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Effects of Vitamin D Addition Levels on Growth Performance, Body Composition and Serum Biochemical Parameters of Mid-Term Tilapia

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Abstract

Twenty hundred and seventy tails of tilapia with an initial body weight of (78.58±1.93) g were randomly divided into 6 groups (3 replicates in each group, 15 fish per replicate), and the levels of vitamin D were respectively fed. 0 (control group), 200, 400, 800, 1600, 3200 IU / kg of 6 kinds of feed (crude protein, crude fat content of 30% and 7.6%, respectively), after 12 weeks of feeding, through Growth performance, body composition, and serum biochemical indicators were determined to determine the appropriate level of vitamin D in the mid-term development of the tilapia feed. The results showed that the weight gain rate of each vitamin D-added group was significantly higher than that of the control group ($P<0.05$), and the feed efficiency of 200, 400, 800, 1600 IU / kg group was significantly higher than that of the control group ($P<0.05$). There was no significant difference in water and crude protein content between the two groups ($P>0.05$). With the increase of dietary vitamin D, the crude fat content of whole fish increased first and then decreased, reaching 200 IU/kg group. The highest, significantly higher than the other 5 groups ($P <0.05$); the total ash content of whole fish in each vitamin D addition group was significantly higher than the control group ($P <0.05$), but there was no significant difference between the vitamin D added groups. Difference ($P>0.05$). With the increase of the level of vitamin D in the feed, the activity of alkaline phosphatase in serum increased first and then decreased, and the group of 200, 400, 800 IU / kg was significantly higher than the control group ($P<0.05$); total cholesterol in serum, total Both protein and albumin levels reached a maximum in the 200 IU/kg group and were significantly higher than the control group ($P < 0.05$). It can be concluded that the proper addition of vitamin D in the feed can promote the growth of the mid-term tilapia. Excessive vitamin D addition may cause liver damage and affect fat metabolism.

Keywords

Tilapia; Vitamin D; Requirement; Growth; Serum Biochemical Index.

Introduction

Vitamin D is a steroid derivative, the most important of which are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D is a fat-soluble vitamin that plays an important role in the metabolism of substances. It is necessary to maintain the normal life activities of aquatic animals, but it cannot be synthesized in animals. The main physiological function of vitamin D is to regulate the calcium and phosphorus metabolism of animals and promote bone growth and calcification (Sahota, 2014). Aquatic animals lacking vitamin D will show some lack of symptoms. For example, the lack of vitamin D in the feed will cause slow growth of rainbow trout, increased hepatic lipids, damage to calcium balance, and submicroscopic structure of muscle spasm and axial white muscle.

Rainbow trout lacks vitamin D and also exhibits symptoms such as lordosis and muscle tissue weakness. After 16 weeks of feeding the channel catfish with a vitamin D-deficient diet, vitamin D deficiency is observed: growth is blocked and the calcium, phosphorus and crude ash content of the fish is reduced. Hybrid tilapia lack of vitamin D will show growth retardation, decreased feed coefficient and decreased hepatocyte index, and alkaline phosphatase activity and hemoglobin content in plasma also decreased. The muscle fiber structure of the abalone disc is affected by the lack of vitamin D.

There are some studies on the need for vitamin D in fish feed. For example, rainbow trout requires 1600 to 2400 IU/ kg of vitamin D, and the demand for vitamin D in large scale salmon is 2400 IU. /kg, the requirement for vitamin D in hybrid tilapia is 374.8 IU/kg; the requirement for vitamin D in juvenile carp is 431.0 IU/kg, the amount of vitamin D required by channel catfish It is 250 to 500 IU/kg. These findings suggest that there is a large difference in the amount of vitamin D required by different cultured species of aquatic animals (Klack and de Carvalho, 2010).

Genetically improved farmed tilapia (GIFT, Oreochromis niloticus) is a genetically modified Nile tilapia with rapid growth, high meat yield, few diseases, and stable genetic traits. Large-scale artificial breeding has been carried out in Guangxi and Hainan (Spirichev, 2015).

