}
Egg Content	8.1^{ac}	8.0^{ac}	7.9^{ac}	7.9^{ac}	9.2^{b}	7.3^{c}	7.9^{ac}	8.3^{ab}	8.6^{ab}
Percent Content	80.3^{ab}	82.7^{abc}	85.5^{a}	82.5^{abc}	85.0^{ab}	77.9^{c}	84.3^{ab}	79.5^{bc}	85.7^{a}
Fatty-Acid (Carbon:Unsaturated)									
12:0	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.0^{a}
14:0	0.0^{a}	0.2^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	1.2^{c}	1.1^{bc}	0.0^{a}	0.2^{abc}
14:1	0.3^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.3^{a}	0.0^{a}	0.1^{a}	0.2^{a}	0.0^{a}
16:0[1]	47.7^{a}	36.6^{a}	35.4^{a}	41.2^{a}	39.3^{a}	39.9^{a}	36.3^{a}	37.3^{a}	36.5^{a}
16:1	0.0^{a}	1.3^{a}	3.5^{a}	0.6^{a}	0.1^{a}	2.5^{a}	0.0^{a}	0.1^{a}	4.5^{a}
16:2	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.0^{a}	0.1^{a}
18:0	8.1^{a}	14.3^{a}	12.1^{a}	19.3^{a}	17.0^{a}	14.6^{a}	15.7^{a}	14.6^{a}	16.8^{a}
18:1[1]	27.0^{a}	29.1^{a}	21.9^{a}	25.7^{a}	25.8^{a}	27.4^{a}	21.2^{a}	25.6^{a}	27.2^{a}
18:2	13.1^{a}	14.3^{a}	16.1^{a}	10.5^{a}	8.7^{a}	13.9^{a}	18.1^{a}	11.7^{a}	10.7^{a}
20:0	1.5^{a}	0.8^{a}	2.7^{a}	2.0^{a}	9.6^{a}	0.0^{a}	7.6^{a}	11.5^{a}	1.0^{a}
Saturated[1]	59.9^{a}	53.8^{a}	56.3^{a}	62.4^{a}	64.6^{a}	55.8^{a}	61.7^{a}	62.3^{a}	56.2^{a}
Unsaturated[1]	40.1^{a}	46.2^{a}	43.7^{a}	37.6^{a}	35.4	44.2^{a}	38.3^{a}	37.7^{a}	43.8^{a}

a,b,c = Least Square mean values with common superscripts within a row are not significantly different (P=0.05).

[1]Statistical analysis performed on raw data. All other data arcsine transformed.

Table 15. Mean egg weight, percent egg content weight and percent composition of individual fatty-acids of EPA small pen test eggs pooled for treatment types with respect to day of collection.

	Collection Day		
	4	8	12
N	15	15	15
Weight (g)			
Whole Egg	9.7[a]	10.3[b]	9.6[a]
Egg Content	8.0[a]	8.5[a]	7.9[a]
Percent Content	82.4[a]	82.4[a]	83.0[a]
Fatty-Acid (Carbon:Unsaturated)			
12:0	0.0[a]	0.0[a]	0.0[a]
14:0	0.1[a]	0.1[a]	0.3[a]
14:1	0.1[a]	0.1[a]	0.1[a]
16:0[1]	41.7[a]	37.7[a]	37.2[a]
16:1	0.1[a]	0.4[a]	3.5[b]
16:2	0.0[a]	0.0[a]	0.1[a]
18:0	13.9[a]	15.3[a]	14.4[a]
18:1[1]	24.6[a]	26.8[a]	25.5[a]
18:2	13.8[a]	11.4[a]	13.4[a]
20:0	3.3[a]	6.0[a]	0.9[a]
Saturated	61.4[a]	60.2[a]	56.1[a]
Unsaturated	38.6[a]	39.8[a]	43.9[a]

a,b = Least Square mean values with common superscripts within a row are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data arcsine transformed.

Table 16. Mean percent composition of fatty-acids and carcass weights of EPA small pen test quail analyzed with respect to a treatment type by sex interaction.

