TRACING THE MINERAL FROM THE SOIL TO THE PLANT TO THE ANIMAL BLOOD

Part II. Effect of Unlimed and Limed Grass on the Chemical Composition of Goats' Blood

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The effect of lime applications on the composition of calcium, magnesium, manganese, phosphorus and iron, in the acid red soil type "Fajardo clay", and on a mixture of "Para and Carib" grass, was discussed in Part I published by Bonnet and Riera (1).

This paper presents information on the effect of the unlimed grass, the limed grass, and the limed grass supplemented with manganese per os, on the weight of the goat; on the grass consumed; and on the hemoglobin, calcium, phosphorus, iron, hematocrits, red-blood cells, and white-blood cells of the goats' blood.

EXPERIMENTAL

The layout of the experimental field was explained in Part I. All the grass cut daily from the strips of the unlimed plots was chopped into small pieces and mixed into a sample labeled "Unlimed grass". An identical sample from the limed plots was labeled "Limed grass".

Fifteen one-year virgin female goats were selected for the experiment. They were given the parasite treatment: twelve grams of phenothiazine per os. The animals were randomized, one for each of fifteen pens (see photo), into five groups for the following three treatments: 1) goats fed with unlimed grass, 2) goats fed with limed grass, 3) goats fed with limed grass, and in addition, fed per os, with manganese sulphate.

The goat experiment covered an eleven-month period. It was started on October 19, 1944 and finished on September 15, 1945. The experiment was divided into four periods as follows:

1. Pre-feeding period (October 19–November 14, 1944)
2. Pre-gestation period (Nov. 15, 1944–January 15, 1945)
3. Gestation period (January 16–July 15, 1945)
4. Lactation period (July 16–September 15, 1945)

Eight pounds of the chopped "Unlimed grass" were fed to each of the goats in treatment No. 1. Eight pounds of the chopped "Limed grass" were fed to each of the goats in treatments No. 2 and No. 3. The feeding box (see photo) in each pen avoided the contamination of the grass with urine and excrement.

Each animal in treatment No. 3 was supplied daily in addition, per os, a solution containing 0.453 gram of manganese sulphate ($MnSO_4H_2O$) per liter, equivalent to .147 milligrams of manganese per milliliter. From November 15 to November 29, 1944, inclusive, and from February 3, 1945 to July 15, 1945, inclusive, each goat received daily, per os, 1 ml. of the manganese solution, but for the period between November 30, 1944 to February 2, 1945, inclusive, each goat received daily, per os, 1.5 ml. of the manganese solution. For the eight-month period, covering pre-gestation and gestation, each goat received 275.5 milliliters of the manganese solution equivalent to 40.5 milligrams of manganese.

The amount of residual grass left daily by each animal was also weighed. A composite sample from the "Unlimed Grass" and the "Limed Grass", was taken daily for moisture analysis. A record was kept of the amount of green and dry grass consumed by each animal.

Rain water from the two concrete wells besides greenhouse No. 5 in the Experiment Station Farm, was supplied daily to each animal. The water consumed daily, however, was very low. The mineral content of this water was as follows:

	parts per million
Calcium	4.0
Phosphorus	2.0
Iron	0.03
Magnesium	None
Manganese	None

Each goat was weighed three times in three consecutive days, around the middle and the end of each month.

To induce breeding of the goats at approximately the same time, each animal was given, per os, on January 29, 1945, 5 milligrams of diethyl stylbestrol.

METHODS OF ANALYSES

Blood samples were taken from each animal at the beginning of the "Pre-Feeding Period" on October 19, 1944; one month after the beginning of the "Pre-Gestation Period" in the middle of December 1945; and thereafter every middle of the month up to August 1945. About 10 ml. of blood were drawn from each animal by a direct puncture of the jugular vein: 2 ml. for the hematological test and 8 ml. for the chemical test. The 2 ml. blood portion was poured into a 10 ml. test tube containing a dry oxalate salt. This salt was prepared by adding 0.1 ml. of a mixed solution of 6 per cent ammonium oxalate and 4 per cent of potassium oxalate to each tube, and evaporating to dryness.

Hematological Test

A 0.1 ml. of the oxalated blood was used for the red-blood cell and white-blood cell counts. A 0.7 ml. portion of this blood was used for hematocrits and 0.1 ml. for hemoglobin.

Hemoglobin

Hemoglobin was determined in a fresh sample of cow's blood by the Van Slyke's method (2). Its content was found to be 9.04 grams hemoglobin per 100 ml. blood. A 1:25 solution was prepared by diluting 2 ml. of this cow's blood to 50 ml. with 0.1 per cent sodium carbonate solution. Transmittances of eleven dilute solutions, prepared from the 1:25 blood solution, are as follows:

SOLUTION NO.	ALIQUOT 1:25 BLOOD SOL	DILUTED WITH 0.1% SODIUM CARBONATE TO	DILUTION RATIO	HEMOGLOBIN MILLIGRAMS PER ML. BLOOD	TRANSMITTANCE
	ml.			*mg.*	%
1	6.00	18.00	1: 75.0	1.2053	15.0
2	5.00	20.00	1: 100.0	0.9040	22.5
3	5.00	23.00	1: 114.7	0.7881	26.7
4	5.00	26.67	1: 133.3	0.6782	31.3
5	5.00	32.50	1: 162.5	0.5563	38.1
6	2.50	20.00	1: 200.0	0.4520	45.1
7	2.50	26.67	1: 266.7	0.3390	54.8
8	1.25	20.00	1: 400.0	0.2260	66.8
9	1.25	26.67	1: 533.4	0.1695	73.6
10	1.25	40.00	1: 800.0	0.1130	81.0
11	1.25	80.00	1:1600.0	0.0565	90.0

The transmittance was determined in a Coleman spectrophotometer, model 11, using a PC-4 filter at a wave length of 540 mμ where maximum color absorption (figure 2) occurs. The sodium carbonate solution was used as reference. It gave a 99.1 per cent transmittance when the instrument was set at zero. The transmittance-concentration curve obtained for hemoglobin is reported in figure 1.

For the determination of hemoglobin in the unknown, 0.1 ml. of oxalated blood was diluted to 20 ml. with 0.1 per cent sodium carbonate solution. The transmittance of the colored solution was read in a Coleman spectrophotometer, model 11, using filter PC-4 at a wave length of 540 mμ. The transmittance from the curve (figure 1) multiplied by 20 gives grams of hemoglobin per 100 ml. blood.

