MINERAL COMPOSITION OF RUMINANT SALIVA 1. PHOSPHORUS, SODIUM AND POTASSIUM VALUES AND THE EFFECT OF PHOSPHORUS SUPPLEMENTATION

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INTRODUCTION

In the early 1920's Sir Arnold Theiler through classical research in South Africa associated the diseases of lamsiekte and styfsiekte in cattle with a deficiency of phosphorus in the diet. This dietary deficiency was correlated with periods of low rainfall and a lack of phosphorus in the soil. These conditions, which resulted in enormous losses to the livestock industry in South Africa, are now known to have a world-wide distribution, including several areas in the United States.

In Oklahoma and other Southwestern areas, the lack of phosphorus becomes an important consideration because of the fundamental nature of the cattle enterprise. Frequently, in these areas, roughages low in phosphorus constitute either part or all of the ration for beef cattle. Low production and inefficient gains are the direct result of a phosphorus deficiency. Death is often the indirect result. The widespread occurrence of these losses and the realization of their economic importance have aroused the interest of many scientific workers. In more recent years much information has become available which helps to elucidate the role of phosphorus in some physiological processes of ruminant nutrition.

In addition to its structural function, phosphorus accounts for 0.2 percent of the soft tissues within the body. According to Morrison (1947), phosphorus is a vital ingredient in the proteins that are present in the nuclei of all body cells. It is also present in the phospholipids which are essential parts of all living protoplasm. Phosphorus is now known to be important in energy transformation, and is believed to be

indispensible in carbohydrate, fat and protein metabolism. It is also a constituent in some of the blood buffers which aid in maintaining the acid-base balance of the body.

Depraved and depressed appetities in cattle are symptoms that are observed often by cattle producers. These have been described as the most obvious clinical symptoms of a phosphorus deficiency in cattle. The economic losses that can be associated with these symptoms are substantial; yet, the relationship between phosphorus deficiency and these symptoms is not known.

It is known that changes in the ration of ruminants alter the microflora of the rumen and consequently the digestive function of this organ. Since saliva aids in maintaining a constant rumen environment, it seems logical to assume that secondary salivary changes might either prevent or prolong the abnormalities that are expressed outwardly as depraved or depressed appetite. The data presented in this thesis constitute results of research initiated to study the role of phosphorus in ruminant digestion. Particular emphasis is placed on the effect of the diet on the salivary phosphorus concentration.

REVIEW OF LITERATURE

Phosphorus in Ruminant Nutrition

Numerous experiments show the economic importance of phosphorus supplementation of low-phosphorus rations. Theiler, Green and DuToit (1928) reported a 29 percent increase in calf crop due to phosphorus supplementation of rations for range beef cattle. Black, Ellis and Jones (1942) in an experiment in Texas found that phosphorus supplementation resulted in a 30 percent larger calf crop, and a net gain of 70 pounds per calf at weaning time. Knox and associates (1942) studied the value of phosphorus supplements for range cattle in New Mexico. They reported that each dollar invested in phosphorus supplement gave a net return of $16.71. Ross et al. (1949), at the Oklahoma station, demonstrated that cows fed phosphorus supplement at a cost of $1.17 produced 166 pounds more gain than cows not fed supplement.

Theiler et al. (1924) indicated that young, pregnant and lactating animals have a higher dietary phosphorus requirement than older and non-producing animals. The response to 3 ounces of bonemeal, daily, was greater in 300 lb. calves than for older animals. A 40 percent increase in milk flow was observed when lactating cows were given 8 ounces of bonemeal daily. Morrison (1947), states that "high-producing cows and young stock are usually most affected by the deficiency, due to their greater needs for the mineral. In phosphorus-deficient areas cows frequently fail to come in heat regularly and often do not calve more than once in two years." Work with sheep also indicates that the dietary phosphorus requirement depends on the class of the animal (Martin and Pierse, 1934;

Briggs et al., 1950).

Phosphorus deficiencies generally occur when animals are fed roughages that are produced on phosphorus deficient soils (Theiler et al., 1924; Whitehair, 1951). Walters and co-workers (1951) observed significant differences in phosphorus retention between lambs that were fed hay from phosphorus fertilized and unfertilized pastures. These differences were in favor of the fertilized pastures.

Theiler et al., (1924) found that phosphorus deficiencies were correlated with periods of low rainfall. In 1937, Watkins presented an immense amount of data pertaining to the composition of native grasses in New Mexico. Phosphorus values of 0.03 and 0.2 percent for winter and summer grasses, respectively, (fresh-weight basis) were not uncommon. Robertson (1948) reported similar seasonal variations at Wilburton, Oklahoma.

Lamsiekte, an enzootic disease in cattle of South Africa, was found to be an indirect consequence of a dietary phosphorus deficiency (Theiler et al., 1924). The direct cause of death was botulism brought about by ingestion of infected carcass debris. Depraved appetites did not occur when dietary phosphorus was adequate, and consequently the debris was left undisturbed. In 1928, these workers reported that 53 percent of 100 phosphorus-deficient cows had died within two years. During the first year 25 of the cows died from botulism, five died from plant poisoning and five from malnutrition. This indicated that the larger percentage of the losses was the indirect result of abnormal appetites. Whitehair (1951) reported a high incidence of traumatic pericarditis, gastritis and liver abscesses among phosphorus-deficient cattle at Wilburton, Oklahoma. These injuries were believed to be due mainly to the ingestion

of foreign materials, such as nails, sand, bones and rocks.

The symbiotic role of the rumen microflora is now recognized as being highly important in ruminant digestion. Experiments at Cambridge University (Phillipson et al., 1942) showed that the volatile fatty acids, acetic, propionic, and butyric, were the end products of carbohydrate digestion in the rumen and that these acids which resulted from bacterial fermentation were absorbed directly from the rumen. Phillipson et al., (1942) also noted that doses of lactic acid introduced into the rumen of sheep caused a rapid disappearance of the above volatile acids. This was presumably due to the cessation of bacterial fermentation. South African workers, Clark, Oyaert and Quinn (1951), stated that the rumen microflora of starved sheep could not produce the acids that were necessary for protection against urea toxicity. Hungate and co-workers (1952) studied the bacterial changes that were associated with acute indigestion in sheep. A lower pH was observed when the metabolic products exceeded the absorptive capacity of the rumen. This condition resulted in reduced growth of the rumen micro-organisms and reduced utilization of glucose.

Burroughs et al. (1949), at the Ohio station, observed that dietary changes could alter the activity of rumen micro-organisms in vivo. The addition of starch reduced the digestion of poor quality roughages; however, it had no effect on the digestion of alfalfa hay. Burroughs and co-workers (1950) noted an increase in corncob digestion when alfalfa ash was added to a basal ration of corncobs, dried skimmilk and corn starch. They suggested that an unknown factor(s) was present in alfalfa hay, and that this factor(s) was either partially or totally associated with the inorganic constituents in the hay. Burroughs and associates (1951) used the in vitro technique to show that both iron and phosphorus

increased cellulose digestion in cultures that were inoculated with rumen micro-organisms. Van der Wath (cited by McDougall, 1948) postulated that phosphorus, when added to a phosphorus-deficient diet, stimulated the growth of micro-organisms.

