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Multiple Sequence Alignment and Phylogenetic Analysis of *Growth Differentiation Factor 9* Gene for Egg Production in Poultry Species

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ABSTRACT

Growth differentiation factor 9 gene (*GDF9)* is an oocyte-secreted factor which controls the functions of the ovarian cells, regulates the development and caliber of the egg. *GDF9* DNA and protein sequences from NCBI were used to computationally investigate molecular genetic variation in poultry species. Results showed that percentage similarities of DNA and protein sequences ranged from 78.45 to 92.80% and 83.63 to 94.74%, respectively. The phylogenetic analysis results showed that chicken and turkey were closely related. The high percent similarities and close relatedness of poultry species show that *GDF9* can be used as a candidate gene for improving egg production in poultry species.

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Introduction

Poultry species refers to a group of birds which include chicken, turkey, Ostrich and they are bred for their ability to produce products including eggs (FAO, 2022). According to Liu *et al.* (2019), egg production plays an essential role in food security and alleviation of unemployment. However, due to the ongoing poor performance in egg production, these needs are barely met (Beesabathuni *et al.,* 2018). This means that there is a constant need to improve egg production performance. *Growth differentiation factor 9* gene (*GDF9*) is an oocyte-secreted factor that is responsible for controlling the functions of the ovarian cells as well as regulating the development and quality of the egg, subsequently making it a good candidate gene for improving egg production performance (Belli and Shimasaki, 2018). According to Zamani *et al.* (2015), studying genes through bioinformatics allows for determination of genetic similarities and variation in their sequences in a faster manner thus allowing researchers to know how these findings affect the production performance of species. Multiple sequence alignment is essential in bioinformatics for identifying homologous regions on biological sequences, such as DNA, and it is a crucial prerequisite for phylogenetic tree construction (Bodenhofer *et al*., 2015). Phylogenetic analysis is a simple bioinformatic method that aid in mapping the lineage of genes across various species, as well as predicting the interactions between the species based on their evolutionary relationship (Singh *et al*., 2023). According to Pramod *et al*. (2019), phylogenetic analysis can provide important data that may assist scientist with implementing effective animal management and conservation strategies. In a scientific study, Egom *et al.* (2019), was able to document that the percentage identity and similarities of insulin-like growth factor-1 gene in poultry species fell in a range of 86-99% and was able to conclude that the gene was highly effective for its protein function improvement.

Furthermore, Karthikeyan *et al.* (2020), documented that the Osteopontin gene in Bos taurus exhibited 99% similarity with the closely related ruminant species. This proves the ability to use bioinformatics for gene comparison within and among species. Additionally, Ewuola *et al.* (2018), has proved that bioinformatics methods, such as multiple alignment and phylogenetic analysis, are viable strategies to analyze variations and similarities in genes of poultry species. However, similarities and differences of *growth differentiation factor 9* gene in poultry species are not yet known. Hence the objective of the current study was to computationally investigate the molecular genetic variation of *growth differentiation factor 9* gene in poultry species. Findings from the current study will provide genetic variation information on percentage similarities of *growth differentiation factor 9* gene in poultry species which may be beneficial to scientists and researchers interested in improving egg production performance of these poultry species.

Materials and methods

Retrieval of deoxyribonucleic acid (DNA) and protein sequences

DNA and protein sequences of the *growth differentiation factor 9* gene of chicken, turkey and ostrich and their accession numbers were retrieved from the National Centre for Biotechnology Information (NCBI), as shown in Table 1.

Table 1. Retrieved DNA sequences of *growth differentiation factor 9* gene of selected poultry species with their accession numbers and sequence lengths

Species	DNA sequence accession number	Base pair	Protein seq. Accession number	Amino acid sequence length
Chicken	NM_206988.3	1707	NP_996871.3	457
Turkey	XM_003210484.4	1903	XP_003210532.1	457
Ostrich	XM_009678924.1	1341	XP_009677219.1	446

Alignment of multiple deoxyribonucleic acid (DNA) and protein sequences

Alignment of the multiple DNA and protein sequences were done using CLUSTAL-W software incorporated by Online Analysis Tools. Through this software utilization, a pairwise comparison was conducted and percentage similarities of all the retrieved sequences were obtained.

