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Achillea Millefolium Based on Bioinformatics Studies and Toxicity Test on The Chicken Embryo

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***Abstract**. In Iranian Traditional Medicine, some herbs like Achillea millefolium, commonly known as yarrow, are implicated as appetite enhancers. However, there is not enough research evidence to prove their actual effect. Achillea millefolium is a dicot which belongs to the family Asteraceae. The purpose of this study was the analysis Achillea millefolium based on a bioinformatic study and toxicity test on the chicken embryo. This research method consisted of conversion of nucleotides into amino acids, analysis of the three-dimensional structure of Achillea millefolium protein, epitope and Allergen Proteins, antigens and toxins and toxicity test on the chicken embryo. In addition, this study also obtained the results of proteins that are epitope, antigenic, non-allergenic and non-toxic and toxicity test on the chicken embryo was 250 ng/egg.. Morphological description of the embryo on the 21st day after injection, at a concentration of 250 ng of Mentha piperita/egg product, an abnormal embryological picture was obtained. Chicken Embryo Weight and Body Length Measurements were carried out in chicken embryos. Need research for other species of plant.*

***Keywords:** Achillea Millefolium, Bioinformatics, Chicken Embryo, Chicken Semicolon, Toxicity Test.*

1. BACKGROUND

Herbs like yarrow (*Achillea millefolium*) are said to stimulate hunger in Iranian traditional medicine. But there isn't enough data to say for sure how effective they are. A member of the Asteraceae family, *Achillea millefolium* is a kind of dicot (Peighambardoust et al., 2019). Currently, *Achillea millefolium* may be found throughout the majority of temperate zones, including northern China, Europe, Canada, and Iran. It has a long history of medicinal usage throughout many cultures, particularly for the treatment of dyspeptic (digestive) issues, problems with the liver and gallbladder, and a lack of hunger. Yarrow, or *Achillea millefolium,* is a common herb in traditional medicine from many different nations that is used as an appetizer and to alleviate dyspepsia (Hartady et al., 2021; Jachimowicz et al., 2022). Results showed that the orexigenic impact of yarrow extract varied with dosage in this investigation. Rats' food intake was shown to be increased at concentrations of 50 and 100 mg/kg of extract 2 hours after gavage, while at 150 mg/kg of extract, the rats' hunger was suppressed for the first 6 hours after gavage and thereafter decreased. It is unclear why 150 mg/kg of extract causes a reduction in hunger. It might be because of the negative effects of A. millefolium when taken in large quantities. A small number of research on yarrow's orexigenic effects have shown that it increases broiler chickens' food intake, which is a growth indicator. Additionally, broiler chicks were fed 10g/kg of powdered yarrow every day and their food intake was assessed once

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a week by Cross and colleagues. Consequently, they ate more and performed better (Fathi Najafi et al., 2021; Hartady et al., 2021). The goal of this research was to examine *Achillea millefolium* using bioinformatics and toxicity testing on embryos of chickens.

2. THEORITICAL STUDY

This is a feasible study because it combines bioinformatic analysis and in vivo toxicity experiment in chicken embryos to examine the possible therapeutic efficacy of *Achillea millefolium* thoroughly. Although other studies have characterized the phytochemical content and overall cardiovascular efficacy of hawthorn extracts, not many studies have integrated the computational forecasting of interactions between bioactive compounds and experimental embryonic toxicity. The current paper gives a more mechanistic insight into the bioactive entities of hawthorn by revealing molecular targets by bioinformatics and validating safety profiles using embryo-based assays. This is an integration of the available literature as the blending of the two evidence (computational and biological) provided a strong scientific foundation on which the therapeutic applications of *Achillea millefolium* can be applied in the future.

3. RESEARCH METHODS

Conversion of nucleotides to amino acids

The Expasy Translate Tool program was used to translate the nucleotides of *Achillea millefolium* from the National Center for Biotechnology Information (NCBI), GenBank: HM590229.1, into amino acids.

Analysis of the three dimensional structure of *Achillea millefolium* Protein

The study was conducted in accordance with the Protein Structure Homology Modeling Using SWISSMODEL Workspace approach in order to anticipate the three-dimensional structure of the *Achillea millefolium* protein via homology:

Epitope and allergen protein analysis

In order to produce epitope-specific proteins, the IEDB program incorporates the amino acids of *Achillea millefolium*. In order to get allergen protein, the Allertop program includes Mentha piperita amino acids.

