STUDIES ON DIGESTION AND NITROGEN METABOLISM OF BEEF CATTLE

PART I. DIGESTION AND NITROGEN RETENTION DATA OBTAINED WITH STEERS IN UNIFORMITY TRIALS

PART II. THE DETERMINATION OF THE METABOLIC FECAL NITROGEN OF STEERS

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TABLE OF CONTENTS

STUDIES ON DIGESTION AND NITROGEN METABOLISM OF BEEF CATTLE

PART I. DIGESTION AND NITROGEN RETENTION DATA OBTAINED WITH STEERS IN UNIFORMITY TRIALS

INTRODUCTION

Reliable digestion and metabolism data can be obtained with large animals only when accepted experimental techniques based upon established principles of animal nutrition are utilized. This is especially true in the case of the ruminant because of the complications associated with unusual digestive systems of these animals. Even when accepted experimental techniques are meticulously followed, the reliability of the data so obtained is often questioned as a result of the normal physiological variations of individual animals in different trials and those existing between animals in same trial. A knowledge of the extent of these variations would be of considerable value in designing digestion and metabolism experiments and in interpreting the results of such experiments. This knowledge would enable the investigator to determine the number of animals needed to detect desired differences between treatments and to determine the length of each collection period. Although studies by Forbes and associates (1946) with sheep have provided data of this type, there has been a virtual lack of similar data obtained with beef cattle. The desirability of such data for application to the numerous digestion and metabolism trials relating to the maintenance of beef cattle in Oklahoma resulted in the conducting of a series of uniformity trials with steers. The data obtained in these studies have made it possible to state the optimum length of collection periods, the minimum number of steers needed to detect the least significant difference in treatments, the major source of variation, and related factors controlling the success of digestion and metabolism trials.

REVIEW OF LITERATURE

A digestion trial represents an accurate accounting of the amounts of feed eaten and feces excreted by an animal in a definite period of time. In conjunction with the proximate chemical analysis of the feed and feces, the data obtained in a digestion trial provide a means of determining the digestibility of each nutrient. From such data the practical nutritive value of a ration or roughage can be expressed in terms of T.D.N. and digestible protein. (Maynard, 1951)

The reliability of digestibility data depends upon factors relating to the judgment shown in the selection of the experimental animals, the skill with which the animals are managed and the accuracy of procedure associated with the quantitative aspects of the trial. These and other factors such as the length of time alloted the animal for adjustment to the ration and environment, the maintenance of a constant feed intake and the special consideration necessitated by the nature of the physiology of digestion in the species being studied, have been thoroughly considered by Armsby (1914), Benedict and Ritzman (1918) and Forbes and Grindley (1923). The application of the principles stressed by these early investigators has been of particular importance in digestion trials with large animals because of the limited number of individuals which economic feasibility permits being used in each trial. When it is recalled that most of the published digestibility data on large animals have been obtained with three and four animals and frequently with only two, the importance of good experimental technique is clearly indicated.

Very often the urinary nitrogen excretion is determined as a supplementary part of a digestion trial. The amount of nitrogen retained per day by an animal is calculated by subtracting the nitrogen excreted in the feces and urine from the nitrogen intake. Although the nitrogen retention is not a necessary value in the determination of digestibility, it can, after correcting for the nitrogen excretion of body origin, be used in the determination of the biological value of proteins and the net protein requirement of animals. The problems involved in obtaining nitrogen metabolism data have been considered in detail by Mitchell (1924)(1926).

It has become apparent in recent years that even when the best techniques of animal nutrition are applied to experiments on digestion and nitrogen metabolism, normal physiological variations of individuals with respect to time, and between individuals at the same time, greatly influence the results of such experiments. Variation in the physiological performance of individuals was realized long ago by Benedict and Ritzman (1918) who determined the digestibility of nutrients with two steers fed only roughage. They reported the mean digestion coefficients for each steer in 16 digestion trials of 7 to 10 days duration, but in the absence of statistical methods no further analysis was made of the data. The introduction of statistics to nutrition studies focused attention on the importance of physiological variations of animals in digestion and metabolism studies. Through the application of special experimental designs it has become possible to eliminate these variations, but in doing so the efficiency was reduced and the

cost per unit observation was consequently increased. Knowledge of the normal variation of animals in different trials and that existing between animals in the same trial could lead to the use of more efficient experimental designs. (Snedecor, 1953).

It is of considerable importance that a sufficient number of animals be used in each treatment of a digestion trial to detect the desired difference between the means, yet it is economically unsound to use more animals than are necessary. In an attempt to provide this information, Forbes and associates (1946) conducted a digestion trial with 22 sheep. From the standard error they calculated the least significant difference between means which could be detected with 1 to 22 sheep per treatment. Although similar data would be highly beneficial in digestion and metabolism experiments with beef cattle, an examination of the literature failed to reveal such information. For this reason, a series of uniform digestion and metabolism trials was conducted by the Departments of Animal Husbandry and Agricultural Chemistry Research of the Oklahoma Agricultural Experiment Station.

EXPERIMENTAL

A series of digestion and nitrogen balance trials was conducted on a group of 12 grade Hereford steers fed a ration of approximately constant composition (10 per cent protein) composed of prairie hay supplemented with cottonseed meal and minerals. The average daily intake and composition of the rations fed to the steers in all trials are given in Table 1. The first trial, which was a digestion trial only, was conducted when the steers were 10 months old and weighed approximately 500 pounds. The second and third trials, involving both digestion and nitrogen retention studies, were conducted when the steers were 19 and 30 months old, respectively. Their average weight at those ages was 685 and 1000 pounds, respectively.

The experimental procedure was the same in all three trials. The animals, after being brought in from the range, were kept in pens for a week or more to permit their adjustment to the ration and environment. Following this adjustment period, the steers were placed in metabolism stalls constructed as described by Briggs and Gallup (1949) and Nelson, Tillman, Gallup and MacVicar (1954).

Preliminary feeding periods were of 10 or more days duration followed by three successive 5-day collection periods. The animals were fed one-half of the ration in the morning and the remainder in the evening. Samples of the prairie hay and cottonseed meal were taken at each feeding. At the end of the collection period these samples were thoroughly mixed and approximately one-fourth of each

Table 1. CONSTITUENTS AND AVERAGE COMPOSITION OF RATIONS FED TO STEERS

5 Day coll. period	Trial I			Trial II			Trial III :		
	First	Second	Third	First	Second	Third	First	Second	Third
Constituents, gm.									
Prairie hay	3178	3178	3178	4086	4086	4086	5902	5902	5902
Cottonseed meal	454	454	454	586	586	586	890	861	886
Salt	30	30	30	30	30	30	30	30	30
Bone meal	12	12	12	20	20	20	20	20	20
Total	3674	3674	3674	4722	4722	4722	6822	6833	6828
Composition, dry matter basis, %									
Organic matter	91.62	91.88	91.85	92.70	92.21	91.92	92.00	91.96	91.98
Protein (Nx6.25)	9.58	9.50	9.96	9.72	10.12	10.03	9.12	9.14	9.21
Ether extract	2.63	2.61	2.58	2.94	3.08	2.98	2.76	2.89	2.61
Crude fiber	29.08	29.62	30.71	29.71	29.39	30.30	28.89	28.48	29.64
N-free extract	49.53	50.15	48.55	50.23	49.61	48.41	51.36	51.36	50.63

was submitted for chemical analysis. Refused feeds were removed from the feed-box at the end of the collection period, dried at 70° C, weighed and analyzed.

Feces were collected in troughs attached to the metabolism stalls (Nelson et al., 1954) and transferred to covered metal containers. Sampling of the feces was made once a day, after the evening feeding. Five per cent of the daily weight of fresh feces was stored in screw-cap jars in a refrigerator. Thymol crystals were added to these samples to inhibit bacterial action.

Urine was collected in 2-gallon bottles during trial 2 and in 2-gallon metal containers with wooden covers during trial 3. The daily amount of urine was made up to 10 kg. with water and a 150 ml. sample stored in a refrigerator. In the event the daily amount of urine exceeded 10 kg., it was made up to 20 kg. with water and a 300 ml. sample taken.

Since the urine was exposed to bacterial contamination during the 24-hour collection, a question was raised regarding nitrogen losses resulting from urea breakdown. To determine whether acidification was necessary, a test was conducted in which samples of urine from 3 steers were divided equally between two 2-gallon bottles and the urine in one bottle of each pair acidified with hydrochloric acid. After storage in open bottle for 24 hours in the Metabolism Room, the urine was analyzed for nitrogen. The lack of significant difference between the nitrogen content of acidified urines and non-acidified urines, as shown in Appendix table F, made it unnecessary to acidify the urines during the 24-hour collection.

At the completion of each 5-day collection period, urine, feces and refused feed for each steer and the feed samples were prepared for proximate analysis. The fresh feces were thoroughly mixed to provide representative samples. Triplicate 10-gm. samples of fresh feces were weighed on a torsion balance for nitrogen determination by the modified Kjeldahl method. Nitrogen in the urine was determined on duplicate 10-ml. samples. A mixture of anhydrous sodium sulfate and anhydrous cupric sulphate (17:1 ratio) was used as the catalyst in all determinations.

The dry matter content of each fecal sample was determined by weighing a 300 gm. sample of fresh feces into a tared metal container and drying at 105° C to constant weight. It was of interest to ascertain the accuracy of fecal dry matter determinations made on duplicate samples of fresh feces from four steers. The results of this test (Appendix table G) showed that the difference between the means was not significant at the 0.05 level.

The moisture content of all feeds and air-dry feces was determined by drying duplicate 2-gm. samples at 100° C for 4 hours. The determinations of ash, ether extract and crude fiber were made according to the Methods of Analysis of the Association of Official Agricultural Chemists (1950).

Since the standard error in nitrogen determinations on triplicate 10-gm. samples of fresh feces is large because of the difficulties involved in adequately mixing this material, it was felt that conducting the nitrogen determination on the ground feces previously dried to constant weight would eliminate sampling difficulties. Although it

was realized that drying at 105° C would cause some reduction in fecal nitrogen it was felt that this loss would be less than the error involved in the sampling of fresh feces. In an attempt to decide whether the sampling of fresh or dried feces yielded the more reliable results, nitrogen determinations were made on both the fresh and oven-dried feces of one steer. The losses in nitrogen due to drying were approximately 8 per cent which is in agreement with the report of Gallup and Hobbs (1944). Losses of this magnitude exceeded the error involved in sampling fresh feces and the means were significantly different. The results of this experiment are shown in Table H of the Appendix. From these results it can be concluded that less error is involved in nitrogen determinations on fresh feces than on dried feces.

