

171°, no depression with an authentic sample of formononetin acetate). The ultraviolet and infrared spectra were also identical with those of an authentic sample.

The paper chromatographic and physical properties of compound VII from fraction 28 agreed with those of authentic daphnoretin, and with that previously isolated from ladino clover by another procedure (4). The ultraviolet ( $\lambda_{\text{max}}^{\text{EtOH}}$  346 m $\mu$ ) and infrared spectra were also found to be identical to those of the authentic sample. Acetylation with acetic anhydride and fused sodium acetate gave a monoacetate (m.p. 235–36° C., no depression with an authentic sample).

The ultraviolet and infrared spectra were also identical.

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## FEED SUPPLEMENTS

### Availability of Calcium in a Synthetic Hydrated Calcium Silicate (Micro-Cel)

The calcium in Micro-Cel, a synthetic hydrated calcium silicate, was found to be available to growing chicks, the degree of availability varying between one half and three quarters of that of the calcium of analytical grade calcium carbonate. The criteria used in this comparison were: body growth, plasma alkaline phosphatase, bone ash, and bone calcium. Bone ash and body growth were the more satisfactory procedures for evaluating calcium availability.

MICRO-CEL is a highly absorptive synthetic hydrated calcium silicate made by the hydrothermal reaction of diatomaceous silica and lime. It is very absorptive and will facilitate a free flow of material when mixed in diets containing a large amount of fat or oil. As much as 2 to 3% Micro-Cel may be used in a feed mixture. Titus *et al.* (6) reported that tallow prepared with Micro-Cel was as well utilized in growing chicks as plain tallow, and that the diet supplemented with Micro-Cel supplied more metabolizable energy than the diet which did not contain Micro-Cel. The question arose whether Micro-Cel could serve as a source of calcium in the diet. In the following study, Micro-Cel was compared to CaCO<sub>3</sub>, which is frequently used as a standard for the comparison of calcium sources in poultry rations. Changes in dietary calcium level have been shown to influence body growth, plasma alkaline phosphatase, bone ash, and the calcium content of bone ash (3); therefore these parameters were used as criteria in this comparison.

#### Experimental Procedure

Day-old Arbor Acre White Rock chicks were distributed into 20 lots on the basis of initial body weight and sex. Two lots, male and female, of 11 birds each, represented each treatment. The birds were housed in electrically heated Wahmann brooders, and at 4 weeks of

age they were transferred to larger unheated cages situated in an air-conditioned room maintained at 22° C. The calculated calcium content for the basal diet NR110, shown in Table I, is 0.11% and the total phosphorus content 0.79%, including not less than 0.23% inorganic phosphorus. The basal ration, therefore, contained an adequate supply of phosphorus as recommended by the National Research Council (4). CaCO<sub>3</sub> or Micro-Cel was added to the ration at the expense of an equal amount of Cellufour. Five levels of both CaCO<sub>3</sub> and Micro-Cel were fed (providing 0.2, 0.4, 0.6, 0.8, and 1.0% calcium). The chicks were individually weighed and food consumption data were collected for each treatment level.

At the end of 3 weeks, approximately half of the chicks of each lot were sacrificed and their serum alkaline phosphatase was determined, using the Sigma method (5). The ash content of the left tibia was determined using the bone ash procedure of Bliss and Gyorgy (7). The calcium content of the ash was determined by an (ethylenedinitrilo)tetraacetic acid (EDTA) titration procedure used by Hurwitz and Griminger (2). The remaining birds were sacrificed at 8 weeks of age and plasma alkaline phosphatase, bone ash, and calcium analyses performed.

The Micro-Cel used in the experimental diets contained 17.5% calcium, by analysis. The figure of 40% was used in the calculation of the calcium

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Table I. Experimental Chick Diet NR110

	% of Diet
Ground yellow corn	42.95
Soybean oil meal, 50% protein	42.00
Cellufour <sup>a</sup>	5.70
Animal fat	3.50
Fish solubles, 50% solids	2.50
KH <sub>2</sub> PO <sub>4</sub>	1.35
NaH <sub>2</sub> PO <sub>4</sub>	1.15
NaCl	0.50
Vitamin mix <sup>b</sup>	0.25
Trace mineral mixture <sup>c</sup>	0.10
Choline chloride	0.05
DL-Methionine	0.05

<sup>a</sup> Cellufour, Chicago Dietetic Supply House, Chicago, Ill.

<sup>b</sup> Vitamin mix, mg./kg. of diet: inositol, 62.5; D- $\alpha$ -tocopheryl succinate, 37.5; nicotinamide, 7.5; thiamine NO<sub>3</sub>, 5; D-Ca pantothenate, 5; riboflavin, 2.5; menadione, 2.1; vitamin A acetate, 1.29; pyridoxine HCl, 1.125; folic acid, 0.20; biotin, 0.0375; cyanocobalamine, 0.015; vitamin D<sub>3</sub>, 0.010.

