THE NUTRITIVE VALUE OF DRIED RUMEN MICROBIOTA

by

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I. INTRODUCTION AND LITERATURE REVIEW

"The biological value is of interest because it is known to represent, to a considerable extent, the protein available to ruminants. Rumen micro-organisms after they leave the rumen, are digested in the true stomach and the small intestine of ruminants. The mixed bacterial population includes many forms which are able to convert non-protein nitrogen into bacterial protein during fermentation in the rumen. Since many of these bacteria also degrade and convert feed protein into bacterial protein, it is of importance to establish the value of the microbial protein." (Nutr. Rev. 16, 115 (1958))

Probably the earliest attempt to investigate this problem was made by Muller (22), who fed a bitch protein isolated from bacteria. He found that nitrogen retention on the ration containing the bacterial protein was similar to that on rations containing albumin and casein. Usuelli and Fiorini (29) observed that the increase in growth of chickens fed rumen protozoa was higher than that of chickens fed rumen bacteria. This observation may lead to the conclusion that the protozoa increase the nutritive value of bacterial protein. Since there is some doubt of the purity of the bacterial and protozoal preparations used by these workers further work is needed. Johnson et al (16) endeavored to obtain further information on this point. They found that the digestibility of the protozoal protein was higher than that of bacterial protein and that the biological values of these two preparations were about equal. The limited amount of the preparations available in their experiments made it impossible to obtain results which could be interpreted with certainty. Two groups of workers (24), (21) reported that the biological value of mixed rumen bacterial protein is equal to or above that of casein. The biological value obtained by these workers was higher than that obtained for L. arabinosus and E. coli (18). This is probably a result of better amino acid balance in the mixed rumen bacterial populations (18).

McNaught et al (20) reported the biological value of both rumen bacteria and protozoa to be similar, but the true digestibility of the protozoal protein was higher than that of the bacteria. They also concluded that part of the bacterial protein is converted to protozoal protein prior to use by the host animal.

Other workers used dried rumen products in feeding trials with ruminants. Williams and Jensen (31), for example, obtained no benefit from feeding replacer containing 2 per cent dried rumen contents to calves. Ronning et al (25) and Tucker et al (27) reported no response from feeding dried rumen products to dairy cattle and sheep respectively. Kamstra et al (17) using a dried rumen product to supply 375 mg. per pound of final ration reported that dried rumen products had no stimulatory effect in lambs on rate of gain, feed efficiency, feed consumption or digestibility of major nutrients with any of the rations under test.

Two reports have appeared which suggest that mixed rumen bacterial populations vary in their amino acid composition, depending upon the ration fed. Rumen bacteria in sheep fed green forage contained more methionine than those in sheep fed dry rations (24). This finding was confirmed by the work of Holmes _et al_ (14). Weller (30) reported that for a wide range of types of diet the amino acid composition of the mixed bacterial proteins is almost constant. Tryptophan was found to be the most deficient amino acid in the protein of bacteria found in sheep that were fed casein or gelatin (12). Based upon the studies of Kaufman _et al_ (18) these variations in amino acid composition of mixed rumen bacterial populations probably represent species shifts within the flora rather than changes in amino acid composition of individual species (18).

"Since it has been difficult, or impossible, to demonstrate any benefit from amino acid supplementation of the ration in ruminants, regardless of the quality of protein used in the ration, there is some question as to the usefulness of the rat as a test animal in evaluating the protein quality of ruminant rations or their fermentation products. It is not impossible that the ruminant is particularly adapted to the protein derived from bacterial fermentation products. Perhaps the young calf in which the rumen fermentation has not yet started, should serve as the test animal in determining the biological value of bacterial protein" (Nutr. Rev. 16, 115 (1958)).

Millions of dollars are spent annually in transportation and dumping slaughter house wastes. At the same time, the most expensive bulk constituent of animal feed is protein. Therefore, it would be of value to find a cheap source of protein in the packing house material now disposed of as wastes. The previous review shows that packing house wastes contain one potentially good source of protein in the form of the micro-flora of the rumen fill. The studies with rats both here and at other laboratories indicate that this source of protein is promising for use in poultry and swine feeds.

This report concerns a study of the proximate analysis, amino acid composition, B-vitamin content and the dietary protein value of lyophilized rumen microbiota. This study furnishes more practical information about protein quality and the amino acid balance of dried rumen microbiota than is presently available.

