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VITAMIN D FORTIFICATION OF EGGS ALONE AND IN COMBINATION WITH MILK IN WOMEN AGED 44-65 YEARS: FORTIFICATION MODEL AND ECONOMIC EVALUATION

OBOGATITEV JAJC Z VITAMINOM D V KOMBINACIJI Z MLEKOM PRI ŽENSKAH, STARIH MED 44 IN 65 LET: MODEL OBOGATITVE IN EKONOMSKO VREDNOTENJE

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ABSTRACT

Introduction: For almost nine decades, the fortification of foods with vitamin D has been proven effective in preventing rickets. This study aims to build and economically evaluate a fortification model based on egg biofortification and milk (including yoghurt) fortification.

Methods: A cross-sectional study was carried out between 1. March and 31. May 2021. Three hundred and nineteen healthy women from the Central Slovenian region aged between 44 and 65 were recruited for the study, with 176 participants included in the final analysis. For the fortification model calculations, the vitamin D contents of unenriched milk (including yoghurt) and eggs were replaced by enriched foods containing vitamin D. The economic evaluation was done using available drug and food supplement prices. Fortification costs were calculated using vitamin D prices provided by suppliers.

Results: Mean vitamin D intake from food was 2.19±1.34 µg/d. With fortification Model 1 (enriched eggs), it would be: 6.49±4.45 µg/d, and with Model 2 (enriched eggs and milk): 10.53±6.49 µg/d. Without fortification, none of the participants would reach a daily vitamin D intake >10 µg. With fortification Model 1 (egg fortification), 15.3% would reach >10 µg and with Model 2 (egg and milk fortification) 46.2% would reach >10 µg. The economic comparison of the annual cost of 10 µg vitamin D/d/person was EUR 6.17 for prescription drugs, EUR 6.37 for food supplements, EUR 0.09 for direct milk fortification and EUR 0.12 for egg biofortification with vitamin D.

Conclusions: Egg and milk (including yoghurt) fortification could cost-effectively increase vitamin D intake in the Slovenian population of women between 44 and 65 by almost five-fold, and could significantly lower the prevalence of vitamin D deficiency. Additional research and changes to legislation are needed before this can be introduced.

Keywords:
vitamin D, 25(OH) D3, pre-menopausal women, post-menopausal women, fortification, economic evaluation

IZVLEČEK

Uvod: *Obogatitev živil z vitaminom D se je kot učinkovita pri preventivi rahitisa izkazala že pred skoraj 90 leti. Namen študije je zgraditi obogatitveni model, temelječ na bioobogatitvi kokošjih jajc preko krme, samostojno in v kombinaciji z mlekom (vključno z jogurtom). Strošek smo ekonomsko ovrednotili v primerjavi z individualnim dodajanjem vitamina D z zdravili ali prehranskimi dopolnili.*

Metode: *V obdobju med 1. marcem in 31. majem 2021 smo izvedli presečno študijo, v katero je bilo vključenih 319 žensk, starih med 44 in 65 let. Ob upoštevanju izločitvenih dejavnikov smo v končno analizo vključili 176 udeleženk. Za izračun obogatitvenega modela smo vsebnost vitamina D v neobogatenem mleku (vključno z jogurtom) in jajcih nadomestili z vsebnostjo v obogatenih živilih. Ekonomsko vrednotenje smo opravili na podlagi razpoložljivih cen zdravil, prehranskih dopolnil in cen surovin.*

Rezultati: *Povprečni vnos vitamina D s hrano je bil 2,19 ± 1,34 µg/d. Pri modelu obogatitve 1 bi bil vnos: 6,49 ± 4,45 µg/d in pri modelu 2: 10,53 ± 6,49 µg/d. Brez obogatitve nobena udeleženka ne dosega dnevnega vnosa vitamina D > 10 µg. Vnos > 10 µg/d bi z modelom obogatitve 1 (obogatitev jajc) in modelom 2 (obogatitve jajc in mleka) doseglo 15,3 % in 46,2 % udeleženk. Primerjava letnih stroškov dodajanja 20 µg vitamina D/osebo je bila 6,17 € za zdravila na recept, 6,37 € za prehransko dopolnilo, 0,09 € za neposredno obogatitev hrane in 0,12 € za obogatitev jajc z vitaminom D. ≥ 1 jajce/teden uživa 88,6 % udeleženk.*

