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# Histological, Histochemical and Gene Expression Analysis of Mucin 1,4 in the Small Intestine of Owl (Tyto Alba)

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**Annotation:** The point of this study is to look at how environmental and dietary factors affect the small intestines of owls by looking at the histological features and gene expression of mucin proteins. We compared the histochemical and gene expression profiles of MUC1 and MUC4 proteins present in the small intestines of owls. The small intestine of the owl was characterised by a layer of simple, columnar epithelium interspersed with goblet cells. The duodenum organizes the muscular mucosa into a single set of longitudinal fibres, while the submucosa constitutes a substantial layer of loose connective tissue. The jejunum exhibited a mucous membrane characterised by numerous large villi, while the ileum had simple columns and goblet cells. The RT-PCR amplification plots of the MUC1 and MUC4 genes in the owl's duodenum, jejunum, and ileum showed different Ct cycle numbers, which were between 20 and 25. The melting peaks for the MUC1 of owl were in the ranges of 65-85°C, while the melting peaks for the housekeeping gene, MUC4, owl were in the ranges of 65-80°C. The results of this study give us a lot of information about the

structure, expression, of MUCs in owls. This helps us learn more about how MUCs work in the digestive system and how they might be linked to elementary canal disorders.

**Keywords:** MUC, Owl, small intestine, gene expression.

## Introduction:

The studies of histological and molecular traits on this species of birds provide a useful instrument for comprehending the structural and functional adaptations that have evolved over time to suit the several environments and means of life that these birds have come to know. The small intestine's mucous membrane controls both nutrient absorption and body defense against germs that cause infections. Apart from preserving mucosal integrity and supporting digestion and absorption, mucin proteins are crucial for building the defensive mucosal barrier in the small intestine (1,2,3). Moreover, improving digestion and absorption are mucin proteins. The owls are carnivores that survive mostly on live prey. The dietary and lifestyle variances between the two species could affect the composition and operation of the intestinal mucosa(4,5,6,7). The aim of this work is to assess the histological features and gene expression of mucin proteins in response to the impact of environmental and dietary elements within the framework of the small intestine.

Gene expression studies allow one to ascertain the functional functions played by MUC1 and MUC4 across several species, therefore exposing their adaptation to fulfil a range of physiological needs. The results of this work offer a fresh understanding of how the intestinal mucosa functions change in birds on different diets. By clarifying the molecular and histological mechanisms behind the functional adaptations seen in the avian small intestine, we hope to improve our knowledge of the biological and physiological diversity existing throughout the planet.

## Material and Method:

Histologically the small intestine of 10 healthy adult slaughtered owl of both sexes were dissected, the small intestine is three-part section (duodenum, jejunum and ileum) were fixed by immersion in 10% neutral formal saline for 48 h., the three parts of small intestine of both birds were dehydrated, cleared and embedded in paraffin. Histochemically techniques were used for the identification of mucin in goblet cells in small intestine of owl were stained with periodic Acid-Schiff (PAS) for glycogen and neutral mucosubstances, alcian blue pH 2.5 for the carboxyl group of acidic mucosubstances,

### RNA extraction and complementary (cDNA) synthesis

owl mRNA was extracted from their small utilising the Accuzol® reagent kit (Bioneer, Korea) as per the manufacturer's instructions. Each segment of three part included 200 mg of tissue, measured in a 1.5 ml Eppendorf tube. Two hundred microlitres of chloroform were thereafter added and agitated on ice for five minutes. The supernatant was collected after 15 minutes of tissue centrifugation at 12,000 rpm and 4°C. Introduce 500 µL of isopropanol; agitate; thereafter, incubate at 4°C for ten minutes. The samples were subjected to centrifugation at 12,000 rpm for 10 minutes at 4°C. Subsequent to the addition of one millilitre of 80% ethanol, the mixture was vortexed and then centrifuged at 12,000 rpm for ten minutes at 4°C, omitting the supernatant. Eliminated the supernatant; air-dried the pellet in Eppendorf tubes; reconstituted RNA pellets in 50 µL DEPC water and stored at -20°C until analysis. In each sample, a Thermo, USA nanodrop spectrophotometer measured RNA concentrations. All samples were processed according to manufacturer instructions utilising the DNase I enzyme kit (Promega Company, USA).The DiaStarTM OneStep RT-PCR Kit (China) utilised directions and thermocycler parameters to