Phylogenetic analysis

Evolutionary relationships using the sequences of *growth differentiation factor 9* gene retrieved from NCBI was conducted using Molecular Evolution and Genetic Analysis (MEGA) software, version 11. The phylogenetic tree was constructed using this software. A scale length of 0.05 was used for the construction of the tree.

Results

Retrieved of deoxyribonucleic acid (DNA) and protein sequences

Table 1 shows the accession, base pair and amino acid length numbers of DNA and protein sequences of the *growth differentiation factor 9* gene of chicken, turkey and ostrich which were retrieved from the National Centre for Biotechnology Information (NCBI). The *growth differentiation factor 9* gene and protein sequences of chicken, turkey and ostrich ranged from 1341 to 1903 bp and 446 to 457 bp, respectively.

Multiple alignment of DNA sequences

Figure 1 shows *growth differentiation factor 9* gene sequences of chicken, turkey and ostrich which were aligned using CLUSTAL-W incorporated by Online Analysis Tools. The percentage similarity of chicken and turkey DNA sequences was found to be 78.45% and 92.80% between chicken and ostrich. On the other hand, turkey and ostrich shared 83% similarity in their *GDF9* gene sequences.

```
Chicken          ------------------------------------------------------------
Turkey           ACGTGCGAAGACTCAGCCCGGCCGCACGCACACACGTGGGTTCCACAGCCAGCTGCGGAG
Ostrich          ------------------------------------------------------------

Chicken          ------------------------------------------------------------
Turkey           CTTTGCCTTTGGTTTCTAATCACAAAGAAATCGCACACAGCTGAAACGTATATAAGGGCT
Ostrich          ------------------------------------------------------------

Chicken          -------------------------GAATGCTTAGGGGGAGGCGTTGTTAGGCTACAGCC
Turkey           CGTGCTCGCATGCTCTTTTTCTCTCGAATGTTTTGGGGGAGGCGTTGTTAGGCTGCAGCT
Ostrich          ------------------------------------------------------------

Chicken          CTGAAGAGCATGGGGGGTACGTGGAGGATCTGTGTTTGTTTCTATTGCTGCCTTCACTGG
Turkey           CCGAAGAGCATGGCGGGTACCTGGAGGATCTGCGTTTGTTTCTATTGCTGCATTCAGTGG
Ostrich          ---------ATGGGGAGTCCTTGGAGAATTTGTGTTTGTTTCTATTGCTGTATTCACTGG
