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INTERRELATIONSHIP AND EFFECTS OF CALCIUM AND VITAMIN D ON GROWTH, FEED EFFICIENCY AND BONE ASH OF WEANLING RATS

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Introduction

The importance of both calcium and vitamin D in the ration of animals is well cited in the literature of animal nutrition. Calcium and phosphorus nutrition mainly depends on the following factors: An adequate supply of each element, a suitable ratio between these two elements and presence of vitamin D.

Different species within the animal kingdom have different requirements of both calcium and vitamin D. These requirements are quite specific even within the species dependent upon age and physiological functions.

Approximately 99 per cent of calcium of the body is present in the bones and teeth. For this reason the primary function of calcium is in the formation of bones and teeth. It is also present in all cells and necessary for their normal functioning; and it is concerned in the coagulation of blood.

Vitamin D increases the absorption of calcium from the intestinal wall, and plays an important role in calcification.

In experiments conducted by Bell (2) on rats, bone growth varied much the same way as body weight with the addition of varying amounts of vitamin D to low calcium diets. He found that the bone ash rats on rachitogenic diets was 30 per cent, while the bone

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ash of rats 60 per cent on normal diets. Harrand et al (6) have shown that vitamin D addition to the diets containing Ca and P at low levels (0,12 and 0,24 %) reduced the fresh weight of humeri. The addition of minerals increased it significantly in the presence or absence of vitamin D. They also observed the addition of vitamin D to the diet with the lower mineral content caused an increase in the lenght of humerus and absolute amount of ash in the humeri.

Gaster et al (5) studied the interrelationship between calcium and vitamin D in young rats and found that the rats gained less body weight and had less ash in their bones on a low-calcium diet. Growth was improved by the addition of vitamin D to this diet, but no effect was observed on bone ash content. Cheesman and others (3) showed that the rats receiving vitamin D on rachitogenic diet gained more had more ash than the rats receiving no vitamin D.

Irving (7) declared that if the dietary intake of calcium and phosphorus is adequate and Ca: P ratio is in normal range, vitamin D requirement of the rat will be very small.

This experiment was conducted to determine the interrelationsship and effects of calcium and vitamin D on growth, feed efficiency and bone ash of weanling rats with milo as the only feed grain.

Materials and Methods

In a 2X2 factorial desing weanling rats are used as experimental animals. The rats weaned at 21 days were randomized into four treatments. One rat was assigned to each cage and one treatment. Each treatment was replicated four times. The rats had their own feeders and waterers separetely. The lighting in the battery room was of the fluorescent type. Sunlight coming into the room passed first through window glass.

The initial weight of each rat was recorded at the time of assigment to its treatment. Body weight for each rat was taken at weekly intervals and recorded. At the same time feed consumptions were also determined and recorded.

The feed ingredients of the basal diet are shown in Table 1. Ground milo was the only natural feed ingredient used in the diets. One per cent animal fat was included in the ration as suggested by Long and Göksu (8). BHT was added to the diets as antioxidant to reduce oxidation and fat rancidity.

TABLE 1
INGREDIENTS IN DIETS

Ingredients	Diets			
	1	2	3	4
Milo	897.50	897.50	880.20	880.20
Casein	78.00	78.00	80.00	80.00
Fat	10.00	10.00	10.00	10.00
Trace mineral mix	0.50	0.50	0.50	0.50
Vitamin premix	5.00	5.00	5.00	5.00
Antioxidant BHT	0.10	0.10	0.10	0.10
$CaCO_3$	——	——	15.30	15.30
Monosodium Phos.	3.90	3.90	3.90	3.90
NaCl	5.00	5.00	5.00	5.00
Vitamin D_2	——	0.11	——	0.11

The vitamin premix was formulated according to the vitamin requirements of the rat and mouse as set forth by Cuthbertson (4). The content of the vitamin premix is shown in Table 2. Vitamin D_2 was added to the diets 2 and 4. The suggested calcium: phosphorus ratio of 1,5:1 was maintained in those diets where calcium was added (Diets 3 and 4). The mineral content of the diets is shown in Table 3.

