Research Article

Production of vitamin D enriched yeast with UV-B irradiation and the degradation of vitamin D in rumen fluid of Thai native cattle

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Abstract

The purposes of this study were to investigate the effect of UV-B exposure condition to produce vitamin D enrich yeast and analyze degradation of UV-B irradiated vitamin D enrich yeast in the rumen fluid from Thai native cattle. The baker's yeast (*Saccharomyces cerevisiae*) was selected from single colony of instant active dry yeast by streak plate method for the investigation of the optimum UV-B irradiation time. Yeasts were exposed to different UV-B irradiation for 0, 2, 4, 8, 10, 12, 16 and 24 hours. This study found that UV-B irradiation of all treated yeast enhanced the amount of vitamin D_2 and the maximum amount of vitamin D_2 at 10 hours (70.5 to 8409277.11 IU/100g DM) was significantly different ($p < 0.05$). The rumen fluid from 4 fistulated Thai native cattle were used for analyze degradation of UV-B irradiated vitamin D enrich yeast. The treatments were divided into 5 groups, which were rumen liquor buffer without vitamin D and yeast supplementation (Control) and those with non-irradiation yeast supplementation (Non UV-B IY), vitamin D_2 (VIT D_2), vitamin D_3 (VIT D_3) and UV-B irradiated vitamin D enrich yeast (UV-B IVDRY). The samples were taken after overnight incubation at 39 °C, shaking at 70 rpm for 24 hours in order to analyze of degradation of vitamin D after incubation in the rumen. This study was found that the VIT D_2 and VIT D_3 groups had decreased vitamin D content from the initial supplementation (411.56 μg compared to 205.48 μg and 393.48 μg compared to 178.44 μg or decreased about 50.07% and 54.64%, respectively). On the other hand, the UV-B IVDRY group had decreased amount of vitamin D at only 18.22 μg or 4.47%. The amount of vitamin D was decreased less than the VIT D_2 and VIT D_3 groups ($p < 0.05$). Therefore, supplementing UV-B irradiated vitamin D enrich yeast as a source of vitamin D to dairy cows can help to decrease the loss of vitamin D that is degraded by the rumen microbes, allowing to be more absorbed and utilized.

Keywords: Vitamin D_2, Vitamin D_3, UV-B irradiated vitamin D enrich yeast

Introduction

Vitamin D is the main function of absorption potassium and phosphorus, which is an essential nutrient for the mineralization of bone and teeth. Prevention of certain diseases such as osteoporosis, osteopenia, hypertension diseases, immune system stimulation and against various cancers [15]. Vitamin D consists of two major compounds, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_3 is found in animal products such as fish liver oil, egg, milk, and cheese, that is mainly generated from 7 - dehydrocholesterol in epidermal layer of skin during exposure to ultraviolet light [6]. Vitamin D_2 is found in plants product, it can be formed from the plant steroid called ergosterol by UV irradiation [9]. The vitamin D_2 can be produced by the convertation of ergosterol or provitamin D_2 which is precursor of vitamin D_2. Generally, yeast cells contain high levels precursor of vitamin D_2 [1], [14]. When exposure to UV-B can be converted to vitamin D_2 [4], [7]. The conversion of vitamin D_2 by UV light consists of three sub-region of wavelengths, including UV-C (190-290nm), UV-B (290-320nm) and UV-A (320-400nm) [8]. The highest yields synthesis of vitamin D is dependent upon the absorption of irradiation in the ultraviolet-B range [19] but the duration of the UV-B irradiation were not certain, so experiment was required. Earlier researches suggested that vitamin D supplemented in the diets of ruminants were degraded in the rumen. Only 10 to 25% of added ergocalciferol and cholecalciferol was recovered after 24 hours of incubation in intact ruminal fluid [16]. Since dairy cattle are ruminants, there are microorganisms in the rumen that convert vitamin D in food to a form that cannot be utilized [15], making animals less utilization. Nowadays, yeast is used as a feed additive in ruminant feed which aims to increases the activity of rumen microflora by helping to remove oxygen, increase the production of volatile fatty acids and protein microorganisms synthesis. This lead to increase the production efficiency of ruminants [5]. Also, yeast can grow in both under aerobic and anaerobic conditions. Usually, yeast can grow and survived in the neutral environment. Even though, the rumen fermentation was sometimes formed acidic environment (pH 3-4), it is also resistant to acidic conditions [20]. Thus, the concept is to apply by using vitamin D enriched yeast to prevent wastage from the activity of microorganisms that degrade vitamin D. If the vitamin D enriched yeast could be bypassed from the rumen to be digested directly in the small intestine. Then dairy cows will be able to utilize vitamin D effectively. Therefore, this research aimed to investigate the optimum UV-B irradiation time to produce of vitamin D enriched yeast and analyze degradation of vitamin D enriched yeast in the rumen fluid of Thai native cattle which have not yet been explored in the Thai cattle.