convert whole RNA into cDNA. All sample cDNA concentrations were subsequently normalised and stored at -20°C.

**Real-time RT-qPCR**

The expression of MUC1 and MUC4 genes was quantified using 2.5 RT-qPCR using Real-Time PCR apparatus (BioRad, USA). This study employs these primers: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping **gene Forward primordial:**

**TGCTGGCATTGCACTGAATG, CACGGTTGCTGTATCCAAACTC.**

**Reverse: MUC1-like (LOC1073175), mRNA (XM_0324487). Forward primer:**

**TAATGCTGCCCCAATTGCTG; Reverse primer: GAGGTTGTATYCAGTGCAG.**

**MUC4 mRNA of Coturnix japonica; code XM_0324465. One forward primer is AATGCAAAGTGCCACAGCTG;**

**one reverse primer is TTGGTGTTCCTCCAAAACGC**.

The SYBER Green dye QPCR master mix (AccuPowerTM 2XGreen Star, Bioneer, Korea) was utilised for the amplification and normalization of GAPDH housekeeping gene, as well as MUC1 and MUC4 gene expression, following the kit instructions. The thermocycler were set up in the following manner:

**Statistical Analysis**

Using RT-qPCR and the 2^Δ CT technique (14), the MUC1 and MUC4 gene expression data were computed statistically under SPSS (IBM SPSS Statistics 24.0) and with significance regarded at the $P \leq 0.05$.

**Results:**

H&E stain showed the small intestine of owl was characterized by a lining of simple, columnar epithelium interspersed with goblet cells. The duodenum organizes the muscularis mucosa into a single set of longitudinal fibres. The submucosa constituted a substantial layer of loose connective tissue. Tunica serosa is a thin layer composed of areolar loose connective tissue (Fig.1). The jejunum exhibited a mucous membrane characterised by numerous large villi, which displayed a blunt apical region and a broad basal region lacking muscularis mucosa (Fig.1). Similar to the jejunum, we identified simple columnar cells and goblet cells in the ileum, but the muscularis mucosa was absent (Fig.1) Histochemically indicated that columnar cells exhibited a negative reaction to the PAS stain, while goblet cells demonstrated a strong positive reaction (Fig1). The wall showed negatively stained epithelial cells when the combined PAS-AB (pH 2.5) was applied, while the goblet cells had a strong positive reaction, indicated by a dark blue coloration (Fig.1). The AB (pH 2.5) had a strong reaction with goblet cells because of their acidic mucopolysaccharides, but it had a weak reaction with columnar epithelium (Fig.1).

Our work primarily investigated the presence. The RT-PCR amplification plots of the MUC1 and MUC4 genes in the duodenum, jejunum and ileum owl distinctive Ct cycle numbers, ranging between 20 and 25. (Fig.1,3,5). The RT-qPCR analysis of housekeeping (GAPDH) genes and MUC1 and MUC4 genes exhibited high specificity and consistent curve amplifications with distinct melting peaks in owl range between 25-30 (Figure2,4, 6). These melting peaks for the MUC1 of owl were in the ranges of 65-85°C, (Fig.2,) while MUC4 in the owl were, 65-80°C (Figures 4). These melting peaks for the housekeeping gene, MUC1,4 of owl were in the ranges of, 65-80°C, (Figures.6) As presented in Tables 1 and 2. Although the concentration of MUC1 was notably higher ileum in campair with l and jejunum and duodenum while the concentration of the MUC4 was notably higher jejunum in campair with ileum and duodenum this difference was not statistically significant (Tables 1 and 2).

