INVESTIGATION OF THE CHANGES IN VITAMIN METABOLISM WITHOUT CAECOTROPHY IN RABBITS*

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Key words: retinoid, carotenoid, metabolism, caecotrophy, rabbit

Introduction

Retinoids (vitamin A) and provitamin A carotenoids are important elements in researches around the world (cytoprotective, immunstimulative, antitumor effects, reproduction, development, proliferation, cell differentiation) (2). In the last decade the effects of high or low dietary vitamin A levels on growth and reproductive performances, the parameters of vitamin A in the blood, liver, placenta and embryonic tissues were investigated in rabbits (4, 7). Despite the wide-range of examinations the requirement of nutritive and biological active substances (for example retinoids/carotenoids) has not been cleared in rabbits. The most important reason for this is the special physiological nutritional phenomenon of the rabbit, the caecotrophy, which is nowadays an importantly investigated area. There is a lack in our knowledge, whether the caecotrophy takes part in the metabolism of vitamins.

The aim of this study was therefore to investigate the retinoid and provitamin A carotenoid metabolism of rabbit prevented from caecotrophy.

Material and methods

Adult New Zealand White rabbits (n = 15) were kept in individual metabolic cages and fed *ad libitum* with commercial rabbit diet (10000 IU/kg vitamin A; 13.1 µg/g total carotenoid). In two experimental periods ("pre-feeding" and 2 x 4 day "collection") the true (hard) and soft faecal matter of rabbits were collected. In the first experiment the animals were free in their cages, while in the second period the caecotrophy was hindered with plastic collars (7.5 cm in diameter). This arrangement was repeated with 7 animals in each group (period 4) after regeneration period without collars (period 3).

At the end of the experiment the retinoid (retinol (ROL) and retinyl palmitate (RP)) and carotenoid (canthaxanthin, ß-carotene, ß-cryptoxanthin, lutein and zeaxanthin) content of

blood, liver, kidney, caecal content and the collected faeces (hard and soft) were measured by high performance liquid chromatographic methods (1, 3, 5, 8, 10, 13).

Results

The vitamin A analysis of the blood represented the dominance of the ROL, the basic compound in the plasma (Table 1). The prevention of the caecotrophy resulted in significant decrease in the retinoid content of the blood. The application of the collars caused significant decrease in the ROL ($P < 0.001$) and RP concentrations ($P < 0.01$), as well. The use of collars did not result in any significant changes in the retinoid concentration of the storage organs (liver and kidneys).

Table 1. Retinoid concentrations and contents of the investigated organs

			1st period *(intact)*	2nd period *(collared)*	3rd period *(intact)*	4th period *intact*	4th period *collared*
Blood (µg/L)	RP	*mean*	543.93	407.31[b]	460.02	461.94	359.33
		±*SEM*	123.86	118.98	172.94	94.75	102.68
	ROL	*mean*	2897.85	1884.36[a]	2415.72[c]	2281.81	1778.51[c]
		±*SEM*	694.15	556.52	513.04	496.35	558.06
Liver (µg/g)	RP	*mean*				88.22	113.13
		±*SEM*				29.15	39.66
	ROL	*mean*				33.14	30.22
		±*SEM*				9.66	10.97
Kidney (µg/g)	RP	*mean*				26.64	27.41
		±*SEM*				5.83	11.37
	ROL	*mean*				7.75	9.86
		±*SEM*				3.67	2.79

Significance between different periods: [a]=P<0.001; [b]=P<0.01; [c]=P<0.05

The caecal content (25.78±6.87 µg/g) and the soft faeces (34.52±10.48 µg/g) contained the retinoids in similar amounts (Table 2). In the retinoid concentrations of the caecal content no significant difference was found between the intact and collared animals. The hard faeces have a significantly different retinoid content compared with the caecum and the soft faeces.