Materials and Methods

Production of vitamin D enriched yeast

Baker's yeast (*Saccharomyces cerevisiae*) was selected from single colony of instant active dry yeast by streak plate method. Baker's yeast culture were plated on YPD agar plate (Yeast Extract Peptone Dextrose) which was grew at 25°C for 48 hours. The randomly selected single colony was inoculated on YPD broth in sterile test tubes at 25 °C for 24 hours and then, yeast culture was maintained in YPD broth containing 25% glycerol at -20 °C on microtubes. For activation, the stock of baker's yeast was streaked on YPD agar plates at 25°C for 48 hours, then randomly selected single colony was inoculated on YPD broth in sterile test tubes at 25 °C for 24 hours. After that, the 1% (v/v)

enrich cultures was inoculated on YPD broth in sterile Erlenmeyer flask at 25 °C with agitation rate of 120 rpm for 16 hours. The 1% (v/v) enrich cultures were moved to YPD broth and incubated at 25 °C with an agitation rate of 120 rpm for 12 hours. After incubating, baker's yeast was exposed to UV-B irradiation at different duration times. Eight unit of UV-B (wavelength 280-315 nm) lamps (311±5 nm, Philips TL 20W/01 RS SLV/25) with 589.8 mm in length were placed 15 cm away from the sample for irradiation in total area 80x120 cm^2. In these experiments, baker's yeast was treated with different UV-B irradiation times in an irradiation chamber including

Treatment 1: Baker's yeast non UV-B irradiation (Control)

Treatment 2: Baker's yeast treated with UV-B irradiation 2 hours

Treatment 3: Baker's yeast treated with UV-B irradiation 4 hours

Treatment 4: Baker's yeast treated with UV-B irradiation 8 hours

Treatment 5: Baker's yeast treated with UV-B irradiation 10 hours

Treatment 6: Baker's yeast treated with UV-B irradiation 12 hours

Treatment 7: Baker's yeast treated with UV-B irradiation 16 hours

Treatment 8: Baker's yeast treated with UV-B irradiation 24 hours

After UV-B irradiation, UV-B irradiated yeast were stored at -20 °C with 50% relative humidity under dark condition immediately. UV irradiated yeast were separately freeze dried and homogenized with a blender and then stored at -20 °C up to 4 week until analysis.

Analysis of vitamin D

UV-B irradiated yeast was extracted and analyzed according to the method of AOAC [1] as modified by Mattila *et al*. [12]. Approximately 0.5 g freeze dried UV-B irradiated yeast was weighed and mixed with 1 g L-ascorbic acid into 250 ml round bottom flask, follow by 50 ml of 95% ethanol and 10 ml of 50% potassium hydroxide and 100 µg of cholecalciferol (vitamin D3; in 1 ml of methanol) was added as an internal standard. The mixture was saponified for 30 min under reflux at 85 °C. The mixture was cool down at the room temperature and transferred into a separating funnel. The mixture was extracted twice times with 10 ml of deionized water and n-hexane of volumes 30 ml. The organic layers were washed three times with 50 ml deionized water until neutralized, then transferred into a round bottom flask, rotary evaporated to dryness at 40 °C and immediately re-dissolved in 1 ml of a mixed solution of eluent (acetonitrile : methanol = 75:25 v/v) and isopropyl alcohol (2:1 v/v). The sample was filtered through a 0.45 µm non pyrogenic filter. Five microliter of filtered sample was injected into a HPLC system (1220 Infinity II LC, Agilent Technologies, USA) and eluted through a reverse phase C18 column (Restek, USA, 5µm, 4.6 x 250 mm). The mobile phase was acetonitrile: methanol (75:25 v/v), at a flow rate of 1.3 ml/min and UV detection was at 264 nm. The qualitative of vitamin D was analyzed by comparing the times of obtained standards and quantification was done by using a calibration curve.

