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Effects of UV-B irradiated vitamin D enrich yeast supplementation on production performance and blood metabolites in dairy cows

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ABSTRACT: The objective of this study was to investigate the effect of UV-B irradiated vitamin D enrich yeast supplementation with total mixed ration (TMR) in dairy cow diets on production performance and some blood parameter profiles at different experimental periods. Twenty-seven Holstein-Friesian cows (average age= 4±0.6 years old, 2nd lactation and day in milk > 90 days) were assigned and analyzed using completely randomized design (CRD). There were divided into 3 treatment groups (n= 9/groups; 7 days of preliminary and 21 days of collection periods). All of the treatment groups were fed with TMR which each group fed with different feed additive were fed only TMR (without yeast supplemented; CON), TMR with 1.6 g/day non UV-B irradiation yeast (Non UV-B IY) and TMR with 1.6 g/day UV-B irradiated vitamin D enrich yeast supplementation (200,000 IU/day; UV-B IVDRY). Milk yield was recorded twice per day, milk samples were collected every week for milk composition analysis. This study found that milk fat and total solid of UV-B IVDRY group at day 14 were significantly higher than day 0 of experimental periods ($P<0.05$). Non UV-B IY group at day 21 had milk protein higher than day 7 were significantly different ($P<0.05$). Additionally, milk lactose at day 0 and 21 of UV-B IVDRY group higher than CON and Non UV-B IY groups were significantly different ($P<0.05$). In respect of blood parameter profiles, blood samples were collected from all cows all at initial and finished collection periods. This study found that alanine aminotransferase (ALT) of Non UV-B IY and UV-B IVDRY groups were significantly lower than CON group ($P<0.05$). Therefore, UV-B irradiated vitamin D enrich yeast supplementation with TMR can increase milk fat and has not affected health in dairy cows.

Keywords: UV-B irradiated vitamin D enrich yeast; milk composition; blood metabolites

Introduction

Vitamin D was the main function of potassium and phosphorus absorption, which was an essential nutrient for the mineralization of bone and teeth. Prevention of certain diseases, such as osteoporosis, osteopenia and other health importance, such as hypertension diseases, immune system stimulation and against various cancers (Schmid and Walther, 2013). Yeasts are the source of valuable bioactive substances, these microorganisms have an important role among other biotechnological used for the production of vitamins and yeasts were considered high sterols producers, especially ergosterol or provitamin D_2 (Fuoli et al., 2008). *Saccharomyces cerevisiae* strains have been received a great attention due to the capacity for ergosterol biosynthesis (Reiner et al., 2005; Abe and Hiraki, 2009). Also, *S. cerevisiae* is widely used for the production of beer, bread, wine,

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nutraceuticals and pharmaceuticals (Nielsen and Jewett, 2008). They contain a high amount of ergosterol which can be converted to vitamin D_2. When yeast are exposed to UV light, ergosterol undergoes photolysis to yield a variety of photoirradiation product, principally previtamin D_2, tachysterol and lumisterol. The previtamin D_2 undergoes spontaneous thermal rearrangement to vitamin D_2 (Braun et al., 1991; Elena et al., 2013). 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) (Teichmann et al., 2007). The highest yields synthesis of vitamin D is dependent upon the absorption of radiation in the ultraviolet-B range (Foss, 2009). However, 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 were recovered after 24 hours of incubation in intact ruminal fluid (Sommerfeldt et al., 1979). Since dairy cattles are ruminants, there are microorganisms in the rumen that convert vitamin D in food to a form that cannot be utilized (Schmid and Walther, 2013), making animals less utilization. The research conceptual that is fed of rumen undegradable vitamin D for increasing utilization of vitamin D in the dairy cows. Yeast supplementation is a once alternative that can produce rumen undegradable vitamin D because yeast cells contain high levels precursor of vitamin D_2 when exposure to UV-B can be converted to vitamin D_2 (Reiner et al., 2005) and yeast can grow in both under aerobic and anaerobic conditions, it 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 (Walker, 1998). Although, yeast can also be used as a feed additive in ruminant feeds which aims to increase 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 (Dias et al., 2017). But vitamin D enrich yeast production needs to undergo UV-B irradiation, which may have negative effects by supplementation to the diets of dairy cows lactation. Consequently, it is wise to check for any disadvantages or side effects of UV-B irradiated vitamin D enrich yeast before using a large field. Therefore, UV-B irradiated vitamin D enrich yeast should increase ruminal digestibility, can be used as source of vitamin D without negative effect on health status of dairy cows. The objectives of this study were to investigate the effect of UV-B irradiated vitamin D enrich yeast supplementation with TMR in dairy cow diets on production performance and some blood parameter profiles at different experimental periods.

