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Article

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Computational identification of conserved microRNA candidates in turkey (*Meleagris gallopavo*) through homology-based mining of transcriptomic data

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Short title:

In silico identification of turkey microRNAs

Abstract

MicroRNAs (miRNAs) are crucial post-transcriptional regulators, yet remain poorly annotated in the turkey (*Meleagris gallopavo*). This study aimed to comprehensively identify conserved turkey miRNAs to elucidate their regulatory architecture. We employed a homology-based *in silico* approach, aligning 1,723 mature miRNAs from four avian reference species against 586 turkey RNA-sequencing libraries (17 tissues, two sexes, five developmental stages; BioProject PRJNA259229). Following stringent BLAST filtering and hairpin structural validation, we identified 244 putative miRNA candidates, predominantly 22 nucleotides in length. Expression analysis revealed a striking inverse relationship between uniqueness and abundance: while tissue-specific miRNAs comprised 41.4% of unique sequences, general miRNAs accounted for 92.7% of total abundance, ensuring broad network stability. Conversely, low-abundance tissue- and sex-specific miRNAs likely function as precise post-transcriptional micro-switches. Phylogenetic analysis confirmed strong evolutionary conservation, and target prediction highlighted highly interconnected regulatory networks. This first comprehensive *in silico* characterization of the

turkey miRNome establishes a foundational resource for developing miRNA-based biomarkers to improve health, welfare, and production traits in this species.

Keywords: microRNA, *Meleagris gallopavo*, homology-based prediction, Omnigenic model, tissue-specific expression

Introduction

MicroRNAs (miRNAs) are small single-stranded non-coding RNA sequences of ~22 nt that have an important role in post-transcriptional regulation of mRNA expression in a broad range of biological processes (Eulalio et al. 2008, Bartel 2004, Leitão and Enguita 2022). The first miRNA (lin-4) was discovered in 1993 during the genetic screening of *Caenorhabditis elegans* with antisense complementarity to the lin-14 gene (Lee RC et al. 1993, Ambros 2008). After a while, the phylogenetic distribution of miRNAs was illustrated in several studies showing conserved miRNAs among different organisms (Lagos-Quintana et al. 2001, Lau et al. 2001).

The technologies of transcriptomic sequencing are increasingly applicable to the analysis of multiple aspects of gene expression (Lowe et al. 2017, Wang et al. 2024, Du et al. 2023, Dong and Chen 2013, Guo et al. 2021). Additionally, integrating the expression of mRNAs and miRNAs and *in silico* computational methods to detect miRNA-target gene pairs (mTPs) is an important approach to deriving indispensable biological information and surmounting technology limitations (Du et al. 2023). Therefore, *in silico* studies can provide useful information and create new insights in certain circumstances, and are essential for generating efficient predictive models (Bofill-De Ros and Vang Ørom 2024, O'Brien et al. 2018, Esfahani et al. 2025).

miRNAs can be found in different tissues, blood, and other body fluids (urine, tears, amniotic fluid, milk) in stable forms and the form of binding to RNA-induced silencing complex (RISC) (Cortez et al. 2011, Gayosso-Gómez and Ortiz-Quintero 2021, Miretti et al. 2020).

miRNAs prevent translation by binding to complementary sequences on mRNA. Depending on whether the binding to the complementary sequence is complete or incomplete, the mRNA is degraded or silenced, respectively (Eulalio et al. 2008, Bartel 2009, Kozomara et al. 2019, Dexheimer and Cochella 2020). In each cell at a certain time, several miRNAs and several genes are active in different biological processes, and each miRNA interacts with multiple genes and miRNAs under a complex interconnected network (Liu et al. 2009, Bahrami et al. 2017, Su et al. 2018, Chang et al. 2020, Hicks and Liu 2021). The expression pattern of miRNAs has a decisive role in determining the cells' function, homeostasis, health, and immunity (Li et al. 2016, Bahrami et al. 2017).

The standard approach to detect mTPs is to analyze the expression of mRNA and miRNA to find differentially expressed genes (DEGs) or miRNAs (DEMs), and then the sequence-based prediction of mTPs through DEGs–DEMs pairs (Huang et al. 2015, Bahrami et al. 2017, Li et al. 2017, Hao et al. 2021, Li et al. 2022, Li et al. 2023, Yu et al. 2023, Mao et al. 2019).

Global multiple sequence alignments of the chicken, turkey, and zebra finch at the genome level reported the alignment at 91.92 and 92.39% in chicken and turkey, respectively. Although this level of genomic similarity does not transmit to the protein level, there is still a high similarity degree in the protein level (Arsenault et al. 2014). Additionally, another comparative analysis revealed a lower rate of nucleotide similarity on micro chromosomes than macro chromosomes in chicken and turkey (Axelsson et al. 2005). Altogether comparative studies suggest that avian genomes have remained relatively more conserved during evolution and have less rate of rearrangement

compared to equivalent mammalian (Griffin et al. 2008, Aslam et al. 2010, Barros et al. 2023). On the other hand, avian miRNAs are crucial posttranscriptional regulators of different biological processes, such as immune responses and the pathogenesis of immunosuppressive diseases, which impact birds' susceptibility and resistance to diseases (Duan et al. 2020, Samir et al. 2016, Yang et al. 2020). Turkey (*Meleagris gallopavo*) is an important domestic species, the second economic bird in the world's poultry meat production (Islam et al. 2023), that has a high genomic similarity with other avian. The lack of miRNA information in Turkey prompted us to predict potential miRNAs through the *in silico* approach using avian miRNAs based on the publicly available Turkey's transcriptomic data in several tissues. Therefore, this research has gained insights into turkey miRNAs and their target genes in different tissues, ages, and sexes.

Results

Potential Turkey miRNAs

To define miRNA selection criteria, the aforementioned features were calculated for 1,381 annotated avian precursor miRNAs from miRBase. Due to skewed frequency distributions, outliers were excluded, and lower and upper threshold values were determined for each feature (Table 1). Sequences falling within the defined thresholds for all features were selected as potential turkey miRNAs.

Table 1. Statistics of various microRNAs-related factors in birds recorded in MiRBase.

Factor	1_{th} Quartile	3_{th} Quartile	IQR (Q_3-Q_1)	Lower limit	Upper limit
Length	73.00	99.00	26.00	34.00	144.50
AMFE	-47.46	-35.28	12.19	-71.83	-17.00
MFEI	0.70	0.93	0.23	0.35	1.43
GC%	43.96	58.06	14.11	22.79	80.64

Interquartile Range (IQR), AMFE (Adjusted Minimum Free Energy), MFEI (Minimum Free Energy Index)

Length Distribution of Identified miRNAs

Using stringent prediction criteria, 244 unique putative mature miRNA sequences were identified. When all replicate alignments were considered, these sequences generated 441,692 alignments, corresponding to a total normalized abundance of 29,557 reads per million (RPM). Across the examined turkey tissues, the identified mature miRNAs ranged from 20 to 27 nucleotides (nt) in length. When the distribution was weighted by total abundance, the 22-nt class was predominant, accounting for 62% of total miRNA abundance. The 21-nt and 23-nt classes were the second and third most abundant, representing 21.3% and 15.3%, respectively. The remaining length classes, comprising 20, 24, 25, and 27 nt, collectively accounted for only 1.4% of total abundance, and no 26-nt miRNAs were detected (Fig. 1).

To determine whether this pattern was influenced by highly abundant sequences, the length distribution of unique miRNAs was also examined by counting each miRNA once, regardless of abundance. The 22-nt class remained the most frequent, comprising 53.7% of unique miRNAs. The 23-, 21-, and 24-nt classes were the next most common, whereas 20-, 25-, and 27-nt miRNAs were rare; no 26-nt miRNAs were detected. However, the length distribution of unique miRNAs differed from that of total miRNA abundance (Fig. 1). Specifically, 22-nt miRNAs represented 53.7% of unique miRNAs but 62% of total abundance, while 21-nt miRNAs represented only 13.1% of unique miRNAs but 21.3% of total abundance. In contrast, miRNAs of 20 nt and 23–27 nt showed lower proportions in the abundance-based distribution than in the unique-miRNA distribution. These results indicate that 21- and 22-nt miRNAs contribute disproportionately to overall miRNA abundance, suggesting relatively higher expression levels, whereas 20-nt and 23–27-nt miRNAs are comparatively less abundant.

