

Original article

**Effect of dietary  $\beta$ -carotene on the accumulation  
of  $\beta$ -carotene and vitamin A in plasma  
and tissues of gilts**

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**Abstract** — The absorption of  $\beta$ -carotene in pigs is limited. Nevertheless  $\beta$ -carotene might positively affect reproduction. In this study the absorption and tissue distribution of  $\beta$ -carotene as well as its function as precursor of vitamin A was investigated in gilts that were fed according to one of three dietary treatments: VA (4000 IU vitamin A), VA + VA (4000 IU + 8300 IU) and VA + BC (4000 IU + 100 mg  $\beta$ -carotene per kg diet) for 14 weeks. Only in the VA + BC group was  $\beta$ -carotene detected in plasma (1–8 ng·mL<sup>-1</sup>), liver, adrenals and corpora lutea, indicating that pigs absorb intact  $\beta$ -carotene at low rates. Liver levels of vitamin A were higher ( $P < 0.01$ ) at comparable levels in the VA + VA and VA + BC group than in the VA group, indicating a conversion rate of  $\beta$ -carotene to vitamin A of 40 to 1 on the basis of weight for  $\beta$ -carotene at this level (100 mg·kg<sup>-1</sup>) in the diet. Higher levels of vitamin A in the uterus of the VA + BC group ( $P < 0.01$ ) as well as the accumulation of  $\beta$ -carotene in adrenals and corpora lutea might reflect some influence of  $\beta$ -carotene on local vitamin A metabolism which might be of importance for reproductive performance in gilts.

**pig / metabolism / absorption / tissue distribution /  $\beta$ -carotene / vitamin A**

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## 1. INTRODUCTION

For herbivorous and omnivorous species,  $\beta$ -carotene is an important source of vitamin A. But beside this function as a systemic or local vitamin A source,  $\beta$ -carotene has attracted attention because of its possible importance in the reproductive performance of different species of farm animals [22]. Positive effects of  $\beta$ -carotene on fertility have been observed in cattle, horses, rabbits and pigs [3, 6]. But results in all species are still somewhat contradictory [8]. The possible mode of action of  $\beta$ -carotene, other than its function as a precursor of vitamin A, is still in question. Besides a local conversion of  $\beta$ -carotene into vitamin A metabolites through central or eccentric cleavage,  $\beta$ -carotene might have a specific independent function or act as an antioxidant. Local effects of  $\beta$ -carotene in peripheral target tissue such as the ovary, the adrenals or the uterus are difficult to explain in pigs or other so called white-fat animals which do absorb  $\beta$ -carotene only to a very limited extent [22].

The present experiment addresses the question as to what extent  $\beta$ -carotene is absorbed during digestion in pigs, the extent of accumulation in plasma and tissues, and how the supplementation effects the vitamin A levels in the liver and different extrahepatic tissues.

## 2. MATERIALS AND METHODS

### 2.1. Animals and feeding

The study involved 36 crossbred primiparous gilts (German Landrace  $\times$  Piétrain) of similar age, weight and genetic background. The pigs were housed in individual pens and were randomly allocated three different dietary supplementations: VA: 4000 IU; VA + VA: 4000 IU vitamin A + 8300 IU vitamin A (Hoffmann-La Roche, Grenzach-Wyhlen, Germany) VA + BC: 4000 IU vitamin A + 100 mg  $\beta$ -carotene (Rovimix 10%,