II. PROCEDURES AND RESULTS

a. Isolation of Rumen Microbiota

Dried rumen microbiota was prepared by removing the rumen contents from fistulated steers and squeezing it through cheese cloth into a cylindrical container. After settling for 1/2 to 1 hour, three layers resulted, one dark greenish which was mostly fibrous plant residues at the top, one gray-whitish which was mostly protozoa and starch grains in the bottom, and one brownish in the middle which was mostly bacteria. The fibrous layer was removed by decantation. The other two layers were harvested at a high speed in a Sharples centrifuge to collect a mixture of rumen bacteria and protozoa which was finally dried by Lyophilization. The yield was about 3 grams of dried rumen micro-organisms per liter of rumen fluid.

b. Chemical Analysis

1. Proximate Analyses

Proximate Analyses were made on the dried preparation for crude protein by the Kjeldahl method, for ash, for moisture by the oven method, for ether extract and crude fiber by acid digestion (1), with the results seen in Table 1.

Table 1

Proximate Analysis of Dried Rumen Microbiota

Expressed as Percentage

Crude Protein	Ash	Moisture	Ether Extract	Crude Fiber	Nitrogen Free Ext.*	Total
36.3	6.8	4.9	6.5	4.8	40.7	100

* Nitrogen free extract obtained by subtracting the crude protein, ash, moisture, ether extract and crude fiber percentages from one hundred

2. Amino Acid Analysis

One gram of the dried preparation was refluxed with 100 ml of 6N hydrochloric acid for 24 hours in an atmosphere of N_2. The hydrolysate was filtered and dried on a rotary evaporator in vacuo. The residue was dissolved in 10 ml of distilled water and dried again. This step was repeated three times to remove the hydrochloric acid. The final residue was then dissolved in 100 ml of citrate buffer pH 2.2, and amino acid determinations were made by the method of Spackman et al (26) using the Beckman/Spinco Model 120 Amino Acid Analizer.

Table 2 shows the amino acid composition of the dried preparation of rumen micro-organisms, of soy bean meal, and of whole egg protein.

Table 2

Amino Acid Composition of Dried Rumen Micro-

organisms, Soybean Meal+ and Whole Egg Protein

Expressed as g. amino acid/100 g. crude protein

Amino Acid	Dried Rumen Preparation	Whole Egg** Protein	Soybean+ Meal
Arginine	2.8	6.7	5.8
Histidine	1.6	2.7	2.3
Lysine	7.5	7.9	5.8
Threonine	3.0	4.5	4.0
Serine	2.3	6.1	—
Aspartic	7.6	10.5	—
Glutamic	9.4	13.2	—
Proline	2.6	4.1	—
Glycine	3.0	2.3	—
Alanine	3.3	6.9	—
Cystine	0.3	2.4+	0.6±1.4
Methionine	1.5	3.0	2.0
Valine	3.3	7.1	4.2
Isoleucine	3.9	6.0	4.7
Leucine	5.1	9.1	6.6
Tyrosine	3.2	3.1	4.1
Phenylalanine	3.8	5.8	5.7
Tryptophan (13)	1.8	1.5+	1.6
Ammonia	1.8	—	—
Total	67.8	102.9	

\+ Block, R. J. and Bolling D. - Amino Acid Composition of proteins and foods. Springfield, Ill. C. C. Thomas 1947

** Dr. Paul D. Lepore (Ph.D. Thesis, Virginia Polytechnic Institute, Blacksburg, Virginia (1962)) average of 4 eggs of two lines of White Rocks.

The true protein content of the dried rumen preparation calculated from its amino acid composition is 24.7 per cent. This indicates that 32 per cent of the crude protein consists of non-protein compounds, mostly nucleic acids. The essential amino acid index is 63.4 per cent and the most limiting amino acids are the sulfur amino acids, cystine and methionine. Table 2 shows clearly that the dried rumen preparation has a higher content of tryptophan and a lower content of cystine, methionine, arginine, isoleucine and phenylalanine than whole egg and soybean meal.

3. B-vitamin content

Various analyses were made on the dried rumen preparation for B-vitamins by the Wisconsin Alumni Research Foundation. The results obtained are given in Table 3 along with the recommended allowances for B-vitamin for starting chickens.

Table 3 shows that the dried rumen preparation has a higher B-vitamin content per pound than those recommended for the starting chickens. Therefore, if the dried rumen preparation were used to supply all the protein required by the starting chickens, there would be no need for supplementing their rations with costly B-vitamins. On the other hand such a ration would be almost 100 per cent microbiota. Dubiski _et al_ (11) in their study of the use of rumen contents as a supplement for Vitamin B_{12} in chick rations found a slight growth response with supplementary rumen contents which was attributed to the Vitamin B_{12}. It should be noticed that these workers used whole rumen

