THE EFFECT OF DIETARY LYSINE LEVEL ON THE ENERGY AND NITROGEN BALANCE OF THE DARK-EYED JUNCO

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Our knowledge of dietary requirements of passerine birds is severely limited, but it has been found (Martin 1968) that Tree Sparrows (*Spizella arborea*) can maintain positive weight, nitrogen balance, and energy balance when fed an 8.7% protein diet which supplies 4,060 calories per gram. Diets with approximately the same caloric content, but lower protein contents (2.5% and 4.9%) are not adequate for maintenance; one of higher protein content (16.9%) results in wastage of protein. Low protein diets (about 9%) also have been found to be adequate for maintenance in House Sparrows (*Passer domesticus*) (Martin, unpubl. data). Nevertheless, standard poultry diets that have been fed to passerine birds in bioenergetic studies in recent years usually contained 14–16% protein with combustible energy contents of about 4,000 cal/g (Kendeigh 1949, Seibert 1949, Zimmerman 1965, Brenner 1966, Brooks 1968, Helms 1968, Kontogiannis 1968).

Although the essential amino acid requirements have been studied extensively in commercially important fowl (NRC 1966), they have not been investigated in passerine birds. The purpose of our study was to investigate the maintenance energy and nitrogen balance of the Dark-eyed Junco (*Junco hyemalis*) when fed amino acid-supplemented 8% protein wheat diets. We chose the requirement for lysine for study because lysine is often a major limiting amino acid in Gramineae seeds (Block and Weiss 1956, Orr and Watt 1957), and such seeds are the predominant winter food of the junco and many other granivorous passerines (Martin et al. 1951).

MATERIALS AND METHODS

Juncos were captured with mist nets in the vicinity of Bowling Green, Ohio, and transferred to 1 × 1 × 1 m cages. Initially, all trapped birds were fed a mixture of wild bird seed (Alber Brothers Co.). After a few days, a chicken laying mash (approximately 15% protein) was blended with the seed in increasing proportions until a complete transition to mash was made.

The birds were weighed at this stage, classified according to visible fat depot (West 1960) and placed in individual cages designed for metabolism studies with small passerine birds (Martin 1967). There they were fed laying mash and subjected for three weeks to the physical conditions of the experiment (photoperiod, temperature, routine servicing, etc.). These conditions were a nine-hour photoperiod provided by standard fluorescent lamps and an air temperature of 24 ± 2°C maintained by a thermostatically-controlled air-conditioner. Six birds were randomly assigned after acclimation to each of the two experimental diets, and maintained on their assigned diets throughout the remainder of this study. Food and water were freely available at all times during the experiment.

The birds were weighed and fed, and excreta were collected at 3- or 4-day intervals. The procedures used in servicing the birds and analyzing feed and excreta samples were similar to those described by Martin (1968). The first chemical analyses were made on the collections from day 9 through 12, or three service periods after the birds were first fed their respective experimental diets. Unavoidably, the 12- through 15-day samples were not analyzed but all subsequent 3- or 4-day excreta collections were analyzed. The combustible energy (CE) of collected excreta and feed samples was measured with an adiabatic Parr oxygen bomb calorimeter, Model 2331. Nitrogen contents of those materials were measured by a Coleman, Model 29A, nitrogen analyzer which had a manufacturer's rated accuracy of ± 0.2% nitrogen (N × 6.25 = 1.25% protein).

EXPERIMENTAL DIETS

The 8% protein diet (corn:soybean oil meal, 30:70) found adequate for maintenance in Tree Sparrows (Martin 1968) was assumed to be adequate for juncos, since stomach analyses have indicated these two species eat similar seeds in the wild (Martin et al. 1951:200–201). The levels of amino acids contained in the diet used by Martin (1968) were calculated from amino acid analyses (Bressani and Mertz 1958:232, Smith et al. 1964:178) of the corn and soybean oil meal in that diet. The calculated values constituted the desired levels of each of the amino acids to be provided in the diets used in this study.

