AN ABSTRACT OF THE THESIS OF

MICHAEL DENNIS CAMPBELL for the MASTER OF SCIENCE
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Title: INVOLVEMENT OF INORGANIC AND ORGANIC COMPONENTS IN THE LAXATIVE EFFECT OF CANE FINAL MOLASSES

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Dr. James E. Oldfield

The objective of this study was to investigate the effect of inorganic and organic components of molasses on the laxative problem encountered when molasses makes up a large portion of livestock rations. In a series of four trials 280 male Long Evans rats weighing approximately 50 grams each were placed on dietary treatments, each supplying or removing a possible causative agent.

In the first trial the involvement of inorganic components and in particular potassium was studied in their relation to the laxative effect. Potassium when supplied at a level calculated to be equivalent to the content of molasses did not cause any laxative effect. However, it did result in increased water consumption by rats. The addition of molasses ash to the diet did not cause any laxative effect in the rats but did cause increased water consumption as did

the potassium.

The second trial was designed to scan other factors which might be involved in the laxative problem. Magnesium when supplied as $MgSO_4$ at a level calculated to be equivalent to the magnesium concentration of the molasses-supplemented diet did cause loose droppings in all rats receiving that diet.

The results of the third trial were quite variable, mainly due to poor experimental technique and were not considered in the final conclusions. The trial pointed out several areas where experimental technique could be refined.

The fourth trial showed that magnesium when supplied as dicalcium magnesium aconitate at a level calculated to be equivalent to its concentration in the molasses-supplemented diet produced dry matter values of the large intestinal content similar to the rats on the molasses-supplemented diet. However, the wet weight of large intestinal contents of rats receiving the dicalcium magnesium aconitate diets was significantly ($P < 0.01$) less than that of the rats on the molasses containing diet, but significantly ($P < 0.05$) more than that of the rats on the basal diet. The addition of potassium to the dicalcium magnesium aconitate diet did not affect the dry matter or wet weight of the large intestinal contents of the rats. It did, as before, cause an increased water consumption by the rats on the high potassium diets. Rats on the deionized molasses and cation-free molasses showed significantly ($P < 0.05$) higher dry matter values for the contents of the large intestine over the dry matter values recorded on the rats on the molasses diet, but still below the value recorded for the rats on the basal diet. The rats on the anion-free molasses showed no differences in dry matter of large intestinal contents from those rats on the molasses containing diet. Wet weight of large intestinal contents of the rats was significantly ($P < 0.01$) reduced by the cation-free molasses and deionized molasses. The wet weight of the contents of the large intestine of the rats on the dicalcium magnesium aconitate diet was significantly ($P < 0.05$) increased over that of the rats on the basal diet but was significantly ($P < 0.01$) less than that of the rats on the molasses-supplemented diet.

Involvement of Inorganic and Organic Components in the Laxative Effect of Cane Final Molasses

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APPROVED:

[redacted]

Professor of Animal Science
In Charge of Major

[redacted]

Head of Department of Animal Science

[redacted]

Dean of Graduate School

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DEDICATED TO MY WIFE

JUDY

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INTRODUCTION

Molasses has been used periodically in rations for farm animals for over 150 years. High initial acceptance in rations for livestock awaited endorsement by a recognized scientific leader, and such was provided by a review of the feeding value of molasses by Otto Kellner in 1909 (Scott, 1953). Following this publication, many papers flooded European literature praising the value of molasses in rations for farm stock. In America, Lindsey reported, in 1905, on the beneficial value of molasses as a feedstuff for livestock. Since that date, molasses has been used continuously in varying amounts in rations for farm animals in this country (Scott, 1953). Its feeding value was compared with all of the conventional grains and the limits of the dietary level were studied with cattle, sheep, swine, and poultry.

Molasses is potentially well-suited to serve as an energy source in livestock rations by reason of its high sugar content (Kowkabany, Binkley, and Wolfrom, 1953) and its high digestibility (Lofgreen and Otagaki, 1960). Moreover it is a by-product material usually available at low cost. Increasing competition for available feed resources dictates maximum usage of such materials.

Molasses also serves as an excellent source of trace minerals and supplies some of the B vitamins (Scott, 1953).

The limiting factor in the use of molasses as a major source of energy in rations for farm animals is the severe laxative effects incurred when it is fed at high levels. This thesis approaches this topic with reference to the specific effects of certain inorganic and organic components of cane final molasses on the diarrhea syndrome.

LITERATURE REVIEW

Molasses has been the subject of much research in the last 65 years. In particular, research efforts have been directed to ascertaining its feeding value as a replacement for the common feed grains and studies on the various claims of its so-called "tonic effect" and unidentified growth stimulant effects. It has generally been concluded that molasses can be profitably included in rations for livestock up to the point where diarrhea begins.

Several workers have shown that the energy value of molasses decreases with increased levels in the diet (Lofgreen and Otagaki, 1960a; Lofgreen and Otagaki, 1960b; Martin and Wing, 1966; Scott, 1953). Molasses when fed at higher levels has also been shown to reduce the digestibility of other feed components (Iwanaga and Otagaki, 1959).

Two things happen when molasses is included in the diet at high levels: 1. Diarrhea increases with increasing levels of molasses, and 2. The feed value of molasses decreases at increasing dietary levels (Kondo and Ross, 1962; Scott, 1953; Lofgreen and Otagaki, 1960). One may conclude that the diarrhea may in part be responsible for molasses' decreased feed value at high levels. Diarrhea is defined as the rapid and frequent propulsion of watery ingesta through the digestive tract. The diarrhea will then cause a faster passage rate with less time for proper digestion and utilization of ingesta.

It has also been shown that the diarrhea presents a problem of sanitation. In one local experiment, Jackets and Oldfield (1964) fed swine diets containing 79 percent molasses with no success. The trial was terminated at the end of the first week due to the severe scouring encountered. Rosenberg and Palafox (1956b) reported greater incidence of soiled eggs from birds fed molasses at levels higher than 16.5 percent of the diet. In 1955 Rosenberg fed five diets, varying in molasses content from zero to 34.5 percent, to chicks for 42 days. The results of the experiment show that the growth rate of chicks was not impeded to three weeks of age among experimental groups that received levels of molasses up to 23 percent of the total ration. In contrast, those fed the ration containing 34.5 percent molasses grew significantly slower ($P < 0.01$). Efficiency of feed conversion decreased progressively as the concentration of molasses was raised. Viability was not adversely affected by these experimental rations.

Inorganic Constituents of Molasses in Relation to its Laxative Effect

Potassium

It is known that certain minerals in cane molasses are responsible for some of the changes which occur in the water metabolism of chicks when molasses is fed. Bice and Dean (1939) reported that potassium which is present in relatively high concentration in cane final molasses is the primary cause of its laxative effect.

Rosenberg and Palafox (1956a) studied the effects of certain cations in cane molasses on fecal moisture of chicks. The experimental rations contained either granulated sugar, cane molasses ash, a synthetic mineral mixture that simulated this ash, potassium chloride, magnesium oxide, or water in amounts equivalent to their respective concentrations in cane molasses where the molasses was fed at 33 percent of the total ration. The results of their trial showed that wherever potassium appeared in this study the fecal moisture levels were raised significantly.

However, it should be pointed out that water consumption data were not collected and the rise in fecal moisture caused by potassium may in fact have been due to increased water consumption. The fowl's anatomical make-up is such that there is no method to remove large quantities of water as urine; rather the bird concentrates the urinary waste. Due to the fact that birds have a common excretory tract, the excess water must be expelled with or as a part of the feces. Kondo and Ross (1962a) presented data that showed conclusively that the mineral component of molasses does increase the water consumption in birds. From their preliminary data on water consumption in diets containing 30 percent molasses or KCl or K_2CO_3 fed at levels calculated to be equivalent to the amount of these salts present in 30 percent molasses and the basal diet, total water consumption was respectively 5.05, 3.97, 4.35, and 2.46 liters. Kondo and Ross' study showed that increasing levels of sugar in the diet resulted in increased fecal moisture although there did not appear to be a corresponding increase in water consumption.

