STUDIES OF THE EFFECTS OF HIGH LEVELS OF NEUTRAL FATS IN RUMINANT RATIONS

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INTRODUCTION

An increased interest in the inclusion of animal fat, as well as the vegetable oils, in practical rations for ruminants has developed in recent years because of an increased availability of inedible tallow and greases, the production of which amounted to over three billion pounds in a recent twelve month period (USDA, 1956). The metabolizable energy of fat is 2.25 times that of carbohydrate and protein, thus the relative energy value of tallow, greases, and plant oils should be much greater than that of the cereal grains. In addition to this nutritive advantage less important benefits from the inclusion of fat are an improved physical appearance of the ration and a reduction in dustiness. When blended into commercial feeds, it decreases the wear of the mixing machinery and facilitates pelleting.

At the present time the outlook for the utilization of fats in ruminant rations is discouraging. Although there may be a slight increase in gain when small amounts of fat are included in the diet, the feeding of large amounts has usually resulted in decreased growth. Digestion trials indicate that this effect is due to a decrease in the digestibility of ration components, especially crude fiber.

The addition of alfalfa ash to rations containing cottonseed hulls or corn cobs seems to counteract the detrimental effects of corn oil upon the utilization of these rations. The first experiment of this investigation was designed to study the effect of a high level of animal fat upon the utilization of a ration containing cottonseed hulls.

Subsequent experiments were planned to yield information as to why additional fat in large amounts is detrimental to ruminants as well as why alfalfa ash, under certain conditions, counteracts the deleterious effects of fat.

REVIEW OF LITERATURE

The Utilization of Fat in Ruminant Rations

Many workers have shown that the addition of fat to the rations of simple stomached animals results in more efficient utilization of feed as measured by the units of feed required to produce a unit of gain. Forbes and Swift (1944) working with mature rats found that the specific dynamic effect was 16% of the gross energy in the case of fat while that of protein and carbohydrates (cerelose) was 32 and 20%, respectively.

The results with ruminants are more confusing. Jones et al. (1942), working with cattle, supplemented silage with 4 lb. cottonseed meal and .18, .58, and .98 lb. per day of cottonseed oil. The average daily gains were 1.76, 1.84, and 1.97 lb. on the respective rations. When cottonseed oil was added to steer fattening rations to increase the fat content from 3% to 7.5%, Willey et al. (1952) found no difference in the rate of gain but noted that the amount of feed necessary for 100 lb. gain dropped from 820 to 710 lb. when cottonseed oil was included in the ration. The value of adding pellets containing 5.5% corn oil or beef tallow to a ration of corn and hay for fattening steers was studied by Matsushima and Dow (1953). When 12 lb. of the pellets were fed with 9 lb. corn and 2 lb. hay, the average daily gains for steers receiving beef tallow and corn oil pellets were 2.00 and 1.74 lb. for the respective rations. The control lot, fed 17 lb. corn, 1 lb. soybean meal, and 2 lb. hay, gained 2.11 lb. per day.

No difference was noted by Pharham et al. (1950) in the gains of two groups of cows receiving solvent extracted and hydraulic processed cottonseed meal as a supplement on grass. Results of work by Hentges et al. (1954) indicate that waste beef fat can replace up to 5% of the concentrates in steer fattening rations. In a 154-day feeding trial conducted by these investigators, rations containing 0, 5, and 10% additional fat produced respective gains of 1.8, 1.9, and 1.5 lb. per day.

Lucas and Loosli (1944) determined the digestibilities of different soybean preparations containing low and high amounts of ether extract (1% vs. 7%). Crude fiber and NFE digestibilities were lowered when the high fat rations of ground soybeans, solvent extracted soybeans plus corn oil, and solvent extracted soybeans plus soybean oil were fed to dairy cows. These workers also noted that added fat decreased milk production without increasing the fat content of the milk.

Studying the effect of different proportions of nutrients upon the digestibility of rations by lambs, Swift et al. (1947) fed rations containing 2.8, 6.4, and 9.8% corn oil and reported dry matter digestibility as 72.8, 76.2, and 74.7%, respectively, for the three treatments. In another trial (Swift et al., 1948) corn oil was fed to mature sheep at levels of 3, 4, 5, 6, 7, and 8%. The increasing levels of fat lowered digestibility of dry matter, NFE, and energy. Gallup et al. (1950) reported that there was no difference in the digestibility of rations containing hydraulic or solvent process cottonseed meal.

Hale and King (1955) separately added corn oil, prime tallow, and hydrogenated animal fat in levels of 0, 4, 8, and 12% to lamb fattening rations and found that dry matter digestibility was reduced in rations containing added fat at the two higher levels.

Increasing the fat from 1 to 12% of the ration decreased the growth of rabbits in a trial conducted by Wooley and Mickelson (1954). However, the addition of calcium, potassium, and sodium, especially the latter, increased growth on the high fat diets although the minerals had no effect when the fat content was low. Brooks et al. (1954) designed a digestion trial to study the effect of two levels of corn oil and lard, with or without alfalfa ash, on the digestibility of cellulose and crude protein. The addition of either 32 or 64 grams of fat, especially corn oil, lowered the apparent digestibility of cellulose. Corn oil also decreased the digestibility of crude protein. The addition of 1.8 gm. alfalfa ash overcame the detrimental effect of 32 gm. fat and improved digestibility of the ration containing 64 gm. corn oil.

Alfalfa ash increased the crude fiber digestibility of rations containing corncobs with corn oil as a part of the concentrate (Swift et al., 1951; Chappel et al., 1955). According to Tillman et al. (1954) the digestibility of a semi-purified diet containing cottonseed hulls, cerelose, corn oil, urea, gelatin, di-calcium phosphate, salt, sodium sulfate, and vitamin A and D oil was improved by the addition of either natural or simulated alfalfa ash.

Ward (1956) studied the effect of fat and/or minerals on low quality roughage utilization by lambs. He found that corn oil depressed growth when added to a semi-purified diet containing cottonseed hulls. The effect of adding alfalfa ash to the basal ration was small, while there was a significant increase in gain when alfalfa ash was included in a ration containing corn oil. However, a lamb fattening ration containing 10% corn oil was not improved by adding 0.85% alfalfa ash. In further work he reported that corn oil, when added to rations containing low quality roughages, lowered the apparent digestibility of NFE, crude fiber, organic matter and dry matter. Alfalfa ash overcame this effect and returned digestibility to normal. Erwin et al. (1956a) showed that 7% tallow added to a steer fattening ration containing alfalfa hay increased gain, but reduced it when fed with wheat straw. The same workers (Erwin et al., 1956b) measured digestibility and found that in rations containing alfalfa hay the addition of tallow did not reduce the digestibility of dry matter and crude fiber.

The work up to this time indicates that the main effect of increasing fat in a ruminant ration is to decrease the digestibility of crude fiber. It is also indicated that an increase in the mineral content of the ration favorably affects digestibility when fat has been added.

The Development of Ketosis in Ruminants

Ketosis, which occurs in cattle and sheep during periods of stress, is due to an abnormal metabolism that produces large amounts of the ketone bodies. An important contribution to the knowledge regarding the formation of the ketone bodies was made when Weinhouse et al. (1944) studied the distribution of C_{13} in the acetoacetate which was formed from the B-oxidation of fatty acids. Later studies by Gurin and Crandall (1948) indicated that butyric acid (or a terminal C_4-unit of B-oxidation) may be directly converted to acetoacetic acid. From acetoacetic acid the other ketone bodies are formed by reduction or decarboxylation (Fruton and Simmonds, 1953). According to the same authorities, ketonemia is due either to a decreased rate of metabolism or to an elevated rate of production of ketone bodies in the tissues. They state that the latter process is the more important in ketosis and is due to an increased metabolism of fat.

The stress of pregnancy in ewes and lactation in high producing cows which preludes ketosis apparently causes a hypoglycemic condition (Dukes, 1955). This stimulates the hypothalmus to release ACTH which, in turn, brings about the secretion of the adrenal cortical hormones (Best and Taylor, 1955). While the initial effect of the cortical hormones is to elevate the glycemic level through gluconeogenesis, the combined actions of the same hormones constitute the endocrine mechanism by which depot fat is mobilized to meet the energy requirements of the animal and to conserve its carbohydrate supplies (Levin and Farber, 1950).

Other conditions which cause an increase in fat metabolism are starvation, diabetes, renal glucosuria, and a diet which is rich in lipid and low in carbohydrate content (White et al., 1954). Daily ketonuria resulted when two healthy men lived exclusively for one year on a low carbohydrate diet of lean and fat meat (Tolstoi, 1929). Cameron and Goss (1940) found that there was a significant lowering of the alkali reserve of pregnant ewes fed a high fat diet of soybean meal plus 50% cottonseed oil. There is a lowering of the respiratory quotient when the fat content of the diet is increased, according to the findings of Forbes et al. (1946a).

