

Effect of a minor vitamin A deficiency on the course of infection with *Ascaridia galli* (Schränk, 1788) and the resistance of chickens

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Summary

The effect of vitamin A deficiency was studied in chickens infected with 500 *Ascaridia galli* eggs and controls. Diet 1 (deficient, 500 IU vitamin A or 172 µg retinol acetate per kg diet), Diet 2 (deficient, 1000 IU vitamin A or 344 µg retinol acetate per kg diet) and Diet 3 (sufficient, 1500 IU vitamin A or 516 µg retinol acetate per kg diet) were assigned to 46 chickens each. Clinical signs, weight gains, livers' weights, vitamin A levels, worm burdens and parasite eggs' excretions were recorded.

Infected chickens had lower weight gains than the controls fed alike. Chickens given Diet 1 stored lesser vitamin A in liver than those fed Diet 3. Although worm counts in the 3 groups did not differ significantly, chickens fed Diet 1 excreted more *A. galli* eggs than those fed the 2 other diets. Female worms harboured by chickens fed Diet 1 had higher fecundity at week 5 pi than those of chickens fed Diet 2. Results indicated that Vitamin A is important for poultry in the moderation of the infection with *A. galli*.

Key words: chicken; *Ascaridia galli*; vitamin A; minor deficiency, resistance

Introduction

Vitamin A is thought to play a major role in the host defence against pathogens by maintaining the integrity of epithelial barriers including the intestinal mucosae (Chauhan, 1993). Vitamin A has also a systemic effect on immunity by boosting its diverse components: maturation of lymphoid organs, proliferation of lymphocytes, production of cytokines, activity of natural killer cells and production of antibodies (Davies & Sell, 1983). Therefore, vitamin A deficiency impairs both cellular and humoral immune res-

ponses in animals, chickens and turkeys included (Bang *et al.*, 1975). Ackert and his co-workers (1931) were the first to demonstrate that chickens affected by avitaminosis A were less resistant to infection with *Ascaridia galli*. Pande and Krishnamurty (1959) noted that under practical farm conditions chickens deficient in vitamin A were more predisposed to *A. galli* infections. Chubb and Wakelin (1963) reported that the resistance to many gastrointestinal helminths including *A. galli* was decreased in vitamin A deficient adult chickens. Vitamin A is also reported to reduce mortality and oocysts excretion of chickens infected with *Eimeria acervulina* and *Eimeria tenella* and to have beneficial effects on their performances (Crévieu-Gabriel & Naciri, 2001). However, to our knowledge, since Ackert (1931) no studies have been performed to investigate the effect of a minor vitamin A deficiency on infections with *A. galli* in growing chickens.

Today, vitamin A is added to poultry fodder in high doses (e.g. 13000 IU or 4472 µg retinol acetate per kg diet while the nutritional requirement of Brown-Eggs Layers is 1420 IU vitamin A or 489 µg retinol acetate per kg diet, NRC, 1994), as it could be destroyed during some industrial processes like pelleting (which combines heat, humidity and pressure) or by sub-optimal conditions of storage. Vitamin A was reported to be sensitive to moisture, heat, light, oxygen and lipoxygenase, an enzyme present in soybeans (Barua & Olsen, 2000). Inappropriate feed storage could decrease vitamin A amount in the diet to levels of minor deficiency, which in turn could affect the production and the immunity to infections. This study aimed to investigate the effect of different dietary levels of retinol acetate (172, 344 and 516 µg per kg diet corresponding to 500, 1000 and 1500 IU vitamin A per kg diet respectively) on a primary infection with *A. galli* in chickens.

Material and Methods

Animals and housing facilities

One hundred and thirty-eight one-day-old female Lohman Brown chicks hatched from eggs of the same parent flock were used for the investigation. They were placed in wooden houses of 6 m² with sawdust litter. Each group of chickens fed the same diet and having the same status regard to infection (infected or not) was hosted in a separate house. The houses were located 5 to 50 m from each other. Houses of infected groups were situated far from those of control groups. The birds had free access to water and feed. All other environmental conditions were similar inside the houses.

Infection material

A. galli eggs were harvested from mature female worms' uteri originating from naturally infected free-range hens and cultivated in 0.1N H₂SO₄ at 18°C, until they became infective, according to the procedure of Permin *et al.* (1997). Eggs collected from 5 female worms constituted a batch.

Experimental design

On the day of delivery, 138 chicks aged one day were randomly divided into 3 groups of 46 chicks, which were given ad libitum one of the 3 diets with different vitamin A levels, each (Table 1):

Diet 1: Vitamin A deficient diet containing 172 µg retinol acetate (500 IU) per kg diet.

