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NOTE

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Japanese rock ptarmigan displays high levels of polyunsaturated fatty acid in egg yolk compared to chicken and quail

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ABSTRACT. Egg yolk from captive and wild Japanese rock ptarmigan were analyzed for fatty acid composition. Compared to commercially reared poultry species, the ptarmigan yolk samples displayed higher level of polyunsaturated fatty acids as opposed to monounsaturated fatty acids. The difference between the commercial controls and ptarmigan were larger than the difference between groups of ptarmigan, indicating that the fatty acid profile of Japanese rock ptarmigan might be partly attributed to genetic factors rather than feed, despite wild and captive birds having vastly different diets, and captive birds having been artificially bred for several generations.

KEYWORDS: conservation, egg quality, fatty acid, rock ptarmigan

The Japanese rock ptarmigan (*Lagopus muta japonica*) is an alpine bird species native to the mountainous region of Honshu, Japan. Due to several factors such as habitat destruction, predation and parasitic infections the population numbers have been steadily declining [2] and the species are now classified as endangered by the Japanese Ministry of Environment (MOE). In order to save the species from extinction in the wild, *in-situ* and *ex-situ* conservation efforts have been initiated in the form of a seasonal cage protection program in several mountains in the Japanese Alps and captive breeding in zoos, respectively [4]. Success of captive breeding of birds relies heavily on the hen's ability to produce viable and healthy progeny, and as such "egg quality" is an important factor which can in turn influence breeding practice protocols. In terms of egg quality, fatty acid (FA) composition of egg yolk is one important parameter. Egg yolk lipids serves as one of the major nutritional components of which the chick will survive on both during embryonic development and the first few days after hatching, before it can feed on its own [8]. In addition, certain FAs, in particular the long chain polyunsaturated fatty acids (PUFAs), linoleic acid (LA, 18:2 n6) and alpha-linolenic acid (ALA, 18:3 n3), as well as their respective derivatives arachidonic acid (AA, 20:4 n6) and docosahexaenoic acid (DHA, 22:6 n3), are important structural components in phospholipid membranes thus affecting neurological and cardiac development. Immunologically these fatty acids also play significant roles [15]. The PUFAs that the hen deposits into the yolk must be of a certain ratio in order to meet the needs of the developing embryo, and the original FA signature of egg yolks of various species are often related to their dietary mode in the wild [12].

The Japanese rock ptarmigan survives on a remarkably varied diet in the wild and has adapted well to the changes in availability throughout the seasons in the harsh alpine environment. Although primarily a herbivorous bird, feeding on leaves, buds, flowers, fruits, seeds and even stalks of various alpine plants, they also supplement their diet with insects during spring and summer. By observation, the birds have been confirmed to utilize 75 kinds of plants [3, 6], and later DNA barcoding and -metabarcoding studies on fecal samples from wild specimens identified several new plant taxa in addition to the previously reported ones [1]. In captivity, the birds are maintained on a diet regime consisting mainly of commercial formula feed supplemented with various vegetables like mustard spinach, cabbage and celery, as well as berries and mealworms. From previous studies comparing avian species held in captivity against their natural counterparts, a change can be seen in the composition of the fatty acid profiles in their egg yolks [5]. This is not only limited to herbivorous species, but also birds utilizing other feeding modes [13]. By domestication of other herbivorous avian species like the ostrich, rhea and prairie chicken, one often sees a shift in the n6:n3 PUFA ratio from a low to a higher one, due to the

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Table 1. Information regarding the Japanese rock ptarmigan individuals used in the current study

