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THE INFLUENCE OF SUPPLEMENTED PROTEIN AND  
ENERGY ON INTAKE AND DIGESTIBILITY OF  
WINTER RANGE FORAGE.

The University of Nebraska, Ph.D., 1969  
Agriculture, animal culture

University Microfilms, Inc., Ann Arbor, Michigan

THE INFLUENCE OF SUPPLEMENTED PROTEIN  
AND ENERGY ON INTAKE  
AND DIGESTIBILITY OF WINTER RANGE FORAGE

by  
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A THESIS

Presented to the Faculty of  
The Graduate College in the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Doctor of Philosophy  
Department of Animal Science

Under the Supervision of Dr. Donald C. Clanton

Lincoln, Nebraska

January, 1969

**TITLE**

THE INFLUENCE OF SUPPLEMENTED PROTEIN AND ENERGY ON INTAKE

AND DIGESTIBILITY OF WINTER RANGE FORAGE

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## ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Donald C. Clanton for his counsel and advise while conducting the study and preparing the manuscript and to Drs. Donald F. Burzlaff and Terry Klopfenstein for editing the manuscript. The author also recognizes David Haskell for his assistance in collection of the field data. Special appreciation is expressed to my wife, Leona, for her patience and sacrifice during the pursuit of this degree and for typing the manuscript.

## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	ii
TABLE OF CONTENTS.....	iii
LIST OF FIGURES.....	v
LIST OF TABLES.....	vii
LIST OF APPENDIX TABLES.....	x
INTRODUCTION.....	1
LITERATURE REVIEW.....	3
The use of esophageal fistulae.....	3
Indicator techniques.....	3
Chromium oxide.....	3
Form administered.....	4
Time of administration of $\text{Cr}_2\text{O}_3$ and sampling of feces.....	7
Nature of feed and feeding regimen.....	8
Lignin.....	9
Factors Influencing Feed Intake and Utilization	11
PROCEDURE.....	22
Experiment I.....	22
Site and animals.....	22
Experimental design.....	22
Experiment II.....	23
Site and animals.....	23
Experimental design.....	26
Feeding regimen and supplements.....	29
Sampling diet and feces.....	29
Preparation of samples.....	32
Chemical analysis.....	32
Expression of date.....	33
RESULTS AND DISCUSSION	
Experiment I.....	34
Experiment II.....	41
Intake of protein and energy.....	41
Sampling feces for $\text{Cr}_2\text{O}_3$ and lignin.....	41

	page
Qualitative intake.....	52
Cell wall constituents.....	52
Lignin.....	55
Nitrogen.....	55
Quantitative intake.....	59
Dry matter digestibility of forage.....	67
Dry matter digestibility of diet.....	72
Energy digestibility of diet.....	74
Meeting the energy requirement of the animal	79
Meeting the protein requirement of the animal.....	83
Expressing protein data.....	90
Laboratory techniques for evaluating winter range forage.....	91
SUMMARY AND CONCLUSIONS.....	95
LITERATURE CITED.....	99
APPENDIX TABLES.....	108

## LIST OF FIGURES

Figure	Page
1. Factors limiting the removal of organic matter from the rumen.....	15
2. Estimates of fecal production using three different methods.....	36
3. The influence of varying levels of supplemented crude protein on cell wall constituents of dietary samples collected via esophageal fistulae.....	53
4. The influence of varying levels of supplemented energy on cell wall constituents of dietary samples collected via esophageal fistulae.....	56
5. The influence of varying levels of supplemented crude protein and energy on lignin content of dietary samples collected via esophageal fistulae.....	57
6. The influence of varying levels of supplemented crude protein on nitrogen content of dietary samples collected via esophageal fistulae.....	58—
7. The influence of varying levels of supplemented energy on nitrogen content of dietary samples collected via esophageal fistulae.....	60
8. The influence of varying levels of supplemented crude protein or energy on voluntary intake of cattle grazing winter range.....	64
9. The influence of varying levels of supplemented crude protein or energy on dry matter digestibility of forage ingested by grazing cattle on winter range.....	69
10. The influence of lignification of acid detergent fiber on dry matter digestibility.....	71