Zaključki: *Obogatitev jajc in mleka (vključno z jogurtom) lahko stroškovno učinkovito poveča vnos vitamina D v slovenski populaciji žensk, starih med 44 in 65 let, za skoraj petkrat in lahko bistveno zmanjša prevalenco pomanjkanja vitamina D. Dodajanje vitamina D preko zdravil in prehranskih dopolnil je učinkovito na individualni ravni, v nasprotju z obogatitvijo živil pa ne doseže najranljivejših populacijskih skupin. Pred implementacijo bodo potrebne dodatne raziskave in prilagoditev zakonodaje.*

Ključne besede:
vitamin D, 25(OH) D3, ženske v predmenopavzi, ženske po menopavzi, obogatitev živil, ekonomsko vrednotenje

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1 INTRODUCTION

The main vitamin D source for humans is the synthesis of cholecalciferol (D3) from 7-dehydrocholesterol in skin exposed to UV rays. To a lesser extent, it is obtained from fatty fish and eggs in the form of cholecalciferol (D3) and from UV-exposed yeast and fungi in the form of ergosterol (D2). Animal products also contain 25-hydroxycholecalciferol (25(OH)D3) (1).

The interest in vitamin D has increased due to the association of vitamin D deficiency with increased risk of SARS-CoV-2 infection and disease severity (2). Vitamin D deficiency is not a new disease, nor is its prevention. Herodotus first described possible vitamin D deficiency and its aetiology in a lack of sun exposure in 525 BC (3). Beginning in the 17th century, industrialisation and increased coal use resulted in air pollution. Particles suspended in the air decreased sun exposure and contributed to the widespread development of rickets. In Germany, Schmorl (4), as cited in Hernigou et al. (3), noted that 96% of the children that died in 1906 had signs of rickets. In the 19th century, the first associations between sun exposure and rickets were made, followed by the discovery that cod liver oil cured and prevented rickets. In 1904 Buchholz reported that UV irradiation of human skin cured rickets (5). Steenbock discovered that milk and yeast could be fortified with vitamin D by irradiation with UV lamps (6). Hess and Windaus first demonstrated that cholecalciferol and ergocalciferol could be synthesised with cholesterol and ergosterol (7). Within 20 years, a wide variety of foods were fortified, from milk, margarine and breakfast cereals to bread and even beer. This led to the rapid eradication of rickets in developed nations (5). However, some errors in fortification and subsequent hypervitaminosis effectively led to the prohibition of mass vitamin D fortification in most countries in the 1950s (8). Prior to EC Regulation 1925/2006 (9), voluntary fortification in many European countries was severely restricted or tightly controlled (10).

Only a few countries currently maintain mandatory fortification of milk (US, Canada: 1–2 µg/100 mL) and fat spreads (Canada: 15–30 µg/100 g) (11). There is mandatory fortification of bread in Jordan (12) and the voluntary fortification of milk (2 µg/100 mL) and fat spreads (20 µg/100 g) in Finland (13). Instead, rickets prevention relies on rickets prophylaxis by vitamin D supplementation in infants (14). The fortification programme in Finland has proved to be particularly successful, as 71% of men and 55% of women reached a dietary vitamin D intake ≥10 µg/d (13).

Various fortification possibilities have been suggested (15, 16): milk (13), flour (17), UV-irradiated yeast for the preparation of bread (18), UV-irradiated mushrooms (19), and the enrichment of eggs through feed (20). The appropriate fortification media should be consumed regularly by most of the population, be affordable, and lead to only a minimal increase in production costs (16).

Hen eggs can be readily enriched with vitamin D (20–26), and cholecalciferol (D3) has been shown to be more effective than ergocalciferol (D2) (25). To achieve even higher vitamin D efficacy in eggs, the 25(OH)D3 form can be used in addition to cholecalciferol (22). This is an issue because most studies have only analysed cholecalciferol (20–26). Food composition data frequently undervalues vitamin D content, as it is commonly based only on cholecalciferol (D3) determination (1, 27). The reported efficacy of dietary 25(OH)D3 to increase serum 25(OH) D is ~5-fold greater than that of cholecalciferol (24). Considering its higher potency, the contribution of 25(OH) D3 intake from eggs may be even greater than that of cholecalciferol (D3) (1, 24).

