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# FEEDS RICH IN GROWTH STIMULATING FACTORS FOR RUMEN ORGANISMS AND CERTAIN VOLATILE FATTY ACIDS AS SUPPLEMENTS FOR RATIONS FOR CATTLE AND SHEEP

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**ON THE COVER . . .**

This is Figure 1 referred to on page 14. It shows the harness used in the metabolism tests on sheep.

# FEEDS RICH IN GROWTH STIMULATING FACTORS FOR RUMEN ORGANISMS AND CERTAIN VOLATILE FATTY ACIDS AS SUPPLEMENTS FOR RATIONS FOR CATTLE AND SHEEP

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Feeds are subject to digestive action by micro-organisms and protozoa in the rumens of cattle and sheep. These digestive processes are thus linked to the well-being of the microbial flora and the various factors influencing their proliferation and activity. It has been established that the micro-organisms require an available source of nitrogen (protein or non-protein nitrogen), minerals, energy both in the form of cellulose and more easily digested carbohydrates, and small amounts of certain nutrients which are present in protein concentrates, good quality roughages fermentation by-products and rumen fluid itself (Arias **et al.**, 1951; Burroughs **et al.**, 1950a, 1951a, 1951b; and Bentley **et al.**, 1955). The role of these latter nutrients has been studied extensively at this Station using the artificial rumen technique.

When rumen micro-organisms are grown in a medium composed of purified ingredients in the artificial rumen a marked response in cellulose digestion is obtained from the addition of these natural materials to the nutrient medium. The bacterial numbers increase more rapidly, especially cellulose digesting bacteria, if protein-rich concentrates, hot water extracts of alfalfa meal, or materials such as dried distiller's solubles, steepwater or other fermentation by-products are added to the artificial rumen medium. Furthermore, research results have shown that a similar response can be obtained from certain volatile fatty acids (valeric, iso-valeric, iso-butyric, iso-caproic, caproic) and amino acids (valine, leucine, proline and iso-leucine). Protein concentrates, hot water extracts of forages, forages themselves and all protein containing feedstuffs contain amino acids. The volatile fatty acids are formed in the rumen by bacteria during the process of digesting carbohydrates such as starch.

Concurrent with the laboratory studies with the artificial rumen, a number of steer and lamb feeding trials were undertaken. The objective of these experiments was to evaluate the effect of adding materials rich in stimulatory nutrients for rumen micro-organisms to the feed by observing the performance of steers and lambs. Secondary objectives were: (1) To study the effect of forage quality on the performance of steer calves or lambs in terms of specific nutrients, and, (2) to further evaluate the concept of feeding the rumen micro-organisms as a means of getting maximum benefit from low grade forages.

## PART I. STEER FEEDING EXPERIMENTS

### EXPERIMENTAL

A series of feeding trials were carried out with small lots of steers during the period from 1953-57. The starting weights of the calves were from 425-525 lb. Many of the calves were individually fed in stanchions. In 1955-56, nine lots of steers (7 per lot) were group fed free-choice.

The rations used are described in Table 1. Since the objective was to evaluate the supplements as sources of nutrients for rumen micro-organisms, poor quality roughages were used and the supplementary nitrogen was supplied as non-protein nitrogen (urea). Attempts were made to balance the rations for calcium, phosphorus, vitamins A and D, sodium chloride and protein in accordance with National Research Council Recommendations (1950). The urea, minerals, vitamins A and D, and special supplements were mixed with enough ground ear corn to make four pounds of ration. As the calves were started on the rations, they were fed this mixture, the roughage and additional ground ear corn being fed **ad libitum.** Water was available from water cups in the stalls. It was observed that calves kept in stanchions and fed these rations started slowly but after becoming adjusted (2-4 weeks) they ate well and, in most experiments, gained quite well. All calves were Herefords selected for uniformity in weights from groups of 100-120 calves purchased from one ranch in Texas.

When valeric acid was added it was neutralized with sodium hydroxide and a concentrated solution added to the ration. Biotin and para-amino benzoic acid were added as solutions.

### RESULTS AND DISCUSSION

#### 1953–1954

The ration used in this trial is described in Table 1. The experiment involved two phases. During the first part of the test the steers received all the roughage they would consume plus an average of 5 lb.