Egg ID	Egg collection date	Hen ID	Hen's origin*	Age of hen at egg collection	Generation** after first founders born in zoos (2015)	Ovulation status	Basal feed***
T-1	2021.05.21	N99	TMFPZ	1 year (hatched at 2020.08.04)	4th generation	1st ovulation	Formula feed (RM-4) and a vitamin supplement (Minevitn), Japanese mustard spinach
T-2	2021.05.24					2nd ovulation	
T-3	2021.05.29					3rd ovulation	
T-4	2021.06.01					4th ovulation	
T-5	2021.06.04					5th ovulation	
T-6	2021.06.07					6th ovulation	
T-7	2021.06.12					7th ovulation	
T-8	2021.06.15					8th ovulation	
T-9	2021.06.18					9th ovulation	
T-10	2021.06.24	N103	TMFPZ	1 year (hatched at 2020.08.04)	4th generation	1st ovulation	
N-1	2021.05.09	N49 or N97	N49:TMFPZ N97:NAK	N49: 3 years (hatched at 2018.06.13) N97: 1 year (hatched at 2020.07.18)	N49: 2nd generation N97: 3rd generation	Unrecorded	Formula feed (Bunny Selection Maintenance), Japanese mustard spinach, Lingonberry fruits, mealworms
N-2	2021.05.27					Unrecorded	
N-3	2021.05.31					Unrecorded	
N-4	2021.05.13	N93	NAK	1 year (hatched at 2020.07.15–16)	3rd generation	1st ovulation	
N-5	2021.05.14					2nd ovulation	
N-6	2021.06.23	N89	NAK	1 year (hatched at 2020.07.15)	3rd generation	1st ovulation	
N-7	2021.06.27					2nd ovulation	
W-1	2020.06.07	Wild solitary Mt Kisokomagatake	Wild: estimated at Japanese Northern Alps	Before 2016 (estimated)	-	Undefined, but 2017-laid eggs were discovered on site in 2018	Alpine vegetation, insects
W-2							
W-3							
W-4							
W-5							
W-6							

*TMPF, Toyama Municipal Family Park Zoo; NAK, Nasu Animal Kingdom Zoo. **Generation is numbered from first chicks, assigned as the 1st domesticated generation, which were hatched from eggs laid in zoos by first founders born in 2015. ***Formula feed was offered 40 g/d. RM-4 (Funabashi farm Co., Ltd., Funabashi, Chiba), Bunny selection Maintenance (Yeaster Co., Ltd., Tatsuno, Hyogo).

wild diet naturally being higher in n3-PUFAs compared to the formula feed, which is higher in n6-PUFAs [5, 7, 9]. Thus the change in yolk composition of FAs could possibly affect how well equipped the newly hatched chick is to survive in its new environment, as well as how efficiently it can cope with the various health challenges often encountered in captivity. However phylogeny can like diet also shape the FA signature of certain species, seen in the hen's enzymatic ability to covert LA and ALA to AA and DHA, respectively.

Captive breeding of Japanese rock ptarmigan has been facing issues related to high chick mortality since its initiation in 2015, of which many cases the cause has not been fully elucidated (unpublished, Japanese Association of Zoos and Aquariums). Elucidating whether egg yolk FA signature in Japanese rock ptarmigans is influenced by diet or species-specific can provide possible clues as to whether diet-associated changes in FA composition could be a contributing factor to the experienced chick mortality. In this study, we have analyzed the egg yolk FA composition from unfertilized captive rock ptarmigan eggs originating from two breeding facilities (Toyama Municipal Family Park n=10 and Nasu Animal Kingdom n=7). In addition, thanks to an egg-exchange experiment in 2020 conducted by the MOE at Mt Kisokomagatake, we secured unfertilized egg yolk samples (n=6) from a solitary wild hen. Diet, age of laying hens as well as ovulation status are listed in Table 1. Eggs laid under captivity were cracked open upon receipt and the yolk carefully punctured with a 1 mL syringe taking care to avoid albumen contamination. 0.5 mL aliquots (3–6 per egg) were collected in microtubes and frozen at −20°C until further analysis. For studying the effect of a commercial formula diet provided to that of captive rock ptarmigan, one chicken egg yolk sample as well as a quail egg yolk sample (both purchased from the supermarket) were utilized as controls. Yolk samples were thawed and weighed accurately before being sent for FA compositional analysis. Samples of formula feed (RM-4 and Bunny Selection Maintenance) were also sent for same type of analysis. Hydrolyzation, methylation and GC-MS analyses were performed by Dojin Glocal Co., Ltd. (Mashikimachi, Kumamoto, Japan). Briefly, hydrolyzation and methylation was performed with a FA methylation kit for glycerides (Nakalai Tesque, Kyoto, Japan). Methylated FA was analyzed by GC8890/MSD5977B GC-MS (Agilent Technologies, Waldbronn, Germany). Separation was achieved by a VF-WAXms column (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies) with He as carrier gas (1 mL/min). The oven temperature program was as follows: initially 60°C held for 4 min, then ramped at 10°C/min to 120°C, and then ramped at 3°C/min to 240°C and held for 10 min. The temperature of the splitless injector was 250°C. The MS analyses were carried out in scan mode. Proportions of individual FAs were calculated, given as relative abundance (% of total FAs) in a yolk or formula feed sample. Only detectable FAs in at least one egg yolk/feed sample were used in the analysis. Individual FAs were pooled into total saturated fatty acid (SFA), total monounsaturated fatty acid (MUFA), total PUFA which were again split into n6 PUFA and n3 PUFA. n6/n3-ratios were also calculated for all samples.

