International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3, 2025
https://www.theaspd.com/ijes.php

MUC1 and MUC4 gene expression and histochemical of small intestine of Quail (Coturnix coturnix) and owl *(Tyto alba)* (Comparative study)

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Abstract
The point of this study is to look at how environmental and dietary factors affect the small intestines of owls and quails 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 and quails. The small intestine of the owl was characterised by a layer of simple, columnar epithelium interspersed with goblet cells. The duodenum organises 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. When quail ate, their small intestine had a simple columnar epithelium with microvilli that made a brush-like border and increased the surface area that could be absorbed. Goblet cells scattered throughout the epithelial cells are responsible for the release of mucus. The muscularis mucosa was different, with a thin layer of smooth muscle from the submucosa. The RT-PCR amplification plots of the MUC1 and MUC4 genes in the quail and owl's duodenum, jejunum, and ileum showed different Ct cycle numbers, which were between 20 and 25. The melting peaks for the MUC1 of quail and owl were in the ranges of 65-80°C and 65-85°C, respectively, while the melting peaks for the housekeeping gene, MUC1,4, of quail and owl were in the ranges of 65-90°C and 65-80°C, respectively. The results of this study give us a lot of information about the structure, expression, of MUCs in quails and 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, Quail, Owl, small intestine, gene expression

INTRODUCTION

Comparative studies of histological and molecular traits among several 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 colon (Birchenough et al .,2015; Brown et al.,2017; Gendler and Spicer,1995). Moreover, improving digestion and absorption are mucin proteins. The histochemical and gene expression profiles of MUC1 and MUC4 proteins present in the small intestines of owls (*Tyto alba*) and quails (Coturnix coturnix) are compared in this work. Unlike quail, which are terrestrial birds that eat a great range of foods, 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(Johnson *et al*., 2020 ; Lee *et al*.,2019 ; Linden *et al*.,2008 ; Smith *et al*., 2018). 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. Comparative 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 20 healthy adult slaughtered owl and quail 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 mucine in goblet cells in small intestine of owl and quail 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

Quail and 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 coturnix: 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 (Almhanna *et al.*,2024), 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 organises 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).
The small intestine of the quail is distinguished by a simple columnar epithelium that contains microvilli. This epithelium serves to create a brush border, which increases the surface area available for absorption. There are goblet cells scattered throughout the epithelial cells. These cells are responsible for the release of mucus. In addition to having villi that are shorter, the duodenum is responsible for organizing the muscularis mucosa into a single arrangement of longitudinal fiber elements. The submucosa is made up of dense connective tissue that is irregular in shape and contains bigger blood arteries, lymphatics, and nerves. A. muscularis. Deepest, most circular layer The diameter of the duodenal lumen is decreased as a result of contraction. The duodenum becomes shorter as a consequence of the constriction of the outer longitudinal layer which occurs during the process. There is a thin layer known as the tunica serosa, which is composed of areolar loose connective tissue (Fig. 2). When compared to the duodenum, which had a large basal region with muscular mucosa, the jejunum displayed a mucous membrane with short villi. Goblet cells were present, but their number was lower than that of the duodenum (Fig. 2). At the same time that we detected simple columnar cells and goblet cells in the ileum, which are similar to those seen in the jejunum, we also found that the muscularis mucosa was different. From the submucosa, the mucosa can be distinguished by a thin layer of smooth muscle, as seen in Figure (2).Histochemically indicated that columnar cells exhibited a positive reaction to the PAS stain, while goblet cells demonstrated amid positive reaction(Fig2). The wall showed negatively stained epithelial cells when the combined PAS-AB (pH 2.5) was applied, while the goblet cells had light positive reaction, indicated by a light blue coloration (Fig.2). The AB (pH 2.5) had a strong reaction with goblet cells, but it had a weak reaction with columnar epithelium(Fig.2).
Our work primarily investigated the presence. The RT-PCR amplification plots of the MUC1 and MUC4 genes in the duodenum, jejunum and ileum of quail and owl distinctive Ct cycle numbers, ranging between 20 and 25. (Fig.3,4,5 and 6). 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 while in the quail 20-25 (Figure 7,8). These melting peaks for the MUC1 of quail and owl were in the ranges of 65-80°C, 65-85°C, respectively(Fig.9,10) while MUC4 in the quail and owl were 65-85°C, 65-80°C (Figures 11,12). These melting peaks for the housekeeping gene, MUC1,4 of quail and owl were in the ranges of 65-90°C, 65-80°C, respectively(Figures.13,14) 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). Notably, concentration of the MUC1 and MUC4 of quail exhibited elevated expression levels jejunum, ileum and duodenum (Tables 3 and 4).

