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Advances in Human Milk Fortification with Added Supplements

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Abstract:- Humans require a variety of critical nutrients, and milk is one of the most significant sources of these nutrients. Farm animal milk, whether in the form of cheese, curd, butter, or other fermented or bio-transformed products, is a common source of nutrients. Proteins and lipids are important components of milk's functional component, and studying them is a difficult task. Caseins, a type of protein found in milk, help to produce micelles that vary in size and casein content depending on the species;They play a significant role in the MFGM (Milk Fat Globule Membrane), a topic of recent, intensive research. When broken down by fermentation or digestive processes, milk proteins can function as enzymes, have antimicrobial effects, and hold latent physiological activity that becomes active. Recent developments in proteomics, peptidomics, and bioinformatics are summarised in this article. These new techniques allow us to analyse the peptidomic makeup of the proteins under study, highlight specific aspects of the milk proteome of farm animal species, and even predict potential nutraceutical properties.

***Keyword:-** Biofortification, Vitamins,Micronutrients.*

I. INTRODUCTION

A significant portion of the world's population has been plagued by severe public health problems that have been associated with micronutrient deficiencies. One of the major contributors to the worldwide burden of diseases, particularly in developing nations, has been identified as iron deficiency. Its absence leads to anaemia, which impairs the body's ability to function. (Dhaliwal SS *et.al.* 2022) (Manjeru *et.al.*2019) (Galani *et.al.*2020) (Cappellini*et.al* 2020).Malnutrition or "hidden hunger" can be a symptom of too little nutritional intake, which is a common problem in wealthy countries even though the amount of digested food and supplied calories is more than necessary. According to some estimates, approximately half of the world's population suffers from iron, zinc, and calcium, iodine, and selenium deficiencies. ‘Malnutrition caused by a shortage of micronutrients can be treated in three ways: direct nutrient supplementation, dietary modification and diversity, and indirect therapies like bio fortification.Bio fortification, which is defined as the act of adding useful chemicals to food to increase the content/density of vital nutrients and/or its bioavailability, is a potential way to boost nutrient intakes. Genetic manipulation and traditional breeding with changed fodder, as well as the agronomic approach, can produce "designer food" with higher nutrient content.(Saeid A *et.al* 2019).

A. Bio-enhancement:

Although traditional fortification strategies, such as adding vitamin D to meals exogenously, will continue to be an essential strategy for improving vitamin D intake, novel vitamin D fortification approaches, such as the use of bio fortification or bio-addition, are gaining traction. (Mattila *et al*,)The effect of cholecalciferol-enriched hen diet on egg quality was investigated. After 8-13 days on the high-cholecalciferol diet,The greatest level of cholecalciferol in egg yolk (about 1200 IU (30 ug)/100 g) was found. whereas after 112 days of feeding, the cholecalciferol level gradually declined to ca 880 IU (22 ug)/100 g. The chickens' health, as well as the sensory characteristics, fatty acid content, and eggshell strength, were unaffected by vitamin D. (Mattila *et al*., 2003). The effect of replacing vitamin D with 25(OH) D3 on the vitamin D content of commercial eggs and chicken flesh was studied in another investigation. Two commercial egg yolk pools had vitamin D3 concentrations of 196 IU/100 g (4.9 0.14 ug/100 g) and 160 IU/100 g (4.0 0.10 ug/100 g), respectively, and 25(OH) D3 contents of 1.3 0.19 ug/100 g and 1.0 0.07 ug/100 g. The vitamin D3 level of the chicken flesh pools was 8-12 IU/100g (0.2-0.3 ug/100 g), whereas the 25(OH) D3 value was 0.2 ug/100 g. 25(OH) D3 was effectively transported from the chickens' diet to the yolk in several tests.(Mattila *et al*., 2011).A total of 162 hens were fed three different amounts of vitamin D3 in combination with three different levels of 25(OH) D3 in the study. There was a considerable rise in vitamin D3 and 25(OH) D3 content in the egg yolk as a result of this, but no significant alterations in egg quality measures were found. Depending on the dietary concentrations employed, this method could result in eggs containing between 100 and 500 IU (2.5 and 12.5 ug) vitamin D, allowing children and adults to achieve their daily vitamin D requirements. (Browning *et al.*) (Browning and Cowieson, 2014).

