ETIOLOGY AND PREVENTION OF RUMINANT BLOAT

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Animal Nutrition

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1964

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REVIEW OF LITERATURE

A comprehensive review of the literature on the subject of bloat would be a combination of the reviews written by the following authors: Blake (1955), Cole et al. (1956), Brown (1959), Johnson (1959), Cole and Boda (1960), Kassir (1962) and Van Horn (1962). This review will include only literature relating to the use of antibiotics in bloat prevention, some physiological responses of cattle to orally administered antibiotics and the hydrolysis of dietary glycerides by rumen micro-organisms.

Bloat Prevention with Antibiotics

The oral administration of antibiotics for prevention of bloat was proposed initially by Barrentine et al. (1956), who found that single doses of 50 mg. of procaine penicillin protected yearling steers (body weight, 500-600 lb.) from bloat for 1- to 3-day periods. Older steers, weighing about 900 lb. required 75 mg. doses for complete protection for the same period. The effect of the penicillin was not manifested, however, until about 12 hours after administration. Potassium penicillin appeared to be as effective as an equivalent amount of procaine penicillin. Chlortetracycline, oxytetracycline, bacitracin and streptomycin in single doses of up to 300 mg. were ineffective. Thomas (1956) found that the oral administration of single doses of procaine penicillin (100 mg.) to cows prevented bloat for periods up to 96 hours. Moore et al. (1957) were able to control bloat 77% of the time in sheep by administering a single dose of penicillin (25 mg. per animal) 1 day

INTRODUCTION

Although bloat is one of the oldest nutritional diseases affecting ruminants and has been intensively studied for many years, it is still one of the serious problems facing the animal husbandman today. Research during the past 20 years has contributed much to our understanding of the etiology and prophylaxis of pasture bloat. During this period, it has been demonstrated that, in pasture bloat, a stable foam is formed in the rumen. The gas is entrapped in the foam and cannot be eructated. It has been shown that anti-foaming agents such as animal fats and plant oils provide very good control of bloat when they are consumed with the plant. The oral administration of various antibiotics, either singly, in rotation, or in various combinations has been proven useful in the control of bloat for periods up to 3.5 months.

During the past decade, Iowa State University has conducted extensive studies on the etiology, prevention and treatment of bloat and has contributed to the North Central Regional Project on "Chemistry and Physiology of Bloat." Workers at this institution introduced the concept of feeding antibiotics in combination or rotation to prolong the period of prophylaxis by orally administered antibiotics. More recent work has shown that the oral administration of a combination of antibiotics is more practical than the use of a rotation. The present studies were designed to find a more practical method of administering antibiotics for the control of pasture bloat and to elucidate certain etiological aspects of this syndrome.

before drenching with alfalfa juice. The efficacy of the treatment declined very rapidly when drenching occurred more than 1 day after the antibiotic was administered.

In later trials, Barrentine et al. (1958) observed that the base and thiocyanate forms of erythromycin controlled bloat equally as well as penicillin. Thiostrepton and bacitracin administered in single doses of up to 300 mg. did not reduce bloat. These researchers also retested oxytetracycline and penicillin in single doses of 50 and 75 mg. per steer. Oxytetracycline reduced the incidence of bloat more than it did in earlier studies but it was still considerably less effective than penicillin. Oxytetracycline did not reduce bloat in the only trial in which a control group was used in evaluating the treatments. In all of the Mississippi trials except this one, no control group was used and the effects of the treatments were judged by the per cent reduction in the incidence of bloat on the 3 days following treatment as compared to the incidence of bloat on the 3 days immediately prior to treatment.

Barrentine et al. (1957) field tested, on 19 farms, a penicillin-salt mixture (containing 50 mg. procaine penicillin per ounce of salt) and concluded that the mixture reduced the incidence of bloat in cattle grazing legume pastures. Salt consumption varied from farm to farm, ranging from 0.3 to 1.37 oz. per animal daily; the average was about 0.7 oz. The penicillin activity of the salt mixture remained stable when stored under dry conditions, in the unopened bag, for periods up to 8 months. However, the activity declined when the mixture was exposed to licking by the animals. The loss in activity was very slight when

exposed for 2 to 3 days, but very marked by the end of 1 week of exposure.

In an extensive field trial involving 739 cows over a period of 150 days, Emery et al. (1958) found that the incidence of pasture bloat was reduced by about 66% when 100 mg. of procaine penicillin was fed per cow daily, either with the grain or in the salt on a free-choice basis. The efficiency of the treatment appeared to decrease as the season progressed, during which time the incidence of bloat in both the treated and control groups declined. These authors recommended feeding 50 mg. procaine penicillin per cow per day when bloat becomes a problem and gradually increasing the dose to 100 mg. as the season progresses. The penicillin was quite stable in this salt mixture (trace mineral salt) as long as it was dry or sealed in the bag. The penicillin activity declined very rapidly after 2 days of exposure to licking by the animals.

The oral administration of 62,500 units of penicillin (equivalent to approximately 65 mg. of procaine penicillin) daily reduced the incidence, but not the severity, of bloat in cows grazing lush alfalfa pasture (Johnson and Bailey, 1958). Jacobson et al. (1957) found penicillin (dosage not given) effective in preventing bloat in all of 13 cows grazing blue grass-white clover pasture.

Procaine penicillin (75 mg. per animal daily) reduced bloat for about 11 days when administered to steers receiving alfalfa soilage (Brown et al., 1958). After this period, the incidence and severity of bloat in the penicillin-fed steers was similar to that in the control steers. Johnson et al. (1958) found that procaine penicillin (75 mg.

per animal daily) reduced bloat for 9 days in steers grazing alfalfa pasture. Subsequently, its effectiveness declined very rapidly. Increasing the dosage to 125 mg. reduced bloat for 2 days, after which the incidence increased sharply.

Several workers (Barrentine et al., 1958; Brown et al., 1958; Emery et al., 1958; Johnson, 1959; Mangan et al., 1959) had observed a decrease in the effectiveness of penicillin and erythromycin in bloat prevention after a short period (1 to 2 weeks) of successful use. This decreased efficiency or "resistance" was thought to be due to the development of resistant strains of bacteria or to changes in the balance of the ruminal microflora (Mangan et al., 1959). Johnson (1959) found that resistance to erythromycin was not reversed by withholding the antibiotic for periods up to 26 days. Mangan et al. (1959) reported that the effectiveness of penicillin was regained after withholding it for 2 months.

The latter observation suggested to Johnson et al. (1960b) the possibility of sustaining the effectiveness of antibiotics by feeding several antibiotics in succession and repeating them after resistance had subsided or by feeding several antibiotics in combinations with the hope that synergistic combinations might extend the duration of effectiveness. With these objectives in mind, they conducted a series of trials in which it was found that a combination of penicillin (35 mg.) and erythromycin (70 mg.) fed daily controlled bloat for a longer period of time (23 days in one trial, 26 days in another) than did the same antibiotics fed in sequence. When fed individually, erythromycin (70 mg.)

(70 mg.) and tylosin (70 mg.) administered in the drinking water daily prevented bloat for about 7 weeks. These results indicated that the feeding of antibiotics in combination offered more promise as a practical bloat prophylactic measure than did the feeding of antibiotics in rotation.

Van Horn et al. (1963) field tested, on 14 farms, a combination of penicillin (40 mg.), erythromycin (70 mg.), tylosin (70 mg.) and streptomycin (70 mg.) in wheat middlings-molasses pellets. Bloat incidence and severity during the grazing season was 67% lower in 474 treated animals than in 362 control animals. The authors suggested that the effectiveness of the antibiotic pellets probably would have been greater had the erythromycin activity been more stable in the pellets and had some animals not refused to eat the pellets. In other studies, the same antibiotic pellets reduced the incidence and severity of bloat in steers grazing legume pasture (Essig et al., 1962; Johnston et al., 1962). Feeding a combination of penicillin (40 mg.), erythromycin (70 mg.), tylosin (70 mg.) and streptomycin (70 mg.) in loose salt daily was not as effective in controlling bloat as the combinations fed in pellets (Van Horn et al., 1963). Neither of these methods was as effective as feeding the combination daily in grain.

Physiological Effects of Orally Administered Antibiotics

Various antibiotics administered orally are effective in preventing pasture bloat in ruminants. Even though certain antibiotics bring about some beneficial responses, under certain circumstances they also can

was effective for about 10 days following penicillin (35 mg.) which also had been effective for about 10 days. Tylosin (70 mg.) and chloramphenicol (140 mg.) fed sequentially were effective for about 7 days each after previous exposure of the animals to penicillin and erythromycin. Subsequently, oxytetracycline (140 mg.) was effective for about 4 days. The levels expressed are in terms of amounts per animal daily. These results suggested a possible sequence including penicillin, erythromycin, tylosin, chloramphenicol and oxytetracycline which could be effective in bloat prevention for a period of about 38 days. They also observed that withholding penicillin for periods of 14 and 17 days was not sufficient to permit the return to effectiveness of this antibiotic.

Van Horn et al. (1961) continued the search for a combination or rotation of antibiotics which would control bloat for a period of several months. They found that a sequence consisting of penicillin (35 mg.), erythromycin (70 mg.), tylosin (70 mg.), chloramphenicol (140 mg.), oxytetracycline (140 mg.) and streptomycin (70 mg.) prevented serious bloat during a 6-week period (each antibiotic was fed for 1 week). In two subsequent repetitions of the cycle, it was observed that penicillin had regained effectiveness in about 6 weeks but that more than 6 weeks were required for the other antibiotics to regain their effectiveness. However, a combination of penicillin (40 mg.), erythromycin (70 mg.), tylosin (70 mg.), chloramphenicol (100 mg.) and oxytetracycline (100 mg.) fed daily in grain provided adequate protection from bloat for a period of 3.5 months. A combination of penicillin (40 mg.), erythromycin

fat and solids-not-fat contents of the milk; or on the iodine value, saponification value, softening point, Reichert value and carotene and vitamin A content of the butterfat. Murnane (as quoted by Johns et al., 1959), in Australia, observed no significant change in either milk yield or fat test of the milk as the result of feeding 400,000 units of penicillin (equivalent to about 400 mg. procaine penicillin) daily for 4 weeks. The only adverse effect was a transient diarrhea in some of the cows during the first few days of treatment.

Shor et al. (1959) fed chlortetracycline to lactating dairy cows at levels of 0, 0.1, 0.5 and 1.0 mg. per pound of body weight per cow daily for a period of 2 weeks. About one-half of the cows receiving the two higher levels of antibiotic showed slightly depressed appetites and reduced milk production shortly after the trial was started. However, feed consumption and milk production returned to normal within a few days. Cannon et al. (1962) reported that cows which had been fed 10,000,000 units of procaine penicillin in a single dose exhibited symptoms of physiological disturbances including severe scouring, loss of appetite, reduction of milk yield and an increase in the fat percentage of the milk.

No adverse effects have been observed when antibiotics were fed singly at the levels (penicillin 35 to 75 mg., erythromycin 70 mg., tylosin 70 mg., chloramphenicol 140 mg. and oxytetracycline 75 to 140 mg. per animal daily) effective in bloat prevention (Barrentine et al., 1956, 1958; Johnson et al., 1958, 1960b; Emery et al., 1958; Van Horn et al., 1961). Clover consumption appeared to be slightly greater in

cause some detrimental effects. The extent to which these adverse effects might occur has not been adequately determined. Horn et al. (1955) fed varying amounts of procaine penicillin to steers and noted no outward ill effects when as much as 800 mg. was fed. However, the only physiological effect observed which could be attributed to the feeding of an antibiotic was a change in the urine from a clear brownish color to a milky looking suspension with a yellowish-green cast. This condition was observed on the third and fourth days when the steers had been fed 400 mg. of penicillin daily for 4 days. Barrentine et al. (1956) observed severe diarrhea in all steers which had received a single dose (1.0 gm.) of either chlortetracycline, oxytetracycline, or procaine penicillin. Single doses of these antibiotics up to 300 mg. did not produce this effect. Emery et al. (1958) found that a marked depression of milk production often occurred for several days after the inclusion of 400 mg. of procaine penicillin in the ration. This depression of milk production was sometimes accompanied by a loss of appetite, discharge at the nostrils and hyperemia of the vaginal mucosa. All of these symptoms disappeared within a few days and milk production returned to normal even though penicillin feeding was continued. These symptoms also were noticed in a herd of cows receiving only 100 mg. procaine penicillin per animal daily.

Johns et al. (1959) fed 100, 200 and 500 mg. doses of procaine penicillin to milking cows every third day over periods of 15 days, 5 months and 12 days, respectively. There was no adverse effect on the body weights of the cows; on the yields of milk and butterfat; on the

steers after treatment with 25 to 50 mg. of penicillin (Barrentine et al., 1956). Johns et al. (1959) noted an occasional marked increase in appetite when penicillin was being given during outbreaks of bloat. Steers receiving various antibiotics singly and in combination for the prevention of pasture bloat usually had greater weight gains than the control steers (Johnson et al., 1958; Van Horn et al., 1961, 1963).

Transient depression of appetite, diarrhea and swelling in the region of the vulva occurred occasionally in animals receiving a combination of penicillin (40 mg.), erythromycin (70 mg.), tylosin (70 mg.) and streptomycin (70 mg.) in wheat middlings-molasses pellets daily (Van Horn et al., 1963). These reactions were restricted almost exclusively to animals receiving 6 to 12 lb. of grain daily. In other studies, steers receiving the same antibiotic pellets for a period of about 2 months did not exhibit any adverse reactions (Essig et al., 1962; Johnston et al., 1962). Similar antibiotic pellets were fed to heifers and lactating cows by Emery (1962). He reported that a small portion of the animals involved exhibited such adverse reactions as transient loss of appetite, dark colored feces containing mucus casts and drastically reduced milk production. Van Horn et al. (1963) demonstrated with animals on high-grain diets that transient depression of appetite and diarrhea sometimes occurred when daily doses of 250 mg. of penicillin, erythromycin and tylosin were fed singly, but that there was no evidence of these reactions when daily doses of 250 mg. of streptomycin was fed alone. Steers receiving a feedlot bloat-provoking ration refused feed for 32 to 48 hr. after the first exposure to 75 or

(Wright and Harold, 1960). Oxytetracycline did not produce any detectable residue in milk when daily doses of 0.5 to 5.0 mg. per pound of body weight were given orally; however, the assay method used was only about one-fifth as sensitive to this antibiotic as it was to chlortetracycline. Streptomycin was not detected in the milk when 1.0 gm. was administered per day. Johns et al. (1959), feeding from 200 to 500 mg. of procaine penicillin per animal per day, and Wright and Harold (1960), administering 1,000 to 5,000 mg. of penicillin orally per cow daily, did not detect any residue in the milk. Conversely, Skaggs and Miller (1959) found that daily doses of 178 and 278 mg. of procaine penicillin in a feed concentrate resulted in residues of 0.05 to 0.15 units of penicillin per milliliter of milk. The oral administration of a single dose of 10.0 gm. of procaine penicillin to lactating cows produced detectable amounts of penicillin in the milk up to 86 hr. after administration (Cannon et al., 1962). However, no detectable residue was found in the milk 96 hr. after administration.

Hydrolysis of Dietary Lipids in the Reticulo-Rumen

It has been known for some time that proteins and carbohydrates undergo hydrolytic and fermentative changes in the rumen, but very little attention has been given to the fate of dietary lipids in the rumen. Recent reviews on the latter subject have been written by Garton (1960) and Hill (1960). It has been established within the last 13 years that rumen microorganisms can hydrogenate unsaturated fatty acids and, more recently, that rumen microorganisms also have the ability to hydrolyze

150 mg. of procaine penicillin per day (Bryant et al., 1961). After feed consumption resumed, further treatment with 50 to 200 mg. of penicillin daily did not result in feed refusals. Conversely, no adverse reactions were observed by Van Horn et al. (1963) when as much as 2.0 gm. of an antibiotic mixture (40 mg. penicillin, 70 mg. erythromycin, 70 mg. tylosin and 70 mg. streptomycin) was fed per animal daily to yearling steers receiving primarily green-chopped forage or alfalfa hay.

Penicillin is the only antibiotic used in bloat prevention for which appreciable milk residue data are available. Even though each antibiotic probably acts as an entity, the milk residue studies conducted with antibiotics which are not useful in bloat prevention may be of value in evaluating those antibiotics which are useful. Penicillin could not be detected in the milk from over 80 cows that had been fed 100 mg. of procaine penicillin daily (Emery et al., 1958). The assay method used was capable of detecting less than 0.5 units of penicillin per milliliter of milk. Shor et al. (1959) found no antibiotic residue in the milk from cows receiving 0.1 mg. of chlortetracycline per pound of body weight daily and detected only slight amounts in the milk from cows receiving 0.5 and 1.0 mg. of chlortetracycline per pound of body weight daily. These slight amounts were completely eliminated from all cows' milk by 48 hr. after feeding of the antibiotic had been discontinued. These results had substantiated those found earlier by Henderson et al. (1957). Chlortetracycline produced significant residues in milk when oral doses of 0.5 to 10.0 mg. per pound of body weight were given daily

Hill et al. (1960) found most of the lipolytic activity to be with the protozoa fraction while Garton et al. (1961) found it to be associated primarily with the bacteria. The latter were unable to prepare a cell-free extract from mixed rumen organisms which would exhibit lipase activity. However, Wright (1961) successfully prepared cell-free extracts from bacteria and mixed protozoa which had considerable lipolytic activity.

Lipolytic bacteria have been found in rumen contents of sheep. Hobson and Mann (1961) isolated several types of bacteria which hydrolyzed linseed oil but apparently could not utilize the resultant long chain fatty acids. These bacteria were strictly anaerobic, Gram-negative rods and were variable in size. Some of the colonies were buried in masses of slime while others were not. None of the bacteria isolated could be identified with any of the rumen bacteria thus far described in the literature.

Lipolysis of ingested glycerides in the rumen of intact sheep was observed in two animals that had been fed for several months on the same ration (Garton et al., 1958, 1961). Sheep no. 1 was fed a ration of hay and concentrates to which 40 gm. of linseed oil was added daily and sheep no. 2 was fed a mixture of concentrates. At slaughter, 7 hr. after the last feeding, 92% and 78% of the lipids in the rumen contents of sheep no. 1 and 2, respectively, consisted of free higher fatty acids. About 92 and 96% of the lipids present in the abomasum and small intestines, respectively, of both sheep were in the form of free higher fatty acids. These authors concluded that dietary lipids can be pre-digested

dietary glycerides. This review will be limited to a discussion of the hydrolysis of triglycerides by rumen microorganisms.

Garton et al. (1958) were the first to demonstrate that hydrolysis of triglycerides occurs in rumen contents of sheep. They incubated (37°C. for 24 hours) 1.0 gm. linseed oil or tung oil with 100 ml. of rumen contents from sheep. More than 75% of the total lipid recovered at the end of incubation was in the form of free higher fatty acids. When rumen contents were incubated without added oil, free higher fatty acids accounted for 50 to 60% of the total lipid recovered.

Further studies were conducted by Garton et al. (1958, 1961) in which linseed oil was incubated with rumen contents obtained from fistulated sheep 4 hr. after the last feeding of hay and concentrates. Hydrolysis usually resulted in the liberation of from 60% to more than 90% of the esterified fatty acid residues of the original oil; occasionally the extent of hydrolysis was as low as 20%. From 18 to 48% of the triglycerides in soybean oil were hydrolyzed by rumen fluid obtained from fistulated steers (Allen et al., 1959). Hill (1960) and Hill et al. (1960) observed that soybean oil was hydrolyzed to the extent of 5 to 96% when incubated with rumen fluid from steers. No lipolysis was observed when the rumen fluid had been heated (90°C. for 1 hour) before incubation (Garton et al., 1958, 1961; Allen et al., 1959; Hill, 1960).

Lipolysis of added oil did not occur when most of the microorganisms were removed from the rumen liquid by centrifugation (Hill, 1960; Garton et al., 1961). By using differential centrifugation to separate the feed particles and large protozoa from the bacteria and small protozoa,

the lipolytic activity of rumen fluid and that the lipolytic activity of rumen fluid may very likely be involved in the etiology of bloat.

in the rumen and that most of the higher fatty acids in the digesta reach the small intestine in the form of free fatty acids. This is in marked contrast to glyceride digestion in adult mon-ruminants in which little or no lipolysis occurs before the lipid reaches the small intestine.

Lipolysis of ingested plant lipids has been implicated in the etiology of bloat in ruminants. Mangan et al. (1959) postulated that under bloating conditions the rumen bacteria modify the chloroplast lipid in some way to lessen its anti-foaming activity - probably by hydrogenation or hydrolysis. It has been observed by Mangan et al., (1959) that the chloroplasts (which contain most of the plant lipids) obtained from rumen ingesta of penicillin-treated animals had much greater anti-foaming properties than did chloroplasts from rumen ingesta of animals not receiving penicillin. This suggests that penicillin inhibits the bacteria which alter the chloroplast lipids in the rumen. Wright (1961) observed that penicillin and oxytetracycline markedly decreased the lipolytic activity of rumen liquor and that neomycin and streptomycin had no effect. Hill (1960) found that the lipolytic activity of rumen fluid was reduced 50% by penicillin, erythromycin and tylosin, 49% by streptomycin and 30% by chloramphenicol. Neomycin did not affect lipolysis. Research at this University showed that penicillin, erythromycin and tylosin are highly effective in controlling bloat, and chloramphenicol and streptomycin are less effective. Mannose, which is toxic to holotrich protozoa, also drastically reduced the lipolytic activity of rumen fluid (Hill, 1960). It can be concluded, on the basis of these data, that bacteria and protozoa contribute to

EXPERIMENTAL PROCEDURE

This section of the dissertation is divided into two parts - one part describing animal studies, and the second describing the methods used in laboratory analyses and miscellaneous procedures.

