MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 00891 4305

This is to certify that the

dissertation entitled

The Influence of Dietary Antimicrobials on Intestinal Fermentation and Mucosal Morphology, Enzyme Activity, and the Turnover Rate of the Intestinal Mucosa in Weanling Pigs

presented by

Steven Victor Radecki

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in **Animal Science**

Eldon R. Mills
Major professor

Melvin J. Yokoyama

Date **September 21, 1990**

MSU is an Affirmative Action/Equal Opportunity Institution

O-12771

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.

DATE DUE	DATE DUE	DATE DUE

MSU Is An Affirmative Action/Equal Opportunity Institution
c:\circ\datedue.pm3-p.1

THE INFLUENCE OF DIETARY ANTIMICROBIALS ON INTESTINAL FERMENTATION AND MUCOSAL MORPHOLOGY, ENZYME ACTIVITY, AND THE TURNOVER RATE OF THE INTESTINAL MUCOSA IN WEANLING PIGS.

By

Steven Victor Radecki

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1990

ABSTRACT

THE INFLUENCE OF DIETARY ANTIMICROBIALS ON INTESTINAL FERMENTATION AND MUCOSAL MORPHOLOGY, ENZYME ACTIVITY, AND THE TURNOVER RATE OF THE INTESTINAL MUCOSA IN WEANLING PIGS.

By

Steven Victor Radecki

Three trials were conducted to investigate the interactions between dietary antimicrobials, the gastrointestinal tract microflora, and the intestinal mucosa in pigs.

In trial 1, intestinal contents from the stomach (S), jejunum (J), cecum (C), and two colon sites (upper and lower, UC and LC, respectively) were collected from six weanling pigs. The production of ammonia, volatile fatty acids (VFA), p-cresol, and cadaverine was determined. Bacteria of the S and J produced more ($P < .05$) ammonia and cadaverine than those of the C, UC, or LC, while VFA and p-cresol production was greater in the C, UC, and LC ($P < .05$).

In trial 2, eight weanling pigs were surgically fitted with cannulae in the J and colon. One of four diets were fed (two pigs/treatment): control (no antimicrobials, CO), CO + 250 ppm copper (CU), CO + 55 ppm carbadox (CARB), CO + 110 ppm chlortetracycline (CTC), for 14 days (period 1), followed by the control diet for 10 days (period 2). Treatment diets

were then imposed for 7 days (period 3). CU increased urea concentrations in the J on days 4, 7, and 9 of period 1 ($P < .05$). CU, CTC and CTC increased ($P < .01$) urea concentrations in the J on day 14 of period 1. Urea concentrations were not affected by treatments in the colon ($P > .10$). Treatments had no affect on ammonia, VFA, or number of anaerobic bacteria in either J or colon contents or during periods 2 and 3.

In trial 3, twenty-four weanling pigs were assigned to one of four pens, and fed either a CO diet, or CO + 250 ppm CU, two pens per treatment. After 14 days, tissue samples obtained from the duodenum (D), two jejunal sites (JA and JB), ileum, cecum and colon. The mucosal activity of glucose-6-phosphatase and alkaline phosphatase tended to decrease in the JB ($P < .11$, $P < .08$, respectively) in pigs fed CU as compared to CO. CU decreased the rate of mucosal turnover in the JB ($P < .05$) and JA ($P < .10$). CU also increased the cell generation interval in the JB ($P < .05$). CU had no affect ($P > .10$) on mucosal morphology.

This disseration is dedicated to those whom I love most: my mom and dad, Rita and Jerry Radecki; my brother, Tom; my sisters, Ann and Mary; and my wife and best friend, Ann Donoghue.

ACKNOWLEDGMENTS

Without the assistance and help of numerous people, the completion of this dissertation would not have been possible. The guidance from my graduate committee, including Drs. Andy Thulin, Maurice Bennink, Mel Yokoyama, and Elwyn Miller, was appreciated and helpful. Mel Yokoyama's role as research advisor helped ensure a successful and meaningful dissertation project. The friendship, guidance, and knowledge shared by my advisor, Elywn Miller, is, and will always be, special to me.

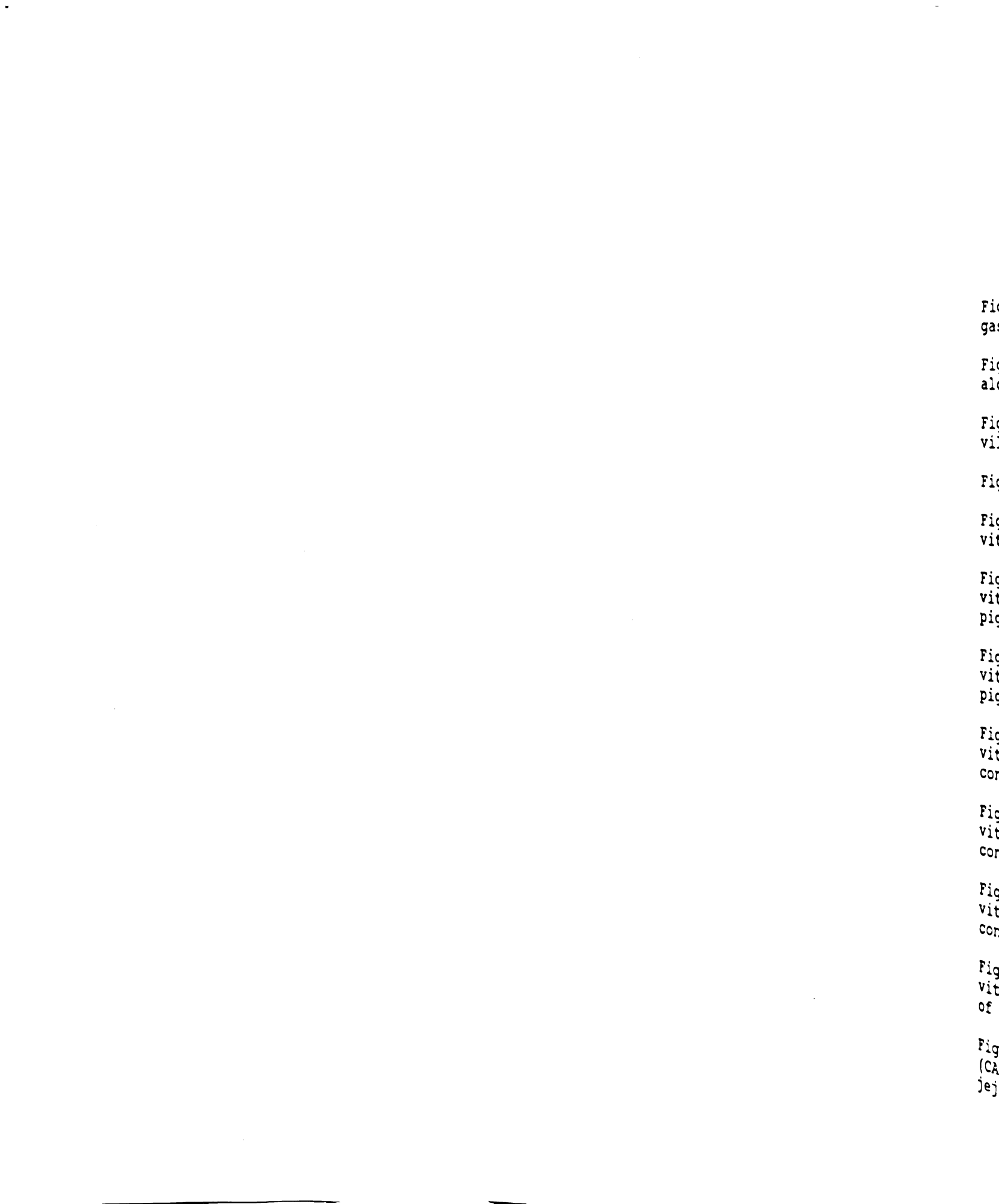
The analytical expertise of Dr. Pau Ku, and surgical skills of Dr. Kent Ames were essential to the completion of this research project.

Friends and colleagues, Bill Weldon, Lee Johnston, Jerry Shurson, and Ormond MacDougald, gave insight to research problems, and support when when the rigors of graduate student life became difficult.

Without the encouragement and unending support from my mom and dad, Rita and Jerry Radecki; my brother, Tom; my sisters, Ann and Mary; and my wife and best friend, Ann Donoghue, the drive for what I have accomplished would not have been instilled in my mind and heart. The love I receive from these special people is very mutual, and I thank them.

TABLE OF CONTENTS

LIST OF TABLES



LIST OF FIGURES

F
e

F.
i
p

F
i
p

Fi

Fi

Fi

Fi

Fi

Fi

ADG
ADM
AP
ATPa
BWT
C
CARB
CDC
CO
CP
CT
CTC
CU
D
d
FID

GE
GIT
GLC
GP
h
HC
HDC
INJ
ip
IU
iv
J
JA,
JB,
LC
LC
LI
MSE
NS
PDV
pHPA
S
SEM
UC
VFA
vol
wk
wt

LIST OF ABBREVIATIONS

ADG	average daily dain
ADM	anaerobic dilution media
AP	alkaline phosphatase
ATPase	adenosine triphosphatase
BWT	body weight
C	cecum
CARB	carbadox
CDC	chenodeoxycholic acid
CO	control
CP	crude protein
CT	column temperature
CTC	chlortetracycline
CU	copper
D	duodenum
d	day
FID	flame ionization detector temperature
GE	gain efficiency
GIT	gastrointestinal tract
GLC	gas liquid chromatography
GP	glucose-6-phosphatase
h	hour
HC	hyocholic acid
HDC	hyodeoxycholic acid
INJ	injection temperature
ip	intraperitoneal
IU	international units
iv	intraveinous
J	jejunum
JA, jejunum A	proximal jejunum
JB, jejunum B	distal jejunum
LC	lower colon
LC	lithocholic acid
LI	labelling index
MSE	mean squre error
NS	not significant
PDV	portal drained viscera
pHPAA	p-hydroxyphenylacetic acid
S	stomach
SEM	standard error of the mean
UC	upper colon
VFA	volatile fatty acid
vol	volume
wk	week
wt	weight

tract

nutri

well-

detri

compo

and v

anima

produ

detri

produ

assoc

tract

and H

compo

the i

pharm

or ar

Visek

hand,

provi

PART I. INTRODUCTION

The fermentation which occurs in the gastrointestinal tract of animals as a result of the microflora acting on nutrients contained in the animal's diet, will influence the well-being of the host. This influence may be either detrimental, as in the production of toxins or toxic compounds, or beneficial, such as the production of vitamins and volatile fatty acids. Promoting fermentation in the animal that results in the formation of beneficial end products, while diminishing the formation of compounds detrimental to the host, may be one method to improve production agriculture.

The detrimental products of fermentation generally associated with the metabolic activity of the intestinal tract microflora include ammonia, amines and p-cresol (Larsen and Hill, 1960; Visek, 1972; Yokoyama et al., 1982). These compounds are capable of altering the metabolic activity of the intestinal mucosa (ammonia, Visek, 1972), can be pharmacologically active (cadaverine, Drasar and Hill, 1976) or are negatively correlated with growth rate (ammonia, Visek, 1972; p-cresol, Yokoyama et al., 1982). On the other hand, the production of VFA in the gastrointestinal tract can provide up to 40% of the maintenance requirements of the

gr

nut

die

gai

195

die

gas

al.,

al.,

in a

1986

1979

anti

anim

demo

gain

impli

the g

gastr

tissu

rapid

(up t

(Yen

parti

rate

growing pig (Friend et al., 1964; Farrell and Johnson, 1970).

Antimicrobials (including antibiotics and other non-nutritive growth-promoting feed additives), when added to the diet at low levels, have been shown to improve the growth and gain efficiency of livestock for over 35 years (Jukes et al., 1950; Carpenter, 1950). Use of these feed additives in the diet of pigs alters the fermentation patterns in the gastrointestinal tract of these animals in vitro (Vervaeke et al., 1979; Dierick et al., 1986a), and in vivo (Dierick et al., 1986b). This altered fermentation includes a decrease in ammonia and cadaverine production (Dierick et al., 1986a,b) and a decrease in VFA formation (Vervaeke et al., 1979). These may be two of the mechanisms by which antimicrobials function to improve the production traits of animals. However, the exact mechanism of action has not been demonstrated.

Germ-free animals show little response in body weight gain to antimicrobials (Coates et al., 1963), further implicating the role of antimicrobials on the microflora of the gastrointestinal tract. The absence of bacteria in the gastrointestinal tract also decreases the rate at which the tissue of this organ turns over (Lesher et al., 1964). The rapid rate of turnover of this tissue demands a large portion (up to 25%) of the animal's maintenance energy requirements (Yen et al., 1988). Reducing the rate of this turnover may partition more energy to body weight gain. If the turnover rate of the intestinal mucosa is lower, fewer cells will be

n

t

re

ga

ep

th

in

bod

in

needed to maintain the integrity of the gastrointestinal tract during a given period of time. Thus the "nutrient requirements" of the gastrointestinal tract may be lower.

Altering the fermentation patterns in the gastrointestinal tract with antimicrobials to a state where epithelial cell life-span is longer and the turnover rate of the mucosa is slower (i.e. decreased ammonia production), may in turn lead to an increased amount of energy available for body weight gain. This hypothesis has not been investigated in pigs.

A.

19
hav
dai
ant
rec
imp
17
con

per
like
(GI
supp
grow
(Whi
et a

anti

PART II: REVIEW OF LITERATURE

A. Introduction.

Since the early 1950's (Jukes et al., 1950; Carpenter, 1950) subtherapeutic levels of antibiotics and antimicrobials have been added to the diets of pigs to increase average daily gain (ADG) and gain efficiency (GE). This response to antimicrobials has continued to be evident, as indicated in a recent review (Zimmerman, 1986, Table 1). ADG and GE are improved with the addition of antimicrobials, an average of 17 and 7%, respectively, over animals consuming diets not containing these feed additives.

The mechanism by which antimicrobials enhance performance traits is not clearly understood. It is very likely that the microflora of the gastrointestinal tract (GIT) is involved, as germ-free animals fed diets supplemented with antimicrobials show no improvement in growth over their conventionally reared counterparts (Whitehair and Thompson, 1956; Coates et al., 1963; Shurson et al., 1990).

Visek (1978) reviewed the mode of growth promotion of antimicrobials, and summarized them as follows:

1. Microorganisms reponsible for mild, unrecognizable infections are suppressed.
2. Absorption of nutrients is enhanced due to a thinner intestinal tract wall.

Ta
th

An

CT
Ca
Co
Vi
Ty

Li
Ch
Ch

[a]Zi
te

[b]Ch
su
li

[c]Fr

Table 1. Pig responses to dietary antimicrobials fed during the starter period - a summary of 3197 pigs[a].

Antimicrobial	Dietary level	% improvement over controls ADG	Feed/gain
CTC-P-S[b]		14.3	5.2
Carbadox	55 ppm	17.1	7.0
Copper[c]	250 ppm	17.2	5.4
Virginiamycin	11 ppm	8.0	3.6
Tylosin-sulfamethazine	44 ppm	32.5	23.1
Lincomycin	22 ppm	4.2	3.6
Chlortetracycline	11 ppm	16.4	1.7
Chlortetracycline	22 ppm	23.9	6.1

[a]Zimmerman, 1986. Pigs weighing less than 18 kg at start of test.

[b]Chlortetracycline : penicillin : sulfamethazine (2:1:2), supplying 110 ppm chlortetracycline, 55 ppm penicillin and 110 ppm sulfamethazine to the diet.

[c]From copper sulfate ($CuSO_4 \cdot 5H_2O$).

It
wc
an
ha
de
gr
ev
ant
198
Gra
res
al.
not

of
inc
dec
ana
ant
the
the
str
chl
the

3. Antimicrobial agents inhibit microbial degradation of nutrients in the gastrointestinal tract.
4. Microbial production of growth depressing toxic compounds is decreased.

Little evidence exists to support the first hypothesis. It is unlikely that pathogenic bacteria present in the GIT would have remained sensitive to the commonly used antimicrobials over the more than 35 years these compounds have been added to diets. These bacteria would have developed a resistance to common antimicrobials, and the growth and gain efficiency responses would no longer be evident. Furthermore, the number of bacteria resistant to antibiotics, in general, is not increasing worldwide (Walton, 1988). The normal, non-pathogenic bacteria of the pig (both Gram positive and Gram negative), however may develop resistance to antibiotics (Christie et al., 1983; Dawson et al., 1984). The practical importance of this resistance is not known.

Antimicrobials have been shown to alter the population of various bacteria in the GIT. In pigs, copper sulfate increased the number of coliforms, molds and yeasts, and decreased the number of lactobacilli, total aerobes, total anaerobes, and streptococci (Hawbaker et al., 1961). The antibiotics oxytetracycline and oleandomycin also increased the coliform, mold, and yeast counts. Oleandomycin lowered the lactobacilli, total anaerobes, total aerobes, and streptococci (Hawbaker et al., 1961). Low levels of chlortetracycline, penicillin, and sulfamethazine prevented the development of the clinical disease and lesions

ass

(Fe

thi

Ful

red

Ant

the

al.,

sugg

Absc

epit

thes

This

matu

Subs

effi

abil

enzy

1977)

fourt

relat

ferme

be th

impro

alter

detr

associated with pigs infected with Salmonella typhisuis (Fenwick and Olander, 1987).

The intestinal wall of germ-free animals is generally thinner than conventionally reared animals (Coates and Fuller, 1977; Shurson et al., 1990), largely due to a reduction in the amount of connective tissue present. Antimicrobials have been shown to decrease the wet weight of the GIT (Braude et al., 1955; Coates et al., 1955; Yen et al., 1985), while having no influence on the length, suggesting a reduction in the thickness of the gut wall. Absorption of nutrients is primarily a function of the epithelial cells of the villus. In the germ-free animal, these cells have a longer life-span (Abrams et al., 1963). This extended life-span may allow these cells to become more mature than cells of a conventionally reared animal. Subsequently, these villi epithelial cells may become more efficient in their role in digestion and absorption, as the ability of epithelial cells to produce certain digestive enzymes increases as the cell matures (Coates and Fuller, 1977).

The remainder of this review will focus on the third and fourth of these theories, as these mechanisms are closely related. A change in the metabolic activity and (or) fermentation pattern of the microflora of the pig's GIT may be the primary route by which antimicrobials function to improve the growth performance of growing swine. These alterations may remove end products of fermentation that are detrimental to the host, either directly (such as a toxic

con

tu

B.

pic

Unc

the

the

pro

as

the

the

ani

com

toxi

High

foun

the

Thes

pyri

(Topp

(Zimm

depr

by V

compound) or indirectly (as in the case of an increase in the turnover rate of the intestinal tissue).

B. Fermentation in the Gastrointestinal Tract (GIT).

Numerous dietary components can be metabolized by the pig's intestinal microflora to various compounds (Table 2). Understanding the function and dynamics of fermentation in the pig's GIT is essential to understanding how changes in these activities may influence the host. Fermentation end products can be beneficial, such as the production of VFA's, as well as detrimental, as in ammonia production. Limiting the amount of "detrimental" fermentation, while increasing the microflora's production of compounds useful to the animal, may be important functions of antimicrobial compounds.

1. Detrimental products of fermentation.

a. Ammonia. Ammonia has long been recognized as a toxic compound in warm-blooded animals (Visek, 1964, 1972). High concentrations of ammonia (10 mM or more), such as those found in the GIT, especially in the colon, are able to alter the metabolic activity of the intestinal epithelial cells. These alterations include an increased synthesis of pyrimidines, and their subsequent incorporation into RNA (Topping and Visek, 1977); an increased rate of DNA synthesis (Zimber and Visek, 1972, Topping and Visek, 1977); a depression of the immune response (Fridlyand, 1959, as sited by Visek, 1978; Dang and Visek, 1968); and an increase in the

Tab
mic

Die

Car

Ami

Table 2. Metabolism of dietary components by the intestinal microflora of the pig: Major end products.

Dietary component		end product
Carbohydrates and fiber	VFAs:	formate
		acetate
		propionate
		isobutyrate
		butyrate
		isovalerate
		valerate
		caproate
	NVFAs:	lactate
		oxaloacetate
		succinate
		fumarate
Amino acids		ammonia, branched chained fatty acids
Histidine		histamine
Arginine		agmatine, putrescine
Lysine		cadaverine
Tyrosine		tyramine, p-cresol
Phenylalanine		phenylethylamine
Tryptophan		skatole, indole

rat

al.

dec

197

syn

Gen

but

bod

tis

dec

amou

leve

furt

inte

lowe

the

cont

GIT

impr

throu

urea

The

vari

cecu

cons

al.,

rate of glycolysis and the tricarboxylic acid cycle (Prior et al., 1974).

The life span of the cells lining the intestinal tract is decreased when exposed to high levels of ammonia (Visek, 1972). Subsequently, a greater number of new cells must be synthesized to maintain the integrity of the mucosa. Generation of this new tissue demands energy and nutrients, but synthesis of this new body tissue is not evidenced as body weight gain. The use of nutrients to maintain this tissue may decrease the efficiency of gain, as well as decrease ADG. The wet weight of the GIT mucosa and the amount of protein synthesis also increases when ammonia levels are high (Topping and Visek, 1977), and may provide further evidence that ammonia increases the proliferation of intestinal epithelial cells. Regardless of the means, lowering the ammonia level in the GIT consistently increased the growth of rats and chicks (Visek, 1972). Thus the control of ammonia production by the microflora in the host's GIT may be one mechanism by which antimicrobials function to improve growth performance in the pig.

Ammonia in the GIT of the pig is produced primarily through the deamination of amino acids and the hydrolysis of urea by the microflora (Visek, 1978; Dierick et al, 1986a). The amount of ammonia that is produced by these two processes varies between the small intestine, cecum and colon. In the cecum and colon, where the concentration of ammonia is considerably higher than in the small intestine (Dierick et al., 1986a), production is primarily a function of amino acid

de

pr

in

de

ar

de

co

aci

pig

car

cul

fer

bac

uni

thes

conc

the

and

majo

prod

virg

ceca

(Her

deamination. Urea hydrolysis and amino acid fermentation produce approximately equal amounts of ammonia in the small intestine of the pig (Dierick et al., 1986a).

The addition of antimicrobials to the diet consistently decreases urea hydrolysis. In rats, the addition of arsanilic acid, chlortetracycline, or penicillin to the diet decreased urea metabolism, as compared to unsupplemented controls (Visek, 1972).

Dierick et al. (1986a), observed a reduction in amino acid degradation and urea hydrolysis from ileal contents of pigs incubated with either virginiamycin, spiramycin, carbadox or copper sulfate (200 ppm Cu) as compared to cultures not containing these antimicrobials. This change in fermentation resulted in less ammonia production. The bacterial activity of duodenal contents was deemed unimportant, as a low ammonia concentration was noted, and these antimicrobials had little influence on the concentration of ammonia, amino acid nitrogen, or urea. Thus the ileum seems to be a major site of amino acid degradation and urea hydrolysis by the microflora, and subsequently a major site where antimicrobials can influence ammonia production.

In young pigs receiving diets containing either virginiamycin or spiramycin, the concentration of ammonia in cecal contents was nearly 50% of that of control-fed pigs (Henderickx and Decuypere, 1972).

Ammonia concentrations in the contents of the small and

large intestine, collected from slaughtered pigs (95 kg), were decreased 17-35% when animals were fed either virginiamycin or spiramycin (Dierick et al., 1986b). From these reports, it appears that antimicrobials can decrease the production of ammonia, and perhaps also influence the availability of amino acids.

Further evidence of the "anti-urease" activity of antimicrobials has been demonstrated in pigs fitted with portal vein catheters. Menten (1988) as well as Yen et al. (1990), found ammonia levels of portal vein blood to be lower in pigs fed either 250 ppm supplemental copper or 55 ppm carbadox, respectively, as compared to unsupplemented pigs.

The number of Streptococcus spp, the major ureolytic bacteria in the pig's GIT (Robinson et al., 1984), decreased in the feces of swine fed either copper sulfate or Aureo SP 250 (a mixture of chlortetracycline, penicillin and sulfamethazine, Varel et al., 1987). Copper sulfate also reduced fecal urease activity. However, fecal ammonia concentations were not different due the addition of antimicrobials to the diet (Varel et al., 1987). This observation (no change in fecal ammonia) is not surprising, as ammonia production in the lower GIT is primarily a function of amino acid deamination, as stated previously (Dierick et al., 1986a).

b. Amines. Amino acids can also be degraded by the microflora to amines. Biogenic amines have various effects on the intestinal mucosa and the host animal as a whole. Many amines have pharmacological activities on the

host (Drasar and Hill, 1974). Putrescine and ethylamine appear to stimulate intestinal epithelial cell proliferation (Ginty et al, 1990; Grant et al., 1990), as well as enhance the intestinal absorption of nutrients (Grant et al., 1990). Mucosal DNA, RNA and protein content are also increased by putrescine (Ginty et al., 1990). Polyamines, in these studies, were considered to be essential growth factors in the development of a functional intestinal mucosa. Grant et al. (1990) suggested that the addition of these polyamines to the diet of very young pigs (neonate) fed high levels of soy protein isolate, improves the absorptive capacity of these animals.

Other amines, such as cadaverine and histamine, can also be toxic or pharmacologically active in mammals (Drasar and Hill, 1974). However, the production of these compounds is likely of little consequence to the animal, as the concentration is low (uM quantities). Perhaps more important is the consequential loss of essential amino acids from the lumen of the GIT, particularly lysine, which may have been absorbed by the host and used for protein synthesis.

Amines are produced mostly by the microflora of the ileum, cecum and colon. Dierick et al. (1986a) found that the antimicrobials virginiamycin, spiramycin, carbadox, and copper sulfate decreased amine production from ileal contents of pigs fermented in vitro. When pigs were fed virginiamycin or spiramycin during the finisher phase (to 95 kg), the total concentration of amines in the contents of the GIT decreased

as compared to unsupplemented control-fed pigs (Dierick et al., 1986b). Pigs supplemented with chlortetracycline also show lower concentrations of amines in the GIT (Larson and Hill, 1960).

c. Phenolic compounds. The production of phenolic compounds may also lead to situations which depress the growth of young animals. Yokoyama et al. (1982) detected the excretion of at least 5 phenolic compounds in the feces and urine of growing swine. These include phenol, 4-methylphenol (p-cresol), skatole, 4-ethylphenol, and indole, of which p-cresol is the major component. These compounds are the end products of GIT anaerobic bacterial degradation of tyrosine and tryptophan in the pig. The effect of chronic exposure to p-cresol is not understood; however, high levels of p-cresol in the urine of pigs was negatively correlated with average daily gain (Yokoyama et al., 1982). Furthermore, the addition of Aureo-SP 250 (providing 110 ppm chlotetracycline, 110 ppm sulfamethazine, and 55 ppm penicillin to the diet) to the diet decreased the amount of p-cresol excreted (on a metabolic weight basis) via the urine and feces over a 30 day period of time.

d. Bile acids. In the pig, primary bile acids produced in the liver are chenodeoxycholic (CDC) and hyocholic (HC) acid. These bile acids can be metabolized to secondary bile acids by the microflora of the pig's intestinal tract, resulting in the production of metabolites that can be toxic to the pig. High concentrations of secondary bile acids can be hepatotoxic and cause inflamation

of the intestinal epithelium (Eyssen et al., 1965; Eyssen, 1973). Bacteria involved in the formation of secondary bile acids include: Clostridium, Lactobacillus, Peptostreptococcus, Bifidobacterium, Fusobacterium, Eubacterium, Streptococcus, and Bacteroides (Hylemon and Stellwag, 1976).

Streptococcus faecium has been shown to attach to the intestinal wall, colonize, and consequently may play a role in depressing the growth of chicks by metabolizing bile acids in the anterior gut, and decreasing nutrient absorption (Cole and Fuller, 1984).

The metabolism of bile acids by the pig's intestinal microflora, CDC to lithocholic acid (LC) and HC to hyodeoxycholic acid (HDC) was decreased by the addition of carbadox to the diet (Tracy and Jensen, 1987). Concomitant with this decrease in CDC and HC degradation is an increase in the rate of hepatic circulation of these two bile acids (Tracy et al., 1986). Bile concentrations of CDC and HC were not influenced by this antimicrobial (Tracy and Jensen, 1987). The activity of the rate limiting enzyme, 7 alpha-hydroxylase, is also decreased when pigs are fed diets containing carbadox, as compared to controls (Tracy and Jensen, 1987). Thus the addition of antimicrobials to the diet may reduce the presence of toxic end products of bile acid degradation, while at the same time improving the recycling of CDC and HC. This improved recovery of bile acids may lead to a decreased requirement for the production

of these compounds (as indicated by a lower activity of 7 alpha-hydroxylase).