## List of Figures (Continued)

Figure		Page
11.	The influence of varying levels of supplemented crude protein or energy on dry matter digestibility of the diet of cattle grazing winter range.....	75
12.	The relationship between the organic matter digestibility of the diet and energy digestibility of the diet or megcal. digestible energy per kg. organic matter.....	77
13.	Relationship between crude protein and digestible crude protein in the diet.....	92



# LIST OF TABLES

Table	Page
1. Climatic measurements for selected dates during the winter of 1967-68.....	25
2. Schedule of dates composing the four periods (1967-68).....	28
3. Nature and calculated amount of supplement fed.	30
4. Composition of supplements used.....	31
5. Fecal production, voluntary intake and dry matter and nitrogen digestibility determined from different methods of estimating fecal production.....	35
6. Daily recovery of chromium oxide, %.....	37
7. Estimates of the minimum number of animals required to limit the 95% confidence interval to $\pm 10\%$ of the trial mean, where $d = 5$ .....	40
8. Actual protein content of the supplements fed as compared to that calculated; adjusted for changes in dry matter.....	42
9. Calculated protein actually fed daily, corrected to a 454 kg. animal.....	43
10. The actual amount of estimated digestible energy supplied by the various supplements, corrected to a 454 kg. animal.....	44
11. Regression statistics for the estimation of fecal production from morning, evening or an average of morning and evening grab samples compared to measured total fecal production, kg.	45
12. Regression statistics for the estimation of lignin in the feces from grab samples as compared with lignin content of a composite sample from a 24 hr. total fecal collection, kg...	47
13. Estimates of the indigestibility of supplements using Van Soest's summative equation.....	48

# List of Tables (Continued)

Table	Page
14. Example calculation of forage dry matter intake and digestibility, using a 336 kg. animal receiving supplement No. 13.....	49
15. Example calculation of forage organic matter intake and digestibility, using a 336 kg. animal receiving supplement No. 13.....	50
16. Regression statistics used in adjusting dry matter and organic matter intake and indigestibility calculated from estimates of fecal production using $\text{Cr}_2\text{O}_3$ , to estimates of fecal production from total collections, kg.....	51
17. Qualitative intake of cattle grazing winter range supplemented with protein and energy (ash-free basis).....	54
18. Forage dry matter and organic matter intake of cattle grazing winter range supplemented with varying levels of protein and energy, gm./kg. metabolic weight.....	61
19. Protein content of the diet of cattle grazing winter range supplemented with varying levels of protein and energy.....	62
20. Voluntary intake predicted from Elliott's (1967a) Multiple Regression equation and measured under range conditions.....	66
21. The dry matter and organic matter digestibility of forage consumed by cattle grazing winter range supplemented with varying levels of protein and energy.....	68
22. The dry matter and organic matter digestibility of the diet of cattle grazing winter range supplemented with varying levels of protein and energy.....	73
23. Digestible energy of the diet of cattle grazing winter range supplemented with varying levels of protein and energy.....	78
24. The ability of winter range supplemented with varying levels of protein and energy to meet the energy requirements of animals for maintenance and growth.....	80

## List of Tables (Continued)

Table	Page
25. Adjusted means of digestible protein intake.....	85
26. Adjusted means of protein digestibility.....	87
27. Adjusted means of fecal nitrogen content.....	89

# LIST OF APPENDIX TABLES

Table		Page
1.	Least squares analysis of variance of fecal production and intake expressed as kg. and gm./kg. metabolic weight, respectively.....	109
2.	Analysis of variance: Lignin content of composite compared to grab samples of feces....	110
3.	Analysis of variance: Qualitative intake.....	111
4.	Analysis of variance: Dry matter and organic matter intake.....	112
5.	Analysis of variance: Dry matter and organic matter digestibility of forage.....	113
6.	Analysis of variance: Dry matter and organic matter digestibility of the diet.....	114
7.	Analysis of variance: Digestible energy in the diet.....	115
8.	Analysis of variance: Digestible protein intake, nitrogen digestibility and fecal nitrogen content.....	116

## INTRODUCTION

Exploiting the natural resources of native range land is often most efficiently accomplished by converting the forage to human food by grazing ruminant animals. Efficient conversion of this natural resource into edible meat products depends on man's ability to supplement deficient nutrients in the forage to allow for optimum performance by the animal.

