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Vitamin A content (retinol and retinyl esters) in livers of different animals

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Abstract

In the present study, 90 animal livers of five different species (pig, cattle, calf, chicken, turkey) were examined for their vitamin A contents. The investigation of extracted vitamin A included all-*trans* retinol, retinyl palmitate, stearate, oleate and linoleate, expressed as retinol equivalents (RE). The separation of the various chemical forms was done using HPLC. The liver vitamin A contents ranged between 6.5 and 18.9 mg RE/100 g in pigs, from 1.1 to 6.7 mg RE/100 g in cattle and from 1.6 to 16.6 mg RE/100 g and 2.7 to 21.5 mg RE/100 g in chickens and turkeys, respectively. The livers of calves contained the smallest amount of vitamin A, with variation from 1.3 to 3.2 mg RE/100 g. Retinyl palmitate was the predominant form of vitamin A in the livers of investigated animals and contributed about 40% (avids) up to 75% (calf) of the total liver vitamin A contents. The results indicated that the lower levels of animal liver vitamin A, observed in our study, could be a result of small-structured agriculture in Austria. The variations of liver vitamin A concentrations among the species were a result of differences in race, age and the different feeding regimen.

Keywords: Retinol; Retinyl esters; Liver; Animals

1. Introduction

Vitamin A is a micronutrient, essential for biological processes such as vision, reproduction, cell growth and differentiation and embryonic development in most mammalian species. The term vitamin A describes a group of lipid-soluble compounds (retinyl esters) related metabolically to all-*trans* retinol (Arnhold, Nau, Meyer, & Rothkoetter, 2002; Got, Gousson, & Delacoux, 1995; Scientific Committee on Food, 2002).

The main sources of vitamin A in the diet are animal origin foods, such as dairy products and liver, fortified foods (e.g., cereals, juices), vitamin supplements and plant-synthesized vitamin A-active carotenoids that are partially converted to retinol during or after absorption. In products of animal origin, vitamin A is found as retinyl esters, mainly retinyl palmitate. Other esters (oleate, stearate, myristate) and retinol also contribute to the dietary vitamin A intake (Anderson, 2002; Bendich & Langseth, 1989; Rothman et al., 1995; van Vliet, Boelsma, de Vries, & van den Berg, 2001).

A high consumption of vitamin A, leading to hypervitaminosis, has been considered to cause a number of adverse effects (Anderson, 2002; Scientific Committee on Food, 2002) and appears to be teratogenic (Arnhold & Nau, 1998; Rothman et al., 1995). Excessive intake of vitamin A is usually a result of the elderly consuming high levels of supplements, but is also associated with the consumption of liver and/or liver products in combination with the daily use of a vitamin A-containing supplement (van den Berg, Hulshof, & Deslypere, 1996). For a more complete risk evaluation, bioavailabilities of vitamin A from various sources were considered. Buss, Tembe, Prendergast, Renwick, and George

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(1994) provided evidence of a lower availability of vitamin A from liver compared with vitamin A-containing supplements after single dosages. Data presented by van Vliet et al. (2001) showed that the bioavailabilities of vitamin A from single doses of liver products (liver paste) and from a vitamin A supplement do not differ. The tolerable upper limit of safety (UL) of vitamin A set by the Scientific Committee on Food (2002) and the Committee on Dietary Reference Intakes of Institute of Medicine (2002) is 3000 μg RE/day, irrespective of the source of the vitamin. Recently, concern has been raised regarding very high levels of vitamin A in livers of different animals (calf, cattle, pork, chicken). The concentrations of vitamin A in livers available in retail markets in the United States appear to be below European levels and are found to be in the non-toxic range (ACOG, 1998). Measurements up to 147 mg vitamin A/100 g in chicken liver or 112 mg/100 g in pig liver were recorded in Germany (BgVV, 1995).

The large variation of the vitamin A content in the liver documented in the literature was the reason for investigating vitamin A levels in the liver of different Austrian animals.