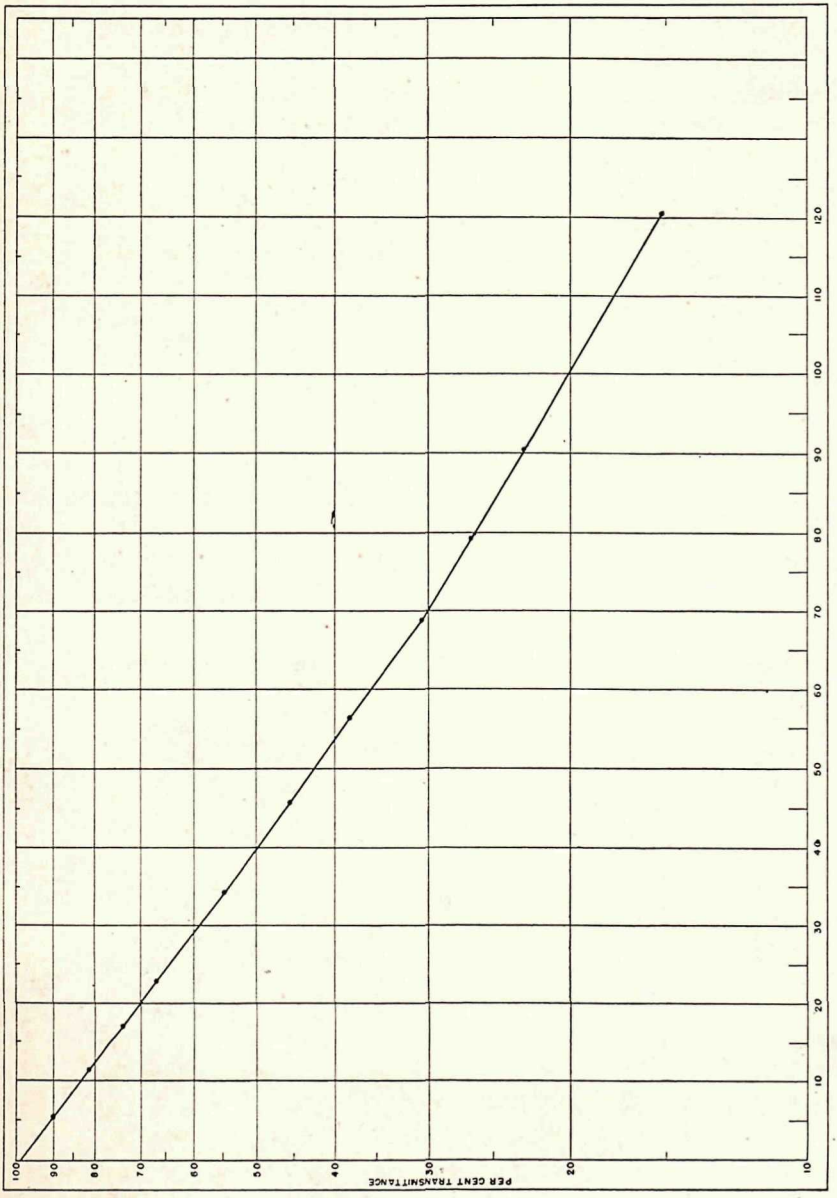


FIG. 1. Hemoglobin in blood. Curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 540 mu, and 0.1 per cent sodium carbonate as reference solution. Abscissa represents milligrams of hemoglobin per milliliter blood. On the basis of 0.1 ml. blood diluted to 20 ml. with 0.1% $NaCO_3$; value in curve $\times$ 20 = grams hemoglobin per 100 ml. blood.

Chemical test

Iron. A sample of 0.5 ml. of oxalated blood was taken for the iron determination. The Wong's (5) modified method was used to develop the color and the transmittance was read in the Coleman spectrophometer with filter PC-4 at a wave length of 480 $m\mu$ using a reagent blank as the reference solution. The method used was as follows: Transfer with an Ostwald pipette 0.5 ml. of blood into a 50 ml. volumetric flask and introduce 2 ml. of iron-free concentrated sulphuric acid. Whirl the flask to agitate the mixture for 1 or 2 minutes. Add 2 ml. of saturated potassium persulfate solution and

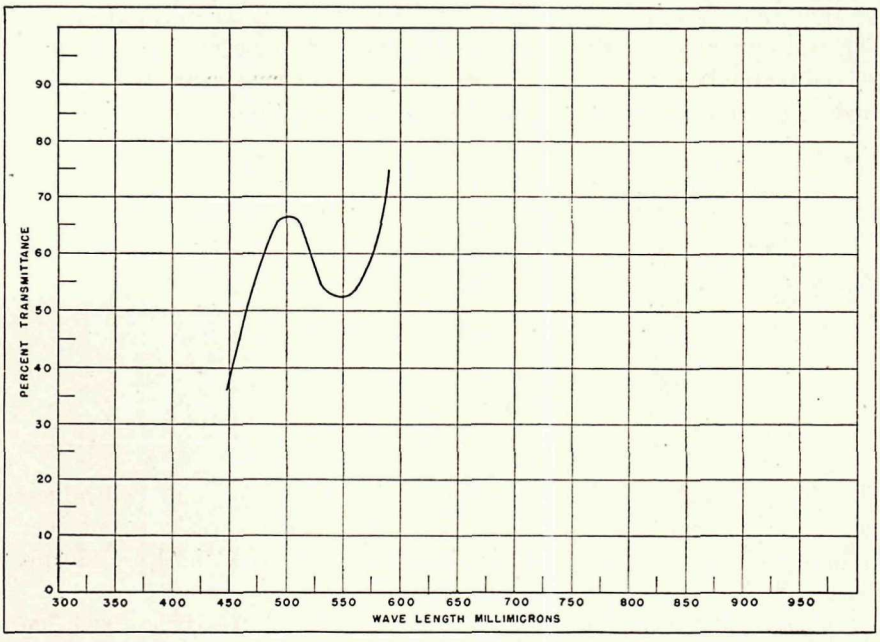


FIG. 2. Spectral-transmittance curve for hemoglobin. Maximum absorption of light at 540 millimicrons.

shake. Dilute to about 25 ml. with distilled water and add 2 ml. of 10 per cent sodium tungstate solution. Mix, cool to room temperature under the tap and then dilute to volume with distilled water. Stopper the flask and invert two or three times to effect thorough mixing. Filter through a dry filter paper into a clean, dry receiving vessel. Pipette 20 ml. of the clear filtrate into a large test-tube graduated at 20 ml. and 25 ml. The color was developed by adding 1 ml. of saturated potassium persulfate solution and 4 ml. of 3 N potassium sulfocyanate, KCNS, solution.

The iron standard solution was prepared as follows: Transfer 0.8635

gram of crystallyzed ferrous ammonium sulphate, $FeNH_4(SO_4)_2 \cdot 12H_2O$, to a small beaker and dissolve in about 50 ml. of water. Add 20 ml. of 10 per cent iron-free sulphuric acid. Transfer quantitatively to a liter volumetric flask and dilute to the liter mark with water. One ml. of this solution contains 0.1 mg. Fe. Dilute 10 ml. of this standard solution to 100 ml. with distilled water. One ml. contains 0.01 mg. Fe. The equivalent amount of this standard iron solution was measured in a pipette and poured into the 25 ml. test tube; 0.8 ml. of iron free concentrated sulphuric acid was added, and diluted to the 20 ml. mark with distilled water. Cool to room temperature under the tap, and develop the color as mentioned above, but develop it at the time of reading in the spectrophotometer to avoid fading. The transmittance obtained, for the various iron concentrations using a PC-4 filter, and a wave length of 480 mμ in the Coleman spectrophotometer, model 11, using the reagent blank as reference solution, was as follows:

IRON CONCENTRATION	TRANSMITTANCE
mg.	%
0.01	83.5
0.02	71.2
0.03	60.0
0.05	42.7
0.07	30.6
0.08	26.0
0.10	19.0

The slope of the standard curve (figure 3) remains constant. Readings from curve give milligrams Fe per 100 ml. blood. The lower transmittance or maximum light absorption was obtained at a wave length of 480 mμ (figure 4).

Calcium and Phosphorus. The non-oxalated blood was centrifuged, immediately after drawn to avoid hemolysis, for 5 minutes at 2800 r.p.m. in an International clinical centrifuge. The fibrin sealing the plasma was loosened carefully with a wooden rod. The plasma was poured down, or was centrifuged again if necessary, to avoid hemolysis since the red-blood cells of goat's blood are quite minute (diameter = 4.1 microns) and fragile.