Animal Studies

Use of a combination of antibiotics in a bolus and of potassium levopropylcillin as bloat prophylactic agents

In a study at Ames, Iowa, 68 dairy and beef animals were allowed to graze alfalfa pasture from 7 to 10 a.m. and from 3:30 to 6:30 p.m. A rotational grazing plan was followed on 60 acres of first- and second-year alfalfa pasture which was divided into 2.5- to 3-acre plots. Several of the plots were irrigated during the dry season. Between grazing periods, the animals were retained in a holding pen near the pasture, where fresh water and iodized salt were available.

The animals were divided into five groups. Groups I, II, III and IV were composed of 14 animals each, with average initial weights of 626, 620, 628, and 627 lb., respectively. Group V, composed of 12 animals with an average initial weight of 669 lb., was the heaviest since the eight smallest animals were allotted to the first four groups. Table 1 shows the treatments and the date on which each treatment was started. The combination of antibiotics was administered by bolus and the potassium levopropylcillin was given by gelatin capsule.

Bloat severity was evaluated by visual observation, using scores from 0 (no bloat) to 5 (terminal) as described by Johnson et al. (1958).

Table 1. Treatments and date when started

Date	Group I	Group II	Group III	Group IV	Group V
May 14	Control	1 Bolus[a]	2 Boluses	3 Boluses	Control
June 27		1 Bolus	1 Bolus	1 Bolus	
Aug. 4			2 Boluses		
Aug. 10					_b
Aug. 28		2 Boluses			
Aug. 31			_c	_c	
Sept. 15		_b			
Sept. 18	Experiment terminated				

[a]Antibiotic combination bolus supplied by Eli Lilly and Company, Greenfield, Indiana. Each bolus weighed approximately 64 gm. with a specific gravity of about 4.5. The total antibiotic activity of each bolus was 6 gm.; the combination of antibiotics was streptomycin sulfate, tylosin phosphate, erythromycin thiocyanate and procaine penicillin (STEP) in a 7:7:7:4 ratio. Boluses were administered by balling gun.

[b]Potassium levopropylcillin (the potassium salt of alphaphenoxypropyl penicillin), which is more resistant to penicillinase than penicillin G or V, was supplied by Eli Lilly and Company. Each animal received 100 mg. in a gelatin capsule by balling gun at 48-hour intervals.

[c]Groups III and IV were removed from experiment because of insufficient bloat-producing pasture.

Bloat scores were recorded hourly while the animals were on pasture. Only the maximum bloat score attained by each animal daily was used in evaluating the efficacy of the treatments. Each animal was weighed at the beginning of the trial and at 4-week intervals throughout the season.

In addition to the trial at Ames, cattle on two other Iowa State University farms (at Napier and Castana) and two herds owned by co-operating farmers (Appendix Table 11) were used in continuous grazing studies. At Napier, the control group was composed of five Angus and seven Hereford steers, and the treated group was composed of three Angus and nine Hereford steers. The groups were permitted to graze together on legume pasture. Two 12-acre fields were used on a rotational grazing basis. Each animal in the treated group was given two boluses on May 4, one bolus on June 21, and two boluses on August 23, 1962. The animals in the control group did not receive boluses. Starting on September 18, a grain mixture was fed to all animals at the average rate of 1 lb. per animal daily. Each pound of grain mixture contained 50 mg. of potassium levopropylcillin during the first 4 days and 100 mg. during the next 4 days. The bloat score of each animal was determined by visual observation and recorded twice daily. Each animal was weighed at the beginning of the trial, during the 6th and 17th weeks, and again when removed from pasture (22nd week).

At Castana, two groups, each composed of 10 Hereford heifers and 8 Hereford steers, allotted on the basis of body weight, were allowed to graze together on alfalfa-brome grass pasture. Each animal in the treated group was given two boluses on May 16, 1962 and at 6-week intervals throughout the grazing season. The control animals did not receive boluses. Each animal was weighed initially and at 3-week intervals thereafter.

The two herds owned by cooperating farmers were divided into two groups and allowed to graze together on legume pasture. Two boluses were administered to each animal in the treated group at the beginning of the trial and at 6-week intervals throughout the season. Seventy-five animals were involved; 39 received boluses and 36 were used as controls.

Some of the cattle were given boluses immediately upon removal from pasture, whereas others were held in the corral for about 3 to 4 hours before receiving the boluses. Animals were not released from the stanchion until the bolus had been swallowed. Regurgitation of boluses was not observed.

In addition to the studies with cattle, trials were conducted with two flocks of sheep owned by cooperating farmers (Appendix Table 11). Flock A, composed of 95 lactating ewes grazing alfalfa-ladino clover pasture, was divided into two groups of 50 (treated group) and 45 (control group) each (Trial I). Each ewe in the treated group received one-half of a cattle bolus at the beginning of the trial and another half 4 weeks later; the controls were given no boluses during this period. After 8 weeks, the ewes remaining in the control group (three had died of bloat) were divided into two groups of 21 each (Trial II). One group received one-half of an antibiotic bolus initially and another half 4 weeks later, while the control group received a placebo bolus in the same manner. The sheep were removed from pasture, promptly bolused, and returned to pasture. Regurgitation of boluses was not observed. After Trial II was completed, a grain mixture was fed to all

ewes at the average rate of 0.75 lb. per ewe daily. Each 0.75 lb. of grain mixture contained 20 mg. of potassium levopropylcillin during the first 6 days and 40 mg. during the next 8 days. Observations on the incidence of bloat among the ewes were recorded twice a day.

Flock B, comprised of 350 ewes, was used for a short-term trial (2 weeks) in mid-September, 1962. Fifty ewes were allotted to the control group which did not receive boluses, while the remainder comprised the treated group wherein each ewe received a sheep bolus which was one-half the size of the cattle bolus described previously (Table 1). The ewes were in dry-lot when they received boluses and were placed on pasture 2 days later. Both groups grazed together on excellent alfalfa pasture. The ewes were observed frequently; bloat severity was recorded several times daily.

Point of deposition, movement and disintegration of antibiotic boluses in the reticulo-rumen

Four steers with rumen fistulas and four intact steers were employed to study the point of deposition, subsequent movement and rate of disintegration of the boluses in the reticulo-rumen. In the first of two experiments with the fistulated steers, four small (34 gm.) boluses were introduced into the reticulo-rumen by balling gun. Four weeks later, in the second experiment, the remnants of the boluses were removed from two of the steers and three large (64 gm.) boluses were administered by balling gun; ultimately, all fragments of the boluses were removed. The animals were fed dry feed (hay and grain) during these two experiments; later, when on pasture, each animal received two large boluses.

and III received one antibiotic capsule at 1-, 2- and 3-day intervals, respectively. Those in Group IV received no antibiotics (control). All animals were not started on the antibiotic at the same time (see Experiment 1 in the following section). Bloat severity was evaluated by the scale of 0 (no bloat) to 5 (terminal) as presented by Johnson et al. (1958).

Feeding regime and response to initiation of antibiotic administration

Experiment 1 During the period from May 3 to May 12 in the foregoing section, the 18 heifers and 18 steers comprising Groups I, II and III were used to determine the effect of various ration changes on the occurrence of adverse reactions of cattle to the antibiotic combination. For several months prior to May 3, each animal received about 4 lb. of grain mixture daily plus corn silage and/or alfalfa hay free-choice. On May 3, two heifers and two steers from each of the three groups were started on their respective STEP administration schedules, and, starting with the next feeding, the ration of all animals was changed to 2 lb. grain mixture per animal daily plus alfalfa hay free-choice. On May 6, another set of two heifers and two steers from each group was started on the antibiotic administration schedule and that afternoon green-chopped alfalfa replaced alfalfa hay in the ration. The remaining set of animals from each group was started on antibiotics on May 9. During this period, all animals were observed for symptoms of diarrhea, depressed appetite and other disorders.

Experiment 2 Twelve dairy steers (average body weight, 340 lb.) were divided into three groups of four animals each. The design of the

Each of the intact steers received two boluses by balling gun at 6-week intervals until each steer had received a total of 14 boluses. During the first 5 months, the animals received hay and grain; subsequently they were put on alfalfa pasture. At periods ranging from 42 to 88 days after the last boluses were administered, the animals were sacrificed and the boluses remaining in the reticulo-rumen were recovered.

A combination of antibiotics administered at 1-, 2- and 3-day intervals for bloat prevention

Thirty-three dairy steers and 21 dairy heifers were divided into 4 groups. Groups I, II and III were composed of 6 heifers and 6 steers each and Group IV was composed of 3 heifers and 15 steers. The average body weights of the heifers and steers were 720 and 555 lb., respectively. The animals were confined to dry-lot and were fed green-chopped alfalfa at 7 a.m. and 2 p.m. daily. The alfalfa was harvested with a flail-type chopper prior to each feeding. Whenever possible, to increase the bloat potential, only the top 4 to 8 in. of the plants were harvested. Forage not consumed within 3 hours after feeding was removed from the bunk. A grain mixture (65% ground shelled corn, 34% rolled oats and 1% dicalcium phosphate) was fed at 6:30 a.m. at the average rate of 2 lb. per animal daily. All animals had access to fresh water and iodized block salt.

A combination of antibiotics (STEP), 70 mg. streptomycin sulfate, 70 mg. tylosin phosphate, 70 mg. erythromycin thiocyanate, and 40 mg. procaine penicillin, was administered in a gelatin capsule with a balling gun. The capsules were given at 1 p.m. from May 3 to May 23 and at 6 a.m. from May 24 to September 17, 1963. Each animal in Groups I, II

experiment is presented in Table 2. The grain mixture was fed twice daily to each steer while confined to a small individual pen. The antibiotic combination (STEP) was fed with the grain at the morning feeding only. Between grain feeding periods, all animals were confined to a paved lot where they had access to alfalfa hay and fresh water. Grain consumption per feeding and incidence of diarrhea were recorded for each steer.

Experiment 3 Nine dairy heifers (weighing about 450 lb. each) were divided into three groups of three animals each. The experimental design was the same as that described for Experiment 2 (Table 2), and the animals were handled in the same manner.

Experiment 4 The same 12 steers used in Experiment 2 were used in this experiment. They were about 200 lb. heavier than when used earlier. The experimental design was the same except for a longer preliminary period (Table 2).

Laboratory Methods and Miscellaneous Procedures

Sampling of rumen fluid

Rumen fluid was obtained from a rumen fistulated steer receiving a ration consisting of 12.6 lb. alfalfa hay and 10.4 lb. grain mixture (ground corn cobs, 130 lb.; rolled corn, 240 lb.; soybean oil meal, 55 lb.; cane molasses, 65 lb.; dicalcium phosphate, 5 lb.; salt, 5 lb.; and Quadrex, 30 gm.) daily. Samples were usually taken prior to the morning feeding. A suction strainer similar to that described by Raun and Burroughs (1962) was used to obtain rumen fluid from the ventral

Table 2. Design of experiments 2, 3 and 4; rations and level of antibiotics fed

	Days	Group I	Group II	Group III
Preliminary period[a]				
Experiment 2	14			
Experiment 3	14			
Experiment 4	54			
Experimental period				
Experiments 2, 3 and 4	1 to 7	H.G.[b]	H.G.	H.G.
	8	H.G. + 250 mg. STEP	L.G.[c]	L.G.
	9	"	"	"
	10	"	"	"
	11	"	"	"

[a]During the first week, grain feeding was gradually increased until each animal was consuming 9 to 10 lb. of grain mixture per day. This level of grain feeding was maintained for the duration of the preliminary period. Alfalfa hay was fed free-choice. The grain mixture was composed of ground shelled corn, 500 lb.; ground oats, 300 lb.; soybean meal, 100 lb.; dicalcium phosphate, 18 lb.; iodized salt, 9 lb.

[b]High grain ration (H.G.) - 9 lb. grain mixture (Experiments 2 and 3) or 10 lb. grain mixture (Experiment 4) per animal daily plus alfalfa hay free-choice.

[c]Low grain ration (L.G.) - 2 lb. grain mixture (Experiments 2, 3 and 4) per animal daily plus alfalfa hay free-choice.

Table 2. (Continued)

Days	Group I	Group II	Group III
12	"	L.G. + 250 mg. STEP	"
13	"	"	"
14	"	"	"
15		"	L.G. + 250 mg. STEP
16		"	"
17		"	"
18		"	"
19			"
20			"
21			"

portion of the rumen. Also, samples of rumen fluid were obtained by squeezing (through 4 layers of cheesecloth) the fibrous material removed from the dorsal part of the rumen. About 2,000 - 3,500 ml. of fluid were obtained at each sampling, placed immediately into a warm vacuum jug and transported to the laboratory.

Preparation of bacterial suspensions

Rumen fluid was centrifuged at 500 x G for 5 min. to remove protozoa and feed particles. The supernatant was decanted and the residue was used in preparing the protozoal suspension described in the next paragraph. The supernatant was then passed through (slow flow rate) a Sharples Super Centrifuge at 25,000 r.p.m. The residue remaining on the inside of the barrel, except the bottom $\frac{1}{2}$ inch, was placed in a 500 ml. Erlenmeyer flask, resuspended in incubation media (Appendix Table 12), gassed with CO_2 and placed in the water bath. The volume of the bacterial suspension was one-tenth the initial volume of rumen fluid.

Preparation of protozoal suspensions

Washed suspensions of mixed protozoa were prepared from the residue resulting from low speed centrifugation of rumen fluid (described in previous paragraph). The residue was resuspended in a buffer solution (Appendix Table 13), transferred to a 500 ml. separatory funnel (no. 1), shaken vigorously, gassed with CO_2 and placed in a 39°C. water bath. After 1 hr. the protozoa layer was drawn off into another separatory funnel (no. 2) containing warm buffer solution, shaken, gassed with CO_2 and placed in the water bath. Separatory funnel no. 1 was also vigorously shaken, gassed with CO_2 and returned to the water bath. After 1

hr. the protozoa layer in funnel no. 2 was drawn off into a flask containing incubation media (Appendix Table 12), gassed with CO_2 and put into the water bath. The protozoa layer in funnel no. 1 was drawn off into funnel no. 2, shaken, gassed with CO_2 and put into the water bath. The fluid remaining in funnel no. 1 was discarded. After 1 hr. the protozoa layer in funnel no. 2 was also drawn off into the flask containing the incubation media, gassed with CO_2 and returned to the water bath. The fluid remaining in funnel no. 2 was discarded. The final volume of the protozoal suspension was one-tenth the initial volume of rumen fluid.

Substrate and incubation flasks

The substrate used for assaying the rumen fluid and rumen microorganisms for lipolytic activity was soybean oil. The soybean oil (bleached and refined) was supplied by Durkee Famous Foods, Chicago, Illinois. Uniform quantities of oil were distributed into the incubation flasks by dissolving 2.5 or 5.0 gm. in 250 ml. of ether (anhydrous) and transferring 5.0 ml. aliquots of solution (50.0 or 100.0 mg. of oil) into each incubation flask (25 ml. Erlenmeyer flasks). The ether was evaporated from the flasks by warming. This method of preparing the incubation flasks compared very favorably with the gravimetric method. When other substances were added, they were weighed and transferred into each flask before the rumen fluid or microorganism suspension was added.

Measurement of lipid hydrolysis

Ten ml. of either rumen fluid, bacterial suspension, protozoal suspension, or 10 ml. each of bacterial suspension and protozoal suspen-

sion were transferred to incubation flasks containing a known quantity of oil. The flasks were incubated at 39°C. for 24 hr. under CO_2 in a Dubnoff Metabolic Incubator Shaker. Continuous shaking was maintained at the rate of 112 oscillations per min. Flasks containing no added rumen fluid or microorganisms were incubated as controls. The unhydrolyzed lipid remaining after incubation as well as that present in similar flasks at the beginning of incubation was estimated by the hydroxamic acid method (Allport and Keyser, 1957).

The incubation mixture was added to 60 ml. of 95% ethanol-ether (3:1) in a 100 ml. volumetric flask. The mixture was heated to boiling in a water bath, cooled, diluted to volume with ethanol-ether and allowed to stand overnight. Two ml. of the supernatant was transferred to a test tube containing 4.0 ml. of ethanol-ether. Then 1.0 ml. of hydroxylamine hydrochloride reagent (2 M) and 1.0 ml. of 3.5 N NaOH were added. After mixing, the tube was stoppered and allowed to stand for 20 min. at room temperature. Then 1.2 ml. of 3.5 N HCl was added, the tube was shaken and 1.0 ml. of 10% (w/v) $FeCl_3 \cdot 6H_2O$ in 0.1 N HCl was added. The solution was mixed well and its optical density was measured in a Beckman Model B Spectrophotometer at 525 millimicrons against a solvent blank prepared in the same manner. The amount of oil was calculated from standard solutions of refined soybean oil which were analyzed with each set of samples.

EXPERIMENTAL RESULTS

Animal Studies

Effect of administration of a combination of antibiotics in a bolus and of potassium levopropylcillin

Bloat incidence and severity The results of the Ames experiment are presented in Figures 1, 2 and 3. The initial administration of either one, two, or three boluses reduced bloat for 3 to 4 weeks. Soon thereafter several cases of mild bloat (bloat score 2) occurred and by the end of the 6th week several animals had bloated severely (bloat score 3 or greater). Subsequent administration of either one or two boluses reduced bloat for approximately 1 week. The administration of 100 mg. potassium levopropylcillin per animal at 48 hr. intervals prevented bloat for 5 weeks (Figure 4); after which, bloat occurred in only one animal. Potassium levopropylcillin also appeared to be an effective bloat prophylactic agent in animals that had been previously exposed to the antibiotic bolus (Figure 1).

The average daily maximum bloat scores (A.D.M.) for Groups I and V during the period from May 14 to August 9 were similar and exhibited a parallel relationship (Figure 4). This suggests that the two groups possessed a similar potential for bloat.

Appendix Tables 14 and 15 show the bloat data collected on each day of the experiment.

The results of the Napier experiment are presented in Appendix Table 16. The initial administration of two antibiotic boluses reduced

Figure 1. Effect of antibiotic boluses and potassium levopropylcillin on the average daily maximum bloat score (L = potassium levopropylcillin. Each animal was given 100 mg. in a gelatin capsule by balling gun at 48-hour intervals)

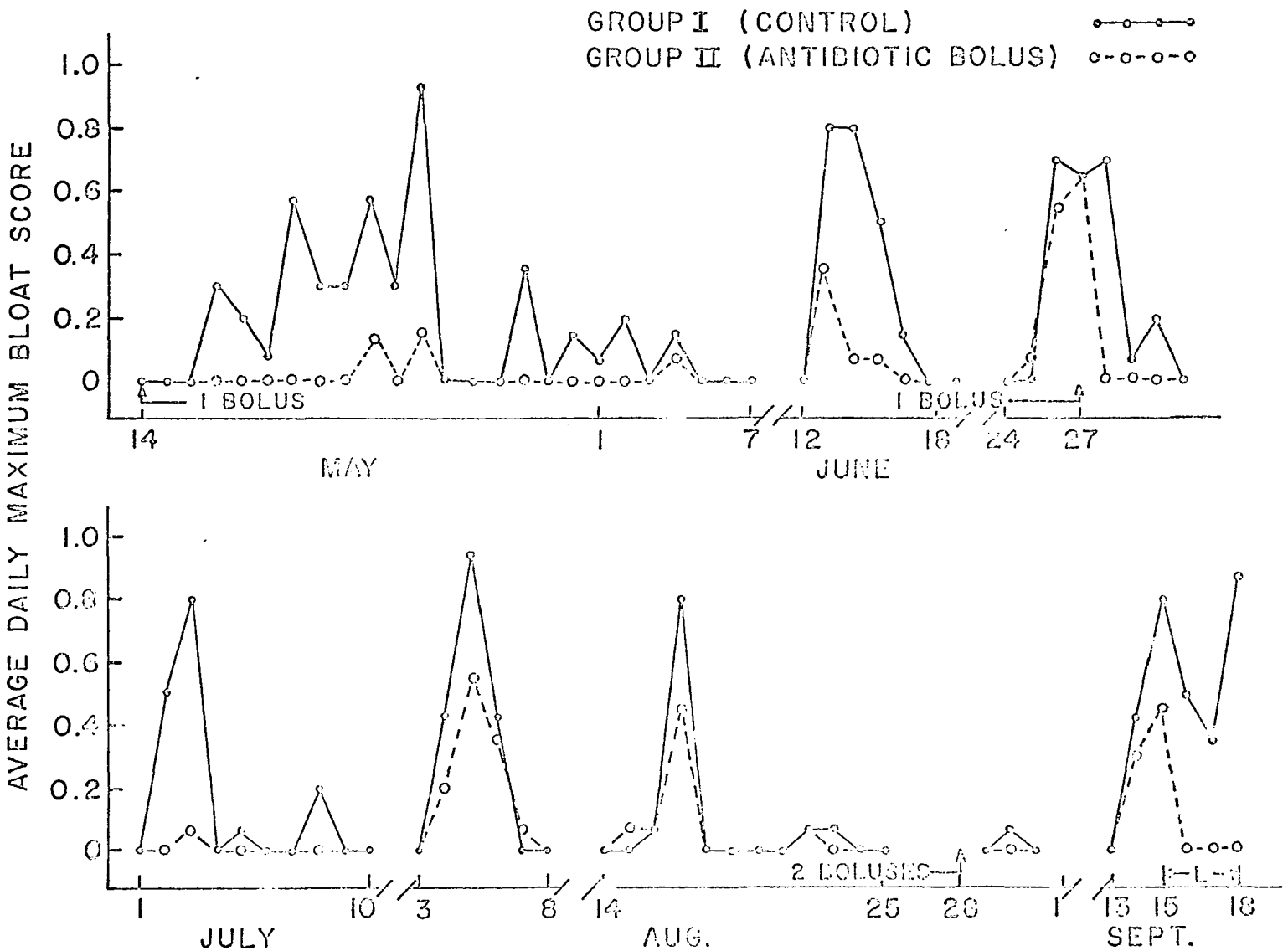

GROUP I (CONTROL)
GROUP II (ANTIBIOTIC BOLUS)
AVERAGE DAILY MAXIMUM BLOAT SCORE
1.0
0.8
0.6
0.4
0.2
0
1 BOLUS
1 BOLUS
14
1
7
12
18
24
27
MAY
JUNE
1
10
3
8
14
25
28
1
13
15
18
2 BOLUSES
JULY
AUG.
SEPT.