**Table 1: Values of gene expression and housekeeping gene of MUC1 of owl which were analyzed using 2^ΔCT method.**

| S | Sample | CT (Muc1 gene) | CT(GAPDH gene) | ΔCT | Gene expression ratio | Mean |
|---|---|---|---|---|---|---|
| | D1 | 25.51 | 29.39 | 3.88 | 14.75 | |
| | D2 | 26.20 | 29.43 | 3.23 | 9.40 | |
| | D3 | 26.03 | 29.24 | 3.21 | 9.27 | 9.70 |
| | D4 | 25.66 | 29.35 | 3.69 | 12.93 | |
| | D5 | 26.22 | 29.24 | 3.02 | 8.13 | |
| | D6 | 26.50 | 28.38 | 1.88 | 3.69 | |
| **Jejunum** | **Sample** | **CT (Muc1 gene)** | **CT(GAPDH gene)** | **ΔCT** | **Gene expression ratio** | Mean |
| | D1 | 25.46 | 29.56 | 4.10 | 17.18 | |
| | D2 | 24.57 | 28.75 | 4.18 | 18.16 | |
| | D3 | 24.34 | 28.96 | 4.62 | 24.64 | 21.44 |
| | D4 | 24.55 | 28.91 | 4.36 | 20.58 | |
| | D5 | 24.19 | 29.07 | 4.88 | 29.51 | |
| | D6 | 24.86 | 29.07 | 4.21 | 18.55 | |
| **ILEUM** | **Sample** | **CT (Muc1 gene)** | **CT(GAPDH gene)** | **ΔCT** | **Gene expression ratio** | Mean |
| | D1 | 25.09 | 28.63 | 3.54 | 11.62 | |
| | D2 | 24.52 | 29.67 | 5.15 | 35.48 | |
| | D3 | 24.34 | 29.48 | 5.14 | 35.24 | 23.33 |
| | D4 | 24.54 | 29.59 | 5.05 | 33.11 | |
| | D5 | 26.02 | 29.48 | 3.46 | 11.00 | |
| | D6 | 24.86 | 28.62 | 3.76 | 13.54 | |

**Table 2: Values of gene expression and housekeeping gene of MUC4 of owl which were analyzed using 2^ΔCT method.**

| Doudenum | Sample | CT (Muc4 gene) | CT(GAPDH gene) | ΔCT | Gene expression ratio | Mean |
|---|---|---|---|---|---|---|
| | D1 | 26.74 | 29.39 | 2.65 | 6.29 | |
| | D2 | 24.47 | 29.43 | 4.96 | 31.19 | |
| | D3 | 25.82 | 29.24 | 3.42 | 10.73 | 9.98 |
| | D4 | 26.49 | 29.35 | 2.86 | 7.28 | |
| | D5 | 27.33 | 29.24 | 1.91 | 3.77 | |
| | D6 | 29.11 | 28.38 | -0.73 | 0.60 | |
| **Jejunum** | **Sample** | **CT (Muc4 gene)** | **CT(GAPDH gene)** | **ΔCT** | **Gene expression ratio** | Mean |
| | D1 | 25.34 | 29.56 | 4.22 | 18.67 | |
| | D2 | 24.36 | 28.75 | 4.39 | 21.01 | |
| | D3 | 24.86 | 28.96 | 4.10 | 17.18 | 10.34 |
| | D4 | 29.13 | 28.91 | -0.22 | 0.86 | |
| | D5 | 27.33 | 29.07 | 1.74 | 3.35 | |
| | D6 | 29.11 | 29.07 | -0.04 | 0.97 | |
| ILEUM | **Sample** | **CT (Muc4 gene)** | **CT(GAPDH gene)** | **ΔCT** | **Gene expression ratio** | Mean |
| | D1 | 24.88 | 28.63 | 3.75 | 13.45 | |
| | D2 | 25.76 | 29.67 | 3.91 | 15.02 | |
| | D3 | 27.06 | 29.48 | 2.42 | 5.35 | 7.62 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | D4 | 27.11 | 29.59 | 2.48 | 5.58 | |
| | D5 | 28.18 | 29.48 | 1.30 | 2.46 | |
| | D6 | 26.66 | 28.62 | 1.96 | 3.89 | |