Supplemental vitamin D and 25(OH)D3 for animal nutrition are commercially available (28). Some legal barriers to the biofortification of animal feed remain, as the maximum levels of added vitamin D in animal feed place a limit on higher levels of biofortification (29).

Perimenopausal and postmenopausal women are at high risk of vitamin D deficiency and its public health consequences. Menopause and age-related changes in vitamin D and calcium metabolism increase the risk of vitamin D deficiency and secondary hyperparathyroidism (29, 30). Milk intake in Slovenia is low; therefore, milk fortification alone would not be an effective fortification medium (31). Consequently, this article aims to build a fortification model based on egg biofortification and the fortification of milk (including yoghurt). Additionally, it seeks to evaluate the costs compared to individual supplementation.

2 METHODS

2.1 Study design and participants

A cross-sectional study of the Health Interview Survey (HIS) type and a Clinical Health Examination Survey (HES) was conducted between 1. March and 31. May 2021. Three hundred and nineteen healthy women from the Central Slovenian region, aged between 44 and 65, were recruited for the study.

Participants were initially recruited by healthcare workers during preventive health visits at Kamnik and Vrhnika health centres (Figure 1). Since recruitment was hindered by Covid-19 preventive measures, the snowball sampling method was successfully employed (32). This meant that after the participants were informed of their vitamin D status, either by post or email, they were asked to invite suitable peers to the study. They were also given a link to an online contact form through which new participants could join.

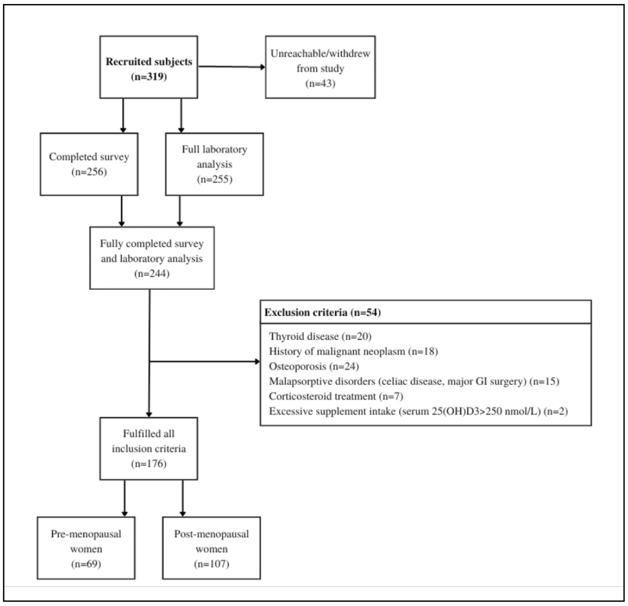


Figure 1. Flow chart of the study in healthy women from the Central Slovenian region aged between 44 and 65, conducted between 1. March and 31. May 2021.

2.2 Inclusion and exclusion criteria

All willing women between the ages of 44 and 65 were included in the study. For the final analysis, the exclusion criteria, summarised according to (33) and supplemented, were: 1) confirmed osteoporosis; 2) current oestrogen therapy (prior oestrogen therapy was not an exclusion criterion if >3 months have elapsed since the last dose); 3) treatment with corticosteroids in the previous 12 months; 4) treatment of hyperthyroidism or hypothyroidism in the previous 12 months; 5) any new or unregulated chronic disease (e.g. unregulated diabetes); 6) malignant diseases at any time in life; 7) hysterectomy was not an exclusion criterion; 8) health conditions associated with vitamin D deficiency (malabsorption, chronic inflammatory bowel disease, gastrointestinal resection, liver disease, acute gallbladder disease, chronic kidney disease grade 3 to 5); 9) institutionalisation and poor physical mobility; 10) excessive vitamin D intake (serum 25(OH)D3 >250 nmol/L).