RESULTS AND DISCUSSION

The digestibility coefficients for dry matter, organic matter, protein, ether extract, crude fiber and nitrogen-free extract were determined for each steer during each 5-day collection period in all trials. Digestibility coefficients for all nutrients by each steer during 10-day collection periods were calculated from results obtained during periods 1 and 2. Similarly the digestibility coefficients obtained in 15-day collection period were calculated from results obtained during period 1, 2 and 3. All digestibility coefficients were calculated after correcting for any feed refusals during a collection period.

Twelve steers were used in all periods, except the third one of trial 3. During that period it was necessary to remove steers 55 and 57 because of badly swollen hocks. The data obtained with steers 2 and 57 in trial 1 were not included in the statistical treatment because the samples from steer 2 were lost and steer 57 refused exceptionally large amounts of feed. As the result of these eliminations, there remained for statistical analysis the data obtained with 10 steers in each period of trial 1, 12 steers in each period of trial 2, and 12 steers in periods 1 and 2, and 10 in period 3, of trial 3.

The digestion coefficients and related digestibility data for individual steers on 5-day collection periods in trials 1, 2 and 3 may be found in Appendix tables A, B and C, respectively. Tables B and C contain in addition to the digestibility data nitrogen

balance data for individual steers on each 5-day collection period within trials 2 and 3, respectively. Digestion coefficients obtained with individual steers on 10-day collection periods in trials 1, 2 and 3 are presented in Appendix table D. Similar data obtained from 15-day collection periods are presented in Appendix table E. Individual nitrogen retention data from 10- and 15-day collection periods in trials 2 and 3 are included in Appendix table D and E.

The mean digestion coefficients, variance, standard deviation, and standard error for the three 5-day collection periods, the 10-day and the 15-day collection periods have been computed and are given in Table 2 of the text. Similar data on nitrogen retention are given in Table 3.

Further statistical treatment is confined to the digestibility coefficients of organic matter, protein, and crude fiber, and the retention of nitrogen. Organic matter digestibility was chosen for further study rather than that of dry matter digestibility because of the greater significance of the former in terms of energy and the elimination of the errors related to the excretion of minerals into the intestinal tract. Data on the apparent digestibility of protein and crude fiber were included in additional statistical analysis because of the importance of these nutrients in digestibility studies. Crude fiber as a fraction in feed analysis has been criticized by Crampton and Maynard (1938). Although this fraction is supposed to contain the cellulose and lignin of plant tissue, loss of lignin in crude fiber determinations has been shown by Moxon and Bentley (1953) to amount to as much as 85 per cent. It has been suggested by Crampton and

Table 2. AVERAGE DIGESTIBILITY COEFFICIENTS OBTAINED WITH STEERS IN DIFFERENT PERIODS IN ALL TRIALS

Trial	Collection period	No. of steers	Dry matter				Organic matter				Protein (Nx6.25)			
			$\bar{x}$	s^2	s	$s_{\bar{x}}$	$\bar{x}$	s^2	s	$s_{\bar{x}}$	$\bar{x}$	s^2	s	$s_{\bar{x}}$
I	1st 5 days	10	56.5	5.65	2.38	0.75	58.2	14.00	3.74	1.18	53.5	4.10	2.02	0.64
"	2nd 5 days	"	56.8	3.61	1.90	0.60	59.8	4.11	2.03	0.65	51.4	5.17	2.27	0.72
"	3rd 5 days	"	56.7	3.06	1.75	0.56	59.3	2.83	1.68	0.53	54.4	4.21	2.05	0.65
"	10 days	"	56.7	1.52	1.24	0.39	59.6	2.81	1.67	0.53	52.3	1.54	1.24	0.39
"	15 days	"	56.7	3.29	1.81	0.58	59.5	3.40	1.84	0.59	53.0	2.17	1.47	0.46
II	1st 5 days	12	62.9	0.75	0.87	0.25	65.9	0.67	0.82	0.23	52.2	6.45	2.54	0.74
"	2nd 5 days	"	61.1	1.49	1.22	0.35	64.2	1.32	1.15	0.33	55.5	3.27	1.81	0.52
"	3rd 5 days	"	63.2	1.67	1.29	0.38	66.1	1.42	1.19	0.35	53.8	4.25	2.06	0.59
"	10 days	"	62.0	0.72	0.85	0.24	65.0	0.63	0.80	0.23	53.7	4.37	2.09	0.60
"	15 days	"	62.4	4.28	2.07	0.60	65.4	0.77	0.88	0.26	53.8	2.30	1.52	0.44
III	1st 5 days	12	56.4	1.83	1.35	0.39	60.2	2.02	1.42	0.41	48.6	3.63	1.91	0.55
"	2nd 5 days	"	57.4	2.23	1.49	0.43	61.0	2.03	1.43	0.41	51.8	1.55	1.25	0.36
"	3rd 5 days	10	58.4	1.22	1.11	0.37	61.8	1.57	1.25	0.42	51.3	3.77	1.94	0.65
"	10 days	12	57.0	1.05	1.03	0.30	60.5	1.48	1.22	0.35	50.1	4.39	2.09	0.61
"	15 days	10	57.4	1.03	1.02	0.32	61.0	1.09	1.04	0.33	50.7	0.99	1.00	0.31

Trial	Collection period	No. of steers	Ether extract				Crude fiber				N-free extract			
			$\bar{x}$	s^2	s	$s_{\bar{x}}$	$\bar{x}$	s^2	s	$s_{\bar{x}}$	$\bar{x}$	s^2	s	$s_{\bar{x}}$
I	1st 5 days	10	53.0	31.35	5.60	1.76	64.3	12.99	3.60	1.14	58.1	6.67	2.58	0.82
"	2nd 5 days	"	54.7	16.20	4.02	1.27	63.8	9.88	3.14	0.99	59.5	9.23	3.04	0.92
"	3rd 5 days	"	68.7	6.31	2.51	0.80	65.0	17.51	4.19	1.32	56.2	3.43	1.85	0.59
"	10 days	"	53.9	7.80	2.79	0.89	64.0	9.30	3.05	0.96	58.8	5.99	2.45	0.77
"	15 days	"	58.8	8.91	2.99	0.95	64.7	6.81	2.61	0.83	57.8	4.42	2.10	0.67
II	1st 5 days	12	53.3	17.02	4.14	1.19	68.6	1.16	1.08	0.31	67.6	1.25	1.12	0.32
"	2nd 5 days	"	43.3	5.27	2.29	0.66	67.2	2.63	1.62	0.47	65.5	1.62	1.27	0.37
"	3rd 5 days	"	43.8	9.26	3.04	0.88	70.5	1.90	1.38	0.40	67.2	1.21	1.10	0.32
"	10 days	"	48.2	4.17	2.04	0.59	67.7	1.57	1.25	0.37	66.5	3.20	1.79	0.52
"	15 days	"	46.8	3.39	1.84	0.53	68.8	1.03	1.02	0.30	66.8	0.64	2.80	0.25
III	1st 5 days	12	50.7	10.75	3.28	0.95	64.9	5.55	2.36	0.68	60.2	3.70	1.92	0.56
"	2nd 5 days	"	50.3	5.29	2.30	0.67	64.2	4.00	2.00	0.58	61.1	1.85	1.36	0.39
"	3rd 5 days	10	48.9	32.08	5.73	1.79	67.0	3.37	1.84	0.58	60.9	3.93	1.99	0.63
"	10 days	12	50.5	5.24	2.28	0.66	64.5	2.60	1.61	0.47	60.8	1.39	1.18	0.34
"	15 days	10	50.0	4.42	2.10	0.66	65.8	0.91	0.96	0.30	60.5	3.52	1.83	0.60

$\bar{x}$, mean
s^2, variance
s, standard deviation
$s_{\bar{x}}$, standard error

Table 3. NITROGEN RETENTION OF STEERS IN TRIALS II AND III

Trial	Collection period	No. of steers	Average N intake	Nitrogen retention			
				Mean	Variance	Standard deviation	Standard error
			gm.	gm.		gm.	gm.
II	First 5 days	12	68.86	+3.37	6.61	2.57	0.68
"	Second 5 days	12	71.52	+7.47	10.91	3.30	0.95
"	Third 5 days	11	70.13	+2.94	4.80	1.72	0.66
"	10 days	12	70.19	+4.82	8.73	2.19	0.86
"	15 days	11	70.14	+4.21	5.64	2.38	0.78
III	First 5 days	12	93.75	+5.56	26.01	5.10	1.54
"	Second 5 days	12	94.26	+8.77	5.26	2.29	0.66
"	Third 5 days	10	94.97	+9.41	7.93	2.82	0.89
"	10 days	12	93.94	+6.65	6.04	2.46	0.50
"	15 days	10	94.16	+7.93	2.55	1.60	0.50

Maynard (1938)that the crude fiber determination be replaced with lignin, hemicellulose and cellulose determinations, but the cost of the extra labor required has prevented wide-spread adoption of this suggestion. The general utilization of crude fiber digestion coefficients in present-day digestibility data justified their inclusion in the present study.

Statistical analysis of the nitrogen retention data obtained in trials 2 and 3 has ben confined to within trial treatment since the difference in age and size of the steers in the two trials would be expected to influence nitrogen retention.

The digestibility and nitrogen retention data of Appendix tables A through E have been compiled in an attempt to determine the optimum length of digestion trials, the major source of variation with trials, the number of animals needed to detect significant difference between means and the effect of slight variations in the composition of rations used in different trials.

Analysis of the digestibility data was first directed toward determining whether the variations in digestion coefficients by the same steers in different periods was greater than that by different steers within the same period. An analysis of variance was made on the digestion coefficients of organic matter, protein and crude fiber obtained in the 5-day collection periods within each trial. The results of these analyses are shown as "Three 5-day periods" in Table 4.

Variation in digestibility of organic matter by the same steers in different periods was less than the variation among steers within a period in trials 1 and 3; the reverse was true in trial 2. Variation in protein

digestibility by the same steers in different periods exceeded that between animals within a period in all three trials. Variation in digestibility of crude fiber by the same steers in different periods was less than among steers in the same period in trial 1; the opposite was true in trials 2 and 3. From the fact that in six of the nine analyses of variance differences between the same steers in different periods was greater than those among steers within the same period, it would appear that these differences which appeared in 5-day periods within trials are generally characteristic of the population. In an attempt to substantiate this conclusion, an analysis of variance was made for the digestion coefficients of the three nutrients obtained in all 5-day collection periods. The sources of variance analyzed were trials, periods within trials, trial x period interaction and error. The results are summarized in the first section of Table 5. Of interest are the significant differences between periods within trials. This and the within trial data previously presented is understood to mean that the variation in physiological performance of an individual as manifested in digestibility data is greater than the variation between individuals within the same period. This leads to the conclusion that 5-day collection periods are not long enough to permit a true sampling of the population.