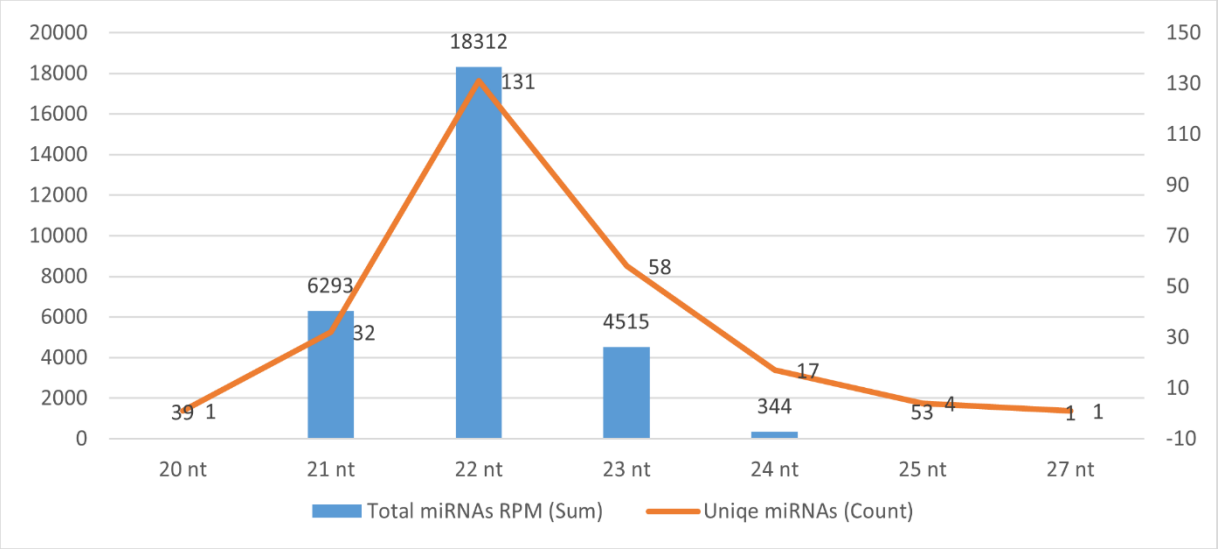


Fig 1. Length-frequency distribution of detected microRNAs in turkey across all studied tissues. The distribution is shown for total miRNA abundance, expressed as reads per million (RPM) and for the number of unique mature miRNAs identified.

Validation of Predicted microRNAs Based on Pre-miRNA Structural Regions

A pre-miRNA adopts a characteristic stem-loop structure comprising three main regions: the 5p arm, the 3p arm, and the terminal loop connecting them. During the maturation process, the loop region is cleaved and degraded by Dicer, yielding only the mature miRNA duplex from the 5p and 3p arms. Consequently, in an RNA-Seq dataset enriched for mature miRNAs, reads originating from the loop region are expected to be significantly less abundant than those mapping to the 5p and 3p arms. This principle can serve as an internal validation criterion for miRNA identification.

In the initial, unfiltered alignment analysis, a total of 46,133,381 alignments were obtained. Of these, 10,997,124 alignments (23.8%) mapped to the Midseq sequences, likely representing pre-miRNA fragments, degradation products, or low-stringency short matches, while the remaining alignments corresponded to the 5p and 3p arms. However, after applying a stringent E-value filter (retaining only alignments with E-value ≤ 0.001), the number of Midseq alignments dropped to

zero, whereas the 5p and 3p arm alignments remained robust. This result confirms that the loop region is indeed eliminated during miRNA maturation and that the predicted mature miRNAs originate exclusively from the stem arms, thereby validating the accuracy of the identification pipeline (Fig. 2)

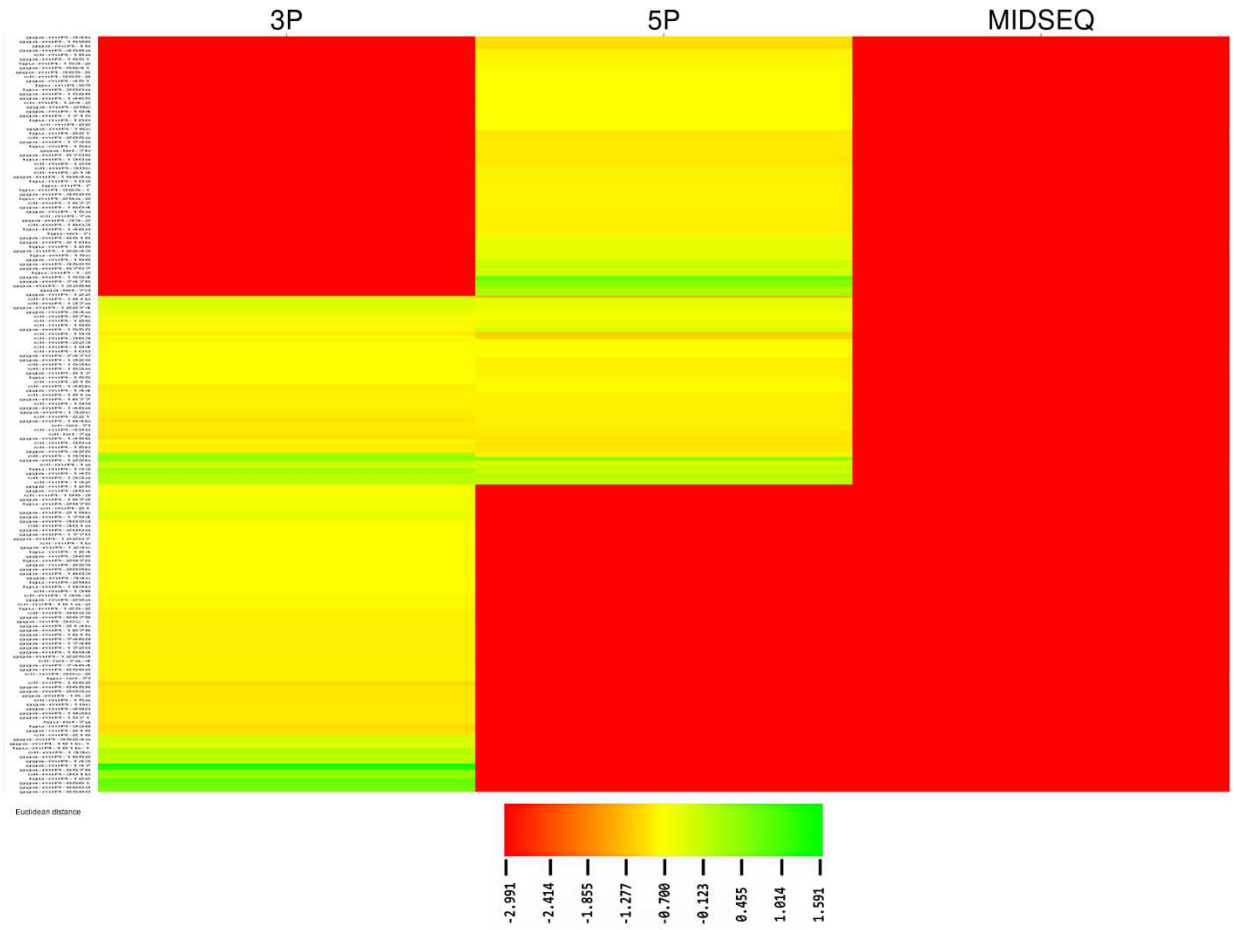


Fig 2. Heat map of the 5′ and 3′ mature miRNA arms (miRNA-5p and miRNA-3p) and the pre-miRNA loop sequence (Midseq). The heat map was generated using the CIMMiner heat-map tool of the NCI Discover platform (https://discover.nci.nih.gov/).

In more detail, of the 244 predicted unique miRNAs, 123 were derived from the 3′ arm (3p) and 121 from the 5′ arm (5p) (Fig. 3a). Consistent with their nearly equal numbers, the total abundances of the two groups were also similar, at 15,063 and 14,494 RPM, respectively. Sex-specific analysis likewise revealed only slight differences between 3′ and 5′ miRNAs: in females, the corresponding

values were 8,093 and 7,437 RPM, and in males, 6,970 and 7,057 RPM (Fig. 3b). Thus, 3′ and 5′ miRNAs were quantitatively comparable both overall and within each sex, with 3′ miRNAs marginally more abundant in females and 5′ miRNAs marginally more abundant in males. Nevertheless, similar overall abundances do not exclude the possibility that the two groups are distributed differently among tissues. To explore this, redundant miRNA records across experimental replicates (sexes, days, and repetitions) were collapsed, retaining a single occurrence of each miRNA per tissue and yielding 1,808 miRNA–tissue occurrences.

The per-tissue analysis of unique miRNAs showed that, with the exception of the gizzard and bursa, the number of unique 3′ miRNAs slightly exceeded that of unique 5′ miRNAs in most tissues (Fig. 3c). In contrast, the total abundances of the two groups varied considerably among tissues (Fig. 3d). Specifically, 3′ miRNA abundance exceeded that of 5′ miRNAs in all three segments of the small intestine (duodenum, jejunum, and ileum), the cecal tonsil, and the pancreas, whereas no marked difference was observed in the proventriculus and gizzard. In the remaining tissues, namely the testes, liver, ovaries, heart, bursa, spleen, thymus, brain, thigh, and breast, 5′ miRNA abundance exceeded that of 3′ miRNAs (Table 2).