Swedish workers, Hoflund and Hedstrom (1949), observed poor appetites among animals fed straw and paper pulp; however, the animals managed excellently when molasses, casein, minerals and cod liver oil were added to the ration. When sugar or water-soluble phosphates were excluded from the ration, the rumen fungi died within two and one-half months. The addition of cultured fungi and sugar or water-soluble phosphate was necessary to restore the health and appetite of the animal. The function of oxygen utilization and maintenance of anaerobic conditions was accredited to the rumen fungi. These workers postulated that dietary water-soluble phosphates were necessary for the maintenance of a normal population of rumen microflora. Clark (1953) presented contradictory evidence concerning the importance of water-soluble phosphates to the ruminant. This evidence will be presented later.

Characteristics of Ruminant Saliva

Saliva functions to assist mastication and deglutition in all domestic animals. In ruminants it has another important role. Saliva serves as a lubricant for the transport of ingesta, both back to the mouth for remastication and onward through the stomach to the small intestine. It also functions as a buffered medium in which the rumen microflora can flourish.

The alkalinity of ruminant saliva indicates its importance in neutralizing acids that are produced in the rumen. Babkin, in his textbook, (1944) stated that Mangold collected 50 liters of mixed saliva from the

ox during a course of 24 hours. This collection was found to contain 300 to 350 gm. of sodium carbonate. Babkin also cited the work of Scheunert and Trautmann who reported that the parotid saliva of sheep contained 0.56 to 0.77 percent sodium carbonate. Popov (cited by Babkin, 1944) found that the pH of parotid saliva was 8.09 to 8.42 in sheep and 8.12 to 8.32 in the goat. McDougall (1948), at Cambridge, conducted comprehensive research to determine the composition of saliva in sheep. Seven parotid saliva samples were found to have an average pH of 8.23.

In addition to other functions, the salivary secretion contributes substantially to the volume of rumen fluid. According to McDougall (1948), Colin observed that the parotid glands of the ox secreted 56 kg. of saliva per day. Markoff's cow (cited by McDougall, 1948) secreted 50 liters of mixed saliva per day. McDougall (1948) reported the rate of parotid saliva secretion in six sheep. A single gland secreted, on the average, 1310 ml. per day. This amount is more than twice that reported in previous observations (Scheunert and Trautmann, 1921; Popov, 1921) cited by McDougall (1948).

Mangold (cited by Babkin, 1944) noted the absence of diastatic enzymes in the parotid saliva of ruminants. Wegner *et al.* (1940) demonstrated the absence of proteolytic enzymes and maltase in mixed saliva collected from the cow. Only slight amylolytic activity was observed.

McDonald (1951) of Cambridge stated that a small but significant amount of nitrogen is added to the rumen contents by the saliva. This nitrogen is secreted chiefly as urea which is readily converted to ammonia and utilized by the rumen micro-organisms. He also suggested that absorbed ammonia may, after passing through the liver, return to the rumen *via* the saliva.

Clark (1953) made a similar postulation regarding phosphorus. Water-soluble phosphorus was thought to circulate from the blood through the saliva to the rumen and back via the small intestine. In support of this postulation the small intestine was found to be the principal site of phosphorus reabsorption. Also, there was a lack of correlation between the amount of phosphorus in the blood and the phosphorus concentration in the rumen contents.

Clark (1953) stated that Brunnich and Winks found the phosphorus concentration in the rumen of sheep to be three times the amount that could be accounted for in the ingested feed. Clark also cited Watson who reported that large amounts of water-soluble phosphates were secreted in the saliva of sheep. Clark's study (1953) indicated that active salivary phosphorus secretion was characteristic of ruminants. The average values were 68.5 and 19.6 mg. of phosphorus per 100 ml. of saliva from the sheep and cow, respectively. The comparable values were 1.1 and 0.2 mg. of phosphorus per 100 ml. of saliva from the horse and dog, respectively. Differences in salivary phosphorus values of phosphorus-deficient versus bonemeal fed animals were significant. The average values were 10 mg. percent for six phosphorus-deficient cows and 18 mg. percent for six bonemeal-fed cows. The average salivary phosphorus values, for three phosphorus-deficient and four normal sheep, were 33.3 and 68.5 mg. percent, respectively.

McDougall (1948) made a detailed analysis of mixed saliva from sheep. Samples collected from five animals contained on the average, 59 mg. of phosphorus, 445.5 mg. of sodium and 33.8 mg. of potassium per 100 ml. of saliva.

DEVELOPMENT OF TECHNIQUES

Saliva Collection

A review of literature indicated that three methods have been used to obtain saliva. Most frequently saliva has been collected from a cannula inserted into the parotid duct. Past observations (McDougall, 1948) indicate that the parotid gland may function abnormally as a result of the cannulation. In two out of six of McDougall's animals, the secretion became negligible on the first and second days after cannulation of the duct. In a third sheep the gland was altered structurally and infection was noted.

A second method for saliva collection involved the use of anesthetized animals.

The third method utilized a sponge gag held in place with a wire nose-piece and suitable ties. An adaptation of this method was selected as being most suitable for the conditions of the following experiments.

Details pertaining to the collection and analysis of saliva were not given in the literature reviewed. When the first attempts were made to collect and analyze saliva, it became obvious that a technique would have to be developed and standardized. Thus, studies were conducted to determine the technique that would give the most reliable results.

Saturated Versus Unsaturated Cellulose Sponges

During the first collection period, records were kept as to whether each sample was collected with a dry, clean individual sponge, or one that had been saturated with saliva from the previous animal. There was considerable doubt that a sponge which had been used to collect from

previous animals would yield saliva that was consistent with that collected with a clean individual sponge. The belief was that residual saliva in the sponge would influence the composition of saliva collected from the next animal. This study was conducted to see if such a condition existed.

DuPont cellulose sponges were used to collect two samples of saliva from each of three steers and one heifer averaging 473 lb. in weight. To obtain the first sample, one sponge was used to collect from all four animals. The sponge had been saturated with saliva from animal number 4 before collecting the first sample from animal number 1. The second sample was obtained by using a dry, clean individual sponge for each animal. The samples were analyzed for phosphorus by the Fiske and Subbarow (1925) method for plasma-inorganic phosphorus.

The results of the above comparison are presented in Table 1. Phosphorus values were significantly higher in samples that were collected with saturated sponges ($P < 0.05$). Apparently significant amounts of phosphorus were adsorbed by the dry sponge on initial contact with the saliva.

Rubber Versus Cellulose Sponges

The same cattle were used in this study as in the previous one. Four sponges, two rubber and two cellulose, were assigned to the animals at random. Four consecutive saliva samples were collected from each animal. A sample consisted of the amount of saliva that could be squeezed by hand from the sponge after one swabbing of the oral cavity.

The results are shown in Table 2. The differences between the two treatments were significant ($P < 0.01$). Samples collected with rubber sponges gave higher phosphorus values than samples collected with

cellulose sponges.

Table 1. Salivary inorganic phosphorus, mg. per 100 ml., as obtained with saturated and unsaturated cellulose sponges.