Figure 2. Effect of antibiotic boluses on the average daily maximum bloat score

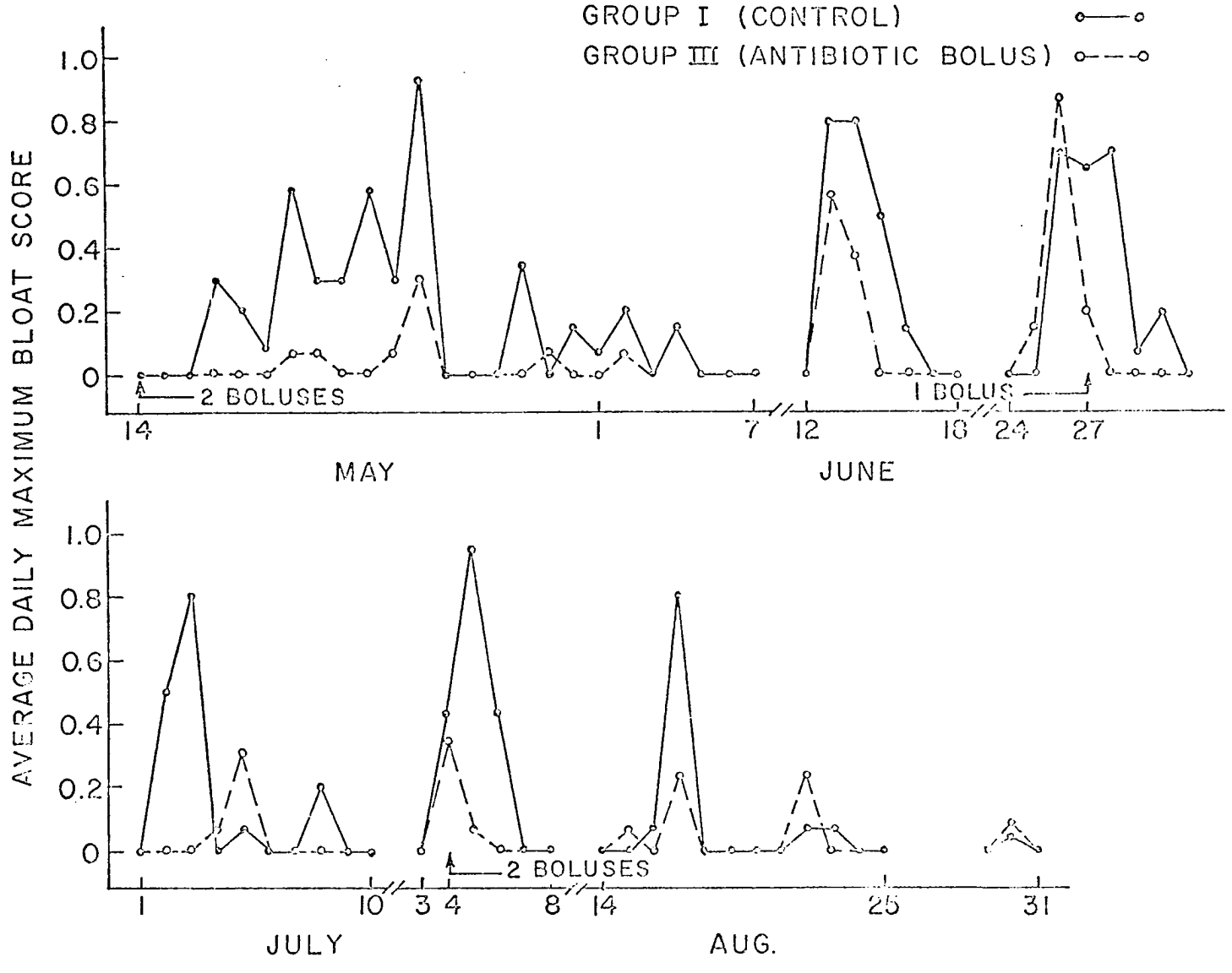
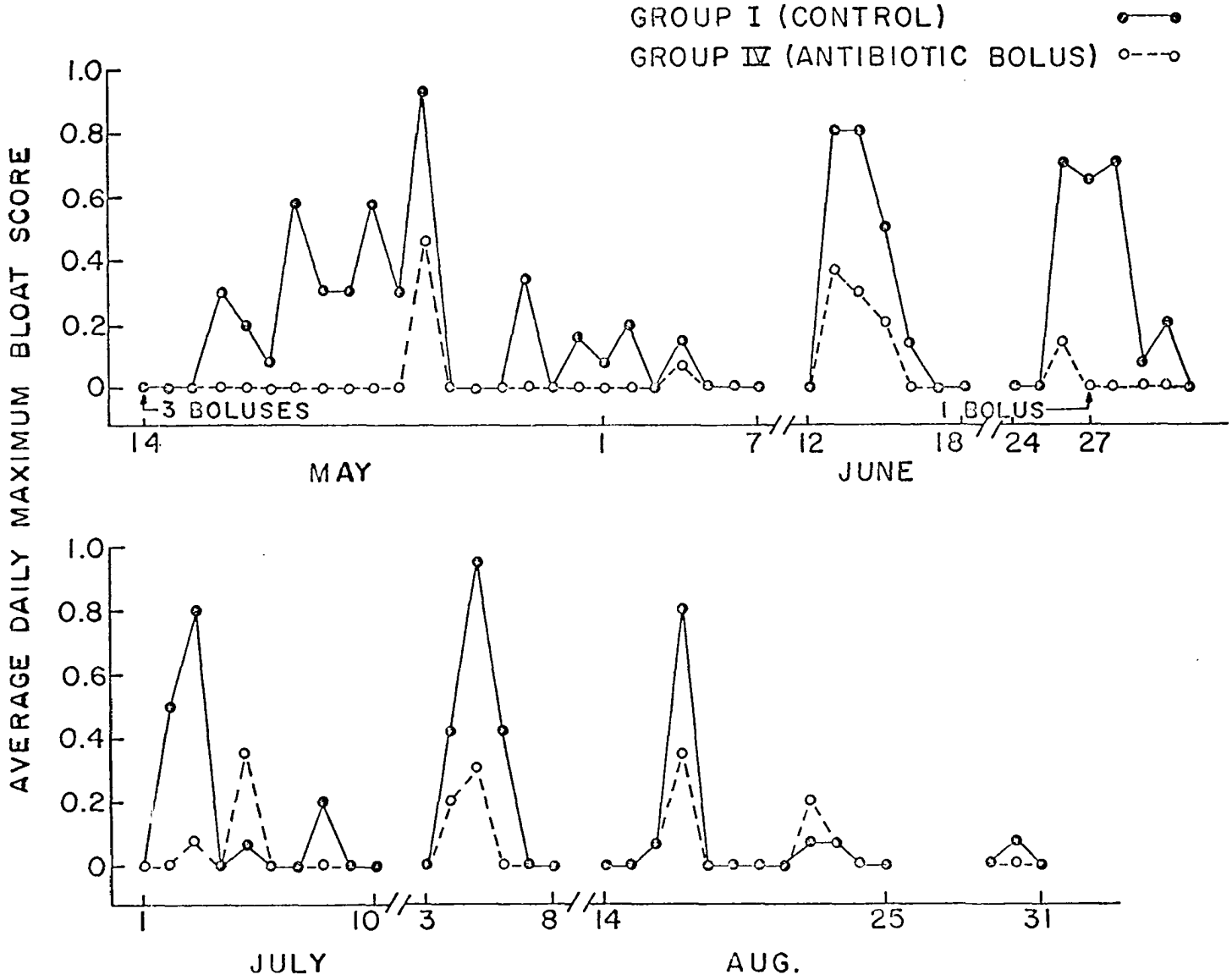

GROUP I (CONTROL)
GROUP III (ANTIBIOTIC BOLUS)
AVERAGE DAILY MAXIMUM BLOAT SCORE
1.0
0.8
0.6
0.4
0.2
0
2 BOLUSES
1 BOLUS
14
1
7
12
18
24
27
MAY
JUNE
1
10
3
4
8
14
25
31
JULY
AUG.

Figure 3. Effect of antibiotic boluses on the average daily maximum bloat score


GROUP I (CONTROL)
GROUP IV (ANTIBIOTIC BOLUS)
AVERAGE DAILY MAXIMUM BLOAT SCORE
1.0
0.8
0.6
0.4
0.2
0
3 BOLUSES
1 BOLUS
14
1
7
12
18
24
27
MAY
JUNE
1
10
3
8
14
25
31
JULY
AUG.

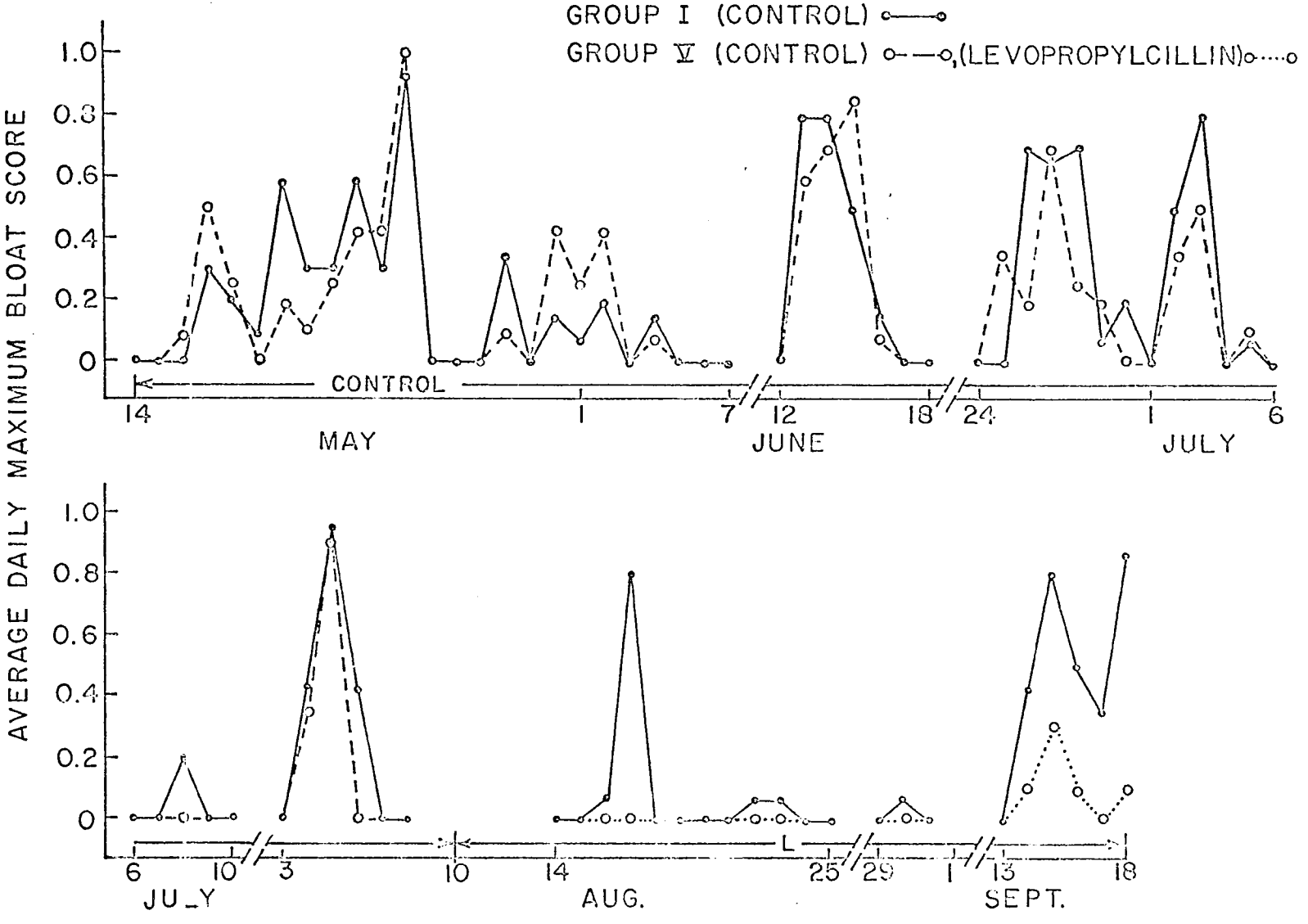

GROUP I (CONTROL)
GROUP V (CONTROL)
(LEVOPROPYLCILLIN)
AVERAGE DAILY MAXIMUM BLOAT SCORE
1.0
0.8
0.6
0.4
0.2
0
CONTROL
14
1
7
12
18
24
1
6
MAY
JUNE
JULY
L
6
10
3
10
14
25
29
1
13
18
JU_Y
AUG.
SEPT.

Figure 4. Effect of potassium levopropylcillin on the average daily maximum bloat score (L = potassium levopropylcillin. Each animal was given 100 mg. in a gelatin capsule by balling gun at 48-hour intervals)

bloat for about 3 to 4 weeks. After this period of bloat reduction, the treated group (Group II) almost always bloated considerably more than the control group (Group I), even after subsequent administration of one or two boluses. Later in the season, bloat declined 30% when each animal received daily an average of 50 mg. of potassium levopropylcillin. When the level was increased to 100 mg., there was an additional 50% decline in bloat. Although the results suggest that the antibiotic depressed bloat, the data cannot be considered conclusive since no control group was employed during this period.

The results obtained with sheep were similar to those obtained with cattle. In Flock A, Trial I, the initial administration of one-half of a cattle bolus to each mature ewe effected a 75% reduction in bloat incidence for 3 to 4 weeks. Subsequent to the administration of the same dosage 4 weeks after the first bolus, the treated group had an incidence of bloat which was 7% higher than that in the control group. Death losses in the control and bolus groups were 6.7 and 8.0%, respectively. In Flock A, Trial II, ewes receiving the antibiotic bolus bloated 15% more frequently than the controls. Death losses were 14 and 19% in the control and bolus groups, respectively. Bloat incidence was not reduced by feeding 20 mg. of potassium levopropylcillin per ewe daily in a grain mixture. However, when the level was increased to 40 mg. daily the amount of bloat declined gradually, and by the sixth day bloat no longer occurred. Mild bloat occurred on the second day after the removal of the potassium levopropylcillin from the grain, and continued for several days. Ewes that had previously received anti-

biotic boluses and those that had not received antibiotic boluses responded similarly to potassium levopropylcillin. In Flock B, the administration of one sheep bolus per ewe resulted in an 81% reduction in bloat incidence during a 2-week period. Death losses in the control and bolus groups were 12.0 and 1.3%, respectively.

Weight gains The effect of the antibiotic bolus on weight gains as determined in the three Iowa State University herds is shown in Table 3. At Castana, the antibiotic treated steers gained significantly ($P < .025$) more than the control steers. The differences obtained in the other experiments were not statistically significant ($P > .05$), even though the antibiotic treated steers at Ames gained more (0.21 lb. per animal daily) than the control steers.

Occurrence of adverse effects No adverse effects attributable to the antibiotics were observed in any of the Iowa State University herds. However, one of the cooperating farmers noticed transient diarrhea in several animals subsequent to the administration of antibiotic boluses.

About 75% of the treated ewes in Flock A, Trial I, exhibited extensive loss of wool after the administration of the second bolus at 4 weeks. The wool was lost, usually in patches, along the dorsal surface of the body. No loss of wool was observed in the control group. This condition did not occur in any of the other trials involving sheep.

Table 3. Effect of antibiotic combination on weight gains of cattle on pasture

Location of experiment	Group	Number of animals	Sex	Number of days	Average daily gain, lb.
Ames	Control	14	Steers	114	1.00
Ames	Bolus	40[a]	Steers	114	1.21
Castana	Control	10	Heifers	126	.91
Castana	Bolus	10	Heifers	126	.94
Castana	Control	8	Steers	126	.71*
Castana	Bolus	8	Steers	126	1.17*
Napier	Control	12	Steers	158	1.40
Napier	Bolus	12	Steers	158	1.36

[a]Two animals in the antibiotic combination bolus group had to be removed from experiment and were not included in the weight gain computations. One animal died of bloat and the other one was sold because of actinomycosis.

*$P < .025$.

Point of deposition, movement and disintegration of antibiotic boluses in the reticulo-rumen

Two minutes after the administration of four small (34 gm.) boluses to each of four fistulated steers on a hay-grain diet, the distribution of the boluses in the reticulo-rumen was as follows: anterior dorsal blind sac - 13, reticulum - 3. Four days and 30 days later, respectively, the locations were: ventral rumen - 3, reticulum - 13, ventral rumen - 4, reticulum - 12. Subsequently, remnants of the boluses were removed from two of the steers and each steer was given three large (64 gm.) boluses. Two minutes after administration, three boluses were found in the anterior dorsal blind sac and three in the reticulum; 4 days later, all were found in the reticulum.

The four fistulated steers later were placed on alfalfa pasture for several months. During that period, two large boluses were administered to each animal. Two weeks after administration, all boluses were found in the reticulum.

The weights of the boluses recovered from the four intact steers are presented in Table 4. The "time in the reticulo-rumen" varied among steers because the interval between the administration of the last boluses and the time of sacrifice was not constant. The boluses gradually eroded, becoming cylindrical with well-rounded ends. The rate of erosion varied considerably among the four animals. Remnants of the boluses remained in the reticulo-rumen for periods of time ranging from 125 to 225 days. The average half-life (weight basis) of the boluses was approximately 60 days. Photographs of the boluses recovered from the reticulo-rumen of animal nos. 12 and 14 are presented in Plate 1.

Effect of a combination of antibiotics administered at 1-, 2- and 3-day intervals

Bloat incidence and severity The administration of 250 mg. of STEP per animal at either 1-, 2- or 3-day intervals reduced bloat during the period from May 6 to June 1, 1963 (Figures 5, 6 and 7). The average daily maximum bloat scores (ADM) for Groups I, II and III were 89, 87 and 91% less, respectively, than that of Group IV (control). Very little bloat occurred during the period from June 2 to July 23. The limited observations obtained on July 20 indicate that the administration of 250 mg. of STEP at 3-day intervals (Group III) was effective for as long as (and perhaps longer than) the same dosage given at 1- or 2-day inter-

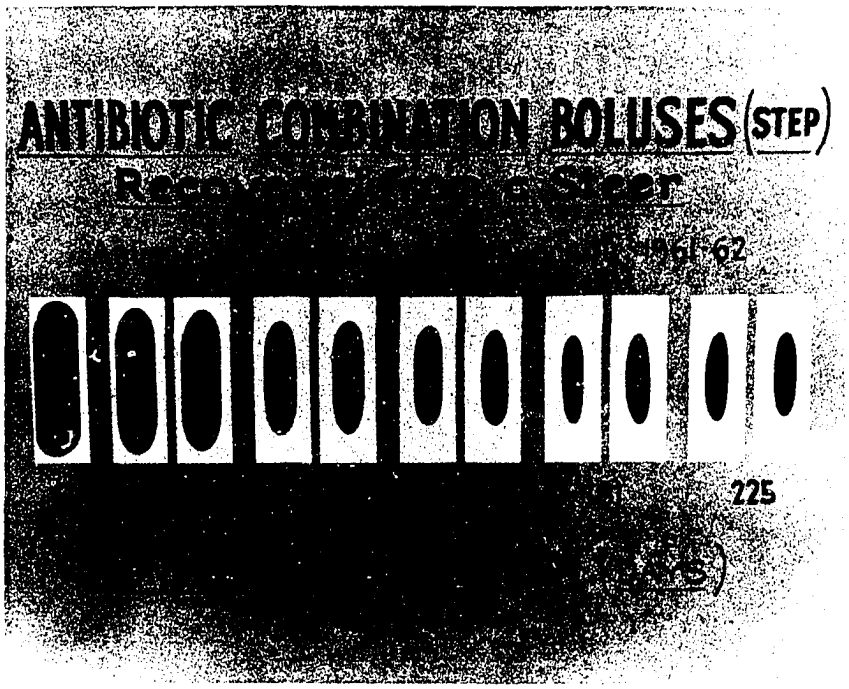
Table 4. Weights of boluses recovered after various periods in the reticulo-rumen[a]

Animal number	Mean initial weight of bolus, gm.	Time in reticulo-rumen, days												
		42	55	67	84	88	97	109	126	130	139	172	181	223
		(Mean weight of recovered boluses, gm.)												
1	64			30.4				19.3						
11	64					17.3				15.2		8.7		
12	64	36.8			14.1				3.8					
14	64		43.6				20.8				12.6		6.8	5.9

[a]Each animal was given 2 boluses at 6-week intervals until a total of 14 boluses had been administered. Animals 1, 11, 12 and 14 were sacrificed at 67, 88, 42 and 55 days, respectively, after the last pair of boluses was administered, and all boluses in the reticulo-rumen were recovered, dried and weighed. Each weight represents the mean of 2 boluses except for animal 1 at 109 days when only one bolus was recovered.

