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DIETARY PROTEIN QUALITY AND β-CAROTENE SUPPLEMENTATION AFFECT THE ACTIVITY OF HEPATIC ACYL CoA: RETINOL ACYLTRANSFERASE IN CHICKS

R. Murwani[1]

Abstract

To study the effect of protein quality and β-carotene supplementation on the activity of intestinal and hepatic enzyme i.e. Acyl CoA: retinol acyl transferase (ARAT), one-day-old male leghorn chicks were used. After feeding the chicks on vitamin-A deprived diet for 10 days to lower and equalise hepatic retinol storage, 28 chicks with similar average body weight were assigned into four groups to receive diet containing: 1) 20% gluten protein supplemented with 1.5 μg β-carotene/g diet, 2) 20% gluten protein supplemented with 15 μg β-carotene/g diet, 3) 20% casein protein supplemented with 1.5 μg β-carotene/g diet, 4) 20% casein protein supplemented with 15 μg β-carotene/g diet. Each experimental group consisted of seven replicate chicks. The experimental diet was performed for two weeks. Serum retinol, hepatic retinol and hepatic β-carotene content and the activity of intestinal and hepatic enzyme i.e. Acyl CoA:retinol acyl transferase (ARAT) were measured to estimate the fate of retinol converted from β-carotene. The results showed that regardless of protein quality, increase β-carotene supplementation raised liver retinol and β-carotene content significantly. These raise was followed by increased activity of intestinal and hepatic ARAT activity. Comparing the effect of protein quality at the same level of β-carotene supplementation it could be observed that there was no significant difference in intestinal ARAT activity. However, a striking difference was found when comparing serum retinol and the activity of hepatic ARAT in gluten groups and casein groups each supplemented with 15 μg β-carotene. Serum retinol content was higher up to 65.0 ± 14.3 μg/dl in casein groups supplemented with 15 μg β-carotene compared to 15.2 ± 4.6 μg/dl in gluten groups supplemented with the same amount of β-carotene. Hepatic ARAT activity was also increased up to 114.2 ±13.3 pmol/minute/mg in these casein groups compared to 51.5 ±9.1 pmol/minute/mg in gluten groups. These results suggested that when the level of β-carotene supplementation and dietary protein is sufficient (20%), good quality of protein enhanced the esterification rate of retinol in liver and might suggest its influence in retinol release from the liver and its consequent increase in serum retinol.

Key words: β-carotene, Retinol, Acyl CoA:retinol acyl transferase, Chick, Protein quality

[1] Laboratory of Nutritional Biochemistry, Faculty of Animal Agriculture, Diponegoro University, Semarang, Indonesia, E-mail: rmurwani@telkom.net

Introduction

β-carotene is one of the most potent source of provitamin A among carotenoids. β-carotene occurs widely in plant product and poultry receives dietary vitamin A from various source including yellow corn, fish oils, or vitamin A acetate supplement. Other rich dietary sources of vitamin A are carrot, which is known to contain high amount of carotenoids especially β-carotene.

The study of β-carotene metabolism in mammalian has been well researched and documented but it is not so with poultry or chicken. However, the activity of β-carotene cleavage has been demonstrated in chick intestinal mucosa (Sklan, 1983). It has also been shown that the activity of retinol esterifying enzyme acyl CoA: retinol acyltransferase (ARAT) is present in chick intestinal mucosa with the optimal condition of the enzyme similar to that present in mouse or rats (Murwani, 1990). Some studies in mammalian indicated that the biosynthesis of vitamin A from β-carotene is influenced by several dietary factors such as lipid, dietary fibre, the level of protein and β-carotene intake itself (van het Hof, 2000; Deming *et al.*, 2000; Lee *et al.*, 2000). Few studies on the biosynthesis of dietary β-carotene in poultry or chicken have been done. A study in male leghorn chicks has demonstrated that level of dietary protein and β-carotene supplementation affected significantly the conversion of β-carotene into retinol (Murwani, 2001). This effect was due to the increase in the activity of retinol esterifying enzyme within the mucosal cells of chicks and followed by increase storage in hepatic retinol and decrease storage of hepatic β-carotene.

Further study in male leghorn chicks was carried out to investigate the metabolism of dietary β-carotene of carrot powder as affected by the quality of dietary protein and the level β-carotene supplementation. Gluten and casein were used as the sole source of different quality of proteins.

Materials and Methods

Experimental design

One-day-old male leghorn chicks were given a vitamin A deprived diet *ad libitum* (Table 1). They were kept under controlled light and warm temperature, and water was provided on free access. After feeding the chicks on vitamin-A deprived diet for 10 days to lower and equalise hepatic retinol storage, 28 chicks with similar average body weight were assigned into four groups to receive diet containing: 1) 20% gluten protein supplemented with 1.5 μg β-carotene/g diet, 2) 20% gluten protein supplemented with 15 μg β-carotene/g diet, 3) 20% casein protein supplemented with 1.5 μg β-carotene/g diet, 4) 20% casein protein

supplemented with 15 μg β-carotene/g diet. Each experimental group consisted of seven replicate chicks. The experimental diet was performed for two weeks. The casein, gluten and vitamin mix were vitamin A free. β-carotene was given in a form of carrot powder mixed thoroughly into the diet. All experimental diets were stored in a cold storage at 4^0C.