Further test diets prepared by Kondo and Ross including blackstrap molasses and partially deionized blackstrap molasses were fed at 5, 10, 15, 20 and 30 percent levels to chicks for six weeks. In addition, the salts: KCl, K_2CO_3, MgO, and $MgSO_4$ were fed at levels calculated to be equivalent to the amounts of these salts present in the 30 percent molasses. Those diets containing partially deionized molasses caused lower water consumption and fecal moisture than diets containing untreated molasses. Potassium was again the major factor in molasses which caused loose droppings, while the chloride ion and magnesium ion had no apparent effect at the levels used. Molasses-fed birds almost always consumed more water than birds on rations containing comparable amounts of KCl and sugar. These workers concluded that other factors besides potassium were involved in the laxative problem since the effects could only be partially matched with potassium. Kondo and Ross' results agreed with an earlier study by Holst and Newlon (1927), later reviewed by Almquist and Jukes in 1935, indicating that owing to its high mineral content, a main constituent of which is potassium, molasses becomes highly laxative at over five percent diet levels.

High positive correlations were observed between the potassium content of feed and fecal moisture; water consumption and fecal moisture and potassium content of feed and water consumption. There were no differences in results between KCl and K_2CO_3 supplemented diet groups.

After the work of Rosenberg and Palafox (1956a) it was shown that the potassium content in molasses increased the water consumption in chicks and also increased incidence of loose droppings. Ross (1960) followed the earlier work of Rosenberg and Palafox by studying the effect of restricting water intake time on chicks being fed on zero, 15 and 30 percent molasses. His study was performed to determine whether water restriction could reduce the fecal moisture of chicks. Restricting the water intake to three one-half hour periods per day resulted in depressed growth and feed consumption at all levels of molasses feeding. The severity of the growth depression increased with increasing molasses concentrations. Water restriction of the zero molasses group increased total water consumption and significantly increased the moisture in the droppings. The chicks receiving the 15 percent molasses ration on the restricted water regime consumed almost as much water as the corresponding control and also produced significantly wetter droppings. At the 30 percent molasses level, the restricted group consumed approximately 69 percent as much water as the corresponding control and showed a tendency to produce drier droppings that approached significance. The water restriction resulted in decreased gain and efficiency of gain of chicks on the 15 and 30 percent molasses diets.

Ross also stated that the results suggest increased water consumption due to inclusion of molasses in the rations is primarily a physiological reaction concerned with electrolyte balance and excretion rather than one involving thirst *per se*.

Ross' results are in conflict with an earlier report by Maxwell and Lyle (1957) stating that wet droppings could be prevented in hens housed in individual pens by restricting the water intake to three 15 minute periods per day. No harmful effects on production were noted from this treatment. There were several differences between Ross' study and the study of Maxwell and Lyle which make a direct comparison difficult. The periods of restriction varied and the dietary treatment varied as well. However, Ross believed that in the case where molasses was included in the diet, water consumption was increased to maintain proper electrolyte balance whereas with the other procedure, water consumption was increased due to thirst.

Interrelationship of Potassium with Other Ions

The importance of sodium and potassium, and the ratio of these ions in relation to water metabolism has been appreciated for some time. While Richards, Godden and Husband (1927) have shown the essentiality of sodium and potassium, the ingestion of four to eight percent dietary NaCl or levels above 2.5 percent dietary KCl have been shown to induce loose droppings (Mitchell, Card and Carman, 1926; Kare and Biely, 1948; Lenkeit and Taupitz, 1954). However in certain other species there seems to be an antagonistic action between one element and the other which reduces the looseness of the droppings and the toxicity to the animal. The toxicity of KCl to mice was decreased by the administration of 10 to 20 mg. NaCl per 10 gm. body weight (Emmens and Marks, 1942). Ray and Talapatra (1945) noted a considerable reduction of diuresis when molasses-fed cattle were allowed free access to salt, suggesting a possible interaction between potassium and sodium.

Merril et al. (1950) reported that the administration of calcium salts and sodium solutions was effective in the treatment of potassium intoxication in humans. In addition, Pfeiffer, Roby and Smith (1941) found that diuresis caused by a salt solution was considerably reduced by the addition of calcium (10 mg. percent). They also reported that the combination of calcium (10 mg. percent) supplied as CaCl and potassium (18 mg. percent) supplied as KCl prevented the diuretic action of each ion. These reports would indicate that there might be an antagonistic relationship between sodium and potassium, and calcium and potassium, in their effects on water metabolism.

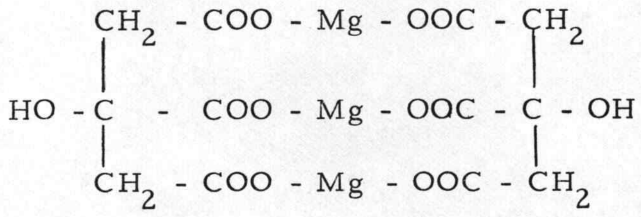
A second report by Kondo and Ross in 1962b showed again that increasing dietary levels of potassium for chicks resulted in increased fecal moisture. They showed that no significant decrease was noted when calcium was added, although there seemed to be a trend toward lower fecal moisture with increasing calcium levels at high potassium levels.

Magnesium

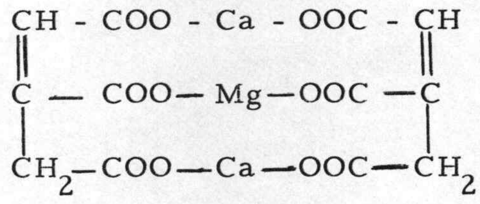
Magnesium is capable of causing diarrhea when excessive amounts are administered. Care (1960) showed that cattle diets supplemented with calcined magnesite at levels of 6 to 12 ounces per day brought about pronounced scouring in all animals tested. The laxative effects of magnesium are further documented by the numerous salts of magnesium that are used as cathartics (Goodman and Gilman, 1965). Some of the salts listed include magnesium hydroxide, magnesium sulfate, magnesium oxide, and magnesium citrate.

Organic Constituents of Molasses in Relation to its Laxative Effect

Cane final molasses contains an average of five percent aconitic acid and may reach as high as seven percent on a dry solids basis (Fort et al. 1952). The aconitic acid is in the form of dicalcium magnesium aconitate hexahydrate (Ambler, Turer, and Keenan, 1945; Fort et al., 1952). The molecule is very stable, resistant to chemical change, insoluble in water and has only low solubility in acids (Ambler, Turer and Keenan, 1945). The chemical structures of dicalcium magnesium aconitate and magnesium citrate which are employed as cathartics are very similar.



Magnesium Citrate



Dicalcium Magnesium Aconitate

Mechanism of Action of Cathartics

The saline cathartics are salts that are slowly and incompletely absorbed from the gastrointestinal tract. Latency and intensity of cathartic effect vary with the salt and dosage. When a saline cathartic is taken orally, a major fraction of the drug is unabsorbed from the gastrointestinal tract. When large amounts of salts are taken, the volume of water retained in the digestive tract by their osmotic effect is thus considerable. The resulting bulk serves as a mechanical stimulus that increases intestinal motor activity, and the intestinal contents are rapidly propelled into the colon. The normal absorption of fluid in the colon is similarly impeded and the colonic contents remain liquid and are promptly expelled (Goodman and Gilman, 1965).

Sugar

In addition to the effects of the mineral component of molasses, the effect of dietary sugar has been studied in relation to water consumption and moisture content of the feces. Rosenberg and Palafox

(1956a) concluded that sugar in feed had little effect on fecal moisture. Jacobs and Scott (1957), however, showed that the liquid intake of six-week old chicks was increased significantly when sucrose was added to water. They concluded that sugar may also play a role in water metabolism, although it seems probable that the increased liquid intake may be a result of a taste preference for the sweetened water and not a direct effect on water metabolism *per se*.

In a 26 week feeding trial, Allen and Leahy (1966) fed adult male rats on diets containing about 80 percent of carbohydrate given as dextrose, fructose, liquid glucose, or sucrose. The animals' performance was compared with that of rats receiving a standard laboratory diet containing 60 percent of carbohydrate as starch. Dextrose was the only sugar that produced a significantly lower weight gain. There was no mention of any digestive problems on the high sugar diets.