<sup>c</sup> Delamix, Limestone Products Corp. of America, Newton, N. J. Contains following trace minerals (as % of Delamix): Mn, 6%; Fe, 2%; Cu, 0.2%; I, 0.12%; Co, 0.02%.

content of diets supplemented with analytical grade CaCO<sub>3</sub>.

#### Results

**Body Weight.** The results in Table II indicate that not less than 1% CaCO<sub>3</sub>

(0.4% Ca) and 2.3 to 3.4% Micro-Cel (0.4 to 0.6% Ca) are required for optimum chick body growth, since the differences observed between these and their higher supplemental levels are not significantly different.

**Feed Efficiency.** There is at 8 weeks a reduction in feed efficiency (Table II) for chicks fed either 0.5% CaCO<sub>3</sub> or 1.15% Micro-Cel (0.2% Ca).

**Bone Composition.** The ash content of the tibia (Table III) was significantly reduced when 1.0% CaCO<sub>3</sub> (0.4% Ca) was fed to 3-week-old chicks. A reduction in tibia ash was also seen in Micro-

Cel-fed birds when 0.4% and 0.6% Ca were fed to pullets and cockerels, respectively. These observations parallel data observed for body growth in Micro-Cel-fed chicks, but suggest a higher calcium requirement for CaCO<sub>3</sub>-fed birds than was indicated by growth data. There was a general increase in the ash content of the tibia, but only a slight change in the tibia ash pattern, at 8 weeks of age.

Eight-week-old chicks generally had a greater tibia calcium content than 3-week-old birds (Table III). The significant differences in the calcium content of

the tibia ash of chicks fed CaCO<sub>3</sub> or Micro-Cel were not consistent with the tibia ash data.

**Plasma Alkaline Phosphatase.** The authors found, as did Hurwitz and Griminger (3), that plasma alkaline phosphatase is higher in young chicks than in older birds. Plasma alkaline phosphatase was higher in chicks fed 0.5 and 1.0% CaCO<sub>3</sub>. An increase in plasma alkaline phosphatase in Micro-Cel-fed chicks began at the 0.6% Ca level (3.4% Micro-Cel) and was significantly greater at the lower Micro-Cel levels (1.15 and 2.3%). The differences reported for 3-week-old chicks were essentially similar to those at 8 weeks.

**Table II. Comparison of CaCO<sub>3</sub> and Micro-Cel in Growth of Arbor Acre White Rock Chicks**

Treatment <sup>a</sup>	Sex <sup>b</sup>	Average Body Weight, Grams		Feed Efficiency <sup>c</sup>	
		3 weeks	8 weeks	3 weeks	8 weeks
NR110 + 2.5% CaCO <sub>3</sub>	M	325	1622	1.67	1.77
	F	313	1313	1.85	2.06
NR110 + 2.0% CaCO <sub>3</sub>	M	312	1681	1.64	1.79
	F	302	1273	1.64	1.97
NR110 + 1.5% CaCO <sub>3</sub>	M	295	1416	1.67	1.88
	F	306	1256	1.69	2.03
NR110 + 1.0% CaCO <sub>3</sub>	M	300	1400	1.83	1.85
	F	294	1146	1.71	2.02
NR110 + 0.5% CaCO <sub>3</sub>	M	265 <sup>d</sup>	878 <sup>d</sup>	1.70	2.11
	F	261 <sup>d</sup>	838 <sup>d</sup>	1.59	2.43
NR110 + 5.7% Micro-Cel	M	322	1427	1.65	1.81
	F	292	1312	1.62	1.98
NR110 + 4.6% Micro-Cel	M	289	1375	1.59	1.80
	F	282	1283	1.58	1.87
NR110 + 3.4% Micro-Cel	M	270 <sup>d</sup>	1344	1.77	1.96
	F	268	1153	1.68	2.05
NR110 + 2.3% Micro-Cel	M	262 <sup>d</sup>	953 <sup>d</sup>	1.72	1.96
	F	228 <sup>d</sup>	926 <sup>e</sup>	1.87	2.23
NR110 + 1.15% Micro-Cel	M	217 <sup>d</sup>	590 <sup>d</sup>	1.86	2.60
	F	188 <sup>d</sup>	601 <sup>d</sup>	1.80	2.49

<sup>a</sup> CaCO<sub>3</sub> calculated to be 40% calcium, Micro-Cel assayed as 17.5% calcium. Calcium levels used: 0.2, 0.4, 0.6, 0.8, 1.0%.