Plate 1. Boluses recovered after various periods in the reticulo-rumen (Each animal was given 2 boluses at 6-week intervals until a total of 14 boluses had been administered. Animals 12 and 14 were sacrificed at 42 and 55 days, respectively, after the last pair of boluses was administered, and all boluses in the reticulo-rumen were recovered)

Figure 5. Effect of administering STEP (250 mg. per animal) at 1-day intervals on the average daily maximum bloat score


ANTIBIOTIC COMBINATION BOLUSES (STEP)
1961-62
225

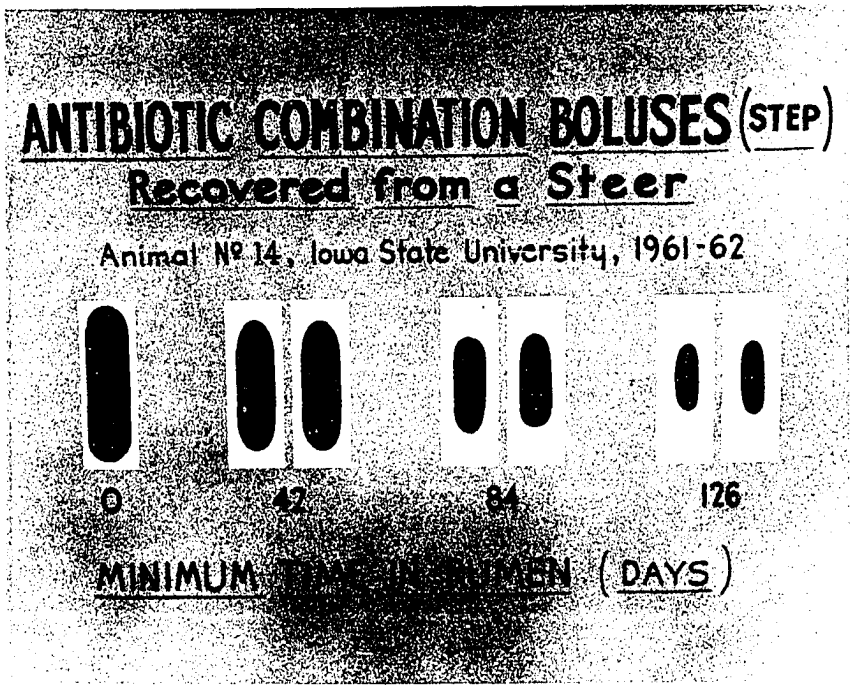

ANTIBIOTIC COMBINATION BOLUSES (STEP)
Recovered from a Steer
Animal Nº 14, Iowa State University, 1961-62
0
42
84
126
(DAYS)

Figure 6. Effect of administering STEP (250 mg. per animal) at 2-day intervals on the average daily maximum bloat score

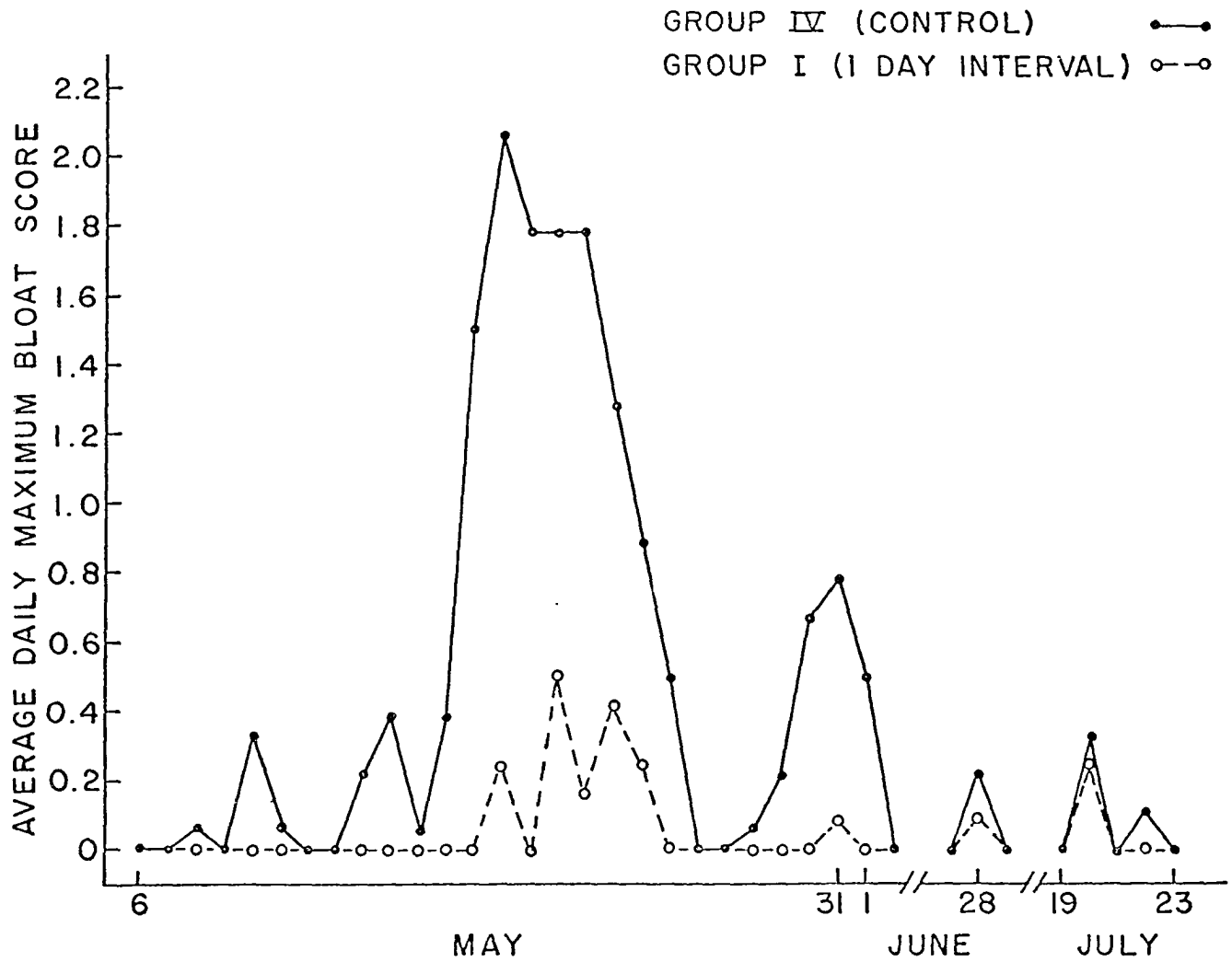
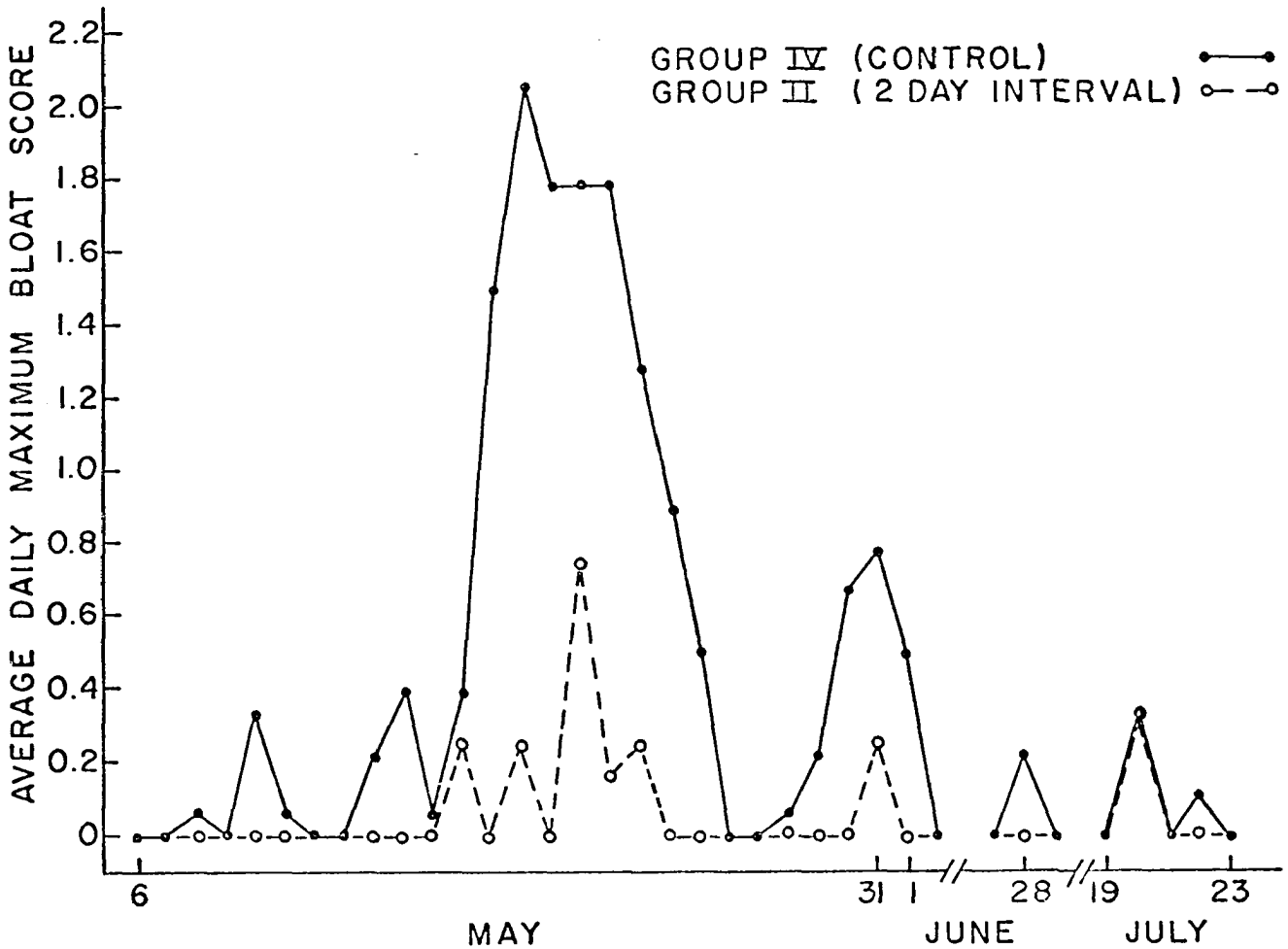
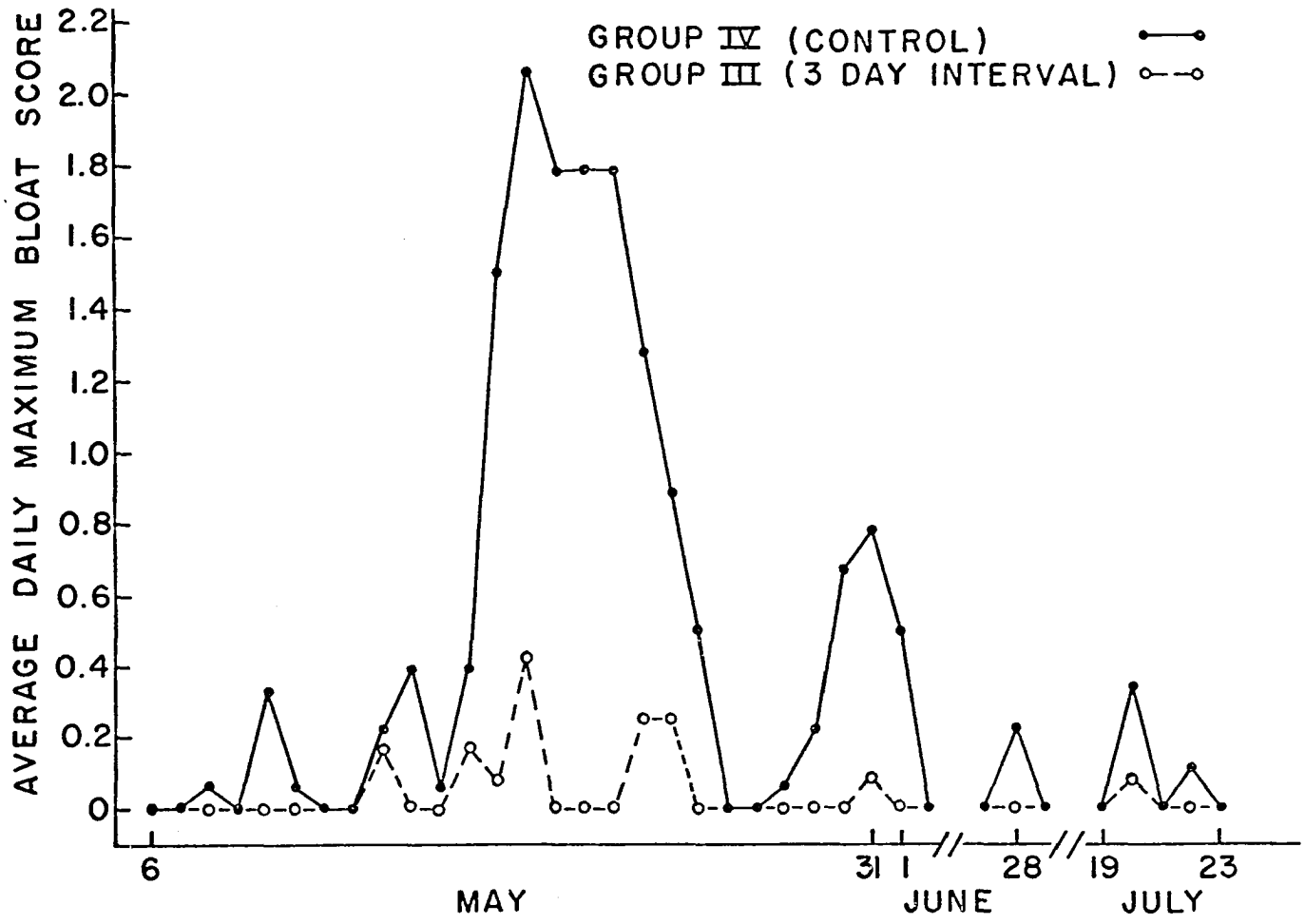

GROUP IV (CONTROL)
GROUP I (1 DAY INTERVAL)
AVERAGE DAILY MAXIMUM BLOAT SCORE
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
6
31
1
28
19
23
MAY
JUNE
JULY

Figure 7. Effect of administering STEP (250 mg. per animal) at 3-day intervals on the average daily maximum bloat score


AVERAGE DAILY MAXIMUM BLOAT SCORE
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
GROUP IV (CONTROL)
GROUP II (2 DAY INTERVAL)
6
31
1
28
19
23
MAY
JUNE
JULY


GROUP IV (CONTROL)
GROUP III (3 DAY INTERVAL)
AVERAGE DAILY MAXIMUM BLOAT SCORE
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
6
MAY
31
1
JUNE
28
19
23
JULY

vals. The long-term effect of these treatment intervals could not be evaluated adequately, however, because no bloat occurred between July 23 and September 18.

Appendix Tables 17 and 18 show the bloat data collected on each day of the experiment.

Weight gains The effect of a combination of antibiotics on the weight gains of cattle is presented in Table 5. The antibiotic treated animals usually gained more than the control animals; however, there were no statistically significant differences among the groups ($P > .05$). The steers gained significantly ($P < .01$) more than the heifers.

Table 5. Effect of a combination of antibiotics on the weight gains of cattle receiving alfalfa soilage[a,b]

Sex	Group I	Group II	Group III	Group IV	Sex means
	Average daily gain, lb.				
Heifers	1.33 (6)	1.33 (6)	1.48 (6)	1.17 (3)	1.35**
Steers	1.92 (6)	1.53 (6)	1.74 (6)	1.65 (15)	1.69**
Group means	1.62	1.43	1.61	1.57	

[a]Numbers in parenthesis indicate the number of animals included in each value.

[b]Length of trial, 142 days.

**Significantly different ($P < .01$).

Effect of feeding regime on the response of cattle to the initiation of antibiotic administration

Experiment 1 No adverse effects attributable to the antibiotic combination were observed in any of the animals used in this experiment. The rations consisted primarily of roughages - alfalfa hay, corn silage and alfalfa hay, or alfalfa soilage.

Experiments 2 and 4 Steers receiving a ration consisting primarily of alfalfa hay (Groups II and III) when 250 mg. of a combination of antibiotics (STEP) was fed daily did not show symptoms of diarrhea or depressed grain consumption (Figures 8 and 9). Only a few of the steers receiving the high grain ration (Group I) exhibited a slight to moderate depression in grain consumption (Figures 8 and 9), but none showed symptoms of diarrhea.