The basic source of protein in the diets for this study (table 1) was soft red wheat (Monon variety) which provided 8% protein (N × 6.25). Amino acid levels in the wheat were calculated from published amino acid analysis of Monon wheat (Waggle et al. 1967:50). The differences between the level of each amino acid provided by the wheat and the level desired to be available in the diets was compensated for by adding crystalline amino acid (L-form) to the formulation. Lysine was added only to the control diet but 0.2% urea was added to the low-lysine diet instead of lysine, as a non-protein nitrogen (Chavez et al. 1966, Fernandez del Pino 1969).

TABLE 1. Percentages of ingredients in the diets.

	Diet	
	Control	Low-lysine
Wheat, Monon variety	66.390	66.390
Corn oil	2.000	2.000
Corn starch	15.650	15.650
Cellulose (Alphacel)	7.990	7.990
Mineral mixture[a]	7.000	7.000
Vitamin mixture[b]	0.190	0.190
Choline chloride	0.200	0.200
Arginine	0.077	0.077
Threonine	0.038	0.038
Tyrosine	0.030	0.030
Leucine	0.149	0.149
Isoleucine	0.059	0.059
Valine	0.027	0.027
Lysine	0.200	—
Urea	—	0.200
Procaine penicillin	11mg/kg	11mg/kg
Tetracycline	11mg/kg	11mg/kg
Total	100.00	100.00

[a] Modified Fox and Briggs Salt using $CaHPO_4 \cdot H_2O$, plus 0.007% $Na_2MoO_4 \cdot 2H_2O$ and 0.0003% Na_2SeO_3.

[b] Supplied the following in mg/kg of diet: thiamine HCl 100; niacin 100; riboflavin 16; calcium pantothenate 20; pyridoxine HCl 6; biotin 0.6; folic acid 4; p-aminobenzoic acid 2; menadione 5; alpha tocopherol acetate 20; vitamin B_{12} 0.02; vitamin D_3 600 IU; vitamin A acetate 10,000 IU per kg of diet.

The mineral mixture was a Briggs salt mixture (Fox and Briggs 1960), modified by additions of trace amounts of molybdenum and selenium salts (elements characteristically deficient in Monon wheat; Nabor and Touchburn, Ohio Agricultural and Research Development Center, 1968, pers. comm.). Those minerals were added to alleviate any possible deficiency symptoms since they have been shown to be essential for poultry (Teekell and Watts 1959, Thompson and Scott 1969).

The average combustible energy (CE) of the control diet was $3,871 \pm 9$ (SD) calories per gram, while that for the low-lysine diet was $4,172 \pm 9$ calories per gram. The control diet provided 8.5 ± 0.4 (SD)% protein; the low-lysine diet provided $10.6 \pm 0.7\%$ protein. We do not know the reason for this greater amount of nitrogen (protein) in the low-lysine diet. Possibly an error was made in the amount of urea added to the low-lysine diet when it was constructed. Since that compound is over 46% nitrogen, a small error in weighing would greatly influence the total nitrogen content in the diet.

The wheat used in the diets was ground in a Standard Model No. 3 Wiley Mill, using a medium screen, and delivered to Nutritional Biochemicals Corporation, Cleveland, Ohio, where the formulation of the diets was completed. The feeds were refrigerated after preparation to preserve their vitamin and nutrient qualities.

STATISTICAL TREATMENTS

The single-factor design of the experiment permitted statistical treatment by randomized-complete-block analysis of variance methods. Individual sets of data were subjected to Student's 't' and linear regression analyses (Sokal and Rohlf 1969). Results of the various tests were considered statistically significant if they equaled or exceeded the table 't' or 'f' values for 0.05 probability (Rohlf and Sokal 1969).

TABLE 2. Average values ($\pm$SE) in cal/g mean body wt/day of energy balance components as affected by diet.

	Diet	
	Control	Low-lysine
Gross energy intake	827.41 ± 15.89	911.55 ± 22.33[a]
Excretory energy loss	277.60 ± 8.76	343.36 ± 15.18[a]
Metabolized energy	549.81 ± 8.88	568.19 ± 9.65
Efficiency of energy utilization	66.45 ± 0.55[a]	62.33 ± 0.75
N = 6 (each level)		

[a] This value is significantly greater at the 0.05 or lower level of probability.