Degradation of vitamin D enriched yeast after *in vitro* incubation

Rumen fluid were collected from four fistulated Thai native cattles (8-10 years old). The cows were fed corn silage *ad libitum* and 2 kg of concentrate per day. The rumen fluid were filtered through two layers of cheesecloth and added to the buffered mineral solution, while maintained in a water bath at 39 °C under anaerobic condition by flushing with CO_2. The degradation of UV-B irradiated vitamin D enrich yeast was applied according to Menke *et al*. [13]. The two hundred thirty milligram of mixed roughage and concentrate (70:30 ratio) were mixed with 30 ml of rumen liquor buffer solution into a serum bottle 125 ml, closed with a rubber stopper and an aluminum cap after that squeeze the cap tightly with the crimper hand seal ring. There were 5 treatments including

Treatment 1: rumen liquor buffer solution (Control)

Treatment 2: rumen liquor buffer with non-UV-B irradiated yeast supplementation (Non UV-B IY)

Treatment 3: rumen liquor buffer with vitamin D_2 supplementation (VIT D_2)

Treatment 4: rumen liquor buffer with vitamin D_3 supplementation (VIT D_3)

Treatment 5: rumen liquor buffer with UV-B irradiated vitamin D enrich yeast supplementation (UV-B IVDRY)

Samples were incubated at 39 ^oC, with an agitation rate of 70 rpm for 24 hours, then stored at -20 °C to analyze of vitamin D, using modified method of AOAC [1] and Mattila et al. [12].

Statistical analysis

Vitamin D quantity of UV-B irradiated yeast and vitamin D degradation in the rumen between treatments groups were statistically analyzed by analysis of variance (ANOVA) in completely randomized design (CRD). Differences among treatment means were tested using Duncan's new multiple range test [18].

Results and Discussion

The content of vitamin D_2 after UV-B irradiation time at 0, 2, 4, 8, 10, 12, 16 and 24 hours were 70.5, 1397041.63, 2912764.33, 4261463.41, 8409277.11, 7659111.67, 7420496.82 and 7231405.21 IU/100g DM, respectively. As the duration of the UV-B irradiation increased, the vitamin D_2 contents of UV-B irradiated yeast were significantly increased (the 10 hour compared with the 8, 4, 2 and 0 hours, respectively) ($p<0.05$), because within yeast cells there were a high accumulation of ergosterol or provitamin D_2. When stimulated by UV-B light, it could be converted into vitamin D2 [7]. They contained a high amount of ergosterol which could be converted to vitamin D_2. When yeast were exposed to UV light, ergosterol undergoes photolysis to yield a variety of photoirradiation products, principally previtamin D_2, tachysterol and lumisterol. The previtamin D_2 undergoes spontaneous thermal rearrangement to vitamin D_2 [4]. After 10 hours of UV-B irradiation period, the amount of vitamin D began to significantly decreases ($p<0.05$). Due to yeast cells began to die and self-decomposition (autolysis), the enzyme inside the yeast cell was independent and

digested various substrates which leads to the thinning of the cell wall and the loss of semi-permeable membrane properties. Yeast cells decompose themselves, until the cell lysis, the intracellular fluid such as cytoplasm, fat, protein and vitamins. It was released outside the cell [3]. In the irradiation at 12, 16 and 24 hours, the amount of vitamin D_2 tended to decrease, respectively (P> 0.05). Therefore, the irradiation time of 10 hours was selected (the amount of vitamin D_2 increased from 70.5 to 8409277.11 IU / 100g DM) to test the degradation of vitamin D in the rumen. as shown in **Figure 1**.

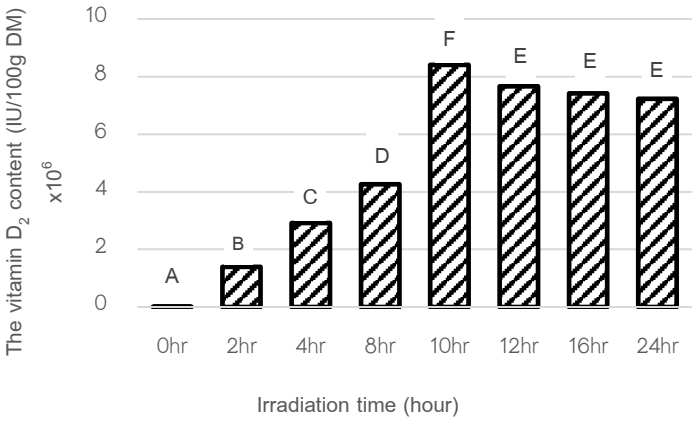

Figure 1 The increasing of vitamin D_2 quantity of yeast at different irradiation times and $^{A, B, C, D, E, F}$ means along columns among irradiated times with different superscripts are significantly different at P < 0.05