Analysis of proteins that are antigenic and toxin

The Vaxijen program incorporates the amino acids of *Achillea millefolium* in order to generate antigenic proteins. The Toxinpred program incorporates the amino acids of *Achillea millefolium* to generate proteins that are toxic and those that are not:

Toxicity test of *Achillea millefolium* on the chicken embryo

In order to find out how *Achillea millefolium* affects the growth of chicken embryos, an experiment was devised in which a number of sprouting chicken eggs were given varying amounts of the plant. Here, the *Achillea millefolium* product doses are divided into six levels.

1. "control (0 ng of *Achillea millefolium* /eggs);
2. 15.6 ng of *Achillea millefolium* /egg products;
3. 31.2 ng of *Achillea millefolium* /egg products;
4. 62.5 ng *Achillea millefolium* /egg product;
5. 125 ng *Achillea millefolium* /egg product;
6. 250 ng *Achillea millefolium* /egg."

For each treatment dosage, 200 ul of solution was injected into an egg via the allantois space route. One hundred twelve-day-old brood chicken eggs were injected with 200 ul/egg of each treatment (treatments 1–6) using the air bag line. The first treatment, which served as a control, consisted of injecting 12-day-old sprouting chicken eggs with a 1 ml solution devoid of honey ingredients. After reinserting liquid paraffin into the injection hole, it was placed in an incubator and monitored daily using candling. At the 21-day mark, two eggs were removed from each treatment to examine the embryo morphology and any potential problems. It was necessary to measure and examine the newborn chicks (DOC) for any observable morphological defects.

4. RESULTS AND DISCUSSION

The amino acid composition of *Achillea millefolium* may be observed in table 1, which is derived from the results of translating nucleotides into amino acids.

Table 1 Nucleotides of *Achillea millefolium* after conversion to amino acids.

No	Protein Sequence
1	"GYWAALKAFSLGKSLSTGTSKSFFVRQTNKIKKVIKKRGKP NNINRPDSKIEPFRFKKKFPNSISWINPEMEFFCLLPNSNKVFL TKKHKILDLHFSIFSFQDGSLIFPINLFVLDFLFCTTFLLLDFLIL ISPYISGHEFTQKHLMKIFWINMCEFIINIFLFIDKTYFWVVLFI KINQKRSIKKMFFGGGLIHSKTCWFYKSPSREESKIYNKNKTL NINEPSSIYLNKFLFIDLNLSYKILHSIEFLNLDDFLFIG
2	AIGRRKHFLGNLFLPGLPKVFLCDKQIKSKNKLLRNKGENLR TIIDLTQKLNPSVSKKNSPIPFPGELIHRKWNSSVYFDRIQTKF

	FQKNTRYSICILVYFLSRMGVLFFPSTYLYYKIFFFVQLSYFWI FYKFLYRAHISLAMNSRKNTKYSGICVNFESKIFFCSLIKLIFG LYFFKLKIKNGQLKKCFLGGAFIVRLAGFTRVPVEKSLKSTIK IKPTKLMNLQVYITSFYLSIIFPTKYCTQLNSITISYS
3	LLGGVKSIFFREISFYRDFQKFFCATNKNQKISYEIKGKTLEQ YKSTLKNTLPFQKKIPQFHFLVNSIGNGILLFTLTEFKQSFFNK KTQDTRFAFYIFFPGWESYFSHQPICTIRFSFLYNFLTFGFSINS YIEPIYLWPIHAKTLNENILDKYVILNNLKYFFVHNLFLGCTF LNNKSKTVNKNVFWGGLNSDLLVLQESQSRRVNLQKNPKL NTFKYIFEQVFIYRFESFLQNIALNILKSRRFLIHR"

The research also included a three-dimensional structural investigation of a *Achillea millefolium* protein, with the outcome being six different three-dimensional models of the protein.

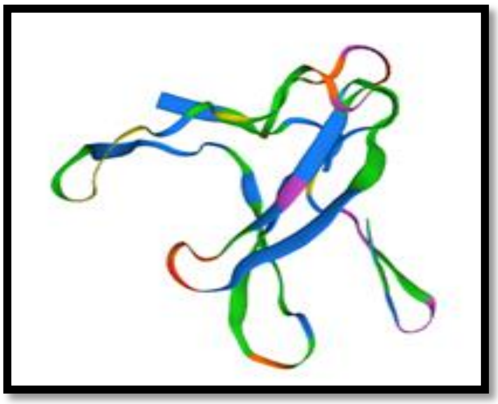

Figure 1. Analysis of the three-dimensional structure of the *Achillea millefolium* protein no 1.