RESULTS

VISIBLE FAT DEPOT AND BODY WEIGHT OF BIRDS

The average fat class (mean = 2.6) was the same for both groups of birds and did not change significantly from the beginning to the end of the experimental feeding period, regardless of diet. Although neither the initial nor the final mean weights of the control (17.15 ± 0.070, 18.42 ± 0.67 g) and low-lysine birds (17.70 ± 0.58, 18.74 ± 0.63 g) differed significantly from each other, the low-lysine birds gradually lost weight during the last two weeks of the experiment. This resulted in a net weight change that was 18% lower for the low-lysine birds than for the control birds.

ENERGY BALANCE

The gross energy intake (GE) and excretory energy loss (EE) were significantly greater ($P < 0.001$) for the low-lysine birds than for the control birds (table 2). The metabolized energy (ME) values for the two groups were not significantly different although the ME for the low-lysine birds was slightly higher. The higher GE of the low-lysine birds resulted from the somewhat higher CE of their diet (about 8%) coupled with their slightly higher food consumption rate (about 2%). This is supported by the fact that food consumption, GE, EE and ME were all significantly greater, on a per bird per day basis, for the low-lysine birds.

The efficiency of energy utilization (UE), determined by dividing the ME by the GE, was significantly greater ($P < 0.001$) for the control birds than the low-lysine birds. Changes in the individual energy components with time are shown in figure 1.
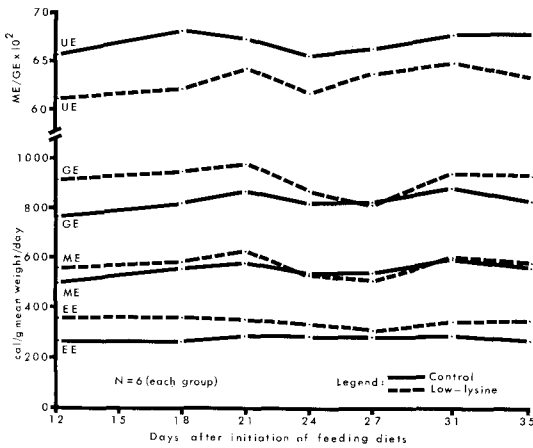
FIGURE 1. Changes in the components (means) of energy balance as affected by diet. Utilization efficiency is indicated by ME/GE.
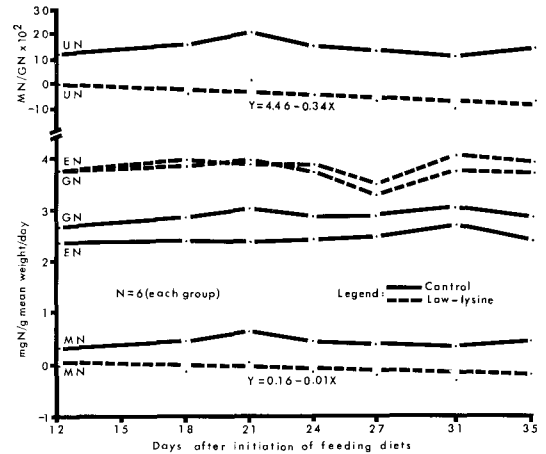

FIGURE 2. Changes in the components (means) of nitrogen balance as affected by diet. Utilization efficiency is indicated by MN/GN.

NITROGEN BALANCE

When data from all service periods were combined, the average gross nitrogen intake (GN) and excretory nitrogen loss (EN) were significantly greater ($P < 0.001$) for the low-lysine birds than for the control birds (table 3). The EN for the low-lysine birds was greater than the GN and resulted in those birds having a negative average metabolized nitrogen (MN). The MN was positive in the control birds and was significantly higher ($P < 0.001$) than that of the low-lysine birds. The MN of the low-lysine birds decreased linearly ($Y = 0.16 - 0.01X$, where X equals days after initiation of feeding the low-lysine diet) over the course of the experiment. A significantly higher ($P < 0.001$) efficiency of nitrogen utilization (UN) was realized in the control birds; the average for the low-lysine birds was a negative value (table 3). The UN of the low-lysine birds also decreased linearly with time ($Y = 4.46 - 0.34X$, where X is days on diet). Changes in the individual nitrogen components showed that the low-lysine birds exhibited near neutral nitrogen

balance during the first half of the feeding period, but that nitrogen balance decreased progressively for the last half (fig. 2).