Since there were significant differences in the digestibility data obtained in 5-day collection periods within the same trial it was of interest to determine whether there were significant differences in the digestion coefficients obtained in 5-, 10- and 15-day collection periods. Results of variance analyses are shown opposite the item "5-, 10- and 15-day periods" in Table 4. These results indicate that significant

Table 4 SUMMARY OF STATISTICAL ANALYSES OF DIGESTIBILITY OF ORGANIC MATTER, PROTEIN, AND CRUDE FIBER WITH RESPECT TO THE LENGTH OF THE COLLECTION PERIOD

Trial	Analysis	Source	Degrees of freedom	Digestibility of organic matter	Apparent digestibility of protein	Digestibility of crude fiber	Nitrogen retention
				mean square	mean square	mean square	mean square
I	Three 5-day periods	Periods	2	0.015	30.25**	3.28	
		Error	27	4.11	5.96	12.85	
I	5, 10 and 15-day periods	Length of period	2	8.50	1.34	3.24	
		Error	27	7.11	2.61	9.70	
II	Three 5-day periods	Periods	2	12.25**	31.30**	39.23**	
		Error	33	1.14	4.66	1.90	
II	5, 10 and 15-day periods	Length of period	2	1.36	12.74	16.26**	6.33
		Error	33	0.69	4.37	1.00	7.03
III	Three 5-day periods	Period	2	6.56	31.23**	22.35*	59.92**
		Error	27	2.07	3.35	4.28	6.06
III	5, 10 and 15-day periods	Length of period	2	0.64	12.60*	4.65	56.63*
		Error	27	1.55	2.40	3.17	11.34
III	5 and 10-day periods	Period	1		13.88*		21.34*
		Error	18		2.88		5.66
III	10 and 15-day periods	Period	1		1.11		8.91
		Error	18		1.51		4.47

* Significant at 5 per cent level.
** Significant at 1 per cent level.

Table 5. SUMMARY OF STATISTICAL ANALYSES OF DIGESTIBILITY OF ORGANIC MATTER, PROTEIN AND CRUDE FIBER

Analysis	Source	Degrees of freedom	Digestibility of organic matter	Apparent digestibility of protein	Digestibility of crude fiber
			mean square	mean square	mean square
5-day collection periods. All trials.	Trial (age)	2	349.60**	100.76**	185.61**
	Periods within trials (age)	2	9.30*	33.71**	54.02**
	Trial x period interaction	4	9.52*	31.64**	3.39
	Error	91	3.25	3.90	5.99
10-day collection periods. All trials.	Trial (age)	2	38.85*	38.56**	40.67**
	Error	27	5.86	3.71	4.58
Covariance analysis (1) of 10-day collection periods. All trials.	Errors of estimate				
	Trial (age)	2	5.75	4.80	2.34
	Error	26	7.18	3.16	3.18

* Significant at 5 per cent level.
** Significant at 1 per cent level.
(1) Digestibility of nutrient as a function of the protein content of the ration.

variations do not occur in the case of organic matter digestibility. Significant variations do occur in the digestion coefficients of protein and crude fiber in most instances. Although differences were significant in comparisons of 5- and 10- day digestibility data, these differences were no longer significant when comparisons were made of the data obtained from 10- and 15-day collection periods. The results of these comparisons are shown as the last two items of Table 4. Thus, it can be concluded that 10-day collection periods are of sufficient length to reduce the variation of individuals with respect to time to the point where it is not significantly different at the 5 per cent level from the variation between individuals. The results of analyses of variance of nitrogen retention data which also appears in Table 4 favor the same conclusions.

The age of the steers was quite different in each of the trials. This raised the question whether the digestion coefficients obtained in one trial would be significantly different from those obtained in the other two trials. While it is possible that environmental factors could cause significant differences in the physiological performance of steers in trials conducted months apart, it is unlikely that this was the case because of the uniform management the steers received throughout the entire experiment including the time between trials. Any differences in digestibility data obtained in the three trials were therefore assumed to be due to differences in the age of the steers. The coefficients of digestibility of organic matter, protein and crude fiber obtained with the same 10 steers in a 10-day collection period in each trial were subjected to analysis of variance. The results of these analyses are given in the second section of Table 5. The results show significant differences

between trials in nutrient digestibility. Before accepting these trial differences as being due to age of steers, it was thought advisable to determine whether these differences were related to differences in the protein content of the rations. Gallup and Briggs (1948) reported a correlation of 0.92 between the apparent digestibility of protein and the percentage protein of the ration and calculated the regression equation of the apparent digestibility of protein as a function of the protein content of the ration. Blaxter and Mitchell (1948) showed a regression to exist between the apparent digestibility of protein and the percentage of protein in the ration when the latter is 5 per cent or more. The digestibility of organic matter and crude fiber would also be expected to be positively correlated with the percentage of protein in the ration. The average protein content of the rations ingested by the steers in trials 1, 2 and 3 were 9.7, 9.9 and 9.1 per cent respectively. Although these differences in the protein content of the ration are not large, it was considered advisable to correct for any effect of regression by subjecting the data to an analysis of covariance. The results of these analyses are presented in the last section of Table 5. It is apparent from these results that the trial differences in digestibility of the nutrients is not the result of differences in age or environmental factors, but resulted from differences in protein content of the rations.

The cost of experimentation is related to the degree of precision with which data are obtained. If the precision of the methods used is inadequate to detect the desired experimental differences, a total financial loss results. The use of expensive methods yielding data whose precision is greater than that required is likewise economically

unsound. Since the avoidance of these circumstances in digestion and nitrogen trials with beef cattle is desirable, an attempt was made to determine the number of animals required to detect differences due to treatment.

To accomplish this, the digestibility and nitrogen retention data of the 10-day collection period in trial 2 were selected for further analysis. These data were selected because 12 steers were used in the trial, the protein content of the ration (9.9 per cent) was highest of all trials and there were no feed refusals. As shown in Table 2, the mean digestion coefficients for organic matter, protein and crude fiber were 65.0, 53.7 and 67.7, respectively. The variances for the digestibility of organic matter, protein and crude fiber were 0.72, 0.63 and 1.57, respectively. The standard error for each nutrient was then calculated for 1 to 12 steers. From the standard error the least significant difference between the means was calculated for each nutrient. The results of these calculations are shown in Table 6.

In Table 6 the least significant difference between means is an approximation of the difference which must exist between digestion coefficients in order for this difference to be significant at the 5 per cent level when a given number of steers are subjected to two treatments. From the data in Table 6, it is apparent that the variation in the apparent digestibility of protein requires a greater number of animals to detect significant differences than is required for comparisons involving organic matter and crude fiber. In the case of apparent digestibility of protein, which in this trial had a mean of 53.7, it is evident

Table 6, NUMBER OF STEERS REQUIRED TO DETECT THE LEAST SIGNIFICANT DIFFERENCE (5 PER CENT) BETWEEN THE MEANS OF COEFFICIENTS OF DIGESTIBILITY OF NUTRIENTS AND OF NITROGEN RETENTION*

	Organic matter		Protein (Nx6.25)		Crude fiber		Nitrogen retention, gm.	
No. of steers	Standard error	Least significant difference between means	Standard error	Least significant difference between means	Standard error	Least significant difference between means	Standard error	Least significant difference between means
1	0.80	2.5	2.09	6.4	1.25	3.9	2.98	9.3
2	0.57	1.8	1.48	4.6	0.89	2.8	2.09	6.5
3	0.46	1.4	1.21	3.7	0.72	2.2	1.71	5.3
4	0.40	1.2	1.04	3.2	0.65	2.0	1.48	4.6
5	0.38	1.1	0.97	3.0	0.56	1.7	1.33	4.1
6	0.32	1.0	0.85	2.6	0.51	1.6	1.21	3.8
7	0.30	0.9	0.80	2.5	0.48	1.5	1.12	3.5
8	0.28	0.9	0.74	2.3	0.44	1.4	1.05	3.3
9	0.26	0.8	0.70	2.2	0.42	1.3	0.99	3.1
10	0.25	0.8	0.66	2.1	0.40	1.2	0.94	2.9
11	0.24	0.7	0.63	2.0	0.38	1.2	0.89	2.8
12	0.23	0.7	0.60	1.9	0.37	1.1	0.86	2.7

*Calculated from the results of Trial II, periods 1 and 2, 10 day collection.

that if four steers are used in each treatment the difference between the means will be significant if it exceeds 3.2. The limits beyond which difference would be detected would be 50.5 and 56.9. An increase in the number of steers to 12 per treatment only narrow the limits, 51.8 to 55.6, so it would appear that the use of four steers per treatment would detect difference of practical value. It is interesting to note how few animals would be required to detect differences in organic matter digestibility. One steer per treatment would permit detection of smaller significant differences between treatments than would four steers in trials in which apparent digestibility of protein was being determined. In trials in which crude fiber digestibility was of first concern, two steers per treatment would permit detection of differences of about the same magnitude as would be detected with four steers per treatment in the case of the apparent digestibility of protein.

It will be observed in Table 3 that average nitrogen retention for the 10-day collection period in trial 2 was +4.82 with a variance of 8.75. The use of these values permitted the calculation of the least significant difference which can be detected for a given number of animals but the coefficient of variation is so large (46 per cent) that the differences between treatment must be very large to be significant. From the data of Table 6, it appears that a significant difference in nitrogen retention could be detected with four steers only if the difference due to treatment differed by as much as 4.6 gm.

SUMMARY

Three digestion and nitrogen metabolism trials were conducted with 12 steers at the ages of 10, 19 and 30 months. They were fed a maintenance type ration of relatively constant composition. Each trial consisted of a 10-day preliminary period and three successive 5-day collection periods. The digestibility coefficients of dry matter, organic matter, protein, ether extract, crude fiber and nitrogen-free extract were determined for each steer in each 5-day collection period. Digestibility coefficients were similarly determined for 10- and 15-day collection periods. The mean, variance, standard deviation and standard error of the digestibility coefficients of each nutrient by the steers on the three 5-day periods, the 10-day and the 15-day periods were determined in each trial. The mean, variance, standard deviation and standard error for nitrogen retention by steers on the three 5-day periods, the 10-day and the 15-day periods were determined in two trials.

Statistical analysis of digestibility data was confined to those nutrients having the greatest physiological significance in the ruminant, namely, organic matter, protein and crude fiber. Statistical analysis of nitrogen retention data was limited to within trial comparisons because of age and weight differences of animals between trials.