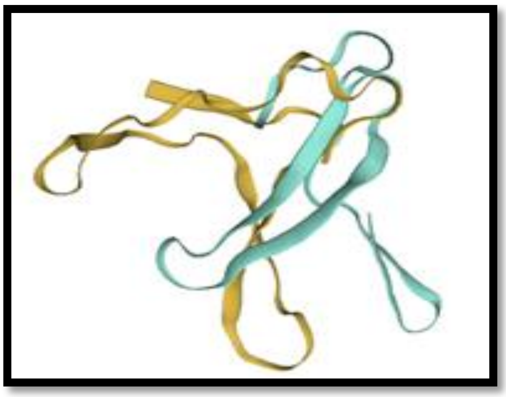

Figure 2. Analysis of the three-dimensional structure of the *Achillea millefolium* protein no 2.

Figure 3. Analysis of the three-dimensional structure of the *Achillea millefolium* protein no 3.

The research also gathered information on the ligand characteristics, GQME, and Qmean values for each protein in *Achillea millefolium*. *Achillea millefolium* protein binding site not conserved ligand was more dominant, and GQME and Qmean values differed, according to the study.

Table 2 Characteristics of *Achillea millefolium* Protein.

No	GQME Value	QmeanDisCo Value
1	0.21	0.13 ± 0.06
2	0.19	0.19 ± 0.09
3	0.05	0.17 ± 0.12

This research used the IEDB to examine the epitope-forming amino acids of *Achillea millefolium*. Also, *Achillea millefolium* was tested for allergens and non-allergens in its protein composition. Based on the study findings, it was discovered that most *Achillea millefolium* proteins were not allergenic. These findings were derived from epitope studies.

Table 3 *Achillea millefolium* Protein which is Epitope and Allergen.

Mentha piperita	Peptides that are Epitopes	Position	Allergen
1	YKILHSIEFLNLDDF	241–255	PROBABLE ALLERGEN
2	FTRVPVEKSLKS	203–214	PROBABLE ALLERGEN
3	FFREISFYRDF	9–19	PROBABLE NON-ALLERGEN

Antigenic and poisonous *Achillea millefolium* proteins were also analyzed in this work using the software packages Vaxijen and ToxinPred. Research conducted utilizing Vaxijen revealed that the vast majority of *Achillea millefolium* proteins exhibited antigenic properties. Furthermore, study employing ToxinPred established that most *Achillea millefolium* proteins are not hazardous. You can view the findings of study on the poisonous and antigenic proteins found in *Achillea millefolium* in table 4.

Table 4 *Achillea millefolium* Protein Which is Antigenic and Toxic.

No.	Epitope Peptides	Antigenic Peptides	Toxic Peptides
1	YKILHSIEFLNLDDF	Probable NON ANTIGEN	Toxin
2	FTRVPVEKSLKS	Probable NON-ANTIGEN	Non-Toxin
3	FFREISFYRDF	Probable ANTIGEN	Non-Toxin

Table 5 Chicken Embryo Weight after being injected with *Achillea millefolium*.

Achillea millefolium concentration	Chicken Embryo Weight (grams)
control 0 ng Achillea millefolium / eggs	7.75 ± 0.01
15.6 ng of Achillea millefolium / egg product	7.71 ± 0.03
31.2 ng of Achillea millefolium / egg product	7.75 ± 0.15
62.5 ng Achillea millefolium / egg product	7.71 ± 0.07
125 ng Achillea millefolium / egg product	7.73 ± 0.19
250 ng Achillea millefolium / egg	7.71 ± 0.11

Table 6 Body length of chicken embryo 21 days after injection with *Achillea millefolium*.

Achillea millefolium concentration	Embryo Length (cm)	Leg Length (cm)	Wing Length (cm)	Bust (cm)
control 0 ng Achillea millefolium / eggs	7.17 ± 0.17	7.20 ± 0.37	4.26 ± 0.51	5.07 ± 0.72
15.6 ng of Achillea millefolium / egg product	7.17 ± 0.14	6.39 ± 0.37	3.95 ± 0.59	5.16 ± 0.78
31.2 ng of Achillea millefolium / egg product	7.67 ± 0.15	6.42 ± 0.34	4.09 ± 0.50	5.35 ± 0.07