	Treatment Type					
	No Pesticide (Control)		Pesticide Applied			
			Surface-Banded		In-Furrow	
	Male	Female	Male	Female	Male	Female
N	5	5	5	5	5	5
Fatty-Acid (Carbon:Unsaturated)						
12:0	0.0^{a}	0.0^{a}	0.0^{a}	0.1^{a}	0.0^{a}	0.2^{a}
14:0	7.4^{a}	7.6^{a}	6.0^{a}	7.8^{a}	10.2^{a}	4.1^{a}
14:1	0.1^{a}	0.8^{a}	0.4^{a}	0.2^{a}	0.4^{a}	0.9^{a}
16:0[1]	21.6^{a}	22.4^{a}	22.2^{a}	22.0^{a}	25.6^{a}	35.1^{a}
16:1	0.2^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
16:2	0.4^{a}	0.0^{a}	1.0^{a}	1.0^{a}	0.0^{a}	0.0^{a}
18:0[1]	27.9^{a}	35.9^{a}	27.8^{a}	32.5^{a}	30.5^{a}	28.7^{a}
18:1[1]	37.1^{a}	32.4^{a}	36.0^{a}	33.8^{a}	27.5^{a}	29.2^{a}
Saturated[1]	58.7^{a}	66.7^{a}	56.8^{a}	62.9^{a}	67.9^{a}	69.4^{a}
Unsaturated[1]	41.3^{a}	33.3^{a}	43.2^{a}	37.1^{a}	32.1^{a}	30.6^{a}
Unknown Fat	0.2^{a}	0.1^{a}	2.2^{a}	0.1^{a}	2.0^{a}	0.0^{a}
Weight (g)						
Carcass	157.4^{bc}	170.2^{ab}	156.3^{bc}	185.4^{a}	163.9^{bc}	145.6^{c}

a,b,c = Least Square mean values with common superscripts are not significantly different (P = 0.05) within a row or column.

[1]Statistical analysis performed on raw data. All other data arcsine transformed

banded on the surface (185.4 g) which was not significantly different from the carcass weight of females on control areas (170.2 g). Females on areas where pesticide was applied in the furrow were significantly lower (145.6 g) in weight than other female Bobwhites. The carcass weights of male Bobwhites did not differ significantly between any treatment type.

The following fatty-acids were present in all EPA small pen test quail: $C_{14:0}$, 14:1, 16:0, 18:0, 18:1, and an unknown fatty-acid or possible contaminant between the fatty-acids $C_{12:0}$ and 14:0 (Table 17). Fatty-acid $C_{12:0}$ was not present in control quail and was present only in small amounts in the other 2 treatment groups (0.1% in female quail on areas banded with pesticide and 0.2% on areas with pesticide in-furrow) (Table 16), while fatty-acid $C_{16:2}$ was not present in quail from the in-furrow pesticide areas. Quail from the in-furrow pesticide treatment had a significantly larger percentage of $C_{16:0}$ (30.3%) than quail from the banded pesticide area (22.1%) or the control area (22.0%) (Table 17). The larger percentage of fatty-acid $C_{16:0}$ in quail from in-furrow treated areas is due to the high amount of this fatty-acid (35.1%) in the female Bobwhites in this treatment (Table 16). In contrast, quail from the in-furrow area had a lower (non-significant) percentage of $C_{18:1}$ (28.3%) than quail from the banded area (34.9%) or the control area (34.8%) (Table 17). Both the male and female Bobwhites had less (non-significant) $C_{18:1}$ than both sexes of quail in the other two treatments (Table 16). Fatty-acids $C_{18:1}$ and 18:0 were present in the greatest amount in the quail and saturated fatty-acids were present in greater quantity than unsaturated fatty-acids (Tables 16,17). Quail from the surface-banded areas had a higher percentage (40.1%) of unsaturated fatty-acids than quail from the in-furrow pesticide areas (31.4%). This difference was not statistically significant (Table 17). When broken down

Table 17. Mean percent composition of fatty-acids and carcass weights of EPA small pen test quail analyzed with respect to treatment type.

	Treatment Type		
	No Pesticide (Control)	Pesticide Applied: Surface Banded	Pesticide Applied: In Furrow
N	10	14	10
Fatty-Acid (Carbon:Unsaturated)			
12:0	0.0[a]	0.0[a]	0.1[a]
14:0	7.5[a]	6.9[a]	6.8[a]
14:1	0.4[a]	0.3[a]	0.6[a]
16:0[1]	22.0[a]	22.1[a]	30.3[b]
16:1	0.1[a]	0.0[a]	0.0[a]
16:2	0.1[a]	1.0[a]	0.0[a]
18:0[1]	31.9[a]	30.2[a]	29.6[a]
18:1[1]	34.8[a]	34.9[a]	28.3[a]
Saturated[1]	62.7[a]	59.9[a]	68.6[a]
Unsaturated[1]	37.3[a]	40.1[a]	31.4[a]
Unknown Fat	0.1[a]	0.9[a]	0.5[a]
Weight (g)			
Carcass	163.8[a]	170.8[a]	154.7[a]

a, b = Least Square mean values with common superscripts within a row are not significantly different (P = 0.05).