Sampson and Boley (1940) reported that in 75% of the blood samples collected from 60 pregnant ewes, the total ketones expressed as acetone were less than 3 mg.%. The same authors stated that the presence of 7 to 10 mg.% acetone in the blood indicates that the ewe is probably afflicted with an early stage of pregnancy disease. Work by Sampson and Hayden (1936) indicates that from 0.50 to 5.00 mg.% ketone bodies (expressed as acetone) occurs in the urine of healthy ewes while the acetone content of urine from ketotic animals may range from 15 to 100 mg.%. From 6 to 9 mg.% acetone was measured in the urine of normal ewes by Sampson et al. (1933) while 300 to 400 mg.% acetone occured in urine associated with a fatal case of pregnancy disease. Roderick and Harshfield (1932) determined that the ketone body content of urine from a normal ewe is less than 1 mg.% while that of pathogenic urine ranges from 10 to 300 mg.%.

While the majority of the investigations of ketosis have been directed toward elucidating its etiology in conjunction with the stress of pregnancy, there is some evidence, supported by the fact that keto-nemia is a metabolic condition, that a relationship exists between nutrition and ketosis.

Factors Affecting Intraruminal pH

The pH of the rumen contents of adult cattle under normal conditions was reported by Kick et al. (1938) to range from 5.5 to 7.7. The pH values observed by Monroe and Perkins (1939) fell within a range of 6.85 to 7.07 while Cason et al. (1954) reported readings that varied from 6.5 to 7.2. Smith (1941) indicates that the ingesta may be somewhat more acid than observed by the above investigators as he found pH values to range from 5.5 to 6.8. The normal pH range is 6.19 to 6.85 in the sheep rumen according to Ammerman and Thomas (1955). In goats the values fall between 6.6 and 7.4 (Kameoka et al., 1953). Kesler et al. (1951) observed that the mean value of the pH of ingesta taken from 8 to 32 day old dairy calves is 5.6 which indicates that the mature rumen is not as acid as the underdeveloped one. Hibbs et al. (1956) reported a significant increase in pH in the rumen of dairy calves from a range of 6.14 to 6.42 at four weeks to 6.50 to 6.76 at twelve weeks.

Clark and Lombard (1951) found that the pH of samples taken from the rumen with a stomach tube and through a fistula measured 7.4 and 6.8, respectively. Turner and Hodgetts (1955) stated that use of a stomach tube produced samples that tested .23 to .42 pH units higher than samples carefully taken through a fistula. The use of a thirty inch electrode to make in vivo readings gave Smith (1941) lower values than when he took samples from the rumen. Subsequent observations by the same investigator indicated that a closed fistula reduced values .30 pH units. Turner and Hodgetts (1955) attributed the difference of in vivo and in vitro readings to the loss of CO_2 from the ingesta. They found an immediate rise in pH when rumen contents were exposed to the air. When these authors opened the fistula of their sheep, there was a drop in intraruminal CO_2 from 58.5% to 3.2% and a corresponding rise in pH at the surface of the ingesta from 6.63 to 6.92 in nine minutes. Since 50% of the total rumen gas was CO_2, the same authors found that they could equilibrate a sample of ingesta with an equal mixture of CO_2 and N_2 and return it to its normal pH value, providing that it had not become more alkaline than 7.5.

Smith (1941) measured pH in different areas of the rumen and reported the mean values of his observations as given in the following table:

	Front	Middle	Rear
Surface	6.27	6.05	6.00
Deep	6.20	6.13	6.13

The work of Hale et al. (1940) and of Monroe and Perkins (1939) indicate that the anterior of the rumen is more acid than the posterior.

Monroe and Perkins (1939) measured the diurnal variation in pH by reading samples at two hour intervals and found that there is a drop in pH for 3 to 4 hours after feeding followed by a gradual rise in pH. The rumen is most alkaline just prior to feeding (Smith, 1941, and Kick et al., 1954). Phillipson (1942) related the diurnal curve in pH to the volatile fatty acid production in the rumen. He fed a mangold and cabbage diet containing a large amount of readily available carbohydrate and noted that the most acid pH occured 3½ hours after feeding. When a slower fermenting bran and oats ration was fed, the pH values were lowest about 6 hours after feeding. The volatile fatty acid production reached a peak 12 hours after feeding when Phillipson fed hay to sheep, but in this trial pH measurements were not made.

Monroe and Perkins (1939) measured the pH values of rumen contents of 16 slaughter animals that had been on a 12 to 18 hour fast and found that the pH ranged from 7.13 to 7.61.

Work by Hale et al. (1940) indicates that different feeds produce ingesta that differs in respective pH values. They found that the ingesta of dairy cows fed alfalfa hay averaged 6.82 while a mixed ration of alfalfa, silage and soybean oil meal resulted in ingesta with a pH of 6.2. Dairy calves fed a 4:1 roughage to grain ratio were more alkaline in the reaction of their ingesta than calves on a 3:2 ratio according to Hibbs et al. (1956). According to Phillipson (1942), the volatile fatty acid production is greater and the pH of the ingesta is lower when green grass is fed instead of dry hay. Hibbs et al. (1956) fed alfalfa and timothy hay to dairy calves and noted a greater amount of fermentation from the former roughage.

When they dispensed 100 gm. of glucose, fructose, or sucrose into the rumen of sheep, Phillipson and McAnally (1942) noticed a marked fall in pH which lasted one hour. When galactose, lactose, or maltose was administered, there was a more gradual fall in pH of the rumen contents for 4½ hours. The addition of 100 gm. baked starch or 30 gm. filter paper lowered the pH values slightly for about eight hours. In vitro studies by Hoflund et al. (1948) showed that there was a decrease in pH when glucose was added to samples of digesting cellulose. The role of fatty acids in maintaining an acid pH was shown by Clark and Lombard (1951) when two ewes were treated with sulphanilamide to stop fermentation. After four days both animals were weak and off feed. The pH of their rumen ingesta measured 7.8.

The diurnal variations in pH, ash content of the ingesta, and fat content of the ingesta was measured by Cason et al. (1954). They found a significant positive correlation of 0.89 between the ash content of the ingesta and the pH in the rumen. The reported correlation between fatty acid content of the rumen and pH was 0.15. Their work showed that sericea lespedeza hay with an ash content of 6.89 consistently produced an ingesta with a higher pH than Korean lespedeza or upland prairie hay with respective ash contents of 6.73 and 5.17. Ammerman and Thomas (1955) state that plant juice from mature forages has a higher buffering capacity than that from young plants.

Clark and Lombard (1951) produced data to show that rumen contents are more strongly buffered toward acids than toward alkalis. When they fed 12 lb. of A.I.V. silage with a pH of 3.6 to a dairy cow, Monroe and Perkins (1939) found that there was not an appreciable lowering of pH in the rumen. However, the administration of 2 oz. of ground limestone raised pH from 6.87 to 6.97. Turner and Hodgetts (1955) state that the fasting rumen is buffered by phosphate and bicarbonate systems, while a moderately fermenting rumen is balanced by phosphate, bicarbonate, and acetate; and the actively fermenting rumen is maintained by a phosphate and acetate buffers. Hoflund et al. (1948) used protein to buffer in vitro digestion of cellulose.

The chief source of rumen buffers is saliva, which is profusely secreted in ruminants. Markoff (1913) calculated that the mature bovine produces 50 liters of saliva per day which contains 300 to 350 gm. of alkali expressed as sodium bicarbonate. The production of large amounts of saliva by ruminants may be the result of a gastric control of secretion caused by innervation of the vagus nerve by mechanical stimulation of the cardiac area of the rumen (Clark and Weiss, 1952). A rise in pH, apparently due to an increase is saliva secretion, was noted immediately after feeding by Phillipson (1942). According to Reid and Huffman (1949), the average pH of saliva is 8.53.

The effect of an abnormal reaction upon the physiological activities of the rumen was studied by Clark and Lombard (1951). Their findings show that rumen motility is decreased when the pH reaches 7.5. The authors concluded from their data that the paresis is caused by the effect on the vagal centers of an upset of the acid-base balance of the blood. There was no lowering of motility when a sheep rumen was dosed with 100 ml. of normal HCl which dropped the pH of the ingesta from 7.3 to 6.7. It was also noted in this study that the maintenance of a high rumen pH caused inappetance and inhibition of both cellulose digestion and sugar fermentation. Meites et al. (1951) measured in vitro cellulose digestion over a range of pH values from 4.53 to 8.30 and found that breakdown of cellulose dropped off sharply when the pH exceeded 7.35. Clark et al. (1951) associated urea poisoning with an increase in pH caused by the release of ammonia. This was followed by a decrease or entire cessation of rumen motility. Dilute acid administered into the rumen or injected intravenously was found to be beneficial in limiting paresis. It was observed that urea was more toxic when given to sheep which had been fasted for 48 hours. The authors explained that this may have been due to insufficient organic acid present to counteract the ammonia or a lack of readily available carbohydrate to utilize the urea.