62.5 ng Achillea millefolium / egg product	8.27 ± 0.21	6.49 ± 0.32	4.80 ± 0.58	5.54 ± 0.79
125 ng Achillea millefolium / egg product	7.27 ± 0.19	6.23 ± 0.34	4.05 ± 0.53	5.31 ± 0.75
250 ng Achillea millefolium / egg	7.17 ± 1.10	6.20 ± 0.36	5.15 ± 0.55	5.25 ± 0.77

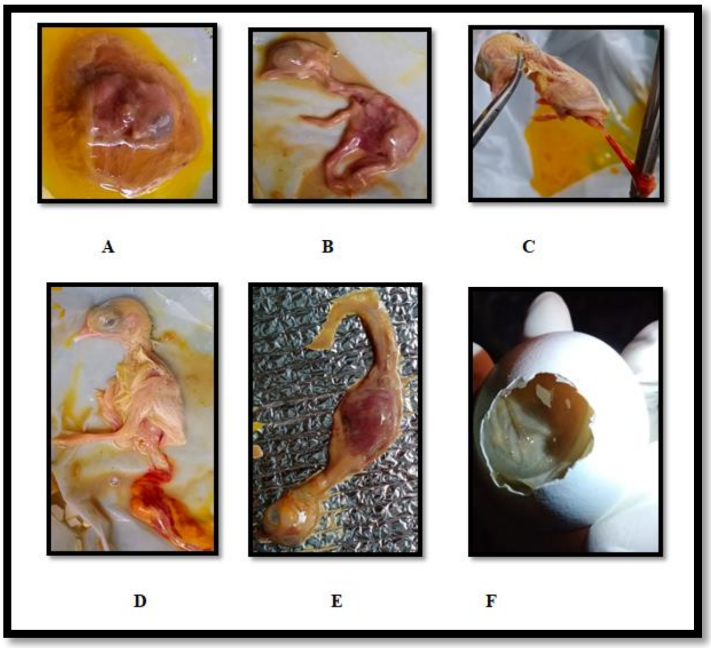

Figure 4 Observation of embryo morphology in each treatment. "A. control 0 ng *Achillea millefolium* /eggs, B.15.6 ng of *Achillea millefolium* /egg product, C.31.2 ng of *Achillea millefolium* /egg product, D.62.5 ng *Achillea millefolium* /egg product, E.125 ng *Achillea millefolium* /egg product, F.250 ng *Achillea millefolium* /egg."

There is an endless supply of potential novel antiviral medications made from natural sources, such as pure chemicals and plant extract scan (Michalczuk et al., 2021; Torki et al., 2021). Viruses that cause infectious illnesses have always been a major problem for people and animals across the world. Because viruses rely on host cells for their survival, there are currently very few antiviral medications that can effectively cure viral infections. The search for novel antiviral compounds with both intracellular and extracellular activity is an urgent matter nowadays. While acting on viruses, these chemicals must be gentle on host cells (Ramana & Mehla, 2020; Zhang et al., 2020). The in vivo and in vitro plant studies are costly and time-consuming, thus the in ovo injection approach was developed as an alternative. This approach began with an in ovo screening of plants for antiviral activity, followed by the selection of the most promising plants for further processing (Bhanja & Mandal, 2005; Oli et al., 2020). Geographical distribution, collecting season, and environmental and climatic conditions of the location all have a role in the quantity of active components found in the plants. Alkaloids, terpenes, flavonoids, naphthoquinones, coumarins, and anthraquinones are among the substances found in abundance in many plants used in traditional medicine to treat viral illnesses, according to studies (Jasemi, 2020; Mahfuz et al., 2021). These chemicals destroy the virus or prevent it from replicating by blocking its enzymes. For attachment and replication, two of NDV's most crucial glycoproteins—fusion proteins and haemaglutinin neuramidase—are required. Because some of these plant chemicals have protease inhibitory action, they may impede the cleavage of these glycoproteins and prevent the virus from attaching to their targets . These days, people are more concerned in the potential uses of plants and their components in conventional and alternative medicine. For thousands of years, people all over the world have turned to the healing properties of the *Achillea millefolium* plant, more often known as yarrow. It seems that A. millefolium first appeared in folk medicine in Europe before making its way to Asia. Researchers have shown that yarrow can fight against inflammation, tumors, antioxidants, bacteria, and viruses (Li et al., 2021; Park & Kim, 2020; Peighambardoust et al., 2019)