                           **** * ** * ***** ** ** ****************  **** ***

Chicken          CTTTCTTCCAGCATCCAGTGCTCCCCCCGCTCCAGGGATCGCACAGCACCTGACAAGGTC
Turkey           CTTTCTTCTAGCATCCAGTGCTCCCCCCGCTCCAGGGATCGCGCAGCACCTGACAAACTC
Ostrich          CTTTCTTCTAGCATCCAGTGTTCCCCCCGCTCCAGGGATCATGTAGTGTCTGACAAGACC
                 ******** ********** ******************    **   *******   *

Chicken          TCCGGGCTCCTGGGAGCTCCTGAGC------TCAGCGAGCTCCACCCGCTGCTGCGGCTG
Turkey           TCCGGGCTCCTGGAAGCTCCTGAGC------TCAGCGAGCTCCACCCGCTGCTGCGGCTG
Ostrich          TCCGGGTTACTGGTAGCTCCTGAGGACTACACCAGTAAGCTAAATCCGTTATTGCGGCTT
                 ****** * **** **********        ***  ****  * *** *  *******

Chicken          CCCAAGGGCGTGAGCCGAGGCTATGCGCTCCTGCCCCCCCTGCTCGAAGTGCTGTCTGAC
Turkey           CCCCAGGGTGCGAGCCGAGGCTGTGCCCTCCTGCCCCCCCTGCTCAAAGTGCTGTCCGAC
Ostrich          CCAAAAGGTGTGAGACGTGGATATGCCCTCCTGCCTCCCCTTCTCAAGGTGCTGTCTGAC
                 **  * ** * *** ** ** * *** ******** ***** *** * ******** ***

chicken          CACAGGGACCCGATTACAGGAGACTTTCACTCGGTGGATTTGCTCTTCAACCTGGATCGT
Turkey           CACAGGGATCTGATTACAGGAGACCTTCACTCGGTGGATTTGCTCTTCAACCTGGATCGT
Ostrich          CACAGGGACCTAATGAAAGGAGACCTTCACTCGGTGGATTTGCTCTTCAACCTGGATCGT
                 ******** *  ** * ******* ************************************

Chicken          GTTACTGCTCTCGAGCACTTACTCAAGTCTGTCTTGCTCTACTCGTTTGACACCTCAGTT
Turkey           GTTACTGCTCTCGAGCACTTACTCAAGTCTGTCTTGCTCTACTCATTTGACACCTCAGTT
Ostrich          GTTACTGCTCTAGAGCACTTACTCAAGTCTGTCTTGCTCTATTCCTTTGACACATCAGTT
                 *********** ***************************** ** ******** ******

Chicken          CAGGGACGTCAGAGCTGGGAAAGCGGGACCCCCAGGCTCCAGCCGGACTCCCGAGCCCTG
Turkey           CAGGGACGTCAGAGCTGGGAAAGCGGGACCCCCAGGCTCCAGCCGGACTCCCGAGCCCTG
Ostrich          CAGGGACACCAGAATTGGGAAAGTGAGACTCCCAGGCTTCAGCCAGACTCCAGAGCCCTT
                 *******  ****  ******** * *** ******** ***** ****** *******

Chicken          AGGTACATGAAGAGGCTGTATAAGATGTACGCCACCAAGGAGGGAATCCCAAAGGCCCAT
Turkey           CGGTACATGAAGAGGCTGTATAAGATGTATGCCACCAAGGAGGGAATCCCGAAGGCCCAC
Ostrich          AGGTACATGAAGAGGCTATATAAGATGTCTGCCACCAAGGAGGGAATCCCAAAGGCCAAC