TABLE 2
VİTAMİN CONCENT OF DİETS

Vitamin	Requirement per Kg. diet	Conversions to Con centration used
Vitamin A	3000 IU	0.1 gm
Vitamin D	1000 IU (omitted)	0.11 gm.
Vitamin E	50 mg.	1.14 gm.
Vitamin K	1 mg.	1 mg.
Thiamine	2 mg.	2 mg.
Riboflavin	5 mg.	5 mg.
Calcium D Pantothenate	12 mg.	12 mg.
Niacin	10 mg.	10 mg.
Pyridoxine	2 mg.	2 mg.
Vitamin B_{12}	30 mg.	0.68 gm.
Choline	1 gm.	4 gm.

TABLE 3
MINERAL CONTENT OF DIETS

Mineral	Diets			
	1	2	3	4
	%			
Calcium	——	——	0.6	0.6
Phosphorus	0.4	0.4	0.4	0.4
Potassium	0.5	0.5	0.5	0.5
Sodium	0.5	0.5	0.5	0.5
Chlorine	0.3	0.3	0.3	0.3
Trace minerals	0.005	0.005	0.005	0.005

At the end of a 10-week experimental period the animals were killed, both right and left femurs were removed and bone ash determinations were made according to a method adapted from A. O. A.-C (1). The procedure used as following:

1 – Animal was killed and femurs were removed,

2 – Adhering tissues were removed.

3 – Femurs were placed in distilled boiling water for 3 minutes,

4 – Tissue was removed by hand scraping and peeling,

5 – Bones were placed in 95 % ethanol for 4 days,

6 – Bones were dried and then cracked,

7 – Fat was extracted using a Goldfisch fat extract apparatus (Fig. 1).

8 – Bone ash of fat-free bone was determined in a muffle furnace by conventional method.

Statistical analyses were made by the methods as outlined by Snedecor (9).

Results

Initial and weekly body weights of the experimental animals are shown in Table 4. Body weight gains of the rats during the experiment are presented in Fig. 2. The addition of calcium with or without vitamin D to the diets significantly ($P < 0.01$) increased body weight gains of animals. The increase in body weight gain with the addition of vitamin D was significant at the third week only. The difference between the diets 3 (with only Ca) and 4 (with Ca and vitamin D) was observed at the third week of the experiment.

TABLE 4

BODY WEIGHTS OF EXPERIMENTAL ANIMALS[1]

Diets	period				
	Initial	1 stwk.	2 nd wk.	3 rdwk.	10thwk.
	gm				
Basal	48.75 a	72.50 a	95.25 a	101.75 a	141.00 a
Basal + Vit. D_2	47.50 a	73.75ab	106..50ab	128.00b	178.50ab
Basal + Ca	47.00 a	77.00 b	117.50ab	147.25 b	241.25b
Basal+Ca+Vit. D_2	48.00 a	78.25 b	126.75c	176.25 c	337.25c