II. MILK BIOFORTIFICATION

In recent years, bio fortification has been proposed and developed as an agricultural modification as a public intervention technique for enhancing the absorbable concentration of micronutrients.Given that micronutrient deficiencies are one of the leading causes of disability and a roadblock to global growth, agriculture must now focus on innovative tactics that not only produce more food, but also offer more nutrient-dense foods to ensure appropriate nutritional health. Bio fortification could be utilized to improve the nutritional content of foods during plant breeding. Bio enriched foods could supply enough amounts of micronutrients for the population if taken on a regular basis. This technique has the potential to be a long-term and cost-effective way to increase the population's micronutrient status. (Hotz and McClafferty, 2007) (Bouis *et al*., 2011).Iron, zinc, and provitamin A are the micronutrients now used in bio fortification initiatives. Feeds are simply fortified with bioavailable micronutrients at specific doses to make bio fortified foods of animal origin. Finally, these nutrients are transported to food products such as milk, meat, and eggs. In order to biofortify milk and cheese with trace elements such as zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn), goats' feed was supplemented with trace element-enriched soy bean meal. Finally, Cu (8.2%), Mn (29.2%), and Zn (14.6%) concentrations were found to be considerably greater in the bio fortified items than in the controls. However, there was no discernible difference in iron concentration between fortified and unfortified milk.Bio enriched milk was suggested as a way to reduce vitamin deficits based on these findings. (Harvest Plus, 2010) (Witkowska *et al*., 2015)Limited data exists to assess the influence of bio fortification of milk on community health indicators, and nutritionists continue to have obstacles in recommending bio fortified foods, including milk, as having good effects on the population's nutritional and health status.

III. PRE-AND PROBIOTICS: MILK FORTIFICATION FOR HEALTH PROMOTION

According to current evidence from systematic reviews, probiotics may significantly contribute to the prevention and treatment of infectious diarrhoea, upper respiratory tract infections, a reduction in allergy symptoms, and cancer prevention.(Roberfroid, 2001) (Bernaola Aponte *et al*., 2010; D'Souza et al., 2002) (Schrezenmeir and de Vrese, 2001)

Efficacy of probiotic-fortified milk on children's height and weight velocity has also been found in several research. It has been observed that giving probiotic milk products to children is a simple and acceptable strategy for reducing the prevalence of respiratory infections in children with no side effects. Probiotic milk, in addition to providing energy, high-quality protein, and minerals, may provide extra health advantages to sick children. (Agustina *et al*., 2013; Sazawal *et al*., 2010a). (Hatakka *et al*., 2001). (Agustina *et al*., 2013).

IV. FORTIFICATION OF MILK TO PREVENT MICRONUTRIENT DEFICIENCY

Micronutrient deficiencies are a significant global public health problem.. Although this is a more serious problem in many low- and middle-income nations where the quantity and quality of dietary intakes are harmed, people in the industrialized world also suffer from various nutritional issues. (Darnton-Hill and Nalubola, 2002.The high incidence of micronutrient deficiency, particularly in young children and women of reproductive age, is a public health issue and a major barrier to socioeconomic advancement.But also because of its economic impact through secondary physical and mental disorders and lower job capacity. (Organization, 2006).There are viable ways for combating micronutrient deficiency, but they require government backing to succeed. Some governments have launched nationwide programs to combat micronutrient deficiency, which include the provision of supplements to certain demographic groups. This technique is thought to be beneficial, especially in high-risk groups; nonetheless, it is normally only used for a short period of time. Food-based solutions, such as food fortification, are cost-effective and long-term approaches to nutritional deficiency prevention and control. The required mass fortification of micronutrients such as iron, vitamin A, and vitamin D in dairy products, especially milk, is generally well-accepted. (Organization, 2006).Previous research has shown the benefits of fortified milk in ensuring enough nutrition for newborns and children. (Oelofse *et al.*,2003; Sazawal *et al.*, 2010a), andsome countries, such as Mexico, have developed national fortification programmes, with milk fortification being a key component. (Rivera *et al.*, 2010).