Calcium. Calcium in blood serum was determined by the method of Roe and Kahn (4) using a Klett-Summerson photoelectric colorimeter No. 2141, test-tube model, with red filter 66, at a wave length range 640–700 mμ, reading against a reagent blank. The procedure for the blank, unknown, and standard, was as follows:

Unknown. Add 1 volume of serum to 4 volumes of 10% trichloroacetic acid in a small flask and shake well. Pour onto a dry calcium-free filter

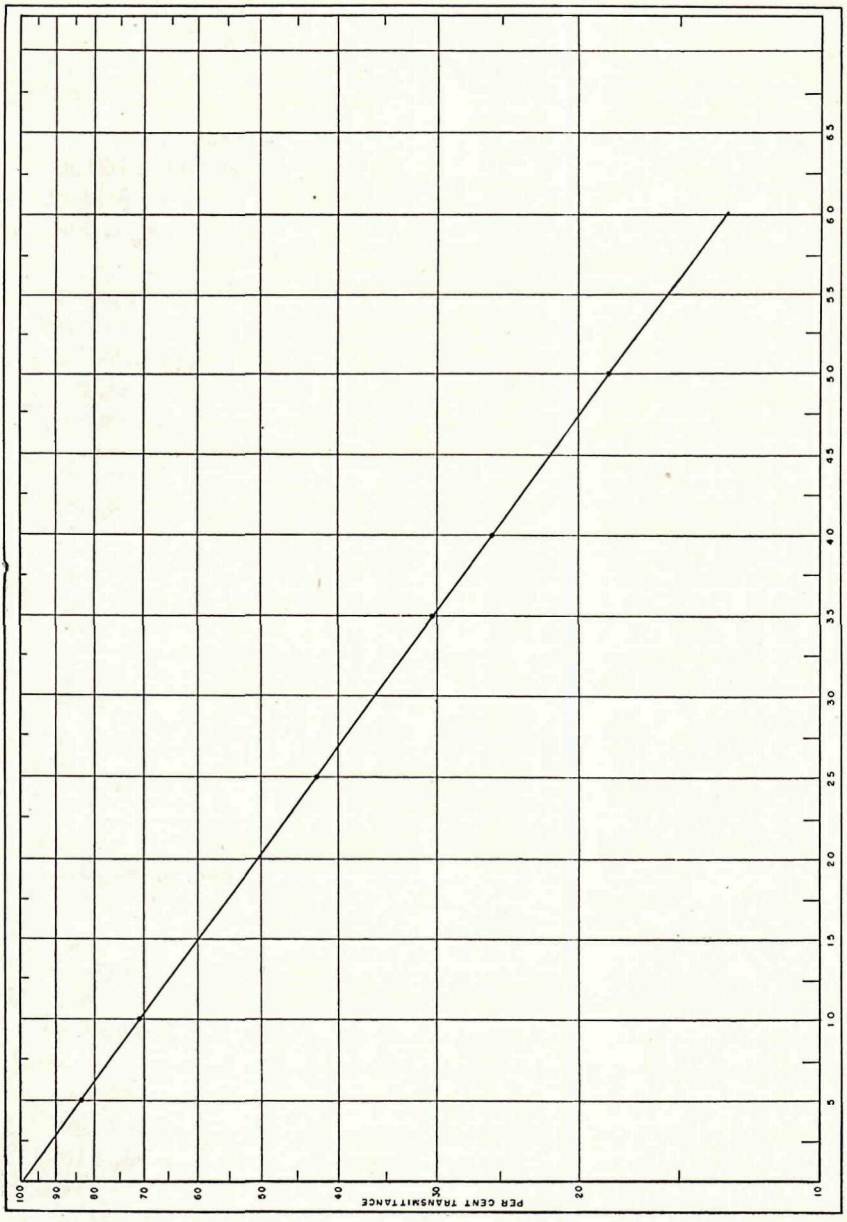


FIG. 3. Iron in blood curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 480 mu, and reagent blank as reference solution. Abscissa represents milligrams of iron as Fe. Readings from curve give milligrams Fe per 100 ml. blood.

paper (Whatman No. 42 or its equivalent) and collect the filtrate in a dry flask. Place 5.0 ml. of the filtrate in a graduated 15 ml. conical centrifuge tube and add 1.0 ml. of 25% sodium hydroxide solution, with mixing by lateral shaking. Allow to stand for 5 minutes, then add 1.0 ml. of 5% trisodium phosphate solution, mix well by lateral shaking, and set aside for an hour. At the end of this time, centrifuge for 2 minutes, and pour off the supernatant fluid, allowing the tube to drain in an inverted position for 2 minutes. Wipe the mouth of the tube dry with a clean cloth. Wash the

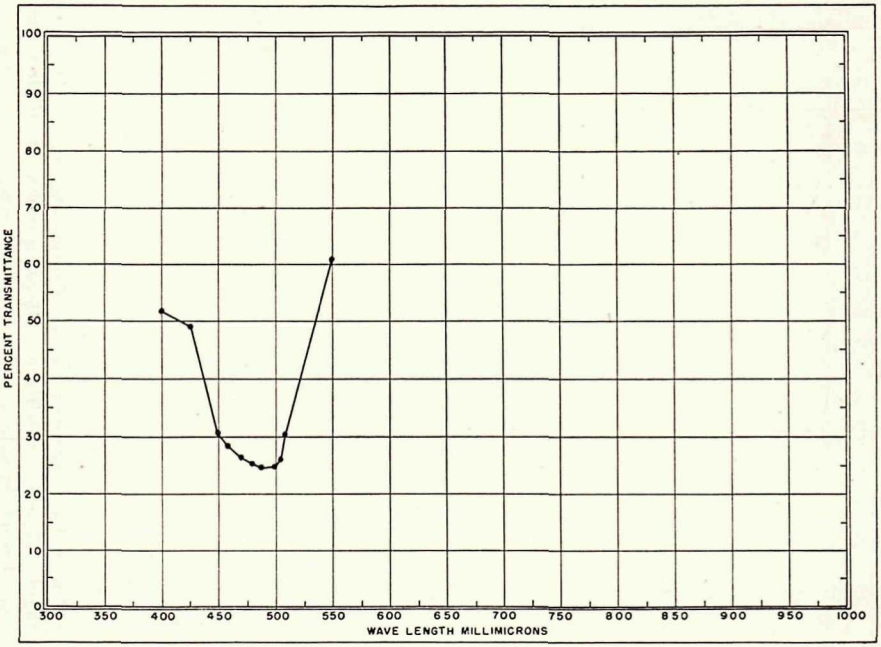


FIG. 4. Spectral-transmittance curve for iron as per method of blood. Maximum absorption of light at a wave length of 480 millimicrons.

precipitate with 5 ml. of alkaline-alcoholic wash reagent (to 10 ml. of amyl alcohol, add 58 ml. of ethyl alcohol, and mix; dilute to 100 ml. with water; add 2 drops of 1 per cent phenolphthalein solution and then, drop by drop, add sufficient 5 per cent sodium hydroxide solution to a distinct pink color; (a few drops should be sufficient); delivered from a pipette with a fine tip, blowing the first portion of wash fluid against the precipitate with such force as to break it up, and using the remainder of wash fluid to rinse down the sides of the centrifuge tube. If necessary use a stirring rod to break up the precipitate. Centrifuge for 2 minutes, pour off the supernatant fluid and allow the tube to drain as before. After draining, wipe the mouth of the

tube dry, and add 2.0 ml. of molybdate reagent (dissolve 25 grams of c.p. ammonium molybdate in 200 ml. water, and pour into a one-liter volumetric flask containing 500 ml. of 10 normal sulfuric acid; dilute to the mark and mix); to dissolve the precipitate and form phosphomolybdate from the phosphate present. After a complete solution of the precipitate, which may be hastened by shaking or stirring, dilute to 10.0 ml. with distilled water, and mix well. Transfer a 5.0 ml. portion from the centrifuge tube to a colorimeter tube and add 0.4 ml. of the aminonaphtholsulfonic acid reagent. Dilute with water to 10.0 ml., mix, and read in the colorimeter after five minutes setting the colorimeter at zero with reagent blank.

