MAGNA MEDIKA
Medical and Health Scientific Periodical
e-ISSN 2774-2318
p-ISSN 2407-0505

Research Article
MAGNA MEDIKA
Medical and Health Scientific Periodical
Journal Pages:https://jurnal.unimus.ac.id/index.php/APKKM

Mineral and Insilico Study of Sapat Siam Fish (*Trichopodus pectoralis*, Regan 1910) on Appetite Regulation

Erwin Rosadi[1], Lia Yulia Budiarti[2], Andifa Anugerah Putra[3], Rezvi Amalia Rahmah[4], Putri Mariatul Qiptiah[5], Isnaini[6]

[1)] Capture Fisheries Study Program, Faculty of Fishery and Marine, University of Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
[2)] Department of Microbiology and Parasitology, Faculty of Medicine, University of Lambung Mangkurat, South Kalimantan, Indonesia
[3)] Student of Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat, South Kalimantan, Indonesia
[4)] Student of Faculty of Medicine, University of Lambung Mangkurat, South Kalimantan, Indonesia
[5)] Student of the Faculty of Medicine, University of Lambung Mangkurat, South Kalimantan, Indonesia
[6)] Department of Pharmacology, Faculty of Medicine, University of Lambung Mangkurat, South Kalimantan, Indonesia.

Article Info

Article history:
Received 08 September 2023
Revised 19 December 2023
Accepted 19 January 2024
Available online 01 February 2024

Keywords:
Sapat Siam Fish;*Trichopodus pectoralis*; Mineral compounds; Appetite; insilico

Correspondence:
isnaini@ulm.ac.id

How to cite this article:
Erwin Rosadi, Lia Yulia Budiarti, Andifa Anugerah Putra, Rezvi Amalia Rahmah, Putri Mariatur Qiptiah, Isnaini. Mineral and Insilico Study of Sapat Siam Fish (*Trichopodus pectoralis,* Regan 1910) on Appetite Regulation. MAGNA MEDIKA Berk Science of Medicine and Health. MAGNA MEDIKA Berk Ilm Kedokt dan Kesehat. 2024; 11(1):38-52

Abstract

Backgrounds: Production of Sapat siam fish is ranked second after Papuyu. Giving fish in cases of wasting can overcome mineral deficiencies in wasting sufferers because fish contains minerals. Wasting sufferers experience Fe, Ca, and Zn deficiencies. There is a relationship between cases of wasting and the incidence of anemia. Anemia sufferers experience a decrease in appetite. Until now, no known effect of giving the minerals Fe, Ca, and Zn on appetite regulation has been known.

Objectives: This research aims to determine the mineral content of Sapat siam fish, specifically Fe, Ca, and Zn, and the influence of those minerals on appetite regulation in silico.

Methods: Analysis of mineral content in Sapat siam fish meat using the AAS method and analysis of the influence of Fe, Ca, and Zn minerals using the in-silico method.

Results: Sapat siam fish meat contains Fe, Ca, and Zn with concentrations of 34.5 ± 0.8485, 1,670 ± 183.8478, and 22.8 ± 0.1414, respectively. Fe, Ca, and Zn in Sapat siam fish meat prediction can be binding with ligand Ghrelin, leptin, NPY (Neuropeptide Y), ARC, IL-1β, and AgRP.

Conclusion: Sapat siam fish prediction can stimulate appetite

e-ISSN 2774-2318
p-ISSN 2407-0505

INTRODUCTION

Inland water areas are a source of fish production. Fisheries production in Indonesia 2021 will be 21 million tons[1], while fisheries production in South Kalimantan was 218,545 tons with a production value of 6.7 Trillion rupiah.[2] This high production rate is accompanied by increased fish consumption in Indonesia by 55.37 kg/capita in 2021. The fish consumption rate in South Kalimantan in 2021 will be 61.74 kg/capita, more significant than the consumption rate in Indonesia. Sapat siam (*Trichopodus pectoralis*, Regan 1910) is one of the fish consumed in South Kalimantan[3]. Sapat siam fish production is ranked second after Papuyu[4].

Fish meat contains water, protein, fat, and ash. Protein is the most significant component in fish scales. 40-90% of the protein is collagen, and the rest is mineral residue and inorganic salts such as magnesium and calcium carbonate.[5] Fish also contains calcium, phos-phorus, Zn, Fe, K, and Na, which benefit humans.[6] Fish is used to treat stunting and wasting in children. In wasting toddlers, there is a deficiency of Fe, Ca, and Zn[7]. Based on research conducted by[8], there is a relationship between wasting sufferers and the incidence of anemia. Anemia sufferers experience a decre-ase in appetite.

Regulation of appetite is influenced by Ghrelin, leptin, NPY (Neuropeptide Y), ARC, IL-1β, and AgRP. Ghrelin is a hormone produced in the stomach.[9] Appetite regulation involves several neuropeptides, such as Agouti-related Peptides (AgRP). AgRP works to stimulate food intake and cause weight gain. Neuro-peptide Y (NPY) works to stimulate appetite. NPY is expressed in the same neurons as AgRP, but NPY is expressed in other regions of the hypothalamus and brain[10–12]. Until now, the influence of the minerals Fe, Ca, and Zn on appetite regulation is unknown. This research aims to determine the mineral content of Sapat siam fish, specifically Fe, Ca, and Zn, and the prediction affinity of those minerals with ligand Ghrelin, leptin, NPY (Neuropeptide Y), ARC, IL-1β, AgRP by in silico method.

METHODS

Materials

Sapat siam fish was obtained from the Martapura market, Banjar District, South Kalimantan, and the determination was carried out at the Faculty of Fisheries and Marine, Lambung Mangkurat University.

Preparation of Sapat siam Fish

The scales of the fish obtained are cleaned with distilled water, and the meat is removed. The fish meat was washed and dried using an oven at 50^0C for three days. The dried fish meat is then ground to obtain meat powder. Then, it is ready for further analysis.