Material and Method

Strains and growth conditions

A strain of yeast (*Saccharomyces cerevisiae*) was obtained from laboratory at Department of Biology, Faculty of Sciences, Chiang Mai University. Yeast culture (*S. cerevisiae*) was plated on Yeast Extract Peptone Dextrose (YPD) agar plates, they grew at 25°C for 48 hours. The single colony of randomly selected for inoculation 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.

UV-B irradiated vitamin D enrich yeast production

Yeast was inoculated on synthetic media broth (Olson and Johnson, 1949) in sterile Erlenmeyer flask which were grown at 25°C for 12 hours. After incubating, yeast cultures were treated with different UV-B irradiation

times in an irradiation chamber including 2, 4, 8, 10, 12 and 16 hours. Eight unit of UV-B 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. After UV-B irradiation, UV-B irradiated yeast were stored at -20°C immediately. UV-B irradiated yeast were separately freeze dried and homogenized with a blender before determination and then stored at -20 °C until analysis. The best irradiation time was selected to vitamin D enrich yeast production for supplementation with TMR fed to the cow during the experimental periods. For vitamin D enrich yeast supplementation in the cows during the experimental period, UV-B irradiated yeasts were randomly collected 4 times out of a total of 10 times after UV-B irradiation for analyze accuracy and precision in vitamin D enrich yeast production before vitamin D enrich yeast were supplemented to the dairy cow diets. This protocol has been approved by Chiang Mai University Institutional Biosafety Committee (CMUIBC0662003, Approval No. A0662002)

Analysis of vitamin D

UV-B irradiated yeast was extracted and analyzed according to the method of AOAC (2000) as modified by Mattila et al. (1994). 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 time with 10 ml of deionized water and n-hexane of volumes 30 ml. The organic layers was 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, 5 μm, 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.

Animals and experimental design

The research was performed at ChiangMai Freshmilk Farm, Ban Hong, Lamphun. Twenty-seven Holstein-Friesian cows were assigned and analyzed completely randomized design (CRD), the average age of 4±0.6 years old, in the second lactation period, day in milk more than 90 days. There were divided into 3 treatment groups. All of the treatment groups were fed with TMR. The ingredients of the experimental diets are listed in **Table 1** which each group fed with different feed additive including:

Treatment 1: fed only TMR without yeast supplementation (Control group; CON)

Treatment 2: TMR with 1.6 g/day non UV-B irradiation yeast (Non UV-B IY)

Treatment 3: TMR with 1.6 g/day UV-B irradiated vitamin D enrich yeast (200,000 IU/day; UV-B IVDRY)

Dairy cows housed in individual stall houses without exposure to sunlight, free access to drinking water, and TMR were fed ad libitum twice daily (06.00 A.M and 16.30 P.M).

Table 1 The ingredients and chemical composition of the TMR fed to the cow during the experimental periods.

Item	Ration (%)
Ingredients	
Corn	64.10
Soybean meal	8.97
Concentrate	8.97
Pineapple peels	7.69
Cassava chip	5.13
Cassava pulp	2.57
Corn kernels	2.57
Total	100
Chemical compositions, DM basis (%)	
Dry matter	46.90
Organic matter	94.20
Crude protein	15.04
Crude fiber	18.47
Ether extract	3.50
Ash	5.80

Sample collection, measurements

The 28 days of experimental period consists 7 days of preliminary and 21 days of collection period, milk yield was recorded twice per day, milk samples were collected every week (milked twice daily at 06.00 A.M and 18.30 P.M) for milk composition analysis. Blood samples were collected after evening milking from all cows all at 0 day and 21 day of experimental periods, by venipuncture from the caudal vessels, using 4 mL Li-heparin treated tubes. Samples were immediately cooled in ice water bath after collection. The blood samples were centrifuged (3500 × g for 20 min at room temperature) and then stored at -20°C until analysis.

