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

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The process of feather replacement (moulting) entails considerable physiological and energetic costs to an avian organism. Even under highly 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 among the exceptions, as many young snipe initiate the post-juvenile moult during their first autumn migration. Here, we evaluated moult-related changes in blood biochemistry of the common snipe during a period of serious trade-off in energy allocation (migration). For this purpose, plasma concentrations of basic metabolites were evaluated in over half a thousand 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 no changes were recorded in the concentrations of uric acid and albumin. Total protein concentration increased significantly in the initial stage of moult, probably as a result of increased keratin production, but it dropped in the advanced stage of moulting to the pre-moult level. Plasma triglyceride concentration decreased over the period of tertial and rectrice moult, which reflected depletion of endogenous fat reserves. By contrast, glucose concentration was found to increase steadily over the course of moulting, which could be due to increased catabolism of triglycerides (via gluconeogenesis) or, alternatively, due to increased glucocorticoids as a stress response. Our results suggest that considerable physiological changes associated with moult may be among the primary determinants of the low pace of migration characteristic for the common snipe.



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#### 13 **ABSTRACT**

14 The process of feather replacement (moulting) entails considerable physiological and energetic costs to 15 an avian organism. Even under highly favourable feeding conditions, endogenous body stores and 16 energy reserves of moulting birds are usually severely depleted. Thus, most species of birds separate 17 moult from other energy-demanding activities, such as migration or reproduction. Common snipe 18 Gallinago gallinago is among the exceptions, as many young snipe initiate the post-juvenile moult 19 during their first autumn migration. Here, we evaluated moult-related changes in blood biochemistry of 20 the common snipe during a period of serious trade-off in energy allocation (migration). For this purpose, 21 plasma concentrations of basic metabolites were evaluated in over half a thousand of young snipe 22 migrating through Central Europe. We found significant changes in the plasma concentrations of total 23 protein, triglyceride and glucose over the course of moult, while no changes were recorded in the 24 concentrations of uric acid and albumin. Total protein concentration increased significantly in the initial 25 stage of moult, probably as a result of increased keratin production, but it dropped in the advanced 26 stage of moulting to the pre-moult level. Plasma triglyceride concentration decreased over the period of 27 tertial and rectrice moult, which reflected depletion of endogenous fat reserves. By contrast, glucose 28 concentration was found to increase steadily over the course of moulting, which could be due to 29 increased catabolism of triglycerides (via gluconeogenesis) or, alternatively, due to increased 30 glucocorticoids as a stress response. Our results suggest that considerable physiological changes associated with moult may be among the primary determinants of the low pace of migration 31 32 characteristic for the common snipe. Keywords Common snipe, Gallinago gallinago, Moult-migration overlap, Moulting, Physiology, Plasma 33 34 biochemistry

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#### 35 INTRODUCTION

Moulting is a process by which the bird maintains feathers of appropriate quality, improves its ability to fly and enhances thermoregulation. However, synthesis of feathers is considered one of the most physiologically costly events in the annual cycle of birds and it requires substantial body stores for completion (*Murphy*, 1996). As birds increase their tissue content and protein synthesis during the moulting period, periodic replacement of feathers demands a lots of energy for whole body protein turnover (*Newton*, 1968; *Murphy & King*, 1992; *Murphy & Taruscio*, 1995).

42 The biochemical analysis of blood is a widely-used technique used to indicate avian body 43 condition and to investigate physiological processes occurring in different life-history phases. In general, 44 plasma metabolites reflect various aspects of physiological state and characterize the feeding state of 45 birds. Earlier studies have shown that total protein and triglyceride levels reliably indicate nutrient 46 status of wild-living and captive birds (Jenni-Eiermann & Jenni, 1998; Jenni-Eiermann, Jenni & Piersma, 47 2002; Albano et al., 2016), although they may also vary in relation to environmental conditions and stress (Artacho et al., 2007; Ibañez et al., 2015). Plasma glucose level is also known to decrease during 48 49 the fasting periods and, thus, may serve as an indicator of short-term changes in food intake (Jenni-50 Eiermann and Jenni, 1998; Totzke et al., 1999; Alonso-Alvarez et al., 2002). Numerous studies indicated 51 that glucose levels positively correlate with different components of condition or broadly-defined 52 individual quality (Alonso-Alvarez et al., 2002; Minias & Kaczmarek, 2013), although increasing the level 53 of glucose may also be associated with increased glucocorticoids as a stress response (Mondal et al., 54 2011). By contrast, plasma concentrations of nitrogenous excretion components, such as uric acid, are known to increase substantially in response to starvation, as tissue proteins are actively mobilized as a 55 source of energy. Plasma concentration of uric acid was demonstrated as a good indicator of condition 56 57 especially in the species with low fat reserves, in which protein catabolism is rapidly activated during the 58 periods of 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*).