A. Calcium:

A study has been done to see how calcium-fortified milk, with or without added vitamin K, affects the indicators of bone production and resorption in premenopausal women. After 16 weeks, fortified milk supplementation did significantly reduce bone turnover, but additional vitamin K had no discernible additive impact. (Kruger *et al.*, 2006). There is evidence that daily drinking of fortified milk with 1000 mg of calcium and 800 IU of vitamin D3 increased blood levels of 25(OH)D, decreased bone loss in males over 50 at the femoral neck, entire hip, and ultra-distal radius, and increased parathyroid hormone levels. (Daly *et al.,* 2006). Another study attempted to answer the clinical question of whether there are any residual skeletal benefits once the intervention is stopped. A total of 109 males aged 50 and up were participated in a two-year study on the intake of fortified milk. After the intervention period, the patients were followed for another 18 months to assess BMD. The findings demonstrated that drinking calcium-D3–fortified milk for two years resulted in some BMD advantages in older males after 18 months. (Daly *et al.,* 2008).

B. Iron:

Iron-fortified milk has demonstrated variable degrees of success in treating iron deficiency according to numerous research from various nations. Contrarily, effectiveness is influenced by the intervention's dosage, duration, and participant's iron status at the outset. According to data from Chile, Brazil, Morocco, India, and Argentina, the use of iron-fortified milk is associated with lowering the prevalence of iron insufficiency and improving iron status in infants and early children. (El Menchawy *et al.*, 2015; Hertrampf *et al.,* 1990; Iost *et al.,* 1998; Rapetti *et al.,* 1997; Sazawal *et al.,* 2010a; Stekel *et al.,* 1988; Torres *et al.,* 1996).

C. Vitamin A:

The 1940s saw the start of fluid milk fortification in the United States. (Murphy *et al.,* 2001).In a randomised controlled clinical trial, it was discovered that daily consumption of 250 mL of fortified milk with vitamin A—which provided 196 retinol equivalents (RE)/day—increased total body vitamin A stores, liver stores, and serum retinol concentration in preschoolers in Mexico who had mild to moderate vitamin A deficiency. This suggests that supplementing milk with vitamin A may be a useful way to treat vitamin A deficiency in young Mexican children. (Lopez-Teros *et al.,* 2013).

D. Zinc:

There are few studies on milk fortification with zinc (Zn). According to a study, adolescent Mexican girls who consumed milk enriched with Zn and other micronutrients had higher plasmahigher Zn concentrations than the controls. As a result, micronutrient-fortified milk was introduced as a successful way to prevent or treat Zn deficiency in adolescent girls. (Méndez *et al.,* 2012).

E. Multi-Micronutrient:

Multiple micronutrient deficiencies are highly frequent in most cases, especially in poorer nations, and are mainly caused by poor-quality diets. (Dijkhuizen et al., 2001; Jiang et al., 2005; Winichagoon, 2008).This problem raises the question of whether delivering multiple micronutrient interventions is more effective than delivering a single nutrient. Providing multiple nutrients at the same time is a more cost-effective and long-term solution to multiple micronutrient deficiencies. Although it would be interesting to see how micronutrients and technological challenges interact.(Allen et al., 2009; Winichagoon, 2008). Few studies have looked into the effects of fortifying milk with multiple nutrients. According to a systematic review of the effects of micronutrient-fortified milk and cereal food on haemoglobin levels in infants and children, the presence of additional micronutrients in fortified milk or cereal seems to be more effective than just iron fortification, indicating that complex micronutrient deficiencies are to blame for health problems. (Eichler *et al.,* 2012).

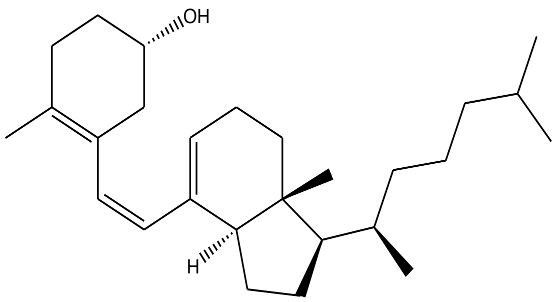
F. Vitamin D:

Vitamin D is a fat-soluble vitamin that is important for bone health as well as non-skeletal health, organ function, and disease prevention. (Christakos *et al.,* 2013). Vitamin D is mostly obtained by skin synthesis in response to ultraviolet Blight exposure, with a diet devoid of fortified foods providing only a small percentage of the required quantity. (Holick, 2017). Vitamin D deficiency, which is caused by a lack of sun exposure, has gotten a lot of attention in recent years because of its link to the risk of significant chronic diseases. Because prolonged exposure to sunlight has been linked to an increased risk of skin cancer, food fortification has emerged as a viable option for ensuring vitamin D sufficiency.