                  **************** **********  ******************** ****** *

Chicken          AAGAGCCACCTCTATAACACTGTTCGACTTTTCACCCCGTGTTCTGAGTGCCAGCACCGC
Turkey           AAGAGCCACCTCTATAACACCGTTCGACTTTTCACCCCGTGTTCCGAGTGCCAGCACCGC
Ostrich          AAAAGTCACCTTTATAACACTGTTCGACTTTTCACTCCGTGTTCTGAATGCAAGCACCGC
                 ** ** ***** ******** ************** ******** ** *** ********
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```
Chicken     CCCACTTCTTC---CTTTACGTGCACGTGCCATTTGTCTGTGAAGGAGCATGATTTTTCT
Turkey      CCCACTTCTTC---CTTTATGTGCACGTGCCATTTGTCTGTTAAGGAGCATGATTTTTCT
Ostrich     CCCATTTCTTCTGCCACTACATGCATGTGCCATTTATCTGTTACGGAATATGATTTTTCT
            **** ******   *  **  **** ********* ***** * ***  **********

Chicken     AGCCAAGTATGTCCCAGCGTTTCACACTCCGTAGCTTTTAGCCTGCACTTTGAAGTTAGA
Turkey      AGCCAAGTATGTCCCAGCGTTTCGCACTCCGTAGCTTTTAGCCTGCACTTTGAAGTTAGA
Ostrich     AGCCAAGTGTGTCCAAGTGTTTCACACTCTATAGCTCTTAGTTTGCACGTTGAAGTTAGA
            ******** ***** ** ***** *****  ***** ****  ***** **********

Chicken     AAGCGCAAGTGGGTTGAGATTGATGTGACTTCTTTTCTGCGGCCTCTCATTGCTACTAAC
Turkey      AAGCGCAAGTGGGTTGAGATCGATGTGACTTCTTTTCTGCAGCCTCTCATTGCTGCTAAC
Ostrich     AAACGCAAGTGGGTTGAGATTGATGTGACTTCTTTTCTCCAGCCTCTAATTGCTACTAAC
            ** ***************** ***************** * ****** ****** *****

Chicken     AGGAGGAATATTCATATGGCTGTGAACTTCACTTGTCTGACGGGTAACCCGCAACATAAC
Turkey      AGGAGGAATATTCACATGGCTGTGAACTTCACTTGTCTTTTGGGTAACCCACAACATAAC
Ostrich     AGGAGGAATATTCATATGGCTGTAAACTTCACTTGTCTAATGGGTGATCCACAACATAAC
            ************** ******** **************   **** * ** *********

Chicken     ACTAAACAGGATAATCTCATTAATGTGGCTCTGGTCCCCCCTTCTCTTCTTCTTTACCTA
Turkey      GCTAAACAGGATAATCTCATTAATATGGCTCTGGTCCCCCCTTCTCTTCTTCTTTACCTA
Ostrich     ACTAAACAGGAAAATTCCATTAACATGGCACTGGTTCCCCCTTCTCTTCTTCTTTACCTG
             ********** ***  ******  **** ***** ************************

Chicken     AATGATACCAGCGAGCAAGCTTATCACAGGTGGAACTCACTTAGACACAGAAGGAAAAGC
Turkey      AATGATACCAGCGAGCAAGCTTATCACAGGTGGAACTCACTCAGGCACAGAAGGAAAAGC
Ostrich     AATGATACCAGTGAGCAAGCTTATCACAGGTGGAACTCACTTAGGCATAGAAGGAAAAAC
            *********** ******************************** ** ** ********** *