Hoffmann-La Roche) per kg diet for 14 weeks. Basal  $\beta$ -carotene levels in the diet were  $< 100 \mu\text{g}\cdot\text{kg}^{-1}$  feed. Gilts were fed 2.5 to 3.0 kg basal diet low in vitamin A and  $\beta$ -carotene daily for four weeks in order to lower body stores of vitamin A. Thereafter they were fed the basal diet with the indicated amounts of vitamin A and  $\beta$ -carotene for 14 weeks until they were slaughtered. Diets were formulated to meet or exceed the nutrient recommendation of the National Research Council for pigs [15]. The diet consisted of wheat (30%), wheat bran (30%), oats (30%) and soybean meal (6.9%). Nutrient and energy content of the basal diet per kg was as follows: dry matter (879 g), metabolizable energy (12.0 MJ), crude protein (141 g), ether extracts (23 g), ash (58 g), crude fiber (57 g), calcium (8.1 g), phosphorus (6.9 g), sodium (1.6 g), magnesium (2.5 g). The amount fed was based on the daily maintenance caloric requirements determined by body weight. Water was available for consumption at all times. The gilts were inseminated and then slaughtered at the University of Leipzig abattoir at day 12 of gestation. Immediately after slaughter, uteri and ovaries as well as various tissues (liver, kidney, adrenals, lungs) were removed and weighed. The tissue samples were frozen following collection and stored at  $-40^\circ\text{C}$  until analysis. At slaughter the gilts were weighed (VA:  $118 \pm 7$  kg, VA + VA:  $121 \pm 7$  kg VA + BC:  $120 \pm 8$  kg). The experimental protocol and housing facilities were reviewed and approved by the institutional and governmental Animal Welfare Committee (Regierungspräsidium Leipzig, Germany – TVV #: A 32).

### 2.2. Analysis of $\beta$ -carotene and vitamin A in plasma and tissue

Blood samples were collected in one to two week intervals from the *ear vein* into heparinized tubes and plasma was harvested by centrifugation at  $1500 \times g$  for 15 min immediately after collection. During

storage and handling the exposure to sunlight was avoided.

Vitamin A and  $\beta$ -carotene were separated and quantified using a reversed-phase HPLC-system (Waters, Eschborn, Germany). Vitamin A (retinol and retinyl esters) and  $\beta$ -carotene were extracted twice from plasma and uterine fluid (200  $\mu$ L) using n-hexane (1 mL) containing 0.05% butylated hydroxytoluene (BHT) as antioxidant after deproteinization with ethanol (200  $\mu$ L). The combined organic extracts were dried under nitrogen and redissolved in methanol:tetrahydrofuran (90:10 vol/vol). Vitamin A and  $\beta$ -carotene were extracted from the various tissues using n-hexane and isopropanol (3:2; vol/vol, 0.05 BHT) to homogenize the samples [17]. The organic extracts were treated as those of plasma and uterine fluid. Vitamin A (retinol and retinyl esters) and  $\beta$ -carotene were extracted twice from blood plasma (200  $\mu$ L) using n-hexane (1 mL) after deproteinization with ethanol (200  $\mu$ L). The tissue samples were homogenized in 15 mL of a mixture of n-hexane and isopropanol (3:2; vol/vol, 0.05 BHT) three times [17]. The organic phases of the plasma and tissue extracts of the respective samples were combined, dried under nitrogen and redissolved in methanol:tetrahydrofuran (90:10 vol/vol). For separation and quantification a modified gradient reversed-phase HPLC-system (Waters, Eschborn, Germany) on an Inertsil-ODS column (5  $\mu$ m, 250  $\times$  4 mm; Grom, Germany) was used [13]. The solvent system consisted of a step-gradient of acetonitrile:methanol (85:15; vol/vol; 0.01% ammonium acetate). After 7 min 30% isopropanol was added. The flow rate was 1 mL $\cdot$ min<sup>-1</sup>. Total run time was 40 min. For peak detection and characterization a photodiode array detector was used (Model 996, Waters). Retinol, retinyl esters and  $\beta$ -carotene were identified and quantified using a photodiode array detector (Model 996, Waters) at 325 nm (vitamin A) and 455 nm ( $\beta$ -carotene) by comparison of retention time and peak areas with external standards (Serva, Heidelberg,

