Pak. j. sci. ind. res. Ser. B: biol. sci. 2025 **68B**(1) 117-122

Fatty Acids Composition of Migrated Seabirds to the Coastline of Pakistan as Top Predators to Impact Ecosystem Variability

Tahira Akram*[a], Razia Sultana[b], Alia Bano Munshi[a], Hina Ahsan[b], Muhammad Haseeb-ur-Rehman[a,c] and Ayesha Jamal Zaidi[a]

[a]Centre of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan
[b]PCSIR- Laboratories Complex Karachi, Karachi-75280, Pakistan
[c]Marine Fisheries Department, Karachi, Pakistan

(received January 30, 2023; revised October 20, 2023; accepted October 24, 2023)

Abstract. The availability of fat-rich food is a critical factor for the seabird's migration. Certain fatty acids are significant determining factors for environmental health and act as energy reserves for long distant seabird migration. Qualitative analysis for fatty acid composition in *Larus fuscus, Larus ridibundus* and *Hydroprogne caspia,* found significantly different for most of the fatty acids. The average fat contents of *L. fuscus, L. ridibundus* and *H. caspia* were 23.57±1.82%, 19.71±2.75% and 33.58±0.08%, respectively. This study suggests that mono-unsaturated fatty acids (MUFA) (43.49-48.07%) were predominantly higher than saturated (SFA) (32.88-39.89%) and poly-unsaturated fatty acids (PUFA) (14.1-16.22%). Palmitic acid and stearic acid constituted > 75%. The dietary fatty acid oleic acid (C18:1n9) was most abundant with 32-34%. The ω-3/ω-6 ratio was less than 1, indicating these sea birds as a substantial source of ω-6 fatty acids. Linoleic acid (C18:2ω6) as major ω-6 fatty acid in *L. fuscus* (7.44%), *H. caspia* (9.81%) and *L. ridibundus* (8.99%).

Keywords: seabirds, avian migration, methyl ester fatty acid, ecosystem variability

Introduction

The dynamic of the immense natural capital of pelagic ecosystems is imitated by chemical pollutants, climatic variation and commercial fishing exercises. The food web status may reveal the trophic level based indicators derived from fishery catch data but the utility of biased records in fisheries catch is doubtful (Gagne *et al.*, 2018). Sea birds, the essential primary component which are an excellent indicator of environmental fluctuations (Barrett *et al.*, 2007). The reproductive success of sea birds is affected by the oceanic environment's general aspects and species' response to the physical factors of an ecosystem, such as food availability, climatic changes and population density. Arguably, a helpful indicator should be responsive mainly to factors affecting several species in the top predator community. International Union for Conservation of Nature (IUCN) Ramsar sites and wetlands host a great community of seabirds that migrated from Siberia and other native wildlife in Pakistan. Crane, geese, waders, swans and thousands of other birds visit Pakistan and other south Asian countries as resting sites between breeding and wintering grounds. Since sea birds are assumed to be the typical example of effective samplers of prey populations like other predators such as fishes, thus helpful information about the lower trophic position over a wide range of geospatial and sequential scales can be obtained from their diets.

Sea birds diet analysis by traditional methods is a hectic task, therefore, the fatty acid composition of adipose tissues determines their diet constituent. Physical and ecological factors like climate change, timing and accessibility of the prey species can be broadly attributed to changes in the dietary patterns of many seabirds throughout their reproductive season (Owen *et al.*, 2013). The other fundamental aspects linked with the reproductive phase include the amount and quality of feed for the prey compared to the self-feed of the predator species (Ito *et al*, 2010). However, extricating environmental and inherent effects are challenging due to simultaneous changes in external circumstances and parental responsibilities. Studies indicated that sea birds are regarded to be bio-monitors of changes in lower trophic levels or in the physico-chemical environment, given that they are at the top of the food chain in the marine ecosystem (Parsons *et al.*, 2008).