Table 2. Fatty acid composition (relative abundance in %) of egg yolk from captive and wild Japanese rock ptarmigan and commercially-bred birds

Fatty Acid	Chicken (n=1)	Quail (n=1)	Captive Rock Ptarmigan (n=17)	Wild Rock Ptarmigan (n=6)	Welch's *t*-test
Myristic acid (C14:0)	0.3	0.4	0.35 ± 0.02	0.30 ± 0.00	**
Pentadecanoic acid (C15:0)	ND	ND	0.11 ± 0.01	0.10 ± 0.00	NS
Palmitic acid (C16:0)	25.9	25.5	27.56 ± 0.21	25.55 ± 0.27	**
Margaric acid (C17:0)	0.1	0.1	0.39 ± 0.01	0.40 ± 0.03	NS
Stearic acid (C18:0)	9.8	8.6	9.42 ± 0.20	9.43 ± 0.13	NS
Total SFA	36.1	34.6	37.84 ± 0.36	35.78 ± 0.40	**
Palmitoleic acid (C16:1)	2.4	4	3.18 ± 0.11	2.65 ± 0.06	**
Heptadecenoic acid (C17:1)	ND	ND	ND	0.05 ± 0.05	-
Oleic acid (C18:1n-9)	47	44.8	29.07 ± 0.74	35.43 ± 1.14	**
Eicosenoic acid (C20:1n-9)	0.2	ND	ND	ND	-
Total MUFA	49.6	48.8	32.25 ± 0.80	38.13 ± 1.12	**
Linoleic acid (C18:2n-6)	11.5	13.6	23.43 ± 0.97	17.32 ± 0.76	**
γ-Linolenic acid (C18:3n-6)	0.1	0.1	0.14 ± 0.01	0.10 ± 0.00	**
Eicosadienoic acid (C20:2n-6)	ND	ND	0.01 ± 0.01	ND	NS
Dihomo-γ-Linolenic acid (C20:3n-6)	ND	ND	0.14 ± 0.02	0.07 ± 0.02	*
Arachidonic acid (C20:4n-6)	1.7	1.7	0.88 ± 0.04	0.60 ± 0.03	**
Total n6-PUFA	13.3	15.4	24.61 ± 1.01	18.08 ± 0.75	**
α-Linolenic acid (C18:3n-3)	0.2	0.2	4.12 ± 0.37	6.47 ± 0.68	*
Eicosapentaenoic acid (C20:5n-3)	ND	ND	0.26 ± 0.02	0.32 ± 0.02	NS
Docosahexaenoic acid (C22:6n-3)	0.8	0.8	0.87 ± 0.03	1.22 ± 0.04	**
Total n3-PUFA	1	1	5.26 ± 0.40	8.00 ± 0.68	**
Total PUFA	14.3	16.4	29.86 ± 0.87	26.08 ± 1.40	*
n6/n3-ratio	13.3	15.4	5.17 ± 0.46	2.30 ± 0.10	**

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid. Values for rock ptarmigan are given as mean ± SEM. ND=not detected. NS=not significant. Welch's *t*-test was applied to compare the composition between captive and wild. **: $P<0.01$, *: $P<0.05$, NS: $P>0.05$.