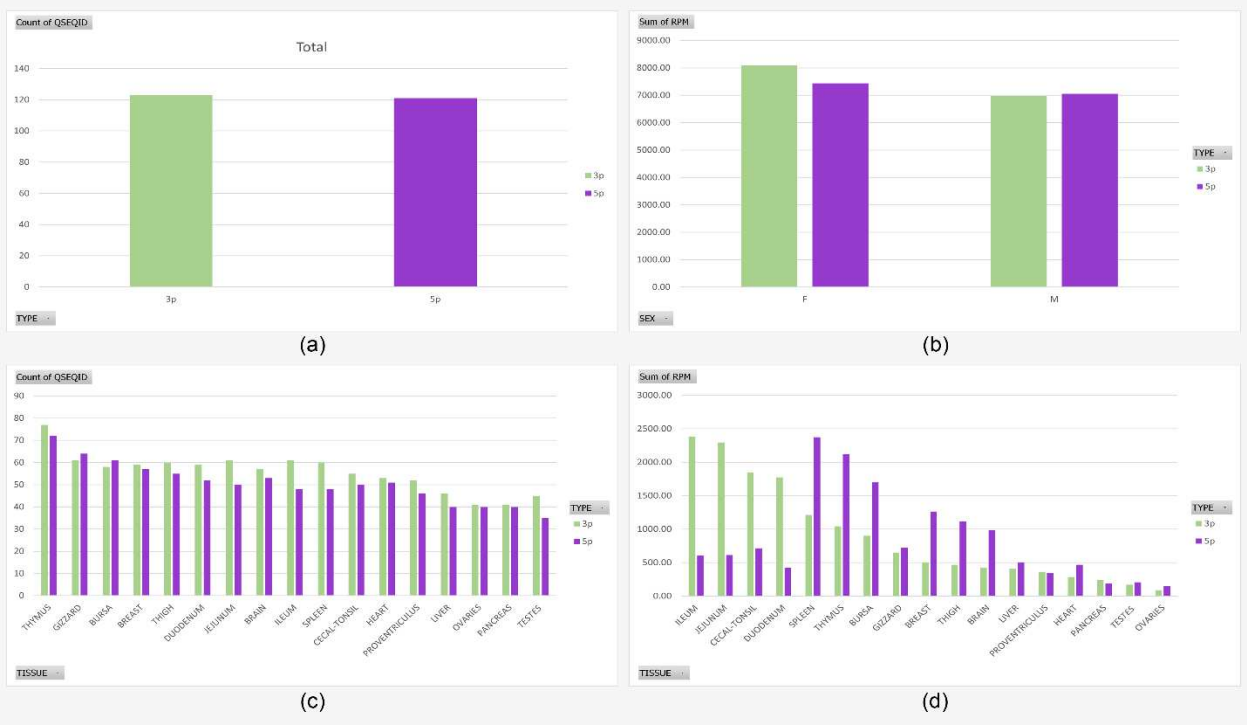


Fig 3. Number of unique miRNAs and total miRNA abundance (RPM) for 3p- and 5p-arm-derived miRNAs by sex and tissue. (a) Total number of unique mature miRNAs derived from the 3′ (3p) and 5′ (5p) arms of pre-miRNAs identified across all samples. (b) Total abundance (sum of RPM) of 3p and 5p miRNAs in female (F) and male (M) turkeys. (c) Number of unique 3p and 5p miRNAs detected in each tissue. (d) Total abundance (RPM) of 3p and 5p miRNAs in each tissue.

Table 2. The total number of 3′ and 5′ miRNAs in different tissues.

miRNA/Tissue	3′		5′		Total	3′/5′	5′/3′
	Total RPM	%	Total RPM	%			
DUODENUM	1773.2	6	422.6	1.43	2195.83	4.2	0.2
ILEUM	2384.4	8.07	609.6	2.06	2994.01	3.9	0.3
JEJUNUM	2295.2	7.77	614.7	2.08	2909.92	3.7	0.3
CECAL-TONSIL	1844.1	6.24	713.7	2.41	2557.87	2.6	0.4
PANCREAS	240.9	0.81	185	0.63	425.86	1.3	0.8
PROVENTRICULUS	363.8	1.23	342.8	1.16	706.61	1.1	0.9
GIZZARD	651.6	2.2	723.8	2.45	1375.34	0.9	1.1
TESTES	169.4	0.57	207.6	0.7	377.03	0.8	1.2
LIVER	406.3	1.37	503.4	1.7	909.67	0.8	1.2
OVARIES	89.7	0.3	146.1	0.49	235.78	0.6	1.6
HEART	284.7	0.96	466	1.58	750.71	0.6	1.6
BURSA	902.2	3.05	1699.8	5.75	2601.99	0.5	1.9
SPLEEN	1213.2	4.1	2374.2	8.03	3587.38	0.5	2
THYMUS	1042.6	3.53	2119.5	7.17	3162.07	0.5	2
BRAIN	426.8	1.44	987.1	3.34	1413.87	0.4	2.3

THIGH	469	1.59	1115.5	3.77	1584.54	0.4	2.4
BREAST	505.7	1.71	1262.9	4.27	1768.6	0.4	2.5
Total	15062.7	50.96	14494.3	49.04	29557.08	1.04	0.96

Analysis of total miRNA abundance across the different ages revealed a distinct temporal pattern separating the first (days 1–14) and second (days 21–28) two-week periods of the first month of life (Fig. 4a, b). In both sexes, 3′ miRNAs were more abundant than 5′ miRNAs at most early time points (days 1, 7, and 14); the only exception was day-7 males, in which 5′ miRNAs slightly exceeded 3′ miRNAs. This trend was reversed on days 21 and 28, when 5′ miRNAs became more abundant than 3′ miRNAs in both sexes (Fig. 4a, b). To examine miRNA diversity independently of abundance, repeated occurrences of each miRNA were collapsed, retaining a single instance of each miRNA per sex per sampling day (Fig. 4c, d). Under this counting scheme, the number of unique 3′ miRNAs consistently exceeded that of unique 5′ miRNAs at every age and in both sexes (Fig. 4c, d).

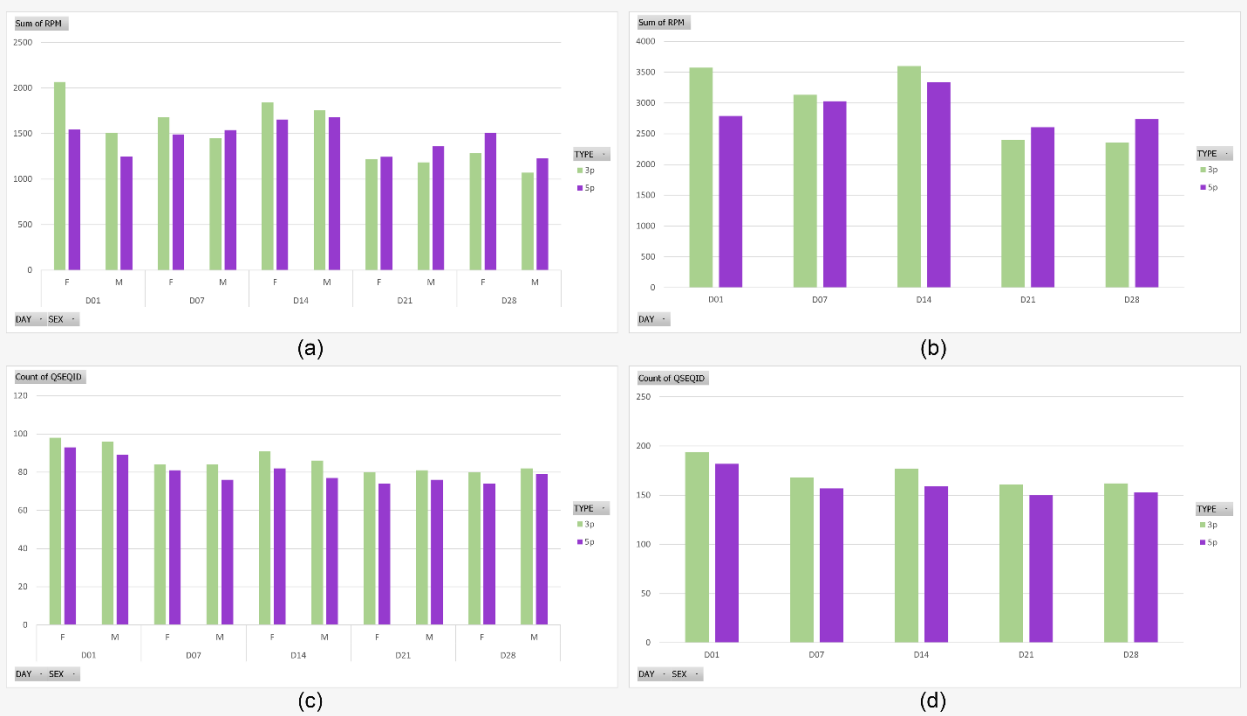


Fig 4. Abundance (sum of RPM) and unique counts of 3p- and 5p-derived miRNAs across sampling days. (a) Total abundance (sum of RPM) of 3p and 5p miRNAs in female (F) and male (M) birds at each sampling day (D01–D28). (b) Total abundance (sum of RPM) of 3p and 5p miRNAs at each sampling day, with both sexes combined. (c) Number of unique 3p and 5p miRNAs detected in each sex at each

sampling day. (d) Number of unique 3p and 5p miRNAs detected at each sampling day, with both sexes combined. D, day; RPM, reads per million; QSEQID, query sequence ID.

Expression Pattern across Tissues and Tissue-Specific miRNA Identification

Counting all unique alignments and standardizing them by dividing by reads per million (RPM) in each database revealed an average range of 0.66 to 3.15 RPM for all miRNAs in all tissues (Fig. 5a). The highest average was observed in the spleen, while the lowest average was observed in the heart, pancreas, and ovaries. Furthermore, summing up the standardized number of miRNAs across different tissues, which ranged from 235.78 to 3587.38 RPM across different tissues, showed that the lowest total number of miRNAs was found in reproductive organs of the ovaries and testis respectively, whereas the spleen still exhibited higher quantity compared to other tissues (Fig. 5b).

Given that tissues vary in their average number (Fig. 5a) and total amount of miRNAs (Fig. 5b), it can be inferred that each tissue possesses a distinct number of unique miRNAs and different levels of miRNAs expression. Considering the quantification of the unique miRNAs in each tissue, the thymus exhibited the highest number of unique miRNAs, indicating the highest diversity as well (Fig. 5c). However, the testis, pancreas, and ovaries had the lowest numbers (Fig. 5c). It was found that the reproductive organs and the pancreas had the smallest number of miRNAs and the least diversity in terms of the unique miRNAs. Conversely, in the point of average and total number of all miRNAs, the spleen and thymus displayed the highest number and diversity of miRNAs, respectively. The average number of miRNAs, the total number of miRNAs, and the number of unique miRNAs in different tissues in percentage are depicted in Fig. 5d.