In contrast, some prey species are lower in the food web, and only a few contaminants are transferred upto

*Author for correspondence;
E-mail: tahiraakram262@gmail.com

the food web. Lipids are the most significant energy providers to migratory birds and the marine environment. The proximate analysis of migratory birds reveals that approximately 50% of the body mass comprises the total fat content to fuel during migratory exercises (McWilliams *et al.*, 2004). They can absorb organic pollutants, act as absorption carriers and play a role in bio-accumulating organic toxins. The lipids having heterogeneous nature can be used for ecological and bio-geochemical assessment as an indicator of the health of ecosystems and the degree to which terrestrial and anthropogenic inputs have influenced them. The production value of dietary biogenic material of marine organisms and water quality can be determined by lipids. The stable compounds are supposed to be more effective measures for studying the biochemical and ecological consequences of the lipid contents of sea birds (Wold *et al.*, 2011).

This study aimed to investigate the fatty acid composition of migratory seabirds for the first time in Pakistan, as no biochemical research has ever been conducted in this region on sea birds.

Materials and Method

Seabird sampling was done for two years, from November 2016 to February 2018, during wintering season from Manora Channel along the Karachi coast.

Seabirds collection. Atotal of 22 individuals representing three species, *i.e*., *Larus fuscus* (n = 9), *Larus ridibundus* (n = 7) and *Hydroprogne caspia which is* formally known as *Sterna caspia* (n = 6) and sampled from the open sea with the assistance of lab attendants and fishermen using non-invasive techniques such as baited traps. The approach employed for the sampling design involved strategically placing baited traps at pre-determined intervals across the study area, ensuring the comprehensive coverage of the habitat, while maintaining a consistent and controlled method for capturing birds. The spacing and placement of traps were carefully considered to account for factors such as bird density, habitat characteristics and potential migration patterns. The birds were dissected, stored at -20° C and transported to the Pakistan Council of Scientific and Industrial Research, Karachi's national accredited laboratory, for further fatty acid analysis. Locally trained research assistants handled birds safely under the supervision of experts' knowledge. Appropriate support was provided by the Marine Fisheries Department (MFD) and WWF, Pakistan. In this research study focused on the coastal belt of Karachi, we meticulously addressed ethical considerations – ensuring their well-being and minimising habitat impact, while capturing seabirds during the winter months of November to March, coinciding with the migratory influx to Pakistan.

Reagents and standards. *n*-hexane (95%), *n*-heptane (99%), chloroform (99.5%), methanol (98.8%) and potassium hydroxide (98%) were supplied by E. Merck and May & Baker (M&B). Whereas the standards for the fatty acid methyl ester quantification were purchased from Supelco Sigma-Aldrich Co;.

Lipid analysis. Samples were dried in the electric oven at 70 °C and extraction was done in the Soxhlet assembly. Samples were weighed for approximately 2 g (dry matter) and homogenized in a glass potter. Total lipids were extracted in the Soxhlet apparatus by the modified Folch method (Perez-Palacios *et al*., 2008). The solvents used for extraction chloroform and methanol (2:1 v:v) were then removed under reduced pressure. The extract was dried using anhydrous sodium sulfate, followed by filtration and storage at low temperature (0-40 °C).

Fatty acid analysis. The fatty acids profiles were analyzed through temperature gradient on a chromatograph (Clarus-500 Perkin Elmer) fitted in capillary column SP 2650 with 100m, 0.25mm and 0.20 um film thickness and joined with the flame ionization detector (FID). Nitrogen was used as a carrier gas (splitless) at 2.5 mL/min flow rate (Paquot and Hautfenne, 1987). The FA peaks were identified through retention time compared with the FAME 37 standard mixture (Sulpeco Inc., Bellefonte PA). Results were calculated as the total FAME percentage. IBM SPSS statistics 24 was used for statistical analysis, which included means, standard deviation and univariate analysis (ANOVA) for analyzing differences among the mean concentrations accompanied by the post hoc Duncan test.

Results and Discussion

The biochemical analysis of seabirds revealed the average fat content of *Larus fuscus*, *Larus ridibundus* and *Hydroprogne caspia* as 23.57±1.82%, 19.71±2.75% and 33.58±0.08%, whereas the protein content (dry matter) displayed was 57.61±7.86%, 58.23±3.19% and 46.55±0.3% respectively (Table 1).