Table 2. Retinoid concentrations of faeces (Values represent means±SEM)

		Slaughter	
		intact	*collared*
Caecal content	RP	28.88±8.61	25.78±6.87
(µg/g wet weight)	ROL	13.14±5.16	19.76±5.14
Soft faeces	RP		34.52±10.48
(µg/g wet weight)	ROL		0.51±0.21
Hard faeces	Retinoid	1.90±0.77	10.25±3.12
(µg/g wet weight)			

Because of the speciality in the carotenoid metabolism of rabbits their tissues (blood, liver and kidney) have no considerable content of these pigments, which is also undetectable

with the routinely applied methods (11). Although in the HPLC analysis various carotenoids were found in significant amounts in the different types of faeces (Table 3). The representation of carotenoids in the samples was identical and proportional with the carotenoids referred in the feed. The total carotenoid concentration was similar in the caecal content and in the caecotroph on the basis of the same dry matter concentration.

Table 3. The carotenoid concentrations of the investigated samples (wet weight)

µg/g	Feed	Blood, liver, kidney	Caecal content	Soft faeces	Hard faeces
Lutein	6.05	-	3.65	5.53	13.38
Zeaxanthin	1.03	-	0.88	1.22	3.50
Keto-lutein	-	-	0.60	0.67	1.96
9/9'-cis-lutein	0.76	-	0.50	0.73	1.95
13/13'-cis-lutein	0.58	-	0.33	0.49	1.49
Canthaxanthin	0.14	-	0.19	0.26	0.94
ß-cryptoxanthin	0.22	-	0.15	0.17	0.59
ß-carotene	2.14	-	1.54	2.80	9.32
Total carotenoid concentration	13.10 µg/g	Ø	11.23 µg/g	13.85 µg/g	37.41 µg/g

Discussion

Existence of the normal physiological procedure of a special coprophagy by the rabbit was first reported by Morot (1882) and later confirmed and reviewed detailed by others (9). The duality in excretion of two types of faeces in the proximal colon: the caecotroph i.e. the soft caecal originated mucous faeces produced by the initial digestion of the food and the firm oval shape pellet is a special physiological mechanism of *lagomorph* species (12, 14). In the caecal content there are certain vitamins intact and in pre-digested form after the first passage, which suggests the possible re-utilization of the vitamin A compounds by the caecotrophy. In literature only few experimental results have been found concerning this problem. But the authors did not investigate the effects of the caecotrophy on the utilisation of vitamins. It is not cleared what extent would affect such a phenomenon the pharmacokinetic profile of the administered retinoid and its metabolites (16).

In our experiment the prevention of the caecotrophy resulted decrease in blood ROL and RP concentrations, but did not cause significant changes in liver and kidney. In the case of prevention the caecotrophy the replacement of the metabolic requirement of vitamin A was covered predominantly from the blood. The comparison of the composition of the soft faeces, caecal content and hard faeces suggested that the soft faeces are of caecal origin. This similarity is available for their RP concentration, but in the ROL content there is a significant variance. The observed differences were caused probably by digestive and absorption processes.

The specific rate of absorption of carotenoids varies among species. There is a marked degree of selectivity in the rate of carotenoid absorption, the plasma level of carotene in some species undetectable low, the tissues have no considerable content of pigments (6, 9, 11, 15).

Therefore in our investigation detailed carotenoid analyses were made exclusively from the caecal content, from the soft and hard faeces, to examine whether in these biological materials of rabbit origin the carotenoids are represent. It should be noted, that the determined main carotenoids, occurred in the highest degree, both the ß-carotene and the ß-cryptoxanthin are provitamin A carotenoids, therefore they would play an important role in the retinoid metabolism, as well. The representations of the identified carotenoids in the samples were identical and proportional with the carotenoids referred in the feed.

According to our presented results, the caecotrophy in the rabbit has an important role in the metabolism of compounds having vitamin A activity. The soft faeces (caecotroph) contain remarkable amounts of retinoids and various carotenoids, which could then contribute as micronutrients succeeding caecotrophy. It could be concluded that the retinoid content of rabbit feed could be lowered in case of adequate caecum function and caecotrophy.

Acknowledgements

This study was supported by Hungarian Scientific Research Fund, OTKA (Project No: F030043).

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