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1983 Animal Science Research Progress Report

Research Report 84-06 March, 1984

Animal Science Faculty

Dept. of Animal Science

FOREWORD

Please accept with our compliments the 1983 Animal Science Research Progress Report. These summaries will provide a partial overview of our departmental research program. Continuing projects are not included every year. Therefore, a complete overview would require two or three years to accomplish. It is fully intended that much of this information will eventually appear in more detailed publications, experiment station bulletins or journal articles, but if you have more immediate need for additional information, feel free to contact the authors.

1983 was both a productive, sometimes very special year and at other times a trying year in the Animal Science Department. The death of Dr. E. W. Swanson during February, 1983; the departures of Dr. J. W. Holloway for a position with Texas A & M and of Dr. Earl Cartee for Auburn University; and the retirement of Mrs. Joyce Goolsby after 37 years of service with the department were noteworthy losses to our program during the last year. On the other hand, we were pleased to have had Dr. B. R. Bell join us in January of 1983, as a dairy cattle geneticist; Dr. J. D. Godkin in June, 1983, as a bovine reproductive physiologist; Dr. B. H. Erickson in August, 1983, as a bovine reproductive physiologist; Dr. R. W. Henry in September, 1983, as a gross anatomist; and Dr. T. W. McDonald in October, 1983, as a hematologist. In addition, we have successfully recruited for a mammary physiology position and a beef cow/calf management position and these individuals will join us in the Spring of 1984.

Although 1983 was an austere year in many ways, we were able to initiate remodeling of laboratories and office space in McCord Hall for a headquarters for our reproductive and mammary physiology group. Items such as the updating of the poultry facilities and modification of other animal facilities remain high on our priority lists.

In summary, I remain very impressed with our faculty and staff and their dedication to our departmental and institutional missions. The last few years have been lean years in a budgetary sense but the faculty and staff morale, loyalty, and dedication to our goals are items that are most rewarding to me as department head. I believe we are blessed with a solid group of dedicated people and it is a pleasure to work with them.

Don O. Richardson
Professor and Head

TABLE OF CONTENTS

BEEF CATTLE PAGE

Passage of gentamicin from the blood into the ejaculate of bulls..... 2

A comparison of rumensin and bovatec in whole corn and whole corn-whole wheat finishing rations..... 4

A look at mineral variability and solubility in fertilized fescue pastures during fall season..... 6

Effects of excessive K intake from fertilized pasture compared with KCl boluses on excretion of Mg by lactating beef cows..... 8

Effects of excess K intake from fertilized pasture compared with KCl boluses on plasma Mg and Ca in lactating beef cows..... 10

Vitamins A, D and E injection effects on pregnant beef cow plasma Ca, Mg, and K on calf viability..... 12

Effect of magnesium supplements on plasma magnesium in beef cows..... 14

The utility of measures of fatness and skeletal size in explaining weight and size differences in yearling Angus and Hereford bulls..... 16

Calfhood weights, body measurements and estimates of body composition and criteria of overall size and shape for prediciting yearling performance in beef cattle..... 18

Relationship of feeder calf size and shape to changes in rumenal characteristics during marketing and transporting..... 20

Comparison of balance and tissue data in hypomagnesemic and normomagnesemic calves..... 22

Fall nitrogen fertilization and spring renovation of fescue pastures..... 24

Effects of K fertilization of pastures and oral KCL on plasma minerals, glucose and insulin levels of lactating beef cows..... 26

DAIRY CATTLE

Response to selection for milk yield in Jerseys..... 28

Evaluation of effects of various calving intervals and female replacement policies on the performance of dairy herd..... 30

The effect of a long half-life, high-activity prostaglandin analogue, fenprostalene, on uterine motility in the bovine..... 32

PAGE

Hypomagnesemia in young calves and effects of magnesium repletion on plasma and CSF mineral levels..... 34

Correlated response in feed efficiency accompanying selection for milk yield in Jerseys..... 36

SWINE

Stem-spermatogonial survival and incidence of reciprocal translocations in the γ-irradiated boar..... 38

Reproductive and genetic effects of continuous prenatal irradiation in the pig..... 40

Hydrocortisone acetate or ACTH administration during diestrus in gilts: I. Effects on peripheral plasma cortisol and progesterone levels..... 42

24-hour peripheral plasma cortisol levels in gilts following once vs twice daily injection with ACTH or hydrocortisone acetate..... 44

Hydrocortisone acetate or ACTH administration during diestrus in gilts: II. Effects on total uterine proteins and corpora lutea LH/hCH binding characteristics..... 46

A comparison of three farrow-finish pork production systems at Ames Plantation..... 48

Effect of rearing environment on subsequent reproductive performance of gilts..... 50

Evaluation of Tennessee feeder pig grades..... 52

POULTRY

The induction of cage layer fatigue in laying hens..... 54

SHEEP

Effects of β-hydroxybutyrate on ketogenesis in fasted ewes..... 56

Effects of β-hydroxybutyrate on ketogenesis in normal and diabetic sheep... 58

Use of "Exotic Breeds" of sheep to increase lamb production in Tennessee... 60

Hypomagnesemia and hypertension in pregnant or lactating ewes..... 62

PAGE

MISCELLANEOUS

Morphometric analysis of mitochondrial alterations induced by selected monoaromatics..... 64

Testing of hyperadnenocorticism in the dog by the combined dexamethasone suppression/ACTH stimulation procedure..... 66

Effects of a thrombocytopoiesis-stimulating factor on megakaryocytopoiesis of mice..... 68

The site of production of thrombopoietin..... 70

Structure-activity correlations of selected azaarenes, aromatic amines, and nitroaromatics..... 72

Electron microscopic analysis of growth phase dependent phagocytosis of Pasteurella hemolytica by bovine pulmonary macrophages in vitro..... 74

The mitigation of acute toxicity of coal-derived liquids by hydro-treatment..... 76

The development and validation of the frog embryo teratogenesis assay: Xenopus..... 78

Teratogenicity and embryotoxicity of monosodium methanearsonate herbicide.. 80

ACKNOWLEDGMENTS..... 82

ANIMAL SCIENCE RESEARCH FACILITIES..... 83

ANIMAL SCIENCE DEPARTMENT RESEARCH FACULTY AND STAFF..... 84

ANIMAL SCIENCE GRADUATE STUDENTS..... 85

PUBLICATION LIST FOR ANIMAL SCIENCE..... 88

ABSTRACTS OF PAPERS PRESENTED..... 91

University of Tennessee
Agricultural Experiment Station

1983 ANIMAL SCIENCE RESEARCH PROGRESS REPORT

RR No. 84-06 March, 1984

DEPARTMENT OF ANIMAL SCIENCE

PASSAGE OF GENTAMICIN FROM THE BLOOD INTO THE EJACULATE OF BULLS

C. S. Armstrong-Backus, R. D. Walker, H. Eiler and W. A. Lyke

The lumen of the seminiferous tubules seems to be guarded from systemic influence by the blood-testes barrier. This barrier partially isolates the developing spermatids from the blood and its immunological components. However, the passage of steroids, amino acids and alkaloids into the ejaculate has been reported in humans, dogs, rams and bulls. The passage of antibiotics into the ejaculate has been reported in humans, dogs and rams, but has not been investigated in bulls. Researchers have found that men with prostatitis, when compared to controls, have higher levels of antibiotics in the semen. This experiment was conducted in order to determine if gentamicin is able to pass into the ejaculate of bulls.

