EFFECT OF COFFEE GROUNDS ON *IN VITRO* RUMEN FERMENTATION, NUTRIENT DIGESTIBILITY AND DIURESIS IN CATTLE AND ISOLATION OF SOME INHIBITORY FRACTIONS USING RATS

by

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TABLE OF CONTENTS

Page

INTRODUCTION

Of all the domestic livestock, ruminants are least competitive with humans for food because of microbial fermentation in the rumen which allows them to utilize cellulosic materials for energy and non-protein nitrogen sources for protein synthesis. Residues from the wood industry, waste paper, municipal organic wastes, food industrial wastes, and other cellulosic waste materials are high in energy (112). These materials are environmental pollutants and it may be possible to convert them to meat and milk by feeding them to ruminants.

One such environmental pollutant is the waste residue from soluble coffee manufacturing. The purpose of this research was to investigate the use of coffee grounds as a feedstuff for ruminants.

REVIEW OF LITERATURE

Review of Coffee Grounds Research

Within the past 35 years several uses have been devised for waste from the soluble coffee industry. In 1939 French workers (32) suggested using coffee grounds with the oil, wax, and resin removed as filler in synthetic resin. In 1943, Johnson, Gore, and Frey (58) found that adding 0.2% or more of the unsaponifiable portion of coffee oil would inhibit rancidity of several fatty food products. Later in the same year, Polin, Nerken, and Wettingfeld (93) patented a tough, resinous material made from coffee oil. Brown in 1944 (27) patented a soap made by grinding waste coffee grounds with a soap-forming fatty material which resulted in saponification. Ward, 1945, (113) suggested using dried coffee grounds as a fuel or fertilizer. In 1946 Dahl-Rode (109) received a patent for using coffee grounds as a water softener. Brazilian workers (94) in 1947 found coffee grounds to contain almost 10% oil and 0.5% caffeine when extracted with petroleum ether. Cheney (33) also suggested producing caffeine from coffee grounds. Gilmont (45) discovered a process in which coffee waste material could yield soluble coffee. He fractionated a solvent extract of ground roasted coffee by molecular distillation into separate aroma and fat fractions, then recombined the aroma component with a water extract to make the beverage. The fatty component is useful as edible fat. The heavier residual fat can be used for soap. In 1954, Indian workers (81) prepared ion exchange material from coffee grounds. Later the same year, a patent for a soluble crystalline coffee from coffee grounds was granted (17). In 1961 Bollen and Lu (24) found increased resistance to general decomposition in soil, decreased nitrogen demands for plants,

and increased availability of nitrogen for plant growth in soil microbial studies using spent and treated coffee grounds. Yersansian (118) patented a procedure for treating coffee grounds with 0.07N NH_4OH at 85 C to obtain a coffee extract of high yield and color. Ligo in 1970 (70) received a patent using coffee grounds as filters in thermosetting materials. In 1971 an industrial use for coffee grounds in an antipiping compound (5% coffee grounds) for ingot making was found. The grounds were noted for their superior heat-retaining properties (80). Indian workers (102) in 1974 used coffee oil extracted from wastes to produce varnishes. The low acid value varnishes produced are rich in monoglycerides.

Coffee Grounds as a Feedstuff

Much of the early work related to the use of coffee grounds in poultry diets. In 1944 Hammond (47) found that the grounds decreased chick-growth and attributed this to a dilution effect. Later in 1962, Portuguese workers (5,6) discovered adverse effects of coffee meal on chick growth, egg production, and hatchability.

Coffee meal (a term commonly used for coffee grounds or the solvent extracted residue of coffee beans) was found by Carew, Alvez, and Marin (28) to contain 15.4% crude protein, 1.4% ether extract, 23.7% crude fiber, 5.4% ash, 11.2% moisture (88.8% dry matter) and 42.9% nitrogen-free extract. They observed growth depression at 2.5% coffee meal and toxicity at 10% in growing chicks. With increasing concentrations of the meal in the diet, there was a progressive decrease in feed intake and growth rate. Autoclaving the raw coffee meal resulted in a small improvement in the growth rate and reduced mortality 50%. Deaths were related to hepatic and biliary lesions, indicating that the toxicity was probably caused by substances other than those causing

growth reduction. Addition of water soluble vitamins and changing the carbohydrate source did not improve growth. They concluded that the growth depression was by way of appetite reduction. They found that neither the growth depressing or toxicity factors were fat soluble and that the growth depressing substance was heat resistant whereas the toxic factor was not.

Japanese workers (108) found that dry coffee grounds had 20% protein. When fed as a protein supplement in poultry rations, it was less valuable than wheat bran. Coffee grounds did not ferment well in silage.

Mather and Apgar (79) fed up to 18% dried grounds and up to 24% of a mixture of 63% liquid molasses dried with coffee grounds to dairy cattle with no significant effect on milk production, milk fat percentage, pulse rate, or milk flavor. The body weight of cows on the coffee grounds was significantly reduced and some cows refused to eat both of the ration types at their highest concentrations. However, two cows ate up to 2.36 kg of the coffee grounds for 100 days without refusal or undesirable effects. Mather and Apgar (79) fed dairy calves 0, 10, and 20% molasses-coffee ground mixture in starter diets from 5 to 25 wk of age. Growth rate was reduced due to decreased food consumption resulting from poor palatability of the ration. The coffee grounds were found to have 10.2± 30% protein digestibility, 1.3± 0.5% digestible protein, and 55.8± 3.6% TDN.

Berglund (18) found "Black X" (a trademark for coffee grounds) was a carrier for molasses, vitamin premixes, and mineral feeds and aided in pelleting, but it depressed growth when used as a roughage source. The product was: 10-13% crude protein, 10-13% fat, 6-10% moisture, 65% fiber, and 1% or less ash. Sales claims were i) improved palataibility of livestock supplements and better acceptance of mineral feeds, ii) 10% improvement

in feed efficiency and gain as compared with same feed without coffee grounds, iii) some improvement and stabilizing value in Vitamin A utilization, iv) slower decrease in milk production during lactation when rations containing coffee grounds are fed, v) an aid in pelleting.

"Cherco," a type of coffee ground product, contains 24% oil, 10% protein, 44% fiber, and 1% ash and has a starch equivalent of 75, similar to barley (20). Blair (21) reported coffee grounds contain 9.3% oil, 10.2% protein, and 21.5% fiber, that the "normal" feeding level in cattle rations was 5%, and that coffee grounds contain 180 mg/kg caffeine which would affect the performance of race horses (22).

The Atlas of Nutritional Data on United States and Canadian Feed (86) gives the following information on coffee grounds and coffee grounds with chicory residue:

Coffee grounds Ref. No. 1 01 576			Mean as fed	Mean dry
Dry Matter		%	73.7	100.0
Ash		%	1.2	1.6
Crude Fiber		%	21.5	29.2
Ether Extract		%	9.3	12.6
N-free Extract		%	31.5	42.7
Protein (N x 6.25)		%	10.2	13.8
Cattle	dig. prot.	%	6.6	8.9
Goats	dig. prot.	%	7.0	9.5
Horses	dig. prot.	%	6.8	9.3
Rabbits	dig. prot.	%	6.9	9.4
Sheep	dig. prot.	%	6.6	9.0
Calcium		%	0.09	0.12
Phosphorous		%	0.06	0.08

Coffee, grounds w chicory residue, dehy

Ref. No. 1 01 575			Mean as fed	Mean dry
Dry Matter		%	92.8	100.0
Ash		%	5.5	5.9
Crude Fiber		%	36.2	39.0
Sheep	dig. coef.	%	38.0	38.0
Ether Extract		%	6.6	7.1
Sheep	dig. coef.	%	82.0	82.0
N-free Extract		%	31.4	33.0
Sheep	dig. coef.	%	14.0	14.0
Protein (N x 6.25)		%	13.2	14.2
Sheep	dig. coef.	%	1.0	1.0
Cattle	dig. prot.	%	8.6	9.2
Goats	dig. prot.	%	9.1	9.8
Horses	dig. prot.	%	8.9	9.6
Rabbits	dig. prot.	%	8.9	9.6
Sheep	dig. prot.	%	0.1	0.1
Energy	GE	Mcal/kg		
Sheep	DE	Mcal/kg	1.34	1.45
Sheep	ME	Mcal/kg	1.10	1.19
Sheep	TDN	%	30.4	32.6

Coffee grounds may inhibit rumen microflora. Johor, Natarajan, and Romochandra (59) in 1958 reported that coffee extracts would support the growth of yeast and mold, but not most bacteria. Others (40) found that neutralized coffee (0.23% and greater) was bacteriostatic to Staphlococcus aureus.

Chemical Composition of Coffee Grounds

A few workers have investigated various chemical components in coffee grounds, but most of the work involves the composition of coffee and roasted coffee beans. The following is a summary of the proximate analysis of published coffee ground data. Differences may be due to roasting procedures, type of coffee grounds analyzed, or analytical procedures.