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

Doudenum	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

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3, 2025
https://www.theaspd.com/ijes.php

	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	

Table 3: Values of gene expression and housekeeping gene of MUC1 of Quail which were analyzed using 2^ΔCT method.

Doudenum	**Sample**	**CT (Muc4 gene)**	**CT(GAPDH gene)**	**ΔCT**	**Gene expression ratio**	**Mean**
	D1	28.04	28.47	0.43	1.35	
	D2	27.47	29.51	2.04	4.12	
	D3	25.89	28.32	2.43	5.41	2.81
	D4	26.53	28.43	1.90	3.72	
	D5	27.33	28.32	0.99	1.99	

	D6	29.22	27.46	-1.76	0.30	
Jejunum	**Sample**	**CT (Muc4 gene)**	**CT(GAPDH gene)**	**ΔCT**	**Gene expression ratio**	Mean
	D1	26.68	28.64	1.96	3.90	
	D2	25.41	27.83	2.42	5.36	
	D3	24.91	28.04	3.13	8.74	5.04
	D4	25.23	27.99	2.76	6.79	
	D5	26.33	28.15	1.82	3.54	
	D6	27.22	28.15	0.93	1.91	
ILEUM	**Sample**	**CT (Muc4 gene)**	**CT(GAPDH gene)**	**ΔCT**	**Gene expression ratio**	Mean
	D1	24.99	27.71	2.72	6.59	
	D2	25.84	28.75	2.91	7.49	
	D3	27.06	28.56	1.50	2.82	3.83
	D4	27.12	28.67	1.54	2.91	
	D5	28.28	28.56	0.27	1.21	
	D6	26.72	27.70	0.98	1.97	

Table 4: Values of gene expression and housekeeping gene of MUC4 of Quail which were analyzed using 2^ΔCT method.

Doudenum	**Sample**	**CT (Muc4 gene)**	**CT(GAPDH gene)**	**ΔCT**	**Gene expression ratio**	**Mean**
	D1	28.04	28.47	0.43	1.35	
	D2	27.47	29.51	2.04	4.12	
	D3	25.89	28.32	2.43	5.41	2.81
	D4	26.53	28.43	1.90	3.72	
	D5	27.33	28.32	0.99	1.99	
	D6	29.22	27.46	-1.76	0.30	
Jejunum	**Sample**	**CT (Muc4 gene)**	**CT(GAPDH gene)**	**ΔCT**	**Gene expression ratio**	Mean
	D1	26.68	28.64	1.96	3.90	
	D2	25.41	27.83	2.42	5.36	
	D3	24.91	28.04	3.13	8.74	5.04
	D4	25.23	27.99	2.76	6.79	
	D5	26.33	28.15	1.82	3.54	
	D6	27.22	28.15	0.93	1.91	
ILEUM	**Sample**	**CT (Muc4 gene)**	**CT(GAPDH gene)**	**ΔCT**	**Gene expression ratio**	Mean
	D1	24.99	27.71	2.72	6.59	
	D2	25.84	28.75	2.91	7.49	
	D3	27.06	28.56	1.50	2.82	3.83
	D4	27.12	28.67	1.54	2.91	
	D5	28.28	28.56	0.27	1.21	
	D6	26.72	27.70	0.98	1.97	

