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Seasonal dietary shifting in yellow-rumped warblers is unrelated to macronutrient targets

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ABSTRACT

Dietary shifting, for example from insects to fruits, is a common mechanism used in migratory songbirds to accumulate fat to fuel migratory flights. We examined a potential underlying cause of dietary shifting in yellow-rumped warblers (*Setophaga coronata*) by comparing energy and protein intake goals of birds during fall migration and winter. We offered captive warblers pairs of three diets differing in macronutrient composition in both the fall and winter. Using the principles of the geometric framework of nutrition we evaluated protein and energy intake to determine if consumption of the diet pairs was adjusted to meet an energy or protein intake target, and if the target differed seasonally. Regardless of season, the warblers preferred the diet with the lowest protein content and highest carbohydrate content. Total energy intake was maintained relatively constant during migration, at around 60 kJ/day, regardless of diet combination, and at about 50 kJ/day during winter. This suggests that warblers consume macronutrients available to them without protein limitations to reach their total energy intake target. When the diet combination offered allows, the warblers mixed their diet intake to consume roughly 0.5 g/day of protein, regardless of season, which suggested a constant protein target. Our findings suggest that songbirds prefer to alter non-protein energy intake proportionally to meet changing energy demand, rather than an overall increase in macronutrient intake. Additionally, they have the ability to shift their diet based on availability, resulting in high flexibility in their macronutrient intakes to maintain energy intake.

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1. Introduction

Migration is an energetically demanding process for birds, and accumulating fuel stores is crucial to migration success (Pond, 1978; McWilliams et al., 2004). Fat accounts for approximately 90% of the energy used for migratory flight, with protein contributing the remaining fuel (Jenni and Jenni-Eiermann, 1998). Fuel storage in preparation for migration is primarily achieved by increasing food intake prior to and during migration (McWilliams and Karasov, 2001). However, migrating songbirds also may use dietary shifting, where birds switch their primary food source, for example from insects to fruits to enhance fueling (Bairlein, 1990).

Many migratory songbirds shift from an insectivorous diet during breeding season to a frugivorous diet during fall migration (Parrish, 1997). Foraging for fruit may require less energy expenditure and expose birds to less predation risk compared with foraging for insects (Parrish, 1997). High fruit abundance at stopover sites allows for rapid energy intake while conserving energy due to opportune foraging (Parrish, 1997). Optimal diet theory usually assumes organisms always

maximize their net rate of energy intake (MacArthur and Pianka, 1966), and therefore would support the preference for fruit. However, this focus on energy neglects other nutrients (Schaefer et al., 2001), such as protein, which may have the potential to limit consumption. High protein diets can lower caloric intake and promote satiety (Davidenko et al., 2013), which may reduce refueling rates by lowering the intake of other macronutrients.

Food preference, rather than food abundance or availability during fall migration, has been found to contribute to dietary shifting from insects to fruits, potentially allowing birds to be better able to seasonally balance their nutrient and energy intakes (Wheelwright, 1988; Bairlein, 1990). High protein-to-calorie ratio foods, such as insects, reduce adipose tissue build up and instead promote lean muscle mass growth (Rosebrough and McMurty, 1993). On the other hand, low protein-to-calorie ratio foods, such as fruits, promote adipose tissue accumulation (Rosebrough and McMurty, 1993). Smith and McWilliams (2009) observed that a high glucose diet promotes fat accumulation in migratory songbirds, regardless of protein or fat content. Migratory songbirds could switch to frugivory to promote fat accumulation rather than muscle growth in order to support the energetic demands of migratory flight. Stopover sites with more fruit available, leading to more fruit intake, had birds with significantly greater body masses and faster rates of body mass gain than stopover sites will little to no fruit availability (Thomas, 1979; Parrish, 1997). Conversely, eating a purely insectivorous

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diet, or high protein diet, may limit the rate of fattening (Bairlein, 2002). The difference in macronutrient content of these diet items may influence intake. In mice, a low protein diet can promote an increased intake of carbohydrates and lipids, whereas a high protein diet can decrease caloric intake potentially due to satiety (Sørensen et al., 2008; Davidenko et al., 2013).