	Reference				
	(113)	(7)	(17)	(27)	(20)
% Moisture	9.45	11.42, 9.45	6-10	11.2	-
% Ash	-	1.71, 2.03	1	5.4	-
% Crude fiber	-	25.03	65	23.7	21.5
% Ether extract	11.64, 12.45	11.64, 12.45	10.13	1.4	9.3
% N-free extract	-	14.81	-	42.9	-
% Crude protein	-	-	10.13	15.4	10.2
% N	11.68, 11.5	11.68, 11.5	-	-	-
% Starches	17.00, 22.47	17.00, 22.47	-	-	-

Barbera (10) found alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryosine, and valine in raw roasted coffee. Manzanilla and Carangol (76) reported 0.96% glutamic acid in air dried coffee grounds. Portuguese workers (25) hydrolyzed coffee grounds with 5% H_2SO_4 at 134 C for 45 min. Total nitrogen was 1.25%, reduced sugar (as glucose) was 32.8%, and glycine, alanine, serine, threonine, proline, hydroxyproline, asparagine, leucine, valine, phenylalanine, tyrosine, aspartic acid and glutamic acid were found.

Other Chemicals Found in Coffee Material

Högl (54) reported that diisopropyl ether extracted coffee yields maltol, hydromethylfurfural, pyrogallol, pyrocatechol and hydroquinone. Pyrocatechol (oral administration to cats and rats) at 50 mg per kg of body weight produces convulsions followed by paralysis and death within 48 h due to respiratory and circulatory failure (103). Hydroquinone can be readily reduced to a potent bacteriostat, quinone, especially in alkaline solutions (104). Adding small quantities of hydroquinone to fat retards rancidity, but this has not been proven safe as an antioxidant for continued consumption in food (103). Long term feeding of hydroquinone to rats had no effect on

growth or blood constituents (103). Pyrogallol (pyrogallic acid) forms methemoglobin, disrupts red blood cells, and leads to acute nephritis (103).

French workers (92) separated the phenolic acids, ferulic, caffeic, chlorogenic, isochlorogenic, neochlorogenic, 3 ferulylquinic, and p-coumaric acids, from roasted coffee using aqueous isopropyl alcohol and lead salt precipitation. P-coumaric acid is a hydroxycoumarin derivative. Other hydroxycoumarin derivatives are effective anticoagulants, including bishydroxycoumarin (dicumarol, a rat poison with long term anticoagulant action), cyclocumarol (2 or 3 times more potent than dicumarol), and coumadin sodium (Warfarin, another potent rat poison) (103). The effects of caffeic, chlorogenic, isochlorogenic, and neochlorogenic acids will be discussed in the xanthine section.

In 1971 the following sterols were reported in coffee beans by a gas chromatograph-mass spectroscopy: cycloartenal, obtusifoliol, citrostadienol, 24-methylenelophenol, stigmasterol, sitosterol, campesterol, stigmastanol, and campestanol (83). Sitosterols are natural plant sterols which enhance fecal cholesterol excretion and thus decrease its blood levels (103). German investigators (62) in the same year identified six humic acid type compounds in roasted coffee, and phenol carboxylic acids, 3,5-dihydroxybenzoic caffeic, 3-hydroxybenzoic, 4-hydroxybenzoic, ferulic, and vanillic acids. In 1974 other German workers (72) found 0.1% atractyligenin (a glucoside) in roasted coffee beans. Levi, Trenk, and Mohn (67) reported the occurrence of ochratoxin A in green coffee beans, approximately 80% of which is destroyed in roasting.

Changes in the Chemical Composition of Coffee Beans During Roasting

Venezuelan workers (26) reported that as roasting time increases,

the moisture and crude fiber decrease while the nitrogen and ash increase. Czechoslovakian workers (100) found that the caffeine gradually decreases during roasting and that tannins undergo change and volatilize. German researchers (111) demonstrated that roasting partially degrades the polysaccharides. Other investigators (57) showed that roasting elevates the free fatty acids by 13.4% to 15.2% and reduces caffeine by 11.9% to 13.4%. Santos Oliveira (98) noted that insoluble high molecular weight protein represents half of the total nitrogen in roasted coffee beans and soluble protein is partially destroyed by roasting. Wurziger (117) indicated that little change occurs in the ether extract content during roasting. Others (60) have shown no difference in the wax, phytoesterol esters, or hydrocarbons due to roasting. Cerma and Baradel (29) in 1967 found that decaffeination decreased stearic, oleic, linoleic, arachidonic, behenic, and myristic acids while palmitic acid increased.

Coffee Oil Analysis

Khan and Brown (61) reported that 20-25% of the water extracted coffee ground is recovered as oil. However, since the oil contains over 5% unique unsaponifiable material, they believed that the oil was inedible. Martinenghi (78) analyzed the oil obtained from coffee grounds and found the following characteristics:

melting point	-	8-10%
acidity as oleic	-	4.3%
iodine number	-	103.3
saponification number	-	183
total fatty acids	-	85.54%
unsaponifibles	-	9.76%

He found that the unsaponifiable fraction was a high molecular weight, waxy material with an iodine number of 157.2. Khan and Brown (61) reported

the following analytical data of oil extracted from coffee grounds:

iodine number	-	97.6
saponification number	-	180.7
free acids (% oleic)	-	0.27
unsaponifiables	-	5.84%
peroxide number	-	5.2
hydroxyl	-	0.245 m moles/g

The component acids were 32.0% palmitic, 0.9% hexadesenoic, 7.6% stearic, 8.2% oleic, 46.3% linoleic, and 5.0% C_{20} and higher. Heiduschka and Khun (49) obtained the following analysis of oil extracted from roasted coffee beans with petroleum ether:

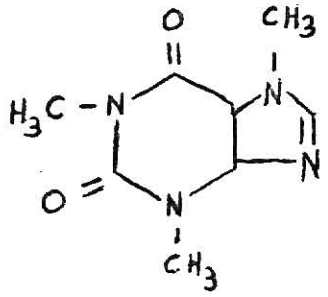
acid number (% oleic)	-	7.93
ester number	-	173.91
iodine number	-	93.48
glycerol	-	9.50
neutral fat	-	93.95

Cerma and Merli (30) extracted ground roasted coffee beans with petroleum ether. Gas chromatographic analysis showed linoleic palmitic oleic stearic arachidic linolenic beheric myristic palmitoleic. They also found that all normal C_{10} to C_{35} paraffins were present in the unsaponifiable portion. Hella (51) reported that the coffee grounds oil contains up to 15% wax.

The antioxidant properties of coffee oil develops during roasting (57). Indian workers (85) found that oil extracted from freshly roasted coffee beans is more stable than from green coffee beans. They found that caffeic acid, quinic acid, roasted coffee powder, and H_2O, ethanol, and $ClCH_2$ extracts of roasted coffee suppressed peroxide formation in the oil.

Coffee Xanthines and Tannins

Xanthines (dioxypurine are weak bases having pK's of 13-14 (55). The most important xanthine found in coffee material is caffeine:



Caffeine (1,3,7-Trimethylxanthine)

Others include theophylline (103), theobromine (103), caffeic acid (92), chlorogenic acid (92), isochlorogenic acid (92), and neochlorogenic acid (92). Methylxanthines have the following distinctive pharmacological actions:

1) Increased irritability of the central nervous system leading to stimulation of the psychic areas (insomnia, etc.), and the medullary centers (respiratory vasomotor, vagus). Large amounts lead to tetanic convulsions and paralysis (103).

2) Increased ease of muscular contraction (heart rate quickens) (103).

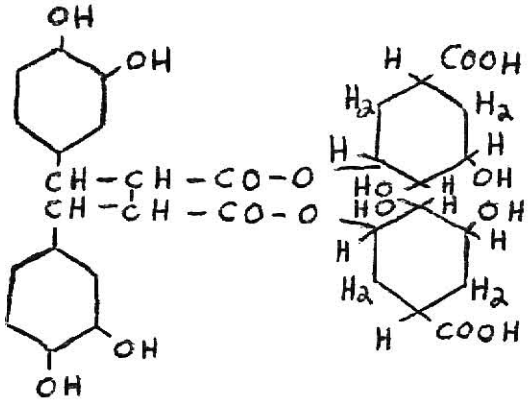
3) Vasodilation (103).

4) Diuresis (103).

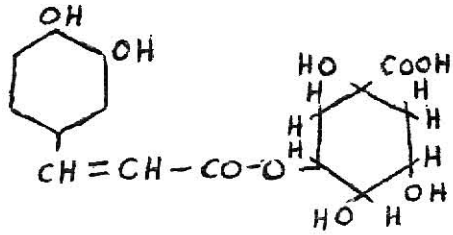
Japanese researchers (56) isolated 4,5-di-O-caffeylquinic acid from coffee beans which on hydrolysis yields caffeic acid and quinic acid. This finding along with others demonstrates that the methyl-xanthines found in coffee material are often associated with other compounds which may modify their effects.