Chicken     CCGGTGCGGCCCAAGCAAAGGAGCAGTCTGTTTGCTGATGTGACAGGTGATGAAGGAAGA
Turkey      CCGGTGCGGCCCAAGCAAAGGAACAGTCTGTTCGCTGATGTGACGGGTGGCCAAGGAAGA
Ostrich     CCAGTGTGGCCCAGGCAAAGGAACAATCTGCTTGCTGATCCTATGGATGGCCAAGGAAAA
            ** *** ****** ******** ** **** * ******   *  * **   ****** *

Chicken     CAGAACACGCAGGGTAAAAGGGCATCTCGGCATCGAAGAGAAGAGAATCTGAAGGAAGCT
Turkey      GAGAACACGCAGGGTAAAAGGGCATCTCGGCATCGAAGGGAAGAGAATCTGAAGGAAGCT
Octrich     GAAAATACACAGAGTAAAAGGGCCTCTCGACGTCGAAGAGATGAGCATTTGAAAGAAGCT
             * ** ** *** ********** ***** * ****** ** *** ** **** ******

Chicken     CCAGCGACTGCGCCTCAAAACTTGAGTGAATATTTCAAACAATTCCTGTTTCCTCAAAAC
Turkey      CCAGCAACTCCGCCTCAAAATTTGAGTGAATATTTCAAACAATTTCTGTTTCCTCAAAAC
Ostrich     CCAGCAGCACCACCTTATAATTTGAGTGAATATTTCAAACAGTTTCTGTTTCCTCAGAAC
            *****  *  * *** * ** ********************** ** *********** ***

Chicken     GAGTGTGAGCTCCACAGCTTCCGTTTAAGTTTTAGCCAATTAAAATGGGACAAATGGATA
Turkey      GAGTGTGAGCTTCACAGCTTCCGTTTAAGCTTTAGCCAACTAAAATGGGACAAATGGATA
Ostrich     GAGTGTGAGCTTCACAACTTTCGTCTAAGTTTTAGCCAACTAAAATGGGACAAATGGATA
            ********** **** *** *** **** ********* ********************

Chicken     ATAGCCCCGCATCGATACAGCCCTCCAGTACTGCAAGGGTGACTGTCCGAGGGTAGTCGG
Turkey      ATAGCACCGCATCGATACAGCCCTC-AGTACTGCAAGGGTGACTGTCCCAGGGTGGTTCG
Ostrich     ATAGCACCTCATAGGTACAGCCCTC-AGTATTGCAAAGGTGACTGCCCAAGAGTAGTTGG
            ***** ** *** * ********** **** ***** ******** ** ** ** **  *
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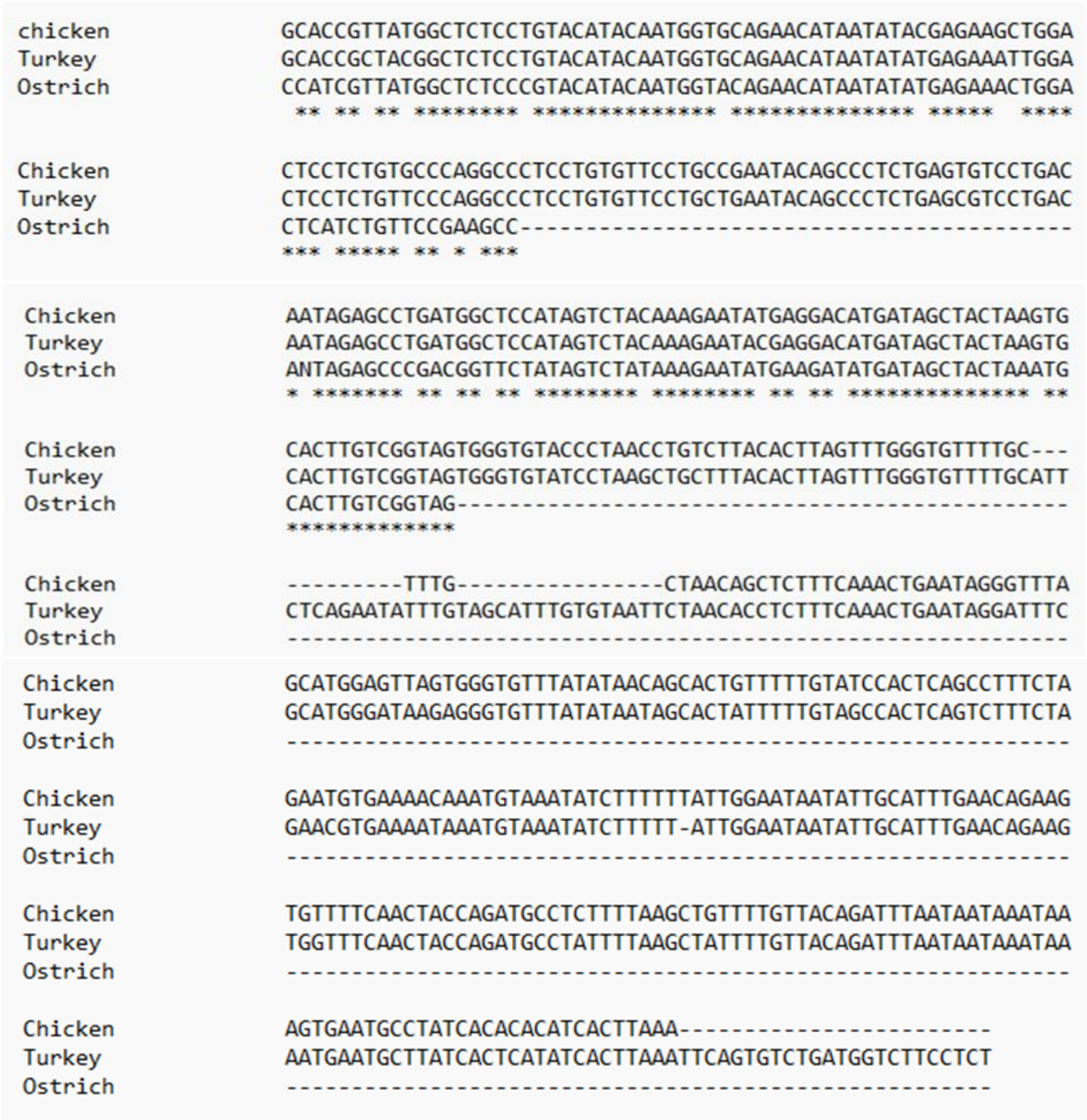

Figure 1. Multiple sequence alignment of *GDF9* DNA sequences of chicken, turkey and ostrich

Multiple alignment of protein sequences

Figure 2 shows g*rowth differentiation factor 9* protein sequences of chicken, turkey and ostrich which were aligned using CLUSTAL-W incorporated by Online Analysis Tools. The results showed that the percentage similarity of *GDF9* protein sequences of the selected poultry species ranged from 84-95% The percentage similarity of chicken and turkey protein sequences was found to be 94.74% and 83.63% between chicken and ostrich. On the other hand, turkey and ostrich shared 84,08% similarity in their *GDF9* protein sequences.