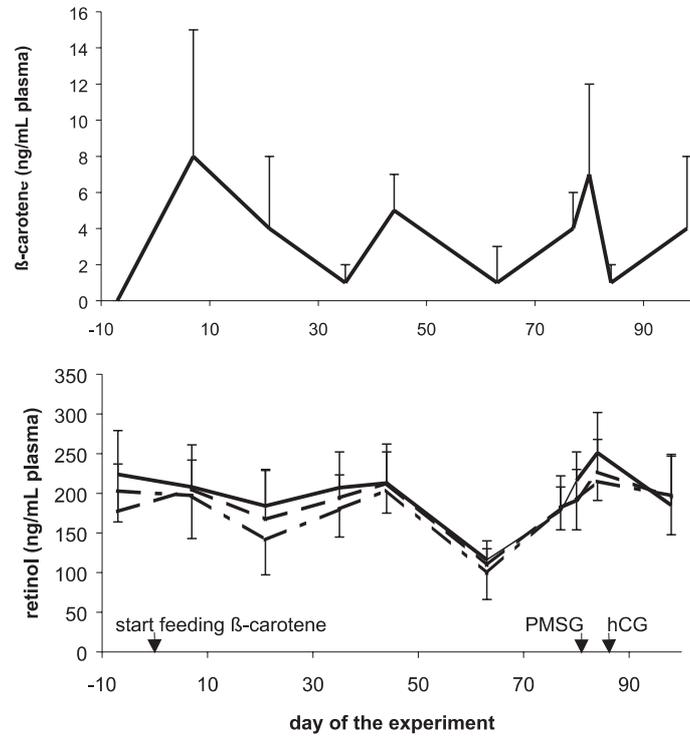
Germany). Since extinction coefficients of retinol and its esters are essentially the same, esters can be quantified with a single calibration curve using retinol as standard [18]. Results for retinyl esters are presented as retinol equivalents. Results were compared to standard reference material 968a (Natl. Inst. Standards Technology, Gaithersburg, USA). All solvents or chemicals used for extraction or HPLC were of high purity commercial grade (Merck, Darmstadt, Germany).

Data are reported as means  $\pm$  standard deviation. Variation in the response variable (retinol, retinyl esters,  $\beta$ -carotene in tissue) were partitioned using an ANOVA procedure of SAS. Where a significant difference was found, a Duncan post-hoc test was used for normally distributed parameters and Kruskal-Wallis for unequally distributed ones to determine the cause of the significant difference. Normal distribution was tested according to Kolmogoroff-Smirnov. Correlations were performed according to Pearson [19]. The probability level at which differences were considered significant was  $P < 0.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1. $\beta$ -Carotene and vitamin A in plasma

Vitamin A was present in blood plasma as retinol and retinyl palmitate. Changes in concentrations of plasma vitamin A (retinol, retinyl palmitate) and  $\beta$ -carotene throughout the experimental period are shown in Figure 1. The plasma retinol concentrations were in agreement with the literature values [1, 3, 27]. Over the whole feeding period there were no differences within or between the differently fed animals concerning retinol. Retinyl palmitate was only present in plasma at low concentrations when the animals were switched to the diet containing either  $\beta$ -carotene or vitamin A. Thus, significantly higher levels of retinyl esters,



**Figure 1.** Changes in the concentration of  $\beta$ -carotene ( $\text{ng}\cdot\text{mL}^{-1}$ ; mean  $\pm$  SD) in the plasma of  $\beta$ -carotene-supplemented gilts (top) and retinol ( $\text{ng}\cdot\text{mL}^{-1}$ ; mean  $\pm$  SD) in plasma (bottom) of differently fed gilts (— VA; - - - - VA + VA; - · - · VA + BC).

representing less than 3% of total vitamin A, were found in the vitamin A (VA + VA)- and  $\beta$ -carotene (BC)-supplemented group compared to the control group ( $P < 0.01$ ). In general, plasma retinyl ester levels in mammals are regarded as low [9], but limited data are available for pigs. The only available study shows a much higher percentage of retinyl esters, which would be interpreted as toxic, but these differences are very probably due to other techniques of analysis such as column chromatography with aluminum oxide vs. HPLC in our study [5]. Retinol levels in the plasma fluctuated significantly over the experimental period ( $P < 0.001$ ). No explanation can be given for the decrease of retinol observed in all groups simultaneously.