Tannins are amorphous, rarely crystalline substances noted for their astringent taste and ability to form colored solutions and precipitates with iron and other metals (74,88). They also precipitate proteins (74,77,88). Tannins are formed from phenolic substances such as pyrogallol, pyrocatechol, phloroglucinol, and gallic acid and are usually esterified with sugars (77).

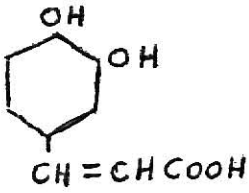
Tannins in coffee (coffetannin) are derived from two molecules of chlorogenic acid by the addition of water (77,88) or by a mixture of several substances which include chlorogenic acid (74). Coffetannins are hydrolyzable andyield caffeic acid, quinic acid, and a residue of unknown constitution (73,88). During roasting, the colorless tannins in coffee are oxidized to colored products (77).



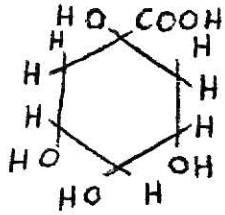
Coffetannin



Chlorogenic acid



Caffeic acid



Quinic acid

Coffetannins do not have the astringent flavor and protein precipitating reactions of other tannin types (103), and are more effective in preserving carotene than other antioxidants tested in a Japanese study (89). An Italian study demonstrated that the addition of 0.5-5% tannins to coffee neutralized the caffeine, pyridine bases, terpenes, and aldehydes (8).

A symposium on the chemistry of vegetable tannins was held in 1956 (115). It was noted that some plants may contain as high as 25% tannin on a dry matter basis. Vegetable tannins modify the effect on plants of bacteria, fungi, and viruses. They inactivate both viruses and extracellular enzymes by insolublizing them and by using various catalytic cations and chelation they create unfavorable conditions for the enzymatic action. Thus, material high in tannins would be undesirable in ruminant rations.

The symposium also reported that in mice the toxicity of hydrolyzable tannins (e.g. coffetannins) is far greater than condensed tannins ($L.D._{50}$ 2 mg/mouse vs 32 mg/mouse respectively, I.M. or S.C.). Since hydrolyzable tannins caused liver damage at physiological pH, they can migrate from the point of application past several proteins (e.g. plasma, muscle, blood) to affect vital organs. Condensed tannins remain fixed by protein. The lethal effect of hydrolyzable tannins is not due to hydrolysis, since gallic acid was non-toxic (up to 32 mg/mouse).

Phenolic components of tannins react with the amino and amido centers of collagen by hydrogen bonding. The symposium proposed that tannins link to collagen by multiple linkage similar to the way carboxypeptidase hydrolyzes peptides and tyrosinase breaks down tyrosine. This phenomenon could explain tannin deactivation of enzymes.

In 1953, workers at the University of Missouri (52) studied the effect of feed tannins on milk production. Using Korean lespedeza they found that the feeding of 18% tannin in the cows' ration did not affect milk yield or composition or the amount consumed. They concluded that the adverse effect of the Korean lespedeza in autumn was due to a decrease in digestibility.

Physiological Effect of Coffee Material and Health Problems

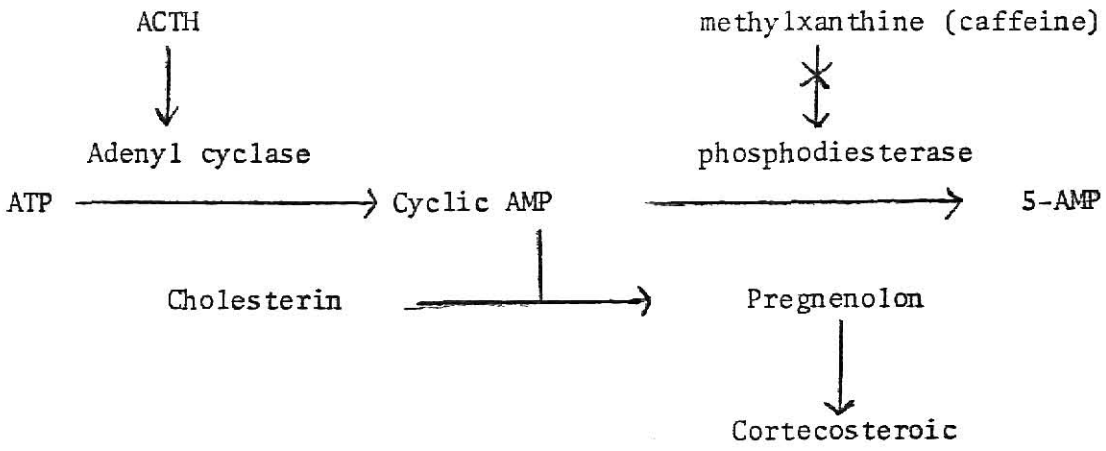
Lehman (65) reported the physiologically important coffee constituents were caffeine, trigonelline, chlorogenic acid, fat, and oil. Czok (37) found the absorption of caffeine from the gastrointestinal tract of rats was complete after 3 to 5 hs and the serum caffeine levels returned to zero except if chlorogenic acid was present after coffee ingestion. Oral doses of chlorogenic acid and trigonelline stimulated gastrointestinal persistalsis. Excitability of the central nervous system was increased by caffeine and chlorogenic acid, coffee oil, and roasting products, but was decreased by trigonelline.

Coffee contains 0.5 to 1.5% caffeine (73). Caffeine affects the central nervous system, muscle activity, and acts as a diuretic. Toxic doses produce convulsions similar to strychnine. Czok (38) noted that the oral caffeine combined with trigonelline, chlorogenic acid, adenine, or theophylline caused greater CNS activity than caffeine alone. Caffeine also enhances alertness, judgment, etc. in humans (73). Of the various methylxanthines of coffee, caffeine penetrated most easily from the blood into the brain and cerebrospinal fluid (110).

Levi (68) found that the ingestion of 75 mg/100 ml H_2O of caffeine increased urinary adrenaline excretion, reflecting a stimulation of adrenocortical activity. German workers (44) noted increased adrenaline and noradrenaline excretion and a small rise in plasma lipids following ingestion of 225 mg of caffeine in humans. They concluded that coffee and caffeine strongly increased sympathoadrenomedullary system, with less pronounced but significant effects on plasma lipid levels. Strubelt and Siegers (106) noted that catecholamines liberated following the intake of coffee or caffeine play a decisive role in the effect of coffee on energy balance and heart rate and

thus may explain the effect of coffee on the autonomic nervous system.

Bellet et al. (15) suggested that caffeine increased the 11-hydroxy-corticoid secretion in dogs by stimulating catecholamine release, thus enhancing corticosteroid secretion. Bieck (19) proposed that caffeine inhibits phosphodiesterase activity which enhances corticosteroid synthesis.



Bellet (13) stated that caffeine elevates free fatty acid and catecholamine concentrations which increase certain lipid fractions particularly triglycerides and cholesterol. The catecholamine stimulation may be on a long term basis following the ingestion of coffee (16).

Several workers have reported an increase in plasma free fatty acids following administration of coffee or caffeine to monogastric animals (15,16,53,82,120). Serum cholesterol, phospholipids, and triglyceride concentrations increased when coffee was fed to rats (2). Decaffeinated coffee did not show this effect. Other workers reported no increases in cholesterol (1,16) or triglycerides (1) in the plasma following coffee ingestion.

Hawkins and Davis (48) studied the plasma free fatty acids and triglycerides in dairy cattle following intraruminal dosing of coffee and caffeine. They noted that 2 h after drenching with 50-100 g coffee there was an increase in free fatty acid and triglyceride concentrations in the

plasma. Ten grams of caffeine citrate showed an increase of 88.6% in plasma free fatty acid levels.

Zeller (119) and Ammon and Estler (4) noted an increase in blood acetone and B-hydroxybutyric acid concentrations and in liver triglycerides in humans following the ingestion of caffeine. Czok and Lang (36) reported an increase in bile production and intestinal motility following the feeding of coffee and decaffeinated coffee to rats. Caffeine in unprocessed coffee accounted for 25% of the bile effect and 33% of the intestinal motility effect.

Stock (105) summarized the influence of methylxanthines on lipolysis. During the enzymatic hydrolysis of triglycerides in adipose tissue, the cleavage products (unesterified fatty acids and glycerol) are released into the blood. Various organs use the fatty acids to meet their energy requirements. Adjustment of the lipolysis rate supplies the organs with a continuous supply of energy since carbohydrate concentrations are limited to rhythmic food intake. The maintenance of caloric homeostasis by unesterified fatty acids is controlled by the sympathetic nervous system and hormones.

Lipolysis is accelerated by the activation of a specific triglyceride lipase via an increase in cyclic AMP formation which is under control of adenylcyclase. Several hormones activate this enzyme including norepinephrin. The cyclic AMP that is formed is metabolized and inactivated by phosphodiesterase. Methylxanthines inhibit this enzyme resulting in an accumulation of cyclic AMP in fat cells and causing an increase in lipolysis.