Blank. Treat a 5.0 ml. portion of distilled water with 1 ml. of molybdate reagent and 0.4 ml. of aminonaphtholsulfonic acid reagent (weigh 0.125 gram of c.p. 1-amino-2-naphthol-4-sulfonic acid, Eastman Kodak 360, into a 250 ml. beaker containing 50 ml. of 15 per cent sodium bisulfite and 1 ml. of 20 per cent sodium sulfite; shake until dissolved; add a little more of the sodium sulfite solution if necessary to bring the powder into solution; add a little more of the sodium sulfite solution, but an excess should be avoided; keep in a brown bottle away from light; this solution should be prepared fresh every two weeks); and dilute to the 10.0 ml. mark. Set the colorimeter at zero with this reagent blank.

Standard. Treat a 5.0 ml. portion of the standard phosphate solution with 1 ml. of molybdate reagent, add 0.4 ml. of aminonaphtholsulfonic acid reagent, dilute with water to the 10.0 ml. mark, and mix. Read in the colorimeter after 5 minutes. 1 ml. standard phosphate solution = 0.001 milligram P $= \frac{9.67}{5}$ or 1.934 milligrams Ca per 100 ml. blood serum. The readings obtained for the standard curve, after setting instrument at zero with reagent blank, were as follows:

STANDARD P SOLUTION	PHOTOCOLORIMETER READINGS	Ca PER 100 ML. SERUM	SLOPE OF CURVE
mg.		*mg.*	
0.002	76	3.87	0.05092
0.004	155	7.74	0.04994
0.005	192	9.67	0.05036
0.006	228	11.60	0.05088
0.008	310	15.47	0.04990

The slope of the standard curve remains constant. Its average value or factor (figure 5) equals 0.05040. Reading of unknown in photocolorimeter $\times$ 0.05040 equals milligrams of calcium per 100 milliliters blood serum.

Inorganic Phosphorus. Inorganic phosphorus in blood serum was deter-

mined by the method explained in Levinson & MacFate (3) but using a Coleman spectrophotometer, model 11, with filter PC-4 and a wave length of 600 mμ (figure 7), reading against a reagent blank. The procedure was as follows: Transfer 4 ml. of 10 per cent trichloracetic acid to a small flask and add 1 ml. of the blood serum with shaking. Larger volumes of the serum may be used in the same proportion. Shake well, let stand for 1 to 2 minutes, and filter through a phosphorus-free filter paper. If small quantities of material are used, pour the mixture into a centrifuge tube, centri-

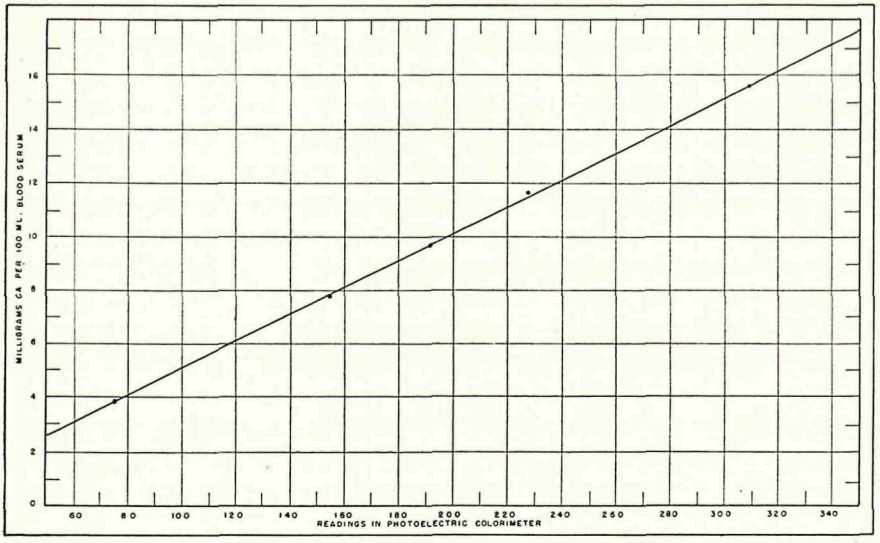


FIG. 5. Calcium in blood serum. Curve obtained in Klett-Summerson photoelectric colorimeter, No. 2141, test tube model, with red filter 66, at a wave length range 640–700 mu, and the instrument at zero with reagent blank. Average slope of curve = 0.0504. Readings of curve $\times 0.0504$ = milligrams of Ca per 100 ml. blood serum.

fuge for a few minutes and filter the clear supernatant fluid through 4.25 centimeter filter paper in a small funnel. Transfer 2 ml. of the filtrate to a 50 ml. Erlenmeyer flask. Add 12 ml. of distilled water, 4 ml. of the molybdic-sulfuric acid reagent A (50 ml. of 10 normal sulphuric acid added to 50 ml. of 7.5 per cent sodium molybdate solution), and 2 ml. of dilute stannous chloride solution (dissolve 10 grams of stannous chloride in 25 ml. of concentrated hydrochloric acid; preserve this stock solution in a brown bottle; dilute 1 ml. of stock solution to 200 ml. with water; preserve in a brown bottle; this dilute solution keeps for 5 days, but is better if prepared fresh each time). Mix well and after 15 minutes read in spectrophotometer.

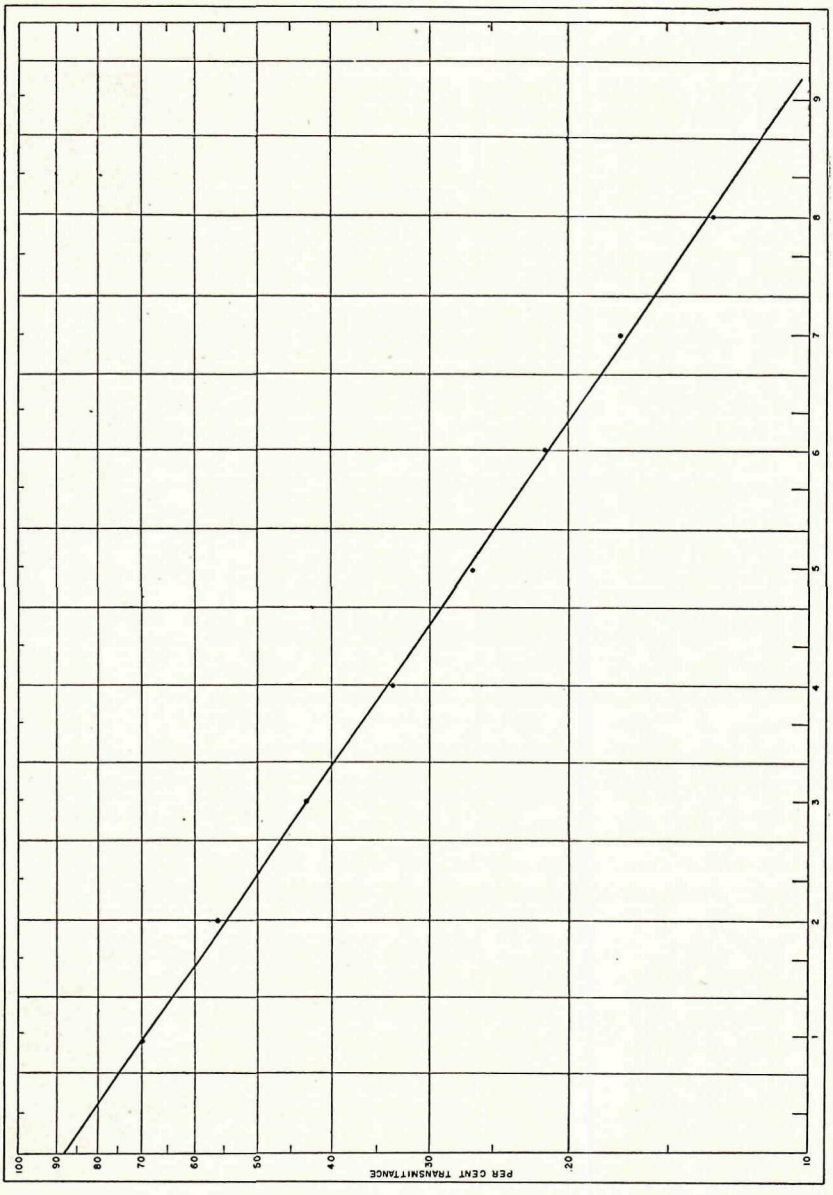


FIG. 6. Inorganic phosphorus in blood serum. Curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 600 mu, and the reagent blank as reference solution. Abscissa represents milligrams phosphorus (P) per 100 ml. serum. Slope of standard curve was not found to be constant. Run at least three standards to check slope.