$\beta$ -Carotene in plasma was only found in the group fed  $\beta$ -carotene at low levels (1 to 8  $\text{ng}\cdot\text{mL}^{-1}$ ). The low levels of carotene in plasma correspond to the results of other studies [26, 27]. Comparisons are difficult, however, since contradictions in reports may be due to differences in feed and differences in detection limit. Reports have been made of 10-fold greater levels in plasma, but they contain no information on the amount of  $\beta$ -carotene in the feed [5]. The same group could not detect any  $\beta$ -carotene in the plasma of gilts fed up to 65.2 mg  $\beta$ -carotene per day [3]. This amount represents approximately one fifth of the amount fed in this study. In general, despite high supplementation in the diet, plasma levels of  $\beta$ -carotene

are much lower than those observed in the plasma of so called yellow-fat species such as cattle, horses and humans [23, 24]. The reasons for low plasma levels in white-fat species might be due to a very efficient conversion of  $\beta$ -carotene into vitamin A in the gut and/or a limited absorption [22, 32].

### 3.2. $\beta$ -Carotene and vitamin A in tissues

Vitamin A levels in liver, kidney, lung, adrenals, corpora lutea and uterine tissue are presented as retinol and retinyl esters in

Table I. Highest values were always observed in the liver, being 1 000-fold higher than in the other tissues investigated, because the liver is the major storage organ for vitamin A [2, 16]. When animals are supplemented above requirements, liver levels are dependent on daily supplementation and are linearly related to the dietary vitamin A level [11, 20]. This could be confirmed by our results as well. Additional vitamin A in the diet in group VA + VA resulted in higher levels of vitamin A in the liver ( $P < 0.01$ ). The obtained values are in accordance with data obtained recently in pigs [1]. In this study total vitamin A in the

**Table I.** Concentration of retinol, retinyl palmitate, retinyl stearate and retinyl oleate (mean  $\pm$  SD) in liver ( $\mu\text{g}\cdot\text{g}^{-1}$  tissue), lung, kidney, adrenals, corpus luteum and uterus ( $\text{ng}\cdot\text{g}^{-1}$  tissue) of gilts fed vitamin A (VA or VA + VA) or  $\beta$ -carotene (BC).

	Retinol	Retinyl palmitate	Retinyl oleate	Retinyl stearate
<b>Liver</b>				
VA	8.3 $\pm$ 5.7	143 $\pm$ 24 <sup>b</sup>	36 $\pm$ 6.3 <sup>b</sup>	23 $\pm$ 4 <sup>b</sup>
VA + VA	13.5 $\pm$ 12.1	234 $\pm$ 32 <sup>a</sup>	60 $\pm$ 11 <sup>a</sup>	33 $\pm$ 6 <sup>a</sup>
VA + BC	13.0 $\pm$ 12.5	242 $\pm$ 49 <sup>a</sup>	65 $\pm$ 13 <sup>a</sup>	36 $\pm$ 7 <sup>a</sup>
<b>Lung</b>				
VA	62 $\pm$ 14	18 $\pm$ 19	1 $\pm$ 3	50 $\pm$ 16
VA + VA	65 $\pm$ 25	34 $\pm$ 33	6 $\pm$ 10	57 $\pm$ 15
VA + BC	70 $\pm$ 16	34 $\pm$ 38	8 $\pm$ 11	48 $\pm$ 11
<b>Kidney</b>				
VA	223 $\pm$ 163	38 $\pm$ 36	19 $\pm$ 24	301 $\pm$ 251
VA + VA	301 $\pm$ 184	54 $\pm$ 44	29 $\pm$ 26	349 $\pm$ 283
VA + BC	300 $\pm$ 148	50 $\pm$ 53	27 $\pm$ 37	241 $\pm$ 303
<b>Adrenals</b>				
VA	90 $\pm$ 18	43 $\pm$ 41	27 $\pm$ 22	50 $\pm$ 34
VA + VA	102 $\pm$ 15	52 $\pm$ 41	18 $\pm$ 14	72 $\pm$ 16
VA + BC	106 $\pm$ 20	28 $\pm$ 10	18 $\pm$ 9	66 $\pm$ 22
<b>Corpus luteum</b>				
VA	103 $\pm$ 58	87 $\pm$ 52	101 $\pm$ 117	105 $\pm$ 37
VA + VA	135 $\pm$ 24	58 $\pm$ 29	46 $\pm$ 33	98 $\pm$ 56
VA + BC	129 $\pm$ 37	77 $\pm$ 76	35 $\pm$ 36	70 $\pm$ 72
<b>Uterus</b>				
VA	75 $\pm$ 26	51 $\pm$ 34 <sup>a</sup>	6 $\pm$ 9 <sup>a</sup>	n. d.
VA + VA	59 $\pm$ 25	78 $\pm$ 40	13 $\pm$ 9	n. d.
VA + BC	76 $\pm$ 25	91 $\pm$ 46 <sup>b</sup>	17 $\pm$ 11 <sup>b</sup>	n. d.