Animal number	Saturated sponge	Unsaturated sponge
1	26.0	19.4
2	20.8	16.6
3	18.2	12.0
4	19.0	16.8

Table 2. Salivary inorganic phosphorus, mg. per 100 ml., as obtained with rubber and cellulose sponges.

Animal number	Sponge used	Phosphorus in four consecutive samples 1	2	3	4
1	rubber	20.4	29.0	31.2	34.4
2	rubber	25.2	24.4	25.4	23.4
3	cellulose	13.4	17.2	20.8	22.4
4	cellulose	15.8	16.0	18.0	16.4

Saturated Rubber Versus Saturated Cellulose Sponges

Four animals, the same as those used in the previous studies, were passed through the chute. Two cellulose sponges were saturated with saliva from animals 3 and 4. These sponges were placed aside for 20 to 30 minutes, while rubber sponges were being used to collect from animals 1 and 2. Animals 3 and 4 were then returned to the chute and saliva samples were obtained by using rubber and cellulose sponges alternately. The rubber sponges were the same as those used to collect from animals 1 and 2 a few minutes earlier. Two to three minutes elapsed between consecutive collections.

The results are summarized in Table 3. The data for animals 1 and

2 show that salivary phosphorus was adsorbed by the unsaturated rubber sponges. The maximum amount retained neared the peak in the fourth sample, and then became constant in samples 5 and 6. The differences between saturated rubber and cellulose sponges were nonsignificant ($P > 0.3$).

Table 3. Salivary inorganic phosphorus, mg. per 100 ml., as obtained with saturated rubber and saturated cellulose sponges.

Animal number	Sponge used	Phosophorus in six consecutive samples 1	2	3	4	5	6
1	rubber	18.3**	26.2	27.8	31.6	32.4	32.4
2	rubber	23.0**	28.8	29.0	30.2	31.0	31.0
3	alternate	25.2	28.2*	29.8	30.0*	31.0	32.2*
4	alternate	33.0*	26.6	29.8*	29.6	28.2*	31.2

* Collected with cellulose sponges.
** Collected with unsaturated (clean, dry) rubber sponges.

Adopted Collection Technique

As a result of the previous studies, the following technique was adopted for the collection of saliva from cattle.

A DuPont cellulose sponge was manipulated with a pair of 20-inch forceps. In each case the sponge was saturated with saliva 20 to 30 minutes before collections were started. The saliva was squeezed from the saturated sponge and discarded when the collections were begun. The oral cavity was then swabbed and the contents of the sponge were squeezed into a clean glass beaker. After this procedure was completed two to three times, the composite sample was transferred to a clean, properly labeled test tube. The saliva sample was frozen until a chemical analysis could be made.

Saliva Analysis

The method of Fiske and Subbarow (1925) for plasma-inorganic phosphorus was adopted for analysis of inorganic phosphorus in saliva. The addition of dilute saliva (1 to 100 aqueous dilution) to trichloroacetic acid resulted in a cloudy solution that could not be cleared by centrifugation. Therefore, dilute saliva was used directly in the analysis without other preliminary treatment. The following study was conducted to determine the effect of dilute saliva on recovery of phosphorus from solutions of known phosphorus concentration.

Effect of Saliva on Recovery of Known Quantities of Phosphorus

Five samples of saliva from cattle were chosen at random. Five ml. of each sample, after a dilution of 1 to 100, was placed in separate colorimeter tubes. One ml. of molybdate I solution and 4 ml. of color reagent (1 to 100 dilution of standard aminonaphtholsulfonic acid reagent) were added to each tube. The light absorption of the colored solution was measured in an Evelyn photoelectric colorimeter using a 660 mu. filter. The phosphorus concentrations were calculated from a chart of previously determined values obtained with solutions of known phosphorus concentration.

The above procedure was repeated with the exception that 2 ml. of water and 2 ml. of a solution containing 0.005 mg. of phosphorus per ml. replaced 4 ml. of dilute saliva.

Previously the rate of recovery of phosphorus from the solution of known phosphorus concentration was ascertained to be 97 percent. Therefore, salivary phosphorus was obtained by subtracting 0.0097 mg. from the total phosphorus in the tube. On comparing the two methods, appropriate adjustment was made for differences in saliva dilution.

The results are presented in Table 4. Evaluation of the two methods for determining salivary phosphorus indicated that the differences were nonsignificant ($P > 0.3$). Thus, the presence of dilute saliva did not affect the recovery of known quantities of phosphorus.

Table 4. Inorganic phosphorus as determined in saliva and saliva plus standard phosphorus solution.

Sample number	Saliva mg./100 ml.	Saliva plus standard mg./100 ml.
10	15.8	17.0
46	9.2	9.0
49	7.8	8.0
51	11.4	12.0
54	15.4	15.0

Adopted Analytical Technique

The composite saliva sample was cleared of sediment by centrifuging at 1800 to 2000 r.p.m. for 30 minutes. One ml. of the clear saliva was placed in a volumetric flask and diluted to 100 ml. with distilled water. From this dilution, a 5-ml. aliquot was taken for analysis and placed in a colorimeter tube. One ml. of molybdate I solution and 4 ml. of color reagent (1 to 10 dilution of standard aminonaphtholsulfonic acid reagent) were added to the tube. A blank solution containing 5 ml. of water, 1 ml. of molybdate I and 4 ml. of color reagent, was prepared at the same time. The contents of each tube were mixed by gentle shaking. The tubes were stoppered and placed aside for 15 minutes.

With the blank solution in place, the colorimeter was set to read 100. The blank was removed and the air setting recorded. Readings were then recorded for each sample. When necessary, adjustments were made in order to maintain the proper air setting.

The technique was adapted to sheep saliva by changing the dilution factor. Sheep saliva was diluted from 1 ml. to 250 ml. In other respects the technique remained the same.

The foregoing technique was used for inorganic phosphorus analysis in all subsequent saliva samples collected in this study.

EXPERIMENTAL PROCEDURES

Past research at the Oklahoma station has indicated an inverse relationship between the plasma carotene and plasma-inorganic phosphorus levels in range beef cattle fed adequate- and low-phosphorus rations (Ross and Gallup, 1949). In a later study Thomas, Gallup and Whitehair (1953) fed limited but equal amounts of carotene to pregnant cows on adequate- and phosphorus-deficient rations. These cows were continued on experiment through the 20th week of lactation. Plasma carotene levels were found to be higher in the phosphorus-deficient cows.

Thomas (1951) observed a similar relationship in Hereford steers fed adequate- and low-phosphorus rations containing adequate carotene. Gallup and associates (1953) increased the carotene intake in steers to ten times their minimum requirement. They stated that the plasma carotene levels were consistently higher in phosphorus-deficient steers than in steers fed adequate phosphorus.

At Montana, Lewis and co-workers (1951) drenched six phosphorus-deficient steers with mono-sodium phosphate in amounts sufficient to provide 7.5 gm. of phosphorus per 100 pounds of body weight. Their report indicates plasma calcium dropped 2 mg. per 100 ml. within 4 hours. Plasma-inorganic phosphorus increased 9.5 mg. per 100 ml. in the same period of time. Severe convulsions were observed in one steer. Incoordination and arched backs were observed in the other five steers.