In the chick, the potential for bile acid metabolism decreased in ileal homogenates when diets contained subtherapuetic levels of efrotomycin, virginiamycin, penicillin, avoparcin, lincomycin, and bacitracin methylenedisalicylic acid, and corresponded to increased growth rates (Feighner and Dashkevicz, 1987). Antibiotics which did not decrease bile acid metabolism (eg. polymyxin) did not improve the growth rate of chicks.

Neomycin, when added to the in vitro cultures of human fecal flora, also inhibited the bacterial metabolism of bile acids (Hirano et al., 1981).

From these studies, it is evident that subtherapeutic levels of antimicrobials in the diet play a role in bile acid metabolism. This role may be a decrease in the production of toxic secondary bile acids (LC and HDC).

2. Beneficial products of fermentation.

a. Volatile fatty acids (VFA). The production of VFA, primarily acetate, propionate, and butyrate, is likely beneficial to the host, as these compounds can be used to supply up to 44% of the maintenance energy required by the growing pig (Friend et al., 1963a; Farrell and Johnson, 1970; Imoto and Namioka, 1978a; Mason, 1979; Kass et al., 1980; Kennelly et al., 1981). However, this benefit is difficult to measure, as the fermentation processes involved may use carbohydrates otherwise used by the host. These carbohydrates are more efficiently converted to energy by the

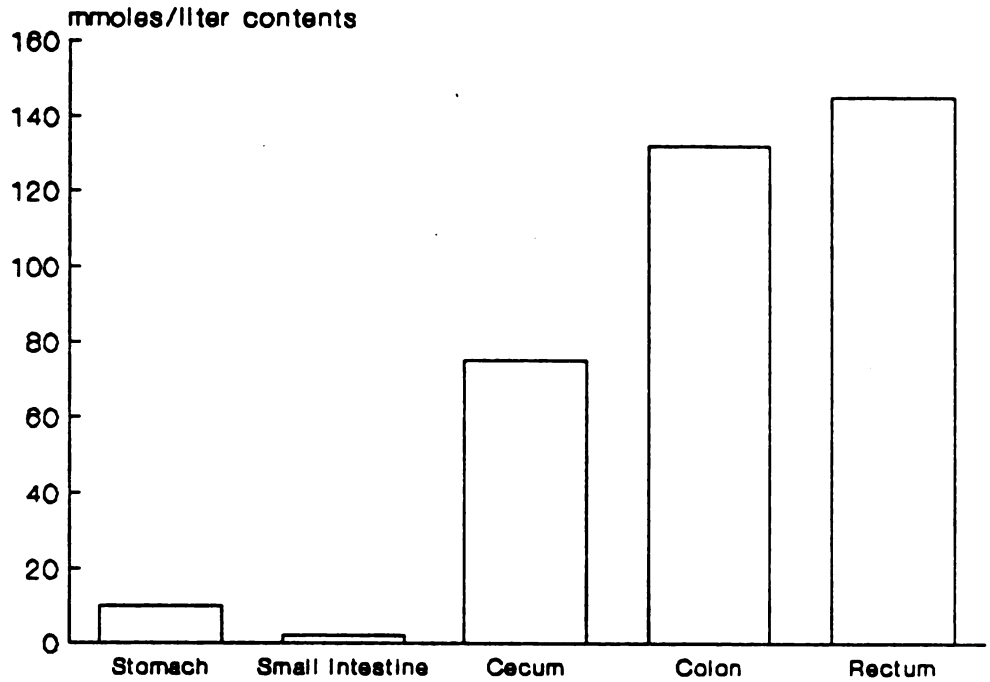
host than VFA would be.

VFA production rates and concentrations vary from one GIT site to the next, with the greatest amount of production occurring in the lower GIT (cecum and colon) as compared to the upper GIT (stomach and small intestine; Argenzio and Southworth, 1974; Imoto and Namioka, 1978a; Kennelly et al., 1981; Radecki et al., 1988a; Robinson et al., 1989; Figure 1). Little information exists on the rate of production of VFAs in the small intestine, especially the duodenum and jejunum. The ratio of acetate:propionate:butyrate at each site also varies (Argenzio and Southworth, 1974; Imoto and Namioka, 1978a; Kennelly et al., 1981; Robinson et al., 1989; Figure 2).

A primary factor contributing to the variation in VFA production rates and concentrations along the GIT is the rate of transit of fermentable substrates. Greater production rates and concentrations are generally associated with the slower passage rate of digesta that are typical of the lower GIT.

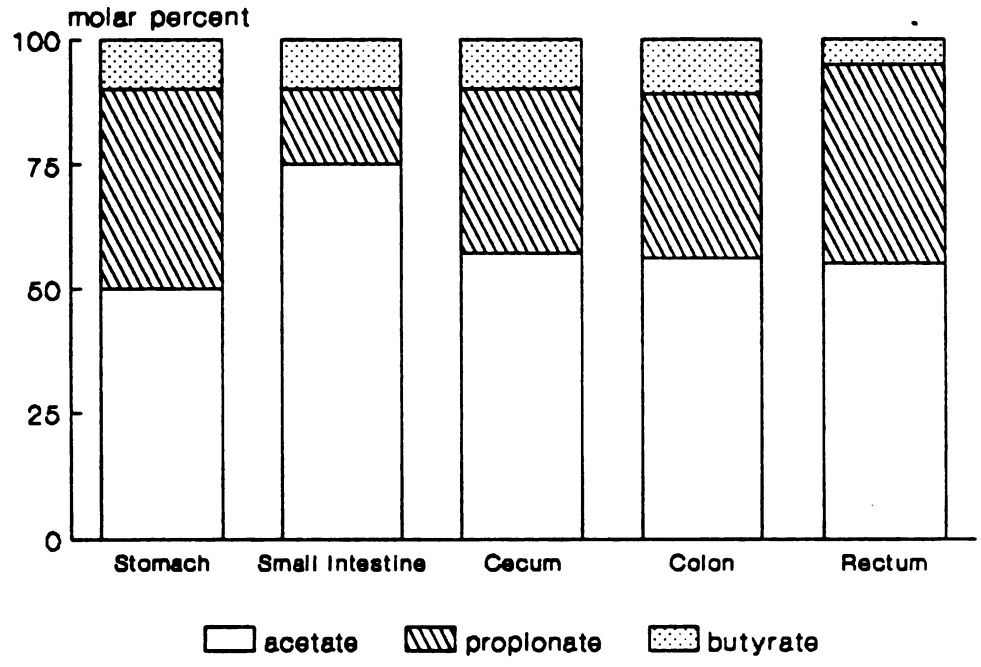
Studies conducted in vitro using ileal contents from cannulated pigs (6-8 weeks of age), indicated that the addition of virginiamycin and spiramycin decreased VFA and lactic acid production, with a concomitant sparing of glucose (Vervaeke et al., 1979). In porcine cecal contents, spiramycin stimulated VFA production, while virginiamycin depressed the production of VFA (Vervaeke et al., 1979; Fernandez et al., 1986). A net sparing of glucose was

Figure 1. Concentration of volatile fatty acids along the gastrointestinal tract of pigs[a].



[a]From Argenzio and Southworth, 1974.

Figure 2. Percentages of acetate, propionate, and butyrate along the gastrointestinal tract of pigs[a].



[a]From Argenzio and Southworth, 1974.

observed for both antibiotics, which corresponded to a higher net energy available for the growth of the pig. These researchers concluded that the addition of these two antibiotics to the diets of young pigs improves the availability of glucose, and subsequently improves the efficiency of gain generally seen when these compounds are added to the diet.

Shurson (1986) observed lower concentrations of VFA in cultures of porcine intestinal contents when these cultures where incubated with 250 ppm copper.

b. Lactic acid. In the nursing pig, lactic acid is the major organic acid produced by the microflora of the stomach (Friend et al., 1963a,b), comprising 80 to 100% of the total organic acids (Cranwell et al., 1976). As the pig is weaned and ages, the amount of lactic acid found in the GIT decreases (Friend et al., 1963a,b; Cranwell et al., 1976), yet lactic acid remains the primary organic acid in the stomach and small intestine (Argenzio and Southworth, 1974; Hedde et al., 1982).

The production of lactate in the stomach and small intestine, from the fermentation of primarily glucose, may represent a small energy loss to pigs, as they can utilize glucose more efficiently than they can lactate (Christie and Cranwell, 1976). Lactic acid can also be further metabolized by the microflora to form VFA (Friend et al., 1963a,b), limiting lactate's use by the pig.

Lactate produced in the lower GIT (cecum and colon) from soluble carbohydrates (especially unabsorbed glucose) of the

digesta, may, on the other hand, be beneficial to the host. Although the amount of lactic acid in the lower GIT is substantially less than that in the stomach and small intestine (Argenzio and Southworth, 1974), lactate can be metabolized to VFA, which can supply energy to the pig. Lactate may also be absorbed in the lower GIT and used directly for energy. It is important to note that lactic acid formation in the lower GIT represents the fermentation of soluble carbohydrates, which if not fermented to organic acids (including lactic acid) would be a net loss of energy to the animal.

Lactic acid production in the ileum can be decreased by the addition of virginiamycin and spiramycin to the diet (Vervaeke et al., 1979). This change in fermentation may be beneficial to the host, as a greater amount of soluble carbohydrates (i.e. glucose) may be available to the animal.

C. Metabolic Activity of Intestinal Epithelial Cells.

The turnover rate of the mucosa of the intestinal tract is the greatest and fastest of any tissue in the mammalian body (LeBlond and Walker, 1956). In rats, it has been estimated that approximately 1% of the animal's body weight is synthesized daily to maintain the integrity of the intestinal mucosa (Leblond, as cited by Visek, 1978). Reducing the turnover rate of the intestinal mucosa may improve the metabolic energy efficiency of the animal, as the synthesis of this tissue is not seen as body weight gain.

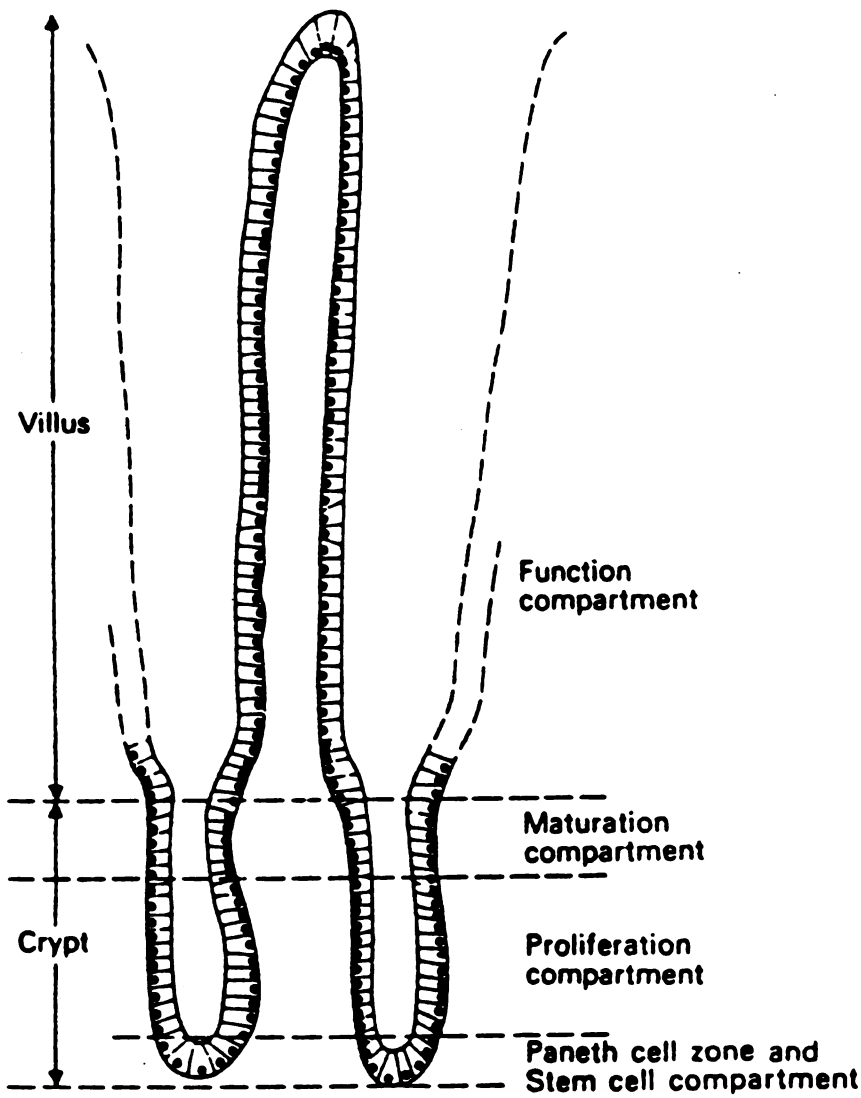
As shown in Figure 3, the intestinal mucosa consists of two macro-structures: the villus and crypt. Cell mitosis and proliferation occurs primarily in the proliferation zone of the crypt (approximately cell positions 1-18) and "older" cells are pushed into the maturation zone (cell positions 19 to the crypt-villus junction; Wright, 1980). Little mitosis occurs in this compartment. Finally, cells enter the functional zone, essentially the villus, where they will become mature epithelial cells and be able to play an active role in the digestion and absorption of nutrients. Cells continue to migrate up the villus until they are extruded from the villus tip. As the epithelial cell migrates, it also matures with regard to its capacity to function. Associated with this functional maturity is an increase in the activity of various enzymes important in digestion (Coates and Fuller, 1977).

1. Turnover of the Intestinal Mucosa.

a. Measuring intestinal tissue turnover rate and cell generation interval: Autoradiography. Since early in the middle 1940's, (Leblonde and Walker, 1946) researchers have used autoradiography as a means to determine intestinal mucosa turnover rate in rats. Since this time, this technique has been used in various species of animals, including humans, chickens, and pigs (Leblond and Walker,1946; Imoundi and Bird, 1956; Lipkin et al., 1966; Moon, 1974), to estimate the turnover rate of the intestinal mucosa.

The cell generation interval (cell cycle time) of

Figure 3. A schematic of the small intestinal crypt and villus (Wright, 1980).

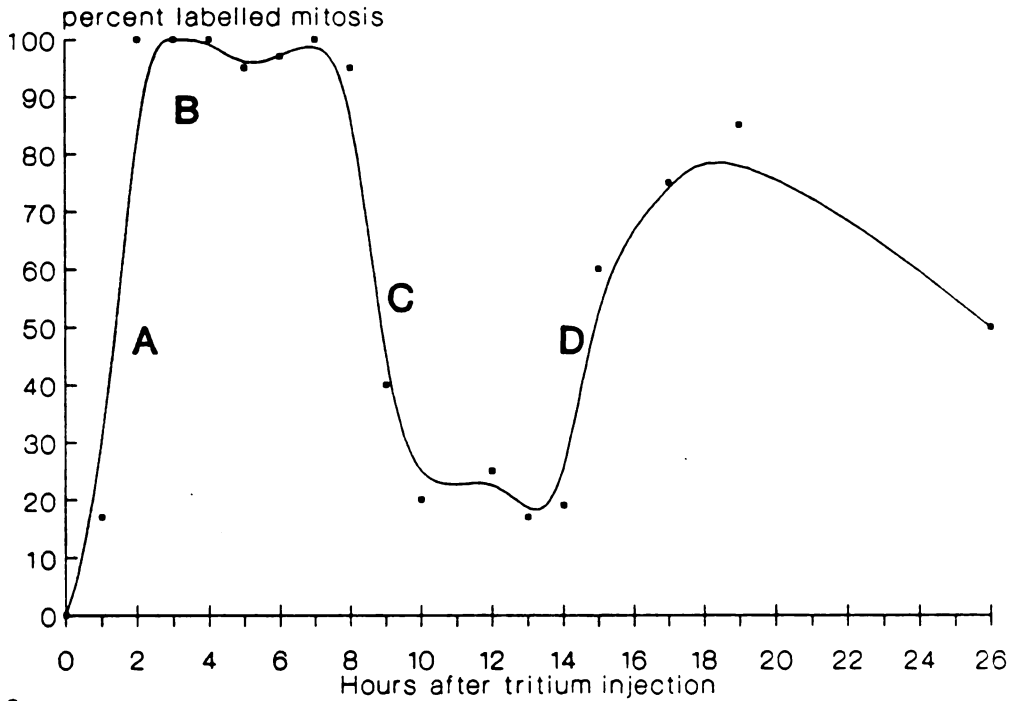


intestinal epithelial cells can also be estimated using autoradiography, as outlined by Lesher et al. (1964) and Cleaver (1967). The cell generation interval of intestinal epithelial cells has not been determined in swine.

This method to estimate tissue turnover rate and cell cycle interval is based on the incorporation of radioactive thymidine (^{3}H or ^{14}C) into the DNA of mitotic cells, which are located solely in the crypt. For complete review of this method, see Cleaver (1967).

The length of the 4 phases of the cell cycle (DNA synthesis, S; the two resting phases, G_1 and G_2; and mitosis, M) can be determined by the autoradiography method described above. Serial sacrifices of animals are used to produce a labelling index curve (the fraction of mitotic cells that are labelled following a pulse dose of radioactive thymidine, Figure 4). The cell generation interval is calculated as the time interval between the 50% labelling level of the first ascending limb (A on Figure 4) and one half the maximum value of that reached by the second ascending limb (D on Figure 4). Mitosis and G_2 are estimated as one value, the point where the labelling index is 100% (B of Figure 4). The DNA synthesis phase (S) is estimated as the time between the 50% labelling intercepts of the ascending and descending limbs (A and C of Figure 4, respectively) of the curve (Lesher et al., 1964; Cleaver, 1967). G_1 is determined by the difference between the cell generation interval and $M + G_2 + S$. The length of these phases in intestinal epithelial tissue from

Figure 4. Percentage labelled mitosis curve[a].



[a]From Tsubouchi, 1983.

various species is shown in Table 3.

The cell generation interval can also be estimated from the length of the DNA synthesis phase (S) and the percent of cells in the crypt that are radioactively labelled at 1 hour post dose. The fraction of the cell population labelled after a brief exposure to the labelled thymidine (LI), is equal to the duration of the DNA synthesis phase divided by the duration of the cell generation interval, as indicated by the following formula:

$$LI = \frac{\text{DNA synthesis phase (hr)}}{\text{cell generation interval}}$$

From this relationship, the length of the cell cycle can be calculated by determing the length of the DNA synthesis phase (S, as determined from Figure 4) and determining the fraction of crypt cells labelled after a short exposure to ^{3}H thymidine (Cleaver, 1967). Manipulating the above formula to the one below:

$$\text{Cell generation interval} = \frac{\text{DNA synthesis phase (hr)}}{LI}$$

allows for the estimation of the cell generation interval.

This relationship is based on the assumption that all cells which become labelled can divide at least once more prior to exiting the crypt.

b. Energy use by the intestinal mucosa. In the cell, the use of available energy, as ATP, is partitioned

Table 3. Duration of intestinal epithelial cell cycle phases (hours)[a].

Animal	G_1	S	G_2	M	GI
Mouse					
conventional	1-9	7.5-8	1.5	1	11.2-19
germ-free	18.5	14.5	3.0	2	38
duodenum	4.5-5.5	5	< 2	-	11.5
forestomach	14	13.5	1-2	1-2	20.5
Rat					
duodenum	<1	8.2	1	-	9.4
jejunum	<1	7.7	1	-	9.0
ileum	<1	7.8	1	1	14.0
Hampster					
jejunum	1-1.75	6.7	4.9	-	13.0
Human					
colon	>10	11-14	1.0	-	24.0
Chicken					
duodenum	5.8	5.0	.7	-	11.5

[a] G_1 and G_2 = the resting phases of the cell cycle. S = the DNA synthesis phase, and M = the length of mitosis. GI represents the length of the cell cycle. From Cleaver, 1967.

into the Na/K dependent ATPase system (which regulates the Na/K balance in the cell) and energy required for the synthesis of other cellular constituents. Oxygen consumption by the cell is a commonly used indicator of cellular energy requirements, as oxygen is required in the synthesis of ATP. Monitoring changes in oxygen uptake by the cell can help to indicate changes in cellular energy requirements.

The oxygen demand of the portal drained visera (PDV, including the GIT, spleen, and pancreas, of which the GIT constitutes the greatest portion by far), has been reported as 24% of the whole body oxygen uptake in pigs (Yen et al., 1988), suggesting that these organs demand nearly one fourth of the animal's maintenance energy needs. Reducing this demand may partition energy from maintenance of this tissue to body weight gain, and subsequently improve gain efficiency.

c. Influence of the microflora on the integrity of the intestinal mucosa. In the germ free animal, villi are generally shorter and narrower, and intestinal crypts are shallower than their conventionally reared counterparts (Abrams et al., 1963; Coates and Fuller, 1977). The greater depth of the proliferative compartment (the crypt) in conventionally reared animals, suggesting a greater rate of cell division, is in agreement with the observations of an increased mitotic index in these animals as compared to their germ-free counterparts (Abrams et al., 1963; Rolls et al.,

1978). The increased rate of cell division in the crypt of animals reared in conventional environments leads to an increase in the migration rate of the epithelial cell up the villus. Consequently, the tissue turnover rate is increased, regardless of the longer villi generally seen in the conventionally reared animal. Thus the turnover rate of the intestinal epithelial tissue is slower in germ-free animals (Abrams et al., 1963; Lesher et al., 1964).

Menten (1988), observed a lower mitotic index of crypt cells throughout the GIT of pigs fed high levels of copper (250 ppm), indicating a decreased production rate of cells, and suggesting a slower turnover rate of the mucosa of the intestinal tract.

Johnson et al. (as cited by Parker and Armstrong, 1987), found longer villi when animals were fed antimicrobials, but also a higher villus:crypt ratio, indicating a lower rate of enterocyte cell migration and tissue turnover.

However, Shurson et al. (1990) suggested that the addition of 250 ppm Cu to the diet of pigs increased the turnover rate of the intestinal mucosa, as indicated by shorter villi and deeper crypts in the small intestine. These researchers concluded that the high level of Cu improved the nutrient availability of the diet, and that this improvement in nutrient availability is a primary factor regulating the turnover rate of this tissue. This observation is contrary to the hypothesis that antimicrobials will produce a situation in the GIT that is similar to that found in the germ-free animal, and will decrease the turnover

rate of the intestinal mucosa.

No reports exist describing the direct influence of dietary antimicrobials on mucosal turnover rates or epithelial cell generation interval as measured by autoradiography in swine. In the rat, the antimicrobial avoparcin, when added to the diet, had no effect on cell turnover rate in the small intestine (Parker et al., 1984). However, this antimicrobial decreased the rate of cell division in the duodenal crypts of sheep (Parker, 1990).

Using oxygen uptake by the PDV as an indication of intestinal cell energy requirements, Yen et al. (1989) observed a decrease in oxygen consumption by these organs (as a percent of the whole body oxygen consumption) when pigs were fed diets containing 55 ppm carbadox. This finding further supports the hypothesis that antimicrobial may function by decreasing the energy requirements of the GIT.

2. Enzyme Activity of the Epithelial Cells.

The activity of various enzymes (alkaline phosphatase, AP; glucose-6-phosphatase, GP; and adenosine triphosphatase, ATPASE) associated with intestinal epithelial cell function is higher in germfree rats than in their conventionally reared counterparts (Kawai and Morotomi, 1978). These three enzymes are important in active transport systems (AP and ATPASE) and glucose transport from the epithelial cell to the portal vein blood (GP). The introduction of various species of Lactobacillus organisms decreased the activity of these enzymes (Kawai and Morotomi, 1978), suggesting a direct role

of bacteria on the activity of the epithelial cell.

The activity of disaccharidases was also greater in germ-free rats as compared to their conventionally reared counterparts (Kawai and Morotomi, 1978). In the pig, Szabo (as cited by Ratcliffe, 1985) observed that germ-free pigs had greater intestinal mucosal activities of peptidases and disaccharidases than did conventionally reared animals.

It has been postulated that antimicrobials function by increasing the activity of various intestinal enzymes. Vonk et al. (1957) found that chlortetracycline increased the amount of protease and amylase in the contents of the small intestine and cecum of growing pigs. The amount of intestinal alkaline phosphatase in the chick increased due to the addition of zinc bacitracin to the diet (Ripley and Brown, 1978). Parker et al. (1984) observed an increase in dipeptidase activity in the small intestine when an antimicrobial was added to the diet of rats.

Decreasing the impact of the microflora on these enzymes may improve the digestive efficiency of the host.

D. Summary.

A plethora of experiments and methods of investigation have been used to determine the exact mechanism of the growth enhancing effect of antimicrobials, yet no definitive answer has been ascertained. Numerous compounds produced by the fermentation process in the GIT may have deleterious effects on the host. Reducing the production of these compounds through the addition of antimicrobials to the diet, may

relieve this burden on the animal, and allow the host to grow faster and more efficiently.

PART III. IN VITRO PRODUCTION RATES OF AMMONIA, 4-METHYLPHENOL (P-CRESOL), CADAVERINE, AND VFA BY MICROFLORA OBTAINED FROM DIFFERENT SITES ALONG THE GASTROINTESTINAL TRACT OF YOUNG PIGS

A. Abstract.

Six pigs were weaned at 28-d of age and placed on a standard fortified corn-soybean meal diet, devoid of antimicrobial compounds. After 2 wk, pigs were sacrificed, and intestinal contents from the stomach (S), jejunum (J), cecum (C), upper colon (UC), and lower colon (LC) were collected. These intestinal contents were diluted (1:10 wt/vol) and inoculated into culture media containing .1% of either urea, p-hydroxyphenylacetic acid (pHPAA), lysine, or glucose, incubated for 24 h at 37^{o} C, then analyzed for ammonia, 4-methylphenol (p-cresol), cadaverine, and VFA (acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate), respectively. Production of ammonia and cadaverine from urea and lysine, respectively, was greatest ($P < .05$) in the S and J as compared to the UC and LC. P-cresol production from pHPAA was only detected in the C, UC and LC, and no differences ($P > .10$) were detected between these sites. In general, the production rate of VFA from glucose was greatest in the lower gastrointestinal tract (C, UC, LC) as compared to the upper gastrointestinal tract (S, J). Total VFA production increased ($P < .05$) from the S to

the LC. From these data we suggest that the ability of the microflora of the S and J to produce ammonia, p-cresol, cadaverine, and VFA from urea, pHPAA, lysine, and glucose, respectively, differs from that of the C, UC, and LC.

B. Introduction.

Fermentation of amino acids and carbohydrates along the gastrointestinal tract (GIT) plays an important role in the health and growth of the young growing pig. End products of fermentation which can influence average daily gain (ADG) and gain efficiency include: ammonia, amines, phenolic compounds, VFA, and non-volatile fatty acids (Visek, 1978; Vervaeke et al., 1979; Yokoyama et al., 1982; Dierick et at., 1986a). These products of microbial metabolism may be produced at different rates and at different sites along the GIT. In growing pigs (45kg), Dierick et al. (1986a) observed that urea hydrolysis is greater in the jejunum than the cecum, but that the overall concentration of ammonia and amines is greater in the lower GIT than the small intestine. Numerous reports have shown differences between carbohydrate fermentation in the small intestine versus the cecum (Argenzio and Southworth, 1975, Imoto and Namioka, 1978a; Vervaeke et al. 1979). From these studies, it is evident that the fermentation patterns along the GIT vary considerably. However, a comprehensive study investigating the production rates of ammonia, amines, phenolic compounds and VFA throughout the GIT of the pig has not been conducted.

Identifying the potential production rates of these microbial metabolic end products, may lead to a better understanding of how the intestinal microflora influences the pig.

In the following study, intestinal contents from young pigs were used to determine ammonia, p-cresol, and cadaverine (end products of urea hydrolysis and amino acid fermentation), as well as acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate (carbohydrate fermentation end products) production rates in vitro along the GIT. Sites along the GIT which were studied included the stomach, jejunum, cecum, and two colon sites. From this information, unique sites of fermentation and metabolites produced by fermentation will be identified that are likely to influence the pig's well-being.

C. Materials and Methods.

1. Animals and Collection of Ingesta.

Six 21-d old crossbred pigs (Yorkshire X Landrace) were weaned and placed in individual 1.5 x 1.5 m stainless steel pens, in an environmentally controlled (29^{o} C) nursery room. Pigs were fed a corn-soybean meal-whey type diet (Table 4) twice daily at a level approximately equal to ad libitum for 14 d. Water was available at all times. On d 14, pigs were sacrificed 2 h post-prandial by a lethal injection of T-61 Euthanasia Solution (Hoechst-Roussel, Somerville, NJ) i.v. Immediately following death (within 10 minutes), the intestinal tract was exteriorized, and 5 sites identified as stomach (S), jejunum (J), cecum (C), upper colon (UC), and

Table 4. Diet composition.

