Effect of Different Sources of Vitamin D on Transfer of Vitamin D to Egg Yolk

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Experiments were conducted to investigate the effect of supplementation of different sources of vitamin D on the transfer of vitamin D to the egg yolk. Vitamin D₂-fortified shiitake, pure vitamin D₂ and pure vitamin D₃ were used as a source of vitamin D. Experimental diets contained each source of vitamin D at the level of 500 µg/kg as the amount of vitamin D in the basal diet. Laying hens (White Leghorn) were divided into three groups of five birds each and fed the experimental diets for 14 days. The initial contents of vitamin D₂ and vitamin D₃ in the egg yolk were undetected and 0.05 µg/g, respectively. At the end of the experiment, vitamin D₂ and vitamin D₃ contents (µg/g) in the egg yolks were as follows: 0.326, 0.080 on the shiitake diet; 0.277, 0.055 on the vitamin D₂ diet and undetected, 0.494 on the vitamin D₃ diet. The transfer ratio of vitamin D (total amount of vitamin D₂ and vitamin D₃) to the egg yolk on the shiitake, vitamin D₂ and vitamin D₃ diets were 10.9%, 8.8% and 12.7%, respectively. The transfer ratio of vitamin D₃ tended to be higher than that of vitamin D₂. The content of 25-OH-D₃ in the egg yolks on the vitamin D₃ diet increased three-fold during the experimental period, and 25-OH-D₂ was detected in the egg yolk on the shiitake diet and vitamin D₂ diet. However, the content of 25-OH-D₂ in the egg yolk was significantly lower than that of 25-OH-D₃. There was no effect of the different sources of vitamin D on the performance, egg shell quality and concentrations of calcium and phosphorus in the plasma of laying hens.

It is well known that fat-soluble vitamins are transferred from the feed to the egg yolk in laying hens¹⁾²⁾. SUNDA reported that feeding of vitamin A-rich ingredients to laying hens markedly influenced the vitamin A content of the egg yolk³⁾. KONNO *et al.* also reported that both vitamin A and vitamin E in the egg yolk were increased by increasing the content of these vitamins in the diet⁴⁾.

The vitamin D-fortified eggs are produced by feeding the vitamin D-fortified shiitake (*Lentinus edodes*) as the source of vitamin D⁵⁾. In economical production of vitamin D-fortified eggs, the transfer ratio of vitamin D from poultry feed to the egg yolk is an important factor. However, there have been few reports on the transfer ratio of vitamin D.

The physiological activity of vitamin D is

much different between vitamin D₂ and vitamin D₃ for the birds⁶⁾⁷⁾. Vitamin D₂ and vitamin D₃ are apparently about equally active for man and other mammals. The physiological activity of vitamin D₂ for the chick was one-tenth or below of that of vitamin D₃⁸⁾. Therefore, it is presumed that there may be a difference between vitamin D₂ and vitamin D₃ in the transfer ratio of vitamin D to the egg yolk.

In this study, vitamin D₂, vitamin D₃ and vitamin D₂-fortified shiitake (*Lentinus edodes*) were used as the source of vitamin D and the transfer ratio of each vitamin D from the different sources of vitamin D to the egg yolk was investigated.

Materials and Methods

Animals

Single Comb White Leghorn hens, 25 weeks old, were used. They were divided into three groups of five birds each on the basis of body weight and previous egg production records, and housed individually in wire cages in an air-conditioned room (22°C) and randomly assigned to each experimental diet. They were fed *ad libitum* for 14 days.

Experimental diets

Vitamin D₂-fortified shiitake was prepared by irradiating dried shiitake (*Lentinus edodes*) powder with ultraviolet light⁹. The contents of vitamin D₂ and ergosterol in the vitamin D₂-fortified shiitake were 125 µg/g and 2 420 µg/g, respectively. The 25-OH-D₂ was undetected in vitamin D₂-fortified shiitake.