**TABLE 1.—Rations Fed Growing-Fattening Steers**
**(Amount per Steer per Day)**

| | 1953-1954 | 1954-1955 | 1955-1956 | 1956-1957 |
|---|---|---|---|---|
| Ground Ear Corn | Full-Fed* | Full-Fed | Full-Fed | Full-Fed |
| Poor Quality Timothy Hay (Chopped) | Full-Fed | Full-Fed | Full-Fed | Full-Fed |
| Ground Corn Cobs, 3/8" Grind | Full-Fed | Full-Fed | Full-Fed | Full-Fed |
| Corn Gluten Meal | | 0.5 lb. | 0.5 lb. | 0.5 lb. |
| Urea† | 0.25 lb. | 0.25 lb. | 0.20 lb. | 0.20 lb. |
| $CaHPO_4$ (Purified) | 26. gm. | 26. gm. | 26. gm. | |
| $CaCO_3$ (Purified) | 19. gm. | 19. gm. | 19. gm. | |
| Trace Mineral Salt Mix‡ | 22.5 gm. | 22. gm. | 22. gm. | 22.0 gm. |
| Stabilized Vitamin A§ | 21,000 I.U. | 21,000 I.U. | 21,000 I.U. | 21,000 I.U. |
| Sodium Sulfate | | 2.5 gm. | 2.5 gm. | 2.5 gm. |
| Vitamin D§ | | 0.5 gm. | 0.5 gm. | 0.5 gm. |
| Stilbestrol Premix | | | 0.01 lb. | |
| Steamed Bone Meal | | | | 50. gm. |
| Feeding Grade Limestone | | | | 10. gm. |

*Restricted to 5 lb. for phase 1 of experiment; full-fed during second phase.

†Crystalline urea (DuPont) used in 1953-56; Feeding grade urea supplied either by Allied Chem. and Dye Corp. or the E. I. DuPont de Nemours Co. used in other years.

‡Designed to supply 0.5 mg. cobalt; 20 mg. manganese, 14 mg. zinc, 25 mg. copper and 800 mg. iron daily and iodized salt.

§Vitamin A., 3,000 I. U./100 lb. body weight, average weight used 700 lb. Product contained 250,000 I. U. per g. (Chas. F. Pfizer & Co.) Vitamin D., 1,500 I. U. equal estimated D. content of 3 lb. of alfalfa hay.

of corn and cob meal. In the second portion of the experiment, corn and cob meal was full-fed along with the roughage.

The results in Table 2 suggest that feeding 50 g. of dried brewer's yeast per steer per day or a hot water extract prepared from 70 g. of alfalfa meal per day increased the average daily gain 13 to 17 percent and decreased the amount of feed required per pound of gain by 13 to 22 percent.

In this trial one half of the steers were fed ground corn cobs and the other half received chopped mature timothy hay (late seed stage). The steers on the cobs gained faster (1.79 lb. per day), ate more roughage (cobs) and had a better appearance than the animals fed the poor timothy; the latter animals gained 1.63 lb. per day during the 238 day test.

**TABLE 2.—The Effect of Dried Yeast and Alfalfa Extract on Steer Performance (1953-54)**

| | Corn and Cob Meal Limited* | | Corn and Cob Meal Full-Fed† | |
|---|---|---|---|---|
| | Basal | Basal plus yeast or alfalfa extract | Basal | Basal plus yeast or alfalfa extract |
| No. of Steers | 4 | 6 | 4 | 6 |
| Av. Daily Gain, lb. | 1.26 | 1.42 | 1.9 | 2.22 |
| Feed/lb. Gain, lb. | 9.37 | 7.27 | 8.93 | 7.81 |
| Feed Intake: | | | | |
| Roughage | 7.0 | 6.3 | 2.5 | 2.9 |
| Concentrate | 5.2 | 5.4 | 14.5 | 13.9 |

*126 days. †112 days

## 1954–1955; 1955–1956

In 1954 (Bentley **et al.**, 1954, 1955) it was found that valeric acid plus biotin and para-aminobenzoic acid were growth factors ("rumen factors") for cellulose digesting rumen microorganisms in the artificial rumen. In this laboratory rumen fermentation, purified ingredients were used to supply the nutrients to the micro-organisms, **e.g.** purified wood cellulose, minerals, etc. The micro-organisms were also separated from their natural environment, rumen liquor, as found in the rumen. The addition of the "rumen factors" to the medium resulted in a 3 to 5 fold increase in the amount of cellulose digested during a 30-hr. fermentation period.

It became of interest to evaluate these rumen factors and other feedstuffs, such as alfalfa meal, which have similar rumen factor activity in the artificial rumen, as supplements to fattening-type rations for steers. In these preliminary studies a basal ration was chosen (see Table 1) made up of ground ear corn and ground corn cobs fed free-choice, plus 0.5 lb. of corn gluten meal and 0.2 lb. of urea per head per day to supply additional crude protein. Calcium, phosphorus, salt, trace minerals, sodium sulfate and dry vitamin A and D were fed to supply recommended amounts of these nutrients. In 1954-1955, the steers were implanted with three pellets which contained 12 mg. each of stilbestrol and in 1955-1956 the steers were fed 10 mg. daily of stilbestrol.

**TABLE 3.—The Effect of "Rumen Factors" on Steer Performance (1954-55)***

| Ration | No. of steers | Ave. daily gain (lbs.) | Feed required per 100 lbs. of gain | | Ave. daily feed intake, lb. | |
|---|---|---|---|---|---|---|
| | | | Concen-trate | Corn cobs | Concen-trate | Corn cobs |
| Control | 5 | 2.44 | 450 | 192 | 11.0 | 4.69 |
| Control + rumen factors† | 5 | 2.60 | 466 | 199 | 12.1 | 5.13 |
| Control + valeric acid | 3‡ | 2.17 | 495 | 225 | 10.74 | 4.79 |

*Steers were individually fed in stanchions for 182 days.