The observed diversity of miRNAs in different tissues in this study ranged from the lowest proportion of 4.42% (80 miRNAs) in testis to highest proportion of 8.24% (149 miRNAs) in thymus. These indicate that the number of unique miRNAs between testis and thymus was less than twofold (1.86 times) (Fig. 5d). However, the average differential expression of miRNAs between testis and thymus - the tissues with the lowest and highest expression- was about 4.74-fold (Table 3). Consequently, it can be inferred that miRNAs exhibit different expression levels across distinct tissues. Therefore, the overlapping miRNAs that were expressed in all studied tissues (28 microRNAs) were selected for further examination of their expression levels. It was determined that these miRNAs displayed significantly different expression levels across tissues.

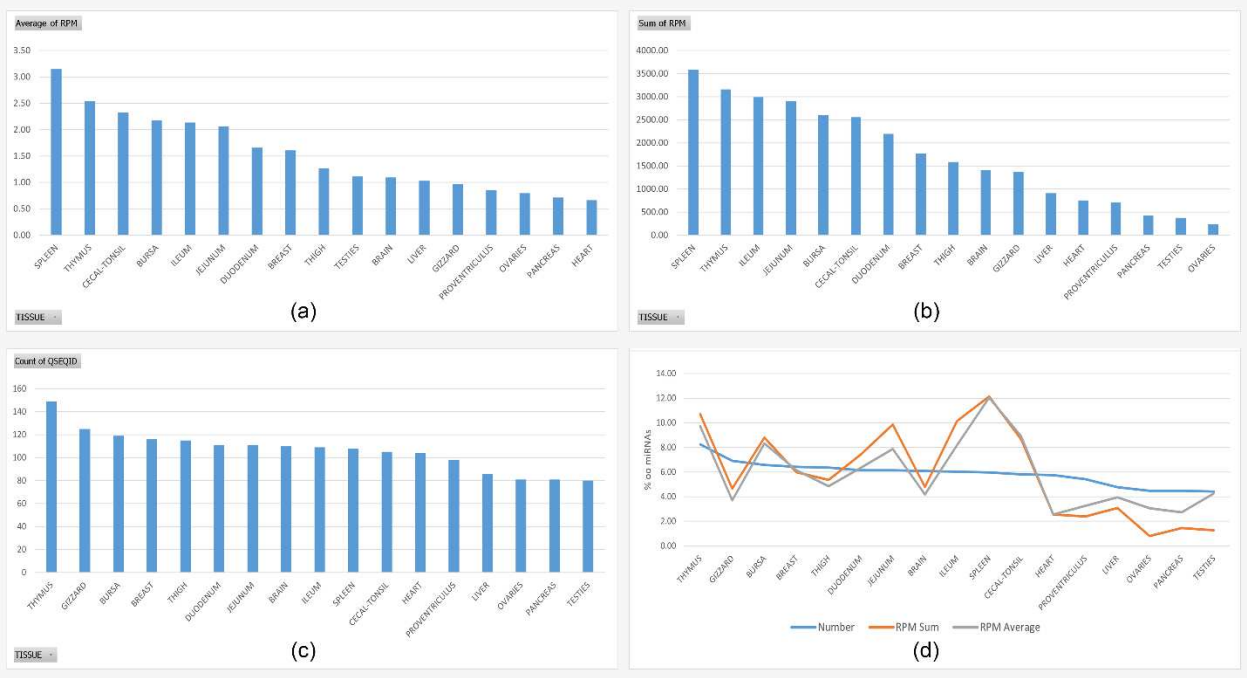


Fig 5. a: Figure 5. Tissue-wise distribution of miRNA abundance and unique miRNA counts. (a) Mean miRNA abundance (average RPM) in each tissue. (b) Total miRNA abundance (sum of RPM) in each tissue. (c) Number of unique miRNAs detected in each tissue. (d) Relative contribution of each tissue, expressed as a percentage of the corresponding grand total, for the unique miRNA count (Number), total abundance (RPM Sum), and mean abundance (RPM Average). RPM, reads per million; QSEQID, query sequence ID.

Table 3. The number of unique microRNAs, total number of microRNAs and average number of microRNAs in different tissues.

Tissue	Unique miRNAs		Mean abundance		Total abundance	
	Count	%	Average RPM	%	Total RPM	%
THYMUS	149	8.24	2.54	9.73	3162.07	10.7
GIZZARD	125	6.91	0.97	3.7	1375.34	4.65
BURSA	119	6.58	2.18	8.32	2601.99	8.8
BREAST	116	6.42	1.61	6.15	1768.6	5.98
THIGH	115	6.36	1.27	4.84	1584.54	5.36
DUODENUM	111	6.14	1.66	6.34	2195.83	7.43
JEJUNUM	111	6.14	2.06	7.88	2909.92	9.85
BRAIN	110	6.08	1.09	4.18	1413.87	4.78
ILEUM	109	6.03	2.13	8.16	2994.01	10.13
SPLEEN	108	5.97	3.15	12.04	3587.38	12.14
CECAL-TONSIL	105	5.81	2.33	8.91	2557.87	8.65
HEART	104	5.75	0.66	2.54	750.71	2.54
PROVENTRICULUS	98	5.42	0.85	3.25	706.61	2.39
LIVER	86	4.76	1.03	3.94	909.67	3.08
OVARIES	81	4.48	0.8	3.05	235.78	0.8
PANCREAS	81	4.48	0.71	2.73	425.86	1.44
TESTES	80	4.42	1.11	4.25	377.03	1.28

To characterize the distribution patterns of miRNAs across the tissue categories from complementary perspectives, Figure 6 was generated. For each tissue category (T1–T17), the total abundance (sum of RPM), mean abundance (average RPM), and number of unique miRNAs are shown in Figure 6a. In addition, a heatmap of miRNA abundance across the studied tissues was constructed from the complete tissue–miRNA matrix (Fig. 6b). Together with Table 4, this heatmap allowed the identified turkey miRNAs to be classified into three groups: (i) general miRNAs, comprising 53 miRNAs (21.73%) detected in nearly all studied tissues (14–17 tissues); (ii) semi-specific miRNAs, comprising 90 miRNAs (36.9%) present in multiple tissues (5–13 tissues); and (iii) tissue-specific miRNAs, comprising 101 miRNAs (41.4%) detected in only 1–4 tissues (Table 4).

By definition, miRNAs in category T1 were detected in a single tissue, those in T2 in two tissues, and so on, with higher category numbers representing more broadly expressed miRNAs. For each category, the total miRNA abundance, mean abundance, and number of unique miRNAs were calculated (Table 4; Fig. 6a), and the 17 categories were then consolidated into the three classifications above. Although the share of unique miRNAs varied across the 17 categories (1.6–16.8%), the mean per-category percentage of unique miRNAs was of the same order of magnitude among the three groups (tissue-specific, 10.35%; semi-specific, 4.1%; general, 5.43%). In contrast, total miRNA abundance differed sharply among categories, reflecting the presence of certain miRNAs in multiple tissues. For instance, category T17, comprising the 28 most ubiquitous miRNAs, accounted for 89.22% of total miRNA abundance across all tissues, while the remaining 16 categories contributed only 10.78%. Mean abundance was therefore used to further assess differences among the three groups. Thus, whereas unique miRNAs were relatively balanced across the 17 categories, a clear trend emerged for expression: the more tissues in which a miRNA was detected, the higher its average expression. This was most evident in the general group, which, despite comprising only 21.73% of all identified miRNAs, accounted for 50.87% of mean abundance and 92.71% of total miRNA abundance across all tissues. Also, the corresponding numbers of sex-specific and shared miRNAs are summarized in Figure 6c. Furthermore, the radar chart displays the distribution of total miRNAs (green) together with male- (blue) and female-specific (red) miRNAs across the 17 tissue categories (Fig. 6d). Overall, miRNAs were detected in all 17 categories; however, male-specific and female-specific miRNAs were observed in up to 8 and 6 categories, respectively (Fig. 6d).