Vitamin D₂ or vitamin D₃ premix was prepared by mixing pure vitamin D₂ or pure vitamin D₃ (Wako Pure Chemical Industries, Ltd.) with cellulose powder (Oriental Yeast Co., Ltd.) at a vitamin D content of 125 µg/g.

Experimental diets were prepared by adding vitamin D₂-fortified shiitake, the vitamin D₂ or the vitamin D₃ premix at 4 g/kg to the basal diet, the composition of which is shown in Table 1 and involved vitamin D₃ in advance at 25 µg/kg. The ergosterol content was 3 840 µg/kg in the basal diet.

Measurement parameters

The egg yolks were separated from the eggs laid by the hens fed experimental diets on the initial and final days of the experimental period and freeze-dried. These egg yolks from each experimental groups were mixed in order to make the representative analytical samples. The vitamin D₂, vitamin D₃, 25-OH-D₂, 25-OH-D₃ and ergosterol contents were determined.

The excreta were collected from each birds for the final two days of the experimental period and freeze-dried. The samples pooled from each experimental group were determined for vitamin D (total amount of vitamin D₂ and vitamin D₃) and ergosterol.

Blood was collected from wing veins of the

Table 1 Composition of basal diet

Corn	56.2%
Sorghum	9.0
Soybean meal	7.0
White fish meal	7.0
Wheat bran	5.0
Defatted rice bran	2.3
Alfalfa meal	2.0
Corn gluten meal	1.8
Tallow	1.0
Calcium carbonate	7.1
Tricalcium phosphate	1.2
Sodium chloride	0.2
Mineral vitamin premix	0.2

birds at the end of the experiment, and the concentrations of 25-OH-D₂, 25-OH-D₃, calcium and phosphorus in the plasma were assayed. All results are given as means and standard errors for five birds.

The performance of laying hens; body weight, egg production, egg weight and feed intake were recorded. The egg shell quality; egg shell weight and egg shell thickness were evaluated on the initial and final days of the experiment.

Analytical methods of Vitamin D, 25-OH-D and ergosterol

The determinations of vitamin D, 25-OH-D and ergosterol in the egg yolk and excreta were carried out by a slightly modified method of OKANO *et al.*^{10)~12)}. The outline is shown in Fig. 1.

About 5 g of sample was accurately weighed and saponified. The extracted unsaponifiable matter was applied to the first HPLC to collect the fractions of vitamin D, 25-OH-D and ergosterol. The first HPLC was performed using a TOSOH ODS-80 T_M column (reversed-phase type, i.d. 21.5×300 mm) with methanol as the mobile phase. The flow rate was 10.0 ml/min.

The second HPLC for determination of 25-OH-D was performed using a TOSOH column (straight-phase type, i.d. 4.6×250 mm) with 5% isopropanol in n-hexane as the mobile phase. The flow rate was 1.0 ml/min. The contents of

Fig. 1 Procedure for determination of vitamin D, 25-OH-D and ergosterol in the egg yolk and excreta. These values were determined by reading the peak height at 265 nm.

25-OH-D₂ and 25-OH-D₃ in the egg yolk were estimated using 25-OH-D₃ (Funakoshi Co., Ltd.) as the reference standard compound.

Determinations of vitamin D and ergosterol were carried out under the same conditions of HPLC, the second HPLC was performed using a TOSOH OH-120 column (straight-phase type, i. d. 4.6×250 mm) with 0.6% isopropanol in n-hexane as the mobile phase at a flow rate of 1.0 ml/min. The third HPLC was performed using a TOSOH ODS 120 T (reversed-phase type, i. d. 4.6×250 mm) with methanol as the mobile phase at a flow rate of 1.0 ml/min.

The contents of vitamin D, 25-OH-D and ergosterol in the egg yolk were expressed in weights per gram of raw egg yolk by conversion of moisture. The content of vitamin D in the excreta was expressed as the total amount of vitamin D₂ and vitamin D₃ by estimating the peak of second HPLC on the dry base.