A relationship between the reaction of the rumen and absorption from it was reported by Pfander and Phillipson (1953) who observed that lowering of the pH of the rumen from 6.4 to 5.0 increased the absorption of volatile fatty acids from 21 millequivalents per hour to 98 meq. per hour. Danielli et al. (1945) also found that an increase in pH decreases absorption, and Gray (1948) demonstrated that there is no absorption of acetic acid or sodium acetate when the pH is 7.5 or greater. The literature indicates that many factors control the reaction of the rumen ingesta. To maintain a favorable rumen environment for the digestion and absorption of nutrients, it is essential that the pH of the ingesta be maintained within an optimum range.

EXPERIMENT I

Effect of Alfalfa Ash and Bicarbonate on the Utilization of Fat by Lambs in a Feeding Trial

In previous tests alfalfa ash has increased rate of gain and dry matter digestibility when ruminants were fed a ration containing added fat. However, Ward (1956) did not obtain an increase in gain by adding 0.85% alfalfa ash to a fattening type ration containing 10% corn oil and suggested that insufficient ash was present. Therefore, in this experiment the amount of ash was increased. Also in this trial the hypothesis that the alkaline alfalfa ash has a neutralizing and buffering effect on fat in the rumen was tested by including rations containing sodium and potassium bicarbonate in the following design.

Procedure

The animals fed in this experiment were kept in five pens 12 feet square that were located in a brick barn. Each lot had access to an outside enclosure 12 by 25 feet at all times. Water and feed were offered *ad libitum*.

The following treatments were used:-

Ration No.	Treatment
1	Basal
2	Basal + 15% Stabilized Animal Fat
3	Basal + 15% Stabilized Animal Fat + 3.6% Alfalfa Ash
4	Basal + 15% Stabilized Animal Fat + 4.7% $NaHCO_3$
5	Basal + 15% Stabilized Animal Fat + 5.6% $KHCO_3$

The composition of the different rations is given in table 1. The calculated crude protein content of the basal ration is 10%; while that of the rations containing additional fat is 12%. This increase in the protein content maintains a calculated TDN/crude protein ratio of 6.5:1. Minerals were added in amounts calculated to be approximately equivalent to the amounts of alkali required to saponify the extra fat. The saponification number of the animal fat was 188.8 which is equal to that of tri-stearin.

The alfalfa ash was prepared prior to the beginning of the experiment by burning good quality alfalfa hay in an open barrel with further ashing in a muffle furnace at 600° C to remove any remaining carbon. Rations were mixed as needed in 100 lb. lots and the stabilized animal fat was added at this time.

Forty-five western-type ewe lambs were drenched with two ounces of a phenothiazine suspension twenty days before the experiment began and ten days before the start of the trial they were sheared. The lambs were distributed into five nearly equal groups on the basis of their shrunk weight. These groups were assigned at random to the different pens which were in turn randomly designated to the various treatments.

Shrunk weights were obtained at the beginning and the end of the experiment by withholding feed and water for 12 hours before weighing. Full weights were taken every two weeks throughout the experiment. The lambs were paint branded for ease of identification. The experiment was terminated on the forty-second day at which time all the lambs were placed on the basal ration during a subsequent twenty-one day recovery period.

Results and Discussion

The results of the experiment, which are summarized in table 2, show that 15% animal fat in the ration caused the lambs to consume less feed and to lose weight. When the variance due to treatment is analyzed and assigned to orthogonal sets of comparisons as described by Snedecor (1946), the detrimental effect of fat is found to be highly significant. A further depression of growth by the addition of bicarbonates approaches significance (P less than 0.10). The only previous report of including bicarbonate in lamb rations is made by Cox and Erhart (1949). These workers added 0.5% $NaHCO_3$ to lamb rations in an effort to control death losses in the feedlot. In this trial $NaHCO_3$ affected neither mortality nor rate of gain.

Difficulty was experienced in starting the lambs on feed, especially when fat was included in the diet; as in these four lots all animals but one lost weight during the first two weeks. Table 1 in the appendix shows that subsequent gains of lambs on these treatments were not great enough to off set the loss in weight during the first period. It seemed apparent that the animal fat used in this study adversely affected palatability since feed consumption of the fat-containing rations was below that of the basal ration. The wool of some of the lambs on the high fat treatments appeared to become detached from the skin easily, and wool-pulling by the lambs was common. Some scouring occured in lot 2; while hard, gray-colored pellets were formed in lot 3.

Seven lambs were lost during the trial; all on fat containing rations. They were generally emaciated and had lost weight for some time preceeding death. Analysis with the chi square test (Snedecor, 1946) indicated that there was an independent distribution of lamb deaths among the different treatments.

When the lambs received the basal ration during the recovery period, the gains of lots 2, 3, 4, and 5 were significantly greater than the gains of the lambs that had been on the basal ration. This may indicate further that the loss of weight during the experiment was due to the presence of the additional fat. The recovery of lots 2 and 3 was significantly faster than the lots that had received the bicarbonates. This suggests that the bicarbonate may have a residual, detrimental effect upon rumen function.

Summary

Forty-five lambs were used in a 42-day group feeding trial to test the value of including 15% stabilized animal fat in a fattening type ration. The effect of adding alfalfa ash, sodium bicarbonate, or potassium bicarbonate to improve the utilization of a fat containing ration was also studied. The addition of fat reduced feed consumption and significantly decreased rate of gain, actually causing the animals to lose weight. Alfalfa ash, potassium bicarbonate or sodium bicarbonate did not improve the utilization of the high fat rations.

When placed on the basal ration during a 21 day recovery period, lambs that had previously received fat significantly outgained those that were fed the basal ration during the experiment. However, the recovery of lambs that had received bicarbonate was significantly less than that of lambs in the other animal fat treatments.

TABLE 1

Rations Used in Experiment I

Feed	Ration 1[a]	2	3	4	5
	%	%	%	%	%
Cottonseed Hulls	35.00	35.00	35.00	35.00	35.00
Ground Milo	58.50	31.20	26.35	24.80	23.60
Cottonseed Meal	4.90	17.20	18.45	18.90	19.20
Limestone	.50	.50	.50	.50	.50
Di-calcium Phosphate	.50	.50	.50	.50	.50
Salt	.50	.50	.50	.50	.50
Quadrex[b]	.10	.10	.10	.10	.10
Stabilized Animal Fat		15.00	15.00	15.00	15.00
Alfalfa Ash			3.60		
$NaHCO_3$				4.70	
$KHCO_3$					5.60

[a] All lambs received ration #1 during the recovery period.

[b] Micratized vitamin A and D supplement containing 10,000 I.U. of vitamin A and 1,250 I.U. of vitamin D_2 per gram.

TABLE 2

Effect of Fat and Mineral Additives in a Lamb Fattening Ration, Experiment I

	Basal	Basal + Fat	Basal + Fat + Alfalfa Ash	Basal + Fat + $NaHCO_3$	Basal + Fat + $KHCO_3$
No. of Animals	9	9	9	9	9
No. Died	0	1	1	2	3
Av. Daily Feed Intake (lb.)*	2.41	1.45	1.67	1.29	1.03
Av. Initial Wt. (lb.)	58.6	59.3	58.9	59.1	59.2
Av. Daily Gain (lb.)	0.17	-0.04	-0.04	-0.12	-0.15
Av. Daily Gain During Recovery (lb.)	.29	.45	.55	.35	.40

* Corrected by dividing total feed by lamb-days.

EXPERIMENT II

The Effect of 15% Animal Fat in Lamb Rations on Dry Matter and Organic Matter Digestibility

It has been shown with digestion trials that corn oil depresses nutrient digestibility (Ward, 1956). In Experiment 1 of this series the addition of 15% stabilized animal fat significantly depressed growth. The purpose of this trial was to ascertain whether the effect of animal fat is similar to that of corn oil on the digestibility of dry matter and organic matter by sheep.

Procedure

Six western-type wether lambs, averaging 86 pounds, were divided on the basis of weight into two groups. The treatments, basal and basal plus 15% stabilized animal fat, were randomly assigned to the two groups. Feed intake was controlled at a low level in an attempt to maintain constant consumption during the preliminary and collection periods. The lambs were fed once a day in individual, stanchion-type feeders. The composition of the rations is shown in table 3. One lb. of ration 1 or 1.2 lb. of ration 2 was given to each lamb daily. The rations for the entire trial were mixed at the beginning of the experiment and stored until used.

