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On the Availability of Phosphorus in Bonemeal, Bonemeal Supplemented with Red Oxide of Iron, and Boneash to White Rats.

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According to correspondence which commenced in the issue of the Farmer's Weekly for 18th April, 1934, good results were obtained by feeding a lick containing 125 lb. salt, 25 lb. red oxide and 1 lb. copper sulphate to lactating cows in Southern Rhodesia.

Although iron is frequently incorporated in phosphatic licks, either as iron sulphate or as iron oxide, the suggested lick would not be recommended in the Union where phosphates are in such short supply in the natural herbage. Furthermore, it is doubtful whether continued good results can be expected with a lick containing 0·6 per cent. copper sulphate instead of the generally recommended one containing only 0·1 per cent. of this constituent.

The work referred to has popularised the use of red oxide in phosphate licks. After the treatment of animals for internal parasites, it is not infrequently found that they may benefit by the addition of a small amount of iron in the lick. However, the solubility of red oxide is relatively low, consequently Government Notice No. 1575 of August, 1943, specifies that any phosphate-salt-iron oxide lick offered for sale should contain a minimum of 13 per cent. of iron oxide. The question arises as to what extent the incorporation of this form of iron in phosphatic licks influences the retention of phosphorus.

From the literature it is evident that iron has a general detrimental effect on phosphorus and calcium metabolism. Rhem and Winters (1940) showed that ferric chloride has a definite adverse effect on phosphorus and calcium utilization. Waltner (1927) showed that the addition of 2 per cent. of reduced iron to McCollum's stock diet produced rickets in rats within four weeks. Analysis of blood serum of rachitic rats showed a low phosphorus but a normal calcium content.

It was accordingly planned to conduct an experiment to test the availability of phosphorus in bonemeal against bonemeal supplemented with red oxide, and at the same time the availability of phosphorus in boneash to white rats.

As regards the availability of phosphorus in several phosphatic supplements, Malan and du Toit (1932) tested these supplements and placed them in the following order of availability of P. :—

(1) Sodium Phosphate.

(2) Precipitated Calcium Phosphate.

(3) Bonemeal and degelatinised bone flour.

Their conclusions were based on the weight increase of the experimental animals.

Otto (1938) tested the availability of P in several commercial phosphates. The phosphates were fed at a level well below that of the P requirement of the class of animal concerned. Throughout a Ca : P ratio of 2 : 1 was maintained. Availability was determined by means of the retention of P.

From the experiments di-sodium phosphate appeared to be slightly more available at the given level of P intake than either di-calcium phosphate or bonemeal. There was no significant difference between the retentions of di-calcium phosphate and bonemeal. Summarising his findings he considers that " taking all the results obtained into consideration it is doubtful whether animals when fed phosphates well below their requirements will retain more P when it is given as di-sodium phosphate than the relatively insoluble di-calcium phosphate or bonemeal ".

Preliminary Experiment.

Animals.

Ten quartos of albino rats of the same age, (27-35 days), sex and weight (44-77 gm.) were chosen. Here it was impracticable to select quartos from litter mates, and as these animals were highly in-bred, quartos were selected with restrictions only to age, sex and weight.

At the beginning of the experiment one rat out of every quarto was sacrificed. The entrails were removed and the digestive tract freed of its contents. The left femur was dissected out and analysed for its ash percentage on a dry fat-free basis. The entire rat was then dried at 100° C. and subsequently ashed at a low temperature and the calcium and phosphorus content determined after addition of the ashed femur.

The remaining three rats out of every quarto were assigned to the three different diets and the paired-feeding method was employed. The food intake of the trio was restricted by the amount consumed by the member eating the least. In the exploratory experiment, tap water was given *ad libitum*.

The animals were kept in cages fitted with screened floors thus preventing access to excreta.

The constituents of the three diets in which bonemeal (Diet A,) bonemeal plus red oxide (Diet B), and bone ash (Diet C) was incorporated, are given in Table 1.

The bonemeal and boneash were both of 70 mesh fineness. The boneash that was used was prepared by ashing bonemeal in an electric furnace, keeping it at a dull red heat for one hour and grinding to 70 mesh fineness.

TABLE 1.