[1]Statistical analysis performed on raw data. All other data arcsine transformed.

into separate sexes (Table 16), male Bobwhite from the control and surface-banded areas both had larger (non-significant) percentages of unsaturated fatty-acids than the male Bobwhites from the in-furrow treated areas. Female Bobwhites from the surface-banded treated area had more (non-significant) unsaturated fats than either of the other treatments.

DISCUSSION

SECTION I

Milo, sumac, and sunflower were chosen to be experimental seeds because together they comprise over 50% of the fall and winter diet of Bobwhites in Kansas (Robel et al., 1974). False sunflower and soybean were added as experimental foods when the quail fed sumac were not surviving the full 20 days. The seeds were to be pelleted to reduce differences in composition and handling, however, only milo was pelleted when it was discovered that the other seeds, because of their composition, would have been subjected to intense heat and pressure which could have altered the amount and composition of the volatile fatty-acids in the seeds.

The weight recovery results of starved quail fed P-18 as a recovery diet agree with the previous data presented by Beardmore and Robel (1976) who state that dietically stressed birds in the laboratory can regain, in 10 to 14 days, their normal weight after a 20% weight loss if a nutritious diet is provided. The larger percentage of unsaturated fatty-acids in starved birds must have been due to a higher utilization of the saturated fatty-acids during the period of food deprivation since there was no dietary input. More specifically, the retention of $C_{20:4}$ was probably the cause of the larger amount of unsaturated fatty-acids in the starved quail. Fatty-acid $C_{20:4}$ (arachidonic acid) is synthesized from $C_{18:2}$ and is considered to be an essential fatty-acid for proper growth and cell maintenance (Suttie, 1972: 57; Hilditch, 1956: 478). Other nutritionally deprived quail (those fed sumac or false-sunflower) also had a very high percentage of $C_{20:4}$ illustrating their similarity to starved quail. This also would most likely be due to the selective retention of this essential fatty-acid. Bobwhites from both the P-18 diet with lengthening times experiment and the different diets for

20 days experiment had the greater percentage of the fatty-acids made up of unsaturated fatty-acids. Bower and Helms (1968: 166) in their study with Juncos calculated the energetic gain of selectively laying down unsaturated fatty-acids and found it to be "of minor adaptive importance." Johnston and West (1973) proposed that the increased mobility of the more unsaturated fats might be of "adaptive significance" during the metabolic demands of migration. This increased mobility may also be of biological significance in regards to the metabolic demands of non-migratory birds in winter. Periods of food deprivation and/or extreme environmental conditions (cold, sleet, etc.) would be better tolerated if energy reserves could be utilized quickly. Small organisms, such as Bobwhites, have a high surface/volume ratio causing a greater heat loss problem. Energy must be available soon to maintain a homeothermic animal in ambient temperatures below the thermoneutral zone (Geers, 1978). How much of an advantage is gained by having more unsaturated fats is not known. Yom-Tov and Tietz (1978) propose a third possible explanation for the large amount of unsaturated fatty-acids seen in animals during winter conditions. Fatty-acids are important in giving flexibility to cell and mitochondrial membranes. They report correlations between the percentage of unsaturated fats and membrane flexibility of mitochondria in homeothermic rats and poikilothermic catfish as possible evidence for this idea. They continue by applying the idea to birds. Though birds are homeothermic, the extremities and surface area are lower than the core temperatures. Therefore, unsaturated fatty-acids, which have a lower melting point, could remain in a more liquid state and give flexibility to cell membranes at lower temperatures. An additional piece of evidence for a relationship between ambient temperatures, which affect the temperature of the surface area of homeotherm, and the amount of unsaturated fatty-acids is seen in the results of the EPA small pen test study (Results III, Discussion III) where quail in summer conditions had a greater percent of <u>saturated</u> fatty-acids.

It is important to note that in the experiment where birds were fed different foodstuff for 20 days, quail fed false sunflower and sumac did not survive the full 20 days. There are then, in addition to the experimental design, two factors which could have influenced the composition of fatty-acids in the Bobwhites; the length of time on the diet, and the nutritional quality of the diet.