Ingredient, %	
Ground shelled corn	55.35
Soybean meal (44%CP)	25.00
Dried whey	15.00
Calcium carbonate	1.00
Mono-dicalcium phosphate	1.50
Vitamin-mineral premix[a]	.75
Selenium/vitamin E premix[b]	1.00
Salt	.25
L-lysine HCl (78%)	.15
	100.00
Calculated analysis:	
Lysine, %	1.12
Calcium, %	.75
Phosphorus,%	.65

[a]Supplied the following amounts of vitamins and minerals/kg of diet: vitamin A, 3,300 IU; vitamin D, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 18 mg; D-pantothenic acid, 13 mg; choline, 110 mg; vitamin B12, 20 ug; Zn, 75 mg; Mn, 34 mg; Fe, 60 mg; Cu, 10 mg; I, .5 mg.

[b]Supplied .1 mg of Se and 17 IU of vitamin E/kg of diet.

lower colon (LC). The jejunal sample was taken from the middle of the small intestine. The colon was divided into two halves, and a sample was taken from each half and identified in relation to the cecum. A 5 cm section of each site was ligated at two ends, removed and placed in a sterile glass beaker, covered, and placed on ice until inoculations of the media could be made.

The use and handling of animals in this study was approved by the Michigan State University Committee on Animal Use.

2. Media Preparation and Inoculation.

To determine which sites along the gastrointestinal tract are capable of producing the various metabolites, and at what rate, media were prepared which would promote the specific production of the various metabolites. This involved formulating media which contained the immediate precursor of the metabolite in question. In turn, this precursor became the only source of that nutrient in the medium, which allowed for the monitoring of the conversion of the precursor to the metabolite in question.

Growth media were formulated to supply .1% (by wt) of either urea (16.7 mM), p-hydroxyphenylacetic acid (pHPAA, 6.6 mM), lysine (5.5 mM), or glucose (5.6 mM, Table 5). These media provided the immediate precusor for the production of ammonia, p-cresol, cadaverine and VFA, respectively. All media were boiled under CO_2, stoppered, and sterilized. Using anaerobic techniques (Hungate, 1970), the sterile media

Table 5. Composition of the growth media, amount per 100 ml distilled water.

Ingredient	Media[a]			
	1	2	3	4
Glucose, g	.125	.125	.125	0.0
Yeast extract, g	.1	.1	.1	.1
Trypticase peptone, g	0.0	.2	0.0	.2
Hemin, ml	.2	.2	.2	.2
Mineral solution 1[b], ml	7.5	7.5	7.5	7.5
Mineral solution 2[c], ml	0.0	7.5	0.0	7.5
Mineral solution 3[d], ml	7.5	0.0	7.5	0.0
Resazurin, ml	.1	.1	.1	.1
Sodium carbonate (8%), ml	.1	.1	.1	.1
Cysteine sodium sulfide (2.5%), ml	2.0	2.0	2.0	2.0
Substrate[e], ml	1.0	1.0	1.0	1.0

[a]Media 1 = urea, 2 = pHPAA, 3 = lysine, 4 = glucose.

[b]Contained 6 g K_2PHO_4/l distilled water.

[c]Contained 6 g KH_2PO_4, 6 g $(NH_4)_2SO_4$, 12 g NaCl, 2.45 g $MgSO_4 \cdot 7H_2O$, 1.59 g $CaCl \cdot 2H_2O$/l distilled water.

[d]Contained 6 g KH_2PO_4, 12 g NaCl, 2.45 g $MgSO_4 \cdot 7H_2O$, 1.59 g CaCl $2H_2O$/l distilled water.

[e]Substrates added in a 10% stock solution.

Table 6. Composition of dilution media, ml per 100 ml distilled water.

Ingredient	
Mineral solution 1[a]	5.25
Mineral solution 2[a]	5.25
Resazurin	.07
Cysteine sodium sulfite (2.5%)	1.40
Sodium carbonate (8%)	3.50

[a]See Table 5.

were pipetted in quadruplicate into sterile culture tubes (13 x 100 mm) in 4.5 ml quantities, and stoppered with butyl rubber stoppers.

Approximately 2 grams of intestinal contents were weighed, and placed in a sterile culture tube containing 18 ml (1:10 dilution, wt/vol) of a sterile dilution media (Table 6), flushed with CO_2, shaken vigorously, then allowed to settle. During this time, diluted samples were stored at 4^{o} C. After the diluted samples had settled, .5 ml of the liquid layer was pipetted into each of the prepared media (1:10 dilution, vol/vol; 4 tubes per site/pig/media).

Two of the four replicate tubes were incubated for 24 h at 39^{o} C then frozen at -20^{o} C until the cultures could be analyzed. The other two replicate tubes were frozen after inoculation, and after laboratory analysis, served as baseline levels of the metabolite in question.

3. Analytical Methods.

Prior to the analysis of the culture media, cultures were thawed to room temperature, and centrifuged at 15,000 X g for 10 minutes.

a. Ammonia. Ammonia was determined by the procedure described by Chaney and Marbach (1962) using Sigma Kit No. 640 (Sigma Chemical Co., St. Louis, MO.). A 50 ul sample was taken from the culture tubes containing the urea substrate, and diluted with 950 ul of distilled water prior to analysis. In this analysis, ammonia reacts with alkaline hypochlorite and phenol, forming the stable blue indophenol. The absorbance (at 625 nm) obtained is proportional to the

amount of ammonia present. Ammonium sulfate was used to generate a standard curve.

b. p-Cresol. A 4 ml aliquot of the culture tubes containing the pHPAA substrate was pipetted into small glass screw-cap tubes (13 x 100 mm). One ml of an internal standard (p-methoxyphenol, 2 mg/ml ethanol) was then added to each tube. Four ml of ethyl ether were used to extract the p-cresol from the sample. After the addition of the ether, tubes were tightly capped and shaken vigorously. Samples were then allowed to settle. The ether layer, containing the p-cresol and the internal standard, was pipetted into 1.5 ml gas chromatography vials. Two ul of this sample was injected into the gas liquid chromatograph (GLC; Hewlet Packard, Model 5840A, Avondale, PA). A stainless steel column (1.8 m X 2 mm) packed with 21% Carbowax 4000 on WAW-DMCS (60/80 mesh, Anspec Company, Ann Arbor, MI) was used to detect p-cresol. Column conditions were as follows: column temperature (CT): 180^{o} C; injection temperature (INJ): 250^{o} C; flame ionization detector temperature (FID): 250^{o} C; and helium flow rate: 50 ml/minute. p-Cresol was identified by relative retention time, as compared to a standard (p-cresol, 1.18 mg/ml ethanol). The concentration of p-cresol in the sample was calculated by the following formula: $C_{unk}=[(A_{unk}C_k)/A_k]$ * %rec, where C_{unk} is the concentration of the unknown, A_{unk} is the area under the peak of the unknown, C_k is the concentration of the standard, A_k is the area under the peak of the standard, and %rec is the percent recovery of the

internal standard (p-methoxyphenol) in the sample. Percent recovery was determined by injecting 2 ul of a standard containing p-methoxyphenol (2 mg/ml) into the GLC. The area of the peak of the internal standard in the sample was then divided by the area of the peak of the internal standard in the standard.

p-Cresol was detected within one minute of injection of the sample, and the internal standard within 3 minutes. Recovery of the internal standard was between 60 and 70%.

c. Cadaverine. Cadaverine was determined by the method of Staruszkiewicz and Bond (1981), using GLC. One ml of methanol, .5 ml of an internal standard (hexanediamine, 10 ul/ml), and .5 ml 1N HCl was added to the sample in a small screw-cap test tube. Samples were then evaporated to dryness at 50° C under nitrogen. One ml ethyl acetate and 300 ul pentafluoropropionic anhydride (Pierce Chemical Co., Rockford, IL) was added to the residue, the tube was capped tightly, mixed, and heated for 30 min at 50° C. Samples were again evaporated under nitrogen at 50° C, and the subsequent residue dissolved in 1 ml of 30% ethyl acetate in toluene. Samples were then washed by passage through an alumina column. Alumina columns were prepared by packing a 24 cm X 10.5 mm i.d. glass column with activated alumina (packed to 8 cm), and covered with about 1 cm of anhydrous Na_2SO_4. Column effluent was concentrated to approximately 2 ml under nitrogen, and placed in 1.5 ml gas chromatography vials for subsequent GLC analysis. Two ul of sample were injected into a 1.8 m X 2 mm stainless steel column packed with 3% OV-225

on 100-200 mesh Gas Chrom Q (Supelco, Bellefonte, PA). Operating conditions were as follows: CT: 180° C; INJ: 200° C; FID: 200° C; and helium flow rate: 28 ml/min. Cadaverine was identified by its retention time (less than 6 min), as indicated by a cadaverine standard (Sigma Chemical Co.). A standard curve was plotted using 5, 10, and 30 ug/ml cadaverine relative to the ratio of the area of standard peak to the area of the internal standard peak. The concentration of cadaverine was then determined by calculating the ratio of areas (unknown/internal standard) and fitting this to the standard curve.

d. Volatile fatty acids. To determine acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate, culture media were acidified with 25% metaphosphoric acid, mixed, then centrifuged at 15,000 X g for 15 min. The subsequent supernatant was then transfered to 1.5 ml gas chromatography vials, and 2 ul of sample injected into a 1.8 m X 2 mm stainless steel column. A column packed with 10% GP SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW (Supelco) effectively separated the VFA. Column conditions were as follows: CT: 115° C; INJ: 170° C; FID: 175° C; and helium flow rate: 15 ml/min. Under these conditions, the six VFA of interest were eluted in less than 20 min. Peaks were identified through the use of a VFA standard (Supleco), and concentrations determined similarly as in p-cresol, but with no internal standard to calculate the percent recovery.

4. Statistical Analysis.

Data were analyzed by the GLM procedure of SAS (1985), in a completely randomized block design, where the pig served as the block (Gill, 1978). Differences between GIT sites were detected using Tukey's all pair-wise t-test (Gill, 1978).

D. Results.

1. Ammonia.

Prior to incubation (time 0), ammonia levels in the UC and LC were greater ($P < .05$) than levels in the S and J (Figure 5). After 24 h of incubation, however, no differences could be detected between intestinal sites. Consequently, the rate of ammonia production was greatest ($P < .05$) in the upper GIT (S and J) as compared to the lower GIT (UC and LC).

2. Cadaverine.

Initial concentrations of cadaverine were below the detectable range prior to incubation of the cultures (Figure 6). Production of cadaverine was greater ($P < .05$) in the upper GIT (S and J) as compared to the lower GIT (UC and LC).

3. P-cresol.

No p-cresol was detected at any site prior to incubation, therefore only production rates are reported (Figure 7). Furthermore, only the C, UC, and LC produced detectable amounts of this metabolite from pHPAA after 24 h of incubation. Production of this metabolite was not different between these three sites.

Figure 5. Influence of gastrointestinal tract site on in vitro ammonia production from intestinal contents of pigs.

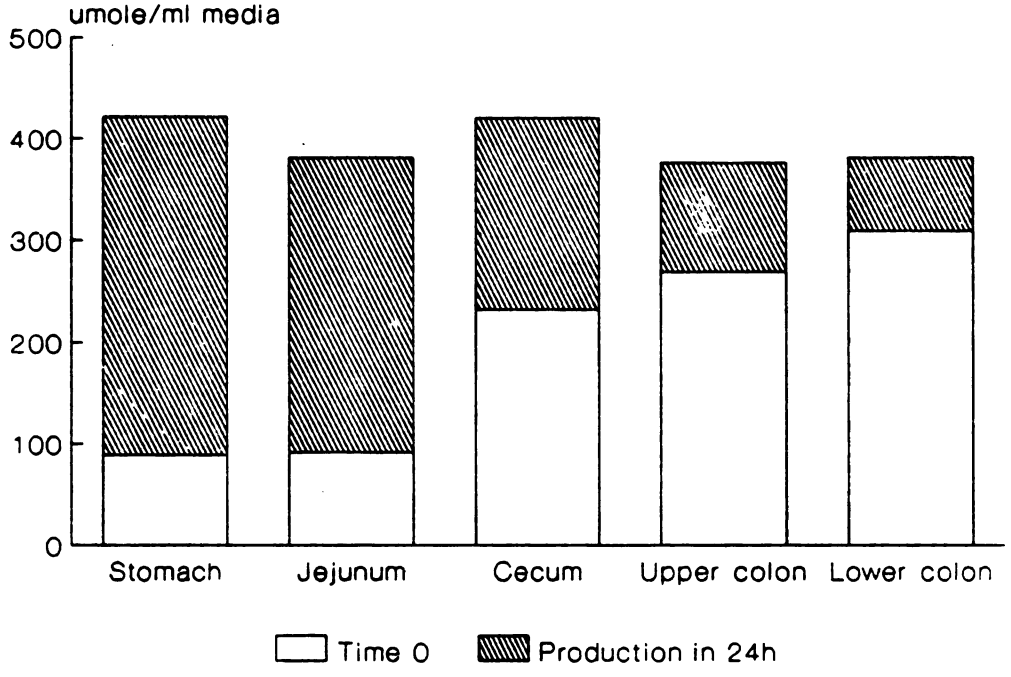
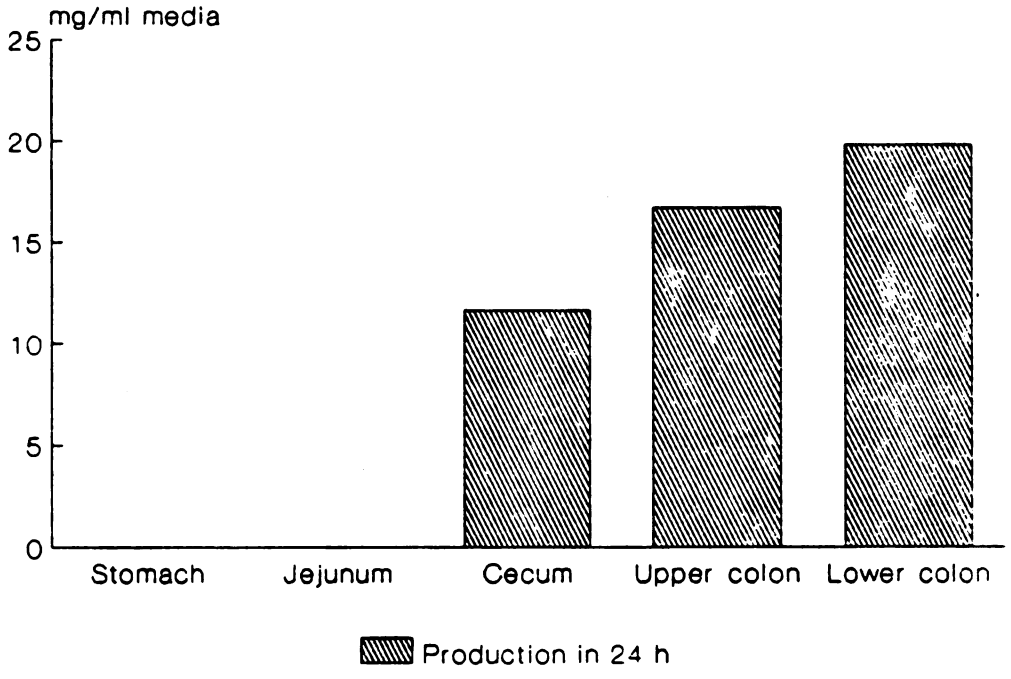


Figure 7. Influence of gastrointestinal tract site on in vitro p-cresol production from intestinal contents of pigs.



4. VFA.

VFA data are presented in Figures 8, 9, 10, and 11. Initial concentrations of valerate (Figure 10) were not different ($P > .10$) across sites, with little to no valerate detected. Acetate (Figure 8) and isovalerate (Figure 10) levels tended to be greater ($P < .06$, $P < .08$, respectively) in the lower intestinal tract prior to incubation. Concentrations of propionate (Figure 8) and butyrate (Figure 9) were greater ($P < .001$, $P < .02$, respectively) at time 0 in the lower GIT. Isobutyrate (Figure 9) was not detected at any site initially, and thus only the production in 24 h is reported. Total VFA concentration prior to incubation tended to increase ($P < .06$, Figure 11) from the S to the LC.

The production rate of acetate per 24 h tended to be greater ($P < .09$) in bacterial cultures isolated from the lower GIT as compared to those the upper GIT. Propionate production was not different ($P > .10$) across sites. Isobutryate, butyrate, isovalerate, valerate ($P < .001$), and total VFA ($P < .05$) production rates were greater from samples collected from the C, UC, and LC than samples collected from the S and J.

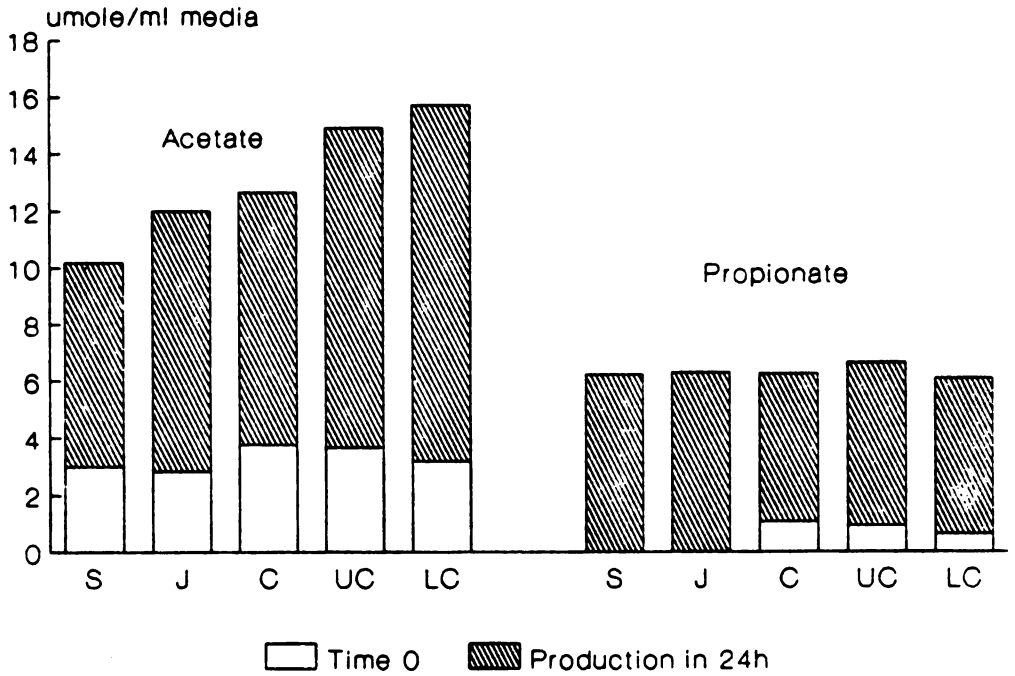
See Appendix 1 for data in tabular form.

E. Discussion.

1. Nitrogen Containing Compounds.

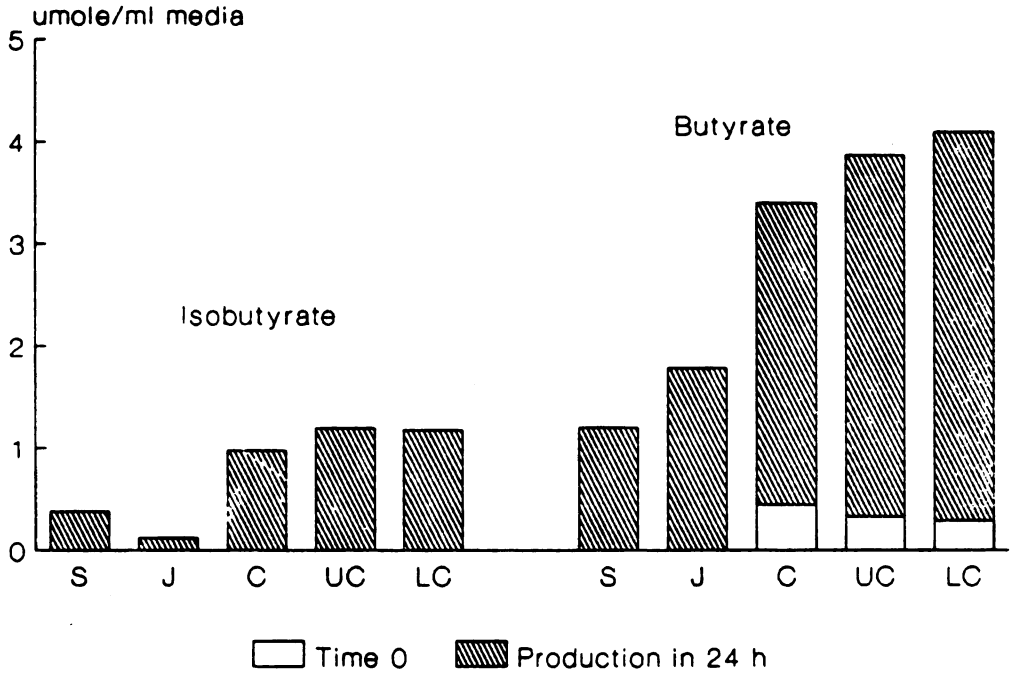
a. Ammonia. Dierick et al. (1986a) concluded that ammonia concentrations of the intestinal contents are higher

Figure 8. Influence of gastrointestinal tract site on in vitro acetate and propionate production from intestinal contents of pigs[a].



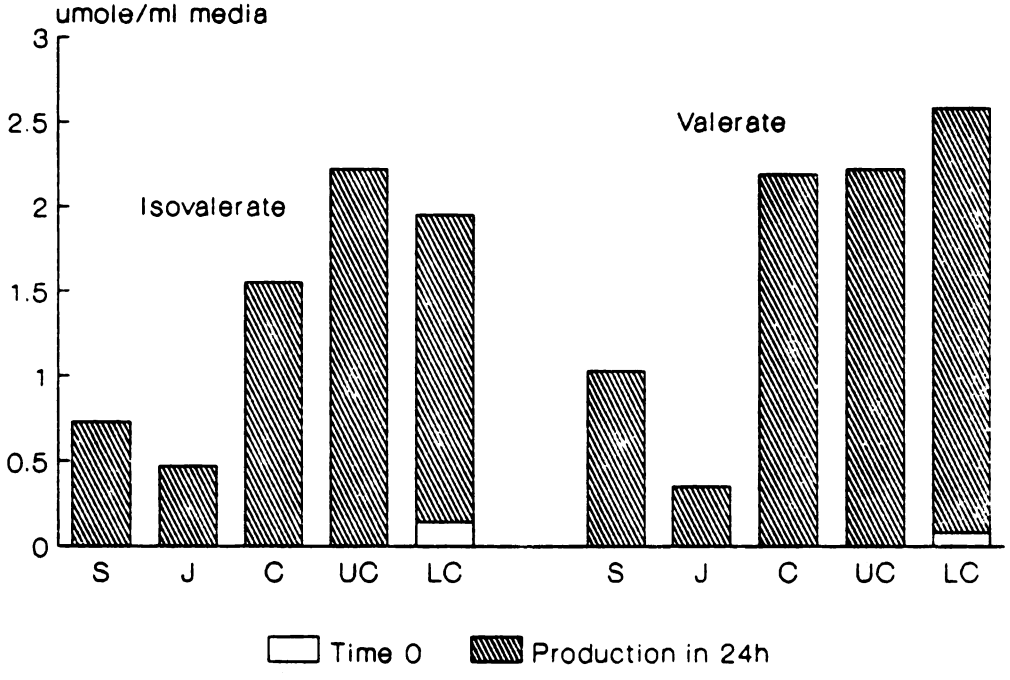
[a]S = stomach, J = jejunum, C = cecum, UC = upper colon, LC = lower colon.

Figure 9. Influence of gastrointestinal tract site on in vitro isobutyrate and butyrate production from intestinal contents of pigs[a].



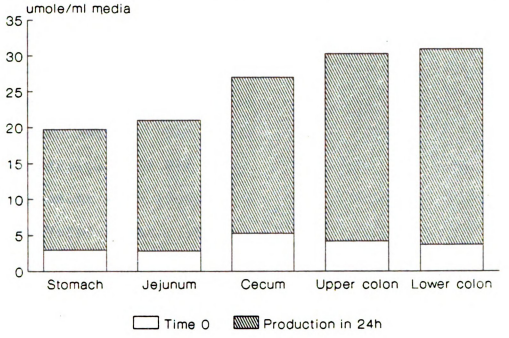
[a]S = stomach, J = jejunum, C = cecum, UC = upper colon, LC = lower colon.

Figure 10. Influence of gastrointestinal tract site on in vitro isovalerate and valerate production from intestinal contents of pigs[a].



[a]S = stomach, J = jejunum, C = cecum, UC = upper colon, LC = lower colon.

Figure 11. Influence of gastrointestinal tract site on in vitro volatile fatty acid production from intestinal contents of pigs.



in the cecum than the small intestine, and that the substrate(s) for the production of this metabolite is likely different between these two sites. Furthermore, ammonia production in the upper GIT is divided between urea hydrolysis and amino acid fermentation and deamination, while in the lower GIT, ammonia is primarily produced from amino acid deamination. The higher rate of ammonia production from urea hydrolysis in the upper GIT seen in the present study is in agreement with findings of other workers, (Dierick et al., 1986a, Varel et al., 1987). Ammonia levels reported here are likely above physiological levels due the level of urea (16.7 mM vs .5 mM in vivo, as estimated from Dierick et al., 1986b) available to the bacteria in our media. The toxic effect of ammonia on cellular metabolism is well documented (Visek, 1979).

b. Cadaverine. Cadaverine appears to be the most abundant amine produced in the GIT (Dierick et al., 1986a). Formation of this compound may indicate a loss of nutrients to the pig, as this metabolite is the decarboxylation product of lysine. Escherichia coli has been identified to be a major bacterium involved in the production of this metabolite from lysine (Cheeseman and Fuller, 1966; Yen et al., 1979). In the current study, it appears that the microflora of the upper GIT have the potential to produce more cadaverine from lysine than the microflora of the lower GIT. The greater availability of amino acids in the small intestine of the live animal lend further support to the theory that this intestinal location may play an important role in the

production of cadaverine.

c. p-Cresol. Research conducted by Yokoyama et al. (1982), indicated that the production of p-cresol by the pig's microflora may be involved in lower growth rates of young pigs. Furthermore, a bacterium was isolated from swine feces that decarboxylates pHPAA to p-cresol (Ward et al., 1987). This compound is readily absorbed by the pig and excreted in the urine (Yokoyama et al., 1982), which may explain the absence of this compound at time 0 in our study. After 24 h of incubation, cultures from the C, UC and LC were the only cultures that produced measurable amounts of p-cresol, suggesting that in nursery pigs, perhaps the only bacterial population capable of producing this metabolite are located in the lower GIT.

From these data it appears that bacterial metabolism of nitrogen containing components, such as urea and amino acids, differs between sites along the GIT.

2. Carbohydrate Metabolism - VFA Production.

The ability of the intestinal microflora of the pig to ferment carbohydrates and produce organic acids is well documented (Friend et al., 1963a; Argenzio and Southworth, 1974; Imoto and Namioka, 1978a; Vervaeke et al., 1979; Kennelly et al., 1981). The total concentration of VFA are greater in the lower GIT of young pigs than in the upper GIT (Argenzio and Southworth, 1974). The results of our study are in agreement with these observations.

The higher rate of fermentation in the lower GIT is not

surprising, given the greater numbers of bacteria present in these locations and the accumulation of fermentable substrates. However, it is interesting to note that when fermentable substrates are available for extended periods of time, the microflora of the upper GIT are capable of producing acetate and propionate at the same rate as the microflora of the lower GIT.

VFA production may benefit the host, as these compounds are readily absorbed by the pig, and may provide an important source of energy for the pig (Argenzio and Southworth, 1974). It has been estimated that up to 44% of the maintenance energy needs of growing pigs may be supplied by VFA's produced by the microflora (Friend et al., 1963a; Farrell and Johnson, 1970). However, the production of VFA is only beneficial to the pig when the bacteria use undigested carbohydrates, or otherwise unused fermentable compounds, to produce VFA.

The importance of the interactions between GIT site, metabolite production, and the well-being of the host is still unclear. A greater understanding and control of these processes, perhaps through the use of antibacterial compounds or other means of modifying fermentation, may enhance growth performance in the pig.