For rock ptarmigan samples, values are given as mean ± standard error of the mean. Due to the small sample size of both controls (n=1), literature reference values for the respective commercial species from a different study by Wang *et al.* have been included for comparison (Supplementary Table 1) [14]. In order to compare captive and wild rock ptarmigan, Welch's *t*-test for unequal variances was applied. All data processing and statistical analysis were made using R version 4.1.2 (R Core Team, 2021) [10].

The results are indicated in Table 2 for the yolk samples, and Supplementary Table 2 for the formula feed samples. Rock ptarmigan egg yolk samples, both from wild and captive specimens, displayed different FA-ratios compared to the commercial controls (Table 2). Although SFA profiles were similar between the three species, the rock ptarmigan yolk samples contained more PUFA (twice as much as poultry species) compared to MUFA (two thirds of poultry species), for which the situation was opposite in the chicken and quail yolk samples. The levels of n6 PUFA and n3 PUFA were also higher in the rock ptarmigan egg yolk which was most evident in the case of n3 PUFA, and this is in turn reflected in the higher n6/n3-ratio seen in the commercial controls. In addition there were significant differences between captive and wild rock ptarmigan for most FA parameters (Table 2), with captive birds displaying a more similar composition to that of commercial species. Formula feed supplied to commercially bred chickens and quails, mainly composed of yellow corn and soybean meal, are naturally higher in n6-PUFAs due to the inclusion of rice bran and fish meal than herbs which the ptarmigan primarily feed on in the wild. The captive diet composition of rock ptarmigans is indeed closer to commercial feeds than to their natural diet (Supplementary Table 2), and this can have affected the slight change observed in yolk FA composition. However compared to the difference against the commercial species (Supplementary Table 1), these differences–although significant–is much smaller. This is evident when comparing battery cage chickens to free-range chickens (Supplementary Table 1), in which the difference in feed has a larger impact on differences especially related to the amount of n3 FAs and thus n6/n3-ratio, being almost 50% lower in free-range birds. Thus at present there seems to be no pressing issue as to whether the current feed in captivity will markedly alter the FA composition in the ptarmigan egg yolk. In addition to diet, genotype is another factor that has been shown to determine yolk FA composition. This can be seen in other bird species that despite completely different feeding modes, have maintained a similar FA composition in their egg yolk [11], and this might also in part hold true for the Japanese rock ptarmigan. Due to seasonal availability, the FA composition of the diet of herbivorous wild birds will likewise not be completely fixed, as seen in the wild Japanese rock ptarmigan [3], thus obtaining a constant FA ratio in the yolk might be mainly influenced by genotype [12]. Indeed, the alpine environment which is prone to extreme seasonal fluctuations in temperature might have shaped the PUFA contents of rock ptarmigan egg yolk. During the egg-laying season the hen will lay her eggs every other day but not start the actual incubation until all eggs have been laid. In order to pass sub-zero temperatures to which the eggs might be exposed until she starts incubating, a higher PUFA content will increase fluidity in the yolk preventing it from freezing. In addition to the FA profiles of the formula feed provided in captivity (Supplementary Table 2), the nutritional composition and FA profile of the various dietary sources in wild environments needs to be further assessed, since such information is presently lacking. This will further help clarify whether the yolk FA profile is mostly due to genetic factors or diet, and if captive diet has had any modifying effect on these parameters in the birds. The captive breeding of Japanese wild rock ptarmigan began in 2015, of which the currently used hens constitute the 3rd and 4th generation born in zoos (Table 1), and have all been hatched by means of incubators. It might be that the timeframe since domestication in this species is still insufficient to have influenced any significant changes in the FA signatures of their egg yolks, however should there be a gradual change over time the situation ideally needs to be monitored in case this can affect the status of the progeny.

CONFLICT OF INTEREST. There are no conflicts of interest to declare.

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