†Valeric acid (0.5 gm.), biotin (5 mg.), para-aminobenzoic acid (5 mg.) per steer per day.

‡One steer removed from this group because of swelling caused by a reaction to resorption of grubs killed by dusting.

Corn cobs were used as the roughage, since it appears that better quality roughages are also sources of rumen factors.

The final results of the 1954-1955 experiment are summarized in Table 3.

From these results it appeared that the rumen factors, i.e. valeric acid in combination with the B-vitamins, but not valeric acid alone had a slightly beneficial effect on average daily gains but not on feed efficiency. The apparent growth depression observed in the valeric acid fed lot was due largely to one steer which did not gain as well as the others.

In 1955-1956, the same control ration was fed to 14 steers, individually fed and kept in stanchions, using the rumen factors and alfalfa meal (0.5 lb. per day) as the supplements to the ration (see Table 1). As in 1954-1955 the steers were carefully selected for uniformity and weighed about 500 pounds at the start of the experiment. The results are summarized in Table 4.

In both experiments these steers consumed an average of from 2.5 to 5.0 lb. of the ground cobs and from 10 to 12 lb. of ground corn per day per head.

The rumen factor supplement did not improve gains or feed efficiency in the 1955-1956 experiment (Table 4). However a positive response in average daily gain was obtained from the addition of the

**TABLE 4.—The Effect of "Rumen Factors" and Alfalfa Meal on Steer Performance (1955-56)***

| Ration | No. of steers | Ave. daily gain (lbs.) | Feed required per 100 lbs. of gain | | Ave. daily feed intake, lb. | |
|---|---|---|---|---|---|---|
| | | | Concen-trate | Corn cobs | Concen-trate | Corn cobs |
| Control | 5 | 2.17 | 526 | 211 | 11.34 | 4.6 |
| Control + rumen factors† | 5 | 2.14 | 532 | 215 | 11.34 | 4.58 |
| Control + 0.5 lb. per day per steer of alfalfa meal | 4 | 2.37 | 512 | 218 | 11 98 | 5.07 |

*224 day experiment.

†Valeric acid (0.5 gm.), biotin (5 mg.), para-aminobenzoic acid (5 mg.) per head per day

alfalfa meal supplement to the corn-corn cob roughage ration. These results would suggest that the steers on this ration were capable of synthesizing sufficient valeric acid and B-vitamins in their rumens to meet the requirements for these compounds as rumen factors. In experiments conducted by Klosterman (Table 5) there was a slight benefit from the factor supplement similar to that observed in the 1954-1955 experiment. In summary, it would appear that the benefit from these rumen factors is not sufficiently large to warrant their inclusion in a supplement at this time.

Alfalfa meal, however, gave a larger response. Previous work at the Ohio Station and at Purdue University would suggest that the alfalfa meal supplementation of rations in which poor quality roughages are used will improve steer performance. The protein, vitamin A, and presumably the minerals supplied by the alfalfa meal should not have been responsible for the stimulation in rate of gain since the control rations were adequately fortified with vitamin A and minerals and the protein content of all rations was equalized. Purified cellulose was added to the control and control plus rumen factor rations to compensate for the cellulose (crude fiber) in the alfalfa. All steers were fed diethylstilbestrol (10 mg. per head per day) which should have adjusted or overcome the possibility of hormone activity supplied by the alfalfa.

## Feedlot Trial 1955–1956

The main objective of this experiment was to study various factors which may influence the amount of protein which needs to be fed to fattening cattle. The factors under study in this experiment were soybean oil meal, rumen factors and good quality alfalfa hay. Good quality alfalfa hay has been shown to be a good source of rumen factors.

The calves were divided at random within weight groups into nine uniform lots. All lots were fed four pounds of concentrates per head daily, a full feed of corn silage, salt and a mineral mixture of two parts steamed bone meal, two parts ground limestone and one part salt. Three levels of protein were fed: no supplement, 0.75 lb. or 1.5 lbs. of soybean oil meal per head daily or its equivalent as supplied by alfalfa hay. The levels of soybean oil meal were fed with and without the addition of rumen factors. These rations were fed for a 98-day period which utilized the corn silage available for this experiment. The steers were then reallotted and used in other feeding experiments.

The rumen factors and amounts fed per head daily were: valeric acid as the sodium salt, 0.5 gram; biotin, 5 mgs.; and para-aminobenzoic acid, 5 mgs. These materials were mixed with ground ear corn in such amounts that one pound per head daily supplied the specified amounts.

The results of this experiment are presented in Tables 5 and 6.