Direct comparisons of the percent fatty-acids of dietary items and of quail fed those diets could not be made for all fatty-acids due to differences in the variances of the seeds and quails' fatty-acids. For the allowable comparisons, no group of quail had fatty-acid profiles which were not significantly different in at least 2 fatty-acids from their corresponding diet's profiles. Based on this information fatty-acid profiles of Bobwhites under simulated winter conditions do not appear to reflect the compositions of their diets based on one-to-one comparisons of percent fatty-acid compositions. However, an absence of a direct one-to-one statistical equality between the diet's fatty-acids and the quails' fatty-acids does not mean that the diet does not have an influence on, or even direct the fatty-acid profile of the quail. Even if quail alter their dietary fatty-acids, there might be a degree of predictability to the manner in which the fatty-acids in the food are altered. Direct comparisons of percent fatty-acid compositions in the diet and quail may not, then, be an appropriate means of analyzing the dietary influences on avian fatty-acid deposition.

Discriminant Analysis (Snedecor and Cochran, 1967; Sokal and Rohlf, 1969) allows one to determine the amount of overlap among populations by simultaneously analyzing two or more variables. In this study, it was used to classify new individuals (quail) into previously recognized groups of quail fed particular diets based on the characteristics of the groups; which are, in this instance, the individual fatty-acid compositions of the quail fed the different diets.

The large number of birds which were correctly classified into their proper dietary group indicated that there is a relationship between the fatty-acid composition of a dietary item and the quail fed that itme, and that it is possible to "predict" the diet of a bird using Discriminant Analysis. Misclassifications between sumac, false sunflower, and starved groups hold a particular interest because they all represent birds that are malnourished. All quail in these three groups had very similar fatty-acid compositions with the exception of $C_{18:2}$. Misclassifications between other groups shows that there are diets which, when altered by the quail, do not produce a distinct fatty-acid profile in the quail and therefore, are not easily distinguishable from the compositions of quail fed other diets. The usefulness of this technique of Discriminant Analysis needs to be tested next on birds of field origin. Presumably, coveys of quail would be feeding on similar dietary items and should all be classified into the same food type. Experiments could be performed in which coveys feeding in known areas in winter (mapped for vegetation and possible food sources) such as grain fields would be trapped and analyzed for fatty-acid composition. The fatty-acid compositions of laboratory quail on known diets would be used by the researcher to predict the diet of the wild quail using Discriminant Analysis.

The high amount of unsaturated fatty-acids in the dietary seeds is typical of that usually found in plant matter. Generally speaking, the most abundant and widely distributed fatty-acids in plant fats are $C_{18:1,}$ 18:2, and the saturated fat; 16:0 (Hilditch, 1956: 437). In this light, the inverse relationship between the amount of linoleic acid ($C_{18:2}$) and palmitic acid ($C_{16:0}$) is not unusual. Since these two are the main fatty-acids found in the seeds analyzed here, if one fatty-acid is found in smaller quantity, it stands to reason that the deficit will be made up of

the remaining major fat. Sunflower seeds are reported by Hilditch (1956: 305,374) to be the most sensitive of all the Compositae plants to variation in fatty-acid composition due to different conditions of growth. The sunflower seed oils from individual ripe heads of sunflowers of the same variety grown in the same plot were found to vary over a range of about 5 units per cent of linoleic acid. While no separate fatty-acid analyses were performed on the fruit coats and the seeds, Hilditch (1956: 163-164) reported data that show the composition of each may be very different from the other. Specifically, the fruit coat of the species Rhus (sumacs) contained an average of 77% $C_{16:0}$, 20-35% $C_{18:1}$, and trace $C_{18:2}$. In contrast, the fat of the seed contained "small" amounts of $C_{16:0}$, and "much" $C_{18:1}$, and 18:2. He concludes to say that whereas seed fats contain "a wide range of specific component acids according to their botanical origins, fruit-coat fats almost invariably include only $C_{16:0}$ and 18:1 with occasional $C_{18:2}$ among their major component acids" (Hilditch, 1956: 164). Because so much of the sumac seeds passed out of the digestive system undigested (Discussion II), the fatty-acids actually available to the quail fed sumac may only have been $C_{16:0}$ and 18:1.