Analysis of Mineral Content

Mineral content analysis was carried out using a Shimadzu AA-6300 atomic absorption spectrophotometer (AAS) and following the procedures carried out by Lopies et al. [13]

Mineral Testing Procedures

The principle of determining minerals is after removing organic materials by dry or wet ashing, and the residue is dissolved in dilute acid. The solution is distributed in the flame inside the atomic absorption spectrophotometer (AAS) to analyze and measure mineral absorption or emission at certain wavelengths.

e-ISSN 2774-2318
p-ISSN 2407-0505

Samples were washed using the wet ashing method. In the wet ashing process, The sample was weighed as much as 1 g, put into a 150 ml Erlenmeyer flask, then 5 ml of HNO3 to the Erlenmeyer flask and left for 1 hour. Then, it was heated on a hotplate for ± 4 hours and cooled. Next, 0.4 ml of concentrated H_2SO_4 was added and heated again. After the color changes from brown to clear yellow, the sample is added mix 3 ml of $HClO_4$ and HNO_3, and reheat for ± 15 minutes, then added 2 ml of distilled water and 0.6 ml of concentrated HCl, then the standard stock solution of each mineral is diluted with distilled water until the concentration is at desired metal working range. Standard solutions can be prepared using chemical material. The blank standard solution and sample were flowed into the AAS. Then, measure the absorbance or peak height of the standard solution, blank, and sample at length waves and parameters corresponding to each mineral with a spectro-photometer.

Mineral content can be calculated using the formula:

$$\text{Mineral content} = \frac{(a-b)\ x\ V\ x\ fp\ x\ 100}{10w}$$

$$\text{Mineral content (mg/100 g dry base)} = \frac{mineral\ content\ wet\ base}{100\%-water\ content}\ \text{x } 100\%$$

Information:
a = sample solution concentration (ppm)
b = concentration of blank solution (ppm)
v = volume of extract
fp= dilution factor
w= sample weight (g)

Insilico analysis

In silico analysis, the computer specification used is Intel(R) Core (TM) i5-8250 1.8 GHz (8 CPUs), 8 GB RAM, NVIDIA GeForce MX150, and Windows 11. The scientific method employed in this research project involved a series of systematic steps. Firstly, ligands and proteins relevant to the research question were identified by conducting thorough searches on databases such as RCSB.org and PubChem. The search criteria included specific structural features, functional properties, and biological relevance to the research focus. Next, the ligand structures were modified by inserting mineral ions, such as calcium (Ca), iron (Fe), and/or zinc (Zn), and non-added ions as a control, using Discovery Study Visualizer (DSV) tools. This software tool facilitated the precise placement of the mineral ions within the ligand molecules, ensuring an accurate representation of the desired mineral-ligand interactions. Subsequently, water molecules and inherent ligands were removed from the protein structure using an official PyMOL build. This step aimed to isolate the protein molecule and eliminate any potential interference or bias caused by solvent molecules or pre-existing ligands. Removing these components focused solely on the interaction between the prepared ligands and the protein receptor. After these preparations, the ion-inserted ligands, non-inserted ligands, and their respective receptor proteins were prepared for docking simulations using DockThor (https://dockthor.lncc.br/v2/). [14]

DockThor is a web server that facilitates protein-ligand docking simulations. The process starts with preparing input files, including the protein and ligand structures. Users can upload a protein file in PDB format and choose to add missing hydrogen atoms, complete side chains, and modify protonation states. Similarly, a ligand file can be uploaded in PDB, SDF, or MOL2 format, with options to add hydrogen atoms, freeze rotatable bonds, and assign atom

e-ISSN 2774-2318
p-ISSN 2407-0505

types and charges. Additionally, users can include cofactor structures if desired. Once the input files are prepared, the next step is to define the binding site. DockThor provides multiple options, such as user-defined binding sites, blind docking across the entire protein, or using predefined test cases. Users can specify the binding site's grid center, size, and discretization parameters. After defining the binding site, users can set the docking parameters, including the search algorithm preci-sion and other specific parameters such as the number of evaluations, population size, and initial seed. These parameters influence the level of detail and accuracy of the docking calculations. DockThor performs the calcula-tions using the selected algorithm and parame-ters upon submitting the docking job. The results include ranked compounds based on their predicted affinity and other energy terms. Each compound is presented with multiple docking poses. To visualize and analyze the docking results using BIOVIA Discovery Studio Visualizer, users must download and install the software. The docking results from DockThor can be exported in a compatible format, such as PDB or SDF. By importing the docking results file into Discovery Studio Visualizer, users can explore the docking poses and predicted complexes in 3D. The software provides various visualization tools to analyze protein-ligand interactions, calculate binding energies, generate interaction diagrams, and perform additional analysis as needed[15].

a. Ghrelin 1-4 ($C_{25}H_{38}N_4O_8$)

IUPAC of Ghrelin is H-Gly-Ser-Ser(octanoyl)-Phe-OH. Ligand Ghrelin used Receptor 7NA8 Chain R. The entry with the identifier 7NA8 in the RCSB Protein Data Bank (PDB) represents the structures of human Ghrelin receptor-Gi complexes with Ghrelin and a synthetic agonist. Ghrelin is a peptide hormone that plays a role in regulating appetite and energy balance. It binds to the ghrelin receptor, a G protein-coupled receptor (GPCR) located on the cell membrane. The complex formed between the ghrelin receptor and Gi protein is particularly interesting because it is involved in signaling pathways regulating various physiological processes, including hunger, satiety, and metabolism. The complex includes the ghrelin receptor, a transmembrane protein, and the Gi protein, a guanine nucleotide-binding protein. The structure provides insights into the interaction between the ghrelin receptor and its ligands, Ghrelin, and a synthetic agonist. The ligands bind to specific regions on the ghrelin receptor, triggering a cascade of signaling events that regulate cellular responses[16].

b. Leptin (93-105) Human

IUPAC of leptin is H-DL-Asn-DL-Val-DL-xiIle-DL-Gln-DL-xiIle-DL-Ser-DL-Asn-DL-Asp-DL-Leu-DL-Glu-DL-Asn-DL-Leu-DL-Arg-OH. Ligand leptin used receptor 1AX8, chain A. The entry 1AX8 in the RCSB Protein Data Bank (PDB) corresponds to the crystal structure of the human obesity protein leptin. Leptin is a cytokine crucial in regulating body weight and energy balance. Mutations in the gene encoding leptin or its receptor can lead to obesity, infertility, and diabetes in mice. The crystal structure of the mutant form of human leptin (leptin-E100) was determined using X-ray diffraction. The resolution of the structure is 2.40 Å, which provides detailed information about the arrangement of atoms in the protein. The structure of leptin-E100 reveals a four-helix bundle, similar to other members of the long-chain helical cytokine family. This structural motif is essential for the biological activity of leptin. Despite being a mutant, leptin-E100

e-ISSN 2774-2318
p-ISSN 2407-0505

retains comparable biological activity to the wild type.[17]

c. ARC (Human Arc C-lobe)