At present, the research on the vitamin D requirement mainly focuses on the hybrid tilapia about 1 g, and the research on the vitamin D requirement of the tilapia in the middle of the cultivation has not been reported. Since different types of fish and the same species of fish have different vitamin D requirements at different stages of growth, this experiment uses vitamin D gradient addition test to study the different levels of vitamin D to develop mid-term tilapia [Initial weight (78.58±1.93) g.

The effects of growth performance, body composition and serum biochemical indicators to determine the appropriate amount of vitamin D in the medium-term tilapia feed, enriching the growth stages of Luo Fei. The nutritional needs data of fish also provides a theoretical basis for the addition of vitamin D to feed.

Materials and Methods

Experimental Feed

Purified diet prepared by using casein and gelatin as protein sources, dextrin as sugar source, corn oil and soybean oil as fat source, the composition and nutritional level are shown in Table 1. Add different levels (0, 200, 400, 800, 1 600, 3 200 IU/kg) of vitamin D to the base feed (vitamin D source is vitamin D3 microparticles for feed supplementation with a vitamin D content of 476,000 IU/g), prepared 6 test feeds.

All raw materials were pulverized and passed through a mesh with a pore size of 0.3 mm, and thoroughly mixed. Then, add appropriate amount of water and then machine into a strip of 2.0 mm diameter with a meat grinder. After being blown dry with an electric fan in a cool and ventilated place, it was crushed and grown by a shredder. Cylindrical particles of 0 mm are stored in a -20 °C freezer for later use.

Test Fish and Feeding Management

The test Jifu tilapia was provided by the Guangxi Tilapia National Breeding Test Site. The test fish was purchased and returned to the Yangtze River Fisheries Research Institute of the Chinese Fisheries Research Institute for disinfection with povidone iodine and cultured in the culture tank of the indoor recirculating aquaculture system (Hohman and Weaver, 2011).

It was first domesticated with basal feed for 2 weeks to adapt it to the test environment and test feed, and to consume vitamin D stored in the body. Before the start of the formal trial, the test fish were subjected to starvation for 24 h, and then 270 tails of robust and well-regulated Gifford tilapia were selected, with an initial average weight of (78.58±1.93) g, randomly assigned to 18 In the bucket, 15 tails per barrel.

The 18 barrels were randomly divided into 6 groups (3 replicates in each group), and 6 kinds of test feeds were fed separately, and fed 3 times a day (08:00, 12:00, 16:00), and the table was fed satiety.

Change the water 1/4 every morning. The weight of the test fish was weighed once every 2 weeks, and the feeding amount was adjusted according to the change in body weight.

The culture test lasted for 12 weeks. Daily observations recorded water temperature, test fish feeding and death. During the feeding period, the water temperature is 27 to 33 °C, the pH is 7.2 to 7.5, the dissolved oxygen concentration is 6.0 mg/L or more, and the ammonia nitrogen concentration is 0.03 mg /L or less (Table 1).

Sample Collection

After 12 weeks of culture, the test fish were subjected to starvation for 24 h, then the total weight of the test fish in each barrel was weighed and the mantissa was recorded, and the terminal weight gain, weight gain rate (WGR) and survival rate (survival rate) were calculated. Rate, SR.

Calculate the amount of feed for each repeated feed and calculate feed efficiency (FE). Three fish were randomly selected from each barrel for the determination of conventional nutrients such as crude protein, crude fat, water and coarse ash.

Three fish were randomly selected from each barrel, body length and body weight were measured after MS-222 anesthesia, and the condition factor (CF) was calculated (Alshahrani and Aljohani, 2013). Then blood was collected from the tail vein and the blood was allowed to stand in a refrigerator at 4 °C for 2 h.

Table 1: Composition and nutrient levels of the basal diet (air-dry basis).

Items	Content
Ingredients	
Casein	30.00
Gelatin	7.50
Dextrin	38.00
Corn oil	4.00
Soybean oil	4.00
Mineral premix	4.00
Vitamin premix	1.00
Choline chloride	0.25
Cellulose	11.25
Total	100.00
Nutrient levels	
Moisture	9.70
Crude protein	30.00
Crude fat	7.60
Ash	2.90

After centrifugation at 3 000 r /min for 10 min, the supernatant was taken for the detection of serum biochemical indicators.