<sup>b</sup> At 3 weeks 11 birds of each sex per treatment; reduced to 5 or 6 birds at 8 weeks.

<sup>c</sup> Grams of diet per gram of body weight gain.

<sup>d</sup> *P* < 0.01, analysis of variance comparing all CaCO<sub>3</sub> treatments to 2.5% CaCO<sub>3</sub> and all Micro-Cel treatments to 5.7% Micro-Cel.

<sup>e</sup> *P* < 0.05.

## Discussion

Multilevel statistical analyses of growth, ash, calcium, and phosphatase data, comparing dose response of Micro-Cel with that of CaCO<sub>3</sub>, are presented in Table IV. These analyses indicated that the calcium present in Micro-Cel can be utilized by the chick; however, it is not as available and/or as efficiently utilized as the calcium in CaCO<sub>3</sub>.

The determination of tibia calcium resulted in an estimation of available calcium in CaCO<sub>3</sub> and Micro-Cel similar to values obtained by tibia ash analysis. Body growth comparisons resulted in an 8-week estimation of available Micro-Cel calcium for pullets similar to the one obtained for tibia ash, but a lower Micro-Cel-estimated calcium potency in 3- and 8-week-old cockerels. The estimated Micro-Cel potency in 8-week cockerels, as measured by plasma alkaline phosphatase, fell between growth and ash estimated values; in pullets, the relative available calcium in Micro-

**Table III. Comparison of CaCO<sub>3</sub> and Micro-Cel in Plasma Alkaline Phosphatase, Tibia Ash, and Tibia Calcium**

Treatment <sup>a</sup>	Sex	3-Week-Old Chicks				8-Week-Old Chicks			
		No. of birds	PAP <sup>b</sup>	% T.A. <sup>c</sup>	% T.C. <sup>d</sup>	No. of birds	PAP <sup>b</sup>	% T.A. <sup>c</sup>	% T.C. <sup>d</sup>
NR110 + 2.5% CaCO <sub>3</sub>	M	5	108	50.7	34.3	6	9.9	52.9	36.2
	F	5	116	50.1	35.2	6	13.5	54.6	34.9
NR110 + 2.0% CaCO <sub>3</sub>	M	4	102	47.2	34.6	4	10.0	51.7	36.6
	F	5	104	48.8	33.5	6	14.1	52.7	35.4
NR110 + 1.5% CaCO <sub>3</sub>	M	4	99	46.6	34.4	4	11.1	51.7	36.4
	F	5	111	47.3	35.1	6	23.9	51.5	35.7
NR110 + 1.0% CaCO <sub>3</sub>	M	4	137	40.7 <sup>e</sup>	33.2	5	19.2	46.5 <sup>e</sup>	36.2
	F	5	166	42.3 <sup>e</sup>	34.9	5	24.3	50.1	35.6
NR110 + 0.5% CaCO <sub>3</sub>	M	5	154	38.7 <sup>e</sup>	33.0	5	50.8 <sup>e</sup>	39.1 <sup>e</sup>	35.4
	F	5	155	40.8 <sup>e</sup>	32.9	5	37.8	44.4 <sup>e</sup>	34.1
NR110 + 5.7% Micro-Cel	M	5	93	48.2	34.6	6	13.3	49.7	36.8
	F	5	115	46.5	32.9	6	12.0	52.8	35.9
NR110 + 4.6% Micro-Cel	M	5	107	45.4	33.2	6	13.9	51.2	36.8
	F	5	113	46.2	33.2	6	17.8	52.4	35.5
NR110 + 3.4% Micro-Cel	M	5	141	39.1 <sup>e</sup>	32.8	5	18.8	46.8	36.7
	F	5	130	44.1	32.2	6	24.3	50.0	35.4
NR110 + 2.3% Micro-Cel	M	5	162 <sup>e</sup>	38.6 <sup>e</sup>	34.0	6	40.6	44.6 <sup>e</sup>	34.9 <sup>e</sup>
	F	5	186	37.8 <sup>e</sup>	32.1	6	24.9	46.7 <sup>e</sup>	34.1
NR110 + 1.15% Micro-Cel	M	5	183 <sup>e</sup>	34.1 <sup>e</sup>	32.8 <sup>e</sup>	5	48.7 <sup>e</sup>	39.8 <sup>e</sup>	34.0 <sup>e</sup>
	F	6	183 <sup>e</sup>	35.1 <sup>e</sup>	33.2	5	64.6 <sup>e</sup>	42.3 <sup>e</sup>	34.0

<sup>a</sup> CaCO<sub>3</sub> calculated to be 40% calcium, Micro-Cel assayed as 17.5% calcium. Calcium levels used: 0.2, 0.4, 0.6, 0.8, 1.0%.