In a series of trials at Oregon State University, mink have been fed a purified diet containing 39 percent sucrose based on modification of the diet prepared by McCarthy *et al*. (1966), without any laxative effect.

Summary of Literature Review

To recapitulate, the review of literature has shown that potassium has been implicated as the causative agent for diarrhea in poultry fed high levels of molasses. However, there are certain anatomical differences between poultry and rats that make direct application of the previous findings difficult. It has also been pointed out that magnesium is capable of causing a laxative effect when present in an appropriate form and amount. Generally, there was no evidence implicating a direct involvement of organic components of molasses, although, the magnesium is present as a part of an organic molecule.

PROCEDURE

The purpose of this thesis was to determine the reason or reasons for the severe scouring associated with high levels of molasses in the diets of animals and to devise means for overcoming this scouring problem. Four separate trials were conducted with laboratory rats to identify the laxative factor of molasses. The results of these trials were subjected to statistical analysis for interpretation of results.

Trial 1

From a series of published papers it has been shown that the potassium content of molasses is responsible for the loose droppings in poultry. This trial was designed to determine if elevated potassium would also cause diarrhea in rats. As stated previously, in the literature review, there are anatomical differences between the fowl and mammal that makes direct inferences difficult. The rat is equipped to remove the increased water intake via a separate urinary channel.

Forty male weanling rats of the Long Evans strain weighing approximately 50 grams each were purchased from Simonsen Laboratories, Gilroy, California. The rats were randomly allotted to one of four dietary treatments until a total of ten rats had been assigned to each treatment. The diets were based upon various modification of purified diets formulated by Oldfield, Bull and Ellis (Aug. 1965) the compositions of which are shown in Table 1. The dietary treatments are defined as follows:

1. Basal
2. Basal modified with molasses as the energy source
3. Basal + molasses ash
4. Basal + KCl

The basal diet's energy source was supplied by sucrose, glucose, and fructose at levels calculated to be equivalent to the levels of the sugars present in the molasses-supplemented diet (2).

The molasses ash was prepared by first drying the molasses in a Thermo-Vac[1] freeze dryer and then ashing the dry matter in a muffle furnace at 500°C for eight hours. The ash was added to diet three at a level calculated to be equivalent to the mineral content of diet two with molasses. Potassium chloride was added to diet four at a level equivalent to the potassium content of diet two. Potassium content of the molasses was determined with a Zeiss PMQ II spectrophtometer[2] with Aztec[3] atomic absorption unit.

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[1] Thermovac Industries Corporation, Copiague, New York.

[2] Carl Zeiss Oberkochen/Wurtt, West Germany.

[3] Aztec Instruments, Inc., Westport, Connecticut.

Table 1. Composition of experimental diets (grams). Trial 1.

Group	1	2	3	4
Ingredients				
molasses[1]		643		
sucrose	307		307	307
glucose	85		85	85
fructose	76		76	76
fibrin, beef blood	180	180	180	180
water	175		98	127
lard	100	100	100	100
mineral mix[2]	40	40	40	40
vitamin mix[3]	10	10	10	10
cellulose	27	27	27	27
KCl				48
molasses ash			77	
Total	1000	1000	1000	1000

[1] Pacific Molasses Company, San Francisco, California.

[2] Jones and Foster (1942).

[3] Vitamin mix:

Vitamin A	200 I.U.	Pyridoxine HCl	0.12 mg.
α-tocopherol	6.0 mg.	Niacin	1.5 mg.
Mendione	0.01 mg.	Ca pantothenate	0.8 mg.
Thiamine HCl	0.25 mg.	Vitamin B_{12}	1.0 mcg.
Riboflavin	0.25 mg.	Choline Cl	75.0 mg.

The above vitamins are weighed out and made up to 1 gram with starch.

Diets one, three, and four were mixed with all ingredients added except the water and refrigerated in plastic bags in quantities sufficient for two weeks feeding. The water was added to the diets at the time of feeding at the proper level and mixed thoroughly. Water was added to these diets to more closely approximate the moisture content of the molasses diet, although exact equality was not achieved. Molasses ash and KCl were added to diets three and four at the expense of water. Diet two was prepared by mixing all components of the diet except molasses; the molasses being added at the time of feeding at the proper level and mixed thoroughly with the other dietary ingredients.

Feed consumption, water consumption and body weights were measured weekly. A visual evaluation of the looseness of the droppings was made daily. The rats were on test for four weeks.

Each rat was housed in an individual galvanized iron cage in a room with temperature controlled between 74^{o} and $80^{o}F$. The diets were provided <u>ad. lib</u>. in a feeder consisting of a stainless steel cup with screen and cover to reduce feed wastage. Water was provided by glass water bottles with 120 ml. capacity using rubber stoppers fitted with glass drinking tubes.

In the second week of the trial five of the ten rats on the molasses diet were randomly selected and given a therapeutic dose of aureomycin by stomach tube for five days. This was done with the thought that the molasses diet formula might be favorable to the rapid build-up of some microorganisms which may cause diarrhea. Visual observations were made on looseness of droppings comparing the aureomycin-treated rats and those not treated.

At the end of the second week of the trial the rats on the diet containing molasses were manually cleaned using dry paper towels and were placed in freshly cleaned cages. This was done to determine if the rats could perform better and to determine the true extent of diarrhea.

At the end of the four week feeding period the rats were killed by ether inhalation. The abdominal cavities of the rats were opened and observations were made on the condition of the gastrointestinal tract and the nature of its contents.

Trial 2

The second trial was designed to be a survey of several treatments with a limited number of animals to give indications of other factors which may be involved with the diarrhea problem. If there were tendencies for one of the dietary treatments to affect the diarrhea significantly, then a larger and more comprehensive study of its effects could be done.

Similar rats were purchased from the same source as in trial one. Three rats were randomly assigned to each of the six dietary treatments as follows:

1. Basal
2. Basal + graded increases in molasses
3. Basal + molasses
4. Basal + $MgSO_4$
5. Basal + molasses with ten percent ethanol
6. Basal + molasses with 20 percent ethanol

(The diet compositions are shown in Table 2.)

Preparation of the basal and the basal + molasses diet was done the same as in trial one. Diet two was designed to determine if the rats could become accustomed to the molasses by gradually increasing the increment fed. Each week of the trial the molasses level was raised at the rate of 10, 25, 40 and 64.3 percent of the diet. Each increase in molasses was compensated by appropriate decreases in the sugar and water level of the diet. Diet four was designed to determine whether magnesium was responsible for the laxative effect. Magnesium sulfate was added at a level calculated to be equivalent to the magnesium level in the molasses diet, as determined by atomic absorption spectrophotometry. Diets five and six were prepared to determine if molasses mixed with ethanol alleviated the laxative effect.

The animal care was the same as described in trial one and the trial feeding period was again four weeks. Records of the

Table 2. Composition of experimental diets (grams). Trial 2.

Group	1	2	3	4	5	6
Ingredients						
molasses*		643	**		597	515
sucrose	307		**	307		
glucose	85		**	85		
fructose	76		**	76		
fibrin, beef blood	180	180	180	180	180	180
water	175		**	150		
lard	100	100	100	100	100	100
mineral mix*	40	40	40	40	40	40
vitamin mix*	10	10	10	10	10	10
cellulose	27	27	27	27	27	27
$MgSO_4$				25		
ethanol					64	128
Total	1000	1000	1000	1000	1000	1000

*See footnotes Table 1.

**Graded increases in molasses.

Week	1	2	3	4
Ingredients				
molasses	100	250	400	643
sucrose	261	189	117	--
glucose	71	52	32	--
fructose	64	46	29	--
water	147	106	65	--

progress were kept as in trial one.

After completing the feeding trial, the rats were killed and visual observations were made on the condition of the gastrointestinal tract and on the contents of the tract.

Trial 3

Upon completion of trial two there seemed to be some indication that magnesium might have been involved in the diarrhea problem. This subsequent study was then designed to determine whether Mg as found in the form of dicalcium magnesium aconitate hexahydrate in molasses is responsible for the loose droppings.