Analysis of variance of digestibility coefficients obtained from all 5-day collection periods pointed to significant differences between trials and between periods within trials. The analysis of variance of the digestibility coefficients obtained in a 10-day collection period in each trial indicated that highly significant differences exist

between trials. Covariance analysis revealed that these differences were due to small differences in protein content of the rations in different trials.

Analysis of digestibility coefficients obtained in three 5-day collection periods within trials indicated a greater variation between different periods than between individuals in the same period. This variation was reduced when 5-, 10- and 15-day periods within the same trial were analyzed. With the exception of crude fiber digestibility in trial 2, any variation between 5-, 10- and 15-day periods was eliminated in a subsequent comparison of 10- and 15-day collection periods. Likewise significant variation in nitrogen balance data was eliminated. On this basis 10-day collection periods are recommended for studies of digestibility and nitrogen retention in steers.

From the digestion and nitrogen balance data obtained in trial 2, the least significant difference between means which could be detected with 1 to 12 animals per treatment was determined.

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APPENDIX

TABLE A. DIGESTIBILITY OF NUTRIENTS BY STEERS ON 5 DAY COLLECTION PERIODS, TRIAL I

5 Day Coll. period	Steer No.	Dry matter intake	Digestibility coefficients						Nitrogen intake	Fecal dry matter	Fecal nitrogen
			Dry matter	Organic matter	Protein (Nx6.25)	Ether extract	Crude fiber	N-free extract			
		gm.							gm.	gm.	gm.
First	1	3412	60.9	63.7	51.3	47.7	71.4	62.2	52.19	1336	25.38
"	53	3484	53.9	56.3	55.6	47.3	61.1	53.9	55.97	1608	24.92
"	54	3413	55.0	58.3	56.2	46.2	63.0	56.6	55.17	1537	24.13
"	55	3484	56.5	59.4	54.1	62.0	64.2	57.6	55.97	1517	25.78
"	60	3484	56.2	58.9	52.8	61.8	63.2	57.4	55.97	1528	26.45
"	61	3484	58.1	62.7	52.3	51.6	67.7	59.6	55.97	1461	26.74
"	63	3484	56.4	57.4	56.4	54.3	64.0	57.3	55.97	1519	24.45
"	65	3484	53.4	56.2	50.6	49.7	59.0	56.0	55.97	1625	27.63
"	66	3412	55.9	59.0	53.1	54.9	61.8	58.7	52.53	1504	24.67
"	67	3373	59.2	60.2	52.5	52.9	67.1	62.0	49.97	1375	23.79
Second	1	3375	59.5	62.5	49.2	47.2	67.9	62.3	46.43	1366	23.50
"	53	3477	54.9	57.6	48.6	60.6	64.0	55.4	52.70	1568	27.13
"	54	3274	54.1	57.6	52.3	51.0	63.8	54.6	51.24	1504	24.36
"	55	3477	58.3	61.4	52.4	53.0	65.9	60.9	52.70	1450	25.09
"	60	3477	56.2	59.2	50.6	55.5	62.9	58.8	52.70	1523	26.03
"	61	3477	58.2	61.6	50.7	58.5	66.2	61.2	52.70	1455	26.04
"	63	3477	54.8	57.6	50.0	58.3	58.7	58.3	52.70	1573	26.43
"	65	3477	57.0	60.2	51.8	56.6	61.8	61.0	52.70	1496	25.56
"	66	3477	55.8	58.9	53.2	52.5	60.6	59.3	52.70	1536	24.73
"	67	3477	58.9	62.3	51.3	53.8	65.8	62.7	52.70	1430	25.60
Third	1	3491	60.1	62.6	56.2	64.6	70.4	58.9	55.61	1393	24.38
"	53	3491	55.8	56.4	54.8	65.5	64.5	54.8	"	1544	25.17
"	54	3491	57.6	60.0	58.9	67.2	65.6	56.2	"	1481	22.96
"	55	3491	57.1	59.7	52.3	70.4	65.2	57.2	"	1497	26.50
"	60	3491	57.3	58.5	55.8	70.6	65.2	55.8	"	1492	24.62
"	61	3491	55.1	58.4	50.3	69.3	66.7	54.1	"	1568	27.60
"	63	3491	55.7	58.4	55.1	71.3	66.2	53.5	"	1546	25.05
"	65	3491	54.2	57.1	53.2	69.6	63.3	58.1	"	1597	26.03
"	66	3491	55.7	58.4	54.0	66.6	63.9	55.3	"	1546	25.66
"	67	3491	58.5	61.5	53.3	71.6	68.6	58.1	"	1448	26.06

TABLE 8. DIGESTION OF NUTRIENTS AND NITROGEN RETENTION BY STEERS ON 5-DAY COLLECTION PERIODS, TRIAL II

5 Day Coll. period	Steer No.	Dry matter intake	Digestibility coefficients						Nitrogen intake	Fecal dry matter	Fecal nitrogen	Nitrogen retention
			Dry matter	Organic matter	Protein (Nx6.25)	Ether extract	Crude fiber	N-free extract				
		gm.							gm.	gm.	gm.	gm.
First	1	4420	65.6	66.3	57.3	53.8	70.0	66.5	68.86	1611	29.31	+6.85
"	2	"	61.4	64.4	51.1	53.2	68.3	65.3	"	1706	33.60	+8.38
"	53	"	62.7	65.7	54.4	49.4	67.8	67.5	"	1648	31.32	-0.06
"	54	"	62.5	65.7	52.8	49.4	67.1	68.3	"	1659	32.51	+1.16
"	55	"	63.6	66.8	53.6	52.0	69.3	68.7	"	1608	31.84	+2.32
"	57	"	61.4	64.6	49.2	45.6	66.4	67.5	"	1706	34.98	-4.20
"	60	"	63.2	66.2	49.9	54.1	68.3	68.8	"	1626	34.47	+2.14
"	61	"	62.7	65.8	47.8	52.0	68.9	68.4	"	1648	35.92	-0.36
"	63	"	65.8	66.8	53.3	60.7	70.0	67.9	"	1600	32.16	+1.35
"	65	"	65.8	66.6	52.4	58.3	68.8	68.4	"	1599	32.78	+3.88
"	66	"	62.2	63.5	53.1	55.3	68.9	66.1	"	1670	32.23	+4.08
"	67	"	63.4	66.4	51.5	56.4	69.3	68.1	"	1617	33.30	+5.16
Second	1	4403	62.6	65.4	60.0	46.1	66.7	66.9	71.52	1648	28.51	+13.91
"	2	"	60.1	63.1	55.3	39.7	65.0	65.1	"	1755	31.84	+11.58
"	53	"	61.5	64.8	56.1	45.0	65.7	67.2	"	1693	31.32	+6.10
"	54	"	59.3	62.7	54.7	44.2	65.1	63.9	"	1790	32.22	+3.90
"	55	"	63.1	66.2	56.2	46.7	70.2	67.1	"	1624	31.18	+4.64
"	57	"	62.0	64.9	54.4	43.9	67.9	66.5	"	1675	32.50	+6.82
"	60	"	62.6	65.6	55.2	54.1	69.6	66.6	"	1648	31.98	+7.99
"	61	"	60.1	63.3	53.7	39.9	67.1	64.4	"	1757	33.04	+5.93
"	63	"	60.9	63.9	56.4	42.6	67.8	64.5	"	1724	31.03	+2.64
"	65	"	60.8	64.0	54.1	41.1	68.5	64.7	"	1724	32.76	+7.56
"	66	"	60.2	63.2	56.2	43.3	66.4	63.9	"	1754	31.22	+11.10
"	67	"	60.1	63.4	53.0	41.3	67.1	64.8	"	1758	33.58	+7.44
Third	1	4387	63.9	66.6	55.4	48.4	71.6	66.9	70.13	1586	31.40	+5.13
"	2	"	63.0	65.9	53.1	45.8	70.1	67.1	"	1622	33.09	+5.34
"	53	"	62.6	65.7	54.1	41.5	70.5	66.5	"	1641	32.32	+0.31
"	54	"	61.6	64.7	52.9	41.4	68.1	66.4	"	1687	32.32	+2.10
"	55	"	63.6	66.5	53.5	44.4	71.0	67.7	"	1599	32.77	-2.64
"	57	"	63.1	66.0	49.7	43.6	70.6	67.7	"	1620	35.48	-0.45
"	60	"	64.2	66.9	51.4	45.3	72.0	68.2	"	1572	34.26	-0.53
"	61	"	63.2	66.1	56.6	40.4	70.1	67.0	"	1615	30.48	+3.25
"	63	"	62.4	65.3	53.2	40.6	70.0	66.4	"	1649	32.98	
"	65	"	65.7	68.3	56.6	48.9	72.0	69.5	"	1604	30.53	+6.90
"	66	"	61.0	63.7	52.9	40.2	67.4	65.0	"	1713	32.24	+1.89