The four main structural components of a protein are the amino acids, peptide bonds, and quaternary ammonia. Amino acid sequences are organized in a linear fashion, much like the arrangement of letters in a word, and there is no branching in the fundamental structure . Hydrogen bonds between =CO and =NH groups along the polypeptide backbone linearly stabilize the combined primary and secondary structures of a protein, which is a two-dimensional structure. According to Taylor et al. (2001), the -helix is a kind of secondary structure (Taylor et al., 2001). A protein's tertiary structure is an extra layer on top of its secondary structure, which is a pattern of twisted bonds between the R groups (side chains) of different amino acids. According to Benkert et al. (2011) and Hardin et al. (2002), this structure is a three-dimensional conformation that describes the unique connection between secondary components (Benkert et al., 2011; Hardin et al., 2002) . So far, the homology/comparative approach, the fold recognition method, and the ab initio method have all been used to predict the three-dimensional structure of proteins (Waterhouse et al., 2018; Zaki & Bystroff, 2008). The target protein's amino acid sequence is aligned with a comparable protein whose three-dimensional structure is known in the lab (the template) in order to model the target protein's three-dimensional structure using the homology modeling approach (Hillisch et al., 2004; Sali & Blundell, 1993; Waterhouse et al., 2018). When compared to the fold identification and ab initio methods, the computing complexity of the homology process is lower. Additionally, compared to other methodologies, working with the homology method is quicker (Hillisch et al., 2004; Waterhouse et al., 2018; Zaki & Bystroff, 2008). The ab initio approach is used to model proteins when there is no template protein in the database. When compared to other approaches, the ab initio method is the most complicated, time-consuming, and challenging. Protein three-dimensional structures are modeled using the energy function as the basis of the ab initio technique (Hardin et al., 2002; Studer et al., 2020). Both the method's applicability and the precision it produces are limited to proteins with modest sizes (Kopp et al., 2007).According to Zaki and Bystroff (2008), fold recognition modeling is more challenging than homology but simpler than ab initio (Zaki & Bystroff, 2008). To create a structural model with the optimal fold value, the fold recognition technique compares the target sequence to a library of template structures (Hung & Samudrala, 2003). So, if you have a template protein structure, the homology modeling approach is the way to go for building an in silico three-dimensional model of a protein (Benkert et al., 2011; Kopp et al., 2007). According to Hillisch et al. (2004) and Kopp and Schwede (2004), homology modeling is extensively used in virtual screening, mutagenesis experiment design, and sequence variation impact analysis (Hillisch et al., 2004; Kopp et al., 2007). Research by Rose (2019), Sobolev et al. (2020), and Tam, Sinha, Wang (2020) revealed that the *Achillea millefolium* protein exhibited three distinct three-dimensional structures, each with its own unique set of properties (Rose, 2019; Sobolev et al., 2020; Tam et al., 2020).