Diet 2: Vitamin A deficient diet containing 344 µg retinol acetate (1000 IU) per kg diet.

Diet 3: An adequate diet containing 516 µg retinol acetate (1500 IU) per kg diet, which was close to the vitamin A requirements of Brown-Eggs Layers Leghorn-Type chickens (1420 IU per kg diet according to NRC (1994) or 489 µg retinol acetate per kg diet).

Except for vitamin A levels, the 3 diets were identical in all other respects and fulfilled all requirements of growing chickens. Before the allotment, 6 chicks aged one day were randomly sacrificed for baseline liver vitamin A level determinations.

The pre-infection adaptation period was 4 weeks in order to let chicks fed vitamin A deficient diets lose their liver reserves of vitamin A. Then, in each group 30 chickens were randomly inoculated orally with 500 ± 57 (SD) invasive *A. galli* eggs; 10 randomly selected chickens were maintained as uninfected controls and 6 chickens were sacrificed in order to determine the level of vitamin A in the liver at the end of the adaptation period, which was also the day of infection with *A. galli*. Every 2 weeks post-infection (pi), 10 infected chickens were sacrificed for worm collection and liver extraction for vitamin A measurement. The control chickens were sacrificed at the end of the experiment for liver vitamin A level measurements. The experiment lasted 9 weeks in total from vitamin A diets application.

Table 1. Composition and chemical analysis of feed for experimental growers

Composition	(%)
Barley	65.15
Oat	20.6
Soybean meal toast, dehulled	2.90
Fishmeal	2.00
Grass pellets	3.30
Soybean oil	3.20
Salt (NaCl)	0.25
Calcium carbonate	0.19
Dicalciumphosphate	1.91
Vitamin premix (without vitamin A) ^a	0.50
Retinol acetate (µg/kg)	172.0 (Diet 1) 344.0 (Diet 2) 516.0 (Diet 3)
Chemical analysis	(% DM)
Dry matter (DM)	92.15
Ash	5.15
Protein (N x 6.25)	13.19
Fat-HCL	4.98
Gross energy (MJ/ kg DM)	18.80
Crude fiber	6.62
Glucose	0.00
Fructose	0.12
Sucrose	0.12
Aminoacids:	(% Protein)
Cysteine	2.79
Methionine	2.43
Aspartic acid	9.66
Threonine	4.71
Serine	6.14
Glutamic acid	25.69
Glycine	6.04
Alanine	5.96
Valine	6.75
Isoleucine	5.15
Leucine	9.12
Phenylalanine	6.06
Histidine	3.07
Lysine	5.80
Arginine	7.49
Proline	10.26

^a Supplying in mg (or IU) per 100 kg of premix: lysine 590; methionine 240; threonine 500; tryptophan 210; calcium 99,320; phosphorus 650; sodium 10; magnesium 220; vitamin E (all-rac tocopherol) 3,750; vitamin E (α-tocopherol) 3,412.5; vitamin B₁ 125; vitamin B₂ 1,000; vitamin B₆ 250; vitamin B₁₂ 2; D-pantothenic acid 2,000; vitamin K₃ 1,250; vitamin D₃ 10,000; niacin 6,250; choline chloride 187,500; folic acid 250; biotin 50; BHT 31,250; FeSO₄ 7 H₂O 11,000; ZnO 21,000; MnO 26,500; CuSO₄ 5 H₂O 2,175; KI 300; Na₂SeO₃ 50; Cobalt 82.5.

Clinical observations and measurements

The chickens were checked every day in the morning and afternoon at the same hours for any clinical signs of ascari-

diosis (loss of appetite, drooping wings, ruffled feathers, anaemia, diarrhoea or mortality according to Permin & Hansen, 1998) and signs of vitamin A deficiency (low feed consumption and growth rate, asthenia, unsteady gait, increased susceptibility to infection, xerophthalmia, epithelial keratinisation in the eyes, and death) according to Olson (1991). All chickens were weighed on a weekly basis with an electronic scale (Geniweigher GM-11K, J.R.O.) having a precision of ± 50 g.

Parasitological examination

Quantitative egg determinations were made on faecal samples by a modified McMaster method (Henriksen & Aagaard, 1976; Permin & Hansen, 1998) with a lower detection limit of 20 eggs per g of faeces. Pooled faecal samples were taken from all groups from week 1 to week 3 pi. Individual faecal samples were taken from infected chickens at weeks 4 and 5 pi. Faecal egg counts from the non-infected control chickens were made on pooled samples too. Fecundity of female worms was estimated in each chicken by dividing the number of *A. galli* eggs per g faeces (EPG) by the number of female worms recovered.