2. Materials and methods

2.1. Materials

In this study, 90 animal livers of five different species (pig, cattle, calf, chicken, turkey) were examined ($n = 18$ of each species; three samples of six different farms). The livers of the slaughtered animals were directly collected at the farms (between November and December 2002), rapidly cooled, transported to the laboratory and frozen at −80 °C. Immediately after thawing, the samples were weighed, homogenized and prepared for the analysis. The information about age, race and feeding was obtained from the farmers (Table 1). The vitamin A content in the mix feed was not stated.

The investigation of vitamin A included all-*trans* retinol and four retinyl esters: all-*trans* retinyl palmitate, oleate, stearate and linoleate.

2.2. Chemicals

Standards of all-*trans* retinol and all-*trans* retinyl palmitate were obtained from Sigma–Aldrich (Vienna, Austria). Retinyl oleate, stearate and linoleate standards were commercially not available and therefore required synthesis.

2.3. Synthesis of retinyl esters

Retinyl esters were synthesized according to the description of Huang and Goodman (1965). In the first step, 0.5 ml of fatty acid chloride, suspended in 2.5 ml of ether, was added dropwise to a mixture of 0.2 g all-*trans* retinol, 5 ml diethyether (dry) and 0.1 ml pyridine (dry). The mixture was stirred on ice for 1 h. The entire procedure was carried out with exclusion of daylight due to the component's light sensitivity. After adding water

Table 1
Animal characteristics

Animals/farm	Race	Age	Type of feed (content of vitamin A in IU/kg)
Chicken			
A, C	TA-58	6 weeks	Triticale, com, grain (13,500 IU/kg)
B	TA-58	6 weeks	Triticale, com, grain (31,000 IU/kg)
D, E	TA-58	12 weeks	Triticale, com, protein concentrate (60,000 IU/kg)
F	Hilbro	12 weeks	race specific Gsellmann-feed (G-7)(12,500 ID/kg)
Turkey			
A, B	T9	21 weeks	Soya, wheat, corn (13,500 IU/kg)
C	BIG 6	21 weeks	Soya, wheat, corn (17,500 ID/kg)
D, E	Bronze	24 weeks	Corn, wheat, forage plants (without fortification)
F	BIG 6	16 weeks	Soya, wheat, corn (22,000 IU/kg)
Pig			
A, C	Landrasse	6 months	Triticale, forage peas, protein concentrate (60,000 IU/kg)
E	Landrasse	6 months	Triticale, barley, corn, pumpkin, protein concentrate (35,000 IU/kg)
B, F	Landrasse	6 months	Triticale, barley, oats, protein concentrate (40,000 IU/kg)
D	Landrasse	6 months	Triticale, barley, forage peas, clover, protein concentrate (60,000 IU/kg)
Calf			
A, B, C	–	2.5–5 months	Commercially available cow's milk substitution (Gav 40) (40,000 IU/kg)
D, E, F		2.5–5 months	Cow's milk (40,000 IU/kg)
Cattle			
A, B, C	Fleckvieh	20 months	Corn, barley, soya, hay, supplementary feed (750,000 IU/kg)
D	Fleckvieh	15 months	Pasture feeding
E	Fleckvieh	15 months	Com, barley, soya, hay, supplementary feed (750,000 IU/kg)
F	Fleckvieh	15 months	Corn, barley, oats, rye, hay (without fortification)

(and shaking), the ether phase (upper phase) was collected, washed twice, filtered through dehydrated sodium sulfate and finally evaporated in a rotary evaporator. The residue was reconstituted in ether, applied on a column filled with silica gel and eluted with hexane/acetic acid ethyl ester (10:1 v/v). The solvent was evaporated again, as before, and the resultant synthesized retinyl ester was redissolved in eluent, stored at −20 °C and later used as a standard.

The confirmation of the chemical structure of synthesized retinyl esters was done by gas chromatography (GC Perkin–Elmer).

2.4. Sample preparation

After being homogenized and weighed, lipid phases from the livers were obtained according to the method described by Folch, Less, and Stoane-Stanley (1957). One gram of each sample was extracted with chloroform/methanol (2:1 v/v). A solution of 0.05 M calcium chloride was added to separate the lipid and the water phase. After filtration through dehydrated sodium sulfate, a 3 ml sample of the lower phase (lipid phase) was evaporated under a nitrogen stream. The residues were reconstituted in 450 μl eluent and injected (50 μl) to the HPLC.