61 Taking all these into account, changes in blood plasma biochemistry may well serve to evaluate 62 physiological costs of moulting. Earlier studies have investigated changes in plasma biochemistry during 63 the moulting process in captive birds (Dolnik & Gavrilov, 1979; Murphy & King, 1984), while others have 64 examined wild-living flightless birds (Ghebremeskel et al., 1989; Cherel, Charrassin & Challet, 1994). 65 However, few, if any, papers have examined moult-related changes in plasma biochemistry occurring 66 during the migration period of wild-living birds. The aim of this study is, therefore, to determine the 67 nutritional and physiological costs of moulting by examining the changes occurring in blood plasma 68 biochemistry during the moult of a migratory wild-living bird, the common snipe Gallinago gallinago. 69 The common snipe breeds in low Arctic and boreal zones throughout entire Palaearctic, and migrates for 70 wintering grounds in South-Western Europe. While most avian species separate moult from other 71 energy-demanding activities, such as migration or reproduction (Pérez-Tris et al., 2001; Rohwer et al., 72 2009), young common snipe typically initiate the post-juvenile moult during their first autumn migration 73 (Minias et al., 2010). The post-juvenile moult of the common snipe is also more extensive and, thus, 74 more energetically expensive, compared to other shorebirds, as it includes replacement of body 75 feathers, lesser and median wing coverts, tertials and rectrices (Włodarczyk et al., 2008). In these 76 respects, the common snipe is a perfect model species for studying moult-related changes in blood 77 biochemistry during a period of serious trade-off in energy allocation, such as migration.

78

#### 79 METHODS

#### 80 General field procedures

81 Common snipe were captured at the Jeziorsko reservoir (51°40'N, 18°40'E), central Poland, during

82 autumn migration (04 August – 25 September). Jeziorsko reservoir is one of the most important

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83 stopover sites for migrating waders in inland Poland and the maximum concentrations of common snipe 84 at the site can exceed a thousand of individuals in August (Janiszewski et al., 1998). In total, we caught 85 1007 first-year common snipe during seven migratory seasons (2009-2015). Snipe were captured in 86 walk-in traps and mist nets, vocal stimulation was occasionally used. All birds were ringed and aged by plumage (Kaczmarek et al., 2007; Włodarczyk et al., 2008). Sex of birds was determined either 87 88 molecularly (only in 2009), following methods described by Kahn, John & Quinn (1998), or by 89 morphological measurements, using discriminant equations developed for the same migratory 90 population of common snipe (Włodarczyk et al., 2011). For the purpose of sexing by morphology, bill 91 length and distance between tips of two outermost rectrices were measured with calipers  $(\pm 0.1 \text{ mm})$ , 92 while vane length of the outermost rectrix was measured with a ruler (± 1 mm). Fieldwork was 93 performed under the annual permissions of the Regional Environmental Protection Directorate in Łódź, 94 Poland (nos. RDOS-10-WPN.I-6630-12/09/db; RDOS-10-WPN.I-6630-23-10/kb; WPN-I.6205.4.2011HG; 95 WPN.6205.13.2012.DB.4; WST-SI.6205.6.2013.MJ; WST-SI.6205.6.2014.MJ; WPN.6205.63.2015.HG). 96 Catching, ringing, and handling of birds was performed under individual annual permissions for ringers 97 issued by the Polish Academy of Sciences, with an approval of the Ministry of the Environment in Poland 98 and General Environmental Protection Directorate (nos. DOPozgiz-4200/III-173/622/09/ls - DZP-99 WG.6401.03.36.2015.km).