2.3 Data collection

After obtaining signed informed consent forms (ICF), a telephone survey was performed by trained registered nurses (RN) or nutritionists (MNutr.) using a purpose-made questionnaire. The questionnaire was tested in a pilot study carried out in 2019 (34). It contained a modified food frequency questionnaire (FFQ), body weight, height, health status, use of food supplements with vitamin D, food intake, menstruation/menopause, sun exposure status, and socio-economic and socio-demographic status. Blood samples were collected during regular working hours at the participating health centres and Ljubljana University Medical Centre between 1. March and 31. May 2021. All samples were transported to a central laboratory at Ljubljana University Medical Centre, where they were analysed the same day or stored at -80°C until analysis. Measurements were performed at the Clinical Institute of Clinical Chemistry and Biochemistry (Ljubljana University Medical Centre). In all participants, S-25(OH)D was measured by an Architect 25-OH D vitamin kit (Abbott Diagnostics, Lake Forest, USA).

2.4 Egg and milk fortification and economic analysis model

There are many studies on the fortification of eggs with vitamin D; the best to date, containing data for both cholecalciferol (D3) and 25(OH)D3, is by Browning et al. (20). The models are made to be safe, cost-effective, simple and feasible.

The input data for the model is: 1) assessment of vitamin D status by 25(OH)D3 determination; 2) assessment of food intake that will be used for vitamin D intake calculation and for the calculation of fortification; 3) food composition data; 4) method for achieving the biofortification of eggs (20); 5) vitamin D ingredient prices.

Vitamin D status was assessed by serum 25(OH)D3 determination. The data for food sources of vitamin D was assessed using an FFQ. Fish composition data was obtained from the Open Platform for Clinical Nutrition (OPEN) database. A mean of 10.01μg/100 g was used for fatty fish and a mean of 1.49 μg/100 g was used for lean fish (35); for eggs, 2.4 μg/1 egg (63.8 g) was used (20). In the fortification model, egg composition data from Browning et al. (20) was used. Eggs were produced using feed containing 250 μg/kg of cholecalciferol and 69 μg/kg of 25(OH)D3. The mean egg weight was 63.8 g and the feed conversion-to-egg ratio was 2.27±0.693) (kg feed/kg egg). For the production of one enriched egg, 144.82 g of feed was used. This amount contained 36.21 μg cholecalciferol and 9.99 μg of 25(OH)D3. Conversion from feed to eggs was 14.32% for cholecalciferol and 13.54% for 25(OH)D3. There were no detrimental effects on animal health or production parameters (20). For the economic analysis, the prices of vitamin D3 and feed grade 25(OH)D3 were provided by DSM Nutritional Products. The milk and yoghurt can be fortified by direct addition during manufacture (16). Milk and yoghurt do not differ significantly in nutrient content and method of vitamin D addition.

We built three models: a no-fortification model, Model 1 (only egg fortification used) and Model 2 (combination of egg fortification and milk and yoghurt fortification). Fortification model input data is presented in Table 1. For the fortification model calculations, vitamin D contents for unenriched milk, yoghurt and eggs were substituted with enriched food vitamin D content. All participants were included in the building of both fortification models even if they did not consume milk, yoghurt and/or eggs.

Table 1. Fortification model input data.

		Cholecalciferol µg/1 egg	**25(OH)D3** µg/1 egg	**Vitamin D efficacy*** µg (IU)/1 egg
No fortification	Unenriched egg	1.07	0.27	2.4 (80 IU)
Model 1	Enriched eggs**	5.18	1.35	11.98 (479 IU)
Model 2	Enriched eggs**	5.18	1.35	11.98 (479 IU)
	Enriched milk and yoghurt	3 µg/100 mL		3.00 µg (120 IU)/100 mL

*Vitamin D efficacy to increase 25(OH)D levels includes cholecalciferol and 25(OH)D3 (20, 24)
**Source of vitamin D efficacy data: (20).
The mean egg weight used was 63.8 g.
1µg vitamin D=40 IU

2.5 Statistical analysis

The variables observed in the analysis were: 1) serum 25(OH)D3 vitamin D (dependent variable); 2) taking (frequency and quantity) of dietary supplements/ medicines with vitamin D; 3) survey questionnaire (eating habits – frequency and quantity, vitamin D content, sun exposure); and 4) body mass index (BMI).

Serum 25(OH)D3 levels were stratified using Endocrine Society cut-off values: Deficiency: <50 nmol/L, Insufficiency: 50–75 nmol/L and target concentration for optimal vitamin D effects 75–125 nmol/L (36, 37).