```
Chicken         MGGTWRICVCFYCCLHWLSSSIQCSPRSRDRTAPDKVSGLLGAPELS--ELHPLLRLPKG
Turkey          MAGTWRICVCFYCCIQWLSSSIQCSPRSRDRAAPDKLSGLLEAPELS--ELHPLLRLPQG
Ostrich         MGSPWRICVCFYCCIHWLSSSIQCSPRSRDHVVSDKTSGLLVAPEDYTSKLNPLLRLPKG
                *...**********::**************:...** **** ***    :*:******:*

Chicken         VSRGYALLPPLLEVLSDQGRQSWESGTPRLQPDSRALRYMKRLYKMYATKEGIPKAHKSH
Turkey          ASRGCALLPPLLKVLSDQGRQSWESGTPRLQPDSRALRYMKRLYKMYATKEGIPKAHKSH
Ostrich         VRRGYALLPPLLKVLSDQGHQNWESETPRLQPDSRALRYMKRLYKMSATKEGIPKANKSH
                . ** *******:******:*.*** ******************** *********:***

Chicken         LYNTVRLFTPCSECQHRHRDPITGDFHSVDLLFNLDRVTALEHLLKSVLLYSFDTSVPTS
Turkey          LYNTVRLFTPCSECQHRHRDLITGDLHSVDLLFNLDRVTALEHLLKSVLLYSFDTSVPTS
Ostrich         LYNTVRLFTPCSECKHRHRDLMKGDLHSVDLLFNLDRVTALEHLLKSVLLYSFDTSVPIS
                **************:***** :.**:******************************** *

Chicken         -SFTCTCHLSVKEHDFSSQVCPSVSHSVAFSLHFEVRKRKWVEIDVTSFLRPLIATNRRN
Turkey          -SFMCTCHLSVKEHDFSSQVCPSVSHSVAFSLHFEVRKRKWVEIDVTSFLQPLIAANRRN
Ostrich         SATTCMCHLSVTEYDFSSQVCPSVSHSIALSLHVEVRKRKWVEIDVTSFLQPLIATNRRN
                 :  * *****.*:************:*:***.***************:****:****

Chicken         IHMAVNFTCLTGNPQHNTKQDNLINVALVPPSLLLYLNDTSEQAYHRWNSLRHRRKSPVR
Turkey          IHMAVNFTCLLGNPQHNAKQDNLINMALVPPSLLLYLNDTSEQAYHRWNSLRHRRKSPVR
Ostrich         IHMAVNFTCLMGDPQHNTKQENSINMALVPPSLLLYLNDTSEQAYHRWNSLRHRRKNPVW
                ********** *:****:**:* **:*****************************.**