Male Long Evans rats weighing approximately 50 grams each were again used. Ten rats were randomly assigned to each of eight dietary treatments, as follows:

1. Basal
2. Basal + molasses as energy source
3. Basal + 5% diCa Mg Aconitate
4. Basal + 2-1/2% diCa Mg Aconitate
5. Basal + 1% diCa Mg Aconitate
6. Basal + 5% diCa Mg Aconitate + KCl
7. Basal + 2-1/2% diCa Mg Aconitate + KCl
8. Basal + 1% diCa Mg Aconitate + KCl

(The diet compositions are shown in Table 3.)

Table 3. Composition of experimental diets (grams). Trial 3.

Group	1	2	3	4	5	6	7	8
Ingredients								
molasses*	--	643	--	--	--	--	--	--
sucrose	418	--	385	402	412	354	370	380
glucose	116	--	106	111	114	98	103	106
fructose	109	--	103	105	107	93	97	99
fibrin, beef blood	180	180	180	180	180	180	180	180
lard	100	100	100	100	100	100	100	100
mineral mix*	40	40	40	40	40	40	40	40
vitamin mix*	10	10	10	10	10	10	10	10
cellulose	27	27	27	27	27	27	27	27
KCl	--	--	--	--	--	48	48	48
diCaMg Aconitate	--	--	50	25	10	50	25	10
Total	1000	1000	1000	1000	1000	1000	1000	1000

*See footnotes Table 1.

The dicalcium magnesium aconitate was prepared in the laboratory by the method described by Ambler, Turer and Keenan in 1945. It was added to the diet at levels calculated to be 5, 2-1/2 and 1 percent of the diet. In this trial water was no longer included in the diet and the sugar component was increased. The diets were mixed and refrigerated in plastic bags. The molasses was added at the time of feeding as before. The feeding period was for 16 days, with animal care the same as in earlier trials. Records of progress were kept as in trial one.

After completing the feeding trial the rats were removed from the test and killed. Observations were made on the condition of the gastrointestinal tract and its contents. Following various unsuccessful attempts to collect feces for dry matter determinations it was concluded that dry matter of the contents of the large intestine would be a satisfactory measure of dry matter of the droppings. The samples were collected in preweighed aluminum dishes by clipping the apex of the caecum and forcing the contents of the large intestine into the dish. Wet weights were taken and the samples were placed in a drying oven at 90°C for 12 hours, then removed to a dessicator to assume a constant weight and dry weights were taken. Percent dry matter was then calculated for each sample and tabulated.

Trial 4

As mentioned earlier, it has previously been demonstrated that partially deionized molasses reduced the looseness of droppings in poultry (Kondo and Ross, 1962a). This study was designed to evaluate various forms of deionized molasses and to further study the role of magnesium and potassium as causative agents of diarrhea. One hundred, 50 gram Long Evans rats were purchased from Simonsen Laboratory. Ten rats were randomly assigned to each of the ten dietary treatments. Dietary treatments were as follows:

1. Basal
2. Basal with molasses as the energy source
3. Basal with cation-free molasses as the energy source
4. Basal with anion-free molasses as the energy source
5. Basal with deionized molasses as the energy source
6. Basal + diCa Mg aconitate
7. Basal + diCa Mg aconitate +KCl
8. Basal + $CaCO_3$
9. Basal + MgO
10. Basal + KCl

(The diet compositions are shown in Table 4.)

The basal diet was prepared as in trial three. All ingredients except the various forms of molasses were mixed and refrigerated

Table 4. Composition of experimental diets (grams). Trial 4.

Group	1	2	3	4	5	6	7	8	9	10
Ingredients										
molasses*		643								
cation-free molasses			643							
anion-free molasses				643						
deionized molasses					643					
sucrose	418					384	353	403	416	386
glucose	116					107	98	112	115	108
fructose	109					100	92	105	108	101
fibrin, beef blood	180	180	180	180	180	180	180	180	180	180
lard	100	100	100	100	100	100	100	100	100	100
mineral mix*	40	40	40	40	40	40	40	40	40	40
vitamin mix*	10	10	10	10	10	10	10	10	10	10
cellulose	27	27	27	27	27	27	27	27	27	27
diCaMg Aconitate						52	52			
MgO									4.7	
$CaCO_3$								23.4		
KCl							48			48
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

*See footnotes Table 1.

in plastic bags with the molasses added at the time of feeding. Diet six had dicalcium magnesium aconitate at a level calculated to be equivalent to the level present in the molasses diet (2). Diet seven was the same as diet six except that KCl was added at a level calculated to be equivalent to its occurrence in the molasses diet. Diets eight and nine were made up to individually supplement the basal diet with the mineral components making up the dicalcium magnesium aconitate. Diet ten contained KCl at a level equivalent to diet two and seven, without the high magnesium level.

The cation-free molasses was prepared by diluting molasses with distilled water until the sugar concentration was approximately 15 percent (Kondo and Ross, 1962a). The diluted molasses was then put through a Dowex 50W-X4 resin[4], 20-50 mesh, in a 40 X 2 inch column. The resulting cation-free molasses was then reconstituted by two methods. The first involved complete drying in a Thermo-Vac freeze dryer and then adding distilled water back to the original consistency. The second method involved evaporating *in vacuo* at 95°C until the cation-free molasses reached its original consistency. The second method proved more efficient than the first because of problems of the molasses bubbling in the freeze dryer.

Anion-free molasses was prepared by running the diluted

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[4]Dow Chemical Company, Midland, Michigan.

molasses through an Amberlite IRA-410 resin[5] 20-50 mesh and reconstituting by the second method described above. The deionized molasses was prepared by the reverse cycle method as described by Fort et al. (n. d.) to minimize sucrose inversion and loss of reducing sugars. The diluted molasses was first put through the strongly basic anion exchange resin (Amberlite IRA-410) followed by the weakly acidic resin (Amberlite IRC-50). The deionized molasses was reconstituted by the second method described above.

At the end of the feeding trial the rats were removed from test and killed. The contents of the large intestine were collected in pre-weighed aluminum dishes by clipping the apex of the caecum and forcing the contents out to be weighed immediately. Dry matter determinations were made, the same as in trial three.

Statistical Analysis

Analyses of variance were performed on all variables measured. Then tables were prepared comparing the differences between every combination of means for each variable, and a separate L. S. D. was calculated for each comparison, adjusting for variation in sample size. A complete record of statistical data is shown in the Appendix.

[5] Rohm and Haas Company, Philadelphia, Pennsylvania.

RESULTS AND DISCUSSION

Trial 1

Observations During the Test

The rats were placed on test June 29, 1966. Group one adjusted rapidly to the diet, showing the characteristics of healthy, growing rats with no apparent problems. The droppings in this group remained firm and were a tan color.

The rats on the molasses-containing diet (2) developed diarrhea within 24 hours of being placed on feed. The droppings were characterized as thin and watery, and dark brown or black in color. The rats became coated with feces on the posterior portion of the body, and had much of the molasses-containing diet adhering to their heads. All of the rats on diet two stayed in a generally poor sanitary condition for the first two weeks of the trial. No beneficial effect was noted by the aureomycin treatment.

After the clean-up period previously described the rats maintained a cleaner appearance. Rat eight of this group had a habit of crawling inside the feeder and thus continued to be coated with the diet material.

The rats on diet three, with molasses ash added, and diet four, with KCl added, did not at any time during the feeding trial show any signs of diarrhea. This is contrary to the results of

Rosenberg and Palafox (1956a) who produced diarrhea in chicks fed molasses ash and potassium. This discrepancy is probably due to a species difference.

Water Consumption

Data on water consumption showed dramatic differences among the four groups. The rats on the molasses-containing diet consumed more water than any of the other three groups ($P < 0.01$). Rats on the diet containing molasses ash consumed significantly ($P < 0.01$) greater quantities of water than did the basal diet-fed animals but significantly less than the molasses ($P < 0.01$) and KCl ($P < 0.05$) groups. The mean total water consumption for the test period was 308.0 ml., 927.7 ml., 664.8 ml. and 736.3 ml. for the four groups respectively.

It is interesting to note that the group with KCl added consumed more water than the group with the molasses ash added. The reason for this is somewhat confusing since the diets were prepared with the intent of duplicating the potassium content. It is possible that during the ashing of the molasses some of the minerals and in particular potassium may have been lost, which would account for the lower water intake. Or more likely, the balance of the mineral make-up of molasses is such that there is less need for water intake.