Each group of animals was housed in a box stall which was approximately 10 feet square. During the time the lambs were not stanchioned, they were allowed the freedom of the stall with free access to water.

Following a 7-day preliminary period, complete collection was made by means of a harness and bag as described by Tillman <u>et al</u>. (1954). Collection was made once daily, and the feces were dried for 48 hours in a forced draft oven from 68° to 72° C. The total collections were stored in open metal containers. After allowing three days for equilibrating with the air, they were sampled for analysis. The feed and feces were chemically analyzed by the methods of the AOAC (1950).

Two lambs on ration 2 started to go off feed the fourth day of the collection period, and collection from this group was stopped at the end of the fifth day. Fecal collections from the lambs receiving the basal ration continued until the seventh day; the total collection period for ration 1 was seven days while that for ration 2 was five days.

The trial was replicated using nine native wether lambs averaging 70 lb. The six lambs allotted to the high fat ration had previously become accustomed to a 10% corn oil diet. The three lambs assigned to receive ration 1 had been fed a ration with no added fat. During the fourteen-day preliminary period, it was necessary to remove a lamb from each group because of feed refusal. Feces were collected for seven days by the method described previously.

Results and Discussion

In addition to reducing the acceptability of the ration, a high level of fat caused the feces to be soft and loose and to have an offensive odor. In the replication the ash content of the feces of three lambs, two on the basal ration and one receiving added fat, was abnormally high (14.6, 20.4, 23.4%). There is a possibility that some dirt may have been consumed by the lambs. Digestion coefficients are shown in appendix table IV. The average values for apparent digestibility of dry matter and organic matter are as follows:

	Dry Matter	Organic Matter
	%	%
Basal	63.8	65.7
Basal plus 15% stabilized animal fat	45.1	49.2

A statistical analysis of the data, summarized in appendix table V indicates that the animal fat significantly (P less than .05) reduced the apparent digestibility of both dry matter and organic matter. This agrees with the findings of Ward (1956) when corn oil was added to the ration.

Summary

Thirteen native and western type wether lambs were used to determine the effect of an addition of 15% stabilized animal fat on the digestibility of dry matter and organic matter in a fattening type ration. The fat was found to significantly (P less than .05) depress, in instances to extremely low levels, the apparent digestibility of dry matter and organic matter.

TABLE 3

Composition of Rations Used in the Digestion Trial, Experiment II

	Ration	
Feed	1	2
	%	%
Cottonseed Hulls	35.00	35.00
Ground Milo	45.00	15.40
Cottonseed Meal	18.40	33.00
Limestone	.50	.50
Di-calcium Phosphate	.50	.50
Salt	.50	.50
Vitamin A and D supplement (Quadrex)	.10	.10
Stabilized Animal Fat		15.00
Daily Allowance (lb.)	1.20	1.00

EXPERIMENT III

The Effect of Separating the Concentrate Portion of the Ration from the Roughage When Corn Oil and Cottonseed Hulls are Included in the Ruminant Diet

The two previous experiments of this series have confirmed the observations of other workers that fat added to a ruminant diet decreases rate of gain and lowers digestibility of the ration. However, it is not known how fat exerts this detrimental effect. It has been suggested by Brooks et al. (1954) that by coating the roughage portion of the ration fat prevents the digestion of complex carbohydrates by rumen microorganisms. The following experiment was designed to test this explanation.

Procedure

Fifteen native-type lambs were divided into five blocks on the basis of weight, sex, and previous treatment. A lamb from each block was randomly assigned to each treatment. The lambs were housed in individual pens that measured 3 by 6 feet. They were fed once a day in metal buckets, and had free access to water at all times. Shrunk weights were taken at the beginning and end of the 42-day trial while full weights were taken during the progress of the experiment. The lambs in group 1 served as controls and received the basal ration, which is described in table 4. The other two groups received a diet that was equal in protein and energy to the basal ration but contained

10% corn oil. In treatment 2 the fat was thoroughly mixed with both the concentrate and roughage just prior to feeding, and the ration was fed in a single receptacle. Treatment 3 differed from this in that the corn oil was mixed with only the concentrate portion and fed in one bucket while the cottonseed hulls were fed at the same time in another bucket.

Results and Discussion

At the beginning of the trial 2.30 lb. of the basal ration and 2.00 lb. of the basal plus corn oil ration were fed daily. However, to maintain an equal consumption of calculated TDN, on the sixth day the amount was cut back to 2.01 and 1.75 lb., respectively, of the basal and basal plus corn oil rations. A lamb on treatment 3 refused to clean up the hull portion of the ration, while another lamb in the same block (on treatment 1) became sick in the latter part of the experiment. Therefore, this block was not considered in the statistical analysis of the experiment.

The results of the trial are given in table 6. The average daily gains of the lambs on treatments 1, 2, and 3 are, respectively, .28, .20, and .19 lb. for the 42-day period. There was no advantage in the practice of feeding the cottonseed hulls separately from the corn oil. Apparently, either sufficient coating of the hulls to impair digestion occurs within the rumen, or the deleterious effect of fat is exerted in some other manner.

The addition of 10% corn oil significantly (P less than 0.05) reduced gain and increased the amount of calculated TDN necessary for one pound of gain.

Summary

The results of the response of 12 lambs were analyzed to determine whether the coating of the hulls when corn oil is mixed into the ration reduces the utilization of the ration. In this trial there was no advantage in not allowing the cottonseed hulls to absorb the fat when the ration was mixed.

When a basal ration was compared with an iso-caloric, iso-nitrogenous, ration containing 10% corn oil, it was observed that fat significantly decreased gain and feed efficiency (defined as pounds of TDN required for one pound of gain).

TABLE 4

Basal and 10% Fat Rations Calculated to Provide Equal Protein and Caloric Intake When Fed in a Ratio of 1.15:1 and Used in Experiments III and IV

Feed	Basal	10% Fat
	%	%
Cottonseed Hulls	35.0	35.0
Ground Milo	45.0	25.4
Cottonseed Meal	18.4	28.0
Corn Oil		10.0
Limestone	.50	.50
Di-calcium Phosphate	.50	.50
Salt	.50	.50
Vitamin A and D Supplement (Quadrex)	.10	.10

TABLE 5

Composition of Basal and Basal plus 15% Corn Oil Rations Fed in Latter Part of Experiment IV

Feed	Basal	Basal + Fat
	%	%
Cottonseed Hulls	35.00	35.00
Ground Milo	45.00	15.40
Cottonseed Meal	18.40	33.00
Corn Oil		10.00
Limestone	.50	.50
Di-calcium Phosphate	.50	.50
Salt	.50	.50
Vitamin A and D Supplement (Quadrex)	.10	.10
Daily Allowance (lb.)	1.81	1.50

TABLE 6

Daily Gain and Feed Efficiency for Experiment III

Treatment	Lamb No.	Initial Wt.(lb.)		Daily Gain(lb.)	Daily Feed Intake (lb. TDN)	Feed Efficiency (lb. TDN per lb. gain)
	8	61.0		.28	1.29	4.61
	23	54.0		.30	1.29	4.30
Basal	27	56.5		.25	1.26	5.04
	3	56.5		.27	1.27	4.70
			Ave.	.28	1.28	4.66
Basal + Corn Oil (Corn Oil Mixed with Concentrate and Roughage)	33	54.0		.27	1.29	4.78
	22	52.5		.24	1.29	5.38
	25	53.5		.12	1.24	10.33
	14	56.5		.15	1.29	8.60
			Ave.	.20	1.28	7.27
Basal + Corn Oil (Corn Oil Mixed with Concentrate Portion Only)	16	58.5		.25	1.26	5.04
	53	58.0		.12	1.22	10.17
	43	55.0		.17	1.27	7.47
	13	69.0		.21	1.29	6.14
			Ave.	.19	1.26	7.20

EXPERIMENT IV

The Effect of a High Fat Ration upon Ketone Body Accumulation in the Blood and Urine of Wether Lambs

The primary effect of fat added to a ruminant ration is to depress digestibility. However, it is plausible that, since ketone bodies are formed from the metabolism of fatty acids, a diet high in fat content might cause ketosis to occur in ruminants. This experiment was carried out to compare the ketone body content of the blood and urine of lambs on high fat diets (10 and 15% added corn oil) with lambs on predominantly carbohydrate rations.