1 Valves with common superscripts are not significanthy different ($P < 05$).

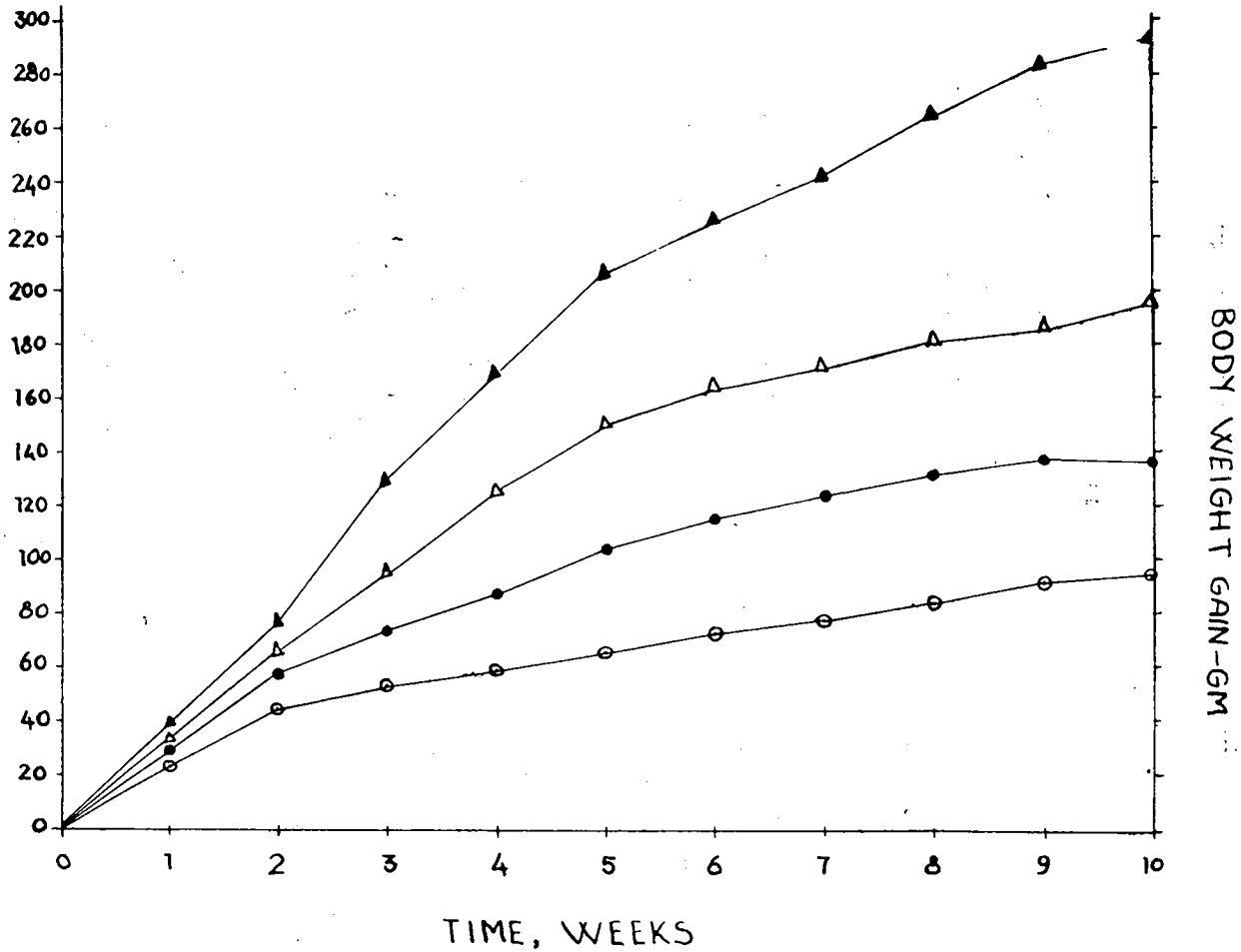

Fig. 2. Mean body weight gains of animals on the diets with neither Ca nor P (o pen circles); with witamin D (solid circles); with only Ca (open triangles) and with both Ca and P (Solid triangles).

Feed efficiency was considerably improved with the addition of calcium to the diets and it was found to be significant at 1 per cent level of probability. The mean values of feed efficiency are shown in Table 5.

TABLE 5

FEED EFFICIENCY AND BONE ASH[1]

Treatment	Feed Efficiency	Bone Ash
	gm DM/gm. gain	gm
Basal	6.27 a	33.82 a
Basal + Vit. D_2	6.50 a	36.61 a
Basal + Ca	5.34 b	60.73 b
Basal + Ca + Vit. D_2	4.34 c	61.30 b

1 Values with common superscripts are not significantly different ($P < 05$).

Table 5 also shows the treatment means for bone ash. The addition of calcium to the diets increased bone ash in femurs significantly ($P < 0.01$). The addition of vitamin D to the diet yielded 8 % more bone ash than the diet with no vitamin D. But it was not significant at 5 % level of probability. There was not any remarkable difference between diet 3 (with Ca) and diet 4 (with Ca plus vitamin D). Figure 3 shows the left femurs of the rats fed different diets.

Discussion

The addition of vitamin D to the diet did not effect weight gain during the first two weeks of the experiment. We suggested that the requirement of vitamin D was supplied from storage in liver during these two weeks. At the third week of the experiment weight gain was effected by the addition of vitamin D to the diet significantly. This suggested that vitamin D stored in the liver during 21 day weanling period was depleted in two weeks. At the tenth week of the experiment again the difference between diet 1 and 2 was not significant. It was thoght that it is due to the the insufficient amount of calcium for normal growth in the diet. The lock of difference in weight gain between diet 2 diet (with Ca) and diet 3 (with vitamin D) shows the close relationship between these two elements. The above observations confirm the results of Caster et al (5) and many others.

The presence of both calcium and vitamin D in the diet improved the weight gain after the third week of the experiment significantly in comparison with the diet 3 which contained calcium. It

indicates that the requirement of calcium for growth increases and it is indispensable after a certain age. The animals receiving both calcium and vitamin D differed significantly ($P < 0.01$) in weight gain than the other groups at the end of the experient. They gained 197 .00, 158 .00 and 95 .00 gm more than the rats which were on diet 1,2 and 3 respectively. These results are in very good agreement with the results of many workers and confirm the previous results indicating the importance of calcium and vitamin D in growth.