Plasma analysis

The concentrations of 25-OH-D₂ and 25-OH-D₃ in the plasma were assayed by the method of OKANO *et al.*¹²⁾ The concentration of calcium in the plasma was determined by the orthocresolphthalein complexone (OCPC) method¹³⁾ using commercial kits (705 CA) from Eiken Chemical Co., Ltd.. The concentration of phosphorus in plasma was determined by

the direct method of molybdc acid¹⁴⁾ using commercial kits (MUKI RIN-HA TEST WAKO) from Wako Pure Chemical Industries, Ltd..

Calculations of transfer ratio and exhaust ratio

The transfer ratio of vitamin D from the diet to the egg yolk was calculated using the following equation; [vitamin D content (total amount of vitamin D₂ and vitamin D₃) in the egg yolk×egg yolk weight×egg production]/[vitamin D content in the diet (525 µg/kg)×feed intake]. The measurements of vitamin D content in the egg yolk and egg yolk weight were performed using the egg at the end of the experiment. Regarding egg production and feed intake, the average values during the experimental period were used. Egg yolk weight on the shiitake, vitamin D₂ and vitamin D₃ diets at the end of the experiment was 15.6, 16.4 and 15.0 g per egg, respectively. The exhaust ratio of vitamin D was calculated using the following equation; [vitamin D content in the freeze-dried excreta×weight of freeze-dried excreta]/[vitamin D content in the diet×feed intake]. The weights of freeze-dried excreta per day per bird on the shiitake, vitamin D₂ and vitamin D₃ diets were 24.7, 25.2 and 23.1 g, respectively. The transfer ratio and exhaust ratio of ergosterol were cal-

culated using the same equations as that for vitamin D.

Results and Discussion

Content of vitamin D in the egg yolk and transfer ratio

The contents of vitamin D₂ and vitamin D₃ in the egg yolk and the transfer ratio of vitamin D from the diets to the egg yolk are shown in Table 2.

The contents of vitamin D₂ and vitamin D₃ in the egg yolk on initial day of the experiment were undetected and 0.05 µg/g, respectively. At the end of the experiment, vitamin D₂ was detected in the egg yolk on the shiitake and vitamin D₂ diets. The increase in vitamin D₂ content in the egg yolk indicated that vitamin D₂ was transferred from the diet to the egg yolk.

The transfer ratio of vitamin D in the vitamin D₃ diet was the highest, and the transfer ratio of vitamin D₃ tended to be higher than that of vitamin D₂. However, there was not much difference between vitamin D₂ and vitamin D₃ in transfer to egg yolk in comparison with that in the physiological activity for birds.

The mechanism of transfer of vitamin D₃ to egg yolk was shown by FRASER, D.R. and EMTAGE, J.S. as follows⁽⁵⁾: Vitamin D₃ absorbed in the hen's body was bound to the plasma vitamin D-binding protein (DBP). These proteins were further bound to the phosvitin protein of the major component in the egg yolk. Therefore, vitamin D was transferred to the egg yolk with DBP and phosvitin. In this experiment, vitamin D₂ and vitamin D₃ were

roughly equal in transfer ratio of vitamin D to the egg yolk. These results suggested that vitamin D₂ was also transferred to the egg yolk by almost the same mechanism as vitamin D₃. There was not much difference between vitamin D₂ and vitamin D₃ bonding potency to DBP.

The transfer ratio of vitamin D on the vitamin D₂ diet was slightly lower than that on the shiitake diet. The reason for this difference was considered to be that the storage stability of vitamin D₂ in shiitake was higher than that of vitamin D₂ in cellulose powder.

Contents of 25-OH-D₂ and 25-OH-D₃ in the egg yolk

The contents of 25-OH-D₂ and 25-OH-D₃ in the egg yolk are shown in Table 3. The contents of 25-OH-D₂ and 25-OH-D₃ in the egg yolk at the start of the experiment were undetected and 0.008 µg/g, respectively. The content of 25-OH-D₃ was close to that given by TAKEUCHI *et al.* who reported a value of 0.01 µg/g as the 25-OH-D₃ content in egg yolk⁽⁶⁾.