Procedure

Six western-type wether lambs weighing from 65 to 75 lb. were used in this experiment. During a 10-day preliminary period the lambs were fed 1.72 lb. per day of the basal ration described in table 4. At the end of this period blood samples were taken and the lambs were placed in metabolism crates described by Briggs and Gallup (1949). After the lambs were allowed two days to become accustomed to the stalls, urine was collected for a 24-hour period. At the end of this collection four of the lambs were placed on the 10% corn oil diet given in table 4. They received 1.51 lb. of this ration while the other two lambs continued to be fed 1.72 lb. of the basal. The rations were fed in a ratio which allowed an equal calculated intake of protein and energy for all lambs.

After 10 days on this regimen the lambs were bled and urine was again collected over a 24-hour period. During the last ten days of the experiment, 15% corn oil was included in the rations of the lambs that had previously received 10% additional fat. In order to attempt to maintain isocaloric, isonitrogenous intake, 1.81 lb. of the basal and 1.50 lb. of the high fat ration were allowed to the lambs. At the end of the ten day standardization period urine was collected and blood samples were taken.

The lambs were fed once a day and the rations were mixed just prior to feeding. Water was available at all times.

After sugar and other substances interfering with the test were removed by the copper sulfate-calcium hydroxide procedure described by Van Slyke (1917), the ketone body content of the urine and blood filtrates was determined by the salicylaldehyde method of Behre (1940). The results are expressed as mg. acetone per 100 ml. blood or urine.

Results and Discussion

Difficulty was experienced in keeping the lambs receiving corn oil on feed. Furthermore, one of the animals receiving the basal treatment (No. 5) became sick and refused feed. This animal was removed from the experiment at the end of the second trial and was replaced with a native-type wether lamb. Lamb no. 5 had been on a fast for over four days when the last blood sample was taken from him. No urine could be obtained.

The results are summarized in table 7. The average concentration of acetone in the urine of lambs fed the basal ration was 3.9%, while it was 2.6% in the urine of animals on the high fat rations. Expressed as mg.% acetone, the blood ketone concentration was 4.80 (disregarding no. 5) in the basal group and 3.30 in the basal plus corn oil group.

The only significant ketonemia noticed was in the case of the no. 5 lamb where a fasting ketosis apparently occured.

Summary

A comparison was made of the quantity of ketones in normal blood and urine and in the blood and urine of lambs receiving 10 and 15% corn oil.

No ketosis that could be attributed to the different treatments was observed although one case of fasting ketosis occured.

TABLE 7

Effect of Added Fat on Ketone Body Content of Blood and Urine

Treatment	Lamb No.	Ketones in urine mg.%[a]	Total ketone excreted per 24 hrs. (mg.)	Ketones in blood (mg.%)
Trial I				
	1	2.7	19.2	--
	2	5.3	47.4	--
	3	2.9	36.8	--
1.72 lb. basal	4	1.9	21.5	--
	5	3.2	15.2	--
	6	4.5	30.6	--
Trial II				
	1	2.0	13.2	2.7
Basal plus 10%	2	3.9	84.6	2.4
corn oil	3	3.0	36.7	2.4
(1.51 lb.)	4	2.0	20.0	4.5
1.72 lb. basal	5	--	--	11.1
(controls)	6	7.8	56.9	3.9
Trial III				
	1	2.15	14.0	2.4
Basal plus 15%	2	2.25	10.8	2.4
corn oil	3	2.35	16.4	3.6
(1.50 lb.)	4	2.57	11.8	6.0
1.81 lb. basal	7[b]	2.00	6.2	6.0
(controls)	6	2.50	36.5	4.5

[a] Ketones expressed as acetone.

[b] Replacement for no. 5.

EXPERIMENT V

A Study of the Effects of Corn Oil, Alfalfa Ash, and $NaHCO_3$ on Intraruminal pH

A shift in the pH of the ingesta may have a definite harmful effect on the microbiological and physiological activities which are carried on in the rumen. There is a decrease in ruminal motility and absorption when the reaction becomes more alkaline. A change in environment also results in a change in the microbial population in the rumen. Such a change could be either harmful or beneficial.

The purpose of this study was to determine the effect of various additives on the pH of the ruminal ingesta. The normal pH of the ruminal contents was determined and compared with the intraruminal pH when corn oil, corn oil plus alfalfa ash, or corn oil plus sodium bicarbonate was added to the basal ration. The effect of the various treatments on the diurnal variation in pH was also studied.

Procedure

Three months before the beginning of this experiment four western-type yearling wethers were fistulated.[1] In each wether a plastic fistula plug (Hentshl et al., 1954) with a 2 inch orifice was permantly inserted. Foam rubber discs were later placed around the fistulas

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[1]Acknowledgement is made to Dr. E. W. Jones, Dept. Veterinary Medicine and Surgery, Okla. A. and M. Coll. for conducting the surgery involved in inserting the fistula plugs.

to decrease leakage. During the ensuing recovery period one animal died from an undetermined cause.

The pH measurements were made within the rumen with a locally-constructed meter[2] with a specially-designed and also locally-constructed glass-electrode system. The instrument was standardized several times each day with pH 6 and 4 buffers. Several measurements were made at the time of each reading to insure that the ruminal ingesta had become equilibrated with the glass-electrode so that the obtained values were repeatable. As it was found to be necessary to insulate the animals from the ground, the lambs were placed on rubber mats while readings were made.

The lambs, which were housed in a box stall approximately 10 feet square, were fed once a day in individual stanchions. When they were not feeding they were allowed the freedom of the stall with free access to water. Their daily ration consisted of cottonseed hulls, 190 gm.; ground milo, 245 gm.; cottonseed meal, 100 gm.; limestone, 3 gm.; salt, 3 gm.; di-calcium phosphate, 3 gm.; and Quadrex, 1 gm. This ration was mixed in bulk and stored until used.

During the first phase of the experiment the lambs were maintained on the basal ration with no additives. Readings of intraruminal pH were made at various times after feeding for 8 days in order to establish the amount of diurnal variation as well as to confirm the values reported by other workers as to the normal pH of rumen ingesta. In

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[2]After the design of Professor Marvin Johnson, Department of Biochemistry, University of Wisconsin.

order to establish a curve for normal duirnal variation of intraruminal pH the several values obtained for each particular time after feeding were converted to their respective antilogarithms and averaged. The mean was then reconverted to its equivalent pH value.

When the basal curve was established, 10% (55 gm.) corn oil was added. Observations of the effects of this treatment were made for 4 days; then 14 gm. alfalfa ash was included in the basal plus corn oil ration. After 3 days the amount of ash was increased to 45 gm. After this diet was fed for six days, the ash was replaced with 25 gm. $NaHCO_3$ which was given to the animals for 4 days. The data gathered for each treatment over a period of several days were combined into a single curve by the same method of computation described previously for establishing a normal curve.

Results and Discussion

In figures 1 through 5 are shown the effects of the various treatments upon the pH in the rumen of each animal. In figure 1 it can be seen that readings taken when the same period of time has elapsed since feeding the basal ration vary greatly among individual animals as well as from day to day, even though the daily ration is kept constant. Since this variation was greater during the early part of the experiment when the basal ration was fed than with the later treatments, part of it may be due to technique.

The data obtained on July 26 deviated strikingly from all other data obtained in this study and, while unexplainable, is considered unreliable and is therefore not included in establishing the composite diurnal curve for the basal ration (figure 5). Animal no. 3 was sick on July 23; therefore, in order to hasten recovery data was not collected from him on this day. Also in figure 1 it can be seen that the ruminal pH of animal no. 2 remained at a low level throughout July 23. It was observed that there was a delay in the passage of food through this rumen as it was still full of ingesta the next morning. The summary of the readings taken while the basal ration was fed (figure 5) indicates that the pH in the rumen drops for 2 to 10 hours after feeding until a pH value of approximately 5.8 is reached. After this initial drop there is a gradual rise in pH until the animal is fed again and reaches about 6.2 twenty-four hours after the previous feeding. Realizing that the conditions under which the values were determined differ, it is still noteworthy that this diurnal change in pH varies inversely with the diurnal change in ruminal fatty acid concentration which was found by McClymont (1951) to reach a peak 2 to 8 hours after feeding.

No change in pH in the rumen or in its characteristic variation was noted when corn oil was added to the diet (figure 2). However, corn oil caused a marked change in the appearance of the ingesta. Six hours after the ration containing it was fed, the rumens were full of a frothy foam within which feed particles were suspended. Although it did fill the entire rumen there was not enough pressure exerted to cause bloat. The foam diminished after 6 to 9 hours after feeding and the rumen was free of it just before the subsequent feed. A putrid odor, reported by Brooks et al. (1954) to occur when corn oil was added to the ration, was not noted; actually the odor was less intense than that associated with normal ingesta. Samples of this material were tested with silicone anti-foaming agents and titrated with dilute

NaOH, but neither of these treatments were observed to affect the foam. When titrated to neutrality with dilute $NaHCO_3$ the foam actually expanded.

