When moult overlaps migration: moult-related changes in plasma biochemistry of migrating common snipe (#12317)

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When moult overlaps migration: moult-related changes in plasma biochemistry of migrating common snipe

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Moult of feathers entails considerable physiological and energetic costs to an avian organism. Even under favourable feeding conditions, endogenous body stores and energy reserves of moulting birds are usually severely depleted. Thus, most species of birds separate moult from other energy-demanding activities, such as migration or reproduction. Common snipe *Gallinago gallinago* is an exception, as during the first autumn migration many young snipe initiate the post-juvenile moult, which includes replacement of body feathers, lesser and median wing coverts, tertials and rectrices. Here, we evaluated moult-related changes in blood plasma biochemistry of the common snipe during a period of serious trade-off in energy allocation between moult and migration. For this purpose, concentrations of basic metabolites in plasma were evaluated in more than 500 of young snipe migrating through Central Europe. We found significant changes in the plasma concentrations of total protein, triglyceride and glucose over the course of moult, while the concentrations of uric acid and albumin did not change. Total protein concentration increased significantly in the initial stage of moult, probably as a result of increased production of keratin, but it decreased to the pre-moult level at the advanced stage of moult. Plasma triglyceride concentration decreased during the period of tertial and rectrice moult, which reflected depletion of endogenous fat reserves. By contrast, glucose concentration increased steadily during the course of moult, which could be caused by increased catabolism of triglycerides (via gluconeogenesis) or, alternatively, due to increased glucocorticoids as a stress response. Our results suggest that physiological changes associated with moult may be considered important determinants of the low pace of migration typical of the common snipe.

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ABSTRACT

Moult of feathers entails considerable physiological and energetic costs to an avian organism. Even under favourable feeding conditions, endogenous body stores and energy reserves of moulting birds are usually severely depleted. Thus, most species of birds separate moult from other energy-demanding activities, such as migration or reproduction. Common snipe *Gallinago gallinago* is an exception, as during the first autumn migration many young snipe initiate the post-juvenile moult, which includes replacement of body feathers, lesser and median wing coverts, tertials and rectrices. Here, we evaluated moult-related changes in blood plasma biochemistry of the common snipe during a period of serious trade-off in energy allocation between moult and migration. For this purpose, concentrations of basic metabolites in plasma were evaluated in more than 500 of young snipe migrating through Central Europe. We found significant changes in the plasma concentrations of total protein, triglyceride and glucose over the course of moult, while the concentrations of uric acid and albumin did not change. Total protein concentration increased significantly in the initial stage of moult, probably as a result of increased production of keratin, but it decreased to the pre-moult level at the advanced stage of moult. Plasma triglyceride concentration decreased during the period of tertial and rectrice moult, which reflected depletion of endogenous fat reserves. By contrast, glucose concentration increased steadily during the course of moult, which could be caused by increased catabolism of triglycerides (via gluconeogenesis) or, alternatively, due to increased glucocorticoids as a stress response. Our results suggest that physiological changes associated with moult may be considered important determinants of the low pace of migration typical of the common snipe.

INTRODUCTION

Moulting is a process by which the birds maintain feathers in good quality, which improves birds' flight performance and enhance thermoregulation. However, synthesis of feathers is one of the most physiologically costly events in the annual cycle of birds and it requires substantial stores of nutrients in body (*Murphy, 1996*). While the apparent nutrient and energy costs of moult associated with deposition of materials in new feathers may be relatively mild when compared with the costs of maintenance or reproduction (*Murphy & King, 1992*), the process of moult requires a wide spectrum of metabolic adjustments that are not directly related to plumage synthesis. These additional metabolic process include recrudescence of the integument, cyclic osteoporosis, and an increased whole-body protein turnover, which may ~~combine to create~~ daily energy costs of peak moult exceeding 50% of basal metabolic rate (*Murphy & King, 1992*). In fact, the energy deposited daily as keratins in feather was estimated to equal only ca. 10% of the energy costs of moult and much higher energy costs were associated with protein metabolism not directly related to keratin synthesis (*Murphy & Taruscio, 1995*).

The biochemical analysis of blood is a technique widely used to indicate avian body condition and to investigate physiological processes during different phases of life. In general, plasma metabolites reflect various aspects of physiological condition and characterize the feeding state of birds. Total protein and triglyceride levels reliably indicate nutrient status of wild and captive birds (*Jenni-Eiermann & Jenni, 1998*; *Jenni-Eiermann, Jenni & Piersma,* 2002; *Albano et al., 2016*), although triglyceride levels may also vary in relation to environmental conditions and stress (*Artacho et al., 2007*; *Ibañez et al., 2015*). Glucose level in plasma decreases during periods of fast and, thus, may serve as an indicator of short-term changes in food intake (*Jenni-Eiermann & Jenni, 1998*; *Totzke et al., 1999*; *Alonso-Alvarez et al., 2002*). Numerous studies indicated that glucose levels positively correlated with different components of condition or with a broadly-defined individual quality (*Alonso-Alvarez et al., 2002*; *Minias & Kaczmarek, 2013*). High level of plasma glucose are also associated with increased glucocorticoids as a

stress response (*Mondal et al., 2011*), although this relationship may be obscured by the processes of protein catabolism, gluconeogenesis, and insulin regulation (Remage-Healey & Romero, 2001; *Cyr et al., 2007).* Plasma concentrations of nitrogenous excretion components, such as uric acid increase substantially in response to starvation, when tissue proteins are actively mobilized as a source of energy. Plasma concentration of uric acid is a good indicator of condition, especially when individuals have low fat reserves, which rapidly activates protein catabolism during food shortage (*Villegas et al., 2002*). Finally, low albumin concentration may reflect acute diseases and chronic infection or inflammation, which may result from decreased allocation of resources to the immune function (*Hõrak et al., 2002*).