Supplementing native winter range forage with protein and energy in accordance with the age and stage of production of the animal, is a common practice. However, there is some question as to the amount of protein and energy that a supplement should provide.

Research at the University of Nebraska North Platte Station and U.S.D.A. Beef Cattle Research Station at Fort Robinson would indicate that this is a complex problem involving the interrelationships between the amount of supplemented protein and the amount and source of carbohydrate from which energy was derived, i.e. the readily hydrolyzable carbohydrates compared to the more lignified, less available holocellulose source. In the above study animals responded to increased levels of supplemented protein, but did not perform as well as might have been expected when

fed high amounts of supplemented energy. Supplements may have influenced both quantitative and qualitative intake and/or digestibility of the forage.

Of the studies reported in this thesis, experiment I was designed to determine the optimum number of days and animals required to estimate fecal production, dry matter and nitrogen digestibility, and quantitative intake using three different methods of estimating fecal production, i.e. total collections using harnesses and bags, chromium oxide ( $\text{Cr}_2\text{O}_3$ ) from aliquots of total collections and  $\text{Cr}_2\text{O}_3$  from grab samples collected via rectal palpation.

Experiment II was designed to (1) evaluate the influence of varying levels of supplemented crude protein and energy on intake and digestibility of dry matter of winter range forage, (2) measure the digestibility of dietary protein and energy and determine how well these met the animal's requirements and (3) develop laboratory procedures to evaluate standing winter forage.

## REVIEW OF LITERATURE

### The Use of Esophageal Fistula

In recent years the esophageal fistula has been used to obtain samples for assessment of the chemical and botanical composition of the diet of grazing animals. Van Dyne and Torell (1964) reviewed the history and development of the use of the esophageal fistula. The validity of the use of esophageal fistulated animals to collect samples of the diet is based on three assumptions as discussed by Streeter (1966):

1. Fistulation does not alter the physiological condition or the forage consumption of the animal.
2. Samples obtained via esophageal fistulae during short periods of time are representative of the botanical and chemical composition of the total forage consumed by the grazing animal.
3. Sampling via esophageal fistulae causes no alternation in the chemical composition of the ingested forage.

### Indicator Techniques

#### Chromium oxide

The use of  $\text{Cr}_2\text{O}_3$  as an external indicator dates back to 1918 when it was used by Edin for determination of digestibility (cited in Schneider et. al., 1955). Brisson (1960) gave two criteria which must be met for a valid estimate of fecal production by the external indicator method:

1. Exact measurement of the amount of indicator administered and its concentration in the feces so as to obtain quantitative recovery of the indicator in the feces.
2. Sampling of the feces so as to yield an aliquot qualitatively representative of all feces excreted during the collection period.

A review of the use of  $\text{Cr}_2\text{O}_3$  as an external indicator for estimating fecal production is given by Schneider et. al. (1955), Streeter (1966) and Prior (1966). Summarizing these reviews one may conclude:

1. The form in which  $\text{Cr}_2\text{O}_3$  is administered is critical.
2. The time of administration of  $\text{Cr}_2\text{O}_3$  and consequent sampling of the feces is important.
3. The nature of the feed consumed and intake relative to the time of dosing must be considered.

