

# Influence of High Dietary Calcium and Phosphorus and Ca:P Ratio on Liver Copper and Iron Stores in Lactating Cows

## Abstract

The copper and iron status of lactating cows fed rations borderline in copper (6 mg/kg) but of high calcium content or with a wide Ca:P ratio was investigated. Liver copper stores decreased on the basal ration (which contained 0.88% Ca and 0.47% P) and also when dietary Ca was elevated to 2.3% (resulting in a 5:1 Ca:P ratio). However, an increase in phosphorus to 1.2% along with 2.3% Ca to maintain a 2:1 ratio significantly alleviated the depression in liver Cu. Iron stores were not affected by treatment.

## Introduction

High calcium intakes (2) and wide ratios of calcium to phosphorus (6) in rations for lactating dairy cows have been associated with increased parturient paresis. Studies with sheep (4) have shown wide Ca:P ratios (8:1), decreased liver stores of iron, but there was no consistent effect on liver copper. Underwood (7) stated that excessive intakes of  $\text{CaCO}_3$  depress liver Cu. Price and Moschler (5) showed that excessive liming of soils markedly decreased copper, iron, and cobalt content of forages harvested from such soils. However, there is little information on the influence of high calcium intakes and wide Ca:P ratios on concentration of Cu and Fe in livers of lactating cows.

## Experimental Procedures

Nine Holstein cows (three first-calf heifers and six cows past their second lactation) were randomly assigned to three treatments approximately 56 days prior to parturition. One first-calf heifer was allotted to each treatment group. Treatment A was the basal ration of corn silage (18.2 kg/day), orchardgrass hay (4.5 kg/day), and a 16% crude protein concentrate fed at 1 kg per 3.5 kg milk. The concentrate was specially prepared to contain low Cu (5.0 mg/kg) for which the composition is shown in Table 1. Copper content of the total ration was 6.1 mg per kilogram. Treatment B differed from A in that  $\text{CaCO}_3$  was added at 3.6% of the total basal ration dry matter by thorough mixing

in the corn silage just prior to feeding. To Treatment C, tricalcium phosphate was added to supply equivalent calcium as in B. Table 1 lists the concentration of various minerals in the rations.

Just before treatment, two liver samples were taken from each cow by the biopsy technique previously described (3). Additional liver biopsies were at two-month intervals. Treatments were for 12 months and covered the complete lactation for all cows except for one cow which was sacrificed during the 11th month of treatment because of a faulty biopsy. Jugular blood was sampled with liver biopsies. Feed and liver samples were analyzed for Cu and Fe and blood for Cu, Ca and P according to AOAC (1). Hemoglobin, hematocrit concentrations, and red blood cell numbers in blood were determined by conventional methods.

## Results and Discussion

Milk yields were similar for all treatments and averaged 17.5 kg per day for the complete lactation. Dry matter intakes did not differ between treatment groups and averaged 12.5 kg per day. Intakes of calcium and phosphorus of cows on the basal ration were 110 and 59 g per day; values for the carbonate treatment (B) were 293 and 58 and those for phosphate (C) were 285 and 140.

Copper concentrations of the pretreatment biopsies averaged about 200 mg per kg dry liver for all groups (Fig. 1). By the end of the experimental period, levels were higher ( $P < .05$ ) for Group C (134 mg/kg) than A (58 mg/kg) or B (77 mg/kg). The reason for the copper-sparing action of the tricalcium phosphate is not apparent. Neither high calcium nor a wide Ca:P ratio explains this effect because calcium intakes were similar for Rations B and C, and Ca:P ratios were equal in A and C. One possibility is that the high phosphorus in Ration C enhanced mineral uptakes in the small intestine through increased intestinal acidity. Depression of liver copper on the basal ration was expected because data from a previous study showed decreases on rations correspondingly low in copper (3).

Liver iron concentrations did not differ between treatments (Fig. 2). This finding con-

TABLE 1. Mineral content of control ration.<sup>a</sup>

Mineral	Feedstuff			Total ration
	Grain <sup>b</sup>	Silage	Hay	
Calcium (%) <sup>c</sup>	1.56	0.32	0.77	0.88
Phosphorus (%)	0.95	0.20	0.22	0.47
Copper (mg/kg)	5.00	7.50	5.63	6.12
Iron (mg/kg)	140.90	127.90	67.90	116.80

<sup>a</sup> Sufficient calcium carbonate was added to Ration B to raise its calcium content to 2.34%. This addition increased the Ca:P ratio to approximately 5:1. To Ration C tricalcium phosphate was added and the calcium and phosphorus levels were 2.28 and 1.23%, respectively.

<sup>b</sup> Ingredient composition (%): Ground shelled corn, 59; rolled oats, 20; meat and bone scraps, 20%; iodized salt, 1%.

<sup>c</sup> All analyses are on a dry matter basis.