Four 18-month-old Angus bulls were injected intravenously with 3.0 gm gentamicin, following collection of baseline blood and semen samples. Blood was collected by electroejaculation. Samples were collected at 20, 40, 60, 120, 160, 180 minutes after injection of gentamicin. Samples of seminal and blood plasma were analyzed by both radioimmunoassay and bioassay procedure for gentamicin.

1. In the radioimmunoassay, plasma levels of gentamicin were highest at 20 minutes after injection and slowly declined over the 180 minute period. Concentrations of gentamicin in the semen were apparent at 20 minutes and reached 83.5 µg/ml at 120 minutes and concentrations were back to baseline at 20 hrs.

2. In the bioassay, plasma levels of gentamicin were highest at 20 minutes after injection and slowly decreased over the testing period. Gentamicin was apparent in the semen at 20 minutes and reached a maximum concentration at 120 minutes. Seminal gentamicin concentration was above baseline at 20 hrs.

3. Results from the bioassay and radioimmunoassay were relatively similiar. See figure 1. Standard error of the mean for different points throughout the experiment fluctuated between 14 and 30 percent of the mean.

4. It appears that gentamicin injected intravenously can gain rapid entry into the ejaculate of bulls. Although the anatomical location of the transfer from blood to ejaculate is unknown, this information may be of value in treating reproductive infections in the male. There may also be beneficial effects of using gentamicin in a breeding program to decrease infections. The effects of gentamicin on metabolism of spermatozoa have yet to be determined.

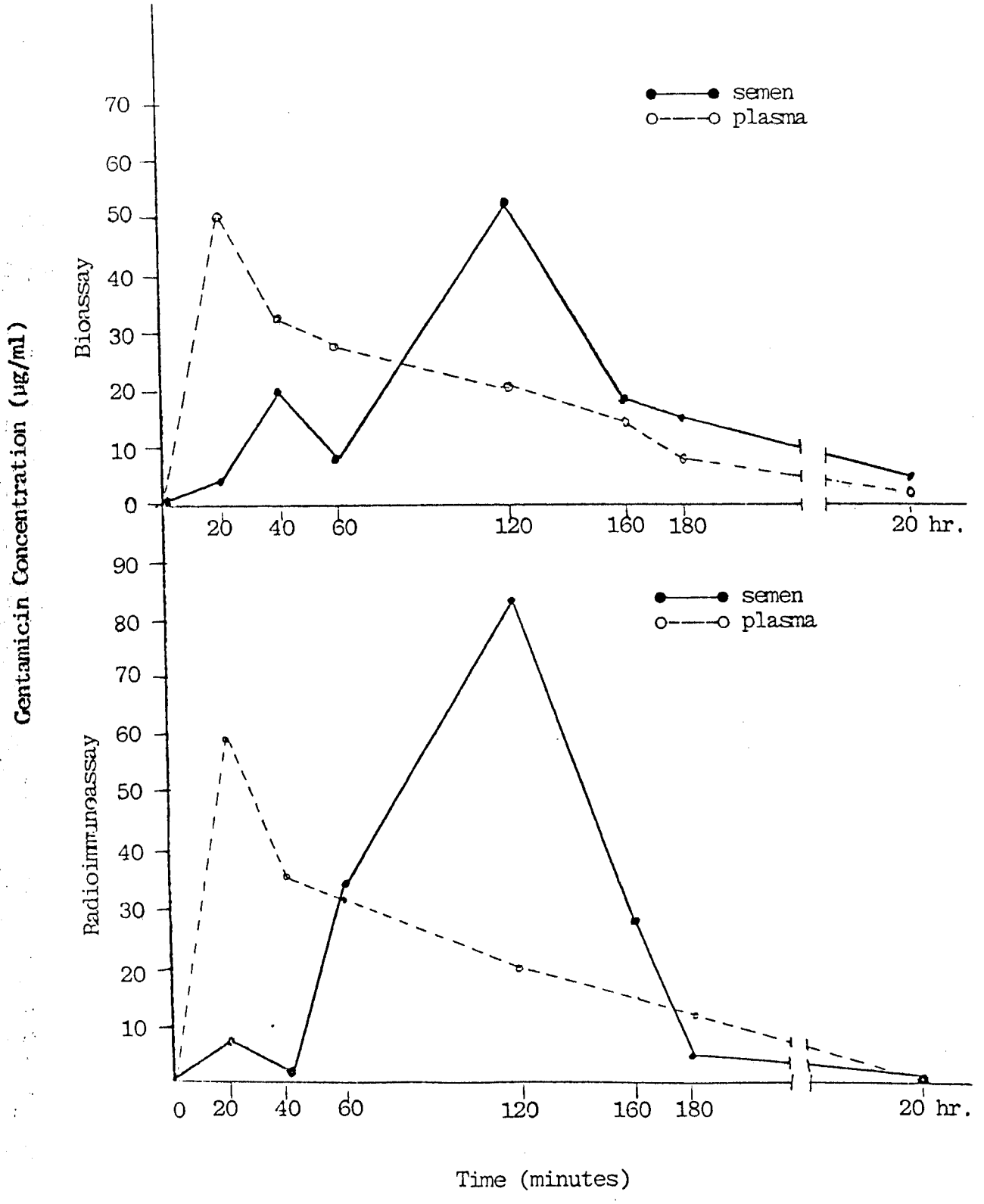


Figure 1. Concentration of gentamicin in blood plasma and semen plasma by radioimmunoassay (bottom) and bioassay (top) at different times after injection (I.V.).

A COMPARISON OF RUMENSIN AND BOVATEC IN WHOLE CORN AND WHOLE CORN-WHOLE WHEAT FINISHING RATIONS

W. R. Backus, B. B. Greene and T. D. Bryson

Rumensin has enjoyed widespread usage in cattle finishing rations, consistently improving feed: gain ratios, while having no effect on ADG. Similar feed intakes are observed, with a 10-15% increase in feed efficiency. Recently, however, Bovatec, a similar product, has been purported to increase both ADG and feed efficiency.