Czok et al. (39) studied the effects of some phenols and phenolcarboxylic acids (most of which are found in roasted coffee) on bile flow of rats and intestinal motility in mice and guinea pigs. Pyrocatechol,

resorcin, and hydroquinone had greater choleretic effects than the standard. Phenol and pyrogallol were less active and the choleretic activity decreased in the following order: ferulic, caffeic, isochlorogenic, trimethoxycinnamic, neochlorogenic, and quinic acids. Intestinal peristalsis was inhibited by pyrocatechol, pyrogallol, hydroquinone, and ferulic and accelerated by cinnamic, caffeic, and hydrocaffeic acids.

In addition to the central nervous system and lipolysis effects resulting from caffeine and coffee ingestion, the heart is also affected. Caffeine may have a direct effect on cardiac muscles and nerves and an indirect effect through the hypothalamus (69). Lynch (73) believes that caffeine acts directly on muscle cells (not on nerve cells) causing acceleration of the heart beat with a decrease in the diastolic period. He suggested that a long time effect on the heart (due to constant caffeine ingestion) may be unfavorable. The increase in cardiac contractibility influenced by methylxanthines is due to an effect on Ca^{2+} exchange (64). The stimulation properties of caffeine and theobromine results from an inhibition of myocardial phosphodiesterase activity which results in an accumulation of cyclic AMP. Cyclic AMP is important for Ca^{2+} exchange in cardiac muscle.

Caffeine and coffee also exhibit a diuretic effect. This aspect will be discussed later.

In addition to the physiological effects of coffee material in animal systems, various health problems may arise from the feeding of coffee material. Boenicke and Czok (23) describes active antithiamine compounds 3,4-dihydroxycinnamic acid, chlorogenic acid, and pyrocatechins in coffee. In some cases as much as 97-98% thiamine was inactivated. No difference was

noted between unheated, decaffeinated, steamed, and soluble coffees with caffeine added. Leushner and Czok (66) noted a high rate of fetal death, increased malformation in the young, and 72.5% dwarfs in pregnant rats treated intragastrically with caffeine (180 mg/kg). The LD_{50} of caffeine in mice with hereditary obese hyperglycemic syndrome was 80 mg/kg whereas in lean mice it was 400 mg/kg (63). German workers (42) noted the ability of mice to convert their energy reserves to mechanical work was reduced by 150 mg/kg/day when caffeine was given for 6 wk in drinking water. Roffo (96,97) discovered that the tar extracted from coffee beans has strong carcinogenic properties. It is believed that roasting produces the carcinogenic hydrocarbons.

Other studies have shown no adverse effects when feeding coffee and caffeine. German researchers (107) found no liver damage, adipose degeneration, or lipolysis when feeding 40 or 80 mg/100 ml coffee or caffeine solutions to rats for 6 to 7 mo. Other researchers (101) found changes in free fatty acids, free glycerol, triglyceride, blood glucose, and immunoreactive insulin to be too small to be important from a pathogenic standpoint. Using human subjects, others (84) indicated no change in immunoreactive insulin, platelet adhesiveness, fibrinogen, or blood clot lysis with the ingestion of 560 mg caffeine per day.

DIGESTION TRIAL

Introduction

Waste material from the soluble coffee industry is increasingly becoming an environmental and economical problem. In 1971, 651,000 metric tons of green coffee (44% of the world's green coffee production) was imported into the United States (91). Approximately 15% of the green coffee imported was processed as soluble coffee in the same year. Thus, it would be advantageous to find an economical outlet for the waste material from this industry. One use for the coffee grounds waste may be as a source of nutrients in cattle rations.

This study was designed to determine if this material could be used by the ruminant as an inexpensive feedstuff. The coffee grounds then could be converted to meat and milk for human consumption.

Experimental Procedure

A basal ration (Table 1) containing 0% coffee grounds was compared with the rations containing 5,10, or 20% grounds. Proximate analysis showed the coffee grounds to contain 91.1% dry matter, 11.8% crude protein, 23.1% ether extract, 42.5% crude fiber, 0.7% ash, and 13.0% nitrogen-free extract. The coffee grounds replaced equal portions of ground sorghum grain and ground corn in the concentrate fraction of the basal ration. The rations were pelleted (1.9 cm die). The basal ration was slightly below the recommended values for the animals in protein and energy according to the National Research Council Requirements (87) for growth of 200 kg steers (Table 2). This was done to insure that the basal ration would not be oversupplying

Table 1. Composition and Chemical Analyses of Rations.

Ingredients	Percentage of Coffee Grounds			
	0	5	10	20
Coffee grounds	0	50	100	200
Prairie hay	450	450	450	450
Ground sorghum grain	243	218	193	143
Ground corn	243	218	193	143
Molasses	50	50	50	50
Dicalcium phosphate	4	4	4	4
Limestone	3	3	3	3
Salt (trace mineralized)	5	5	5	5
A & D supplement[1]	2	2	2	2
	1000	1000	1000	1000
Analysis				
Dry matter (%)	89.5	89.0	89.0	89.5
Crude protein (%)	7.8	7.7	7.7	8.0
Ether extract (%)	2.5	3.6	5.1	7.0
Crude fiber (%)	13.0	15.4	17.1	20.4
Ash (%)	5.8	5.8	6.1	5.7

[1]A & D supplement: 2,000,000 IU of A and 250,000 IU of D per 908g of supplement. Made up with ground sorghum grain to 908g.

nutrients and thereby permit a true evaluation of the nutrients supplied by the grounds.

National Research Council Requirements for Growth of 200 kg Steers and Nutrients Supplied by Basal Ration at Comparable Dry Matter Intake.

	Intake D.M. (kg)	Protein Total (g)	M.E. (Mcal)	TDN (kg)	Ca (g)	P (g)	Vit.A (IU)	Vit.D (IU)
Requirement	5.0	500	12.3	3.4	18	14	11	1.3
Basal ration	5.0	424.8	10.5	3.5	20.9	17.1	22.0	2.7

A 3 x 3 Latin square design was used to test the digestibility of the rations containing 0, 5, and 10% coffee grounds. The 0 and 20% coffee ground rations were tested in a separate trial using a 2 x 2 Latin square design. Six Holstein steers of approximately equal size and weight (200 kg starting weight) were used. Each animal was allowed a 3 wk adjustment period following each ration change. During the adjustment period each animal was allowed to reach its maximum intake. A feeding level 10% below maximum intake was selected for use in the collection period. This was to insure total ration consumption. A 6 day collection followed the adjustment interval. During the collection period, daily quantities of feces and urine voided were recorded and samples were obtained for proximate analysis. Each animal was also weighed before and after the collection period. Following the established experimental design for the trials, each of the four rations was tested on each of the six steers.

Sample collections were made using metabolism cages. Seventy-five milliliters of 12N hydrochloric acid was added to each urine collection pan to preserve the urine for nitrogen analysis. The total urine volume was measured and a 1% sample was taken and frozen until needed. At the end of the 6 day collection period, the daily urine samples were thawed and pooled.

A 5 ml aliquot of the pooled sample was used for each Kjeldahl nitrogen determination (8).

The daily quantity of feces voided by each animal was weighed and mixed for sampling. A 5% aliquot of the total daily feces was placed on a drying pan and the sample was dried for 48 h in a forced-draft drying oven at 60 C. The weight of the feces samples was determined before and after drying to calculate dry matter values. Each dried daily feces sample was ground in a Wiley mill and the ground samples from each animal were pooled. Proximate and bomb calorimetric analyses were performed on the pooled samples (8). The digestibility coefficients and nitrogen balance values were calculated in the manner described by Church (34,35).

Results and Discussion

Data on the apparent digestibility of nutrients are presented in Table 2. In Trial I coffee grounds significantly ($P < .05$) reduced dry matter digestibility. Although differences between the rations containing 5 and 10% coffee grounds were not significantly different in digestibility of dry matter, they were lower than those of the controls. In Trial II the digestibility of dry matter for the 20% coffee ground ration was lower than that of the control, but this difference was not statistically significant.

Trial I data indicate a difference ($P < .01$) between the digestibility of crude protein in the control and the 10% coffee ground rations. An animal variation was observed in the analysis of the crude protein digestibility coefficients in this trial. Also, the data indicate a decrease in crude protein digestibility with increasing coffee ground concentrations. Trial II data show a highly significant ($P < .005$) decrease in crude protein digestibility with the addition of 20% coffee grounds to the basal ration.

Table 2. Mean Digestibility Coefficients Obtained in Digestibility Trials I and II.

Treatment (% coffee grounds)	Digestibility Coefficient					
	Dry matter	Crude protein	Crude fiber	Ash	Ether Extract	Energy
Trial I -						
0	53.0[a]	45.4[c]	20.1	23.1	58.8[c]	50.1[a]
5	48.0[b]	40.5[cd]	16.4	16.2	60.7[cd]	48.8[a]
10	47.2[b]	37.4[d]	20.6	16.8	66.9[d]	44.8[b]
Trial II -						
0	48.6	40.9[c]	11.9	11.4	37.3[a]	47.5
20	42.1	16.0[d]	29.7	-19.3	61.4[b]	40.1

[ab]Means within each column with different superscripts differ significantly ($P < .05$).