	Diet A. gr./Kg.	Diet B. gr./Kg.	Diet C. gr./Kg.
Egg White (heated)	150	150	150
Gelatine	30	30	30
Butterfat	80	80	80
Bacto Agar	20	20	20
Harris Yeast	15	15	15
Cod Liver Oil	20	20	20
Sucrose	40	40	40
Dextrinized Starch	596·5	592	604.5
Salt Mixture	30	30	30
Bone Meal	18·5	18·5	—
Red Oxide	—	4·5	—
Bone Ash	—	—	10·5
Percentage Ca	0·4673	0·4670	0·4558
Percentage P	0·2370	0·2368	0·2321
Ca/P	1·97	1·97	1·96

The weight of iron oxide added was equal to the weight of phosphorus pentoxide in the supplement, which is in the same proportion as that incorporated in phosphate-iron oxide licks.

Supplementing of the diets was made at the expense of the dextrinized starch. The salt mixture of Day and McCollum (1939) with the omission of calcium carbonate, was employed.

The basal diets contained 0·05 per cent. P and 0·06 per cent. Ca and were accordingly supplemented to contain 0·23 per cent. P and 0·46 per cent. Ca, giving a Ca/P ratio of 2 to 1. The rats throve on these diets as was manifested by their general appearances and gain in weight.

The experimental feeding was continued for 42 days when the rats were killed, the percentage ash on the left femurs and the calcium and phosphorus content of the entire rat determined as before.

The calcium was determined by the volumetric method of McCrudden (1911), while the phosphorus was determined by the method of Fiske and Subbarow (1925) using photo-electric technique.

Results.

Results of the preliminary experiment are given in Table 2.

The phosphorus content of the experimental rats at the beginning of the experiment was estimated from the analysis obtained from the controls. From the data the average ash percentages of the femurs of the three groups are 54·90, 55·15, and 54·93, which manifest a reasonable good bone formation. The average percentage retentions of phosphorus for the three groups are 53·03, 51·76 and 55·70.

Applying the analysis of variance technique to the individual ash percentages and percentages of phosphorus retentions, no significant differences were found, which was to be expected from the highly variable individual data.

TABLE 2.

Diet.	Rat No. and Sex.	Rat Weight (gm.)			Food Consumed (gm.)	Total P in Food (gm.)	P in rat at end (gm.)	P in rat at beginning (gm.)	P gained from food (gm.)	Percentage P gained.	Average percentage gained.	Ash percentage of femur	Average Ash percentage.
		Initial.	Final.	Gain.									
A.	1 ♂	73	130	57	307	·7276	·8104	·4349	·3755	51·61		57·06	
	4 ♂	49	90	41	231·5	·5486	·5299	·2948	·2351	42·85		54·18	
	7 ♂	47	92	45	255·8	·6062	·5701	·3050	·2651	43·73		55·31	
	10 ♂	53	107	54	253·3	·6003	·6601	·3553	·3048	50·77		54·94	
	13 ♀	57	112	55	257·5	·6103	·6802	·3601	·3201	52·45		54·84	
	16 ♂	45	100	55	237·6	·5631	·6405	·2800	·3605	64·02		55·49	
	19 ♀	49	108	59	251	·5949	·6405	·3052	·3353	56·36		52·79	
	22 ♀	48	113	65	256	·6067	·6252	·3150	·3102	51·12		53·46	
	25 ♀	77	152	75	318	·7537	·9205	·4950	·4255	56·45		56·04	
	28 ♀	70	146	76	315	·7465	·8904	·4355	·4549	60·93	53·03	54·92	54·90
B.	2 ♂	73	124	51	308	·7293	·7602	·4349	·3253	44·60		59·80	
	5 ♂	49	102	53	238	·5636	·5802	·2948	·2854	50·63		54·29	
	8 ♂	47	108	61	258	·6109	·5902	·3050	·2852	46·68		52·86	
	11 ♂	53	107	54	263	·6228	·6903	·3553	·3350	53·78		56·24	
	14 ♀	57	102	45	255·5	·6050	·6802	·3601	·3201	52·90		55·09	
	17 ♂	45	96	51	232·8	·5513	·5701	·2800	·2901	52·62		51·95	
	20 ♀	49	122	73	260	·6157	·7200	·3052	·4148	67·37		52·53	
	23 ♀	48	94	46	250·1	·5922	·5701	·3150	·2551	43·07		54·50	
	26 ♀	77	149	72	318	·7530	·9105	·4950	·4155	55·17		57·65	
	29 ♀	70	128	58	303·5	·7187	·8004	·4355	·3649	50·77	51·76	56·65	55·15
C.	3 ♂	73	140	67	310	·7195	·8904	·4349	·4555	63·30		56·96	
	6 ♂	49	102	53	238	·5524	·6003	·2948	·3055	55·20	55·30	54·79	
	9 ♂	47	106	59	258	·5988	·6200	·3050	·3250	54·27		58·20	
	12 ♂	53	103	50	263	·6104	·6955	·3553	·3402	55·73		55·01	
	15 ♀	57	122	65	262	·6081	·7602	·3601	·4001	65·79		52·48	
	18 ♂	45	108	63	243	·5640	·6003	·2800	·3203	56·79		53·10	
	21 ♀	49	106	57	249·2	·5784	·5553	·3052	·2501	43·23		50·94	
	24 ♀	48	104	56	256	·5942	·6099	·3150	·2949	49·62		53·79	
	27 ♀	77	160	83	318	·7381	·9302	·4950	·4352	58·96		57·97	
	30 ♀	70	145	75	315	·7311	·8305	·4355	·3950	54·02	55·70	56·10	54·93