TABLE 2. Whole blood calcium and phosphorus concentrations as affected by supplemental calcium and phosphorus.

Ration Pretreat- <sup>a</sup> ment	Calcium			Phosphorus		
	Pretreat- <sup>a</sup> ment	Treat- <sup>b</sup> ment (10 months)	Average change <sup>c</sup>	Pre- <sup>a</sup> treat- ment	Treat- <sup>b</sup> ment (10 months)	Average change <sup>c</sup>
	(%)					
A, Control	12.25	12.83	+ .58	5.76	6.48	+ .72
B, Carbonate	12.08	14.00	+2.00	7.28	7.22	- .08
C, Phosphate	14.08	10.00	-4.00	5.86	7.43	+1.57

<sup>a</sup> Pretreatment samples at about 15 days prior to parturition.

<sup>b</sup> Treatment samples at 10 months after parturition.

<sup>c</sup> None of the changes was significant ( $P < .05$ ).

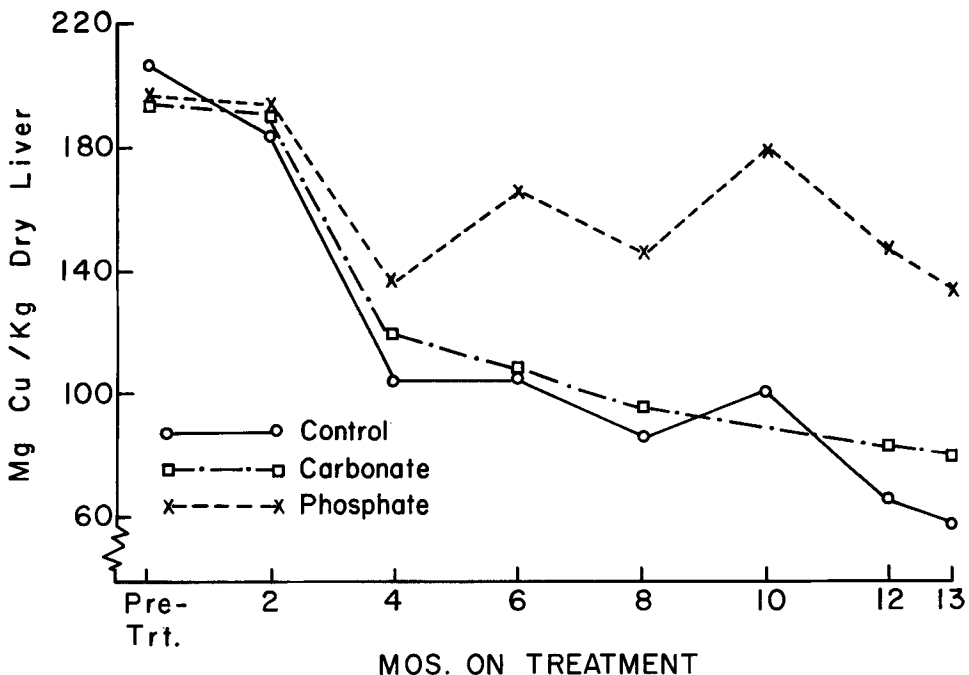


FIG. 1. Liver copper concentrations during a complete lactation for cows fed rations: A, Control; B, Carbonate; C, Phosphate.

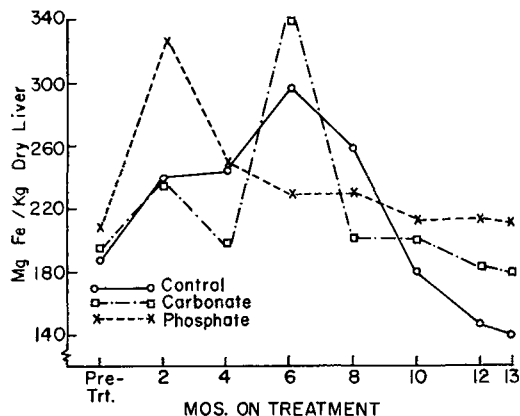


FIG. 2. Liver iron concentrations during a complete lactation for cows fed rations: A, Control; B, Carbonate; C, Phosphate.

trasts with data of Fontenot et al. (4), who observed that high Ca:P ratios (8:1) decreased liver iron but had no effect on copper. The high iron content of our ration (117 mg/kg), due primarily to the meat scraps in the concentrate, may account for the lack of response of liver iron to the high-calcium rations. Rations in the sheep study (4) were similar to our basal ration except that cottonseed meal was the protein supplement, which is lower in iron than meat scraps.

Copper in whole blood, as shown in Figure 3, followed the same general trend as copper in

liver with cows on Ration C maintaining higher levels than shown for Rations A and B, but differences between treatments were not significant ( $P > .05$ ). Calcium and phosphorus concentrations in blood (Table 2) were within the normal range during the entire experimental period and were not significantly affected by treatment, but there was a trend towards depressed calcium on Ration C and lower phosphorus on Ration B. Neither were hemoglobin, hematocrit or red blood cell counts altered by treatment.