[cd]Means within each column with different superscripts differ significantly ($P < .01$).

Both trials show no significant decrease in the digestibility coefficients for crude fiber although decreases were noted with increasing coffee ground concentrations. Trial II data show an increase (not significant) in the digestibility of crude fiber with the addition of 20% coffee grounds.

Although a decrease in the digestibility coefficients for ash was noted with the addition of coffee grounds to the ration, the difference was not statistically significant.

The energy digestibility values show a significant decrease ($P < .01$) between the basal and 10% coffee ground rations in Trial I. Trial II data also show a decrease in energy digestibility with the addition of 20% coffee grounds to the basal ration, but this difference was not statistically significant.

Data from both trials indicate an increase in the digestibility coefficient of the ether extract portion of the ration with increasing coffee ground concentrations. Trial I data show a significant increase ($P < .05$) in the ether extract digestibility with the addition of 10% coffee grounds and Trial II data show a significant ($P < .05$) increase with the addition of 20% coffee grounds. A period and animal effect was noted in the analysis of the data for ether extract digestibility coefficients in Trial I. The environmental temperature may have had an effect on the ability of some of the animals to utilize the ether extract portion of the ration since the trial was conducted during the early summer months.

It was noted during the feeding of the rations in both trials that ration consumption decreased as coffee ground concentration increased. However, reduction of feed intake following a ration change to a higher coffee ground concentration ration did not occur until the third or fourth

day following the change. This suggests that the palatability of the coffee ground rations was not the cause of the reduced ration intake. The coffee grounds may have had an effect on the rumen fermentation process or appetite regulation process or both.

The results of the digestibility study indicate that the addition of coffee grounds to the basal ration causes a decrease in the digestibility of dry matter, crude protein and energy. No significant difference was observed in the digestibility of crude fiber and ash fractions with the addition of coffee grounds. An increase in the ether extract digestibility was observed with the addition of coffee grounds. According to Lucas and Loosli (71), the apparent digestibility of ether extract fractions in cattle rations increases as the ether extract fraction increases. This may explain the increase in the ether extract digestibility coefficients with increasing concentrations of coffee grounds.

The nitrogen balance data are shown in Table 3. Data in Trial I indicate a significant decrease ($P < .05$) in nitrogen intake between the control and 10% coffee ground rations. A slight decrease (not statistically significant) in nitrogen intake was observed between the control and 5% coffee grounds rations.

These decreases in nitrogen intake were a result of decreased total ration consumption with increasing coffee ground concentrations. Trial I data indicate a significant increase in fecal ($P < .05$) and urinary ($P < .005$) nitrogen loss and decrease in nitrogen retention ($P < .005$) with the addition of 10% coffee grounds to the ration. No significant differences were noted between the 0 and 5% and 5 and 10% coffee ground rations although decreases were observed with increasing coffee ground concentrations.

Table 3. Results of Nitrogen Balance Trials I and II.

Treatment (% coffee grounds)	Nitrogen balance					
	Intake	Fecal	Urinary	Retained	Retained as % of Intake	Retained as % of Absorbed
	(g)	(g)	(g)	(g)		
Trial I -						
0	624.8[c]	340.3	104.0	180.5[c]	28.8	63.4[a]
5	578.2[c]	337.7	114.8	125.7[cd]	21.5	52.1[a]
10	468.7[d]	289.6	120.0	59.1[d]	16.5	35.6[b]
Trial II -						
0	479.2[c]	282.1	77.4	119.7[c]	24.6[c]	59.3[a]
20	278.5[d]	233.3	94.9	-49.7	-18.0[d]	-143.2[b]

[ab]Means within each column with different superscripts differ significantly ($P < .05$).

[cd]Means within each column with different superscripts differ significantly ($P < .005$).

No significant differences were noted between the nitrogen retained as a percentage of the nitrogen intake even though decreases in this value were observed with increasing coffee ground concentrations in Trial I. However, a significant decrease ($P < .05$) was noted between the control and 10% coffee ground rations in the nitrogen retained as a percentage of that absorbed. Decreases (not significant) were noted in this value between 0 and 5% and 5 and 10% coffee ground rations.

Trial II data indicate a highly significant ($P < .005$) decrease in the nitrogen intake, increase in fecal and urinary nitrogen loss, and decrease in the amount of retained nitrogen with the addition of 20% coffee grounds to the basal ration. The nitrogen retained as a percentage of the total nitrogen intake also show a highly significant ($P < .005$) decrease with the addition of 20% coffee grounds. The nitrogen as a percentage of the nitrogen absorbed decreased ($P < 0.05$) with the 20% coffee ground ration.

The results of these two trials indicate that coffee grounds may have a detrimental effect on the protein status of the ruminant. Concentrations of coffee grounds of 20% of higher may cause severe protein imbalance since the animals in Trial II exhibited negative nitrogen balance with the 20% coffee ground ration.

Data in Table 4 show the weight gains and urinary outputs obtained in Trials I and II. The data indicate no significant difference between the weight gains among the animals in Trials I and II. There were perhaps too few animals involved in the trial to show any significant difference in weight gain. However, the animals did show a decrease in body weight gain with increasing coffee ground concentrations. Trial II data indicate the same results.

Table 4. Mean Steer Weights and Urinary Outputs of Digestibility Trials I and II.

Treatment (% coffee grounds)	Starting wt.	Ending wt.	Gain	Urinary output
	(kg)			
Trial I -				
0	278.0^{cd}	282.4^{e}	3.6	20.28^{c}
5	283.6^{c}	283.9^{e}	0.3	32.62^{d}
10	269.1^{d}	265.8^{f}	-3.4	73.26^{d}
Trial II -				
0	314.7	313.5^{a}	-1.3	19.73^{a}
20	291.7	284.0^{b}	-7.8	88.73^{b}

^{ab}Means within each column with different superscripts differ significantly ($P < .05$).

^{cd}Means within each column with different superscripts differ significantly ($P < .01$).

^{ef}Means within each column with different superscripts differ significantly ($P < .005$).

Both trials demonstrate a significant ($P < 0.5$) increase in urinary output with increasing coffee ground concentrations. A significant increase ($P < .05$) in urine output was noted in Trial I between the control and 10% coffee ground rations. Trial II data also show a significant increase ($P < .05$) in urinary output with the addition of coffee grounds to the 20% concentration.

The results of the two trials show that the body weight gains of steers decrease with increasing coffee ground concentrations. Also, the trials indicate that coffee grounds have a diuretic effect on cattle.

DIURESIS STUDY

Introduction

An observation that resulted from the digestibility trials with steers was that coffee grounds have a diuretic effect. The following study was designed to investigate the diuretic effect of coffee grounds.

Experimental Procedure

Six Holstein steers of approximately equal size, age, and weight were selected for the single reversal experiment. The basal ration used in the digestibility trials was used in this trial. The test ration contained 15% coffee grounds. The coffee grounds replaced equal portions of the ground corn and ground sorghum grains in the basal ration. The steers were allowed a 2 wk adjustment period following each ration change followed by a 5 day collection period.

During the collection period, daily feed and water consumption were recorded. Water meters were attached to the water cups on the metabolism crates. Daily urinary output was measured and samples were obtained for urinalysis. Urine samples were obtained by cementing a rubber funnel made from a respirometer pouch to the belly of the steer and covering the sheath of the penis. A tube, attached to the bottom of the funnel, carried the urine to a plastic collection container secured beneath the metabolism crate.

Microscopic examination of the daily urine samples was made (46,99, 114). Robert's albumin ring test (90) and a test for tannins (74) in urine were performed on daily urine samples. Specific gravity and total solids determination were made. Specific gravity data were obtained with a pycnometer. Kjeldahl nitrogen (8) values were determined on each sample. Sodium,

potassium, and calcium concentrations were obtained by flame emission and atomic absorption spectrophotometric techniques.

Caffeine analysis was made of the coffee grounds using the Mannelli (75) method. Caffeine determinations were also made on the defatted, hot water extracted, defatted-hot water extracted, and defatted-0.2% NaOH extracted coffee grounds. Coffee grounds were defatted using a 24 h extraction with petroleum ether in a modified Soxhlet apparatus. The defatted grounds were air dried and placed in a 60 C oven to remove traces of solvent. A portion of the defatted grounds was placed in boiling water for 1 h, rinsed in hot water, and allowed to air dry. Untreated coffee grounds were also extracted by the hot water treatment without the ether extraction. Another portion of the defatted grounds was extracted for 1 h with 0.2% NaOH, rinsed in cold water, and allowed to air dry. A Bechman DU spectrophotometer was used for the caffeine analysis.