Means in the same row for each organ or tissue with different superscripts are significantly different ( $P < 0.05$ ). n. d. = below detection limit.

liver calculated as retinol was  $103 \mu\text{g}\cdot\text{g}^{-1}$  when pigs were fed  $2000 \text{ IU}\cdot\text{kg}^{-1}$  feed and  $876 \mu\text{g}\cdot\text{g}^{-1}$  at  $20000 \text{ IU}\cdot\text{kg}^{-1}$ . Irrespective of the total amount of vitamin A in the liver or the form ( $\beta$ -carotene or vitamin A) in which vitamin A is supplemented in the diet, the dominant form of vitamin A in the liver is retinyl palmitate, followed by retinyl oleate and retinyl stearate. Retinyl esters represent 97% of the total vitamin A in the liver. Both the retinyl esters pattern as well as the ratio of retinol and retinyl esters corresponds to the results in the literature [2, 10].

Estimates of vitamin A potency of  $\beta$ -carotene were based on total liver vitamin A stores. For this purpose, the vitamin A reserves of pigs were reduced by previously feeding a ratio low in vitamin A. No obvious differences in the concentration of vitamin A in the liver of the animals supplemented additionally with vitamin A (VA + VA) or with  $\beta$ -carotene (VA + BC) ( $408 \pm 65$  vs.  $427 \pm 86 \mu\text{g}\cdot\text{g}^{-1}$  tissue) compared to the VA group ( $250 \pm 37 \mu\text{g}\cdot\text{g}^{-1}$ ) were found. This observation strongly indicates that this amount of 100 mg  $\beta$ -carotene in the diet can substitute 8300 IU of vitamin A. This would indicate that 1000  $\mu\text{g}$  of  $\beta$ -carotene equals 83 IU or 24.9  $\mu\text{g}$  of vitamin A. Based on these results, a conversion efficiency of  $\beta$ -carotene into vitamin A of 40:1 on a weight basis can be calculated. This is much lower than the one observed (1 mg correspond to 123 to 174 IU) when 10 mg  $\beta$ -carotene $\cdot\text{kg}^{-1}$  of diet was fed [33]. This lower conversion rate can be explained by the observation that the conversion rate of  $\beta$ -carotene varies greatly with the level of intake [32]. Starting at a carotene to vitamin A ratio of 6:1 at levels of intake to provide freedom from night blindness, little to no storage of vitamin A was observed. Significant storage of vitamin A was achieved with approximately  $600 \mu\text{g} \beta$ -carotene $\cdot\text{kg}^{-1}$  body weight with a conversion ratio of 10:1 in pigs [14, 21]. The highly variable conversion rate of  $\beta$ -carotene into vitamin A not only depends on the amount of

$\beta$ -carotene in the diet but also on possible interaction with other nutrients and on the source of  $\beta$ -carotene, and not so much on the species [33].

Vitamin A levels in other tissues investigated (Tab. I) such as the kidney, lung, adrenals, corpora lutea and the uterine tissue were one thousand times lower compared to those observed in the liver, which is in accordance with observations in other species [31]. Except for uterine tissue, no differences in total vitamin A could be observed among the different feeding regimes. In the uterine tissue (endometrium and myometrium) levels of total vitamin A were highest in sows supplemented with  $\beta$ -carotene ( $P < 0.01$ ). These higher levels were due to greater levels of retinyl esters. In all tissues, the percentage of retinol was higher than in the liver, representing approximately 50% of total vitamin A. Concerning the pattern of retinyl ester obvious differences were observed between the different tissues. While in lung, kidney, adrenals, and the corpus luteum the dominant retinyl ester was retinyl stearate, this ester was not detectable in the uterus.