A 106 day feeding trial was conducted to compare the effects of Bovatec and Rumensin in whole grain finishing regimes. Eight-four Angus or Angus X Hereford backgrounded calves were stratified according to breed and weight, then randomly assigned to one of four treatments groups: 1) 100% whole corn + Rumensin, 2) 50% whole corn + 50% whole wheat + Rumensin, 3) 50% whole corn + 50% whole wheat + Bovatec, or 4) 100% whole corn + Bovatec. Following a 14-day adjustment period, feed bunks were managed to ensure ad libitum grain intake. Rumensin or Bovatec was supplied in Tend-R-Lean, a 36% protein supplement produced by Tennessee Farmers Co-operative. Normal production data and carcass characteristics were collected and means are reported in Table 1.

The results of the trial revealed the following:

1. Production parameters were similar across all treatments.

2. Following a 106-day trial, 79% of cattle graded C$^-$ or above.

3. Bovatec produced similar results to Rumensin; usage under whole-grain regimes should be based on factors other than performance advantages, i.e., availability, price, personal preference, etc.

4. Cattle exhibited no acceptability problems associated with Rumensin or Bovatec.

Table 1. Performance and carcass data by treatments

Treatments	100% whole corn + Rumensin	50% whole corn: 50% whole wheat + Rumensin	50% whole corn: 50% whole wheat + Bovatec	100% whole corn + Bovatec
Initial wt. (lbs)	829	824	799	824
Ending wt. (lbs)	1158	1137	1113	1124
ADG (lbs/d)	3.11	2.93	2.95	2.82
Feed intake (lbs/d)	23.11	24.01	23.90	23.99
Feed: gain	7.75	8.19	8.09	8.50
Carcass wt. (lbs)	681	674	659	663
Dressing %[1]	61.23	61.71	61.70	61.47
Marbling[2]	5.78	5.95	5.29	5.71
% KHP	1.88	1.71	1.67	1.83
Yield grade	2.52	2.81	2.62	2.79
Quality grade[3]	12.10	12.48	11.52	12.10
Carcass fat (mm)	11.5	12.48	12.62	12.67

[1]Based on $\frac{\text{hot carcass weight}}{\text{Feedlot wt. - 4\% pencil shrink}}$

[2]Marbling scale: 4 = Slight, 5 = Small, 6 = Modest.

[3]USDA grade: Good^{+} = 11, Choice^{-} = 12, Choice Avg. = 13, etc.

A LOOK AT MINERAL VARIABILITY AND SOLUBILITY IN FERTILIZED FESCUE PASTURE DURING FALL SEASON

J. A. Bacon, M. C. Bell and J. K. Miller

The variability of mineral content of pasture grasses have been noted by many workers and include responses from seasonal change, root temperature, rate of growth, maturity, dry matter, fertilization practices, etc. It is common practice to report these observations as total amount of the mineral element per unit of dry matter. A major medium of movement through the bovine gastrointestinal tract for calcium, potassium and magnesium is an aqueous solution of these minerals and/or a water suspension of plant juices. Since mineral absorption from the rumen is known to occur and some have proposed that it may be a major site of magnesium absorption, there is need to study water solubility of fresh grasses compared to their total mineral content as seasonal and growth changes occur. Two pasture sites that have received different fertilization for ten years were used to collect fresh grass samples. The period of collection was from the time of annual fertilization (in 1983 it was on Sept. 22) through a period of stockpiling (Sept. 22 - Nov. 28) and through a period of heavy grazing (Nov. 28 - Dec. 15). The collections of nine sampling periods were examined by water extraction and a successive 85% acetone extraction for forages from each of the two pasture sites.

Analyses were made of Ca, Mg and K to determine the ratio $\left(\frac{\text{Meq K}}{\text{Meq Mg + Meq Ca}}\right)$ which often used as an index to hypomagnesemia conditions of forages. The larger values reflect greater danger of this condition.

1. Using this ratio to compare total vs water extractable minerals revealed an inverse relationship (Fig. 1). Dry matter content of forage was also related directly to ratios calculated from total but inversely to ratios from water extractable minerals. Water solubility of both Mg and Ca decreased moderately whereas solubility of K decreased sharply as moisture content of rapidly growing grass increased.

2. The ratio $\frac{\text{K extracted in 85\% acetone}}{\text{K extracted in water}}$ increased sharply during the growing or "danger" period but returned to a normal range after 1st freeze and during the grazing period (Fig. 2).

3. If Mg, Ca and K extracted from forage by rumen fluid are the absorbable forms of these minerals, percentages of total forage minerals which are water extractable should be considered when evaluating the tetany producing potential of forage.

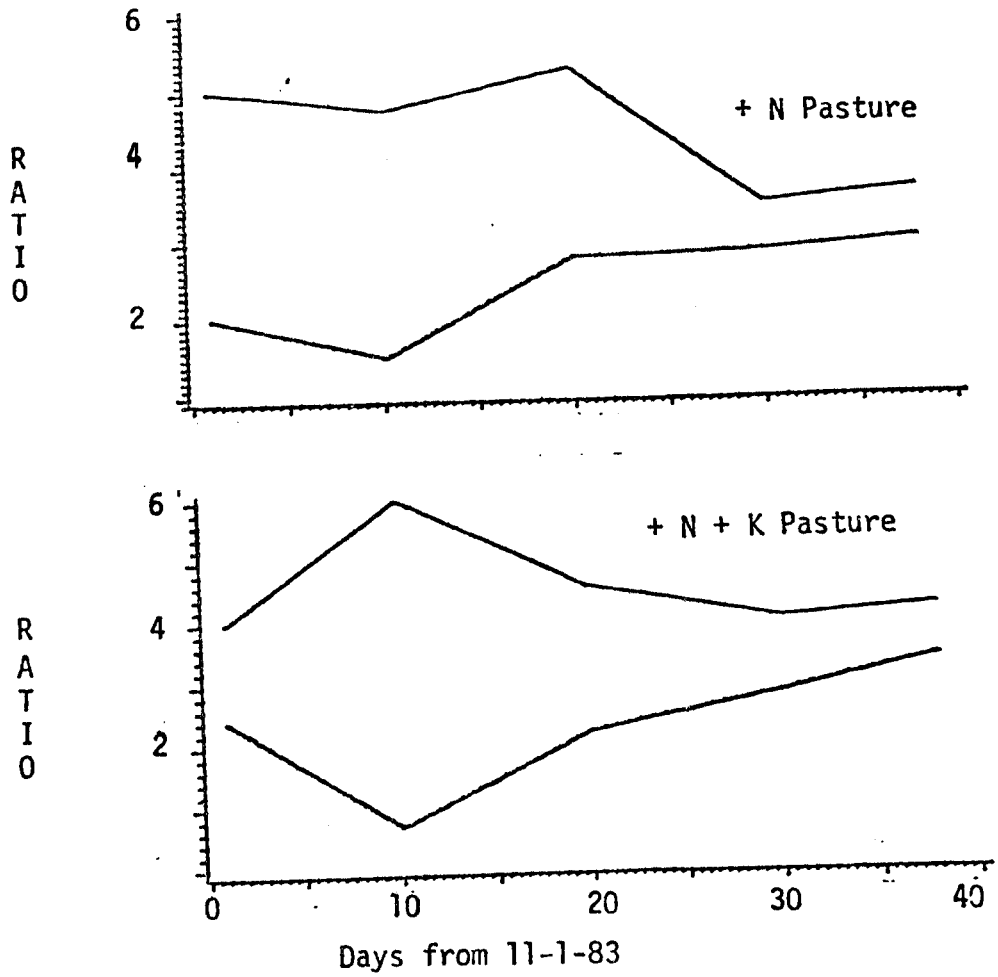


Fig. 1. Ratio of minerals in total ash——and ratio of minerals in water extract-----. Meq K ÷ Meq Ca + Meq Mg.