The drop in percent ether extractable fat of seeds and subsequent rise during the winter months is very curious and difficult to explain. The loss of fats in the exposed seeds could easily be due to the oxidation of fats, but the replenishment of them in the seeds is another matter. Because it happens in all the seeds, and because there were 3 replications per seed type each month, this cannot be blamed on miscalculations or improper sample size. Instead, I would like to pose that it would be possible to add fat to the seeds if bacteria or fungus were present which were converting carbohydrates or other nutrients into fats. The fats measured may be those in the micro-organisms themselves. Analysis on the change in carbohydrate levels or protein levels in the seeds would provide more information in solving this problem.

SECTION II

The gross energy values of the milo (4.3 Kcal/g), smooth sumac (5.1 Kcal/g), and sunflower (5.8 Kcal/g) agree with the values given by Robel (1979) of 4.304 Kcal for sorghum, 5.23 Kcal/g for smooth sumac, and 6.041 Kcal/g for sunflower (Helianthus annuus). The metabolizable energy values (metabolic efficiency times gross energy (Kcal/g of food) of sunflower (3.82 Kcal/g) and milo (3.64 Kcal/g) were comparable to those values given by Robel (1979) of 3.64 Kcal/g and 3.564 Kcal/g, respectively. The soybean metabolizable energy value (2.61 Kcal/g) was lower than the 3.776 Kcal/g reported by Robel (1979). The differences in the soybean values could be due to different strains of commercial soybean or a poorer crop used during this study.

The inner seed and the outer hull of sumac both contain the same ($P > 0.05$) amounts of gross energy (Kcal/g). An examination of the droppings of sumac-fed quail, however, revealed many inner seeds from the sumac which had passed through the gut undigested. Since the inner seed constitutes 51% of the seed by dryweight, at least half of the available energy in some of the sumac was untouched. In addition, uncrushed sumac had only 43.4% of the total ether extractable fat. This is comparable to the amount of fat a quail might receive if the inner seed passes through undigested. Not only is 56.6% of the available fat in a seed lost, while the gut is full of sumac there is less room in the gut for more nutritious foods. Exactly how much of sumac is wasted has been studied by Nestler and Bailey (1944). These investigators report one experimental quail fed dwarf sumac (Rhus latifolia) which passed 47.1% of the seeds out in the excretement. Dwarf sumac was shown by Nestler and Bailey (1944: 695) to contain 12.00% fat and 27.64% crude fiber. Smooth sumac used in this study had a comparable 12.9% ether extractable fat.

False sunflower also has a very high (18.8%) fat content and gross energy (5.6 Kcal/g) value, but it was also observed undigested in the feces. These small seeds are, like sumac, very hard and may be difficult to crush in the gizzard to digest. As with sumac, the high fat content is useless to a wild bird with limited feeding time and gut space when part of it cannot be utilized.

P-18-fed quail exhibited a high metabolic efficiency (73.7%), similar to the 73.2% reported by Bisset (1972: 27) which was determined under similar simulated conditions of 1°C temperature and 10L:14D photoperiod. P-18 proved to be a nutritious diet as evidenced by the weight gain of the Bobwhites fed P-18. The efficiency of assimilation for male Bobolinks (Dolinchonyx oryzivorus) under simulated natural photoperiods and fed Purina Game Bird Starter (4.260 Kcal/g dryweight) was 63% during pre-fat deposition, but 58% during rapid fat deposition in fall and slow or fast fat utilization in winter (Gifford and Odum, 1965: 391). Milo, a component part of P-18 (Appendix Table 1), also proved to be a highly nutritious food. The metabolic efficiency of quail fed milo was higher than that of P-18-fed quail. There was not as large a weight gain as in the P-18-fed quail but this can be attributed possibly to less nutrients and minerals in an all-milo diet, or more likely, the adjustment of the body to digesting and utilizing a different diet. These two diets were nutritionally complete for caged Bobwhites kept indoors in simulated winter conditions, but how might they compare to wild birds and their energy needs? For caged birds, the main energy requirement is thermoregulation as there is little activity. West (1968: 1043) estimated that, for willow ptarmigan, the "added cost of maintenance and winter activity in outdoor cages is about 20 Kcal/day." Kendeigh (1949: 118) notes that for English Sparrows, there is less efficient digestion and a less complete utilization of food energy at lower temperature.