G. Vitamin D chemistry and metabolism:

Vitamin D is a broad term that refers to a group of biologically active secosteroids that play a role in calcium and bone metabolism, as well as other vital biological functions. Vitamin D comes in two forms: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2). Vitamin D is a 9, 10-secosteroid, a steroid derivative in which the B ring of the steroid molecule is expanded and the connection between C9 and C10 is severed. The natural form of the vitamin, cholecalciferol, is produced by a non-enzymatic mechanism in the skin of vertebrates by the action of UV radiation (290-315 nm) on the precursor molecule 7-dehydrocholesterol. (Christakos et al., 2016; Holick, 1987, 2017). Irradiation of the plant sterol ergosterol produces ergocalciferol. Ergocalciferol's significance stems from its widespread use as a food supplement and fortifier. Due to the limited number of such sources and the low vitamin D concentration in most of them, vitamin D cannot usually be obtained in sufficient amounts through natural dietary sources. As a result, in most nations, exposure to sun radiation is the primary source of vitamin D. (Holick, 2017). In this view, vitamin D isn't actually a vitamin because it may be made from sunshine and doesn't need to be obtained through dietary sources. Vitamin D is a prohormone of the steroid hormone 1, 25-dihydroxyvitamin D (1, 25(OH) 2D), which is the active form of vitamin D. (Christakos *et al.,* 2016; DeLuca, 2004).

V. STRUCTURE OF VITAMIN D



(1S)-4-Methyl-3-[(1Z)-2-{(1R,3aR,7aR)-7a-methyl-1-[(2R)-6-methylheptan-2-yl]-2,3,3a,6,7,7a-hexahydro-1H-inden-4-yl}ethen-1-yl]cyclohex-3-en-1-ol

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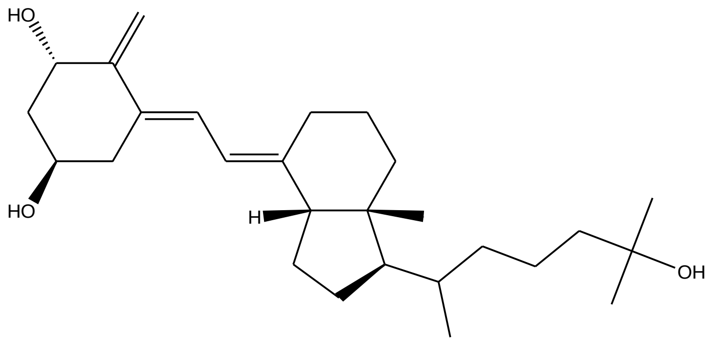
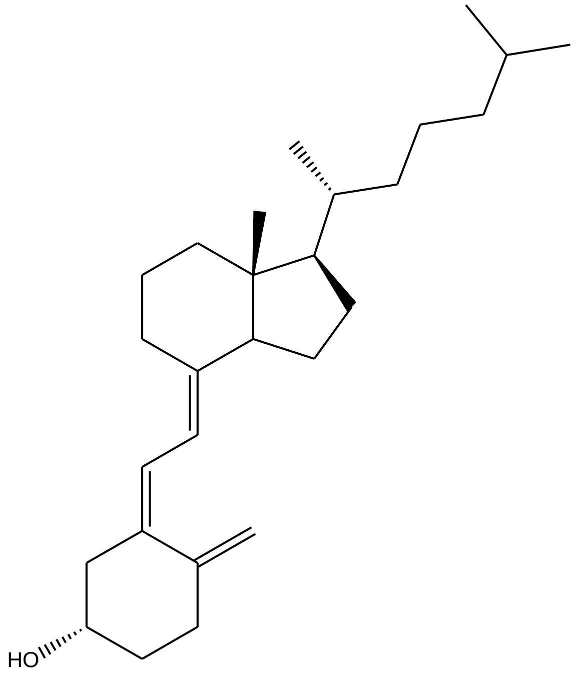
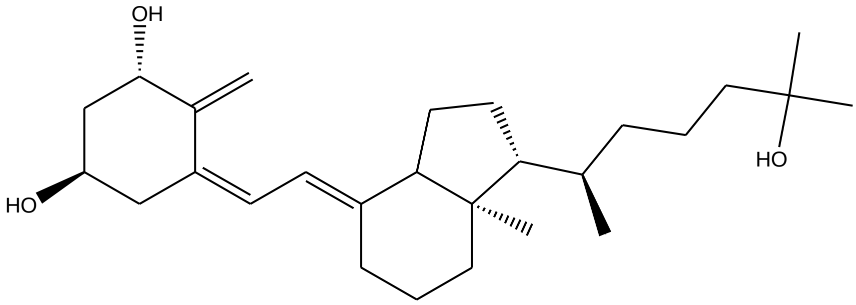