The presented literature shows that changes in blood plasma biochemistry may well serve to evaluate physiological costs of moult. Earlier studies investigated changes in plasma biochemistry during moult in captive birds (*Dolnik & Gavrilov, 1979*; *Murphy & King, 1984*) and others in wild-living but flightless birds (*Ghebremeskel et al., 1989*; *Cherel, Charrassin & Challet, 1994*). However, few, if any, papers have examined moult-related changes in plasma biochemistry of wild birds during migration. Most avian species separate moult from other energy-demanding activities, such as migration or reproduction, but several species of birds have been reported to show a moult-migration overlap to a varying degree (*Pérez-Tris et al., 2001*; *Rohwer et al., 2009*), including the common snipe *Gallinago gallinago* (*Podlaszczuk et al., 2016*). Adult common snipe start post-breeding moult at breeding grounds, as soon as they conclude reproductive activities, and continue moulting during migration. Young common snipe typically begin the partial post-juvenile moult during their first autumn migration, although probably some individuals can delay moulting until arrival at wintering grounds (*Podlaszczuk et al., 2016*). The post-juvenile moult of the common snipe is more extensive and, thus, more energetically expensive than in other waders, as it includes replacement of body feathers, lesser and median wing coverts, tertials, and rectrices (*Włodarczyk et al., 2008; Minias et al., 2010a*). In these respects, the common snipe provide a good opportunity to study moult-related changes in blood plasma biochemistry

during a period of serious trade-off in energy allocation. The aim of this study was to determine physiological consequences of moult in migrating common snipe. For this purpose, we measured plasma concentrations of basic metabolites in over half a thousand of moulting and non-moulting young common snipe at their final phase of migration through Central Europe.

METHODS

Study site and species

Common snipe were captured at the Jeziorsko reservoir (51°40'N, 18°40'E), central Poland, during autumn migration (04 August – 25 September) to the south-west. Jeziorsko reservoir is one of the most important stopover sites for migrating waders and waterfowl in inland Poland, due to the water management policies which ensure considerable seasonal oscillations of water level. In autumn, water level at the reservoir decreases at a constant rate, continuously exposing new areas of mudflats, which provide abundant food resources and attract large flocks of migrating waders. The maximum concentrations of common snipe at the site exceed a thousand of individuals in August (*Janiszewski et al., 1998*).

The common snipe breeds in low Arctic and boreal zones throughout entire Palaearctic, and migrates to the wintering grounds in South-Western Europe (*Cramp & Simmons, 1986*). As indicated by ringing recoveries, common snipe migrating through inland Poland originate mostly from Central Russian populations (Fig. 1; *Minias et al., 2010b*). Although common snipe also breed in Poland and neighbouring Central European countries, we have no evidence that local individuals use Jeziorsko reservoir as a fuelling site prior to autumn migration, as they probably depart on migration before the suitable feeding habitats (mudflats) start to appear at the reservoir (usually in early or mid-August). While the common snipe is known to migrate according to the strategy of energy minimization, which is characterized by the low pace of migration and frequent stopovers (*Włodarczyk et al., 2007*), Jeziorsko

105 reservoir is likely to be one of the last staging sites for birds wintering in France and other West-
106 European countries.
107
108 **General field procedures**
109 In total, we caught 1007 first-year common snipe during seven migration seasons (2009-2015). Snipe
110 were captured in walk-in traps and mist nets, occasionally with vocal stimulation. All birds were ringed
111 and aged according to plumage (*Kaczmarek et al., 2007*; *Włodarczyk et al., 2008*). The sex of birds was
112 determined either molecularly (in 2009) from blood samples, following protocols developed by *Kahn,*
113 *John & Quinn* (*1998*), or by morphological measurements, using discriminant equations developed for
114 the same migratory population of the common snipe (*Włodarczyk et al., 2011*). For sexing by
115 morphology, bill length and distance between the tips of two outermost rectrices were measured with
116 calipers (± 0.1 mm) and the vane length of the outermost rectrix was measured with a ruler (± 1 mm).
117 Fieldwork was performed under the annual permissions from the Regional Environmental Protection
118 Directorate in Łódź, Poland. Catching, ringing, and handling birds was performed under individual annual
119 permissions for ringers by the Polish Academy of Sciences, with an approval of the Ministry of
120 Environment in Poland and General Environmental Protection Directorate in Poland.
121
122 **Recording moult**
123 In all captured snipe we quantified the stage of post-juvenile moult. During post-juvenile moult snipe
124 change their natal feathers (body feathers, lesser and median wing coverts, tertials, and rectrices) to an
125 adult-type plumage (Fig. 2). Thus, when post-juvenile moult is completed, first-year birds become
126 indistinguishable from adults based on the plumage characteristics. However, few young birds (if any)
127 finish their post-juvenile moult before they reach wintering grounds. Throughout seven years of study
128 we captured only 43 individuals in fresh, (recently moulted) adult-type plumage, most of which were