Table 3

B-Vitamin Content of the Dried Rumen Preparation

and the Recommended Allowances for Starting Chickens*

Vitamin	Dried Rumen Preparation	Dried Rumen Preparation mgm/lb.	Recommended Allowance mgm/lb.
Choline (15)	1.98(mgm/g)	899.9	600
Folic Acid (2)	0.696(γ/g)	0.32	0.25
Pantothenic (23)	37.8(γ/g)	17.18	4.2
Vitamin B_{12} (28)	1.84(γ/g)	0.84	0.004
Vitamin B_6 (6)	8.0(γ/g)	3.64	1.3
Biotin (32)	1.04(γ/g)	0.47	0.04
Niacin (3)	72.6(γ/g)	32.99	12.0
Thiamine (4)	6.42(γ/g)	2.92	0.8
Riboflavin (5)	30.7(γ/g)	13.95	1.3

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* "Nutrient Requirements of Poultry" (1961) Publication No. 827 of the National Academy of Sciences - National Research Council

contents rather than the rumen microbiota. It is very possible that the microbiota has a higher content of vitamin B_{12} than the whole rumen contents.

C. Protein Quality Tests

A preliminary feeding trial was conducted to determine the protein efficiency ratio (PER)(grams gain in weight per gram protein consumed) and the supplementary value of dried rumen microbiota with corn which is very widely used in chicken rations.

Three litters of 4 male Sprague-Dawley albino rats weighing 35 ± 5g were divided into four groups in such a way that each group had one rat from each litter. The first group received a diet containing 10.6 per cent crude protein from vitamin-free casein, the second 10.6 per cent crude protein from the dried rumen preparation, the third 7.7 per cent crude protein from corn meal plus 2.9 per cent crude protein from vitamin-free casein, and the fourth 7.7 per cent crude protein from corn meal plus 2.9 per cent crude protein from the dried rumen preparation. All diets had the same composition except for the source and protein which was added at the expense of sucrose to the protein-free basal diet shown in Table 4.

Table 4

Composition of Protein-free Basal Diet

Ingredient	Percentage
Salt IV Mixture*	4
Vitamin Mixture**	1
Crisco	10
Sucrose	85

To each 1 Kilogram of diet 5 ml of 20 per cent choline chloride solution was added. Two drops of vitamins A, D, and E were given in oil to each rat weekly.

The animals were fed *ad libitum*. The food was changed daily, and food intake was recorded. The body weights were measured at three or four day intervals. Growth curves were plotted and PER - values were calculated. The results are seen in Figures 1, 2, 3 and 4 and in Table 5.

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* Salt IV Mixture: $CaCO_3$, 300 g; $CaHPO_4{:}2H_2O$, 75 g; K_2HPO_4, 322.52 g; NaCl, 167.5 g; $MgSO_4.7H_2O$, 102 g; $Fe(C_6H_5O_7)2.6H_2O$, 27.5 g; $MnSO_4.2H_2O$, 5 g; KI, 0.8 g; $ZnCl_2$, 0.25 g; $CuSO_4.5H_2O$, 0.3 g.

** Vitamin Mixture: L-cystine, 200 g; Ca-pantothenate, 2 g; Niacin, 1 g; Inositol, 1 g; Thiamine, 0.5 g; Vitamin B_{12}, 0.0001 g; Menadione, 0.4 g in 794.5 g sucrose to make 1 Kg vitamin mixture.

Table 5

PER-Values for the Four groups

Casein	Corn plus Casein	Dried Rumen Preparation	Corn plus dried Rumen preparation
4.7[a]	3.3[a]	2.8[b]	2.8[b]
—	—	1.3[c]	1.3[c]
—	—	1.8[d]	1.1[d]
—	—	3.1[e]	4.1[e]
—	—	3.4[f]	4.8[f]
—	—	3.4[g]	3.1[g]
—	—	4.0[h]	3.8[h]
—	—	3.5[i]	3.3[i]

a- average of 3 rats and 27 days period

b- average of 3 rats and 7 days period

c- for one rat and 5 days period, given 1 mg tryptophan per g. diet

d- for one rat and 5 days period, given 1 mg tryptophan + 2 mg lysine HCl

e- for one rat and 3 days period, the amount of the dried preparation was doubled in the diet

f- for one rat and 3 days period, the amount of the dried preparation was doubled and histidine and tryptophan were added to meet the amount required by the albino rat

g- for one rat and 3 days period, double dried rumen preparation and histidine to meet the requirements for the albino rat