PART IV. INFLUENCE OF DIETARY COPPER, CARBADOX, AND CHLORTETRACYCLINE ON AMMONIA, UREA, AND VFA CONCENTRATIONS, AND ANAEROBIC BACTERIA NUMBERS IN THE JEJUNAL AND COLONIC DIGESTA OF YOUNG PIGS

A. Abstract.

Cannulas were surgically fitted into the colon and jejunum of eight 22-d old nursing pigs to determine the influence of dietary antimicrobials on the intestinal microflora. Treatments included a control diet (CO) containing no antimicrobials, CO + 250 ppm Cu (CU), CO + 55 ppm carbadox (CARB), and CO + 110 ppm chlortetracycline (CTC). Pigs were fed the treatment diets for 14 d (period 1), followed by a 10 d re-adaptation period (period 2) during which time the CO diet was fed. A second 14 d treatment period followed (period 3). During period 1, urea concentrations of jejunal digesta were greater ($P < .05$) on days 4, 7, and 9 when pigs were fed the CU diet as compared to jejunal contents from pigs fed the other three diets. CTC, CARB, or CU increased ($P < .01$) urea concentrations of jejunal contents on day 14 of period 1. Urea concentrations in jejunal digesta were not affected ($P > .10$) by the addition of these antimicrobials to the diet during period 3 . Concentrations of urea in colonic digesta were not affected ($P > .10$) by treatment during any of the experimental periods. Antimicrobials had no effect ($P > .10$) on the number of

anaerobic bacteria, ammonia concentration, or VFA concentration in jejunal or colonic digesta during any of the experimental periods. From these data we conclude that the shift in fermentation due to antimicrobial addition to swine diets is intestinal site dependent with regard to urea hydrolysis. The concentrations of ammonia, VFA, and the numbers of anaerobic bacteria in the intestinal digesta do not seem to be sensitive indicators of the action of antimicrobials on the microflora.

B. Introduction.

The mechanism by which antimicrobials improve the growth rate of young animals is not clearly understood. Dierick et al. (1986a,b) have observed that spiramycin, virginiamycin, carbadox, and copper sulfate may decrease the production of potentially toxic compounds, including ammonia and amines, in the gastrointestinal tract of pigs, both in vitro and in vivo. Spiramycin and virginiamycin may also decrease VFA production by the intestinal microflora of the pig, and consequently increase the availability of carbohydrates to the host (Vervaeke et al., 1979). However, studies investigating the mechanism of action of antimicrobials (including the studies cited above) are often conducted in vitro, and the results are difficult to interpret because numerous physiological variables present in the intestinal tract can not be simulated in vitro. Secretions of digestive enzymes by the animal, absorption of metabolites,

concentration of antimicrobial present in the digesta, and the intestinal site from which the bacteria are isolated, are some of the variables that need to be considered when interpreting these data. Radecki et al. (1988a,b) indicated that the fermentation by the jejunal and colonic microflora differs greatly in regard to ammonia, VFA, p-cresol, and cadaverine production.

The following study was designed to investigate the influence of dietary copper, carbadox, and chlortetracycline on the fermentation processes within the intestinal tract of young pigs. The design of the experiment allowed in vivo physiological processes to interact with fermentation and fermentation end products.

C. Materials and Methods.

1. Animals and Sample Collection.

Eight crossbred pigs from two litters were weaned at 22 d of age (approximately 6 kg BW) and surgically fitted with Silastic™ t-cannulas in the jejunum and colon at the Michigan State University Large Animal Clinical Center. Pigs were anesthetized for surgery with ketamine and stresnil, i.v., and maintained on halothane. After surgery, pigs were placed in individual 1 x 1 m stainless steel pens, with heat lamps to provide additional warmth. Within 12 h post-surgery, all animals seemed to be fully recovered from the anesthesia. No therapeutic injections of antibiotics were administered during the recovery period. Pigs were allowed to recover from surgery for one week before the commencement

of the experiment. The diet fed during this period did not contain antimicrobial compounds (Control, CO, Table 7). This diet, as well as water, were available ad libitum. Animals were weighed 3 times each week, and ADG was calculated. The use and handling of animals in this study was approved by the Michigan State University Committee on Animal Use.

The addition of 250 ppm Cu (from $CuSO_4 \cdot 5H_2O$, CU), 55 ppm carbadox (CARB), or 110 ppm chlortetracycline (CTC) to the CO diet produced the experimental diets. Pigs were fed once each day an amount approximately equal to ad libitum intake. Water was available at all times. One animal from each litter was randomly assigned to one of the four treatment diets (two pigs per treatment). Treatment diets were fed for 14 d (period 1, d 0 to 14). All pigs were then fed the CO diet (period 2, d 14 to 24). After period 2, treatment diets were again fed (period 3, d 24 to 38), with the pigs receiving the same antimicrobial in the diet as they received in period 1.

Daily samples of jejunal (1 to 5 g) and colonic (.5 to 2 g) contents were taken starting on d 0 and ending on d 14 during period 1. During period 2, digesta samples were collected on d 7 and 10 of this period. Digesta samples were collected on d 3, 7, 10 and 14 of period 3. After period 3, pigs were fed the CO diet, and after 7 d a final sample was collected. Samples were collected immediately (within 30 min) after feeding (0930) and held on ice until all samples were collected for the day. Collections were then diluted

Table 7. Composition of the basal diet[a]

Ingredient	%
Ground shelled corn	55.2
Soybean meal, 44% CP	25.0
Dried whey	15.0
Ground limestone	1.0
Mono-dicalcium phosphate	1.5
Selenium-Vitamin E premix[b]	1.0
Vitamin-mineral premix[c]	.75
L-lysine HCl (78%)	.30
Salt	.25
	100.00
Calculated analysis:	
Lysine, %	1.25
Calcium, %	.75
Phosphorus, %	.65

[a]Treatment diets were formed by adding 250 ppm Cu, 55 ppm CARB, or 110 ppm CTC at the expense of ground shelled corn.

[b]Provided .1 mg of Se and 17 IU vitamin E per kg of diet.

[c]Provided the following amounts of vitamins and minerals/kg of diet: vitamin A, 3300 IU; vitamin D, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 18mg; D-pantothenic acid, 13 mg; choline, 110 mg; vitamin B_{12}, 20 ug; Zn, 75 mg; Mn, 34 mg; Fe, 60 mg; Cu, 10 mg; I, .5 mg.

(1:10 wt/vol) with distilled water. Diluted samples were vortexed and centrifuged at 27,000 x g for 10 min, and the supernatant collected for analysis. Ammonia was determined at this time (within 1 h after collection), and the remaining supernatant frozen (-20° C) until VFA and urea analysis could be performed.

2. **Sample Analysis.**

a. **Ammonia and Urea.** Ammonia concentrations were determined by the colorimetric procedure described by Chaney and Marbach (1962) using Sigma Kit No. 640 (Sigma Chemical Co., St Louis, MO). A colorimetric procedure (Sigma Kit 535) in which diacetyl monoxime reacts directly with urea, was used to determine urea concentrations.

b. **VFA.** VFA were determined by GLC (Hewlett-Packard, model 5840A). One milliliter samples were acidified with .25 ml of 25% metaphosphoric acid and placed in 1.5 ml crimp top GLC vials. A 2 ul aliquot was injected into a 2 mm x 1.8 m stainless steel column, packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W-AW (Supelco, Inc., Bellefonte, PA). Operating conditions were as follows: column temperature, 115° C; flame ionization detector temperature, 175° C; injection temperature, 170° C; and helium flow rate, 14 ml/min. Peaks for acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were identified by their relative retention times, and concentrations determined using a VFA standard (Supelco, Inc.).

c. **Anaerobic Bacterial Counts.** On d 0 and 14 of periods 1 and 3, digesta samples were also collected to

determine anaerobic bacteria counts. Total viable anaerobic bacteria counts were determined using the roll tube technique described by Hungate (1970). One gram of intestinal contents was diluted (1:10 wt/vol) with an anaerobic dilution medium (Table 8, Bryant and Robinson, 1962), and serially diluted to 10^{-10}. Dilutions between 10^{-4} to 10^{-10} were inoculated in duplicate into a non-selective medium (GCS-RF, Table 8, Bryant and Robinson, 1962), and incubated for 72 h at 39° C. All visible colonies were counted in roll tube dilutions which contained 30 to 100 colonies.

3. Statistical Analysis.

Data were analyzed in a split-plot design (Gill, 1978) using the GLM procedure of SAS (1985). The influence of treatment period was also tested to determine whether the results from the two treatment periods (periods 1 and 3) could be pooled. When a treatment effect and (or) a time X treatment interaction was observed ($P < .10$), treatment means were separated using Tukey's t-test, testing all pairs of means.

D. Results.

Results from treatment periods 1 and 3 could not be pooled, as evidence for a treatment X period interaction was moderately strong ($P < .10$) for each variable.

During all periods, animals rapidly consumed their daily allotment of feed, and no feed remained from day to day. Animal performance was slightly depressed due to the

Table 8. Composition of media for bacterial counts.

Item, %	ADM[a]	GCS-RF
Agar	0	2.0
Glucose	0	.07
Cellibiose	0	.07
Starch	0	.07
Yeast extract	0	.2
Trypticase	0	.5
Clarified rumen fluid	0	30.0
Mineral 1[b]	8.3	3.8
Mineral 2[c]	8.3	3.8
Resazurin	0	.1
Sodium carbonate (8%)	5.0	5.0
Cysteine-sodium sulfide (2.5%)	2.0	2.0
Distilled water	76.4	52.39

[a]Anaerobic dilution media.

[b]Supplied 49.8 mg K_2HPO_4 per 100 ml media.

[c]Supplied 49.8 mg KH_2PO_4, 49.8 mg $(NH_4)_2SO_4$, 99.6 mg NaCl, 20.3 mg $MgSO_4 \cdot 7H_2O$, and 13.2 mg $CaCl_2 \cdot 2H_2O$ per 100 ml media.

experimental protocol (average across time and treatments, 322.7 g per d).

1. Ammonia and Urea.

Ammonia concentrations in jejunal and colonic digesta were unaffected ($P > .10$) by the addition of antimicrobials to the diet when compared to the CO diet (Figures 12 and 13, respectively). Daily variations in digesta ammonia concentrations, especially in the jejunum, were quite high (2 to 27 umol/g digesta).

On d 14 of period 1, pigs consuming CTC, CARB or CU had greater ($P < .01$) urea concentrations in jejunal digesta than pigs fed the CO diet (Figure 14). Pigs receiving the CU diet also had higher ($P < .05$) urea concentrations in jejunal digesta on d 4, 7 and 9 than pigs consuming other diets.

The concentration of urea in colonic digesta was not affected by antimicrobials ($P > .10$, Figure 15).

2. VFA.

The total concentration of VFA (the sum of acetate, propionate, isobutyrate, butyrate, isovalerate and valerate) in the digesta of either the jejunum or colon was not affected ($P > .10$) by the addition of antimicrobials to the basal diet (Figures 16 and 17). These levels are similar to those reported for swine by other researchers (Argenzio and Southworth, 1974; Kennelly et al., 1981). Acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate concentrations in the digesta of the jejunum and colon were also not affected ($P > .10$) by antimicrobial addition to the basal diet.

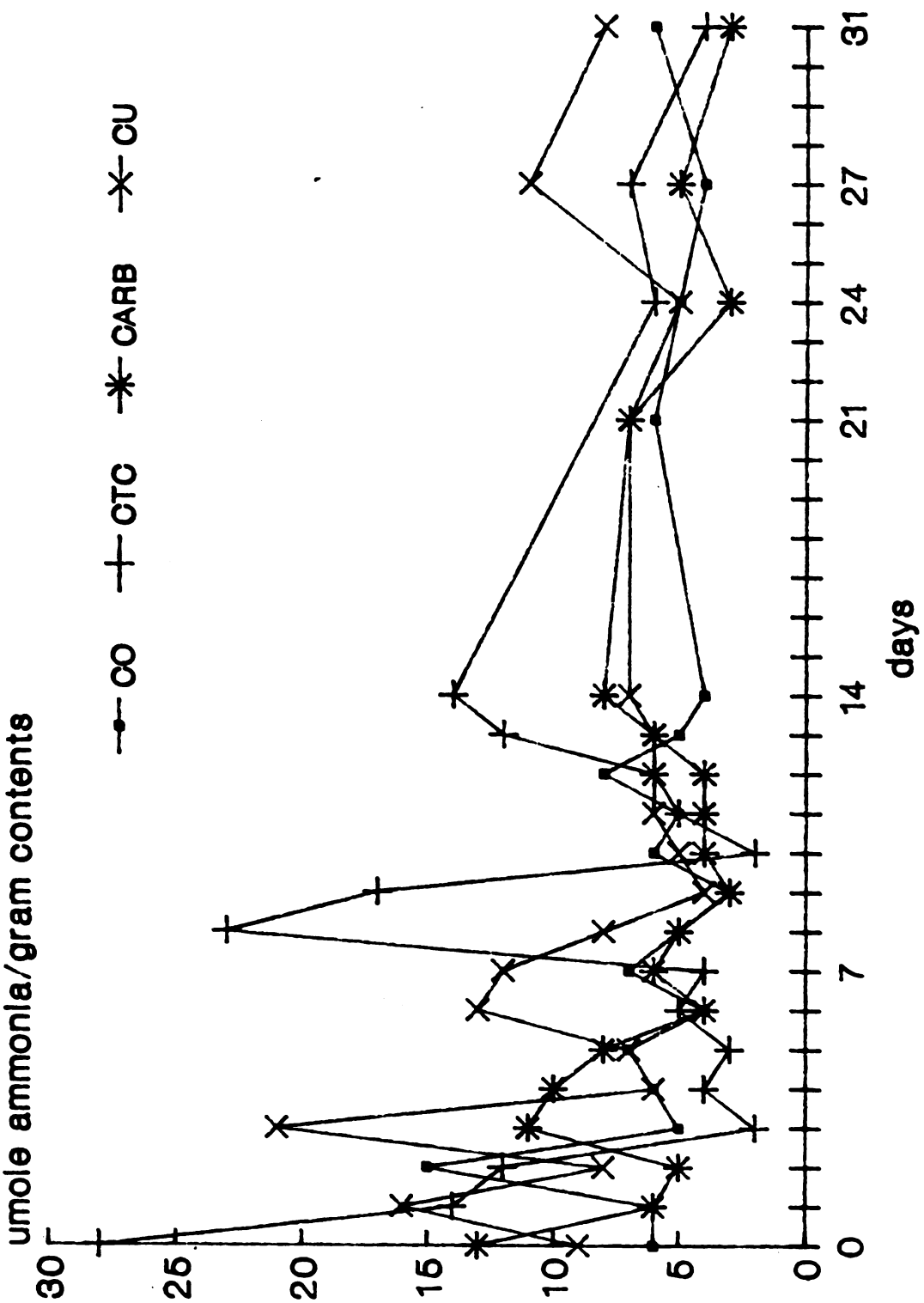


Figure 12. Influence of chlortetracycline (CTC), carbadox (CARB), or copper (CU) on the concentration of ammonia in jejunal contents of weanling pigs.

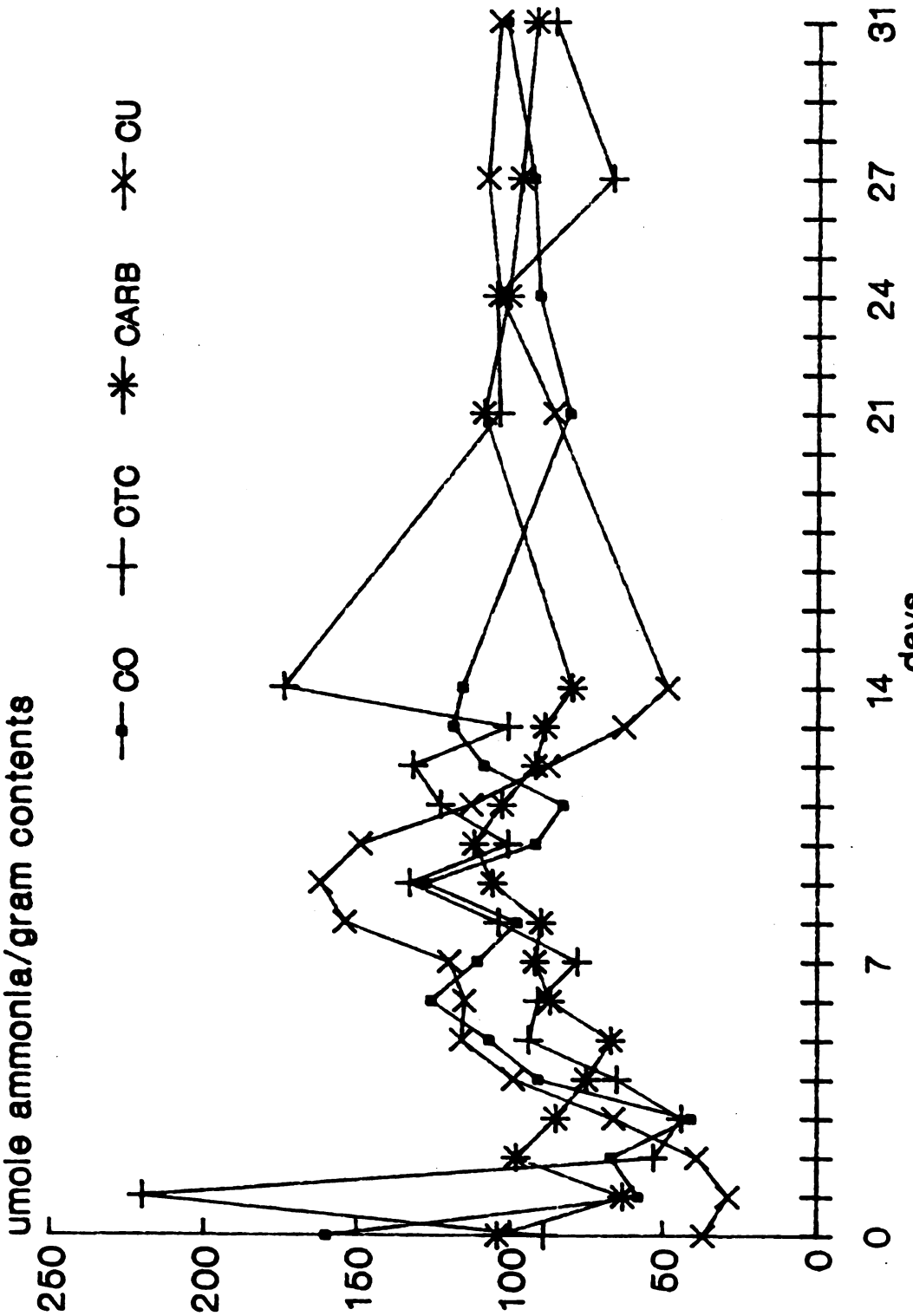
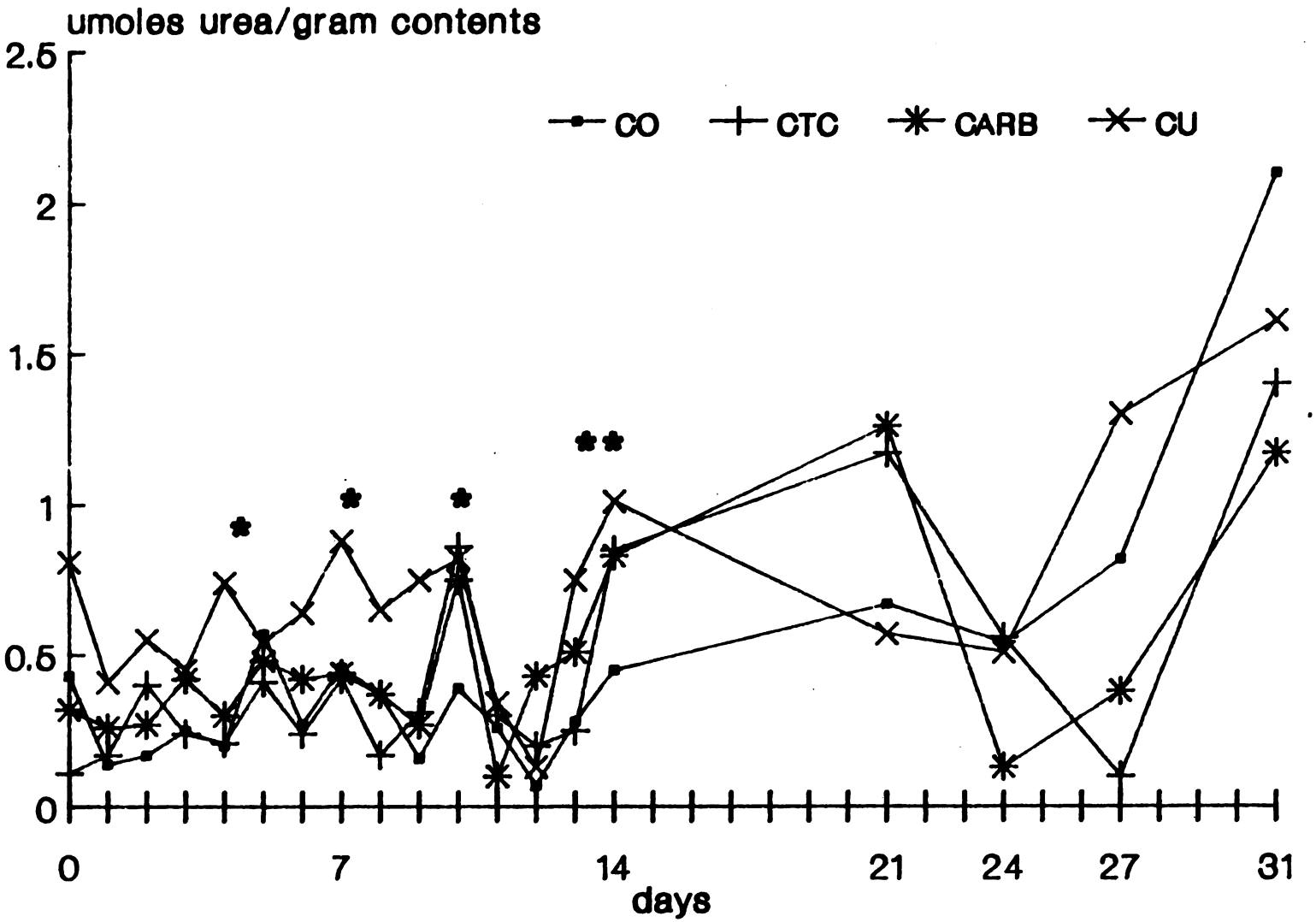


Figure 13. Influence of chlortetracycline (CTC), carbadox (CARB), or copper (CU) on the concentration of ammonia in colonic contents of weanling pigs.

Figure 14. Influence of chlortetracycline (CTC), carbadox (CARB), or copper (CU) on the concentration of urea in jejunal contents of weanling pigs[a].



[a](*) - CU effect, P < .05. (**) - antimicrobial effect, P < .01.

Figure 15. Influence of chlortetracycline (CTC), carbadox (CARB), or copper (CU) on the concentration of urea in colonic contents of weanling pigs.

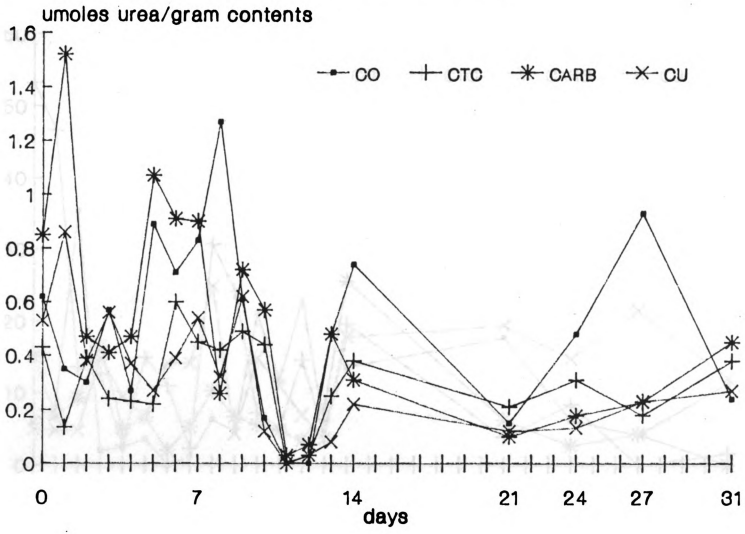
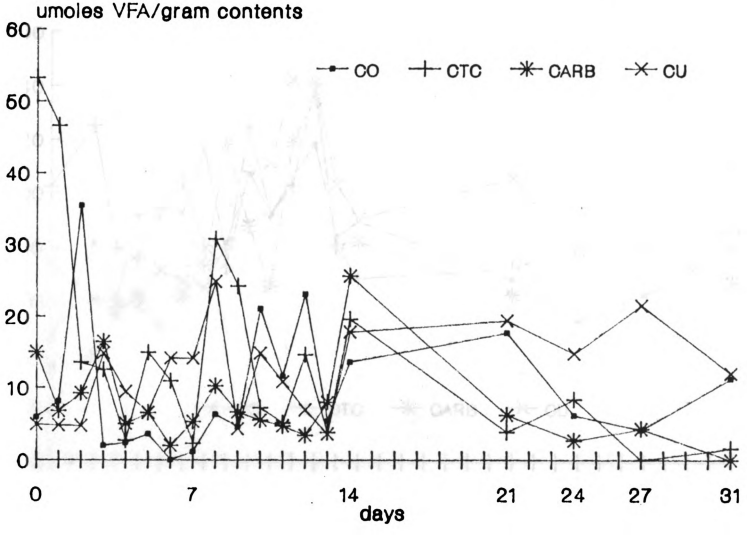


Figure 16. Influence of chlortetracycline (CTC), carbadox (CARB), or copper (CU) on the concentration of volatile fatty acids in jejunal contents of weanling pigs.



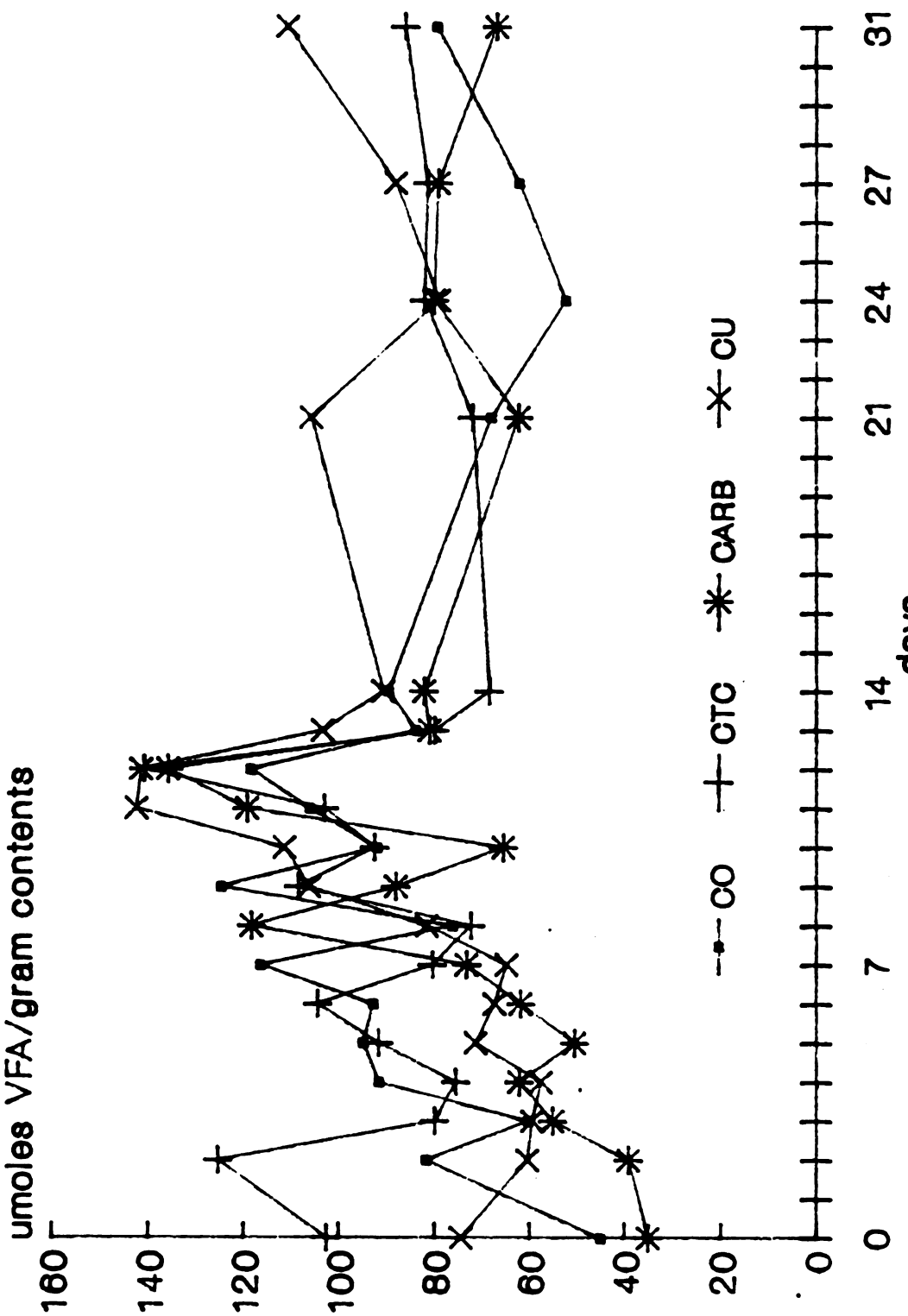


Figure 17. Influence of chlortetracycline (CTC), carbadox (CARB), or copper (CU) on the concentration of volatile fatty acids in colonic contents of weanling pigs.

