tid and chromosome fragments were also observed, although rarely.

Such insecticides or fungicides, when applied, may increase hereditary changes in cultivated varieties (''pure lines''), leading thus to more rapid degeneration of the highly bred, uniform varieties. This means that when such insecticides or fungicides are applied the seeds of the propagated varieties should be changed more often so as to secure new nondegenerated stocks.

Rumen Bacteria in Cobalt-Deficient Sheep

Lorraine S. Gall,[1] S. E. Smith, D. E. Becker, C. N. Stark, and J. K. Loosli

Cornell University, Ithaca, New York, and Ohio Experiment Station, Wooster

In 1944, McCance and Widdowson (*4*) suggested that cobalt may function in connection with the rumen bacteria. This view was based on a communication from C. J. Martin who stated that sheep suffering from ''Coast Disease'' were cured by feeding cobalt but not by cobalt injection. Cobalt deficiency does not occur in horses, according to Marston (*3*). Attempts to produce cobalt deficiency in rats (*3, 8*) and in rabbits (*7*) have been unsuccessful. Thompson and Ellis believe that cobalt is needed only by ruminants and that it may be concerned with biological processes in the rumen. Recently, Ray *et al.* (*5*) observed a definite response in hemoglobin and gain in weight in cobalt-deficient sheep injected with cobalt, but the response was much less than in animals fed cobalt.

In an attempt to obtain direct evidence which might show with some certainty whether or not cobalt deficiency influenced the bacteria of the rumen, studies were undertaken to measure the bacterial activity of rumen samples. Twenty-one Western yearling sheep became cobalt-deficient after being fed a ration low in cobalt for 8 months. At this time the sheep were losing weight, and had poor appetites and low hemoglobin values (*6*). These sheep were then divided into three equal groups. The first group was fed 1 mg of cobalt per day, while the second received the same amount of cobalt by injection. The remaining seven sheep were kept on the deficient ration. After 1 week sheep fed cobalt were gaining weight, and had good appetites and increased hemoglobin values, while those on the basal ration alone or those injected with cobalt continued to decline. At this time the rumen contents of four sheep from each group were sampled by stomach tube for bacteriological study, and the remaining sheep were sampled the following week. Six additional sheep were maintained for 1 month on a restricted feed intake comparable to that of the cobalt-deficient sheep. These sheep were then sampled to determine the effect of lowered feed intake *per se* on rumen bacteria. The sheep received adequate cobalt in the ration and ate 60% or more of the ration eaten by the sheep fed cobalt. The kinds and number of bacteria present in the rumen contents of the four groups of sheep were compared by means of Gram stains, direct slide counts, and anaerobic cultures (*1*).

The results of the slide counts and cultural tests are summarized in Table 1.

TABLE 1

INFLUENCE OF DIET ON RUMEN BACTERIA

Dietary group	No. of animals	Bacterial slide count	Cultural results—No. growing in dilutions 10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}
		billions/gm					
Cobalt-deficient	7	30.2	7	7	2	0	0
Cobalt injected	7	30.7	7	5	3	0	0
Cobalt fed	7	54.6	7	7	7	5	2
Restricted feed intake	6	56.3	6	6	5	3	1

Culturally, there were major differences between the dietary groups. The samples from sheep fed cobalt gave the highest cultural counts, all cases showing growth in the 10^{-9} dilution, 5 in 10^{-10}, and 2 in 10^{-11} dilution of rumen contents. In marked contrast, only 2 cobalt-deficient and 3 cobalt-injected animals showed bacterial growth in the 10^{-9} dilution, and neither group gave growth above this dilution. Bacteriologically, sheep on restricted feed intake but fed sufficient cobalt resembled those fed cobalt and unlimited feed. Both the kinds and numbers of bacteria in the rumen content of sheep fed cobalt resembled those of sheep fed normal rations (*1*).

It can be seen by slide count that cobalt-fed sheep had almost twice as many bacteria per g of rumen contents as cobalt-deficient and cobalt-injected animals. The bacterial slide count of sheep on restricted feed intake was about the same as that of sheep fed cobalt.

Gram stains of samples were identified only by number, which had no significance to the examiner, but after microscopic study it was possible, on the basis of the stains alone, to separate cobalt-deficient sheep from cobalt-fed animals. In 5 out of 7 cases, Gram stains from cobalt-injected sheep were grouped with cobalt-deficient sheep; while in the other two instances, the bacterial picture was not clearly typical of either group.