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#### 101 Moult

In all captured snipe we quantified the stage of post-juvenile moult. During post-juvenile moult snipe change their natal feathers (body feathers, lesser and median wing coverts, tertials, and rectrices) to an adult-like plumage (Fig. 1). Thus, when post-juvenile moult is completed, first-year birds become indistinguishable from adults based on the plumage characteristics. However, very few young birds (if any) finish their post-juvenile moult before reaching wintering grounds and throughout all seven years

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107 we captured only 43 individuals in fresh (recently moulted) adult-like plumage, most of which were 108 likely adults. All these birds were excluded from further study procedures. The remaining young birds 109 were classified into one of three moult categories: 1) before moult (no feathers moulted); 2) initial stage 110 of moult (only body/wing coverts in active moult); 3) advanced stage of moult (tertials or rectrices in 111 active moult). An exact moult progress was also quantified for birds that moulted tertials or rectrices. 112 For this purpose, each tertial (n = 8) and rectrix (n = 14) was given a moult score according to the feather 113 scoring system developed by Ginn & Melville (1983), where: 0 – old feather remaining, 1 – old feather 114 missing or a new feather completely in a pin, 2 – new feather up to one third grown, 3 – new feather 115 between one and two thirds grown, 4 – new feather more than two thirds grown, 5 – new feather fully 116 developed. A sum of all individual feather moult scores was used as a general moult score (max. 110, 117 when all tertials/rectrices are fully renewed).

118

#### 119 Plasma biochemistry

120 Ca. 50% of captured young snipe (n = 538 individuals) were selected for plasma biochemistry 121 measurements. Between 20 and 40 µl of blood was collected into heparinized capillary tubes from the 122 ulnar vein of each bird. Blood sampling was performed under the permission of the Local Bioethical 123 Commission in Łódź, Poland (no. Ł/BD/278). Samples were centrifuged at 6000 rpm for 5 min within an 124 hour of collection. Plasma was separated from blood cells and kept at -20°C until analysis. 125 Concentrations of the following plasma metabolites were measured (methods in parentheses): total 126 protein (biuret reaction), albumin (bromocresol green), triglycerides (glycerol phosphate 127 oxidase/peroxidase), glucose (glucose oxidase/peroxidase), and uric acid (uricase/peroxidase). All 128 parameters were analysed with a spectrophotometer (BTS-330, BioSystems Reagents & Instruments, 129 Barcelona, Spain) using commercial kits and reagents recommended by the manufacturer. The applied 130 biochemical methods followed the standard methodology used in avian studies (e.g. Artacho et al.,

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131	2007). Since the amount of plasma collected from each birds was often not sufficient to measure all five
132	plasma biochemistry parameters, we obtained varying sample sizes for each parameter (Table 1).
133	
134	Statistical analyses
135	Differences in plasma biochemistry parameters between consecutive stages of post-juvenile moult were
136	analysed with the general linear models (GLMs), separately for each parameter. In each model, we
137	controlled for the confounding effects of sex, year, date of capture, and hour. Date was standardized
138	within each season to account for annual variation in the timing of migration. To obtain more
139	parsimonious reduced models, we removed non-significant (p > 0.15) predictors from initial full models.
140	All statistical analyses were performed with Statistica 10.0 (StatSoft, Tulsa, OK, USA). All values are
141	presented as means ± SE.
142	
143	RESULTS
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143 144 145 146 147 148 149 150 151 152 153	RESULTS43.7 % of young common snipe showed signs of post-juvenile moult (n = 538). Most moulting snipe (74.9 %, n = 235) were in the initial stage of moult (moulting body/wing coverts, no tertials or rectrices) moulted), while the remaining 25.1 % were in the advanced stage of moult (moulting tertials and rectrices).We found significant differences in plasma concentrations of total protein and glucose between the consecutive stages of post-juvenile moult (Table 2, 3). Total protein concentration increased (33.33 ± 0.42 g/l; Tukey: p < 0.001; Fig. 2a). Afterwards, it significantly dropped in the advanced stage of moult (32.62 ± 0.90 g/l; Tukey: p = 0.011), returning to the low pre-moult level (Tukey: p = 0.67; Fig. 2a).By contrast, glucose concentration increased from before moult to the advanced stage of moult (454.8 ±