Chicken         PKQRSSLFADVTGDEGRQNTQGKRASRHRREENLKEAPATAPQNLSEYFKQFLFPQNECE
Turkey          PKQRNSLFADVTGGQGRENTQGKRASRHRREENLKEAPATPPQNLSEYFKQFLFPQNECE
Ostrich         PRQRNNLLADPMDGQGKENTQSKRASRRRRDEHLKEAPAAPPYNLSEYFKQFLFPQNECE
                *:**..*:**  ..:*::***.*****:**:*:******:.* ****************

Chicken         LHSFRLSFSQLKWDKWIIAPHRYSPQYCKGDCPRVVGHRYGSPVHTMVQNIIYEKLDSSV
Turkey          LHSFRLSFSQLKWDKWIIAPHRYSPQYCKGDCPRVVRHRYGSPVHTMVQNIIYEKLDSSV
Ostrich         LHNFRLSFSQLKWDKWIIAPHRYSPQYCKGDCPRVVGHRYGSPVHTMVQNIIYEKLDSSV
                **.******************************** **********************

Chicken         PRPSCVPAEYSPLSVLTIEPDGSIVYKEYEDMIATKCTCR
Turkey          PRPSCVPAEYSPLSVLTIEPDGSIVYKEYEDMIATKCTCR
Ostrich         PKPX--------------EPDGSIVYKEYEDMIATKCTCR
                *:*              **********************
```

Figure 2. Multiple sequence alignment of *GDF9* protein sequences of chicken, turkey and ostrich

Phylogenetic analysis

The evolutionary relationship among the DNA and protein sequences of *GDF9* gene of chicken, turkey and ostrich and other selected livestock species such as sheep, goat and cattle, are shown in Figure 3 and Figure 4, respectively. The relative taxa were grouped together in the bootstrap test (1000 replicates) and the percentage of the replicate trees is exhibited near the branches. The phylogenetic study revealed that the phylogenetic relationship of the chicken, turkey and ostrich grouped together with a bootstrap probability of 100%, equivalent to a confidence level of 99%. Chicken and turkey were found to share the last internal node on the phylogenetic trees

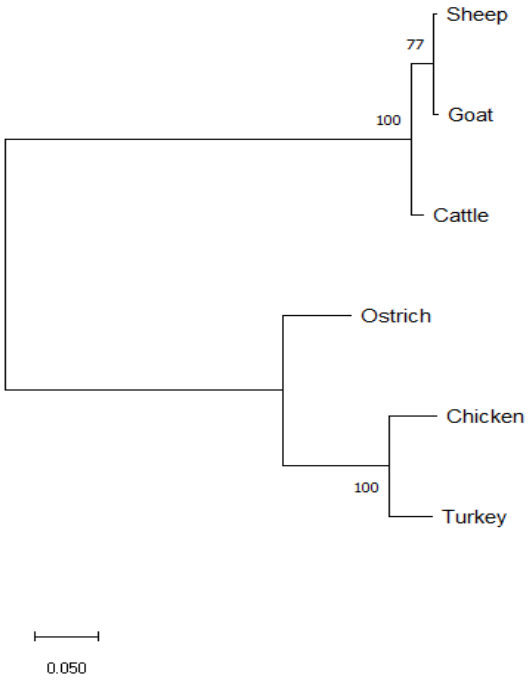

Figure 3. Phylogenetic tree showing the evolutionary relationship among poultry species and other selected livestock species using mRNA sequences.

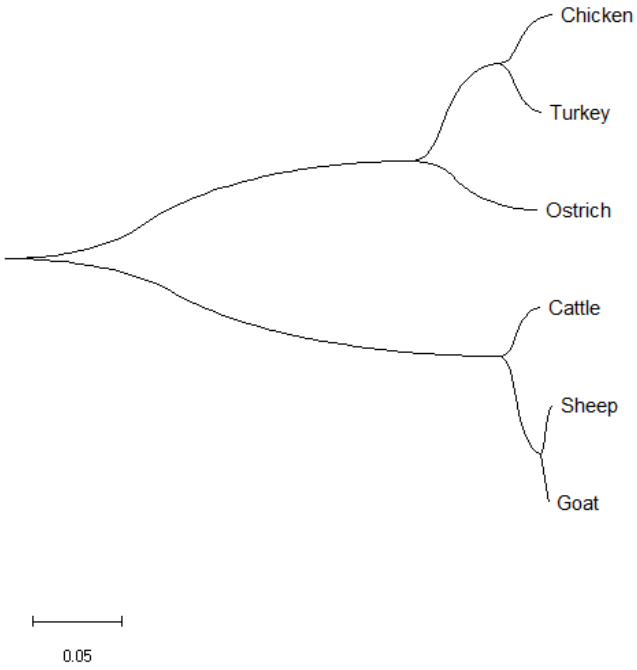

Figure 4. Phylogenetic tree showing the evolutionary relationship among poultry species and other selected livestock species using protein sequences.

Discussions

Bioinformatic tools, such as multiple sequence alignment and phylogenetic analysis, involve the use of computer softwares to make sense of great amounts of biological data (Madiajagan and Raj, 2019). The objective of the current study was to computationally investigate the molecular genetic variation of *growth differentiation factor 9* gene in poultry species using multiple sequence alignment and phylogentic analysis whereby CLUSTAL-W was used to align DNA, and protein sequences and determine the percentage similarities while MEGA11 software was used to carry out the evolutionary study. Percentage similarity values between the *GDF9* mRNA and Protein sequences of chicken, turkey, and ostrich ranged from 78.45-92.80% and 83.63-94.74%, respectively, meaning that the species are highly similar