probably adults. All these birds were excluded from analyses. The remaining young birds were classified into one of three moult categories: 1) pre-moult (no feathers moulted); 2) initial stage of moult (only body feathers and wing coverts in active moult); 3) advanced stage of moult (tertials or rectrices in active moult). An exact moult progress was also quantified for birds that moulted tertials or rectrices. For this purpose, each tertial (n = 8) and rectrix (n = 14) was given a moult score according to the feather scoring system developed by *Ashmole* (*1962*), where: 0 – old feather, 1 – old feather missing or a new feather in a pin, 2 – new feather up to one third grown, 3 – new feather between one and two thirds grown, 4 – new feather more than two thirds grown, 5 – new feather fully developed. A sum of all moult scores for individual feathers was used as a general moult score (max. 110, when all tertials/rectrices were renewed).

Plasma biochemistry

About 50% of captured young snipe (n = 538 individuals) were selected for plasma biochemistry measurements. Between 20 and 40 µl of blood was collected from the ulnar vein of each bird into heparinized capillary tubes. Blood sampling was performed under temporal permissions of the Local Bioethical Commission in Łódź, Poland. Samples were centrifuged at 6000 rpm for 5 min within an hour of collection to separate plasma from blood cells, and kept at -20°C until analysis. Plasma metabolite concentrations (total protein, albumin, triglycerides, glucose, and uric acid) were analysed with a spectrophotometer (BTS-330, BioSystems Reagents & Instruments, Barcelona, Spain) using commercial kits of the same manufacturer (BioSystems Reagents & Instruments, Barcelona, Spain). All analyses were conducted according to the manufacturer protocols using the following methods: total protein (biuret reaction), albumin (bromocresol green), triglycerides (glycerol phosphate oxidase/peroxidase), glucose (glucose oxidase/peroxidase), and uric acid (uricase/peroxidase). Absorbance of each sample was measured in a flow cuvette against a blank reagent at the following wave lengths: 500 nm (glucose,

153 triglycerids), 520 nm (uric acid), 545 nm (total protein), and 630 nm (albumin). Run-to-run repeatability
154 (R) and linearity limits (LL) were specified as follows: total protein (R: 1.85%; LL: 150 g/L), albumin (R:
155 1.90%; LL: 70 g/L), triglycerides (R: 2.15%; LL: 600 mg/dL), glucose (R: 2.3%; LL: 500 mg/dL), and uric acid
156 (R: 2.00 %, LL: 25 mg/dL). The applied biochemical methods followed the standard methodology used in
157 avian studies (e.g. *Artacho et al., 2007*). Since the amount of plasma collected from each birds was often
158 not sufficient to measure all five plasma biochemistry parameters, sample sizes for each parameter are
159 different (Table 1). Distributions of all plasma metabolite concentrations were reasonably close to
160 normal (skewness: 0.08 – 0.69) and were not ~~subject to any transformations~~.
161
162 **Statistical analyses**
163 Differences in plasma biochemistry parameters between consecutive stages of post-juvenile moult were
164 analysed with the general linear models (GLMs), separately for each parameter. In each model, we
165 controlled for the effects of sex, year, date of capture (Julian day), and hour of capture. Date was
166 standardized to equal unit variances (z-scores) within each season to account for annual variation in the
167 timing of migration. For birds at the advanced stage of moult, we also used GLMs to investigate the
168 effect of moult score on plasma metabolite concentrations. In these models, the general moult score
169 calculated for tertials and rectrices was entered as a covariate. To obtain more parsimonious reduced
170 models, we removed non-significant ($p > 0.15$) predictors from initial full models. All statistical analyses
171 were performed with Statistica 10.0 (StatSoft, Tulsa, OK, USA). All values are presented as means ± SE.
172
173 **RESULTS**
174 43.7 % of young common snipe showed signs of post-juvenile moult (n = 538). Most moulting snipe
175 (74.9 %, n = 235) were at the initial stage of moult, while the remaining 25.1 % were at the advanced
176 stage of moult.