During migration, birds have an increased energy demand, but protein requirements do not increase proportionately (Langlois and McWilliams, 2010). Migratory birds have the same nitrogen excretion per day as non-migratory birds but consume more food overall, providing migrants with a more positive nitrogen balance which decreases the minimum dietary protein content required (Langlois and McWilliams, 2010). The dietary shift from an insectivorous diet to a frugivorous diet during fall migration could be a response to decreased dietary protein requirements, as migrant birds satisfy their protein requirements by eating a greater amount of lower protein food (Langlois and McWilliams, 2010).

An additional factor that may alter diet preference towards carbohydrates over protein during migration may be related to differences in nutrient absorption in the gut. Paracellular absorption of monosaccharides, amino acids and dipeptides occurs in the gut, but differ in the fractional absorption rates (Chediack et al., 2006). Monosaccharides have a higher rate of paracellular absorption compared to dipeptides, where peptide electroaffinity influences paracellular absorption (Chediack et al., 2006). This could influence the absorptions costs depending on diet composition. High protein diets, such as insects, increase transporter-mediated amino acid uptake in the small intestine (Afik et al., 1997a; Karasov and Levey, 1997). However, when fed a high carbohydrate fruit diet, no increase in transporter-mediated uptake capacity is observed (Afik et al., 1997a), while passive absorption of glucose is increased (Afik et al., 1997b). Passive absorption allows for higher absorption rates at lower energetic costs, which can be beneficial to fruit-eating migrating songbirds that need to accumulate fat stores quickly.

Migrating songbirds that shift their diet to fruit benefit from the greater energy efficiency to digest and utilize glucose, and this is aided by their ability to distinguish nutritional values of their food. Birds can detect subtle differences in nutritional values and select their diet accordingly (Wheelwright, 1988; Whelan and Willson, 1994), to the point of distinguishing between diets differing in carbohydrates by 1%, lipids by 2%, or cysteine by 4% (Schaefer et al., 2001). This precision for food nutrient evaluation allows birds to meet their macronutrient and energy goals by mixing the foods they consume.

The geometric framework of nutrition (GFN) is a graphical modeling approach used to assess how animals mix foods to meet their nutritional goals or targets (Simpson and Raubenheimer, 1994). By examining nutrient intakes within a nutritional space, the GFN allows visualization of the potential combinations of macronutrient and energy intakes an organism may choose to satisfy requirements. Each axis represents a nutrient of choice (e.g. protein, carbohydrate, or energy) (Simpson and Raubenheimer, 1994). Food consumption data can be plotted within the nutrient space created by different food options to evaluate potential nutrient targets, and to determine how animals consume their different food options to reach these targets. Nutrient or energy targets are the amount per day the animal is choosing to consume. The targets can be diet dependent or defended if different diet combinations lead to the same targets. The GFN analysis allows one to evaluate foraging goals of animals in their current physiological state, rather than minimum nutrient requirements or overall preference (Schaefer et al., 2001).

The GFN differs from the optimal diet theory, as it considers physiological state in the analysis. The optimal diet theory lacks this physiological perspective, and therefore may produce misleading results, for example, concluding that migratory birds switch to a fruit diet strictly due to fruit abundance and energy costs rather than a change in nutritional targets. Previous studies of migratory bird nutrition have evaluated diet complementation, and seasonal changes in diet based on nutritional reward and requirements (Moore and Simm, 1985; Wheelwright, 1988; Whelan and Willson, 1994; Parrish, 1997). However, seasonal diet shifting and changes in preference may be related to changing protein and energy targets during migration.

Our objective was to examine protein and energy targets of a migratory songbird, the yellow-rumped warbler (*Setophaga coronata*), during fall migration and winter using the GFN, and to understand how this relates to diet preference. Yellow-rumped warblers shift their diet seasonally, consuming insects and other small invertebrates during the spring breeding season, and eating a mixture of insects and fruit, and sometimes exclusively fruit during fall migration and winter (Hunt and Flaspohler, 1998). We hypothesized that yellow-rumped warblers would have different nutrient targets depending on their migratory condition. Overall, we predict higher energy intake during migration. Additionally, we predicted that protein requirements would decrease when the warblers were in a migratory condition, and high protein intake would lower total energy intake due to potential satiation.