Case (1971: 76) found no predictable pattern for efficiency of food utilization for Bobwhites as influenced by ambient temperature. Egg-laying females had a higher efficiency than males at cold temperatures but their efficiency did not change significantly with temperature. West (1968: 1044) reports that the caloric "equivalent of willow ptarmigan droppings collected in the wild is much higher than that of captives at any season." From this information, he calculates that the "approximate digestive efficiency of wild birds living on a natural diet" is approximately 33 to 50%.

Kendeigh (1949: 117) reported than an increasing amount of energy lost in the feces is to be expected at decreasing temperatures because of the larger amounts of food ingested and from "the increased rate of metabolism in the body generally." Brody (1945: 79-86) is cited by Kendeigh (1949) to say that the completeness of digestion and absorption "decreases as the mass of food ingested increases." This is contrary to the results of this study (Figure 6) which show metabolic efficiency increasing as ingestion decreases. Figure 6 is the result of information from various diets but still illustrates that increasing the amount of energy ingested does not curb the efficiency with which it is digested for Bobwhites under simulated winter conditions.

SECTION III

The composition of fatty-acids in eggs generally tend to "reflect" that of the diet of the hens which laid them (Machlin et al., 1962). While this may be a working generalization, a comparison of the composition of fatty-acids in P-18 (Table 5) with those of the eggs (Table 14) shows at least three areas where this is not the case: the amounts of $C_{16:0}$, $C_{18:2}$, and unsaturated or saturated fats. Cruickshank (1934) and Feigenbaum and Fisher (1959: 305) propose that egg fats are influenced only by unsaturated fats. It seems safer to say that the composition of the eggs reflects that of the Bobwhite hens. While comparing the fatty-acid compositions of the hens and the eggs may explain the general fatty-acid pattern, it does not explain the changes in $C_{14:0}$ or 20:0 mentioned previously (Results III). P-18 was not the only dietary item the experimental quail had access to. Animal matter, such as insects or worms, and corn plant seedlings were available to the quail and were observed to have been consumed. The corn seedlings were available to all three experimental groups of quail and, unless the composition of fatty-acids in the plants was affected by the pesticide, would have been a consistent food addition to all pens. Table 3 of the Appendix shows the mean number of uneaten corn seedlings per pen for each treatment on days 4, 8, 12, and 16 of the experiment. The pesticide may have affected both the number of invertebrates available and their fatty-acid composition. This extraneous animal and plant matter would have been an important factor in considering the fatty-acid composition of both the Bobwhites and the eggs laid by the hens. The presence of the carbamate pesticide and its mode of application does then seem to influence the fatty-acid composition of the eggs and the Bobwhites, but whether it is a direct influence or indirect by means of extraneous dietary matter is unknown.

The presence and mode of application of this pesticide also influenced the weight of the female Bobwhites, either directly or indirectly. The low weight of the hens on the in-furrow treated areas may be due in part to continued egg laying (Appendix - Table 4). Females on in-furrow pesticide areas laid more eggs than the females of the other groups and continued to lay eggs up to the end of the experiment. This cannot be the entire explanation, however, because females on control areas, which quit laying before the other females, were lower in weight than the females on the surface-banded pesticide areas. Wehther or not the presence of pesticide or the mode of application influenced the amount and duration of egg laying is uncertain. Again, the indirect influences on other dietary items could also have affected the weights of the females, but curiously, the males did not change in weight. If the pesticide somehow altered the behavior of the quail, the amount of food consumed or amount of energy utilized by one sex could be altered. This idea shows itself in the greater weight of males on the in-furrow treated area with the marked low weight of the females on the same area (Table 16).

The dominance of saturated fatty-acids in all three treated groups of quail is of interest since the quail in simulated winter conditions contained more unsaturated fatty-acids. The factors which could have influenced the larger amount of saturated fats (extraneous dietary items, temperature, and photoperiod) would need to be controlled for in addition research to determine their influence.

CONCLUSIONS

Through the use of controlled, laboratory experiments, it has been shown that:

1. Food deprived quail fed a nutritious diet (P-18) regain their original stable weight, fat levels, percent fat levels, and their original percent $C_{20:4}$ levels in 10 to 20 days. A period of 40 days was needed to restore the percent unsaturated fat to the pre-starved levels.

2. The percent compositions of fatty-acids in quail under simulated winter conditions do not directly reflect that of the diet (P-18, milo, sunflower, soybean, false sunflower, and sumac). The use of direct comparisons of fatty-acids in the quail and the diet does illustrate the similarities and differences between them, but does not indicate the degree of influence the diet has on the fatty-acid profile of the quail.