Feed Consumption

Feed consumption for this trial showed that the rats on diets with molasses and molasses ash consumed a significantly ($P < 0.01$) greater amount of feed than either of the other two groups. It should also be pointed out that both the molasses and molasses-ash groups wasted more feed than the other two groups, so the measure of feed consumption may be somewhat misleading. The basal diet group (1) and the basal + KCl group (4) consumed similar amounts of feed with average total feed consumptions of 380.0 and 375.2 grams, respectively.

Weight Gain

Analysis of variance showed no significant differences in the gains of the four groups. However, the group on the molasses diet had the highest average total gain of the four groups and the average also included rat eight of this group which performed below the average of the group throughout the trial. It is interesting to note that despite the severe diarrhea of the molasses group, their gain was not adversely affected by a diet containing 64.3 percent molasses. The efficiency of gain of the basal group was better than that of the molasses group (2.97 grams of feed per gram of gain versus 3.5 grams of feed per gram of gain). This agrees with the results

Rosenberg and Palafox in 1956b where they experienced a decrease in efficiency of gain with increased levels of molasses.

A summary of the results of the feeding trial 1 is given in Table 5.

Table 5. Summary of the results of trial 1.

Group	1	2	3	4
Average total water consumption/ rat (ml.)	308.0	927.7	664.8	736.3
Average total feed consumption/ rat (gm.)	380.0	460.9	475.1	375.2
Average total gain/rat (gm.)	128.1	135.3	131.4	134.6

Intestinal Observations

The rats on the molasses diet had greatly enlarged large intestines. The large intestine extended anteriorly along the ventral side of the rat nearly up to the area of the stomach. It has been reported that in cases of diarrhea the colon appears large and distended and atonic (Alvarez, 1948) and these results certainly bear out that observation. The large intestines of the rats on the molasses diet were completely filled with a thin watery material which appeared very similar to the droppings in this group. The other three groups showed no abnormalities.

Summary of Trial 1

The results of trial one show that:

1. The potassium is not directly responsible for the laxative problem in rats, as was the case in poultry.
2. The mineral component of molasses as supplied by molasses ash is not directly responsible for the laxative effect.
3. Both the potassium and molasses ash group showed increased water consumption but not to the extent that molasses *per se* increased water consumption.
4. There were no adverse responses noted from the basal diet.
5. Gain was not adversely affected by any of the dietary treatments.

Trial 2

Observations During the Test

The rats were placed on test August 24, 1966. Group one adjusted rapidly to the diet and gained well throughout, showing no symptoms of diarrhea or any other problems. Group two, on the molasses diet, developed diarrhea within 24 hours after being placed on the molasses diet. Group three on the increasing levels of molasses showed no signs of diarrhea until the third week when the molasses was increased from 25 to 40 percent of the diet. Then diarrhea occurred within 24 hours after the increase. The droppings from the third week until termination of the trial appeared nearly identical to those of the rats in group two. There was no apparent increase in the severity of the diarrhea when the molasses concentration was increased from 40 to 64.3 percent. Group four with the $MgSO_4$ added to the diet also began to show loose droppings within 24 hours, but they were not as watery as the droppings of those on the molasses diet. Groups five and six with the molasses + 10 and 20 percent ethanol, respectively, showed the same response in consistency of droppings as did group two.

Water Consumption

The average total water consumption for feeding trial two is shown in Table 6.

Again, the group on the high molasses diet consumed the most water--far exceeding any other group. The group on the increasing level of molasses showed an increasing water intake with each increase in molasses. The average weekly water consumption per animal for group three was 79, 182, 224, and 272 ml. for weeks one through four. Group four, with $MgSO_4$ added, showed greater water consumption than the basal group but still far below the consumption of any group receiving molasses. Groups five and six with

Table 6. Summary of the results of trial 2.

Group	1	2	3	4	5	6
Average total water consumption/ animal (ml.)	371	1110	764	478	791	780
Average feed consumption/rat (gm.)	475	480	453	458	430	385
Average total gain/rat (gm.)	166.3	142.6	135.6	173.6	117.3	116.6

molasses + ethanol showed lower water consumption than the group on the molasses alone. The probable reasons for this are that the animals on this diet consumed less feed and the molasses was diluted by the addition of the ethanol.

Feed Consumption

Feed consumption results for trial two are shown in Table 6.

The main point of interest shown by the records is the decline in feed consumption in groups five and six. As the concentration of ethanol increased, the feed consumption decreased.

Weight Gain

The average total gain for the feeding trial two is shown in Table 6.

In terms of gain it appeared that there was no advantage to the gradual increases in molasses concentration as the average gain for that group was lower than the average for group two which started at 64.3 percent molasses. Group four on the diet with added $MgSO_4$ actually outgained the basal group, so the loose droppings caused by the $MgSO_4$ evidently did not effect gain. The gains of group five and six are lower than that of the molasses-fed group. The difference is probably due to the lowered feed intake on the diets containing ethanol.

Observations of the Intestinal Tract

Inspection of the intestinal tract showed, as in trial one, a greatly distended and enlarged large intestine in rats receiving the molasses diet and molasses + ethanol diets. The contents of the large intestine were thin and watery as was the case for rats on the molasses diet in trial one. Even though diarrhea occurred in group four, the diet containing $MgSO_4$, the large intestine was not nearly as distended as those receiving the molasses diets. However, the large intestine of group four was more distended than the rats on the basal diet. The contents of the large intestine in group four were much like those of the molasses group but in lesser amounts than the rats on the molasses diet.

Summary of Trial 2

The results of trial two showed that:

1. Magnesium when supplied as $MgSO_4$ in amounts equivalent to the magnesium level of the molasses diets is capable of producing diarrhea in rats, although not to the extent that the molasses did.
2. The addition of ethanol to molasses did not in any way reduce the laxative effect of molasses. Rather, its addition

resulted in lower feed consumption and gain as well as the diarrhea.

3. There was an early advantage shown to the gradual increase in molasses concentration, but as the level of molasses reached 40 percent, diarrhea again resumed.
4. As observed by Alvarez in 1948, the large intestine was distended and in an atonic state in animals showing signs of diarrhea.

At this point some conclusion may be drawn concerning the laxative effect of molasses. It has been demonstrated that the potassium content of molasses does cause increased water consumption, and that the magnesium content is capable of causing diarrhea. However, when molasses ash was added to the diet no diarrhea occurred. Therefore if magnesium is the causative agent it is likely bound to an organic molecule or it undergoes some change in its form during ashing. A search of the literature showed that the molecule in question may well be dicalcium magnesium aconitate (Ambler, Turer and Keenan, 1945; Fort _et al._, 1952). The results of trials three and four evaluate dicalcium magnesium aconitate as the causative agent in the laxative effect.

Trial 3

The results of this trial are subject to questions in that problems occurred in the preparation of the dicalcium magnesium aconitate. It was learned that the aconitic acid used as a starting material in the synthesis was only 85 percent pure. In addition, it appeared that there was an error made while adjusting the pH due to the fact that the MgO was not in complete solution. Further complicating this trial, due to problems with air freight, the rats suffered excessive handling in coming to Corvallis, and as a result death losses were very high. Thus the results of this trial will be presented as a matter of interest, but not weighed too heavily in the final conclusions.

Observations During the Test

The rats on the molasses diet showed signs of diarrhea after 24 hours on test, however, the diarrhea or the soiling due to the diet did not hamper their performance. No other group showed any signs of diarrhea throughout the trial. On completion of the trial, magnesium and calcium analyses were performed on the dicalcium magnesium aconitate used in this trial. The results showed that magnesium made-up 3.56 percent of the molecule whereas theoretical magnesium level is 5.38 percent. Calcium made-up 115.67 percent of the theoretical level. The levels of magnesium and calcium found do not equal the theoretical total. The remainder may be made-up of some unidentified portion of the impure aconitic acid used in the synthesis. The fact that a pure product was not used to supply the magnesium may explain the lack of diarrhea on the dicalcium magnesium aconitate treatments.

Water Consumption

The water consumption averages are shown in Table 7.