in function. These high levels of similarities in *GDF9* gene sequences of chicken, turkey and ostrich is proof that the gene is very effective for its biological function improvement (Egom *et al*., 2019). The high similarity percentages also imply that the *GDF9* gene at which each poultry species evolved is due to divergence, meaning that the species sharing similar ancestry acquired differences, gradually leading to speciation throughout the course of evolution (Gautam, 2020). The results are supported by Koonin and Galperin (2003), who documented that the likelihood that two sequences began independently of one another and became similar purely by coincidence decreases with increasing similarity between them. The phylogenetic studies revealed that since the selected poultry species clustered together into the same taxon, they have evolved from a common ancestor (Egom *et al.,* 2019). The results further showed that chicken and turkey share a most recent common ancestor, as depicted by the last internal node which is furthest from the root of the evolutionary tree.

According to Egom *et al*. (2019), species sharing a most recent common ancestor will have a high percentage similarity value. This scenario is observed in the findings of the phylogenetic analysis of protein sequences in this study, whereby chicken and turkey share the highest similarity value and the most recent common ancestor. The reports, however, are in contradiction with the phylogenetic analysis of the DNA sequences which shows that chicken and ostrich do not share a most recent common ancestor regardless of having high percentage similarity of their *GDF9* gene sequences. Egom *et al*. (2019) and Divya *et al*. (2018) further reported that chicken and turkey do not share a most recent common ancestor thus in line with the phylogenetic analysis of the protein sequences and in contradiction with those of the DNA sequences. These differences may occur as a result of conducting the study with different genes. The study only used three poultry species; hence further studies can be done to improve egg production through *growth differentiation factor 9* gene using other poultry species.

Conclusion

The study concluded that *GDF9* mRNA and protein sequences of chicken, ostrich and turkey were highly similar, and that the species were derived from a common ancestor thus these species can be improved similarly or through the other. The current study recommends that researchers focusing on *GDF9* for improving egg production in chicken might also improve egg production in ostrich and vice versa. It is also recommended that researchers may improve chicken and turkey for egg production under similar conditions.

Competing interest

There is no conflict of research interest.

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