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intermediate level of glucose concentration (Fig. 2b). Other plasma parameters showed no variation with moult stage (Table 4). Only plasma triglyceride concentration changed with moult score of snipe that moulted tertials or rectrices ( $F_{1,61} = 4.10$ , p = 0.047), and we found that it significantly decreased over the course of tertial/rectrice moult ( $\beta = -0.29 \pm 0.14$ ; Fig. 3). All other plasma parameters showed no variation with moult score of tertials and rectrices (all p > 0.05).

160

#### 161 **DISCUSSION**

Changes were observed in the total protein, triglyceride and glucose concentrations of plasma during 162 163 the post-juvenile moult of the common snipe. While total protein concentration increased significantly 164 in the initial stage, it significantly dropped in the advanced stage of moult. Glucose concentration was 165 found to increase over the course of moulting, with higher values observed in the more advanced 166 stages. In contrast, plasma triglyceride concentration significantly decreased over the period of tertial 167 and rectrice moult. At least some of these changes in blood plasma biochemistry are likely associated with the energy required by the process of feather replacement. No moult-related changes were found 168 169 in plasma concentrations of uric acid and albumin.

170 Total protein concentration increased significantly in the initial stage of moulting but fell later 171 during the advanced stage, returning to the low pre-moult level. The snipe has probably the highest 172 protein demand at the beginning of the moult, with whole body protein turnover accelerating during 173 successive stages of feather replacement. Deposition of protein as keratins can equal one-quarter or 174 more of the total protein mass of the bird (Newton, 1968; Murphy & Taruscio, 1995; Roman et al., 2009). However, as well as playing a role in plumage replacement, plasma proteins perform a variety of 175 176 immunological and transport duties and are important indicators of nutritional state and health (Jenni-177 Eiermann & Jenni, 1996). Plasma proteins also carry a range of metabolites. Reduction of total protein

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content is an indicator of many pathological changes (malnutrition), as proteins contribute to a pool of
amino acids for protein synthesis and can act as a source of energy.

180 Similar to the present study, Dolnik & Gavrilov (1979) found total protein level to increase in the 181 initial state of moulting, which was associated with much more intensive synthesis of protein as material 182 for new feather production. This initial rise was followed by a decrease over the next stages. Similar 183 findings have been made in a number of other bird species, with decreases in total protein content 184 observed during moulting in sea birds (Work, 1996), passerines (Newton, 1968; DeGraw & Kern, 1985) 185 and anseriformes (Driver, 1981; Roman et al., 2009). In waterfowl, accumulation of muscle protein 186 occurred simultaneously with the growth of new wing feathers (Ankney, 1984). Other studies show that 187 the level of total protein is significantly higher after moulting than during feather replacement 188 (Thompson & Drobney, 1996). Nevertheless Ghebremeskel et al. (1989) found total plasma protein to be 189 significantly lower in the post-moult than the pre-moult stage in rockhopper Eudyptes crestatus and 190 Magellanic penguins Spheniscus magellanicus. Differentiation in protein level can be seen between 191 species, and this may result from variations in the supply of amino acids and energy. Most species rely 192 mostly on their diet to meet the growing demand for protein during moulting, but some birds, such as 193 penguins, use endogenous nutrients to synthesize feathers (Cherel, Charrassin & Challet, 1994). While it 194 remains unknown whether the common snipe primarily use endogenous or exogenous nutrients for 195 feather synthesis, it has been found that snipe depend on endogenous energy from adipocyte cells 196 during moult period (Minias et al., 2010). The falling levels of total protein observed in blood serum are 197 believed to occur as a result of protein accumulation in the muscles and feathers while moulting and in 198 response to less intensive synthesis in the liver; the protein requirement is also greater at the beginning 199 of the moulting period than at the end (Murphy & King, 1984). In an advanced state of feather 200 replacement, some proteins delivered with food could be catabolized for amino acids and keto acids, 201 and then used primarily as energy or for fatty acid synthesis (Artacho et al., 2007).