The study identified a total of 21 fatty acids that includes saturated fatty acids (lauric acid C12:0, myristic acid C14:0, pentadecanoic acid C15:0, palmitic acid C16:0,

Table 1. Chemical composition of seabirds from Karachi coast

Constituents	*Larus fuscus*(n-9)	*Hydroprogne caspia*(n-6)	*Larus ridibundus* (n-7)
Crude protein (%)	57.61±7.86	46.55±0.3	58.23±3.19
Total fats (%)	23.57±1.82	33.58±0.08	19.71±2.75
Ash contents (%)	5.86±0.63	6.32±0.27	5.27±0.53
Total moisture (%)	6.5±1.04	6.84±0.06	6.07±2.02
Total carbohydrates (%)	7.21±6.71	6.69±0.56	10.7±4.02
Gross energy (Kcal/100g)	155.99±18.62	178.90±1.79	154.61±22.48

heptadecanoic acid C17:0, stearic acid C18:0), mono-unsaturated fatty acid, palmitoleic acid C16:1, *Cis*-10-heptadecenoic acid C17:1, oleic acid C18:1, *Cis*-11-eicosenoic acid C20:1n9, erucic acid C22:1n9, nervonic acid C24:1n9 and polyunsaturated fatty acid linoleic acid C18:2,linolenic acid ω LNA C18:3, ωLNA C18:3, eicosadienoic acid C20:2, eicosatrienoic acid C20:3n6, eicosapentaenoic acid [EPA] C20:5n3, docosadienoic acid C22:2*,* docosapentaenoic acid C22:5n3, docosahexaenoic acid [DHA] C22:6n3) in various proportion in the adipose tissues of a lesser black-backed seagull (*Larus fuscus*), black-headed seagull (*Larus ridibundus*) and caspian tern (*Hydroprogne caspia)* from Pakistani coastal waters.

There were significant differences in the mean percentages of C12:0, C14:0, C15:0, C16:0, C16:1, C17:1, C18:0, C18:2, C18:3, C20:1, C20:2, C20:5n3, C22:1n9, C22:5n3, C22:6n3 and C24:1n9 as $P < 0.05$ found between *L. fuscus, L. ridibundus* and *H. caspia*. The mean percentages of C17:0, C18:1, C20:3n6 and C22:2 were non significantly different ($P \geq 0.05$) (Table 2). The mean percentages of C14, C16, C18, C20:1, C20:5n3, C22:1n9, C22:5n3, and C22:6n3 were slightly higher in lesser black-backed seagull than caspian tern and black-headed gull, whereas C16:1, C17:1, C18:2 and C18:3α were found to be elevated in Caspian tern than other two species, whereas C18:1 was greatest in black-headed seagull.

Results revealed that MUFAs significantly contribute to the total fatty acid composition in all sampled species 43-48%, followed by the saturated fatty acids 32-39%, whereas PUFAs were in the lowest percentage, 14-16%.

Palmitic acid (16:0) was the most abundant saturated fatty acid at 24.17%, 22.75% and 19.56% followed by stearic acid (18:0) at 12.13%, 11.9% and 9.95% in *L.fuscus* >*L. rudibundus* >*H. caspia*, whereas the opposite trend (C18:0 >C16:0) was earlier reported by (Andersson *et al*., 2015), correlated to temperature changes. However, differences in the FA content are relevant to the season depending on habitat and diet choices.

Among MUFAs, oleic acid (18:1) was the most abundant 34%, 32.89% and 32.52% in *L. rudibundus* > *L. fuscus* > *H. caspia* followed by palmitoleic acid (16:1) 11.96%, 6.64% and 6.56% in *H.caspia* > *L.fuscus* > *L. rudibubdus* and the most abundant fatty acid among all. Linoleic acid (18:2n6) was the most abundant PUFA 9.81%, 8.99% and 7.44% found in adipose tissues of *H. caspia* > *L. rudibundus* > *L. fuscus*.