In each instance, the literature reviewed dealt with cattle fed low-phosphorus rations. Reports of past research have not been found to show the effects of large doses of phosphorus on carotene and phosphorus metabolism in cattle previously fed high-phosphorus rations.

Therefore, a series of experiments were conducted to study these effects and also the effect(s) on the composition of saliva.

The results are presented with each experiment.

Experiment I

Phosphorus Administered to Normal Cattle as Mono-sodium Phosphate in the Feed

The animals used were three steers and one heifer averaging 473 pounds in weight. These animals were group fed 5 pounds of bright prairie hay and one-half pound of cottonseed meal per head twice each day. Block salt and water were available ad libitum. After 15 days on feed the salt was replaced by a mixture containing two parts salt and one part steamed bonemeal.

After the animals had been on feed for 50 days, blood samples were taken by venous puncture and the four animals were paired on the basis of their plasma carotene levels. One animal of each pair was chosen at random to receive treatment. The other member of each pair served as a control. The experimental period extended from 8:00 A.M. to 4:30 P.M.

At 7:15 A.M. April 28, 1955, blood and saliva samples were obtained by methods previously described. The animals were divided as to treatment and placed in two pens. At 8:00 A.M. the animals on treatment were offered one pound of cottonseed meal mixed with sufficient monosodium phosphate to provide 3.75 gm. of phosphorus per 100 pounds of body weight. These animals consumed the ration reluctantly, therefore, feed was withheld from the control animals until it was apparent that both groups would finish eating at approximately the same time. The control steers were fed the same amount of cottonseed meal without added phosphorus. Hay was withheld from both groups. Blood and saliva

samples were obtained at 2, 4 and 8 hours after the rations were consumed.

The plasma was analyzed for carotene and vitamin A. (Kimble, 1939) and inorganic phosphorus (Fiske and Subbarow, 1925). The saliva was analyzed for inorganic phosphorus as described under analytical techniques.

The results of the experiment are summarized in Table 5. Salivary phosphorus values increased rather sharply between the zero hour and 2 hours after treatment. Values in subsequent samples showed a gradual decrease from the two-hour to the eight-hour collection period. This trend was apparent in all four animals; therefore, it can not be accredited to mono-sodium phosphate feeding. A similar trend of less magnitude was observed in plasma vitamin A.

The plasma-inorganic phosphorus values of the control animals remained rather constant while the value of the treated animals increased 1.46 mg. per 100 ml. in 8 hours. A trend toward a direct relationship between plasma-inorganic phosphorus and plasma carotene values was observed. Plasma carotene in animal 1 increased from 96.8 mcg. initially to 108.8 mcg. per 100 ml. 2 hours after treatment. It then decreased to 101.2 mcg. per 100 ml. at 4 hours and remained constant until the 8th hour. Plasma carotene in control animal 4, of the same pair, showed a reverse trend. It decreased from 124 mcg. initially to 113.2 mcg. per 100 ml. at 2 hours and then increased gradually until the 8th hour. No similar trend was observed in the animals of pair 2; however, their initial plasma carotene values were, on the average, 37 mcg. per 100 ml. lower than the corresponding values of pair 1.

Table 5. Plasma and saliva values of cattle given 3.75 gm. of phosphorus per 100 lb. of body weight. Experiment I. Phosphorus administered as NaH_2PO_4 in the feed.

Animal number	Pair number	Body Weight (lb.)	Hours after admin.	Plasma			Saliva
				Carotene** mcg./100 ml.	Vitamin A** mcg./100 ml.	Inorg. P*** mg./100 ml.	inorg. P mg./100 ml.
1	1*	455	0	96.8	26.0	6.80	26.0
1			2	108.8	28.6	7.40	34.8
1			4	101.2	28.3	7.40	35.6
1			8	101.2	28.3	9.00	31.0
2	2*	515	0	69.6	13.4	6.92	21.8
2			2	71.2	14.5	6.80	29.8
2			4	69.6	14.6	7.04	26.6
2			8	69.6	10.1	7.64	26.2
3	2	525	0	78.0	10.0	7.64	21.2
3			2	76.8	14.1	7.20	24.8
3			4	76.8	14.7	6.64	25.0
3			8	75.2	14.2	7.40	24.8
4	1	575	0	124.0	13.8	7.84	22.4
4			2	113.2	16.6	7.72	34.2
4			4	121.2	13.3	7.40	31.6
4			8	124.0	15.8	7.84	32.4

* Received 3.75 gm. of phosphorus per 100 lb. of body weight.
** Courtesy of Dr. J. P. Fontenot, formerly of the Department of Physiology and Pharmacology.
*** Courtesy of Dr. W. D. Gallup and Mr. Bill Davis, Department of Agricultural Chemistry.

Experiment II

Phosphorus Administered to Normal Cattle as Mono-sodium Phosphate in Capsules

The animals used in this experiment were the same as those used in experiment I. The pairs remained the same; however, animal 4 in pair 1 received the treatment due to random choice. There was no change in pair 2.

Because the animals were reluctant to consume feed containing mono-sodium phosphate in experiment I, in this experiment the mono-sodium phosphate was administered in gelatin capsules in such amounts as to furnish 7.5 gm. of phosphorus per 100 pounds of body weight. The dose required from 6 to 8 capsules per animal. Feed was offered the evening previous to the experiment and withheld thereafter until the experiment was complete (6:30 A.M. to 5:00 P.M.).

At 6:30 A.M. May 4, blood and saliva samples were taken for analysis. Immediately afterwards, capsules of mono-sodium phosphate were administered to animals 2 and 4. Blood and saliva samples were collected at 1, 4, 8 and 10¼ hours after treatment. The plasma was analyzed for calcium by the Clark-Collip modification of the Kramer-Tisdall method as described by Hawk and Bergeim (1926). Other analytical techniques were the same as mentioned in experiment I.

The results of experiment II are presented in Table 6. These results indicate that phosphorus, when administered at the rate of 7.5 gm. per 100 pounds of body weight, has no effect on plasma carotene and vitamin A levels in cattle previously fed adequate phosphorus rations. Saliva and plasma-inorganic phosphorus values remained relatively constant in the control animals; however, they changed substantially in the treated

Table 6. Plasma and saliva values of cattle given 7.5 gm. of phosphorus per 100 lb. of body weight. Experiment II. Phosphorus administered as NaH_2PO_4 in capsules.