2.5. High-performance liquid chromatography

Retinol and retinyl esters were determined according to the slightly modified isocratic HPLC method of Jakob and Elmadfa (1995).

The separation of all-*trans* retinol and retinyl esters in the liver samples was done using an analytical column (AQUASIL C18/5 μm, 150 × 4 mm) with acetonitrile/methanol (85:15 v/v) as mobile phase (Fig. 1). Peak responses were measured at 320 nm, for all retinoids, using a diode array detector (Merck HITACHI Diode Array Detector L-7455). The concentrations were calculated by peak areas, using a linear regression curve from standard solutions.

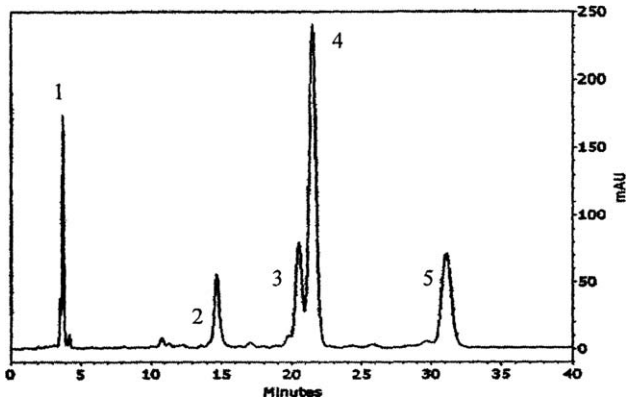

Fig. 1. HPLC separation of all-*trans* retinol (1) and retinyl esters: all-*trans* retinyl linoleate (2), all-*trans* retinyl oleate (3), all-*trans* retinyl palmitate (4), all-*trans* retinyl stearate (5) in the liver sample (for condition see Section 2).

The inter-assay variation was about 3.5% for all-*trans* retinol, all-*trans* retinyl palmitate, stearate and linoleate and 6.4% for all-*trans* retinyl oleate. The intra-assay variations ranged between 4% and 6% for all parameters. Detection limits were found at concentrations of 7.1×10^{-4} mg/100 g for all-*trans* retinol, 6.7×10^{-4} mg/100 g for retinyl palmitate, 8.1×10^{-4} mg/100 g, 3.1×10^{-4} mg/100 g and 7.3×10^{-4}mg/100 g for retinyl oleate, stearate and linoleate, respectively.

2.6. Calculations and statistics

Results of vitamin A in the livers were expressed as milligrames retinol equivalents (RE) per 100 g liver (means ± SD). The RE was calculated as follows: mg RE = mg all-*trans* retinol + mg all-*trans* retinyl palmitate/1.83 + mg all-*trans* retinyl oleate/1.92 + mg all-*trans* retinyl stearate/1.93 + mg all-*trans* retinyl linoleate/1.92. The calculation of the conversion factors (1.83; 1.92; 1.93) was done by dividing the molecular weight of retinyl ester by molecular weight of retinol.

The statistical evaluation, to determine differences between vitamin A contents of animal livers from various farms, was done by employing the *t*-test. Differences were regarded as statistically significant at $p < 0.05$.

3. Results

3.1. General

Vitamin A levels in the investigated livers showed a large variation in both, individuals of the same species and between the different species (Table 2). Race, age and feeding of an animal were found to have a very strong influence on liver vitamin A contents.

3.2. Vitamin A contents of pig liver

The vitamin A contents observed in the pig livers varied from 6.51 up to 18.9 mg RE/100 g (mean: 11.5 ± 3.81 mg RE/100 g). The investigated livers were all from pigs of the same race (Landrasse), at the average age of 6 months and similar weight (115 ± 16 kg). Therefore, the results could reflect a relationship between the amount of vitamin A offered in the diet and vitamin A in the liver. The mean vitamin A content of 7.45 ± 0.72 mg RE/100 g (farm B, F) was observed in livers of pigs fed with corn and clover or with a low vitamin A-fortified potato protein (40,000 IU/kg) in addition to their basal diet. Employing a lesser vitamin A-fortified potato protein (35,000 IU/kg) (farm E) in addition to a β-carotene rich diet (corn, pumpkins) to

Table 2
Vitamin A contents in the livers of animals from different farms mg RE/100 g[a]