The vitamin A concentration in uterine tissue corresponds to values for other gilts during early gestation [25]. In this study it has been shown that vitamin A content and distribution in uterine tissue was dependent on the stage of gestation. Within uterine tissue the highest level, primarily as retinol, was observed in the endometrium, while in the myometrium retinyl ester was the predominant form of vitamin A.

Despite differences in the vitamin A content of the uterine tissue, no differences in the concentration of vitamin A in the uterine fluid were observed. Vitamin A was exclusively present as retinol at a concentration of  $380 \pm 261$ ,  $358 \pm 205$  and  $374 \pm 244 \text{ ng}\cdot\text{mL}^{-1}$  for the VA, VA + VA and VA + BC group, respectively.

With regard to the distribution of  $\beta$ -carotene among the different tissues investigated, generally low levels were observed

**Table II.** Concentration of  $\beta$ -carotene (mean  $\pm$  SD) in liver, lung, adrenals and corpus luteum ( $\text{ng}\cdot\text{g}^{-1}$  tissue) of gilts fed vitamin A (VA or VA + VA) or  $\beta$ -carotene (BC).

	Liver	Lung	Adrenals	Corpus luteum
VA	n.d.	n.d.	9 $\pm$ 13 <sup>a</sup>	34 $\pm$ 50 <sup>a,e</sup>
VA + VA	n.d.	n.d.	11 $\pm$ 29 <sup>a</sup>	25 $\pm$ 35 <sup>a,c</sup>
VA + BC	487 $\pm$ 282	12 $\pm$ 8	217 $\pm$ 111 <sup>b</sup>	172 $\pm$ 126 <sup>d,f</sup>

Means in the same row with different superscripts are significantly different <sup>a,b</sup> at  $P < 0.001$ ; <sup>c,d</sup> at  $P < 0.01$ ; <sup>e,f</sup> at  $P < 0.05$ .

n. d. = below detection limit.

(Tab. II). In the groups which were not supplemented with  $\beta$ -carotene, low levels of  $\beta$ -carotene could only be observed in the adrenals and the corpora lutea whereas nothing was observed in the liver or the lung. In gilts supplemented with  $\beta$ -carotene,  $\beta$ -carotene could be detected at greater levels not only in adrenals or corpora lutea but also in the liver and lung. Traces of  $\beta$ -carotene could only be detected in the uterine tissue of one gilt supplemented with  $\beta$ -carotene. While an accumulation of  $\beta$ -carotene in those animals receiving  $\beta$ -carotene supplementation was expected, the observation that low concentrations of  $\beta$ -carotene was present in the adrenals and in the corpora lutea is difficult to explain. Both tissues are endocrine glands which strongly depend on exogenous cholesterol for the synthesis of steroids. This uptake is mediated via the low density lipoprotein (LDL)-receptor. Since LDL is not only involved in the plasma transport of cholesterol, phospholipids and triglycerides but also in that of  $\beta$ -carotene and vitamin E, an accumulation of these components in steroid producing glands has been observed [4, 7, 12]. The highly selective accumulation is obvious since  $\beta$ -carotene was also found in those animals receiving less than 100  $\mu\text{g}$   $\beta$ -carotene per kg of diet. Similar levels were observed in corpora lutea obtained from the slaughter house [5]. Based on the observation that not only the gut or the liver but also other peripheral tissues such as the corpora lutea [29, 30] and the granulosa

cells of the bovine follicle [28] have the capacity to convert  $\beta$ -carotene into vitamin A, it might be speculated that these local accumulations could be of importance in the contribution to the local vitamin A supply of reproductive structures. This indicates that only little  $\beta$ -carotene might be necessary to effect steroid hormone metabolism in sows.

#### 4. CONCLUSION

In conclusion the performed study shows that, under the indicated conditions,  $\beta$ -carotene can be absorbed in pigs at low levels and does accumulate in their tissues, where it might influence the local vitamin A metabolism. This might be of importance in tissues associated with reproduction and might explain some of the effects of  $\beta$ -carotene observed in porcine reproduction. With 100 mg of  $\beta$ -carotene a similar provitamin A effect was observed as in the group which was treated with additional vitamin A, indicating a conversion rate of  $\beta$ -carotene to vitamin A of 40 to 1 on a weight basis.

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