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UTILIZATION OF VITAMIN A FROM A STABILIZED DRY PREPARATION. COMPARATIVE EXPERIMENTS WITH CHICKENS [1])

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SUMMARY

The utilization of vitamin A (continuously administered in low doses) from a stabilized dry preparation and from fish liver oil has been compared in experiments with chickens.

The preparations have been administered by mixing through the feed and by direct dosing into the beaks of the animals respectively.

Two criteria have been used:

a storage of vitamin A in the livers,

b growth.

Modifications, for both methods permitting a quantitative statement of the results, have been developed.

In order to obtain reliable figures, it was necessary to exclude any influence of differences in stability of the preparations investigated. Therefore, in the experiments during which the preparations were administered through the feed, this mixing was performed daily.

The results, obtained by means of both criteria, lead to the conclusion that, aside from the question of stability, 1 I.U. of vitamin A in the form of the dry preparation, mixed through the feed, has the same biological activity in chickens as 1.3 I.U. of vitamin A in the form of fish liver oil.

An experiment in which the mixing took place only once (5 weeks before the start of the experiment) resulted in an "activity ratio" of about 4.

INTRODUCTION

Many data have become available about the fact that the "biological value" of vitamin A highly depends on the nature of the carrier. For instance, many experiments have shown, after oral administration, a better availability of vitamin A, from aqueous dispersions than from solutions in oil.

Some years ago all aspects of this subject have been summarized in an excellent way by LUTHER c.s. (9). In their review many references are given.

Most of the data on the availability have been based on the vitamin A levels obtained in the livers of experimental animals some time after administering this vitamin as a massive dose.

Recently, in experiments with rats, we compared this liverstorage of vitamin A from an aqueous dispersion and from a solution in oil after various methods of administering (10).

The fact that stabilized dry vitamin A preparations (the so called "mineral stable" preparations) are used in a growing extent, especially in feed mixtures, has made the problem of availability of vitamin A more important from the practical point of view.

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BRÜGGEMANN and TIEWS (1) compared the liverstorage of vitamin A after administration to rats of single doses of 14 different commercial preparations and were able to establish appreciable differences in utilization.

KRING and REKLING (8) examined in a similar way some preparations and obtained the best results with a gelatin coated product. By the performance of these liverstorage tests much higher doses of vitamin A have been used, than in the normal feeding practice and it is evident that comparative experiments, during which continual administration on normal levels has been used, are important.

Results of experiments with chickens by HALPERN c.s. (3, 4) and with dairy calves by JACOBSON c.s. (7) enable us to draw conclusions concerning the superiority of aqueous dispersions of vitamin A under somewhat more practical circumstances than those of the liverstorage tests.

In experiments with chickens under practical conditions, HARMS c.s. (5) demonstrated the superiority of vitamin A from a stabilized vitamin preparation to that from a solution in oil with regard to the growth of the animals and the storage of the vitamin in the liver. In similar experiments GLEDHILL and SMITH (2) came to the same conclusion.

In 1957 SCOTT c.s. (12) had the opinion that with the widespread use of antioxidants and new manufacturing processes for stabilizing vitamin A supplements, it could be desirable to re-evaluate vitamin A requirements using vitamin A preparations stabilized by envelopment of the vitamin droplets in gelatin, fat or wax and further stabilized by the use of antioxidants.

The above mentioned experiments (2, 5) have been performed in such a way, that it was impossible to differentiate the effect caused by the better stability of the dry vitamin A preparations from the effect caused by differences in utilization (availability, resorption).

The value of experiments in which only differences in utilization, aside from the question of stability, are investigated, is evident. A trial in this direction has been performed by NIJVELD (11).

In experiments with chickens he proved the superiority of vitamin A from stable dry preparations to that from a solution in oil in performing a storage of this vitamin in the liver by a continuous dosage of 4.5 I.U. of vitamin A per gram of feed.

By preparing his rations freshly every three days he excluded the influence of differences in stability to a great extent.

In a recent publication, HOCHBERG (6) concluded from experiments with chickens that, aside from the question of stability, vitamin A from a stable dry preparation was more effective than from fish oil or vitamin A palmitate from a solution in oil.