Calibration Curve. Dilute 20 ml. of standard phosphorus solution (1 ml. = 0.01 mg. P) to 25 ml. with distilled water. Dilute to 14 ml. Add 4 ml.

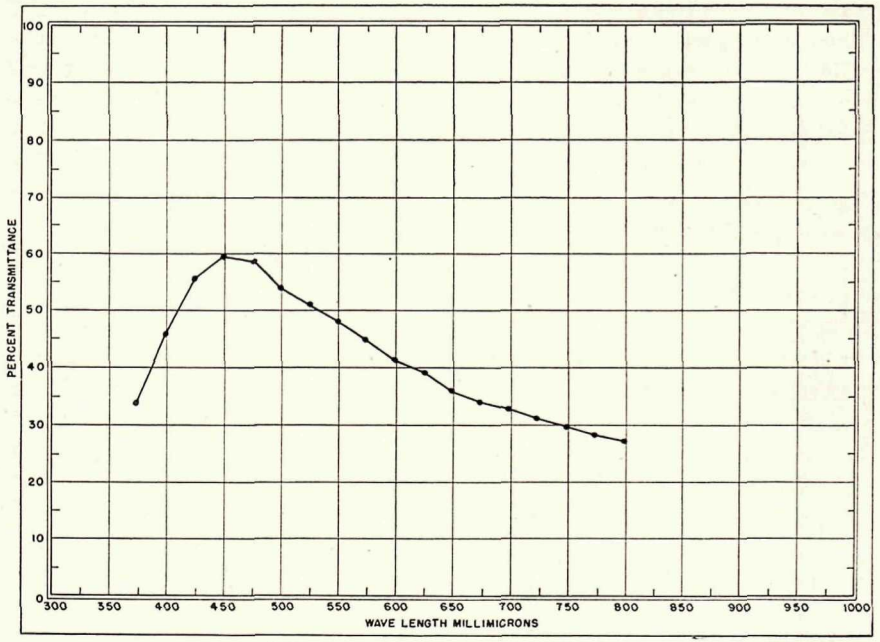


FIG. 7. Special-transmittance curve for phosphorus as per method of blood serum. The PC-4 filter was used for wave lengths, from 350 to 700 mu, and the PC-5 filter, from 700–800 mu.

of the molybdic-sulfuric acid reagent A and 2 ml. of the stannous chloride solution. Read after 15 minutes and plot in semi-log paper.

The following readings were obtained for transmittance:

P-STANDARD	P IN STANDARD	P PER 100 ML. SERUM	TRANSMITTANCE
ml.	*mg.*	*mg.*	%
0.5	0.004	1	70.0
1.0	0.008	2	56.3
1.5	0.012	3	43.7
2.0	0.016	4	34.4
2.5	0.020	5	27.0
3.0	0.024	6	21.9
3.5	0.028	7	17.5
4.0	0.032	8	13.2

Prepare three sets of standards with 1, 5, 8 mgm. P per 100 ml. serum, respectively, simultaneously with the unknown, since it was found that the slope of the standard curve (figure 6) is not constant.

PRESENTATION OF DATA AND DISCUSSION

The monthly weights in pounds per goat and the average monthly weights for the five goats in the treatments: unlimed grass, limed grass, and limed grass plus manganese, are reported for a period of eleven months in table 1. The changes in average weight of the animals in each treatment before and after the pre-gestation period and after the gestation period are condensed in table 2. There was no significant increase in the weight of the goats for the two-month pre-gestation period. However, there was a higher significant decrease of about 8.5 pounds in weight after the six-month gestation period.

The total amount in pounds of the Para-Carib grass mixture and minerals, on the dry basis, eaten by each goat and the average eaten by the five goats in each of the three treatments during the pre-gestation and gestation periods are reported in table 3. The per cent of calcium, phosphorus, manganese, iron and magnesium used for the calculation is that reported previously (1) in part I for the third and fourth grass crops, respectively, in the field at the time of sampling. The total amount of dry grass eaten in the pre-gestation and gestation periods and the total minerals eaten for both periods by the five goats in each treatment are condensed in table 4.

There were significant differences for the pre-gestation period between the grass intake of the goats under the three treatments. The goats fed with unlimed grass ate less than those fed with limed grass. However, for the gestation period the difference for the grass intake between the treatments was not significant.

The average daily grass and mineral intake, in grams per five goats, of about 52 pounds in average weight, is reported in table 5. These data were calculated for a total of 243 days covering the pre-gestation and gestation periods.

The average intake of unlimed grass was about 6 pounds of dry grass per five goats per day and of limed grass was about 7 pounds for animals weighing around 52 pounds.

The goats fed with limed grass received daily about 4 grams more of calcium, 2 grams more of phosphorus, 0.2 grams more of iron, 1 gram more of magnesium, and about 0.2 grams less manganese than the goats fed with unlimed grass. The additional manganese added, per os, to each goat fed with the limed grass, which amounted to 41 milligrams of manganese for the 243 days covering the pre-gestation and gestation periods, was too small to account for an increase in the manganese content of the limed grass.

The average hemoglobin, iron in blood, calcium and phosphorus in blood serum, and blood count per goat in each treatment is reported in table 6. There were no significant differences in the pre-gestation period between the mean red or white-blood cells, the hematocrits, the hemoglobin and iron

TABLE 1

Monthly weights of goats fed with unlimed grass and limed grass with or without extra manganese, for a ten-month period

GROUP	TREATMENT	ANIMAL NUMBER	1944		1945								
			Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
			lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
I	Unlimed grass	81	57.2	56.7	54.8	53.5	51.3	49.5	46.2	48.8	D*	—	—
		90	69.5	68.0	68.8	64.0	64.5	60.2	59.3	54.2	57.7	60.3	58.2
		91	49.5	50.7	49.3	49.0	48.8	46.5	44.8	44.8	43.3	45.2	45.7
		92	48.0	50.0	47.8	45.2	42.7	39.8	37.0	40.0	38.8	39.8	40.3
		94	52.9	54.8	54.0	51.0	52.3	48.8	47.0	48.8	48.0	51.8	51.5
Ave.			55.4	56.0	55.0	52.5	51.9	49.0	46.9	47.3	47.0	49.3	48.9
II	Limed grass	72	39.3	43.3	43.7	41.5	38.7	37.0	35.0	37.3	39.2	39.2	40.5
		80	63.0	64.5	65.8	66.8	63.7	61.3	59.0	63.3	59.7	57.0	53.7
		83	66.8	66.8	67.5	64.5	62.3	57.2	57.3	59.2	44.5	50.2	51.2
		95	52.0	54.2	51.0	49.7	45.5	44.3	39.5	46.5	44.2	45.8	42.0
		97	55.5	55.8	54.8	52.3	50.8	45.5	43.5	48.0	45.8	48.8	48.5
Ave.			55.3	56.9	56.6	55.0	52.1	49.0	46.9	50.9	46.7	48.7	47.2
III	Limed grass & manganese	78	63.5	62.5	63.0	63.2	60.8	58.7	55.8	59.5	57.5	57.5	54.8
		84	49.5	54.2	51.0	50.3	48.3	42.7	42.3	46.7	42.0	45.0	40.0
		87	54.0	54.5	52.0	53.5	52.5	51.8	47.8	51.0	39.8	45.3	48.0
		88	68.2	71.0	65.8	66.7	62.8	61.2	57.3	60.5	60.2	60.0	59.5
		93	46.5	48.2	44.5	45.3	43.3	39.2	37.5	41.5	39.7	43.2	39.5
Ave.			56.3	58.1	55.3	56.0	53.5	50.7	48.1	51.8	47.8	50.2	48.4

* D = died.