**TABLE 5.—The Effect of Rumen Factors and Alfalfa Hay on the Performance of Feedlot Steers Fed Varying Levels of Protein (1955-56)—Summary**

| | Average daily gains | | | |
|---|---|---|---|---|
| | Control | Rumen factors | Alfalfa hay | Average |
| No soybean oil meal | 1.43 | 1.37 | 1.54 | 1.45 |
| 0.75 lb. soybean oil meal or equivalent | 1.66 | 1.73 | 1.50 | 1.63 |
| 1.50 lb. soybean oil meal or equivalent | 1.67 | 1.79 | 1.70 | 1.72 |
| Average | 1.59 | 1.63 | 1.58 | |
| | Feed cost per hundredweight of gain | | | |
| No soybean oil meal | $13.60 | $13.96 | $13.39 | $13.65 |
| 0.75 lb. soybean oil meal or equivalent | 12.53 | 12.10 | 14.99 | 13.21 |
| 1.50 lb. soybean oil meal or equivalent | 13 74 | 12.51 | 14.96 | 13.74 |
| Average | 13.29 | 12.86 | 14.45 | |

**TABLE 6.—The Effect of Rumen Factors and Alfalfa Hay on the Performance of Feedlot Steers Fed Varying Levels of Protein (1955-56)—Detailed Data**

| | Soybean oil meal | | | Soybean oil meal plus rumen factors | | | Alfalfa hay | | |
|---|---|---|---|---|---|---|---|---|---|
| Lot number | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Number in lot | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Average weight, November 8 | 506 | 516 | 516 | 516 | 517 | 508 | 527 | 511 | 506 |
| Average weight, February 14 | 647 | 679 | 679 | 650 | 686 | 683 | 677 | 659 | 672 |
| Average daily gain, 98 days | 1.43 | 1.66 | 1.67 | 1.37 | 1.73 | 1.79 | 1.54 | 1.50 | 1.70 |
| Average daily ration: | | | | | | | | | |
| Corn and cob meal | 4.00 | 3.25 | 2.50 | 4.00 | 3.25 | 2.50 | 4.00 | 4.00 | 4.00 |
| Soybean oil meal | | .75 | 1.50 | | .75 | 1.50 | | | |
| Silage | 16.50 | 16.50 | 16.80 | 15.60 | 16.80 | 16.80 | 19.70 | 15.70 | 13.40 |
| Hay | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | | | |
| Alfalfa hay | | | | | | | 1.50 | 3.75 | 6.00 |
| Minerals, oz. | .5 | .6 | .7 | 1.1 | 6 | .8 | .5 | 5 | .4 |
| Salt, oz. | 5 | 2 | .3 | 3 | 4 | 3 | .5 | 4 | 7 |

**TABLE 6.—The Effect of Rumen Factors and Alfalfa Hay on the Performance of Feedlot Steers Fed Varying Levels of Protein (1955-56)—Detailed Data—Continued**

| | Soybean oil meal | | | Soybean oil meal plus rumen factors | | | Alfalfa hay | | |
|---|---|---|---|---|---|---|---|---|---|
| Feed per hundredweight, gain: | | | | | | | | | |
| Corn and cob meal | 279 | 196 | 150 | 292 | 188 | 140 | 260 | 266 | 236 |
| Soybean oil meal | | 45 | 99 | | 43 | 84 | | | |
| Silage | 1151 | 993 | 1008 | 1135 | 971 | 940 | 1280 | 1042 | 788 |
| Hay | 174 | 151 | 150 | 182 | 144 | 140 | | | |
| Alfalfa hay | | | | | | | 97 | 250 | 354 |
| Minerals | 2 | 2 | 3 | 5 | 2 | 3 | 2 | 2 | 2 |
| Salt | 2 | .5 | 1 | 1.5 | 1 | 1 | 2 | 2 | 2.5 |
| Feed cost per hundredweight of gain | $13.60 | $12.53 | $13.74 | $13.96 | $12.10 | $12.51 | $13.39 | $14.99 | $14.96 |

**FEED PRICES USED**

Corn ________________________________$ 1.40 per bu. (70 lb.)
Soybean oil meal _______________________ 75.00 per ton
Corn silage __________________________ 10.00 per ton
Mixed hay ___________________________ 25.00 per ton
Alfalfa hay ___________________________ 35.00 per ton
Minerals ____________________________ 3.00 per cwt.
Salt ________________________________ 1.50 per cwt.

The average rates of gain for the three lots fed soybean oil meal, soybean meal plus rumen factors or alfalfa hay were quite similar (Table 5). The rumen factors used had no apparent sparing effect on the protein requirement. When fed the low-protein ration, the steers fed the rumen factors did not gain quite as rapidly as the control steers. However, when fed with soybean oil meal they gained slightly faster. There was a marked increase in rate of gain from the addition of 0.75 lb. soybean oil meal to the ration but little additional advantage from feeding 1.5 lb. soybean oil meal. The cheapest gains were obtained when 0.75 lb. soybean oil meal was fed. The value placed on alfalfa hay would influence the feed costs per hundredweight of gain for the cattle fed this ration.

The detailed results of this experiment are given in Table 6. In the calculations of feed costs, no charge was made for the rumen factors fed.

### 1956–1957

The positive results obtained from feeding alfalfa meal in 1955-56 trial suggested a continuation of the research with the meal. If a positive response could be demonstrated for an alfalfa extract one could then proceed with the chemical identification of the compounds derived from the alfalfa. In a previous experiment (1953-54) an indication that hot water extracts could be prepared which had growth promoting activity for steers had been obtained (Table 2).