3. Dietary fatty-acids alter the fatty-acid compositions of Bobwhites in a predictable manner. Discriminate Analysis can be used to predict the diet of Bobwhites. Nutritionally deficient quail were occasionally misclassified into another group of nutritionally deficient quail.

4. False sunflower- and sumac-fed quail are nutritionally deficient and have a larger percent of arachiodonic acid ($C_{20:4}$), and essential fatty-acid, as do starved (food deprived) quail.

5. Sunflower and sumac, exposed to natural winter weathering, have a significant decline ($P < 0.05$) in ether extractable fat (based on dryweight) from November to January with a subsequent rise in March.

6. The metabolic efficiency of quail fed 6 different food items ranks from high to low as follows: milo, P-18, sunflower, soybean, false sunflower, and sumac. Quail died within 1 to 6 days when fed false sunflower or sumac if not previously starved. Quail died within 1 to 4 days when fed false sunflower or sumac if they had been previously starved (food deprived).

7. The presence of a carbamate insecticide and its mode of application had some effects on Bobwhites and their eggs, either directly or indirectly through differences in the compositions of fats in extraneous insect and plant dietary matter. No quail died. There was a significant influence ($P < 0.05$) of treatment type (no pesticide, pesticide applied surface-banded, or in-furrow) on the egg content weight, percent content weight, and percent $C_{14:0}$ but this effect was not independent of the day of collection. Non-significant changes were seen in the levels of $C_{20:0}$ in the same eggs. Quail caged on in-furrow pesticide sites had significantly more $C_{16:0}$, specifically in females, not males. There were significant treatment differences ($P < 0.05$) in the carcass weights of female Bobwhites, these differences were independent of sex.

8. Quail under simulated Kansas winter conditions had more unsaturated fatty-acids than quail under summer field conditions.

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APPENDICES

Appendix 1. Formulation of the P-18 pelletized feed used in this study. Percentages expressed on a weight basis.

INGREDIENTS	PERCENT	CUMULATIVE PERCENT
Soybean oil meal (44%)	28.0	28.0
Alfalfa meal (17%)	2.0	30.0
Ground sorghum grain	46.0	76.0
Ground corn	15.0	91.0
Fish meal	1.0	92.0
Meat and bone scraps	1.0	93.0
Distillers dried solubles	2.0	95.0
Ground limestone	0.5	95.5
Dicalcium phosphate	3.0	98.5
Salt	0.5	99.0
Vitamin B_{12} (Proferm 20)	1.0	100.0
Vitamin D_3 (15,000 ICU/g	-	-
Vitamin A (10,000 IU/g)	-	-

Appendix 2. Percent composition of fatty-acids of five quail repeated on subsequent days.[a]

Percent Fatty-Acid (Carbon:Unsaturated)	QUAIL NUMBER									
	201		273		345		410		412	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
12:0	0.0	3.4	0.0	2.1	0.0	0.0	0.0	0.0	0.0	4.0
14:0	4.9	11.0	0.8	7.0	29.4	15.3	7.9	2.6	4.7	4.0
14:1	0.0	0.0	0.7	4.9	0.0	0.0	0.0	7.9	0.0	0.0
16:0	36.9	23.4	18.7	21.1	23.8	23.0	23.5	21.8	11.2	23.8
16:1	0.0	0.0	0.0	0.0	5.9	8.0	0.0	0.0	0.0	0.0
16:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	3.5	7.9
18:0	22.6	27.6	39.9	25.4	11.2	20.4	26.7	32.4	35.2	27.8
18:1	35.5	34.5	39.9	39.4	29.7	33.2	41.8	32.4	45.4	32.4

[a]The GLC analysis on day 2 was done at increased sensitivity.

Appendix 3. Mean number of uneaten corn seedlings per pen on days 4, 8, 12, and 16 of the EPA Small-Pen-Test.

	Treatment Type		
	No Pesticide	Pesticide Applied	
	Control	Surface Banded	In-Furrow
Day of Experiment	Mean number of seedlings per pen		
4	0.0	0.0	0.0
8	0.0	0.0	0.2
12	0.0	0.3	0.0
16	1.3	2.3	5.3

Appendix 4. Mean number of eggs laid per female Bobwhite during four 4-day periods of the EPA Small-Pen Test.