Milk composition and blood chemistry analysis

Milk samples were analyzed by automated milk analyzer (MilkoScan FT 2, FOSS, Denmark). The measurement of some blood parameter profiles in plasma were analyzed by automated clinical chemistry analyzer (BX-3010, Sysmex, Singapore) using the technique described by Schlebusch and Hicks, (1995); Artiss and Zak, (1997); Thomas, (1998); Johnson et al. (1999); Young, (2000); Guder and Zawta, (2001); Bakker and Mucke, (2007).

Statistical analysis

Data on milk composition and blood parameter profiles were analyzed by using the R program (Version R-4.0.4). Analysis of variance (ANOVA) for completely randomized design (CRD) was analyzed differences in means of dairy cow products. Shapiro-Wilk test and Levene's test were used to evaluate the normality and homogeneity of

variance assumptions. Differences among treatment means were tested using Duncan's new multiple range test (Steel and Torrie, 1960). Blood parameter profiles of initial and finished experimental were analyzed by comparing the mean values of two paired sample experimental groups (paired sample t-test).

Results and Discussion

Vitamin D enrich yeast production

The content of vitamin D_2 after UV-B irradiation time at 2, 4, 8, 10, 12 and 16 hours were 2676285.09, 7570023.52, 17381019.26, 14909505.19, 14350428.62, 12820223.51 IU/100g DM, respectively. As the duration of the UV-B irradiation increased, the vitamin D_2 contents of UV-B irradiated yeast were increased (the 8 hour compared with the 4 and 2 hours, respectively) ($p<0.05$), because within yeast cells there were a high accumulation of ergosterol or provitamin D_2. When yeast were exposed to UV light, ergosterol undergoes photolysis to yield a variety of photoirradiation product, principally previtamin D_2, tachysterol and lumisterol. The previtamin D_2 undergoes spontaneous thermal rearrangement to vitamin D_2 (Braun et al., 1991; Elena et al., 2013). After 8 hours of UV-B irradiation period, the amount of vitamin D began to decreases. 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 (Arnold, 1972). In the irradiation at 10, 12 and 16 hours, the amount of vitamin D_2 tended to decrease, respectively ($p>0.05$). Therefore, 8 hours of the irradiation time was selected to fed with TMR to the cow during the experimental periods, as shown in Figure 1. The 4 times random measurement of vitamin D enrich yeast products after UV-B irradiation were 213899.08, 188139.02, 196193.85 and 219173.64 IU/1.6 g DM, respectively. Accuracy and precision of vitamin D enrich yeast production were presented in **Table 2**.

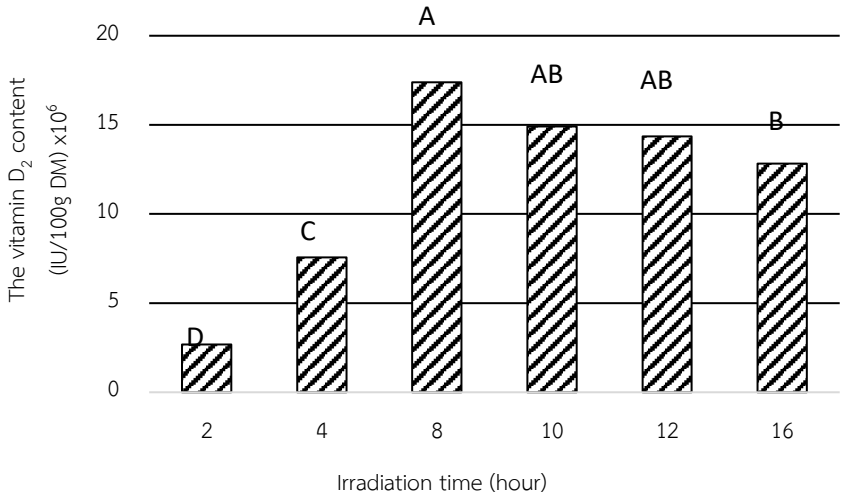

Figure 1 The quantity of vitamin D_2 of yeast at different irradiation times and $^{A, B, C, D}$ means along columns among irradiated times with different superscripts are significantly different at $P < 0.05$