In 1956-57 another group of 14 Hereford steers were fed the basal ration described in Table 1 supplemented as indicated in Table 7. The hot water extracts were prepared by extracting 1 lb. of 17 percent alfalfa meal with 7 lb. of water heated with steam for 30 minutes. The slurry was then filtered through cheesecloth and the liquid pressed out with a fruit press. The cake (residue) was then re-suspended in the minimum amount of water needed to form a slurry and re-pressed. The extract was concentrated in a drying oven, weighed, and an aliquot equal to 0.5 lb. of meal fed to the animals where indicated. The residue was dried and, on the average, 1 lb. of meal yielded 0.72 lb. of dried residue.

The results of a 174-day experiment are given in Table 7.

These results again indicated that the addition of alfalfa meal to a corn-gluten meal-urea-corn cob-minerals ration increased the rate of gain of steers slightly. The substitution was made on an iso-nitrogenous basis and purified cellulose was added to the ration containing the hot water extract to adjust the fiber content of the rations. No progress

TABLE 7.—The Effect of Alfalfa Extracts on Steer Performance (1956-57)

| | No. of steers | Ave. daily gain, lb. | Feed per lb. gain, lb. | Ave. feed intake, lb. | |
|---|---|---|---|---|---|
| | | | | Conc. (cob free) | Corn cobs |
| Basal plus Water Ext. equal to 0.5 lb. meal | 4 | 2.18 | 816 | 13.2 | 4.5 |
| Basal plus 0.5 lb. 17% protein alfalfa meal per day per steer | 4 | 2.30 | 816 | 13.7 | 4.9 |
| Basal plus residue from water extraction of meal | 4 | 2.20 | 808 | 13.1 | 4.5 |

was made on the separation or extraction of the activity in meal, **i.e.** the extract and residue from the hot water extracted meal gave comparable results.

One drawback to this experiment was that lack of space did not permit the inclusion of a lot of cattle fed the basal ration without the alfalfa fractions or meal itself.

## SUMMARY OF PART I.

The results of the steer feeding experiments indicate that alfalfa meal and yeast may improve the growth performance of steer calves fed essentially a corn-corn cob-urea-minerals ration. The addition of the "rumen factors", valeric acid, biotin, and para-aminobenzoic acid, did not consistently improve steer performance.

## PART II. LAMB EXPERIMENTS

### EXPERIMENTAL

Several feeding experiments were conducted using small lots (6–7) lambs group-fed the rations described in Table 8. As with the steers, attempts were made to formulate rations based on poor quality feeds inasmuch as the objective of these trials was to evaluate supplements that might replace, wholly or in part, the "growth factors" of good forages.

The lambs were procured either from the Station flock or purchased. Before they were started on test, the lambs were wormed with phenothiazine. Allocation of the lambs to the various nutritional treatments was made on the basis of sex, weight, type and breeding. The lambs were weighed at 2-week intervals and the feed intake measured. Water was available at all times.

**TABLE 8.—The Composition of the Rations Fed to Growing-Fattening Lambs**

| | 1954-1955* | 1955-Fall† | 1955-Summer† |
|---|---|---|---|
| Ground Corn | Full-Fed | | |
| Poor Quality Timothy Hay | Full-Fed | Full-Fed | Full-Fed |
| Supplement: | | | |
| Urea, Feed Grade | 8.0 % | 2.0 % | 2.0 % |
| $CaCO_3$ | 2.0 % | | |
| $CaHPO_4$ | 0.5 % | | |
| Vitamin D (Delstrol) | 0.2 g/lb. | 0.6 g/100 lb. | 0.6 g/100 lb. |
| Vitamin A (Pfizer's) 250,000 I.U./g | 0.01 g/lb. | 0.75 g/100 lb. | 0.75 g/100 lb. |
| Ground Corn | 89.5 % | 79.0 % | 79.78 % |
| Corn Gluten Meal | | 15.0 % | 15.0 % |
| Steamed Bone Meal | | 1.5 % | 1.5 % |
| Ground Limestone | | 0.5 % | 0.5 % |
| Sodium Sulfate | | 0.2 % | 0.22 % |
| Trace Mineralized Salt | | 1.0 % | 1.0 % |

*0.5 lb. of supplement fed per head per day.
†Fed as a complete mixed ration.

Metabolism experiments were carried out with wethers in the conventional manner using crates which permitted separate total collection of the feces and urine. A harness was used to collect the feces as illustrated in Figure 1. Nitrogen in the wet feces sample and in the urine was determined by the Kjeldahl method. Cellulose was determined by the Crampton-Maynard procedure (1938) and the proximate analyses of the feed and feces by the conventional A.O.A.C. procedures.

Valeric and caproic acids were added to a portion of the ration as their sodium salts in a solution. This facilitated mixing the volatile fatty acid into the feed. (Figure 1 appears on the cover.)

A mineral mixture composed of three parts steamed bone meal or dicalcium phosphate mixed with one part of trace mineralized salt was available in each pen at all times.

## RESULTS AND DISCUSSION

### 1954–1955 Lamb Feeding Experiment

Merino wether lambs weighing between 51 and 52 lb. were started on the ration described under the heading of 1954 in Table 8 on September 10, 1954. They were continued on this ration supplemented as indicated in Table 9 for 161 days or to an average weight of 91 to 93 lb.