Water consumption of the rats on the basal group was significantly ($P < 0.01$) lower than that on any diet containing molasses or KCl. The animals on the diet containing molasses consumed significantly ($P < 0.01$) greater quantities of water than did any of the other groups. There were no significant differences between the basal group and any of the three groups receiving dicalcium magnesium aconitate. The rats receiving dicalcium magnesium aconitate + KCl consumed significantly ($P < 0.05$) greater quantities of water than did the basal or the groups receiving dicalcium magnesium aconitate without added potassium, but significantly ($P < 0.01$) less than group two receiving molasses. There were no significant differences among the three groups receiving diets high in potassium. In summary, the data show a similar picture to those from trials one and two in that molasses increased water consumption the greatest and potassium increased it but not to the same extent as molasses.

Feed Consumption

Average feed consumption data are shown in Table 7.

Feed consumption of rats on the molasses diet was significantly ($P < 0.05$) greater than that of group six or eight both of which contained KCl. Likewise the basal group and the group receiving five percent dicalcium magnesium aconitate consumed significantly ($P < 0.05$) greater quantities of feed than did groups seven and eight.

Weight Gain

Average total weight gain per rat is shown in Table 7.

No other groups in this trial gained as rapidly as the rats on the basal diet. Contrary to the results of earlier trials the rats on the molasses diet gained significantly ($P < 0.05$) less than did the basal group. Groups three, four and five gained significantly

($P < 0.05$) more weight than group six. The general tendency, though not significant in all cases, was toward increased gain by those groups receiving the basal and dicalcium magnesium aconitate diets without potassium over that of the groups with added potassium.

Observations on the Intestinal Tract

The rats on the molasses diet again showed the greatly distended large intestine filled with thin watery contents. No abnormalities were noted in any other group.

Dry Matter of the Contents of the Large Intestine

The average dry matter of the contents of the large intestine are shown in Table 7.

The rats receiving the molasses diet had significantly ($P < 0.05$) lower intestinal dry matter contents than any other group in the trial. In interpreting these results concerning intestinal dry matter, one must consider the collection procedure. The samples were taken at the Small Animal Laboratory and then brought to the Nutrition Laboratory to take wet weights. The time between when the first rat was killed and when the first weight was taken was approximately four hours. In this time the samples had an opportunity to dry, and the drying was uneven because sample size varied from rat to rat and group to group.

Table 7. Summary of results of trial 3.

Group	1	2	3	4	5	6	7	8
Average total water consumption/rat (ml.)	160.2	425.5	183.8	201.6	203.2	322.9	326.1	291.9
Average total feed consumption/rat (gm.)	168.0	174.3	168.2	154.1	149.4	143.3	153.6	139.4
Average total weight gain/rat (gm.)	63.4	44.5	51.2	50.8	49.1	26.7	47.0	42.3
Average percent dry matter of large intestinal contents/rat	31.8	22.0	28.9	37.1	30.9	30.2	36.5	34.8

Summary of the Results of Feeding Trial 3

The results of trial three although inconclusive for reasons given previously, suggest:

1. Again the rats on the molasses diet showed the same characteristic signs of severe diarrhea and the greatly distended large intestine.
2. There was no evidence of a tendency toward diarrhea in the other groups.
3. The results showed areas of the experimental technique that need improvement. Included in these are better preparation of dicalcium magnesium aconitate and the taking of intestinal content weights at the time of collection.

Trial 4

Observations During the Test

Groups two, three, four, five, six, seven, and nine showed signs of diarrhea, varying in severity, 24 hours after being placed on test. Group two, with molasses added, showed severe diarrhea as described before in earlier trials. Groups three and five receiving cation-free molasses and deionized molasses respectively, showed signs of diarrhea for the first two days of the trial, then gradually the droppings firmed up and resembled those of the basal group. The droppings of the rats on the anion-free molasses diet continued to be loose throughout the trial, though not to the same extent as those of the rats fed molasses. Groups six, seven and nine on dicalcium magnesium aconitate, dicalcium magnesium aconitate + KCl, and magnesium oxide respectively, showed signs of severe diarrhea throughout the trial. No other group showed any signs of diarrhea at any time during the trial.

Table 8 summarizes the results of the feed trial.

Water Consumption

There are some interesting results on water consumption. As expected, the animals receiving the molasses consumed the most water and the animals receiving the basal diet consumed the least. Unexpected however, were the high values recorded for the various forms of ion-free molasses. The rats on the molasses diet consumed significantly ($P < 0.05$) more water than all groups except four and five, suggesting that there is something besides the ionic components of molasses responsible for the increased water consumption. The animals on the cation-free molasses diet drank significantly ($P < 0.05$) less than the animals on the molasses diet. In this case, the cation-free molasses was prepared using the Dowex 50W-X4 resin which is a strongly acidic cation resin. The differences in the removal qualities of the Amberlite and the Dowex resins may be involved in the differences in water consumption. However, the mean water consumption for the cation-free molasses-fed group was very close to the means of those groups receiving high potassium, including the anion-free molasses; all of which consumed significantly ($P < 0.01$) greater quantities of water than the basal group. Thus, there appears to be other factors involved in water consumption besides the ionic components. There were no significant differences among groups one, six, eight, and nine.

Feed Consumption

Rats on the anion-free molasses diet consumed significantly ($P < 0.01$) greater quantities of feed than any other group. The rats on the remaining forms of molasses consumed similar (lesser) amounts of feed and these in turn were significantly ($P < 0.05$) greater than those consumed by groups one, six, seven, nine and ten. These latter groups consumed similar quantities of feed except group eight ($CaCO_3$ added), which consumed significantly ($P < 0.05$) greater amounts of feed than either group containing dicalcium magnesium aconitate. It should be noted that all groups fed molasses wasted more feed than any of the other groups.

Weight Gain

Group four, fed the anion-free molasses, had the highest mean gain of any group. The gain of the animals on the anion-free molasses was significantly ($P < 0.05$) greater than all other groups except the animals on the deionized molasses which would also be anion-free. It has been shown by Ambler, Turer, and Keenan (1945) that the Amberlite IRA-410 resin removes over 95 percent of the aconitic acid. Burau and Stout (1965) reported that trans-aconitic acid is a competitive inhibitor to the Kreb's Cycle, so its removal should logically result in increased gain. Also pointed out in the above section, rats on the anion-free molasses consumed more feed than any other group. Group two on molasses diet gained significantly ($P < 0.05$) more weight than did the basal group. Both groups on the dicalcium magnesium aconitate diets gained significantly ($P < 0.01$) less weight than did the animals in the basal group.

Observations on the Intestinal Tract

Again, the rats on the molasses diet had greatly distended large intestines, filled with thin watery material, as did the group on the anion-free molasses. The large intestine of the rats in the cation-free molasses and deionized molasses-fed groups approached normal as compared to the basal group but some distension was detectable. The three groups on the high magnesium diets did not show the distension which characterized the rats on the molasses diet, even though diarrhea was severe in these groups. A clearer understanding of the condition of the large intestine may be realized by an evaluation of the next two sections.

Dry Matter of Large Intestinal Contents

From the data presented, the dry matter of the large intestinal contents was significantly ($P < 0.01$) lowered by the molasses diet and the three diets containing high magnesium. The animals on the three forms of deionized molasses had significantly ($P < 0.05$) higher dry matter figures than the molasses diet but still significantly ($P < 0.05$) lower than the basal group. The rats on the various forms of the deionized molasses have large intestinal dry matter values midway between the values of the rats on the molasses and the rats on the basal diet. The dry matter of the large intestinal contents of the rats on the deionized molasses came closest to the value recorded for animals on the basal diet (see Table 8). The high dietary potassium levels did not significantly lower the intestinal content dry matter figures. An interesting point appears in group eight on the diet containing $CaCO_3$ which shows a significantly ($P < 0.01$) higher dry matter value than any other group. This

Table 8. Summary of results of trial 4.