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202 Plasma triglyceride level is a well-known indicator of malnutrition or fasting, and the level has 203 been found to decrease rapidly, even during overnight fasting (e.g. Jenni-Eiermann & Jenni, 1996; Jenni 204 & Schwilch, 2001; Jenni-Eiermann, Jenni & Piersma, 2002). In the present study, plasma triglyceride 205 levels were found to significantly decrease over the course of moulting. This is consistent with the 206 previous findings showing that the fat reserves of snipe decreased by approximately 50% between the 207 initial stage and the end of the advanced stage of the post-juvenile moult (Minias et al., 2010). The 208 falling plasma triglyceride level observed over the course of the moult is probably an indicator of 209 increasing problems with food supply. To compensate for energy demand, snipe rely on their fat 210 reserves and probably catabolised protein obtained from dietary sources. It is known that birds 211 catabolise fat reserves to compensate for energy deficiencies in food intake and moulting is a highly 212 demanding energetic process (Jenni-Eiermann & Jenni 1996; Klasing 1998; Jenni & Schwilch, 2001; Jenni-213 Eiermann, Jenni & Piersma, 2002; Artacho et al., 2007). For example, considerable energy usage has 214 been observed in moulting chaffinches Fringilla coelebs (Dolnik & Gavrilov, 1979). A number of studies 215 have found metabolized energy level to increase during the first part of the moult, but then fall during 216 moulting, finally settling at a level below initial values upon completion (Newton, 1968; Myrcha & 217 Pinowski, 1970; Dolnik & Gavrilov, 1979; Jenni-Eiermann & Jenni, 1996; Artacho et al., 2007). 218 In contrast to plasma triglyceride levels, our findings indicate that glucose concentration steadily 219 increases from the start of the moulting period until the advanced stage. Glucose is the main metabolite 220 of the carbohydrate metabolism and is obtained from the diet. Some studies indicate that better body

condition is associated with increased glucose level (Minias & Kaczmarek, 2013). It is also known that a

falling glucose level in birds could be an indicator of short fasting periods (*Jenni-Eiermann & Jenni, 1994,* 

- 223 1997), however, in some species plasma glucose concentration has been reported to negatively
- 224 correlate with body mass (Kaliński et al., 2014). During starvation, glucose is produced from stored

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225 glycerol and amino acids or by gluconeogenesis (Herzberg et al., 1988) and may also occur with 226 increased glucocorticoids as stress-induced hyperglycaemia (Remage-Healey & Romero, 2001). 227 There are two likely explanations for the increasing levels of plasma glucose over the course of 228 moult in the common snipe. First, snipe use a considerable amount of their fat reserves in the final 229 stages of moulting (Minias et al., 2010), which is supported by decreasing plasma triglyceride 230 concentrations, as shown in this study. Thus, the increasing glucose level may occur as an effect of the 231 catabolism of triglycerides, which are stored in adipocyte cells. During lipolysis, the triglycerides are split 232 into monoacylglycerol units which are then converted to free fatty acids and glycerol. Glycerol can be 233 then converted into glucose by conversion into dihydroxyacetone phosphate and then into glyceraldehyde 3-phosphate: the process of gluconeogenesis (Herzberg et al., 1988). Consequently, we 234 235 cannot exclude that the increased catabolism of fat may lead to higher plasma glucose levels in the 236 advanced stage of moult. 237 A second reason for rise in glucose levels may be an increase in the levels of corticosteroids, as 238 these can raise the level of glucose and increase the rate of protein breakdown. Glucocorticoids increase 239 glucose level by working as an insulin antagonist, and stimulating lipolysis in adipose tissue, resulting in 240 an increase in plasma free fatty acids and glycerol levels (Remage-Healey & Romero, 2001). This scenario 241 seems likely, as migrating birds are known to express higher levels of baseline corticosterone that may 242 be related to the development or maintenance of migratory condition (Ramenofsky, Piersma & Jukema,