Finally, the test fish was dissected, the viscera and liver were separated, and weighed to calculate the hepatosomatic index (HSI). Compare with the viscerosomatic index (VSI) and save the liver sample, serum and liver samples were stored in a -80 °C freezer for subsequent determination (Burild et al., 2015).

Determination Method

(i) Determination of Conventional Nutrients

The moisture content of feed and whole fish is determined by the constant weight loss method (GB/T 5009.3-2016) at 105 °C.

And the crude protein content is determined by Kjeldahl method (GB/T 5009. 5-2016) Determination, crude fat content was determined by Soxhlet extraction method (GB/T 5009.6-2016). And crude ash content was determined by burning weighing method (GB/T 5009. 4 - 2016).

(ii) Determination of Growth Performance Indicators

The growth performance related indicators are calculated as follows: weight gain rate (%) = 100 × (end weight average - initial average weight) / initial average weight; feed efficiency = (final total weight - initial total weight) / total feed consumption Survival rate (%) = 100 × terminal mantissa / initial mantissa; liver to body ratio (%) = 100 × liver weight / body weight; visceral ratio (%) = 100 × visceral weight / body weight; fatness (g / cm_3) = 100 ×body weight / body $length_3$.

(iii) Determination of Serum Biochemical Indicators

Serum alkaline phosphatase (ALP) activity and total protein (TP), total cholesterol (T-CHO), and albumin (ALB) levels were determined using a Sysmex automatic biochemical analyzer (CHEMIX-800). The reagents were purchased from Sysmex Corporation.

Data Processing

The test data were analyzed by one-way ANOVA program in SPSS 18.0 statistical software, and the difference significance test was performed by Duncan's multiple comparison method.

The test results were expressed as "mean ± standard deviation", and the difference was significant when P<0.05.

Results and Analysis

The Effect of Vitamin D Addition Level on the Growth Performance of Mid-Term Tilapia

The effect of vitamin D addition levels on the growth performance of tilapia is shown in Table 2. After 12 weeks of indoor culture, the survival rate of each group of G. tilapia was 100%.

The addition of vitamin D in the feed promoted the growth of tilapia, and the weight gain rate of each vitamin D addition group was significantly higher than that of the control group (P < 0.05).

In addition, the 400 IU / kg group was significantly higher than 1 600. There was no significant difference between the 200 and 800 IU/kg groups (P>0.05).

With the increase of vitamin D in the feed, the feed efficiency increased first and then decreased, with the highest in the 400 IU / kg group, and significantly higher in the 200, 400, 800 and 1 600 IU / kg groups (P < 0. 05).

There was no significant change in liver-to-body ratio, visceral ratio and plumpness of G. tilapia with the increase of vitamin D in feed (P>0.05) (Table 2).

In the same row, values with no letter or the same letter superscripts mean no significant difference (P > 0.05), while with different small letter superscripts mean significant difference (P < 0.05). The same as below.

The regression equation of feed vitamin D (x) and the growth rate of tilapia (y) was obtained by regression analysis of the line model: y = 0.153 x + 237.4, R2 = 0.808 (Figure 1).

From the regression equation, the level of vitamin D added to the maximum growth of G. tilapia was 259.8 IU / kg.

Table 2: Effects of vitamin D supplemental level on growth performance of GIFT in growth mid-stage.

Items	Vitamin D supplemental level / (IU /kg)					
	0	200	400	800	1600	3200
IBW/g	78.73±1.74	78.04±1.11	78.71±1.14	77.57±0.49	80.06±3.99	78.30±2.08
FBW/g	259.34±5.81[a]	299.85±3.57[bc]	307.38±1.92[c]	297.27±7.36[bc]	296.17±16.06[b]	292.24±8.06[b]
WGR/%	229.40±2.60[a]	284.23±5.00[bc]	290.58±6.76[c]	273.17±12.49[bc]	269.88±6.89[b]	266.57±9.65[b]
FE	0.65±0.05[a]	0.79±0.05[bc]	0.83±0.04[c]	0.80±0.06[c]	0.79±0.03[bc]	0.73±0.04[a]
CF / (g /cm^3)	4.44±0.41	4.52±0.10	4.47±0.15	4.46±0.21	4.56±0.34	4.72±1.04
VSI /%	7.67±0.35	8.02±0.82	8.12±0.51	8.04±0.65	7 .97±0.73	7.13±0.60
HSI /%	1.65±0.04	1.77±0.34	1.80±0.34	1.71±0.22	1.61±0.29	1.78±0.30
SR/%	100	100	100	100	100	100