The low phosphorus retention may indicate that this element was fed in the diet at a level higher than that required for normal growth and phosphorus retention. According to Utley and Macleod (1935) a diet containing 0·16 per cent P and a Ca/P ratio of 3 to 1 would supply either the minimal or slightly under the minimal amount of phosphorus for normal growth and phosphorus retention.

Second Experiment.

The low phosphorus retentions observed in the preliminary experiment which may indicate that the P in the diets of the preliminary experiment was in excess of the minimal requirements of the animals, might cloud any differences in the assimilation of P from the three diets. Accordingly an identical experiment with seven quartos of albino rats was conducted as before, except that the phosphorus in the diets was supplied at a level of 0·16 per cent P. The Ca/P ratio of these diets was 1·87 in every case. Distilled water was given *ad libitum*. The rations were made up as before. Supplementing was made according to Table 3.

Table 3.

Diet.	Basic Ration.			Supplement/Kg.					Total/Kg.		
	Weight (gm.)	Ca (gm.)	P (gm.)	Bone-meal (gm.)	Bone-ash (gm.)	FeO (gm.)	Ca (gm.)	P (gm.)	Ca (gm.)	P (gm.)	Ca/P
A	989·18	·5935	·4954	10·82	—	—	2·3890	1·0993	2·9825	1·5947	1·87
B	986·58	·5919	·4932	10·82	—	2·6	2·3890	1·0993	2·9809	1·5925	1·87
C	993·67	·5962	·4968	—	6·325	—	2·3837	1·0999	2·9799	1·5967	1·87

The red oxide in diet B was added in the same amount as the P_2O_5 in the phoshate supplement. Feeding was continued for 42 days. The animals fed well and their general appearances were good. Except that several rats knibbled at the solder of the metal screens nothing abnormal was observed.

Results of the experiment are summarised in Table 4.

The average ash percentages on a dry fat-free basis of the femurs of the three groups are 51·69, 52·34 and 51·05. The individual ash percentages exhibit no significant difference on statistical analysis.

As was expected the percentage phosphorus retentions were considerably higher than in the preliminary experiment. The average percentage phosphorus retentions were 76·07, 73·36 and 71·52. On applying the analysis of variance technique to the individual retentions between the three groups, no significant differences were found.

Since the analysis of variance showed no significance for pairings, significance of treatment differences were adjudged by the variation within groups only. No differences were found. This was applied to the preliminary experiment with the same result.

TABLE 4.