In figure 3 it is shown that the first day alfalfa ash was added there was no change in pH. However on subsequent days the rumen ingesta became more alkaline. When the amount of ash was increased the pH values remained higher than normal. It was also noted that the ash caused the ingesta to take on a gray-colored appearance. However, the production of foam, associated with the ingestion of corn oil, did not diminish with the feeding of alfalfa ash.

When 25 gm. $NaHCO_3$ were fed, the pH values, indicated in figure 4, remained high. When the additional minerals were included in the ration, the pH in the rumen averaged 6.8 prior to feeding and did not become more acid than 6.2 during the day. Figure 5 indicates that the $NaHCO_3$ resulted in a more alkaline reaction in the rumen. However this may have been due to the fact that this treatment followed the feeding of alfalfa ash and reflects the accumulation of minerals in the rumen. The observations for all five days that the ash was fed were combined into the composite curve for this treatment. Therefore, values indicated in figure 5 for the intraruminal pH when alfalfa ash was fed are lower than if the curve had been based on readings taken the last three days after the ash had become stabilized in the rumen.

During the time $NaHCO_3$ was fed, there was more water ingested, which appeared to diminish the amount of foam in the rumen. The ration with the added $NaHCO_3$ was readily consumed the first two days it was offered but was partially refused the third and fourth days it was fed. At times part of the ration was administered through the fistula in order to maintain equal intake among sheep. This did not appear to adversely affect the animals although digestion, as evidenced by the passage of food through the rumen, was delayed. Some wool was occasionally found in the ingesta indicating that wool-pulling occurred.

Because of the number of variables affecting pH and the sources of error involved in measuring pH, combined with the short time and small number of animals allotted to each treatment, this must be considered a preliminary study of the effect of the diet upon rumen function as indicated by the pH of the ingesta. Few, if any, conclusions are justified on the basis of these limited observations. The observed rise in intraruminal pH caused by the mineral additives may prove to be real and may be sufficient to affect ruminal microorganisms. Further work is needed to verify and evaluate such an effect. There may be a close relationship between the foam produced when corn oil was fed and the overall effect of fats upon digestibility. Perhaps the foam physically blocks the absorption of nutrients through the ruminal wall. The observation that water breaks down the foam in the rumen may indicate that further studies should be made of the effect of mineral additives on water intake.

Summary

A study was made of the effect on intraruminal pH of adding corn oil, corn oil plus alfalfa ash, or corn oil plus sodium bicarbonate to a basal ration. The pH values were determined <u>in vivo</u> through the fistulas of three sheep.

Alfalfa ash and sodium bicarbonate caused the pH of the rumen contents to become more alkaline. The addition of corn oil did not

appear to change the pH inside the rumen but did cause the ingesta to become very foamy shortly after feeding. The ingestion of large amounts of water seemed to break down this foam.

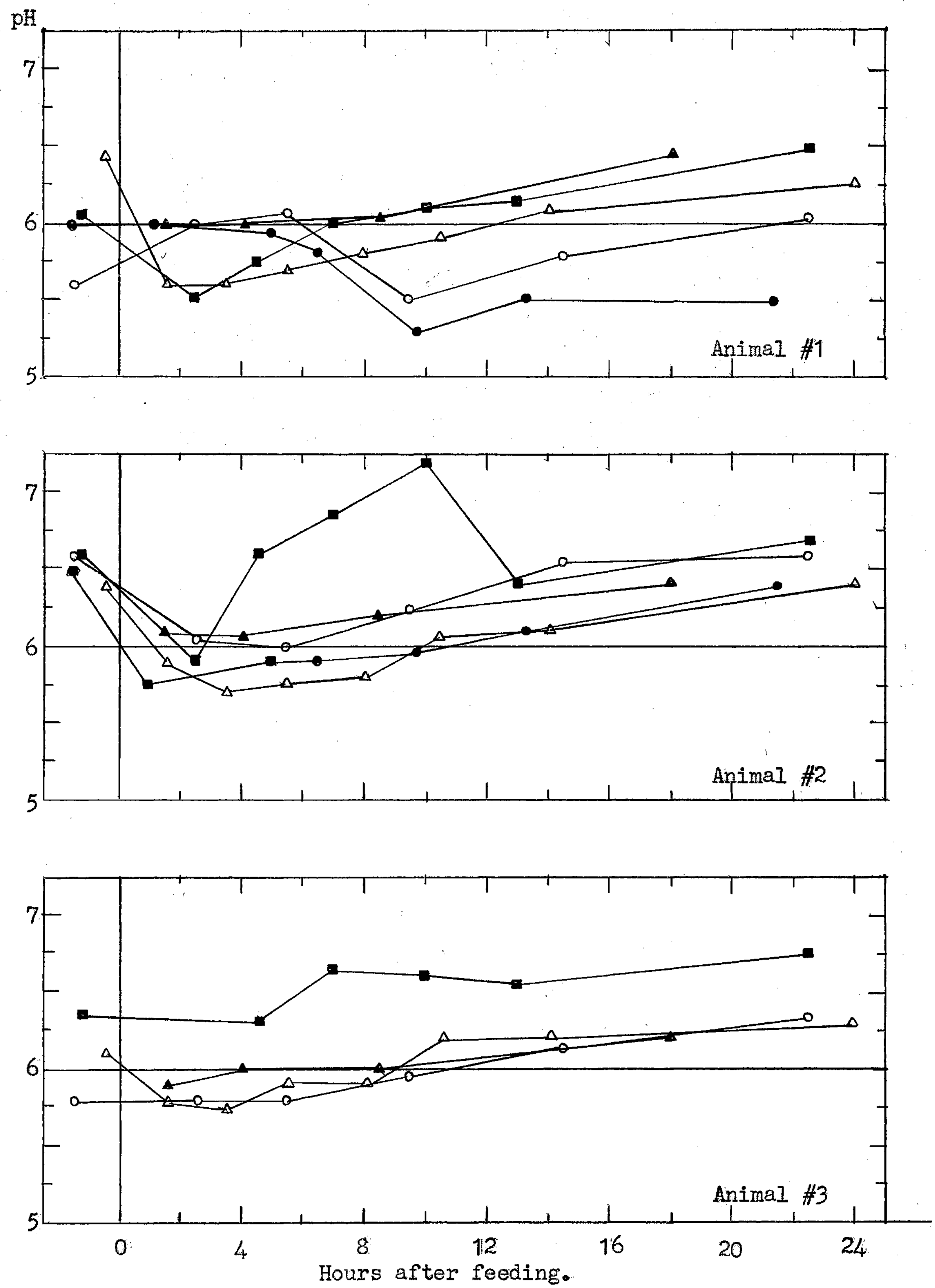

Figure 1. Diurnal variation of intraruminal pH in sheep receiving the basal ration in Experiment V. July 23 ●—●, July 25 ○—○, July 26 ■—■, Aug. 9 ▲—▲, Aug. 10 △—△.