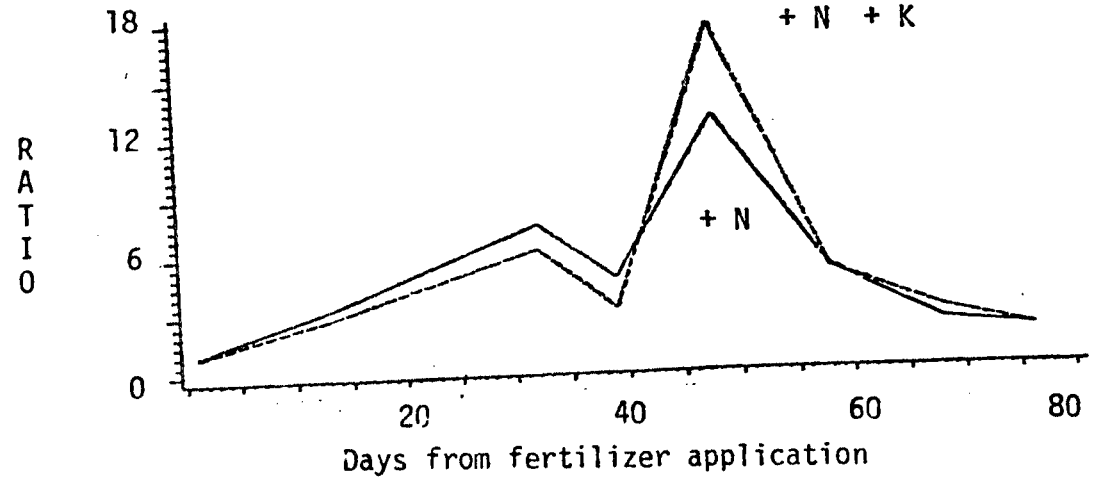


Fig. 2. Ratio of K extracted by 85% acetone: H_2O on dry matter basis.

EFFECTS OF EXCESSIVE K INTAKE FROM FERTILIZED PASTURE COMPARED WITH KCl BOLUSES ON EXCRETION OF Mg BY LACTATING BEEF COWS

S. R. Banet, M. C. Bell and T. Johnson

Concentration of Mg in urine has been suggested as a better monitor of Mg status than plasma Mg in grazing ruminants. Two 14-day pasture studies were conducted in February and March in 1982 and 1983 involving 48 lactating beef cows. In each trail 6 cows were on a fescue pasture fertilized with 39 kg N/ha, and 6 more cows were on another similar fescue pasture fertilized the same plus 117 kg of K/ha. Half of the cows on each pasture was bolused twice daily with 140 g of KCl during the 14-day period. Cows were allotted to treatment to minimize differences in cow weight, age, plasma Mg and age of calf. Urinary creatinine concentration was used to estimate total urinary volume. Calves were kept separate from cows and were allowed to suckle twice daily to estimate milk production using the calf weight-suckle-weight technique. Milk and urine samples were analyzed for Mg, Ca and K.

1. Milk production varied but was inconsistent with pasture and bolus treatment.

2. Excretion of Mg in milk was related to milk production and not to pasture and K bolus treatment.

3. Urinary Mg excretion varied inconsistently with cow treatment in 1982. In 1983, urinary excretion of Mg was lower for cows on the K fertilized pasture. In 1982, urine volume was increased for cows on the K fertilized pasture but not in 1983.

4. Urinary Mg concentration was not as consistent as plasma Mg in showing the effect of pasture fertilization on Mg status of beef cows in early lactation.

Table 1. Milk production (kg/day)

Year	Trial	Pasture and Cow Treatment			
		Control	+K bolus	+K	+K +K bolus
1982[2]	1	5.90[b]	5.54[b]	6.74[a]	5.40[b]
1982[2]	2	5.50[b]	5.48[b]	6.74[a]	5.10[b]
1983[1]	1	9.42[a]	9.22[a]	8.07[b]	7.92[b]
1983[2]	2	7.23[a]	5.42[b]	7.05[a]	7.37[a]

[1]Means within this row with different superscripts are different (P<.01).
[2]Means within this row with different superscripts are different (P<.05).

Table 2. Excretion of Mg in milk (g/day)

Year	Trial	Pasture and Cow Treatment			
		Control	+K bolus	+K	+K +K bolus
1982[2]	1	.62[a]	.51[bc]	.56[ab]	.44[c]
1982[2]	2	.45[b]	.45[b]	.73[a]	.51[b]
1983[1]	1	1.00[a]	.94[a]	.92[a]	.76[b]
1983[2]	2	.79[a]	.59[b]	.82[a]	.81[a]

[1]Means within this row with different superscripts are different (P<.01).
[2]Means within this row with different superscripts are different (P<.05).

Table 3. Urinary Mg excretion (g/day)

Year	Trial	Pasture and Cow Treatment			
		Control	+K bolus	+K	+K +K bolus
1982[2]	1	.13[a]	.08[ab]	.04[b]	.15[a]
1982[2]	2	.10[a]	.03[a]	.50[a]	.01[a]
1983[1]	1	.45[a]	.40[a]	.10[b]	.02[b]
1983[2]	2	.44[a]	.50[a]	.03[b]	.13[ab]

[1]Means within this row with different superscripts are different (P<.01).
[2]Means within this row with different superscripts are different (P<.05).

EFFECTS OF EXCESS K INTAKE FROM FERTILIZED PASTURE COMPARED WITH KCl BOLUSES ON PLASMA Mg AND Ca IN LACTATING BEEF COWS

S. R. Banet, M. C. Bell and T. Johnson

Hypomagnesemia has been shown to be associated with excess K intake in ruminants. Two 14-day pasture studies were conducted in February and March, 1982 and 1983 involving 48 lactating beef cows. In each trail, 6 cows were on a fescue pasture fertilized with 39 kg N/ha, and 6 more cows were on another similar fescue pasture fertilized the same plus 117 kg of K/ha. Half of the cows on each pasture was bolused twice daily with 140 g of KCl during the 14-day period. Cows were allotted to treatment to minimize differences in cow weight, age, plasma Mg and age of calf. Blood samples were taken on day 0, 3, 5, 7, 9, 11 and 13 of each trail for analysis of plasma Mg, Ca and K.