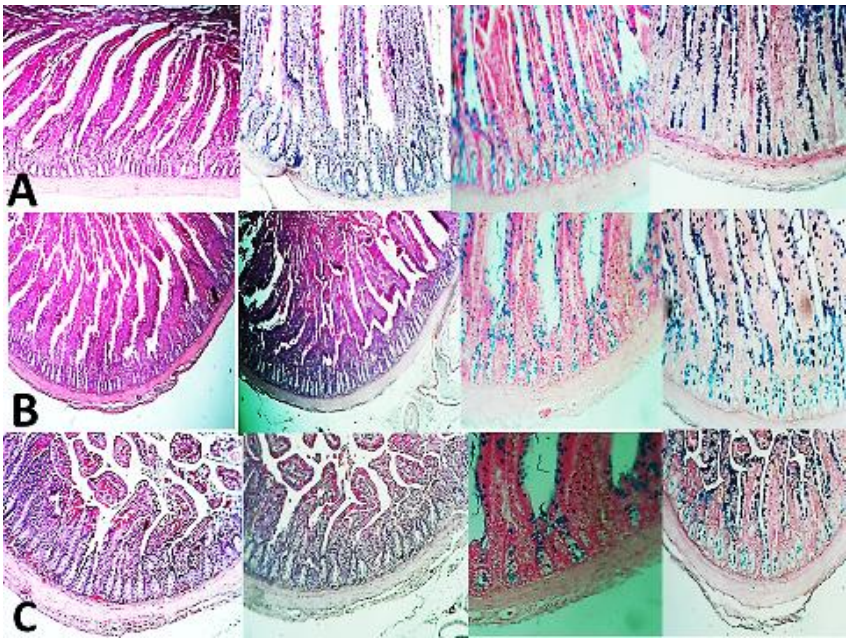


**Figure.1. Histology of owl intestines. Representative histology sections of Duodenum(A), jejunum(B) and ileum(C) of owl stained with H & E, and PAS, Alcian blue (2.5 ph.) combination Alcian blue/ PAS (100x magnificence).**

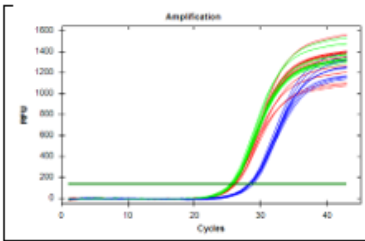


Fig1: qPCR plots of muc1 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

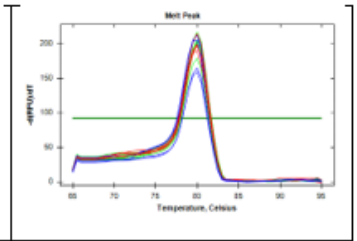


Fig2: qPCR melt peak of of muc1 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

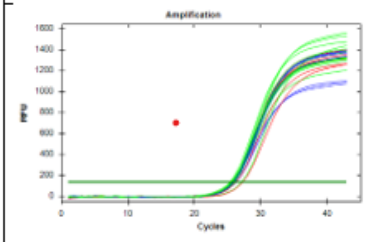


Fig3: qPCR plots of muc4 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

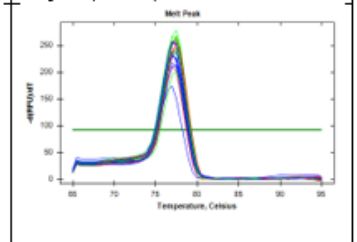


Fig4: qPCR melt peak of of muc4 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