Five criteria have been used: growth, feed efficiency, prevention of deficiency symptoms, prevention of mortality due to deficiency and liverstorage. According to the author, the amount of feed in the feeding troughs was regulated to last a maximum of 4 to 5 days in order to insure against loss of potency.

In experiments with chickens described hereafter, we compared the utilization of vitamin A (applied in low practical doses) from a mineral-stable dry preparation with that from a solution in oil.

In mixing every day freshly and, in other experiments, in administering the preparations directly into the beak of our experimental animals, we believe to have excluded any possible influence of differences in stability in a more accurate way than has been done so far.

As criteria we used:

a liverstorage,

b growth.

For both methods modifications have been worked out, which permitted a quantitative statement of the results.

Experimental part

1 *Basal diet and vitamin A-preparations*

The basal diet used had the following composition:

Casein (acid, commercial quality)	19	kg
Product T *)	25	„
Buckwheatgroatsmeal	25	„
Rolled oatmeal	11	„
Wheat bran	6.5	„
Dried baker's yeast	9	„
Sucrose	2	„
Minerals **)	2	„
Vitamin mixture ***)	0.5	„
	100	kg

*) A partially hydrolyzed potato starch manufactured by N.V. Scholten, Foxhol, Holland.

**) The composition of the mineral mixture for chickens was:

calcium carbonate	70	kg
dicalcium phosphate	25	„
iodized salt	6.25	„
ferrous sulfate	0.75	„
copper sulfate	0.10	„
manganese sulfate	0.60	„
	102.70	kg

***) This vitamin mixture provided per kg of diet:

vitamin D_3	1500	I.U.
vitamin K_3	1	mg
dl-α-tocopherolacetate	10	„
vitamin B_{12}	5	μg

The vitamin A-preparations used were:

a *Fish liver oil.* The potency of the oil used in the various experiments varied from 2495 to 2830 I.U. of vitamin A per gram. If necessary, dilutions were made by means of small amounts of purified peanut oil.

b *Mineral stable vitamin A preparation.* The preparations used varied in potency from 342.000 to 350.000 I.U. of vitamin A per gram [2]).
Premixes were prepared with the aid of rolled oatmeal at the expense of rolled oatmeal of the basal diet.
In order to make all experiments comparable, we have corrected all diets which contained the dry preparation with the same amounts of oil (peanut oil) as were present in the fish oil containing mixtures.

To be sure that the right doses were administered to our experimental animals, chemical vitamin A determinations in the preparations were frequently carried out. These determinations were based on the general principle: saponification, chromatography on activated aluminium oxide and measuring of the absorption in u.v. light.

In the experiments during which any influence of differences in stability of the preparations used was excluded, the preparations were freshly mixed every day in the basal diet.

In some experiments the direct dosing into the beak of the chickens was daily performed in amounts corresponding to the concentrations used in the diets.

To be able to work in this way, the food consumption was determined daily during these experiments.

2 *Liverstorage experiments*

One-day North Holland Blue chickens were used (males plus females) in these experiments. After a pre-period of 4 days, during which time the animals received the basal diet, they were divided into comparable groups of 25 chickens and the administration of the vitamin A preparations was started and continued for a period of 3 weeks.

After that time, 7 pairs of 2 livers (one from a male and one from a female chicken) were formed from each group and the vitamin A content in these pairs was determined colorimetrically, after saponification and extraction, by treating the vitamin A containing extract with the reagent of CARR and PRICE.

As in the performed liverstorage experiments any influence of differences in stability of the preparations used had to be excluded, both preparations were freshly mixed every day in the basal diet.

In these experiments the same quantities were administered also by dosing the preparations directly into the beak of the animals, according to the feed quantity consumed the day before.

This was possible only because the exact feed consumption was determined per group day by day. According to the mean feed consumption the chickens were dosed individually with the corresponding quantity of the vitamin A preparations.

In the first experiment the results obtained with vitamin A from fish liver oil and from the mineral stable preparation were compared using a dosage of 4.5 I.U. of vitamin A per gram of feed.