The data has a normal distribution. The differences between users and non-users of supplements were examined using a two-sample t-test. The statistical analysis was conducted using MS Excel and SPSS, version 27 (IBM, 2020).

2.6 Ethical approval

The study protocol was approved by the Slovenian National Medical Ethics Committee, identification no KME 0120-68/2019/9 (approval letter ID 0120-68/2019/9, date of approval: 22. March 2019). The study was performed in compliance with the requirements of local authorities. All subjects signed a written informed consent form (ICF) before participating in the study.

3 RESULTS

The population characteristics, vitamin D status, supplement use and food intake are presented in Table 2.

Table 2. Population characteristics, vitamin D status, supplementation and food intake in healthy women from the Central Slovenian region aged between 44 and 65, carried out between 1. March and 31. May 2021 (n=176).

Variables	Category/ Unit	All participants n=176	Users of supplements n=108	Non-users of supplements N=68	p- value
Age	year	53.83±4.99	54.25±4.83	53.14±5.17	0.150
BMI	kg/m²	25.74±4.36	25.47±4.18	26.14±4.63	0.322
	18.5-24.9	51.70%	54.63%	47.06%	
	25.0-29.9	32.39%	31.48%	33.82%	
	30.0-34.9	10.80%	8.33%	14.71%	
	35.0-39.9	4.55%	4.63%	4.41%	
	over 40.0	0.57%	0.93%	0.00%	
LABORATORY ANALYSIS					
25(OH)D	nmol/L	66.42±27.35	76.24±27.06	50.82±19.55	**0.000**
	<30	8.52%	3.70%	16.18%	
	30-50	15.91%	6.48%	30.88%	
	50-75	43.18%	44.44%	41.18%	
	>75	32.39%	45.37%	11.76%	
Education	Primary and secondary school	32.39%	29.63%	36.76%	
	Higher education	67.61%	70.37%	63.24%	
MILK AND EGG INTAKE					0.0400
Milk and yoghurt intake (mL/d)		134.58±160.46	154.26±171.29	103.33±137.03	
Milk consumers (≥200 mL /week)		72.16%	79.63%	73.53%	0.895
Egg intake (pcs/week		3.17±2.44	3.19±2.52	3.14±2.34	
Egg consumers (≥1 egg/week)		88.6%	86.1%	92.7%	
VITAMIN D INTAKE AND SUPPLEMENTATION					0.532
Mean food intake (µg/d)		2.19±1.34	2.24±1.41	2.11±1.22	
Supplement use (≥5 µg vitamin D /d)		61.36%	100%	0.00%	
Mean supplemental intake (µg/d)		21.72±26.24	35.4±25.26	0±0.08	**0.000**
Mean intake of all sources (µg/d)		24.06±26.19	37.64±25.17	2.48±3.33	**0.000**
FORTIFICATION MODEL					

	Fortified eggs (Model 1)	**Fortified eggs and milk (Model 2)**	**Fortified eggs (Model 1)**	**Fortified eggs and milk (Model 2)**	**Fortified eggs (Model 1)**	**Fortified eggs and milk (Model 2)**
Mean vitamin D intake all sources (µg/d)	29.49± 26.56	33.52± 27.24	43.33± 25.33	47.96± 25.40	7.49± 4.22	10.59± 6.13

All values are presented as Mean ±SD or %. T-test analysis was performed to compare variables among users and non-users of supplements. Values are presented as mean ±SD, ¤<0.05 was considered statistically significant (¤ values of significant variables are in bold print).

Figure 2 shows the difference in serum 25(OH) D3 concentrations between users and non-users of supplements. The 5 µg vitamin D cut-off was chosen because it is the lowest level found in multivitamin supplements.

3.1 Fortification model

Mean vitamin D intake from food without fortification was 2.19±1.34 µg/d. With fortification Model 1, it would be6.49±4.45 µg/d and with Model 2 it would be 10.53±6.49 µg/d. Without fortification, none (0%) of the participants would reach a daily vitamin D intake >10 µg. With fortification Model 1 (egg fortification), 15.3% would reach >10 µg and with Model 2 (egg and milk fortification) 46.2% would reach >10 µg. Vitamin D supplementation was not included.