At the end of the experiment, the content of 25-OH-D₃ in the egg yolk on the vitamin D₃ diet increased about three-fold, and 25-OH-D₂ was detected in the egg yolk on the shiitake and vitamin D₂ diets.

The presence of 25-OH-D₂ in the egg yolk indicated that vitamin D₂ absorbed in the hen's body was metabolized to 25-OH-D₂ in the liver and this 25-OH-D₂ was transferred to the egg yolk. However, the content of 25-OH-D₂ in the egg yolk was considerably lower than that of 25-OH-D₃.

The physiological activity of vitamin D₂ for the birds was one-tenth or below of that of vitamin D₃, unlike that for mammals. The

Table 2 Contents and transfer ratio of vitamin D₂, vitamin D₃ in egg yolk

	Vitamin D ₂ (µg/g)	Vitamin D ₃ (µg/g)	Transfer ratio (%)
Initial	N.D.	0.050	—
Shiitake	0.326	0.080	10.9
Vitamin D ₂	0.277	0.055	8.8
Vitamin D ₃	N.D.	0.494	12.7

Table 3 Contents of 25-OH-D₂, 25-OH-D₃ in egg yolk

	25-OH-D ₂ (µg/g)	25-OH-D ₃ (µg/g)
Initial	N.D.	0.0080
Shiitake	0.0133	0.0063
Vitamin D ₂	0.0102	0.0067
Vitamin D ₃	N.D.	0.0244

reason for the difference in physiological activity was demonstrated by OKANO *et al.* as follows¹⁷⁾: The bonding potencies of vitamin D₂ metabolites to human plasma vitamin D-binding protein were equal to these of vitamin D₃ metabolites; however, bonding potencies of vitamin D₂ metabolites to chick plasma vitamin D-binding protein showed a low tendency in comparison with these of vitamin D₃ metabolites. Especially the bonding potency of 25-OH-D₂ was lowest and less than half the bonding potency of 25-OH-D₃.

The content of 25-OH-D₂ in the egg yolk was lower than that of 25-OH-D₃, suggesting that the bonding potency of 25-OH-D₂ to chick plasma vitamin D-binding protein was lower compared with that of 25-OH-D₃.

Vitamin D content in the excreta and exhaust ratio

The vitamin D content in the excreta and the exhaust ratio were highest on the shiitake diet followed by the vitamin D₂ diet and lowest on the vitamin D₃ diet as shown in Table 4. The exhaust ratio of vitamin D₂ tended to be higher than that of vitamin D₃. The high exhaust ratio of vitamin D on the shiitake diet was probably due to the low digestibility of shiitake and the high stability of vitamin D₂ originating from shiitake in excreta.

Table 4 Contents and exhaust ratio of vitamin D in excreta

	Vitamin D ($\mu\text{g/g}$)	Exhaust ratio (%)
Shiitake	1.220	53.9
Vitamin D ₂	0.830	34.9
Vitamin D ₃	0.565	22.7

Transfer ratio and exhaust ratio of ergosterol

The transfer ratio and exhaust ratio of ergosterol are shown in Table 5. Ergosterol is the precursor of vitamin D₂, and it is known that egg yolk contains ergosterol¹⁸⁾. The content of ergosterol in the egg yolk on the shiitake diet was higher than that on the other diets. This result suggested that the ergosterol content in egg yolk increased with the addition of shiitake to the basal diet, that is, ergosterol was also transferred from the diet to the egg yolk. However, the transfer ratio of ergosterol was significantly lower than that of vitamin D.

The transfer ratio of ergosterol was lowest on the shiitake diet. This means that the shiitake diet seem to approach the limitative level of the transfer of ergosterol.

