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Identification, Structure and Phylogenetic Tree Progression of Mucin-4 Gene in Pig and Other Mammals Using Avian as an Out-Group

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ABSTRACT: The objective of this paper was to identify the Structure and phylogenetic tree progression of Musin-4 (MUC-4) gene in pig and with other mammals and avian as out-group using appropriate bioinformatics tools. The results obtained revealed that the identity of mammalian species ranged from 70 -100% while the chicken as an out-group ranged from 42 -50%. The pig MUC-4 gene is closer to the Bovine family (0.34-0.35) and goat (0.282-0.286). The result from the structural prediction of mucin4 by phyre2, 467 residues have been accurately modelled with 100% confidence using a single template. Additionally, 738 residues (65%) have been modelled with over 90% confidence using multiple templates. From various amino acid substitutions obtained from protein effect variation in the three mammalian species, only one was deleterious in pigs, only two were deleterious in bovine, and only three were deleterious in goat. The dendrogram obtained from the phylogenetic tree revealed that different species were separated from each other based on their different taxonomic classes. In conclusion, these mammalian species had a high genetic relationship and conservation. The deleterious variants observed in the pig need to be investigated to prevent the malfunction of the reproductive, digestive and disease-resistant traits. This study gives basic information that may be useful in the genetic improvement of livestock species.

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Disease management and prolificacy in pigs is a very important aspect in livestock improvement programmes. Preventing infectious diseases in pigs is crucial for animal welfare, economic productivity, food safety, and public health (Alarcons *et al.*, 2021). Enterotoxigenic Escherichia coli ETEC expressing F4ab and F4ac fimbriae is a major cause of diarrhea outbreaks in pig breeding. Susceptibility to this infection is inherited as a dominant trait, with resistance observed in some pigs. Identifying the genetic basis of this trait on pig chromosome 13 can help improve pig welfare and breeding practices by enabling selection against susceptible animals, potentially reducing diarrhea outbreaks (Jacobsen *et*

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al., 2011). According to Bertch (2020), gastrointestinal disorders, specifically diarrhea, are significant issues in pig production affecting productivity and profitability. The causes of diarrhea in pigs are diverse, including bacteria, viruses, parasites, and nutritional factors. Different types of diarrhea occur at various stages of pig production, such as neonatal, post-weaning, and gray-fattening diarrhea. Overuse of antibiotics in industrialized production can lead to endemic diarrhea, damaging intestinal health and increasing bacterial resistance. However, the World Health Organization (WHO) has restricted the use of antibiotics and growth promoters to preserve the effectiveness of antibiotics crucial for human medicine. Thereby alternatives such as plant extract have been used to replace the use of Zinc oxide and growth promoters but the genes responsible for this disease can be worked on and targeted for disease resistance (Bertch, 2020). Mucus, primarily made up of glycoproteins called mucins, is a rheological substance that coats the intestinal epithelium. It serves as a protective barrier against harmful molecules, microbial infections, and fluctuating conditions within the intestinal lumen.