FOOD CONSUMPTION AND EXCREMENT ANALYSIS

Food consumption was not significantly different between the two groups of birds when based on grams eaten per gram of body weight of bird per day (table 4). Excreta production, likewise, was not significantly different when based on grams excreted per gram of body weight of bird per day (table 4).

DISCUSSION

CORRELATION OF ENERGY AND NITROGEN BALANCE WITH DIETARY LYSINE

Although both groups of birds showed slight weight gains at the termination of the experiment, indicating positive energy balance for both groups, the mean weight increase

TABLE 3. Average values ($\pm$SE) in mgN/g mean body wt/day of nitrogen balance components as affected by diet.

	Diet	
	Control	Low-lysine
Gross nitrogen intake	2.91 $\pm$ 0.06	3.72 $\pm$ 0.09[a]
Excretory nitrogen loss	2.47 $\pm$ 0.05	3.85 $\pm$ 0.09[a]
Metabolized nitrogen	0.44 $\pm$ 0.03[a]	-0.13 $\pm$ 0.04
Efficiency of nitrogen utilization	15.12 $\pm$ 0.88[a]	-3.49 $\pm$ 1.00
N = 6 (each level)		

[a] This value is significantly greater at the 0.05 or lower level of probability.

TABLE 4. Food consumption and excreta production ($\pm$SE) with results of chemical analyses of excrement.

	Diet	
	Control	Low-lysine
Food consumption, g/g body wt/day	0.214 $\pm$ 0.004	0.219 $\pm$ 0.005
Excreta production, g/g body wt/day	0.091 $\pm$ 0.002	0.093 $\pm$ 0.004
MgN per gram excreta	27.53 $\pm$ 0.34	42.53 $\pm$ 0.82[a]
Kcal per gram excreta	3.056 $\pm$ 0.016	3.764 $\pm$ 0.343[a]

[a] This value is significantly greater at the 0.05 or lower level of probability.

of the control birds (7.4%) was about 20% greater than that of the low-lysine birds (5.9% increase). Furthermore, the control birds were found to demonstrate a small linear weight gain during the last 11 days of the experiment, while the low-lysine birds exhibited a linear weight loss during the same period. The loss of weight in the low-lysine birds correlates well with the occurrence of a negative nitrogen balance over the last two weeks of the study (fig. 2).

The significantly higher efficiency of energy utilization (UE) and efficiency of nitrogen utilization (UN) of the birds fed the control diet, and the fact that the birds fed the low-lysine diet had negative mean values for MN and UN, indicate a greater efficiency of utilization of the control diet. March et al. (1950) found similar increases in feed and nitrogen utilization in chicks fed all-wheat diets supplemented with the essential amino acid lysine.

The demonstration of a negative nitrogen balance in the low-lysine birds (0.2% lysine and the higher utilization efficiencies of the control birds (0.4% lysine) imply that lysine is an essential amino acid for Dark-eyed Juncos. The maintenance of nitrogen balance by the low-lysine birds during the first three weeks of the experimental feeding period (see fig. 2), was probably a result of protein (lysine) storage when those birds were fed the high protein (15%) chicken laying mash, because birds are known to store reserve protein when fed higher than normal protein diets (Fisher 1967). Tarver (1951: 842) stated that when the protein content of an animal's diet is reduced from a high to a low level, there is a lag of several days or even weeks before attainment of a new and constant level of nitrogen balance because of excess protein storage. Fisher and Ashley (1967) found that young and adult chickens previously fed high protein diets survived longer than those fed normal diets when both groups were subsequently subjected to protein-free diets. They concluded that the birds which had been fed high protein survived longer because they were able to utilize excess protein that had been stored in the body. Harms et al. (1971) have shown that specific amino acids may be stored as well, since laying hens fed methionine levels above their normal requirements were able to continue laying when later subjected to methionine-deficient diets.