These experimental diets were performed for two weeks. At the end of the experimental diets chicks were sacrificed for blood, liver and intestinal mucosa samples.

Table 1. Composition of purified basal diet (vitamin A deprived) for chicks

Ingredient	Amount (%)
Casein or Gluten (vitamin A free)	20.0
Starch	67.6
Soybean oil	4.5
Vitamin mix (vitamin A-free) [a]	0.25
Mineral mix [b]	5.0
Fat soluble vitamin (A[c], D, E)	0.5
Cellulose	2.0
Choline Chloride	0.15

[a, b] The kinds and amount of vitamin and mineral mix was given according to NRC 1978
[c] Vitamin A for basal diet was given at 40 IU/100 g diet as vitamin A-palmitate

β-carotene source

β-carotene source was given as carrot powder which was analysed for β-carotene content. The powder was stored at -20^0C and mixed with basal diet prior to feeding.

Sample preparation for retinol and β-carotene determination

Serum samples were prepared without saponification and extracted with hexane. Liver samples was prepared by saponification in 60% ethanolic KOH containing pyrogallol and extracted with hexane.

HPLC analysis for β-carotene and retinol

β-carorotene were separated on Waters RCM 8x10 Millipore column and detected at 450 nm. Eluting solvent of Acetonitril:Methanol:THF (58:35:7) was pumped at 2 ml/minute. Retinol was identified on Licrosorb RP-18 column by Fluorescence Spectrophotometer Detector at 325 nm (exitation) and 470 nm

(emission) as a single peak. Eluting solvent of Methanol:H_2O (98:2) was pumped at a flow rate of 1.5 ml/minute.

Microsome preparation from intestinal mucosa and liver

Intestinal microsome was obtained from intestinal mucosa, which was collected using a glass rod, and frozen directly until further preparation. Mucosa and liver samples were homogenized in Tris HCl buffer containing sucrose, $MgSO_4$ and KCl pH 7.5 and then centrifuged at 20,000 g for 15 minutes. The supernatant was collected and further centrifuged at 105,000 g for one hour. The resulting pellet was resuspended in Potassium Phosphate buffer pH 7.4, washed once with the same buffer and centrifuged as before. Washing was repeated twice and the last resuspension was frozen and stored at –80°C. Part of each sample was prepared for protein determination.

Protein determination

Protein concentration was measured following Lowry method using Bovine Serum Albumin as standard (Alexander *et al.*, 1985).

Assay for ARAT activity

ARAT was assayed by measuring the formation of labelled [^{3}H] retinyl palmitate after incubating radicactive 50,000 cpm [^{3}H] retinol and 10 nmol unlabeled retinol with unlabelled palmitoyl-CoA in the presence of microsomal ARAT. The assay procedures were basically the same as that described by Helgerud *et al.* (1982, 1983), and modified so that radioactive retinol was dried under nitrogen and redissolved in bovine serum albumin (BSA) containing potassium phosphate buffer. Incubation volume was 0.5 ml containing 2.5 mg BSA, 30 μM palmitoyl-CoA, and 200 μg microsomal protein. Each microsomal preparation was tested in duplicate and corrected with blank using inactive microsomes (heated at 80°C for 30 minutes). The reaction was carried out at optimal time, pH, and temperature i.e. 15 minutes, 6.6, and 43°C respectively for chick intestinal ARAT (Murwani, 1992). [^{3}H] retinyl palmitate formed was extracted and subjected to HPLC. HPLC fraction co-eluted with retinyl palmitate standard was collected, and the radioactivity was counted. The radioactivity count was converted into pmol of the actual retinyl palmitate formed.

[^{3}H] retinol purification

To ensure the experimental result of ARAT activity assay, commercial labelled retinol was purified by HPLC. The [^{3}H] retinol fraction was collected and stored at –20°C until use.

Statistical analysis

Differences between groups were determined using one-way ANOVA and followed by Student Newman Keuls Test. Differences were considered significant at $P<0.05$ or $P<0.01$.

Results and Discussion

The results in Table 2 showed that serum retinol content in gluten groups supplemented with different level of β-carotene were not significantly different. In contrast, the casein groups supplemented with 15 μg β-carotene/g diet showed higher level of serum retinol compared to groups supplemented with 1.5 μg β-carotene/g diet.

Table 2. The concentration of serum retinol, hepatic retinol and β-carotene in the experimental groups

Experimental groups	Serum Retinol (μg/dl)	Liver	
		Retinol (total μg)	β-carotene (total μg)
Gluten groups:			
1.5 μg β-carotene/g diet	12.0 ± 5.5	3.1 ± 2.3 c	0.5 ± 0.4 f
15 μg β-carotene/g diet	15.2 ± 4.6 b	365.3 ± 61.2 c,d	3.3 ± 1.7 f
Casein groups:			
1.5 μg β-carotene/g diet	19.7 ± 6.0 a	4.1 ± 1.6 e	0.4.± 0.2 g
15 μg β-carotene/g diet	65.0 ± 14.3 a,b	245.2 ± 56.13 e,d	2.7 ± 0.9 g

Each value represented as mean ± SD of seven chicks analysed individually
Same letter indicates statistically different ($P<0.01$)

The retinol and β-carotene storage in liver showed that in either gluten or casein groups, higher β-carotene supplementation (15 μg/g diet) resulted in more retinol and β-carotene storage compared to low (1.5 μg/g diet) β-carotene supplementation. However, different quality of protein accompanied by high β-carotene supplementation (15 μg/g diet) resulted in lower storage of hepatic retinol in casein groups.