Ligand ARC used receptor 6TN7, chain B. The entry 6TN7 in the RCSB Protein Data Bank (PDB) corresponds to the crystal structure of the human Arc C-lobe. The Arc protein, also known as activity-regulated cytoskeleton-associated protein, plays a crucial role in synaptic plasticity and the brain's normal functioning. It interacts with various neuronal postsynaptic proteins. The crystal structure of 6TN7 provides insights into the structural properties and peptide ligand binding of the C-terminal domain of Arc, which consists of tandem domains known as the N-lobe and C-lobe. The N-lobe contains a peptide binding site capable of binding multiple targets. The researchers measured the affinity of human Arc towards various peptides derived from stargazin and guanylate kinase-associated protein (GKAP), which are known interactors of Arc. They refined the specificity determinants of Arc and identified two binding sites in the GKAP repeat region. These interactions were confirmed through X-ray crystallography. GOL is a small molecule that interacts with the human Arc C-lobe protein. Glycerol is commonly used as a cryoprotectant in X-ray crystallography experiments to prevent damage to the protein crystals during freezing. The presence of GOL in the crystal structure suggests that it may play a role in stabilizing the protein or mediating protein-ligand interactions.[18]

d. Human Neuropeptide Y (5ZBH)

Ligand NPY used Human Neuropeptide Y (5ZBH) chain A. The entry 5ZBH in the Protein Data Bank (PDB) refers to the crystal structure of the Human Neuropeptide Y Y1 Receptor in complex with the ligand BMS-193885. The structure provides insights into the binding mode of the ligand and the receptor's conformation. The Human Neuropeptide Y Y1 Receptor is a signaling protein that plays essential roles in food intake, anxiety, and cancer biology. It belongs to the G-protein-coupled receptor superfamily. The receptor is a membrane protein and is expressed in Homo sapiens (human) and Enterobacteria phage T4. The expression system used for this structure is *Spodoptera frugiperda*. The ligand in the complex with the receptor is BMS-193885, which is a small molecule compound of 9AF. The ligand interacts with the receptor through specific binding sites, and the crystal structure provides information about the interactions between the ligand and the receptor residues. The 5ZBH entry and its associated publication provide valuable information for understand-ding the binding behavior of the Neuropeptide Y Y1 Receptor and its potential as a target for structure-based drug discovery. The crystal structure and the insights gained from it can contribute to the development of therapeutics targeting the NPY receptor system.[19]

e. Human Interleukin-1 Beta (91LB)

The structure with the PDB code 9ILB corresponds to the protein Human Interleukin-1 Beta (IL-1β). Interleukin-1 Beta is a signaling protein involved in the human body's immune response and inflammation processes. It is crucial in mediating the body's response to infection, injury, and other pathological conditions. The protein's structure was determined using X-ray crystallography, a technique that allows scientists to visualize the arrangement of atoms in a crystal. The resolution of the structure is 2.28 Å, which refers to the level of detail at which the atoms are resolved in the structure.

e-ISSN 2774-2318
p-ISSN 2407-0505

The 9ILB structure consists of a single protein chain, the monomeric form of Interleukin-1 Beta. The protein chain comprises 153 amino acids, and its sequence corresponds to the human IL-1β protein. The protein's amino acid sequence provides essential information about its biological function and the interactions it can form with other molecules. In addition to the protein structure, you mentioned a ligand associated with 9ILB: Interleukin-1beta (163-171). A ligand is a small molecule or another protein that binds to a specific site on the protein of interest, affecting its function. In this case, Interleukin-1beta (163-171) is a specific region or peptide derived from Inter-leukin-1 Beta that interacts with the protein.[20]

f. AgrP (1HYK)

The binding between AgrP (AGOUTI-RELATED PROTEIN) (87-132) and heparan sulfate has been shown to affect appetite regulation. AgrP is known to play a role in modulating feeding behavior and energy homeostasis, while heparan sulfate is a polysaccharide found on cell surfaces involved in various cellular processes. The presence of mineral ions such as Ca, Fe, and Zn can influence the binding interaction between AgrP and heparan sulfate. These mineral ions can potentially affect the appetite-regulating properties of AgrP, either by stimulating or inhibiting appetite. The binding site of AgrP (87-132) on heparan sulfate and the key residues responsible for the interaction have been identified through structural analyses. The positively charged regions of AgrP, often due to the presence of lysine or arginine residues, interact with the negatively charged sulfate groups on heparan sulfate. These interactions stabilize the AgrP-heparan sulfate complex and play a crucial role in mediating its biological functions. The binding of AgrP to heparan sulfate may modulate signal transduction pathways, cellular adhesion, or other processes involved in cell communication and tissue development.

RESULTS

1. Mineral levels in Sapat siam fish

The dried fish meat content of Fe, Ca dan Zn. The result showed that the meat has a high calcium level and a lower Zn level. The Fe, Ca, and Zn levels in dried fish meat are 34.5 ± 0.8485, 1,670 ± 183.8478, and 22.8 ± 0.1414, respectively.

Table 1. Results of analysis in silico of Ca, Fe, Zn with Ghrelin

Mineral	Affinity	Total Energy	vdW Energy	Elec. Energy
Ca	-8.076	-20.322	-21.838	-8.475
Fe	-7.610	-25.884	-15.550	-11.431
Zn	-8.107	-22.257	-19.794	-10.678
All Mineral	-8.304	-26.545	-20.200	-9.088
Non-added (control)	-7.064	-2.794	-12.215	-12.156