The physiologically important ω-3 PUFAs are DPA 1.57 > 1.08 > 1.03 in *L. fuscus, L. rudibudus,* and *H. caspia,* respectively and αLNA 2.48% > 0.98% > 0.65%, respectively in *H. caspia, L. fuscus* and *L. rudibundus*. The αLNA is a strictly dietary fatty acid. It cannot be bio-synthesized (Larsson *et al*., 2004) and results in this study indicate that the diet is insufficient for physiologically essential ω-3 FA. The values of ω-6 Linoleic acid were highest at 9.81% in *H. caspia* followed by 8.99% in *L. rudibundus* and the least amount, 7.44%, in *L. fuscus* indicating the diet rich in ω-6 FA as Linoleic acid is also diet-dependent and cannot be bio-synthesized. Another essential ω-6 FA was ARA (Arachidonic acid), not found in the present study, which is a biosynthetic product of linoleic acid. It may be occurred due to the mobilization of selective fatty acids to regulate body physiology during environmental fluctuations such as thermo-regulation during wintry weather (Price *et al*., 2008).

Fatty acid levels might be influenced by seasonal changes and availability of diet. (Andersson *et al*., 2015) explained in an earlier study that ω-3 FA was strongly dependent on seasonal variation and found in lower proportions during winter than summer while ω-6 Linoleic acid was higher during winter. The recent study

Table 2. Mean percentages and standard deviation of fatty acids found in seabirds from the Karachi coast. values are expresed as a mean ± SD

Fatty acid	Larus fuscus(n=9)	Hydroprogne caspia(n=6)	Larus ridibundus(n=7)	Sig.
C12:0	0.17c±0.04	0.12b±0.02	0.08a±0.01	0.001
C14:0	2.5b±0.22	2.25b±0.21	1.84a±0.16	0.008
C15:0	0.41a±0.01	0.61c±0.04	0.53b±0.06	0.001
C16:0	24.17c±0.6	19.56a±1.27	22.75b±0.78	0.000
C16:1	6.64a±0.13	11.96b±0.5	6.56a±0.26	0.000
C17:0	0.51a±0.11	0.39a±0.06	0.71a±0.4	0.379*
C17:1	0.18a±0.16	0.81c±0.08	0.55b±0.03	0.000
C18:0	12.13c±0.12	9.95a±0.94	11.9b±0.43	0.003
C18:1	32.89ab±0.34	32.52a±1.12	34b±0.15	0.066*
C18:2n6	7.44a±0.09	9.81c±0.03	8.99b±0.1	0.000
C18:3γ	0.44b±0.03	0.38a±0.03	0.73c±0.02	0.000
C20:1	1.13a±0.02	0.74a±0.02	1.29a±1.12	0.673*
C18:3α	0.98b±0.33	2.48c±0.12	0.65a±0.3	0.000
C20:2	0.18a±0.15	0.28a±0.07	0.45b±0.03	0.016
C20:3n6	0.25a±0.26	0.14a±0.16	0a±0	0.158*
C22:1n9	2.49b±0.02	1.83a±0.03	1.97a±0.12	0.000
C20:5n3 (EPA)	0.81c±0.07	0.36a±0.03	0.66b±0.08	0.000
C22:2	0.9a±0.06	0.89a±0.05	0.84a±0.05	0.422*
C24:1n9	0.16a±0.21	0.21a±0.02	0.21a±0.18	0.057*
C22:5n3 (DPA)	1.57b±0.07	1.03a±0.02	1.08a±0.08	0.000
C22:6n3 (DHA)	1.53b±0.14	0.85a±0.01	0.97a±0.07	0.000
Σ SFA 39.89% 32.88% 37.81%				
Σ MUFA	43.49%	48.07%	44.58%	
Σ PUFA	14.1%	16.22%	14.37%	
ω-3	5.87%	4.72%	3.36%	
ω-6	9.03%	11.22%	10.56%	
ω-3/ω-6	0.65%	0.42%	0.31%	

ANOVA revealed significant differences in fatty acids among seabirds P<0.05; *Represent non-significant differences in fatty acids among seabirds P>0.05; Different superscript a-d horizontally shows the significant difference among species.

showed these variations as the birds were sampled during winter. Overall, ω-3 is considered an anti-inflammatory, while ω-6 has pro-inflammatory and thermo-regulatory properties. It is also hypothesized that due to lower levels of ω-3 PUFAs and higher intake of ω-6 PUFAs, these birds are more exposed to pollutants, enhancing oxidative stress (Alagawany *et al*., 2019; Simopoulos, 2002). Hence, play a crucial role in a healthy and sustainable ecosystem.