Table 3. The activity of intestinal and hepatic Acyl CoA: retinol acyl transferase (ARAT) in the experimental groups

Experimental groups	The activity of ARAT (pmol/minute/mg)	
	Small Intestine	Liver
Gluten groups:		
1.5 μg β-carotene/g diet	536.1 ± 18.6 a	29.3 ± 3.9 c
15 μg β-carotene/g diet	1598.0 ± 199.6 a	51.5 ± 9.1 c,e
Casein groups :		

1.5 μg β-carotene/g diet	755.4 ± 102.3 b	44.2 ± 10.2 d
15 μg β-carotene/g diet	1424.0 ± 138.2 b	114.2 ± 13.3 d,e

Each value represented as mean ± SD of seven chicks analysed individually
Same letter indicates statistically different (P<0.01)

Table 3 showed that in either gluten or casein groups, high β-carotene supplementation (15 μg/g diet) resulted in significantly higher activity of intestinal and hepatic ARAT. However, groups receiving casein as a good quality of protein when accompanied by high β-carotene supplementation (15 μg/g diet) resulted in higher activity of hepatic ARAT compared to gluten groups supplemented with the same high amount of β-carotene.

Three intestinal enzymes involved in the conversion of dietary β-carotene into retinol. The central cleavage route involved 15,15' dioxygenase enzyme which catalysed the splitting of β-carotene into retinal. Retinal reductase reduced the retinal into retinol (Barua and Olson, 2000). The third enzyme i.e. ARAT esterified retinol with long chain fatty acid to form retinyl ester (Helgerud *et al.*, 1982, 1983; Ball, 1985). The retinyl ester was then incorporated into chylomicron for transport by lymphatic route and removed from the circulation by the liver. The activity of the three enzymes in chicks is coordinated, and modulation of one enzyme can affect correspondingly to the others (Tajima *et al.*, 1999). Thus the activity of this retinol esterifying enzyme measured in this study could reflect the amount of retinol released from dietary β-carotene as the source of retinol. The results showed that in both gluten and casein groups the activity of intestinal ARAT were affected by the level of β-carotene supplementation. This suggests that regardless of protein quality the conversion of β-carotene into retinol in intestinal mucosa increases significantly with increase amount of β-carotene supplementation. This increase conversion of β-carotene was also reflected in the concomitant increase in liver retinol store. Although the amount of the remaining unconverted β-carotene transported into and stored in liver was higher in groups supplemented with 15 μg/g β-carotene regardless of protein quality, the amount in both groups remained smaller when compared to the amount in groups receiving low amount of protein (5%) (Murwani, 2001). These results were in line with previous results which showed that the level of β-carotene supplementation only affect the conversion of β-carotene into retinol when dietary protein level was adequate.

In rats, circulating retinyl esters from intestinal metabolism is taken up by the liver and mostly stored in non-parenchymal cells. Hydrolysis and reesterification occurs in the liver involving hydrolase and retinol esterifying enzymes respectively resulting in retinyl ester store (Lakshman *et al.*, 1988). From this liver store, retinol is mobilized bound to retinol binding protein back into the circulation. No reports have been found regarding the effect of dietary factors on the activity of ARAT in chicks. The present results showed that hepatic ARAT activity was also present in

chicks. In each gluten and protein groups increase dietary β-carotene supplementation (from 1.5 to 15 μg/g diet) raised significantly the activity of hepatic ARAT. At the same amount of β-carotene supplementation (15 μg/g diet), this hepatic ARAT activity was significantly higher in casein groups than in gluten groups. However, this increase in hepatic ARAT acitivity was not followed by concomitant increase in liver retinol store (Table 2). Further results which showed a significant increase in serum retinol level of these casein groups compared to gluten groups supplemented with the same amount of β-carotene (15 μg/g diet) lend a possible mobilisation of retinyl ester store in casein groups. This finding was different than that found in mammalian which maintained their serum retinol in a steady state level regardless of dietary treatment (Kelley and Green, 1998; Sulaeman *et al.*, 2002). However, this increase in serum retinol level was similar to that found in human receiving dietary β-carotene (Ncube *et al.*, 2001).

Conclusion

In male leghorn chicks different quality of dietary protein i.e. gluten and casein affects significantly serum retinol level and the activity of hepatic acylCoA:retinol acyl transferase. Serum retinol level and hepatic ARAT activity was higher in groups receiving dietary casein compared to groups receiving dietary gluten supplemented with the same amount of dietary β-carotene in the form of carrot powder (15 μg/ g diet).

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