The volatile fatty acids (valeric and caproic) used as a supplement had no effect on the performance of these lambs. However, the hay used and the breeding of the lambs resulted in relatively slow gains. It was thought the possible supplementary benefit of these acids as rumen factors might be studied more readily with more rapid gaining animals.

**TABLE 9.—The Effect of Valeric and Caproic Acids on Body Weight Gains, Feed Required per Pound of Gain, and Average Daily Ration of Lambs (1954)**

| | No. of Lambs | Ave. daily Gain, lb. | Ave. daily ration, lb. | | Feed Req./lb. Gain, lb. | |
|---|---|---|---|---|---|---|
| | | | Poor Hay | Concentrate | Poor Hay | Concentrate |
| Basal | 7 | .26 | .91 | 1.46 | 3.49 | 5.81 |
| Basal + 0.01% Valeric acid | 7 | .25 | .92 | 1.46 | 3.68 | 6.07 |
| Basal + 0.05% Valeric acid | 7 | .25 | .91 | 1.46 | 3.66 | 6.03 |
| Basal + 0.01% Caproic acid | 7 | .25 | .88 | 1.46 | 3.61 | 6.10 |

## 1955 Lamb Feeding Experiment (Summer)

Thirty-five lambs of Suffolk × Columbia × Merino or Suffolk × Dorset × Merino breeding were placed on the basal ration described in Table 8 and continued for 79 days. The supplements to the basal ration and the performance of the lambs are summarized in Table 10. A hormone implant composed of 160 mg. progesterone and 4 mg. estradiol (Synovex as available in 1955) was placed under the skin under the jaw of one lot of lambs (see Table 10).

The response to the hormone implant and the hormone-antibiotic treatment was from 0.13 to 0.18 lb. per day in daily gains with about a 21 percent saving in feed (basal lot vs. hormone treated animals).

A slight response was obtained with the valeric acid-biotin-PABA supplement but the difference in average daily gain was small while the feed saving was about 16 percent. However, the feed requirement data for this lot may have been influenced by the removal of one lamb near the end of this test. The average feed consumed per animal was deducted from the total to adjust the feed consumption data. This adjustment would not necessarily bias the results but it should be considered.

**TABLE 10.—The Effect of Rumen Factors, Synovex, and Antibiotics on Lamb Performance (1955)**

| Ration | No. of lambs per treatment | Ave. daily gain, lb.* | Ave. daily ration, lb. | | Feed/lb. gain, lb. | |
|---|---|---|---|---|---|---|
| | | | Poor quality timothy hay | Concentrate | Hay | Concentrate |
| Basal | 7 | 0.33 | 0.53 | 2.09 | 1.58 | 6.27 |
| Basal plus 50 mg. biotin, 100 mg. PABA† per 100 lb. of ration | 7 | 0.31 | 0.50 | 2.19 | 1.63 | 6.81 |
| Basal plus biotin and PABA plus 10 g. valeric per 100 lb. | 6 | 0.39 | 0.51 | 2.06 | 1.31 | 5.31 |
| Basal plus biotin, PABA and valeric acid plus synovex treatment of lambs | 7 | 0.46 | 0.60 | 2.28 | 1.31 | 4.99 |
| Basal plus 0.42 lb. of aureofac 2A per 100 lb. ration‡ plus synovex | 7 | 0.51 | 0.61 | 2.39 | 1.19 | 4.70 |

*All lambs graded choice at the Cleveland market when sold at the termination of the experiment.

†PABA represents para-aminobenzoic acid.

‡15 mg. of chlortetracycline per lb. of ration.

## 1955 Lamb Experiment (Fall)

Forty Colorado lambs (ewes and wethers) were placed on the rations described in Table 8 and supplemented as indicated in Table 11. The objective of the experiment was to evaluate the valeric-biotin-PABA supplement as fed to Synovex treated lambs as contrasted to untreated lambs. This was done since the hormone treatment gave an added growth boost to the lambs and also increased feed intake which might result in an increased need for these rumen factor supplements. Chopped poor quality hay was used.

The results of this experiment follow the pattern of those from the 1955 Summer experiment. A small response was obtained from the valeric-biotin-PABA supplement (lot 3) for one treatment but no

**TABLE 11.—The Effect of Rumen Factors on the Performance of Untreated and Hormone Treated Lambs (1955)**

| Ration No. | Ration | Synovex* implant | No. of lambs | Ave. daily gain, lb. | Ave. daily ration, lb. | | Feed/lb. gain, lb. | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Hay | Concentrate | Hay | Concentrate |
| 1 | Basal ration | — | 8 | 0.29 | .89 | 1.75 | 3.06 | 5.88 |
| 2 | Basal ration | + | 7 | 0.44 | .93 | 1.88 | 2.13 | 4.21 |
| 3 | Basal + 50 mg. biotin and PABA and 10 gm. valeric acid per 100 lb. ration | — | 8 | 0.33 | .90 | 1.88 | 2.71 | 5.57 |
| 4 | Same as 3 | + | 8 | 0.41 | .89 | 2.01 | 2.17 | 4.98 |
| 5 | Basal + 15 mg./lb. ration chlortetracycline | — | 7 | 0.35 | .80 | 1.87 | 2.34 | 5.24 |

*The implant contained 4 mg. of estradiol and 160 mg. progesterone.

response when lot 4 and 2 were compared. The hormone implant alone gave a response in feed saving and rate of gain.