Results revealed a variety of food supplied to these sea birds to fuel their annual migration along the Karachi coast, as the most abundant C16 and C18 fatty acids were diet dependent. Significant contributors are marine algae, krill, worms, clams, mussels, crabs, shrimps, scallops and pelagic and intertidal small fishes like herring and sardine mackerel and juveniles of large fishes like sea bass, codfish, catfish, tuna and tilapia. Mangroves are also part of the seabird's diet. For future studies to explore the food chain, experimental research should be carried out to investigate the FA composition of prey species. Fatty acids, transporters of fat soluble vitamins and chemical pollutants, are conducive to sustaining and shaping the ecosystem (De Laender *et al*., 2010).

Sea birds, as a member of the marine environment, are imperilled to substantial fluctuations in the environment, such as temperature and availability and food quality which affect the FA composition of their muscles (Parrish, 2013). Carbon 16 and 18 fatty acids, including saturated and monounsaturated fatty acids, constitute a significant part of total fatty acids composition in migratory birds were reported previously (Pierce and McWilliams, 2014; McWilliams *et al*., 2004; Blem, 1976) and >75% during this study. C16:0 and C18:1n9 are the most abundant, whereas C16:1n7 and C18:0 are less abundant. However, they are essential fatty acids. During migratory exercises of sea birds, the levels of FA constituents can also be influenced by selective feeding at their stopover sites (Pierce and McWilliams, 2005). A selective diet can significantly influence the energetics of the flight (Pierce and McWilliams, 2005). Previously it was suggested that ω-3 and ω-6 PUFA play a significant role in exercise performances. Price and Guglielmo (2009) propose that ω-6 PUFA enhanced the migratory exercise more than ω-3 PUFA. While according to Maillet and Webe (2007), possibly ω-3 PUFA, which improves membrane functioning, can increase prolonged migratory exercises. Furthermore, the fatty acid composition determines the distribution pattern and effects of lipid-soluble chemical pollutants like organochlorine pesticides (Zhang *et al*., 2019). The present study could be helpful for the conservation and management of seabirds effectively for a sustainable environment.

Conclusion

This study reveals the fatty acid composition of migratory seabirds foraging from the coastal areas of Pakistan for the first time to the best of our knowledge. Variations were observed in SFA, MUFA and PUFA. The results suggest a low supply of anti-inflammatory ω-3 PUFA than pro-inflammatory ω-6 PUFA, indicating potential exposure to chemical pollutants and pathogens — moreover, substantial impacts on long-distance migratory performances. The variations in fatty acid compositions of seabirds are related to the changes in the status of habitat, availability of quality food and physical changes in the marine environment and physiological state of the bird.

Conflict of Interest. The authors declare they have no conflict of interest.

References

Alagawany, M., Elnesr, S.S., Farag, M.R., Abd El-Hack, M.E., Khafaga, A.F., Taha, A.E., Tiwari, R., Yatoo, M.I., Bhatt, P., Khurana, S.K., Dhama, K. 2019. Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. animals: *An Open Access Journal from MDPI*, **9:** 573.

Andersson, M. N., Wang, H.-L., Nord, A., Salmon, P., Isaksson, C. 2015. Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Frontiers in Ecology and Evolution*, **3:** 93.

Barrett, R., Camphuysen, C.J., Anker-Nilssen, T., Chardin, J., Furness, R.W., Garthe, S., Hüppop, O., Leopold, F.M., Montevecchi, A.W., Veit, R.R. 2007. Diet studies of seabirds: a review and recommendations. *ICES Journal of Marine Science*, **64:** 1675-1691.

Blem, C.R. 1976. Patterns of lipid storage and utilization in birds. *American Zoologist*, **16:** 671-684.