Animal number	Body weight (lb.)	Hours after admin.	Plasma				Saliva
			Carotene** mcg./100 ml.	Vitamin A** mcg./100 ml.	Inorg. P** mg./100 ml.	Calcium*** mg./100 ml.	inorg. P mg./100 ml.
1	470	0	96.8	26.0	7.68	9.4	25.4
1		1	92.4	26.1	6.84	11.3	26.2
1		4	94.0	25.6	6.64	11.3	----
1		8	91.2	28.5	6.04	11.2	27.4
1		10¼	----	----	----	----	23.4
2*	515	0	76.8	15.4	6.82	10.8	21.8
2		1	74.0	16.3	7.20	10.0	28.6
2		4	78.0	21.3	8.66	9.6	30.8
2		8	80.8	17.1	10.08	9.9	38.4
2		10¼	76.8	16.8	10.08	9.2	----
3	545	0	78.0	21.3	8.04	10.7	25.4
3		1	79.6	15.2	7.64	10.9	25.2
3		4	75.2	15.5	7.64	8.7	27.8
3		8	78.0	14.6	7.88	10.8	27.8
3		10¼	80.8	16.4	7.68	10.1	27.8
4*	585	0	122.4	15.9	7.32	11.3	22.4
4		1	119.6	16.1	8.00	7.4	24.2
4		4	114.8	14.4	9.12	10.7	39.2
4		8	119.6	14.8	10.68	9.4	35.2
4		10¼	121.2	20.0	11.04	10.3	37.4

* Received 7.5 gm. of phosphorus per 100 lb. of body weight.
** Courtesy of Dr. J. P. Fontenot, formerly of the Department of Physiology and Pharmacology.
*** Courtesy of Dr. W. D. Gallup and Mr. Bill Davis, Department of Agricultural Chemistry.

animals. The upward trend for plasma phosphorus was gradual but consistent. The average initial plasma phosphorus value was 7.7 mg. per 100 ml. Ten hours after treatment the average was 10.56 mg. Apparently collections were not continued long enough to establish a peak for plasma phosphorus. Changes in saliva phosphorus values, from zero to the 10th hour, were approximately 4.5 times greater than the corresponding interval changes in plasma phosphorus. No consistent differences were noted in the plasma calcium values; however, a slight downward trend may have existed in animal 2.

Experiment III

Phosphorus Administered to Normal Cattle as Mono-sodium Phosphate in Capsules

The results of experiment II indicated that saliva and plasma-inorganic phosphorus in cattle could be increased by administering large doses of phosphorus orally; however, evidence was not sufficient to determine the extent of these increases. Therefore, collections were extended through a 24-hour period in an effort to establish a peak in saliva and plasma-inorganic phosphorus levels. Saliva studies were extended to determine the effect(s) of large phosphorus doses on the sodium and potassium in the salivary secretion.

The animals, the conditions and the treatments in this experiment were the same as those in experiment II except that the collection intervals were changed and the period of observation extended.

After the initial blood and saliva samples were taken, phosphorus as mono-sodium phosphate, was administered at the rate of 7.5 gm. per 100 pounds of body weight to animals 2 and 4. Additional blood and saliva

samples were taken at 6, 8, 10, 12, 14 and 24 hours after treatment.

The saliva was analyzed for sodium and potassium by the internal standard method of flame photometry. Previous reference has been made to the analytical methods for determining plasma calcium, and plasma- and saliva-inorganic phosphorus.

The results are shown in Table 7. Average plasma phosphorus values in the control animals decreased nearly 1.5 mg. per 100 ml. during the first 6 hours and remained constant at about 7 to 7.5 mg. during the remainder of the period. An upward trend was observed in the plasma phosphorus of the treated animals. The increase in the average value was from 8.25 mg. at the zero hour to 10.66 mg. per 100 ml. 6 hours after treatment. During the next 4 hours it increased to 11.04 mg. The subsequent values remained at about 11.04 mg. and fluctuated only slightly during the remainder of the 24-hour period.

Salivary phosphorus increased steadily in the treated animals from the average initial value of 25.9 mg. per 100 ml. to a peak of 41.5 mg. at the 10th hour. A gradual decline was then observed. The average value at 24 hours was 32.3 mg. per 100 ml. The increase in the average salivary phosphorus value, from zero to the 10th hour was five times the corresponding increase in plasma phosphorus. A similar ratio of 4.5:1 was reported in experiment II. A significant ($P < 0.05$) correlation was obtained between saliva and plasma phosphorus values in the treated cattle.

Plasma calcium values in control animal 1 decreased from an initial value of 9.8 mg. to 9.3 mg. per 100 ml. in 8 hours. From this point onward they increased gradually and were above 10 gm. per 100 ml. at 14 and 24 hours. A different trend was observed in treated animal 4. Plasma

Table 7. Plasma and saliva values of cattle given 7.5 gm. of phosphorus per 100 lb. of body weight. Experiment III. Phosphorus administered as NaH_2PO_4 in capsules.

Animal number	Body weight (lb.)	Hours after admin.	Plasma*		Saliva		
			Inorg. P mg./100 ml.	Calcium mg./100 ml.	Inorg. P mg./100 ml.	Sodium mg./100 ml.	Potassium mg./100 ml.
1	490	0	9.44	9.8	25.2	580	43
1		6	6.12	9.5	28.6	590	46
1		8	6.96	9.3	23.0	560	50
1		10	6.64	9.4	23.6	530	34
1		12	7.08	9.4	29.8	580	53
1		14	6.28	10.2	24.2	580	60
1		24	6.12	10.5	22.0	540	47
2**	530	0	7.74	----	26.0	580	42
2		6	10.44	----	35.2	610	60
2		8	9.92	----	37.0	590	49
2		10	10.08	----	39.2	630	50
2		12	11.04	----	38.4	600	54
2		14	11.04	----	35.6	570	60
2		24	10.80	----	31.4	560	47
3	555	0	7.68	----	24.6	600	38
3		6	8.24	----	30.8	590	60
3		8	8.24	----	28.2	590	49
3		10	8.00	----	29.6	630	55
3		12	7.48	----	28.8	490	62
3		14	7.48	----	25.8	---	--
3		24	8.04	----	24.8	530	56
4**	605	0	8.76	9.4	25.8	580	48
4		6	10.88	8.7	37.6	560	56
4		8	11.64	9.0	41.6	610	49
4		10	12.00	8.7	43.8	610	57
4		12	10.80	8.3	39.2	590	56
4		14	11.36	8.8	36.0	580	50
4		24	10.80	8.6	33.2	570	48

** Received 7.5 gm. of phosphorus per 100 lb. of body weight.
* Courtesy of Dr. W. D. Gallup and Mr. Bill Davis, Department of Agricultural Chemistry.

calcium in this animal dropped to 8.7 mg. at 6 hours and ramained at 9 mg. or below throughout the rest of the experiment. A low calcium value of 8.3 mg. per 100 ml. was observed 12 hours after treatment. These values closely approximate those associated with a calcium deficiency.

No apparent differences were observed in the salivary potassium or sodium of the two groups. The average initial values for four animals were 42.7 and 585.0 gm. per 100 ml. for potassium and sodium respectively.

Experiment IV

Phosphorus Administered to Phosphorus-deficient and Normal Cattle as Mono-sodium Phosphate in Capsules

Previous reference has been made to the work at Montana which indicated that abnormalities such as severe convulsions, incoordination and arched backs were the result of drenching phosphorus-deficient steers with large doses of phosphorus. Thus far, studies in which steers on adequate phosphorus rations were used had not verified the Montana results. Phosphorus-deficient steers became available, and were used to study their response to large doses of phosphorus.

Four steers were used. Two of the steers, which averaged 431 pounds, had been fed a ration containing 0.094 percent phosphorous for 176 days. The remaining two steers averaged 538 pounds and had been fed the same ration for 66 days. During the next 110 days this ration had been supplemented with 0.1 percent phosphorus as mono-sodium phosphate. The initial blood and saliva samples were obtained at 6:00 A.M. June 21. The steers were dosed by capsule with 7.5 gm. of phosphorus, as mono-sodium phosphate, per 100 pounds of body weight. Samples of both blood and saliva were taken at 4, 6, 8 and 10 hours and additional blood samples

were taken at 12 hours after the capsules were administered.