h- for one rat and 10 day period, 2 per cent α-soy protein added to the diet

i- for one rat and 10 day period, 2 per cent casein added to the diet

Figure 1. Growth of the three rats fed the Casein Diet

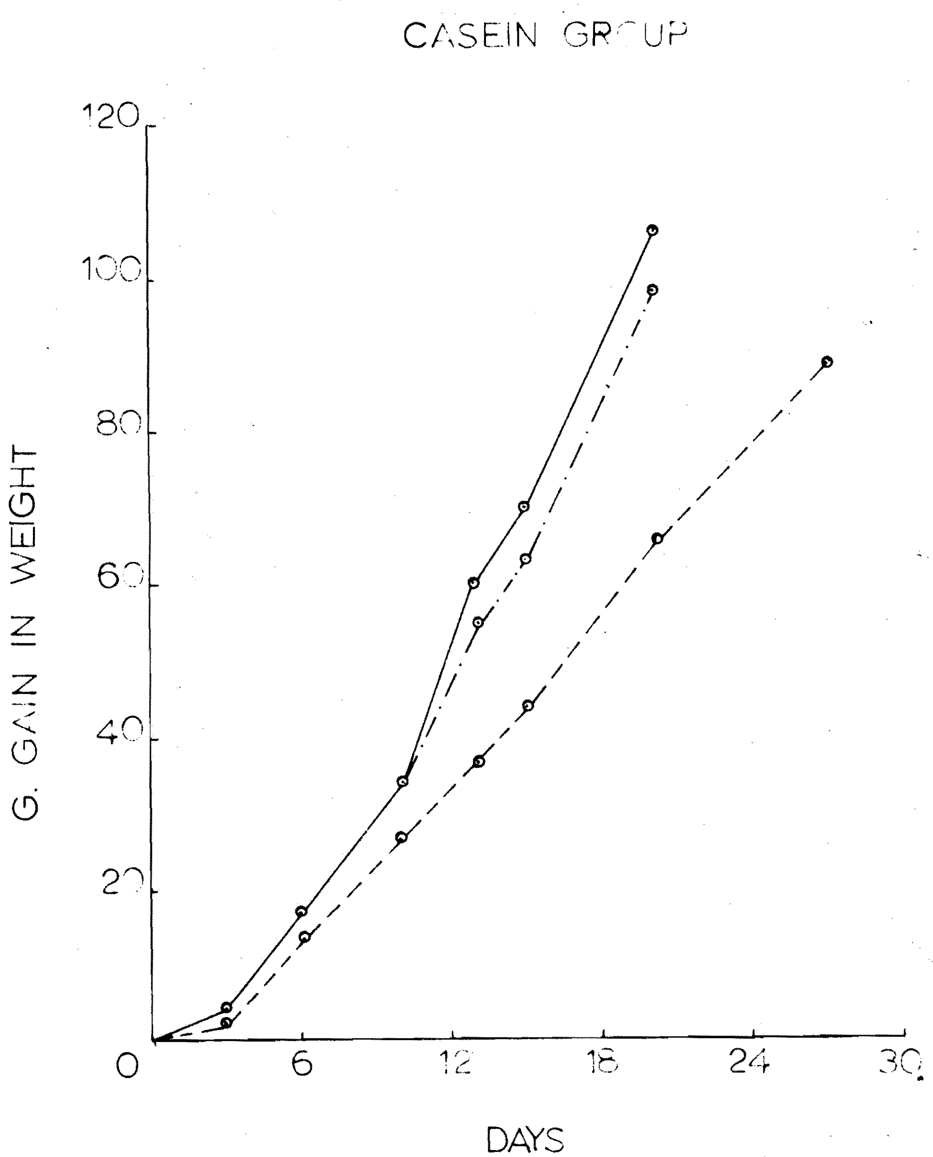

CASEIN GROUP
120
100
80
60
40
20
0
6
12
18
24
30
G. GAIN IN WEIGHT
DAYS