Experiment 3 Feeding 250 mg. of a combination of antibiotics (STEP) daily caused a drastic and abrupt decline in grain consumption by all of the heifers receiving a high grain ration (Figure 10, Group I). Two of the three heifers returned to normal grain consumption by the seventh feeding period after the antibiotic feeding had started. However, the third heifer consistently refused to eat most of the grain which contained the antibiotics (morning feeding) but readily ate grain not containing the antibiotics (evening feeding). This same heifer showed symptoms of diarrhea by the evening of the first day; these symptoms continued for 3 days. One of the other heifers also showed symptoms of mild diarrhea by the evening of the first day but had recovered by the next morning. None of the heifers in Groups II and III

Figure 8. Effect of a combination of antibiotics on the grain consumption of steers in Experiment 2 (There were four steers per group; each steer was fed 250 mg. of STEP daily with the grain at the morning feeding)

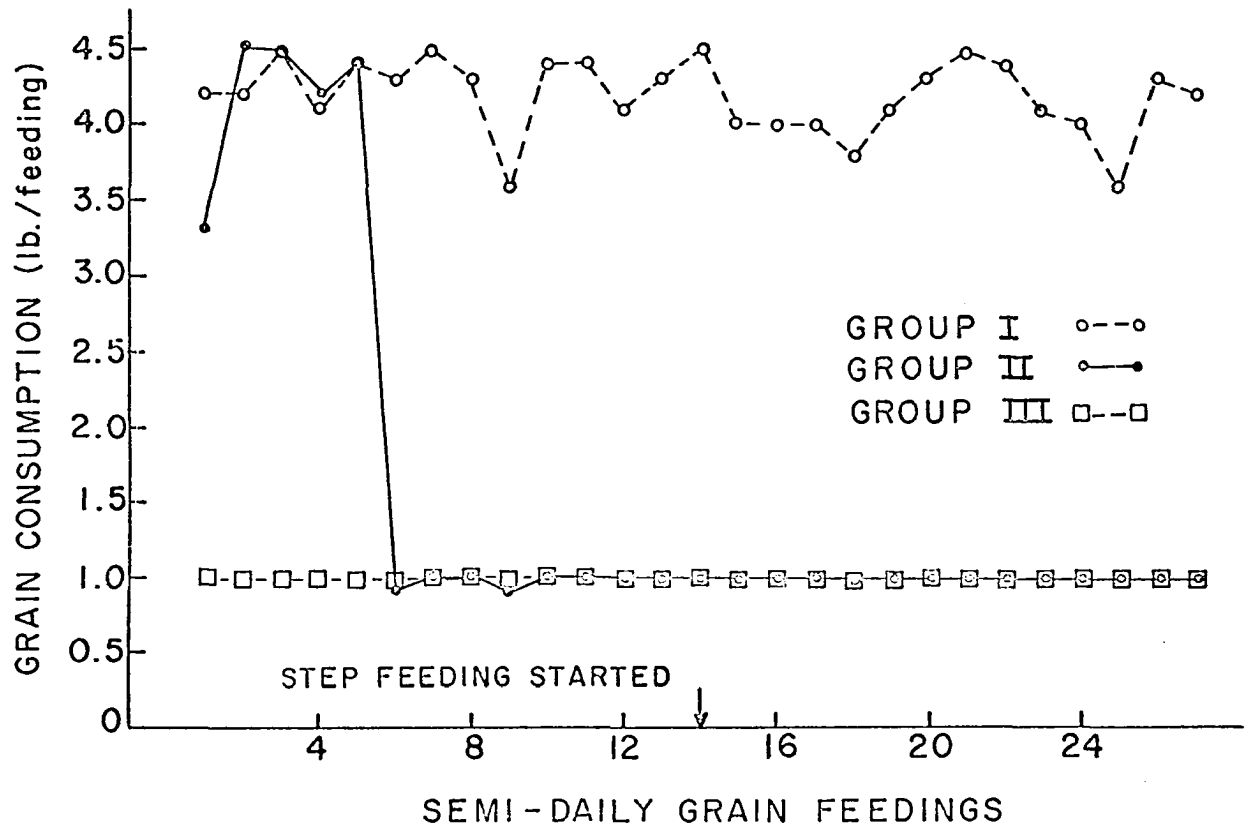

GRAIN CONSUMPTION (lb./feeding)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
GROUP I
GROUP II
GROUP III
STEP FEEDING STARTED
4
8
12
16
20
24
SEMI-DAILY GRAIN FEEDINGS

Figure 9. Effect of a combination of antibiotics on the grain consumption of steers in Experiment 4 (There were four steers per group; each steer was fed 250 mg. of STEP daily with the grain at the morning feeding)

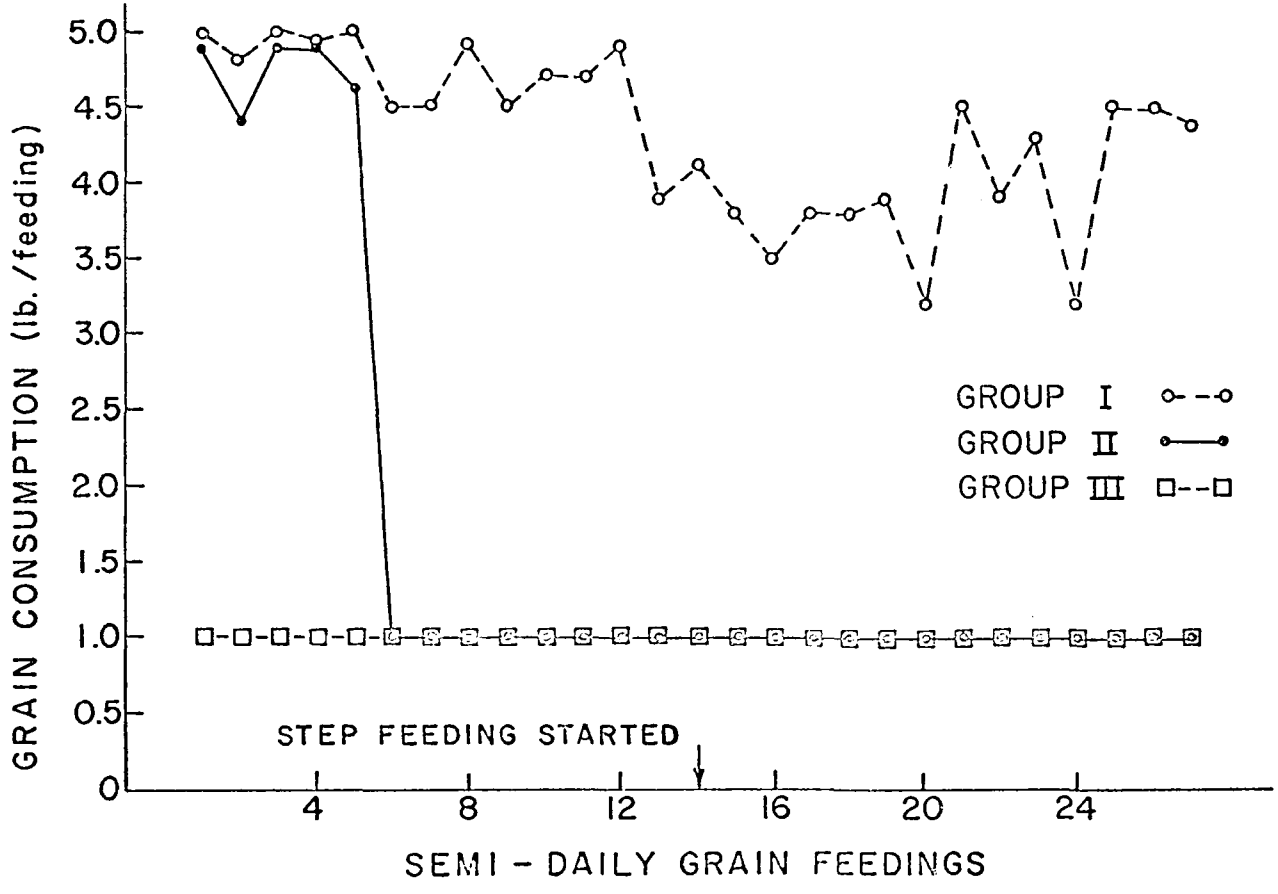
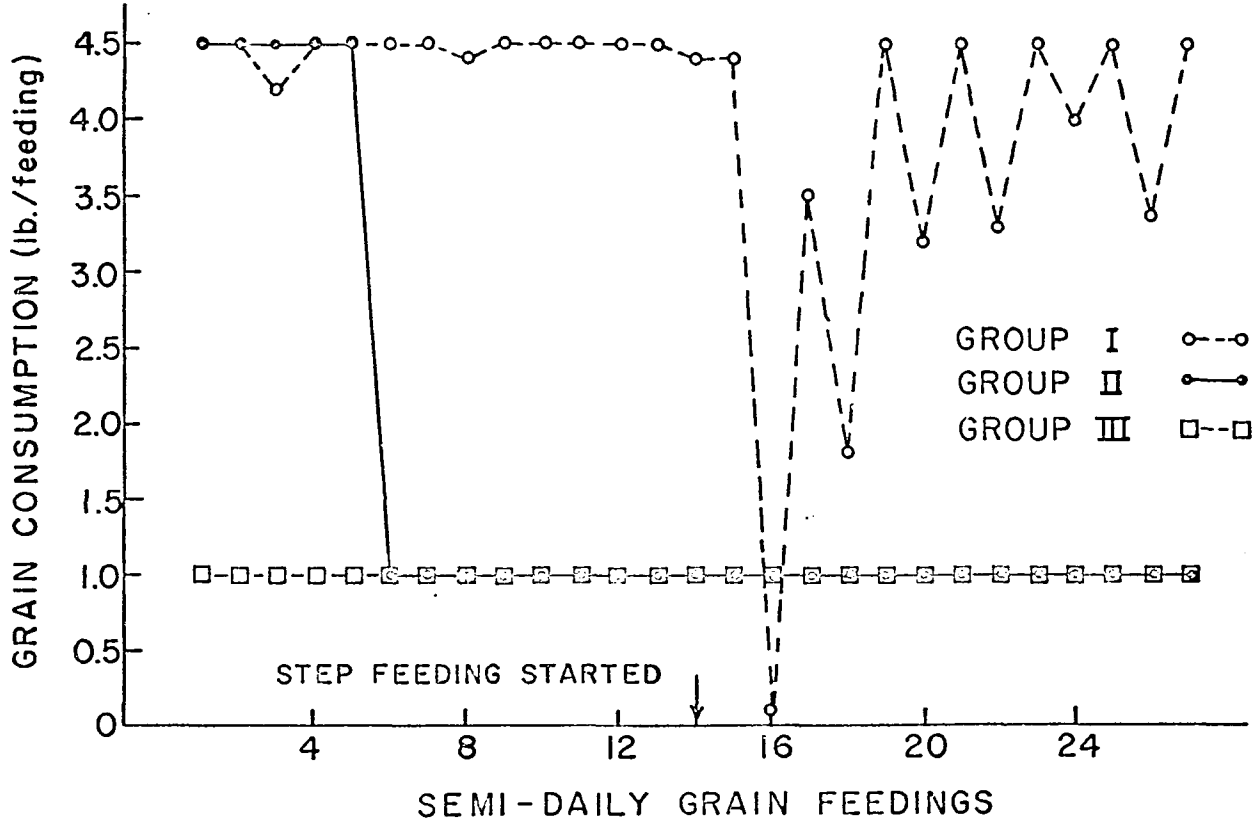

GRAIN CONSUMPTION (lb./feeding)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
GROUP I
GROUP II
GROUP III
STEP FEEDING STARTED
4
8
12
16
20
24
SEMI - DAILY GRAIN FEEDINGS

Figure 10. Effect of a combination of antibiotics on the grain consumption of heifers in Experiment 3 (There were three heifers per group; each heifer was fed 250 mg. of STEP daily with the grain at the morning feeding)


GRAIN CONSUMPTION (lb./feeding)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
STEP FEEDING STARTED
4
8
12
16
20
24
SEMI-DAILY GRAIN FEEDINGS
GROUP I
GROUP II
GROUP III

(receiving primarily alfalfa hay rations) exhibited symptoms of diarrhea or depressed appetite.

Laboratory Studies

Effect of added energy and form of soybean oil on the lipolytic activity of rumen fluid

A factorial design was used in this experiment to determine the effect of added energy (100 mg. of gum cellulose, 100 mg. of dextrose and 100 mg. of maltose) and of form of soybean oil (refined, emulsified and crude) on the lipase activity of rumen fluid. The crude soybean oil was purchased from Cargill, Inc., Des Moines, Iowa, and the emulsified oil was prepared according to the procedure of Johnson et al. (1960a). For this trial only, equivalent amounts of esterified lipid in the form of crude or emulsified oil replaced the refined soybean oil in the enzyme assay method described earlier. The amount of esterified lipid added to each flask before incubation was approximately equal to that contained in 55 mg. of refined soybean oil.

The results are presented in Table 6 and Appendix Tables 19 and 20. The addition of 100 mg. of dextrose or maltose to the rumen fluid before incubation significantly reduced ($P < .01$) the lipolytic activity of the rumen microorganisms. The form of soybean oil had no significant effect ($P > .05$) on the lipolytic activity of rumen fluid, even though crude oil was hydrolyzed to a slightly greater extent than was either the emulsified or refined oil, the refined oil showing the least amount of hydrolysis.

Table 6. Effect of various sources of added energy and of form of soybean oil on the lipolytic activity of rumen fluid

Oil	Added energy				Mean
	None	Gum cellulose (100 mg.)	Dextrose (100 mg.)	Maltose (100 mg.)	
		(% hydrolysis)[a]			
Refined	60.3	61.0	36.0	34.8	48.0
Emulsified	67.9	66.6	35.7	33.9	51.0
Crude	68.1	69.2	41.2	37.7	54.0
Mean[b]	65.4	65.6	37.6	35.5	

[a]Each value is an average of four observations.

[b]Any two means not underscored by the same line are significantly different ($P < .01$) by Duncan's Multiple Range Test.

An additional experiment was conducted to determine the effect of various amounts of several sources of added energy on the lipase activity of rumen fluid. In this trial, either 200, 400, 600, or 800 mg. each of gum cellulose, dextrose and sucrose were added to assay flasks before incubation. Refined soybean oil was used as the substrate for the enzyme assay.

Table 7 and Appendix Table 21 present the data obtained in this experiment. There was a linear decrease in lipolysis as the amount of added energy increased. The addition of gum cellulose caused an increase in lipolysis while the addition of dextrose and sucrose caused a decrease in lipolysis. Sucrose (at all levels used) and dextrose (more than 200 mg.) not only decreased the hydrolysis of soybean oil but caused an apparent synthesis of ester because more ester was recovered at the end of incubation than was present at the start. The

Table 7. Effect of source and level of added energy on the lipolytic activity of rumen fluid

Treatment	Mean[a,b] (% hydrolysis)[c]	
Control	24.9	FG
Gum cellulose - 200 mg.	55.6	G
Gum cellulose - 400 mg.	45.2	FG
Gum cellulose - 600 mg.	48.5	G
Gum cellulose - 800 mg.	38.9	FG
Dextrose - 200 mg.	9.5	EFG
Dextrose - 400 mg.	-58.8	BC
Dextrose - 600 mg.	-96.7	AB
Dextrose - 800 mg.	-143.9	A
Sucrose - 200 mg.	-6.9	DEF
Sucrose - 400 mg.	-33.4	CDE
Sucrose - 600 mg.	-48.7	BCD
Sucrose - 800 mg.	-76.9	BCD

[a]Each value is an average of two observations.

[b]Any two means not having common superscripts are significantly different ($P < .01$) by Duncan's Multiple Range Test.

[c]Negative values indicate an apparent net synthesis of ester.

addition of 200 and 600 mg. of gum cellulose significantly increased ($P < .01$) the lipolytic activity of rumen fluid and the 400, 600 and 800 mg. additions of sucrose and dextrose significantly increased ($P < .01$) the apparent synthesis of ester.

Effect of various antibiotics on the lipolytic activity of rumen microorganisms

Rumen microorganisms were removed from rumen fluid and separated into bacterial and protozoal fractions by differential centrifugation methods discussed earlier. Various antibiotics were added to aliquots of the culture media prior to incubation at the rate of .003% (w/v).

The antibiotics used were streptomycin sulfate, tylosin phosphate, erythromycin thiocyanate and procaine penicillin. Lipid hydrolysis was determined as previously described. The data are presented in Table 8 and Appendix Tables 22 and 23. None of the antibiotics had any significant effect ($P > .05$) on the lipolytic activity of rumen microorganisms. However, penicillin, tylosin and streptomycin appeared to produce a slight stimulatory effect even though the individual responses were quite variable. The bacterial culture possessed significantly more ($P < .01$) lipolytic activity than did the protozoal culture. The activity of the mixed culture was significantly greater ($P < .01$) than that of the bacterial or protozoal cultures alone. A possible synergistic relationship between the bacteria and protozoa is suggested because the activity of the mixed culture was greater than the sum of the activities of the bacteria and protozoa when assayed separately.

Since none of the antibiotics had any apparent effect on the lipolytic activity of the microorganisms when added to the culture immediately prior to incubation for enzyme assay, it was thought that the lack of sufficient time for the antibiotics to exert their effects on the microorganisms might be responsible. Thus, another trial was conducted in which the microorganisms were subjected to more concentrated antibiotic solutions (.006%, w/v) for 22 hours (39°C. under CO_2) prior to the addition of the enzyme substrate (refined soybean oil). The enzyme assay was subsequently conducted according to the previously described procedure. The results are presented in Table 9 and Appendix Tables 24 and 25. Under these conditions, streptomycin significantly

Table 8. Effect of streptomycin, tylosin, erythromycin and penicillin on the lipolytic activity of rumen microorganisms

Organisms	Antibiotics					Mean[a]
	None	Streptomycin (.003%)	Tylosin (.003%)	Erythromycin (.003%)	Penicillin (.003%)	
			(% hydrolysis)[b]			
Protozoa	20.9	24.1	19.6	17.7	26.3	21.7[A]
Bacteria	39.0	37.1	50.3	40.9	44.5	42.4[B]
Protozoa and bacteria	83.3	84.3	80.2	84.0	83.8	83.1[C]
Mean	47.7	48.5	50.0	47.5	51.6	

[a]Any two means not having common superscripts are significantly different ($P < .01$) by Duncan's Multiple Range Test.

[b]The value for each treatment is a mean of four observations.

Table 9. Effect of 22 hours of incubation with streptomycin, tylosin, erythromycin and penicillin on the subsequent lipolytic activity of rumen microorganisms

Organisms	Antibiotics					Mean[a]
	None	Streptomycin (.006%)	Tylosin (.006%)	Erythromycin (.006%)	Penicillin (.006%)	
			(% hydrolysis)[b]			
Protozoa	39.2	65.7	34.1	45.8	34.1	43.8^{A}
Bacteria	42.1	46.5	39.6	46.2	51.4	45.1^{A}
Protozoa and bacteria	78.1	82.3	81.6	79.9	80.6	80.5^{B}
Mean[c]	53.1^{C}	64.8^{D}	51.7^{C}	57.3^{C}	55.3^{C}	

[a]Any two means not having common superscripts are significantly different ($P < .01$) by Duncan's Multiple Range Test.

[b]The value for each treatment is a mean of two observations.

[c]Any two means not having common superscripts are significantly different ($P < .05$) by Duncan's Multiple Range Test.

increased ($P < .05$) the lipolytic activity of the microorganisms. Penicillin and erythromycin increased the activity slightly and tylosin reduced the activity but none of these differences were significant ($P > .05$). The protozoal and bacterial cultures, when incubated separately, had similar lipolytic activities; each of which was significantly lower ($P < .01$) than that for the mixed culture. The activity of the mixed culture was lower than the sum of the activities of the single cultures.

An additional experiment was conducted to determine whether the rumen fluid obtained from the fibrous mat (dorsal region) of the rumen and that obtained from the ventral region of the rumen would respond similarly to antibiotic treatment. For this study, rumen fluid was removed from the two areas just mentioned and each sample was processed separately. The lipolytic activity of the rumen fluid and various fractions thereof for each sample was determined according to the procedures already described. The data are presented in Table 10 and Appendix Tables 26 and 27. The point of collection of the rumen fluid had no significant effect ($P > .05$) on the lipase activity. The addition of penicillin (.003%, w/v) appeared to reduce the lipase activity of the fluid from the ventral rumen and increase the activity of the fluid from the dorsal region. Over-all, the addition of penicillin (.003%, w/v) to rumen fluid or fractions thereof had no significant effect ($P < .01$) on lipase activity. The mixed culture had a significantly greater ($P < .01$) lipase activity than did the single cultures. The activity of the mixed culture was approximately equal to the sum of the activities of the single cultures.

Table 10. Effect of penicillin and point of collection of rumen fluid on the lipolytic activity of rumen microorganisms

Enzyme preparations	Point of collection of rumen fluid		Means[a]
	Ventral rumen	Dorsal rumen	
	(% hydrolysis)[b]		
Rumen fluid	64.4	51.7	58.0[A]
Rumen fluid plus .003% penicillin	53.6	65.7	59.6[A]
Protozoa	36.3	41.2	38.7[B]
Bacteria	37.9	51.8	44.8[B]
Bacteria plus .003% penicillin	35.2	38.7	37.0[B]
Protozoa and bacteria	82.4	82.4	82.4[C]
Protozoa and bacteria plus .003% penicillin	80.1	81.6	80.8[C]
Point of collection means	55.7	59.0	

[a]Any two means not having common superscripts are significantly different ($P < .01$) by Duncan's Multiple Range Test.

[b]Each value is a mean of four observations.

DISCUSSION

Previous research at Iowa State University (Johnson et al., 1960b; Van Horn et al., 1961) has shown that feeding a combination of antibiotics in grain daily is a practical method of controlling bloat in cattle grazing alfalfa pasture. However, when a combination of antibiotics was fed in wheat middlings-molasses pellets, some of the animals refused to eat the pellets after the first day of feeding (Van Horn et al., 1963). This occurrence of "palatability problems" associated with the antibiotic pellets led to the development of an antibiotic-containing bolus which, when introduced into an aqueous solution, released the antibiotic slowly. The bolus could be administered to the animal by balling gun thus eliminating any possible palatability troubles. It was postulated that the antibiotic(s) would "pay out" from the bolus at a relatively constant rate and might control bloat for an extended period of time after one dose. The main question regarding the slow-release bolus was this: would the continuous release of antibiotics into the rumen produce the same prophylactic effect as the daily oral administration of small amounts of the same antibiotic combination? Data presented herein demonstrate that the combination of antibiotics contained in the bolus controlled bloat for about 3 to 4 weeks following the initial administration. Subsequent administration of additional boluses controlled bloat for only 1 week. After this, bloating occurred as frequently as and, in some cases, more frequently than in the controls. These observations suggest that the slow continuous release of

antibiotics into the rumen leads to a more rapid development of "resistance" than the daily feeding of small amounts of the antibiotics since the same antibiotics administered in grain or pellets were effective for a considerably longer period. Nevertheless, the bolus may be quite useful for bloat control over relatively short periods. Moreover, it is possible that improvements can be introduced which will prolong its effectiveness.

Although appetite of the animals was not visibly altered, transient mild diarrhea occurred in several animals subsequent to the administration of the boluses. The reason(s) for this effect is unknown. In one of the sheep trials, about 75% of the ewes which had received antibiotic boluses lost some wool from the dorsal surface of the body. In some cases, only small patches were involved while in others the complete dorsal surface became bald, sun burned and sometimes blistered. This alopecia was definitely attributable to the antibiotic bolus because none of the control animals were affected. All of the ewes had been sheared only 2 days before the first bolus was administered and most of the alopecia occurred subsequent to the administration of the second bolus 4 weeks later. Wass and Hoyt (1963) have discussed the pathogenesis of porphyria in cattle. This condition is usually congenital but cases of "acquired porphyria" have been produced in experimental animals by the administration of various toxic chemicals. The chemicals interfere with the metabolism of porphyrins in the body, and thus, the concentration of porphyrins in the blood and tissues becomes greater. Since the porphyrin molecule has a highly resonating ring structure

which makes it very photodynamic, the animal tissues become very sensitive to the direct rays of the sun and severe skin lesions may occur. White animals appear to be more sensitive than black or nearly black animals. It is possible that the antibiotic(s) and/or the inert ingredients of the bolus interfered with the porphyrin metabolism in the ewes and an "acquired porphyria" condition occurred. If this condition can be repeated, additional work should be done to determine the exact nature and causes of this syndrome. It might then be possible to modify the bolus to eliminate its undesirable characteristics.