Worm recoveries were performed in 3 steps (Permin & Hansen, 1998):

1. The abdominal cavity of each bird was opened; the digestive tract was removed and the intestine was opened with scissors from the gizzard down to the cloacae. Large worms visible macroscopically were removed, counted and stored in 70 % v/v ethanol.
2. The intestinal contents were then washed over a sieve with a mesh aperture of 38 μ m. The sieve retentate was washed into a Petri dish with tap water for later examination and counting of L4 larvae under a microscope at a magnification of 40.
3. The mucosae of the intestine was gently scraped off with a pair of forceps and washed into a sieve with a mesh aperture of 20 μ m, the retentate then washed into a Petri dish and examined for the presence of histotrophic larvae (L3) with a microscope at a magnification of 40. Lumen and histotrophic larvae were counted and stored in 70 % v/v ethanol.

The lengths of the adult worms were measured with a ruler (Linex, 1025 M, Denmark).

Vitamin A analysis

Vitamin A in liver was analysed according to the procedure described by Jensen *et al.* (1998). To liver samples (approximately 3 g) 96 % v/v ethanol was added as twice the amount (w/w). An Ultra Turrax on an ice bath homogenized the samples for 1 min. From this homogenate 100 mg from the for vitamin A deficient groups and 50 mg for the adequate group were transferred into a saponification tube and mixed with 2.00 ml of 96 % v/v ethanol, 0.50 ml of methanol and 1.00 ml of 20 % w/w ascorbic acid solution. These tubes were stored overnight on ice in a refrigerator at 5°C. Next day, 1.00 ml of KOH-water (1:1 w/w) was added to each tube, and was saponificated for 20 min at 80°C in the dark and cooled. Finally, retinol was extract-

ed with 2 portions of 5.00 ml heptane-di-isopropyl ether (3:1 v/v) and 100 μ l of this extract was subsequently injected into High Performance Liquid Chromatography (HPLC; Jensen, 1994).

The HPLC equipment consisted of a Perkin Elmer LC pump Series 200, a Perkin Elmer Advanced LC Sample Processor Series 200 and a Perkin Elmer LC Series 200 fluorescence detector (Perkin-Elmer GmbH, D-7770 Überlingen, Germany). Identification and quantification of retinol was obtained by comparison of retention time as well as peak areas with external standards. The extinction coefficient $A_{1\text{cm}}^{1\%} = 1550$ for retinol acetate in 2-propanol was used. The HPLC column consisted of a 4.0 x 125 mm Perkin Elmer HS-5-Silica column. The mobile phase consisted of heptane modified with 2-propanol (60 ml/l). Fluorescence detection was performed with an excitation wavelength of 344 nm and an emission wavelength of 472 nm. Vitamin A in feed was determined by the same procedure.

Statistical analyses

Statistical analyses were performed with the software package SAS version 8.2 (SAS Institute Inc., Cary, NC, USA, 1999). Weight gains were compared by a repeated measurements procedure with animal as random, using the model:

$$Y_{ij} = \mu + \beta (\text{vitamin}_{ij}, \text{infection}_{ij}, \text{week}_{ij}) + \gamma \text{wd0} + \Sigma_{ij}$$

with *i* for grower, *j* for week pi and the weight at the day of infection (wd0) as covariate. Vitamin A concentration in liver and weights of livers were also compared by a repeated measurements procedure. Worm counts, faecal egg counts, and fecundities were compared by non-parametric methods (Kruskal-Wallis test for each week) because of highly non-normal variation. The level of significance for all calculations was $P < 0.05$.

Results

Clinical measurements

The experiment was terminated a week earlier (at week 5 pi) for technical and ethical reasons: the remaining feed for the most deficient group (where a lot of food wastage was noticed) was insufficient to feed the chickens the whole following week. No clinical signs of ascaridiosis or vitamin A deficiency were observed in chickens in all experimental diet groups. No deaths imputable to other diseases were observed throughout the experiment. Furthermore, no macroscopic pathological lesions in the abdominal cavity or in the intestine of the sacrificed birds were observed. Although no significant differences in weight gains were detected within the 3 different infected chickens diet groups ($P = 0.2$), infected chickens of each diet group exhibited significantly lower weight gains than their matched, uninfected controls ($P = 0.02$; Fig. 1). However, this difference may be slight, as it is not clear in Fig. 1. The interaction between vitamin A and parasite infection with regard to weight gain was also statistically significant ($P = 0.02$).