3. Anaerobic Bacterial Numbers.

The addition of CU or CARB to the basal diet had no effect (P >.10) on anaerobic bacterial numbers of the digesta from either the jejunum or colon after 14 d (period 1) as compared with that of pigs fed the CO diet (Table 9).

On d 10 of period 3, a jejunal and colonic cannula became non-functional from one pig fed the CO diet and one pig fed the CU diet respectively, and these animals were removed from the study. Consequently, bacterial changes due to antimicrobials could not be assessed during this period. Furthermore, metabolite levels could be analyzed statistically only up to 7 d of period 3.

See appendix 2 for data in tabular form.

E. Discussion.

The interaction of treatment with periods suggests that the age of the pig is an important factor involved in the influence of antimicrobials on fermentation in the jejunum and colon. This observation is further evidenced by the relatively smaller impact antimicrobials have on ADG and GE in older pigs (Zimmerman, 1986).

Period 2, during which all animals were fed the CO diet, was included in the trial to identify shifts in fermentation that may occur when CU, CARB, or CTC are removed from the diet. When changes in fermentation were detected in period 1 due to antimicrobials, the microflora tended (P <.20) to re-adapt to the level of fermentation observed when pigs were

Table 9. Influence of dietary chlortetracycline (CTC), carbadox (CARB), or copper (CU) on anaerobic bacterial counts in weanling pigs[a].

Item	Treatment			
	Control	CTC	CARB	CU
Jejunum, period 1				
Initial	10.84	b	8.65	9.78
Final	8.43	b	9.15	8.44
Colon, period 1				
Initial	11.00	10.61	11.28	12.26
Final	9.49	9.63	11.71	8.95

[a]Log value/g intestinal contents. SEM within time: jejunum = .61, colon = .98; within treatments, jejunum = .94, colon = 1.36.

[b]Cultures from one pig on CTC were lost due to environmental contamination of the culture medium, thus CTC was not included in the analysis of jejunal bacterial counts.

fed the CO diet during period 2.

The lack of an effect of antimicrobials on the number of anaerobic bacteria has been observed by other researchers. Varel et al. (1987) have observed that total colony counts from swine fecal samples were not different due to the addition of 250 ppm Cu or the combination of chlortetracycline, sulfamethazine and penicillin, to a basal diet; however, the number of streptococci (a major urease containing bacteria) was significantly reduced. Thus it is likely that a shift in the population occurs, as opposed to an absolute reduction in total numbers of anaerobic bacteria.

In vitro, virginiamycin, spiramycin, carbadox, and copper sulfate decreased the production of ammonia from amino acids and urea by the microflora in the small intestine (Dierick et al., 1986a). Furthermore, the concentration of ammonia in portal vein plasma was determined to be lower in pigs fed either 250 ppm of dietary copper (Menten, 1988) or carbadox (Yen et al., 1990). This evidence appears to be contrary to our findings, however, it is possible that antimicrobials do indeed decrease ammonia production in vivo, but this response is not demonstrated in our study due to the rapid disappearance (via absorption) of this metabolite. Thus, the greater amount of ammonia produced in pigs fed diets devoid of antimicrobials may be rapidly absorbed by the pig.

This hypothesis is further supported by the results obtained from the analysis of urea in jejunal contents. The addition of the antimicrobials to the diet significantly

increased the amount of urea in the jejunum. Other workers have also shown this "anti-urease" activity of copper (Visek, 1978; Varel et al., 1987; Menten, 1988).

The variation in ammonia levels may be due to daily changes in endogenous secretions, with a subsequent diluting effect on the level of ammonia detected.

Vervaeke et al. (1979) observed spiramycin and virginiamycin decreased the production of VFA in vitro. Furthermore, Argenzio and Southworth (1974) have indicated that the absorption of VFA in the large intestine of pigs is quite efficient. From these observations, if antimicrobials do indeed alter carbohydrate fermentation in vivo, the absorption of end products quickly removes them from the contents of the intestinal tract, as indicated previously in regard to ammonia.

From this study, it is evident that urea hydrolysis (with the subsequent production of ammonia) in the jejunal digesta is a common activity inhibited by CU, CARB and CTC. This action of antimicrobials may play a key role in the mechanism by which these feed additives improve the rate of gain and gain efficiency of growing pigs. Other products of fermentation (VFA) may also be affected by these antimicrobials, but the concentration of these compounds is confounded with their absorption by the pig. Consequently, changes in the concentration of these compounds by the addition of antimicrobials to the diet may not be detectable in vivo.

PART V. INFLUENCE OF DIETARY COPPER ON INTESTINAL MUCOSA ENZYME ACTIVITY, MORPHOLOGY, AND TURNOVER RATES, IN WEANLING PIGS

A. Abstract.

Twenty four pigs were weaned from 4 litters at 21 d of age (6 kg BWT) to evaluate the influence of 250 ppm dietary copper on intestinal mucosa glucose-6-phosphatase (GP), alkaline phosphatase (AP), and adenosine triphosphatase (ATPase); mucosal morphology; and the turnover rate of the intestinal mucosa, throughout the gastrointestinal tract (GIT). Pigs were lotted into 4 pens of 6 pigs each based on sex, litter, and weight. Pens were then assigned to one of two treatments: 1) corn-soybean meal-whey type diet with no antimicrobials (CO), or 2) CO + 250 ppm copper (CU). Pigs were fed twice each day an amount approximately equal to ad libitum for 14 d. On day 14, pigs were injected with 50 uCi/kg BWT ^{3}H-thymidine i.p., 10 h after the morning meal. One pig from each pen was sacrificed at 1, 6, 12, 20, 32, and 44 h post injection, and intestinal tissue was collected from the duodenum, two jejunum sites (proximal and distal), ileum, cecum and colon. CU tended to decrease the activity of GP and AP in the proximal jejunum ($P < .11$, $P < .08$, respectively). ATPase activity was not affected by treatment ($P > .10$). CU had no effect on crypt depth, villus height, or

epithelial cells up the villus was also not affected by treatment (P >.10). Turnover rate of the intestinal mucosa of the proximal and distal jejunum was slower (P <.10; P <.05, respectively) and the distal jejunal cell generation interval longer (P <.05) in pigs fed the CU diet. From these data we conclude that the addition of 250 ppm copper to the diet of weanling pigs alters the metabolism of jejunal intestinal mucosa, which may result in a lower energy requirement for the maintenance of this tissue.

B. Introduction.

The addition of antimicrobials to the diet of pigs may change the microbial fermentation along the gastrointestinal tract (GIT), and reduce the formation of potentially toxic compounds, i.e. ammonia (Menten, 1988; Yen et al., 1990), which may explain the growth response generally associated with these feed additives. A reduction in the concentration of these harmful compounds may alter the metabolic activity of the intestinal mucosa in a manner which would be beneficial to the pig. This change in metabolism of the mucosa may result in the conservation of energy, mediated through a decrease in the turnover rate of the intestinal epithelium, and (or) a decrease in the metabolic activity of the epithelial cell.

The addition of the antimicrobial carbadox to the diet of growing pigs may decrease the energy requirements of the portal drained visera (including the GIT), as evidenced by a

lower oxygen uptake by these organs as compared to non-antimicrobial fed animals (Yen et al., 1989).

The presence of bacteria in the GIT of rats decreased the cell generation interval of intestinal epithelial cells (Lesher et al., 1964). The microflora also decreased the activity of alkaline phosphatase, adenosine triphosphatase, (enzymes important in active transport), and glucose-6-phosphatase (which is essential in glucose transport from within the epithelial cell to the portal vein blood, Kawai and Morotomi, 1978). Thus, it is apparent that the microflora of the GIT can influence the GIT environment and the ability of the animal to absorb nutrients. Reducing the concentration of bacterial metabolites (especially those considered toxic) in the GIT may reduce the turnover rate and energy used by intestinal epithelial cells. Also, since enzymes important in digestion and absorption become functional as the cell matures (Coates and Fuller, 1977), increasing the life-span of these cells may improve digestion and cellular metabolic efficiency.

In the following study, the influence of 250 ppm dietary copper (a growth promoting feed additive) on the intestinal mucosa will be investigated. Understanding how supranutritive levels of copper functions to improve gain performance in the young growing pig may lead to the development of alternative, non-antibiotic feed additives, which increase average daily gain and feed efficiency. Furthermore, to my knowledge, the cell generation interval of porcine intestinal epithelial cells has not been

determined.

C. Materials and Methods.

1. Animals.

Twenty-four crossbred pigs were weaned at 21 d of age (6 kg BWT) and assigned to one of 2 treatments based on litter, sex, and weight. Treatments included a control diet (CO, Table 10), devoid of antimicrobials, and CO + 250 ppm Cu, as copper sulfate (CU). Pigs were fed treatment diets for 14 d. During this time, pigs were housed in 4, 1 x 2 m stainless steel pens (6 pigs per pen) in an environmentally controlled (27^{o} C) nursery. Pigs were fed twice daily (0800 and 1700h) an amount approximately equal to ad libitum (6-8% of body weight). Water was available at all times.

On 14 d, the morning meal was fed to each pen at 1 h intervals starting at 0600 to facilitate subsequent tissue collection. Ten hours post-prandial, 50 uCi/kg body weight ^{3}H-thymidine (specific activity 43 Ci/mmole, Amersham Corporation, Arlington Heights, IL) was injected intraperitoneally. The ^{3}H-thymidine was diluted with sterile saline (.9%) to 100 uCi/ml prior to injection, to achieve an injection volume of approximately 5 cc.

2. Sample Collection.

Four pigs (2 per treatment, 1 per pen) were sacrificed at 1, 6, 12, 20, 32, and 44 h following injection. Animals were sacrificed by asphyxiation with CO_2 in a gassing chamber. The GIT was quickly removed, and samples collected

Table 10. Composition of the basal diet[a]

Ingredient	%
Ground shelled corn	55.2
Soybean meal, 44% CP	25.0
Dried whey	15.0
Ground limestone	1.0
Mono-dicalcium phosphate	1.5
Selenium-Vitamin E premix[b]	1.0
Vitamin-mineral premix[c]	.75
L-lysine HCl (78%)	.30
Salt	.25
	100.00
Calculated analysis:	
Lysine, %	1.25
Calcium, %	.75
Phosphorus, %	.65

[a]Copper diet was formed by adding 250 ppm Cu.

[b]Provided .1 mg of Se and 17 IU vitamin E per kg of diet.

[c]Provided the following amounts of vitamins and minerals/kg of diet: vitamin A, 3300 IU; vitamin D, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 18mg; D-pantothenic acid, 13 mg; choline, 110 mg; vitamin B_{12}, 20 ug; Zn, 75 mg; Mn, 34 mg; Fe, 60 mg; Cu, 10 mg; I, .5 mg.

from the duodenum (D), jejunum (2 sites), ileum, cecum, and colon. The D was defined as the small intestine between the stomach and the point at which the bile duct entered the intestinal tract. The ileal-cecal valve and the ileal-artery defined the endpoints of the ileum. The jejunum samples were taken from the anterior (J-A) and posterior (J-B) half of the section of small intestine between the D and ileum. Samples collected from the cecum were obtain from the end of the cecum, and the colon sample was taken from the spiral colon. One to two gram tissue samples for enzyme analysis were quickly frozen in a dry ice/acetone bath. Lengths of gastrointestinal tract measuring 2 to 4 cm were removed, tied at both ends with string, and distended with 10% buffered formalin using a syringe. Distended tissue were placed in a 10% formalin buffer bath.

3. Enzyme analysis.

Approximately .5 g of tissue from each GIT site was minced with scissors, and placed in 18 x 150 mm glass culture tubes. Appropriate amounts of 50 mM Tris buffer (Sigma Chemical Co., St Louis, MO) were added to each tube to achieve the desired dilution of enzyme. Tissues were then homogenized with a Polytron Homogenizer (Brinkman Instruments, Westbury, NY) for 15-30 seconds. The homogenate was centrifuged at 850 x g for 10 minutes at 4^{o} C. The resulting supernatant was used for enzyme and protein analysis.

a. Alkaline phosphatase. Alkaline phosphatase activity was determined by monitoring the appearance of p-

nitrophenyl from p-nitrophenyl phosphate using Sigma Kit 245 (Sigma Chemicals, St. Louis, MO). Enzyme activity was expressed as umole Pi liberated/minute/mg protein.

b. Adenosine triphosphatase. The activity of adenosine triphosphatase was measured by the method described by Hirschhorn and Rosenberg (1968). Briefly, .1 ml of the supernatant was added to 2.4 ml of one of two media in duplicate. One medium contained 140 mM NaCl, 16 mM KCl, while the second medium contained no added NaCl or KCl. Both media contained 2.5 mM ATP (dipotassium salt) and 30 mM Tris. The final pH of both media was 7.4. Reactions were carried out at 37^{o} C for 60 minutes. At this time, the reaction was stopped with 1.5 ml 6 M perchloric acid. The amount of Pi in the media (indicating the activity of the phosphatase) was determined colorimetrically (Gomori, 1942). Na/K dependent ATPase activity was calculated by subtracting the activity of the enzyme in media 2 (no added Na or K, unstimulated ATPase) from the activity detected in media 1 (added Na and K, total ATPase activity). Enzyme activity was expressed as ug Pi liberated/hour/mg protein.

c. Glucose - 6 - phosphatase. The activity of glucose-6-phosphatase was determined by monitoring the release of Pi from glucose-6-phosphate in 30 minutes (modified from Cori and Cori, 1955). The media contained 0.5 ml of .01 M glucose-6-phosphate, .3 ml of .1 M K-citrate, and 0.2 ml of the homogenate. The reaction was incubated for 30

minutes at 37° C then stopped with 10% TCA. A second set of media, also containing the homogenate, were acidified immediately. Acidified media were then centrifuged at 2000 x g for 5 minutes, and the concentration of Pi liberated by the reaction was determined (Gomori, 1942). Enzyme activity was then calculated as the difference between the amount of Pi detected in these two media for each GIT site, and expressed as ug Pi liberated/minute/100 mg protein.

d. Protein. Total soluble protein was determined by the colorimetric procedure of Lowry et al. (1951).

4. Cell morphology and mucosa turnover rate.

Formalin fixed tissue was embedded in paraffin, sliced into 6 um sections, and mounted on glass slides by the Michigan State University Animal Health Diagostic Laboratory. Prepared slides were dipped in Kodak NBT-2 (Eastman Kodak, Rochester, NY) emulsion in a darkroom, and placed in light-proof boxes. Boxes were stored at 4° C during the exposure period. Between day 14 and 38, of the exposure period, the emulsion film was developed with a photograph developer (Dektol, Eastman Kodak) solution, and the reaction stopped with a photograph fixer solution (Eastman Kodak). Slides were then stained with hematoxylin and eosin, and coverslipped with permount.

The number of cells in crypt and villus columns were counted, and the length of these columns measured with an ocular micrometer. The position of the labelled cells along the columns was also noted. Ten columns per sample were counted when possible and these counts averaged. The number

of cells in mitosis was also determined, as well as the number of mitotic cells that were labelled with the tritidated thymidine. The percent of cells labelled at 1 h post-dose was also determined, by counting the total number of cells labelled in the crypt and dividing by the total number of cells in the crypt.

The mucosal turnover rates were calculated as the amount of time necessary for the leading labelled cells in the crypt or villus to reach the tip of the villus. Turnover rates were estimated from the velocity (slope of the regression line) of the labelled cells up the crypt or villus.

To calculate epithelial cell generation interval, a labelling index curve was generated. This curve maps the percent of cells in mitosis that have been labelled. Only those cells in mitosis will be labelled, therefore, the time between points when 50% of cells in mitosis are labelled, will equal the cell generation interval (Cleaver, 1967).

5. Statistics.

The influence of treatment and (or) GIT site on mucosal morphology and epithelial cell enzyme activity was detected using a repeated measure analysis (Gill, 1978). When the variation among pens was trivial, pen variation was pooled with the residual (animal) error, and animal served as the experimental unit. Otherwise, pen served as the experimental unit. The influence of treatment and (or) GIT site on the generation interval was determined similarly, however, pen served as the experimental unit.

Mucosal turnover rates were estimated using regression equations determined for each pen at each GIT site. The slope of these regression equations was a measure of the rate of migration of the epithelial cell up the villus. An inverse prediction equation was then formulated to calculate the turnover rate. The influence of treatment on the turnover rate of the intestinal mucosa was tested according to Gill (1978).

D. Results.

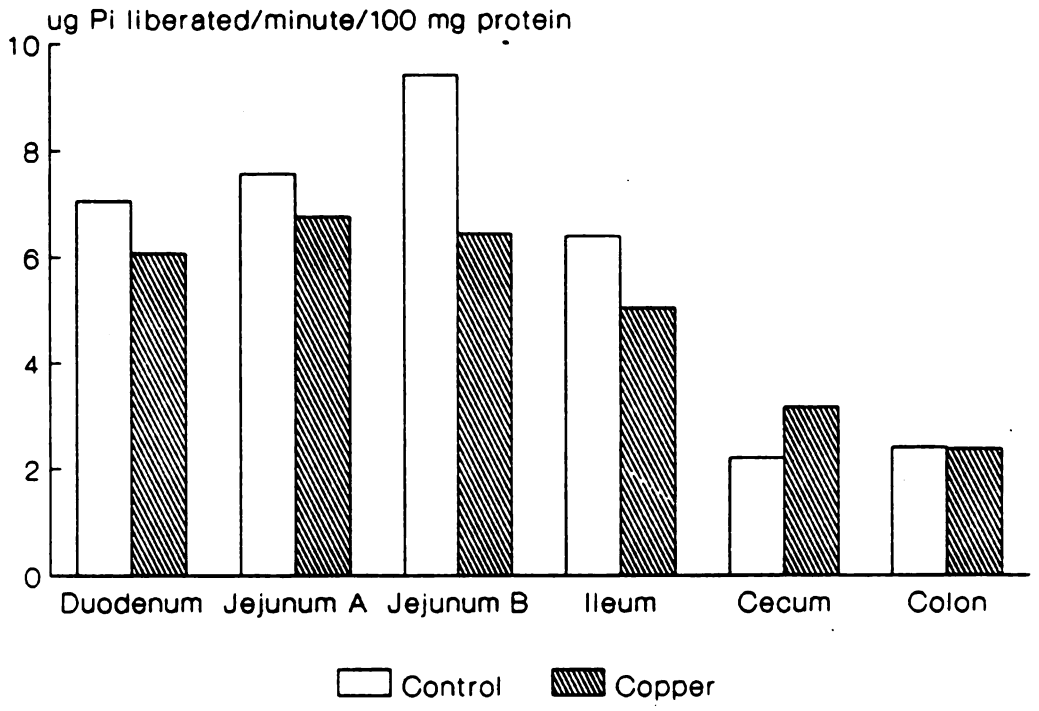
1. Cell enzyme activity.

Glucose-6-phosphatase activity tended to be greater ($P < .11$) in the mucosa of the distal jejunum of pigs fed the CO diet as compared to the CU diet (9.42 vs 6.45 ug Pi liberated/minute/100 mg protein, respectively, Figure 18). When the activity of this enzyme was pooled across small intestine sites, the increase in activity due to CU was more evident ($P < .01$). Glucose-6-phosphatase activity was affected by GIT site ($P < .001$), regardless of treatment.

The activity of alkaline phosphatase also tended to be greater ($P < .08$) in the mucosa of the distal jejunum when pigs were fed the CO diet as compared to the CU diet (168.09 vs 109.84 umole Pi liberated/minute/mg protein, Figure 19). Again, when small intestine sites were pooled, the increase in activity due to CU was more evident ($P < .02$). The activity of this enzyme was also affected by GIT site ($P < .001$).

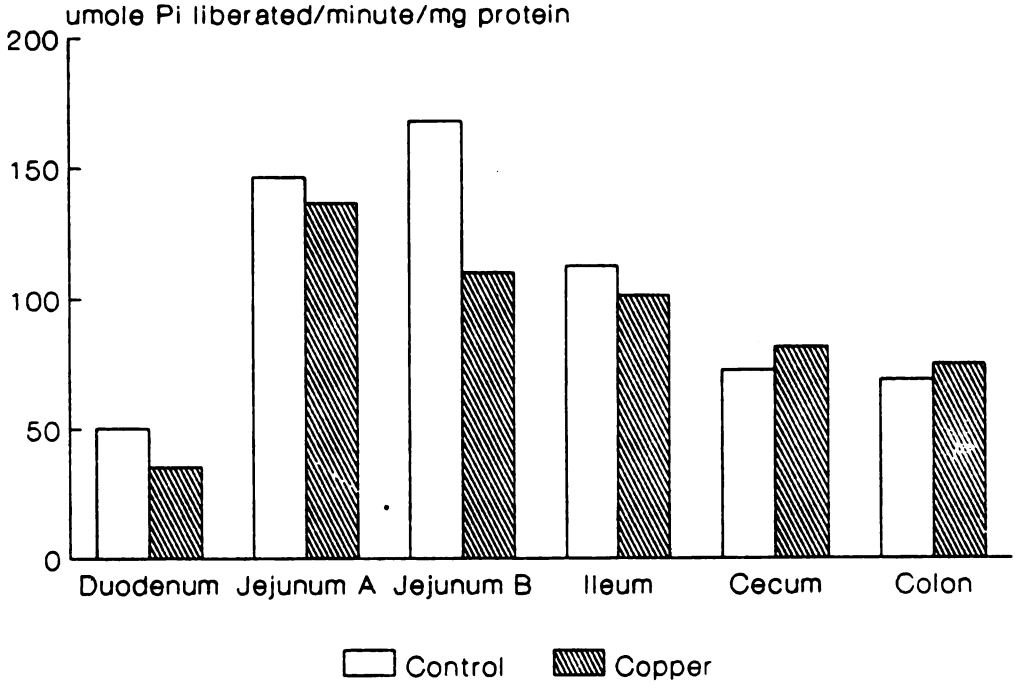
Total ATPase, Na/K stimulated ATPase, and unstimulated

Figure 18. Influence of dietary copper on intestinal mucosa glucose - 6 -phosphatase activity in weanling pigs[a].



[a]SEM, within treatments = 1.01; within site = 1.19.

Figure 19. Influence of dietary copper on intestinal mucosa alkaline phosphatase activity in weanling pigs[a].



[a]SEM, within treatments = 13.35; within site = 17.65.

ATPase activity (ug Pi liberated/hour/mg protein) were unaffected by treatment (P >.10, Figures 20, 21, and 22).

2. Intestinal mucosa morphology.

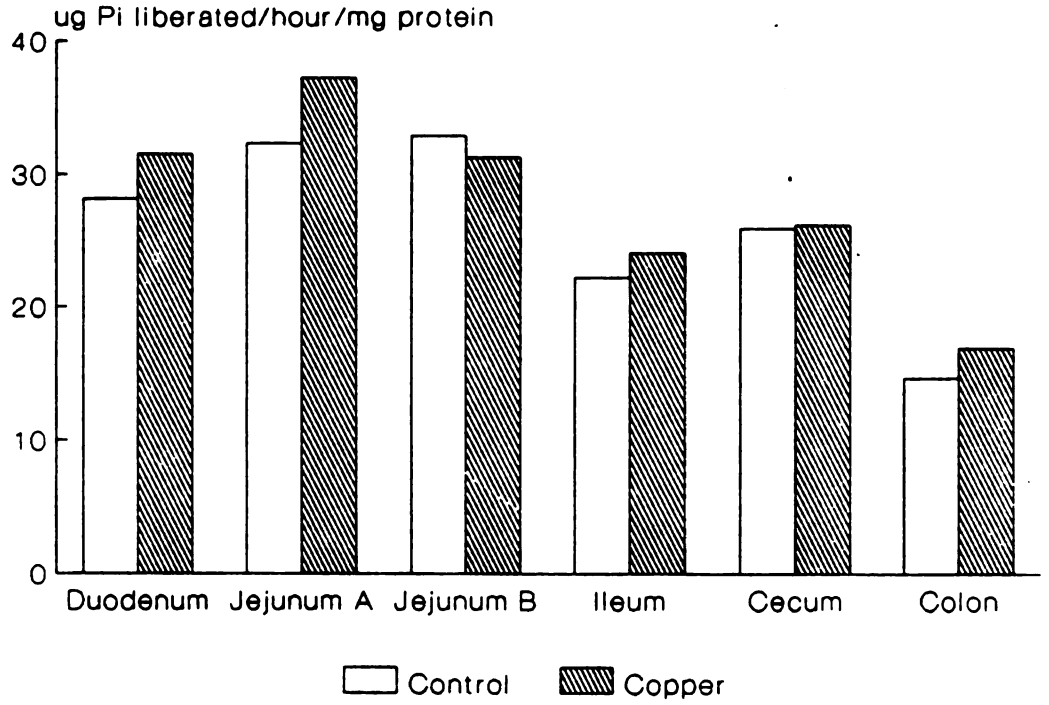
The addition of 250 ppm CU to the diet had no effect on crypt depth (number of cells or microns), villus height (number of cells or microns), total height of the villus/crypt structure (number of cells or microns) or cell size (microns; Figures 23 to 29). However, these measurements were different between sites. Crypt depth increased (P <.001) from the proximal jejunum to the colon. Villi were shorter (number of cells, P <.10; microns, P <.03) in the ileum than the jejunum. Total height of these two structures also decreased from the jejunum to the ileum (number of cells, P <.07; microns, P <.04). Epithelial cell size was also affected by GIT site (P <.004), with cells increasing in size from the proximal jejunum to the colon.

Duodenal estimates of these measures of the mucosal morphology could not be determined due to a lack of well positioned crypts and villi.

3. Intestinal mucosa turnover rates and cell generation interval.

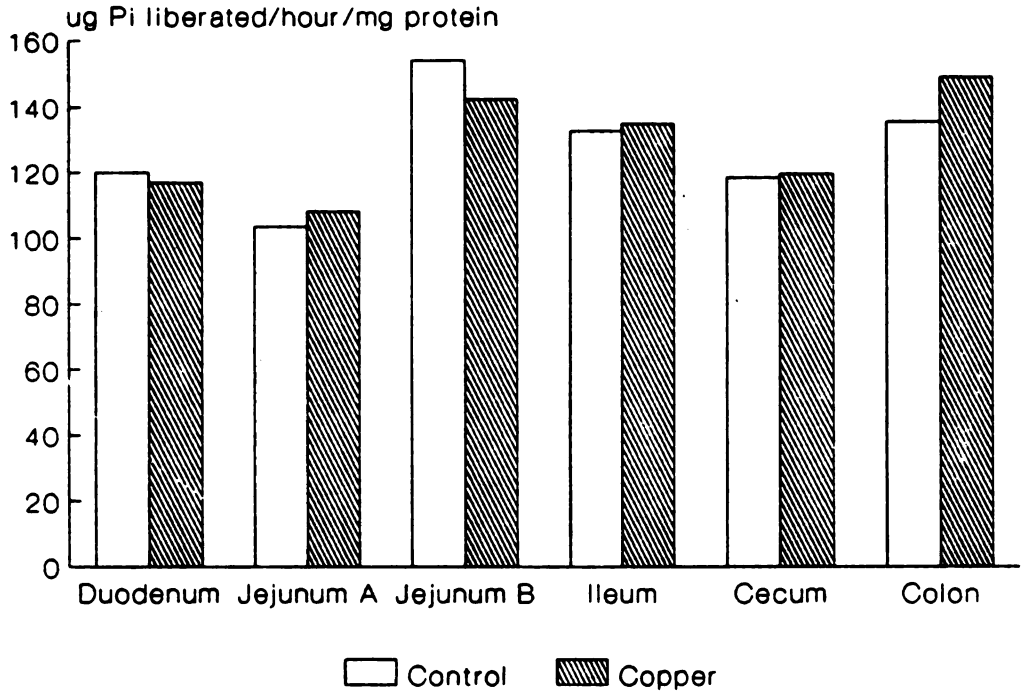
a. Cell generation interval. Due to the lack of well defined labelling index curves (appendix 3), especially in the latter stages of these curves and in the cecum and colon, the cell generation interval was calculated from the length of the DNA synthesis phase and the percent of cells labelled one hour post dose of the ^{3}H-thymidine (Cleaver,

Figure 21. Influence of dietary copper on intestinal mucosa Na/K independent ATPase activity in weanling pigs[a].