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

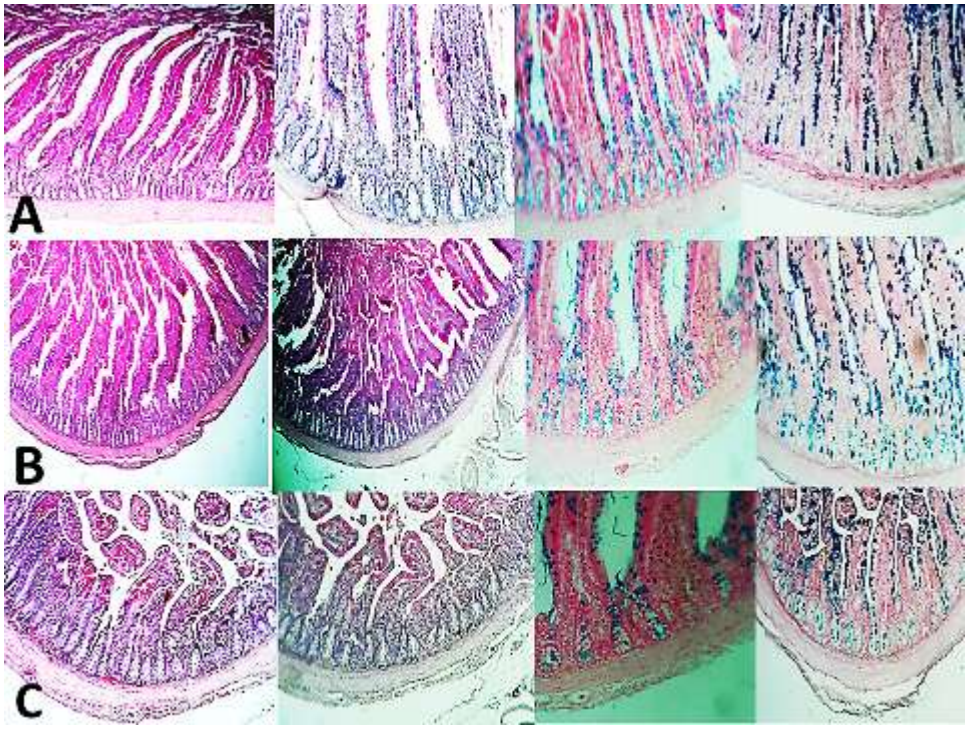

Figure.2. Histology of owl intestines. Representative histology sections of Duodenum(A), jejunum(B) and ileum(C) of owl stained with H & E, and PAS, Alcian blue (2.5 ph.) combination Alcian blue/ PAS (100x magnificence).

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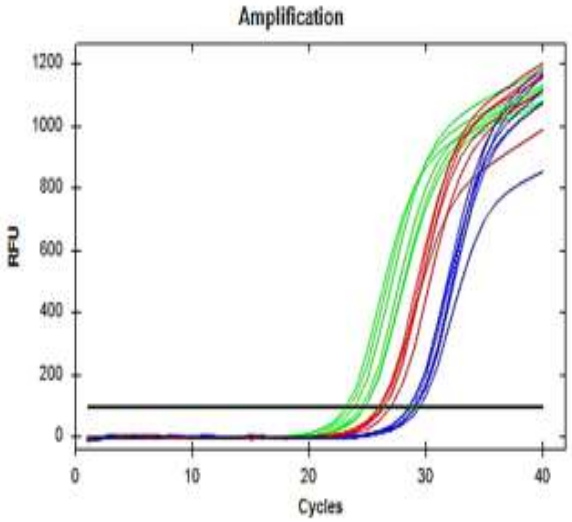

Fig3: qPCR plots of muc1 gene in quail tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (Ileum).

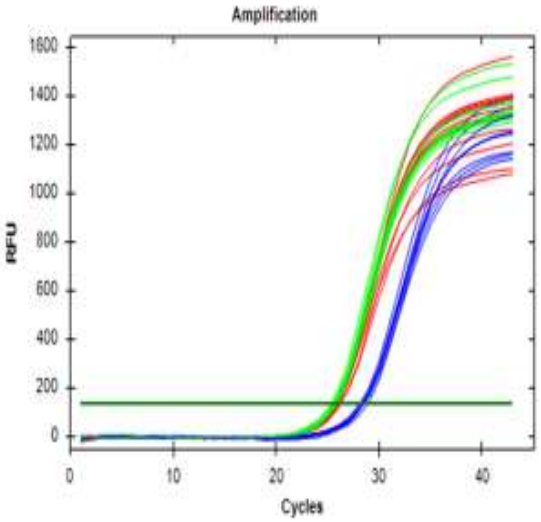

Fig4: qPCR plots of muc1 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (Ileum).