2. Material and methods

2.1. Animals and housing

We used 12 yellow-rumped warblers (11 juveniles: 5 females, 1 male and 5 unknown sex; and 1 adult female). The warblers were caught from late September to early October 2014, at Long Point, Ontario, and housed at the Advanced Facility for Avian Research at the University of Western Ontario. Animal collection and care protocols followed the Canadian Council on Animal Care guidelines and were approved by the University of Western Ontario's Animal Care and Use Sub-Committee (protocol 2010–216), and by the Canadian Wildlife Service (permit CA 0256).

During the feeding trials, the birds were housed individually in cages measuring 70 cm wide by 50 cm deep and 50 cm height. Upon arrival in the fall, the birds were maintained on a natural fall photoperiod (12 h light: 12 h dark), and switched to a short day winter photoperiod (8 h light: 16 h dark) in late November (over one month prior to the winter feeding trial period). To ensure that the warblers were in the appropriate physiological state during each feeding trial, the birds were filmed overnight using infrared cameras, and we confirmed the presence of migratory restless behavior (Gwinner, 1986) during the fall and its absence during the winter trial.

2.2. Diets and feeding trials

The experiment consisted of two 15-day feeding trial periods; a fall migratory feeding trial period ran from October 15–November 1, and a winter feeding trial period ran from December 28–January 12. To reduce any potential food preferences based on familiarity, the warblers were fed a different synthetic diet to the experimental diets and mealworms before and between the feeding trial periods. In both feeding trial periods, we fed the warblers three experimental diets: a high carbohydrate diet (HC), a high protein diet (HP), and an intermediate diet (I) (Table 1). The diets varied in the amount of casein, the primary protein source, and dextrose, the primary carbohydrate source, to achieve the varied macronutrient compositions. All other ingredients were kept consistent between the three different diets (Table 1).

During each feeding trial period, the warblers were rotated through a series of three different diet combinations, with two different diets offered together in each rotation ((i) high carbohydrate diet and intermediate diet (HCI), (ii) high protein diet and intermediate diet (HPI), (iii) high protein diet and high carbohydrate diet (HCHP)). The placement of the dishes in the cage was random each day to reduce a side bias for food selection. The warblers were randomly assigned into three groups of four warblers, with each group rotating through the combination in a different sequence. Each combination was fed ad libitum for five days, with the first day being excluded from data

Table 1
Diet formulation and calculated percent composition of the semisynthetic diets used during the fall migratory and wintering feeding periods.

	Intermediate diet	High carbohydrate diet	High protein diet
	Diet Formulation, g		
Casein	256	66	456
Dextrose	280	510	120
Briggs-N salt mixutre[a]	44	44	44
Cellulose	24	24	24
Amino acid mix[b]	24	24	24
AIN-76 vitamin mix[a]	12	12	12
Canola oil	120	80	80
Agar	40	40	40
Water	2000	2000	2000
Estimated diet composition			
Protein (% DM)	27.7	8.9	47.5
Fat (% DM)	19.6	10.3	11.9
Simple carbohydrate (% DM)	46.6	64.6	20.7
Total energy (kJ/g DM)[c]	16.9	15.6	16.1
Non-protein energy (kJ/g DM)[c]	11.0	13.7	6.05

[a] MP Biomedicals, Solon, Ohio.
[b] Amino acid mix composition: 85.5 g arginine hydrochloride, 34.5 g cystine, 39 g glycine, 19.5 g histidine hydrochloride, 6.6 g isoleucine, 19.5 g leucine, 10.8 g methionine, 6.6 g phenylalanine, 24 g threonine, 4.2 g tryptophan, 5.4 g valine, 44.4 g lysine hydrochloride (Murphy and King, 1982).
[c] Calculated using diet ingredient nutritional information.

analysis due to lower food intake. Additionally, body mass and composition were measured on the first and last day of each feeding rotation within both the fall migratory and winter feeding trial. Quantitative magnetic resonance (Guglielmo et al., 2011) was used to measure body fat and wet lean mass.

2.3. Diet, protein and energy intakes

Dry matter (DM) intake of each diet was calculated as the difference between DM offered and DM left uneaten each day. Daily diet samples and two sub-samples of each bird's leftover diet were oven dried at 65 °C, and used to determine the total dry mass of the offered and leftover diet. Diet preference was determined for each diet combination throughout each feeding trial period and was calculated as the DM intake of each diet over the total DM intake of the two diets. From the daily DM intake of each diet and each diet's composition, total energy (kJ), protein (g) and non-protein energy (combined energy from carbohydrate and fat, kJ) intake were calculated for each warbler daily. Daily intake of total energy, protein and non-protein energy for each diet combination was then determined for each feeding trial period.