The plasma and saliva were analyzed for inorganic phosphorus by methods that were used in previous experiments.

Table 8 summarizes the results of this experiment. The average initial phosphorus values in the phosphorus-deficient steers were 2.32 and 12.2 mg. per 100 ml. of plasma and saliva, respectively. The corresponding values for the normal steers were 6.32 and 19.6 mg. per 100 ml. of plasma and saliva, respectively. An upward trend was observed in plasma and saliva phosphorus in both normal and deficient steers. The salivary and plasma phosphorus in the phosphorus-deficient steers and the plasma phosphorus in the normal steers reached the high level at the 10-hour period. The salivary phosphorus in the normal steers reached a peak at the 8-hour period. The high phosphorus levels, in the plasma and saliva of the normal steers and the saliva of deficient steers were twice the initial levels. The high plasma phosphorous level in the phosphorus-deficient steers was 2.6 times that of the initial level.

When plotted, the curves for plasma phosphorus, in both normal and deficient steers, ascended in a parallel manner. Both curves were level from the 6th to the 8th hour. From the 8th to the 10th hour, the ascent was rather sharp and was followed by a downward trend to the 12th hour. The parallelism between the saliva curves for the two groups was not noticeable after the 6-hour collection period.

From the zero to the 8-hour period, the salivary phosphorus in the normal steers increased 4.2 times as fast as the plasma phosphorus. Similar ratios 4.5 and 5.0:1 were reported in experiments II and III, respectively. In the phosphorus-deficient steers, the ratio of the

Table 8. Plasma and saliva phosphorus values of steers after dosing with 7.5 gm. of phosphorus per 100 lb. of body weight. Experiment IV Phosphorus administered to phosphorus-deficient and normal cattle as NaH_2PO_4 in capsules.

Animal number*	Body weight (lb.)	Hours after Admin.	Plasma inorg. P mg./100 ml.	Saliva Inorg. P mg./100 ml.
2	430	0	2.32	10.2
2		4	4.24	17.2
2		6	4.44	20.4
2		8	4.00	19.0
2		10	6.80	26.6
2		12	4.20	----
4	432	0	2.32	14.2
4		4	2.88	20.4
4		6	3.80	17.2
4		6	4.28	25.6
4		10	5.32	21.8
4		12	5.80	----
5	515	0	7.04	19.6
5		4	11.72	25.2
5		6	10.64	27.4
5		8	11.84	34.2
5		10	11.72	25.8
5		12	11.92	----
7	562	0	5.60	19.6
7		4	6.64	33.2
7		6	9.56	28.2
7		8	10.36	47.6
7		10	12.52	35.2
7		12	11.92	----

* Animals 2 and 4 were on the phosphorus-deficient rations.

salivary phosphorus increase to the plasma phosphorus increase at 10 hours, when the high phosphorus levels were reached, was 3.3.

No apparent abnormality was produced in these steers by this level of phosphorus administration.

Experiment V

The Effect of Two Phosphorus Supplements Upon Salivary and Plasma Phosphorus in Phosphorus-deficient Cattle

Twenty-four heifers, 12 Hereford and 12 Angus-Hereford crossbred, averaging 411 pounds were started on a phosphorus-deficient ration on December 27, 1954. The ration contained 0.094 percent phosphorus in addition to adequate amounts of the other known essential nutrients. These animals were continued on the phosphorus-deficient ration until they were allotted to three groups according to their body weight. At this time, March 3, 1955, the heifers had gained an average of 79 pounds per head in 66 days.

Just prior to allotment, both blood and saliva samples were obtained from each animal. Both the plasma and saliva were analyzed for inorganic phosphorus by methods that have been described previously. The analytical data are presented in Table 9.

All of the heifers were self-fed. Those in lot 1 were continued on the phosphorus-deficient ration. Those in lots 2 and 3 were fed the same ration plus 0.05 percent phosphorus. The phosphorus was provided by adding colloidal clay to the ration of lot 2, and dicalcium phosphate to the ration of lot 3.

On June 11, 1955, samples of blood and saliva were again obtained and the experiment was terminated. On June 11, the average weights were

Table 9. Saliva and plasma phosphorus values obtained 66 days after 24 heifers averaging 411 lb. were started on the phosphorus depletion phase of experiment V.

Animal number	Saliva inorganic phosphorus mg./100 ml.	Plasma** inorganic phosphorus mg./100 ml.
1	8.4	2.52
2	9.0*	3.68
3	10.4	2.48
4	10.6	3.20
5	9.0	2.64
6	3.0*	2.64
7	13.6	2.72
8	9.2	2.84
9	11.4	3.28
10	15.8	3.76
11	11.8	1.76
12	8.8	3.04
46	9.2	2.24
47	10.8	2.76
48	6.6	3.44
49	7.8	3.64
50	14.4	2.68
51	11.4	2.60
52	12.0	2.52
53	14.8	3.20
54	15.4	2.48
55	13.2	2.60
56	14.6	2.40
57	7.2	3.40
Average	11.22	2.85

* Omitted from the average due to deviations from the adopted collection technique.

** Courtesy of Mr. Ted Long, graduate fellow, Department of Animal Husbandry.

503, 524, and 614 pounds for lots 1, 2, and 3, respectively. Data obtained from the analysis of samples from the two collection periods are shown in Table 10.

An analysis of variance indicated that salivary phosphorus was significantly higher in the supplemented lots than in the basal lot. ($P < 0.05$ and $P < 0.01$ at 45 and 110 days after allotment, respectively).

A comparison between the lots showed that salivary phosphorous in lot 3 was significantly higher than in lot 2, ($P < 0.05$ at both collections).

The difference in average salivary phosphorus values between lots 1 and 2 was 5.5 times the difference in plasma phosphorus between these lots. A similar difference, 5.6 times the difference in plasma phosphorus, was observed between lots 2 and 3.

The ratio of salivary phosphorus to plasma phosphorus was 3.9:1 at the initial collection. The ratios at the final collection were 3.0, 3.7 and 4.0:1 for lots 1, 2 and 3, respectively.

Experiment VI

The Effect of High Dietary Protein and Potassium Levels on the Composition of Saliva in Sheep

A group of lambs being used in a balance study to determine the effect(s) of high levels of dietary protein and potassium on metabolism of various nutrients became available for saliva studies.

The sheep consisted of 12 wether lambs averaging 70 pounds in body weight. The lambs had been in metabolism stalls and fed a ration consisting of chopped alfalfa hay and ground corn for approximately 20 days. At the end of that time, February 6, 1955, two rations were allotted to the lambs at random. Six of the lambs were fed a control ration

Table 10. Saliva and plasma phosphorus values for three lots of heifers that were fed phosphorus from variable sources.