Since the antibiotic bolus, in its present form, has some shortcomings as a bloat prophylactic agent, and since considerable time may be required to make the modifications necessary for its improvement, additional research on the oral administration of antibiotics and the elimination of undesirable effects during the initiation of antibiotic feeding seemed highly desirable.

Early research by Barrentine et al. (1956) demonstrated that a single dose of penicillin controlled bloat in steers grazing ladino clover for periods of 1 to 3 days. Later research (Barrentine et al., 1958; Brown et al., 1958; Johnson, 1959; Mangan et al., 1959) showed that animals developed a "resistance" to penicillin and erythromycin after a short period (1 to 2 weeks) of continued use. Studies by Johnson et al. (1960b) and Van Horn et al. (1961, 1963) demonstrated that the development of "resistance" to antibiotics was delayed by daily feeding a combination of antibiotics continuously during the grazing season or intermittently, whenever bloat was likely to occur.

Results reported herein show that the administration of a combination of antibiotics (STEP) at 1-, 2- and 3-day intervals reduced bloat by 89, 87 and 91%, respectively, during a 4-week period. There was an indication that the administration of STEP at 3-day intervals controlled bloat for as long as, and possibly longer than, STEP administered at 1- and 2-day intervals. It is possible that the administration of antibiotics at 3-day intervals may retard the development of "resistance" to the antibiotics. If this is true, the period of prophylaxis by an antibiotic or combination of antibiotics could be extended by employing this system and the cost of bloat prophylaxis could be reduced. More research is necessary to determine the mode of action of antibiotics in bloat prevention, which would be of great value in characterizing the nature of the "resistance" and in development of methods by which it can be delayed.

The administration of an antibiotic combination at 3-day intervals could be accomplished in several ways. One method would be to administer the antibiotics in gelatin capsule by balling gun. This would probably not be very practical since it would require the frequent handling of cattle and demand extra labor. Another method would be to supply the antibiotic in a pre-mix which could be incorporated into a small amount of grain and fed at regular intervals. It would probably be necessary to feed the same grain, but without antibiotics, on the days when antibiotics are not fed in order to maintain regular grain consumption by the cattle. This method would require that grain be fed daily, thus demanding more labor and expense than may usually be required. A third

method would be the use of a salt-mixture containing the antibiotic combination. A quantity of this antibiotic-salt could be put into covered salt boxes at 3-day intervals. Sufficient salt boxes would be required so that all animals would have adequate access to the salt. A minimum amount of labor would probably be required with this method of administration, making it more appealing to the animal husbandman.

Studies reported here also demonstrate that yearling dairy steers and heifers consuming a ration consisting primarily of alfalfa hay, alfalfa hay and/or corn silage and alfalfa soilage, did not exhibit adverse reactions (diarrhea and depressed appetite) to the daily feeding of a combination of antibiotics (STEP). On the other hand, similar animals consuming a ration consisting primarily of concentrates (grain) exhibited symptoms of mild to severe diarrhea for as long as 3 days and a slight to marked decline in grain consumption for several days following the initiation of antibiotic feeding. These observations support those made by Van Horn et al. (1963) in which adverse reactions to antibiotics fed singly and in a combination were confined to animals consuming a high grain ration. Van Horn (1962) proposed that since the antibiotics used in bloat prevention are active primarily against Gram positive organisms, the occurrence of adverse effects during antibiotic feeding may depend on the relative proportion of Gram positive organisms in the rumen, higher proportions being present in animals fed high grain rations than in those fed high roughage rations. The data presented herein support this theory since all of the adverse reactions occurred in animals consuming high grain rations during antibiotic feeding.

The mode of action of antibiotics in bloat prevention is controversial. One postulation, made by Mangan et al. (1959), states that penicillin controls bloat by inhibiting the bacteria which normally modify the plant chloroplast lipid, either by hydrogenation or lipolysis, to lessen its anti-foaming activity. Thus, the plant lipids become implicated in the etiology of bloat. Data presented by Hill (1960) and Wright (1961) support this theory. These workers also have studied various factors which effect the lipase activity of rumen fluid and which might be involved in the etiology and prevention of bloat.

Wright (1961) routinely added 0.2% (w/v) glucose to rumen liquor before incubation with linseed oil to determine its lipase activity. The present studies demonstrate that the addition of 1% (w/v) of various sources of readily available energy (maltose, dextrose and sucrose) to the incubation mixture caused a decrease in the lipolytic activity of rumen fluid, while 1% (w/v) gum cellulose, a source of less readily available energy, increased the activity. These data suggest that the microorganisms utilize the readily available energy in preference to hydrolyzing the esterified lipids. On the other hand, it is possible that certain microorganisms ferment the readily available energy with the formation of acids which increase the acidity and inhibit the organisms which produce the enzyme or directly inhibit the enzyme. Gum cellulose is probably not utilized as readily and thus the increase in acidity may not be so drastic as to effect the enzyme system.

Excessive amounts of readily available energy (2 to 8%, w/v) cause an apparent synthesis of ester or ester-like compounds which are detected

by the hydroxamic acid method used in these studies. However, similar amounts of gum cellulose caused an increase in lipase activity. The author is unaware of any possible explanation for either of these responses.

It is a well known fact in non-ruminant digestion that esterified lipids must be emulsified (normally by bile salts) before they can be efficiently hydrolyzed by pancreatic and enteric lipases. Emulsification of the substrate used in the assay for the lipase activity of rumen fluid may improve this procedure. Hill (1960) found that taurocholate, when used to emulsify soybean oil, decreased the lipolytic activity of rumen fluid. An emulsification agent (Johnson et al., 1960a) used to emulsify crude soybean oil in the present studies slightly decreased the lipase activity of rumen fluid. It is suggested that soybean oil either readily disperses in rumen fluid so that the lipase can readily attack it or that the esterified lipids do not have to be finely dispersed before rumen lipase can hydrolyze them. On the other hand, it is possible that the ingredients in the emulsifying agent were toxic to some of the rumen microorganisms. Additional research on the enzyme mechanisms involved in the hydrolysis of esterified lipid by rumen lipase is needed to help elucidate this aspect.

Previous studies by Hill (1960) demonstrated that the antibiotics penicillin, erythromycin, tylosin and streptomycin reduced by 50% the lipase activity of rumen fluid. Wright (1961) found that penicillin and oxytetracycline reduced the lipase activity of rumen fluid and that streptomycin had no apparent effect. Conversely, the present studies

showed that penicillin, erythromycin, tylosin and streptomycin had no consistent significant effect upon the lipolytic activity of rumen fluid or rumen microorganisms. In most cases, each antibiotic appeared to slightly increase the activity and in one experiment, streptomycin significantly increased the activity. The only feasible explanation for these differences is that Hill (1960) and Wright (1961) obtained the rumen fluid and rumen microorganisms for their studies from animals grazing alfalfa pasture and fed red clover soilage, respectively, and the source of rumen fluid and rumen microorganisms for the present studies was an animal receiving a hay-grain ration. Animals on these two types of rations would have different rumen microbial populations which would probably react differently to these antibiotics.

Hill (1960) concluded that the Gram positive bacteria (those sensitive to penicillin, erythromycin and tylosin) of the rumen contribute to the rumen lipase activity. The present studies show that the Gram positive bacteria apparently do not contribute to the rumen lipase activity. The latter results support the work of Hobson and Mann (1961) who have isolated Gram negative lipolytic bacteria from the rumen of sheep.

Considerably more research should be conducted on the lipase enzyme system in rumen microorganisms. Of utmost importance is the relationship between lipase activity and bloat. If this is a cause and effect relationship, and the work of Wright (1961) indicates that it is, the mode of action of the lipase in the etiology of bloat should be determined. Involved in these studies would be the isolation of specific

organisms responsible for the production of the lipase and the purification and identification of the enzyme.

SUMMARY

During 1962, 203 dairy and beef animals and 445 sheep were used in controlled pasture bloat studies in which the effects of a bolus containing a combination of streptomycin sulfate, tylosin phosphate, erythromycin thiocyanate and procaine penicillin were evaluated. The data obtained from cattle show that the initial administration of either one, two, or three boluses reduced bloat for 3 to 4 weeks. Administration of either one or two boluses 6 weeks after the first boluses were given usually effected a reduction in bloat for about 1 week. Average weight gains were greater (0.14 lb. per animal daily) in 70 animals receiving antibiotic boluses than in 44 controls.

In sheep, the initial administration of one-half bolus reduced bloat for about 3 to 4 weeks, whereas administration of the same dosage 4 weeks after the first bolus effected no reduction. Wool loss from the dorsal surface of the body was noted in about 75% of the treated group in one flock. No wool loss occurred in the control group or in the other treated sheep.

Studies with four rumen fistulated steers demonstrated that the majority of the boluses, when administered by balling gun, are deposited initially in the anterior dorsal blind sac of the rumen. Observations on these fistulated steers and four intact steers showed that most of the boluses subsequently migrate to the reticulum. The bolus, gradually dissipated by erosion, has a half-life (weight basis) of approximately 60 days.

Preliminary observations suggest that oral administration of potassium levopropylcillin reduces the incidence and severity of bloat for a substantially longer period of time when fed singly than other antibiotics that have been tested.

In 1963, 33 dairy steers and 21 dairy heifers were used in an experiment to determine the effect of a combination of streptomycin, tylosin, erythromycin and penicillin (STEP) administered at 1-, 2- or 3-day intervals upon legume bloat in cattle. Bloat was 89, 87 and 91% less when 250 mg. STEP was administered at 1-, 2- and 3-day intervals, respectively, than in the controls. There was an indication that the administration of STEP at 3-day intervals was effective for as long as (and perhaps longer than) STEP given at 1- or 2-day intervals.

In addition, 30 dairy steers and 27 dairy heifers were used to study the effect of various feeding regimes on the reactions of cattle to the initiation of antibiotic administration. When STEP administration was started, transient depression of appetite and diarrhea occurred in a few of the animals receiving a high grain ration. Heifers reacted more severely than steers, indicating a possible sex difference. No adverse reactions were observed in heifers or steers fed rations consisting primarily of alfalfa hay, corn silage and alfalfa hay, or green chopped alfalfa.

The in vitro lipase activity of rumen fluid and of rumen microorganisms was studied by measuring the disappearance of ester, measured by the hydroxamic acid method, during incubation with soybean oil. The addition of 100 mg. of maltose or dextrose to 10 ml. rumen fluid reduced

the lipase activity while the addition of 100 mg. of gum cellulose to 10 ml. rumen fluid produced no apparent effect. Adding greater amounts (200 to 800 mg.) of dextrose or sucrose caused an apparent synthesis of ester. Similar amounts of gum cellulose caused as much as a two-fold increase in the lipase activity of rumen fluid. Emulsification of the soybean oil substrate had no significant effect on the lipase activity of rumen fluid.

By using differential centrifugation and sedimentation techniques to separate rumen protozoa and rumen bacteria, it was shown that the rumen lipase is associated with both the protozoa and bacteria.

Lipase activity of rumen bacteria and rumen protozoa was not affected by adding .003% (w/v) penicillin, erythromycin, tylosin or streptomycin. However, the lipase activity was significantly increased by adding .006% (w/v) streptomycin.

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ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. N. L. Jacobson for his guidance and assistance in the planning of this investigation, the preparation of the dissertation, and valuable suggestions throughout graduate study; and to Dr. R. S. Allen for his consultation and aid in planning parts of this investigation.

Gratitude is also expressed to Doctors P. A. Hartman and A. D. McGilliard for their assistance and cooperation; and to A. Coletti, Dr. J. W. Rust, J. M. Bryant and J. V. DeBarthe for their assistance in working with experimental animals and help in management.

The author also wishes to thank his wife, Joan, for her help in the preparation of the thesis and for her understanding cooperation during graduate study.

APPENDIX

Table 11. Names and addresses of cooperating farmers, 1962

Name	Address	Livestock employed
Jay Bower	Danbury, Iowa	35 Angus cows with calves
Barney Feldhacker	Fostoria, Iowa	40 dairy heifers
Riley Gillette	Spencer, Iowa	350 ewes
Daniel Turner	Marshalltown, Iowa	95 ewes with lambs

Table 12. Incubation media[a]

Compound	gm./l.
NaCl	3.0
KCl	2.0
$NaC_2H_3O_2$	3.0
$KC_2H_3O_2$	2.0
$NaHCO_3$	2.0
K_2SO_4	1.0
$NaHPO_4 \cdot 7H_2O$	3.0
KH_2PO_4	2.5
$MgSO_4 \cdot 7H_2O$	0.1
Starch (potato)	8.0
Starch (soluble)	2.0
Cellulose	2.0
Sucrose	1.0

[a]Christiansen et al. (1962).

Table 13. Buffer solution

Compound	gm./l.
NaCl	3.0
KCl	2.0
$NaHCO_3$	2.0
K_2SO_4	1.0
$NaHPO_4 \cdot 7H_2O$	3.0
KH_2PO_4	2.5
$MgSO_4 \cdot 7H_2O$	0.1

Table 14. Daily bloat summary for Groups I and II, 1962

Date	Group I					Group II				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
5-15	14	0	0	0	0.00	14	0	0	0	0.00
5-16		0	0	0	0.00		0	0	0	0.00
5-17		0	2	0	0.29		0	0	0	0.00
5-18		0	0	1	0.21		0	0	0	0.00
5-19		1	0	0	0.07		0	0	0	0.00
5-20		2	3	0	0.57		0	0	0	0.00
5-21		2	1	0	0.29		0	0	0	0.00
5-22		2	1	0	0.29		0	0	0	0.00
5-23		6	1	0	0.57		2	0	0	0.14
5-24		0	2	0	0.29		0	0	0	0.00
5-25		1	3	2	0.93		0	1	0	0.14
5-26		0	0	0	0.00		0	0	0	0.00
5-27		0	0	0	0.00		0	0	0	0.00
5-28		0	0	0	0.00		0	0	0	0.00
5-29		0	1	1	0.36		0	0	0	0.00
5-30		0	0	0	0.00		0	0	0	0.00
5-31		0	1	0	0.14		0	0	0	0.00
6-1		1	0	0	0.07		0	0	0	0.00
6-2		3	0	0	0.21		0	0	0	0.00
6-3		0	0	0	0.00		0	0	0	0.00
6-4		2	0	0	0.14		1	0	0	0.07
6-5		0	0	0	0.00		0	0	0	0.00
6-6		0	0	0	0.00		0	0	0	0.00
6-7		0	0	0	0.00		0	0	0	0.00
6-8		0	0	0	0.00		0	0	0	0.00
6-9		0	0	0	0.00		0	0	0	0.00
6-10		0	0	0	0.00		0	0	0	0.00
6-11		0	0	0	0.00		0	0	0	0.00
6-12		0	0	0	0.00		0	0	0	0.00
6-13		5	3	0	0.79		5	0	0	0.36
6-14		2	1	2[a]	0.79		1	0	0	0.07
6-15		2	1	1	0.50		1	0	0	0.07
6-16		0	1	0	0.14		0	0	0	0.00
6-17		0	0	0	0.00		0	0	0	0.00
6-18		0	0	0	0.00		0	0	0	0.00
6-19		0	0	0	0.00		0	0	0	0.00

[a]One animal bloated to a score of 4.

Table 14. (Continued)

Date	Group I					Group II				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
6-20	14	0	0	0	0.00	14	0	0	0	0.00
6-21		0	0	0	0.00		0	0	0	0.00
6-23		0	0	0	0.00		0	0	0	0.00
6-24		0	0	0	0.00		0	0	0	0.00
6-25		0	0	0	0.00		1	0	0	0.07
6-26		2	2	1[a]	0.71		3	1	1	0.57
6-27		3	3	0	0.64		1	4	0	0.64
6-28		3	2	1	0.71		0	0	0	0.00
6-29		1	0	0	0.07		0	0	0	0.00
6-30		0	0	1	0.21		0	0	0	0.00
7-1		0	0	0	0.00		0	0	0	0.00
7-2		2	1	1	0.50		0	0	0	0.00
7-3		4	2	1	0.79		1	0	0	0.07
7-4		0	0	0	0.00		0	0	0	0.00
7-5		1	0	0	0.07		0	0	0	0.00
7-6		0	0	0	0.00		0	0	0	0.00
7-7		0	0	0	0.00		0	0	0	0.00
7-8		0	0	1	0.21		0	0	0	0.00
7-9		0	0	0	0.00		0	0	0	0.00
7-10		0	0	0	0.00		0	0	0	0.00
7-11		0	0	0	0.00		0	0	0	0.00
7-12		0	0	0	0.00		0	0	0	0.00
7-13		0	0	0	0.00		0	0	0	0.00
7-14		0	0	0	0.00		0	0	0	0.00
7-15		0	0	0	0.00		0	0	0	0.00
7-16		0	0	0	0.00		0	0	0	0.00
7-17		0	0	0	0.00		0	0	0	0.00
7-18		0	0	0	0.00		0	0	0	0.00
7-19		0	0	0	0.00		0	0	0	0.00
7-20		0.	0	0	0.00		0	0	0	0.00
7-21		0	0	0	0.00		0	0	0	0.00
7-22		0	0	0	0.00		0	0	0	0.00
7-23		0	0	0	0.00		0	0	0	0.00
7-24		0	0	0	0.00		0	0	0	0.00
7-25		0	0	0	0.00		0	0	0	0.00
7-26		0	0	0	0.00		0	0	0	0.00
7-27		0	0	0	0.00		0	0	0	0.00
7-28		0	0	0	0.00		0	0	0	0.00
7-29		0	0	0	0.00		0	0	0	0.00
7-30		0	0	0	0.00		0	0	0	0.00

Table 14. (Continued)

Date	Group I					Group II				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
7-31	14	0	0	0	0.00	14	0	0	0	0.00
8-1		0	0	0	0.00		0	0	0	0.00
8-2		0	0	0	0.00		0	0	0	0.00
8-3		0	0	0	0.00		0	0	0	0.00
8-4		1	1	1	0.43		1	1	0	0.21
8-5		2	4	1	0.93		3	1	1	0.57
8-6		1	1	1	0.43		0	1	1	0.36
8-7		0	0	0	0.00		1	0	0	0.07
8-8		0	0	0	0.00		0	0	0	0.00
8-9		0	0	0	0.00		0	0	0	0.00
8-10		0	0	0	0.00		0	0	0	0.00
8-11		0	0	0	0.00		0	0	0	0.00
8-12		0	0	0	0.00		0	0	0	0.00
8-13		0	0	0	0.00		0	0	0	0.00
8-14		0	0	0	0.00		0	0	0	0.00
8-15		0	0	0	0.00		1	0	0	0.07
8-16		1	0	0	0.07		1	0	0	0.07
8-17		3	1	2	0.79		4	1	0	0.43
8-18		0	0	0	0.00		0	0	0	0.00
8-19		0	0	0	0.00		0	0	0	0.00
8-20		0	0	0	0.00		0	0	0	0.00
8-21		0	0	0	0.00		0	0	0	0.00
8-22		1	0	0	0.07		1	0	0	0.07
8-23		1	0	0	0.07		0	0	0	0.00
8-24		0	0	0	0.00		0	0	0	0.00
8-25		0	0	0	0.00		0	0	0	0.00
8-26		0	0	0	0.00		0	0	0	0.00
8-27		0	0	0	0.00		0	0	0	0.00
8-28		0	0	0	0.00		0	0	0	0.00
8-29		0	0	0	0.00		0	0	0	0.00
8-30		1	0	0	0.07		0	0	0	0.00
8-31		0	0	0	0.00		0	0	0	0.00
9-1		0	0	0	0.00		0	0	0	0.00
9-2		0	0	0	0.00		0	0	0	0.00
9-3		0	0	0	0.00		0	0	0	0.00
9-4		0	0	0	0.00		0	0	0	0.00
9-5		0	0	0	0.00		0	0	0	0.00
9-6		0	0	0	0.00		0	0	0	0.00
9-7		0	0	0	0.00		0	0	0	0.00
9-8		0	0	0	0.00		0	0	0	0.00

Table 14. (Continued)

Date	Group I					Group II				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
9-9	14	0	0	0	0.00	14	0	0	0	0.00
9-10		0	0	0	0.00		0	0	0	0.00
9-11		0	0	0	0.00		0	0	0	0.00
9-12		0	0	0	0.00		0	0	0	0.00
9-13		0	0	0	0.00		0	0	0	0.00
9-14		2	2	0	0.43		2	1	0	0.29
9-15		5	0	2	0.79		0	0	2	0.43
9-16		2	1	1	0.50		0	0	0	0.00
9-17		3	1	0	0.36		0	0	0	0.00
9-18		5	2	1	0.86		0	0	0	0.00