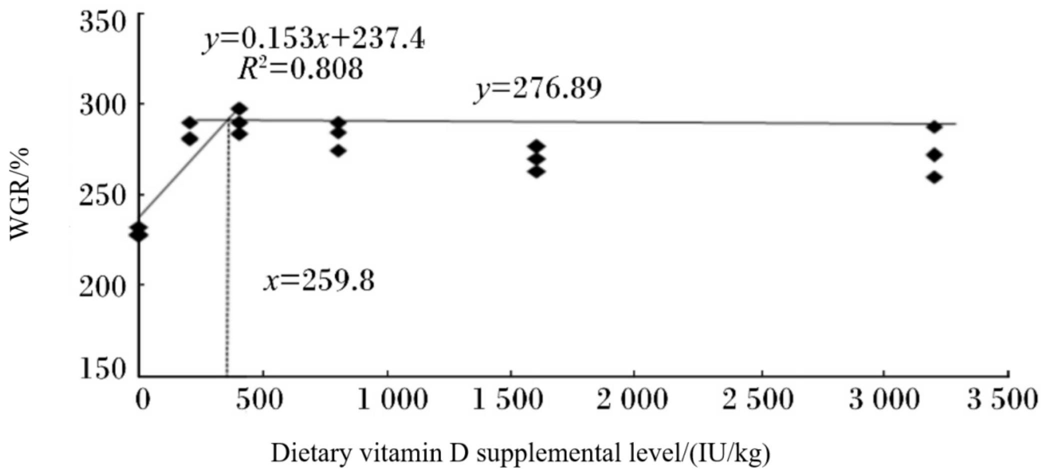

Figure 1: Regression relationship between dietary vitamin D supplemental level and WGR of GIFT in growth mid-stage.

Table 3: Effects of vitamin D supplemental level on body composition of GIFT in growth mid-stage.

Items	Vitamin D supplemental level / (IU /kg)					
	0	200	400	800	1600	3200
Moisture	68.71±0.98	67.62±1.24	67.78±0.69	67.55±2.44	67.33±0.81	67.53±1.08
Crude lipid	9.16±0.64[a]	12.09±0.23[c]	11.06±0.47[b]	11.74±0.61bc	9.56±0.93[a]	9.26±0.78[a]
Crude protein	16.41±0.75	16.91±0.53	16.69±1.55	16.16±0.19	16.76±0.91	16.69±0.74
Ash	4.03±0.28[a]	4.91±0.72[b]	5.32±0.23[b]	5.04±0.31[b]	4.83±0.18b	5.04±0.14[b]

Effects of Vitamin D Addition Levels on Serum Biochemical Parameters of Mid-Term Tilapia

The effect of vitamin D addition levels on serum biochemical parameters of tilapia is shown in Table 4.

The addition of vitamin D to the diet increased the activity of alkaline phosphatase in the serum of tilapia, which was significantly higher in the 200, 400 and 800 IU / kg groups than in the control group (P < 0.05).

With the increase of vitamin D in the feed, the contents of total cholesterol, total protein and albumin in the serum fluctuated.

And the maximum value was obtained in the 200 IU/kg group, which was significantly higher than that in the control group (P<0. 05) (Table 4).

Table 4: Effects of dietary vitamin D level on serum biochemical indices of GIFT in growth mid-stage.