1. Plasma Mg was lower and plasma K was higher ($P<.01$) in 1982 than in 1983.

2. In 1982, KCl bolus had no effect on plasma Mg but plasma Mg was significantly lower for cows on pasture fertilized with excess K compared with cows on the control pasture.

3. In 1983, pasture and bolus effects were noted but inconsistent with trial and treatments.

4. Plasma Ca was lower ($P<.05$) in cows grazing pasture fertilized with excess K compared with control pasture in 1982 and 1983.

5. From these data, we conclude that excess K reduced the availability of forage Mg when applied as fertilizer to pasture but not when administered to cows as KCl boluses.

Table 1. Effect of treatment on plasma Mg (mg/dl) in Trial I and II in 1982

Pasture and cow treatment	Trial 1	Trial 2
Control	1.98[a]	2.36[c]
Control + K bolus	1.78[a]	2.06[c]
+K	1.36[b]	1.46[d]
+K +K bolus	1.30[b]	1.54[d]

[a,b]Means with different superscripts within columns are different (P<.05).
[c,d]Means with different superscripts within columns are different (P<.01).

Table 2. Effect of treatment on plasma Mg (mg/dl) in Trial I and II in 1983

Pasture and cow treatment	Trial 1	Trial 2
Control	2.19[b]	2.32[a]
Control + K bolus	2.69[a]	1.98[b]
+K	1.83[c]	1.88[b]
+K +K bolus	1.87[b,c]	1.58[c]

[a,b,c]Means with different superscripts within columns are different (P<.05).

Table 3. Effect of treatment on plasma Ca (mg/dl)

Pasture and cow treatment	Year 1982	Year 1983
Control	10.24[a]	10.51[a]
Control + K bolus	9.89[b]	10.28[ab]
+K	9.81[b]	10.17[b]
+K +K bolus	10.16[ab]	10.36[ab]

[a,b]Means with different superscripts within columns are different (P<.05).

VITAMINS A, D AND E INJECTION EFFECTS ON PREGNANT BEEF COW PLASMA Ca, Mg, AND K ON CALF VIABILITY

M. C. Bell, R. Q. Synder, J. E. Martin,
P. P. Hunter, J. A. Bacon and T. Johnson

Vitamin injections are being used routinely in many beef cattle herds. Reasons given include calf viability, more live calves born, need for extra vitamin A due to possible nitrate interference, to help prevent "pinkeye", and to prevent vitamin A deficiency. Most vitamin A injection solutions also include vitamin D, and vitamin E is included as a preservative. The purpose of this research was to determine the effects of vitamin A, D and E injections on cows and their calves.

Blood samples were taken from 339 pregnant beef cows at three different experiment stations to determine the effect of vitamin A, D and E injections on plasma vitamin A, carotene, minerals, and on calf viability. Injections were made between December 7 and December 16, 1982. One hundred sixty-eight of the cows were injected with a solution containing 4.5 million I.U. of vitamin A, 675,000 I. U. of D_3 and 450 I. U. of E, while 171 of the cows were injected with the same volume of physiological saline. Calves born to injected cows were scored on viability ranging from 0, being a healthy calf and 5, being a dead calf. Forage samples taken when blood samples were obtained at the three stations were analyzed for beta carotene. Plasma samples were also analyzed for Ca, Mg, K, vitamin A and carotene. Calf viability was scored at birth

1. Vitamin injections had no significant effect on plasma minerals. Research in other states using higher levels of vitamin D had found depressed plasma Mg levels.

2. Plasma samples taken 24 hrs after vitamin injections contained higher ($P<.01$) levels of vitamin A than controls (Table 1). Vitamin A in plasma samples taken 28 days later were lower in preliminary samples.

3. Even though cows were randomly assigned to treatment groups, the control cows at Greeneville had lower initial vitamin A levels which persisted throughout the tests.

4. More calving problems were in the vitamin injected group and more dead calves were in the saline (control) group (Table 2).

Table 1. Effects of vitamin injections on plasma vitamin A in µg/100 ml

Injection	Days after injection 0	1	28
	Greeneville		
Saline	36.51 ± 2.22	37.60 ± 1.20	34.65 ± 1.31
Vitamin A	43.30 ± 1.54	44.56 ± 2.10	41.00 ± 1.24
	Crossville		
Saline	59.82 ± 1.89	58.16 ± 1.75	55.80 ± 2.64
Vitamin A	53.82 ± 2.01	64.38 ± 2.17	48.05 ± 3.22
	Alcoa		
Saline	41.31 ± 1.23	38.48 ± 1.05	34.80 ± 1.47
Vitamin A	40.12 ± 1.89	51.42 ± 2.23	28.31 ± 1.31

Table 2. Effects of cow injections on calves at birth totals— 1982

Calf score	Vitamin	Saline	Total
0 = healthy	131	146	277
1 = minor problem	12	5	17
2 = weak	7	4	11
3 = weak + minor	0	0	0
4 = weak + mechanical assistance	14	8	22
5 = dead calf	4	8	12
	168	171	339

EFFECT OF MAGNESIUM SUPPLEMENTS ON PLASMA MAGNESIUM IN BEEF COWS

M. C. Bell, Lawson Safley, Tina Johnson and J. B. McLaren

Hypomagnesemia and hypomagnesemic tetany usually referred to as grass tetany is a problem with some beef cows in early lactation grazing cool season grasses. Cattle in the Springfield area of Tennessee appear to be more prone to this malady than in other areas of Tennessee. Therefore, at the Highland Rim Experiment Station, 54 beef cows on tall fescue and clover pastures plus hay were used to compare commercial Mg supplements from January through April, 1983. Six control cows received salt, four replicates of 6 each had free access to "Cattle Mag Blocks," and four replicates of 6 each had free access to "High Mag Mix." Seventy other cows at HRES were included for blood data but were not on a controlled experiment.

1. Cows fed blocks consumed an average of .35 lb per day which supplied about 11.9 g of supplemental Mg/day.

2. Cows fed the high Mag mineral mix consumed an average of .11 lb per day which supplied about 7 g of supplemental Mg/day.

3. The average plasma Mg was not significantly different between cows fed mix vs block but more cows and more plasma Mg samples were below 1.5 mg/dl (Table 1) for cows fed mix.

4. Cows not supplemented with Mg had significantly lower plasma Mg and significantly higher plasma K than supplemented cows on all sampling dates except 4-6-83 when no differences were found.

5. Pasture and hay samples averaged low in Mg throughout the sampling period and pasture Mg was very low averaging 0.12% on 2-23-83.

6. One control cow died from what was diagnosed as hardware disease. The other control cows not supplemented with Mg would probably had tetany if they had remained on the original pasture or if they had not been supplemented on 4-29-83.