Diet.	No. and Sex.	Rat Weight. (gm.)			Food consumed (gm.)	Total Ca in Food (gm.)	Ca in rat at begin. (gm.)	Ca in rat at end. (gm.)	Ca gained from food (gm.)	Percentage Ca gained	Total P in food (gm.)	P in rat at begin. (gm.)	P in rat at end. (gm.)	Percentage P gained from food (gm.)	Percentage P gained	Ash Percentage of femur.
		Initial.	Final.	Gain.												
A	1 ♀	60	145	85	294·5	·8783	·5209	·9982	·4773	54·34	·4696	·3800	·7400	·3600	76·66	51·32
	4 ♀	40	110	70	240	·7158	·3406	·7209	·3803	53·12	·3827	·2800	·5600	·2800	73·16	48·42
	7 ♀	60	163	103	327	·9752	·5189	1·1724	·6535	67·01	·5214	·4200	·8000	·3800	72·88	54·50
	10 ♀	58	162	104	333	·9932	·4674	1·1249	·6575	66·20	·5310	·3800	·7900	·4000	75·32	53·00
	13 ♀	53	123	70	262	·7814	·4615	·8397	·3782	48·40	·4718	·3600	·6600	·3000	71·80	46·48
	16 ♂	58	127	69	292·8	·8733	·5625	1·0932	·5307	60·76	·4669	·4200	·7600	·3400	72·82	52·68
	19 ♂	60	128	68	293	·8738	·5466	1·1328	·5862	67·08	·4672	·3860	·8000	·4200	89·89	55·49
Average										59·56					76·07	51·69
B	2 ♀	60	143	83	296	·8823	·5209	1·0338	·5129	58·13	·4714	·3800	·7600	·3800	80·61	52·78
	5 ♀	40	106	66	240	·7154	·3406	·7882	·4476	62·56	·3822	·2800	·5600	·2800	73·26	51·91
	8 ♀	60	160	100	327	·9747	·5189	1·0971	·5782	59·32	·5207	·4200	·7600	·3400	65·29	51·11
	11 ♀	58	156	98	328·7	·9798	·4674	1·1011	·6337	64·67	·5234	·3800	·7600	·3800	72·60	51·67
	14 ♀	53	130	77	265	·7899	·4615	·8793	·4178	52·89	·4220	·3600	·6800	·3200	75·82	53·33
	17 ♂	58	134	76	301	·8972	·5625	1·1091	·5466	60·92	·4793	·4200	·8060	·3800	79·28	52·08
	20 ♂	60	122	62	282·6	·8424	·5166	1·0615	·5149	61·12	·4500	·3800	·6860	·3060	66·66	53·53
Average										59·94					73·36	52·34
C	3 ♀	60	154	94	296	·8820	·5209	1·0496	·5287	59·94	·4726	·3800	·7200	·3460	71·94	51·76
	6 ♀	40	106	66	240	·7151	·3406	·7684	·4278	59·82	·3832	·2800	·5600	·2800	73·06	50·90
	9 ♀	60	158	98	327	·9744	·5189	1·0853	·5664	58·12	·5221	·4200	·7600	·3400	65·12	50·27
	12 ♀	58	144	86	333	·9923	·4674	·9247	·4753	47·89	·5317	·3800	·6800	·3000	56·42	46·26
	15 ♀	53	138	85	265	·7896	·4615	1·0536	·5921	74·98	·4231	·3600	·7800	·4200	99·26	50·38
	18 ♂	58	128	70	301	·8969	·5625	1·0457	·4832	53·87	·4806	·4200	·7600	·3400	70·74	54·28
	21 ♂	60	128	68	292	·8731	·5466	1·0615	·5149	58·97	·4678	·3800	·6800	·3000	64·12	53·53
Average										59·08					71·52	51·05

Summary and Conclusions.

Two experiments have been carried out with albino rats in which the availability of phosphorus in bonemeal, bonemeal supplemented with red oxide of iron and boneash have been tested out. In the preliminary experiment the P was supplied at a level of 0·23 per cent., the Ca/P ratio being 2 : 1.

From this experiment no significant differences between the ash percentages of the femurs of the rats and the phosphorus retentions could be shown.

Low phosphorus retentions indicated that the P was supplied in excess of the minimal requirements of the animals.

In the second experiment P was supplied at a level of 0·16 per cent., the Ca/P ratio being 1·87. The percentage P retentions were considerably higher than in the preliminary experiment, the average percentage retentions being 76·07, 73·36 and 71·52. Again no significant differences could be shown by applying statistical analysis to the ash percentages of the femurs and the P retentions, which implies that under the conditions of the experiment the availability to white rats of the P in the three supplements, are similar.

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