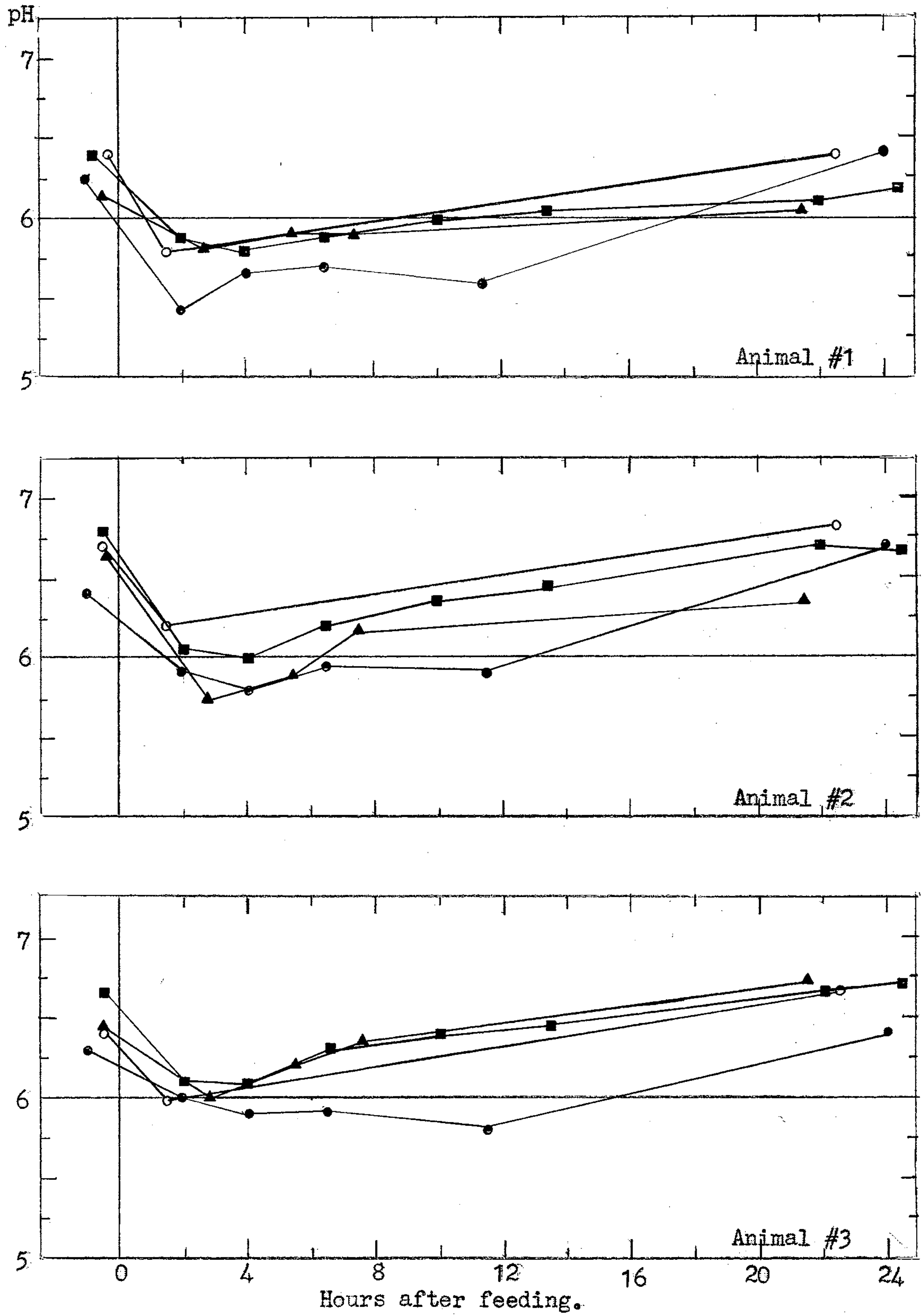

Figure 2. Effect on pH and diurnal variation of adding corn oil to the basal ration in Experiment V. Aug. 11 ●—●, Aug. 12 ○—○, Aug. 13 ■—■, Aug. 14 ▲—▲.

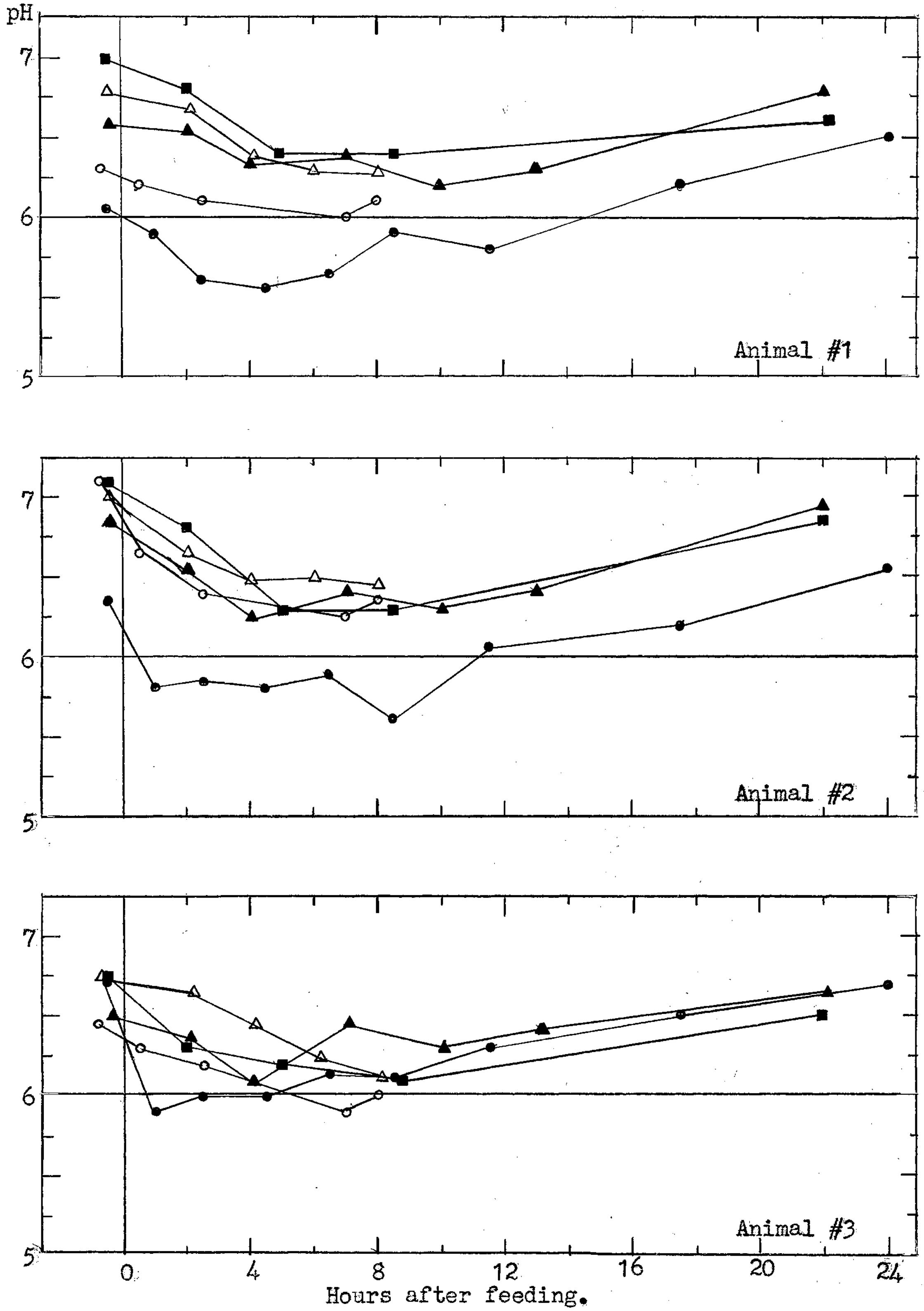

Figure 3. Effect on pH and diurnal variation of adding corn oil and alfalfa ash to the basal ration, Experiment V. Aug. 15 ●—● , Aug. 17 ○—○ , Aug. 20 ■—■ , Aug. 21 ▲—▲ , Aug. 23 △—△ .