177 Plasma concentrations of total protein and glucose differed significantly between the
178 consecutive stages of post-juvenile moult (Table 2, 3). Total protein concentration was significantly
179 higher at the initial stage of moult (35.57 ± 0.52 g/l) when compared to the pre-moult stage (33.33 ±
180 0.42 g/l; Tukey test: $p < 0.001$; Fig. 3a) and to the advanced stage of moult (32.62 ± 0.90 g/l; Tukey test:
181 $p = 0.011$). There was no significant difference in the total protein concentration between the pre-moult
182 and advanced-moult stages (Tukey test: $p = 0.67$; Fig. 3a). By contrast, glucose concentration was higher
183 at the advanced stage of moult than during the pre-moult stage (511.6 ± 20.6 mg/ dl vs. 454.8 ± 7.1
184 mg/dl.; Tukey test: $p = 0.039$; Fig. 3b). Snipe at the initial stage of moult had an intermediate
185 concentration of glucose (Fig. 3b). Other plasma parameters showed no variation with the moult stage
186 (Table 4). Only triglyceride concentration in plasma changed with the moult score of snipe that moulted
187 tertials or rectrices ($F_{1,61} = 4.10$, $p = 0.047$), and it significantly decreased during moult of tertials and
188 rectrices (β = -0.29 ± 0.14; Fig. 4). The other plasma parameters (total protein, albumin, glucose, and uric
189 acid concentrations) showed no variation related to the moult score of tertials and rectrices (all $p >$
190 0.05).
191
192 **DISCUSSION**
193 Concentrations of total protein, triglycerides and glucose in plasma changed significantly during the
194 post-juvenile moult of the common snipe. At least some of these changes in blood plasma biochemistry
195 are likely associated with the use of energy and nutrients during plumage synthesis or during other
196 moult-related metabolic processes, which greatly contribute to the overall costs of moult (e.g.
197 vascularization of integument or alterations to bone metabolism; *Murphy & King*, 1992).
198 Total protein plasma concentration increased significantly in the initial stage of moulting but fell
199 later during the advanced stage of feather replacement, returning to the low pre-moult level. Snipe have
200 probably the highest protein demand at the beginning of moult, due to the rapid acceleration of keratin

201 synthesis for feather production and other metabolic processes associated with early phases of moult,
202 such as vascularization of the active feather follicles, pulp formation, and an increase of erythrocytes
203 (*deGraw & Kern*, 1985; *Murphy & King*,1992). It has been shown that deposition of protein as keratins of
204 feathers may equal a quarter or more of the total protein mass of the bird (*Newton, 1968*; *Murphy &*
205 *Taruscio, 1995*; *Roman et al., 2009*). Production of keratin depends largely upon sulphur containing
206 amino acids (cysteine and cystine), which, thus, may be critical for plumage synthesis. For example,
207 *Murphy & King* (1984) showed that moulting white-crowned sparrows *Zonotrichia leucophrys gambelii*
208 require large amounts of glutathione, which primarily consists of sulphur containing amino acids.
209 However, besides playing a role in feather synthesis, plasma proteins have a variety of immunological
210 and transport functions and are important indicators of nutritional state and health of a bird (*Jenni-*
211 *Eiermann & Jenni, 1996*). Plasma proteins also carry a range of metabolites (*Jenni-Eiermann & Jenni,*
212 *1996*). Reduction of total protein content is an indicator of many pathological changes (malnutrition), as
213 proteins contribute to a pool of amino-acids for protein synthesis and can act as a source of energy
214 (*Jenni-Eiermann & Jenni, 1996*).
215 Our findings are similar to those of *Dolnik & Gavrilov* (*1979*) who found that total protein level
216 increased at the initial stage of moulting in the chaffinch *Fringilla coelebs*, which was due to intensive
217 synthesis of protein as material for new feather production. This initial rise was followed by a decrease
218 over the next stages of moult, similarly as in our study. A decrease in total protein concentration during
219 moult was also recorded in seabirds (*Work, 1996*), passerines (*Newton, 1968*; *deGraw & Kern, 1985*),
220 ducks and geese (*Driver, 1981*; *Roman et al., 2009*). Other studies showed that the level of total protein
221 was significantly higher after moult than during feather replacement (*Thompson & Drobney, 1996*).
222 Nevertheless, *Ghebremeskel et al.* (*1989*) found total plasma protein to be significantly lower in the
223 post-moult than the pre-moult stage in rockhopper *Eudyptes crestatus* and Magellanic penguins
224 *Spheniscus magellanicus*. Species vary in their baseline protein level and this may result from variations

in the supply of amino acids and energy. Most species rely mostly on their diet to meet the growing demand for protein during moulting, but some birds, such as penguins, which do not feed during moult, use endogenous nutrients to synthesize feathers (*Cherel, Charrassin & Challet, 1994*). While it remains unknown whether the common snipe primarily use endogenous or exogenous nutrients for feather synthesis, it was found that snipe depend on endogenous energy from adipocyte cells during moult period (*Minias et al., 2010a*). The decreased levels of plasma total protein observed during the final stages of moult result from an ongoing protein accumulation in feathers or muscles, as well as from less intensive synthesis in the liver (*Roman et al., 2009*). At the advanced stage of moult, some proteins obtained with food could be also catabolized into amino acids and keto acids, and then used primarily as energy or for synthesis of fatty acids (*Artacho et al., 2007*).