Items	Vitamin D supplemental level / (IU /kg)					
	0	200	400	800	1600	3200
ALP / (U /mL)	21.66±1.52[a]	27.33±0.57[c]	26.50±2.12[bc]	25.00±1.41[bc]	24.00±1.73[ab]	24.50±0.70[abc]
T-CHO / (mmol /L)	3.40±0.30[a]	5.10±0.19[c]	4.62±0.20[b]	3.62±0.11[a]	4.30±0.34[b]	3.73±0.05[a]
TP / (mmol /L)	28.00±0.00[a]	32.33±1.15[b]	32.33±2.08[b]	28.33±2.51[a]	29.00±1.00[a]	29.00±1.00[a]
ALB / (mmol /L)	6.66±0.57[a]	7.66±0.57[b]	7.00±0.00[ab]	6.33±0.57[a]	6.33±0.57[a]	6.33±0.57[a]

Discussion

Effects of Vitamin D Addition Levels on the Growth Performance of Mid-Term Tilapia

The effect of vitamin D on fish growth varies with the species, age, culture cycle and environment of the study population. Lovell reported that after 16 weeks of feeding the channel catfish in a diet lacking vitamin D, the weight gain rate was significantly reduced; Shiau reported that the lack of vitamin D in the hybrid tilapia would show poor growth and reduced feeding efficiency; The feed of different levels of vitamin D_3 was fed to the blue tilapia for 24 weeks. The level of vitamin D_3 in the feed had a significant effect on the growth of blue tilapia in the first 12 weeks, but did not show significant growth in the last 12 weeks (Dawodu and Tsang, 2012).

The impact of the study; the lack of vitamin D_3 in P. vannamei will show a lack of appetite, poor growth and incomplete mineralization of shrimp shells. This experiment found that the addition of vitamin D in the feed significantly increased the weight gain rate and specific growth rate of the tilapia, and the control group of tilapia did not show significant vitamin D deficiency.

The reason for this result may be that the test fish used is a medium-sized tilapia, which has a relatively large size and a large amount of vitamin D accumulated in the body tissues, which is insufficient to meet the maximum growth of the test fish, but can prevent it. In addition, the culture cycle of this test is 12 weeks, and the effect of vitamin D deficiency in the feed on the growth performance of the test fish may not be significantly demonstrated during the culture cycle (Singhellakis et al., 2011).

At the same time, the addition of high levels of vitamin D to the feed did not inhibit the growth of tilapia, which may be due to the larger size of the tilapia used in this test and the higher tolerance to high levels of vitamin D. Feeds that fed high levels of vitamin D to the Atlantic salmon and channel catfish did not show inhibition of growth, and only a few reported high levels of vitamin D in the diet inhibited fish growth (Atabek, 2012).

In this experiment, the addition of different levels of vitamin D to the feed did not have a significant effect on the survival rate of tilapia, which was consistent with the results of research on Chinese shrimp and the results of other studies on juvenile carp; Studies have shown that the mortality of hybrid tilapia in the vitamin D_3 group added to the feed is significantly lower than that of the control group. This difference may be due to the species, age, culture cycle and environment of the study (Biancuzzo et al., 2010).

Effects of Vitamin D Addition Levels on the Composition of Mid-Term Tilapia

The lack of vitamin D in the feed can cause an increase in the fat content of the rainbow trout, the scorpionfish, and a decrease in the crude ash content in the bone. The results of this experiment showed that the crude ash content of the whole fish in the control group without vitamin D was significantly lower than that in the vitamin D addition group, and there was no significant difference between the vitamin D addition groups, indicating that the vitamin D of the feed can promote minerals in the body (Tsiaras and Weinstock, 2011).

However, the level of crude ash in the whole fish decreased when the level of vitamin D was too high, indicating that excessive vitamin D may inhibit the formation of osteoblasts and reduce the absorption of calcium and phosphorus, resulting in a decrease in the crude ash content of whole fish. Studies on livestock and poultry have shown that when the level of vitamin D_3 in the diet is 4 to 10 times the normal requirement, long-term feeding of the diet can cause poisoning. The highest level of crude ash in the whole vitamin D addition group in this trial is still high, probably due to the larger size of the test fish, the greater tolerance, and the feeding time is not long enough (Thiele et al., 2013).

In this experiment, the crude fat content of whole fish was significantly affected by the level of vitamin D added in the feed. The crude fat content of whole fish increased first and then decreased with the increase of vitamin D addition level, indicating the vitamin D in the feed. Addition affects the fat metabolism of tilapia, but the pathways that affect metabolism require further research (Kaza and Moulton, 2014).