Figure 3 shows vitamin D intake without fortification, with fortified eggs, and fortified eggs in combination with fortified milk (including yoghurt).

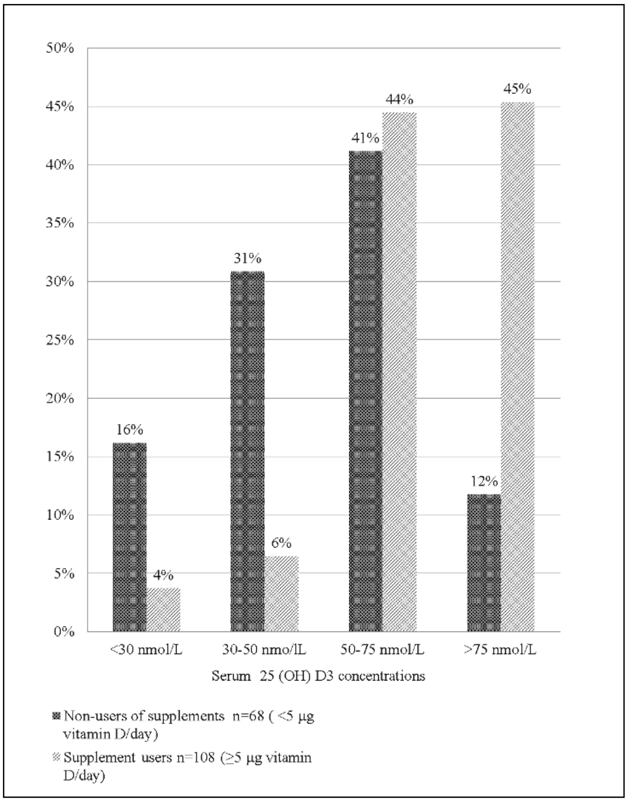


Figure 2. The differences of serum 25(OH)D3 concentrations between users and non-users of supplements in healthy women from the Central Slovenian region aged between 44 and 65 were assessed between 1 March and 31 May 2021 (n=176).

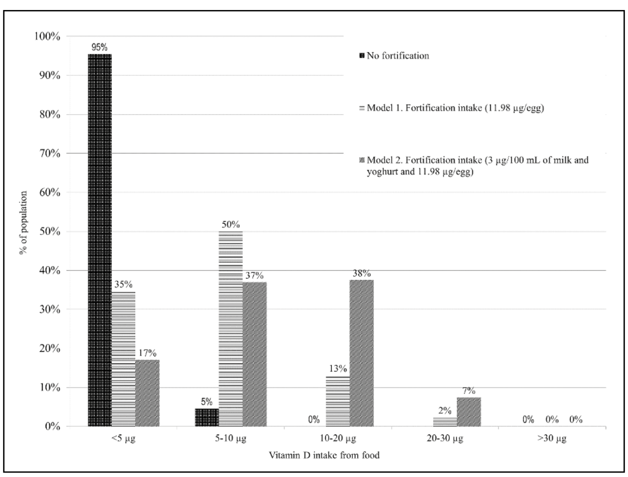
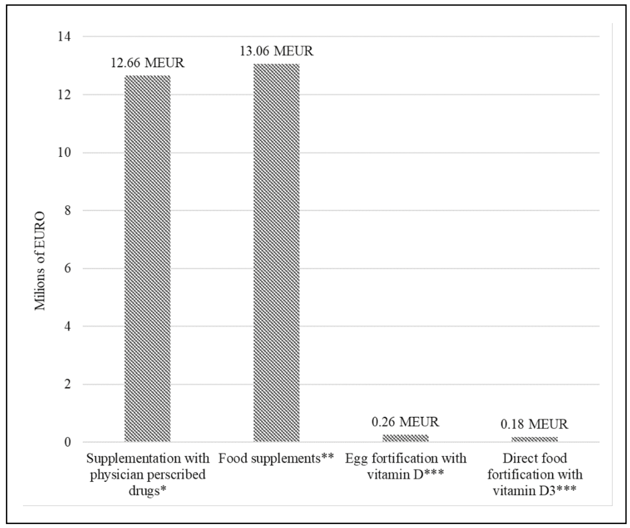


Figure 3. Vitamin D intake from food, without fortification with projection using fortified eggs and fortified eggs and milk (including yoghurt) in healthy women from the Central Slovenian region aged between 44 and 65, was assessed in the period between 1 March and 31 May 2021 (n=176).