	Treatment Type		
	No Pesticide	Pesticide Applied	
4-Day Period	Control	Surface Banded	In Furrow
Of Experiment	Mean number of eggs laid per female Bobwhite		
1-4	1.1[a]	1.2[a]	1.7[a]
5-8	1.6[a]	1.2[a]	1.6[a]
9-12	0.9[a]	0.9[a]	1.7[a]
13-16	0.0	0.4[a]	0.4[a]

[a]Values in a row not significantly different based on a t-test; 18df, 0.05 significance level.

Appendix 5. Results of t-test[a] on mean number of eggs laid per female Bobwhite during the EPA Small-Pen Test.

Days	Comparison	t-value
1-4	C. and IF	1.381
	C. and B.	0.234
	B. and IF	1.387
5-8	C. and IF.	0.209
	C. and B.	0.744
	B. and IF.	0.972
9-12	C. and IF.	1.840
	C. and B.	0.000
	B. and IF.	1.960
13-16	C. and IF.	-----
	C. and B.	-----
	B. and IF.	0.000

[a] t value = 2.101 for 18 df and 0.05 significance level.

METABOLIZABLE ENERGY IN SIX FOODS AND EFFECT OF DIET ON BODY FATTY-ACIDS IN BOBWHITES

by

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The question of the influence of dietary fatty-acid content on the fatty-acid compositions of adult male Bobwhites (Colinus virginanus) under controlled, simulated-winter conditions; the metabolic efficiency of quail on those diets; and the fatty-acid compositions of male and female Bobwhites and their eggs as influenced by a carbamate pesticide during a summer EPA small pen test were the problems of interest in this research.

Adult male Bobwhites were housed individually in an environmental chamber with a 9L:15D photoperiod and 5°C temperature. One set of quail were used to determine the influence of time on quail fatty-acids and a second set of quail were fed either mash, milo, sunflower, sumac, soybean, or false-sunflower seeds for 20 days to determine the influence of dietary fats on the quails' fats. The percent fatty-acid compositions in quail and seeds were analyzed using Gas Liquid Chromatography (GLC) and the amount of total fats were determined using a Goldfisch ether extraction unit. Analysis of Variance and Discriminant Analysis were the statistical tests used. Fatty-acids $C_{18:1}$ and 18:2 comprised 41-54% of the fatty-acids in quail fed mash over different lengths of time. Fatty-acids $C_{16:0}$ and 20:4 comprised another 26-32%. Fatty-acid $C_{20:4}$ made up 39-49% of the fats in starved, sumac-, or false sunflower-fed quail, none of which survived past 4 days. Fatty-acids $C_{18:2}$ and 20:4 were equally prominent in milo-fed (23.4 and 25.6%, respectively) and soybean-fed quail (25.1 and 21.7%, respectively). Fatty-acid $C_{18:2}$ made up 45.5% of the fats in sunflower-fed quail. The use of discriminant analysis to predict the diets of quail correctly identified the diets of birds fed mash, milo, false sunflower, and sumac; but misidentified the diets of some soybean- and sumac-fed and starved birds.

Adult male quail in an environmental chamber were fed the aforementioned diets for 2, 3-day periods to determine the metabolic efficiency (energy)

consumed-energy excreted x 100/energy consumed) of quail on those diets. The energy content of foods and excreta were analyzed by bomb calorimetry and Analysis of Variance. The metabolic efficiency of quail was higher when fed milo (84.6%), P-18 (72.7%), or sunflower (64.7%), and lower when fed false sunflower (48.2%), soybean (45.0%), or sumac (31.2%).

Adult Bobwhites were housed as male/female pairs outdoors on cultivated soil during a summer, EPA small-pen test. Ten pairs of quail were placed on each of three corn-planted areas: no pesticide, carbamate pesticide applied on the surface, and pesticide applied in the furrow. Quail killed after sixteen days were analyzed for fatty-acid content using GLC and total fat content using a Goldfisch fat extraction unit. Eggs collected every 4 days were also analyzed for fatty-acids and total fat. The caloric content of eggs was determined by bomb calorimetry. Data was analyzed using Analysis of Variance. No EPA test quail died. The weight of females was different between the treatments and the level of $C_{16:0}$ was greater in quail from the in-furrow pesticide area. Saturated fatty-acids were the most abundant in quail from the summer test while unsaturated fatty-acids were most abundant in quail from the simulated winter experiments.

It is concluded that dietary fatty-acids do influence the fatty-acids in Bobwhites and that the use of discriminant analysis can identify the diets in many cases. The carbamate pesticide did have an affect on the quail, either directly or indirectly through dietary matter.