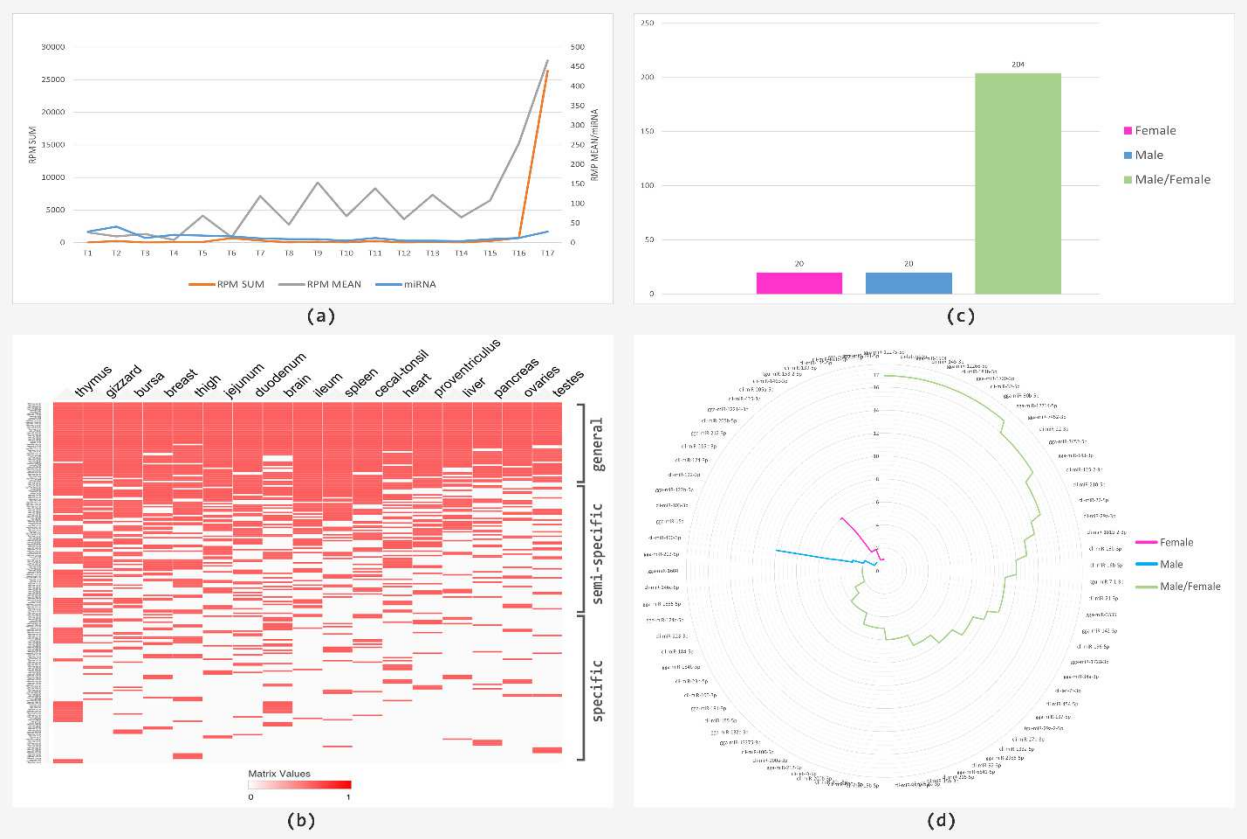


Fig 6. Tissue-wise summary of miRNA abundance and diversity, clustering of abundance patterns, and sex specificity of miRNAs. (a) Total abundance (sum of RPM), mean abundance (average RPM), and number of unique miRNAs in each tissue (T1–T17). (b) Heatmap of miRNA abundance across the studied tissues, generated with Clustergrammer (https://maayanlab.cloud/clustergrammer/). (c) Number of sex-specific and non-sex-specific (shared) miRNAs. (d) Radar chart showing, separately for each sex, the distribution of miRNAs according to the number of tissues (1–17) in which each miRNA was detected. RPM, reads per million.

Table 4. The number of unique microRNAs, average number of microRNAs and total number of microRNAs in 3 different groups of miRNAs: Tissue specific, semi-specific and general miRNAs.

Tissue group/ Category		Unique miRNA		Mean abundance		Total abundance	
		Number	%	Abundance	%	Total RPM	%
Tissue Specific	T1	28	11.48	26	1.48	18.85	0.06
	T2	41	16.8	16	0.91	260.93	0.88
	T3	12	4.92	22	1.25	24.24	0.08
	T4	20	8.2	7	0.4	87.08	0.29
Semi Specific	T5	18	7.38	69	3.93	106.81	0.36
	T6	16	6.56	14	0.8	676.69	2.29
	T7	11	4.51	119	6.78	322.71	1.09

	T8	9	3.69	46	2.62	45.48	0.15
	T9	9	3.69	154	8.77	127.02	0.43
	T10	5	2.05	68	3.87	51.79	0.18
	T11	12	4.92	139	7.92	268.38	0.91
	T12	5	2.05	60	3.42	31.08	0.11
	T13	5	2.05	122	6.95	134.4	0.45
General	**T14**	4	1.64	65	3.7	30.84	0.1
	T15	9	3.69	108	6.15	268.93	0.91
	T16	12	4.92	254	14.47	732.05	2.48
	T17	28	11.48	466	26.55	26369.8	89.22
	Total	244	100	1755	100	29557.08	100

Expression Pattern across Ages/Sexes and Age/Sex-Specific miRNA Identification

Based on their occurrence in each sex, the miRNAs were classified into three groups: male-specific, female-specific, and shared (detected in both sexes) (Fig. 6c; Table 5). Of the 244 putative miRNAs, 20 were male-specific and 20 female-specific, together representing 40 sex-specific miRNAs (16.4%), whereas the remaining 204 (83.6%) were detected in both sexes. Although sex-specific miRNAs accounted for 16.4% of the unique miRNAs, they contributed only 0.04% of total miRNA expression (4.97 and 6.99 RPM for female- and male-specific miRNAs, respectively, versus 29,545.12 RPM for shared miRNAs; Table 5), indicating a marked disparity between their occurrence and their expression levels.

The expression patterns and levels of unique miRNAs in each sex and at each sampling day were then visualized (Fig. 7). Presence/absence heatmaps (Fig. 7a, e, i), abundance heatmaps (Fig. 7b, f, j), and miRNA similarity matrices (Fig. 7c, g, k) were generated for females, males, and both sexes combined, respectively. Based on the number of sampling days on which they were detected, the miRNAs were further classified into five categories, ranging from age-specific (detected at a single day) to age-ubiquitous (detected at all five days) (Fig. 7d, h, l). In each sex, approximately

half of the unique miRNAs were detected at all five days (Fig. 7d, h), whereas in the combined dataset this proportion exceeded 60% (Fig. 7l). In all three groups, miRNAs detected at all time points showed substantially higher expression levels than those detected only at specific ages, with only a few exceptions. Consequently, when expression levels rather than presence/absence were considered, the contribution of age-ubiquitous miRNAs increased markedly, reaching 96%, 97.3%, and 97.45% of total miRNA expression in females, males, and both sexes, respectively (Table 6).

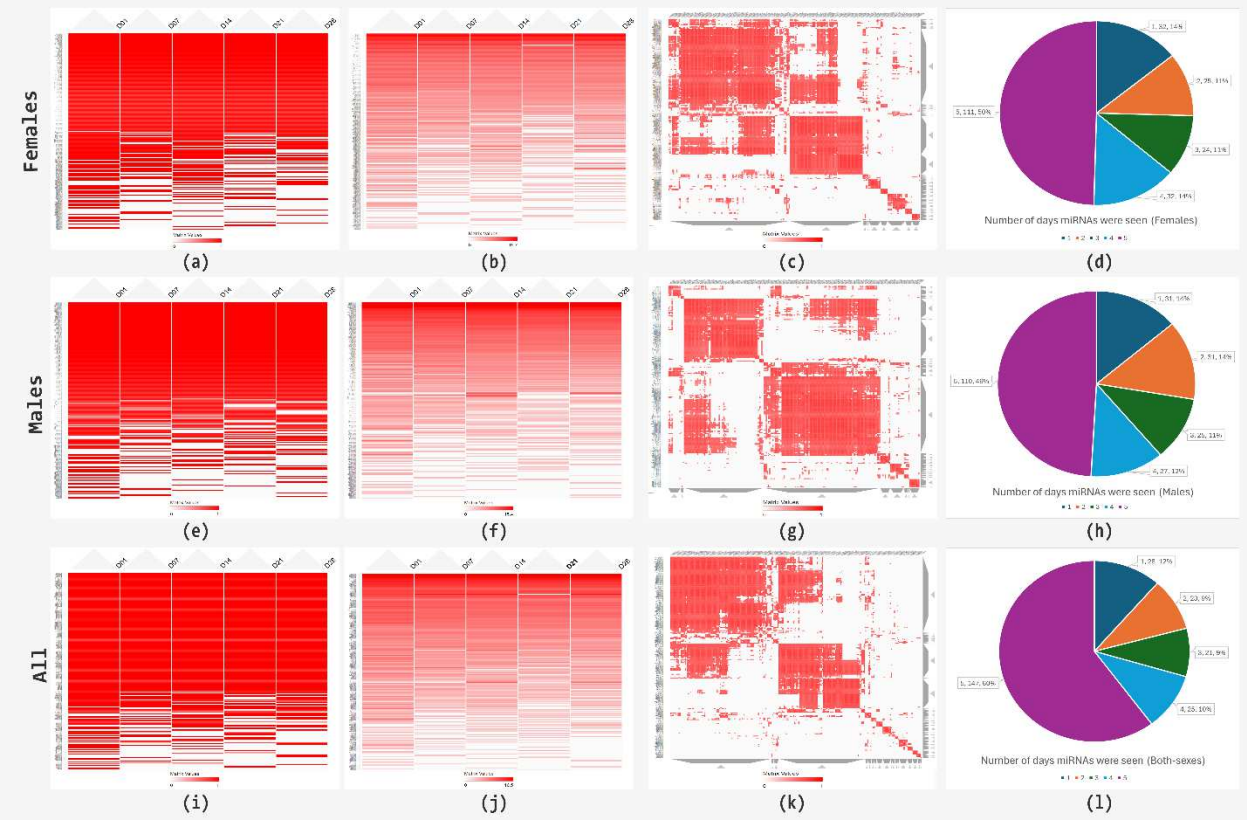


Fig 7. Age-dependent expression of miRNAs in females, males, and both sexes combined. (a, e, i) Presence/absence heatmaps of miRNAs across the five sampling days (D01–D28) in females, males, and both sexes, respectively. (b, f, j) Corresponding heatmaps of miRNA abundance scaled by RPM. (c, g, k) miRNA–miRNA similarity matrices based on Pearson correlation of abundance profiles across samples. (d, h, l) Distribution of miRNAs according to the number of sampling days (1–5) on which each miRNA was detected in females, males, and both sexes, respectively; slice labels indicate the number of days, the number of miRNAs, and the corresponding percentage. D, day; RPM, reads per million.