(1R,3S,5Z)-5-((E)-2-((1R,7aR)-hexahydro-1-((R)-6-hydroxy-6-methylheptan-2-yl)-7a-methyl-1H-inden-4(7aH)-ylidene)ethylidene)-4-methylenecyclohexane-1,3-diol



Cholecalciferol



Calcitriol

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A. Vitamin D's biological roles include:

Compounds of vitamin D have both genomic and non-genomic effects. The hormonal form of vitamin D, 1, 25(OH) 2D3, works by attaching to the vitamin D receptor (VDR), a nuclear receptor family member (Pike *et al.*, 2017). 1,25(OH)2D3 activates a heterodimeric complex of the VDR and the Retinoic X Receptor (RXR) that binds to specific vitamin D response elements (VDREs) in the regulatory area of the gene controlled by 1,25(OH)2D and either upregulates or downregulates the expression of target genes by binding to the VDR. (Lin, 2016; Pike *et al.*, 2017). The main biological roles of 1, 25(OH) 2D3 are to regulate bone metabolism and maintain skeletal health, which are mostly accomplished by increasing calcium and phosphorus absorption in the intestine and encouraging bone mineralization. VDR is found in the colon, bones, kidneys, and parathyroid glands, which is not surprising. VDR, on the other hand, is found in a wide range of cells and tissues throughout the human body, suggesting that 1, 25(OH) 2D3 may play a function in non-skeletal health. (Lin, 2016; Pike *et al.*, 2017; Wang *et al.*, 2012). Experiments in animal models, as well as epidemiological and clinical data, point to vitamin D's significance in infection prevention, cancer prevention, cardiovascular health, and the treatment of some autoimmune illnesses. (Christakos *et al.*, 2013).

Vitamin and chemical name	Source	Function	Deficiency Disease	Daily requirement
Vitamin A (Retinol)	Milk, butter, cheese, egg yolk, fish liver oil, green leafy vegetables and ripe fruits like mango, papaya, and tomatoes	Maintenance of healthy epithelial tissues, proper functioning of retinal and vision	Stunted growth, night blindness, dry eyes, xerophthalmia and keratinization	750µg
Vitamin B1	Yeast, liver, germ of cereals, nuts, pulses, rice polishing, egg yolk, and legumes	Proper utilization of carbohydrate in food, and nutrient of nerve cells	General fatigue and loss of muscle tone, ultimately leads to beriberi	1-1.5mg
Vitamin B2 (Riboflavin)	Liver , yeast, milk, egg, green vegetables	Necessary for tissue oxidation and growth	Angular stomatitis, Cheilosis, Dermatitis and eye lesion	1.5-2 mg
Vitamin B3 (Niacin nicotinic acid)	yeast, offal, fish, pulses and whole meal cereals; synthesis in the body from tryptophan	Metabolic function in cells necessary for the tissue oxidation	Prolonged deficiency causes, pellagra	10-20mg
Vitamin B5 (Pantothenic acid)	Liver, yeast, egg yolk, and fresh vegetables	Formation of RBCs	Dermatitis and adrenal insuffiency	Unknown
Vitamin B6 (Pyridoxine)	Meat, liver, vegetable, bran of cereals egg yolk, beans and soya bean	Protein metabolism and formation of RBCs and WBCs	Rarely observed because of wide distribution in food	1-2mg
Vitamin B7 (Biotin)	Yeast, liver, kidney, pulses and nuts	Carbohydrate and fats metabolism, and growth of bacteria	Dermatitis and conjunctivitis	Unknown
Vitamin B9 (folic acid)	Dark green vegetables, liver, kidney , and eggs	Formation of RBCs	Megaloblastic anemia and diarrrhoea	200gm
Vitamin B12 (cyasnocobalamin)	Liver, Milk, Mould,Fermenting liquors, and egg	Maturation of RBCs	Pernicious anemia, megaloblastic anemia, and degeneration of nerve fibre of spinal cord	2-3gm
Vitamin C (Ascorbic acid)	Citrus fruit, raisins, berries, green vegetables, potatoes ,liver, and glandular tissues of animal	Formation and maintenance of healthy intercellular matrix, and maturation of RBC	Multiple haemorrhages, slow wound healing, anemia, gross deficiency causes scurvy, bleeding gums, and increased capillary fragility	30-20mg
Vitamin D (Calciferol)	Fish liver oil, milk, cheese, and egg yolk	Facilitates absorption and utilization of	Rickets in children, and osteomalacia in adult	2.5 µg