Mucins are O-glycosylated glycoproteins that protect and lubricate cell surfaces, as well as modulate the immune response, inflammation, adhesion, and tumorigenesis (Lagow *et al*., 1999; Moniaux *et al*., 1999; Govindarajan *et al* 2010). Immunostimulants are being used in animals to improve their immune system and protect them from specific pathogens (Veterinarial Digital, 2019). These compounds help to boost the development and strengthen the disease resistance of animals, and their effectiveness depends on their structure and function. It has been reported that a combination of natural immunostimulants with vaccines for pregnant sows improves the quality of colostrum and enhances protection for piglets against infection (Maguregui 2019). Purified porcine gastric mucin (PGM) has been found to activate immune responses, provide disease resistance against pathogenic bacteria, improve heat tolerance, and scavenge reactive oxygen species. These findings suggest potential new applications for PGM as a multifunctional immunomodulator (Thiloma *et al*., 2020). Mucins are essential for forming protective barriers in mucous membranes and have various functions such as regulating solute transport, creating binding sites for microbes, and influencing cellular processes like regeneration, differentiation, and apoptosis. It plays roles in lubrication, protection, cell functions, immune response, adhesion, and cancer development. A study examined the evolution of the porcine MUC4 gene by analyzing nucleotide variability and linkage disequilibrium in Chinese indigenous and Western commercial pig breeds (Ming Yang *et al*, 2012). They are classified into secreted and membrane-bound groups based on their structure and function. The alternating hydrophilic glycosylated and hydrophobic un-glycosylated regions give mucins their amphiphilic character. Factors such as chronic stress, infectious agents, and nutritional deficiencies can affect the immune system of pigs, leading to immunodeficiency and secondary conditions. Pro-nutrients found in various plant species can enhance the physiological functioning of pigs by increasing protein synthesis and stimulating the immune system without causing a pharmacological effect. These molecules are essential for optimal organic performance and can even enhance the transmission of immunoglobulins from mothers to piglets during lactation (Magureji 2019). Adding certain active molecules to pig feed can enhance their immune system and improve the transmission of protective antibodies from mothers to piglets via lactogenic, thus protecting the animals from factors that can cause immunodeficiencies (Devine *et al.,* 1992). Mucin is also located within the confidence interval of a prolificacy quantitative trait loci (QTL). It is associated with reproductive traits such as the total number born and the number born alive (Noguera *et al.,* 2009). This study's objectives are to identify the Gene MUC4 of pig; to predict its protein structure and compare chemical properties of selected mammals and chicken; to predict the functional effect of amino acids substitution as deleterious and normal of small insertions and deletions of protein sequences; to construct a phylogenetic tree of MUC4 of pigs and other mammalian species with avian as an outgroup.

MATERIALS AND METHODS

The nucleotide and amino acid sequence (AAS) of Mucin 4 of pig were downloaded from the Universal Protein Resources (Uniprot) database United Kingdom for identifying the gene and downloaded the protein sequences (Uniprot, 2023). The Protein mucin 4 of other species were downloaded from the same database. The Identity percentage of Mucin 4 of pigs and other mammals was identified by conducting a pairwise comparison of their aas using two or more sequences of the Basic Local Alignment Search Tool (BLAST). Analyses were conducted using the Poisson correction model (Zuckerkand *et al.,* 1965). The analysis involved 23 amino acid sequences. All positions containing gaps and missing data were eliminated. There was a total of 875 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*, 2018). Functional analysis of pigs and other mammals such as bovine and caprine were obtained by using the Protein Variation Effect Analyzer (PROVEAN) online (PROVEAN, 2015,

Choi and Chan, 2015) with a threshold value of -2.5 to predict the functional effect of amino acids substitution as deleterious and neutral of small insertions and deletions. Any protein sequence variants having a PROVEAN scores below the threshold value of -2.5 were termed deleterious and a scores above the same threshold value were considered normal. The phylogenetic tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The study used the Neighbor-Joining method to infer the evolutionary history and presented the optimal tree (Saitout *et al*., 1987). The tree's reliability was tested using the bootstrap method with 1050 replicates. The tree was drawn to scale, and the evolutionary distances were calculated using the Poisson correction method (Zuckerkandl and Pauling, 1965). The analysis included 23 amino acid sequences and eliminated positions with less than 95% site coverage. A total of 875 positions were included in the final dataset, and the evolutionary analyses were conducted using MEGA X (Kumar *et al*, 2018).

RESULTS AND DISCUSSIONS

Mucin protein, a high-molecular-weight glycoprotein mucin, is encoded by MUC-4 gene. It was located on the chromosome 13 in pigs and has 29 exon counts (NCBI, 2024). MUC-4 is a membrane-anchored glycoprotein found in mucus covering various tissues such as the cervix. The organisms and the accession numbers of their amino acid (AA) sequence (AAS) with their sequence length that were retrieved from UniProt are shown in Table 1. All the animal's AAS length ranged from 986 to 2613. AAS length is the number of bases or AA in the canonical sequences. The sequence variations observed, were probably by deletion or insertion of some AA sequences due to convergent evolution. The percent identity of mammalian species ranged from 70- 100% while the chicken as an outgroup ranged from 42 -50%. This is an indication that they are homologous, perform the same function and have high conservation in the MUC4 gene. This is in agreement with Durosaro *et al*. (2016) and Akinyemi *et al*., (2017). According to Joshi and Xu (2007), if two sequences have more than 70% Identity, they have a probability of more than 90% to share biological function. They all have low expected values (e-value), therefore they were significant (<0.05) and homologous. This result corroborated with Lesk, 2002; Durosaro *et al*., 2016; Oluwole *et al*. 2021). The genetic distances between the pig and other mammalian species are shown in Table 1. The greater the genetic distance value the far the closeness, while the smaller the genetic distance value the closer the genetic distance between the species (Kang *et al*., 2008). The pig MUC-4 gene is closer to the Bovine family (0.34-0.35) and goat (0.282-0.286). Among the mammalian species, the pig is far from the human with a value of 0.278-0.279 and very far from the chicken with a value of 0.536-0.854 as an outgroup. This was in line with Oluwole *et al*., (2018) and Oluwole *et al*. (2019) where the pig was always closely related with the bovine and the caprine.