Results

The results of the diuresis study are shown in Table 5. A significant ($P < .005$) decrease in feed intake occurred with the addition of 15% coffee grounds to the control ration. There were no significant differences between the rations with respect to water consumption. A highly significant ($P < .005$) increase in urinary output was noted with the 15% coffee ground ration. Urinary solids and minerals decreased significantly ($P < .005$) with the 15% coffee grounds concentration. There was a significant increase in urinary N ($P < .01$) and Na ($P < .05$) losses with the 15% coffee grounds ration.

The urinary albumin screening test showed no difference between the two rations. Both rations resulted in an occasional positive albumin test with the majority being negative. The majority of the positive urine tannin

Table 5. Effect of 0 or 15% Coffee Ground Concentrations in the Ration on Urine Production and Composition.

Item (daily basis)	Percentage of coffee grounds 0%	15%
Feed intake (kg)	3.49	2.47[c]
H_2O intake (l)	8.67	8.90[NS]
Urine output (l)	1.08	3.64[c]
Urinary N (g)	4.83	7.19[b]
Urinary N (%)	0.47	0.21
Urinary Na (g)	0.77	2.10[a]
Urinary Na (%)	0.33	0.09
Urinary K (g)	1.47	1.97[NS]
Urinary K (%)	0.58	0.06[a]
Urinary Ca (%)[1]	-	-
Urinary solids (%)	6.09	1.63[c]
Urinary minerals (%)	2.34	0.45[c]
Urinary albumin	±	±
Urinary tannin	±	-
Urethral and/or bladder cells	-	+
Renal epithelial cells	-	+

NS - not significantly different.
a - significantly different ($P < .05$).
b - significantly different ($P < .01$).
c - significantly different ($P < .005$).
1 - Ca concentrations are below detection limits.

screening test resulted with samples from animals receiving the control ration. However, too few were found to be significant.

Microscopic examinations showed urethral or bladder cells and renal epithelial cells in the urine of the steers on the 15% coffee grounds ration. Generally, the number of the three cell types increased during the last days of the collection period. None of these cell types was observed in the urine of the animals on the control ration. The cells were found only in the urine of steers on the coffee grounds ration.

The results of the caffeine analysis are shown in Table 6. Untreated coffee grounds contain approximately 0.13% caffeine. The treated coffee grounds contain similar quantities of caffeine.

Discussion and Conclusion

The diuresis study supports the findings of the digestion trials in that feed consumption decreased and urinary output increased with the addition of coffee grounds to the ration. Although there was a slight increase in water consumption with the addition of coffee grounds to the control ration, there was no significant difference in water consumption between the two rations. However, the water consumed by the animals on the 15% coffee grounds ration was higher than those on the control ration when based on dry matter intake. The dry matter intakes for the coffee grounds and control rations were 2.20 and 3.10 kg/day, respectively. The water intake per dry matter intake per day values for the coffee grounds and control rations were 4.04 and 2.79 kg water/kg dry matter/day, respectively (significant at $P<.05$). According to Church (35) the expected water intakes for steers of the size which consumed the coffee grounds and control rations are 7.70-12.10 and 10.85-17.05 kg water/kg dry matter intake, respectively.

Table 6. Caffeine Analysis of Untreated and Treated Coffee Grounds.

Treatment	Caffeine (%)
Untreated	0.13
Defatted	0.14
Hot-water extracted	0.14
Defatted-hot water extracted	0.15
Defatted-.2% NaOH extracted	0.15

Therefore, the steers consumed less water on the control ration than expected.

Cattle excrete 17-45 ml of urine per kg body weight per day (3). Therefore, the expected urinary outputs for the steers consuming the coffee grounds and control rations are 1844-4882 and 1972-5215 ml/day, respectively. Thus, the steers excreted less urine on the control ration than expected.

Nitrogen excretion via the urine increased with the addition of coffee grounds to the ration. This observation was also noted in the digestion trials. Cattle excrete 40-450 mg N per kg body weight per day (3). Therefore, the expected urinary nitrogen losses for the coffee grounds and control rations are 4.34-47.80 and 4.58-52.18 g/day, respectively. The steers lost N via the urine within the expected ranges, however, those on the coffee grounds ration had the greatest urinary nitrogen losses.

Cattle excrete 0.2-1.1 mEq Na and 0.3-0.6 mEq K per kg body weight per day in the urine (3). The expected losses for the steers used in the trial were 0.50-2.74 g Na and 1.27-2.53 g K per day when fed the coffee grounds ration and 0.53-2.93 g Na and 1.35-2.71 g K per day when fed the control ration. The animals excreted these minerals within the expected ranges. However, the animals receiving the coffee grounds ration had a significantly greater ($P<.05$) loss of urinary Na and a slightly greater (not significantly) loss in K than those receiving the control ration.

Urinary solids and mineral percentages were lower for the coffee grounds ration ($P<.005$) than for the control. This was perhaps due to dilution caused by an increase in water output.

The screening tests for albumin and tannins in the urine showed no significant albuminuria (proteinuria) or urinary tannin problems. These tests were designed to determine if any chemical poisoning resulting from coffee ground ingestion (particularly tannin) was causing renal damage.

Microscopic analysis of the daily urine samples indicated possible urethral, bladder, and/or renal damage due to the ingestion of coffee grounds. The results indicated an increase in the number of cells expelled in the urine as the time on the ration increased. Long term feeding of coffee grounds at a concentration of 15% or higher may result in severe damage to these tissues.

Analysis indicated that the coffee grounds contained 0.13% caffeine. Caffeine is used as a cardiac and respiratory stimulant for cattle with doses varying from 5-10 g caffeine per day (104). The steers consumed an average of 370.5 g of coffee grounds each day or 0.48 g of caffeine. Therefore, they consumed well below the amount of caffeine used as a stimulant.

The caffeine study also indicated that the caffeine in the coffee grounds is in a bound form. Caffeine is readily soluble in water. One gram of caffeine dissolves in 46 ml of water and 1.5 ml of boiling water (104). However, no difference was noted in the caffeine analysis of boiling water (212°F) extracted, coffee grounds and untreated coffee grounds. Other treatments listed showed no differences in caffeine concentrations.

IN VITRO GAS PRODUCTION ANALYSIS

Introduction

el-Shazly and Hungate, 1965 (41) described a technique for measuring the rate of fermentation of rumen microorganisms. This technique proved to be a quick, simple method for estimating the net growth of microorganisms on the assumption that with an excess of substrate the rate of fermentation is proportional to the total number of microbial cells.

A modification of the el-Shazly-Hungate gas production technique was used to test the effect of coffee grounds on rumen microbial fermentation. A modification of the technique by Barr, 1973, (12) which improved the method of estimating gas production was used. This technique was also used to study various extraction processes that may be useful in improving coffee grounds utilization by rumen microorganisms.

Experimental Procedure

Rumen fistulated cattle were used as donor animals for rumen fluid inoculum. The rations fed to the donor cattle are described in Table 7. The rumen fistulas were closed by a cannula and bung containing a hole through which a rubber sampling tube could pass. A strainer was attached to the end of the tube and located in the ventral sac of the rumen. The strainer filtered the rumen fluid inoculum and anchored the tube in the desired position of rumen. A stainless steel syringe was used to obtain rumen fluid inoculum and an insulated container was used to transport the rumen fluid to the laboratory.

The rumen fluid inoculum was filtered through four layers of cheese cloth. The filtered inoculum was kept in an incubator (37 C) during the

Table 7. Grain Ration Fed to Donor Cattle[1]

Ingredient	Amount
	(%)
Grain[2] (1/2 ground sorghum grain and 1/2 ground corn)	74
Soybean meal	10
Starea	9
Dehydrated alfalfa pellets	5
Salt mixture	1
Dicalcium phosphate	1
Total	100

[1]Cattle were fed alfalfa hay as a roughage. The proportion of hay to grain given to each animal was adjusted periodically to maintain a rumen pH of approximately 6.8 (12 h after feeding) for the sample inoculum.

[2]Trials 1 and 2 utilized this mixture of grain whereas Trial 3 used donor animals fed only ground sorghum grain.

preparation of the sample for gas production analysis. Samples were tested using the Barr (12) modification of the el-Shazly-Hungate method. A water bath (39 C) was used to incubate the samples. A four gram sample, 50 ml of filtered rumen fluid, and 100 ml of buffer (41) were mixed in the sample bottle and incubated for 4 h. A water displacement (12) apparatus was used to measure the gas produced at 30 min intervals for 4 h.

The in vitro gas production study was divided into two trials. The first was designed to investigate several extraction processes and their ability to improve coffee grounds utilization by rumen microorganisms. This study involved the use of ground corn as the control substrate and coffee grounds replacing ground corn at concentrations of 0 to 50% in increments of 5%. Nonextracted, defatted, defatted-100 C extracted, and defatted-110 C (15 psi) extracted (autoclaved) coffee grounds were tested.