Figure 2. Growth of the three rats fed the casein plus corn diet

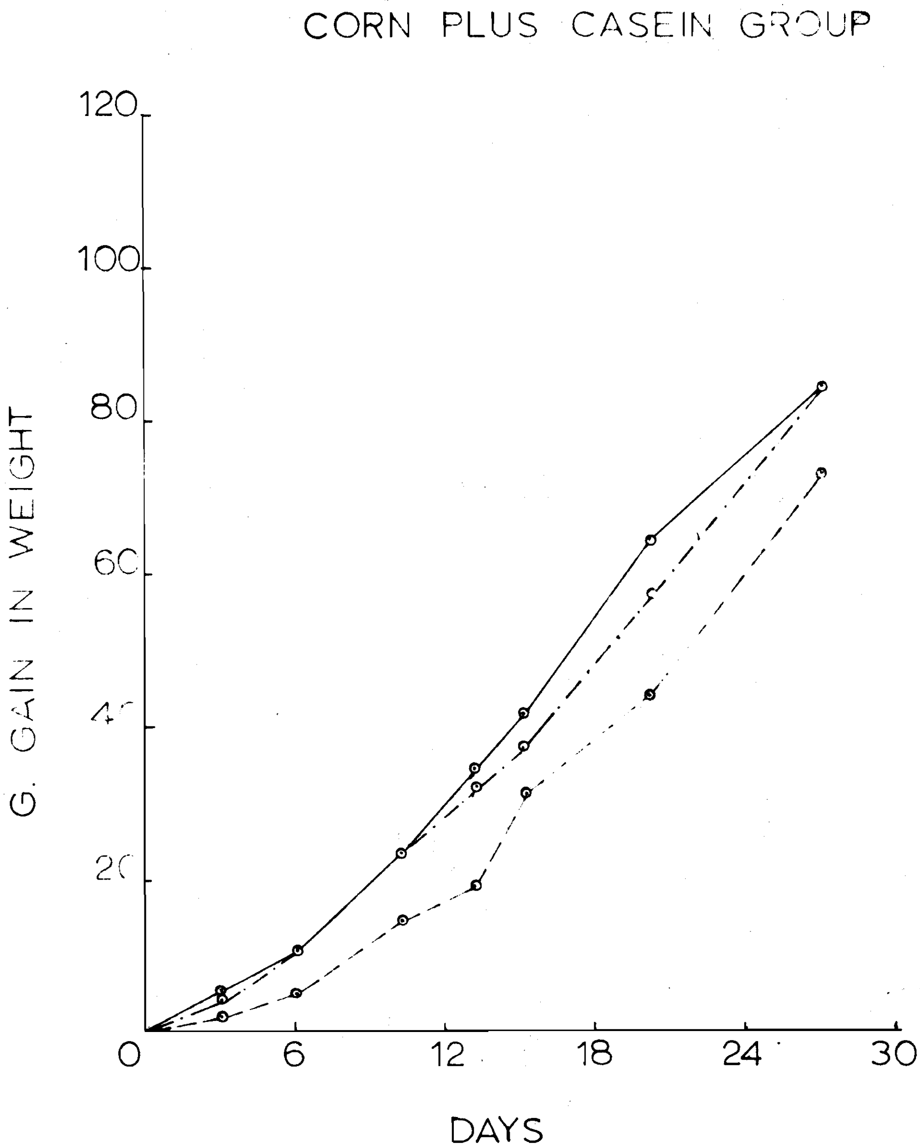

CORN PLUS CASEIN GROUP
120
100
80
60
40
20
G. GAIN IN WEIGHT
0
6
12
18
24
30
DAYS

Figure 3. Growth and the supplementation effect on the growth of the rats fed the dried rumen preparation diet

(See notes on page 21)