2.4. Statistical analysis

Statistical analysis was conducted using linear mixed models in SAS® v.9.3 (SAS Institute INC., Cary, NC). The analysis of body mass and composition initially included the season and day as a repeated measure and individual as random effect. Diet preference and intake was analyzed for each combination between the seasons and included rotation, with individual as a random effect and season as a repeated measure. The analysis of intake of crude protein, non-protein energy and total energy included season, diet combination, the interaction between season and diet combination, diet rotation, sequence, and individual included as a random effect and season as a repeated measure. If rotation, sequence or the interaction between season and diet combination was not significant, it was removed from the model. P-values less than 0.05 were considered to be significant. For significant effects in a model, least significant different (LSD) post hoc tests were performed. Values are expressed as least squares means ± standard error.

3. Results

Season ($F_{1,10} = 2.01$, $P = 0.187$, Table 2), diet combination ($F_{2,10} = 0.60$, $P = 0.569$), and day ($F_{1,11} = 1.73$, $P = 0.216$) did not affect body mass. For fat mass, a seasonal effect was found ($F_{1,10} = 6.30$, $P = 0.031$), with fall warblers (1.399 ± 0.152 g) having a greater fat mass than winter (0.968 ± 0.157 g). Season by day interaction on fat mass was not significant ($F_{1,10} = 3.76$, $P = 0.081$). With regards to lean mass, there was a seasonal effect ($F_{1,10} = 33.70$, $P = 0.0002$), with the warblers have more lean mass in the winter (8.335 ± 0.122) than in the fall (7.970 ± 0.121). A season by day interaction was also found ($F_{1,10} = 22.09$, $P = 0.0008$, Table 2), with warblers gaining lean mass throughout the winter feeding trial ($P = 0.0002$).

For each diet combination, warblers showed a preference for the diet with the lowest protein content and highest non-protein energy content (Fig. 1). Warblers seasonally altered the intake of the lowest protein diet by consuming more during the fall (HCHP: $F_{1,10} = 3.68$, $P = 0.0042$, HCI: $F_{1,10} = 7.79$, $P < 0.0001$, HPI: $F_{1,10} = 5.15$, $P = 0.0004$). When comparing diet preference seasonally, the strength of the preferences changed (HCHP: $F_{1,10} = 6.40$, $P = 0.0299$, HCI: $F_{1,10} = 7.23$, $P = 0.0228$, HPI: $F_{1,10} = 7.10$, $P = 0.0237$, Fig. 1). During the winter feeding trial there was an increase in the preference for the diet with the highest protein content.

There was a significant seasonal effect on daily totally energy intake, which was higher in the fall than in the winter ($F_{1,10} = 68.87$, $P < 0.0001$, Table 3). Rotation ($F_{2,22} = 10.75$, $P = 0.0006$) and diet combination ($F_{2,22} = 7.52$, $P = 0.0032$, Table 3) effects were found where the HCHP diet combination had the highest total energy intake (Fig. 2). There was no season by diet combination interaction for total energy intake ($F_{2,20} = 0.30$, $P = 0.743$).

A rotation effect ($F_{2,22} = 8.70$, $P = 0.0016$), diet combination effect ($F_{2,22} = 153.00$, $P < 0.0001$), and season effect ($F_{1,10} = 13.62$, $P = 0.0042$) were found for daily average protein intake. A season by diet combination interaction was also found ($F_{2,20} = 7.16$, $P = 0.0045$). Intake was highest for the HPI diet combination, which had the highest protein intake during both seasons (Table 3; Figs. 2 and 3). When on either the HCI diet or HCHP diet combinations, the warblers ate within a narrow range of protein intake, with the HCHP diet combination during the winter feeding trial having slightly higher protein intake (Table 3; Figs. 2 and 3).

Rotation ($F_{2,22} = 10.29$, $P = 0.0007$), season ($F_{1,10} = 72.76$, $P < 0.0001$, Table 3) and diet combination ($F_{2,22} = 56.21$, $P < 0.0001$, Table 3) affected daily average non-protein energy intake. A season by diet combination interaction was not found ($F_{2,22} = 1.30$, $P = 0.294$). Daily average non-protein intake was higher during the fall season, and lower when warblers were on the HPI diet combination (Fig. 3). There was no difference in non-protein energy intake for the HCI diet combination or the HCHP diet combination during both feeding trials.