Table 15. Daily bloat summary for Groups III, IV and V, 1962

Date	Group III					Group IV					Group V				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
5-15	14	0	0	0	0.00	14	0	0	0	0.00	12	0	0	0	0.00
5-16		0	0	0	0.00		0	0	0	0.00		1	0	0	0.08
5-17		0	0	0	0.00		0	0	0	0.00		2	2	0	0.50
5-18		0	0	0	0.00		0	0	0	0.00		3	0	0	0.25
5-19		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
5-20		1	0	0	0.07		0	0	0	0.00		2	0	0	0.17
5-21		1	0	0	0.07		0	0	0	0.00		1	0	0	0.08
5-22		0	0	0	0.00		0	0	0	0.00		1	1	0	0.25
5-23		0	0	0	0.00		0	0	0	0.00		3	1	0	0.42
5-24		1	0	0	0.07		0	0	0	0.00		3	1	0	0.42
5-25		0	2	0	0.29		0	3	0	0.43		6	3	0	1.00
5-26		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
5-27		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
5-28		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
5-29		0	0	0	0.00		0	0	0	0.00		1	0	0	0.08
5-30		1	0	0	0.07		0	0	0	0.00		0	0	0	0.00
5-31		0	0	0	0.00		0	0	0	0.00		5	0	0	0.42
6-1		0	0	0	0.00		0	0	0	0.00		3	0	0	0.25
6-2		1	0	0	0.07		0	0	0	0.00		3	1	0	0.42
6-3		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-4		2	0	0	0.14		1	0	0	0.07		1	0	0	0.08
6-5		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-6		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-7		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-8		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-9		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00

Table 15. (Continued)

Date	Group III					Group IV					Group V				
		No. bloated to score:					No. bloated to score:					No. bloated to score:			
	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.
6-10	14	0	0	0	0.00	14	0	0	0	0.00	12	0	0	0	0.00
6-11		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-12		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-13		5	2	0	0.57		3	1	0	0.36		4	2	0	0.58
6-14		5	0	0	0.36		1	0	1	0.29		2	3	0	0.67
6-15		0	0	0	0.00		1	1	0	0.21		4	3	0	0.83
6-16		0	0	0	0.00		0	0	0	0.00		1	0	0	0.08
6-17		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-18		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-19		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-20		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-21		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-22		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-23		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-24		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
6-25		2	0	0	0.14		0	0	0	0.00		4	0	0	0.33
6-26		4	1	2	0.86		0	1	0	0.14		2	0	0	0.17
6-27		1	1	0	0.21		0	0	0	0.00		2	3	0	0.67
6-28		0	0	0	0.00		0	0	0	0.00		1	1	0	0.25
6-29		0	0	0	0.00		0	0	0	0.00		2	0	0	0.17
6-30		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-1		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-2		0	0	0	0.00		0	0	0	0.00		1	0	1	0.33
7-3		0	0	0	0.00		1	0	0	0.07		6	0	0	0.50
7-4		1	0	0	0.07		0	0	0	0.00		0	0	0	0.00

Table 15. (Continued)

Date	Group III					Group IV					Group V				
		No. bloated to score:					No. bloated to score:					No. bloated to score:			
	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.
7-5	14	1	0	1	0.29	14	0	0	1[a]	0.36	12	1	0	0	0.08
7-6		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-7		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-8		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-9		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-10		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-11		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-12		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-13		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-14		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-15		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-16		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-17		0	0	0	0.00		0	0	0	0.00	11[b]	0	0	0	0.00
7-18		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-19		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-20		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-21		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-22		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-23		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-24		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-25		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00

[a]Bloat score of 5, rumenotomy with knife.

[b]Removed a cryptorchid bull from the experiment.

Table 15. (Continued)

Date	Group III					Group IV					Group V				
		No. bloated to score:					No. bloated to score:					No. bloated to score:			
	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.
7-26	14	0	0	0	0.00	14	0	0	0	0.00	11	0	0	0	0.00
7-27		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-28		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-29		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-30		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
7-31		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-1		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-2		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-3		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-4		0	0	1[c]	0.36		0	0	1	0.21		2	1	0	0.36
8-5	13	1	0	0	0.08		0	2	0	0.29		3	2	1	0.91
8-6		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-7		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-8		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-9		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-10		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-11		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-12		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-13		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-14		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-15		1	0	0	0.08		0	0	0	0.00	10[d]	0	0	0	0.00
8-16		0	0	0	0.00		1	0	0	0.07		0	0	0	0.00

[c]Died, due to bloat.

[d]Removed one animal from the experiment, too wild to handle.

Table 15. (Continued)

Date	Group III					Group IV					Group V				
		No. bloated to score:					No. bloated to score:					No. bloated to score:			
	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.
8-17	13	1	1	0	0.23	14	0	1	1	0.36	10	0	0	0	0.00
8-18		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-19		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-20		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-21		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-22		3	0	0	0.23		1	1	0	0.21		0	0	0	0.00
8-23		0	0	0	0.00		1	0	0	0.07		0	0	0	0.00
8-24		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-25		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-26		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-27		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-28		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-29		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
8-30		1	0	0	0.08		0	0	0	0.00		0	0	0	0.00
8-31[e]		0	0	0	0.00		0	0	0	0.00		0	0	0	0.00
9-1												0	0	0	0.00
9-2												0	0	0	0.00
9-3												0	0	0	0.00
9-4												0	0	0	0.00
9-5												0	0	0	0.00
9-6												0	0	0	0.00
9-7												0	0	0	0.00
9-8												0	0	0	0.00

[e]Groups III and IV were removed from the experiment.

Table 15. (Continued)

Date	Group III					Group IV					Group V				
		No. bloated to score:					No. bloated to score:					No. bloated to score:			
	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.	No. of cattle	1	2	3	A.D.M.
9-9											10	0	0	0	0.00
9-10												0	0	0	0.00
9-11												0	0	0	0.00
9-12												0	0	0	0.00
9-13												0	0	0	0.00
9-14												1	0	0	0.10
9-15												0	0	1	0.30
9-16												1	0	0	0.10
9-17												0	0	0	0.00
9-18												1	0	0	0.10

Table 16. Daily bloat summary for Napier experiment

Date	Group I					Group II[a]				
	No. of cattle	No. bloated to score:			A.D.M.	No. of cattle	No. bloated to score:			A.D.M.
		1	2	3			1	2	3	
5-8	12	0	0	0	0.00	12	0	0	0	0.00
5-9		0	0	0	0.00		0	0	0	0.00
5-10		0	0	0	0.00		0	0	0	0.00
5-11		0	0	0	0.00		0	0	0	0.00
5-12		0	0	0	0.00		0	0	0	0.00
5-13		0	0	0	0.00		0	0	0	0.00
5-14		0	0	0	0.00		0	0	0	0.00
5-15		0	0	0	0.00		0	0	0	0.00
5-16		0	0	0	0.00		0	0	0	0.00
5-17		0	0	0	0.00		0	0	0	0.00
5-18		0	0	0	0.00		0	0	0	0.00
5-19		0	0	0	0.00		0	0	0	0.00
5-20		0	0	0	0.00		0	0	0	0.00
5-21		0	0	0	0.00		0	0	0	0.00
5-22		0	0	0	0.00		1	0	0	0.08
5-23		0	1	0	0.17		0	0	0	0.00
5-24		2	5	1	1.25		1	1	1	0.50
5-25		0	0	0	0.00		0	0	0	0.00
5-26		0	0	0	0.00		0	0	0	0.00
5-27		4	0	0	0.33		1	1	0	0.25
5-28		3	1	0	0.42		2	0	0	0.17
5-29		7	0	0	0.58		4	1	0	0.50
5-30		2	1	0	0.33		1	2	0	0.42
5-31		2	1	0	0.33		1	1	0	0.25
6-1		2	0	0	0.17		1	3	0	0.58
6-2		1	0	0	0.08		0	1	0	0.17
6-3		0	0	1	0.25		0	1	0	0.17
6-4		1	1	0	0.25		0	0	0	0.00
6-5		2	1	0	0.33		4	1	0	0.50
6-6		2	1	0	0.33		2	0	0	0.17
6-7		0	0	0	0.00		0	1	0	0.17
6-8		1	0	0	0.08		9	0	0	0.75
6-9		2	0	0	0.17		5	2	0	0.75
6-10		3	0	0	0.25		5	0	0	0.42
6-11		0	0	0	0.00		2	1	0	0.33
6-12		0	0	0	0.00		3	3	0	0.75

[a]Each animal in this group received two antibiotic boluses on May 4, one bolus on June 21 and two boluses on August 23.

Table 16. (Continued)

Date	Group I					Group II[a]				
	No. of cattle	No. bloated to score:			A.D.M.	No. of cattle	No. bloated to score:			A.D.M.
		1	2	3			1	2	3	
6-13	12	1	0	0	0.08	12	2	1	0	0.33
6-14		1	0	0	0.08		4	0	0	0.33
6-15		1	1	0	0.25		3	2	1	0.83
6-16		1	0	0	0.08		1	0	0	0.08
6-17		4	0	0	0.33		2	0	0	0.17
6-18		1	0	0	0.08		4	1	0	0.50
6-19		3	2	0	0.58		4	2	0	0.67
6-20		1	0	0	0.08		1	1	0	0.25
6-21		3	0	0	0.25		4	2	1	0.92
6-22		0	1	0	0.17		1	1	0	0.25
6-23		0	0	0	0.00		0	0	0	0.00
6-24		0	0	0	0.00		0	0	0	0.00
6-25		0	0	0	0.00		0	0	0	0.00
6-26		0	0	0	0.00		1	0	0	0.08
6-27		0	0	0	0.00		0	1	0	0.17
6-28		0	0	0	0.00		2	1	0	0.33
6-29		0	0	0	0.00		0	0	0	0.00
6-30		0	0	0	0.00		0	0	0	0.00
7-1		0	0	0	0.00		0	0	0	0.00
7-2		0	0	0	0.00		0	0	0	0.00
7-3		0	0	0	0.00		0	0	0	0.00
7-4		0	0	0	0.00		0	0	0	0.00
7-5		0	0	0	0.00		0	0	0	0.00
7-6		1	0	0	0.08		3	1	0	0.42
7-7		0	0	0	0.00		0	0	0	0.00
7-8		0	0	0	0.00		0	0	0	0.00
7-9		0	0	0	0.00		0	0	0	0.00
7-10		0	0	0	0.00		0	0	0	0.00
7-11		0	0	0	0.00		0	0	0	0.00
7-12		0	0	0	0.00		0	0	0	0.00
7-13		0	0	0	0.00		0	0	0	0.00
7-14		0	0	0	0.00		0	0	0	0.00
7-15		0	0	0	0.00		0	0	0	0.00
7-16		0	0	0	0.00		0	0	0	0.00
7-17		1	0	0	0.08		0	0	0	0.00
7-18		0	0	0	0.00		0	0	0	0.00
7-19		0	1	0	0.17		0	1	0	0.17
7-20		0	0	0	0.00		1	0	0	0.08
7-21		0	0	0	0.00		1	0	0	0.08

Table 16. (Continued)

Date	Group I					Group II[a]				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
7-22	12	0	0	0	0.00	12	0	0	0	0.00
7-23		0	0	0	0.00		0	0	0	0.00
7-24		2	0	0	0.17		2	1	0	0.33
7-25		2	0	0	0.17		3	0	0	0.25
7-26		0	0	0	0.00		0	0	0	0.00
7-27		0	0	0	0.00		0	0	0	0.00
7-28		0	0	0	0.00		0	0	0	0.00
7-29		0	0	0	0.00		0	0	0	0.00
7-30		0	0	0	0.00		1	0	0	0.08
7-31		0	0	0	0.00		0	0	0	0.00
8-1		0	1	0	0.17		1	0	0	0.08
8-2		2	0	0	0.17		1	0	0	0.08
8-3		2	0	0	0.17		0	1	0	0.17
8-4		3	0	0	0.25		1	1	1	0.50
8-5		2	1	0	0.33		3	2	0	0.58
8-6		2	0	0	0.17		2	1	1	0.58
8-7		2	0	0	0.17		2	2	0	0.50
8-8		1	0	0	0.08		4	0	0	0.33
8-9		1	0	0	0.08		3	2	0	0.58
8-10		1	0	0	0.08		3	0	0	0.25
8-11		6	0	0	0.50		7	0	0	0.58
8-12		0	0	0	0.00		0	0	0	0.00
8-13		1	0	0	0.08		6	0	0	0.50
8-14		1	0	0	0.08		1	0	0	0.08
8-15		0	0	0	0.00		3	1	1	0.67
8-16		4	0	0	0.33		2	4	1	1.08
8-17		2	0	0	0.17		6	0	0	0.50
8-18		1	0	0	0.08		5	0	0	0.42
8-19		1	0	0	0.08		1	0	0	0.08
8-20		0	0	0	0.00		0	0	0	0.00
8-21		0	0	0	0.00		0	0	0	0.00
8-22		0	0	0	0.00		3	0	0	0.25
8-23		1	0	0	0.08		1	0	0	0.08
8-24		0	0	0	0.00		0	0	0	0.00
8-25		0	0	0	0.00		0	0	0	0.00
8-26		0	0	0	0.00		0	0	0	0.00
8-27		0	0	0	0.00		0	0	0	0.00
8-28		0	0	0	0.00		0	0	0	0.00
8-29		0	0	0	0.00		0	0	0	0.00

Table 16. (Continued)

Date	Group I					Group II[a]				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
8-30	12	0	0	0	0.00	12	0	0	0	0.00
8-31		0	0	0	0.00		0	0	0	0.00
9-1		0	0	0	0.00		0	0	0	0.00
9-2		0	0	0	0.00		0	0	0	0.00
9-3		1	0	0	0.08		2	0	0	0.17
9-4		3	0	0	0.25		1	0	0	0.08
9-5		0	0	0	0.00		0	0	0	0.00
9-6		0	0	0	0.00		0	0	0	0.00
9-7		0	0	0	0.00		0	0	0	0.00
9-8		0	0	0	0.00		0	0	0	0.00
9-9		0	0	0	0.00		0	0	0	0.00
9-10		0	0	0	0.00		0	0	0	0.00
9-11		0	0	0	0.00		0	0	0	0.00
9-12		0	0	0	0.00		0	0	0	0.00
9-13		0	0	0	0.00		0	0	0	0.00
9-14		0	0	0	0.00		0	0	0	0.00
9-15		0	0	0	0.00		0	0	0	0.00
9-16		0	0	0	0.00		0	0	0	0.00
9-17[b]		4	0	0	0.33		7	1	0	0.75
9-18		2	1	0	0.33		6	1	1	0.92
9-19		2	0	0	0.17		4	2	0	0.67
9-20		2	2	0	0.50		6	1	0	0.67
9-21[c]		1	1	0	0.25		5	1	0	0.58
9-22		1	0	0	0.08		2	0	0	0.17
9-23		0	0	0	0.00		0	0	0	0.00
9-24		1	0	0	0.08		0	0	0	0.00
9-25[d]		1	0	0	0.08		3	0	0	0.25
9-26		1	0	0	0.08		2	0	0	0.08
9-27		0	0	0	0.00		0	0	0	0.00
9-28		0	0	0	0.00		0	0	0	0.00
9-29		0	0	0	0.00		0	0	0	0.00

[b]Started to feed 50 mg. potassium levopropylcillin in 1 lb. grain per animal daily at 4:00 p.m.

[c]Increased the antibiotic level to 100 mg. per animal daily.

[d]Discontinued antibiotic feeding.

Table 16 (Continued)

Date	Group I					Group II[a]				
	No. of cattle	No. bloated to score: 1	2	3	A.D.M.	No. of cattle	No. bloated to score: 1	2	3	A.D.M.
9-30[e]	12	0	0	0	0.00	12	0	0	0	0.00
10-1		0	0	0	0.00		0	0	0	0.00
10-2		0	0	0	0.00		0	0	0	0.00
10-3		0	0	0	0.00		0	0	0	0.00
10-4		0	0	0	0.00		0	0	0	0.00
10-5		0	0	0	0.00		0	0	0	0.00
10-6		0	0	0	0.00		0	0	0	0.00
10-7		0	0	0	0.00		0	0	0	0.00
10-8		0	0	0	0.00		0	0	0	0.00
10-9		0	0	0	0.00		0	0	0	0.00
10-10		0	0	0	0.00		0	0	0	0.00

[e]Discontinued grain feeding.

Table 17. Daily bloat summary for Groups I and II, 1963

Date	Group I					Group II				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
5-6	12	0	0	0	0.00	12	0	0	0	0.00
5-7		0	0	0	0.00		0	0	0	0.00
5-8		0	0	0	0.00		0	0	0	0.00
5-9		0	0	0	0.00		0	0	0	0.00
5-10		0	0	0	0.00		0	0	0	0.00
5-11		0	0	0	0.00		0	0	0	0.00
5-12		0	0	0	0.00		0	0	0	0.00
5-13		0	0	0	0.00		0	0	0	0.00
5-14		0	0	0	0.00		0	0	0	0.00
5-15		0	0	0	0.00		0	0	0	0.00
5-16		0	0	0	0.00		0	0	0	0.00
5-17		0	0	0	0.00		0	0	1	0.25
5-18		0	0	0	0.00		0	0	0	0.00
5-19		0	0	1	0.25		0	0	1	0.25
5-20		0	0	0	0.00		0	0	0	0.00
5-21		0	0	2	0.50		0	0	3	0.75
5-22		0	1	0	0.17		0	1	0	0.17
5-23		0	1	1	0.42		0	0	1	0.25
5-24		0	0	1	0.25		0	0	0	0.00
5-25		0	0	0	0.00		0	0	0	0.00
5-26		0	0	0	0.00		0	0	0	0.00
5-27		0	0	0	0.00		0	0	0	0.00
5-28		0	0	0	0.00		0	0	0	0.00
5-29		0	0	0	0.00		0	0	0	0.00
5-30		0	0	0	0.00		0	0	0	0.00
5-31		1	0	0	0.08		0	0	1	0.25
6-1		0	0	0	0.00		0	0	0	0.00
6-2		0	0	0	0.00		0	0	0	0.00
6-3		0	0	0	0.00		0	0	0	0.00
6-4		0	0	0	0.00		0	0	0	0.00
6-5		0	0	0	0.00		0	0	0	0.00
6-6		0	0	0	0.00		0	0	0	0.00
6-7		0	0	0	0.00		0	0	0	0.00
6-8		0	0	0	0.00		0	0	0	0.00
6-9		0	0	0	0.00		0	0	0	0.00
6-10		0	0	0	0.00		0	0	0	0.00
6-11		0	0	0	0.00		0	0	0	0.00
6-12		0	0	0	0.00		0	0	0	0.00
6-13		0	0	0	0.00		0	0	0	0.00

Table 17. (Continued)

Date	Group I					Group II				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
6-14	12	0	0	0	0.00	12	0	0	0	0.00
6-15		0	0	0	0.00		0	0	0	0.00
6-16		0	0	0	0.00		0	0	0	0.00
6-17		0	0	0	0.00		0	0	0	0.00
6-18		0	0	0	0.00		0	0	0	0.00
6-19		0	0	0	0.00		0	0	0	0.00
6-20		0	0	0	0.00		0	0	0	0.00
6-21		0	0	0	0.00		0	0	0	0.00
6-22		0	0	0	0.00		0	0	0	0.00
6-23		0	0	0	0.00		0	0	0	0.00
6-24		0	0	0	0.00		0	0	0	0.00
6-25		0	0	0	0.00		0	0	0	0.00
6-26		0	0	0	0.00		0	0	0	0.00
6-27		0	0	0	0.00		0	0	0	0.00
6-28		1	0	0	0.08		0	0	0	0.00
6-29		0	0	0	0.00		0	0	0	0.00
6-30		0	0	0	0.00		0	0	0	0.00
7-1		0	0	0	0.00		0	0	0	0.00
7-2		0	0	0	0.00		0	0	0	0.00
7-3		0	0	0	0.00		0	0	0	0.00
7-4		0	0	0	0.00		0	0	0	0.00
7-5		0	0	0	0.00		0	0	0	0.00
7-6		0	0	0	0.00		0	0	0	0.00
7-7		0	0	0	0.00		0	0	0	0.00
7-8		0	0	0	0.00		0	0	0	0.00
7-9		0	0	0	0.00		0	0	0	0.00
7-10		0	0	0	0.00		0	0	0	0.00
7-11		0	0	0	0.00		0	0	0	0.00
7-12		0	0	0	0.00		0	0	0	0.00
7-13		0	0	0	0.00		0	0	0	0.00
7-14		0	0	0	0.00		0	0	0	0.00
7-15		0	0	0	0.00		0	0	0	0.00
7-16		0	0	0	0.00		0	0	0	0.00
7-17		0	0	0	0.00		0	0	0	0.00
7-18		0	0	0	0.00		0	0	0	0.00
7-19		0	0	0	0.00		0	0	0	0.00
7-20		0	0	1	0.25		1	0	1	0.33
7-21		0	0	0	0.00		0	0	0	0.00
7-22		0	0	0	0.00		0	0	0	0.00
7-23		0	0	0	0.00		0	0	0	0.00