		calcium and phosphorus for healthy bones and teeth		
Vitamin E (Tocopherols)	Egg yolk, milk butter, green vegetables, nuts, and oils of germs of cereals like wheat.	Maintains a healthy muscular	Anaemia in pregnant women, and neurological disorders	8-10 mg.
Vitamin K (Phylloquinone)	Cabbage, cauliflower, fish liver, fruits, and leafy vegetables	Formation of prothrombin and factors VII ,IX, and X in liver	Slow blood clotting, and hemorrhages in new born	70-14- gm.

Table1: Vitamin: Source, function, deficiency disease, daily requirement
(Dr. Sunil jawla, Thakur publication, Social and preventive pharmacy)

VI. FOODS THAT HAVE BEEN FORTIFIED WITH VITAMIN D

A. *Food fortification*

The adding of nutrients or non-nutrient bioactive components to food and food constituents is referred to as fortification. Fortification can be used as a public health measure to encourage the consumption of a nutrient in order to prevent or correct deficiencies, but it can also be used to attract customers. (Dwyer et al., 2015).Instead of adding the vitamin during food processing, bio fortification, or bio-addition, refers to boosting the nutrient content of a meal of plant or animal origin by selective breeding, feeding, treating, or genetically engineering, or by adding another food rich in a nutrient. (Calvo and Whiting, 2013; Cashman, 2015)