Form administered. Chromium oxide has been given in powder form in gelatin capsules (Barnicoat, 1945), as a drench form in a suspension of bentonite and water (Raymond and Minson, 1955), in a paste mixture of flour and  $\text{Cr}_2\text{O}_3$  or a collodion- $\text{Cr}_2\text{O}_3$  mixture (Miller et. al., 1957), as a "sustained release pellet" of  $\text{Cr}_2\text{O}_3$ ; a commercial dental plaster and water (Pigden and Brisson, 1957), as  $\text{Cr}_2\text{O}_3$  impregnated bleached sulfite wood pulp paper (Corbett et. al., 1958) and administered as shredded paper (Corbett and Greenhalgh, 1960) or a pellet (Troelsen, 1963), as a  $\text{Cr}_2\text{O}_3$  powder-Solca-Floc mixture (Raleigh, 1964) and mixed with concentrates (Wheeler, 1962) or the entire ration (Kane et. al., 1952; Elam et. al., 1962).



Chromium oxide impregnated paper-either in the shredded or pelleted form-appears to be a significant improvement over the powder form. Corbett and Greenhalgh (1960) found that the coefficient of variation of fecal concentrations of  $\text{Cr}_2\text{O}_3$  was reduced by about one-third by paper administration. This would have been more meaningful had  $\text{Cr}_2\text{O}_3$  been expressed in terms of recovery rather than concentration.

Under confined conditions excellent recoveries of  $\text{Cr}_2\text{O}_3$  administered in paper form, have been reported. Border (1962; cited in Prior, 1966) obtained average fecal recoveries of 99.98 and 100.49% from sheep. Coefficient of variation of two-day recoveries was 6.13%. Streeter (1966) reported mean recoveries of 100.1 and 99.5% on steers fed brome grass hay and brome grass clippings, respectively. Coefficients of variation were 5.5 and 1.6%, respectively. Low recoveries obtained from cows (82.8%; C.V.-7.7%) in another study were attributed to the regurgitation of capsules. Rittenhouse and Clanton<sup>1</sup> found recoveries of 101.9% from esophageal fistulated steers receiving a chopped meadow grass hay. More variation was found between sets of identical twins than within sets.

Under grazing conditions recoveries have been less consistent. Streeter reported recoveries of 82.6 to 95.1%. Coefficients of variation ranged from 1.9 to 11.0%. The low recoveries were attributed to loss of feces from the bags.

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<sup>1</sup> Unpublished data.

Langlands et. al. (1963) recovered 93.3% of the  $\text{Cr}_2\text{O}_3$  administered to grazing steers and 98.0% from grazing wethers. Low recoveries were attributed to regurgitation of capsules and/or incomplete fecal collections. Data from Carter et. al. (1960) emphasized the necessity of accounting for regurgitation of capsules and loss of feces from the collection bags. Lesperance (1968) feels that many of the low (85%) recoveries reported have been the result of interference of silica in the determination of  $\text{Cr}_2\text{O}_3$  in fecal samples. This problem was overcome by adding fecal ash to the standards.

Chromium oxide-cellulose mixtures have shown good results under grazing conditions in Oregon studies. Wheeler 1962; cited in Prior, 1966) reported more accurate estimates of fecal production with  $\text{Cr}_2\text{O}_3$ -cellulose mixtures than with powder even though recoveries were low (85%). In a later study on steers fed  $\text{Cr}_2\text{O}_3$ -cellulose with and without supplement recoveries of 99.6 and 102.2%, respectively, were recorded. Prior (1966) reported recoveries of 76.7 and 82.5% from steers on crested wheatgrass (Agropyron desertorum) in May and 98.7 to 103.8% recoveries in July-August. It is interesting to note that Streeter (1966) also noted higher recoveries with advance in season, i.e. 82.6 to 95.1% in late June and late July, respectively. Streeter attributed this to a decreasing likelihood of losing feces from the bag, however, factors of rate of passage may have been equally important (Raymond and Minson, 1955).

Time of administration of  $\text{Cr}_2\text{O}_3$  and sampling of feces.

