Reprinted from JOURNAL OF DAIRY SCIENCE, March, 1957, Vol. XL, No. 3, pages 240-251.

EFFECTS OF VARIOUS HAY:CONCENTRATE RATIOS ON NUTRIENT UTILIZATION AND PRODUCTION RESPONSES OF DAIRY COWS. II. OBSERVATIONS ON RATION DIGESTIBILITY AND ON THE EXCRETION PATTERN OF CHROMIC OXIDE[1]

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A number of investigators *(6, 12, 30)* have reported that efficiency of digestion does not vary greatly among cattle. However, Jarl *(17)* reported slight variation in the digestive powers of cows (3.5% difference between the most and least efficient cows) and Baker *et al. (3)* reported that beef steers which used the least feed per pound of gain seemed to be superior in their digestion of crude fiber.

This phase of the current study was designed primarily to determine digestibility values for the various rations described previously *(4)* and, thereby, to provide further information on the relationship between ration digestibility and efficiency of milk production. The large number of cows involved in this study and the facilities available precluded total fecal collections; therefore, the chromic oxide indicator method was employed. A number of reports *(7, 13, 14, 20, 27)* have provided data that stress the importance of collecting fecal grab samples at specified times of the day, if reliable estimates of ration digestibility, or of total fecal output in the case of grazing animals, are to be obtained. The secondary objective of this work was to study the fecal excretion pattern of chromic oxide under the wide range of dietaries imposed.

EXPERIMENTAL PROCEDURE

A digestion trial was run on each of 36 cows, described in the experimental design in the preceding paper *(4)*, approximately four to five months after

Received for publication August 8, 1956.

[1] Journal Paper No. J-3016, Iowa Agricultural Experiment Station, Ames. Project No. 1195.

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each cow started the experimental period. Since the cows entered the experiment over a wide time interval, they were placed in eight convenient groups varying in size from three to seven cows each. Digestion trials for animals within each group were run simultaneously. During the digestion trial, the daily feed allowance, which had been determined at the start of the trial, was continued at a constant rate for a 10-day preliminary and a three-day collection period. When necessary to insure complete consumption, high hay or high concentrate rations were adjusted downward in amounts to maintain the particular hay-to-concentrate ratio. The regular weekly adjustments in feeding implicit in the experimental design were discontinued during these trials, and necessary adjustments in feeding to conform to the regular feeding schedule were made at the end of the digestion trial.

During the three-day collection period, nine fecal samples were obtained from each cow, usually by stimulation of defecation, according to the following schedule: first and third days—9 A.M. and 5 P.M.; second day—5 A.M., 9 A.M., 1 P.M., 5 P.M., and 9 P.M. Three 250-g. portions of each sample were immediately and directly weighed into polyethylene plastic bags, sealed, packed in cardboard containers, and placed in a freezer. All samples were stored in the frozen state until analyzed.

Chromic oxide, the digestibility indicator employed, was prepared for feeding according to the procedure of Kane *et al. (19)*, except that leavening was added before baking, drying, and subsequent grinding of the material. The chromic oxide–flour mixture was incorporated into a premix with the concentrate mixture. The premix was so formulated that each pound contained approximately 15 g. of chromic oxide. Before each digestion trial and for each cow, 13 one-pound portions of the chromic oxide–flour-concentrate premix were weighed into paper bags. During the digestion trial, one bag of the premix was incorporated into each total daily concentrate allowance, from which one pound had been deducted to allow for the pound of premix. This mixture was fed in four equal portions at 7 A.M., 4 P.M., 7 P.M., and 4 A.M.

During each trial one-per cent aliquots of the total hay and concentrates fed each day for the last six days of the 13-day digestion trial were composited and sampled. Proximate analyses of the feeds and feces were conducted as described by the A.O.A.C. *(2)*, except that a Goldfisch Extractor was used for estimation of ether extracts. Chromic oxide in the feces was determined by the colorimetric method described by Schürch *et al. (26)*. Data on the chemical composition of the feeds used and the cows involved in each of the eight digestion trials are given (Table 1).