Plasma triglycerides are a well-known indicator of malnutrition or fasting, and their concentration decreases rapidly even during overnight fasting (e.g. *Jenni-Eiermann & Jenni, 1996*; *Jenni & Schwilch, 2001*; *Jenni-Eiermann, Jenni & Piersma, 2002*). We found that plasma triglyceride levels decreased in moulting common snipe, which is consistent with previous findings that fat reserves of snipe decreased by ca. 50% between the initial and final stages of the post-juvenile moult (*Minias et al., 2010a*). The decreasing plasma triglyceride level observed during moult is probably an indicator of increasing problems with food supply. To satisfy high energy demand, snipe rely on their fat reserves (*Minias et al., 2010a*) and probably on catabolised protein obtained from dietary sources. Birds catabolise fat reserves to compensate for energy deficiencies in food intake, which is especially likely during such energy-demanding processes as moult (*Jenni-Eiermann & Jenni 1996*; *Klasing 1998*; *Jenni & Schwilch, 2001*; *Jenni-Eiermann, Jenni & Piersma, 2002*; *Artacho et al., 2007*). A number of studies showed that the level of metabolized energy increases during the initial stages of moult, but decreases in the next phases of moult and finally settle at a level below initial values upon moult completion

248 (*Newton, 1968*; *Myrcha & Pinowski, 1970*; *Dolnik & Gavrilov, 1979*; *Jenni-Eiermann & Jenni, 1996*;
249 *Artacho et al., 2007*).

250 In contrast to triglycerides, plasma glucose concentration in the common snipe steadily
251 increased from the start of the moult until its advanced stage. Glucose is the main product of the
252 carbohydrate metabolism and it is obtained from the diet. Some studies indicate that ~~high~~ body
253 condition is associated with increased glucose level (*Minias & Kaczmarek, 2013*). A decrease in glucose
254 level in birds could be an indicator of short fasting periods (*Jenni-Eiermann & Jenni, 1994, 1997),*
255 however, in some species plasma glucose concentration negatively correlated with body mass (*Kaliński*
256 *et al., 2014*). During starvation, glucose is produced from stored glycerol and amino acids or by
257 gluconeogenesis (*Herzberg et al., 1988*) and may also occur as a stress-induced hyperglycaemia with
258 increased glucocorticoids (*Remage-Healey & Romero, 2001*).

259 There are two likely explanations for the increasing levels of plasma glucose during moult in the
260 common snipe. First, snipe use their fat reserves during moult (*Minias et al., 2010a*), which is supported
261 by decreasing plasma triglyceride concentrations and, thus, the increasing glucose level may be an effect
262 of the catabolism of triglycerides, stored in adipocyte cells. During lipolysis, the triglycerides are split
263 into monoacylglycerol units which are converted to free fatty acids and glycerol. Glycerol can be then
264 metabolised into glucose by conversion into dihydroxyacetone phosphate and then into glyceraldehyde
265 3-phosphate in the process of gluconeogenesis (*Herzberg et al., 1988*). Consequently, we cannot exclude
266 that increasing catabolism of fat may simultaneously elevate plasma glucose levels during moult.

267 The second reason for increasing plasma glucose concentration may be associated with elevated
268 levels of corticosteroids. Glucocorticoids increase glucose level by working as an insulin antagonist and
269 stimulating lipolysis in adipose tissue, which results in an increase in plasma free fatty acids and glycerol
270 levels (*Remage-Healey & Romero, 2001; Ramenofsky, 2011).* Several studies have shown that
271 glucocorticoid activity is associated with migration (*Landys, Ramenofsky & Wingfield, 2006; Ramenofsky,*

2011) and high levels of plasma corticosterone have been well documented in many long-distance migrants (*Falsone, Jenni-Eiermann & Jenni, 2009; Landys-Ciannelli et al., 2002; Reneerkens et al., 2002*). Thus, it seems likely that migrating young common snipe may show higher levels of corticosterone required for the maintenance of migratory condition (*Ramenofsky, Piersna & Jukema, 1995*; *Holberton, 1999; Landys-Ciannelli et al., 2002; Reneerkens et al., 2002; Falsone, Jenni-Eiermann & Jenni, 2009).* On the other hand, there is no agreement on how corticosterone level is affected by moult. While baseline and stress-induced levels of corticosterone were lower during moult in the common starlings *Sturnus vulgaris* (*Romero & Remage-Healey, 2000*), some other studies suggested that corticosterone suppression is not a prerequisite for synthesis of high-quality feathers (*Buttemer, Addison & Astheimer, 2015)*. Regardless of the mechanism responsible for plasma glucose regulation in moulting common snipe, both pre-moult and moult levels of plasma glucose in snipe were very high when compared to glycemic levels in other bird species (*Prinzinger & Misovic, 1994*; *Beuchat & Chong, 1998*). This suggests that plasma glucose concentration in moulting snipe was above the threshold of glycemic requirement and may not be indicative of catabolic compromise.

In conclusion, our study indicates significant changes in blood plasma biochemistry during the post-juvenile moult in the common snipe. These changes, which indicate high nutritional and physiological costs of moult, might be among the primary determinants for the low pace of migration in this species. The common snipe minimizes energy expenditure during autumn migration, a strategy characterized by low refuelling rates, accumulation of small fat reserves, and migrating by short migratory “hops” between a large number of stopover sites (*Włodarczyk et al., 2007*). Our results suggest that physiological changes associated with moult and a trade-off in energy allocation between moult and migration may prevent the common snipe from adopting migration strategy of energetically-expensive long-distance migratory flights.

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299 for constructive comments on the earlier drafts of the manuscript.