Streeter (1966) stated that variation in qualitative fecal sampling for  $\text{Cr}_2\text{O}_3$  may arise from three sources:

1. Diurnal variation in the excretion of  $\text{Cr}_2\text{O}_3$ .
2. Random variation in the excretion of  $\text{Cr}_2\text{O}_3$  by different animals.
3. Random daily variation in the excretion of  $\text{Cr}_2\text{O}_3$ .

Data from Streeter (1966) and others (Lambourne and Reardon, 1963; Brisson, 1960) would indicate that there is no advantage of one time over another for taking rectal grab samples. However, most authors agree that there is usually associated with the excretion of  $\text{Cr}_2\text{O}_3$  a definite diurnal variation. In general it is thought that much of this variation can be overcome by twice daily dosing of  $\text{Cr}_2\text{O}_3$  (Coup, 1950; Pigden and Brisson, 1956; Davis et. al., 1958; Lambourne and Reardon, 1963). Under range conditions twice daily dosing may be impractical, as suggested by Prior (1966).

Putnam et. al. (1958) obtained digestibility values within  $\pm 5\%$  of those obtained by the total collection procedure using four animals and a 12 hr. grab sampling schedule. Morning grab samples usually contain a higher concentration of  $\text{Cr}_2\text{O}_3$  and are less variable than evening grab samples (Raleigh, 1964 and 1965; Prior, 1966; Kane et. al., 1952).

Random variation exists in the excretion of  $\text{Cr}_2\text{O}_3$  by different animals and among days. Streeter (1966) suggested that from six to nine animals over a period of six days are necessary to detect significant difference of  $\pm 10\%$  of the

mean with 90% confidence. Using stalls, Clanton (1961) found no advantage of 10 compared to 7 day collections to estimate recovery of  $\text{Cr}_2\text{O}_3$ . Clanton (1962) emphasized the need of sampling with sufficient number of days in order to obtain reliable estimates of fecal production, particularly when grab samples are used.

Nature of feed and feeding regimen. Variable recoveries of  $\text{Cr}_2\text{O}_3$  have been obtained in stalls when animals were receiving either high concentrate or roughage rations, e.g. Kane et. al. (1952) fed a mixed concentrate ration and recovered 99.9%; Streeter (1966) recovered 100.1% fed to steers receiving brome grass hay.

Data from Kane et. al. (1952) would indicate that the variation in  $\text{Cr}_2\text{O}_3$  excretion is not associated with the time of ingestion of food. In this case food was offered twice daily, but only one prominent peak in  $\text{Cr}_2\text{O}_3$  excretion occurred.

Balch et. al. (1957) and Lambourne (1957) observed greater diurnal variation when  $\text{Cr}_2\text{O}_3$  was administered after as compared to before feeding. Several investigators have reported that grazing animals exhibit much greater variation in  $\text{Cr}_2\text{O}_3$  excretion than those fed hand-clipped samples at regular intervals (Raymond and Minson, 1955; Hardison and Reid, 1953). This has been observed but not documented by Nebraska workers (Streeter, 1966; Rittenhouse and Clanton<sup>1</sup>).

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<sup>1</sup> Unpublished data.

## Lignin

The use of lignin as an indicator of indigestibility is dependent on the following assumptions:

1. The lignin fraction of the diet is not altered nor digested by the animal.
2. The lignin can be quantitatively recovered in the feces.
3. A representative sample of the feces and diet can be obtained.

Apparently the most difficult problem in the use of lignin as an internal indicator is defining the fraction being used. Varying degrees of digestibility of lignin fractions have been reported (Forbs et. al., 1943; Davis et. al., 1947; Ely et. al., 1953; Elam et. al., 1962; Van Dyne and Meyer, 1964a and b). Many of these discrepancies in the digestibility of lignin can be traced back to the method of isolation. Van Soest (1964) concluded that in general, three artifacts were present in many of these isolated fractions: (1) proteinaceous material, (2) hemicellulose and (3) products of non-enzymatic browning reaction from heat drying, particularly at temperatures above 50° C. He proposed the use of acid detergent to remove interfering proteins and hemicellulose.