The study found that the crude fat content of the carp juvenile fish increased with the increase of the vitamin D level in the feed, and the lowest fat content in the liver fat group showed the lowest fat content in the fish. Excessive addition of D may cause excessive accumulation of vitamin D in the liver of the carp, which may cause liver phospholipid synthesis disorder or apolipoprotein synthesis disorder, so that triglycerides are difficult to transport out of the liver, and a large amount of accumulation in the liver causes lesions.

When the liver fat content is abnormally elevated, the body fat content of the fish is significantly reduced (Laaksi, 2012). It can be seen that the decrease of crude fat content in the whole fish of the tilapia in the high-level vitamin D-added group may be due to liver disease; while the vitamin D addition level has no significant effect on the water and crude protein content of the whole fish of the tilapia, indicating that water and crude protein are not sensitive indicators for vitamin D (Niino and Miyazaki, 2015).

Effects of Vitamin D Addition Levels on Serum Biochemical Parameters of Mid-Term Tilapia

Alkaline phosphatase is an important metabolic regulation enzyme in organisms, which is closely related to the transfer of phosphate groups and calcium and phosphorus metabolism. Alkaline phosphatase is present in various tissues such as liver, intestine and bone. It is an important indicator of bone metabolism. When bone cell metabolism is active, alkaline phosphatase secretion increases, and it is easily released into the blood. Alkaline phosphatase activity is increased (Rajakumar et al., 2011).

In this test, the addition of vitamin D in the feed resulted in a significant increase in serum alkaline phosphatase activity, but this study also found that excessively high levels of vitamin D resulted in a decrease in serum and alkaline phosphatase activity. Similar reports have also appeared in the study of abalone, grass carp and P. monodon. The reason may be that vitamin D has a two-way effect on bone cell metabolism.

When the vitamin D content in the feed is appropriate, it can promote bone cell metabolism. A part of the alkaline phosphatase produced in the blood enters the blood to increase the activity of serum alkaline phosphatase.

However, when the content of vitamin D in the feed is too high, toxicity and inhibition are produced. Therefore, the serum of the high-level vitamin D addition group in this test is added. The decrease in alkaline phosphatase activity may be due to excessive vitamin D inhibiting the metabolism of bone cells (Binkley et al., 2012).

The content of total protein and albumin in serum reflects the state of protein absorption and metabolism, and albumin and globulin combine to be called total protein. Serum albumin maintains plasma colloid osmotic pressure and repairs tissue. If the liver is damaged, serum albumin levels are reduced. In this test, the addition of vitamin D to the feed had a significant effect on serum total protein content. The appropriate addition level (200 IU/kg) of vitamin D significantly increased the serum total protein content of the test fish, and its mechanism may be the addition of vitamin D (Pilz et al., 2016).

It promotes the digestion and absorption of protein in food by the tilapia, resulting in an increase in serum total protein content. However, with the further increase in the level of vitamin D addition, serum total protein and albumin levels decreased, probably due to the high level of vitamin D caused by liver damage in the tilapia. Serum total cholesterol can reflect the lipid metabolism of fish, and it is a reflection index of body fat accumulation (Temmerman, 2011).

It is generally believed that 70% to 80% of cholesterol in the blood is derived from the liver, and a small amount is derived from the digestive tract. When the liver is damaged, the amount of cholesterol in the serum will change accordingly.

In this test, the serum total cholesterol content generally increased first and then decreased. The possible reason is that the proper amount of vitamin D promotes the metabolism of fat in the liver of the fish, accelerates its decomposition, and the amount of saturated fatty acids is relatively increased.

Increased cholesterol levels in the body, resulting in higher serum total cholesterol levels than the control group, but high levels of vitamin D may cause liver damage in the tilapia, hindering fat metabolism, resulting in reduced cholesterol into the serum (Dawodu and Wagner, 2012).

Conclusion

The proper addition of vitamin D in the feed can promote the growth of mid-term tilapia. Excessive vitamin D addition may cause liver damage and affect fat metabolism. Based on the weight gain rate, the appropriate addition level of vitamin D in the medium-term tilapia feed was 259.8 IU/kg.

References

Alshahrani, F. and N. Aljohani. 2013. Vitamin d: deficiency, sufficiency and toxicity. *Nutrients*, 5 (9): 3605-3616.

Atabek, M.E. 2012. Understanding less than nothing: high-dose vitamin d therapy for treating vitamin d deficiency. *Journal of Pediatric Endocrinology & Metabolism*, 25 (7-8): 809-810.