RUMEN PREPARATION

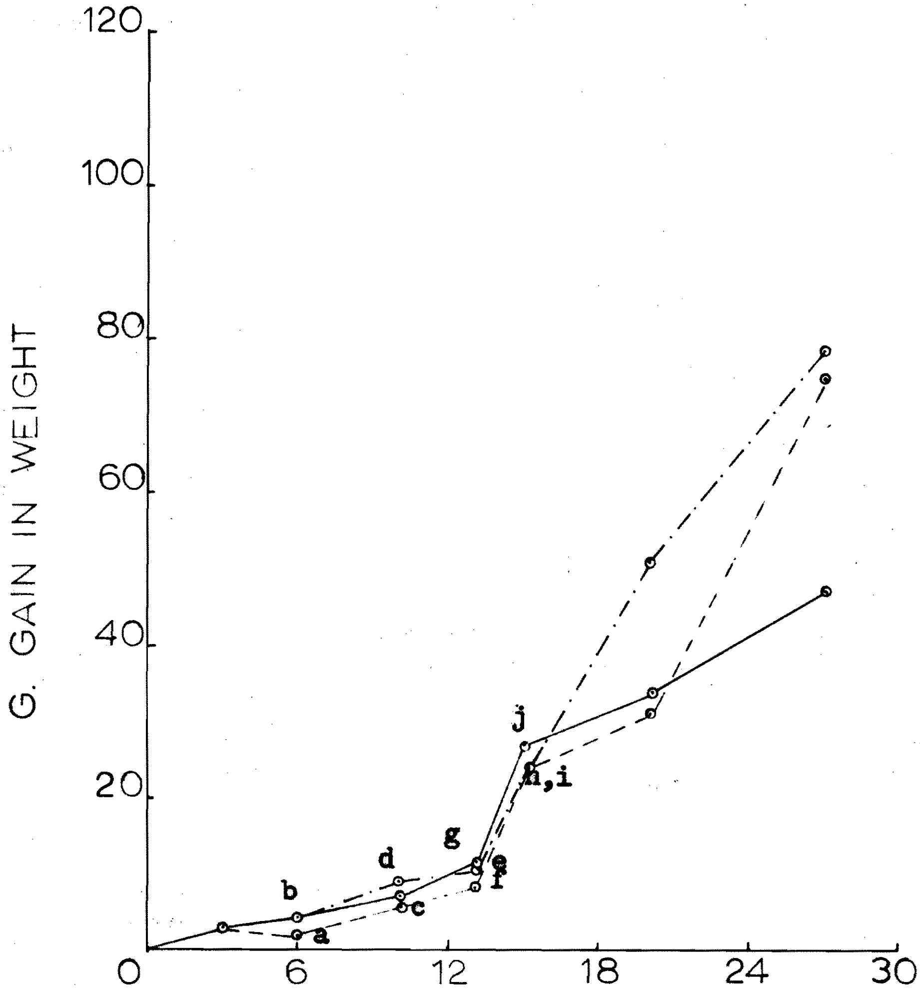
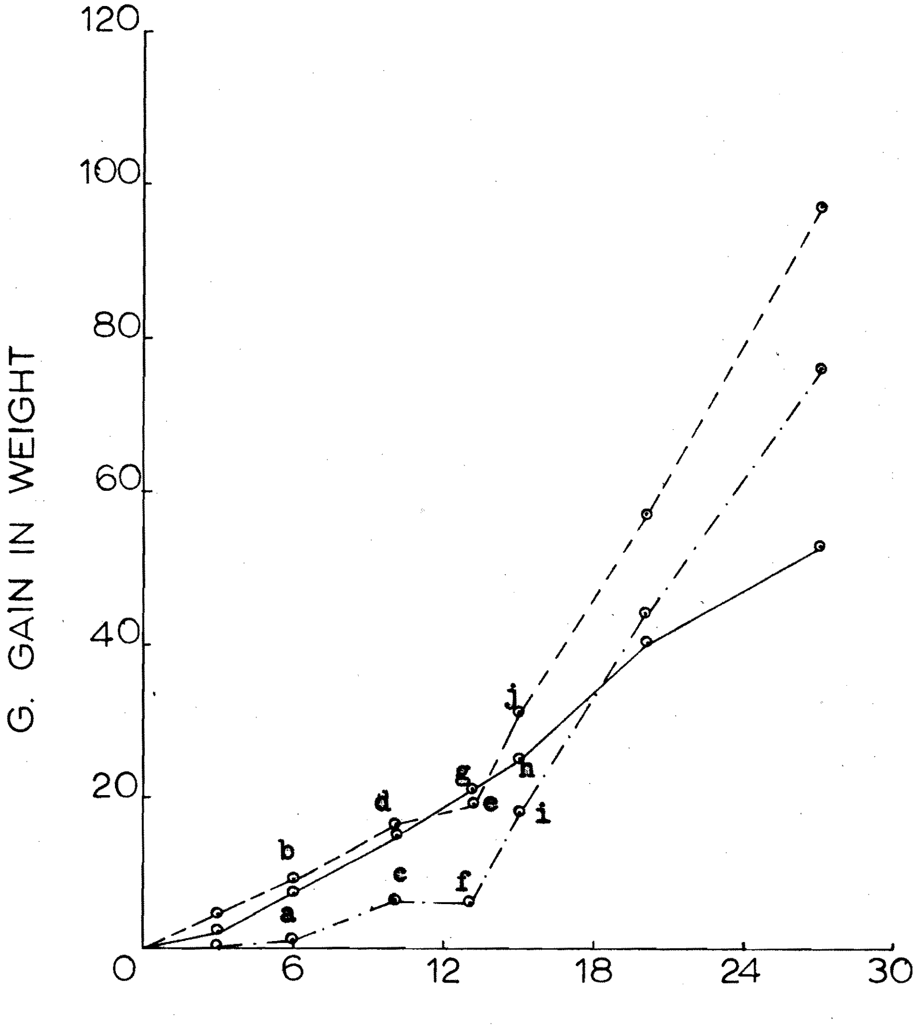


Figure 4. Growth and the supplementation effect on the growth of the rats fed dried rumen preparation plus corn diet (See notes on Page 21).

CORN PLUS RUMEN PREPARATION



The following supplementations were made for the dried rumen group and the dried rumen preparation plus corn group:

a- 1 mg L-tryptophan plus 2 mg L-Lysine hydrochloride per gram diet

b- 1 mg L-tryptophan per gram diet

c- L-histidine plus L-tryptophan added in amounts to meet the requirements of the albino rat

d- L-histidine added in amount to meet the requirements of the albino rat

e- The amount of dried rumen preparation is doubled in the diet

f- Doubling the dried rumen preparation plus L-histidine plus L-tryptophan to meet the requirements for the albino rat

g- Doubling the dried rumen preparation plus L-histidine to meet the requirement of the albino rat

h- The original diet without supplementation

i- The original diet plus 2 per cent casein protein

j- The original diet plus 2 per cent α-soy protein

The low PER values for the animals on diets containing 10.6% crude protein from the dried rumen preparation are due to the low true protein content in these diets. This becomes clear since the chemical analysis showed that 32 per cent of the crude protein is non-protein compounds (mostly nucleic acids), and also supplementation with lysine, lysine plus tryptophan, histidine and histidine plus tryptophan did not increase the rate of growth, nor did it increase the PER value. Doubling the amount of the dried rumen preparation or adding 2 per cent protein from vitamin-free casein or α-soy protein increased both the rate of growth and the PER values making them about equal to those on the casein diets.