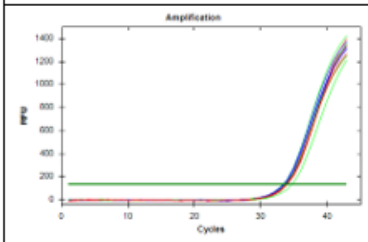


Fig5: qPCR plots of gapgh gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

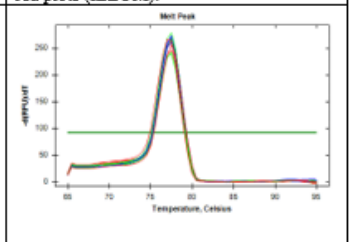


Fig6: qPCR melt peak of of gapdh gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

## Discussion:

Especially important for nutrition absorption and processing, animals' small intestine exhibits obvious structural variation depending on dietary and physiological adaptations. With an eye towards epithelial shape, mucin composition, and the expression patterns of mucin-related genes (MUC1 and MUC4), this work studies the histological and molecular characteristics of the small intestine in owls (a raptor species). Goblet cells mixed with basic columnar epithelial cells generates mucus in the owl's small intestine. Whereas the duodenum is distinguished by a single layer of longitudinal muscle fibres in the muscularis mucosa, the submucosa comprises of loose connective tissue. Large villi defined by a reduced apical segment and a vast basal space devoid of muscularis mucosa identify the jejunum. Though it does not have muscularis mucosa, the ileum has many identical simple columnar cells and goblet cells. Good nutrient absorption is needed since the food's structural characteristics match owls' high-protein eating(8,9). to Periodic Acid-Schiff (PAS) and Alcian Blue (AB). Conversely, columnar epithelial cells responded negatively to PAS, suggesting a deficit in neutral mucins. This propensity helps break down foods heavy in protein, therefore satisfying the demand of protective mucus in carnivorous birds(10,11). Though the intensity was smaller than that of owls, Goblet cells showed a good sensitivity to PAS and AB. The good response of columnar epithelial cells to PAS revealed neutral mucins. Using cycle threshold (Ct) values between 20 and 25, the RT-PCR study revealed that MUC1 and MUC4 genes were expressed all across the small intestine. The ileum obviously had a greater MUC1 concentration than the duodenum and jejunum; MUC4 expression was most obvious in the jejunum. These results imply, considering their position, specific functions of mucins in mucosal protection and absorption of nutrients. The melting values for MUC1 and MUC4 amply demonstrated by their range of 65 to 83°C.

carnivorous avians—such as owls—have longer villi and a higher density of goblet cells, which aids to breakdown diets heavy in protein. Similarly,(12,13,14) found from their less abrasive diets that granivorous birds, particularly quails, have shorter villi and lower mucin synthesis. Furthermore consistent with(15), who showed that the expression of mucin genes varies based on the functional needs of different intestinal sites, our data exposes unique expression of MUC1 and MUC4.

This study emphasises the morphological and functional alterations of the small intestine in owls considering their different physiological and dietary needs. The molecular investigation revealed region-specific expression patterns of the MUC1 and MUC4 genes while the histological and histochemical tests uncovered variations in epithelial architecture and mucin composition. These results provide a basis for further comparative studies and help to clarify the evolutionary adaptations of the avian digestive system. . Further research could investigate at how these changes affect their general digestive efficiency and health. Knowing these adaptations will enable researchers to grasp how different bird species have evolved to fit their distinct environments. Furthermore, depending on this knowledge could be understanding the digestive systems of other avian species and maybe guiding

## Conclusion:

The findings of this study provide substantial insights into the structure, expression, network interactions, and binding locations of MUCs in owls, enhancing the understanding of MUC-related mechanisms in gastrointestinal physiology and their possible association with gastrointestinal disorders.