RESULTS

Results of the digestion study are shown (Table 2), where the data have been summarized as follows: Each entry is the average of the analyses of the 27 fecal samples, nine for each of three cows at each feeding level and at each ability group. These data are presented for each hay-to-concentrate feeding

TABLE 1
Proximate analysis of the feeds in each digestion trial

Sample[a]	Nutrients					
	Prot.	E.E.	C.F.	Ash	Moist.	N.F.E.
	%					
Concentrates						
I	15.8	2.9	6.5	5.2	11.0	58.6
II	18.8	2.5	8.8	8.2	12.8	48.9
III	17.2	2.2	9.0	7.1	10.7	53.8
IV	20.8	1.8	8.6	6.8	12.5	49.5
V	20.4	2.9	8.7	5.8	11.3	50.9
VI	18.5	3.0	9.9	5.6	10.9	52.1
VII	20.8	2.5	7.9	6.2	10.6	52.0
VIII	19.7	2.7	9.1	4.9	11.8	51.8
Hay						
I	11.6	1.3	33.6	7.9	7.6	38.0
II	14.2	2.0	22.9	7.6	10.4	42.9
III	14.8	2.1	33.7	8.2	9.5	31.7
IV	12.1	2.0	36.5	7.8	10.9	30.7
V	11.8	1.7	36.6	7.3	11.8	30.8
VI	12.2	1.6	40.8	7.8	10.2	27.4
VII	10.2	1.5	43.1	7.0	10.6	27.6
VIII	13.3	1.6	37.8	7.1	11.2	29.0

[a] Cow groups corresponding to the various samples were as follows: I–3529, 3516, 2710, 3538, 3266, 2378; II–2553, 2392, 2159; III–2643, 3302, 3440; IV–3160, 3128, 2600; V–3444, 3469, 3432, 3174, 3263; VI–2982, 3294, 3157, 2963, 3450; VII–3291, 2606, 3439, 2976; VIII–3142, 3483, 3493, 3597, 3632, 2649, 3272.

ratio. The average for ration is that of all 81 samples from the nine cows on each ration. Table 3 presents the final combination of the data on the basis of the 12 cows at each feeding level and at each ability level, and then presents the over-all digestibility of each feed constituent for the 36 cows.

An analysis of variance was computed for the data on digestibility and on the fecal excretion pattern of the chromic oxide. Analysis of each feed constituent (Table 4), brought within its scope five sources of variance in the data, namely, ration, level of feeding, ability, day of collection, and time of day at which the collection was made. Only the fecal collections made at 9 A.M. and 5 P.M. for each of the three days of the digestion trial were used in these analyses. The estimate of error for tests of significance in each analysis of variance is the weighted average of the interactions with days. For the few times when an individual fecal sample could not be obtained, values for the missing data were calculated according to the method of Snedecor *(28)*.

Of the three major factors, ration, level of feeding, and ability, the widely varying rations had the greatest effect upon dry matter digestibility (Table 4). From the high to the low hay rations, digestibility coefficients were, respectively, 54.5, 58.5, 55.2, and 63.1%. The nine cows at the 35:65 hay-to-concentrate ratio showed a lowering of dry matter digestibility against the general rise, as the proportion of hay in the rations was decreased. Level of feeding and ability exerted no appreciable influence.

Digestibility of protein ($N \times 6.25$) generally followed a pattern similar to that for dry matter, across the four hay-to-concentrate ratios, and showed

TABLE 2

Digestion coefficients averaged as to level of feeding, producing ability, and ration[a]