Table 5. The number of unique microRNAs, average number of microRNAs and total number of microRNAs in 3 different groups of miRNAs: sex specific and those which were observed in both sexes.

Sex groups	Unique miRNAs		Mean abundance		Total abundance	
	Number	%	Abundance	%	Total RPM	%
Female	20.00	8.20	0.15	7.74	4.97	0.02
Male	20.00	8.20	0.14	7.33	6.99	0.02
Both sexes	204.00	83.61	1.65	84.93	29545.12	99.96
Total	244.00	100.00	1.95	100.00	29557.08	100.00

Table 6. Expression pattern and levels of unique and all miRNAs in both sexes across different ages

Groups/Sexes		Age-specific	General				Total
		1	2	3	4	5	
Females	Count	32	25	24	32	111	224
	Count %	14.3	11.2	10.7	14.3	49.6	100
	RPM	26.9	24.4	18.8	547.5	14913.1	15530.7
	RPM %	0.2	0.2	0.1	3.5	96	100
Males	Count	31	31	25	27	110	224
	Count %	13.8	13.8	11.2	12.1	49.1	100
	RPM	103.2	165.8	59.5	47.8	13650.1	14026.4
	RPM %	0.7	1.2	0.4	0.3	97.3	100
Both Sexes	Count	28	23	21	25	147	244
	Count %	11.5	9.4	8.6	10.2	60.2	100
	RPM	6.8	106.2	67.3	572	28804.7	29557.1
	RPM %	0.02	0.36	0.23	1.94	97.45	100

MicroRNA prediction across the five sampling days identified 214, 188, 196, 187, and 187 high-confidence miRNAs on days 1, 7, 14, 21, and 28, respectively. After excluding overlapping miRNAs, 28 age-specific miRNAs were identified: 13 on day 1 (mga-let-7g-3p, mga-miR-101-5p, mga-miR-132c-3p, mga-miR-132c-5p, mga-miR-146c-5p, mga-miR-153-2-5p, mga-miR-15a-3p, mga-miR-1684a-5p, mga-miR-204-3-3p, mga-miR-212-3p, mga-miR-212-5p, mga-miR-223-5p, and mga-miR-363-5p), 3 on day 7 (mga-miR-144-5p, mga-miR-216b-5p, and mga-miR-33-3p), 4 on day 14 (mga-let-7a-2-3p, mga-miR-1807, mga-miR-205b-5p, and mga-miR-92-2-

5p), 4 on day 21 (mga-miR-135-5p, mga-miR-139-5p, mga-miR-191-5p, and mga-miR-33-2-5p), and 4 on day 28 (mga-miR-1456-3p, mga-miR-1456-5p, mga-miR-1677-3p, and mga-miR-1677-5p). In total, 147 miRNAs (60.2% of the 244 unique miRNAs) were detected at all five ages and were therefore considered age-ubiquitous (general) miRNAs.

Phylogenetic analysis of the identified miRNAs

To create a phylogenetic tree and analyze the evolutionary relationships, all sequences of the mature miRNAs were subjected to multiple sequence alignment (MSA) and phylogenetically analyzed with the CLC software. The Neighbor-joining (NJ) following 10000 bootstrapped replicates and maximum likelihood approaches were used to create a phylogenetic tree from the aligned sequences displayed in a radial layout (Fig. 8).

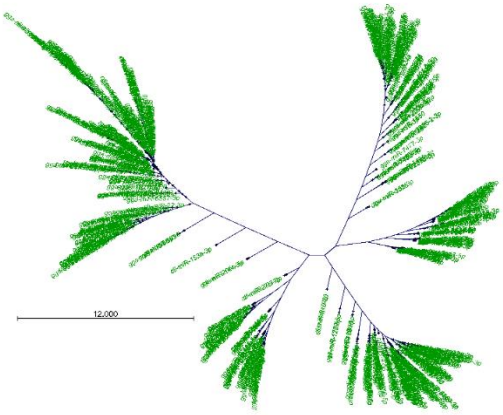


Fig 8. Radial phylogenetic tree of discovered miRNAs in turkey.

Target Gene Analysis

Functional miRNA targets were predicted separately for each tissue using the miRDB database (http://mirdb.org/) (Chen and Wang 2020). To increase confidence in the predictions and reduce the complexity of the interaction lists, only miRNA–target interactions with a target score ≥ 90 were retained. This analysis yielded a total of 26,393 target gene occurrences across the 17 tissues, comprising 1,718 in breast, 1,315 in thigh, 1,840 in brain, 1,309 in heart, 1,578 in liver, 1,691 in spleen, 1,664 in gizzard, 1,407 in ovaries, 1,275 in testes, 1,636 in duodenum, 1,625 in ileum, 1,514 in jejunum, 1,107 in pancreas, 1,379 in proventriculus, 1,755 in bursa, 1,436 in cecal tonsil, and 2,144 in thymus (Fig. 9). After excluding genes shared among tissues, tissue-specific target genes were identified in only seven tissues: 36 in thigh, 1 in brain, 14 in liver, 36 in testis, 44 in pancreas, 1 in bursa, and 17 in thymus; no tissue-specific target genes were detected in the remaining ten tissues.

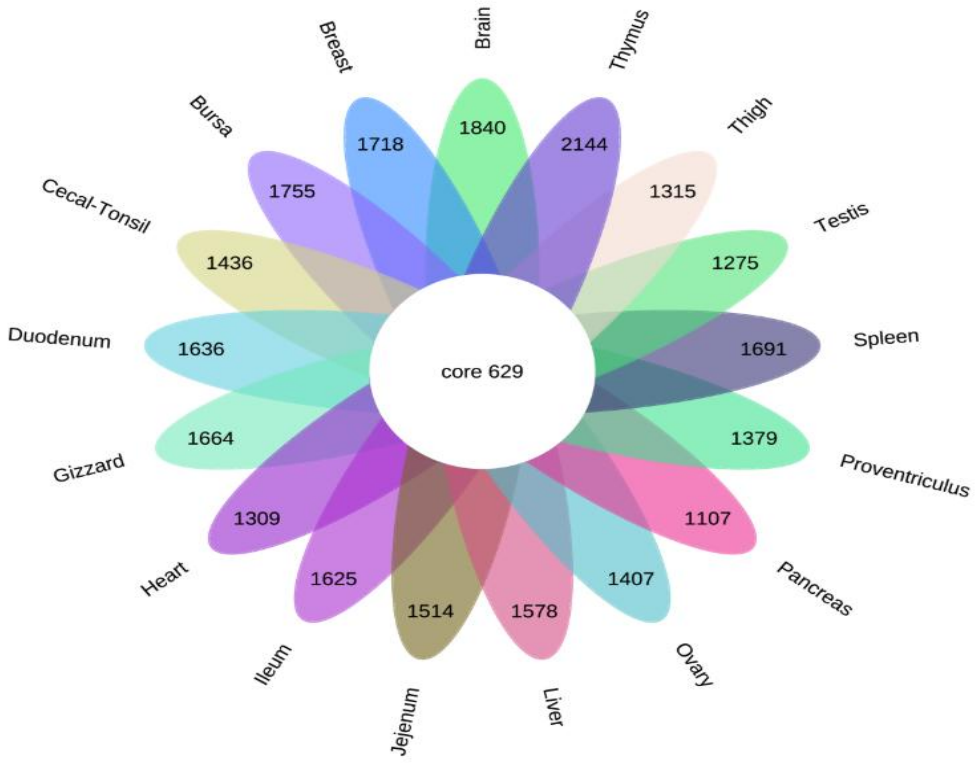


Fig 9. The number of identified target genes for each studied tissue with 629 overlapping genes among all tissues.

Discussion

Length heterogeneity of mature miRNAs

The present study identified 244 unique putative mature miRNAs exhibiting length heterogeneity (20–27 nt; Table 1; Fig. 1), a feature that reflects variability in Drosha/Dicer processing, differential turnover, and evolutionary constraints (Lin et al. 2026). The length distribution of the identified miRNAs closely resembled that of miRBase-registered miRNAs, in which 22-, 21-, and 23-nt species predominate (Griffiths et al. 2008, Kozomara et al. 2019), and is consistent with human studies showing that stable 22-nt isoforms preferentially regulate disease-associated transcripts, whereas longer isoforms target distinct gene sets (Fang et al. 2013, Rolle et al. 2016). The higher abundance of 21- and 22-nt miRNAs relative to less common lengths likely reflects their optimal loading into the RNA-induced silencing complex (RISC) and greater functional stability, whereas longer or shorter isoforms are processed less efficiently or degraded more rapidly (Bartel 2004, Chatterjee et al. 2025, Lin et al. 2026).

Structural validation of predicted miRNAs and 3p/5p arm usage

Structural validation of the predicted pre-miRNAs showed that, after application of the filtering criteria, all loop-derived (Midseq) alignments were completely eliminated, strongly supporting the accuracy of the mature miRNA set identified in this study (Fig. 2). Most miRNAs exhibited a single predominantly active arm (5p or 3p), whereas in some pre-miRNAs both arms were active at different levels, consistent with reported arm-switching behavior (Tam et al. 2015, Pinhal et al. 2025). The relative abundance of 3p and 5p miRNAs was also remarkably consistent across structurally related tissues, including the three small-intestinal segments (duodenum, jejunum, and

ileum), the cecal tonsil, and the pancreas (Table 2; Fig. 3). Because strand selection during miRNA biogenesis is inherently asymmetric, this concordance suggests that shared cellular environments, developmental origins, or functional demands stabilize arm usage in tissues with similar architecture (Zaporozhchenko et al. 2020, Lin et al. 2026).