TABLE 2

Average weights of the goats, before and after the treatment

TREATMENT	PRE-GESTATION PERIOD		GESTATION PERIOD
	Before	2 months after	6 months after
	lb.	*lb.*	*lb.*
Unlimed grass	55.4	55.0	47.0
Limed grass	55.3	56.6	46.7
Limed grass plus manganese	56.3	55.3	47.8

in the blood, and the calcium and phosphorus in the serum of the goats in the three treatments.

The goats were healthy and vigorous at the end of the pre-gestation pe-

TABLE 3

Grass and minerals eaten by goats during pre-gestation and gestation periods (dry basis), pounds

TREATMENT	GOAT NO.	THIRD CROP	FOURTH CROP	TOTAL	CALCIUM (Ca)			PHOSPHORUS (P)			MANGANESE (Mn)			IRON (Fe)			MAGNESIUM (Mg)		
					a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Unlimed grass	81	162	116	278	.33	.34	.67	.45	.28	.73	.03	.04	.07	.03	.01	.04	.30	.24	.54
	90	176	163	339	.35	.47	.82	.48	.39	.87	.03	.04	.07	.04	.02	.06	.32	.33	.65
	91	156	152	308	.31	.44	.75	.43	.36	.79	.02	.04	.06	.03	.02	.05	.29	.31	.60
	92	130	124	254	.26	.36	.62	.36	.30	.66	.02	.03	.05	.03	.02	.05	.24	.26	.50
	94	162	158	320	.33	.46	.79	.45	.38	.83	.03	.04	.07	.03	.02	.05	.30	.33	.63
Total		786	713	1499	1.58	2.07	3.65	2.17	1.71	3.88	.13	.19	.32	.16	.09	.25	1.45	1.47	2.92
Limed grass	72	160	148	308	.54	.50	1.04	.49	.43	.92	.01	.03	.04	.03	.04	.07	.35	.31	.66
	80	195	170	365	.65	.57	1.22	.59	.50	1.09	.02	.03	.05	.03	.04	.07	.43	.35	.78
	83	194	154	348	.65	.52	1.17	.59	.45	1.04	.02	.03	.05	.03	.04	.07	.43	.32	.75
	95	183	132	315	.61	.45	1.06	.56	.39	.95	.02	.02	.04	.03	.03	.06	.41	.28	.69
	97	184	147	331	.62	.50	1.12	.56	.43	.99	.02	.03	.05	.03	.04	.07	.41	.31	.72
Total		916	751	1667	3.07	2.54	5.61	2.79	2.20	4.99	.09	.14	.23	.15	.19	.34	2.03	1.57	3.60
Limed grass plus manganese	78	188	196	384	.63	.66	1.29	.57	.57	1.14	.02	.04	.06	.03	.05	.08	.42	.41	.83
	84	159	139	298	.53	.47	1.00	.49	.41	.90	.01	.03	.04	.03	.03	.06	.35	.29	.64
	87	188	169	357	.63	.57	1.20	.57	.49	1.06	.02	.03	.05	.03	.04	.07	.42	.35	.77
	88	206	189	395	.69	.64	1.33	.63	.55	1.18	.02	.03	.05	.03	.05	.08	.46	.39	.85
	93	154	133	287	.52	.45	.97	.47	.39	.86	.01	.02	.03	.03	.03	.06	.34	.28	.62
Total		895	826	1721	3.00	2.79	5.79	2.73	2.41	5.14	.08	.15	.23	.15	.20	.35	1.99	1.72	3.71

a = third crop, b = fourth crop, c = total.

riod; but were skinny, bony, and weak at the end of the gestation period (see photo). All of them, irrespective of treatment, showed at the end of gestation a reduction in red- and white-blood cells, in hematocrits, in hemoglobin, and in blood iron. However, no change was evidenced in the calcium and phosphorus of the blood serum.

Of the total of fifteen goats; ten aborted, and one died. Each of two goats, fed with limed grass, delivered a weak kid but did not produce milk

TABLE 4

Total dry grass and minerals eaten by the five goats, in each respective treatment, covering the eighth-month pre-gestation and gestation periods

TREATMENT	DRY GRASS EATEN			MINERALS EATEN				
	Pre-gestation period, 2-months	Gestation period, 6-months	Total, 8-months	Ca	P	Mn	Fe	Mg
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Unlimed grass.....	344	1155	1499	3.65	3.88	0.32	0.25	2.92
Limed grass.....	419	1258	1677	5.61	4.99	0.23	0.34	3.60
Limed grass plus 203 milligrams of manganese supplied per os to 5 goats.....	404	1317	1721	5.79	5.14	0.23	0.35	3.71

TABLE 5

Average intake of dry grass in pounds and minerals in grams per day per group of five goats of about 52 pounds each

TREATMENT	DRY GRASS EATEN	MINERALS EATEN				
		Ca	P	Mn	Fe	Mg
	lb.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Unlimed grass.....	6.17	6.8	7.2	0.60	0.47	5.5
Limed grass.....	6.90	10.5	9.3	0.43	0.64	6.7
Limed grass plus manganese.....	7.08	10.8	9.6	0.43	0.65	6.9

even for the newborn. Stylbestrol, at the rate of 5 milligrams per os, which was given to the goats to induce even ovulation, might have been the cause of upsetting their endocrine balance and affecting in general the health of the animal. A marked increase was noticed in the calcium and phosphorus contents of the blood serum one-month after the stylbestrol application (table 7).

Iron combined with hemoglobin and uncombined are reported in table 8. This table contains data for 147 determinations made for iron and hemo-

globin in goat's blood taken monthly for a period of nine months, from each of the fifteen goats in the experiment. The color of the iron was developed by Wong's (5) method, and both, the iron and hemoglobin were determined separately in the spectrophotometer, as already reported. The combined iron in table 8 was calculated by multiplying the hemoglobin by the factor 3.35, as proposed by Wong (5) on the basis that

TABLE 6

Hematological data, iron in blood, calcium and phosphorus in blood serum, for goats in each treatment