The IDDT score and the global QMEANDisCo score, which is the average of the two, showed a strong correlation. The offered error estimates are derived from the root mean of squares (i.e. standard deviation) disparity between the global QMEANDisCo and IDDT scores (basic truth), which in turn are based on the global QMEANDisCo scores determined for several models. According to Studer et al. (2020) and Benkert et al. (2011), a given error estimate is determined using a model that is the same size as the input, as the reliability of the prediction is dependent on the size of the model (Benkert et al., 2011; Studer et al., 2020). The values that were acquired from the investigation are 0.13 ± 0.06, 0.19 ± 0.09, and 0.17 ± 0.12. This demonstrates that the mistake rate of the three *Achillea millefolium* bee proteins is modest. One such quality estimate is the Global Model Quality Estimate, or GMQE. It takes into account both the template-target alignment and template structural features. In order to forecast IDDT scores, they were fused using a trained multilayer perceptron. In order to choose the best template for the current modeling challenge, GMQE is accessible before the actual model is built. Research findings yielded values after model creation (Studer et al., 2020; Waterhouse et al., 2018).An immunoinformatics analysis was performed on the Mentha piperita protein in this work. This approach may be useful in the search for peptide vaccines, which are those that stimulate the immune system with just a little amount of antigen (about 8–15 amino acids) (He et al., 2010; Vita et al., 2018).A survey of immunogenic proteins was compiled from the investigations that were undertaken. The research also found that the proteins extracted from *Achillea millefolium* were safe and did not cause any allergic reactions. Molecular weights ranging from 10 to 70 KDa are characteristic of water-soluble glycoproteins, which are often allergenic compounds (Ipek et al., 2004) Mild symptoms including itching, watery eyes and nose, and swelling might be triggered by allergies. According to Oli et al. (2020), Ramana and Mehla (2020), and Chakraborty et al. (2021), severe responses like anaphylaxis may be fatal when caused by allergies (Chakraborty et al., 2014; Oli et al., 2020; Ramana & Mehla, 2020). Injecting eggs with amino acids, hormones, carbohydrates, or pollen extracts did not impact hatchability, according to Bhanja & Mandal (2005), Nowaczewski et al. (2012), Moore et al. (1994), Shafey et al. (2012), and Coskun et al. (2014), respectively (Bhanja & Mandal, 2005; Coskun et al., 2014; Moore et al., 1994; Nowaczewski et al., 2012; Shafey et al., 2012). Researchers found no significant influence on embryonic mortality from in-ovo injections of vitamin C, ascorbic acid, carbs, or glucose (Ipek et al., 2004; Nowaczewski et al., 2012; Sgavioli et al., 2015; Shafey et al., 2012). Results showed that both the weight and length of the embryonic body at 21 days post-honey injection were significantly different between treatments in this research. When certain nutrients are injected into the eggs during incubation, it might lead to nutritional imbalances and, as a result, restrict the embryo's maximum growth and development. There was no statistically significant change in weight between the control group and the group that received in-ovo vitamin E injections, according to Shafey et al. (2012) (Shafey et al., 2012). Taha et al. (2019) reported that RJ injection had a good impact on all metrics. The investigated parameters were unaffected by the various chicken strains (Taha et al., 2019). Researchers found that different chicken strains had different reactions to RJ inoculation. The fundamental objective of the novel in ovo technique is to introduce bioactive chemicals into eggs while the bird is still an embryo. As a result, we need to figure out how to put in ovo feeding technologies to good use in the poultry industry. This exploratory work has the potential to pave the way for further investigations into the optimal location for manipulating nutritional solutions and carriers for in ovo injection of embryos in laying hens (Pawłowska et al., 2022).The reproductive tract shape, egg production rate, and internal egg quality features of old laying hens may be improved by RJ therapy, according to El-Tarabany, Nassan, Salah (2021) (El-Tarabany et al., 2021)

5. CONCLUSIONS AND SUGGESTIONS

Based on the GQME and QmeanDisCo values, the validity of the six *Achillea millefolium* three-dimensional structures studied is high. Proteins that are epitope-specific, antigenic, hypoallergenic, and non-toxic were also identified in this research. On the twenty-first day after injection, a morphological analysis revealed an anomalous embryological image at a concentration of 250 ng of *Achillea millefolium*/egg product. Researchers measured the length and weight of chicken embryos. Research on other plant species is necessary.

REFERENCE LIST

Benkert, P., Biasini, M., & Schwede, T. (2011). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics, 27*, 343-350. https://doi.org/10.1093/bioinformatics/btq662

Bhanja, S. K., & Mandal, A. B. (2005). Effect of in-ovo injection of critical amino acids on pre- and post-hatch growth, immunocompetence and development of digestive organs. *Asian-Australasian Journal of Animal Sciences, 18,* 524-531. https://doi.org/10.5713/ajas.2005.524

Chakraborty, C., Sharma, A. R., Bhattacharya, M., Sharma, G., & Lee, S. S. (2014). Immunoinformatics approach for the identification and characterization of T cell and B cell epitopes towards the peptide-based vaccine against SARS-CoV-2. *Archives of Medical Research*, *52*(4), 362-370. https://doi.org/10.1016/j.arcmed.2021.01.004

Coskun, I., Cayan, H., Yılmaz, O., Taskin, A., Tahtabicen, E., & Samli, H. H. (2014). Effects of in-ovo pollen extract injection to fertile broiler eggs on hatchability and subsequent chick weight. *Turkish Journal of Agricultural and Natural Sciences, 1*, 485-489.

El-Tarabany, M. S., Nassan, M. A., & Salah, A. S. (2021). Royal jelly improves the morphology of the reproductive tract, internal egg quality, and blood biochemical parameters in laying hens at the late stage of production. *Animals, 11*(7). https://doi.org/10.3390/ani11071861

Fathi Najafi, T., Khadem, N., Bahri, N., Meshkat, M., & Sadri, S. (2021). The fertility outcome of royal jelly versus intra uterine insemination: A pilot randomized controlled trial study. *Jundishapur Journal of Natural Pharmaceutical Products, 16*(3), 107420. https://doi.org/10.5812/jjnpp.107420

Hardin, C., Pogorelov, T. V, & Luthey-Schulten, Z. (2002). Ab initio protein structure prediction. *Current Opinion in Structural Biology, 12*(2), 176-181. https://doi.org/10.1016/S0959-440X(02)00306-8