Farm[b]	Pig	Cattle	Calf	Chicken	Turkey
A	12.1 ± 0.49	4.99 ± 1.47	1.48 ± 0.25	2.19 ± 0.70	9.04 ± 0.4
B	7.75 ± 0.47	5.19 ± 1.02	2.38 ± 0.78	4.11 ± 1.92	10.9 ± 0.68
C	11.0 ± 0.21	5.59 ± 1.26	2.36 ± 0.22	3.44 ± 0.34	19.5 ± 1.72
D	18.2 ± 1.10	3.02 ± 1.31	1.58 ± 0.29	5.03 ± 0.30	3.22 ± 0.63
E	12.7 ± 0.71	4.32 ± 0.38	1.76 ± 0.39	5.78 ± 1.38	3.84 ± 0.24
F	7.14 ± 0.90	1.50 ± 0.39	2.47 ± 0.75	13.0 ± 3.43	18.2 ± 1.49
Mean ± SD ($n = 18$)	11.5 ± 3.81	4.10 ± 1.72	2.01 ± 0.55	5.60 ± 3.89	10.8 ± 6.35
Min.–max.	6.51–18.9	1.08–6.70	1.28–3.22	1.62–16.6	2.72–21.5

[a] mg RE/100 g = mg all-*trans* retinol + mg all-*trans* retinyl palmitate/1.83 + mg all-*trans* retinyl oleate/1.92 + mg all-*trans* retinyl stearate/1.93 + mg all-*trans* retinyl linoleate/1.92.
[b] Each value of farms A, B, C, D, E, F is a mean of three different samples.

the basal feeding, or a higher vitamin A-fortified protein concentrate (60,000 IU/kg) (farm A, C) resulted in a 1.6-fold higher mean vitamin A concentration in the liver (12.0 ± 0.87 mg RE/100 g). An average liver vitamin A value of 18.2 ± 1.10 mg RE/100 g was measured in livers of pigs fed both, a highly vitamin A-fortified protein concentrate (60,000 IU/kg) and β-carotene rich plants (farm D) (Tables 1 and 2).

3.3. Vitamin A contents of cattle liver

In cattle livers, a mean vitamin A value of 4.10 ± 1.72 mg RE/100 g was observed. Single concentrations ranged from 1.08 to 6.70 mg RE/100 g. The variations of the analyzed vitamin A contents occurred in animal livers from different farms and among the same farms. The biggest fluctuation of vitamin A concentrations, due to mix problems, was observed in livers of animals fed a diet enriched with a vitamin supplement (750,000 IU vitamin A/kg feed; farms A, B, C: mean 5.25 ± 1.13 mg RE/100 g) or kept extensively (pasture style, farm D: 3.02 ± 1.31 mg RE/100 g). The smallest standard deviation between the vitamin A contents was found in livers of animals having obtained a commercial fortified feed diet (farm E: 4.32 ± 0.38 mg RE/100 g). Since the animals were of the same race (Fleckvieh), the differences in liver vitamin A values of the cattle from the farms A, B, C (mean: 5.25 ± 1.13 mg RE/100 g; at the age of 20 months) and of the cattle from the farms D, E, F (mean: 2.95 ± 1.41 mg RE/100 g; at the age of 15 months) reflected not only the influence of the feeding practice, but also the different age of the animals (Tables 1 and 2).

3.4. Vitamin A contents of calf liver

With a mean value of 2.01 ± 0.55 mg RE/100 g, calf livers contained the lowest vitamin A amounts of all examined animals. Though the investigated calf liver samples were derived from animals of different races and ages (2.5–5 months), the analyzed concentrations of liver vitamin A showed only a small variation, ranging from 1.26 to 3.22 mg RE/100 g. One explanation for this could be that, the animals were very young and did not therefore indicate any race-specific characteristics. Additionally, the vitamin A content in feed milk and milk substitution showed the same value (40,000 IU/kg). Therefore, vitamin A contents detected in livers of calves fed native cow milk (farms D, E, F; mean: 1.94 ± 0.6 mg RE/100 g) and those observed in livers of animals fed formula milk (farms A, B, C; mean: 2.07 ± 0.52 mg RE/100 g) indicated small, statistically non-significant differences (Tables 1 and 2).