4. Discussion

Studying nutritional targets in migratory songbirds by allowing them to mix nutritionally distinct diets and analyzing intake targets via the GFN provided insight into songbird nutritional targets

Table 2
Body mass and body composition estimates (least squares mean ± SEM) for the first and last day of the fall and winter feeding trial periods.

	Fall		Winter	
	Day			
	0	15	0	15
Body mass (g)	11.16 ± 0.29	11.10 ± 0.30	11.63 ± 0.29	11.13 ± 0.30
Fat mass (g)	1.16 ± 0.19	1.63 ± 0.19	1.06 ± 0.20	1.87 ± 0.20
Wet lean mass (g)	8.01 ± 0.13[b]	7.93 ± 0.13[b]	8.09 ± 0.13[b]	8.58 ± 0.13[a]

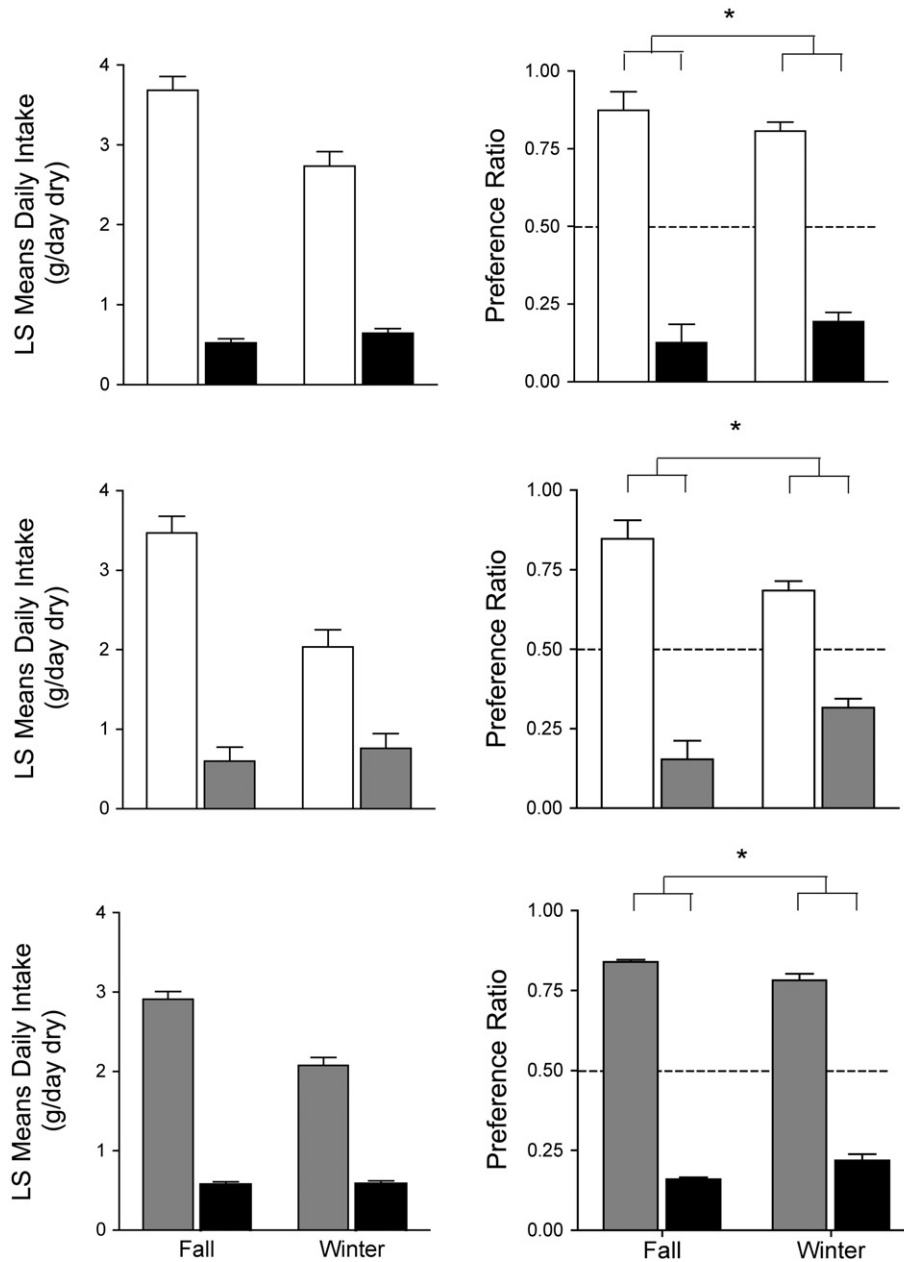Values followed by different letters are significantly different (P < 0.05).