Two other independent feeding trials were conducted essentially in accordance with Bender-Miller method (7). Eight litters of three weanling male albino rats of the Sprague-Dawley strain weighing 40 ± 5 g were kept on a stock diet (8 g cottonseed oil, 5 g salt mixture USP XVI, 2.4 g solka floc, 1 vitamin mixture, 5 g water, 15 g vitamin-free casein, and 63.6 g sucrose), for one week. At the end of this period the food was taken away, and the animals were starved for 18 hours to free their intestinal tracts of food. The litters having the highest and lowest weights were discarded. The remaining 6 litters weighing 57 ± 5 g per rat were divided into three groups in such a way that each group had one rat from each litter and fed *ad libitum* diets containing 10 per cent true protein from α-soy protein, 10 per cent true protein from the dried rumen preparation, and a protein-free diet. The diets were made up according to the AOAC method (10) as shown in Table 6.

Table 6

Composition of Basal Diets

Ingredient	%
Sample	A
Cottonseed Oil	$8\left[\frac{(A)(\%\ \text{Ether Extract})}{100}\right]$
Water	$5\left[\frac{(A)(\%\ \text{Moisture})}{100}\right]$
Salts, USP XVI[2]	$5\left[\frac{(A)(\%\ \text{Ash})}{100}\right]$
Solka Floc	$2.4\left[\frac{(A)(\%\ \text{crude fiber})}{100}\right]$
Vitamin Mixture[3]	1
Sucrose	to make 100

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1 $A = \frac{10 \times 100}{\%\ \text{true protein}}$

2 Salts USP XVI: NaCl, 139.3 g; KI, 0.79 g; KH_2PO_4, 389.0g; $MgSO_4 \cdot 7H_2O$, 57.3 g; $CaCO_3$, 381.4 g; $FeSO_4 \cdot 7H_2O$, 27.0 g; $MnSO_4 \cdot H_2O$, 40.1 g; $ZuSO_4 \cdot 7H_2O$, 0.548; $CuSO_4 \cdot 5H_2O$, 0.447 g; and 0.023 g $CoCl_2 \cdot 6H_2O$.

3 Vitamin Mixture: the same as mentioned the first feeding trial but without L-cystine. See footnote to Table 4.

Food and water were given *ad libitum*. The animal's weights were recorded at the beginning and the end of the experimental period (7 days). The food was changed daily and the intake was recorded. After 7 days the animals were starved for 18 hours to free their digestive tracts of food prior to weighing. After the starvation, the feces, urine and carcasses were analyzed for total nitrogen.

Chemical Analysis

The rats were killed with chloroform and dried in the oven for 48 hours at 90° C. Then they were dissolved in hot 50% sulfuric acid. Duplicate aliquots were taken to determine body nitrogen by the Kjeldahl method (1). The data from these two experiments are summarized in Table 7.

Table 7

Comparison of NPU, digestibility, biological value, replacement value, NPR, PRE and PER of dried rumen microbiota and α-soy protein

	Protein-free Group		α-Soy Protein Group		Dried Rumen Preparation Group	
	1st Expt.	2nd Expt.	1st Expt.	2nd Expt.	1st Expt.	2nd Expt.
N-Intake (g)	00.00	0.00	0.74	0.73	2.10	2.05
Fecal-N (g)	0.04	0.07	0.10	0.13	0.59	0.48
Urinary-N (g)	0.10	0.10	0.35	0.43	0.81	0.71
Body-N (g)	1.21	1.52	1.42	1.77	2.23	2.73
NPU[1]	—	—	0.28	0.34	0.49	0.59
Digestibility[2]	—	—	0.92	0.92	0.74	0.80
Biological Value[3]	—	—	0.26	0.31	0.36	0.47
% Replacement Value[4]	—	—	—	—	155.00	194.00
Weight Gain (g)	-8.7	-8.7	-0.83	4.5	40.7	40.5
NPR[5]	—	—	0.72	2.90	3.80	3.80
PRE[6]	—	—	27.20	46.40	60.8	60.8
PER[7]	—	—	-0.18	0.99	3.57	3.64

[1] Net Protein Utilization (NPU) $= \frac{\text{Nitrogen retained in the body}}{\text{Nitrogen Intake}}$

[2] Digestibility $= \frac{\text{Nitrogen Intake} - [(\text{fecal-N}) - (\text{Metabolic-N})]}{\text{N-Intake}}$

[3] Biological Value = (NPU)(Digestibility)

[4] Replacement Value $= 100 - \left[\frac{\text{Difference in Nitrogen Balance}}{\text{Nitrogen Intake}} \times 100\right]$

5 Net Protein Ratio (NPR) $= \frac{\text{Weight loss of Non-protein group + weight gain of the test group}}{\text{Protein Intake } (N \times 6.25)}$

6 Protein Retention Efficiency (PRE) = NPR X 16

7 Protein Efficiency Ration (PER) = Weight gain (g) per gram protein consumed

Table 7 shows that the dried rumen preparation is a better source of protein than α-soy protein which is the major protein in soybean which is widely used in chicken rations. The replacement value suggests that the dried rumen preparation has a better amino acid balance than the α-soy protein.