Biancuzzo, R.M., A. Young, D. Bibuld, M.H. Cai, M.R. Winter, E.K. Klein, A. Ameri, R. Reitz, W. Salameh, T.C. Chen and M.F. Holick. 2010. Fortification of orange juice with vitamin d-2 or vitamin d-3 is as effective as an oral supplement in maintaining vitamin d status in adults. *American Journal of Clinical Nutrition*, 91 (6): 1621-1626.

Binkley, N., R. Ramamurthy and D. Krueger. 2012. Low vitamin d status: definition, prevalence, consequences, and correction. *Rheumatic Disease Clinics of North America*, 38 (1): 45.

Burild, A., H.L. Frandsen, M. Poulsen and J. Jakobsen. 2015. Tissue content of vitamin d-3 and 25-hydroxy vitamin d-3 in minipigs after cutaneous synthesis, supplementation and deprivation of vitamin d-3. *Steroids*, 98: 72-79.

Dawodu, A. and C.L. Wagner. 2012. Prevention of vitamin d deficiency in mothers and infants worldwide - a paradigm shift. *Paediatrics and International Child Health*, 32 (1): 3-13.

Dawodu, A. and R.C. Tsang. 2012. Maternal vitamin d status: effect on milk vitamin d content and vitamin d status of breastfeeding infants. *Advances in Nutrition*, 3 (3): 353-361.

Hohman, E.E. and C.M. Weaver. 2011. Vitamin d bread could help solve insufficiency problem. *Agro Food Industry Hi-Tech*, 22 (5): 24-25.

Kaza, P.L. and T. Moulton. 2014. Severe vitamin d deficiency in a patient with sickle cell disease: a case study with literature review. *Journal of Pediatric Hematology Oncology*, 36 (4): 293-296.

Klack, K. and J.F. de Carvalho. 2010. High frequency of vitamin d insufficiency in primary antiphospolipid syndrome. *Joint Bone Spine*, 77 (5): 489-490.

Laaksi, I. 2012. Symposium 4: vitamins, infectious and chronic disease during adulthood and aging vitamin d and respiratory infection in adults. *Proceedings of the Nutrition Society*, 71 (1): 90-97.

Niino, M. and Y. Miyazaki. 2015. Genetic polymorphisms related to vitamin d and the therapeutic potential of vitamin d in multiple sclerosis. *Canadian Journal of Physiology and Pharmacology*, 93 (51): 319-325.

Pilz, S., N. Verheyen, M.R. Gruebler, A. Tomaschitz and W. Maerz. 2016. Vitamin d and cardiovascular disease prevention. *Nature Reviews Cardiology*, 13 (7): 404-417.

Rajakumar, K., M.F. Holick, K. Jeong, C.G. Moore, T.C. Chen, F. Olabopo, M.A. Haralam, A. Nucci, S.B. Thomas and S.L. Greenspan. 2011. Impact of season and diet on vitamin d status of african american and caucasian children. *Clinical Pediatrics*, 50 (6): 493-502.

Sahota, O. 2014. Understanding vitamin d deficiency. *Age and Ageing*, 43 (5): 589-591.

Singhellakis, P.N., F.C. Malandrinou, C.J. Psarrou, A.M. Danelli, S.D. Tsalavoutas and E.S. Constandellou. 2011. Vitamin d deficiency in white, apparently healthy, free-living adults in a temperate region. *Hormones-International Journal of Endocrinology and Metabolism*, 10 (2): 131-143.

Spirichev, V.B. 2015. Vitamin d-3. *Yakut Medical Journal* (3): 169-178.

Temmerman, J.C. 2011. Vitamin d and cardiovascular disease. *Journal of the American College of Nutrition*, 30 (3): 167-170.

Thiele, D.K., J.L. Senti and C.M. Anderson. 2013. Maternal vitamin d supplementation to meet the needs of the breastfed infant: a systematic review. *Journal of Human Lactation*, 29 (2SI): 163-170.

Tsiaras, W.G. and M.A. Weinstock. 2011. Factors influencing vitamin d status. *Acta Dermato-Venereologica*, 91 (2): 115-124.