TABLE C. DIGESTIBILITY OF NUTRIENTS AND NITROGEN RETENTION BY STEERS ON 5-DAY COLLECTION PERIODS, TRIAL III

5 Day Coll. period	Steer No.	Dry matter intake	Digestibility coefficients						Nitrogen intake	Fecal dry matter	Fecal nitrogen	Nitrogen retention
			Dry matter	Organic matter	Protein (Nx6.25)	Ether extract	Crude fiber	N-free extract				
		gm.							gm.	gm.	gm.	gm.
First	1	6319	57.3	60.9	50.3	50.8	64.7	62.2	88.07	2701	43.76	+3.55
"	2	6446	57.5	61.2	48.7	49.7	65.9	61.4	94.74	2742	48.53	+5.17
"	53	6446	56.1	60.2	48.3	48.4	65.4	60.0	94.74	2831	48.98	+2.80
"	54	6446	55.3	59.3	49.5	45.6	63.9	59.2	94.74	2882	47.84	+3.50
"	55	6446	53.5	57.2	44.6	46.7	61.5	57.7	94.74	2998	52.47	+3.39
"	57	6446	55.0	58.9	49.2	52.5	61.3	59.7	94.74	2900	48.14	+3.60
"	60	6384	57.2	61.1	48.5	52.3	66.4	60.9	94.25	2730	48.59	-0.55
"	61	6433	58.5	62.1	51.1	51.5	66.7	62.1	93.98	2672	45.96	+9.74
"	63	6374	55.3	59.0	45.1	48.6	63.6	59.3	90.77	2847	49.82	+0.83
"	65	6446	56.0	59.8	49.5	56.2	69.6	56.3	94.74	2834	47.89	+8.85
"	66	6446	57.0	60.8	49.4	55.0	63.0	61.9	94.74	2771	47.93	+5.77
"	67	6446	57.9	62.0	48.7	52.0	66.4	62.3	94.74	2716	48.62	+5.72
Second	1	6353	54.5	58.5	51.5	48.6	60.3	58.8	90.81	2863	44.11	+10.06
"	2	6448	57.7	61.4	51.9	47.7	64.8	62.0	94.69	2727	45.54	+9.95
"	53	6448	55.5	59.1	51.3	49.9	61.9	59.5	94.69	2869	46.19	+9.10
"	54	6448	57.0	60.7	52.0	48.4	64.9	60.6	94.69	2770	45.43	+7.26
"	55	6448	57.5	61.5	50.8	50.7	64.6	62.6	94.69	2708	46.58	+7.31
"	57	6448	57.0	60.5	50.5	51.2	63.6	60.9	94.69	2773	45.86	+9.25
"	60	6448	58.6	62.4	53.1	53.7	66.4	62.3	94.69	2657	44.37	+3.42
"	61	6440	59.2	62.9	52.9	51.0	66.7	63.2	94.19	2625	44.36	+12.03
"	63	6518	59.1	62.5	54.4	49.2	66.0	62.7	92.63	2627	42.29	+9.34
"	65	6448	57.0	60.5	52.2	46.5	63.7	61.0	94.69	2771	45.17	+11.32
"	66	6448	56.5	59.8	51.1	53.1	61.9	60.5	94.69	2808	46.33	+9.36
"	67	6432	58.6	62.2	49.9	53.0	65.8	59.8	94.01	2664	47.15	+6.86
Third	1	6389	59.3	62.2	54.9	51.1	67.2	61.2	95.45	2599	42.10	+13.15
"	2	6447	59.1	62.5	50.0	47.0	67.7	62.1	95.43	2638	47.75	+8.99
"	53	6447	59.2	61.3	53.1	59.8	65.5	60.4	95.43	2696	44.75	+12.48
"	54	6447	56.7	60.1	50.3	46.8	65.4	59.5	95.43	2793	47.48	+8.35
"	55	6447	58.5	61.9	51.8	49.0	68.8	60.3	95.43	2677	46.04	+9.78
"	61	6432	61.2	64.5	53.7	53.7	70.4	63.6	94.50	2498	43.72	+12.38
"	63	6436	57.3	62.6	49.3	44.7	67.1	60.0	94.89	2747	48.07	+4.02
"	65	6392	56.7	59.9	50.8	38.5	64.3	59.8	94.51	2770	46.54	+9.17
"	66	6447	57.9	61.1	50.7	47.4	65.7	61.1	95.43	2715	47.24	+9.91
"	67	6447	58.3	61.9	48.5	52.6	68.1	61.2	95.43	2680	48.49	+6.86

TABLE D. DIGESTIBILITY OF NUTRIENTS AND NITROGEN RETENTION BY STEERS ON 10-DAY COLLECTION, PERIODS 1 AND 2

Trial	Steer No.	Dry matter intake	Digestibility coefficients						Nitrogen intake	Fecal dry matter	Fecal nitrogen	Nitrogen retention
			Dry matter	Organic matter	Protein (Nx6.25)	Ether extract	Crude fiber	N-free extract				
		gm.							gm.	gm.	gm.	gm.
	1	3394	60.4	63.1	50.3	47.5	69.7	62.3	50.10	1351	24.49	
	53	3480	54.4	57.0	52.2	53.9	62.9	54.9	54.39	1588	26.02	
	54	3344	54.5	57.6	54.4	49.1	65.4	55.6	53.21	1521	24.29	
	55	3480	57.4	60.4	53.2	57.6	65.1	59.2	54.39	1484	25.44	
I	60	3480	56.2	58.9	51.7	58.8	63.0	58.1	54.29	1525	26.28	
	61	3480	58.1	61.4	51.4	54.9	66.9	60.3	54.39	1458	26.38	
	63	3480	55.6	58.3	53.3	56.3	61.4	57.8	54.39	1546	25.40	
	65	3480	55.2	58.2	51.1	52.5	59.9	58.5	54.39	1561	26.60	
	66	3445	55.9	58.8	53.1	53.6	61.2	59.0	52.67	1520	24.68	
	67	3426	59.1	61.7	52.0	54.2	66.5	62.3	51.40	1403	25.17	
	1	4412	63.1	65.8	58.7	50.0	68.4	66.7	70.19	1630	28.93	+10.38
	2	4412	60.8	63.8	53.2	46.2	66.6	65.2	"	1731	32.75	+9.98
	53	4412	62.1	65.2	55.3	47.0	66.7	67.3	"	1671	31.30	+3.02
	54	4412	60.9	64.2	53.8	46.6	66.1	66.1	"	1725	32.37	+2.53
	55	4412	63.4	66.5	55.0	49.6	69.7	67.9	"	1616	31.54	+3.73
	57	4412	61.7	64.7	51.9	44.7	67.1	67.0	"	1690	33.71	+1.31
II	60	4412	62.9	65.9	52.6	49.2	68.9	67.7	"	1637	33.21	+5.04
	61	4412	61.4	64.6	50.8	45.9	66.1	66.4	"	1703	34.46	+2.79
	63	4412	62.3	65.4	53.3	51.5	68.8	66.2	"	1662	31.59	+2.02
	65	4412	62.3	63.3	53.3	49.6	68.7	66.5	"	1662	32.70	+5.42
	66	4412	61.2	64.2	54.7	49.5	67.7	65.0	"	1712	31.72	+7.59
	67	4412	61.8	64.9	52.3	48.5	68.1	66.4	"	1686	33.44	+6.30
	1	6326	56.5	59.6	50.9	49.6	62.5	60.5	80.46	2752	43.93	+7.81
	2	6447	57.6	61.3	50.3	48.9	65.3	61.7	94.74	2735	47.04	+7.56
	53	6447	55.8	59.7	49.8	49.1	63.6	59.7	94.74	2850	47.58	+5.95
	54	6447	56.2	60.0	50.8	46.9	64.4	59.9	94.74	2826	46.63	+5.36
	55	6447	55.8	58.7	47.7	48.0	63.1	60.0	94.74	2853	49.51	+5.35
	57	6447	56.0	59.1	49.0	52.0	62.4	60.3	94.74	2837	48.30	+6.42
III	60	6416	58.0	61.8	50.7	53.1	66.4	61.6	94.51	2894	42.13	+1.44
	61	6437	58.9	62.5	52.1	51.1	66.7	62.6	94.11	2699	45.12	+10.89
	63	6396	57.3	60.8	49.8	48.6	64.8	61.0	91.72	2737	46.06	+5.09
	65	6447	56.5	60.2	50.9	51.4	66.6	58.7	94.74	2803	46.54	+10.09
	66	6447	56.7	60.3	50.3	54.3	62.3	61.2	94.74	2790	47.13	+7.57
	67	6439	58.2	62.1	49.3	52.7	66.1	62.6	94.39	2690	47.85	+6.29

TABLE 2. DIGESTIBILITY OF NUTRIENTS AND NITROGEN RETENTION BY STEERS ON 15-DAY COLLECTION, PERIODS 1, 2 AND 3

Trial	Steer No.	Dry matter intake	Digestibility coefficients						Nitrogen intake	Fecal dry matter	Fecal nitrogen	Nitrogen retention
			Dry matter	Organic matter	Protein (Nx6.25)	Ether extract	Crude fiber	N-free extract				
		gm.							gm.	gm.	gm.	gm.
	1	3426	60.2	62.9	52.5	55.2	69.9	61.2	51.41	1365	24.45	
	53	3484	54.8	57.4	53.1	57.7	63.2	54.7	54.80	1573	25.72	
	54	3393	55.6	58.7	55.9	55.2	64.2	55.8	54.02	1507	23.82	
	55	3484	57.3	60.2	52.9	61.7	65.1	58.6	54.80	1488	25.81	
I	60	3484	56.5	58.9	52.9	62.8	63.8	57.3	54.80	1514	25.80	
	61	3484	57.1	60.4	50.1	59.5	66.9	58.3	54.80	1495	26.79	
	63	3484	55.6	58.4	53.9	61.1	63.1	56.3	54.80	1546	25.26	
	65	3484	54.9	57.9	51.8	58.4	61.4	56.8	54.80	1573	26.44	
	66	3460	55.8	58.7	53.4	57.6	62.2	57.8	53.66	1529	24.99	
	67	3484	59.3	62.2	53.2	60.2	62.2	61.0	54.80	1418	25.12	
	1	4403	63.3	66.1	57.6	49.6	69.5	66.8	70.14	1615	29.75	+7.68
	2	"	61.5	64.5	53.2	46.2	67.8	65.8	"	1694	32.85	+8.43
	53	"	62.3	65.4	54.9	45.1	68.1	67.1	"	1661	31.65	+2.12
	54	"	61.1	64.3	53.5	44.8	66.8	66.2	"	1712	32.63	+2.38
	55	"	63.4	66.5	54.5	47.9	70.2	67.9	"	1610	31.95	+1.61
II	57	"	62.1	65.1	51.1	44.3	68.5	67.3	"	1667	34.28	+0.72
	60	"	63.3	66.2	52.2	47.8	70.0	67.9	"	1815	33.54	+3.18
	61	"	62.0	65.1	52.7	44.1	68.8	66.2	"	1676	33.15	+2.94
	63	"	62.4	65.4	54.3	47.9	69.2	66.3	"	1658	32.04	
	65	"	63.5	66.3	54.4	49.4	69.8	67.5	"	1609	31.99	+6.11
	66	"	61.1	64.1	54.1	46.4	67.6	65.0	"	1712	32.19	+5.69
	67	"	63.7	65.7	53.4	47.6	69.5	67.0	"	1642	32.70	+5.48
	1	6347	57.4	60.5	52.5	50.1	65.9	60.7	90.79	2701	43.36	+9.89
	2	6447	58.1	61.7	51.3	48.3	66.1	61.8	94.97	2702	47.28	+7.70
	53	6447	56.6	60.2	50.9	52.5	64.8	60.0	94.97	2799	46.64	+8.13
	54	6447	56.3	60.1	50.6	46.9	64.7	59.8	94.97	2815	46.91	+6.37
	55	6447	56.7	60.2	49.1	48.3	65.0	60.1	94.97	2794	48.36	+6.83
III	61	6435	57.6	63.2	52.6	51.8	67.9	62.9	94.25	2598	44.68	+11.38
	63	6409	57.2	61.4	49.6	48.3	65.6	60.7	92.75	2740	46.74	+4.73
	65	6425	56.6	60.2	50.9	49.4	65.8	56.0	94.67	2792	46.53	+9.78
	66	6447	57.1	60.6	50.4	52.1	63.6	61.2	94.97	2765	47.11	+8.35
	67	6434	58.3	62.0	49.0	52.7	66.8	62.1	94.32	2687	48.09	+6.48

Table F. THE EFFECT OF ACIDIFICATION ON RECOVERY OF NITROGEN IN URINE[1]

Sample No.	Acidified urine N per 10 ml.	Non-acidified urine N per 10 ml.	Difference
	mg.	mg.	mg.
1	40.0	40.4	-0.4
1	39.9	39.7	+0.2
2	33.4	34.3	-0.9
2	33.8	34.4	-0.6
3	34.4	34.6	-0.2
3	34.1	34.7	-0.6
Mean	35.93	36.35	

[1] Stored for 24 hours in unstoppered 2-gallon bottles in the metabolism room of the Animal Husbandry Department.