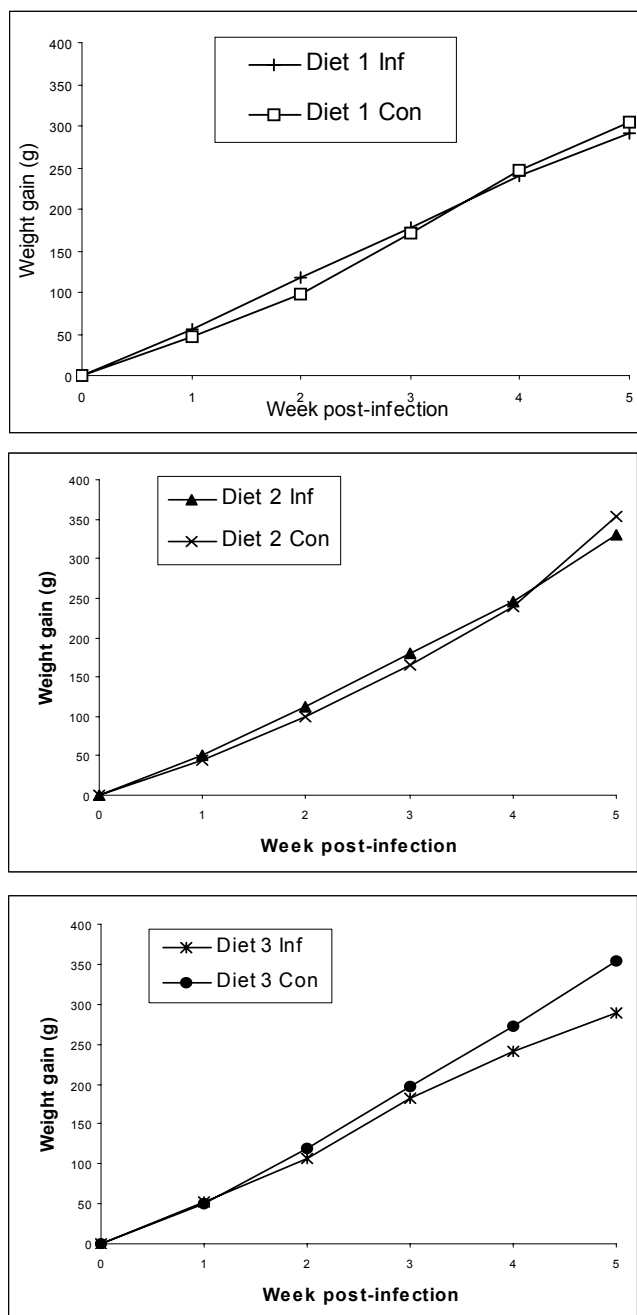


Fig. 1. Weight gain of infected growers and controls in all groups (n = all remaining chickens)

Vitamin A in liver

Diet levels of vitamin A affected the amount of vitamin A stored in the livers of both infected and control birds. At week 5 pi, control chickens fed Diet 1 stored lesser vitamin A in the liver than the control chickens fed Diet 3 ($P < 0.01$). Infection did not alter this relationship; infected chickens fed Diet 1 (low in vitamin A) had a lower level of vitamin A in the liver compared to the infected birds fed Diet 3 (sufficient in vitamin A) throughout the experiment ($P = 0.03$; Fig. 2). At week 5 pi the level of vitamin A in the livers of uninfected controls in each group was lower

than that measured in the livers of infected chickens fed the same diet ($P = 0.004$ for Diets 1 and 2; $P = 0.01$ for Diet 3).

Weight of the livers

The livers' weights of uninfected control chickens fed Diet 1 were lower than those of control chickens fed Diet 2 ($P < 0.05$) at week 5 pi. Liver weight was also affected by infection: at week 5 pi the livers of infected chickens fed Diet 1 were heavier than those of their non-infected controls fed the same diet ($P = 0.02$; Fig. 3); however, the liver weight-body weight ratio of infected chickens and non-infected controls fed Diet 1 did not differ significantly ($P > 0.05$). The level of dietary vitamin A had no effect on the weights of livers of infected chickens throughout the experiment ($P > 0.05$).