Table 1: The percent identity and AAS of Mucin Protein of Pig, other Mammals and Avians.

Animal	Accession number	Class of Animal	Amino acid length	Identity %	E-value	Genetic distance
Pig	A0A8W4F860	Mammalian	1129	100	1.4e-34	0.000
Pig	A0A287B5M2	Mammalian	1180	100	1.4e-34	0.000
Bovine	F1N4E4	Mammalian	1095	76.5	8.8e-23	0.235
Cattle Hybrid	A0A4W2EPU7	Mammalian	1125	76.5	8.8e-23	0.234
Bovine	A0A3Q1MTB5	Mammalian	1141	76.5	8.8e-23	0.234
Cattle Hybrid	A0A4W2D019	Mammalian	1175	74.5	8.8e-23	0.235
Bovine	A0A3Q1LPY0	Mammalian	2613	76.5	8.8e-23	0.234
Cattle (Zebu)	A0A6P5C6I2	Mammalian	1017	74.5	2e-21	0.234
Human	A0A0G2JSB1	Mammalian	2613	71.2	5.5e-21	0.279
Human	A0A0G2JQC1	Mammalian	1098	71.2	5.5e-21	0.278
Human	A0A0G2JRU8	Mammalian	1125	71.2	5.5e-21	0.278
Human	A0A0G2JQJ2	Mammalian	1143	71.2	5.5e-21	0.279
Human	A0A0G2JNJ5	Mammalian	1149	71.2	5.5e-21	0.278
Human	A0A0G2JSC3	Mammalian	1176	71.2	5.5e-21	0.278
Donkey	A0A8C4LDK0	Mammalian	1125	72.5	4.4e-20	0.279
Donkey	A0A8C4LDF7	Mammalian	1173	72.5	5.5e-21	0.279
Horse	A0A5F5PQU9	Mammalian	1125	70.6	1.7e-19	0.273
Goat	A0A452EHU4	Mammalian	1040	71.4	1.7e-19	0.286
Goat	A0A452EIE7	Mammalian	1069	71.4	1.2e-10	0.282
Goat	A0A452EHY5	Mammalian	1083	71.4	1.2e-10	0.286
Turkey	G1N772|G1N772	Avian	986	50.0	0.00024	0.562
Chicken	A0A8V0YK80	Avian	1788	46.2	4.8e-7	0.854
Chicken	A0A8V0YRK0	Avian	986	50.0	0.00096	0.536

The AAS of the mucin 4 protein of pig was submitted into phyre2 online (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi) for protein structure prediction and analysis. 467 residues (41% of the sequence) have been accurately modeled with 100% confidence using a single template as shown in Figure 1 (a). Additionally, 738 residues (65%) have been modeled with over 90% confidence using multiple templates. The result can be viewed at http://www.sbg.bio.ic.ac.uk/phyre2/phyre2 output/54206b769e7b8a88/summary.htmli. The secondary structure disorder prescription result was 2% disorder, Alpha Helix of 8% and 28% of Beta strand. Transmembrane helices have been predicted in the sequence to adopt a specific topology as shown in Figure 1(b). The protein parameters of the pig, goat, bovine (mammals) and chicken as an outgroup were obtained from Expasy Bioinformatics resource portals where the Protfam tool was used to obtain the protein chemical properties as shown in Table 2. The results obtained show that mammals with an instability index of less than 40 caused the protein to be stable. The dendrogram obtained from the phylogenetic tree revealed that the MUC-4 gene of different species was separated from each other based on their different taxonomic classes (Figure 2).