## Digestion Trials with Yearling Wethers

Since nutrients needed in small amounts by the rumen flora are present in natural feedstuffs, a direct approach to the problem seemed to be the use of a ration made up from purified ingredients. A ration palatable to sheep was devised and fed to four wethers. It was necessary to feed 0.5 lb. of wheat straw per day per head to keep the animals on feed. The ration composition is given in Table 12. It will be noted that all ingredients of the ration with the exception of corn gluten meal and wheat straw are purified ingredients. The corn oil reduced the dustiness of the ration and thereby improved palatability of the ration.

A series of digestion trials were carried out using the basal ration described in Table 12 supplemented with 10 gm. valeric acid, and 50 mg. each of biotin and PABA per 100 lb. of ration.

**TABLE 12.—Composition of the Basal Semi-synthetic Type Ration**

| Constituent | Composition, % |
|---|---|
| Urea | 2 |
| Corn gluten meal | 22 |
| Cellulose* | 25 |
| Starch | 25 |
| Corn Sugar (Cerelose) | 18 |
| Corn oil purified | 2 |
| Mineral mix† | 6 |
| Sodium sulfate | 1 g./lb. |
| Vitamin A acetate (250,000 I.U./g.) | 0.007 g./lb. |
| Vitamin D (3,000 I.U./g.) | 0.05 g./lb. |

*Solka Floc BW-40, Brown Company, Berlin, N. H.
†3% $CaHPO_4.2H_2O$ and 3% trace mineralized salt.

The results are summarized in Tables 13 and 14. The wethers fed this purified type ration increased from an average of 107 to 130 lb. in weight indicating that the ration was palatable.

The protein equivalent of the rations used was approximately 13 percent crude protein with the major portion supplied from the concentrate. Of the nitrogen in the concentrate, approximately 40 percent was contributed by urea. This then gives a protein equivalent slightly above the National Research Council recommendation of 12 percent for

**TABLE 13.—Apparent Digestibility Coefficients and Nitrogen Balance in Metabolism Trials with Lambs Fed Purified-type Rations**

| Ration | Dry Matter Digestibility % | Cellulose Digestibility % | Protein Digestibility % | Nitrogen‡ Balance g. |
|---|---|---|---|---|
| Basal | 70.34 | 54.15 | 70.92 | —2.2 |
| Basal + Supplement* | 67.58 | 48.11 | 75.12 | —4.1 |
| Basal | 70.12 | 50.15 | 74.38 | —0.0 |
| Basal + Supplement | 72.62 | 60.75 | 75.87 | —5.7 |
| Basal + B-vitamins† | 72.10 | 57.54 | 75.28 | —1.4 |
| Basal | 70.77 | 62.21 | 74.75 | —2.9 |

*Valeric acid, biotin and PABA, see table 10 and table 11 for amounts.
†Biotin and PABA.
‡Nitrogen Balance-g./head/day.

**TABLE 14.—Consumption of Digestible Nutrients in Grams by Lambs During Metabolism Trials**

| Ration | Digestible Dry Matter/day g. | Digestible Cellulose/day g. | Digestible‡ Protein/day g. |
|---|---|---|---|
| Basal | 664 | 153 | 93.8 |
| Basal + Supplement* | 753 | 180 | 131.3 |
| Basal | 725 | 143 | 108.3 |
| Basal + Supplement | 782 | 198 | 112.5 |
| Basal + B-vitamins† | 847 | 202 | 125.0 |
| Basal | 645 | 171 | 95.0 |

*Valeric acid, biotin and PABA.
†Biotin and PABA.
‡Nitrogen Balance-g./head/day.

best urea nitrogen utilization but is well within their statement that urea is satisfactorily used as long as 25 percent of the total protein equivalent is composed of preformed protein.

This semi-synthetic type ration which, except for the corn gluten meal and straw was composed of pure ingredients, supported very satisfactory lamb performance. The animals gained an average of almost 30 lb. in 110 days, (.27 lb./day) which is a good gain for an animal that size. This ration has many possible uses in investigating nutritional requirements for lambs.

The apparent digestibility coefficient of dry matter, cellulose and protein and the daily consumption of dry matter, cellulose and protein were analyzed statistically by analysis of variance.

The results showed that the digestible dry matter intake was significantly increased ($P<0.01$) by the addition of valeric acid, biotin and PABA to the basal or the addition of biotin and PABA without valeric acid. The digestible cellulose intake was significantly increased ($P<0.01$) by the addition of valeric acid, biotin and PABA or biotin and PABA alone, and the digestible protein intake was significantly increased ($P<0.05$) by the addition of valeric acid, biotin and PABA and significantly increased ($P<0.01$) by biotin and PABA additions alone.