Table 2 Accuracy and precision of vitamin D enrich yeast production

Treatment	Vitamin D_2 (UI/100g DM)
Non UV-B IY	1,832.50
UV-B IVDY	12,771,962.38
SEM	456,542.83
P-value	<0.01

SEM= standard error of the mean, Non UV-B IY= Non UV-B irradiation yeast, UV-B IVDY= UV-B irradiated vitamin D enrich yeast

Milk yield and milk composition

Results of the effect of vitamin D enrich yeast supplementation on the milk composition in dairy cows were presented in Table 3. Evaluating the milk composition of dairy cow products among treatment groups in each days it was found that milk fat of Non UV-B IY and UV-B IVDRY groups tend to be higher than CON group at day 14 and 21 of the experimental periods. Because yeast was potent as a source of probiotics in ruminants, which can stimulate the growth of microbes in the rumen (Jouany et al., 1999). As a result, yeast culture supplementation may promote increasing digestibility of fibers (Moallem et al., 2009; Yalcm et al., 2011). Similarly. Kim et al. (1992) reported that yeast culture supplementation can increase digestibility of CF, NDF and ADF. Thus, it can increase total volatile fatty acids (VFA's) from high fiber digestibility in the rumen (Galip et al., 2006) When there are high acetate lead to increases the milk fat in dairy cows (Dijkstra, 1994). UV-B IVDRY group had milk lactose higher than CON and Non UV-B IY groups at day 21 of experimental period (P<0.05). Corresponding to glucose plasma of UV-B IVDRY group tend to be higher than initial period due to lactose was two molecule of sugar found in milk, formed by one molecule of glucose combined with one galactose molecule, conjugated by 1,4 β-galactoside bond. This synthesis requires up to 80% of glucose from the blood. While, yeast can increases the digestion of CF, NDF and ADF (Plata et al., 1994; Chaucheyras-Durand and Fonty, 2001) lead to enhance the total VFA's when the high proportion of propionate, propionates were converted to glucose and enter to lactose synthesis process. Lactose was further transported through the cells into the alveoli by the process of osmotic pressure (Newbold et al., 1990; Lila et al., 2004). However, milk yield, milk protein, total solid and solid not fat (SNF) of all treatment group were non significantly different (P>0.05).

The estimation of milk composition of dairy cow products among the experimental periods in each treatment it was found that milk fat of UV-B IVDRY group tended to increase higher than after supplementation. Day 14 of the experimental period, milk fat of UV-B IVDRY group higher than day 0 (5.67 compared 4.52) were significantly different (P< 0.05), the reason of milk fat increasing were presented as described above. While, milk fat at day 21 were significantly lower than day 14 (4.70 compared 5.67) (P<0.05). Milk protein of Non UV-B IY group at day 21 were significantly higher than day 7 of the experimental period (3.26 compared 3.06) (p<0.05). Effect of UV-B IVDRY supplementation tended to total solid higher than without supplementation. At 14 day of the experimental period, total solid higher than day 0 were significantly different (13.80 compared 12.80) (P< 0.05). Corresponding to the researches of Alshaikh et al. (2002); Longuski et al. (2009) and Moallem et al. (2009) reported that yeast supplementation were increased milk fat, milk protein and total solid in dairy cows. After Non UV-B IY

and UV-B IVDRY supplementation doesn't affect on milk yield, lactose and solid not fat (SNF) in dairy cow product at different experimental periods (P>0.05).