Since the replacement value depends on both the digestibility and the intermediary metabolism of protein, and since the dried rumen preparation has lower digestibility but higher replacement value than the α-soy protein, it can be safely concluded that the dried rumen preparation has a better amino acid balance than the α-soy protein.

The results of these experiments also suggest no more than a moderate amino acid deficiency in the dried rumen preparation, because the rats were normal and showed very satisfactory growth rates.

Comparison of Nutritive Value of Different

Sources of Protein+(8) with Dried Rumen Preparation

Source of Protein	PER	NPU	NPR
Dried Defatted egg	4.56	99	5.9
Meat Meal	1.05	41	2.56
Dried Milk	2.77	63	3.8
Wheat Glutin	0.09	34	1.61
Sesame Oil	0.68	54	3.39
Fish Meal	3.16	66	4.45
Dried Rumen Preparation	4.9*	54**	5.9*

+ From Brit. J. Nutr. 11, 140 (1957) Bender, A. E. and Doel, B. H.

* average of the two trials and the calculations based on true protein intake

** average of the two trials and the calculations based on total nitrogen intake

III DISCUSSION

The experimental data presented in this report are consistent with data from similar trials in other laboratories for determining the biological value of dried rumen microbiota. There is no evidence of sulfur amino acid deficiency in feeding trials although chemical analysis indicated quite low levels of S-amino acids.

Comparison with similar values recorded for other sources of protein as in Table 8 shows that the dried rumen preparation is comparable to dried defatted eggs, meat meal, dried milk and wheat gluten.

The feeding trials with rats, which are monogastric animals, showed that the dried rumen preparation has protein of good quality and eliminated the possibility of the presence of toxic concentrations of any material for the rat. Also, Since the B-vitamin content of this preparation is enough to meet the recommended allowances of the starting chick as shown in Table 3, it would be of value to use the dried rumen preparation as a supplement for B-vitamin and protein both of which are very costly in poultry rations. Experiments with chickens should be done to confirm this possibility.

The price of the dried rumen preparation depends mainly on the cost of drying, so different ways of drying such as by heat or by lyophilization should be compared and their effect on the protein quality and B-vitamin contents should be studied with chickens. It should be borne in mind that drying by heat increases the digestibility of many proteins.

Accordingly it increases the replacement value and the net protein utilization, but it decreases the biological value due to a partial destruction of some of the amino acids, particularly lysine. This last effect is probably of no significance on the dried rumen microbiota since the lysine is not the limiting amino acid in this microbial protein.

After performing this type of investigation, the final word is left to the agricultural economist to decide whether the use of dried rumen microbiota as a supplement for protein and B-vitamin in chicken rations would be economically advantageous or not.

IV SUMMARY

Dried Rumen Microbiota were obtained from fistulated steers. Proximate analysis, amino acid analyses, and B-vitamin analyses were performed on the dried rumen microbiota.

Balance trials, using male weanling rats of the Sprague-Dawley strain, were also conducted. These trials suggest that the protein in the dried preparation is not grossly deficient in any of the amino acids. The growth rate, net protein utilization, biological value, PER, NPR and PRE values for the dried rumen preparation were much higher than those for α-soy protein. The NPR and PER values of the dried rumen preparation are similar to those recorded by Bender and Doel (18).

The low digestibility and the high replacement value of the dried rumen preparation in comparison with α-soy protein suggest that the dried rumen microbiota has a better amino acid balance than α-soy protein.

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VII VITA

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THE NUTRITIVE VALUE OF DRIED RUMEN MICROBIOTA

by

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ABSTRACT

Dried rumen microbiota were isolated from fistulated steers. Proximate analyses were conducted and the amino acid composition and B-vitamin content were determined. Protein quality tests were carried out using the Bender-Miller method.

The data obtained from the investigation indicated that the protein quality of dried rumen microbiota is comparable with that of dried defatted egg, dried milk, fish meal and meat meal, but it is better than that of α-soy protein and wheat gluten. No amino acid deficiency appeared in the feeding trials even though the amino acid composition showed that the dried rumen microbiota might be deficient in sulfur-containing amino acids.