Hartady, T., Syamsunarno, M. R. A. A., Priosoeryanto, B. P., Jasni, S., & Balia, R. L. (2021). Review of herbal medicine works in the avian species. *Veterinary World*, *14*(11), 2889-2906. https://doi.org/10.14202/vetworld.2021.2889-2906

He, Y., Xiang, Z., & Mobley, H. L. (2010). Vaxign: The first web-based vaccine design program for reverse vaccinology and applications for vaccine development. *Journal of Biomedical Biotechnology*, *297505*. https://doi.org/10.1155/2010/297505

Hillisch, A., Pineda, L. F., & Hilgenfeld, R. (2004). Utility of homology models in the drug discovery process. *Drug Discovery Today*, *9*(15), 659-669. https://doi.org/10.1016/S1359-6446(04)03196-4

Hung, L.-H., & Samudrala, R. (2003). PROTINFO: Secondary and tertiary protein structure prediction. *Nucleic Acids Research*, *31*(13), 3296-3299. https://doi.org/10.1093/nar/gkg541

Ipek, A., Sahan, U., & Yilmaz, B. (2004). The effect of in-ovo ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. *Archiv Für Geflügelkunde*, *63*, 132-135. https://doi.org/10.1016/S0003-9098(25)00035-9

Jachimowicz, K., Winiarska-Mieczan, A., & Tomaszewska, E. (2022). The impact of herbal additives for poultry feed on the fatty acid profile of meat. *Animals*, *12*(9), 1054. https://doi.org/10.3390/ani12091054

Jasemi. (2020). Medicinal plants and phytochemicals for the treatment of pulmonary hypertension. *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2020.00145

Kopp, J., Bordoli, L., Battey, J. N. D., Kiefer, F., & Schwede, T. (2007). Assessment of CASP7 predictions for template-based modeling targets. *Proteins*, *69*(Suppl. 8), 38-46. https://doi.org/10.1002/prot.21753

Li, S., Tao, L., Yu, X., Zheng, H., Wu, J., & Hu, F. (2021). Royal jelly proteins and their derived peptides: Preparation, properties, and biological activities. *Journal of Agricultural and Food Chemistry*, *69*(48), 14415-14427. https://doi.org/10.1021/acs.jafc.1c05942

Mahfuz, S., Shang, Q., & Piao, X. (2021). Phenolic compounds as natural feed additives in poultry and swine diets: A review. *Journal of Animal Science and Biotechnology*, *12*, 48. https://doi.org/10.1186/s40104-021-00565-3

Michalczuk, M., Holl, E., Möddel, A., Jóźwik, A., Slósarz, J., Bień, D., Ząbek, K., & Konieczka, P. (2021). Phytogenic ingredients from hops and organic acids improve welfare and gut health in broilers. *Animals*, *11*(11), 3249. https://doi.org/10.3390/ani11113249

Moore, R. W., Dean, C. E., & Hargis, P. S. (1994). Effects of in-ovo hormone administration at day eighteen of embryogenesis on post-hatch growth of broilers. *Journal of Applied Poultry Research*, *3*, 31-39. https://doi.org/10.1093/japr/3.1.31

Nowaczewski, S., Kontecka, H., & Krystianiak, S. (2012). Effect of in-ovo injection of vitamin C during incubation on hatchability of chickens and ducks. *Folia Biologica*, *60*, 93-97. https://doi.org/10.3409/fb60_1-2.93-97

Oli, A. N., Obialor, W. O., Ifeanyichukwu, M. O., Odimegwu, D. C., Okoyeh, J. N., Emechebe, G. O., Adejumo, S. A., & Ibeanu, G. C. (2020). Immunoinformatics and vaccine development: An overview. *ImmunoTargets and Therapy*, *9*, 13-30. https://doi.org/10.2147/ITT.S241064

Park, J. H., & Kim, I. H. (2020). Effects of dietary *Achyranthes japonica* extract supplementation on the growth performance, total tract digestibility, cecal microflora, excreta noxious gas emission, and meat quality of broiler chickens. *Poultry Science, 99*(1), 463-470. https://doi.org/10.3382/ps/pez533

Pawłowska, J., Sosnówka-Czajka, E., & Skomorucha, I. (2022). Effect of the in ovo injection site of electrolytes on some biochemical blood parameters and quality of layer chicks. *Animals*, *12*(4), 532. https://doi.org/10.3390/ani12040532

Peighambardoust, S. H., Yaghoubi, M., Hosseinpour, A., Alirezalu, K., Soltanzadeh, M., Dadpour, M., & Hosseini, H. (2019). Development and application of dual-sensor labels with chitosan-based coating incorporating yarrow essential oil for chicken fillet shelf-life extension.