Blood parameter profiles

Effect of UV-B irradiated vitamin D enrich yeast supplementation fed to the cow during the experimental period found that total protein, total cholesterol, creatinine, Blood urea nitrogen (BUN), globulin, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and glucose in blood parameters investigated were non significantly different in CON, Non UV-B IY and UV-B IVDRY groups (P>0.05). Similarly, Piva et al. (1993) reported that glucose, cholesterol, total protein and globulin of blood plasma were not affected by supplementation with yeast culture. Bagheri et al. (2009) reported that the levels of glucose and BUN were not affected by live yeast addition to the diets of Holstein dairy cows lactation. In addition, Yalcm et al. (2011) reported that the ALT, total protein, BUN and cholesterol were not affected by supplementation with live yeast culture in dairy cows. Blood parameter profile as referred to above were frequently used to monitor the metabolic health status of dairy herds (Ametaj et al., 2009). Therefore, UV-B irradiated vitamin D enrich yeast in dairy cow diets doesn't have a negative effect on metabolic health status of dairy cows. The differences between some previous studies and the results in this study may be due to the stage of lactation, feeding strategy, environmental conditions, diet composition, type of forage, type and dose of yeast and type of yeast feeding. Some researchers (Arambel and Kent, 1990; Moallem et al., 2009) reported that yeast products might be more effective under stress rather than in the normal conditions.

The some blood parameter profiles of all treatment groups after experimental period was found that the total protein, total cholesterol, creatinine, BUN, globulin, ALP and glucose were non significantly different after day 21 of experimental period (P>0.05). According to the many researchers were presented as described above. While, the ALT of Non UV-B IY and UV-B IVDRY groups were significantly lower than the initial period (P<0.05). In the liver, ALT is most often determined if there is a suspicion of acute and chronic liver disease (Zvonko et al., 2005) though the author considers that the role of ALT in predicting liver damage in ketosis is not significant (Tainturier et al., 1984). It may not actually demonstrate the health of the dairy cows, but it is an indicator that showed that UV-B irradiated vitamin D enrich yeast supplementation hasn't affected dairy cows health.

Table 3 Milk yield and milk composition of dairy cow products with UV-B irradiated vitamin D enrich yeast supplemented at different experimental periods.

Item	Treatment	Experimental periods				SEM	P-value
		0 day	7 day	14 day	21 day		
Milk yield (kg/day)	CON	8.61	8.44	8.54	8.47	0.66	0.764
	Non UV-B IY	8.71	8.28	8.56	8.52	0.51	0.430
	UV-B IVDY	8.21	8.16	8.13	8.35	0.57	0.349
	SEM	0.52	0.65	0.65	0.57		
	P-value	0.160	0.109	0.383	0.093		
Milk fat (%)	CON	4.87	4.91	4.89	4.81	0.25	0.993
	Non UV-B IY	4.86	5.46	5.27	4.53	0.27	0.338
	UV-B IVDY	4.52^{Y}	5.34^{XY}	5.67^{X}	4.70^{Y}	0.30	0.041
	SEM	0.28	0.29	0.31	0.25		
	P-value	0.616	0.483	0.234	0.763		
Milk protein (%)	CON	3.18	3.10	3.26	3.25	0.07	0.414
	Non UV-B IY	3.12^{XY}	3.06^{Y}	3.19^{XY}	3.26^{X}	0.05	0.050
	UV-B IVDY	3.06	2.86	3.08	3.07	0.07	0.229
	SEM	0.06	0.06	0.07	0.07		
	P-value	0.427	0.068	0.182	0.111		
Lactose (%)	CON	4.35^{B}	4.21	4.33	4.31^{B}	0.06	0.459
	Non UV-B IY	4.31^{B}	4.34	4.30	4.30^{B}	0.04	0.901
	UV-B IVDY	4.51^{A}	4.35	4.41	4.49^{A}	0.05	0.199
	SEM	0.04	0.05	0.06	0.05		
	P-value	0.018	0.145	0.436	0.044		
Total solid (%)	CON	13.10	13.40	13.10	13.3	0.32	0.885
	Non UV-B IY	13.30	13.70	13.50	13.0	0.26	0.224
	UV-B IVDY	12.80^{Y}	13.40^{XY}	13.80^{X}	12.80^{Y}	0.29	0.047
	SEM	0.27	0.29	0.27	0.34		
	P-value	0.481	0.621	0.211	0.608		
SNF (%)	CON	8.18	7.92	8.19	8.11	0.10	0.240
	Non UV-B IY	8.04	8.04	8.15	8.22	0.08	0.405
	UV-B IVDY	8.25	7.91	8.12	8.22	0.10	0.090
	SEM	0.08	0.09	0.11	0.08		
	P-value	0.268	0.564	0.908	0.655		