## Authors' Contributions

The final manuscript has been read, reviewed, and approved by all authors. HA designed the study, drafted and revised the manuscript, AMMA conducted the literature review (Veterinary World, EISSN: 2231-0916 1236, , interpreted the data, and drafted the manuscript; ABK carried out the laboratory work and provided an explanation of the gene expression data in the

manuscript; AHSK served as the project advisor, interpreted the data, and drafted and reviewed the manuscript.

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**Competing Interests**

The writers affirm that none of their interests conflict**.**

**REFRENCES**

1. Birchenough, G. M., Johansson, M. E., Gustafsson, J. K., Bergström, J. H., & Hansson, G. C. (2015). New developments in goblet cell mucus secretion and function. Mucosal Immunology, 8(4), 712-719. https://doi.org/10.1038/mi.2015.32
2. Brown, T., et al. (2017). Dietary adaptations in the digestive system of raptors. Journal of Comparative Physiology B, 187(4), 567-579.
3. Gendler, S. J., & Spicer, A. P. (1995). Epithelial mucin genes. Annual Review of Physiology, 57(1), 607-634. https://doi.org/10.1146/annurev.ph.57.030195.003135
4. Johnson, R., et al. (2020). Mucin composition in the gastrointestinal tract of raptors. Avian Pathology, 49(2), 123-134.
5. Lee, H., et al. (2019). Expression of mucin genes in the small intestine of birds. Poultry Science, 98(5), 2345-2353.
6. Linden, S. K., Sutton, P., Karlsson, N. G., Korolik, V., & McGuckin, M. A. (2008). Mucins in the mucosal barrier to infection. Mucosal Immunology, 1(3), 183-197. https://doi.org/10.1038/mi.2008.5
7. Smith, J., et al. (2018). Comparative histology of the small intestine in birds. Journal of Avian Biology, 49(3), 345-356.
8. Van Klinken, B. J., Dekker, J., Büller, H. A., & Einerhand, A. W. (1995). Mucin gene structure and expression: protection vs. adhesion. American Journal of Physiology-Gastrointestinal and Liver Physiology, 269(5), G613-G627. https://doi.org/10.1152/ajpgi.1995.269.5.G613
9. Garcia, A., et al. (2018). Mucin dynamics in the avian intestine. Journal of Avian Biology, 49(3), 123-134.
10. Johansson, M. E., Thomsson, K. A., & Hansson, G. C. (2009). Proteomic analyses of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the Fcgbp protein. Journal of Proteome Research, 8(7), 3549–3557. https://doi.org/10.1021/pr9002504
11. Snyder, J. D., & Walker, A. (1987). Structure and function of intestinal mucin: developmental aspects. International Archives of Allergy and Immunology, 82(3-4), 351–356. https://doi.org/10.1159/000234225
12. Thornton, D. J., & Sheehan, J. K. (2004). From mucins to mucus: toward a more coherent understanding of this essential barrier. Proceedings of the American Thoracic Society, 1(1), 54–.
13. Dharmani, P., et al. (2009). Role of intestinal mucins in innate host defense mechanisms against pathogens. Journal of Innate Immunity, 1(2), 123–135. https://doi.org/10.1159/000163037

14. Almhanna, H., Al-Mahmodi, A. M. M., & Kadhim, A. B. (2024). Network and structural analysis of quail mucins with expression pattern of mucin 1 and mucin 4 in the intestines of the Iraqi common quail (Coturnix coturnix). Veterinary World, 17(6), 1227. https://doi.org/10.14202/vetworld.2024.1227-1237

15. Kim, Y. S., & Ho, S. B. (2010). Intestinal goblet cells and mucins in health and disease: recent insights and progress. Current Gastroenterology Reports, 12, 319–330. https://doi.org/10.1007/s11894-010-0131-2