243 *1995; Holberton, 1999*). This may also explain why moult-related glucose level increase has not been

recorded in the studies on blood biochemistry of moulting birds kept in captivity (Romero & Remage-

245 Healey, 2000).

In conclusion, our study indicates considerable changes in blood plasma biochemistry during the post-juvenile moult in the common snipe. We believe that these nutritional and physiological costs of moulting could be among the primary determinants of the low pace of migration in this species. The

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249	common snipe is known to minimize energy expenditure during autumn migration, a strategy
250	characterized by low refuelling rates, accumulation of small fat reserves, and short migratory flights with
251	a large number of stopover sites (Włodarczyk et al., 2007). Our results may suggest that considerable
252	physiological changes associated with moult and a trade-off in energy allocation between moult and
253	migration may prevent the common snipe from adopting migration strategy of energetically-expensive
254	long-distance migratory flights.
255	
256	ACKNOWLEDGEMENTS
257	We would like to thank all participants of fieldwork at Jeziorsko reservoir, especially Tomasz Iciek, Anna
258	Piasecka, and Przemysław Wylegała.
259	
260	Funding
261	The authors received no funding for this work.
262	
263	Competing Interests
264	The authors declare that they have no competing interests.
265	
266	Author Contribution
267	Patrycja Podlaszczuk performed the experiments, contributed materials/analysis tools, wrote the paper,
268	prepared the figures or/and tables, reviewed drafts of the paper.
269	Radosław Włodarczyk performed the experiments, reviewed drafts of the paper.
270	Tomasz Janiszewski designed the experiments, performed the experiments, reviewed drafts of the
271	paper.



- 272 Krzysztof Kaczmarek designed the experiments, performed the experiments, reviewed drafts of the
- 273 paper.
- 274 Piotr Minias designed the experiments, performed the experiments, analyzed the data, contributed
- 275 materials/analysis tools, reviewed drafts of the paper.
- 276
- 277 Data Deposition
- 278 The raw data has been supplied as a Supplemental Dataset.
- 279
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**Table 1** Sample sizes for different plasma parameters of young common snipe in relation to the stage of

383 post-juvenile moult.

#### 384

Plasma		Total			
parameter	Before	Initial	Advanced	TOTAL	
Total protein	299	171	58	528	
Triglycerides	267	146	49	462	
Glucose	213	103	37	353	
Albumin	191	96	35	322	
Uric acid	75	37	21	133	

386 **Table 2** Plasma total protein concentration in relation to 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).

#### 389

Predictor	F	Р	
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 post-juvenile moult and confounding variables in
- 393 young common snipe migrating through central Poland. Reduced model  $R^2 = 0.16$  ( $F_{8,344} = 7.96$ , p <

394 0.001).

#### 395

Predictor	F	р	
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	

396

### 398 **Table 4** Plasma concentrations of albumin, triglycerides, and uric acid in relation to post-juvenile moult

and confounding variables in young common snipe migrating through central Poland.

400

Dradictor	Albumin		Triglycerides		Uric acid	
Predictor	F	р	F	р	F	р
Moult stage	1.42	0.24	0.12	0.89	0.44	0.65
Sex	1.58	0.21	0.09	0.77	0.01	0.92
Year	9.01	< 0.001	10.23	< 0.001	0.30	0.58
Date	0.02	0.88	0.27	0.60	22.03	< 0.001
Hour	15.77	< 0.001	3.87	0.049	2.50	0.12

- 402 Figure 1 The extent of the post-juvenile moult in wing and tail of the common snipe. Plumage areas
- 403 bounded by white contours are moulted. LC lesser wing coverts, MC median wing coverts, TR –
- 404 tertials, R rectrices.
- 405
- 406
- 407



408 **Figure 2** 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 arepresented.



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- 412 Figure 3 Changes in plasma triglyceride concentration with moult score of young common snipe in the
- 413 advanced stage of post-juvenile moult.