3.5. Vitamin A contents of chicken liver

Vitamin A levels in chicken livers were 5.60 ± 3.89 mg RE/100 g on average. Observed variations of vitamin A stored in chicken livers (1.62–16.6 mg RE/100 g), could be result of differences in age, the amount of vitamin A contained in feed (farms A, B, C, D, E) or the race of the animals (farm F). The chickens of farms A, B, C, D, E were of the same race (''TA 58'') and the detected liver vitamin A concentration found to be about twice as high in animals at the age of 12 weeks, being given feed with 60,000 IU vitamin A/kg (farms D, E: mean: 5.40 ± 0.98 mg RE/100 g), than in those at the age of 6 weeks fed grain fortified with 13,500 IU vitamin A/kg (farms A, C: mean 2.82 ± 0.88 mg RE/100 g). The mean liver vitamin A content of 6 week-old chickens from the farm B, which employed feed enriched with 31,000 IU vitamin A/kg, indicated that the amount of vitamin contained in the feed had a greater influence on the value of the vitamin A stored in the liver than the age of the animals. Another situation was observed in 12 week-old chickens of different races. The broilers of the race ''Hillbro'' (farm F), receiving feed containing 12,500 IU vitamin A/kg, showed the mean vitamin A value of 13.0 ± 3.43 mg RE/100 g liver, while animals of the race ''TA 58'' (farms D, E), though being given higher fortified feed rations (60,000 IU/kg), had an average liver vitamin A content of 5.40 ± 0.98 mg RE/100 g (Tables 1 and 2).

Table 3
Composition (%) of retinol and retinyl esters in the livers of different animals

	Pig ($n = 18$)	Cattle ($n = 18$)	Calf ($n = 18$)	Chicken ($n = 18$)	Turkey ($n = 18$)
Retinol	20.0	3.0	6.0	26.0	22.0
Retinyl palmitate	50.5	54.0	75.0	40.0	36.0
Retinyl oleate	12.0	8.0	4.0	9.0	12.0
Retinyl stearate	7.5	32.0	13.0	18.0	18.0
Retinyl linoleate	10.0	3.0	2.0	7.0	12.0

3.6. Vitamin A contents in turkey liver

In turkey livers, vitamin A contents ranged from 2.72 to 21.5 mg RE/100 g. On the average, the vitamin A concentration was 10.8 ± 6.35 mg RE/100 g. The large variations of the liver vitamin A value of turkeys depended particularly on the different amounts of vitamin A in the supplied feed. The influence of the age of the animals appeared to be of less importance. Turkeys of the race "Big 6" showed similar values of liver vitamin A (19.5 ± 1.72 mg RE/100 g vs. 18.2 ± 1.49 mg RE/100 g) at the age of 21 weeks (farm C) and at the age of 16 weeks (farm F), caused by similar amounts of vitamin A contained in their feed. Although animals of the race "Bronze" (farms D, E) were the oldest (24 weeks), they showed the smallest mean liver vitamin A value of 3.53 ± 0.54 mg RE/100 g, resulting from their diet without vitamin A fortification (Tables 1 and 2).

3.7. Retinyl esters as components of total liver vitamin A content

In the examined livers, vitamin A was present as retinol, retinyl palmitate, oleate, stearate and linoleate. Retinyl palmitate was the predominant form of vitamin A in the livers of all investigated animals. Expressed as a percentage, this ester contributed about 40% (avids: chicken, turkey), 50–55% (pig, cattle) up to 75% (calf) to the total liver vitamin A contents. In the livers of avids (chicken, turkey) and herbivores (cattle), retinyl stearate dominated (18% and 32% of total vitamin A, respectively) over retinyl oleate and retinyl linoleate, while pig livers contained more retinyl oleate (12%) and retinyl linoleate (10%) than retinyl stearate (7.5%). The contribution of retinol to the total vitamin A in the liver ranged from about 3–6% (cattle, calf) up to 20–26% (pig, turkey, chicken) (Table 3).