Fig. 1. Seasonal daily average intakes of each diet within each diet combination (A–C). Asterisks represent significant seasonal differences in diet intake for that combination. Seasonal diet preferences for each diet combination (D–F). The dashed line at preference ratio 0.5 represents no preference, with equal an amount of each diet option consumed. Daily average intake and preference ratio for each season followed by an asterisk are significantly different (P < 0.05). Values presented are in least square means ± SEM (white: high carbohydrate diet; gray: intermediate diet; black: high protein diet).

and body composition changes. This allowed us to evaluate both the preference towards different diet items, and also how these preferences are related to preferred nutritional targets and physiological demands. Body mass did not differ between the fall migratory and winter feeding trial periods, regardless of increased total energy consumption when the warblers were in a migratory condition. As nocturnal migrants, warblers during the fall were highly active at night, expressing migratory restlessness (T. Marshall personal observations). This added nocturnal activity may have increased daily energy expenditure, and thus energy intake during the fall when compared with the winter, where the warblers ate less, were not active at night, and had longer nights. Our experimental design only allotted 5 days for each diet combination and may have provided insufficient time to allow for changes in body mass to become apparent. Compared with wintering birds, migrants typically have an increased fat mass to fuel their migratory flights (McWilliams et al.,

2004), which we observed in this study. Throughout the fall migratory feeding trial the warblers maintained their lean mass, but continued to gain fat mass due to their migratory state. Over the course of the winter feeding trial period, the warblers gained lean mass, but maintained their fat mass. Migratory birds catabolize fat and protein stores for fuel during migration, obtaining protein by catabolizing a portion of the digestive tract. Protein can only be catabolized from structural or functional forms, and these must be rebuilt post migration (Jenni and Jenni-Eiermann, 1998). Fat stores that were expended during migration do not need to be rebuilt to high levels once migration is over, as these fat stores were accumulated to provide fuel for migratory flight (Bairlein, 2002). This may explain why the winter warblers increased lean mass, but maintained fat mass. During migration, a decrease in digestive organs helps to reduce body mass for migratory flight, and therefore reduces flight energy expenditure (Piersma and Gill, 1998).

Table 3
Daily average intake of total energy, non-protein energy, and crude protein (least squares mean ± SEM). No season by combination effect was found for total energy and non-protein energy. A season by combination effect was found for crude protein.

	Season			Diet combination		
	Fall	Winter		HCHP	HCI	HPI
Total energy (kJ)	62.79 ± 1.60[a]	48.47 ± 1.66[b]		59.42 ± 1.84[a]	56.15 ± 1.83[ab]	51.34 ± 1.84[b]
NPE (kJ)	47.75 ± 1.25[a]	35.45 ± 1.30[b]		47.46 ± 1.46[a]	46.47 ± 1.46[a]	30.88 ± 1.46[b]

	Fall			Winter		
	HCHP	HCI	HPI	HCHP	HCI	HPI
CP (g)	0.57 ± 0.04[c]	0.47 ± 0.04[de]	1.08 ± 0.04[a]	0.55 ± 0.04[cd]	0.44 ± 0.04[e]	0.85 ± 0.04[b]

Values followed by different letters are significantly different (P < 0.05).

This may explain why the warblers had a greater lean mass during the winter, as non-migratory warblers can afford maintenance energy costs of larger organs.