7. High Mag Mineral Mix reduced incidence of hypomagnesemia but Cattle Mag Block reduced hypomagnesemia ever more.

Table 1. HRES LOW COW PLASMA MG SAMPLES for 1983

	Mg/dl Less Than		
	.5	1.0	1.5
Block	$\frac{0}{24}$	$\frac{1}{24}$	$\frac{9}{24}$ (6)*
Mix	$\frac{0}{24}$	$\frac{4}{24}$	$\frac{21}{24}$ (13)
Control	$\frac{1}{5}$	$\frac{6}{5}$ (4)	$\frac{13}{5}$ (5)
Others	$\frac{1}{70}$	$\frac{11}{70}$ (8)	$\frac{70}{70}$ (27)

*$\frac{\text{Number of samples}}{\text{Total number of cows}}$ (number of cows)

THE UTILITY OF MEASURES OF FATNESS AND SKELETAL SIZE IN EXPLAINING WEIGHT AND SIZE DIFFERENCES IN YEARLING ANGUS AND HEREFORD BULLS

A. L. Eller, Jr. and R. R. Shrode

Records from 669 yearling Angus and 871 yearling Hereford bulls from three central test stations and seven Land Grant University of USDA research herds were used in a study designed to determine needed refinements in procedures for performance testing of young bulls. Generalized least-squares multiple regression analyses were conducted to determine the value of measures of fatness and measures of skeletal size in defining or explaining weight and size differences over and above the effects of age and age of dam, which are commonly used for this purpose. Principal-component analysis also was used in an effort to assess its usefulness in evaluating bulls as to size and shape. Results of the study warrant the following conclusions:

1. Weight alone is not a sufficient measure of size.

2. Selection for conventional adjusted yearling weight alone will tend to favor fatter animals and produce animals of several different skeletal sizes and body shapes.

3. There is a positive association between measures of fatness (either visual, subjective condition score or ultrasonically measured subcutaneous fat thickness) and final weight.

4. Wither height and body length are valuable as measures of skeletal size, and variation in them explains large portions of the variation in final weight.

5. Wither height, body length and subjective frame-size scores are useful in explaining differences in body type and maturity rate.

6. Wither height and body length, when available, reduce the value of age in explaining weight differences.

7. Width between points of shoulders is highly correlated positively with weight but is correlated also with fatness.

8. Because of individual-animal variation, accurate measurement of weight, skeletal size and fatness is essential in classifying bulls as to potential breeding value.

9. It is inappropriate to adjust weight to a fat-constant basis using regression procedures based on group statistics.

10. The first principal component is useful in ranking bulls as to general, overall size.

11. The second principal component indicates the existence of two extremes in body shape (long, tall, narrow and short, low-set, wide) and is useful for ranking animals between these extremes.

12. Post-weaning performance tests of bulls for estimation of breeding value should be terminated at a constant physiological age rather than at a constant chronological age, physiological age being determined by fatness and skeletal-size measures.

CALFHOOD WEIGHTS, BODY MEASUREMENTS AND ESTIMATES OF BODY COMPOSITION AND CRITERIA OF OVERALL SIZE AND SHAPE FOR PREDICTING YEARLING PERFORMANCE IN BEEF CATTLE

S. P. Hammack and R. R. Shrode

Data were collected from 223 Polled Hereford calves (120 bulls and 103 heifers) and 583 Angus calves (291 bulls and 292 heifers). Eight variables were recorded: age at time of data collection (AGE), live weight (WT), average daily gain (ADG), conventional visual, subjective condition score (COND), ultrasonic estimate of subcutaneous fat thickness at 12th rib (FAT), body length from midpoint of scapula to posterior prominence of pin bones (LT), hearth girth (HG) and hip width (HIP).

Weights were recorded at birth (B), at weaning (W), approximately 230 days of age and at a year of age (Y), approximately 390 days of age. The other variables were recorded at W and Y. Principal components (PC) of weaning variables were calculated as criteria of size and shape (PC_1 through PC_5) and treated as were the recorded variables in stepwise multiple regression analyses to construct equations for predicting birth to yearling average daily gain (BYADG), weaning to yearling average daily gain (WYADG) and yearling condition score (YCOND). Since the Polled Hereford and Angus herds involved are maintained at different locations (resulting in complete confounding of breed and location), and since there was some heterogeneity of correlations in the four breed-location-sex data sets, analyses were conducted separately on the four data sets. The four sets of results were quite similar and warrant making the following statements.

1. COND was the most valuable complement to AGE and WT with LT of some value for predicting WYADG.

2. In equations including PC, PC_1 (a measure of overall size) entered the equations first after AGE, generally followed PC_2, which contrasts animals with respect to overall shape.

3. Coefficients of multiple determination (R^2's) were largest for equations predicting BYADG (greater than .50), followed by those for equations to predict YCOND (.15 to .50) and those for equations to predict WYADG (less than .15).

RELATIONSHIP OF FEEDER CALF SIZE AND SHAPE TO CHANGES IN RUMENAL CHARACTERISTICS DURING MARKETING AND TRANSPORTING

P. J. Hamlett and J. B. McLaren

Feeder calves (360) subjected to one of three market-management systems were used to evaluate the relationship of calf size and shape to changes in rumen characteristics resulting from the stresses of marketing. The management systems included: 1) weaning and feeding a concentrate diet during the last 30 days before marketing and feeding hay at the orderbuyer barn (PW), 2) allowing the calves to graze pasture with their dams during this period and feeding hay (NI) or a 50% concentrate diet at the orderbuyer barn (HE). The calves were weighed and sampled at auction barn arrival (AAB) and orderbuyer barn departure (DOBB). Rumen characteristics including rumen liquid volume (V), volatile fatty acids (VFA), differential protozoal counts, pH and gas producing potential (GPP) of the rumen fluid, were determined. Orthogonal size and shape indices were generated from the body weight and measurements by use of a multivariate procedure, principal-component analysis. Changes in rumen characteristics during market was calculated as the DOBB value minus the AAB value for each trait.

Calf size ranged from light, small-framed (size = -2.5) to heavy, large-framed (size = 2.5). Shape ranged from short, fat, wide calves (shape = -2.5) to thin, narrow and long calves (shape = 2.5). Total protozoal (TPROT) concentration in light-small framed HE calves increased during marketing but decreased in heavy-large framed HE calves. In light-small framed PW calves, TPROT concentration decreased and the decrease was extreme in intermediate and large calves. The AAB protozoal population of PW calves differed from those of NI and HE calves. This was the result of the preshipment diet which probably altered rumen development and influenced the size-rumen relationship. Rumen VFA concentration was related to calf shape. In NI calves the relationships of market changes and calf shape were small ($P>.1$) regardless of calf shape. In HE calves, change in propionate concentration ranged ($P<.02$) from an increase of 10 mmol per ℓ in short-fat-wide calves, to a small increase in calves of intermediate shape and an intermediate increase in long-thin-narrow calves. In PW calves, market change in propionate was greatest ($P<.05$) in short-fat-wide calves and less in long-thin-narrow calves. Change in Holotricha (HO) concentration was related to calf shape. The HO change was smaller in PW calves than in HE or NI calves. In HE calves the decrease in rumen HO concentration during market was greater as calf shape progressed from fat-short calves to calves of intermediate shaped calves. As shape increased from intermediate to long-tall-thin calves, little difference in change was observed.