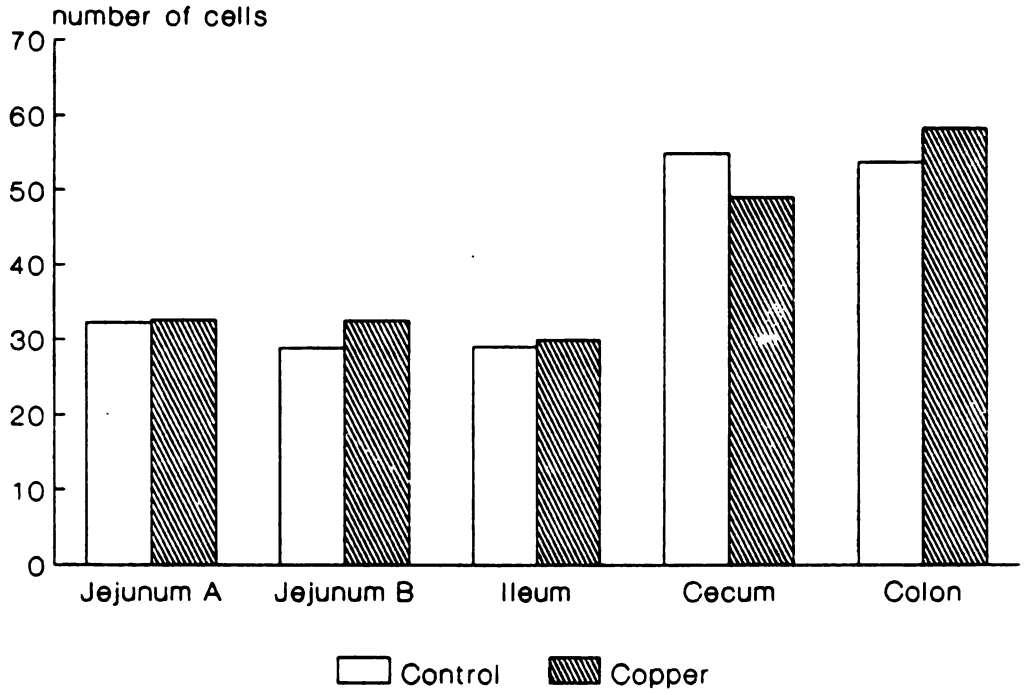
[a]SEM, within treatment = 2.64; within site = 4.38.

Figure 22. Influence of dietary copper on intestinal mucosa total ATPase activity in weanling pigs[a].



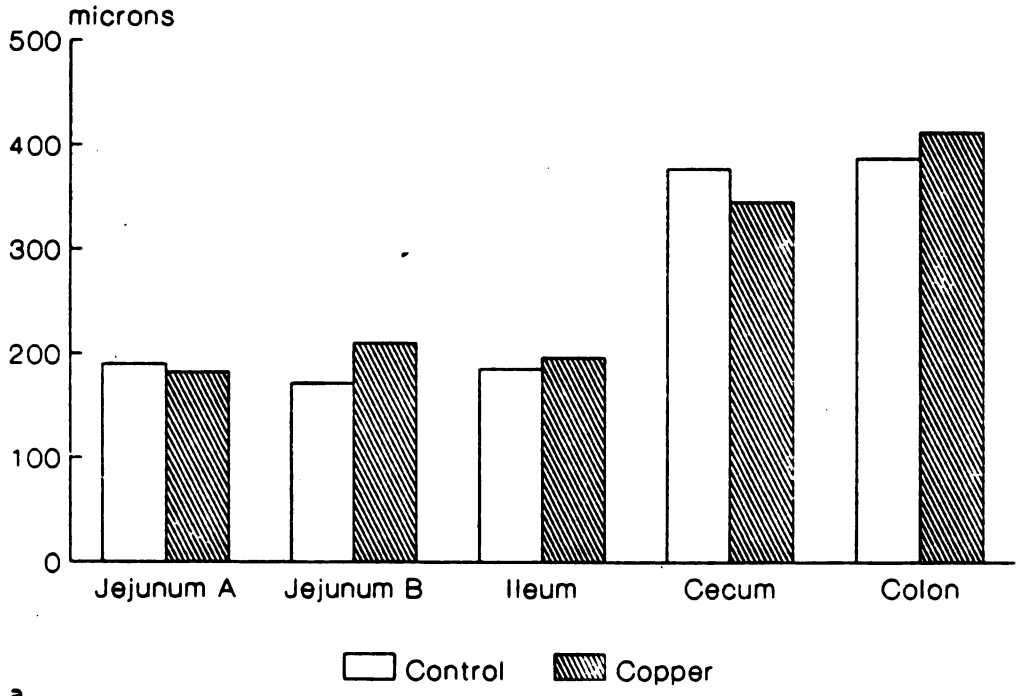
[a]SEM, within treatment = 5.6; within site = 10.37.

Figure 23. Influence of dietary copper on the depth of intestinal crypts in weanling pigs[a].



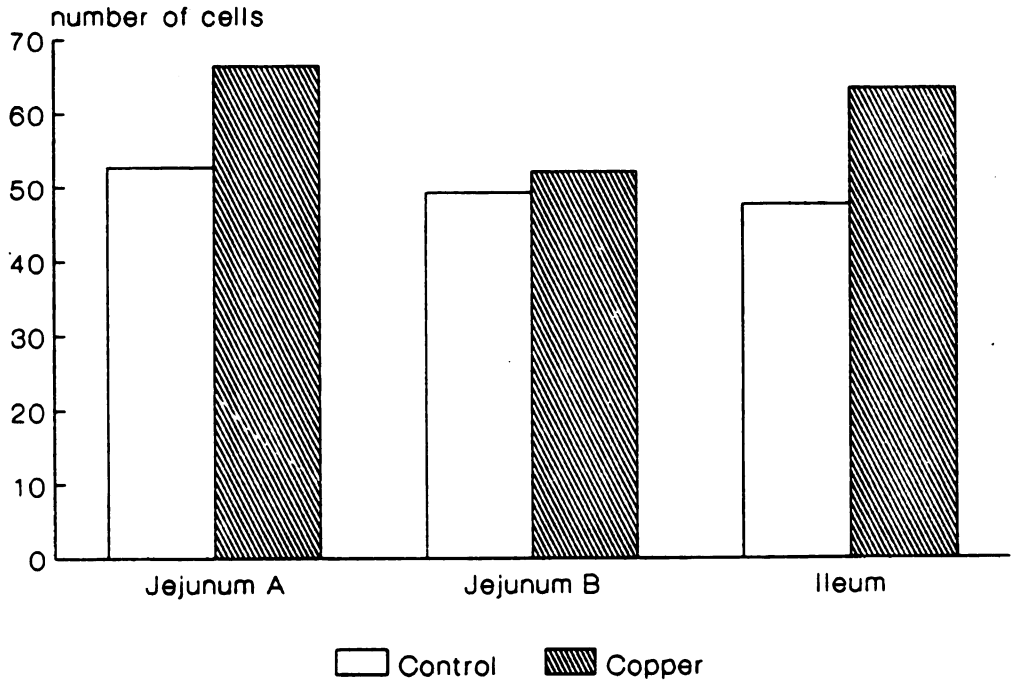
[a]SEM, within treatment = 2.67; within site = 3.15.

Figure 24. Influence of dietary copper on the depth of intestinal crypts in weanling pigs[a].



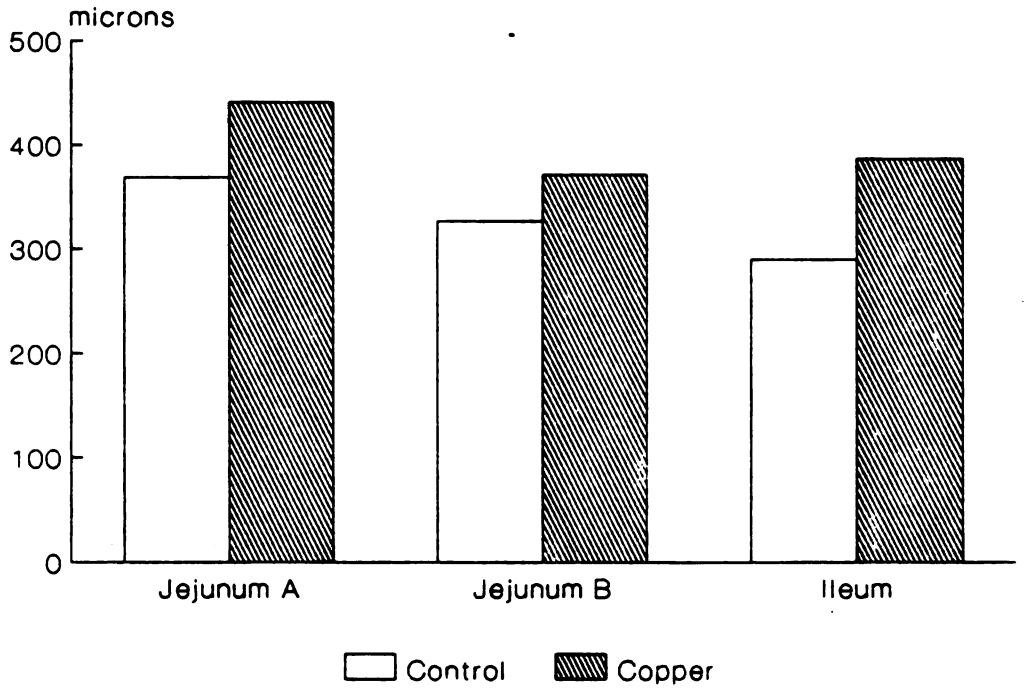
[a]SEM, within treatments = 18.4; within site = 21.99.

Figure 25. Influence of dietary copper on the height of intestinal villi in weanling pigs[a].



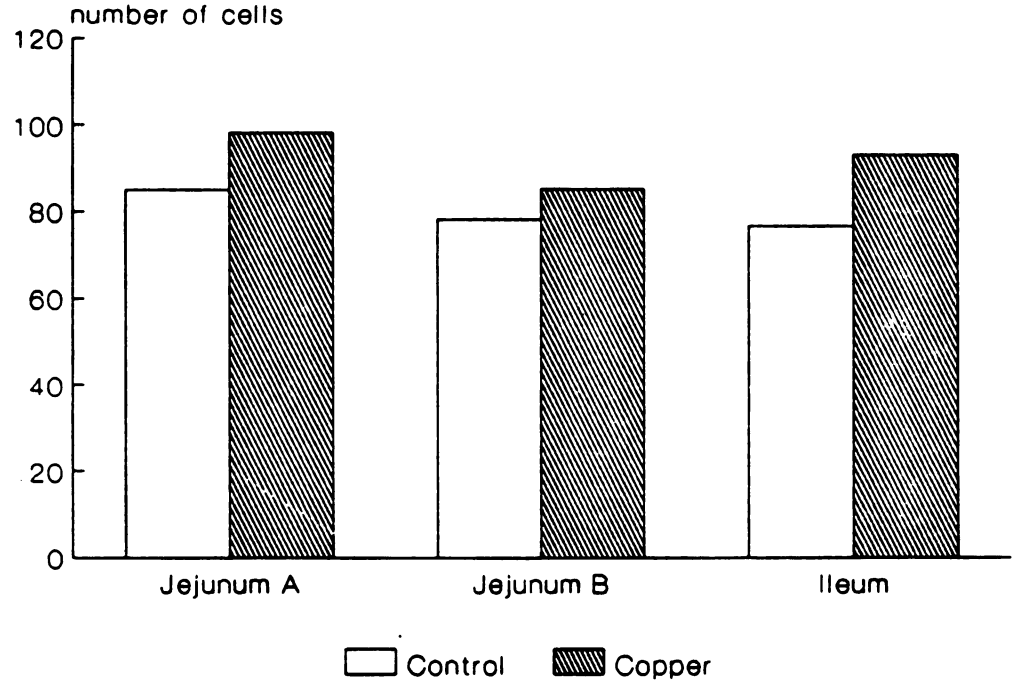
[a]SEM, within treatment = 6.4; within site = 6.94.

Figure 26. Influence of dietary copper on the height of intestinal villi in weanling pigs[a].



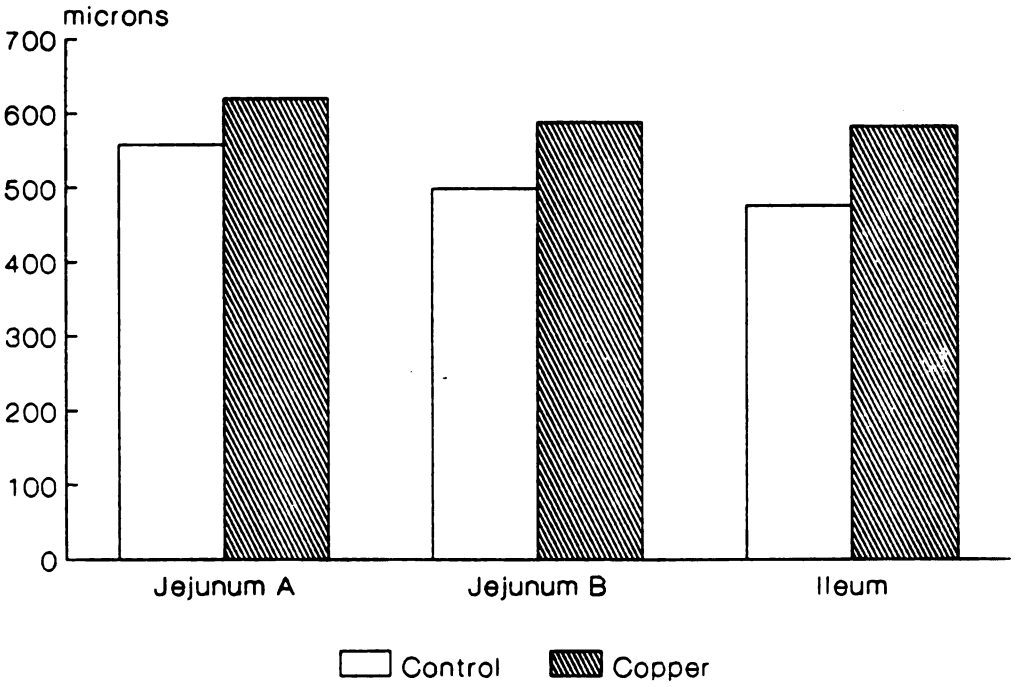
[a]SEM, within treatment = 44.04; within site = 42.7.

Figure 27. Influence of dietary copper on the total number of intestinal epithelial cells (crypt + villus) in weanling pigs[a].



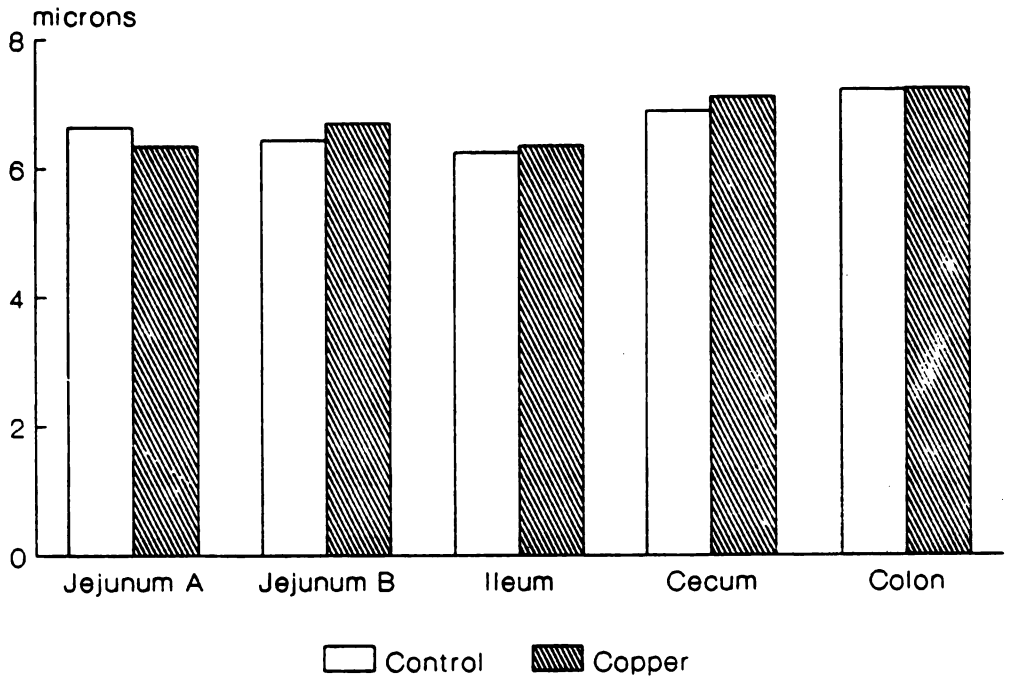
[a]SEM, within treatments = 6.19; within site = 6.45.

Figure 28. Influence of dietary copper on the total height of the intestinal epithelium (crypt + villus) in weanling pigs[a].



[a]SEM, within treatment = 43.45; within site = 41.75.

Figure 29. Influence of dietary copper on intestinal epithelial cell size in weanling pigs[a].



[a]SEM, within treatment = .34; within site = .40.

1967). The generation interval was calculated as follows:

$$\text{Generation interval} = \frac{\text{length of DNA synthesis phase}}{\text{\% of cells labelled at 1 hour}}$$

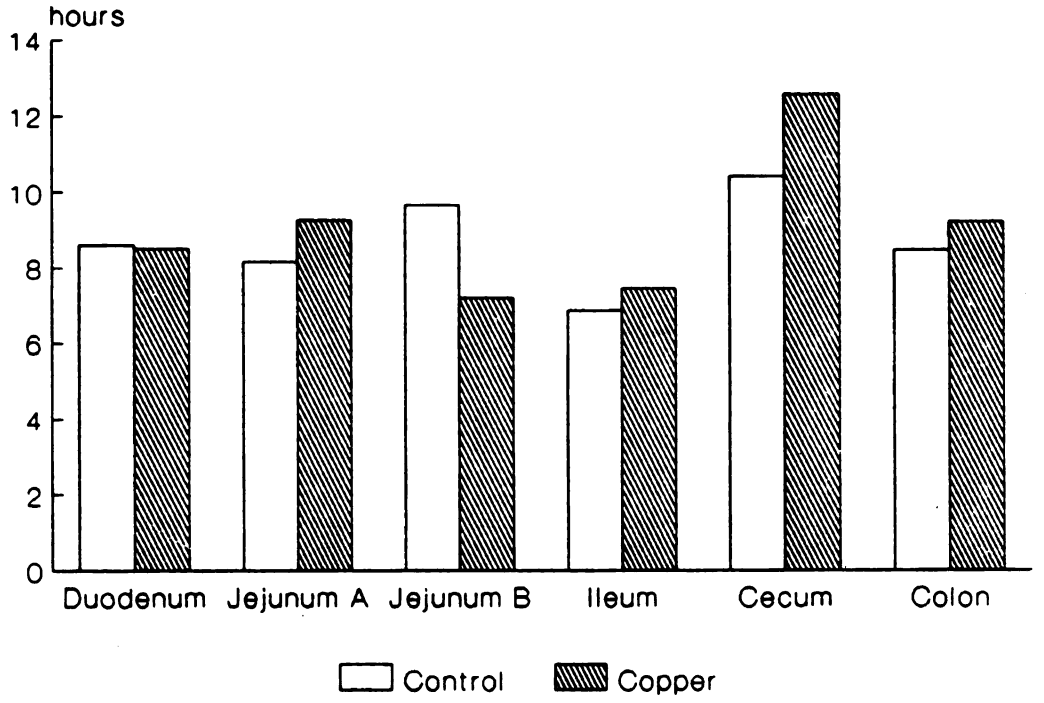
The effect of CU on the length of the DNA synthesis phase is shown in Figure 30. CU tended to decrease ($P < .10$) the DNA synthesis phase (7.2 hours vs 9.65 hours) in the distal jejunum, however, CU also tended to increase ($P < .10$) this measure in the cecum (12.55 hours vs 10.4 hours).

The cell generation interval of animals fed the CU diet was greater (44.55 hours vs 20.37 hours, $P < .05$) in the distal jejunum as compared to pigs receiving the CO diet (Figure 31). Generation interval was not affected by treatment at other GIT sites ($P > .10$).

b. Mucosal turnover rates. The rate of cell migration (number of cell positions/h) is shown in Table 11. CU tended to decrease the rate of cell migration (.21 vs .52 cells/hour, $P < .08$) in the cecum.

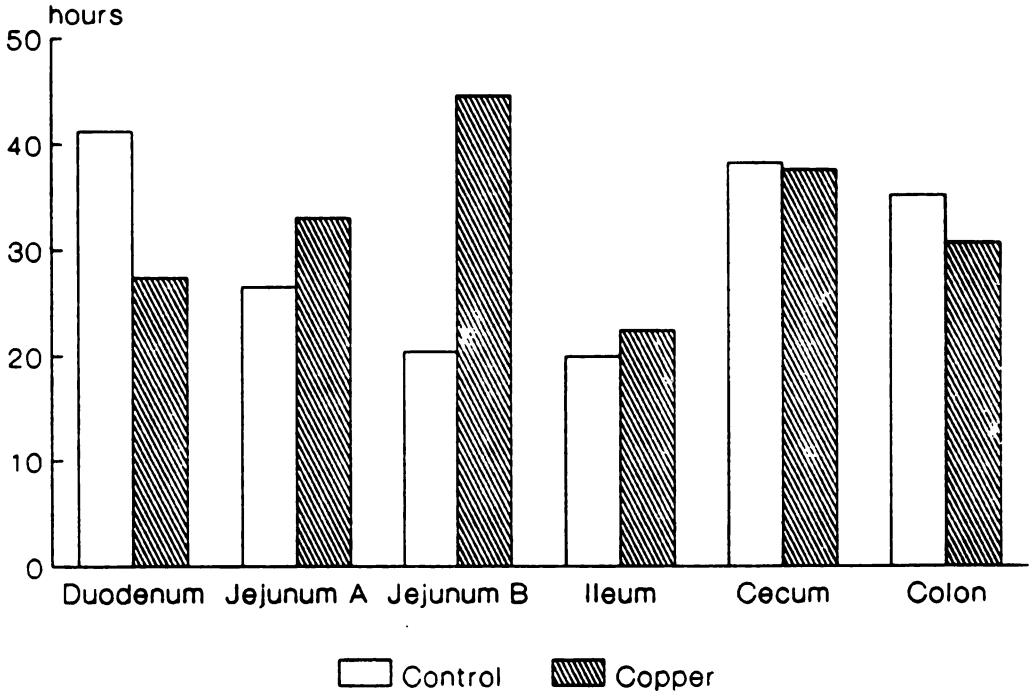
The turnover rate (Table 11) of the intestinal mucosa of proximal jejunum tended to be slower (64.33 h vs 48.56 h; $P < .10$) when pigs were fed the CU diet as compared to animals consuming the CO diet. The turnover rate of the mucosa of the distal jejunum was slower (43.34 h vs 33.8 h; $P < .05$) when pigs were fed CU.

Figure 30. Influence of dietary copper on the length of the intestinal epithelial cell DNA synthesis phase in weanling pigs[a].



[a]SEM, within treatment = .43; within site = .77.

Figure 31. Influence of dietary copper on the length of the intestinal epithelial cell generation interval in weanling pigs[a].



[a]SEM, within treatment = 5.65; within site = 5.71.

Table 11. Influence of dietary copper on intestinal tissue turnover rates in weanling pigs.

	Control		Copper		P
Migration rate, cells/hour					
Jejunum A	1.18	(.35)[a]	1.11	(.37)	NS[b]
Jejunum B	1.42	(.30)	1.27	(.30)	NS
Ileum	.77	(.24)	.77	(.38)	NS
Cecum	.52	(.13)	.21	(.09)	.08
Colon	.57	(.15)	.66	(.05)	NS
Turnover rate, hours					
Jejunum A	48.56	(7.11)[c]	64.33	(8.08)	.10
Jejunum B	33.80	(4.57)	43.34	(4.57)	.05
Ileum	60.83	(48.10)	101.59	(48.10)	NS
Cecum	83.84	(16.39)	61.49	(18.23)	NS
Colon	47.61	(.82)	49.07	(.82)	NS

[a]Standard error of the average slope.

[b]Not significant, P >.10.

[c]Confidence interval.

E. Discussion.

1. Cell enzymes activity.

The activity of glucose-6-phosphatase, alkaline phosphatase, and adenosine triphosphatase have been shown to be greater in germ-free mice as compared to conventionally reared mice (Kawai and Morotomi, 1978). If antimicrobials function to produce a situation in the GIT similar to that in the germ-free animal, antimicrobials may similarly increase the activities of these enzymes. However, the addition of CU to the diet did not increase the activity of these enzymes. CU tended to decrease the activity of glucose-6-phosphatase (distal jejunum; $P < .11$) and alkaline phosphatase (distal jejunum; $P < .08$). The activity of these enzymes at the other GIT sites, together with the activity of ATPase, were not affected by the addition of CU to the diet ($P > .10$).

The influence of GIT site on the activity of GP, AP, and ATPase is not surprising, as the digestive and absorptive capacity of the small intestine is known to be greater than that of the cecum and colon.

2. Intestinal mucosa morphology.

In previous work with high copper diets, Shurson et al. (1990), found this feed additive to increase villus height and crypt depth when fed to weanling pigs. However, changes in morphology due to the addition of CU to the diet were not significant in our study. Values for the different measures observed fall within the range of previously reported values (Moon, 1970; Shurson et al., 1990). No information, however,

exists on the number of cells in the crypt or villus for pigs. The effect of site on the various morphological measures was expected, as this observation has been reported for the chick (Imondi and Bird, 1966) and mouse (Cooper et al., 1974).

3. Intestinal cell generation interval and mucosal turnover rates.

The decrease in the rate of cell migration due to CU observed in the cecum is difficult to explain, and was unexpected at this site. The biological importance of this finding is also difficult to explain. However, the increased rate of epithelial cell migration in the cecum of pigs fed the CO diet would suggest an increased rate of mucosal tissue turnover.

Perhaps of more relevance is the effect of CU on the estimated turnover rate of the mucosal tissue in the pig. The addition of CU to the diet of pigs appears to reduce the rate at which the mucosa of the jejunum is turned over by increasing the length of time required for the epithelial cells of the intestinal mucosa to traverse the villus and be extruded from the tip of the villus. This observation lends support to the hypothesis that antimicrobial compounds are able to decrease the energy requirements of the GIT. The reduction in turnover rate observed in the jejunum of pigs fed CU may allow for more dietary energy to be used for body weight gain, instead of body weight maintenance. CU also increased the length of the cell generation interval in the distal jejunum, supporting the observation of a slower

turnover rate in this GIT site, as a longer cell generation interval would lead to a slower rate of cell renewal. A decrease in the mitotic index (an estimate of the rate of cell division) has been observed in the intestinal mucosa of pigs fed a diet containing 250 ppm Cu (Menten, 1988). Estimates of turnover rate reported here are similar to those reported by Moon (1970) in week old pigs.

Carbadox, another antimicrobial, has been shown to decrease the amount of oxygen used by the portal drained viscera (which includes the GIT, Yen et al., 1989). This suggests that this compound also changes the energy reguirements of the GIT, as oxygen comsumption can be used as an indication of energy use by the tissue.

From this study, it appears that 250 ppm dietary Cu can influence the metabolic activity of the intestinal mucosa (especially the jejunum) in a manner that likely benefits the pig. This change in metabolism may lead to a reduction in the amount of energy required to maintain the GIT, subsequently increasing the amount of energy available for body weight gain.

PART VI. SUMMARY

1. In the weanling pig, fermentation patterns of the small intestine are different than those of the large intestine.
2. The addition of 250 ppm copper, 55 ppm carbadox, or 110 ppm chlortetracycline to the diet of weanling pigs decreases the amount of urea that is hydrolyzed in the jejunum. These feed additives have no significant affect on this reaction in the colon.
3. The addition of 250 ppm copper, 55 ppm carbadox, or 110 ppm chlortetracycline to the diet of weanling pigs has no significant affect on the concentration of ammonia or volatile fatty acids, or the number of anaerobic bacteria in the jejunum or colon.
4. The addition of 250 ppm copper to the diet of weanling pigs has no significant affect on the morphology of the intestinal mucosa in the jejunum, ileum, cecum or colon.
5. The addition of 250 ppm copper to the diet of weanling pigs tends to decrease the activity of glucose-6-phosphatase and alkaline phosphatase in the mucosa of the distal jejunum. The activity of these enzymes is not significantly affected by copper in the duodenum,

proximal jejunum, ileum, cecum, or colon. Adenosine triphosphatase activity in the mucosa is not significantly affected in the duodenum, proximal and distal jejunum, ileum, cecum, or colon.

6. The length of the mucosa cell generation interval is longer in the distal jejunum when pigs are fed 250 ppm dietary copper.
7. The migration rate of epithelial cells up the villus is not significantly affected by the addition of 250 ppm copper to the diet in the proximal and distal jejunum, ileum, cecum, or colon.
8. The addition of 250 ppm copper to the diet of weanling pigs reduces the turnover rate of the intestinal mucosa of the jejunum. This feed additive has no significant affect on the turnover rate of the mucosa of the ileum, cecum, or colon.