Hay-to-conc. ratio	Level of feeding			Ability			Av. for ration
	High	Medium	Low	High	Medium	Low	
	%						
H-75:C-25							
D.M.	54.5	51.4	57.7	53.2	55.5	54.8	54.5
Prot.	57.8	50.7	63.5	54.5	56.6	60.9	57.3
E.E.	11.9	9.3	15.3	12.5	5.2	18.8	12.2
C.F.	23.5	35.7	45.5	28.7	36.7	39.3	34.9
N.F.E.	65.4	58.9	61.1	63.3	61.4	60.7	61.8
H-55:C-45							
D.M.	57.5	61.6	56.3	57.1	59.7	58.7	58.5
Prot.	63.9	66.6	63.9	61.4	67.6	65.4	64.8
E.E.	31.0	45.8	23.4	26.5	38.6	35.2	33.4
C.F.	37.2	46.4	40.8	43.6	40.8	40.1	41.5
N.F.E.	61.4	65.9	60.3	60.3	63.7	63.6	62.5
H-35:C-65							
D.M.	58.3	55.9	51.5	58.8	55.4	51.4	55.2
Prot.	58.2	59.9	62.3	60.6	60.1	59.8	60.2
E.E.	37.5	35.0	31.4	38.3	34.7	30.8	34.6
C.F.	31.0	24.1	26.0	34.1	25.6	21.4	27.0
N.F.E.	67.6	65.2	55.5	67.0	63.3	58.0	62.8
H-15:C-85							
D.M.	60.2	62.1	67.1	63.8	61.8	63.8	63.1
Prot.	69.3	72.8	75.6	74.0	76.5	67.2	72.6
E.E.	49.8	54.6	58.8	60.7	48.5	54.0	54.4
C.F.	19.9	13.6	28.7	28.4	16.9	16.8	20.7
N.F.E.	65.3	68.8	72.4	67.5	66.2	72.8	68.8

[a] Each of the levels of feeding and producing ability coefficients is an average of three cows; each ration coefficient is an average of nine cows.

a similar depression in digestibility at the 35:65 ratio. Differences in protein digestibility due to levels of feeding and of ability were not significant.

Average digestion coefficients for ether extract increased from 12.2% on the high ration to 33.4, 34.6, and 54.4%, respectively, as the hay in the ration decreased. As with the results presented to this point, the fairly orderly increase in ether extract digestibility was interrupted at the 35:65 hay-to-concentrate ratio, where it remained at about the same level as at the 55:45 feeding ratio. As was observed for dry matter and protein, levels of feeding and of ability had no significant effect upon ether extract digestibility.

TABLE 3

Summary of mean digestibility coefficients

Level of feeding	Ability	D.M.	Prot.	E.E.	C.F.	N.F.E.
		%				
High		57.6	62.3	32.6	27.9	64.9
Medium		57.8	62.5	36.2	30.0	64.7
Low		58.2	66.4	32.2	35.3	62.3
	High	58.2	62.6	34.5	33.7	64.5
	Medium	58.1	65.2	31.7	30.0	63.7
	Low	57.2	63.3	34.7	29.4	63.8
Over-all average		57.8	63.7	33.6	31.0	64.0

TABLE 4
Analysis of variance for digestibility data

Source of variation	Degrees of freedom	Mean squares				
		D.M.	Prot.	E.E.	C.F.	N.F.E.
Ration (R)	3	816.84[a]	2425.37[b]	15288.21[b]	4332.84[a]	636.94
Level (L)	2	9.21	228.96	610.30	709.13	240.40
Ability (A)	2	9.77	59.08	590.44	420.92	32.09
RL	6	344.51	326.50	1131.46	1127.48	554.87
Error (a)	22	194.69	310.69	652.52	997.47	272.39
Days (D)	2	88.38	20.21	287.21	462.78[a]	48.31
Time of day (T)	1	1311.18[b]	1227.70[b]	4606.60[b]	4310.87[b]	673.14[b]
TR	3	17.79	64.43	8.80	157.78	35.63
TL	2	41.38	45.28	145.02	33.79	100.50
TA	2	42.15	19.66	9.59	120.05	67.83
TRL	6	36.65	42.17	178.48	107.89	45.81
TRA	6	8.75	12.37	145.17	40.18	22.62
TLA	4	12.74	11.34	19.63	103.71	19.45
RLAT	12	39.12	22.22	118.11	175.34	59.97
Error (b)	142	34.45	24.27	185.62	121.68	33.75
Total	215					

[a] $P < 0.05$.
[b] $P < 0.01$.

Effect of ration upon crude fiber digestibility was significant ($P < 0.05$) and the values from the high to the low hay rations were, respectively, 34.9, 41.5, 27.0, and 20.7%. Although the effect of level of feeding was not significant, there was a trend for greater crude fiber digestibility by the cows at the low level of feeding. Effect of ability upon crude fiber digestion was not significant, but at three of the four hay-to-concentrate ratios (Table 2) animals of highest ability had the highest crude fiber digestibility coefficients, with a reversal of this pattern occurring at the highest hay ration.