Group	1	2	3	4	5	6	7	8	9	10
Average total water consumption/rat (ml.)	161.9	367.0	295.6	389.3	334.2	191.8	291.9	200.6	198.1	282.0
Average total feed consumption/rat (gm.)	122.3	159.7	152.1	217.4	168.0	108.8	103.4	135.8	113.8	123.9
Average total gain/rat (gm.)	48.8	50.2	37.0	63.7	58.2	15.1	15.1	51.8	39.7	53.9
Average dry matter percentage of large intestinal contents/rat	36.95	18.17	26.12	23.77	27.83	20.49	21.64	46.28	20.31	33.67
Average wet weight of large intestinal contents/rat (gm.)	0.73	5.10	2.70	4.9	1.93	1.59	1.56	0.53	2.23	0.74

agrees with the results of Kondo and Ross (1962), that high levels of calcium tended to counteract the loose droppings caused by high potassium in poultry diets. The data on intestinal dry matter in group eight were subject to more error, due to the small sample size, variation in weighing would reflect a greater error in percent dry matter.

Weight of Large Intestinal Contents

The mean total weights of the wet contents of the large intestine of the rats on the molasses diet versus those on the basal diet were 5.096 gm. and 0.725 gm. These data serve to emphasize the differences in the sizes of the large intestines. The animals on the molasses group had significantly ($P < 0.01$) heavier intestinal contents than did all groups except the rats on the anion-free molasses diet. The cation-free and deionized molasses-fed groups showed significantly ($P < 0.01$) less intestinal content weight than the molasses-fed group but significantly more than the rats on the basal group. The animals on the three forms of high magnesium and deionized molasses had similar weights of large intestinal contents, all significantly ($P < 0.01$) greater than the rats on the basal diet but significantly ($P < 0.01$) less than the rats on the molasses-fed diets. The high potassium diet did not cause a significant increase in weight of large intestinal contents. It appears that some other factors besides the ionic components of molasses are responsible for the increased weight of the contents of the large intestine.

Summary of Trial 4

1. Water consumption remained high among rats fed the various forms of deionized molasses, indicating other factors governing water consumption.
2. Feed consumption by rats was increased on the anion-free diet and deionized molasses diet.
3. Gain was increased by the anion-free molasses and deionized molasses diets and lowered by the addition of dicalcium magnesium aconitate to the diet.
4. Moisture content of the large intestinal contents was high on the molasses and high magnesium diets and moderately high on the three forms of deionized molasses.
5. The same pattern follows on weight of large intestinal contents as did moisture content. The highest weight was on the molasses diet but a high weight was also recorded on the anion-free molasses diet. Moderately high weights were recorded for the contents on the high magnesium diets and the cation-free molasses and deionized molasses diets.

SUMMARY AND CONCLUSIONS

The results of the four trial have shown:

1. Diarrhea was severe among rats receiving the diet containing 64.3 percent molasses.
2. Gain was not adversely affected by the high molasses diets although the rats consumed more feed.
3. Water consumption was greatest by those rats receiving the molasses-supplemented diets.
4. Water consumption was increased in rats receiving high potassium diets but not to the extent that molasses supplemented diets increased water consumption.
5. Potassium was not directly responsible for the laxative effect of molasses in rats, as was the case in poultry.
6. Magnesium when supplied in the form found in molasses, dicalcium magnesium aconitate, was capable of causing loose droppings, though not to the extent as the molasses-supplemented diet.
7. The laxative effect was reduced in rats receiving cation-free molasses and deionized molasses.
8. Organic components of molasses were not directly responsible for the laxative effect <u>per se</u>.

At this point a possible explanation of the mechanism involved in the laxative problem is appropriate. As stated by Goodman and Gilman (1965) in the case of saline cathartics, water is added to the intestinal tract until the contents are iso-osmotic with body fluids. This added water may come from body fluid in which case dehydration occurs or from increased water consumption. In the case of molasses, it appears that the dicalcium magnesium aconitate builds up the osmotic gradient and water is supplied because of the high potassium content of molasses and other factors involved in the high water consumption. The result is the severe diarrhea without excessive dehydration. However, this argument has some drawbacks in that the response could not be completely duplicated with the addition of dicalcium magnesium aconitate and potassium or completely alleviated with the deionized molasses.

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APPENDIX

Appendix Table 1. Feed consumption for trial 1.

Analysis of Variance				
Source	d. f.	SS	MS	F
Trt-Var	3	81348. 3546	27116. 1182	10. 84**
Error	35	87555. 3892	2501. 5825	
Total	38	168903. 7435		

**highly significant P< 0. 01

group	1	2	3	4
N	10	9	10	10
Trt. Means	380. 00	460. 889	475. 100	375. 200

LSD P < 0. 05 for N of 10 x 10 = 45. 4051
LSD P < 0. 05 for N of 9 x 10 = 46. 6539

LSD P < 0. 01 for N of 10 x 10 = 60. 9278
LSD P < 0. 01 for N of 9 x 10 = 62. 6036

Appendix Table 2. Water consumption for trial 1.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	3	1939988.6079	646662.8693	104.25**
Error	35	217095.6997	6202.7343	
Total	38	2157084.3076		

**highly significant

group	1	2	3	4
N	10	9	10	10
Trt. Means	308.000	927.667	664.800	736.300

LSD $P < 0.05$ for N of 10 x 10 = 71.4972
LSD $P < 0.05$ for N of 9 x 10 = 73.4637

LSD $P < 0.01$ for N of 10 x 10 = 95.9400
LSD $P < 0.01$ for N of 9 x 10 = 98.5788

Appendix Table 3. Gain for trial 1.

		Analysis of Variance			
Source	d.f.	SS	MS	F	
Trt-Var	3	320.1974	106.7325	0.224	N.S.
Error	35	16687.7000	476.7914		
Total	38	17007.8975			

group	1	2	3	4
N	10	9	10	10
Trt. Means	128.100	135.333	131.400	134.6

Appendix Table 4. Feed consumption for trial 3.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	7	8652.7881	1236.1126	2.46*
Error	62	31152.0833	502.4530	
Total	69	39804.8714		

*significant P < 0.05

group	1	2	3	4	5	6	7	8
N	10	4	10	9	10	9	9	9
Trt. Means	168.00	174.25	168.20	154.11	149.40	143.33	153.56	139.44

LSD P < 0.05 for N of 10 x 10 = 20.03
LSD P < 0.05 for N of 9 x 10 = 20.58
LSD P < 0.05 for N of 9 x 9 = 21.11
LSD P < 0.05 for N of 4 x 10 = 26.50
LSD P < 0.05 for N of 4 x 9 = 26.91

Appendix Table 5. Water consumption for trial 3.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	7	406504.6541	58057.9506	19.35**
Error	62	185994.6888	2999.9143	
Total	69	592400.3429		

**highly significant P < 0.01

group	1	2	3	4	5	6	7	8
N	10	4	10	9	10	9	9	9
Trt. Means	160.20	425.50	183.80	201.56	203.20	322.89	326.11	291.89

LSD $P < 0.05$ for N of 10 x 10 = 48.94
LSD $P < 0.05$ for N of 9 x 10 = 50.28
LSD $P < 0.05$ for N of 9 x 9 = 51.59
LSD $P < 0.05$ for N of 4 x 10 = 64.74
LSD $P < 0.05$ for N of 4 x 9 = 65.76

LSD $P < 0.01$ for N of 10 x 10 = 65.06
LSD $P < 0.01$ for N of 9 x 10 = 66.84
LSD $P < 0.01$ for N of 9 x 9 = 68.58
LSD $P < 0.01$ for N of 4 x 10 = 86.06
LSD $P < 0.01$ for N of 4 x 9 = 87.41

Appendix Table 6. Gain for trial 3.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	7	5923.4159	846.2023	4.46**
Error	62	11763.4556	189.7332	
Total	69	17686.8714		

**highly significant P < 0.01

group	1	2	3	4	5	6	7	8
N	10	4	10	9	10	9	9	9
Trt. Means	44.50	63.40	51.20	50.78	49.10	29.67	47.00	42.33

LSD $P < 0.05$ for N of 10 x 10 = 12.31
LSD $P < 0.05$ for N of 9 x 10 = 12.65
LSD $P < 0.05$ for N of 9 x 9 = 12.97
LSD $P < 0.05$ for N of 4 x 10 = 16.28
LSD $P < 0.05$ for N of 4 x 9 = 16.54

LSD $P < 0.01$ for N of 10 x 10 = 16.36
LSD $P < 0.01$ for N of 9 x 10 = 16.81
LSD $P < 0.01$ for N of 9 x 9 = 17.24
LSD $P < 0.01$ for N of 4 x 10 = 21.64
LSD $P < 0.01$ for N of 4 x 9 = 21.98

Appendix Table 7. Percent dry matter of large intestinal contents for trial 3.