Although the number of animals in this study was relatively small, these data show clearly that elevated phosphorus intake lessens liver depletion of copper on rations borderline in copper concentration. This finding has practical implication in rations which may have wide Ca:P ratios or are low in phosphorus.

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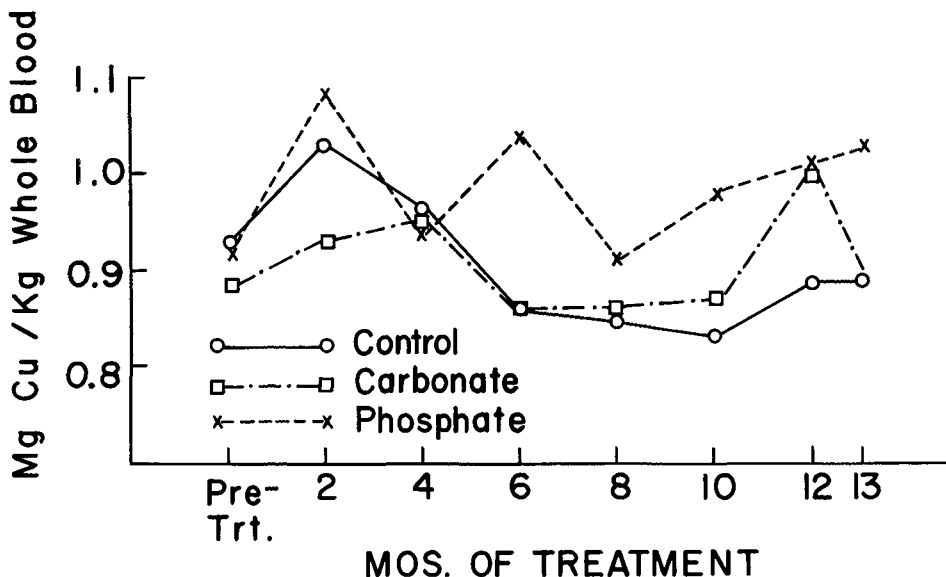


FIG. 3. Level of copper in whole blood of cows fed rations: A, Control; B, Carbonate; C, Phosphate.

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## Effect of Ovariectomy on Pituitary Gonadotropins in the Cow<sup>1</sup>

### Abstract

To study changes in pituitary follicle-stimulating and luteinizing hormone contents following ovariectomy, 24 heifers were spayed on the day of estrus and sacrificed on Day 17 or Day 42. Twelve intact heifers were sacrificed on Day 17 of the estrous cycle. Individual anterior pituitary glands from all animals were assayed for follicle-stimulating hormone by biological assay, and for luteinizing hormone by radioimmunoassay. Pituitary glands from ovariectomized heifers did not differ significantly in fresh or dry weight from those of control animals. Follicle-stimulating hormone content in pituitary glands from all spayed heifers was higher ( $P < .001$ ) than in intact heifers, whereas pituitaries from spayed heifers at Day 42 had more ( $P < .05$ ) follicle-stimulating hormone than did those at Day 17. Luteinizing hormone content for the pituitary glands of the intact heifers was significantly ( $P < .005$ ) higher than the average for glands from all spayed heifers.

### Introduction

An increase in gonad-stimulating activity of the anterior pituitary of the rat following

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ovariectomy was noted as early as 1929 (4). Individual hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) continue to increase for weeks above the general level in the pituitaries of the nonpregnant rat (2,5,13). Changes in the pig (9) have been similar when ovariectomy was near the middle of the estrous cycle but the levels, while greater than those for the estrous cycle, did not appear to exceed those at Day 25 of pregnancy or post-hysterectomy.

Cyclic changes in pituitary gonadotropins and the greater role of the corpus luteum in species with long estrous cycles make it desirable that the studies on the influence of ovariectomy be related to the stage of the estrous cycle when the ovaries are removed and also to the day of the cycle when intact animals are studied. The anterior pituitary gland of the heifer appears to be relatively high in FSH and LH at Day 17 of the estrous cycle (3,10). The following experiment was designed to determine if spaying would increase FSH and LH more than a normal cycle.

### Experimental Procedures

Thirty-six nulliparous Holstein heifers weighing 280 to 360 kg were in this study. Twenty-four heifers were totally ovariectomized approximately 12 hr after beginning of estrus. Following ovariectomy two groups of 12 heifers each were sacrificed at Day 17 and Day 42. A control group of 12 intact heifers was sham-operated on the day of estrus and sacrificed on Day 17 of the cycle. Ovariectomy was through an incision in the dorsal wall of the vagina.

At autopsy anterior pituitary glands from all heifers were individually weighed, homogenized and freeze-dried. Follicle-stimulating hormone activity was tested by a modification