A plausible explanation for the above reversal at the highest hay ration may be as follows: The three animals of low ability on the high hay ration generally consumed smaller total amounts of hay than the high- and medium-ability animals on the same ratio of feeds. The greatest total consumption of hay seemed related to a lowered crude fiber digestibility. Possibly this condition may have been due to the more rapid passage of feed through the digestive tract and, consequently, to the shorter time for bacterial action in the rumen. This relationship of high hay intake and lowered crude fiber digestibility was supported by cows No. 2553 and No. 2392 (high- and medium-ability cows, respectively, at the high level of feeding). These cows consumed the greatest amounts of hay during the digestibility trial and had the lowest crude fiber digestion coefficients of any of the nine cows on this ration.

From the high hay to the low hay rations, the nitrogen-free extract digestion coefficients were, respectively, 61.8, 62.5, 62.8, and 68.8%. Effects of ration, level of feeding, and ability were nonsignificant. Only at the highest concentrate ratio (15:85), in which the greatest proportion of the nutrients was in the form of NFE, did this fraction increase in its digestibility percentage from the high to the low level of feeding. The 35:65 hay-to-concentrate ratio interrupted the generally ascending NFE values from the high to the low hay rations.

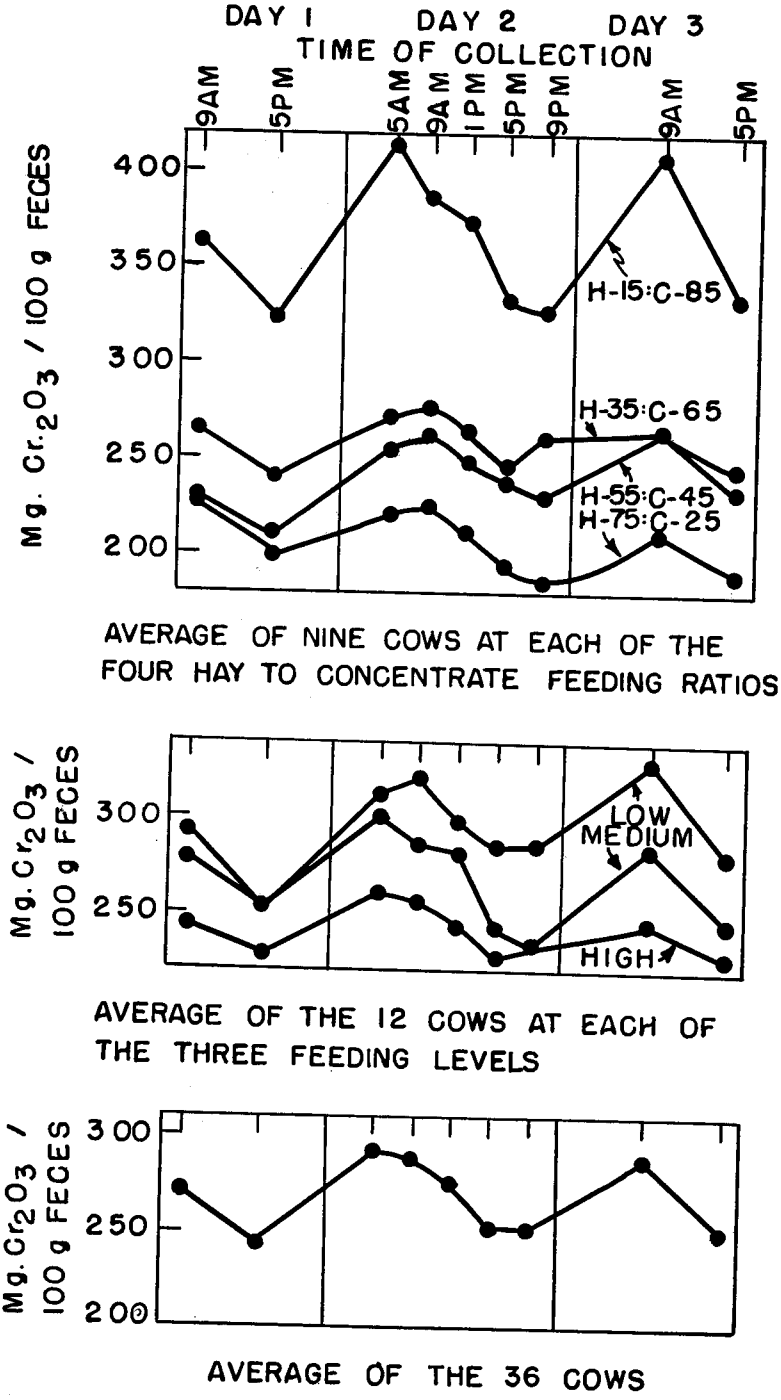

FIG. 1. Summary of fecal excretion patterns of chromic oxide over the three-day collection period.

This ratio had an NFE digestibility value almost equal to that of the 55:45 ratio, although the total amount of the components considered in the NFE fraction had increased considerably, in moving from a ratio of 2 pounds of hay to one pound of concentrate (55:45) to a ratio of one pound of hay to one pound of concentrate (35:65).

Patterns of fecal excretion of chromic oxide (shown Figure 1) are presented successively on the basis of the average curves for each of the four hay-to-concentrate ratios, those for each of the three feeding levels, and finally, that for all 36 cows in this study. For more than two-thirds of the cows, the maximum excretion of chromic oxide was observed in samples at 5 A.M. or 9 A.M.; whereas, the minimum excretion was observed in samples at 5 P.M. or 9 P.M. The orderly decrease in excretion from 5 A.M. to 9 P.M. is shown by the descending curve during the second day of the trial, when more frequent collections were made. Digestibility data for each feed constituent used in the statistical analyses were functions of the amount of chromic oxide present in the feeds and, subsequently, in the feces. Analyses showed that the effect of collection time upon digestibility of the nutrients was significant ($P < 0.01$).