Parasitological results

At weeks 4 and 5 pi, *A. galli* faecal egg counts of chickens fed Diet 1 were higher than those of chickens fed Diet 2 ($P = 0.04$ at week 4 pi and $P = 0.001$ at week 5 pi; Fig. 4). At week 5 pi, *A. galli* faecal egg counts of chickens fed Diet 1

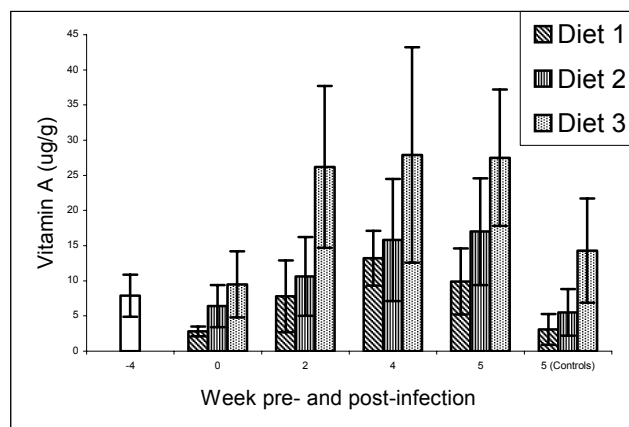


Fig. 2. Vitamin A concentration in liver ($\pm$ SD) in all groups (n = 6 before infection and n = 10 at weeks 2 – 5 pi)

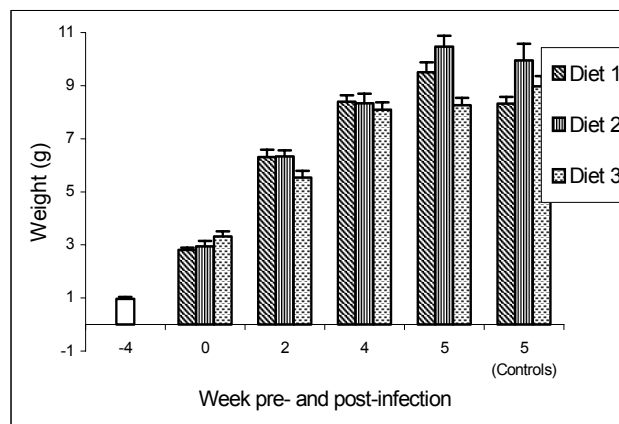


Fig. 3. Livers' weight ($\pm$ SEM) for all groups (n = 6 before infection and n = 10 at weeks 2 – 5 pi; a, b: different superscripts indicate significant difference, $P < 0.05$)

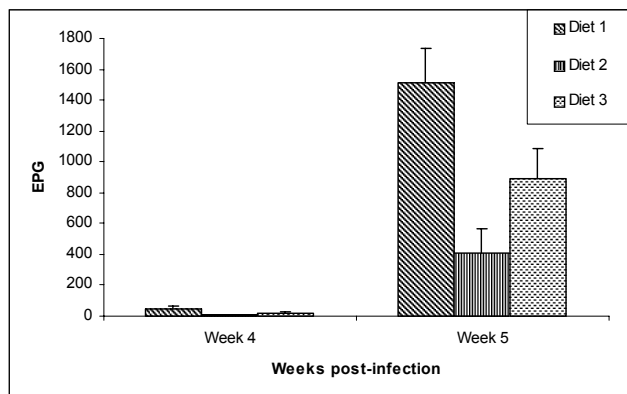


Fig. 4. *Ascaridia galli* faecal egg count (+ SEM) for all groups (n = 10; a, b, c, d: different superscripts indicate significant difference, $P < 0.05$)

were also higher than those of chickens fed Diet 3 ($P = 0.048$). Furthermore, at week 5 pi, female worms harboured by chickens fed Diet 1 had higher fecundity than those harboured by chickens fed Diet 2 ($P = 0.03$). However, no significant differences in larvae and adult worm counts (Table 2) between infected chickens fed the 3 diets were found at week 2 pi ($P = 0.1$), week 4 pi ($P = 0.2$) and week 5 pi ($P = 0.07$). In addition, the calculated male-female worm ratios (Table 3) in the 3 groups were similar ($P = 0.4$), as were the lengths of the worms collected from infected chickens fed the 3 diets (female worms, $P = 0.6$ and male worms, $P = 0.9$).