The utilization of feed was significantly ($P < 0.01$) improved with the addition of calcium, and calcium plus vitamin D to the diets. This might be due to the providing of calcium requirement of the cells for their normal functioning.

The results of bone ash determinations showed again the great importance of calcium in bone formation in this experiment. The bone ash was almost twice as much for the diet with calcium than the one without calcium. The difference between diet 1 and 2 was greater than the difference between diet 3 and 4 in bone ash content. This indicates that the requirement for vitamin D is very small in a diet in which the proportion of calcium: phosphorus is in normal range and in adequate amount. These results are in agreement with the results of Irving (7) Chesman et al (3) and Gaster et al (5).

Summary

An experiment was conducted to determine the importance of calcium and vitamin D and the interrelatinoship of these two elements in growth, bone formation and feed efficiency in rats.

In this experiment 16 weanling rats were used on four treatments in a 2x2 factorial design. The experimental period was 10 weeks.

Basal diet contained casein, animal fat, minerals, vitamins and milo as the only feed grain. Calcium was added to the diets 3 and 4; vitamin D_2 to the diets 2 and 4. Rations were fed *ad libitum*.

The rats were weighted every week and feed consumptions were recorded. At the end of the experiment the rats were killed and bone ash was determined in femurs.

The results obtained from this experiment indicated the following:

1 – Body weight gains were significantly improved by the addition of calcium to the diets with or without vitamin D.

2 – Addition of vitamin D to the basal diet effected body weight gain only on the third week of the experiment.

3 – Feed efficiency was markedly improved with the addition of calcium to the diets, no effect was observed on the feed efficiency by the inclusion of vitamin D to the diets.

4 – Bone ash was also increased with the addition of calcium to the diets while there was no effect of vitamin D addition.

Özet

Kalsiyum ve vitamin D nin büyüme, kemik formasyonu ve yemden yararlanmadaki önemini ve biribiri ile olan ilgisini ortaya koymak üzere bir deneme yapılmıştır.

Denemede 21 günlük iken sütten kesilen 16 rat 2x2 faktöriyel düzende dört gruba ayrılarak kullanılmıştır. Her gruba rasgele dörder rat ayrılmış olup hayvanların her biri bir batarya sisteminde ve ayrı bölmelerde 10 haftalık deneme süresince muhafaza edilmiştir. Deneme hayvanlarının bulunduğu oda fluoresans ile aydınlatılmış, pencereler camlı olduğundan direk güneş ışığı odaya girmemiştir.

Kontrol rasyonu kazein, hayvani yağ, mineraller (kalsiyum hariç), vitaminler (vitamin D hariç) ve dane yem olarak yalnızca süpürge darısından ibaretti. Kalsiyum 3 ve 4 üncü, vitamin D ise 2 ve 4 üncü rasyonlara ilave edilmiştir. Yemlikte yem, sulukta da su temiz olarak her zaman hazır bulundurulmuştur.

Deneme hayvanları denemenin başlangıcında ve müteakiben de her hafta tartılarak canlı ağırlıkları kayıt edilmiştir. Her hayvanın yem tüketimi deneme süresince verilen ve artan yemler kayıt edilmek suretiyle hesap edilmiştir.

Deneme sonunda hayvanlar öldürülmüş sağ ve sol femurları çıkarılarak bu kemiklerde kül tayini yapılmıştır.

Denemeden elde edilen neticeler şöylece sıralanabilir:

1 – Kontrol rasyonuna vitamin D ile veya yalnız olarak ilâve edilen kalsiyum, canlı ağırlığı ehemmiyetli olarak artırmıştır.

2 – Kontrol rasyonuna vitamin D ilâvesi denemenin yalnızca 3. haftasında canlı ağırlık artışını etkiliyebilmiştir.

3 – Kontrol rasyonuna kalsiyum ilâvesi yemden yararlılık derecesini artırmış, vitamin D ilâvesinin ise bir etkisi olmamıştır.

4 – Kemikteki kül miktarı kontrol rasyonuna kalsiyum ilâvesi ile artmış, vitamin D nin ehemmiyetli bir etkisi görülmemiştir.

Bibliography

1 – **A.O.A.C.** (1960): *Official Methods of Analysis* (9th ed.). Association of Official Agricultural Chemists, Washington, D. C.