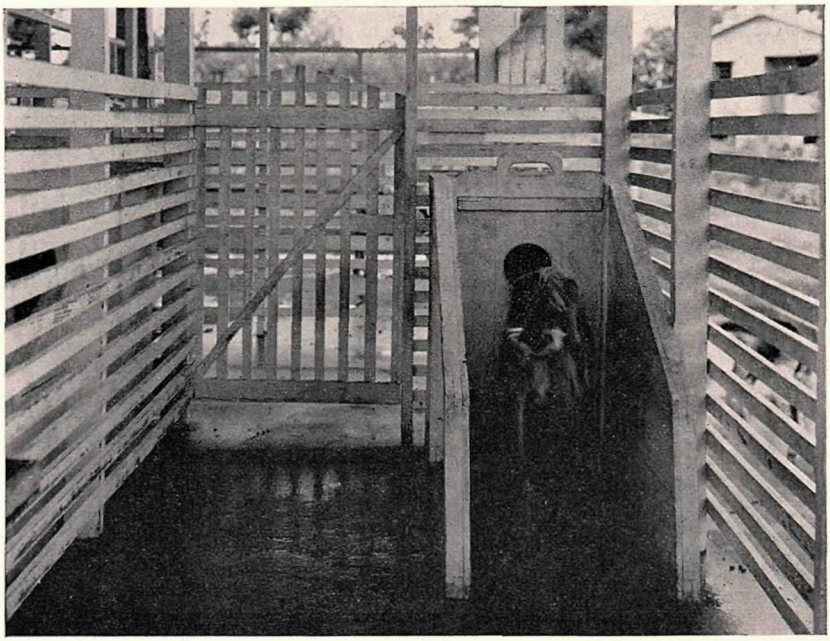
TREATMENT	PERIOD	RED BLOOD CELLS PER CU. MM.	WHITE BLOOD CELLS PER CU. MM.	HEMATOCRITS	HEMOGLOBIN	Fe IN BLOOD	Ca IN SERUM	P IN SERUM
		$\times 10^3$		*% vol.*	*gm. %**	*mg. %†*	*mg. %*	*mg. %*
Unlimed grass	Before pregestation	17,626	19,980	31.9	10.5	48.2	11.4	4.9
	End of pregestation	16,894	15,980	30.2	10.1	37.1	12.5	4.0
	End of gestation	15,043	13,475	25.3	8.2	35.4	13.8	4.2
Limed grass	Before pregestation	19,680	19,560	34.2	11.5	51.2	10.6	5.5
	End of pregestation	17,646	18,880	32.5	10.7	39.0	12.2	4.2
	End of gestation	14,662	13,790	23.7	7.9	34.2	14.1	5.2
Limed grass plus manganese	Before pregestation	20,508	16,490	35.7	11.8	51.9	9.9	6.2
	End of pregestation	18,206	15,690	33.0	11.1	40.1	10.2	5.9
	End of gestation	12,624	13,910	22.5	7.6	34.1	13.9	5.2

* Grams hemoglobin per 100 ml. blood.

† Milligrams Fe per 100 ml. blood.

hemoglobin contains 0.0335 per cent iron as Fe. Results in table 8 reveal that considerable of the iron in the blood is not combined with hemoglobin. The uncombined iron varied from 0.1 to 19.9 milligrams per 100 milliliters of blood in 129 blood tests. In 18 tests the iron calculated from the hemoglobin was from 0.79 to 13.0 milligrams per 100 milliliters of blood higher than that found. The low iron content of the blood and the iron deficit in the hemoglobin occurred at regular intervals; the first, at the fifth month period of the experiment; the second, at the ninth month period.

Wong's proposal for calculating hemoglobin from the iron content of the blood does not give, therefore, a true value for hemoglobin, nor does the calculation of blood iron from the hemoglobin content gives a true value for the iron in blood.



A GOAT IN ITS PEN EATING GRASS FROM THE FEEDING BOX
Note poor physical appearance of goat during the gestation period

TABLE 7

Mean calcium and phosphorus contents of blood serum from fifteen female goats before and after stylbestrol application

MINERAL	STYLBESTROL APPLICATION		
	Before	One month after	Two months after
	%	%	%
Calcium, Ca	11.6	17.8	11.6
Phosphorus, P	4.7	6.2	5.7

It has been mentioned that the animals sustained their weight and were healthy and vigorous at the end of the pre-gestation period; but were skinny, bony, and weak at the end of the gestation period. A reduction in red and white blood cells, in hematocrits, in hemoglobin and blood iron,

TABLE 8

Hemoglobin; iron calculated from hemoglobin, iron determined, and uncombined iron, in blood samples taken monthly for a period of nine months from fifteen female goats

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1. Goats receiving unlimed grass	81	0	10.20	34.17	46.8	12.63
		1	9.96	33.37	42.7	9.33
		2	9.24	30.95	35.3	4.35
		3	9.00	30.15	35.6	5.45
		4	9.62	32.23	36.8	4.57
		5	9.80	32.83	34.3	1.47
		6	8.32	27.87	33.2	5.33
		7	6.00	20.10	23.3	3.20
		8	—	—	—	—
		9	—	—	—	—
	90	0	11.50	38.53	49.2	10.67
		1	10.20	34.17	44.8	10.63
		2	9.82	32.90	36.2	3.30
		3	9.96	33.37	38.5	5.13
		4	8.30	27.81	35.2	7.39
		5	9.20	30.82	31.4	.58
		6	8.64	28.94	33.2	4.26
		7	6.60	22.11	25.7	3.59
		8	7.44	24.92	33.6	8.68
		9	7.40	24.79	17.5	−7.29
	91	0	9.20	30.82	50.7	19.88
		1	8.66	29.01	43.7	14.69
		2	8.66	29.01	32.5	3.49
		3	10.08	33.77	39.5	5.73
		4	8.90	29.82	38.2	8.38
		5	9.00	30.15	31.2	1.05
		6	8.20	27.47	32.4	4.93
		7	7.38	24.72	29.5	4.78
		8	8.38	28.07	37.5	9.43
		9	8.00	26.80	17.7	−9.10
	92	0	10.50	35.18	47.0	11.82
		1	10.40	34.84	44.8	9.96
		2	11.00	36.85	40.5	3.65
		3	9.98	33.43	38.5	5.07
		4	9.02	30.22	35.3	5.08
		5	9.70	32.50	33.0	.50
		6	8.00	26.80	32.5	5.70
		7	7.78	26.06	28.0	1.94
		8	7.90	26.47	33.8	7.33
		9	7.94	26.60	16.8	−9.80

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1. Goats receiving unlimed grass —*Continued*	94	0	11.00	36.85	47.5	10.65
		1	11.54	38.66	48.5	9.84
		2	11.54	38.66	40.8	2.14
		3	11.22	37.59	43.3	5.71
		4	9.68	32.43	38.7	6.27
		5	10.80	36.18	32.6	−3.58
		6	10.02	33.57	41.6	8.03
		7	8.16	27.34	—	—
		8	9.20	30.82	36.5	5.68
		9	9.00	30.15	22.8	−7.35
2. Goats receiving limed grass	72	0	12.10	40.54	55.5	14.96
		1	10.80	36.18	43.0	6.82
		2	10.40	34.84	37.7	2.86
		3	11.00	36.85	41.0	4.15
		4	10.16	34.04	42.0	7.96
		5	9.60	32.16	33.6	1.44
		6	9.02	30.22	35.5	5.28
		7	7.00	23.45	28.7	5.25
		8	8.30	27.81	36.4	8.59
		9	7.76	26.00	13.0	−13.00
	80	0	11.80	39.53	49.5	9.97
		1	10.56	35.38	42.5	7.12
		2	11.30	37.86	41.3	3.44
		3	11.38	38.12	42.5	4.38
		4	9.96	33.37	39.7	6.33
		5	10.00	33.50	33.6	.10
		6	9.84	32.96	36.8	3.84
		7	7.46	24.99	40.0	15.01
		8	9.00	30.15	42.3	12.15
		9	8.24	27.60	19.2	−8.40
	83	0	11.00	36.85	54.3	17.45
		1	10.80	36.18	42.7	6.52
		2	10.20	34.17	36.7	2.53
		3	10.80	36.18	41.0	4.82
		4	10.20	34.17	43.4	9.23
		5	10.80	36.18	36.4	.22
		6	9.24	30.95	35.7	4.75
		7	6.80	22.78	24.2	1.42
		8	7.20	24.12	28.0	3.88
		9	7.00	23.45	17.3	−6.15