e-ISSN 2774-2318
p-ISSN 2407-0505

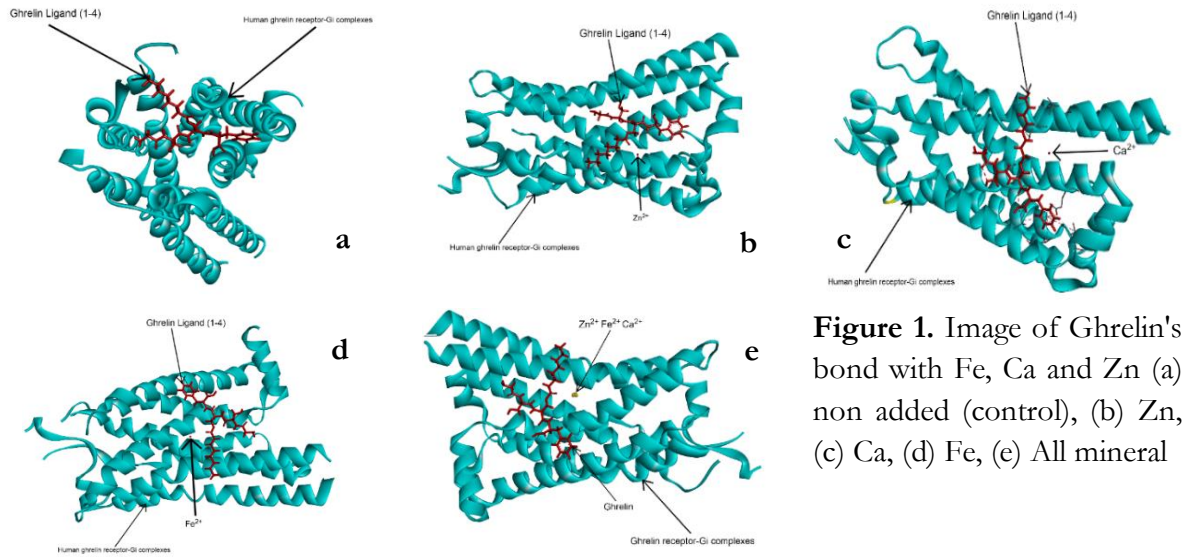

Figure 1. Image of Ghrelin's bond with Fe, Ca and Zn (a) non added (control), (b) Zn, (c) Ca, (d) Fe, (e) All mineral

Table 2. Results of analysis in silico of Ca, Fe, Zn with Leptin

Mineral	Affinity	Total Energy	vdW Energy	Elec. Energy
Ca	-7.355	-70.925	-17.112	-33.032
Fe	-7.171	-70.925	-17.112	-33.032
Zn	-6.960	-70.285	-2.841	-62.477
All Mineral	-7.248	-72.662	-0.991	-85.542
Non-Added (control)	-6.870	-89.622	-6.023	-60.255

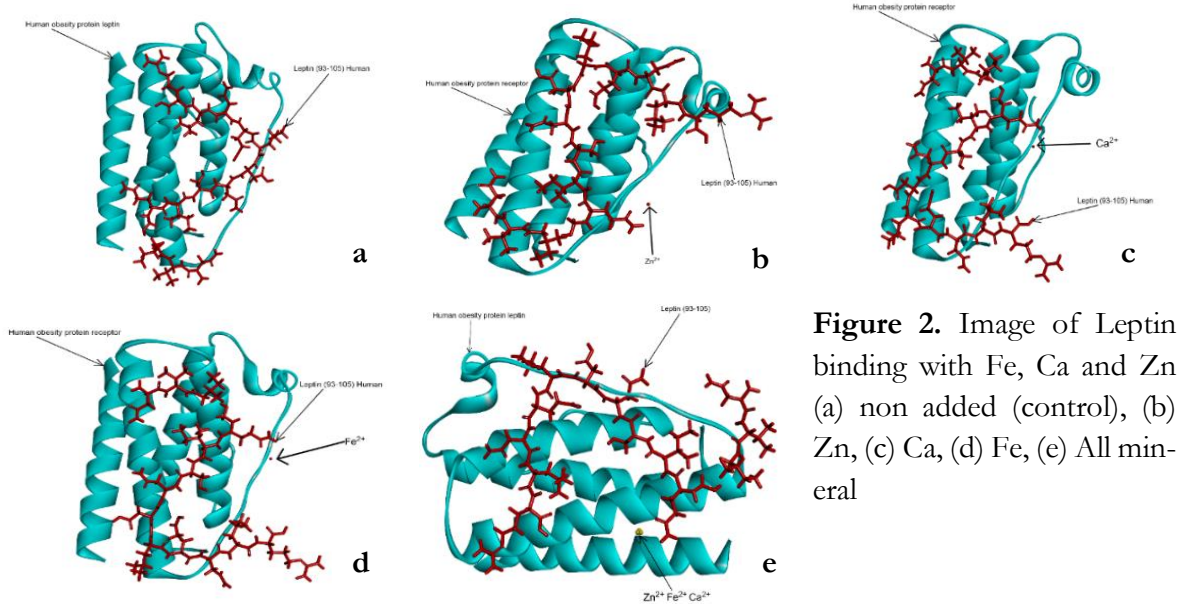

Figure 2. Image of Leptin binding with Fe, Ca and Zn (a) non added (control), (b) Zn, (c) Ca, (d) Fe, (e) All mineral

e-ISSN 2774-2318
p-ISSN 2407-0505

2. Insilico Analysis

The results of in silico analysis of the minerals Ca, Fe, and Zn for compounds that influence appetite, such as ghrelib, leptin, ARC, NPY, IL - 1β, and AgRP, can be seen in Table 1 and Figure 1. All minerals show a smaller affinity value than non-added ones, so it can be predicted that minerals bind more easily to Ghrelin. These minerals have synergistic activity, which can be seen from the small amount of energy required when given simultaneously.

The affinity of Ca is smaller than that of other minerals and all minerals, so it can be predicted that Ca will quickly bind to leptin compared to other minerals. The datas are listed at Table 2. In addition, that indicates the activity of all minerals, if given together, is not synergistic toward leptin, as showen on Figure 2.

The prediction of a bond between Fe, Ca, and Zn with ARC shows that the affinity value for Zn is the smallest compared to Fe, Ca, and all minerals combined (Table 3). This result shows that Zn binds more efficiently to ARC than the others. When given simultaneously, the prediction of mineral activity is not synergistic because the affinity value of all minerals combined is more significant when compared to the Zn affinity value (Figure 3).

The result of prediction binding with NPY is the same binding with leptin, as showen on Figure 4. Zn more easily binds with NPY than another mineral, and if all minerals are given together, the affinity value is more significant than Zn (Table 4).

Table 5 shows if all minerals given together have a smaller affinity value than those given separately. The prediction has the same result as Grelin.