The excretion pattern of chromic oxide was quite consistent from day to day, especially when all animals are considered (Figure 1). This was substantiated by the statistical analyses in which, for example, the mean square for the effect of the day of collection (D) upon dry matter digestibility was one-fifteenth as great as that for the time of collection. This indicated that the chromic oxide had been fed in a uniform manner, that it had been thoroughly distributed in the ingesta and that, over the collection period, it had been excreted uniformly from day to day.

The variability in chromic oxide excretion increased from the high to the low hay rations and from the high to the low levels of feeding (Figure 1). Thus, there appears to be a definite relationship between the amount and type of gastrointestinal fill and the degree of variability found in the chromic oxide content of individual collections. Despite this increasing variability among individual collections, under the above cited conditions, the typical daily excretion pattern was apparent.

DISCUSSION

These data indicated that neither the ability (genetic effect) nor the feeding level exerted important influences upon the powers of digestion. Thus, the observed differences in milk production among the cows of different producing abilities on similar rations, as previously reported *(4)*, can not be explained on the basis of differences in digestibility. A possible explanation of these results was found in the work of Armsby *(1)* and Mumford *et al. (25)*, who showed that, although a probable error of three to four per cent was common in the determination of apparent digestibility, there were cases in which the variation became as high as eight to nine per cent among cattle receiving the same test ration. In this study, samples were taken for a comparatively short period,

and if more frequent collections had been made, the differences might have been much smaller for cows of different abilities on identical rations. Nevertheless, the average results in this work revealed a total difference in dry matter digestibility of but one per cent in favor of the high-ability cows and of but one-half of one per cent in favor of the cows at the low level of feeding (Table 3).

Other studies approaching the pattern of this report, though with different experimental designs and other goals, support the current findings. Hansson *(12)*, in digestibility studies with monozygous cattle twins fed at four levels, found that the total difference in organic matter digestibility over these levels was one per cent in favor of the high-fed twins and that there were no larger differences between individual twin pairs. Watson *(30)*, in a summarization of 15 years of experimentation with various feeding levels, also stated that the levels had no effect upon digestibility or, where differences existed, they were of small magnitude. Hansson *(12)* also indicated that heredity did not markedly affect the animals' capacity to digest the feed.