PART VII. CONCLUSIONS

The decrease in urea hydrolysis in the jejunum due to the addition of antimicrobials to the diet of weanling pigs, very likely leads to a decrease in ammonia production by the microflora at this gastrointestinal tract site. A decrease in ammonia production may lead to a slower replacement rate of the intestinal mucosa, which was observed when 250 ppm copper was added to the diet. This reduction in the replacement rate of the intestinal mucosa likely reduces the energy and nutrient needs for maintenance of the gastrointestinal tract, making more energy and nutrients available for body weight gain and improving gain efficiency in the pig. This may be one of the mechanism by which supranutritive levels of copper improve the growth performance of the pig.

PART VIII. APPENDICES

APPENDIX 1: DATA FROM TRIAL 1

Table 12. Production of ammonia, cadaverine, and p-cresol at different sites along the gastrointestinal tract in pigs[a].

Item	Intestinal site S	J	C	UC	LC	P	MSE
Ammonia, umol/ml							
n	6	6	5	6	6		
Initial	88.55[b]	91.27[b]	232.11[bc]	268.53[c]	308.53[c]	.001	10033.3
Final	426.27	381.27	419.77	375.69	380.88	.380	3075.9
Production per 24h	332.72[b]	290.00[bc]	187.65[bcd]	107.16[cd]	72.35[d]	.001	11845.2
Cadaverine, umol/ml							
n	6	6	5	5	5		
Production per 24h	7.96[b]	9.21[b]	5.54[bc]	3.35[cd]	1.06[d]	.001	5.5
P-cresol, mg/ml							
n	6	6	5	6	6		
Production per 24h	ND[e]	ND	11.59	16.68	19.78	.001	40.2

[a]Expressed on a per ml of culture media basis. S = stomach, J = jejunum, C = cecum, UC = upper colon, LC = lower colon.

[bcd]Means with different superscripts differ, P <.05.

[e]None detected.

Table 13. Production of VFA at different sites along the gastrointestinal tract, umole/ml media[a].

Item	Intestinal Site					P	MSE
	S	J	C	UC	LC		
n	6	6	5	6	6		
Acetate							
Initial	2.98	2.83	3.76	3.65	3.18	.06	.27
Final	9.19^{b}	11.99^{bc}	13.66^{c}	13.69^{c}	14.62^{c}	.004	5.43
Production per 24 h	7.21	9.16	8.88	11.25	12.51	.09	11.35
Propionate							
Initial	0.00^{b}	0.00^{b}	1.07^{c}	.93^{c}	.63^{c}	.001	.07
Final	6.22	6.28	7.07	6.49	5.98	.922	4.09
Production per 24 h	6.22	6.28	5.18	5.71	5.45	.91	5.50
Isobutyrate							
Production per 24 h	.38^{b}	.12^{b}	.97^{c}	1.19^{c}	1.17^{c}	.001	.11
Butyrate							
Initial	0.00	0.00	.44	.33	.29	.02	.05
Final	1.20^{b}	1.78^{b}	3.39^{c}	3.86^{c}	4.09^{c}	.001	.71
Production per 24 h	1.20^{b}	1.78^{bc}	2.95^{bcd}	3.53^{cd}	3.80^{d}	.001	1.23

[a]S = stomach, J = jejunum, C = cecum, UC = upper colon, LC = lower colon.

[bcd]Means with different superscripts differ, P <.05.

Table 14. Production of VFA at different sites along the gastrointestinal tract, umole/ml media[a].

Item	Intestinal Site					P	MSE
	S	J	C	UC	LC		
n	6	6	5	6	6		
Isovalerate							
Initial	0.00	0.00	0.00	0.00	.14	.08	.01
Final	.73^{b}	.47^{b}	1.55^{c}	2.22^{c}	1.95^{c}	.001	.37
Production per 24 h	.73^{bc}	.47^{b}	1.55^{bcd}	2.22^{d}	1.81^{cd}	.001	.51
Valerate							
Initial	0.00	0.00	0.00	0.00	.08	.46	.006
Final	1.03^{b}	.35^{b}	2.19^{c}	2.22^{c}	2.58^{c}	.001	.44
Production per 24 h	1.03^{bc}	.35^{b}	2.19^{cd}	2.22^{cd}	2.50^{d}	.001	.63
Total							
Initial	2.98	2.83	5.27	4.18	3.67	.06	1.61
Final	18.73	21.00	25.24	29.61	30.29	.03	48.71
Production per 24 h	16.75	18.17	21.73	26.12	27.23	.05	46.65

[a]S = stomach, J = jejunum, C = cecum, UC = upper colon, LC = lower colon.

[bcd]Means with different superscripts differ, P <.05.

APPENDIX 2: DATA FROM TRIAL 2

Table 15. Influence of antimicrobials on jejunal ammonia[a].

Day	Treatment			
	CO	CTC	CARB	CU
Period 1[b]				
0	6	28	13	9
1	6	14	6	16
2	15	12	5	8
3	5	2	11	21
4	6	4	10	6
5	7	3	8	7
6	4	5	4	13
7	7	4	6	12
8	5	23	5	8
9	3	17	3	4
10	6	2	4	5
11	5	5	4	6
12	8	6	4	6
13	5	12	6	6
14	4	14	8	7
Period 2[c]				
14	4	14	8	7
21	6	-	7	7
24	5	6	3	5
Period 3[d]				
24	5	6	3	5
27	4	7	5	11
31	6	4	3	8

[a]Data expressed as umole/gram intestinal contents.
[b]SEM, treatment = 2.4; period = 2.63.
[c]SEM, treatment = .67; period = .81.
[d]SEM, treatment = 1.51; period = .84.

Table 16. Influence of antimicrobials on colonic ammonia[a].

Day	Treatment			
	CO	CTC	CARB	CU
Period 1[b]				
0	160	89	104	37
1	58	220	63	29
2	67	53	98	39
3	41	44	85	66
4	91	65	75	99
5	107	94	67	116
6	126	91	87	115
7	111	78	92	120
8	98	104	90	154
9	128	133	106	162
10	92	101	112	149
11	83	123	103	113
12	109	132	92	88
13	119	101	89	63
14	116	174	80	49
Period 2[c]				
14	116	174	80	49
21	81	104	109	86
24	91	105	101	104
Period 3[d]				
24	91	105	101	104
27	93	67	97	108
31	102	86	92	104

[a]Data expressed as umole/gram intestinal contents.
[b]SEM, treatment = 7.91; period = 19.03.
[c]SEM, treatment = 23.19; period = 14.57.
[d]SEM, treatment = 19.23; period = 10.27.

Table 17. Influence of antimicrobials on jejunal urea [a].

Day	Treatment			
	CO	CTC	CARB	CU
Period 1[b]				
0	.43	.11	.32	.81
1	.14	.17	.26	.41
2	.17	.40	.27	.55
3	.25	.24	.42	.45
4	.20	.21	.30	.74
5	.57	.41	.48	.54
6	.27	.24	.42	.64
7	.46	.42	.44	.88
8	.37	.17	.37	.65
9	.16	.31	.27	.75
10	.39	.86	.75	.82
11	.26	.30	.10	.34
12	.07	.20	.43	.13
13	.28	.25	.51	.75
14	.45	.85	.83	1.01
Period 2[c]				
14	.45	.85	.83	1.01
21	.67	1.17	1.26	.57
24	.54	.56	.13	.51
Period 3[d]				
24	.54	.56	.13	.51
27	.82	.10	.38	1.30
31	2.10	1.40	1.17	1.61

[a]Data expressed as umole/gram intestinal contents.
[b]SEM, treatment = .06; period = .09.
[c]SEM, treatment = .36; period = .26.
[d]SEM, treatment = .26; period = .25.

Table 18. Influence of antimicrobials on colonic urea[a].

Day	Treatment			
	CO	CTC	CARB	CU
Period 1[b]				
0	.62	.43	.85	.53
1	.35	.14	1.52	.86
2	.30	.39	.47	.38
3	.57	.24	.41	.56
4	.27	.23	.47	.37
5	.89	.22	1.07	.27
6	.71	.60	.91	.39
7	.83	.45	.90	.54
8	1.27	.42	.26	.32
9	.61	.49	.72	.62
10	.17	.44	.57	.12
11	0	0	.03	0
12	0	.03	.07	.03
13	.48	.25	.48	.08
14	.74	.38	.31	.22
Period 2[c]				
14	.74	.38	.31	.22
21	.15	.21	.10	.12
24	.48	.31	.18	.13
Period 3[d]				
24	.48	.31	.18	.13
27	.93	.18	.23	.23
31	.24	.38	.45	.27

[a]Data expressed as umole/gram intestinal contents.
[b]SEM, treatment = .14; period = .14.
[c]SEM, treatment = .10; period = .08.
[d]SEM, treatment = .07; period = .06.

Table 19. Influence of antimicrobials on total jejunal VFA[a].

Day	Treatment			
	CO	CTC	CARB	CU
Period 1[b]				
0	5.90	53.20	14.97	4.90
1	8.10	46.60	6.76	4.77
2	35.40	13.50	9.20	4.70
3	2.00	12.50	16.40	14.80
4	2.40	2.70	5.00	9.40
5	3.60	14.90	6.50	6.50
6	.03	10.90	2.00	14.10
7	1.16	2.30	5.30	14.10
8	6.30	30.70	10.20	24.80
9	4.60	24.20	6.60	4.30
10	21.10	7.20	5.50	14.80
11	11.60	5.10	4.70	10.80
12	23.10	14.60	3.40	6.90
13	5.40	3.80	7.95	3.60
14	13.60	19.60	25.60	17.80
Period 2[c]				
14	13.60	19.60	25.60	17.80
21	17.70	3.83	6.20	19.40
24	6.15	8.35	2.68	14.76
Period 3[d]				
24	6.15	8.35	2.68	14.76
27	4.15	0	4.29	21.53
31	11.25	1.60	0	11.99

[a]Data expressed as umole/gram intestinal contents.
[b]SEM, treatment = 4.93; period = 5.06.
[c]SEM, treatment = 6.67; period = 6.18.
[d]SEM, treatment = 5.10; period = 3.50.

Table 20. Influence of antimicrobials on total colonic VFA[a].

Day	Treatment			
	CO	CTC	CARB	CU
Period 1[b]				
0	44.96	102.50	35.00	74.70
1	NA[c]	NA	NA	NA
2	81.81	125.30	39.10	60.40
3	60.27	79.90	55.00	59.40
4	91.52	75.50	62.10	57.50
5	94.84	91.60	50.40	71.30
6	92.65	104.30	61.80	67.30
7	116.34	80.40	73.20	64.80
8	76.27	72.30	118.20	81.50
9	124.58	108.30	88.00	106.00
10	91.88	92.40	65.50	111.30
11	105.94	102.90	119.10	142.30
12	118.40	140.70	135.70	141.30
13	83.85	79.90	81.00	103.20
14	89.55	68.42	82.14	90.61
Period 2[d]				
14	89.55	68.42	82.14	90.61
21	68.08	71.94	62.31	105.57
24	52.35	82.00	80.00	79.00
Period 3[e]				
24	52.35	82.00	80.00	79.00
27	62.20	81.35	79.15	88.00
31	79.41	86.00	66.87	110.45

[a]Data expressed as umole/gram intestinal contents.
[b]SEM, treatment = 7.73; period = 10.58.
[c]Not available.
[d]SEM, treatment = 10.72; period = 8.38.
[e]SEM, treatment = 11.78; period = 12.31.

APPENDIX 3: DATA FROM TRIAL 3

Table 21. Influence of copper on the length of the DNA synthesis phase[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	8.50	8.60	NS
Jejunum A	9.25	8.15	.20
Jejunum B	9.65	7.20	.10
Ileum	7.45	6.85	NS
Cecum	12.55	10.40	.10
Colon	9.20	8.45	NS

[a]Hours. SEM: treatment = .43; site = .77.
[b]Not significant, P >.25.

Table 22. Influence of copper on the labelling index[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	31.23	21.37	.10
Jejunum A	29.76	32.22	NS
Jejunum B	23.38	36.93	.05
Ileum	33.27	34.41	NS
Cecum	33.53	28.05	.25
Colon	30.57	26.61	.25

[a]Percent. SEM: treatment = 3.62; site = 3.09.
[b]Not significant, P >.25.

Table 23. Influence of copper on the cell generation interval[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	27.41	41.18	.15
Jejunum A	33.00	26.49	NS
Jejunum B	44.55	20.37	.05
Ileum	22.39	19.93	NS
Cecum	37.49	38.14	NS
Colon	30.62	35.08	NS

[a]Hours. SEM: treatment = 5.65; site = 5.71.
[b]Not significant, P >.25.

Table 24. Influence of copper on mucosal protein[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	8.36	8.07	NS[b]
Jejunum A	7.23	7.07	NS
Jejunum B	7.13	6.89	NS
Ileum	6.37	6.41	NS
Cecum	4.32	4.99	NS
Colon	4.01	4.21	NS

[a]Mg/gram tissue. SEM: treatment = .47; site = .59.
[b]Not significant, P >.25.

Table 25. Influence of copper on glucose-6-phosphatase[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	6.08	7.05	NS[b]
Jejunum A	6.76	7.56	NS
Jejunum B	6.45	9.42	.11
Ileum	5.04	6.38	NS
Cecum	3.16	2.20	NS
Colon	2.37	2.39	NS

[a]Ug Pi liberated/minute/100 mg protein.
SEM: treatment = 1.01; site = 1.19.
[b]Not significant, P >.25.

Table 26. Influence of copper on alkaline phosphatase[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	35.19	50.08	NS[b]
Jejunum A	136.59	146.57	NS
Jejunum B	109.84	168.09	.08
Ileum	101.35	112.49	NS
Cecum	81.35	72.62	NS
Colon	74.83	68.72	NS

[a]Ug Pi liberated/minute/mg protein.
SEM: treatment = 13.35; site = 17.65.
[b]Not significant, P >.25.

Table 27. Influence of copper on Na/K dependent ATPase[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	31.50	28.14	NS[b]
Jejunum A	37.26	32.35	NS
Jejunum B	31.27	32.90	NS
Ileum	24.07	22.17	NS
Cecum	26.13	25.91	NS
Colon	16.82	14.60	NS

[a]Ug Pi liberated/hour/mg protein.
SEM: treatment = 2.64; site = 4.38.
[b]Not significant, P >.25.

Table 28. Influence of copper on Na/K independent ATPase[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	85.50	91.76	NS[b]
Jejunum A	70.97	71.22	NS
Jejunum B	111.13	121.30	NS
Ileum	110.67	110.49	NS
Cecum	93.42	92.43	NS
Colon	132.26	120.89	NS

[a]Ug Pi liberated/hour/mg protein.
SEM: treatment = 4.53; site = 9.15.
[b]Not significant, P >.25.

Table 29. Influence of copper on total ATPase[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Duodenum	116.95	119.91	NS[b]
Jejunum A	108.23	103.57	NS
Jejunum B	142.39	154.19	NS
Ileum	134.74	132.65	NS
Cecum	119.54	118.34	NS
Colon	149.07	135.50	NS

[a]Ug Pi liberated/hour/mg protein.
SEM: treatment = 5.60; site = 10.37.
[b]Not significant, P >.25.

Table 30. Influence of copper on epithelial cell size[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	6.35	6.64	NS[b]
Jejunum B	6.70	6.44	NS
Ileum	6.35	6.34	NS
Cecum	7.10	6.87	NS
Colon	7.23	7.21	NS

[a]Microns. SEM: treatment = .34, site = .40.
[b]Not significant, P >.25.

Table 31. Influence of copper on crypt depth[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	181.72	189.57	NS[b]
Jejunum B	209.93	171.50	.20
Ileum	195.77	184.87	NS
Cecum	345.31	376.61	.25
Colon	412.43	387.30	NS

[a]Microns. SEM: treatment = 18.4, site = 21.98.
[b]Not significant, P >.25.

Table 32. Influence of copper on crypt depth[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	32.58	32.20	NS[b]
Jejunum B	32.48	28.82	NS
Ileum	29.96	29.05	NS
Cecum	48.99	54.89	.20
Colon	58.29	53.78	.25

[a]Number of cells. SEM: treatment = 2.67, site = 3.15.
[b]Not significant, P >.25.

Table 33. Influence of copper on villus height[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	441.28	368.49	.25
Jejunum B	371.33	327.02	NS
Ileum	386.28	290.02	.15

[a]Microns. SEM: treatment = 44.04, site = 42.70.
[b]Not significant, P >.25.

Table 34. Influence of copper on villus height[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	66.42	52.70	.20
Jejunum B	52.06	49.24	NS
Ileum	63.32	47.58	.15

[a]Number of cells. SEM: treatment = 40.99, site = 40.17.
[b]Not significant, P >.25.

Table 35. Influence of copper on crypt + villus height[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	98.18	84.91	.20
Jejunum B	85.10	78.05	NS
Ileum	93.03	76.63	.15

[a]Number of cells. SEM: treatment = 6.19, site = 6.45.
[b]Not significant, P >.25.

Table 36. Influence of copper on crypt + villus height[a].

Gastrointestinal tract site	Treatment		P-value
	copper	control	
Jejunum A	620.53	558.06	.25
Jejunum B	587.23	498.51	.20
Ileum	582.07	474.90	.15

[a]Microns. SEM: treatment = 43.45, site = 41.75.
[b]Not significant, P >.25.

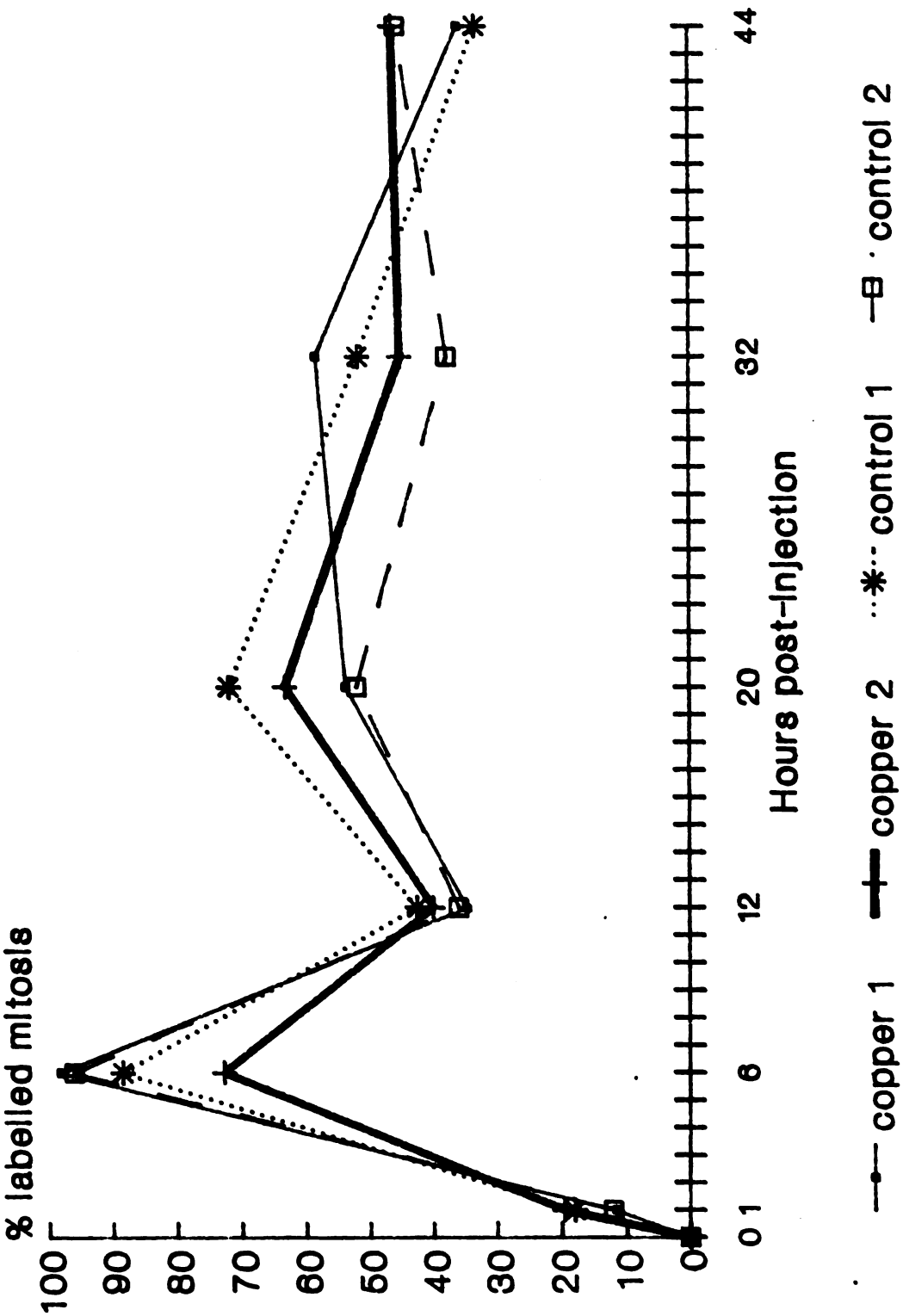


Figure 32. Percentage labelled mitosis curve, duodenum.

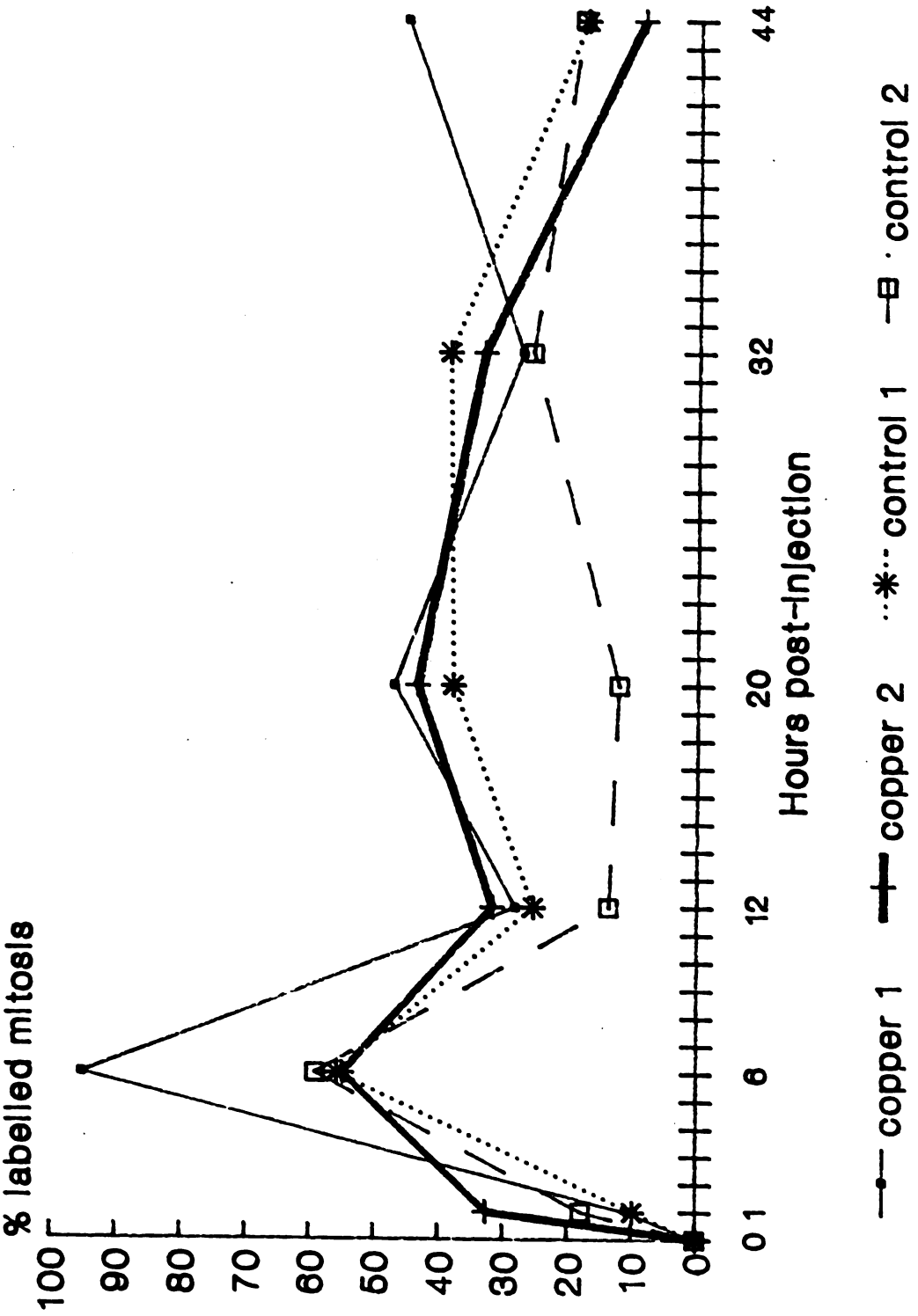


Figure 33. Percentage labelled mitosis curve, jejunum A.

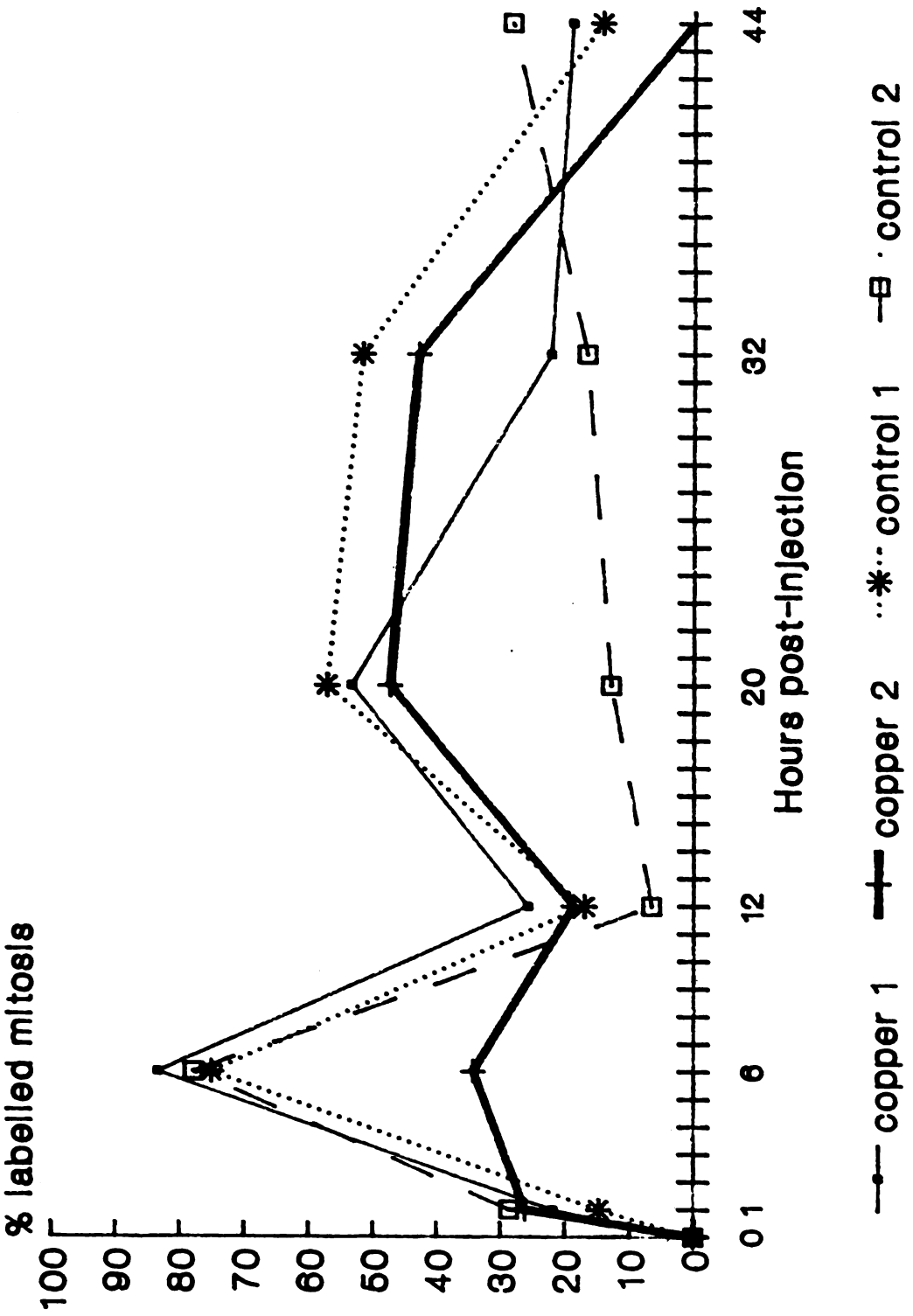


Figure 34. Percentage labelled mitosis curve, jejunum B.