Table 3. Results of analysis in silico of Ca, Fe, Zn with ARC

Mineral	Affinity	Total Energy	vdW Energy	Elec. Energy
Ca	-5.914	-7.387	-2.938	-27.119
Fe	-5.706	-7.375	-7.153	-31.070
Zn	-6.234	-7.191	-2.680	-20.862
All Mineral	-6.098	-6.961	-1.296	-25.110
Non-Added (control)	-6.138	-7.508	-4.196	-28.811

Table 4. Results of analysis in silico of Ca, Fe, Zn with NPY

Mineral	Affinity	Total Energy	vdW Energy	Elec. Energy
Ca	-9.450	-80.121	-25.860	-0.037
Fe	-9.060	-80.163	-27.288	-0.855
Zn	-9.693	-79.973	-28.561	-1.27
All Minerals	-9.315	-51.129	-27.553	-1.056
Non-added (control)	-8.845	-51.725	-22.117	-6.548

e-ISSN 2774-2318
p-ISSN 2407-0505

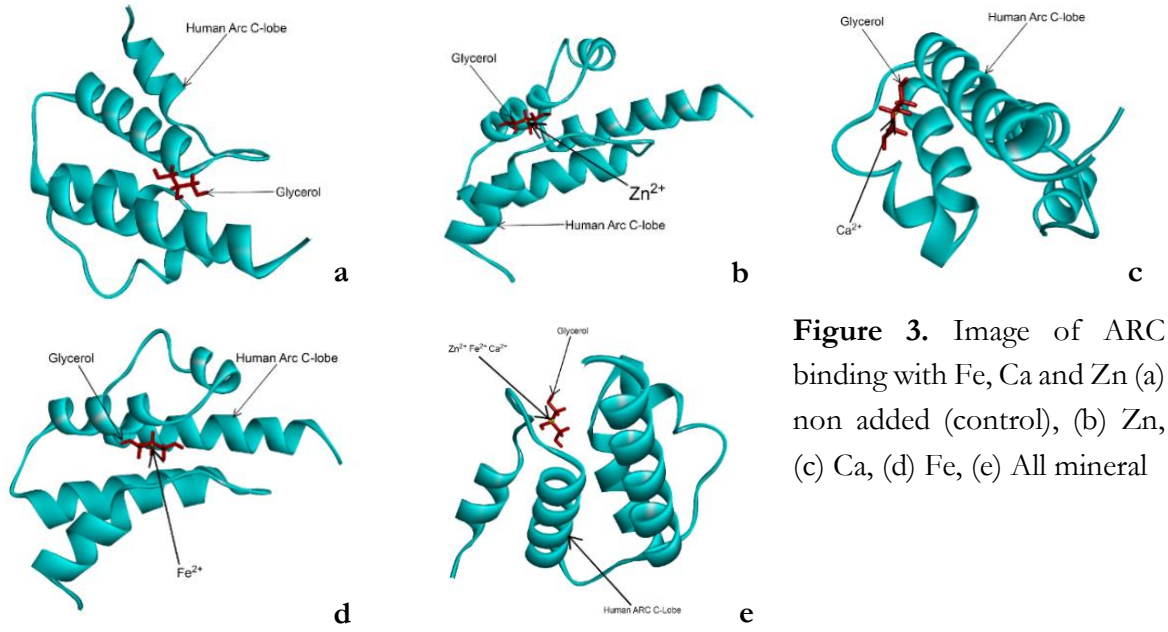

Figure 3. Image of ARC binding with Fe, Ca and Zn (a) non added (control), (b) Zn, (c) Ca, (d) Fe, (e) All mineral

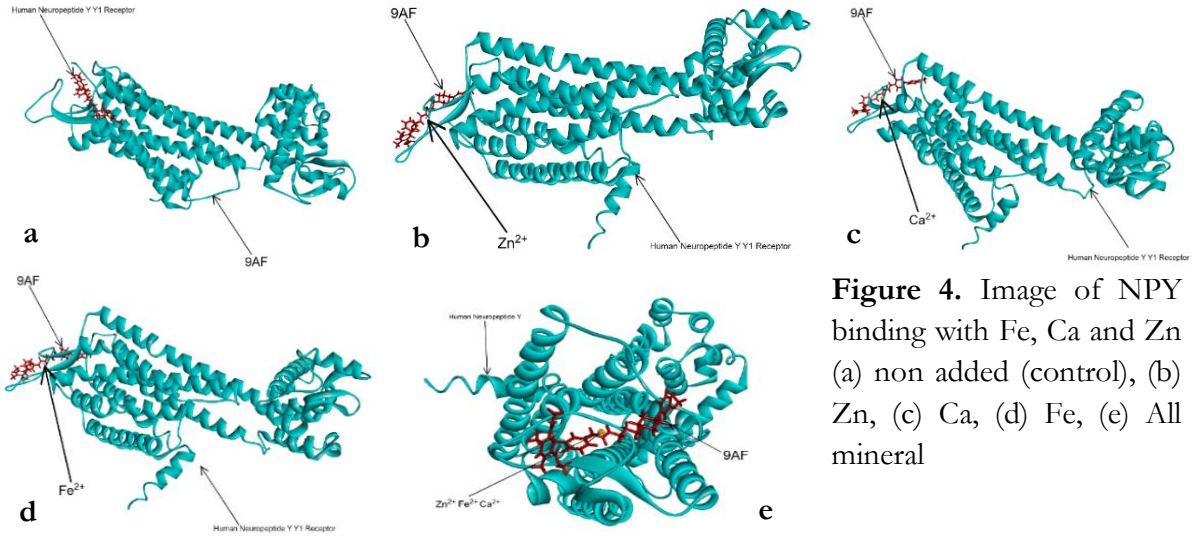

Figure 4. Image of NPY binding with Fe, Ca and Zn (a) non added (control), (b) Zn, (c) Ca, (d) Fe, (e) All mineral

Table 5. Results of analysis in silico of Ca, Fe, Zn with IL-1β

Mineral	Affinity	Total Energy	vdW Energy	Elec. Energy
Ca	-6.999	-34.512	-5.006	-51.432
Fe	-7.214	-32.758	-6.825	-46.162
Zn	-7.513	-31.924	-14.722	-39.644
All Minerals	-8.157	-35.859	-26.021	-19.322
Non-added (control)	-7.770	-36.420	-20.568	-34.769