## GENERAL DISCUSSION

Studies with the artificial rumen have shown that rumen microorganisms will respond by increased cellulolytic activity to specific nutrient additions to the medium (Burroughs **et al.**, 1950a, b; Bentley **et al.**, 1955; Dehority **et al.**, 1957). The practical significance of this nutrient-microorganism relationship is indicated in the results summarized by Klosterman **et al.** (1953). Supplementation of a poor hay ration with trace minerals improved the rate of gain and feed efficiency of steers much the same as feeding molasses or alfalfa meal. Bentley **et al.** (1954) concluded that cobalt was the major limiting trace element. The role of cobalt in the synthesis of vitamin $B_{12}$ by rumen organisms is now well understood (Underwood, 1956).

These specific nutrient-activity relationships for the microflora are presented to illustrate the type of an approach being made in the studies discussed herein. As is pointed out both in the introduction of the publication and throughout the text, these materials were being tested as sources of unidentified or known-growth factors for rumen microorganisms and **not** merely as sources of energy, nitrogen or the major minerals. An attempt was made to design rations that would accomplish this objective.

Some general conclusions seem to be justified on the basis of these results. First, rumen microorganisms and protozoa appear to be able to synthesize enough valeric acid or related acids with cellulolytic factor activity to meet the needs of the cellulose digesting organisms in steers and lambs fed rations made up of 50 to 80 percent corn.

This conclusion must be qualified to the extent that only one level of valeric acid supplementation has actually been studied extensively. Lassiter **et al.** (1958b) reported that a mixture of valeric and iso-valeric acids in a supplement to rations for dairy heifers improved the rate of gain. A possible explanation for the above conclusion is the report by Cline **et al.** (1958) that in the artificial rumen the addition of starch to the medium increased the synthesis of valeric acid. Thus one could theorize that in the rumen of animals being fed a hay-corn-supplement ration, valeric acid (and the related acids known to be active as cellulolytic or growth factors for rumen microorganisms) would be formed in ample amounts.

Second, the observations by Bentley **et al.** (1955) and Dehority **et al.** (1957 and 1958) that certain amino acids have growth promoting activity by virtue of being converted to fatty acids may explain the presence of ample amounts of valeric acid in the rumen to support cellulose digestion, assuming that adequate nitrogen, minerals and energy substrates are available also.

A third consideration is illustrated by the metabolism studies using sheep fed the purified-type ration. The addition of the valeric acid-B-vitamin supplement appeared to increase feed intake but not the apparent digestibility coefficients. It would appear that the **rate** of digestion of cellulose and the entire ration may have been enhanced by feeding the supplement. This is a possible interpretation. Feed intake seems to be an important key to the efficiency of digestion in the ruminants. The illustration used by Doctor Crampton at the Grasslands Conference in 1952 is appropriate. A 1,000 lb. dairy cow will consume an average of 2.5 lb. per 100 lb. body weight of a good forage but only 1.5 lb. of a low quality forage. The activity level in the rumen may be a partial explanation for this observation and could be related to the relationship being studied here. Lassiter **et al** (1958a) found that valeric acid increased the digestibility of dry matter, organic matter, crude fiber and nitrogen free extract and also increased nitrogen retention. Hungate and Dyer (1956) found no increase in performance when steers were given a straw ration supplemented with valeric acid but did observe an improvement in appetite.

## GENERAL SUMMARY

Five different steer feeding experiments and three lamb feeding experiments were carried out to evaluate the supplements previously shown to stimulate the activity of the rumen microflora **in vitro** (artificial rumen).

A supplement composed of valeric acid, biotin and para-aminobenzoic acid (PABA) gave only a slight increase in gain in three of these experiments while in the remainder no benefit was obtained. When this same supplement was added to a purified-type lamb ration, digestibility coefficients were not improved but feed intake was significantly increased. It is concluded that feeding 0.5 gm. per day of valeric acid to a steer receiving a high corn ration will not improve the efficiency of feed conversion and the rate of gain of the animal. A similar conclusion was reached for lamb rations containing 10 gm. of valeric acid per 100 lb.

There was no indication that the addition of biotin or PABA gave a beneficial effect on animal performance.

Alfalfa meal when added to fattening rations for steers improved the rate of gain. The forage used was of low quality (corn cobs or mature timothy hay) but the ration was adequately supplemented with minerals, vitamins A and D and protein and the steers were either fed diethylstilbestrol or pellets of the hormone were implanted under the skin. This would suggest that the alfalfa meal improves growth by a mechanism not now understood. Hot water extraction of alfalfa meal

**did not** yield a fraction as active as the meal itself but this approach to the identification of a factor in alfalfa meal needs more study before a definite conclusion can be made.

When properly supplemented, good performance can be obtained with steers and lambs fed rations formulated to include poor quality timothy hay or corn cobs.

Feeding a good ration, adequate in protein, either from natural sources or non-protein nitrogen, energy from grains or forage and pasture, and adequate minerals should provide the proper amounts of nutrients to support good microbial growth and activity in the rumen.


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