Table 6, DRY MATTER DETERMINATIONS MADE ON STEER FECES DRIED AT 105° C TO CONSTANT WEIGHT

Steer No.	Weight of fresh feces	Loss of weight	Moisture	Dry matter	Difference*
	gm.	gm.	%	%	%
2	300	227.0	75.67	24.33	
"	"	227.4	75.80	24.20	0.13
58	300	230.3	76.77	23.23	
"	"	230.9	76.97	23.03	0.20
59	300	231.3	77.10	22.90	
"	"	231.4	77.13	22.87	0.03
65	300	242.4	80.80	19.20	
"	"	245.4	81.13	18.87	0.03
66	300	226.7	75.56	24.41	
"	"	227.3	75.77	24.23	0.18
67	300	229.5	76.50	23.50	
"	"	230.0	76.66	23.34	0.16

x = 0.12
Standard error, 0.095
t = 1.26, difference between duplicates not significant at 0.05 level.

Table H. THE EFFECT OF DRYING AT 105° C. ON THE RECOVERY OF NITROGEN FROM STEER FECES[1]

Nitrogen content of fresh feces converted to dry matter basis	Nitrogen content of feces dried for 3 days at 105° C
%	%
1.72	1.64
1.75	1.63
1.73	1.54
1.83	1.62
1.79	1.61
1.65	1.65
1.97	1.64
1.67	1.72
1.70	1.67
1.59	
1.60	
Mean 1.727 ± 0.04	1.636 ± 0.016

[1] Eleven 10-gram samples of fresh feces from one steer on a 10-day collection period were taken for nitrogen determination. After drying and grinding nine 2-gram samples of the same feces were analyzed for nitrogen. Copper was used as the catalyst in these determinations by the Kjeldahl method.

Difference between means, 0.091
Pooled variance, 0.138
Standard error, 0.04
t = 2.29*
*Significantly different at 0.05 level.

STUDIES ON DIGESTION AND NITROGEN METABOLISM OF BEEF CATTLE

PART II. THE DETERMINATION OF THE METABOLIC FECAL NITROGEN OF STEERS

INTRODUCTION

In spite of the importance of the metabolic nitrogen of the feces in determinations of the true digestibility and biological value of protein, there are very few published values for the metabolic fecal nitrogen excretion of beef cattle. The determination of this fraction of the total fecal nitrogen excretion presents so many difficulties and is subject to such error that most workers are content to use a value obtained by Swanson and Herman (1943) with dairy heifers. Although it was felt improbable that significant differences would be found between the metabolic fecal nitrogen of beef and dairy cattle, the desirability of knowing the magnitude of this fraction of the fecal nitrogen of beef cattle was thought to justify the experimentation needed for its determination. In the course of this study an opportunity was taken to ascertain whether a difference exists between the metabolic fecal nitrogen value obtained by feeding a low-protein ration and that resulting from the application of regression analysis to the data from several digestion trials.

LITERATURE REVIEW

The nitrogen of the feces contains in addition to undigested food nitrogen, a fraction originating in the body which is referred to as metabolic fecal nitrogen. This fraction is comprised of nitrogenous compounds of the digestive juices, secretory products such as the bile pigments, and abraded cells of the gastro-intestinal mucosa. Mitchell (1924) (1926) has stated that the most important factors determining the amount of metabolic nitrogen excreted in the feces are the amount of dry matter consumed and the concentration of indigestible non-nitrogenous material in the ration. Schneider (1934) has shown that the metabolic fecal nitrogen of rats is dependent in part on the body size of the animal and upon the quantity and fiber content of the ration. Although recognizing the existence of a constant fraction of the metabolic fecal nitrogen which is dependent upon body size, Blaxter and Mitchell (1946) concluded that this fraction is small and not a major consideration, as it is included in the ratio expressing grams of fecal nitrogen per 100 grams of dry matter ingested.

Numerous attempts have been made to determine by feeding nitrogen-free rations, the metabolic nitrogen of the feces of several species of animals. It is less difficult to determine this value in man, mouse, rat and swine than in ruminants because simple-stomach animals can be fed nitrogen-free diets without the occurrence

of a drastic abnormal physiological state of the digestion mechanism. Since nitrogen-free diets are unpalatable, Mitchell and Carmen (1924) have found it expedient to add to such diets a small amount of a completely digestible protein. Such addition does not increase the fecal nitrogen excretion. The success of this method in the determination of fecal nitrogen of endogenous origin of simple-stomach animals is attested to by the rather close agreement of many reports in the literature for each species.

In sharp contrast to the simple-stomach animal is the ruminant. The difficulty associated with determining the metabolic fecal nitrogen of ruminants is indicated by the limited number of observations that have appeared in the literature. The mature ruminant, dependent as it is upon the biochemical activities of the rumen bacteria for the digestion of complex carbohydrates and synthesis of nutrients and growth factors, is not in a normal physiological state on a nitrogen-free ration. The inability to control the feed intake of sheep and cattle, due to the low palatability of nitrogen-free rations, has forced most investigators to use low-protein rations. It would appear that feeding sufficient quantities of low-protein rations containing a high percentage of roughage would permit normal, though reduced, bacterial activity. This would be quite different from the complete change of flora that would be expected on feeding nitrogen-free, purified rations.

The use of low-protein rations has enabled investigators to obtain estimated values for the metabolic fecal nitrogen of sheep, which are in general agreement. Turk, Morrison and Maynard (1931)

found 0.56 gm. of nitrogen per 100 gm. of dry matter intake, which compares favorably with the value of 0.55 reported by Miller and Morrison (1939), and Harris and Mitchell (1941). Sotola's (1930) value of 0.65 seems to be high on the basis of the above results. Recently Blaxter and Mitchell (1946) obtained a value of 0.55 through the use of a low-protein ration and a regression method to be discussed later in this paper.

A similar value for the metabolic fecal nitrogen of cattle can not be accepted with confidence because of the marked variation in reported values and the limited number of animals used in most studies. Swanson and Herman (1943) fed a low-protein ration (1.58%) to 20 dairy heifers and found the fecal nitrogen excretion per 100 gm. of dry matter intake to be 0.53 gm. These Missouri workers accepted this as the metabolic fecal nitrogen without accounting for the undigested portion of the ingested protein. Likewise, Hutchinson and Morris (1936) interpreted the fecal nitrogen excretion per 100 gm. of low-protein ration as being the metabolic fecal nitrogen. Their values, obtained with three dairy cows, without correcting for undigested dietary protein, were 0.42, 0.44, and 0.48 gm. Further disagreement is seen in the reported value of 0.63 obtained by Steenbock, Nelson and Hart (1915) with dairy calves, and the value of 0.33 obtained by Morris and Wright (1935) with one steer.

Studies by Mukherjee and Kehar (1949) emphasize the error resulting from the use of metabolic fecal nitrogen values obtained with low-protein rations. These workers showed that manually separated

fibers from the feces of cattle fed low-protein rations contained between 8 and 12 per cent of the total nitrogen of the feces. Their rations, containing between 0.56 and 1.05 per cent protein were lower in nitrogen than those used by Swanson and Herman (1943), and Hutchinson and Morris (1936). The presence of undigested protein in the feces might have contributed to the high values for metabolic fecal nitrogen reported by the latter two groups of workers. Corrected metabolic fecal nitrogen values obtained by Mukherjee and Kehar on two breeds of Indian cattle fed "nitrogen-free" (0.02% N), and two low-nitrogen rations (0.09 and 0.1%) were 0.33, 0.38 and 0.44, but differences between these values were not significant.

In a comparison of different methods of determining the metabolic fecal nitrogen Kehar and Mukherjee (1949) utilized a regression method proposed years before by Titus (1927). Titus plotted the fecal nitrogen excretion per 100 gm. of dry matter intake of steers against the protein content of their rations and found a linear relationship over the range of 5 to 20 per cent protein. The rations were composed of varying proportions of alfalfa and paper pulp. Titus did not feel justified in extending the line from 5 per cent protein to the intercept at zero per cent to obtain the true metabolic fecal nitrogen.

Bosshardt and Barnes (1946) in studies with mice observed a significant difference between the metabolic fecal nitrogen obtained by feeding a nitrogen-free diet and by regression method. It would appear from their results that in the mouse a nitrogen-free diet depresses the nitrogen metabolism to such an extent that the metabolic fecal nitrogen is reduced. Such a depression is apparently not a

general phenomenon, as studies by Mitchell and Bert (1954) on the rat, and Armstrong and Mitchell (1955) on swine, did not reveal significant differences between the metabolic fecal nitrogen obtained by feeding nitrogen-free diets and by regression analysis. Likewise, Maxter and Mitchell (1946) with sheep failed to find significant differences between results obtained with a low-nitrogen ration and by regression analysis.

In the following section of this thesis, a value for the metabolic fecal nitrogen of beef cattle obtained by feeding a low-protein ration will be compared with that found by regression analysis.

EXPERIMENTAL

In an attempt to determine the metabolic fecal nitrogen of beef cattle, six two-year-old grade Hereford steers were fed a low-protein ration (2.58 ± 0.07%) composed of prairie hay, starch, dextrose and minerals. The average daily intake and chemical composition of the ration eaten by each steer during the entire 16-day collection period is presented in Table 1. The average weight of the steers is given in Table 2. After the steers had become adjusted to the feeding regimen, they were fed the low-protein ration during a 10-day preliminary period followed by four successive 4-day collection periods.

The type of metabolism stalls, the manner of collecting feces and urine, the sampling of feeds and refused feeds, and the analytical procedures were the same as those used for the uniformity trials reported in Part I.