e-ISSN 2774-2318
p-ISSN 2407-0505

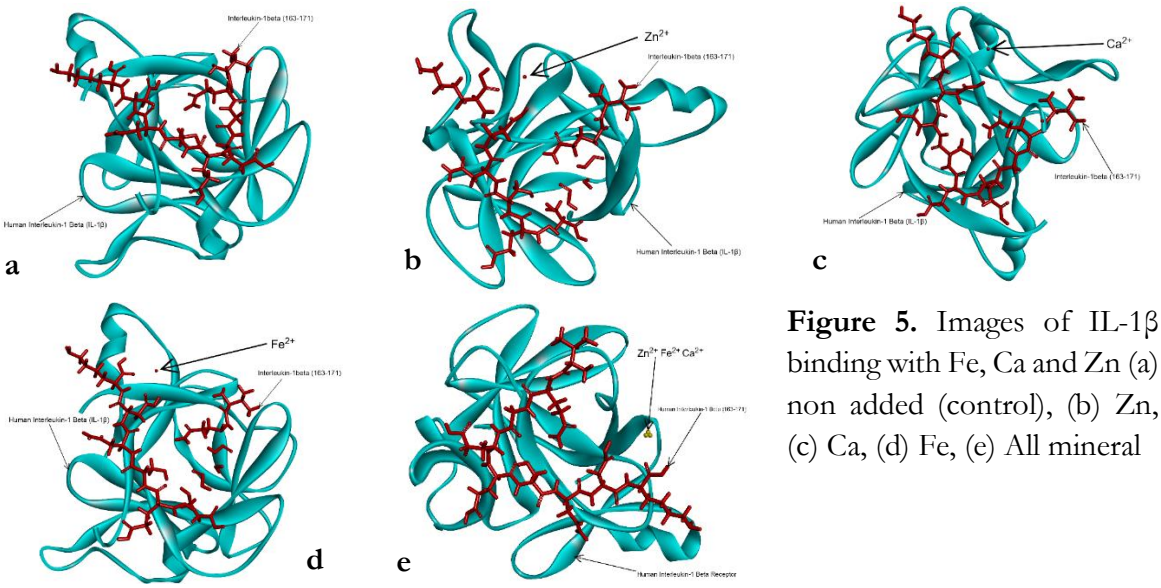

Figure 5. Images of IL-1β binding with Fe, Ca and Zn (a) non added (control), (b) Zn, (c) Ca, (d) Fe, (e) All mineral

Table 6. Results of analysis in silico of Ca, Fe, Zn with AgRP

Metal	Affinity	Total Energy	vdW Energy	Elec. Energy
Ca	-6.778	-29.762	-6.463	-37.509
Fe	-6.741	-31.902	-6.669	-43.870
Zn	-6.818	-32.761	-6.350	-41.729
All Metal	-6.792	-36.700	-7.143	-43.458
Non-added (control)	-6.685	-29.626	-9.949	-36.551

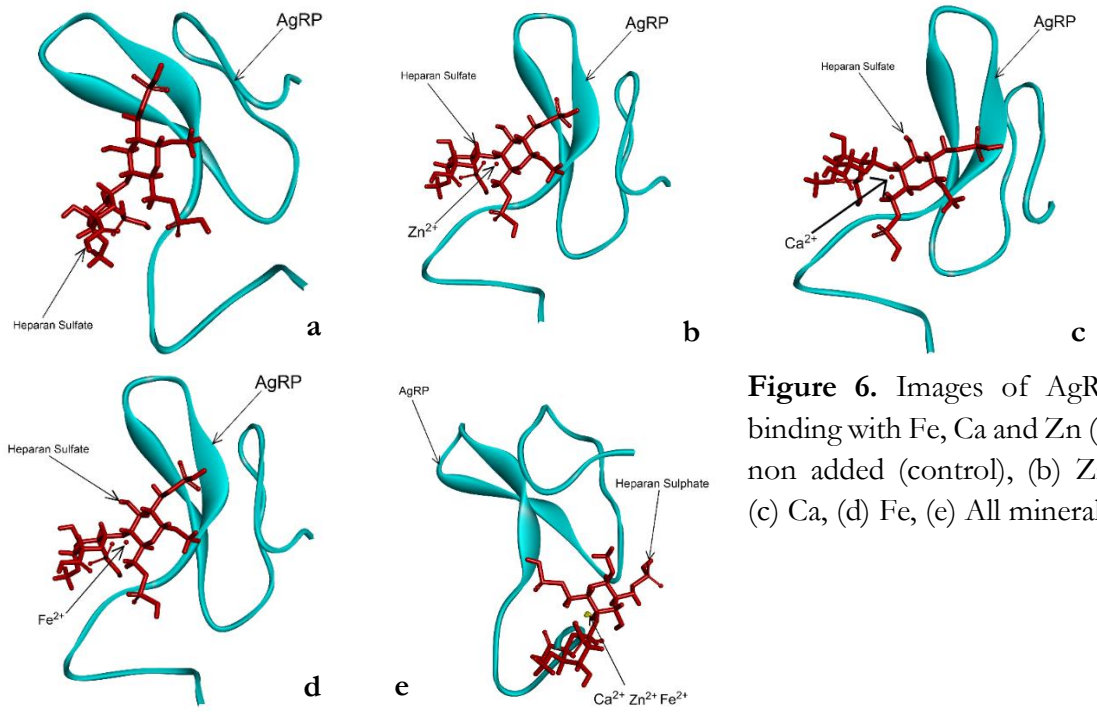

Figure 6. Images of AgRP binding with Fe, Ca and Zn (a) non added (control), (b) Zn, (c) Ca, (d) Fe, (e) All mineral

e-ISSN 2774-2318
p-ISSN 2407-0505

The bond prediction results with AgRP show the same results as the bond with NPY. In a study investigating the docking interactions between Ghrelin, NPY, Leptin, and their respective receptors or ligands, the presence of Ca2+, Fe2+, or Zn2+ ions notably impacted the resulting affinity values. The docking simu-lations that included these metal ions consistently yielded smaller affinity values than those conducted without the added metal ions. The study suggests that the presence of these metal ions can affect the binding interactions between the appetite-stimulating molecules (Ghrelin, NPY, AgRP) and their receptors or ligands. These appetite-stimulating molecules exhibited more negative results in terms of affinity values when compared to the appetite-unstimulating molecules (IL-1B, Leptin, ARC) during the docking simulations.

DISCUSSION

In a study investigating the docking interactions between Ghrelin, NPY, Leptin, and their respective receptors or ligands, the presence of Ca2+, Fe2+, or Zn2+ ions notably impacted the resulting affinity values. The docking simulations that included these metal ions consistently yielded smaller affinity values than those conducted without the added metal ions. This finding is particularly relevant to understanding wasting in underweight children and their deficiencies in essential minerals such as iron (Fe), calcium (Ca), and zinc (Zn). The study suggests that the presence of these metal ions can affect the binding interactions between the appetite-stimulating molecules (Ghrelin, AgRP, NPY) and their receptors or ligands. These appetite-stimulating molecules exhibited more negative results in terms of affinity values when compared to the appetite-unstimulating molecules (IL-1B, Leptin, ARC) during the docking simulations.