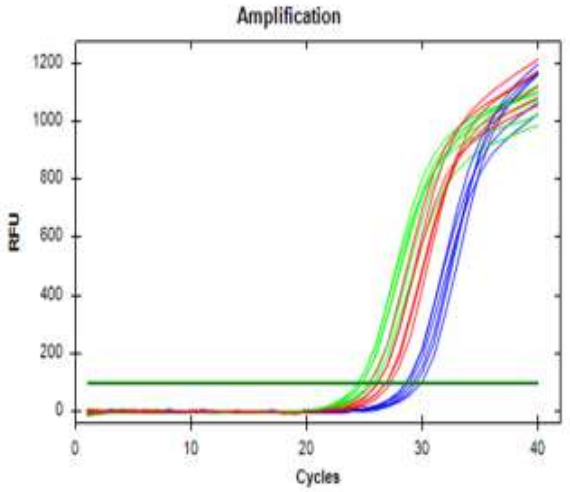

Fig5: qPCR plots of muc4 gene in Quail tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM

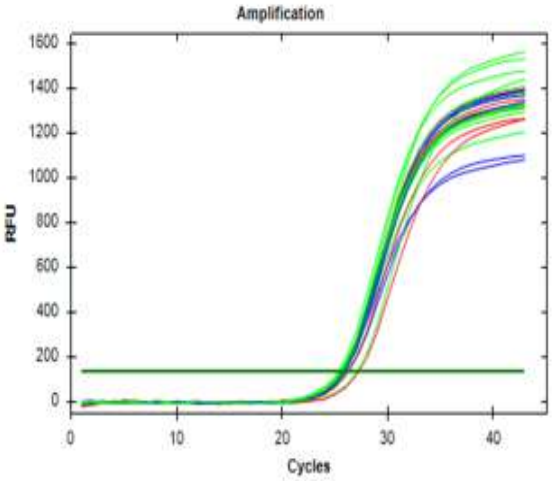

Fig6: qPCR plots of muc4 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM

International Journal of Environmental Sciences
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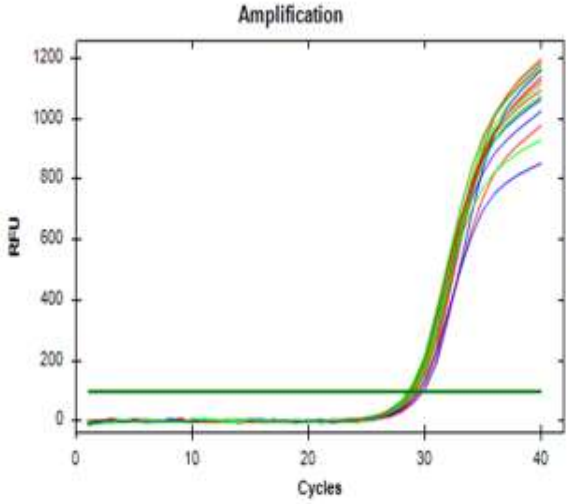

Fig7: qPCR plots of gapgh gene in quail tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