A comparable 3p/5p balance was also observed in the proventriculus and gizzard, two functionally related compartments of the avian gastrointestinal tract. In these organs, miRNAs are implicated in compartment-specific processes, including regulation of digestive enzyme and hydrochloric acid secretion, gut immune responses, and enteric neuronal development in the proventriculus, and control of muscular contraction and mechanical digestion in the gizzard (Yoshimura et al. 2022, Singh et al. 2021, Vishnuraj et al. 2021). The concordant arm usage in such functionally specialized tissues underscores the tissue-specific character of miRNA-mediated regulation.

Functionally, the arm from which a miRNA is processed shapes its target repertoire: miRNAs preferentially recognize the 3′ UTR of target mRNAs, leading to mRNA destabilization or translational repression, whereas binding within the 5′ UTR can modulate translation initiation in either direction (Gu et al. 2007, Lee et al. 2009). These interactions are dynamic, being influenced by subcellular localization, the relative abundance of miRNAs and target transcripts, and the affinity of miRNA–mRNA pairing (Pitchiaya et al. 2019, Diener et al. 2024).

At the sex level, total miRNA abundance was broadly similar between females and males (15,530.7 vs. 14,026.4 RPM; Fig. 3b), with only modest arm-level differences. This contrasts with the pronounced sex-biased miRNA expression reported in other species and in human disease, which has been attributed to sex chromosome dosage, hormonal milieu, and sex-specific epigenetic mechanisms (Sharma and Eghbali 2014, Ko et al. 2022, Karere et al. 2021, Llera-Oyola et al. 2024). Together with the small proportion of sex-specific miRNAs identified here (16.4%),

these results suggest that the core architecture of the turkey miRNome is shared between the sexes, with sex-biased regulation confined to a limited subset of miRNAs.

Tissue-specific and general miRNAs

Consistent with the well-established observation that miRNAs are non-uniformly distributed among tissues(Liang et al. 2007, Yang et al. 2022), the present study identified both broadly expressed (general) and tissue-restricted miRNAs in turkey (Fig. 5). Tissue-specific miRNAs, defined here as those detected in only 1–4 tissues and comprising 41.4% of the unique miRNAs (Table 4), are expected to fine-tune tissue identity by regulating mRNAs underlying development, differentiation, and homeostasis, as likewise reported in other species, including the pig miRNAome (Martini et al. 2014, Preusse et al. 2016, Seida et al. 2025).

Importantly, the abundance distribution contrasted sharply with the distribution of unique miRNAs. Whereas the per-category contribution of unique miRNAs varied modestly, the mean per-category abundance increased progressively from tissue-specific (1.01%) to semi-specific (5.0%) and general miRNAs (12.72%), with individual categories spanning 0.4–26.5% (Fig. 6; Table 4). This inverse relationship, many lowly expressed tissue-restricted miRNAs versus a few highly expressed general miRNAs, indicates that tissue specialization is achieved largely through low-abundance, precise regulators, whereas a small set of general miRNAs dominates the cellular miRNA pool, in line with recent evidence that a large fraction of annotated miRNAs is expressed at low levels yet retains regulatory relevance (Kachooei et al. 2025).

Linking miRNA expression breadth to the Omnigenic model

The observed inverse relationship between tissue specificity and abundance is reminiscent of the Omnigenic model of complex traits (Boyle et al. 2017, Davoodi et al. 2022). In this framework, "core" genes exert direct, trait-specific effects, whereas "peripheral" genes shape phenotypes through broad network interactions. By analogy, tissue-specific miRNAs, low in abundance but precise in function, may act as core regulators for organ identity, whereas general miRNAs, highly expressed and widely distributed, may serve as peripheral modulators that stabilize gene-regulatory networks across tissues (Liu et al. 2019). This conceptual parallel suggests that the turkey miRNome, like polygenic traits, is shaped by a small set of dominant general regulators and a larger set of specialized, low-abundance miRNAs.

Sex- and age-dependent expression patterns

Total miRNA abundance was broadly similar between females and males (15,530.7 vs. 14,026.4 RPM; Table 5), with only modest sex-related differences. However, sex-specific miRNAs exhibited a striking decoupling between their occurrence and their expression: although they represented 16.4% of unique miRNAs and 15.07% of mean per-miRNA abundance (Table 5), they contributed only 0.04% of total miRNA expression. This pattern indicates that sex-specific miRNAs are expressed at very low levels, consistent with their role as fine-tuning regulators in sex-dependent circuits rather than as dominant players (Ko et al. 2022, Lin et al. 2026).

Age-dependent expression patterns were also evident (Fig. 7). Age-specific miRNAs have been implicated in growth, cellular senescence, and age-related diseases (Noren et al. 2013, Hicks and Liu 2021), and recent evidence highlights the dynamic reprogramming of the miRNome across developmental stages in vertebrates (Yu et al. 2023, Zhang et al. 2026). These findings suggest that

miRNA expression is dynamically adjusted to meet the changing biological demands of the organism at different life stages.

Comparison with disease-associated miRNAs in poultry

Comparison of the identified miRNAs with those reported in avian viral diseases revealed substantial overlap. Several miRNAs previously associated with Marek's disease (gga-miR-15b, gga-miR-221, gga-let-7d), avian influenza (gga-miR-181a-5p, gga-miR-10a-5p, gga-miR-146b-5p, gga-miR-451), and avian leukosis subgroup J (gga-let-7b, gga-let-7i, gga-miR-23b-3p) were detected in healthy turkeys across multiple tissues and developmental stages (Duan et al. 2020, Chowdhury et al. 2024, Kappari et al. 2024). A comprehensive list of these disease-associated miRNAs, together with their tissue, sex, and age distributions in the present study, is provided. This overlap suggests that many miRNAs reported as disease-associated are constitutively expressed under physiological conditions and likely represent baseline components of the immune and regulatory network rather than specific disease markers. Consequently, differential expression rather than mere presence appears to be the more informative diagnostic criterion, reinforcing the potential of tissue-resident miRNAs as sensitive welfare biomarkers in poultry (Miranda et al. 2018, Zhou and Zheng 2019). Among the identified miRNAs, the miR-30 family (gga-miR-30b-3p and -5p), detected in breast muscle, has been linked to metabolic regulation across vertebrates, highlighting its conserved role in energy homeostasis (Miranda et al. 2018).

Evolutionary conservation and regulatory implications of identified miRNAs

Phylogenetic reconstruction of the 244 identified putative miRNAs resolved six major clades, one represented by a single branch and five comprising two or more subgroups (Fig. 8). The close clustering of sequences within these clades is consistent with shared evolutionary origins and suggests functional similarity among members of the same family, as closely related miRNAs are expected to target overlapping mRNA repertoires (Huang et al. 2015, Ling et al. 2024). Mature miRNA sequences are generally highly conserved across distantly related vertebrates, providing a robust framework for investigating both the diversification of regulatory roles within a species and the evolutionary trajectories of miRNA families across lineages, a process implicated in the emergence of body-plan diversity during animal evolution (Ling et al. 2024).

Target gene network and regulatory complexity

Consistent with this view of miRNAs as post-transcriptional micro-switches, target prediction revealed a highly interconnected regulatory network: each miRNA potentially targets multiple mRNAs, and each mRNA may be co-regulated by several miRNAs across different tissues (Fig. 9)(Lim et al. 2005, Chen and Wang 2020). The large number of predicted targets per tissue reflects the extensive influence of miRNAs on both the expression and evolution of protein-coding genes (Bartel 2009, Lin et al. 2026). This dense, pleiotropic interaction network is also conceptually aligned with the Omnigenic model, in which a core set of specialized regulators cooperates with a broader periphery of highly connected modulators to shape complex phenotypes (Boyle et al. 2017). Beyond their fundamental regulatory role, the identified miRNAs represent a valuable resource for translational applications, as circulating and tissue-resident miRNAs are increasingly recognized as sensitive biomarkers of health, welfare, and disease status in livestock and poultry (Miretti et al. 2020, Kappari et al. 2024, Metcalf 2024, Zhou and Zheng 2019).

While formal statistical testing for tissue- and sex-specificity was not performed due to the exploratory nature of this in silico study, the observed patterns are consistent with established principles of miRNA biology and provide testable hypotheses for future experimental validation.

Materials and Methods

Rationale for homology-based miRNA prediction in turkey

Because turkey (*Meleagris gallopavo*) miRNAs are not yet comprehensively annotated in miRBase, a homology-based prediction strategy was adopted, exploiting the high evolutionary conservation of miRNA sequences among birds. Mature miRNA sequences of four phylogenetically representative avian species, *Gallus gallus* (Galliformes), *Anas platyrhynchos* (Anseriformes), *Columba livia* (Columbiformes), and *Taeniopygia guttata* (Passeriformes), were retrieved from miRBase (Griffiths et al. 2008, Kozomara et al. 2019). The initial 1,997 entries were collapsed into a non-redundant reference set of 1,723 unique mature miRNAs, which served as query sequences for homology searches against turkey RNA-seq data.