Table 1 AVERAGE DAILY AMOUNT AND PERCENTAGE COMPOSITION OF LOW-PROTEIN RATIONS EATEN BY STEERS DURING 16-DAY COLLECTION PERIOD

Items	Steer 54	55	57	61	63	66
Ration constituents, gm.						
Prairie hay	1875	1938	2220	2270	2128	2019
Dextrose	840	842	560	783	787	806
Starch	840	842	560	783	787	806
Salt	24	25	25	23	24	24
Dicalcium phosphate	24	25	25	23	24	24
Monosodium phosphate	15	15	15	15	15	15
Dry matter, %	93.92	94.39	94.39	93.89	93.84	92.58
Percentage composition of dry matter						
Organic matter	96.20	94.37	93.36	94.09	94.20	94.14
Protein (Nx6.25)	2.39	2.40	3.05	2.72	2.52	2.54
Ether extract	1.38	1.42	1.75	1.56	1.56	1.47
Crude fiber	17.25	17.93	21.78	19.39	19.31	18.39
Nitrogen-free extract	73.30	74.28	66.86	70.73	72.42	71.74

RESULTS AND DISCUSSION

Data on dry matter intake and fecal nitrogen excretion for each steer during each 4-day collection period are given in Table 2. The mean fecal nitrogen excretion per 100 gm. of dry matter intake was 0.527 ± 0.017 gm. To accept this value as the metabolic fecal nitrogen would be to assume 100 per cent true digestibility of the ration protein. Mukherjee and Kehar (1949) however have shown such an assumption to be invalid for wheat straw rations. In feeding wheat straw rations containing only 0.56 per cent and 1.05 per cent protein to Indian cattle, these investigators recovered sufficient undigested fiber in the feces to account for 8 per cent and 12 per cent, respectively, of the total fecal nitrogen. Since the prairie hay-starch-dextrose ration fed in the present experiment contained 2.59 per cent protein, it would seem logical to assume that the undigested feed protein in the feces would amount to at least 15 per cent of the total. On this basis, if a correction of 15 per cent is subtracted from the total fecal nitrogen the remainder will be the metabolic fecal nitrogen. This remainder is 0.448 gm. of nitrogen per 100 gm. of dry matter intake.

It is of interest to note that if the fecal nitrogen value of 0.530 reported by Swanson and Herman (1943) is corrected for 12 per cent undigested nitrogen it becomes 0.467, which is not significantly different from the value of 0.448 obtained in the present experiment.

Table 2. DIGESTIBILITY AND METABOLISM DATA OBTAINED WITH STEERS FED A LOW-PROTEIN RATION

Steer No.	Trial	Average steer weight	Dry matter intake	Hay in ration	Y Protein (Nx6.25)	Nitrogen intake	Dry matter digestibility	Fecal dry matter	Fecal nitrogen	Y Fecal N per 100 gm. dry matter	Adjusted Y, for Y = 2.55%
		lb.	gm.	%	%	gm.	%	gm.	gm.	gm.	
54	I		3817		2.41	14.67	64.2	1367	17.90	0.469	0.482
	II		3304		2.38	12.58	57.9	1391	17.00	0.515	0.570
	III		3264		2.44	12.70	57.3	1391	15.30	0.470	0.480
	IV		3312		2.32	11.92	58.2	1342	16.00	0.498	0.514
Mean		873	3397	55.3	2.39	12.97	59.6	1373	16.55	0.487	0.501
55	I		3112		2.31	11.49	60.2	1239	19.00	0.611	0.631
	II		3286		2.36	12.42	61.8	1256	16.10	0.490	0.505
	III		3586		2.45	14.14	52.6	1700	20.23	0.564	0.564
	IV		3935		2.49	15.70	53.3	1838	18.70	0.475	0.482
Mean		964	3480	55.2	2.41	13.44	57.0	1508	18.51	0.532	0.545
57	I		3226		3.04	15.70	59.5	1308	19.81	0.614	0.582
	II		3614		2.71	15.70	66.1	1225	19.87	0.550	0.542
	III		3434		2.86	15.70	65.8	1175	15.90	0.463	0.445
	IV		2744		3.58	15.70	86.3	1300	17.81	0.649	0.642
Mean		943	3255	67.6	3.05	15.70	63.1	1202	18.35	0.564	0.533
61	I										
	II		3144		3.15	15.70	62.8	1171	16.61	0.528	0.496
	III		3709		2.64	15.70	60.5	1465	18.24	0.492	0.469
	IV		4148		2.36	15.70	66.4	1393	17.41	0.420	0.435
Mean		893	3657	62.0	2.68	15.70	63.3	1343	17.42	0.476	0.469
63	I		3753		2.44	14.67	69.7	1139	14.30	0.386	0.395
	II		3694		2.44	14.40	51.2	1892	21.30	0.577	0.586
	III		3640		2.70	15.70	58.1	1524	18.30	0.503	0.491
	IV		3039		2.42	11.78	46.9	1615	20.80	0.684	0.696
Mean		1000	3532	60.6	2.51	14.14	57.0	1520	18.73	0.530	0.536
66	I		2712		2.44	10.58	56.3	1185	18.61	0.686	0.695
	II		3614		2.49	14.58	59.5	1464	18.61	0.515	0.521
	III		3616		2.39	14.99	67.2	1185	14.80	0.409	0.409
	IV		3732		2.63	15.70	56.1	1637	20.30	0.544	0.542
Mean		916	3419	64.0	2.54	13.96	60.0	1368	18.08	0.529	0.533
All steers all trials, mean					2.59 ± 0.06					0.527 ± 0.017	0.530 ± 0.017

The lack of information on the validity of a regression method for the determination of the metabolic fecal nitrogen of cattle has prompted further investigation. For the regression method of determination to be considered valid it should yield values which do not differ significantly from those obtained by feeding a low-protein ration and correcting for undigested feed nitrogen. To compare the value of 0.448 with that obtained by regression, data were compiled from digestion trials conducted with steers fed prairie hay rations of increasing protein content. In these trials there was a total of 87 collection periods involving 24 grade Hereford steers ranging from 10 months to 2 years of age. The composition of the rations is presented in Table 3. The nitrogen metabolism data are given in Table 4.

The values for nitrogen excretion per 100 gm. dry matter intake were plotted against the percentage protein in the ration. The results are shown in Fig. 1. The slope of the regression was found to be 0.042. Since the mean protein content of all rations was 8.40 per cent and the mean fecal nitrogen excretion per 100 gm. of dry matter intake was 0.667 gm., the regression equation is $Y = 0.314 + 0.042x$. Statistical data are given in Table 5.

In Figure 1, the line expressing fecal nitrogen excretion per 100 gm. dry matter intake intercepts the Y axis (0 per cent protein) at 0.314. At this point the standard error is 0.036. At 8.4 per cent protein, fecal nitrogen excretion per 100 gm. dry matter intake is 0.663, with a standard error of 0.008. Thus as x approaches $\bar{X}$, the mean of the protein intake, the standard error of Y decreases markedly.

Table 3 AVERAGE DAILY AMOUNT AND PERCENTAGE COMPOSITION OF RATIONS USED IN CALCULATING THE METABOLIC FECAL NITROGEN BY REGRESSION ANALYSIS

Trial	I	II	III	IV	V	VI	VII	VIII	IX	X
Ration constituents, gm.										
Prairie hay	3570	2724	5902	5902	5902	2604	2604	2604	2604	3629
Cottonseed meal		270	870	881	886	404	600	404	600	
Dextrose								1050	700	
Cane molasses										1361
Salt	30	25	30	30	30	25	25	25	25	30
Bone meal	60		20	20	20	16	14	16	14	60
Monosodium Phosphate		10				16	8	16	8	
Dicalcium phosphate		26								
Dry matter, %	94.59	94.86	94.47	94.41	94.28	95.29	95.24	93.86	94.95	78.65
Percentage composition of dry matter										
Organic matter	92.75	90.52	92.00	91.96	91.98	92.28	92.64	92.87	93.92	92.65
Protein (Nx6.25)	5.36	8.40	9.12	9.14	9.21	9.75	11.80	6.30	9.75	5.44
Ether extract	2.18	2.74	2.76	2.69	2.51	2.74	2.94	2.03	2.45	2.13
Crude fiber	32.49	30.19	28.80	28.48	29.94	30.03	28.98	22.69	23.92	27.56
N-free extract	52.72	49.19	51.32	51.65	50.64	49.76	46.92	61.85	57.82	57.52

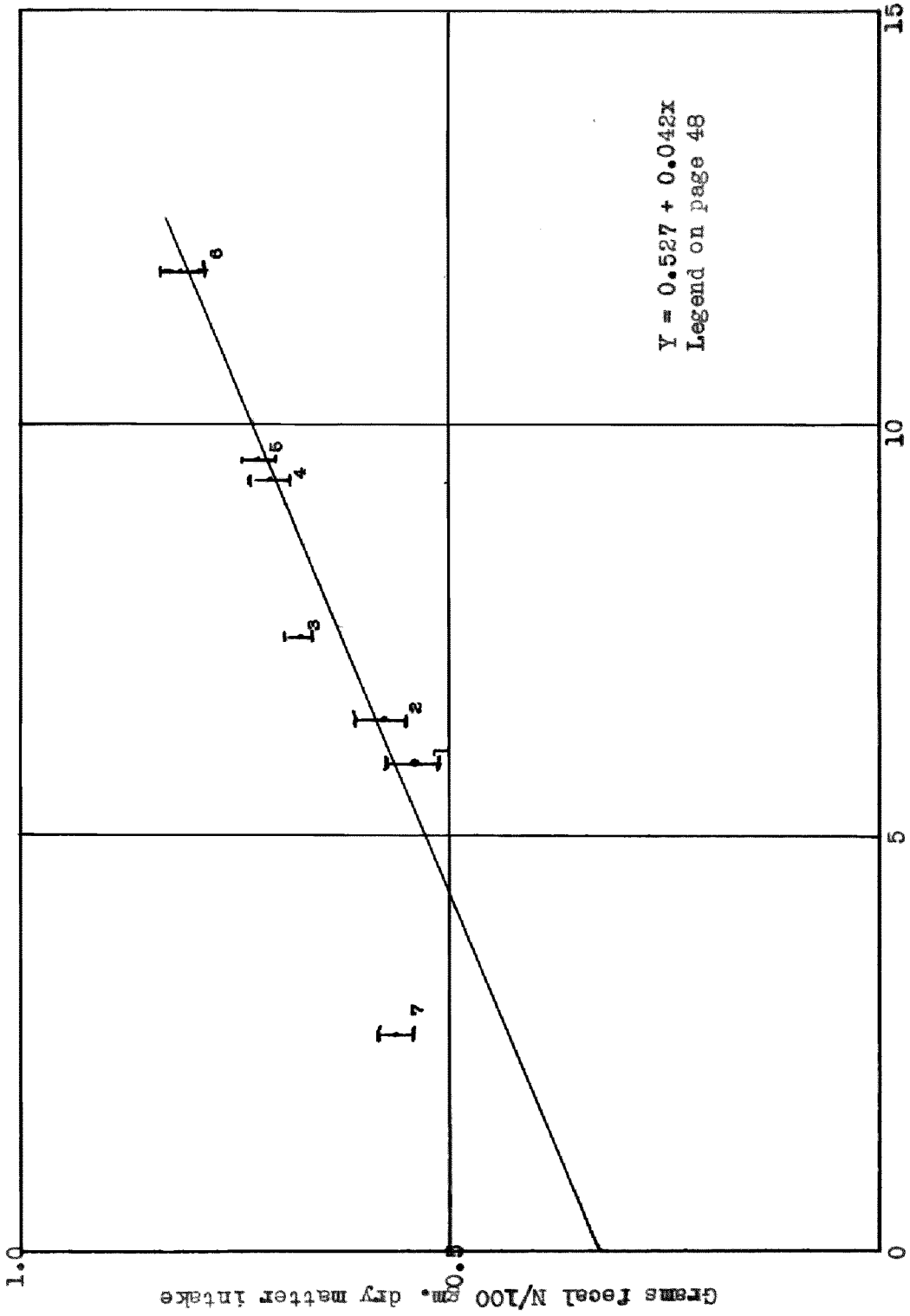
Y = 0.527 + 0.042x
Legend on page 48
Grams fecal N/100 gm. dry matter intake
Per cent protein in ration
1.0
0.5
0
5
10
15