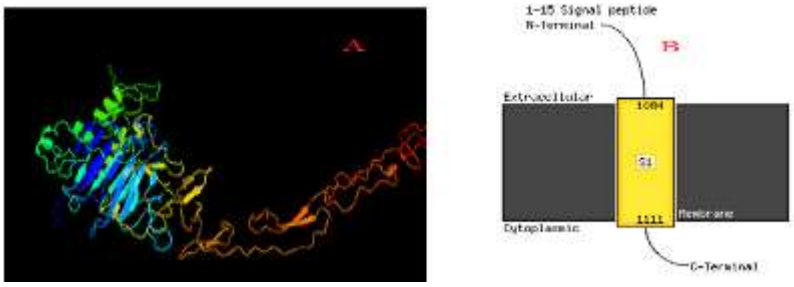

Fig 1: (a) Predicted pig mucin structure and (b) Predicted Transmembrane helices

Table 2: Protein parameters of pig and other mammals

Protein Parameters	Pig	Bovine	Goat	Chicken
Carbon	136	152	138	114 191
Hydrogen	208	229	221	191
Nitrogen	26	31	29	23
Oxygen	27	29	28	27
Sulphur	1	0	0	2
Total Atoms	398	441	416	357
Instability index	3.96	28.73	26.18	10.56
Stability	Stable	Stable	Stable	Stable
Aliphatic index:	172.69	173.33	195	195.2
Hydropathicity (GRAVY)	2.477	2.244	2.543	2.404
N-terminal	Leucine	Alaline	Leucine	Leucine

AAS =Amino acid sequence, c=carbon, H =hydrogen, N=nitrogen, O=oxygen, Sulphur

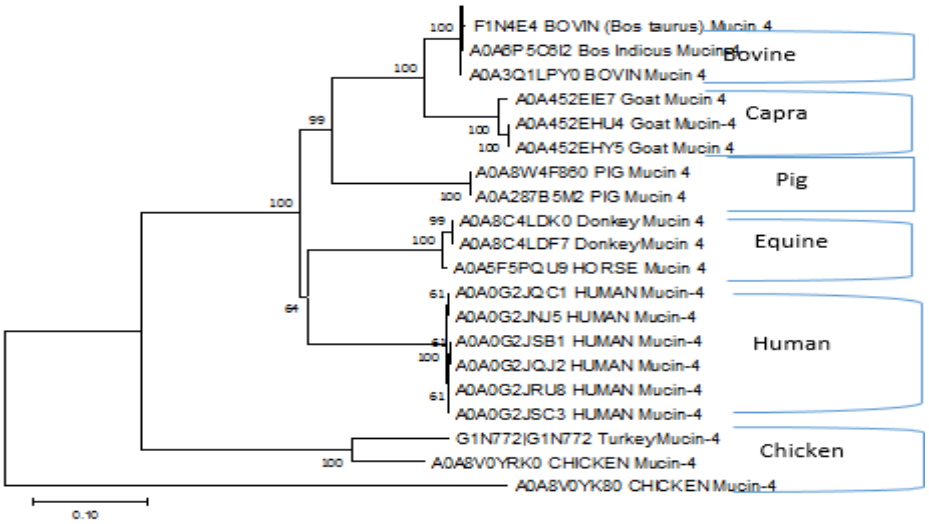

Fig 2: Phylogenetic Tree of MUC-4 of pig, other mammals and Avian as an outgroup

PROVEAN (Protein Variation Effect Analyser) results obtained for the functional analysis of missense in pig, bovine and goat were shown in Table 3. From six aa substitutions obtained in pig, only one was deleterious. This means that mutations at these sites were harmful. From eight aa substitutions obtained from bovine, only two were deleterious, while only three were deleterious in goat. Amino acid substitutions due to single nucleotide polymorphisms (SNPs) can disrupt protein folding and stability, leading to structural changes that impact protein function, interactions, and expression levels (Akinyemi *et al*., 2017; Oluwole *et al*., 2018., Oluwole *et al.,* 2019 Oluwole *et al.*, 2021).