Brumby and Hancock *(5)*, in a recent study of the galactopoietic role of growth hormone in dairy cattle, found that when daily subcutaneous injections of growth hormone were administered to identical-twin cattle there was a marked increase in milk and butterfat production, with an apparent increase in the efficiency of production. Digestibility studies yielded almost identical digestion coefficients for both the treated and untreated twins, which eliminated this possible explanation of the observed increase in efficiency of production. Baker *et al. (3)* found wide differences in the efficiency of feed utilization in beef cattle, but small differences in their efficiencies of digestion. The current work and the above supporting evidence lead to the postulation that differences among animals exist in the efficiency of utilization of nutrients after absorption, but that differences in digestion and absorption of nutrients probably are of lesser magnitude.

The only indication, although not statistically significant, of the relationship of animal ability to the digestion of nutrients in these data was observed with crude fiber (Table 3). Baker *et al. (3)* demonstrated a similar superiority in crude fiber digestion in beef steers of high feed efficiency. These workers reported a total range of difference of 4.9% between steers of low and high efficiency, which compared closely with the 4.3% difference in crude fiber digestion between low- and high-ability cows in the present work. This indicates that in digestibility studies, crude fiber digestion may be a factor related to productive efficiency. Further study is indicated, in order to elucidate this relationship.

While the total energy fed was held constant at each hay-to-grain ratio, the dry matter digestibility increased from high to the low hay rations with an interruption of this trend at the 35:65 hay-to-concentrate ratio, in which the two feeds were offered in approximately equal proportions by weight. This depression of dry matter digestibility perhaps may best be explained by combining the results of the literature, relative to proportions of nutrients in the

ruminant ration and to bacteriological studies on various combinations of hay and readily available carbohydrates. Addition of starch, sugar, and similar substances may cause a depression in the digestibility of the materials with which they are fed *(11, 23, 29)*.

Swift *et al. (29)* and Hamilton *(11)* related the decreased digestibility to the preference of the rumen microorganisms for the simpler carbohydrates. In digestion studies with sheep, wherein the proportions of hay and concentrates approached most closely the 35:65 hay-to-concentrate ratio in this study, Lindsey and Smith *(21)* reported an average decrease in ration digestibility of eight per cent, and Louw and van der Wath *(22)* observed a nine per cent decrease in cellulose digestion when maize was added in nearly equal proportions to a poor veld hay. Bacteriological studies by these workers *(22)* showed that small amounts of maize and meatmeal added to veld hay favored the growth of rumen organisms; whereas, almost equal amounts of hay and maize tended to reduce the number of organisms in the ruminant. Gall *et al. (9)* found, with cattle on practical farm rations, that a high grain ration (proportions of hay and grain not indicated) stimulated an increase in types of organisms already present in moderate numbers in the rumen, rather than encouraged development of different types of bacteria. Later work by Gall *et al. (10)*, employing sheep on purified rations which closely approached the 15:85 hay-to-concentrate ration in this work, showed that cultures of rumen flora consisted almost entirely of fast-growing organisms. Tests for the presence of slower-growing organisms, usually associated with cellulose digestion, were largely negative.

In applying these previous observations to the results of the present study, it can be postulated that on the high concentrate ration (15:85) the microorganisms had adjusted their types to the great predominance of available carbohydrates and that the 55:45 ratio (approximately two pounds of hay to one pound of concentrates) stimulated development of microorganisms capable of greater roughage use. The 35:65 hay-to-concentrate ratio, however, may have represented an unfavorable zone of nutrient combination for satisfactory microorganism activity upon, and perhaps adjustment to, available substrates. At this ratio the microorganisms were presented with sufficient available carbohydrates to decrease their attack upon the roughage in this ration, but with an insufficient amount to achieve their complete adjustment to the high concentrate type of ration.

The present work and the studies of Kane *et al. (20)* and Edin *(7)* have yielded daily chromic oxide fecal excretion curves remarkably similar in nature, despite differences in the mode of chromic oxide administration, time required to consume the ration containing the indicator, time intervals between feedings, length of the preliminary feeding period of the indicator, and the time span over which the trials were conducted. Hardison *et al. (13)* also observed a similar diurnal pattern when the chromic oxide was administered once daily by capsule. However, when the marker was given twice daily, the diurnal

variation was markedly reduced and the diurnal pattern was altered. On the other hand, an appreciable diurnal variation was evident in the present study, even though the marker was fed four times daily (in the concentrate mixture). In work with goats, Kameoka *et al. (18)* observed only one distinct peak in chromic oxide excretion when the marker was fed at 9 A.M. and 4 P.M.; whereas, two peaks were noted when feedings were at 12-hour intervals. These observations were at more frequent intervals than those in the present study, which may account, at least in part, for the apparent difference in chromic oxide excretion patterns.