300

301 **Data Deposition**

302 The raw data has been supplied as a Supplemental Dataset.

303

304 **REFERENCES**

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Figure 1: Map of ringing recoveries from common snipe migrating through inland Poland. Ringing sites are marked with yellow triangles, ~~spring/summer~~ recoveries are marked with ~~blue~~ dots, and autumn/winter recoveries are marked with ~~green~~ dots. Figure adapted from *Minias et al.,* (*2010b*).

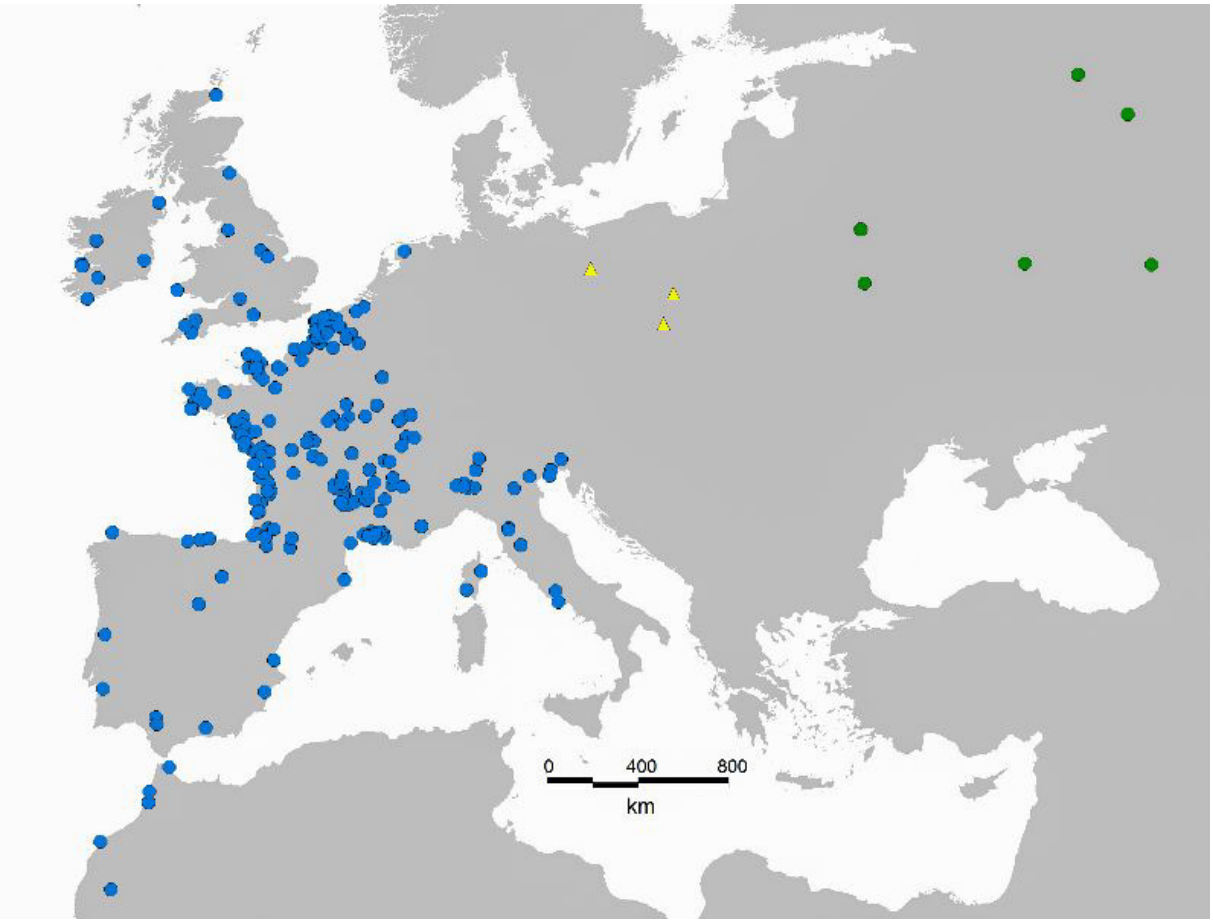

Figure 2: The extent of the post-juvenile moult in wing and tail of the common snipe. Plumage areas marked by white contours are moulted. LC – lesser wing coverts, MC – median wing coverts, TR – tertials, R – rectrices.