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2) The preparation used was "Dohyfral Extra A-325", a dry gelatin sugar coated mineral-stable product, manufactured by N.V. Philips-Roxane, Weesp, Holland.

The second experiment was performed in the same way, except that besides a dosage of 4.5 I.U. of vitamin A during this experiment, also a dosage of 9.0 I.U. of vitamin A per gram of feed was used.

3 *Growth experiments*

Animals of the same origin were used during these experiments. After a pre-period of 10 days, during which all animals received only the basal diet, they were divided into comparable groups of 49 chickens (males plus females) and the administration of the vitamin A preparations was started and continued for a period of 4 weeks.

After this time the animals were weighed individually.

In these experiments, both fish liver oil and the stable vitamin A preparation were used in 5 different dosage groups, respectively amounting to 0.20; 0.32; 0.51; 0.82 and 1.31 I.U. of vitamin A per gram of feed.

From the mineral-stable vitamin A preparation only the sieve fractions consisting of the smaller particles were used in order to achieve a reliable partition of the vitamin in these very low concentrations.

In the first experiment, where any influence of differences in stability of the preparations used was excluded, both preparations were mixed freshly every day in the basal diet in the above mentioned amounts.

In the second experiment an estimation was made of the influence of differences in stability and for this reason both preparations were mixed in the basal diet in the above mentioned amounts only once, 5 weeks before the experiment started.

During this whole second experiment the same mashes were used.

Results and discussion

In table 1 the average vitamin A contents in the livers (male + female) : 2, obtained in the liverstorage experiments are given.

The standard deviations of these average values have, as far as possible, also been stated in this table. As mentioned above the vitamin A determinations have been performed in pairs of livers.

The results show that in both experiments vitamin A from the stable dry preparation has yielded a higher storage of this vitamin in the liver than vitamin A from fish oil.

The differences obtained are considerable. This is true for the experiments during which the preparations have been mixed through the feed as well as for the experiments during which the preparations have been administered directly into the beaks of the animals.

Moreover it is clear that dosing directly into the beak has resulted in lower liverstorages of vitamin A than administration through the feed, though only if the vitamin is provided as fish liver oil the differences are considerable.

This result is in contrast to the findings of Nijveld (11).

The first experiment contained also groups in which the preparations were dosed directly into the beaks of the chickens after they had been fasting for at least two hours before dosing.

It appeared, that this measure has not affected the liverstorage of vitamin A.

Table 1 Average vitamin A content (I.U.) per liver, obtained in the liverstorage experiments.

	Mode of administration	Fish liver oil		"Dohyfral Extra A-325"	
		4.5 I.U. per gram of feed	9.0 I.U. per gram of feed	4.5 I.U. per gram of feed	9.0 I.U. per gram of feed
Experiment I	In the feed	126 ± 30	–	248 ± 25	–
	Directly into the beak	< 50 *)	–	238 ± 28	–
	Directly into the beak (2 hours without feed)	< 56 *)	–	201 ± 23	–
Experiment II	In the feed	121 ± 40	424 ± 54	186 ± 22	646 ± 34
	Directly into the beak	< 58 *)	200 ± 46	158 ± 26	554 ± 62

*) In some pairs of livers the amount of vitamin A present was too low to give an exact value with the used chemical method.

In using two dosages of administration in the second experiment, we were able to compare quantitatively the "biological values" of vitamin A in both preparations.

Following the method as mentioned in U.S.P. XV (13) we calculated an "activity ratio" from our results: the quotient of the biological value of 1 I.U. of vitamin A from the stabilized dry preparation and the biological value of 1 I.U. of vitamin A from fish liver oil.

The calculation based on the experiment, in which the preparations were given through the feed had the following result: activity ratio = 1.30 (P 0.95 limits: 86–117 %).

The calculation from the experiment in which the preparations were dosed directly into the beak of the animals was only possible graphically on logarithmic paper using the value obtained with the high dose fish liver oil; the resulting activity ratio was: 1.86.

Table 2 Average weights (gms), obtained in the growth experiments.