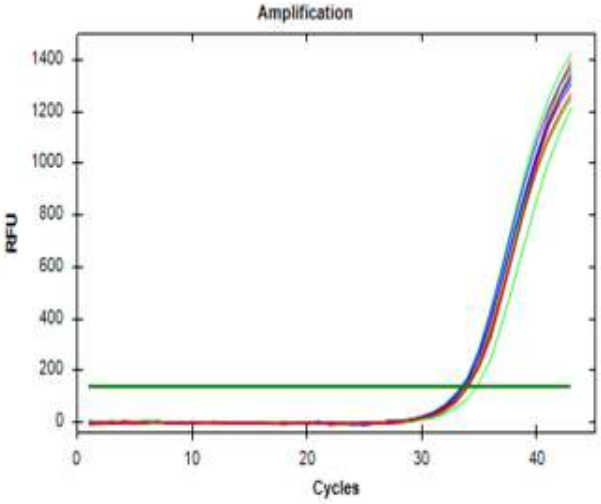

Fig8: qPCR plots of gapgh gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

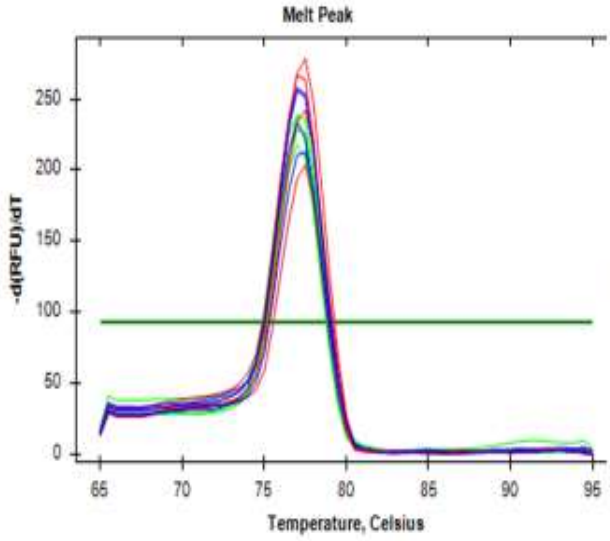

Fig9: qPCR melt peak of of muc1 gene in quailtissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

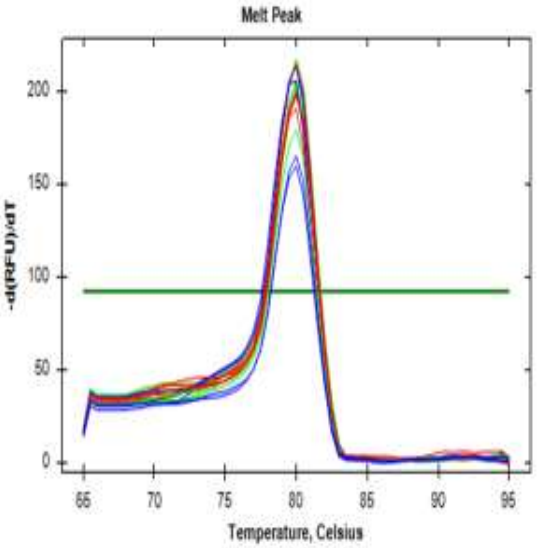

Fig10: qPCR melt peak of of muc1 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

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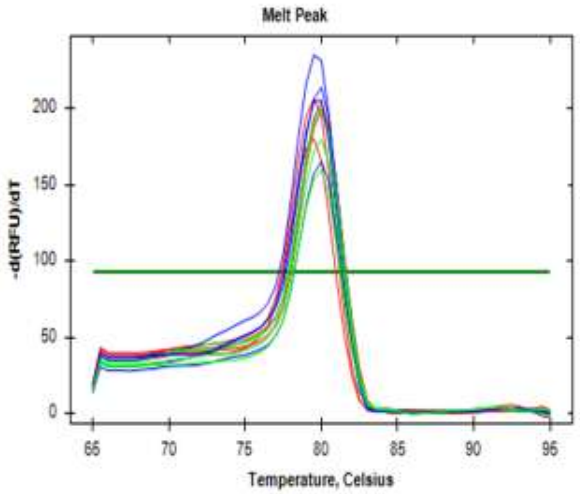

Fig11: qPCR melt peak of of muc4 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM)

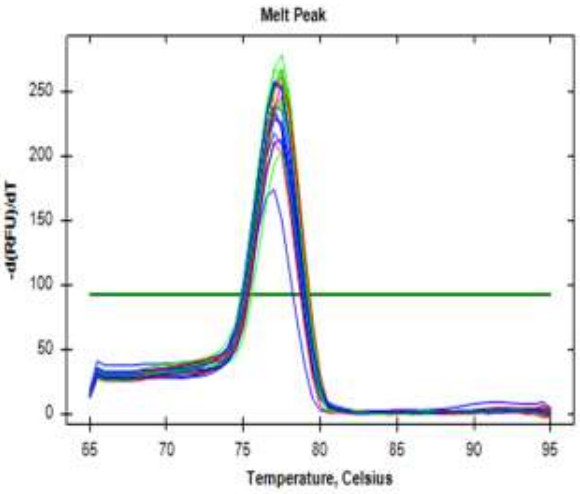

Fig12: qPCR melt peak of of muc4 gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM)