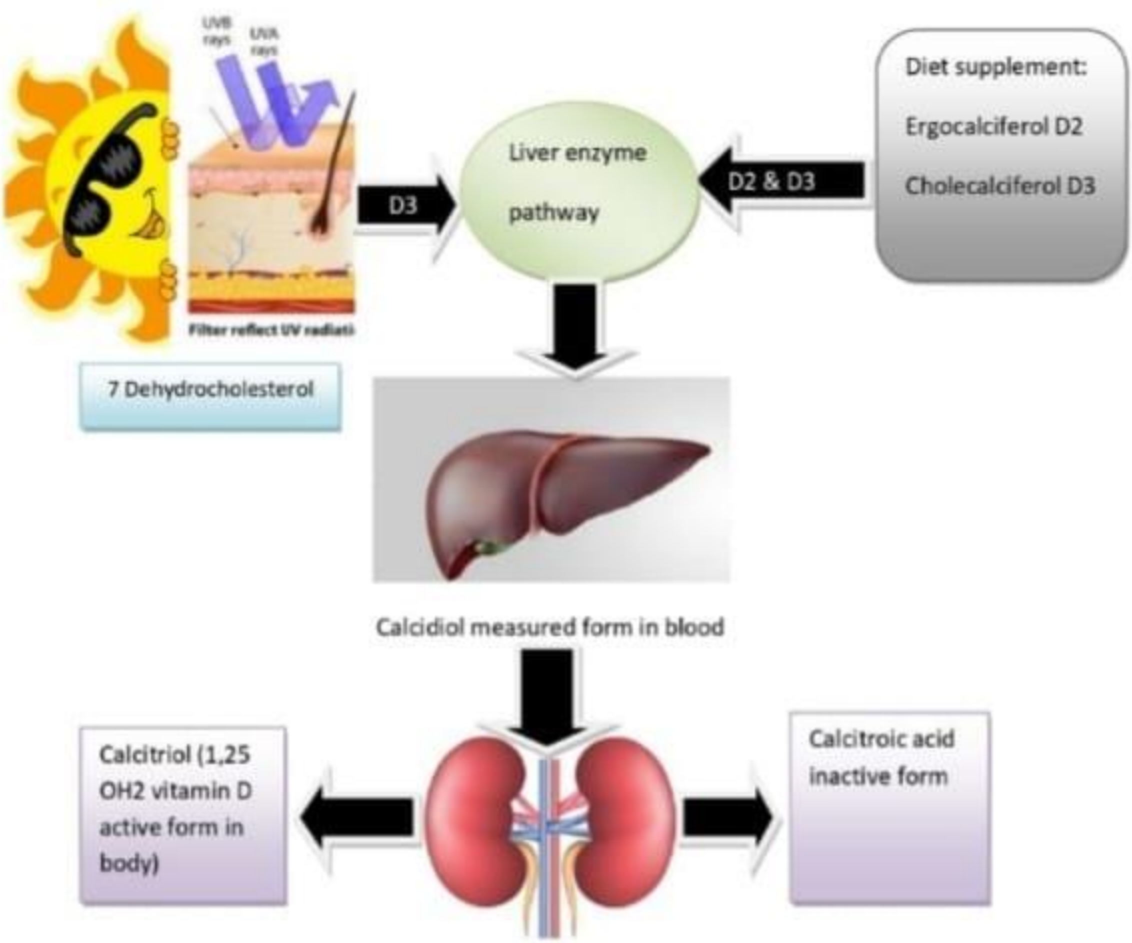

Fig. 1: A picture of demonstrating the source and form of vitamin D

(Mostafa Z. et.al 2015)

B. Fortification strategies

Food fortification has been done with cholecalciferol, ergocalciferol, and their 25-hydroxylated metabolites. Two recent studies summarise the findings of various intervention trials comparing the efficacy of vitamins D2 and D3 in boosting 25(OH)D levels, with the majority indicating that vitamin D3 is more successful in raising 25(OH)D levels. (Silva and Furlanetto, 2018; Wilson *et al.*, 2017).

Following the discovery of vitamin D's anti-rachitic properties in the early twentieth century, a number of foods, including milk and dairy products, margarine, and even beer, were fortified. (Holick, 2004) Since the 1940s, cow's milk has been the primary source of vitamin D in the United States and Canada, and a carefully designed fortification policy has been implemented to eradicate rickets as a public health issue. In the United States and Canada, two different fortification procedures, a voluntary approach and a mandated approach, are used, but both provide fortified foods with proven efficacy. Currently, it is estimated that fortified foods provide for 60% of vitamin D intake from foods in the United States and Canada. Fluid milk and cereals are the main sources of vitamin D in the United States. As seen by the greater 25(OH) D levels found in Canadians who consume fortified milk compared to those who do not consume fortified milk, fortified milk and fortified margarine are the leading contributors to vitamin D intake in Canada. (Calvo and Whiting, 2013).

C. Vitamin D level and food fortification:

Only if the goals of boosting vitamin D intake and 25(OH) D levels are met can food fortification efforts be considered successful. Vitamin D fortification of milk is a safe and effective strategy for dealing with vitamin D deficiency. (Piirainen *et al.*)Only 30.6 percent of 4-year-old children in Finland attained the recommended intake after fortification, although mean blood 25-hydroxyvitamin D concentration increased from 22 ng/mL (54.7 nmol/L) to 26 ng/mL (64.9 nmol/L) following fortification. (Piirainen *et al*., 2007).713 healthy school children aged 10-14 years were assigned to receive either unfortified milk (group A) or milk fortified with 600 IU (15 ug) or 1,000 IU (25 ug) of vitamin D 13 per day for 12 weeks in another prospective double-blind randomised control experiment. Following supplementation, 5.9% of patients in group A, 69.95% in group B, and 81.11 percent in group C had serum 25(OH)D levels greater than 20 ng/ml (50 nmol/L), compared to 6.32 percent, 4.9 percent, and 12 percent, respectively, at baseline. (Khadgawat *et al.,* 2013).

VII. CONCLUSION

Bio fortification is a straightforward, non-invasive, and accessible technique to enhance nutritional consumption among participants, and it may provide long-term health advantages. A comprehensive fortification strategy should encompass a diverse range of staple foods and take into account the dietary habits of all population groups in each country.

A public health issue that affects people of all ages is vitamin D insufficiency. GendersS, socioeconomic status, educational attainment, and ethnicity, and has enormous potential human and economic costs for many nations in Europe and elsewhere. As a result, there is a pressing need to rectify this shortcoming. Food fortification can help to address vitamin D deficiency, which is a major public health problem. Food fortification with vitamin D is technically viable using both conventional and bio fortification technologies, and can be used to address insufficiency in large populations without requiring changes in lifestyles or eating habits.

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