Table 2. Mean adult worm and larvae counts ($\pm$ SD) in all groups throughout the experiment

Week post-infection	Diet 1	Diet 2	Diet 3
2	17.8 $\pm$ 8.4	25.4 $\pm$ 13.8	16.6 $\pm$ 8.6
4	16.0 $\pm$ 11.1	6.5 $\pm$ 8.9	10.7 $\pm$ 8.3
5	21.6 $\pm$ 9.0	10.1 $\pm$ 10.0	16.9 $\pm$ 9.1

Table 3. Means of male-female ratio, parasite eggs excretion and female worms fecundity ($\pm$ SD) in all groups throughout experiment

Week post-infection		4	5
Diet 1	Male-female ratio	0.3 $\pm$ 0.5	0.8 $\pm$ 0.8
	Eggs per g feces	24.6 $\pm$ 62.8	412.7 $\pm$ 514.4
	Fecundity	0.0 $\pm$ 0.0	52.2 $\pm$ 35.6
Diet 2	Male-female ratio	0.7 $\pm$ 0.2	0.7 $\pm$ 0.5
	Eggs per g feces	1606.1 $\pm$ 795.5	25.0 $\pm$ 51.3
	Fecundity	113.7 $\pm$ 47.1	6.7 $\pm$ 14.4
Diet 3	Male-female ratio	Not determined	0.7 $\pm$ 0.6
	Eggs per g feces	1.5 $\pm$ 5.5	886.7 $\pm$ 690.7
	Fecundity	Not determined	80.5 $\pm$ 16.5

Discussion

The present investigation showed that *A. galli* infection, combined with a minor deficit of vitamin A deficiency,

reduced weight gains and increased faecal egg counts and female worms fecundity of infected chickens. Dubinský *et al.* (1975) also reported that chickens infected with *A. galli* and fed diets deficient in vitamins A, D, E, K, riboflavin and pantothenic acid suffered higher weight loss, morbidity and mortality than their infected fellows fed a vitamin-adequate diet. However, others have reported that chickens infected with a low dose of *A. galli* but fed adequate diets compensated for the infection without showing any clinical signs (Dubinský *et al.*, 1973; Permin *et al.*, 1998, Idi *et al.*, 2004). The effect of *A. galli* infection on chicken performance in the present investigation was aggravated by vitamin A deficiency, without apparent compensation. Ackert *et al.* (1931) reported that growing chickens infected with *A. galli* and fed vitamin A deficient diets for 5 weeks harboured greater worm burdens, and larger worm size than those recovered from infected chickens given diets adequate in vitamin A. However, both Ackert *et al.* (1931) and Dubinský *et al.* (1975) utilized natural or semi-synthetic diets which were more deficient in vitamin A than those utilized in this study; and which caused pathognomonic symptoms of vitamin A deficiency or mortality in infected chickens.

Further evidence of the importance of vitamin A in host resistance to *A. galli* was produced by Leutschaya *et al.* (1993) who found that supplementation of diet with orally administered vitamin A (2,500 to 5,000 IU per bird) reduced *A. galli*'s establishment rate by 40 to 78 % in adult chickens fed balanced diets. Higher doses (10,000 to 50,000 IU vitamin A per bird) yielded worm burdens in which 70 to 100 % of the female worms were stunted and immature with a reduced male-female ratio.

Although these previous studies did not report the effects of vitamin A on parasite fecundity, this study clearly demonstrated that worm reproduction is sensitive to vitamin A levels, even in chickens on diets with only a moderate deficiency in vitamin A and exhibiting no signs of vitamin A deficiency; parasite egg output and fecundity of worms collected from chicken fed diet the most deficient diet was higher because of a minor vitamin A deficiency, which reduced the resistance of chickens to infection. This observation is supported by the results of Taylor *et al.* (1947) who concluded that based on histological examinations of pathological lesions in the secretory and glandular epithelium of the nasal cavity, chickens are affected by vitamin A deficiency even when they received dietary amounts of vitamin A only slightly less than their nutritional requirement. Measurement of hepatic vitamin A level was used in the present study to assess the body status in vitamin A. Liver contains more than 90 % of the body reserves of vitamin A (Bondi & Sklan, 1984; Blomhoff *et al.*, 1991). In addition, vitamin A level in the plasma remains constant despite large variations of vitamin A intake and storage (Norum & Blomhoff, 1992). Therefore, hepatic vitamin A level is the best indicator for vitamin A status of the body (Aye *et al.*, 2000). At week 5 pi, infected chickens fed each of the 3 diets had stored more vitamin A in the liver than the controls fed the same diet. The bioavailability of vitamin A