In general, calf size was related to changes in protozoal concentrations and calf shape was related to changes in VFA concentration. It can be concluded that significant relationships of calf size and shape to change in rumen characteristics of weanling calves and changes in rumen characteristics during market do exist. Also, the relationships were affected by market-management or preconditioning system to which the calves were subjected.

Larger calves tend to be more resistant to market-transit stressors than do small calves. Calves that appear to be most desirable to feeder-calf purchasers, feedlot operators, and feeder calf graders tend to be intermediate with respect to shape.

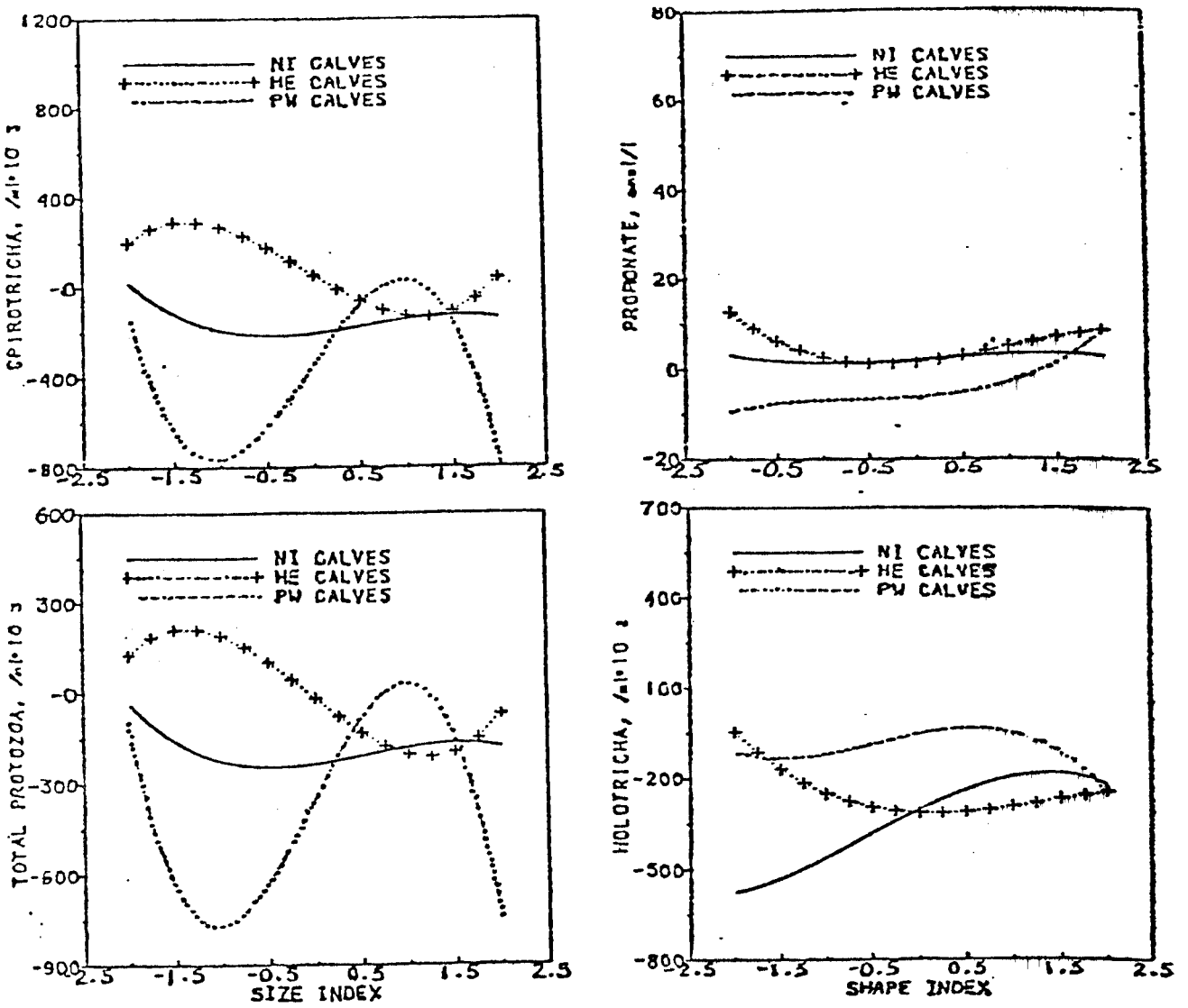


Figure 1. The relationship of calf size and shape to changes in rumen protozoa concentration during marketing.

COMPARISONS OF BALANCE AND TISSUE DATA IN HYPOMAGNESEMIC AND NORMOMAGNESEMIC CALVES

K. A. McKim, M. C. Bell, J. K. Miller, C. K. Reynolds, M. H. Sims and N. Ramsey

Researchers are continually looking for hypomagnesemia models for lactating beef cows. Young dairy calves were utilized in this study to better understand the etiology of this disorder. The objectives of this study, therefore, was to produce hypomagnesemia in calves and to determine the effects of excess potassium (K) on magnesium (Mg) metabolism. Methodology included a mineral comparison of hypomagnesemic and normomagnesemic calves through the collection of balance data and tissue analyses.

Three trials were conducted with 8 Holstein bull calves per trial. The calves were randomly assigned to one of four semipurified diets, diet 1 was Mg deficient, diet 2 was adequate in Mg, diet 3 was Mg deficient plus 4% K, and diet 4 was adequate in Mg plus 4% K. After the calves on the Mg deficient diets depleted to a plasma level less than 1.0 mg of Mg/dl a balance trial was conducted. Following the collection of data all calves were sacrificed and tissue samples were obtained. Tissues collected included brain, kidney, heart, skeletal muscle, rib, liver, spleen, and lung. All data collected in both phases of the study underwent mineral analysis for Mg, calcium, and K.

1. Calves fed diets supplemented in Mg had a higher ($P<.05$) Mg intake, fecal excretion, and balance than those calves fed Mg deficient diets.

2. Calves fed the Mg deficient, Mg deficient plus supplemental K and Mg adequate plus supplemental K diets excreted less ($P<.05$) Mg in the urine than calves fed the Mg adequate diet.

3. Calves dying in tetany exhibited lesions and hemorrhages typically associated with tetany convulsions.

4. Tissue data compiled resulted in no differences in Mg concentrations across treatments for brain, heart, liver, spleen, skeletal muscle, kidney, or lung.

5. Rib Mg was lower ($P<.05$) for calves fed diets not supplemented in Mg compared with calves fed Mg supplemented rations.