The role of Ca, Fe, and Zn in appetite regulation is crucial. Calcium is vital in neurotransmitter release and neuronal signaling, which are involved in appetite control. Iron is necessary to synthesize neurotransmitters and hormones that influence appetite regulation. Zinc is involved in the production and secretion of several appetite-related hormones, including Ghrelin and leptin. Deficiencies in these minerals can disrupt the normal functioning of appetite regulation pathways, potentially leading to imbalances in energy intake and wasting in underweight children. These findings suggest that adding Ca^{2+}, Fe^{2+}, or Zn^{2+} ions can enhance the binding affinity between the studied ligands and their receptors or ligands. The presence of these metal ions likely contributed to the formation of additional coordination bonds or electrostatic interactions, leading to a more favorable docking outcome.[21–23]

The study's relevance to wasting in underweight children and their deficiencies in Fe, Ca, and Zn is crucial for understanding the potential implications of the docking results. Ghrelin, NPY (Neuropeptide Y), Leptin, IL-1 Beta, and ARC are all bioactive molecules that play essential roles in various physiological processes, including appetite regulation, metabolism, and immune response.[24] Wasting in underweight children deficient in Fe, Ca, and Zn, it becomes relevant to investigate how the addition of these compounds affects the interaction between these bioactive molecules and their respective receptors or ligands. The docking results suggest that the presence of metal ions (Ca^{2+}, Fe^{2+}, or Zn^{2+}) during the binding of

e-ISSN 2774-2318
p-ISSN 2407-0505

these bioactive molecules may influence their affinity for their receptors or ligands. Considering the deficiencies in Fe, Ca, and Zn in underweight children, it is plausible that adding these metal ions could modulate the interaction between the studied molecules and their receptors, leading to altered signaling pathways or biological responses.[25]

The role of iron, calcium, and zinc in metabolic processes, their impact on health, and how the presence of these metal ions can affect the binding of bioactive molecules related to appetite regulation, immune response, and other physiological processes such as iron is a crucial component of hemoglobin, the protein in red blood cells that carries oxygen throughout the body. It is also involved in various enzymatic energy production, DNA synthesis, and immune function reactions. Iron deficiency can lead to anemia, fatigue, impaired cognitive development, and a weakened immune system. In the context of wasting and underweight conditions, iron deficiency may contribute to reduced energy production and impaired immune response, further exacerbating the state of undernutrition.[26] Calcium is essential for bone health, muscle contraction, nerve signaling, and blood clotting. It also plays a role in regulating hormone secretion and cellular processes. Inadequate calcium intake can lead to weakened bones, increased risk of fractures, muscle cramps, and impaired nerve function. Calcium deficiency may affect the regulation of appetite-related molecules such as Ghrelin, NPY, and Leptin, potentially influencing hunger and satiety signals and contributing to disrupted appetite regulation in underweight children.[27] Zinc is involved in numerous enzymatic reactions and is essential for immune function, protein synthesis, wound healing, and DNA synthesis. Zinc deficiency can impair immune response, delay growth and development, and increase infection susceptibility. Wasting and underweight conditions caused by zinc deficiency may compromise immune function, impair appetite regulation, and disrupt the signaling pathways involving bioactive molecules such as IL-1 Beta and ARC.[28]

Fe, Ca, and Zn ions during the binding of bioactive molecules can potentially influence their conformation, stability, and affinity for their receptors or ligands. These metal ions may directly interact with the bioactive molecules or indirectly affect the binding through allosteric modulation or structural changes in the receptor. The altered binding affinity or signaling pathways due to the presence of these metal ions can impact appetite regulation, immune response, and other physiological processes related to wasting and underweight conditions. Understanding the interplay between mineral deficiencies and the binding of bioactive molecules provides valuable insights into the potential mechanisms underlying wasting in underweight children. It highlights the complex relationship between nutrition, metabolic processes, and the regulation of various physiological functions. Further research and experimental studies are needed to elucidate the specific molecular mechanisms and functional consequences of metal ion interactions with these bioactive molecules in the context of wasting and mineral deficiencies.[29]

AgrP is a protein involved in regulating pigmentation and energy homeostasis. It has been previously observed that AgrP interacts with heparan sulfate, a linear polysaccharide found on cell surfaces and extracellular matrices.[30] The binding of AgrP with heparan sulfate is of

e-ISSN 2774-2318
p-ISSN 2407-0505

significant interest due to its potential implications in various physiological processes. Additionally, minerals such as Ca, Fe, and Zn are known to modulate protein interactions and play essential roles in cellular functions. The structure of AgrP (87-132) and its binding with heparan sulfate was determined using X-ray crystallography or other relevant structural biology techniques. The binding site and key residues involved in the interaction were identified based on the complex structure. Molecular dynamics simulations or other computational approaches were employed to investigate the stability of the AgrP-heparan sulfate complex in the presence of metal ions. The structural analysis revealed specific binding interactions between AgrP (87-132) and heparan sulfate. Electrostatic interactions, hydrogen bonding, and hydrophobic contacts were observed at the binding interface. The introduction of metal ions (Ca, Fe, Zn) influenced the stability of the complex and resulted in changes in the affinity. This result suggests that the binding of AgrP to heparan sulfate in the presence of Ca may stimulate appetite regulation, potentially leading to increased food intake.

Interestingly, when all three minerals (Ca, Fe, and Zn) are simultaneously added to the AgrP-heparan sulfate, it has been observed that the resulting Affinity value is also lower compared to the control condition. This result indicates that the presence of these minerals stabilizes the binding interaction between AgrP and heparan sulfate, suggesting a potential modulation of appetite regulation. The simultaneous presence of Ca, Fe, and Zn ions may create a unique composition that enhances the stability of the AgrP-heparan sulfate complex, leading to a stronger binding affinity. This stabilized complex could potentially have a synergistic effect on appetite regulation, resulting in increased food intake. However, it is essential to consider that the effects of metal ions on appetite regulation are complex and multifaceted. The concentration and ratio of the metal ions and the cellular context and interplay with other signaling pathways may all influence the ultimate impact on appetite modulation.