2 – **Bell, G. H.** (1952): *Bone as a skeletal Structure.* The British Journal of Nutrition, 6, 405.

3 – **Cheesman, E. M., Copping, A. M. and Prebble, P. M.,** (1964): *The Effect of Vitamin D on Enzyme Activities in the Rats.* The British Journal of Nutrition, 18, 147.

4 – **Cuthbertson, W. F. J.,** (1957): *Nutrient Requirements of the Rat and Mouse.* Proceedings Nutr. Soc., 16,70.

5 – **Gaster, D., Havivi, E., and Guggenheim, K.,** (1967): *Interrelations of Calcium, Fluorine and Vitamin D in Bpne Metabolism.* The British Journal of Nutrition. 21, 413.

6 – **Harrand, R. B., Green, R. M., and Hartles, R. L.,** (1966): A Study in the Rat of the Interaction between the Effects of *Calcium and Phophorus Content of the Diet at Two Different Levels and the Presence or Absence of Vitamin D.* The British Journal of Nutrition, 20, 55.

7 – **Irving, J. T.,** (1957): *Calcium Metabolism.* Methuen, London.

8 – **Long, J., and Göksu S.,** (1962): *Sequebce in which Protein, Minerals and Vitamins of Milo Become Limiting for Growth of The Weanling Rat.* (Unpublished data).

9 – **Snedecor, G. W.,** (1956): *Statistical Methods* (Fith ed.) The Iowa State College Press, Ames, Iowa.

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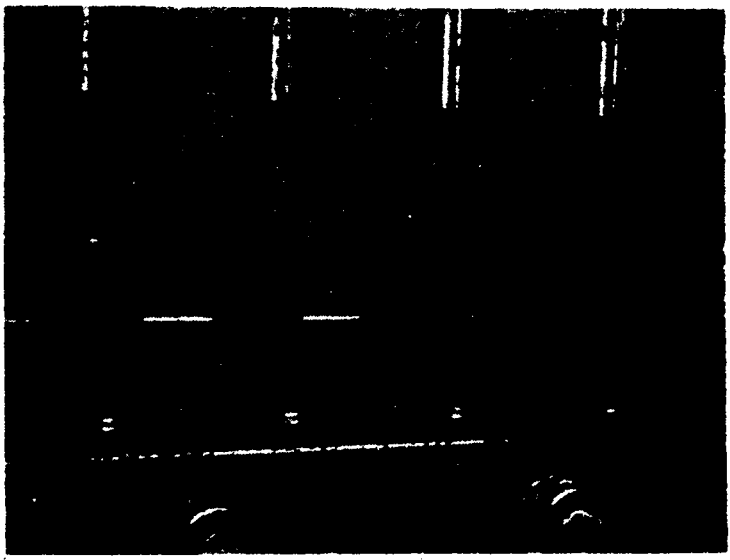

Fig. 1 : Goldfisch Fat Extraction Apparatus

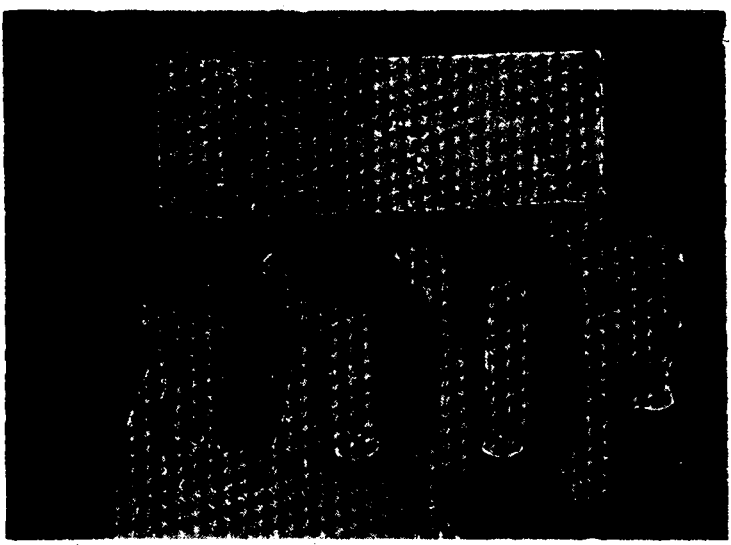

Fig. 3 : The left femurs of rats from each treatment.