Trial 2 involved the rat diets tested in a later experiment. The diet composition is presented in the experimental procedure section of the rat feeding trial section.

Results

Trial 1. In vitro gas production showed no significant differences among the various treatments (Table 8). The treated mixtures did not produce more gas than the untreated coffee grounds mixtures.

Data in Table 9 show no significant differences in gas production among the coffee grounds (treated or untreated) mixtures containing 0 to 35% coffee grounds. Mixtures containing 40, 45, and 50% coffee grounds significantly ($P < .05$) reduced gas production.

Trial 2. In vitro gas production of the rat diets showed no significant differences among the means of the various rations (Table 10).

Table 8. *In Vitro* Gas Production Means of Treated and Untreated Coffee Grounds - Ground Corn Mixtures (Trial 1).

Treatment	Gas production[1]
	(% of control)
Untreated coffee grounds	88.7
Defatted coffee grounds	91.1
110 C (15 psi) - defatted grounds	91.2
100 C - defatted grounds	89.1

[1]No significant differences between treatment means.

Table 9. Trial 2 *In Vitro* Gas Production as a Percentage of Control Means (Treated and Untreated Coffee Grounds) of Coffee Ground Concentrations from 0 to 50%.

Coffee grounds	Mean gas production[1]
(%)	(%)
5	94.2^{ab}
10	91.4^{abc}
15	95.1^{a}
20	93.7^{ab}
25	90.8^{bc}
30	91.8^{abc}
35	88.0^{cd}
40	85.0^{d}
45	78.4
50	74.1

[1]Means with different superscripts differ significantly ($P < .05$).

Table 10. Trial 3 Mean In Vitro Gas Production (As a Percentage of the Control) of the Rat Rations.

Coffee grounds rations	Gas production[1]
	(%)
Untreated coffee grounds	105.6
Defatted coffee grounds	99.3
Defatted-water extracted grounds	116.3
Water extracted grounds	107.3
Defatted-0.2% NaOH extracted grounds	112.0
1% coffee oil ration	104.0
3% coffee oil ration	100.3
5% coffee oil ration	97.6

[1]No significant differences among the means.

Discussion and Conclusion

The *in vitro* gas production was designed to determine to what extent coffee grounds may affect rumen fermentation and be used as a tool for studying various coffee grounds treatments. The results indicated that in the *in vitro* system coffee grounds had no effect on microbial cell growth up to a 35% concentration in the control substrate. The detrimental effect at higher concentrations may partially explain the drop in feed intake 2 to 3 days following a change from control to a 10 or 20% coffee grounds ration noted in the digestibility trials. Toxic substance(s) contained in the grounds may require a 2 to 3 day period to reach toxic levels as a result of slow removal from the rumen. Coffee grounds float on top of the liquid surface in the fermentation bottles. Likewise, the coffee grounds may float on the top of the rumen contents. Thus, with a slow removal rate the coffee grounds could build up to levels that would affect rumen fermentation and cause a lowered feed intake.

These results may also partially explain the inhibitory effect of coffee grounds on digestibility. Although the rumen microorganisms utilize coffee grounds up to a 35% concentration in the *in vitro* system, higher concentrations inhibit fermentation. A gradual increase in the coffee grounds content of the rumen digesta may result in enzymatic inhibition or inhibition of other metabolic processes of the microorganisms once a toxic concentration has been reached.

Coffee grounds may contain toxic substance(s) that diffuse into the rumen liquid as the coffee grounds particles break down. With a slow rate of removal and thus an increase in the concentration in the rumen, coffee grounds could reach a concentration where the toxic substance(s) reach their

toxic concentration. Therefore, a simple extraction procedure may be needed to remove the toxic material and improve coffee grounds utilization. Such an extraction procedure should be inexpensive to make the product economical. If the toxic substance(s) is in the oil, then an oil extraction process would be necessary (most coffee processing plants are equipped with hexane oil extractors). Also, a hot water extraction would be another inexpensive extraction procedure if the substance(s) is soluble in hot water. The results, however, indicated no improvement by petroleum ether, hot water, or hot water under pressure extractions of the coffee grounds.

Trial 3 was designed to investigate the utilization of the rat rations (used in a later study) by rumen microorganisms. The rations differed either by the type of treated coffee grounds or the amount of coffee oil replacing the soybean oil. The results showed no differences among the treatments indicating that either some other extraction is required or coffee grounds must be limited as a feedstuff in cattle rations.

RAT FEEDING TRIALS

Introduction

Carew, Alvarez, and Marion (28) reported that the toxic effect of coffee grounds on growing chicks was a depression in growth rate resulting from an effect on appetite regulation. Barr (11) at Kansas State University studied the utilization of coffee grounds by rats. He found that if 25% or greater of the protein was supplied by coffee grounds in a basal wheat ration a marked decrease in feed intake resulted with rapid weight loss. In a second experiment he found that concentrations of coffee grounds protein replacing 6.25% of the basal wheat diet protein resulted in decreased feed intake and growth rate. Carew et al. (28) reported a progressive decline in the growth of chicks fed increasing concentrations of coffee oil meal. A 2.5% concentration caused a significant reduction in weight gain. They also found that concentrations above 10% coffee oil meal were toxic to chicks and 20% coffee oil meal produced an 84% mortality in 4 wk old chicks. Chen (31) added coffee to the diets of rats either twice weekly or daily. He found that the average life span for coffee fed rats was 809±50 and 503±199 days for the twice weekly and daily fed rats, respectively. The study reported here was designed to study the effects of coffee grounds on the growth of rats and to investigate simple extraction procedures which may improve the utilization of coffee grounds.

Experimental Procedure

Trial 1. A preliminary study was designed to investigate the type of ration and coffee grounds concentration to be tested in the rat trial.

Twenty-four weanling rats (12 males and 12 females of the Sprague-Dawley strain from Carworth, Wilmington, Mich.) were distributed in groups of two males and two females each and housed in individual all-wire-screen cages. Three test diets consisting of 15, 30, and 45% coffee grounds were prepared. The basic diets contained casein, wheat starch, soybean oil, cellulose (Alphacel), minerals, and vitamins. Each test diet was compared with a basal diet that was equal in dry matter, crude protein, crude fiber, ether extract, nitrogen-free extract, and ash content. Table 11 shows the composition of the diets. The preliminary feeding trial lasted 14 days. Food was administered ad libitum in meal form and drinking water was available at all times. The rats were weighed once each week.

Trial 2. Fifty-four male weanling rats (Sprague-Dawley strain obtained from Carworth, Wilmington, Mich.) were distributed among nine groups of six rats each. The experimental procedure was similar to that of Trial 1. The feeding trial lasted 28 days.

The composition of the diets are shown in Table 12. A coffee grounds concentration of 22.5% was selected on the basis of the results of the preliminary study. The basal diet contained casein, wheat starch, cellulose (Alphacel), minerals, and vitamins. The untreated and treated (defatted, defatted-hot water extracted, hot water extracted, defatted-0.2% NaOH extracted) coffee grounds comprised 22.5% of the test diets. All diets were adjusted to similar dry matter, crude fiber, ether extract, crude protein, nitrogen-free extract, and ash values.

In three of the test rations coffee oil replaced 20, 60, or 100% of the soybean oil in the basal ration which was equivalent to 1, 3, or 5% coffee oil in the total ration.

Table 11. Diet Compositions and Proximate Analyses (Rat Trial 1).

	Diet 1		Diet 2		Diet 3	
Ingredients (%)	Basal 1	Basal 1 & grounds	Basal 2	Basal 2 & grounds	Basal 3	Basal 3 & grounds
Casein	22.4	20.5	22.4	18.5	22.4	16.6
Coffee grounds	-	15.0	-	30.0	-	45.0
Wheat starch	61.0	58.0	51.2	46.5	40.2	34.4
Soybean oil	5.6	2.0	7.6	0.3	11.6	-
Cellulose[a]	6.5	-	13.9	0.2	21.3	-
Mineral mix[b]	4.5	4.5	4.9	4.5	4.5	4.0
Vitamin mix[c]						
Proximate analyses (%)						
Crude protein	20.4	20.4	20.4	20.3	20.4	20.4
Ether extract	5.7	5.5	7.7	7.3	11.7	11.2
Crude fiber	6.5	6.3	13.9	13.0	21.3	19.1
N-free extract	62.0	63.4	52.3	51.3	41.2	41.1
Ash	5.3	5.2	5.7	5.4	5.3	4.9

a - Cellulose levels adjusted using Alphacel commercial cellulose.

b - Hegsted et al. (49).

c - All diets supplemented with 2.2g/100g diet with commercial vitamin fortification mixture (National Biochemicals Corp., Cleveland, Ohio, 44128).