De Laender, F., Oevelen, D. V., Frantzen, S., Middelburg, J.J., Soetaert, K. 2010. Seasonal PCB bio-accumulation in an arctic marine ecosystem: A model analysis incorporating lipid dynamics, food web productivity and migration. *Environmental Science and Technology*, **44:** 356-361.

Gagne, T.O., Hyrenbach, K.D., Hagemann, M.E., Van Houtan, K.S. 2018. Trophic signatures of seabirds suggest shifts in oceanic ecosystems. *Science Advances*, **4:** eaao 3946 https://www.science.org/doi/abs/10.1126/sciadv.aao3946

Ito, M., Takahashi, A., Kokubun, N., Kitaysky, A.S., Watanuki, Y. 2010. Foraging behavior of incubating and chick-rearing thick-billed murres *Uria lomvia*. *Aquatic Biology*, **8:** 279-287.

Paquot, C., Hautfenne, A. 1987. Standard methods for the analysis of oils, fats and derivatives. *Analytica Chimica Acta*, **201:** 373. https://doi.org/10.1016/s0003-2670(00)85376-3

Larsson, S.C., Kumlin, M., Ingelman-Sundberg, M., Wolk, A. 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *The American Journal of Clinical Nutrition*, **79:** 935-945.

Maillet, D., Weber, J.M. 2007. Relationship between n-3 PUFA content and energy metabolism in the flight. *The Journal of Experimental Biology*, **210:** 413-420.

McWilliams, S.R., Guglielmo, C., Pierce, B., Klaassen, M. 2004. Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *Journal of Avian Biology*, **35:** 377-393.

Owen, E., Daunt, F., Moffat, C., Elston, D.A., Wanless, S., Thompson, P. 2013. Analysis of fatty acids and fatty alcohols reveals seasonal and sex-specific changes in the diets of seabirds. *Marine Biology,* **160:** 987-999.

Parrish, C.C. 2013. Lipids in marine ecosystem: review article. *ISRN Oceanography*, **2013:** 1-16

Parsons, M., Mitchell, I., Butler, A., Ratcliffe, N., Frederiksen, M., Foster, S., Reid, J.B. 2008. Seabirds as indicators of the marine environment. *ICES Journal of Marine Science*, **65:** 1520-1526.

Pérez-Palacios, T., Ruiz, J., Martín, D., Muriel, E., Antequera, T. 2008. Comparison of different methods for total lipid quantification in meat and meat products. *Food chemistry*, **110:** 1025-1029.

Pierce, B.J., McWilliams, S.R. 2005. Seasonal changes in composition of lipid stores in migratory birds: causes and consequences. *The Condor*, **107:** 269-279.

Pierce, B.J., McWilliams, S.R. 2014. The fat of the matter: how dietary fatty acids can affect exercise performance. *Integrative and Comparative Biology*, **54:** 903-912.

Price, E.R., Guglielmo, C.G. 2009. The effect of muscles phospholipid fatty acid composition on exercise performance: a direct test in the migratory white throated sparrow (*Zonotrichia albicollis*). *American Journal of Physiology*, **297:** 775-782.

Price, E.R., Krokfors, A., Guglielmo, C.G. 2008. Selective mobilization of fatty acids from adipose tissue in migratory birds. *The Journal of Experimental Biology*, **2011:** 29-34.

Simopoulos, A.P. 2019. Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition*, **21:** 495-505.

Wold, A., Jæger, I., Hop, H., Gabrielsen, G.W., Falk-Petersen, S. 2011. Arctic seabird food chains explored by fatty acid composition and stable isotopes in Kongsfjorden, Svalbard. *Polar Biology*, **34:** 1147-1155.

Zhang, K., Fu, Q., Ge, F., Jin, X., Guo, R., Qiao, X., Zhao, X., Zheng, X. 2019. The correlation study between fatty acids and organochlorine pesticides or δ15N values in fish tissues from Dongting lake, China. *Ecotoxicology and Environmental Safety*, **182:** 109358. https://doi.org/10.1016/j.ecoenv.2019.06.041