The significant negative linear curves of both MN and UN of the low-lysine birds are further evidence that the high protein chicken

TABLE 5. Comparison of mean energy values[a] of experimental diets as determined by two different techniques, and calorie:protein ratios computed from each set of values.

	Diet	
Chemically analyzed protein content	Control 8.5%	Low-lysine 10.6%
Total bombed energy	3,871	4,172
Total energy C/P ratio	455	394
Experimental metabolized energy	2,572	2,596
Metabolized C/P ratio	302	245

[a] In calories per gram of feed.

layer diet provided reserve protein stores. The gradual depletion of those lysine stores was followed by a concomitant negative nitrogen balance since the low-lysine diet did supply sufficient amounts of lysine.

THE CONTROL DIET AS A MAINTENANCE DIET FOR JUNCOS

The high efficiencies of utilization of energy and nitrogen show that the control diet (calorie:protein ratio (C/P) = 455; table 5) provided adequate energy and nitrogen for laboratory maintenance of juncos. Although it is likely that all of the supplemental amino acids contributed to the nutrient qualities of the control diet, the level of lysine (0.4%) in that diet was apparently sufficient to maintain positive nitrogen balance and permit high efficiency of utilization. The metabolized energy level determined in this study for juncos (apparently 10,100 cal/bird/day, based on a body weight of 18.4 g; see table 2) is similar to that determined for Tree Sparrows (approximately 11,600 cal/bird/day) by Martin (1968). That the ME is slightly lower for juncos than for the smaller Tree Sparrows, is probably related to the negative correlation of energy requirement and proportional body surface area. These data support the conclusion by Martin (1968) that an approximate 8% protein diet with a caloric content of about 4,000 cal/g (C/P = 466) constitutes an adequate maintenance diet for small passerine birds. We emphasize that single cereal-type grains, at an 8% protein level, may not be adequate because of probable deficiencies of lysine and/or other essential amino acids. Therefore, we recommend that grain combinations, similar to those employed by Martin (1968), be utilized in constructing 8% protein laboratory diets for small passeriforms. That precaution will ensure the necessary balance of essential amino acids required for effective utilization of the diet. Juncos in the wild probably accomplish this same ef-

fect by the mixed variety of seeds that they eat.

AVAILABILITY OF LYSINE IN NATURAL FOODS

Of the genera of seeds eaten by Tree Sparrows and other small passerine birds (Martin et al. 1951, West, 1967), *Setaria, Panicum* and *Triticum* are low in lysine content (< 0.4%), while *Amaranthus* and *Chenopodium* have relatively high amounts of lysine (> 0.8%); *Panicum* also is extremely low in tryptophan (0.047%) (Block and Weiss 1956, Orr and Watt 1957). The low amounts of lysine in both *Panicum* and *Setaria* probably are not adequate for maintenance of nitrogen balance, since lysine-deficient *Triticum* caused a negative nitrogen balance in juncos in the present study. MacMillen and Snelling (1966) also found that White-crowned Sparrows (*Zonotrichia leucophrys*) could not maintain weight on a high-caloric seed diet. Although Fisher (1972) suggested that the sparrows may have been immature and thus, required higher protein concentrations, it is possible that since most commercial seed mixtures are composed mostly of millet, which has less lysine than found in wheat (Block and Weiss 1956), lysine may have been limiting for nitrogen balance. The recent report of Allaire and Fisher (1975) that Bachman's Sparrows (*Aimophila aestivalis*) eat *Panicum* and *Setaria* seeds exclusively in the summer, suggests that the lysine requirement for these birds may be lower, or that, more likely, they obtain sufficient lysine from the supplemental amounts of lysine-rich invertebrates (Block and Weiss 1956) that they consume during the summer breeding season.