3.2 Economic analysis

Figure 4 presents the annual cost of fortification compared with vitamin D-containing prescription drugs and food supplements. The annual cost of 10 µg vitamin D/d/person is shown in Table 3.



*25(OH)D3 analysis, physician visits and dispensing costs are not included. The cheapest drug was used.
**Cheapest food supplement per µg, cost with VAT.
***No additional cost is predicted.

Figure 4. Comparison of the annual cost of 10 µg of vitamin D daily for Slovenians >1 year of age (n=2,050,916) (38). (Key: MEUR – millions of Euros).

4 DISCUSSION

The results of our study indicate that egg biofortification in combination with milk and yoghurt fortification could lead to a 4.8-fold increase in vitamin D intake. In Slovenia, vitamin D drug prescriptions amounted to EUR 6,029,647 in 2020 (39). Considering the low cost of fortification, even a slight decrease in dosage from fortified food would bring substantial savings.

Vitamin D intake and status in Slovenia were assessed in 2017/2018 by Hribar et al. (40–42). The supplementation rate in our study was increased due to the Covid-19 pandemic. Consequently, we report higher serum 25(OH) D3. However, a study of a population of people aged between 18 and 74 that looked at the intake of the specific foods addressed in this study produced very similar findings. The intake of eggs in adults and older adults was between three and four eggs/week. The daily intake of milk and dairy products in was 169 g in older adults and 174 g in adults (31, 40, 41). Consequently, their milk-based fortification model was ineffective because milk intake in the Slovenian population is low (41).

Table 3. Annual costs of 10 μg vitamin D/d/person.

Variables	Cost per 10 μg (EUR)	Annual costs of 10 μg vitamin D/d/person (EUR)
Supplementation with physician-prescribed vitamin D drugs*	0.016911	6.17
Vitamin D food supplements**	0.017440	6.37
Egg biofortification with vitamin D***	0.000342	0.12
Direct food (milk) fortification with vitamin D3***	0.000240	0.09

*Serum 25(OH)D3 analysis, physician visits and dispensing costs are not included.
**Cheapest food supplement per μg, cost with VAT.
***No additional cost is predicted.

The impact of Model 2 (eggs and milk) compared to milk and fat spread fortification in Finland would be lower but still substantial: fortification Model 2 would result in a mean vitamin D intake of 10.53±6.49 μg/d and 46.2% of women reaching vitamin D intake >10 μg/d, compared with the Finnish intake of 12 μg/d (95% CI: 11, 12 μg/d) and 58% of women reaching vitamin D intake >10 μg/d (13).

Similar economic evaluations of food fortification have been performed in Germany. A nationwide fortification programme with 20 μg of vitamin D and 200 mg of calcium per day would prevent 36,705 fractures in women aged 65 and older and save EUR 315 million in health expenditure in this part of the population alone. The yearly cost of a fortification programme for Germany would be EUR 41 million. The cost would be EUR 0.11 per person for cholecalciferol (D3) and EUR 0.22 per person for calcium (43). In our study, the calculated costs were higher, as a small volume of ingredient prices were used in the case of egg biofortification; the higher cost reflects a <15% carryover of dietary vitamin D from hen to egg (15).

4.1 Effectiveness and safety considerations

Food supplements are an effective strategy for preventing vitamin D deficiency on an individual level. However, there are significant limitations. Supplements have a higher risk of excessive intake than food fortification (16, 44). Typically, they do not reach people at the highest risk of deficiency, as supplement intake is positively associated with a healthier lifestyle and higher socioeconomic status (16).