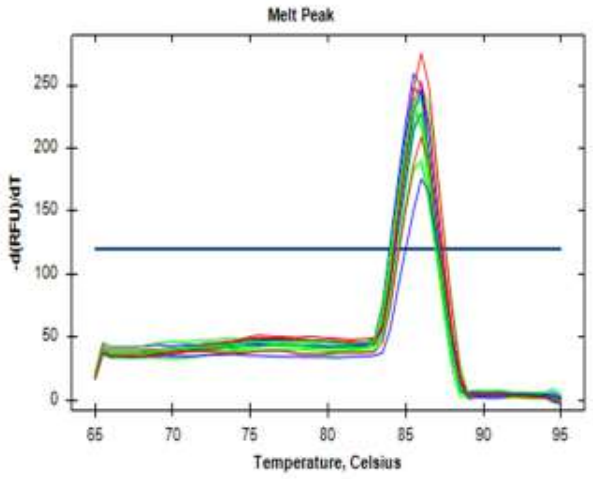

Fig13: qPCR melt peak of of gapdh gene in quail tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

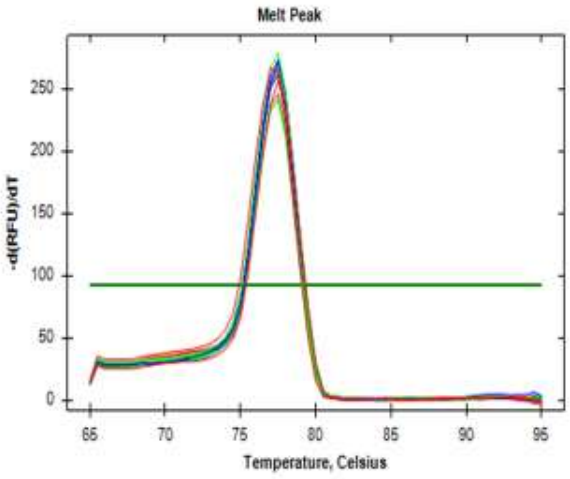

Fig14: qPCR melt peak of of gapdh gene in owl tissue sample. The blue plots (Doudenum), the green plots (Jejunum) and red plots (ILEUM).

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) and quails (a granivorous bird). 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(Van Klinken et al .,1995 ; Garcia *et al.*, 2018). Quails have simple columnar epithelial cells in their small intestines, shorter than those of owls. Whereas the submucosa comprises thick irregular connective tissue with more blood vessels and neurones, the muscularis mucosa is a single layer of longitudinal fibres arranged by the duodenum. The ileum has a thin smooth muscle barrier separating the mucosa from the submucosa, whereas the jejunum has less goblet cells and shorter villi

than the duodenum. These features complement quails' diet, which calls for less absorption surface than that of carnivores(Kim et al .,2020 ; Smith et al .,2015 ; Osinaga, 2007). Goblet cells in owls at pH 2.5 revealed acidic mucopolysaccharides by histochemical labelling, thereby implying strong positive reactivity 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(Johansson and Hansson,2009;Snyder and Walker,1987). Though the intensity was smaller than that of owls, Goblet cells and quails also showed a good sensitivity to PAS and AB. The good response of columnar epithelial cells to PAS revealed neutral mucins. Reduced need for protective mucus in granivorous birds could suggest differences in mucin composition, compared to carnivorous species(Petrou and Crouzier,2018). 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. In quails, Ct values for MUC1 and MUC4 in the jejunum, ileum, and duodenum ranged from 20 to 30 suggesting strong expression levels. Like those of owls, the melting points for both genes fall between 65 and 83°C. Because of variations in dietary content and gut flora, quails exhibit greater general MUC1 and MUC4 expression levels than owls apparently (Smith et al., 2018). The histology and molecular results of this work enhance earlier studies on avian digestive systems. According to(Baos *et al.*,2012 ; Lillehoj et al.,2013).carnivorous avians—such as owls—have longer villi and a higher density of goblet cells, which aids to breakdown diets heavy in protein. Similarly,(Thornton and Sheehan,2004 ; Dharmani *et al.*,2009 ; Almhanna *et al.*,2024) found from their less abrasive diets that granivorous birds, particularly quails, have shorter villi and lower mucin synthesis. Furthermore consistent with(Kim *et al* .,2010), 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 and quails 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. The findings show that owls and quails have evolved original methods to meet their dietary needs. 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 quails and 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.

Acknowledgments

We would like to sincerely thank the whole University of Al-Qadisiyah Department of Anatomy and

Histology for their steadfast support throughout our research project. For this work, the authors did not receive any funding.

Competing Interests

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

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