The exhaust ratios of ergosterol on the vitamin D₂ and vitamin D₃ diets were approximately 30% and were shown to be about the same level as the exhaust ratios of vitamin D on these diets. The high exhaust ratio of ergosterol on the shiitake diet was due to the low digestibility of shiitake.

Influence of different sources of vitamin D on laying hens

The concentrations of 25-OH-D₂, 25-OH-D₃, calcium and phosphorus in the plasma of laying hens are shown in Table 6. The concentrations of 25-OH-D₂ and 25-OH-D₃ in the plasma of laying hens at the start of the experiment were undetected and 18.5 ng/mL, respectively. At the end of the experiment, the concentration of 25-OH-D₃ in the plasma on the vitamin D₃ diet increased 1.5-fold, and 25-OH-D₂ was detected on the shiitake and vitamin D₂ diets. It was indicated that vitamin D₂ was

Table 5 Contents of ergosterol in egg yolk and excreta

	Egg yolk		Excreta	
	Contents ($\mu\text{g/g}$)	Transfer ratio (%)	Contents ($\mu\text{g/g}$)	Exhaust ratio (%)
Shiitake	1.350	1.4	24.452	41.9
Vitamin D ₂	0.835	3.0	5.340	30.7
Vitamin D ₃	0.920	3.2	4.960	27.3

Table 6 Plasma levels of 25-OH-D₂, 25-OH-D₃, calcium and phosphorus of laying hens

	25-OH-D ₂ (ng/ml)	25-OH-D ₃ (ng/ml)	Ca (mg/dl)	P (mg/dl)
Initial	N.D.	18.5± 3.0	23.2±2.9	4.0±0.9
Shiitake	6.4±1.3	6.9± 1.8	23.4±3.5	3.7±0.5
Vitamin D ₂	5.0±1.2	8.0± 2.7	24.6±2.1	4.2±0.5
Vitamin D ₃	N.D.	28.1±10.5	24.4±5.2	4.3±1.0

(n=5)

Table 7 Effect of vitamin D₂-fortified shiitake powder, vitamin D₂ and vitamin D₃ on the performance of laying hens

	Shiitake	Vitamin D ₂	Vitamin D ₃
Body weight (kg) Initial	1 658±104	1 661±94	1 661±87
Final	1 705±117	1 643±93	1 699±77
Egg production (%)	96.0±5.9	97.2±3.8	98.6±3.1
Feed intake (g/day)	106.5±6.1	114.1±3.9	109.3±6.1
Egg weight (g)	52.1±6.6	54.7±3.9	53.3±6.3
Feed conversion	2.13	2.15	2.08

(n=5)

Table 8 Effect of vitamin D₂-fortified shiitake powder, vitamin D₂ and vitamin D₃ on the egg shell quality

	Shiitake	Vitamin D ₂	Vitamin D ₃
Initial			
Egg shell weight (g)	0.357±0.015	0.355±0.012	0.355±0.032
Egg shell thickness (mm)	5.12 ±0.33	5.12 ±0.28	5.12 ±0.27
Egg shell weight ratio	9.96 ±0.27	9.41 ±0.31	9.54 ±0.66
Final			
Egg shell weight (g)	0.344±0.021	0.316±0.024	0.342±0.009
Egg shell thickness (mm)	5.00 ±0.46	4.86 ±0.61	5.06 ±0.14
Egg shell weight ratio	9.49 ±0.56	8.44 ±0.72	9.17 ±0.06

(n=5)

certainly metabolized to 25-OH-D₂. The concentrations of calcium and phosphorus in the plasma were kept within normal levels for laying hens and were not affected by the different sources of vitamin D.

The performance and egg shell quality are shown in Table 7 and 8, respectively. There was no effect of the different sources of vitamin D on the performance. Egg shell quality on the vitamin D₂ diet was shown to be at a

slightly low level in all parameters. However, it was quite insignificant. Therefore, it was considered that there was no effect of the different sources of vitamin D on egg shell quality in this experiment.