In both seasons, the diet with the most non-protein energy content and the least protein content was always preferred within a diet combination. The HP diet, comparable to an insect diet, was never preferred. Yellow-rumped warblers are known to eat primarily fruit during the fall migratory and winter seasons (Hunt and Flaspohler, 1998). The preference for the highest non-protein energy content may be related to the diet's similarity in composition to fruit. Biochemically, non-protein energy (carbohydrates and fats) can be stored in the body as fat, which can be readily used for energy when needed (Jenni and Jenni-Eiermann, 1998). Protein does not have an energy storage form, and is kept in the body in a functional form within muscles and organs (Jenni and Jenni-Eiermann, 1998). For protein to be stored non-functionally it must be ultimately converted to fat, which is very costly and inefficient. Synthesizing triglycerides from protein requires twice the energy compared with carbohydrates (Reeds et al., 1982). To build fat stores efficiently, carbohydrates and fat are the best substrates (Reeds et al., 1982; Jenni and Jenni-Eiermann, 1998). From this point of view, warblers should prefer macronutrients that can be used for energy readily or stored easily (carbohydrates and fat) when not having to build muscle or organ tissue. Simple sugars also have the benefit of

having higher fractional paracellular absorption rates compared to peptides of passive absorption, which allow for high absorption rates at decreased cost (Afik et al., 1997b; Chediack et al., 2006). This may be the reason warblers preferred the diet with the most non-protein energy as these macronutrients can be readily absorbed, utilized and stored. The warblers in this study were not molting at the time of the experiment, therefore their protein requirements should have been stable with changes in energy intake targets shifting seasonally.

Examining dietary intake of protein and energy demonstrates that the warblers mixed the diets offered to meet an energy target, with seasonally higher intakes during fall migration. When on the HPI diet combination the warblers reached a slightly lower, but comparable, total energy intake when related to the other two diet combinations, which offered less protein. This finding suggests that protein intake could be limiting total energy intake, but did not significantly alter body composition during the five days of the experiment. A factor to take into consideration is that the warblers in this experiment were already in a migratory state with pre-existing fat stores at the start of each trial, meaning the warblers may have had to only maintain their body fat composition (Pond, 1978). A limiting protein effect on energy intake may be found during the transition from non-migratory to migratory condition when adipose tissue building first begins or refueling after a migratory flight.
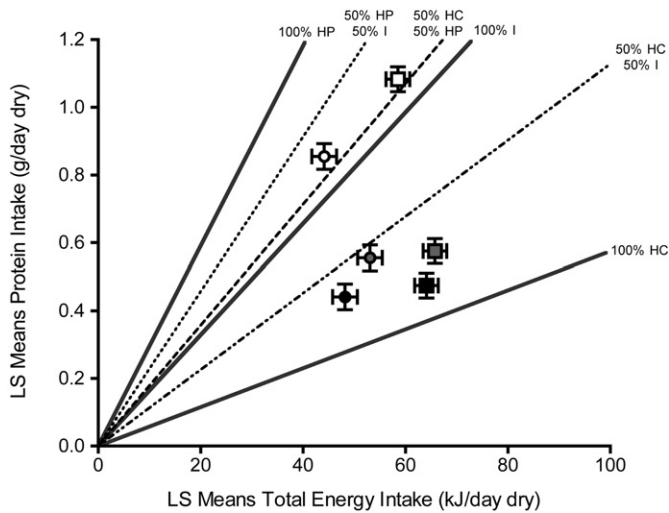

Fig. 2. Average daily protein and total energy intake for each diet combination are plotted in a nutritional space designed from the synthetic diets. The solid lines represent the different synthetic diets; the black dashed lines represent the different diet combinations if each diet was consumed equally. Symbols represent the calculated daily least squares means of protein and total energy (with SEM) of food disappeared for each diet combination. Squares represent the fall migratory feeding trial period intakes and circles represent the winter feeding trial period intakes. Within symbols, white represents the HPI diet combination, gray represents HCHP combination and black represents the HCI diet combination.
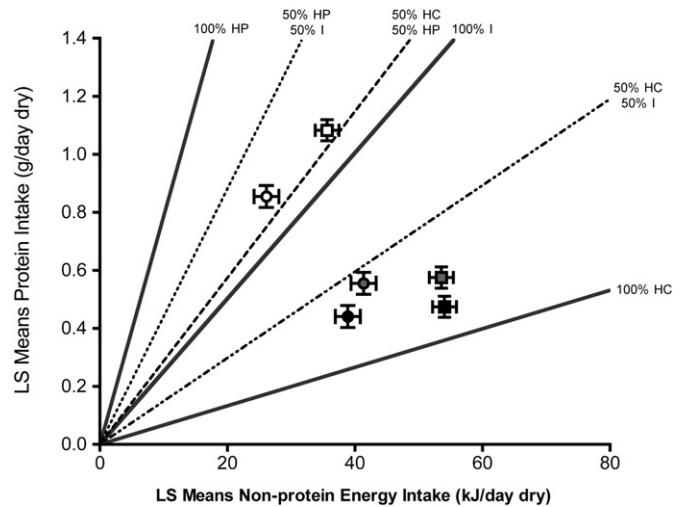
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Total energy intake during the fall feeding trial was significantly greater than in the winter. Migratory birds must gain fuel stores in order to fuel their endurance flights (Pond, 1978), with hyperphagia being one of the primary mechanisms by which migrants build up these fuel stores (McWilliams and Karasov, 2001). The HCHP and HCI diet combinations during the fall feeding trial had very similar high total energy intakes. These diet combinations offer more energy per gram of diet when compared to the HPI diet combination, as they offer more non-protein energy content, which is more energetically efficient to digest and metabolize into fat compared with protein. Warblers appeared to maximize their total energy intake during migration to accumulate fat stores, which may explain why they ate more total energy when on the HCHP and HCI diet combinations in a migratory condition. The HPI diet combination consistently had the lowest total energy intake throughout the experiment, suggesting that consuming high levels of protein may reduce or limit total energy intake.