Achieving 25(OH)D3 levels >50 nmol/L in the entire population by fortification is unrealistic and unsafe, as it would mean a very high median intake. The food fortification goal set out in the World Health Organization (WHO) guidelines is 'to provide most (97.5%) of individuals in the population group(s) at greatest risk of deficiency with an adequate intake of specific micronutrients, without causing a risk of excessive intakes in this or other groups. Inadequate intake is thus defined as intake below Estimated Average Requirements (EAR) and not the recommended intake (RI) value (45). Therefore, based on the WHO guidelines (45), the target intake should be at least EAR (46), which is 10 μg (16). The RI of vitamin D to reach serum 25(OH)D3 levels >50 nmol/L for people over one year of age is set at 20 μg (800 IU) by DACH (45, 46,47), at 15 μg (600 IU) by the European Food Safety Agency (EFSA) and the Institute of Medicine (IOM) (46,47), and at 25 μg (1000 IU) by the Endocrine Society (36, 37,47). It is important to note that supplementation with 10 μg of vitamin D/day is not enough to achieve vitamin D sufficiency (49). As in the Finnish case, food fortification effectively prevented 25(OH)D levels <30 nmol/L, but 13.7% of non-users of supplements still did not achieve levels >50 nmol/L. Importantly, it did not cause a decline in vitamin D supplementation, as supplementation with vitamin D increased almost fourfold (13).

The EFSA report concluded that a long-term daily dose of 250 μg (10,000 IU) is considered to reflect a no observed adverse effect level (NOAEL). Using a safety factor, the upper tolerable intake level (UL) was established at 100 μg (4,000 IU) per day (48). In our fortification models, none of the participants would exceed >30 μg/day even with the maximum modelled fortification.

The effect of the fortification can be difficult to predict as serum 25(OH)D3 levels do not increase proportionally; in populations with lower baseline concentrations of serum 25(OH)D3, there is a higher increase than in populations with higher baseline levels. In Finland, mean serum levels of 65 nmol/L 25(OH)D3 were achieved by systematic vitamin D food fortification and with guidelines suggesting supplementation. This was sufficient to decrease the prevalence of 25(OH)D3 concentrations <30 nmol/L below 1% (15).

4.2 Strengths and limitations of the study

Our study is the first to implement fortification modelling to calculate the effects of egg biofortification on vitamin D intake. Previous studies have been concerned only with egg fortification (19–25). The main limitation of this study is that it is based on data about egg vitamin D content from a single study (20), as other studies lacked 25(OH)D3 data (20–26). Before implementation, additional studies on the transfer of vitamin D from feed to eggs that would include 25(OH)D3 would need to be conducted.

Additionally, before biofortified eggs can be put on the EU market, feed legislation will need to be changed to allow higher vitamin D levels (29), as current legislation allows for vitamin D content in eggs to a maximum of 4.54±1.38 μg of vitamin D/egg (16). Currently, such eggs would be illegal in the EU. Since changes were made in 2006 to vitamin D legislation concerning the addition of vitamins and minerals in 2006 (9), feed legislation could also see changes in the future, especially if research provides the basis for doing so.

Enrolment in the study was offered to all women coming for preventive medical examinations; however, those who chose to enrol in this voluntary study had significantly higher education and lower BMI than the general population (50). Additionally, socio-economic factors, such as language barrier, access to transport and flexibility of working hours, influenced withdrawal from the study.
These fortification models are simple and could be readily applied to any country with significant egg intake. In countries with very high egg intake, such as Mexico (>350 eggs/capita/year) or Japan (>325 eggs/capita/year), egg fortification would be viable even with lower (51) legal levels of fortification (16).

The models could also be applied to already-collected data from representative populations older than one year to confirm suitability for the entire population (31). Additionally, simultaneous fortification of eggs with several other nutrients, e.g. iodine, would also be possible (52).

5 CONCLUSION

Due to the Covid-19 pandemic, almost two thirds of our subjects took vitamin D supplements. However, less than one third of subjects had optimal 25(OH)D3 levels, and 25(OH)D3 levels were particularly low in those who did not use supplements. Vitamin D biofortification of hens' eggs, especially in combination with milk fortification, could be a simple, safe and cost-effective way of increasing vitamin D intake in the population.

CONFLICTS OF INTEREST

No conflict of interest.

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ETHICAL APPROVAL

The study protocol was approved by the Slovenian National Medical Ethics Committee, identification no KME 0120-68/2019/9 (approval letter ID 0120-68/2019/9, date of approval: 22. March 2019). The study was performed in compliance with the requirements of local authorities. All subjects signed a written informed consent form (ICF) before participating in the study.

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