	I.U. per gm of feed	Males		Females	
		Fish liver oil	"Dohyfral Extra A-325"	Fish liver oil	"Dohyfral Extra A-325"
Expmt. I (without influence of stability)	0.20	264 ± 28 (9)	297 ± 19 (19)	266 ± 18 (17)	264 ± 20 (16)
	0.32	299 ± 17 (15)	342 ± 16 (26)	278 ± 20 (17)	339 ± 21 (17)
	0.51	365 ± 19 (25)	404 ± 22 (25)	354 ± 16 (18)	382 ± 19 (22)
	0.82	429 ± 17 (24)	447 ± 19 (26)	416 ± 16 (23)	433 ± 16 (19)
	1.31	482 ± 16 (26)	481 ± 19 (28)	453 ± 18 (20)	456 ± 15 (19)
Expmt. II (with influence of stability)	0.20	199 (1)	233 ± 22 (11)	138 (2)	205 ± 17 (14)
	0.32	240 (2)	260 ± 21 (12)	129 (2)	264 ± 18 (15)
	0.51	194 (2)	307 ± 13 (22)	182 ± 22 (6)	296 ± 16 (26)
	0.82	218 ± 19 (14)	395 ± 14 (22)	192 ± 15 (11)	366 ± 15 (27)
	1.31	247 ± 19 (19)	433 ± 18 (21)	277 ± 17 (23)	422 ± 12 (24)

The difference between the two values has to be regarded in relation to the fact that following the last way of administration, especially for the fish liver oil, the amounts of vitamin A stored in the livers were much lower than those following administration through the feed.

In table 2 the average weights of the chickens at the end of the growth experiments are summarized with their standard deviations and the number of animals still present at that time.

Following the method as mentioned in U.S.P. XV (13) the activity ratios between vitamin A in the stabilized dry preparation and in fish liver oil have been calculated with the following results:

Experiment I,

males: 1.31 (P 0.95 limits: 80–124%)
females: 1.28 (P 0.95 limits: 80–125%)
weighed average: 1.29 (P 0.95 limits: 86–116%)

Experiment II,

males: 4.64 (P 0.95 limits: 72–140%)
females: 3.56 (P 0.95 limits: 77–130%)
weighed average: 3.95 (P 0.95 limits: 81–123%)

Comparing the results of experiment I with those of the experiment in which the storage of vitamin A in the livers has been compared for the two preparations, mixed through the feed, the excellent correlation will be clear.

Summarizing the results, obtained by means of both criteria, it can be stated that 1 I.U. of vitamin A in the form of the stabilized dry preparation, mixed through the feed, has shown the same biological activity in chickens as 1.3 I.U. of vitamin A in the form of fish liver oil.

Once more we want to accentuate the fact that this conclusion is valid, aside from the question of differences in stability in the feed.

It will be clear that the fact, that vitamin A in some vitamin A preparations is highly stable, does not necessarily mean, that the vitamin is also in a highly utilizable form.

In this relation we will not omit to lay stress upon the importance of the micro-structure of the dry preparation under investigation.

As mentioned above, "Dohyfral Extra A-325", is a gelatin sugar coated product. In each particle of this preparation the vitamin A-activity (some Int. Units per particle) is distributed into a large number of micro-particles, the diameter of each being well below 5 μ.

The results of experiment II show the important influence of differences in stability. If both preparations have been mixed only once through the feed, 5 weeks before the start of this growth experiment, the calculated activity ratio appeared to be about 4.

From this result and from the striking differences in mortality of the chickens it can be concluded that the vitamin A from the fish liver oil has been destructed to a large extent during the time between the mixing of the feed and its consumption.

The results of this second experiment are convincing as to the necessity of mixing daily through the feed the preparations to be compared, if the utilization has to be studied, aside from the question of stability differences.

In our opinion, e.g. weekly mixing will not be sufficient to exclude all influences of differences in stability.

All our experiences lead to the conclusion that a (variable) part of vitamin A from fish liver oil disappears within one week after mixing the same through a mash, especially if this latter contains substances harmful to vitamin A, e.g. trace elements.

LITERATURE

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