4. Discussion

The variations of vitamin A concentrations found in different European countries were expected to occur in livers from Austrian animals. However, mean liver vitamin A contents analyzed in our study appeared to be below the levels observed by other authors (BgVV, 1995; Howells & Livesey, 1998; Kessler, Arrigo, Guidon, Frigg, & Rettenmair, 1992; Kusev et al., 2002; Leth, 1994). In the small- and medium-sized farms (dominating in the agricultural production in Austria), animal breeding is being practised less extensively, without higher vitamin A supplementation, than in other European countries, such as Germany or the UK. Many of the farms tend to be quite self-sufficient and only a few of them buy modest amounts of commercial feeding supplies for the animals. Moreover, the slaughter of the animals was done on the farm or in the closed slaughterhouse. Therefore the application of the vitamin A preparation to minimise the stress before slaughter could be excluded.

It was observed that the average amount of vitamin A measured in Austrian pig livers was similar to the mean value reported by Leth (1994) (15 mg RE/100 g), but just a third of that found in German pig livers (36.3 mg RE/100 g) (BgVV, 1995). The maximum liver vitamin A concentration of the Austrian pigs was similar to the mean level given by Howells and Livesey (1998) (17.4 mg RE/100 g) but about 3–6-times lower than reported in other countries (Denmark: 52 mg Re/100 g; Germany: 112 mg RE/100 g). The Austrian cattle showed a mean liver vitamin A content about 4.5–5.5-times lower than those of German (17.9 mg RE/100 g) and Danish cattle (22.3 mg RE/100 g), while the minimum level was similar to the German minimum value (1.6 mg RE/100 g) (BgVV, 1995; Leth, 1994).

The mean amount of vitamin A observed in Austrian calf livers was one-fifth (1/5) of the Danish (10.3 mg RE/100 g), one-ninth (1/9) of the British (18.8 mg RE/100 g) and one-fifteenth (1/15) of the German (28.8 mg RE/100 g) average liver vitamin A contents. The maximum values of vitamin A determined in Danish (27 mg RE/100 g), Swiss (64.4 mg RE/100 g) and German (79 mg RE/100 g) calf livers were 8–25-times higher than the greatest amount of calf liver vitamin A measured in our investigation (BgVV, 1995; Howells & Livesey, 1998; Kessler et al., 1992; Leth, 1994).

The average content of vitamin A found in chicken livers was about 2, 4 and 6-times lower than the mean values reported for British (9.7 mg RE/100 g), for Slovak Republic (17–20 mg RE/100 g) and German chickens (33.5 mg RE/100 g), respectively. The highest

vitamin A value was 8-times lower than the maximum concentration for German chickens (147 mg RE/100 g) but was at a level similar to the maximum content of the liver from Slovak Republic livers (28 mg RE/100 g) (BgVV, 1995; Howells & Livesey, 1998; Kusev et al., 2002).

In turkey livers, the mean content of vitamin A analyzed in our study was in the range of the lowest concentration found in German animals (11.1 mg RE/100 g) and the maximum value was 3-times lower than those found in turkeys from Germany (69.1 mg RE/100 g) (BgVV, 1995).

The observed variations between the species with regard to the relative individual retinyl ester contribution to total vitamin A might be species-specific or could possibly be attributed to differences in feeding. The composition of retinyl esters in the pig liver reflected the fatty acid profile in the diet, which is typical for omnivores. Based on bacterial modification of dietary fat in the rumen (the free released unsaturated fatty acids are hydrogenated to saturated fatty acids), which is typical for ruminants (Engelhardt, 2000), retinyl palmitate and stearate were predominant (54% and 32% of total vitamin A) in the livers of cattle. The high concentrations of free retinol found in the livers of chickens, turkeys and pigs were concluded to be a result of stress related to imminent slaughter, known to trigger the mobilisation of stored vitamin A (Kolb, Gürtler, Ketz, Schröder, & Seidl, 1989).

5. Conclusion

The results indicated that the comparatively lower levels of animal liver vitamin A, observed in our study, could be a result of the small structured agriculture in Austria. Although the detected amounts of liver vitamin A were generally below values found in other European countries, single concentrations, analyzed in our samples, were very high at the level of 20–22 mg RE/100 g.

The variations of liver vitamin A concentrations among the species were a result of differences in race and age, but especially of different contents of vitamin A in the supplied feed.

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