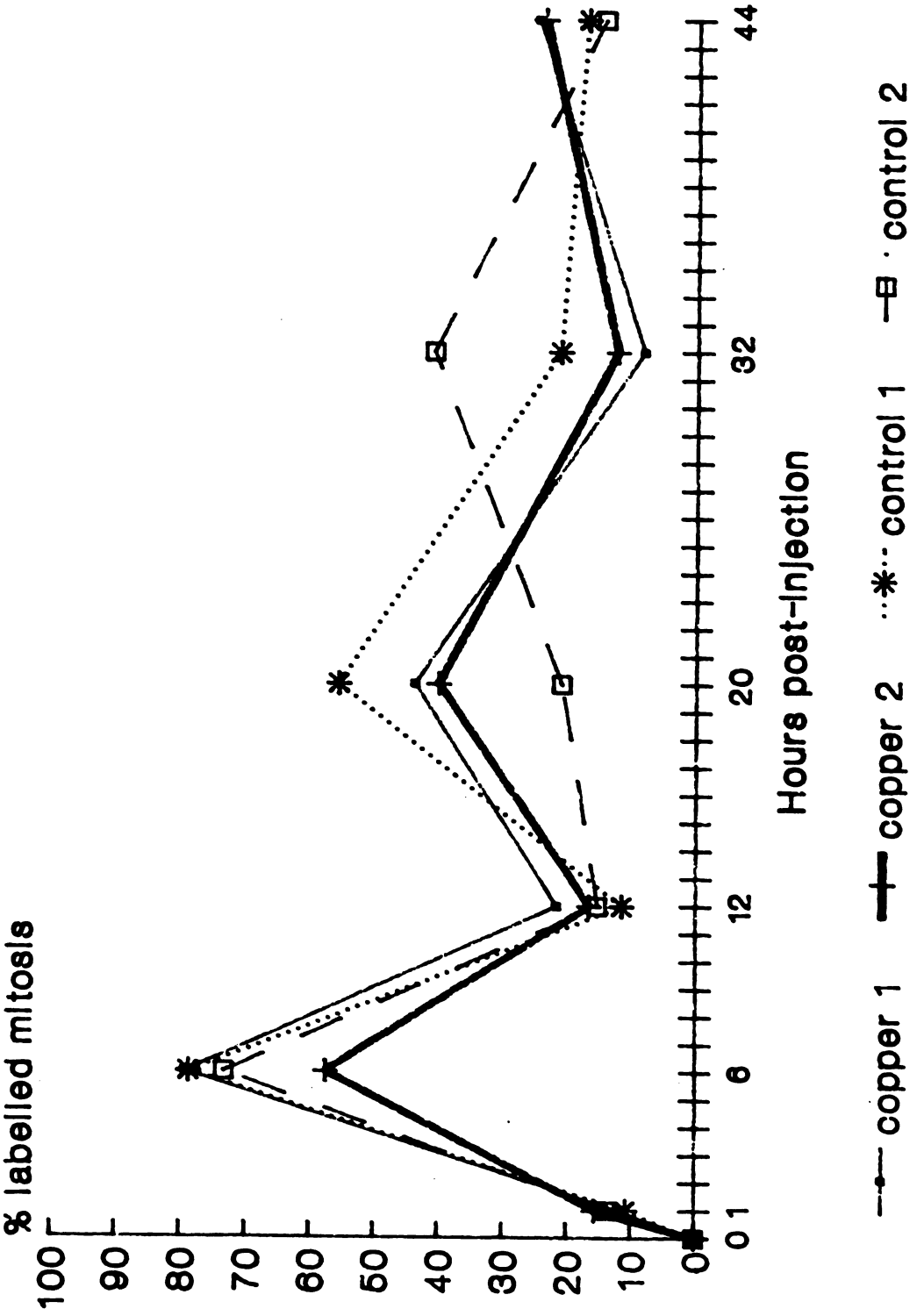


Figure 35. Percentage labelled mitosis curve, ileum.

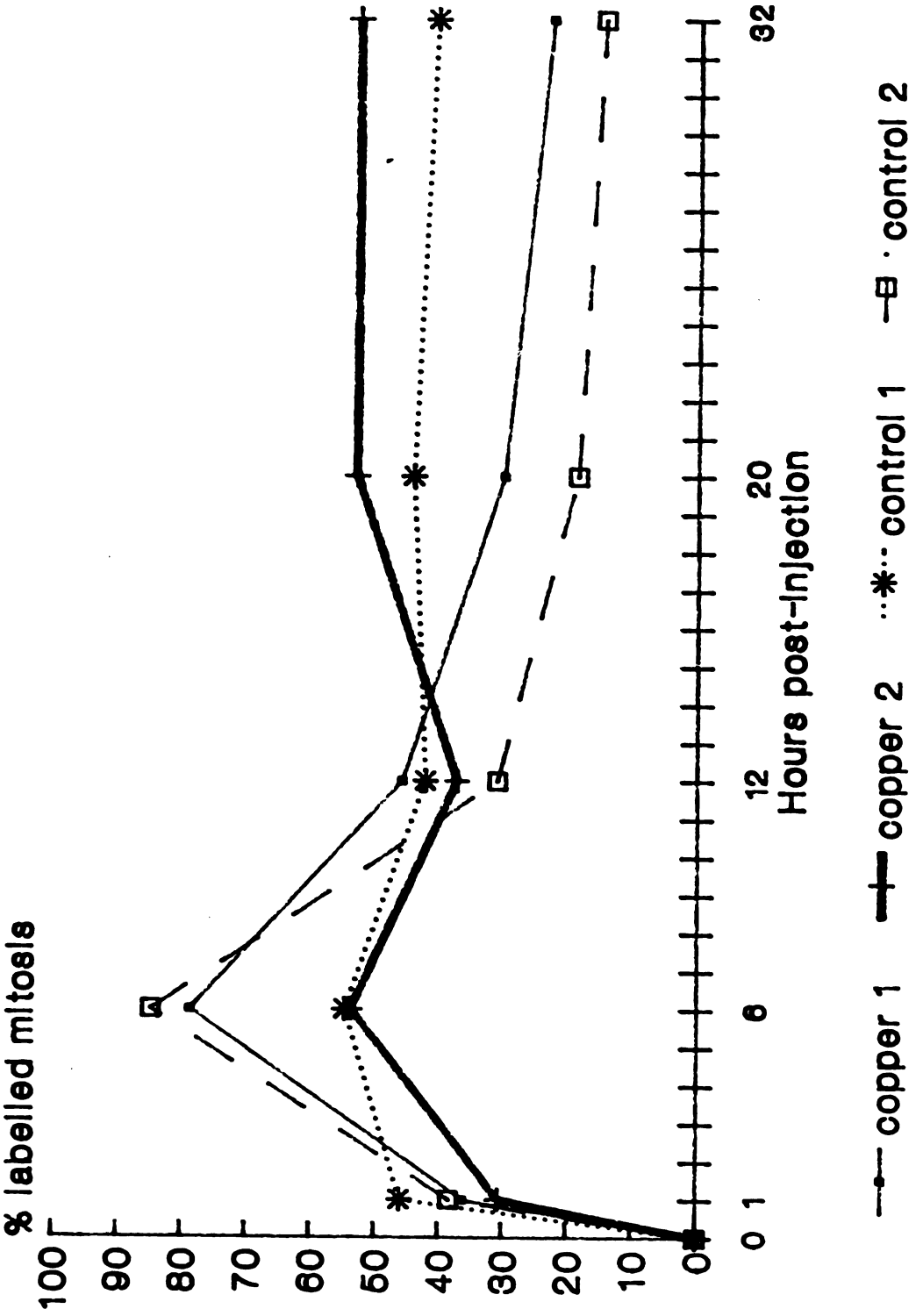


Figure 36. Percentage labelled mitosis curve, cecum.

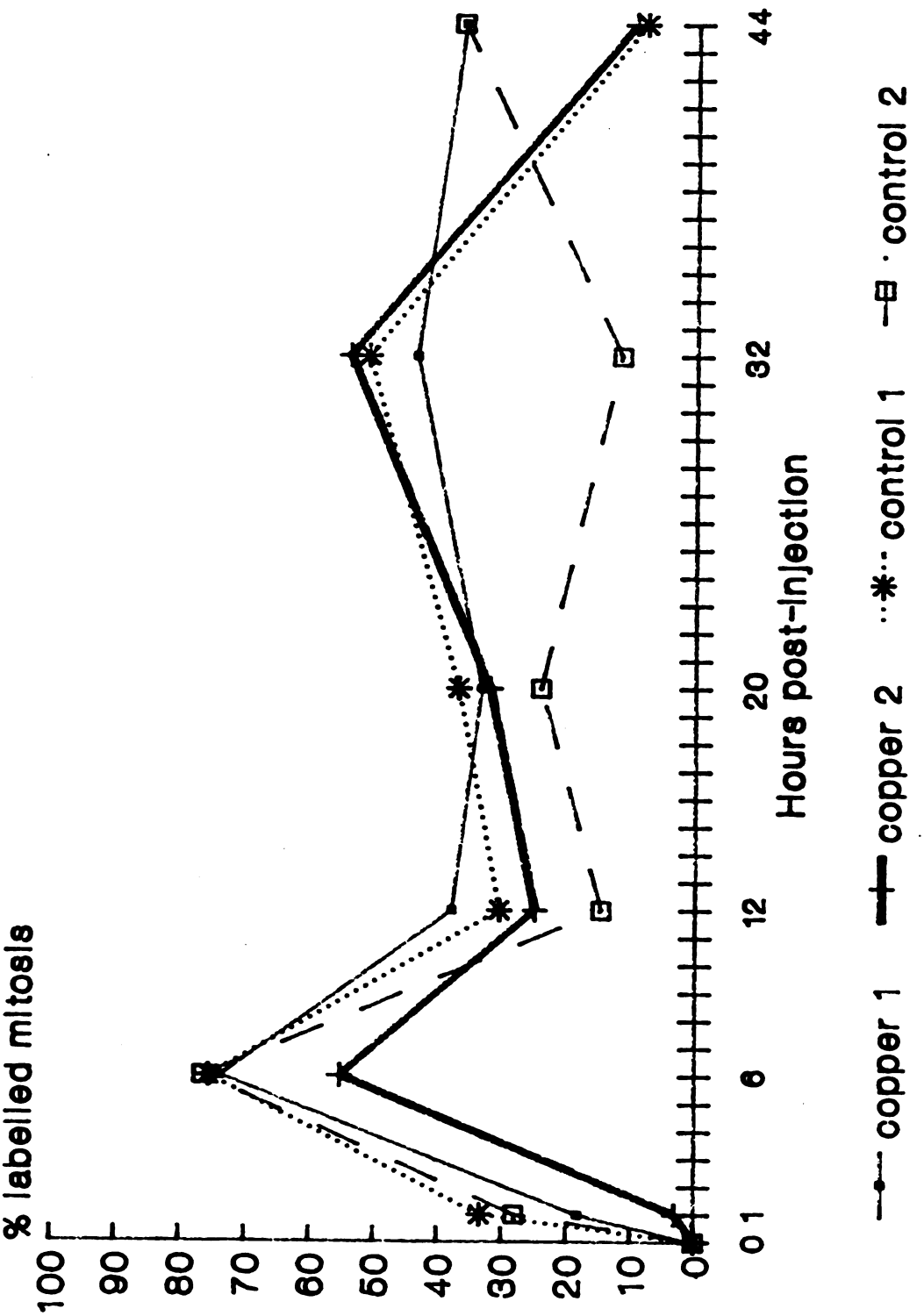


Figure 37. Percentage labelled mitosis curve, colon.

PART IX. BIBLIOGRAPHY

BIBLIOGRAPHY

Abrams, G.D., H. Bauer and H. Sprinz. 1963. Influence of the normal flora on mucosal cell morphology and cellular renewal. Lab. Invest. 12:355.

Argenzio, R.A. and M. Southworth. 1974. Sites of organic acid production and absorption in gastrointestinal tract of the pig. Amer. J. Physiol. 228:454.

Braude, R., M.E. Coates, M.K. Davies, G.F. Harrison, and K.G. Mitchell. 1955. The effect of aureomycin on the gut of the pig. Brit. J. Nutr. 9:363.

Bryant, M.P. and I.M. Robinson. 1961. An improved non-selective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446.

Carpenter, L.E. 1950. Effects of aureomycin on the growth of weaned pigs. Arch. Biochem. 27:469.

Chaney, A.L. and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130.

Cheeseman, G.C. and R. Fuller. 1966. A study by high voltage electrophoresis of the amino acid decarboxyases and arginine dihydrolase of bacteria isolated from the alimentary tract of pigs. J. Appl. Bacteriol. 29:596.

Christie, A. and P.D. Cranwell. 1976. Lactic acid utilization by the baby pig. Proc. Nutr. Soc. 35:27A.

Christie, P.J., J.N. Davidson, R.P. Novick and G.M. Dunny. 1983. Effects of tylosin feeding on the antibiotic resisitance of selected gram-positive bacteria in pigs. Amer. J. Vet. Res. 44:126.

Cleaver, J.E. 1967. Thymidine metabolism and cell kinetics. In: Frontiers of Biology, Vol 6. A. Neuberger and E.L. Tatum, Eds. North-Holland Publishing Co., Amsterdam.

Coates, M.E., M.K. Davies, and S.K. Kon. 1955. The effect of antibiotics on the intestine of the chick. Brit. J. Nutr. 9:110.

Coates, M.E., R. Fuller, G.F. Harrison, M.Lev, and S.F. Suffolk. 1963. A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Brit. J. Nutr. 17:141.

Coates, M.E. and R. Fuller. 1977. The gnotobiotic animal in the study of gut microbiology. In: R.T.J. Clarke and T. Bauchop (Ed.) Microbial Ecology of the Gut. Academic Press, New York.

Cole, C.B. and R. Fuller. 1984. Bile acid deconjugation and attachment of chicken gut bacteria: Their possible role in growth depression. Brit. Poult. Sci. 25:227.

Cooper, J.W., R.F. Hagemann, and G.J. Brodmerkel, Jr. 1974. On the determination of intestinal epithelial cell generation time from labeling index and DNA synthesis duration. Experientia. 30:186.

Cori, G.T. and C.F. Cori. 1952. Glucose-6-phosphatase of the liver in glycogen storage disease. J. Biol. Chem. 199:661.

Cranwell, P.D., D.E. Noakes and K.J. Hill. 1976. Gastric secretion and fermentation in the suckling pig. Brit. J. Nutr. 36:71.

Dang, H.C. and W.J. Visek. 1968. Some characteristics of blood in normal and immune rabbits after urease injection. Amer. J.Physiol. 215:502.

Dawson, K.A., B.E. Langlois, T.S. Stahly and G.L. Cromwell. 1984. Some characteristics and antibiotic resistance of anaerobic bacteria from the ceca and colons of pigs fed chlortetracycline-containing and unmedicated diets. App. Environ. Micorbiol. 47:210.

Dierick, N.A., I.J. Vervaeke, J.A. Decuypere and H.K. Henderickx. 1986a. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. I. Studies in vitro. Livestock Prod. Sci. 14:161.

Dierick, N.A., I.J. Vervaeke, J.A. Decuypere and H.K. Henderickx. 1986b. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. II. Studies in vivo. Livestock Prod. Sci. 14:177.

Drasar, B.S. and M.J. Hill. 1974. Human intestinal flora. Academic Press, Inc., London, England.

Eyssen, H., M. Vandeputte, and E. Evard. 1965. Effect of various dietary bile acids on nutrient absorption and on liver size in chicks. Arch. Int. Pharmacodyn. 158:292.

Eyssen, H. 1973. Role of the gut microflora in metabolism of lipids and sterols. Proc. Nutr. Soc. 32:59.

Farrell, D.J. and K.A. Johnson. Utilization of cellulose by pigs and its effect on caecal function. Anim. Prod. 14:209.

Feighner, S.D. and M.P. Dashkevicz. 1987. Subtherapeutic levels of antibiotics in poulty feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Appl. Environ. Microbiol. 53:331.

Fenwick, B.W. and H.J. Olander. 1987. Experimental infection of weanling pigs with Salmonella typhisuis: Effect of feeding low concentrations of chlortetracycline, penicillin and sulfamethazine. Amer. J. Vet. Res. 48:1568.

Fernandez, J.A., H. Jorgensen, A, Just, and J.O. Andersen. 1982. The influence of differently treated barley straw, nutrient density and virginiamycin on the fermentation potential of hind gut digesta. In: Proceedings of the 3rd International seminar on digestive physiology in the pig. A.Just, H. Jorgensen and J.A. Fernandez, Eds. Beretning fra Statens Husdyrbrugsforsog. 580:284.

Friend, D.W., H.M. Cunningham and J.W.G. Nicholson. 1963a. The production of organic acids in the pig. 2. The effect of diet on the levels of volatile fatty acids and lactic acid in sections of the alimentary tract. Can. J. Anim. Sci. 43:156.

Friend, D.W., H.M. Cunningham and J.W.G. Nicholson. 1963b. Volatile fatty acids and lactic acid in the alimentary tract. Can. J. Anim. Sci. 43:174.

Gill, J.L. 1978. Design and analysis of experiments. The Iowa State University Press, Ames.

Ginty, D.D., M. Marlowe, P.H. Pekala, and E.R. Seidel. 1990. Multiple pathways for the regulation of ornithine decarboxylase in intestinal epithelial cells. Am. J. Physiol. 258: G454.

Gomori, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. J. Lab. Clin. Med. 27:955.

Grant, A.L., J.W. Thomas, K.J. King, and J.S. Liesman. 1990. Effects of dietary amines on small intestinal variables in neonatal pigs fed soy protein isolate. J. Amer. Sci. 68:363.

Hawbaker, J.A., V.C. Speer, V.W. Hays, and D.V. Catron. 1961. Effects of copper sulfate and other chemotherapeutics in growing swine rations. J. Anim. Sci. 20:163.

Hedde, R.D., K.J. Ermillo, R. Quach, T.O. Lindsey, and R.C. Parish. 1982. Microbial aspect of swine's digestion studied. Feedstuffs. December, pp. 28.

Henderickx, H.K. and J.A. Decuypere. 1972. In: Germfree Research-Biological effect of Gnotobiotic Environments. (J.B. Heneghan, Ed.) Academic Press, New York. p. 361.

Hirano, S., N. Masuda and H. Oda. 1981. Effect of neomycin on the in vitro transformation of bile acids by mixed microbial flora of human feces. Microbiol. Immunol. 25:1191.

Hirschhorn, N., and I. H. Rosenberg. 1968. Sodium-potassium adenosine triphosphatase of the small intestine of man: Studies in cholera and diarrheal disease. J. Lab. Clin. Med. 72:28.

Hungate, R.E. 1970. A roll tube method for cultivation of strict anaerobes. In: Methods in Microbiology, Vol 3. Norris, J.R. and D.W. Ribbons (Eds). Acad. Press, Inc. NY.

Hylemon, P.B. and E.J. Stellwag. 1976. Bile acid transformation rates of selected gram-positive and gram-negative intestinal anaerobic bacteria. Biochem. Biophys. Res. Commun. 69:1088.

Imondi, A.R. and F.H. Bird. 1965. The turnover of intestinal epithelium in the chick. Poultry Sci. 45:142.

Imoto, S. and S. Namioka. 1978a. VFA production in the pig large intestine. J. Anim. Sci. 47:467.

Imoto, S. and S. Namioka. 1978b. VFA metabolism in the pig. J.Anim. Sci. 47:479.

Jukes, T.H., E.G.R. Stokstad, R.R.Taylor, T.J. Cunha, H.M. Edwards, and G.B. Meadows. 1950. Growth promoting effect of aureomycin on pigs. Arch. Biochem. 26:324.

Kass, M.L., P.J. Van Soest, and W.G. Pond. 1980. Utilization of dietary fiber from alfalfa by growing swine. 2. Volatile fatty acid concentrations in and disappearance from the gastrointestinal tract. J. Anim. Sci. 50:192.

Kawai, Y. and M. Morotomi. 1978. Intestinal enzyme activities in germfree, conventional, and gnotobiotic rats associated with indigenous microorganisms. Infect. Immunol. 19:771.

Kennelly, J.J., F.X. Aherne, and W.C. Sauer. 1981. Volatile fatty acid production in the hindgut of swine. Can. J. Anim. Sci. 61:349.

Kim, K., D.E. Jewell, N.J. Benevenga, and R.H. Grummer. 1978. The fraction of dietary lactose available for fermentation in the cecum and colon of pigs. J. Anim. Sci. 46:1658.

Larson, N.L. and E.G. Hill. 1960. Amine formation and metabolic activity of microorganisms in the ileum of young swine fed chlortetracycline. J. Bacteriol. 80:188.

Leblond, C.P. and B.E. Walker. 1956. Renewal of cell populations. Physiol. Rev. 35:255.

Lesher, S., H.E. Walburg, and G.A. Sacher. 1964. Generation cycle in the duodenal crypt cells of germ-free and conventional mice. Nature. 202:884.

Lipkin, M., P. Sherlock, and B.M. Bell. 1962. Generation time of epithelial cells in the human colon. Nature. 195:175.

Lowry, O. H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265.

Mason, V.C. 1979. Role of the large intestine in the processes of digestion and absorption in the pig. In: Current Concepts of Digestion and Absorption in Pigs. A.G. Low and I.G. Patridge, Eds. National Institute for Research in Dairing, Reading, England. Page 112.

Menten, J.F.M. 1988. Effects of high dietary copper on the utilization of nutrients and on blood and intestinal variables of starter pigs. Ph.D. Dissertation, Michigan State University, East Lansing.

Moon, H.W. 1970. Epithelial cell migration in the alimentary mucosa of the suckling pig. Proc. Soc. Exp. Biol. Med. 137:151.

Parker, D.S., R.C. MacGregor, H.J. Finlayson, P. Stockill, and J. Balos. 1984. The effect of including avoparcin in the diet on cell turnover and enzyme activity in the mucosa of the rat small intestine. Can. J. Anim. Sci. 64(Suppl): 136.

Parker, D.S. and D.G. Armstrong. 1987. Antibiotic feed additives and livestock production. Proc. Nutr. Soc. 46:415.

Parker, D.S. 1990. Manipulation of the functional activity of the gut by dietary and other means (antibiotics/probiotics) in ruminants. J. Nutr. 120:639.

Prior, R.L., D.C. Topping, and W.J. Visek. 1974. Metabolism of isolated chick small intestinal cells. Effects of ammonia and various salts. Biochem. 13:178.

Radecki, S.V., E.R. Miller, and M.T. Yokoyama. 1988a. In vitro microbial production of volatile fatty acids from gastrointestinal contents of weaned pigs. J. Anim. Sci. 66(suppl. 1): 319.

Radecki, S.V., E.R. Miller, and M.T. Yokoyama. 1988b. In vitro microbial production of p-cresol and ammonia from gastrointestinal contents of weaned pigs. J. Anim. Sci. 66(suppl. 1):138.

Ratcliffe, B. 1985. The influence of the gut microflora on the digestive processes. In: Proceedings of the 3rd International seminar on digestive physiology in the pig. A. Just, H. Jorgensen and J.A. Fernandez, Eds. Beretning fra Statens Husdyrbrugsforsog. 580:245.

Ripley, P.H., and D.J. Brown. 1978. The effect of an antibacterial growth promoter on the levels of alkaline phosphatase. Proc. Nutr. Soc. 38:10A.

Robinson, I.A., S.C. Whipp, J.A. Bucklin, and M.J. Allison. 1984. Characterization of predominant bacteria from the colon of normal and dysenteric pigs. Appl. Environ. Microbiol. 48:964.

Robinson, J.A., W.J. Smolenski, M.L. Ogilvie, and J.P. Peters. 1989. In vitro total-gas, CH_4, H_2, volatile fatty acid, and lactate kinetics studies on luminal contents from the small intestine, cecum, and colon of the pig. Appl. Environ. Microbiol. 55:2460.

Rolls, B.A., A. Turvey, and M.E. Coates. 1978. The influence of the gut microflora and of dietary fibre on epithelial cell migration in the chick intestine. Brit. J. Nutr. 39:91.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

Shurson, G.C. 1986. Mode of action of high dietary copper in promoting growth of weanling pigs. Ph.D. Disseration. Michigan State University.

Shurson, G.C., P.K. Ku, G.L. Waxler, M.T. Yokoyama, and E.R. Miller. 1990. Physiological relationships between microbiological status and dietary copper levels in the pig. J. Anim. Sci. 68:1061.

Staruszkiewicz, W.F. and J.F.Bond. 1981. Gas chromatographic determination of cadaverine, putrescine, and histamine in foods. J. Assoc. Off. Anal. Chem. 64:584.

Topping, D.C. and W.J. Visek. 1977. Synthesis of macromolecules by intestinal cells incubated with ammonia. Amer. J. Physiol. 233:E341.

Tracy, J.D., R.V. Allhands, D.C. Savage, and A.H. Jensen. 1986. The effects of a dietary antimicrobial on the biological half-life of chenodeoxycholic acid and plasma bile acid concentration patterns in the young pig. J. Anim. Sci. 62:997.

Tracy, J.D. and A.H. Jensen. 1987. Effects of a dietary antimicrobial (carbadox) on liver cholesterol 7 alpha-hydroxylase activity and bile acid patterns in the young pig. J. Anim Sci. 65:1013.

Tsubouchi, S. 1983. Theoretical implications for cell migration through the crypt and the villus in labelling studies conducted at each position within the crypt. Cell Tissue Kinet. 16:441.

Varel, H.V., I.A. Robinson, and W.G. Pond. 1987. Effect of dietary copper sulfate, aureo SP-250 or clinoptilolite on ureolytic bacteria found in the pig large intestine. Appl. Environ. Microbiol. 53:2009.

Vervaeke, I.J., J.A. Decuypere, N.A. Dierick, and H.K. Henderickx. 1979. Quantitative in vitro evaluation of the energy metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. J. Anim. Sci. 49:846.

Visek, W.J. 1964. Urease immunity in liver disease: A new approach. Gastroenterology. 46:326.

Visek, W.J. 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. 31:1178.

Visek, W.J. 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447.

Vonk, M.A.A., L.W. McElroy, and R.T. Berg. 1957. The effect of ingested chlortetracycline on some hydrolases and organs associated with the digestive process in growing pigs. III. Protease, amylase, and cellulase activity of the contents of the small intestine and of the cecum: weights of small intestine and mucosa. Can. J. Biochem. Physiol. 35:195.

Walton, J.R. 1988. Bacterial resistance to antibiotics-the present position. Pig News Inform. 9:June: 125.

Ward, L.A., K.A. Johnson, I.M. Robinson, and M.T. Yokoyama. 1987. Isolation from swine feces of a bacterium which decarboxylates p-hydroxyphenylacetic acid to 4-methylphenol (p-cresol). Appl. Environ. Microbiol. 53:189.

Whitehair, C.K. and C.M. Thompson. 1956. Observations on rearing disease-free swine. J. Amer. Vet. Med. Assoc. 128:94.

Wright, N.A. 1980. Cell proliferation in the normal gastrointestinal tract. Implications for proliferative responses. In: Cell Proliferation in the Gastrointestinal Tract, D.R. Appleton, J.P. Sunter, and A.J. Watson, Eds. Pitman Press, Bath, Great Britain. Pg. 3.

Yen ,J.T., T.L. Veum, and R. Lauscen. 1979. Lysine-sparing effect of carbadox in low protein diets for young pigs. J. Anim. Sci. 49 (suppl 1):257.

Yen, J.T., J.A. Nienaber, W.G. Pond, and V.H. Varel. 1985. Effect of carbadox on growth, fasting metabolism, thyroid function and gastrointestinal tract in young pigs. J. Nutr. 115:970.

Yen, J.T., J.A. Nienaber, D.A. Hill, and W.G. Pond. 1988. Oxygen consumption by whole body and by portal drained organs in swine. J. Anim. Sci. 66(1):331.

Yen, J.T., J.A. Nienaber, D.A. Hill, and W.G. Pond. 1989. Effect of carbadox on fasting oxygen consumption by whole body and by portal vein drained organs in swine. J. Anim. Sci. 67(2):127.

Yen, J.T. and W.G. Pond. 1990. Effect of carbadox on net absorption of ammonia and glucose into hepatic portal vein of growing pigs. J. Anim. Sci. In press.

Yokoyama, M.T., C. Tabori, E.R. Miller, and M.G. Hogberg. 1982. The effects of antibiotics in the weanling pig diet on growth and the excretion of volatile phenolic and aromatic bacterial metabolites. Amer. J. Clin. Nutr. 35:1417.

Zimber, A. and W.J. Visek. 1972. Effect of urease injections on DNA synthesis in mice. Amer. J. Physiol. 223:1004.

Zimmerman, D.R. 1986. Role of subtherapeutic levels of antimicrobials in pig production. J. Anim. Sci. 62 (suppl 3):6.