Fig. I

LEGEND FOR FIGURE I

Point number	Data from trial number	Number of steers	Mean protein content of ration	Mean of Y fecal N per 100 gm. dry matter intake	Adjusted Y
			%	gm.	gm.
1	I and X	12	5.40	0.520 ± 0.009	0.541 ± 0.015
2	VIII	12	6.30	0.583 ± 0.050	0.579 ± 0.013
3	II	12	8.40	0.638 ± 0.012	0.667 ± 0.008
4	III, IV and V	34	9.13	0.722 ± 0.014	0.677 ± 0.009
5	VI, IX	11	9.75	0.729 ± 0.056	0.724 ± 0.011
6	VII	6	11.80	0.741 ± 0.026	[illegible] ± 0.017
7 [1]	Low-protein ration	23	2.59	0.527 ± 0.017	

1. Not included in regression analysis.

Table 4 — DIGESTIBILITY AND METABOLISM DATA USED IN CALCULATING THE METABOLIC FECAL NITROGEN OF STEERS BY REGRESSION

Items	Trial I	II	III	IV	V	VI	VII	VIII	IX	X
Number of animals	6	12	12	12	10	5	6	12	6	6
Length of trial days	10	10	5	5	5	10	10	10	10	10
Average steer weight		515	1010	970	940	530	540	515	540	
Dry matter intake, gm.	3468	2884	6423	6439	6451	2870	3096	3754	3720	3903
Protein (Nx6.25) %	5.36	8.49	9.12	9.14	9.21	9.75	11.80	6.30	9.75	5.44
Nitrogen intake, gm.	29.86	38.50	93.75	94.26	94.87	44.71	58.43	37.88	58.18	33.70
Fecal dry matter, gm.	1414	1152	2802	2740	2661	1166	1229	1622	1459	1634
Fecal nitrogen gm.	17.81	18.41	43.23	45.37	46.22	20.06	22.98	21.84	27.96	20.53
Digestibility coefficients										
Dry matter	59.25	60.1	56.4	57.4	58.3	59.4	60.3	56.8	60.8	58.1
Protein (Nx6.25)	40.35	52.2	48.6	51.9	51.3	55.1	60.6	42.4	51.9	39.1
Crude fiber	66.91	71.6	64.9	64.2	67.0	64.6	64.3	49.2	53.2	54.0
Statistical data										
SX (1)	32.17	100.76	109.45	109.71	92.19	48.73	70.82	75.64	58.74	32.64
SX^2 (2)	172.53	846.16	998.53	1003.08	849.92	475.51	836.00	476.92	575.12	178.03
SY (3)	3.08	7.65	9.00	8.46	7.19	3.30	4.45	7.00	4.52	3.15
SY^2 (4)	1.59	4.90	6.76	5.97	5.17	2.45	3.30	4.41	3.41	1.66
SXY (5)	16.51	64.25	82.11	77.37	66.26	34.11	52.54	44.14	44.26	17.11

(1) SX is the sum of the protein content of the ration fed each steer on the trial.
(2) SX^2 is the sum of the square of item (1).
(3) SY is the sum of the grams of fecal nitrogen per 100 gm. of dry matter excreted per steer daily.
(4) SY^2 is the sum of the squares of item (3).
(5) SXY is the sum of the product of the protein content of the ration fed each steer multiplied by the fecal nitrogen excretion per steer daily.

STATISTICAL DATA

THE REGRESSION OF THE FECAL NITROGEN PER 100 GM. OF DRY MATTER AS A FUNCTION OF THE PROTEIN CONTENT OF THE RATION

Table 5

Quantity	Value
Sx^2	272.66
Sy^2	11.46
Sxy	0.94
slope b	0.042
$\bar{x}$, (mean protein content of ration)	8.40
$\bar{Y}$, (mean of fecal N. per 100 gm. D. M. intake)	0.667
Intercept (metabolic fecal N)	0.314 gm. N. per 100 gm. D.M. intake
Regression equation $Y = 0.314 + 0.042x$	
$Sd^2y.x$	0.45
s^2yx	0.0033
syx	0.073
sb	0.00002, reject hypothesis that B=0.

The confidence limits of Y at a value of 2.58 per cent protein are

l_1 (Y) = 0.483 at 5 per cent level

l_2 (Y) = 0.361 at 5 per cent level

The confidence limits of Y at zero per cent protein are

l_1 (Y) = 0.387 at 5 per cent level

l_2 (Y) = 0.241 at 5 per cent level

To determine whether the values of Y obtained at zero and at 2.58 per cent protein in this regression analysis differ significantly from corresponding Y values obtained by feeding the low-protein ration, the variances were pooled and subjected to statistical analysis. The pertinent data of this analysis are given in Table 6. At zero per cent protein the difference between the mean obtained by regression and that obtained by feeding the low-protein ration and correcting for undigested nitrogen was 0.448 - 0.314 or.134. The pooled variance was 0.120 and the standard error was 0.026; hence, the difference between the means was found to be highly significant. It must be concluded that the value for metabolic fecal nitrogen obtained by regression analysis, 0.314, was significantly lower than the value of 0.448 obtained by feeding a low-protein ration and correcting for 15 per cent undigested feed nitrogen in the feces. At the 2.58 per cent level of protein intake, the difference between the means was 0.527 - 0.432 or 0.095, with a standard error of 0.026. This difference between the means was found to be significant at the 5 per cent level.

In determining the metabolic fecal nitrogen by regression analysis, the mean per cent protein of the ration remains so high that, unless it is possible to include several low-protein rations, at zero per cent intake the standard error becomes very large. In the present experiment in which the mean per cent protein of the ration was 8.40, the standard error for metabolic fecal nitrogen was twice as large as that obtained by feeding a low-protein ration and correcting for undigested feed protein.

Table 6 FECAL NITROGEN PER 100 GM. OF DRY MATTER OBTAINED BY REGRESSION ANALYSIS AND BY FEEDING LOW-PROTEIN RATIONS

	From regression Y = 0.314 + 0.042x			From low-protein ration (2.58%)						
	Y	No. of animals	Variance	Y	No. of animals	Variance	Pooled variance	Difference between means	Standard error	t .05
At zero per cent protein	0.314	87	0.0053	0.448(1)	23	0.0067	0.0120	0.134	0.026	5.15*
At 2.58 per cent protein	0.432	87	0.0053	0.527	23	0.0067	0.0120	0.095	0.026	3.65*

* Significant at 5 per cent level.
(1) Corrected for undigested nitrogen.

Although there was a significant difference between the metabolic fecal nitrogen value obtained by regression analysis and that obtained by feeding a low-protein ration and correcting for undigested dietary nitrogen, it is impossible, on the basis of available information, to ascertain which value is correct. The value obtained by the former method is lower than most published values; but, it should be noted that the assumed 15 per cent undigested feed nitrogen in the feces of steers fed low-protein ration may be much too small.

It is apparent in the digestion studies of Swanson and Herman (1943) with dairy heifers that the digestibility of crude fiber of wheat straw is markedly reduced in low-protein rations. The effect of undigested fiber is to increase the bulk of the feces and thereby increase metabolic fecal nitrogen. Further, the reduced digestibility of crude fiber has the effect of making the protein in the roughage less readily available for digestion and absorption. Such undigested protein appears in the feces. It is quite possible that the undigested protein of the prairie hay of the present experiment might exceed the conservative 15 per cent estimated on the basis of the report by Mukherjee and Kehar. If the quantity of undigested feed protein comprised as much as 30 per cent of the total fecal nitrogen excretion on the low-protein ration, then correcting for this quantity would yield a metabolic fecal nitrogen value which is not significantly different from that obtained by regression analysis. It would appear advisable, however, to defer decision as to the method of determining the metabolic fecal nitrogen until additional information is available on the true digestibility of protein of low-protein high-roughage rations.

SUMMARY

The total fecal nitrogen excretion of two-year-old steers fed a prairie hay-starch-dextrose ration containing 2.58 per cent protein was 0.527 ± 0.017 gm. per 100 gm. of dry matter intake. To obtain a measure of the metabolic nitrogen, the total fecal nitrogen was corrected for its content of dietary nitrogen on the assumption that 15 per cent of the nitrogen in the hay was undigested. The resulting value was 0.448.

Regression analysis of fecal nitrogen per 100 gm. dry matter intake of steers fed high-roughage rations containing increasing amounts of protein (5.35 to 11.80 per cent) was found to yield values of zero and 2.58 per cent protein which were significantly lower than those obtained by low-protein feeding. The uncertainty associated with the extent of undigested fecal nitrogen in the feces of steers makes it impossible to state which method yields the more reliable value for metabolic fecal nitrogen.

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Doctor of Philosophy

Thesis: STUDIES ON DIGESTION AND NITROGEN METABOLISM OF BEEF CATTLE
PART I. DIGESTION AND NITROGEN RETENTION DATA OBTAINED WITH STEERS IN UNIFORMITY TRIALS
PART II. THE DETERMINATION OF THE METABOLIC FECAL NITROGEN OF STEERS

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