Ramana, J., & Mehla, K. (2020). Immunoinformatics and epitope prediction. In *Methods in Molecular Biology* (Vol. 2131, pp. 155-171). https://doi.org/10.1007/978-1-0716-0389-5_6

Rose, G. D. (2019). Ramachandran maps for side chains in globular proteins. *Proteins, 87*(5), 357-364. https://doi.org/10.1002/prot.25656

Sali, A., & Blundell, T. L. (1993). Comparative protein modelling by satisfaction of spatial restraints. *Journal of Molecular Biology, 234*(3), 779-815. https://doi.org/10.1006/jmbi.1993.1626

Sgavioli, S., Matos Júnior, J. B., Borges, L. L., Praes, M. F. F. M., Morita, V. S., & Zanirato, G. L. (2015). Effects of ascorbic acid injection in incubated eggs submitted to heat stress on incubation parameters and chick quality. *Brazilian Journal of Poultry Science, 17*, 181-190. https://doi.org/10.1590/1516-635x1702181-190

Shafey, T. M., Alodan, M. A., Al-Ruqaie, I. M., & Abouheif, M. A. (2012). In-ovo feeding of carbohydrates and incubation at a high temperature on hatchability and glycogen status of chicks. *South African Journal of Animal Science, 42*, 210-220. https://doi.org/10.4314/sajas.v42i3.2

Sobolev, O. V, Afonine, P. V, Moriarty, N. W., Hekkelman, M. L., Joosten, R. P., Perrakis, A., & Adams, P. D. (2020). A global Ramachandran score identifies protein structures with unlikely stereochemistry. *Structure, 28*(11), 1249-1258. https://doi.org/10.1016/j.str.2020.08.005

Studer, G., Rempfer, C., Waterhouse, A. M., Gumienny, R., Haas, J., & Schwede, T. (2020). QMEANDisCo-distance constraints applied on model quality estimation. *Bioinformatics, 36*, 1765-1771. https://doi.org/10.1093/bioinformatics/btz828

Taha, A. E., AbdAllah, O. A., Attia, K. M., El-Karim, R. E. A., El-Hack, M. E. A., El-Edel, M. A., Saadeldin, I. M., Hussein, E. O. S., & Swelum, A. A. (2019). Does in ovo injection of two chicken strains with royal jelly impact hatchability, post-hatch growth performance and haematological and immunological parameters? *Animals, 9*(8), 486. https://doi.org/10.3390/ani9080486

Tam, B., Sinha, S., & Wang, S. M. (2020). Combining Ramachandran plot and molecular dynamics simulation for structural-based variant classification: Using TP53 variants as model. *Computational and Structural Biotechnology Journal, 18*, 4033-4039. https://doi.org/10.1016/j.csbj.2020.11.041

Taylor, W. R., May, A. C., Browne, N. P., & Aszodi, A. (2001). Protein structure: Geometry, topology and classification. *The Ridgeway*. https://doi.org/10.1088/0034-4885/64/4/203

Torki, M., Mohebbifar, A., & Mohammadi, H. (2021). Effects of supplementing hen diet with *Lavandula angustifolia* and/or *Mentha spicata* essential oils on production performance, egg quality and blood variables of laying hens. *Veterinary Medicine and Science, 7*(1), 184-193. https://doi.org/10.1002/vms3.343

Vita, R., Mahajan, S., Overton, J. A., Dhanda, S. K., Martini, S., Cantrell, J. R., Wheeler, D. K., Sette, A., & Peters, B. (2018). The immune epitope database (IEDB): 2018 update. *Nucleic Acids Research, 47*, 339-343. https://doi.org/10.1093/nar/gky1006

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., & Schwede, T. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Research, 46*, 296-303. https://doi.org/10.1093/nar/gky427

Zaki, M. J., & Bystroff, C. (2008). *Protein structure prediction* (2nd ed.). Humana Press/Springer. https://doi.org/10.1007/978-1-59745-574-9

Zhang, L. L., Zhang, L. F., & Xu, J. G. (2020). Chemical composition, antibacterial activity and action mechanism of different extracts from hawthorn (*Crataegus pinnatifida* Bge). *Scientific Reports, 10*, 8876. https://doi.org/10.1038/s41598-020-65802-7