$^{A-B}$ Means along column among treatments with different superscripts are significantly different at $P < 0.05$, $^{X-Y}$ means along rows among experimental period with different superscripts are significantly different at $P < 0.05$, SEM= standard error of the mean, CON= control group, Non UV-B IY= Non UV-B irradiation yeast, UV-B IVDY= UV-B irradiated vitamin D enrich yeast 200,000 IU/day, SNF= Solid Not Fat.

Table 4 Blood parameter profiles of dairy cow with and without UV-B irradiated vitamin D enrich yeast supplementation

Item	Periods	Treatment			SEM	P-value
		CON	Non UV-B IY	UV-B IVDY		
Total protein (g/dL)	0 day	9.35	8.83	8.57	0.16	0.435
	21 day	8.70	8.75	8.67	0.20	0.363
	SEM	0.25	0.18	0.17		
	P-value	0.057	0.281	0.978		
Total cholesterol (mg/dL)	0 day	218.14	190.33	191.33	11.36	0.735
	21 day	208.22	192.11	199.44	12.96	0.642
	SEM	10.30	15.16	9.89		
	P-value	0.835	0.636	0.872		
Creatinine (mg/dL)	0 day	1.39	1.24	1.22	0.035	0.090
	21 day	1.40	1.37	1.29	0.036	0.453
	SEM	0.053	0.062	0.054		
	P-value	0.898	0.531	0.724		
BUN (mg/dL)	0 day	11.97	11.88	13.24	0.62	0.632
	21 day	12.11	10.72	12.07	0.60	0.580
	SEM	1.01	0.93	0.75		
	P-value	0.675	0.769	0.734		
Globulin (g/dL)	0 day	4.90	5.14	5.05	0.26	0.937
	21 day	4.82	5.06	4.97	0.20	0.634
	SEM	0.45	0.15	0.70		
	P-value	0.253	0.506	0.443		
ALP (U/L)	0 day	30.66	30.87	29.28	2.05	0.957
	21 day	30.57	29.87	30.55	2.76	0.963
	SEM	3.50	2.30	2.61		
	P-value	0.229	0.405	0.602		
ALT (U/L)	0 day	22.60	24.20^{A}	23.40^{A}	1.12	0.854
	21 day	19.12	20.22^{B}	18.77^{B}	0.97	0.827
	SEM	1.50	0.67	1.45		
	P-value	0.799	0.032	0.046		
Glucose (mg/dL)	0 day	60.25	62.20	58.57	0.78	0.165
	21 day	58.60	62.87	61.00	0.98	0.189
	SEM	2.38	1.51	1.31		
	P-value	0.097	0.644	0.842		

$^{A-B}$ Means along column among treatments with different superscripts are significantly different at $P < 0.05$, SEM= standard error of the mean, CON= control group, Non UV-B IY= Non UV-B irradiation yeast, UV-B IVDY= UV-B irradiated vitamin D enrich yeast 200,000 IU/day, BUN= Blood urea nitrogen, ALP= Alkaline phosphatase, ALT= alanine aminotransferase.

Conclusion

UV-B irradiated vitamin D enrich yeast supplementation with total mixed ration in dairy cow diets can increase milk fat and tend to escalate the production performance were, milk protein, total solid and milk lactose. In the same way, UV-B irradiated vitamin D enrich yeast supplementation doesn't have a negative effect on health status of dairy cows. However, this still requires further study to the UV-B irradiated vitamin D enrich yeast supplementation in the dairy cow diets on the quantity of vitamin D in milk and metabolism of vitamin D in plasma.

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