<sup>b</sup> Mean plasma alkaline phosphatase reported as micromoles of *p*-nitrophenol per ml. of plasma.

<sup>c</sup> Mean % tibia ash in defatted and dried left tibia.

<sup>d</sup> Mean % tibia calcium in ash of tibia.

<sup>e</sup> Confidence limit 99% or greater. All CaCO<sub>3</sub> treatments compared to 2.5% CaCO<sub>3</sub>, and all Micro-Cel treatments compared to 5.7% Micro-Cel.

**Table IV. Multilevel Assay Expressing Relative Estimation of Available Calcium in Micro-Cel as Related to CaCO<sub>3</sub> Calcium**

Criteria <sup>a</sup>	Sex	3 Weeks			8 Weeks		
		Estimated available Ca, %	Lower <sup>b</sup> limits, %	Upper <sup>b</sup> limits, %	Estimated available Ca, %	Lower <sup>b</sup> limits, %	Upper <sup>b</sup> limits, %
Plasma alkaline phosphatase	M	N.s. <sup>c</sup>	N.s. <sup>c</sup>	N.s. <sup>c</sup>	77	57	104
	F	60	32	113	59	28	122
Tibia ash	M	80	56	114	95	81	112
	F	59	44	79	75	54	104
Tibia calcium	M	79	54	116	85	69	105
	F	54	41	73	72	55	96
Body growth	M	53	31	91	62	46	83
	F	N.s. <sup>c</sup>	N.s. <sup>c</sup>	N.s. <sup>c</sup>	70	46	109

<sup>a</sup> Log transformation of all data used in analyses.

<sup>b</sup> Upper and lower 95% confidence limits.

<sup>c</sup> N.s. = not significant, since slopes did not approach significance.

Cel, as measured by alkaline phosphatase, was similar to that of tibia ash at 3 weeks and below it at 8 weeks. Several of the analyses indicate low precision because of their wide confidence limits. The results suggest that a tibia ash analysis is satisfactory in evaluating calcium availability in 3- and 8-week-old pullets, whereas body growth comparisons are adequate for cockerels of comparable age. This conclusion was reached because a satisfactory dose response curve is obtained with each of these two analyses, and their confidence limits are least variable in the sex of selected use. Plasma alkaline phosphatase data may not give as precise results as body growth and tibia ash. Calcium analysis of the tibia ash is not suggested, since it is more cumbersome than a tibia ash determination.

In the present experiment the calcium present in Micro-Cel was 53 and 59% utilized by 3-week-old Arbor Acre White Rock cockerels and pullets, respectively, when compared to CaCO<sub>3</sub> containing an equivalent amount of calcium. At 8 weeks, the value increased to 62% for cockerels and 75% for pullets. The sex difference in utilization of calcium is believed to be due to the different growth patterns; in the pullet the apparent utilization of calcium from Micro-Cel may be accentuated because of slower growth. The difference between cockerels and pullets in early growth is not great, but by 8 weeks it is appreciable.

It is possible that these interpretations of the data may apply only to Arbor Acre White Rock chicks and that other

strains of chickens have different growth patterns, and may therefore have different calcium requirements.

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## CATTLE AS FALLOUT MONITORS

### Cesium-137 Concentrations in Desert Range Cattle

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Cesium-137 concentrations in desert range cattle showed a declining trend from 1959 through 1961, probably due to decreasing amounts of Cs<sup>137</sup> available in the biosphere. In 1962 the concentrations increased in response to renewed nuclear weapons testing. Correlation between liver and muscle Cs<sup>137</sup> concentrations was highly significant. The slope of the equation relating liver and muscle Cs<sup>137</sup> concentrations was 0.42, indicating the possibility of different mechanisms of Cs<sup>137</sup> uptake in these two tissues.

CESIUM-137 is the only fission product of appreciable half life found in the edible tissues of meat animals. Other fission products are either poorly absorbed from feed or are highly localized in portions of the animal not normally used for human food—for example,

zirconium-95 and ruthenium-106 are not physiologically absorbed by the animal (16), strontium-90 is localized in osseous tissues, and iodine-131 is confined largely to the thyroid gland. The iodine-131 concentrations in the thyroid glands of the cattle used in this study have been reported (3).

This study was undertaken to monitor the concentration of cesium-137 in live-

stock grazing two divergent desert range areas.

#### Methods

Three herds of cattle were studied, all grade or purebred Herefords. The cattle in the two southern locations, DV (Deleamar Valley) and NTS (Nevada Test Site), subsisted on range alone except for occasional concentrate feed-

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