Ewing and Smith *(8)* and Mumford *(24)* reported that the rate of feed passage became more rapid as the level of feeding was increased, and that the smaller and finely ground feeds passed through the animal more quickly than larger and more coarse feeds. The above data suggest that in the present experiment there were variations in the rate of passage of feed, owing to the type of feed and to the interaction of the levels of feeding and diverse physical natures of the rations fed. Despite these added variables, the observed maxima and minima of chromic oxide excretion occurred at similar time intervals.

Observations by Edin *(7)* suggested a correlation between the time of the greatest amounts of rumination and the subsequent maximum excretion of chromic oxide. In the current work, however, cows with depressed rumination on the high concentrate rations exhibited the same general time of the observed maximal excretion as those on the high hay rations. It is possible that the periodicity in the chromic oxide excretion pattern is difficult to alter. Some support for this hypothesis may be gained from the observations of Herring and Brody *(16)*, who found that the diurnal rhythm associated with the heat increment in feeding (SDA) in the rat could be extinguished only after a week of continuous feeding (one-eighth of the daily ration every three hours) and continuous light; whereas other measures, used singly or in combination, failed to disturb this diurnal rhythm.

Considering the many metabolic activities subject to a basic diurnal rhythmicity, these and the earlier observations on the nature of the chromic oxide excretion pattern suggested the following tentative explanation:

Although digestion in the ruminant is a continuous process, the absorption of nutrients may proceed at varying rates over a daily period. Such differential rates in absorption of the gut contents would then be reflected in the varying quantities of chromic oxide excreted over a daily period, in relation to the remaining residue in the tract. During periods of high absorptive activity, the amount of chromic oxide would increase in relation to the smaller relative amount of ingesta remaining in the tract, the reverse being the case during a period of low absorptive activity. This proposal, though tenuous, receives some credence from a report which presented evidence of diurnal rhythmicity in the digestive tract. In a study of the production of gastric juice in the human fasting stomach, Hellebrandt et al. *(15)* found that although secretion was continuous throughout a 24-hour period, the total acidity of gastric juice liberated spontaneously during sleep at night was higher than that produced in the daytime.

The bulk of evidence indicates that diurnal variation or a "periodicity" in the excretion of chromic oxide is to be anticipated when this indicator substance is used in digestibility trials. The indiscriminate adoption of time (s) for obtaining fecal samples is precluded in this type of investigation, and it is of interest that Edin *(7)* made this observation in the first reported use of this indicator with dairy cattle. This early work suggested, as have a number of more recent reports, that fecal samples should be taken at 12-hour intervals, or at those hours when the variation of the chromic oxide in the feces averages to a mean value for the daily period.

SUMMARY

A digestibility study made with the chromic oxide ratio technique was conducted with 36 dairy cows. Four ratios of hay to concentrates (replacement on an Estimated Net Energy basis), three feeding levels, and three ability levels were employed. The nature of the fecal excretion pattern of chromic oxide, under the wide range of conditions imposed by the experimental design, also was studied.

From the high to the low hay rations, the dry matter digestibilities were, respectively, 54.5, 58.5, 55.2, and 63.1%. Dry matter digestibilities for high, medium, and low ability cows were 58.2, 58.1, and 57.2%, respectively, and for high, medium, and low feeding levels were 57.6, 57.8, and 58.2%. Digestibility values for the individual nutrients also are reported.

In this study, the fecal excretion of chromic oxide followed a diurnal pattern. Maximum excretion of chromic oxide was observed in samples taken at 5 A. M. or 9 A. M. and the minimum excretion was observed in samples at 5 P. M. or 9 P. M. This diurnal excretion pattern was largely independent of the wide variation in the physical nature of the rations and of the feeding level.

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