depends on the vitamin A status of the animal (Christensen *et al.*, 1978; Bondi & Sklan, 1984; Grolier *et al.*, 1995). This is due to intestinal cleavage of β -carotene, which was reported to be lower in rats fed higher quantity of vitamin A or supplemented with β -carotene (van Vliet *et al.*, 1996). Infected chickens, which were affected by both vitamin A deficiency and ascariidiosis were, therefore, more efficient in converting β -carotene in retinol. For instance, Zoltowska *et al.* (1995; 1996) showed that chickens infected with 500 *A. galli* invasive eggs and then supplemented with vitamin A or β -carotene were able to compensate for the infection by increasing the activity of α -amylase, lipase and trypsin. Another explanation of our results could be that infected chickens fed deficient diets ate more feed to compensate both the deficiency and the infection and subsequently, stored higher amounts of vitamin A in the liver. Except for chickens fed Diet 1, the weights of livers of infected chickens and their matched controls were similar although the infected chickens fed each of the 3 diets stored higher amounts of vitamin A in liver than the controls given the same diet. This was in favour of a higher efficiency in absorption and storage of vitamin A in chickens infected with a low dose of *A. galli* probably because of an increased depletion of nutrients. In addition, we noticed that at weeks 2, 4 and 5 pi, infected chickens fed vitamin A deficient diets had put more liver weight than infected chickens fed the adequate diet even if the differences were not statistically significant. This again suggested that infected chickens fed vitamin A deficient diets consumed more feed to compensate both infection and deficiency. Further studies on the metabolism and the mechanism of action of vitamin A are needed for a full comprehension. Poultry farmers are recommended to pay attention to the conditions of feed storage in order to preserve vitamin A.

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References

ACKERT, J. E., MCILVAINE, M. F., CRAWFORD, N. Z. (1931): Resistance of chickens to parasitism affected by vitamin A. *Am. J. Hyg.*, 13: 320 – 336
 AYE, P. P., MORISHITA, Y. T., SAIF, Y. M. O., LATSHAW, D., HARR, S. B., CHILA, B. F. (2000): Induction of vitamin A deficiency in turkeys. *Avian Dis.*, 44: 809 – 817
 BANG, F. B., BANG, B. G., FOARD, M. (1975): Acute Newcastle disease virus infection of the upper respiratory tract of the chicken. 2. The effects of diets deficient in vitamin A on the pathogenesis of the infection. *Am. J. Pathol.*, 79: 417 – 424
 BARUA, B. A., OLSEN, A. J. (2000): Vitamin A and caro-

tenoids. In DELEENHEER, A. P., LAMBERT, W. E., VAN BOCXLAER, J. F. (Eds): *Modern Chromatographic Analysis of Vitamins*, Marcel Dekker, New York, USA, pp. 1 – 73
 BLOMHOFF, R., GREEN, H., GREEN, J. B., BERG, T., NORUM, K. R. (1991): Vitamin A metabolism: new perspectives on absorption, transport, cellular uptake, and storage. *Physiol. Rev.*, 71: 951 – 990
 BONDI, A., SKLAN, D. (1984): Vitamin A and carotene in animal nutrition. *Food Nut. Sci.*, 8: 165 – 191
 CHAUHAN, H. V. S. (1993): *Poultry diseases, diagnosis and treatment*. Willey Eastern Limited, New Delhi, India
 CREVIEU-GABRIEL, I., NACIRI, M. (2001): Effet de l'alimentation sur les coccidiooses chez le poulet. *INRA Prod. Anim.*, 14: 1 – 18
 CHRISTENSEN, K., DAUGAARD, J., HENNEBERG, U., HJARDE, W., JENSEN, K., AALUND, O. (1978): *Vitamin A and Beta-carotene to cattle. Evaluation of requirements under Danish conditions*. Beretning fra Statens Hus dyrbugsforsøg, 470. Lanhusoldnings Selskabets Forlag, Copenhagen, Denmark
 CHUBB, L. G., WAKELIN, D. (1963): Nutrition and helminthiasis in chickens. *Proc. Nut. Soc.*, 22: 20 – 25
 DAVIES, C. Y., SELL, J. L. (1983): Effect of all-trans retinol and retinoic acid nurture on the immune system of chicks. *J. Nut.*, 113: 1914 – 1919
 DUBINSKÝ, P., LEŠTAN, P., RYBOŠ, M. (1973): The effect of a full-value diet upon experimental *Ascaridia galli* infestations. *Biologia*, 25: 403 – 409
 DUBINSKÝ, P., LEŠTAN, P., RYBOŠ, M. (1975): Effect of vitamin deficiency in the feed of chickens on the course of experimental ascariidiosis. *Pol'nohospodárstvo*, 21: 1001 – 1008
 GROLIER, P., AGOUDAVI, S., AZAIS-BRAESCO, V. (1995): Comparative bioavailability of diet-, oil- and emulsion-based preparations of vitamin A and β -carotene in rat. *Nut. Res.*, 15: 1507 – 1516
 HENRIKSEN, S. A., AAGAARD, K. (1976): A simple flotation and McMaster method. *Nord. Vet. Med.*, 28: 392 – 397
 HONOURS, S., KENNEDY, S., TRUDEU A., WOBESTER, G. (1995): Vitamin A status of wild mallard wintering in Saskatchewan. *J. Wild. Dis.*, 31: 289 – 298
 IDI, A., PERMIN, A., MURRELL, K. D. (2004): Host age only partially affects resistance to primary and secondary infections with *Ascaridia galli* (Schrunk, 1788) in chickens. *Vet. Parasitol.*, 122: 221 – 231
 JENSEN, S. K. (1994): Retinol determination in milk by HPLC and fluorescence detection. *J. Dairy Res.*, 61: 233 – 240
 JENSEN, S. K., JENSEN, C., JAKOBSEN, K., ENGBERG, R. M., ANDERSEN, J. O., LAURIDSEN, C., SORESENSEN, P., SKIBSTED, L. H., BERTELSEN, G. (1998): Supplementation of broiler diets with retinol acetate, β -carotene or canthaxanthin: effect on vitamin status and oxidative status of broilers in vivo and on meat stability. *Acta Agric. Scand.*, 48: 28 – 37
 LEUTSKAYA, Z. K., DRYUCHENCO, E. A., ERANOVA, N. A. (1993): Vitamin A and the establishment of helminths. *Trudy Gel'mintologicheskoi Laboratorii*, 39: 84 – 92
 MATSUMOTO, M., STRAIN, G. J., ENGEL, H. N. (1991): The