Table 12. Diet Composition (Rat Trial 2)

Ingredients (%)	Basal	Diets 1-5	6	7	8
Casein	22.0	19.3	22.0	22.0	22.0
Coffee grounds	-	22.5[1]	-	-	-
Wheat starch	59.5	54.4	59.5	59.5	59.5
Soybean oil	5.0	-	4.0	2.0	-
Coffee oil	-	-	1.0	3.0	5.0
Cellulose[2]	9.5	-	9.5	9.5	9.5
Mineral mix[3]	4.0	3.8	4.0	4.0	4.0
Vitamin mix[4]					

1 - Diet 1 contains untreated coffee grounds
Diet 2 contains defatted coffee grounds
Diet 3 contains defatted-hot water extracted grounds
Diet 4 contains hot water extracted grounds
Diet 5 contains defatted-0.2% NaOH extracted grounds

2 - Cellulose levels adjusted using Alphacel commercial cellulose.

3 - Hegsted et al. (49).

4 - All diets supplemented with 2.2g/100g diet with commercial vitamin fortification mixture (National Biochemical Corp., Cleveland, Ohio, 44128).

Results

Trial 1. The results of the preliminary study are shown in Table 13. Coffee grounds concentrations of 30% and greater were detrimental to the health of the rats and resulted in high mortality within the first week. The rats consuming the 15% coffee grounds ration gained less weight than did the controls. The rats fed the 30 and 45% coffee grounds rations became highly irritable and prior to death exhibited weakness, muscular incoordination, and tetany. The rats fed the diets containing the two highest coffee grounds concentrations consumed very little food.

Trial 2. The data (Table 14) show that rats fed the defatted coffee grounds ration had the greatest weight gains followed by those receiving the control ration. There were no significant differences in the weekly body weight gains between the rats receiving the treated coffee grounds rations and those fed the control ration. There was a significant difference ($P < .05$) between weight gains of rats fed the untreated coffee grounds ration, and those fed the control, defatted, defatted-water extracted, and 1% coffee oil rations. A significant difference ($P < .005$) in weight gains was observed between the mean values of the first 2 and the last 2 wk for rats fed the untreated coffee grounds diet. The rats had a smaller body weight gain when fed this diet during the first half of the trial than during the second half. A significant decrease ($P < .01$) in weight gain of the rats fed the 1% coffee oil diet was observed during the final week of the trial. A reduction ($P < .05$) was also observed in the weekly gains of rats fed the 3% coffee oil ration during the first wk. The 5% coffee oil diet significantly ($P < .005$) decreased gains during the first and last wk of the trial. No significant animal variation was found.

Table 13. Effect of Diets Containing Coffee Grounds on Mean Total Weight Gain of Rats (Trial 1).

Diet	Coffee grounds	Initial Weight		Weight gain		Mortality
		Males	Females	Males	Females	
	(%)	(g)	(g)	(g)	(g)	(%)
Basal 1	0	71.2	63.2	92.8	66.6	0
Diet 1	15	75.7	62.6	78.4	52.8	0
Basal 2	0	78.9	65.2	91.2	58.0	0
Diet 2	30	76.3	65.1	10.6[1]	-	75
Basal 3	0	77.9	68.1	80.8	64.0	0
Diet 3	45	73.0	65.0	-	-	100

1 - One male survived with -0.3 and 13.3 weekly gains for wk one and two, respectively.

Table 14. Effect of Treated or Untreated Coffee Grounds or Coffee Oil on Mean Total Gains in Rats (Trial 2).

Diet	Initial weight (g)	Weight gain[1] (g)
Control	74.4	186.7^{a}
1 (Untreated coffee grounds)	68.1	75.2^{b}
2 (Defatted coffee grounds)	67.5	191.2^{a}
3 (Defatted-H_2O extracted grounds)	68.1	185.6^{a}
4 (H_2O extracted grounds)	68.4	136.0^{ab}
5 (Defatted-0.2% NaOH extracted grounds)	68.1	162.0^{ab}
6 (1% coffee oil)	75.7	174.8^{a}
7 (3% coffee oil)	73.7	157.6^{ab}
8 (5% coffee oil)	70.0	135.2^{ab}

1 - Means with different superscripts differ significantly ($P < .05$).

Discussion and Conclusion

The results of the preliminary study indicate that coffee grounds at a concentration of 30% or greater in the diet are lethal to weanling rats. The rats receiving diets containing 30 and 40% coffee grounds died within the first wk (with one exception). Poor feed consumption was observed with rats fed rations containing these concentrations of coffee grounds. The rats became highly irritable and later developed muscular weakness and incoordination before death. Starvation due to feed refusal was believed to be the cause of death although toxicity may have been a contributing factor as well.

A coffee grounds concentration of 22.5% was selected for use in Trial 2. This value was a compromise between the 15 and 30% coffee grounds concentrations used in Trial 1. Based on the results of the first trial and those of Barr (11), it is believed that a 22.5% coffee grounds concentration is the maximum concentration that rats can tolerate.

No significant differences were found between the means of the weight gains of the control and treated coffee grounds diets. However, the rats consuming the untreated coffee grounds diet gained significantly ($P < .05$) less weight than did those fed control, defatted, defatted-water extracted, and 1% coffee oil diets. This suggests that the oil in the coffee grounds may cause at least part of the toxicity problem. Adding the coffee oil to the control diet decreased (not significantly) body weight gains which corresponded with the effect of increasing the coffee oil concentrations of the diet.

Although the treated coffee grounds diets did not differ significantly in body weight gains from each other, the defatting treatment produced the highest gains followed by the control and the defatted-water extracted treatment. The defatted-0.2% NaOH extraction was next followed by the hot water

extraction alone. Therefore, the rat utilized coffee grounds better when the fraction extractable with ether was removed from the grounds.

SUMMARY

When the concentration of coffee grounds is increased in cattle rations a progressive decline in the intake, dry matter and crude protein digestibility, and energy utilization of the ration results. Nitrogen balance data indicate that coffee grounds concentrations of 20% or higher may have a detrimental effect on the protein status of the ruminant. Coffee grounds also have a diuretic effect on cattle.

Coffee grounds diuresis results in increased urinary N and Na loss. Microscopic examination of steers fed rations containing 15% coffee grounds showed renal, urethral, and bladder irritation.

In vitro gas production analysis showed no significant decrease in rumen microbial fermentation with the addition of 35% or lower coffee grounds concentrations to the substrate. Ether or hot water extractions of coffee grounds did not improve the in vitro gas production.

A rat feeding trial showed an improvement in coffee grounds utilization by using ether extracted grounds. Hot water extraction and a 0.2% NaOH extraction of defatted coffee grounds showed smaller improvements in coffee grounds utilization by the rat than did ether extraction alone.

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EFFECT OF COFFEE GROUNDS ON *IN VITRO* RUMEN FERMENTATION, NUTRIENT DIGESTIBILITY AND DIURESIS *IN CATTLE* AND ISOLATION OF SOME INHIBITORY FRACTIONS USING RATS

by

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Studies have shown that the inclusion of coffee grounds in cattle rations may have detrimental effects on feed intake and body weight gain. Consequently, experiments were conducted to determine the effect of coffee grounds on nutrient digestibility and nitrogen balance in cattle.

Six Holstein steers were used in Latin square designs to compare 0, 5, 10, and 20% coffee grounds which replaced grain in a ration containing equal parts of grain and hay. The coffee grounds analyzed (%) dry matter 91.1, protein 11.8, fat 23.1, fiber 42.5, ash .7, and caffeine .13. Digestibility coefficients for dry matter, crude protein, and energy decreased progressively as coffee grounds concentrations increased. Ether-extract digestibility increased with increasing coffee grounds increments. Nitrogen retained as a percentage of that absorbed was 63.4, 52.1, and 35.6 for 0, 5, and 10% coffee grounds concentrations. Twenty percent coffee grounds concentrations resulted in negative nitrogen balance. Coffee grounds depressed feed intake and increased urinary output. Water consumed by the animals receiving 15% coffee grounds was greater than ($P < .05$) that of the controls based on dry matter intake.

Coffee grounds diuresis in cattle resulted in increased nitrogen and sodium urinary losses. Microscopic examination of the urine of steers fed 15% coffee grounds showed renal, urethral, and bladder irritation.

Rumen microbial fermentation as measured by in vitro gas production indicated no depression in fermentation with the addition of 35% or lower coffee grounds to the substrate. Coffee grounds floated on top of the liquid surface in the fermentation bottles. Likewise, the coffee grounds may float on the top strata in the rumen. Thus, with a slow removal rate the coffee

grounds could build up to concentrations that would affect rumen fermentation and lower feed intake. Ether and hot water extractions did not improve *in vitro* coffee grounds utilization.

The effect of ether extraction, hot water extraction, and 0.2% NaOH extraction on the nutritive value of coffee grounds was evaluated in rats. The rats consuming the untreated coffee grounds diet showed significantly ($P < .05$) lower weight gains than those fed control, defatted, defatted-water extracted, and 1% coffee oil diets. This suggests that the oil in the coffee grounds may cause at least part of the toxicity problem. Adding the coffee oil to the control diet decreased (not significantly) body weight gains which corresponded with the effect of increasing coffee oil concentrations of the diet.