Animal number	Allotment plus 45 days Saliva inorganic P mg./100 ml.	Allotment plus 45 days Plasma* inorganic P mg./100 ml.	Allotment plus 110 days Saliva inorganic P mg./100 ml.	Allotment plus 110 days Plasma* inorganic P mg./100 ml.
		Lot 1--Basal ration		
4			5.2	2.44
6			6.2	1.72
10	10.4	3.08	5.6	2.12
12	14.2	3.36	7.0	2.24
51			6.8	1.52
52	9.2	2.64	4.0	2.12
55			7.2	2.20
56			7.4	1.95
Average	11.27	3.02	6.17	2.04
		Lot 2--Basal ration plus colloidal clay phosphate		
3			9.2	3.68
5	15.8	3.28	10.2	2.52
8			8.8	3.08
11	16.4	3.96	13.0	3.36
47			11.2	2.56
50			10.8	2.92
53			10.6	2.56
57	12.2	2.60	8.2	1.56
Average	14.8	3.28	10.25	2.78
		Lot 3--Basal ration plus dicalcium phosphate		
1			10.2	2.40
2	16.6	4.16	15.8	3.80
7	30.2	6.28	20.8	4.48
9			19.8	4.36
48			9.4	2.32
46			11.2	3.68
49			9.2	2.80
54	26.6	6.40	14.2	5.52
Average	24.4	5.61	15.07	3.67

* Courtesy of Mr. Ted Long, graduate fellow, Department of Animal Husbandry.

containing 12.61 and 0.58 percent of crude protein and potassium, respectively. The other six were fed an experimental ration containing 41.69 and 5.0 percent of crude protein and potassium, respectively. The amounts fed were about 400 gm. of concentrate (Table 11) and 300 gm. of chopped alfalfa hay per day.

Table 11. Feed constituents in two concentrate rations used in experiment VI

Ration constituents	Control ration* (lb.)	Experimental ration** (lb.)
Ground corn	36.00	36.00
Drackett protein	6.00	68.68
Cerelose	65.20	-----
KH_2PO_4	2.54	-----
$CaCO_3$	0.16	-----
$KHCO_3$	-----	1.34
D.L. Methionine	-----	3.98

* 12.1 % crude protein and 0.58% potassium.
** 41.87% crude protein. $KHCO_3$ was added to provide a total of 5.0% potassium.

Saliva samples were taken with sponge gags 48 days after the experiment was started. The analytical data obtained from these samples are presented in Table 12.

After 77 days the experiment was terminated. Samples of both blood and saliva were taken for analysis. The plasma was analyzed for inorganic phosphorus (Fisk and Subbarow, 1925). The saliva was analyzed for sodium, potassium and inorganic phosphorus by methods described previously. The analytical results are summarized in Table 13.

Table 12. Salivary inorganic phosphorus values of sheep fed high dietary levels of protein and potassium for 48 days.

Control ration		Experimental ration	
Animal number	Saliva P mg./100 ml.	Animal number	Saliva P mg./100 ml.
3	95.5	4	111.0
8	65.5	5	112.5
9	97.0	6	92.5
12	106.5	7	99.0
13	107.5	10	66.5
15	85.5	14	136.0

Table 13. Plasma and salivary inorganic phosphorus, and salivary sodium and potassium values of sheep fed high dietary levels of protein and potassium for 77 days.

Sheep number	Plasma*	Saliva*		
	Phos.	Phos.	Sodium	Potassium
Control Ration				
3	8.32	141.0	640	152.5
8	6.80	129.5	580	132.5
9	5.80	87.0	640	207.5
12	7.20	138.0	600	147.5
13	7.84	129.0	630	207.5
15	5.64	90.0	590	95.0
Average	6.93	119.0	613	157.1
Experimental Ration				
4	7.36	148.0	700	162.5
5	8.08	145.5	720	215.0
6	8.80	162.0	710	152.5
7	6.52	126.0	640	250.0
10	6.36	146.5	680	170.0
14	7.12	136.0	540	232.5
Average	7.37	144.0	681	197.1

* mg. per 100 ml.

Significant differences were observed between the two groups in salivary phosphorus values obtained during the first collection ($P < 0.05$). These differences were more pronounced during the second collection period ($P < 0.01$). Average values at the second collection were 119 and 144 mg. per 100 ml. for the control and experimental groups, respectively.

Differences in sodium values between the two groups were also significant ($P < 0.01$). The average values were 613 and 681 mg. per 100 ml. for the control and experimental groups, respectively.

Differences in potassium values were non-significant; however, an average value of 157 mg. per 100 ml. was observed in the control group. This value is 40 mg. lower than the average of the experimental group.

All saliva values obtained in this study are consistantly higher than those reported by previous workers (McDougall, 1948; Clark, 1953). The ratio of salivary phosphorus to plasma phosphorus in the final collection was 19.5:1. This may be compared with the ratio of 18.5:1 as reported by Clark (1953).

McDougall (1948) reported salivary sodium and potassium values for sheep. His values of 445.5 and 44 mg. per 100 ml. for sodium and potassium, respectively, are much lower than those obtained in this study.

DISCUSSION OF RESULTS

The Effect of Large Doses of Phosphorus on Plasma Constituents in Cattle

Carotene and Vitamin A

The results of experiments I and II indicate that large doses of orally administered phosphorus as mono-sodium phosphate has no effect on the plasma carotene and vitamin A levels in cattle previously fed adequate phosphorus rations. One possible exception was observed in pair 1 of experiment I. In this pair there was an apparent increase in plasma carotene and plasma phosphorus as a result of feeding 3.75 gm. of phosphorus per 100 pounds of body weight.

Phosphorus and Calcium

Plasma phosphorus in experiment I was influenced slightly by administering 3.75 gm. of phosphorus per 100 pounds of body weight. The average plasma-inorganic phosphorus values in the treated cattle increased from 6.86 to 8.32 mg. per 100 ml. in 8 hours. All plasma phosphorus values in experiment I were within the range of 4 to 9 mg. per 100 ml. which, according to Maynard (1947), is normal for healthy cattle. In later experiments with larger doses (7.5 gm. of phosphorus per 100 pounds of body weight) plasma-inorganic phosphorus values were observed which exceeded the normal range by 2 to 3 mg. per 100 ml.

Individual plasma phosphorus values, in some instances, reached 12 mg. per 100 ml.; however, levels this high were observed only in cattle previously fed adequate phosphorus rations. Plasma phosphorus in phosphorus-deficient steers increased to values that were only half the

values of normal steers. Thus it is apparent that the normal and phosphorus-deficient steers may have utilized the phosphorus dose in a different manner. Evidence was not sufficient to indicate whether the difference in utilization was due to different absorption rates or a difference in metabolism after the phosphorus was absorbed.

The average initial plasma phosphorus value in phosphorus-deficient steers in experiment IV was 2.32 mg. per 100 ml. These values increased to 6.06 mg. per 100 ml. in 10 hours and remained above 4.0 mg. on the 3rd and 4th days after the phosphorus was administered. Thus, there is an indication that phosphorus-deficient steers may have been able to utilize and store a substantial amount of the phosphorus that was administered.

Results of four experiments in this study did not confirm the results of Lewis et al. (1951). No clinical symptoms were observed after large doses of phosphorus were administered to cattle. Also, the plasma phosphorus levels of deficient steers in this study reached a high value that was scarcely more than half the 12.3 mg. per 100 ml. as reported by Lewis and co-workers. Trends in plasma calcium levels were similar. Both these experiments and the Montana report indicate that plasma calcium may become lower after cattle are dosed with 7.5 gm. of phosphorus per 100 pounds of body weight.