ENVIRONMENTAL INFLUENCE ON DIET

The intake of adequate amounts of nutrients by small passerine birds is subject to environmental and seasonally related influences. For example, food consumption and ME are higher in Tree Sparrows exposed to 15-hour photoperiods than those exposed to 10-hour photoperiods (West 1960, Martin 1968). Many authors have found an inverse relationship between food consumption and metabolizable energy, and environmental temperature (Kendeigh 1949, West 1960, Martin 1968). It is also known that dietary preferences change during the different seasons, and that invertebrates become increasingly important as nutrient sources during the spring and summer breeding seasons in most small passerine birds (Martin et al. 1951). Winter is a critical season when small passerine birds are taxed to meet their energy and protein needs by decreases in temperature and photoperiod, and when lack of invertebrate food supplies make it necessary for juncos and related species to become chiefly granivorous. This is a season when they are susceptible to malnutrition and potential amino acid deficiencies. That problem is apparently overcome in nature by the consumption of relatively small amounts of several different seed types which, because of their similar caloric values (Kendeigh and West 1965) and dissimilar amino acid qualities (Block and Weiss 1956, Orr and Watt 1957), provide an adequate overall nutritional balance for maintenance of wintering birds in the temperate zones of the United States.

Helms (1968) found large accumulations of waste materials in the excretory tracts of high protein-fed (C/P = 250) sparrows which were subjected to low temperature regimes. Martin (1972) found that Tree Sparrows fed a 16% protein diet (C/P = 251) were not able to survive as long at low temperatures as birds fed an 8% protein diet (C/P = 470). He also found that House Sparrows fed a 5% protein diet (C/P = 740) were able to withstand lower temperatures for a longer time than those fed a 9% protein diet (C/P = 454). These findings suggest that high protein poultry diets (low C/P) should not be used with small passeriform species subjected to high energy-demanding situations, especially low temperature stress.

This and other studies show that the quality of the diet influences the existence of juncos and other small passeriform birds. The level of protein in relation to caloric content must be appropriate. The quality of protein should be considered so that adequate levels of lysine and other amino acids are supplied. The study of minimum dietary amino acid requirements of wild birds in several stages of their life cycle should be continued so that future studies of the biology of captive birds can be conducted under optimum nutritional conditions.

SUMMARY

Dark-eyed Juncos were fed 8.5% protein wheat diets supplemented with amino acids. The control diet was supplemented with lysine, tyrosine, threonine, leucine, isoleucine, and valine. The low-lysine diet was similarly supplemented except that 0.2% urea was substituted for 0.2% lysine. Neither group of birds

showed significant changes in either body weight or body fat depots.

Food consumption ($\bar{x} = 0.22$ g/g body wt/day) and excreta production ($\bar{x} = 0.092$ g/g body wt/day) were not significantly different between the two groups of birds.

The birds fed the control diet had a significantly higher efficiency of energy utilization, although there was no significant difference in the metabolized energy between the two groups.

The birds fed the control diet (0.4% lysine) had significantly higher metabolized nitrogen utilization than those fed the low-lysine diet (0.2% lysine). In fact, the metabolized nitrogen and efficiency of nitrogen utilization were both negative for the low-lysine birds. The inability of the low-lysine birds to maintain nitrogen balance demonstrates the essentiality of lysine for Dark-eyed Juncos.

Wintering juncos, as well as other small passeriforms, may maintain proper nutritional balance by eating a variety of seeds, each of which by itself may be deficient in lysine and/or other amino acids, but which, in combination, provide adequate amino acid and energy balance for subsistence.

The 8.5% protein control diet with a combustible energy content of 3,871 cal/g (calorie:protein ratio (C/P) $= 455$) was considered adequate for laboratory maintenance of juncos, since it permitted maintenance of body weight, high efficiencies of energy and nitrogen utilization, and maintenance of positive nitrogen balance.

Diets with 8–9% protein and C/P's of 450–500 appear to be best as laboratory diets for juncos and probably other small passerine birds. The possibility of deleterious effects of 15–16% protein poultry diets with C/P's near 250 are discussed.

ACKNOWLEDGMENTS

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