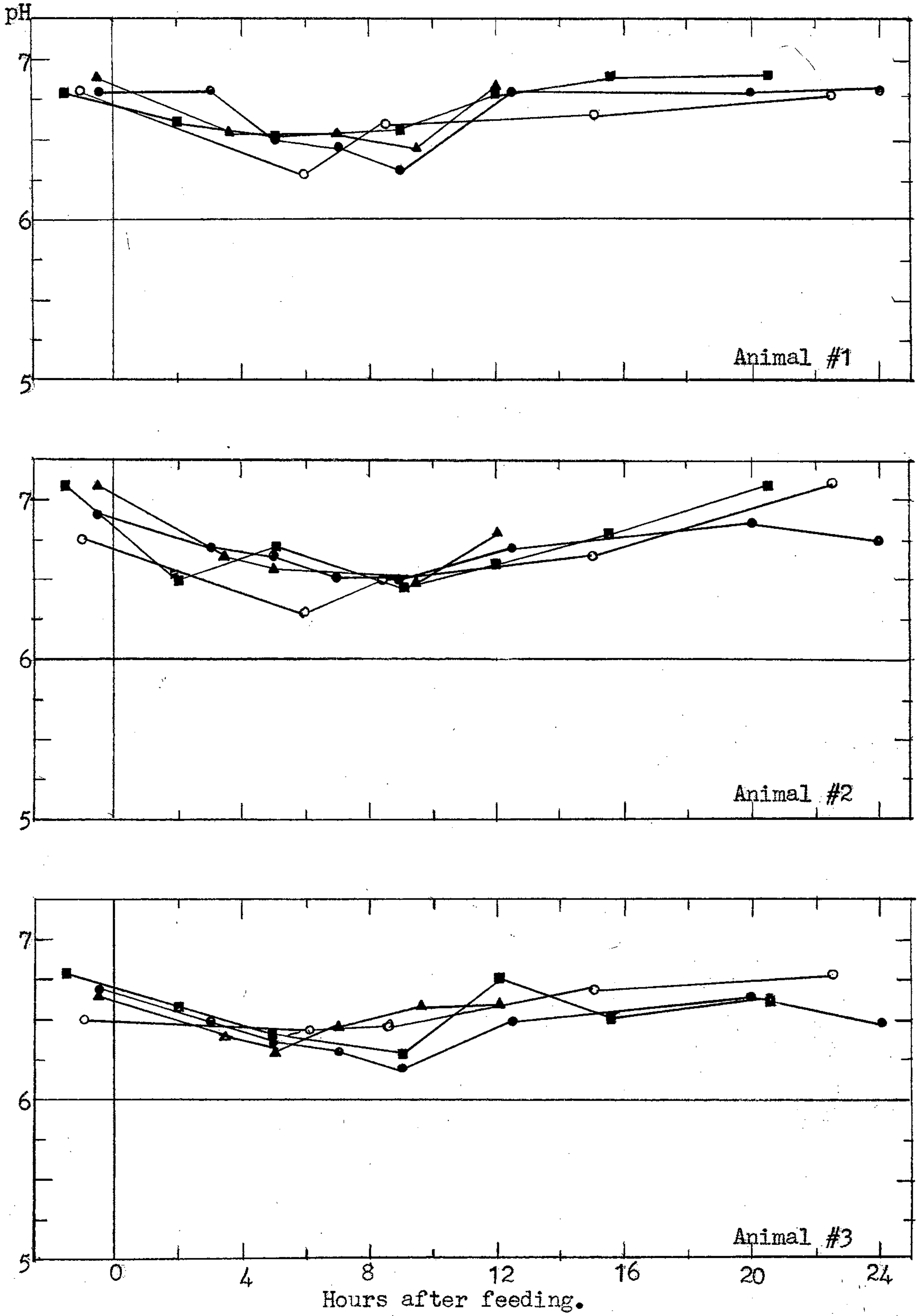

Figure 4. Effect on pH and diurnal variation of adding corn oil and sodium bicarbonate to the basal ration, Experiment V. Aug. 24 ●—●, Aug. 25 ○—○, Aug. 26 ■—■, Aug. 27 ▲—▲.

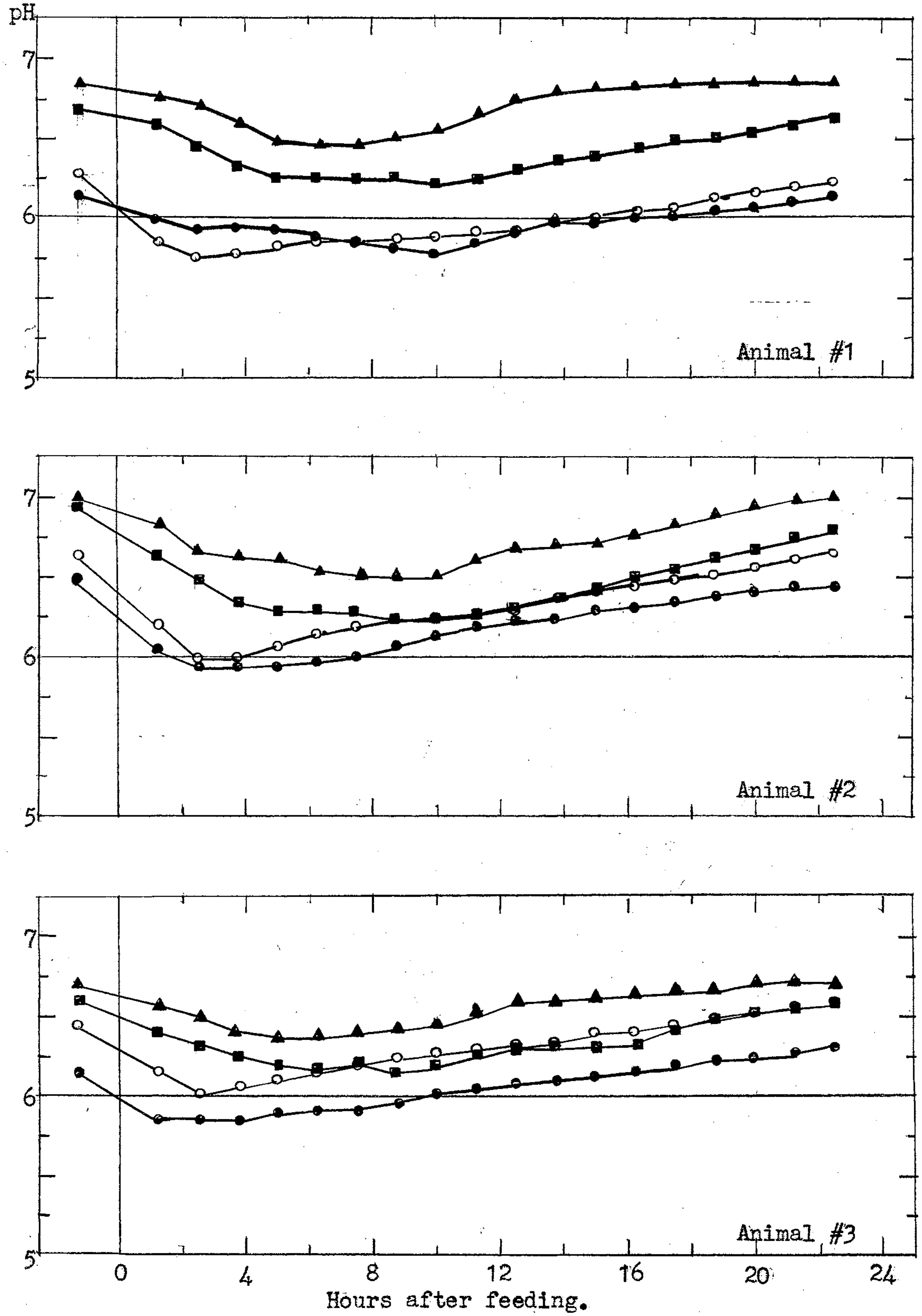

Figure 5. Composite curves comparing diurnal variation in pH resulting from each treatment, Experiment V. Basal ●—● , Basal plus corn oil o—o , Basal plus corn oil plus alfalfa ash ■—■ , Basal plus corn oil plus $NaHCO_3$ ▲—▲ .

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APPENDIX

TABLE I

Average Daily Gains of Lambs in Experiment I, by Periods

	Basal	Basal + Fat	Basal + Fat + Alfalfa Ash	Basal + Fat + $NaHCO_3$	Basal + Fat + $KHCO_3$
Av. Daily Gain (lb.)					
First 14 Day Period	.08	-.29	-.56	-.54	-.79
Second 14 Day Period	.30	.05	.15	.04	.14
Third 14 Day Period	.14	.08	.16	-.08	-.01
For 42 Days	.17	-.04	-.04	-.12	-.15

TABLE II

Analysis of Variance of Average Daily Gains in the 42 Day Feeding Trial of Experiment I

Source of Variance	d/f	Mean Square	F
Total	37		
Treatment	4	224.08	7.70**
Lot 1 vs. Lots 2, 3, 4, 5	1	782.03	26.88**
Lots 2 and 3 vs. Lots 4 and 5	1	110.65	3.80[a]
Lot 2 vs. Lot 3	1	.25	.01
Lot 4 vs. Lot 5	1	3.39	.12
Error	33	29.09	

[a] Significant at 10% level.

TABLE III

Analysis of Variance of Average Daily Gains in Recovery Period of Experiment I

Source of Variance	d/f	Mean Square	F
Total	37		
Treatment	4	36.65	6.51**
Lot 1 vs. Lots 2, 3, 4, 5	1	74.48	13.23**
Lots 2 and 3 vs. Lots 4 and 5	1	51.99	9.23**
Lot 2 vs. Lot 3	1	16.00	2.84
Lot 4 vs. Lot 5	1	4.13	.73
Error	33	5.63	

TABLE IV

Apparent Digestion Coefficients of Dry Matter and Organic Matter in Experiment II

Ration	Replication	Lamb No.	Dry Matter	Organic Matter
Basal	Western Lambs	1	66.1	67.3
		8	62.8	64.9
		15	63.5	64.2
	Mean		64.1	65.5
	Native Lambs	3	64.2	65.5
		8	62.5	66.5
	Mean		63.4	66.0
Basal Plus 15% Stabilized Animal Fat	Western Lambs	2	53.4	54.6
		6	52.9	54.8
		14	47.1	49.3
	Mean		51.1	52.9
	Native Lambs	13	15.6	19.0
		14	23.5	38.9
		16	57.3	59.4
		18	52.1	51.4
		33	59.0	58.9
	Mean		41.4	45.5

TABLE V

Analysis of Variance of Apparent Digestion Coefficients of Dry Matter and Organic Matter in Experiment II

		Dry Matter		Organic Matter	
Source of Variance	d/f	Mean Square	F	Mean Square	F
Total	12				
Treatment	1	1076.83	6.93*	930.76	8.59*
Replication	1	316.84	2.03	197.16	1.82
Error	10	155.37		108.39	

VITA

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