The method of drying has been found to be important in analyzing for lignin by the acid detergent fiber- $\text{H}_2\text{SO}_4$  digestion method of Van Soest (1964) by Lesperance and Bohman (1964), Smith et. al. (1967) and Theurer (1968). Freeze

drying or handling samples wet appears to give the best results. Other variables are involved, as low recoveries of lignin are not uncommon. Using these chemical procedures, Lesperance et. al. (1967) reported digestibilities of -49 and -29% on oven-dried and freeze-dried samples, respectively. The method of preparation does not appear to be as critical when the  $\text{KMnO}_4$  method (Van Soest, 1967a) is used, although this needs further study and evaluation, particularly with feces samples.

Representative samples of dietary lignin may be obtained by once daily collection of samples via esophageal fistulae (Obioha, 1967; Arnold et. al., 1964; Van Dyne and Torell, 1964). It is generally agreed by these authors that adding more collection days will decrease sampling errors more efficiently than increasing sampling frequency within days.

Since under range conditions it is desirable to collect less than a days total fecal production, a representative sample must be taken. This is usually accomplished by taking grab samples once or twice daily. Kane et. al. (1952) found considerable variation in the lignin content of feces samples throughout a 24 hr. period. Highest values were found at about 9:00 a.m. and lowest values at about 9:00 p.m. An  $(n24 + 12)$  hr. lag in the excretion of lignin compared to  $\text{Cr}_2\text{O}_3$  was found. This may have occurred because of differences in the density of the concentrate (containing  $\text{Cr}_2\text{O}_3$ )

and hay resulting in different rates of passage. The determination of times for sampling the  $\text{Cr}_2\text{O}_3$  and lignin content of feces should be considered independently.

Elam and Davis (1961) found a coefficient of variation in the lignin content among grab samples of only 2 to 4%. In general little attention has been given to the variation in the lignin content of grab samples, i.e. either a constant rate of passage of lignin has been assumed or composites of a day's total collection of feces have been used to estimate lignin excretion.

#### Factors Influencing Feed Intake and Utilization

Interrelationships between supplemented protein and energy on animal performance are well documented (Clanton et. al., 1966). However, little is known of the cause and effect relationships involved. In these studies it was found that increasing crude protein in the diet resulted in increased performance; but increasing the amount of energy fed within a specific level of supplemented crude protein did not always increase daily gains. In one study (Ft. Robinson) animals receiving 182 gm. supplemented protein and 3.2, 5.6 and 8.0 megcal. estimated digestible energy per day gained 0.19, 0.12 and 0.03 kg. per day, respectively. Increasing the supplemented crude protein to 363 gm. resulted in weight gains of 0.27, 0.34 and 0.36 kg. per head daily, respectively. When 272 gm. supplemented crude protein was given the previous

year, no increased gains were observed by increasing estimated supplemented digestible energy from 2.4 to 7.2 megcal. per day. Similar results have been recorded at North Platte.

The feed intake of low quality forage is in most cases limited to the greatest extent by the presence of indigestible material in the rumen and alimentary tract. Campling and Balch (1961) found that if hay was removed from the rumen as the cow ate ad lib., the length of the eating period was almost doubled and cows consumed 177% of their normal voluntary intake.

The amount of bulk in the rumen is to a large extent dependent on the digestibility of the feed and the retention time in the lower tract. Campling and co-workers (Campling et. al., 1962; Freer and Campling, 1963) have shown that the intake of roughages is inversely related to the retention time of residues in the alimentary tract. Van Soest (1965) has shown a highly significant negative correlation between cell wall constituents (CWC) and voluntary intake over a wide variety of grasses and legumes. This confirms work by Blaxter (1961) showing a negative influence of undigested residue in the rumen on eating capacity. Indigestibility has been shown to be a result of the lignification of the cell wall by Van Soest (1967b). Colburn et. al. (1968b) found that the CWC fraction accounted for 11% of the variation in intake. Seventy percent of the variation in intake ( $\text{gm./kg. BW}^{0.54}$ ) was explained by variation in body weight and CWC. The