Gram stains from sheep fed cobalt were characterized by the usual wide variety of bacteria with large numbers of *Gram-positive, slender curved rods,* and *coccoid types* of bacteria covering the fibers, which were in an advanced stage of decomposition. The slides from cobalt-deficient and cobalt-injected sheep were recognized because of a complete lack of slender curved rods, and a great reduction in the numbers of coccoid forms on the fibers. The fibers were only slightly disintegrated. The differences in the bacterial picture between the groups lay more in the absence of these bacteria in cobalt-deficient and cobalt-injected animals than in a complete change of flora. Gram stains from sheep on

[1] Formerly at Ohio Experiment Station. (Present address: National Dairy Research Laboratories, Inc., Oakdale, Long Island, N. Y.)

restricted feed intake were similar to those of animals fed cobalt.

These data show that in cobalt deficiency marked alterations occur in the types and numbers of bacteria in ruminants and that these bacteriological changes are not caused by lowered feed intake of the deficient animals.

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Spectrophotometric Determination of Amino Acids by the Ninhydrin Reaction

William H. Fitzpatrick

Medical Nutrition Laboratory, U. S. Army, Chicago

In view of the growing interest in the separation of amino acids by partition paper chromatography (*1, 2, 3, 4, 7, 8*), studies have been made to determine if a quantitative relationship might be established in the colorimetric reaction between ninhydrin and the amino acids. Harding and MacLean (*5*) first developed this reaction and later (*6*) condemned it as a colorimetric method for amino acid determination. In mixtures of amino acids they found a lack of specificity and a variation in the red and blue colors produced in the reaction, since ammonia and amines other than amino acids formed similar colors with ninhydrin.

Solutions of 13 amino acids were prepared by dissolving 2 mg of each in 80% ethanol. The ninhydrin reagent was prepared by dissolving 200 mg of ninhydrin in 100 ml of isobutanol. Fifty γ of each amino acid were placed into a test tube, 2 ml of ninhydrin reagent added, and the total volume made up to 10 ml with isobutanol. It was observed in many trials that the color would develop by itself at room temperature. However, to make conditions uniform, all tubes were incubated at 80° C for 3 min, removed, and cooled under running water to 22° C for 3 min. Each tube was then immediately placed in a Coleman No. 6 spectrophotometer, and the transmission determined at 10 mμ intervals between 400 and 700 mμ against a standard containing 2 ml of ninhydrin reagent and 8 ml of isobutanol until the inflection point was approached when the measurements were made at 5 mμ intervals.

The wavelengths corresponding to the inflection points in the wavelength-% transmission curves of amino acids are presented in Table 1.

TABLE 1

WAVELENGTH OF MAXIMUM ABSORPTION OF AMINO ACIDS REACTED WITH NINHYDRIN

Amino acid	Wavelength in mμ	Amino acid	Wavelength in mμ
Phenylalanine	530	Glycine	555
Lysine	545	Methionine	560
Threonine	550	Valine	560
Tryptophane	550	Arginine	560
Alanine	550	Norvaline	560
Asparagine	550	Isoleucine	565
Leucine	555		

Serial dilutions of the amino acids were made and, after reaction with ninhydrin, measured spectrophotometrically at the appropriate wavelength. The quantitative limits in γ, within which it appears possible to measure spectrophotometrically amino acids which have reacted with ninhydrin under the described conditions, that is, the limits at which the points of a plot of concentration vs. logarithm of transmission fall on a straight line, are presented in Table 2.

TABLE 2

LIMITS OF THE SPECTROPHOTOMETRIC DETERMINATION OF AMINO ACIDS REACTED WITH NINHYDRIN*

Amino acid	Concentration (γ per 100 ml)	Amino acid	Concentration (γ per 100 ml)
Phenylalanine	10–140	Threonine	20–130
Isoleucine	20–125	Tryptophane	20–200
Leucine	10–100	Glycine	10– 80
Lysine	5– 50	Alanine	10– 80
Methionine	10–100	Asparagine	20–180
Valine	10–100	Norvaline	10– 70
Arginine	20–100		

* Within these limits the transmission was a straight line.

From the results of these determinations it appears feasible to adapt these studies to the quantitative estimation of amino acids separated by the partition paper chromatographic method. Transmission curves could be determined, and the quantities of specific amino acid present thereby measured in appropriate dilution.

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