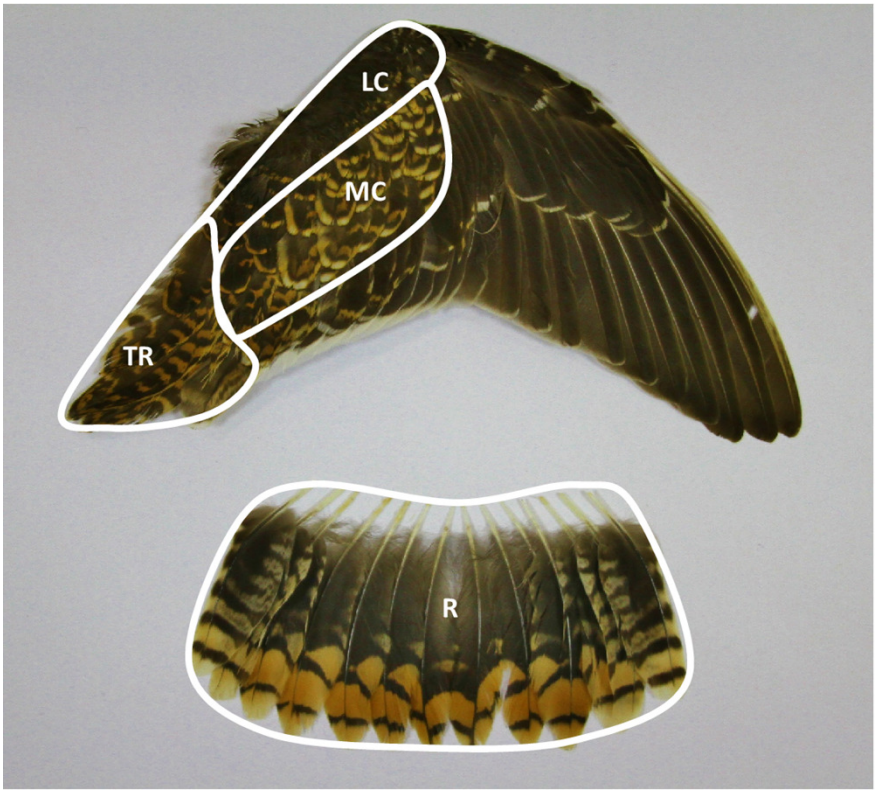

Figure 3: Changes in plasma concentrations of total protein (a) and glucose (b) between the consecutive stages of post-juvenile moult in young common snipe migrating through central Poland. Means ± SE are presented.

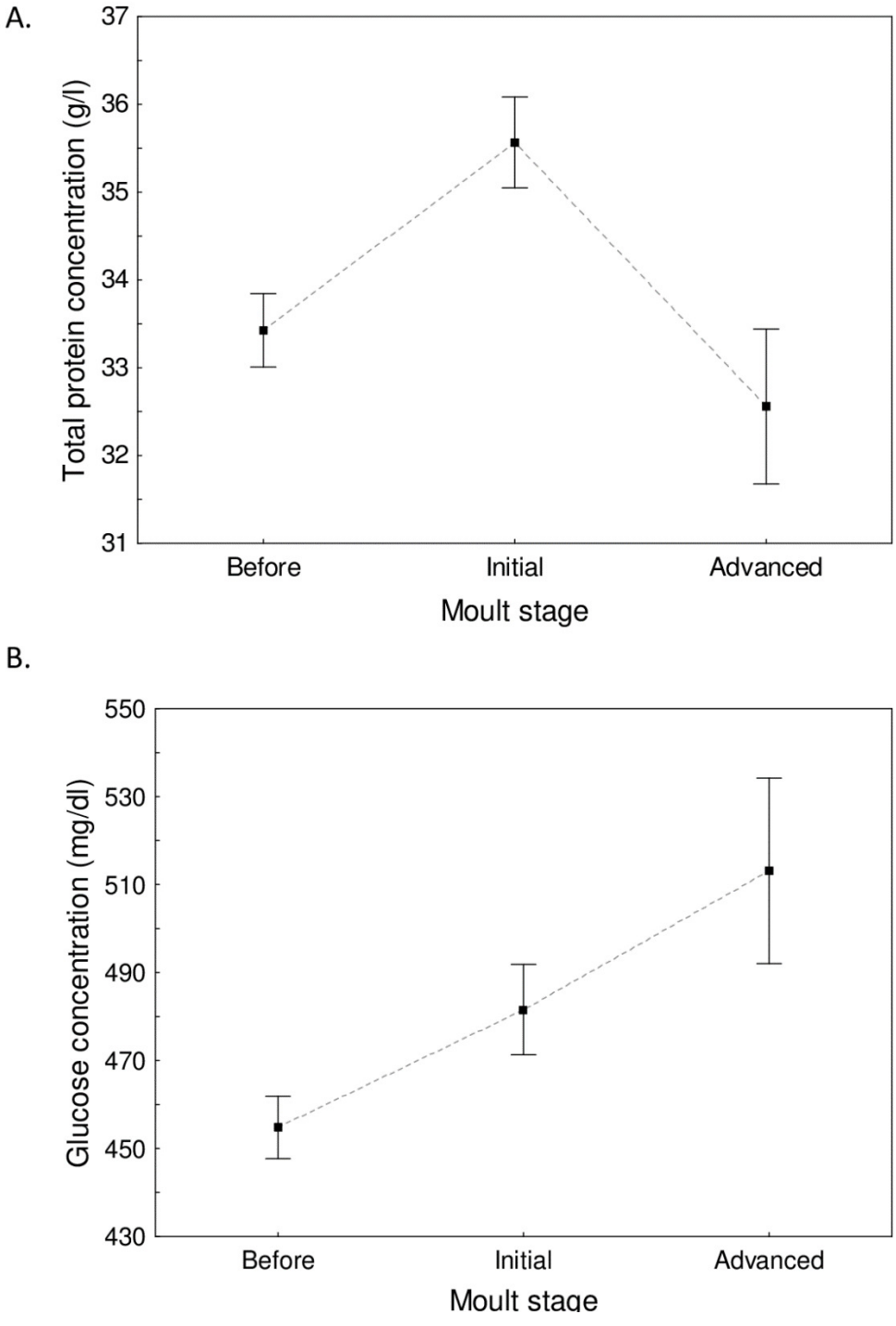

Figure 4: Changes in plasma triglyceride concentration with moult score of young common snipe in the advanced stage of post-juvenile moult. The line indicates a fitted regression ($y = -0.29*x + 78.13$; $R^2 = 0.063$).