References

- 1) NABER, E.C. : *Poultry Science*, **58**, 518 (1979).
- 2) STANDELMAN, W.J. and PRATT, D.E. : *World's Poultry Science Journal*, **45**, 247 (1989).

- 3) SUNDA, M.L. : *Poultry Science*, **41**, 532 (1962).
- 4) KONNO, T., ASADA, T. and KATSUO, T. : *Nihon Chikusan Gakkai-ho*, **56**, 414 (1985).
- 5) KAWAZOE, T., YUASA, K., YAMAZAKI, M. and ANDO, M. : *Nippon Shokuhin Kogyo Gakkaishi*, **41**, 891 (1994).
- 6) BETHKE, R.M., RECORD, P.R. and KENNARD, D.C. : *J. Nutr.*, **6**, 413 (1933).
- 7) WADDELE, J. : *J. Biol. Chem.*, **105**, 711 (1934).
- 8) CHEN, P.A. and BOSMANN, H.B. : *J. Nutr.*, **83**, 133 (1964).
- 9) KAWAZOE, T. and YUASA, K. : *Nippon Shokuhin Kogyo Gakkaishi*, **42**, 262 (1995).
- 10) OKANO, T., TAKEUCHI, A. and KOBAYASHI, T. : *Journal of Nutritional Science and Vitaminology*, **27**, 539 (1981).
- 11) TAKEUCHI, A., OKANO, T., TERAOKA, S., MURAKAMI, Y. and KOBAYASHI, T. : *Journal of Nutritional Science and Vitaminology*, **30**, 11 (1984).
- 12) OKANO, T., MIZUNO, N., SHIDA, S., TAKAHASHI, N., KOBAYASHI, T., KURODA, E., KODAMA, S. and MATSUO, T. : *Journal of Nutritional Science and Vitaminology*, **27**, 43 (1981).
- 13) CONNERY, H.V. and BRIGGS, A.R. : *The American Journal of Clinical Pathology*, **45**, 290 (1966).
- 14) DALY, J.A. and ERTINGSHAVSEN, G. : *Clinical Chemistry*, **18**, 263 (1972).
- 15) FRASER, D.R. and EMTAGE, J.S. : *Biochem. J.*, **160**, 671 (1976).
- 16) TAKEUCHI, A., OKANO, T. and KOBAYASHI, T. : *Vitamins (Japan)*, **55**, 337 (1981).
- 17) OKANO, T. : *Vitamins (Japan)*, **66**, 65 (1992).
- 18) TU, C., POWRIE, W.D. and FENNEMA, O. : *J. Food Sci.*, **35**, 601 (1970).

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ビタミン D の卵黄中への移行に及ぼす

ビタミン D 給源の影響

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ビタミン D₂ 強化シイタケ、ビタミン D₂ 及びビタミン D₃ をビタミン D 給源として用いた。それぞれビタミン D 量として基礎飼料中に 500 µg/kg の割合で混合し、各区 5 羽ずつ 2 週間飼育した。

卵黄中のビタミン D 量 (µg/g) は、試験開始前にビタミン D₂ は不検出、ビタミン D₃ は 0.050 であった。試験終了時、ビタミン D₂、D₃ 量は、それぞれシイタケ区で 0.326, 0.080, ビタミン D₂ 区で 0.277, 0.055, ビタミン D₃ 区で不検出, 0.494 であった。ビタミン D の移行率はシイタケ区、ビタミン D₂ 区、ビタミン D₃ 区でそれぞれ 10.9%, 8.8%, 12.7% であり、ビタミン D₂ よりビタミン D₃ の方が卵黄中に移行しやすい傾向にあった。

ビタミン D₃ 区の 25-OH-D₃ 量は試験開始前の約 3 倍であり、シイタケ区、ビタミン D₂ 区では 25-OH-D₂ が検出された。しかし卵黄中の 25-OH-D₂ 量は 25-OH-D₃ 量に比較してかなり少なかった。

産卵成績、卵殻質、血漿中 Ca、P の値は、試験区間に大差なかった。