Table 2: Functional effect of protein sequence variations results

Species	Variants	Provean Score	Prediction (cutoff=-2.5)
Pig	L101F	5.360	Neutral
	E504Y	-2.768	Deleterious
	E544K	-1.667	Neutral
	R646K	-1.897	Neutral
	H649N	-1.700	Neutral
	T918S	0.997	Neutral
Bovine			
	189Q	-3.983	Deleterious
	S359V	-1.303	Neutral
	I360S	-2.977	Deleterious
	S361G	0.992	Neutral
	R362L	2.778	Neutral
	S504N	-2.007	Neutral
	Q709R	-2.060	Neutral
	A736T	-0.227	Neutral
Goat			
	L58N	-5.953	Deleterious
	W59N	-0.853	Neutral
	N60C	-3.340	Deleterious
	T156S	1.017	Neutral
	S281K	-1.233	Neutral
	R282Q	-0.105	Neutral
	Q346H	-0.997	Neutral
	P347C	-4.699	Deleterious
	P348T	-1.051	Neutral
	T349G	-0.839	Neutral
	P350G	-1.503	Neutral
	P352S	0.590	Neutral
	P353A	4.350	Neutral
	K574A	0.831	Neutral

A = Alanine, G = Glycine, S = Serine, R = Arginine, F = Phenylalanine, I = Isoleucine, C = Cysteine, L = Leucine, P = Proline, Q = Glutamine, V = Valine, D =Aspartic acid I = Isoleucine, Y =Tyrosine, R = Arginine

Conclusion: In conclusion, there was a high genetic relationship and conservation of the MUC-4 gene among the mammalian species investigated. The deleterious variants observed in the pig and other mammalian species need to be investigated to prevent the malfunction of the protein especially in economic traits such as reproductive traits such as liter size, fertility, disease resistance etc. Amino acid substitutions caused by single nucleotide polymorphisms (SNPs) can disrupt protein folding and stability, resulting in changes to the protein's structure. This can ultimately lead to alterations in protein function, interactions, and expression levels. MUC4 is a membrane-anchored glycoprotein found in the mucus covering epithelial surfaces of various tissues such as the trachea, cervix and colon. It is involved in lubrication, digestion, protection, cell processes, and immune response to foreign bodies. Their function is very important in pig reproduction, digestion and disease resistance.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author.

Abbreviations: Amino acid sequences (AAS); Protein Variation Effect Analyzer (PROVEAN);

REFERENCES

Alarcón, LV; Allepuz, A; Mateu, E (2021). Biosecurity in pig farms: A review. *Porc. Health Manage.* 7(5). https://doi.org/10.1186/s40813-020-00181-z

Bertsch, G (2020). Main causes of diarrhea in pigs; Traditional prevention methods and natural solutions of the diarrhea in pigs. *Veterinaria Digital*. https://www.veterinariadigital.com/en/articulos/main-causes-of-diarrhea-in-pigs/

Durosaro, SO; Ilori, BM; Sanda, AJ; Onagbesan OM; Peters SO; Ozoje MO (2016). Mutational and evolutionary Analysis of Bovine Reprimo Gene. *Nig. Soci. Anl. Prod.* 43(1): 10-19.

Choi, Y; Chan, AP (2015). PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 31(16): 2745-2747.

Devine, PL; McKenzie, IF (1992) Mucins: Structure, function, and associations with malignancy. *BioEssays*, *14*, 619–625.

Govindarajan, B; Gipson IK (2010). Membrane-tethered mucins have multiple functions on the ocular surface. *Exp. Eye. Res.* 2010; 90:655–663. doi: 10.1016/j.exer.2010.02.014.

Jacobsen, M; Cirera, S; Joller, D *et al.* (2011). Characterization of five candidate genes within the ETEC F4ab/ac candidate region in pigs. *BMC Res Notes* **4**, 225 https://doi.org/10.1186/1756-0500-4-225

John, G; Mesalhy, S; Rezk, M; El-Naggar, G; Fathi, M (2007). Effect of some immunostimulants as feed additives on the survival and growth performance of Nile tilapia, *Oreochromis niloticus* and their response to artificial infection. *Egypt. J. Aquat. Biol. Fish. 11*, 1299–1308.

Kebouchi, M; Zeeshan, H; Yves Le, R; Dary-Mourot A; Magali G (2020). Importance of digestive mucus and mucins for designing new functional food ingredients. *Food Res. Inter*. 131, 108906, https://doi.org/10.1016/j.foodres.2019.108906

Kumar, S; Stecher, G; Tamura, K (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Bio. Evolut.* 33:1870-1874.