In both seasons, the warblers maintained a narrow range of daily protein intake when on either the HCI or HCHP diet combination. Although the two diet combinations differed significantly in available macronutrients, the close proximity and seasonal stability of this protein intake suggests that warblers combine diets to meet a protein intake target, which does not change during migration. This builds on the findings of Langlois and McWilliams (2010), who concluded that protein requirements decreased during migration due to the sheer increase in food consumption coupled with no change in nitrogen excretion, leading to more nitrogen being stored due to faster accumulation than excretion (Langlois and McWilliams, 2010). However, their study focused on minimum protein requirements, compared with intake targets chosen by the birds. When given the HPI diet combination, the warblers had dramatically higher average daily protein intake, and the highest protein intake occurred during the fall. This indicates that the warblers were feeding towards a total energy intake target, and were willing to eat the macronutrients available to them to achieve this.

Daily non-protein energy intake was higher during the fall feeding trial, consistent with total energy intake. Again, this can be explained by the warbler's migratory condition and the demand to accumulate fat (Pond, 1978). When on the HPI diet combination, the warblers always ate less non-protein energy. A stated previously, this diet combination offered the least amount of non-protein energy, which can account for the reduced non-protein energy intake. As the total energy intake of the HPI diet combination was either the same or comparable to the other two diet combinations seasonally, and total protein intake was dramatically higher, it can be concluded that a high non-protein energy intake is not required to reach energy intake targets.

Plotting energy and protein intakes using the GFN can help one to visualize and evaluate the diet mixing decisions birds make (See Figs. 2 and 3). Protein intake plotted with total energy intake demonstrates how the warblers mixed the diets offered within the diet combination to reach a total energy intake target rather than a set protein goal. During the migratory season the energy target was close to 60 kJ/day. Daily dry protein intake ranged around 0.5 g when the warblers were given the HCI and HCHP diet combination during both feeding trial periods. When the warblers were on the HCHP diet combination, the difference in the diets macronutrient composition gave the birds a much larger nutritional space to mix diet within, but they still remained close to a dry protein intake of 0.5 g/day. This suggests that the warblers consume food to meet a protein target when given the opportunity. The relationship between protein intake and non-protein energy showed that protein intake was dramatically higher when on the HPI combination, which had the lowest non-protein energy intake. The additional protein consumed when compared with the other diet combinations would allow these birds to have a closer total energy intake to the other diet combinations.

The GFN method can help us to understand the mechanisms that underlie dietary shifting in birds, and more specifically to determine whether a diet shift is primarily in response to optimal foraging considerations, changes in food abundance, or shifting nutritional targets. More broadly, it can be used to test foraging decisions of birds under different environmental conditions, such as ecological barriers or changing climates that may alter the types and quantities of food items available and fuel mix during flight. By better understanding the dietary flexibility of migratory songbirds we can better predict how environmental variation will affect migration, diet and geographic range. Songbirds with the ability to shift their diet may have a high flexibility in how they meet macronutrient and energy targets. Species like yellow-rumped warblers may be well adapted to a changing environment.

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