	Analysis of Variance			
Source	d. f.	SS	MS	F
Trt-Var	7	967.8585	138.2655	3.64**
Error	59	2241.6646	37.9943	
Total	66	3209.5231		

**highly significant P < 0.01

group	1	2	3	4	5	6	7	8
N	10	4	10	9	10	9	7	8
Trt. Means	22.03	31.75	28.92	37.07	31.00	30.22	36.49	34.83

LSD $P < 0.05$ for N of 10 x 10 = 5.51
LSD $P < 0.05$ for N of 9 x 10 = 5.66
LSD $P < 0.05$ for N of 9 x 9 = 5.81
LSD $P < 0.05$ for N of 9 x 8 = 5.98
LSD $P < 0.05$ for N of 9 x 7 = 6.21
LSD $P < 0.05$ for N of 4 x 10 = 7.29
LSD $P < 0.05$ for N of 4 x 9 = 7.40
LSD $P < 0.05$ for N of 4 x 8 = 7.54
LSD $P < 0.05$ for N of 4 x 7 = 7.72

LSD $P < 0.01$ for N of 10 x 10 = 7.32
LSD $P < 0.01$ for N of 9 x 10 = 7.42
LSD $P < 0.01$ for N of 9 x 9 = 7.72
LSD $P < 0.01$ for N of 9 x 8 = 7.95
LSD $P < 0.01$ for N of 9 x 7 = 8.25
LSD $P < 0.01$ for N of 4 x 10 = 9.69
LSD $P < 0.01$ for N of 4 x 9 = 9.84
LSD $P < 0.01$ for N of 4 x 8 = 10.03
LSD $P < 0.01$ for N of 4 x 7 = 10.26

Appendix Table 8. Feed consumption for trial 4.

Analysis of Variance				
Source	d.f.	SS	MS	F
Trt-Var	9	99291.9278	11032.4364	19.80**
Error	83	46258.5238	557.3316	
Total	92	145550.4516		

**highly significant P < 0.01

group	1	2	3	4	5	6	7	8	9	10
N	10	9	10	9	10	9	10	10	7	9
Trt. Means	122.30	159.67	152.10	217.44	168.00	108.78	103.40	135.80	113.86	123.89

LSD $P < 0.05$ for N of 10 x 10 = 21.09
LSD $P < 0.05$ for N of 9 x 10 = 21.67
LSD $P < 0.05$ for N of 9 x 9 = 22.37
LSD $P < 0.05$ for N of 7 x 10 = 23.24
LSD $P < 0.05$ for N of 7 x 9 = 23.72

LSD $P < 0.01$ for N of 10 x 10 = 27.92
LSD $P < 0.01$ for N of 9 x 10 = 28.69
LSD $P < 0.01$ for N of 9 x 9 = 29.44
LSD $P < 0.01$ for N of 7 x 10 = 30.77
LSD $P < 0.01$ for N of 7 x 9 = 31.47

Appendix Table 9. Water consumption for trial 4.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	9	510310.1375	56701.1264	17.86**
Error	82	260314.6017	3174.5683	
Total	91	770624.7391		

** highly significant P < 0.01

group	1	2	3	4	5	6	7	8	9	10
N	9	9	10	9	10	9	10	10	7	10
Trt. Means	161.89	367.00	295.60	389.33	334.20	191.78	291.90	200.60	198.14	282.00

LSD P < 0.05 for N of 10 x 10 = 50.34
LSD P < 0.05 for N of 9 x 10 = 51.73
LSD P < 0.05 for N of 9 x 9 = 53.07
LSD P < 0.05 for N of 7 x 10 = 55.48
LSD P < 0.05 for N of 7 x 9 = 56.74

LSD P < 0.01 for N of 10 x 10 = 66.64
LSD P < 0.01 for N of 9 x 10 = 68.48
LSD P < 0.01 for N of 9 x 9 = 70.25
LSD P < 0.01 for N of 7 x 10 = 73.44
LSD P < 0.01 for N of 7 x 9 = 75.11

Appendix Table 10. Gain for trial 4.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	9	24009.1080	2667.6787	16.49**
Error	83	13424.4619	161.7405	
Total	92	37433.5699		

group	1	2	3	4	5	6	7	8	9	10
N	10	9	10	9	10	9	10	10	7	9
Trt Means	48.80	50.22	37.00	63.67	58.20	15.11	15.10	51.80	39.71	53.89

LSD $P < 0.05$ for N of $10 \times 10 = 11.36$
LSD $P < 0.05$ for N of $9 \times 10 = 11.68$
LSD $P < 0.05$ for N of $9 \times 9 = 11.98$
LSD $P < 0.05$ for N of $7 \times 10 = 12.52$
LSD $P < 0.05$ for N of $7 \times 9 = 12.81$

LSD $P < 0.01$ for N of $10 \times 10 = 15.04$
LSD $P < 0.01$ for N of $9 \times 10 = 15.45$
LSD $P < 0.01$ for N of $9 \times 9 = 15.86$
LSD $P < 0.01$ for N of $7 \times 10 = 16.58$
LSD $P < 0.01$ for N of $7 \times 9 = 16.95$

Appendix Table 11. Percent dry matter of large intestinal contents for trial 4.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	9	6677.7658	741.9740	30.07**
Error	80	1974.0155	24.6752	
Total	89	8651.7813		

**highly significant P < 0.01

group	1	2	3	4	5	6	7	8	9	10
N	10	9	10	9	10	8	10	10	6	8
Trt. Means	36.95	18.17	26.12	23.77	27.83	20.49	21.64	46.27	20.31	33.67

LSD P < 0.05 for N of 10 x 10 = 4.44
LSD P < 0.05 for N of 9 x 10 = 4.56
LSD P < 0.05 for N of 8 x 10 = 4.70
LSD P < 0.05 for N of 6 x 10 = 5.12
LSD P < 0.05 for N of 9 x 9 = 4.68
LSD P < 0.05 for N of 8 x 9 = 4.82
LSD P < 0.05 for N of 6 x 9 = 5.23
LSD P < 0.05 for N of 8 x 8 = 4.96
LSD P < 0.05 for N of 6 x 8 = 5.36

LSD P < 0.01 for N of 10 x 10 = 5.86
LSD P < 0.01 for N of 9 x 10 = 6.04
LSD P < 0.01 for N of 8 x 10 = 6.23
LSD P < 0.01 for N of 6 x 10 = 6.78
LSD P < 0.01 for N of 9 x 9 = 6.19
LSD P < 0.01 for N of 8 x 9 = 6.38
LSD P < 0.01 for N of 6 x 9 = 6.92
LSD P < 0.01 for N of 8 x 8 = 6.56
LSD P < 0.01 for N of 6 x 8 = 7.09

Appendix Table 12. Wet weight of large intestinal contents for trial 4.

		Analysis of Variance		
Source	d.f.	SS	MS	F
Trt-Var	9	218.6697	24.2966	50.10**
Error	82	39.7630	.4849	
Total	91	258.4327		

**highly significant P < 0.01

group	1	2	3	4	5	6	7	8	9	10
N	10	9	10	9	10	9	10	10	6	9
Trt. Means	0.725	5.096	2.702	4.858	1.931	1.587	1.558	0.527	2.230	.738

LSD P < 0.05 for N of 10 x 10 = .622
LSD P < 0.05 for N of 9 x 10 = .639
LSD P < 0.05 for N of 9 x 9 = .656
LSD P < 0.05 for N of 6 x 10 = .718
LSD P < 0.05 for N of 6 x 9 = .733

LSD P < 0.01 for N of 10 x 10 = .824
LSD P < 0.01 for N of 9 x 10 = .846
LSD P < 0.01 for N of 9 x 9 = .868
LSD P < 0.01 for N of 6 x 10 = .951
LSD P < 0.01 for N of 6 x 9 = .970