Table 17. (Continued)

Date	Group I					Group II				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
7-24	12	0	0	0	0.00	12	0	0	0	0.00
7-25		0	0	0	0.00		0	0	0	0.00
7-26		0	0	0	0.00		0	0	0	0.00
7-27		0	0	0	0.00		0	0	0	0.00
7-28		0	0	0	0.00		0	0	0	0.00
7-29		0	0	0	0.00		0	0	0	0.00
7-30		0	0	0	0.00		0	0	0	0.00
7-31		0	0	0	0.00		0	0	0	0.00
8-1		0	0	0	0.00		0	0	0	0.00
8-2		0	0	0	0.00		0	0	0	0.00
8-3		0	0	0	0.00		0	0	0	0.00
8-4		0	0	0	0.00		0	0	0	0.00
8-5		0	0	0	0.00		0	0	0	0.00
8-6		0	0	0	0.00		0	0	0	0.00
8-7		0	0	0	0.00		0	0	0	0.00
8-8		0	0	0	0.00		0	0	0	0.00
8-9		0	0	0	0.00		0	0	0	0.00
8-10		0	0	0	0.00		0	0	0	0.00
8-11		0	0	0	0.00		0	0	0	0.00
8-12		0	0	0	0.00		0	0	0	0.00
8-13		0	0	0	0.00		0	0	0	0.00
8-14		0	0	0	0.00		0	0	0	0.00
8-15		0	0	0	0.00		0	0	0	0.00
8-16		0	0	0	0.00		0	0	0	0.00
8-17		0	0	0	0.00		0	0	0	0.00
8-18		0	0	0	0.00		0	0	0	0.00
8-19		0	0	0	0.00		0	0	0	0.00
8-20		0	0	0	0.00		0	0	0	0.00
8-21		0	0	0	0.00		0	0	0	0.00
8-22		0	0	0	0.00		0	0	0	0.00
8-23		0	0	0	0.00		0	0	0	0.00
8-24		0	0	0	0.00		0	0	0	0.00
8-25		0	0	0	0.00		0	0	0	0.00
8-26		0	0	0	0.00		0	0	0	0.00
8-27		0	0	0	0.00		0	0	0	0.00
8-28		0	0	0	0.00		0	0	0	0.00
8-29		0	0	0	0.00		0	0	0	0.00
8-30		0	0	0	0.00		0	0	0	0.00
8-31		0	0	0	0.00		0	0	0	0.00

Table 17. (Continued)

Date	Group I					Group II				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
9-1	12	0	0	0	0.00	11[a]	0	0	0	0.00
9-2		0	0	0	0.00		0	0	0	0.00
9-3		0	0	0	0.00		0	0	0	0.00
9-4		0	0	0	0.00		0	0	0	0.00
9-5		0	0	0	0.00		0	0	0	0.00
9-6		0	0	0	0.00		0	0	0	0.00
9-7		0	0	0	0.00		0	0	0	0.00
9-8		0	0	0	0.00		0	0	0	0.00
9-9		0	0	0	0.00		0	0	0	0.00
9-10		0	0	0	0.00		0	0	0	0.00
9-11		0	0	0	0.00		0	0	0	0.00
9-12		0	0	0	0.00		0	0	0	0.00
9-13		0	0	0	0.00		0	0	0	0.00
9-14		0	0	0	0.00		0	0	0	0.00
9-15[b]	6	0	0	0	0.00	6	0	0	0	0.00
9-16		0	0	0	0.00		0	0	0	0.00
9-17		0	0	0	0.00		0	0	0	0.00

[a]Removed one heifer from the experiment, sore mouth.

[b]Removed all heifers from the experiment because of insufficient forage.

Table 18. Daily bloat summary for Groups III and IV, 1963

Date	Group III					Group IV				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
5-6	12	0	0	0	0.00	18	0	0	0	0.00
5-7		0	0	0	0.00		0	0	0	0.00
5-8		0	0	0	0.00		0	0	1	0.06
5-9		0	0	0	0.00		0	0	0	0.00
5-10		0	0	0	0.00		3	0	1	0.33
5-11		0	0	0	0.00		0	0	1	0.06
5-12		0	0	0	0.00		0	0	0	0.00
5-13		0	0	0	0.00		0	0	0	0.00
5-14		2	0	0	0.17		4	0	0	0.22
5-15		0	0	0	0.00		4	0	1	0.39
5-16		0	0	0	0.00		1	0	0	0.06
5-17		2	0	0	0.17		4	0	1	0.39
5-18		1	0	0	0.08		4	4	5	1.50
5-19		0	1	1	0.42		1	2	10[a]	2.06
5-20		0	0	0	0.00		2	6	6	1.78
5-21		0	0	0	0.00		3	4	7	1.78
5-22		0	0	0	0.00		3	1	9	1.78
5-23		0	0	1	0.25		1	2	6	1.28
5-24		0	0	1	0.25		2	1	4	0.89
5-25		0	0	0	0.00		1	1	2	0.50
5-26		0	0	0	0.00		0	0	0	0.00
5-27		0	0	0	0.00		0	0	0	0.00
5-28		0	0	0	0.00		1	0	0	0.06
5-29		0	0	0	0.00		2	1	0	0.22
5-30		0	0	0	0.00		5	2	1	0.67
5-31		1	0	0	0.08		6	1	2	0.78
6-1		0	0	0	0.00		7	1	0	0.50
6-2		0	0	0	0.00		0	0	0	0.00
6-3		0	0	0	0.00		0	0	0	0.00
6-4		0	0	0	0.00		0	0	0	0.00
6-5		0	0	0	0.00		0	0	0	0.00
6-6		0	0	0	0.00		0	0	0	0.00
6-7		0	0	0	0.00		0	0	0	0.00
6-8		0	0	0	0.00		0	0	0	0.00
6-9		0	0	0	0.00		0	0	0	0.00
6-10		0	0	0	0.00		0	0	0	0.00
6-11		0	0	0	0.00		0	0	0	0.00
6-12		0	0	0	0.00		0	0	0	0.00

[a]One animal bloated to a score of 5.

Table 18. (Continued)

Date	Group III					Group IV				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
6-13	12	0	0	0	0.00	18	0	0	0	0.00
6-14		0	0	0	0.00		0	0	0	0.00
6-15		0	0	0	0.00		0	0	0	0.00
6-16		0	0	0	0.00		0	0	0	0.00
6-17		0	0	0	0.00		0	0	0	0.00
6-18		0	0	0	0.00		0	0	0	0.00
6-19		0	0	0	0.00		0	0	0	0.00
6-20		0	0	0	0.00		0	0	0	0.00
6-21		0	0	0	0.00		0	0	0	0.00
6-22		0	0	0	0.00		0	0	0	0.00
6-23		0	0	0	0.00		0	0	0	0.00
6-24		0	0	0	0.00		0	0	0	0.00
6-25		0	0	0	0.00		0	0	0	0.00
6-26		0	0	0	0.00		0	0	0	0.00
6-27		0	0	0	0.00		0	0	0	0.00
6-28		0	0	0	0.00		1	0	1	0.22
6-29		0	0	0	0.00		0	0	0	0.00
6-30		0	0	0	0.00		0	0	0	0.00
7-1		0	0	0	0.00		0	0	0	0.00
7-2		0	0	0	0.00		0	0	0	0.00
7-3		0	0	0	0.00		0	0	0	0.00
7-4		0	0	0	0.00		0	0	0	0.00
7-5		0	0	0	0.00		0	0	0	0.00
7-6		0	0	0	0.00		0	0	0	0.00
7-7		0	0	0	0.00		0	0	0	0.00
7-8		0	0	0	0.00		0	0	0	0.00
7-9		0	0	0	0.00		0	0	0	0.00
7-10		0	0	0	0.00		0	0	0	0.00
7-11		0	0	0	0.00		0	0	0	0.00
7-12		0	0	0	0.00		0	0	0	0.00
7-13		0	0	0	0.00		0	0	0	0.00
7-14		0	0	0	0.00		0	0	0	0.00
7-15		0	0	0	0.00		0	0	0	0.00
7-16		0	0	0	0.00		0	0	0	0.00
7-17		0	0	0	0.00		0	0	0	0.00
7-18		0	0	0	0.00		0	0	0	0.00
7-19		0	0	0	0.00		0	0	0	0.00
7-20		1	0	0	0.08		2	2	0	0.33
7-21		0	0	0	0.00		0	0	0	0.00

Table 18. (Continued)

Date	Group III					Group IV				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
7-22	12	0	0	0	0.00	18	2	0	0	0.11
7-23		0	0	0	0.00		0	0	0	0.00
7-24		0	0	0	0.00		0	0	0	0.00
7-25		0	0	0	0.00		0	0	0	0.00
7-26		0	0	0	0.00		0	0	0	0.00
7-27		0	0	0	0.00		0	0	0	0.00
7-28		0	0	0	0.00		0	0	0	0.00
7-29		0	0	0	0.00		0	0	0	0.00
7-30		0	0	0	0.00		0	0	0	0.00
7-31		0	0	0	0.00		0	0	0	0.00
8-1		0	0	0	0.00		0	0	0	0.00
8-2		0	0	0	0.00		0	0	0	0.00
8-3		0	0	0	0.00		0	0	0	0.00
8-4		0	0	0	0.00		0	0	0	0.00
8-5		0	0	0	0.00		0	0	0	0.00
8-6		0	0	0	0.00		0	0	0	0.00
8-7		0	0	0	0.00		0	0	0	0.00
8-8		0	0	0	0.00		0	0	0	0.00
8-9		0	0	0	0.00		0	0	0	0.00
8-10		0	0	0	0.00		0	0	0	0.00
8-11		0	0	0	0.00		0	0	0	0.00
8-12		0	0	0	0.00		0	0	0	0.00
8-13		0	0	0	0.00		0	0	0	0.00
8-14		0	0	0	0.00		0	0	0	0.00
8-15		0	0	0	0.00		0	0	1	0.17
8-16		0	0	0	0.00		0	0	0	0.00
8-17		0	0	0	0.00		0	0	0	0.00
8-18		0	0	0	0.00		0	0	0	0.00
8-19		0	0	0	0.00		0	0	0	0.00
8-20		0	0	0	0.00		0	0	0	0.00
8-21		0	0	0	0.00		0	0	0	0.00
8-22		0	0	0	0.00		0	0	0	0.00
8-23		0	0	0	0.00		0	0	0	0.00
8-24		0	0	0	0.00		0	0	0	0.00
8-25		0	0	0	0.00		0	0	0	0.00
8-26		0	0	0	0.00		0	0	0	0.00
8-27		0	0	0	0.00		0	0	0	0.00
8-28		0	0	0	0.00		0	0	0	0.00
8-29		0	0	0	0.00		0	0	0	0.00
8-30		0	0	0	0.00		0	0	0	0.00

Table 18. (Continued)

Date	Group III					Group IV				
	No. of cattle	No. bloating to score: 1	2	3	A.D.M.	No. of cattle	No. bloating to score: 1	2	3	A.D.M.
8-31	12	0	0	0	0.00	18	0	0	0	0.00
9-1		0	0	0	0.00		0	0	0	0.00
9-2		0	0	0	0.00		0	0	0	0.00
9-3		0	0	0	0.00		0	0	0	0.00
9-4		0	0	0	0.00		0	0	0	0.00
9-5		0	0	0	0.00		0	0	0	0.00
9-6		0	0	0	0.00		0	0	0	0.00
9-7		0	0	0	0.00		0	0	0	0.00
9-8		0	0	0	0.00		0	0	0	0.00
9-9		0	0	0	0.00		0	0	0	0.00
9-10		0	0	0	0.00		0	0	0	0.00
9-11		0	0	0	0.00		0	0	0	0.00
9-12		0	0	0	0.00		0	0	0	0.00
9-13		0	0	0	0.00		0	0	0	0.00
9-14		0	0	0	0.00		0	0	0	0.00
9-15[b]	6	0	0	0	0.00	15	0	0	0	0.00
9-16		0	0	0	0.00		0	0	0	0.00
9-17		0	0	0	0.00		0	0	0	0.00

[b]Removed all heifers from the experiment because of insufficient forage.

Table 19. Effect of various sources of added energy and of form of soybean oil on the lipolytic activity of rumen fluid

Oil		Added energy: None	Gum cellulose (100 mg.)	Dextrose (100 mg.)	Maltose (100 mg.)	Total for oil
			(% hydrolysis)			
Refined		75.7[a] 40.1	61.1 47.0	32.6 35.4	24.2 33.9	
		75.5 49.8	78.7 57.1	44.1 31.8	38.5 42.7	
	Sum	241.1	243.9	143.9	139.3	768.2
	Mean	60.3	61.0	36.0	34.8	
Emulsified		81.7 56.2	72.4 53.6	23.7 34.2	23.3 43.7	
		89.1 44.5	81.9 58.4	55.5 29.3	40.3 28.5	
	Sum	271.5	266.3	142.7	135.8	816.3
	Mean	67.9	66.6	35.7	33.9	
Crude		82.7 49.8	81.4 61.3	39.5 43.9	27.2 43.2	
		87.4 52.4	81.2 53.0	46.7 34.5	40.6 39.6	
	Sum	272.3	276.9	164.6	150.6	864.4
	Mean	68.1	69.2	41.2	37.7	
Total for energy		784.9	787.1	451.2	425.7	

[a]Vertical pairs are replicates.

Table 20. Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	
Total	47	17,003.05			
Treatments	11	10,499.03	954.46	5.28	$F_{.01} = 2.78$
Source of energy	3	10,093.21	3,364.40	18.62	$F_{.01} = 4.38$
Oil	2	289.20	144.60	.80	$F_{.05} = 3.26$
Source of energy x oil	6	166.62	27.77	.15	$F_{.05} = 2.36$
Error	36	6,504.02	180.67		

Table 21. Effect of source and level of added energy on the lipolytic activity of rumen fluid

Treatment	Replicates[a,b]		Sum
	(% hydrolysis)		
Control	18.5	31.3	49.8
Gum cellulose - 200 mg.	53.4	57.8	111.2
Gum cellulose - 400 mg.	38.3	52.0	90.3
Gum cellulose - 600 mg.	51.5	45.5	97.0
Gum cellulose - 800 mg.	39.5	38.3	77.8
Dextrose - 200 mg.	-0.9	19.9	19.0
Dextrose - 400 mg.	-72.4	-45.2	-117.6
Dextrose - 600 mg.	-109.7	-83.8	-193.5
Dextrose - 800 mg.	-110.4	-177.5	-287.9
Sucrose - 200 mg.	-13.7	-0.2	-13.9
Sucrose - 400 mg.	-38.8	-28.1	-66.9
Sucrose - 600 mg.	-39.5	-58.0	-97.5
Sucrose - 800 mg.	-66.6	-87.3	-153.9

[a]Each flask contained 56.9 mg. of esterified lipid before incubation.

[b]Negative values indicate an apparent synthesis of ester.

Table 22. Effect of streptomycin,[a] tylosin, erythromycin and penicillin on the lipolytic activity of rumen microorganisms

Organisms		Antibiotics					Total for organisms
		None	Streptomycin (.003%)	Tylosin (.003%)	Erythromycin (.003%)	Penicillin (.003%)	
				(% hydrolysis)			
Protozoa		18.1	27.9	41.4	26.4	23.3	
		44.4	38.4	18.1	26.4	62.4	
		10.8	15.1	10.1	9.4	6.6	
		10.1	15.1	8.7	8.7	12.9	
	Sum	83.4	96.5	78.3	70.9	105.2	434.3
	Mean	20.9	24.1	19.6	17.7	26.3	
Bacteria		59.6	68.9	61.2	58.1	83.7	
		79.0	48.8	99.2	55.0	55.0	
		10.1	17.5	15.3	24.8	19.7	
		7.2	13.1	25.6	25.6	19.7	
	Sum	155.9	148.3	201.3	163.5	178.1	847.1
	Mean	39.0	37.1	50.3	40.9	44.5	

[a]Each flask contained approximately 108 mg. of esterified lipid before incubation.

Table 22. (Continued)

Organisms		Antibiotics: None	Streptomycin (.003%)	Tylosin (.003%)	Erythromycin (.003%)	Penicillin (.003%)	Total for organisms
				(% hydrolysis)			
Protozoa and bacteria		78.5	85.5	79.3	87.5	80.6	
		85.5	78.5	77.8	83.4	85.5	
		85.6	87.6	81.5	81.5	84.9	
		83.5	85.6	82.2	83.5	84.3	
	Sum	333.1	337.2	320.8	335.9	335.3	1662.3
	Mean	83.3	84.3	80.2	84.0	83.4	
Total for antibiotics		572.4	582.0	600.4	570.3	618.6	

Table 23. Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	
Total	59	58,068.14			
Treatments	14	39,724.87	2,837.49	6.96	$F_{.01} = 2.50$
Antibiotics	4	140.00	35.00	.09	$F_{.05} = 2.57$
Organisms	2	39,048.98	19,524.49	47.90	$F_{.01} = 5.10$
Antibiotics x Organisms	8	535.89	66.99	.16	$F_{.05} = 2.14$
Error	45	18,343.27	407.63		

Table 24. Effect of extended incubation with streptomycin, tylosin, erythromycin and penicillin on the subsequent lipolytic activity of rumen microorganisms[a]

Organisms		Antibiotics: None	Streptomycin (.006%)	Tylosin (.006%)	Erythromycin (.006%)	Penicillin (.006%)	Total for organisms
				(% hydrolysis)			
Protozoa		37.0	64.6	30.5	56.6	36.3	
		41.4	66.7	37.7	34.9	31.9	
	Sum	78.4	131.3	68.2	91.5	68.2	437.6
	Mean	39.2	65.7	34.1	45.8	34.1	
Bacteria		47.2	53.1	40.7	50.2	48.8	
		37.0	39.9	38.5	42.1	53.9	
	Sum	84.2	93.0	79.2	92.3	102.7	451.4
	Mean	42.1	46.5	39.6	46.2	51.4	
Protozoa and bacteria		80.5	82.6	82.6	82.6	82.6	
		75.7	82.0	80.5	77.1	78.5	
	Sum	156.2	164.6	163.1	159.7	161.1	804.7
	Mean	78.1	82.3	81.6	79.9	80.6	
Total for antibiotics		318.8	388.9	310.5	343.5	332.0	

[a]Each flask contained approximately 106 mg. of esterified lipid before incubation.

Table 25. Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	
Total	29	10,731.75			
Treatments	14	10,224.02	730.29	21.57	$F_{.01} = 3.56$
Antibiotics	4	629.87	157.47	4.65	$F_{.05} = 3.06$
Organisms	2	8,659.12	4,329.56	127.90	$F_{.01} = 6.36$
Antibiotics x Organisms	8	935.03	116.88	3.45	$F_{.01} = 4.00$
Error	15	507.73	33.85		

Table 26. Effect of penicillin and point of collection of rumen fluid on the lipolytic activity of rumen microorganisms[a]

Enzyme preparations		Point of collection of rumen fluid: Ventral rumen	Dorsal rumen	Total for preparations
		(% hydrolysis)		
Rumen fluid		55.9 60.9 66.5 74.2	45.7 53.8 53.5 53.8	
	Sum	257.5	206.8	464.3
	Mean	64.4	51.7	
Rumen fluid + .003% penicillin		51.7 66.6 54.5 41.5	72.2 62.3 68.4 59.8	
	Sum	214.3	262.7	477.0
	Mean	53.6	65.7	
Protozoa		29.1 46.8 23.0 46.2	37.9 45.8 37.9 43.2	
	Sum	145.1	164.8	309.9
	Mean	36.3	41.2	
Bacteria		27.5 47.9 34.8 41.4	42.8 69.2 52.3 42.7	
	Sum	151.6	207.0	358.6
	Mean	37.9	51.8	
Bacteria + .003% penicillin		28.3 37.2 28.3 47.1	31.9 51.1 31.9 39.9	
	Sum	140.9	154.8	295.7
	Mean	35.2	38.7	

[a]Each flask contained approximately 110 mg. esterified lipid before incubation.

Table 26. (Continued)

Enzyme preparations		Point of collection of rumen fluid: Ventral rumen		Dorsal rumen		Total for preparations
Protozoa + bacteria		88.1	76.8	83.7	82.6	
		84.7	80.0	80.6	82.6	
	Sum	329.6		329.5		659.1
	Mean	82.4		82.4		
Protozoa + bacteria + .003% penicillin		78.2	81.4	85.6	74.8	
		81.5	79.4	81.3	84.5	
	Sum	320.5		326.2		646.7
	Mean	80.1		81.6		
Total for point of collection		1559.5		1651.8		

Table 27. Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	
Total	55	20,376.00			
Treatments	13	17,899.61	1,376.89	23.35	$F_{.01} = 2.54$
Point of collection	1	152.13	152.13	2.58	$F_{.01} = 7.27$
Enzyme preparations	6	16,825.11	2,804.19	47.56	$F_{.01} = 3.26$
Interaction	6	922.37	153.73	2.61	$F_{.01} = 3.26$
Error	42	2,476.39	58.96		