Lagow, E; DeSouza, MM; Carson DD (1999). Mammalian reproductive tract mucins. *Hum Reprod.* **5**:280–292. doi: 10.1093/humupd/5.4.280.

Lehrer, S; Sanchez, M; Song, HK; Dalton, J; Levine, F; Savoretti, P; Thung, SN; Schachter, B (1990). Oestrogen receptor B-region polymorphism and spontaneous abortion in women with breast cancer. *Lancet* 335, 622-624.

Lehninger, AL; Nelson, LD; Cox, MM (1993). Principle of Biochemistry 2nd edition. Worth publisher Inc. USA. 432pp

Lesk, AM (2002). Introduction to Bioinformatics 2nd Edition. Oxford University press, *Oxford*, 255pp.

Linville, RC; Pomp, D; Johnson, RK; Rothschild, MF (2001). Candidate gene analysis for loci affecting litter size and ovulation rate in swine. *J. Anim. Sci.* 79, 60–67.

Maguregui, E (2019). Causes of immune Deficiency in pig. *Veterinaria Digital*.

Ming, Y; Bin, Y; Xueming, Y; Jing, O; Weihong, Z; Huashui, A; Jun, R; Lusheng, H (2012). Nucleotide variability and linkage disequilibrium patterns in the porcine MUC4 gene. *BMC Genet.* 13:13:57

Moniaux, N; Nollet, S; Porchet, N; Degand, P; Laine, A; Aubert, JP (1999). Complete sequence of the human mucin MUC4: a putative cell membrane-associated mucin. *Biochem J.* 338:325–333. doi: 10.1042/0264-6021:3380325.

Noguera, JL; Rodriguez, C; Varona, L; Tomas, A; Munoz, G; Ramirez, O; Barragan, C; Arque, M; Bidanel, JP; Amills, M; Ovilo, C; Sanchez, AA (2009). Bi-dimensional genome scan for prolificacy traits in pigs shows the existence of multiple epistatic QTL. *BMC Genomics.* 2009; 10:636. doi: 10.1186/1471-2164-10-636.

Oluwole, OO (2018). Computational Genomics of RELA Gene of Pig with Other Mammals and Its Protein Prescription. *Nig. J. Anlm. Prod.* 45 (1), 9-17.

Oluwole, OO; Olupona, AO; Tiamiyu, AK; Oladele MO; Olorungbounmi, TO; Boladuro, B.A; Omodewu, I; Adeduro P.A (2019). Comparative Genomic Variation in Porcine Species Could Lead to Species-specific Responses to ASF. *Nig. J. Anim. Sci.* 21 (1), 87-95

Oluwole, OO (2019). Computational Molecular Analysis of the 5 Candidate Genes of Suidae Family Related to African swine fever Occurrence. *Asian J. Biotechnol and Bioresour. Technol*, *5* (2), 1-7

PROVEAN (2015). http://provean.jcvi.org/index.php

Saitou, N; Nei, M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Uniprot 2017. Protein database. http://www.uniprot.org/uniprot/C3S7K5

Thiloma, D; Liyanage, PS; Dahanayake, SL; Edirisinghe, CN; Gang-Joon, H; Mahanama, DZ; Ilson, W (2020). Biological Activity of Porcine Gastric Mucin on Stress Resistance and Immunomodulation. *Molecules.* 25 (13): 2981.

UniProt. 2023. Consortium, TU; Bateman, A; Martin, M; Orchard, S; Magrane, M; Ahmad, S; Alpi, EH; Britto, R; Cukura, A; Denny, P; Dogan, T; Ebenezer, T; Fan, J; Garmiri, P; Jose, L; Hussein, A; Ignatchenko, A; Insana, G; Zhang, J. 2023. The Universal Protein Knowledge base in 2023. *Nucleic Acids Res*, *51*,523-D531. https://doi.org/10.1093/nar/gkac1052

Vetenarial Digital (2019). Porcine epidemic diarrhea: effect of immunostimulant pro-nutrients on the quality of the colostrum. *Vetenarial Digital.*

Zuckerkandl, E; Pauling, L (1965). Evolutionary divergence and convergence in proteins. Edited in Evolving Genes and Proteins by V. Bryson; H.J. Vogel, pp. 97-166. Academic Press, New York.