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg/100 ml.*	*mg./100 ml.*
2. Goats receiving limed grass—*Continued*	95	0	11.00	36.85	49.1	12.25
		1	11.10	37.19	47.0	9.81
		2	10.60	35.51	40.0	4.49
		3	9.92	33.23	39.5	6.27
		4	7.86	26.33	32.7	6.37
		5	9.00	30.15	30.3	.15
		6	7.50	25.13	31.0	5.87
		7	6.80	22.78	28.1	5.32
		8	7.04	23.58	30.5	6.92
		9	6.16	20.64	15.2	−5.44
	97	0	11.40	38.19	47.5	9.31
		1	12.56	42.08	54.0	11.92
		2	10.76	36.05	39.4	3.35
		3	10.08	33.77	39.8	6.03
		4	9.62	32.23	38.7	6.47
		5	9.60	32.16	31.5	−.66
		6	9.50	31.83	34.4	2.57
		7	7.38	24.72	27.8	3.08
		8	—	—	33.8	—
		9	7.90	26.47	18.7	−7.77
3. Goats receiving limed grass plus manganese	78	0	13.20	44.22	61.5	17.28
		1	12.20	40.87	50.5	9.63
		2	12.40	41.54	45.0	3.46
		3	12.18	40.80	46.2	5.40
		4	9.80	32.83	44.0	11.17
		5	12.10	40.54	39.8	−.74
		6	11.06	37.05	41.5	4.45
		7	8.56	28.68	33.8	5.12
		8	8.60	28.81	30.0	1.19
		9	7.92	26.53	18.8	−7.73
	84	0	10.40	34.84	47.6	12.76
		1	12.70	42.55	54.8	12.25
		2	12.20	40.87	43.5	2.63
		3	12.38	41.47	44.8	3.33
		4	8.96	30.02	35.8	5.78
		5	10.40	34.84	35.6	.76
		6	10.20	34.17	40.4	6.23
		7	8.56	28.68	32.8	4.12
		8	7.60	25.46	43.3	17.84
		9	3.60	12.06	5.5	−6.56

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
3. Goats receiving limed grass plus manganese—*Continued*	87	0	11.04	36.98	49.0	12.02
		1	10.10	33.84	40.0	6.16
		2	9.36	31.36	36.2	4.84
		3	8.40	28.14	34.6	6.46
		4	9.00	30.15	37.8	7.65
		5	9.40	31.49	30.7	−.79
		6	8.10	27.14	31.7	4.56
		7	6.18	20.70	24.4	3.70
		8	5.86	19.63	25.3	5.67
		9	5.14	17.22	12.5	−4.72
	88	0	12.80	42.88	52.5	9.62
		1	11.80	39.53	47.0	7.47
		2	11.40	38.19	38.5	.31
		3	9.92	33.23	39.6	6.37
		4	10.06	33.70	38.7	5.00
		5	9.40	31.49	34.5	3.01
		6	8.48	28.41	31.4	2.99
		7	7.46	24.99	30.3	5.31
		8	8.60	28.81	37.2	8.39
		9	7.60	25.46	18.6	−6.86
	93	0	11.50	38.53	49.0	10.47
		1	10.90	36.52	43.5	6.98
		2	10.34	34.64	37.4	2.76
		3	9.60	32.16	39.0	6.84
		4	9.80	32.83	38.5	5.67
		5	9.90	33.17	33.6	.43
		6	8.90	29.82	35.1	5.28
		7	7.60	25.46	29.3	3.84
		8	7.20	24.12	34.5	10.38
		9	6.40	21.44	15.2	−6.24

was also reported for the gestation period. It has also been mentioned that two thirds of the goats aborted and that the two goats delivering weak kids produced no milk. Presumably the stylbestrol application upset the endocrine balance and affected the general health of the animals. All indications pointed to malnutrition of the goats during gestation, probably due to an inadequate protein intake.

A composite sample of the grass fed to the animals was not collected during the pre-gestation and gestation periods. The mineral intake for the goats was calculated from the mineral composition of the grass at time of sampling (table 4). The protein intake (table 9) was calculated similarly.

During the pre-gestation period the goats were fed exclusively with grass from the third crop (1) that received a nitrogen application at the rate of 500 pounds of ammonium sulphate per acre. The protein content of this grass at time of sampling, or 36 days after nitrogen application, was 11.2 per cent.

During the gestation period the goats were fed 72 days with high protein grass from the third crop and 110 days with a low protein grass from the fourth crop that received no nitrogen fertilization.

The daily intake of protein per goat for the pre-gestation period was estimated to be 0.143 pounds or about 65 grams, and for the gestation period was .089 pounds or about 40 grams (table 9). The goats ate per day about the same amount of grass in the pre-gestation and gestation periods, but the

TABLE 9

Estimated protein intake per goat per day during the pre-gestation and gestation periods

PERIOD	TIME OF PERIOD	AMOUNT OF GRASS EATEN PER GOAT PER		PROTEIN CONTENT OF GRASS AT TIME OF SAMPLING	ESTIMATED AMOUNT OF PROTEIN EATEN PER GOAT PER DAY
		Period	Day		
	days	*lbs.*	*lbs.*	%	*lb.*
Pre-gestation	61	77.8	1.28	11.2	0.143
Gestation	72	98.4	1.37	11.2	0.153
	110	150.3	1.37	3.5	0.048
	182	248.7	1.37		0.089

daily intake of protein was 25 grams less in the gestation period. This protein deficiency might have been the cause for the malnutrition of the goats during the gestation period.

CONCLUSION

Female goats kept in their pens, and fed exclusively with a mixture of unlimed and limed Para or "Malojillo" grass (*Panicum purpurascens*) and Carib or "Malojilla" grass (*Eriochloa polystachya*), suffered malnutrition effects during the six-month gestation period. The low normal protein content of the grass mixture, around 3.5 per cent, was probable the main factor involved. Malnutrition was unnoticed during the two-month pre-gestation period because, due to adequate and timely nitrogen fertilization the protein content of the grass mixture was raised to a higher level, around 11 per cent.

Nutrition studies involving mineral in animal blood require that the animal be fed with an adequate protein level during the whole experimental period.

SUMMARY

This paper reports spectrophotometric methods and transmittance-concentration curves for hemoglobin and iron in blood and phosphorus in blood serum and a photocolorimeter method and curve for calcium in blood serum.

Fifteen cross-bred virgin female goats were randomized in groups of five and fed according to three treatments: unlimed grass, limed grass, and limed grass plus manganese per os. Amounts of grass fed to each animal and their monthly weights were recorded for a one-month prefeeding period, a two month pre-gestation period, and a six-month gestation period. Blood samples were also drawn monthly from each animal for determination of hemoglobin, iron, hematocrits, red blood cells and white blood cells in blood, and calcium and phosphorus in blood serum.

The data are reported in nine tables. The important results are as follows:

1. There was no significant increase in the weight of the goats for the two-month pre-gestation period; however, there was a highly significant decrease in weight after the six-month gestation period.
2. There were significant differences for the pre-gestation period between the grass intake of the goats under the three treatments; the goats fed with unlimed grass ate less than those fed with limed grass. However, for the gestation period, the differences for the grass intake between the treatments was not significant.
3. The average intake of dry grass in pounds, and mineral in grams per day per group of five goats, covering the 243 days of the pre-gestation and gestation periods, are reported in table 5.
4. There were no significant differences in the pre-gestation between the mean red or white blood cells, the hematocrits, the hemoglobin and iron in the blood, and the calcium and phosphorus in the serum of the goats in the three treatments.
5. The goats were healthy and vigorous at the end of the pre-gestation period; but were skinny, bony and weak at the end of the gestation period (see photo).
6. The goats at the end of gestation, showed a reduction in red and white blood cells, in hematocrits, in hemoglobin, and in blood iron. However, no change was evidenced in the calcium and phosphorus of the blood serum.
7. Of the total of fifteen goats; ten aborted, and one died. Each of two goats delivered a weak kid, but produced no milk.
8. Stylbestrol, applied at the rate of 5 milligrams per os to each goat, to induce even ovulation, might have been the cause of upsetting the endocrine balance and affecting the health of the animal. A

marked increase was noticed in the calcium and phosphorus contents (table 7) of the blood serum after the stylbestrol applications.

9. Not all of the iron present in the blood is combined with hemoglobin (table 8).
10. The estimated protein intake per goat during the pre-gestation period was calculated to be about 65 grams per day (table 9), while that during the gestation period was about 40 grams per day. This protein deficit in the gestation period might have been an important factor causing malnutrition in the goats.

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