CONCLUSION

Sapat siam fish have Fe, Ca, and Zn minerals. The presence of Fe, Ca, and Zn in Sapat siam fish meat prediction can stimulate appetite through binding with ligand Ghrelin, leptin, NPY, ARC, IL-1β, AgRP

REFERENCES

1. Kementerian Kelautan dan Perikanan RI. Data Volume Produksi Perikanan Indonesia (Ton). 2021.
2. Kementerian Kelautan dan Perikanan RI. Data Volume Produksi Perikanan Tangkap per Provinsi (Ton). 2021.
3. Yasinta. IHK di Kalsel pada Desember 2022 Menjadi 117,15. 2023.
4. Dinas Perikanan Provinsi Kalimantan Selatan. Produksi Ikan Darat menurut Jenis Perairan dan Jenis Ikan (Ton) Tahun 2009. 2018.
5. Fadilla EN, Darmanto YS, Purnamayanti L. Characteristics of Dry Noodles with the Addition of Different Fish Scales. J Perikan Univ Gadjah Mada. 2019;21(2).
6. Panthum T, Laopichienpong N, Kraichak E, Singchat W, Nguyen DHM, Ariyaraphong N, et al. The Snakeskin Gourami (Trichopodus pectoralis) Tends to Exhibit XX/XY Sex Determination. Fishes. 2021;6(43):1–17.

e-ISSN 2774-2318
p-ISSN 2407-0505

7. Amare B, Moges B, Fantahun B, Tafess K, Woldeyohannes D, Yismaw G, et al. Micronutrient levels and nutritional status of school children living in Northwest Ethiopia. Nutr J. 2012;11(1):1–8.
8. Patimah S. Hubungan KEK dan Wasting dengan Kejadian Anemia Pada Remaja Putri di Kabupaten Majene. Wind Public Heal J. 2021;2(1):110–9.
9. Diz-Chaves Y. Ghrelin, Appetite Regulation, and Food Reward: Interaction with Chronic Stress. Int J Pept. 2011;
10. Sohn JW. Network of hypothalamic neurons that control appetite. BMB Rep. 2015;48(4):229–33.
11. Inui M, Miyado M, Igarashi M, Tamano M, Kuno A, Yamashita S, et al. Rapid generation of mouse models with defined point mutations by the CRISPR/Cas9 system. Sci Rep. 2014;23(4):5396.
12. Essner RA, Smith AG, Jamnik AA, Ryba AR, Trutner ZD, Carter ME. AgRP Neurons Can Increase Food Intake during Conditions of Appetite Suppression and Inhibit Anorexigenic Parabrachial Neurons. J Neuurosci. 2017;37(36):8678–87.
13. Loopies C, Apituley D, Soukotta D. Scales Mineral Composition of Red Snapper Fish (Lutjanus Sp.) and Parrotfish (Scarus Sp.) with Acid Immersion. Pros Simp Nas VII Kelaut dan Perikan 2020. 2020;
14. Guedes IA, Costa LSC, Dos Santos KB, Karl ALM, Rocha GK, Teixeira IM, et al. Drug design and repurposing with DockThor-VS web server focusing on SARS-CoV-2 therapeutic targets and their non-synonym variants. Sci Rep. 2021;11(1):5543.
15. Santos KB, Guedes IA, Karl ALM, Dardenne LE. Highly flexible ligand docking: Benchmarking of the DockThor program on the LEADS-PEP protein–peptide data set. J Chem Inf Model. 2020;60(2):667–83.
16. Liu H, Sun D, Myasnikov A, Damian M, Baneres JL, Sun J, et al. Structural basis of human ghrelin receptor signaling by ghrelin and the synthetic agonist ibutamoren. Nat Commun. 2021;12(1):6410.
17. Zhang F, Basinski MB, Beals JM, Briggs SL, Churgay LM, Clawson DK, et al. Crystal structure of the obese protein Ieptin-E100. Nature. 1997;387(6629):206–9.
18. Hallin EI, Bramham CR, Kursula P. Structural properties and peptide ligand binding of the capsid homology domains of human Arc. Biochem Biophys Reports. 2021;26:100975.
19. Yang Z, Han S, Keller M, Kaiser A, Bender BJ, Bosse M, et al. Structural basis of ligand binding modes at the neuropeptide Y Y1 receptor. Nature. 2018;556(7702):520–4.
20. Yu B, Blaber M, Gronenborn AM, Clore GM, Caspar DLD. Disordered water within a hydrophobic protein cavity visualized by x-ray crystallography. Proc Natl Acad Sci. 1999;96(1):103–8.
21. Ataie NJ, Hoang QQ, Zahniser MPD, Tu Y, Milne A, Petsko GA, et al. Zinc coordination geometry and ligand binding affinity: the structural and kinetic analysis of the second-shell serine 228 residue and the methionine 180 residue of the aminopeptidase from Vibrio proteolyticus. Biochemistry. 2008;47(29):7673–83.
22. Geng Y, Mosyak L, Kurinov I, Zuo H, Sturchler E, Cheng TC, et al. Structural mechanism of ligand activation in human calcium-sensing receptor. Elife. 2016;5:e13662.
23. Jevtovic V, Alshamari AK, Milenković D, Dimitrić Marković J, Marković Z, Dimić D. The Effect of Metal Ions (Fe, Co, Ni,

e-ISSN 2774-2318
p-ISSN 2407-0505

and Cu) on the Molecular-Structural, Protein Binding, and Cytotoxic Properties of Metal Pyridoxal-Thiosemicarbazone Complexes. Int J Mol Sci. 2023;24(15):11910.
24. Raiten DJ, Ashour FAS, Ross AC, Meydani SN, Dawson HD, Stephensen CB, et al. Inflammation and nutritional science for programs/policies and interpretation of research evidence (INSPIRE). J Nutr. 2015;145(5):1039S-1108S.
25. Dwivedi SL, Garcia-Oliveira AL, Govindaraj M, Ortiz R. Biofortification to avoid malnutrition in humans in a changing climate: Enhancing micronutrient bioavailability in seed, tuber, and storage roots. Front Plant Sci. 2023;14:1119148.
26. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci Off J Isfahan Univ Med Sci. 2014;19(2):164.
27. Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW, et al. Calcium deficiency worldwide: Prevalence of inadequate intakes and associated health outcomes. Wiley Online Library; 2022.
28. Bonaventura P, Benedetti G, Albarède F, Miossec P. Zinc and its role in immunity and inflammation. Autoimmun Rev. 2015;14(4):277–85.
29. Rytter MJH, Kolte L, Briend A, Friis H, Christensen VB. The immune system in children with malnutrition—a systematic review. PLoS One. 2014;9(8):e105017.
30. Chen J, Chen V, Kawamura T, Hoang I, Yang Y, Wong AT, et al. Charge Characteristics of Agouti-Related Protein Implicate Potent Involvement of Heparan Sulfate Proteoglycans in Metabolic Function. Iscience. 2019;22:557–70.