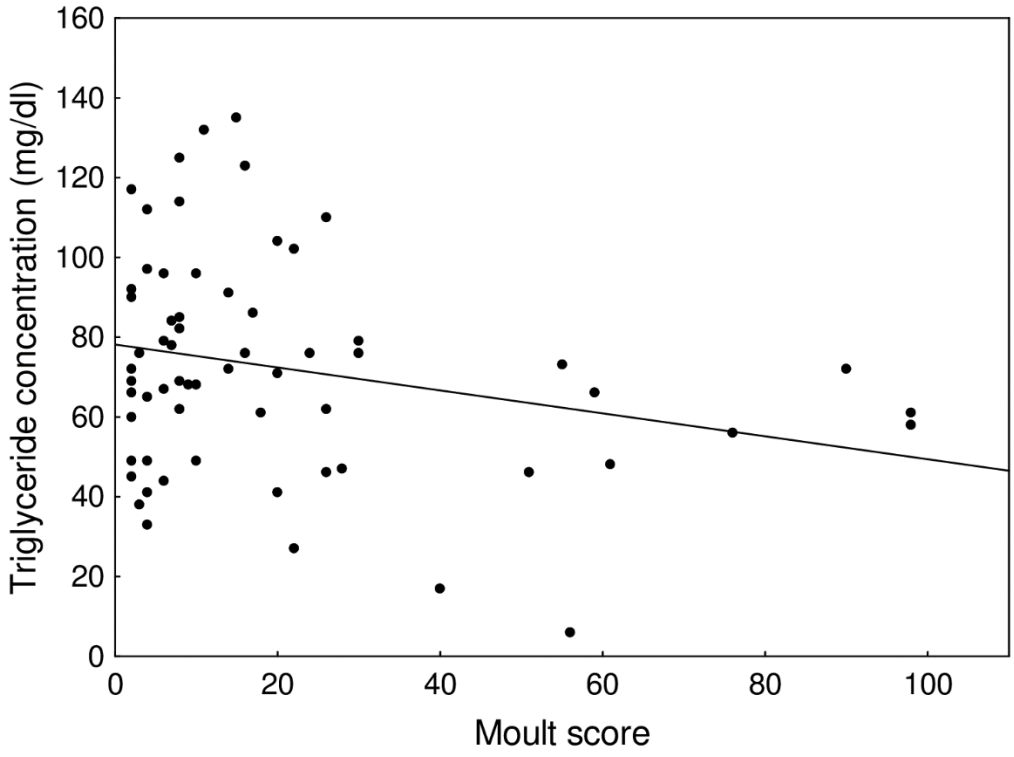

Table 1: Numbers of young common snipe in which different plasma parameters were analysed at three stages of the post-juvenile moult.

Plasma parameter	Moult stage		
	Before	Initial	Advanced
Total protein	299	171	58
Triglycerides	267	146	49
Glucose	213	103	37
Albumin	191	96	35
Uric acid	75	37	21

Table 2: Total plasma protein concentration in relation to the stages of post-juvenile moult and confounding variables in young common snipe migrating through central Poland. Reduced model R^2 = 0.41 ($F_{10,517}$ = 35.85, $p < 0.001$). Significant predictors are marked in bold.

Factor	F	P
Full model		
Moult stage	**3.46**	**0.032**
Sex	2.30	0.13
Year	**7.47**	**<0.001**
Date	1.51	0.22
Hour	**9.68**	**0.002**
Reduced model		
Moult stage	**3.13**	**0.045**
Sex	2.12	0.15
Year	**7.54**	**<0.001**
Hour	**9.14**	**0.003**

Table 3: Plasma glucose concentration in relation to the stages of post-juvenile moult and confounding variables in young common snipe migrating through central Poland. Reduced model $R^2 = 0.16$ ($F_{8,344} = 7.96$, $p < 0.001$). Significant predictors are marked in bold.

Factor	F	p
Full model		
Moult stage	**3.60**	**0.028**
Sex	0.41	0.52
Year	**14.21**	**< 0.001**
Date	**4.59**	**0.033**
Hour	3.23	0.07
Reduced model		
Moult stage	**3.74**	**0.025**
Year	**14.35**	**< 0.001**
Date	**4.82**	**0.029**
Hour	3.38	0.07

Table 4: Plasma concentrations of albumin, triglycerides, and uric acid in relation to the stages of post-juvenile moult and confounding variables in young common snipe migrating through central Poland. Significant predictors are marked in bold.

Factor	Albumin		Triglycerides		Uric acid
	F	p	F	p	F
Moult stage	1.42	0.24	0.12	0.89	0.44
Sex	1.58	0.21	0.09	0.77	0.01
Year	**9.01**	**< 0.001**	**10.23**	**< 0.001**	0.30
Date	0.02	0.88	0.27	0.60	**22.03**
Hour	**15.77**	**< 0.001**	**3.87**	**0.049**	2.50