The control group and the non UV-B IY supplementation group were unable to measure the amount of vitamin D (non detected). After 24 hours incubating, vitamin D content of VIT D_2 and VIT D_3 groups decreased from the initial supplementation (411.56 μg to 205.48 μg and 393.48 μg to 178.44 μg or decreased about 50.07% and 54.64%, respectively), as shown in table 1. Because the rumen contains microorganisms that can degraded or converted vitamin D into other substances that animals cannot be utilizable. As a result, the vitamin D content of VIT D_2 and VIT D_3 decreased from the initial supplementation. Corresponding with Sommerfeldt et al. [17] which study on the degradation of vitamin D in the rumen it was found that the experimental group supplemented with vitamin D in the non-sterilized rumen liquor buffer had vitamin D content decreased 50–75% compared to the sterilized rumen liquor buffer group. The different initial doses in each group did not correlation with a reduction of vitamin D content (Correlation Coefficient (r) = -0.161) (P> 0.05).

Evaluating the reduction of vitamin D content among treatment groups it was found that the reductions of the VIT D_2 and VIT D_3 groups from the initial supplementation (206.08 μg compared to 215.04 μg or 50.07% compared to 54.64%, respectively) were non significantly different (P> 0.05). On the other hand, the vitamin D reductions in the UV-B IVDRY groups showed lower decreases of vitamin D content than the VIT D_2 and VIT D_3 groups (4.47% compared to 50.07% and 54.64%, respectively) (p<0.05). As shown in table 1, the UV-B IVDRY group had the remaining vitamin D content after 24 hours of incubation higher than the VIT D_2 and VIT D_3 groups (95.53 compared to 49.93% and 45.36%, respectively). Because yeast can grew in both an

aerobic and anaerobic conditions. Even though, the rumen fermentation was sometimes formed acidic environment (pH 3-4), it was resistant to acidic conditions [20]. Yeast can utilized glucose and oligosaccharide produced by the digestion of amylolytic bacteria in the production of energy for the growth. They can survived in the rumen without being degraded by microbes in the rumen [10, 11]. Therefore vitamin D in yeast cells is not degraded by the rumen microorganisms.

Table 1 The vitamin D content and degradation of 24 hours *in vitro* rumen liquor buffer incubation with or without vitamin D_2, vitamin D_3, yeast culture and UV-B irradiated vitamin D enrich yeast.

Item	Treatment					SEM	P-value
	Control	Non UV-B IY	$VITD_2$	$VITD_3$	UV-B IVDRY		
Initial of vitamin D (μg)	-	-	411.56±1.75	393.48±1.26	406.68±1.67	-	-
Remaining of vitamin D							
(μg)	ND.	ND.	205.48^{A}±3.64	178.44^{A}±13.58	388.46^{B}±1.70	36.94	<0.001
(%)	ND.	ND.	49.93^{A}±1.10	45.36^{A}±3.48	95.53^{B}±0.78	8.94	<0.001
Disappearance of vitamin D							
(μg)	ND.	ND.	206.08^{B}±5.39	215.04^{B}±13.99	18.22^{A}±3.29	35.70	<0.001
(%)	ND.	ND.	50.07^{B}±1.55	54.64^{B}±3.48	4.47^{A}±0.78	8.94	<0.001

$^{A, B}$ Means along row among treatment groups with different superscripts are significantly different at $P < 0.05$, SEM: standard error of the mean, ND: non detection.

Conclusion

The irradiation time at 10 hours, barker's yeast could produce the highest amount to vitamin D_2. Vitamin D supplementation to cattle using UV-B irradiated vitamin D enrich yeast, can help prevent the loss of vitamin D that was degraded by the rumen microorganisms. This causes vitamin D to be delivered directly to the small intestine, allowing animals to absorb vitamin D to be fully utilized. However, this still requires further studies on the UV-B irradiated vitamin D enrich yeast supplemented in the dairy cow diets to animals can utilize vitamin D that bypass from the rumen.

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