The Effect of Large Doses of Phosphorus on Salivary Constituents in Cattle

Phosphorus

The salivary phosphorus levels in experiments II, III, and IV fluctuated in a manner similar to the plasma phosphorus levels. Results of experiment III, which are considered as typical, indicate that salivary phosphorus reached a peak at or near 10 hours after dosing.

The ratios of the increases in salivary phosphorus to the increases in plasma phosphorus indicate that salivary phosphorus in the normal animals increased 4 to 5 mg. per 100 ml. for each mg. of increase in plasma phosphorus. In the phosphorus-deficient steers in experiment IV, this increase was only 3.3 mg. per mg. increase in plasma phosphorus. The curves for average values in this experiment showed a definite tendency for saliva and plasma phosphorus to follow parallel courses after the two had been scaled according to the ratio of their increases. At 10 hours and thereafter, the salivary phosphorus of the treated animals deviated from the parallel course and decreased gradually.

Sodium and Potassium

No apparent differences were observed in salivary sodium or potassium after dosing normal cattle with mono-sodium phosphate in amounts sufficient to provide 7.5 gm. of phosphorus per 100 pounds of body weight.

The average salivary sodium and potassium values for the control animals in experiment III were 568.4 and 50.2 mg. per 100 ml., respectively. These values for cattle are considerably higher than the values of 445.5 and 44 mg. per 100 ml. for sheep as reported by McDougall (1948).

The Effect of Two Phosphorus Supplements Upon the Salivary and Plasma Phosphorus Concentrations in Phosphorus-Deficient Cattle

The average plasma inorganic phosphorus value of a group of cattle fed phosphorus-deficient rations was 2.85 $\pm$ 0.51 mg. per 100 ml. (Table 9). A comparison of this value with the range of 4 to 9 mg. per 100 ml., which Maynard (1947) states is normal for healthy cattle, indicates that these cattle were in a state of phosphorus deficiency. A comparison of the average salivary phosphorus value of 11.22 $\pm$ 2.76 mg. per 100 ml. with the value of 10 mg. reported for six phosphorus-deficient cows

(Clark, 1953), also indicates their close proximity to a state of phosphorus deficiency.

Tables 9 and 10 show that plasma and salivary phosphorus values of lot 1 (basal) decreased 0.81 and 5.05 mg. per 100 ml., respectively, from the initial to the final collection period. Decreases of 0.07 and 0.97 mg. per 100 ml. were observed for plasma and salivary phosphorus, respectively, in lot 2 (basal plus colloidal clay). These values for lot 3 (basal and dicalcium phosphate) increased 0.82 and 4.85 mg. per 100 ml.

Significant differences were observed in the salivary phosphorus content between the control and the supplemented lots ($P < 0.01$) and also between the two supplemented lots ($P < 0.05$) at the final collection period.

These data indicate that the phosphorus-depleted animals utilized the phosphorus in the form of dicalcium phosphate significantly better than that in the form of colloidal clay.

Statistically, the results obtained in the saliva and plasma studies were very similar; therefore, there is an indication that salivary phosphorus levels may be a relatively accurate index of the plane of phosphorus nutrition.

The ratios of salivary phosphorus to plasma phosphorus at the final collection period were 3.0, 3.7 and 4.0:1 for lots 1, 2 and 3, respectively. Clark (1953) obtained a ratio of 4.5:1 for normal cattle.

The Effect of High Dietary Protein and Potassium Levels on the Composition of Saliva in Sheep

Table 13 indicates that salivary phosphorus values were increased significantly ($P < 0.05$) in sheep fed high levels of dietary protein and

potassium.

The average salivary phosphorus values (Table 13) of 119 and 144 mg. per 100 ml. for the control and experimental lots respectively are higher than the values reported by McDougall (1948) and Clark (1953). However, the ratio of the salivary phosphorus to the plasma phosphorus, was 17.2:1 in the control lot and 19.5:1 in the experimental lot. These ratios are comparable to the ratio of 18.5:1 which, according to Clark (1953), is a normal ratio for healthy sheep.

The sodium and potassium values (Table 13) obtained in this study are extremely high. These values may be in error as some difficulty was experienced with the flame photometer when the determinations were made. However, repeated determinations with sodium and potassium standards gave consistent results.

Normal Salivary Phosphorus Values in Cattle Fed Wintering Rations

Twenty-three saliva samples were obtained under normal conditions from 450 to 585 pound cattle that were being fed a wintering ration containing adequate phosphorus. This ration consisted of a 2:1 salt to bonemeal mixture fed ad libitum and a daily allowance of 1 pound of cottonseed meal plus 2 pounds of bright prairie hay per 100 pounds of body weight.

The average salivary phosphorus value of the 23 samples was 25.42 ± 3.97 mg. per 100 ml. This value is 5.82 mg. per 100 ml. higher than other values that have been reported for cattle fed bonemeal and grazing winter pasture (Clark, 1953). This difference might be expected as the plasma phosphorus values in this study were 2.98 mg. per 100 ml. higher than those reported by Clark (1953).

The Relationship of Salivary Phosphorus to Plasma Phosphorus

During the course of these studies, it was apparent that the salivary phosphorus concentration increased in a direct manner with the plasma phosphorus concentration; therefore, all salivary and plasma phosphorus values, that were obtained from cattle in which the plasma phosphorus levels were stable, were used to calculate regression and correlation coefficients.

The regression coefficient for salivary phosphorus on plasma phosphorus was 3.3 with a standard error of the estimate of 3.19. The correlation coefficient of 0.924 with 81 degrees of freedom was highly significant ($P < 0.01$). Therefore, it is evident that salivary inorganic phosphorus in these experiments increased 3.3 mg. per 100 ml. for each mg. per 100 ml. increase of plasma inorganic phosphorus.

SUMMARY

Techniques were standardized for the collection of saliva and for the analysis of inorganic phosphorus in saliva. DuPont cellulose sponges were found to be satisfactory for saliva collection if saturated with saliva 20 to 30 minutes prior to the beginning of collections. Aqueous dilutions of saliva were found to yield accurate phosphorus values and were used in preference to the acid filtrate.

Oral administration of 7.5 gm. of phosphorus per 100 pounds of body weight to normal and phosphorus-deficient cattle resulted in no visible symptoms. This treatment had no effect on plasma carotene and vitamin A levels; however, the salivary and plasma-inorganic phosphorus levels increased to high levels within 10 hours after dosing. The utilization of the phosphorus dose may have been different in normal and phosphorus-deficient cattle. Plasma-inorganic phosphorus levels in the deficient cattle were above 4.0 mg. per 100 ml. 4 days after dosing.

Salivary inorganic phosphorus values in phosphorus-deficient heifers varied with the availability of phosphorus in the ration.

Salivary inorganic phosphorus values increased 3.3 mg. per 100 ml. for each mg. per 100 ml. increase in plasma-inorganic phosphorus. The correlation between plasma-inorganic phosphorus and salivary inorganic phosphorus was highly significant. High protein and high potassium diets increased the phosphorous content of sheep saliva.

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