- fate of *Pasteurella multocida* after intra-tracheal inoculation into turkeys. *Poult. Sci.*, 70: 2259 – 2266
- NATIONAL RESEARCH COUNCIL (1994): *Nutrient requirements of poultry*. National Academy Press, Washington, USA
- NORUM, K. R., BLOMHOFF, R. (1992): Vitamin A absorption, transport cellular uptake and storage. *Am. J. Clin. Nut.*, 56: 735 – 744
- OLSON, J. A. (1991): Vitamin A. In MACHLIN, J. L. (Ed) *Handbook of Vitamins*, Marcel Dekker, New York
- PANDE, P. G., KRISHNAMURTY, D. (1959): Inter-relationship between hypovitaminosis A and *Ascaridia galli* infestations in poultry. *Poult. Sci.*, 38: 13 – 25
- PERMIN, A., HANSEN, J. W. (1998): *Epidemiology, diagnosis and control of poultry parasites*. Food and Agriculture Organisation of the United Nations, Rome, Italy, pp. 74 – 105, 111 – 115
- PERMIN, A., NANSEN, P., BISGAARD M., FRANDSEN, F. (1998): *Ascaridia galli* infections in free-range layers fed on diets with different protein contents. *Br. Poult. Sci.*, 39: 441 – 445
- PERMIN, A., PEARMAN, M., NANSEN, P., BISGAARD, M., FRANDSEN, F. (1997): An investigation on different media for embryonation of *Ascaridia galli* eggs. *Helminthologia*, 34: 75 – 79
- TAYLOR, M. W., STERN, J. R., RUSSEL, W. C., JUNGHER, E. (1947): The provitamin A requirement of hens. *Poult. Sci.*, 26: 243 – 256
- VAN VLIET, T., FENTENER-VAN VLISSINGEN, M., VAN SCHAIK, F., VAN DER BERG, H. (1996): β -carotene absorption and cleavage in the rat is affected by the vitamin A status. *J. Nut.*, 126: 499 – 508
- ZOLTOWSKA, K., DZIEKONSKA-RYNKO, J., OLENDER, H., JABLONOWSKI, Z. (1995). Effect of vitamin A and protein levels in the diet of chickens infected with *Ascaridia galli* on activity of digestive enzymes in pancreas and small intestine. *Wiad. Parazytol.*, 41: 421 – 428
- ZOLTOWSKA, K., OLENDER, H., DZIEKONSKA-RYNKO, J., JABLONOWSKI, Z. (1996): The influence of various levels of carotene and protein in the diet on the index of invasion and digestive enzyme activity in chickens infected with *Ascaridia galli*. *Wiad. Parazytol.*, 42: 65 – 69

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