RNA-sequencing data acquisition

A total of 586 publicly available RNA-seq libraries from turkey were retrieved from the NCBI SRA (BioProject PRJNA259229); (Katz et al. 2022). The dataset encompasses 17 tissues, two sexes (female and male), and five post-hatch developmental stages (days 1, 7, 14, 21, and 28), with a variable number of biological replicates (typically 3; range 2–4) per tissue–sex–age combination. The difference between the actual library count (586) and the theoretical maximum of 510 ($17 \times 2 \times 5 \times 3$) reflects the natural heterogeneity of the original BioProject, namely

unequal replicate numbers across combinations and the absence of certain tissues at specific stages (testis unavailable at day 21; cecal tonsil unavailable at day 28).

Quality control and preprocessing

Raw FASTQ files from all 586 RNA-seq libraries were trimmed using Cutadapt v4.9 (Martin 2011) to remove adapter sequences and low-quality bases (Phred < 20), discarding reads shorter than 18 nucleotides. Quality assessment was performed using FastQC v0.12.1 (Andrews 2010), and aggregated reports were generated with MultiQC v1.25 (Ewels et al. 2016) (Fig 10). High-quality reads (mean Phred ≥ 20) were retained and converted to FASTA format for downstream homology-based alignment.





Fig 10. Quality assessment of RNA-seq libraries. (**a**) Mean per-base sequence quality scores across all sequenced libraries, summarized from individual FastQC reports and aggregated using MultiQC. (**b**) Distribution of sequence quality scores as a function of read length, showing the consistency of base-calling accuracy across the dataset.

Homology-based alignment using BLAST

To identify turkey miRNAs homologous to the reference avian miRNA set, local alignment was performed using NCBI BLAST (Johnson et al. 2008). The 586 preprocessed turkey RNA-seq FASTA files were compiled into a single BLAST-formatted database using makeblastdb, and the 1,723 non-redundant reference mature miRNAs were used as query sequences. Alignment was performed using blastn with the -task blastn-short parameter optimized for sequences shorter than 50 nucleotides. This homology search yielded 46,133,381 initial alignments.

Loop-region (Midseq) extraction and parallel alignment

To exclude non-miRNA hairpin-derived small RNAs, a parallel alignment strategy targeting both the mature miRNA arms (5p and 3p) and the terminal loop region (Midseq) of precursor miRNAs was implemented, following the approach described by Zeng and Cullen (2003) (Zeng and Cullen 2003). For each annotated pre-miRNA in the reference set, an 18–24 nucleotide Midseq was extracted from the terminal loop region flanked by the corresponding 5p and 3p

mature sequences. BLAST alignments were then performed independently against both the mature miRNA sequences and the Midseq set.

Alignment filtering and exclusion of protein-coding sequences

Given the unique biogenetic features of miRNAs, extremely stringent filtering criteria were applied to the initial alignments (Zhu et al. 2016). In the first filtering step, alignments with E-value > 0.001, length differences exceeding 2 nucleotides relative to the query miRNA, gaps, mismatches, or alignment lengths shorter than 18 nucleotides were removed. This reduced the alignment count to 790,127, from which cross-sample duplications were collapsed to yield 671 unique alignments across all tissues.

Subsequent filters retained the longest alignment for each miRNA name (accounting for minor length variation among species) unless shorter alignments showed higher occurrence counts, ultimately producing 441,692 retained alignments corresponding to 244 unique candidate miRNAs with a total normalized abundance of 29,557 RPM.

To exclude protein-coding transcripts, hairpin structures of candidate precursors were predicted using the Zuker algorithm implemented in the mfold web server v3.0 (Zuker 2003), and the transcript with the lowest minimum free energy (MFE) was retained for each candidate. All candidate sequences were then examined using BLASTX against the NCBI non-redundant protein database with an E-value threshold of 1e-5, and any alignments to known protein-coding genes were discarded.

Identification of Conserved miRNAs in Turkey Using Precursor's Hairpin Characteristics

The hairpin structures of the precursors-miRNAs, in general, possess distinct characteristics that not only differentiate them from other types of RNAs (tRNA, rRNA, and mRNA) (Zhang et al.

2006) but also set them apart from miRNAs of other species due to a specific features (Zhu et al. 2016), Therefore, the factors AMFE (Adjusted Minimum Free Energy), MFEI (Minimum Free Energy Index), GC content, and sequence length were initially calculated for discovery of the potential sequences. The formula of AMFE and MFEI are as follows (Zhang et al. 2006, Chaudhary 2018, Ahmed et al. 2021):

$$AMFE = [MFE / \text{length of precursor sequence (LP)}] * 100.$$

$$MFEI = AMFE/ (G + C)\%$$

To compare the length distribution of the identified miRNAs with the complete list of miRNAs in miRBase, all 48,696 registered miRNAs in miRBase were downloaded, and their lengths and the percentage of each group were determined. Entries shorter than 18 nucleotides, longer than 27 nucleotides, and those with a length of 26 nucleotides were removed, leaving a total of 47,148 miRNAs.

Expression Pattern Analysis

To monitor the expression patterns of predicted miRNAs in turkeys in different tissues, ages, and sexes all counts of unique alignments were standardized by dividing by reads per million (RPM). To demonstrate the expression levels, matrices of actual expression values were generated. Given the wide range of expression levels among different miRNAs, log2 transformation was applied to facilitate better visualization (Tam et al. 2015).

Phylogeny Analysis of Identified miRNAs

To study phylogenetic and evolutionary relationships among all predicted miRNAs, multiple sequence alignment (MSA) was performed in the CLC genomics software. The Neighbor-joining with 1000 Bootstrap values and Jukes-Cantor approaches were used to create a rooted radial phylogenetic tree from the aligned sequences (Reyes-Haro et al. 2024, CLC 2020)

Target Gene Discovery for Identified miRNAs

The miRDB, an online database for miRNA target prediction and functional annotations, was used for target gene discovery in this study. Then the target genes with a target score of more than 90% were selected for each miRNA in each tissue (Liu and Wang 2019, Chen and Wang 2020). The hidden parameters employed were as follows, target duplex free energy $\Delta G \leq 6$ -25 kcal/mol; no space for mismatches in the target region and mature miRNA and only one G: U pairing was allowed.

Limitations

Several limitations warrant careful interpretation of our findings. First, the transcriptomic libraries used in this study (PRJNA259229) were generated using total RNA-seq rather than small RNA-enriched protocols. Consequently, miRNA-derived reads represent only a small fraction of the total sequencing depth, limiting the quantitative reliability of expression estimates. The RPM values reported should therefore be interpreted as relative occurrence metrics rather than absolute expression levels. Second, despite stringent filtering based on homology, structural validation, and conservation, computational predictions require experimental validation (e.g., small RNA-seq, RT-qPCR, northern blotting) to confirm the biogenesis and functional relevance of the identified candidates. Third, statistical inference for tissue- and sex-specificity was not performed; the reported patterns should be considered descriptive and hypothesis-generating rather than

conclusive. Finally, the absence of a curated turkey miRNA reference in miRBase necessitated reliance on homology to other avian species, which may bias the catalog toward evolutionarily conserved miRNAs and underrepresent lineage-specific or rapidly evolving miRNA families.

Conclusion

This study presents the first comprehensive in silico characterisation of the turkey miRNome, identifying 244 high-confidence miRNAs across 17 tissues, two sexes, and five post-hatch developmental stages through homology-based prediction using transcriptomic data from 586 RNA-seq libraries. The integration of stringent filtering criteria with structural validation confirmed the reliability of the identified miRNAs, while revealing a distinct regulatory architecture: general miRNAs, though representing only 21.7% of unique miRNAs, accounted for 92.7% of total miRNA abundance, paralleling the role of peripheral genes in the Omnigenic model, whereas tissue-specific miRNAs functioned as precise, low-abundance regulators of organ identity. Several limitations warrant consideration. The reliance on publicly available RNA-seq data precluded direct experimental validation, and the stringent filtering criteria, while ensuring high confidence, likely excluded genuine miRNAs with sequence variations, mutations, or gaps relative to reference avian species. Future studies incorporating less restrictive filters, expanded reference sets from additional organisms, and experimental approaches such as RNA-seq in turkey tissues will substantially expand the annotated turkey miRNA repertoire.

Despite these constraints, this work provides a foundational resource for turkey functional genomics, with potential applications in biomarker development for disease diagnosis, welfare assessment, and selective breeding programmes. The identified miRNAs and their tissue- and age-

specific expression patterns offer testable hypotheses for investigating the post-transcriptional regulation of economically important traits in this poultry species.

Authors' contributions

All authors contributed significantly to this study. AE was responsible for data collection, methodology development for microRNA prediction, and data analysis. PD contributed to validation, interpretation, target gene analysis, and handled manuscript drafting and revisions. AAM and RVT provided supervision, guidance, and critical feedback throughout the research process. Their expertise contributed significantly to the study design, methodology refinement, and manuscript review. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

We disclose the slight use of AI-assisted language editing tools to paraphrase and enhance the clarity of the manuscript text. All scientific interpretations, analyses, and conclusions remain the responsibility of the authors.

Competing interests

The authors declare that they have no competing interests that could have influenced the research, analysis, or conclusions presented in this study.

Data availability

The datasets are available in the Bio project PRJNA259229.

Ethics declarations

This study did not require ethics approval as it exclusively utilized publicly available data. No human participants, animals, or personally identifiable information were involved in the research. All data were obtained from open-access sources that comply with ethical and legal standards.

Consent to participate/Consent to publish

All participants involved in this study provided informed consent before their inclusion.

All authors and participants involved in this study have provided explicit consent for the publication of this research in the Scientific Reports.

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