Table 1. Least square means of daily Mg balance in grams

Component	Treatment			
	1	2	3	4
No. of calves	3	5	7	4
Intake	.162[a]	2.530[b]	.165[a]	2.093[b]
Urinary excretion	.009[a]	.219[b]	.010[a]	.095[c]
Fecal excretion	.295[a]	1.485[b]	.305[a]	1.439[b]
Balance	-.142[a]	.826[b]	-.150[a]	.559[b]

[a,b,c]Means within a row superscripted with different letters are different (P<.05).

Table 2. Least square means of rib minerals in mg/gram dry basis

Treatment	Minerals		
	Magnesium	Calcium	Potassium
Mg deficient	2.71[a]	201.52	1.23[a]
+ Mg	4.27[b]	197.47	1.49[a,b]
+ K	2.23[a]	194.39	1.98[b]
+ Mg + K	3.88[b]	193.38	1.20[a]

[a,b]Means within a column superscripted with different letters are different (P<.05).

FALL NITROGEN FERTILIZATION AND SPRING RENOVATION OF FESCUE PASTURES

J. B. McLaren and L. M. Safley

Six 10-acre pastures at the Highland Rim Experiment Station were used to compare fescue pastures that were either (1) fertilized with 75 lb N/A each spring and fertilized with 75 lb N/A each fall, (2) renovated with ladino clover and lespedeza (legumes) and overseed with legumes each spring, or (3) fertilized with 75 lb N/A each fall and overseed with legumes each spring. During the winter (Nov. 1 to April 1) each pasture was grazed by five mature, spring-calving Angus-Hereford cows which were each fed 12 to 15 lb/day of grass hay when residual or stockpiled forage was insufficient for their support. These cows, their calves, and extra cow-calf pairs, added to consume any forage above that needed by the five test cows, grazed the pastures during spring (April 1 to July 1) and summer (July 1 to Nov. 1).

The calves, sired by Red Poll bulls, were born in February, March, and April and weaned about October 15 each year. All pastures were fertilized with phosphate, potassium, and lime according to annual soil test recommendations and ammoniun nitrate (230 lb/A) was applied in late March and/or late August to pasture designated in the design only. All cows and calves were weighed and scored for body condition each fall (about Nov. 15), at the beginning of each season, and at weaning. All other animal management practices conformed to those recommended by the University of Tennessee, Animal Science Department, and in general use by Tennessee cow-calf producers. Each fall, in October, all cows were pregnancy checked and open cows were replaced.

RESULTS

1. Cows grazing spring-nitrated fescue pastures weighed less each fall when calves were weaned than they did the previous fall but cows grazing renovated pastures weighed more. Cows and calves grazing renovated pastures tended to be fatter each fall than those grazing nitrated fescue pastures.

2. Hay required by cows grazing stockpiled fescue was less than that needed for cows grazing non-nitrated fescue pastures.

3. The 240-day weight of calves grazing renovated fescue pastures were 73 lb greater than those grazing nitrated pastures without legumes. The 240-day weights of calves grazing renovated fescue pastures with and without fall nitrogen were similar (529 and 515 lb, respectively).

4. Data presented in Table 1 shows that increased production of fall stockpiled forage for winter grazing and higher quality forage necessary for increased spring-summer calf gains can be accomplished in the same pasture when the fescue sod is nitrated with 75 lg N/A in the fall and overseeded with legumes each following spring. For these practices to be compatible, intense animal-forage management is necessary.

Table 1. Effects of Fall nitrogen fertilization and Spring renovation of the same fescue sod on cow and calf performance (1974-1978)

Item	Fertilized 75 lb N/A Spring 75 lb N/A Fall	Renovated with ladino clover lespedeza	Renovated with ladino clover lespedeza + 75 lb N/A Fall
No. test cows (5 yr)	50	50	50
Cow performance, lb			
Initial weight	951	1003	1049
Final weight	938	1072	1100
Winter gain[1,6]	-29[a]	-70[b]	-51[ab]
Spring gain[2,6]	-1[a]	86[b]	56[c]
Summer gain[3,6]	2[a]	44[b]	38[b]
Cow condition[4]	9.2[a]	10.4[b]	9.5[a]
Percent cows diagnosed pregnant[6]	78[a]	87[b]	93[b]
Percent cows calved[6]	64[a]	80[a]	81[b]
Calf performance, lb			
Birth weight	66	66	70
Adjusted 240-day wt.[5,6]	442[a]	515[b]	529[b]
Spring daily gain[2,6]	1.37[a]	1.66[b]	1.67[b]
Summer daily gain[3,6]	1.45[a]	1.73[b]	1.76[b]
Calf condition score[4,6]	8.3	9.2	9.4
Calf gain per acre, lb	380	278	338
Cow grazing days/A	179	114	133

[a,b,c]Means in the same row supercripted with different letter are different ($P<.05$).

[1]Winter gain from Nov. 15 to April 1.

[2]Spring gain from April 1 to July 1.

[3]Summer gain from July 1 to Nov. 1.

[4]Average good = 10, average choice = 13.

[5]Adjusted 240-day weight was adjusted to remove variation due to differences in weaning age, sex of calf, and age of day.

[6]These are least-squares means.

EFFECTS OF K FERTILIZATION OF PASTURES AND ORAL KCL ON PLASMA MINERALS, GLUCOSE AND INSULIN LEVELS OF LACTATING BEEF COWS

C. K. Reynolds, J. K. Miller, M. C. Bell, S. Banet and N. Ramsey

Hypomagnesemic tetany is a metabolic disorder which continues to be a major concern of cattle and sheep producers in temperate regions. When lactating cows consuming stored feed are switched to early spring pasture, hypomagnesemia may develop within a few days. Rapidly growing spring pastures are often low in Mg and high in K. Excess dietary K has been shown to reduce Mg absorption from the rumen; however, the rapid onset of hypomagnesemia in some individuals suggests the involvement of other factors besides decreased Mg absorption alone. Numerous interrelationships between K, Mg, insulin and glucose have been determined, both in vitro and in vivo; therefore, our objective was to determine the effects of K fertilization of fescue pastures and oral doses of KCl on plasma, insulin, glucose, Mg and K of lactating beef cows during early spring grazing.

Forty-eight mature beef cows in the early stages of lactation were used in 4 trials involving 12 cows each during 2 years. Trial 1 was conducted during late February and trial 2 during early March of 1982, while trials 3 and 4 occurred during the same periods of 1983. Cows were maintained on stockpiled fescue pasture and fescue hay before being placed on one of 2 experimental pastures. Both pastures were fertilized with 112 kg N, 74 kg P and zero (control pasture) or 186 kg (high-K pasture) K per hectare. Blood samples were taken from these cows before and during 2 weeks after they were moved to the experimental pastures. Plasma from these samples was saved for analysis of insulin, glucose, Mg and K. In addition, half the cows on each pasture were given 280 g KCl via oral gelatin bolus daily during the second week on the pastures.

1. Plasma K levels were not affected by pasture fertilization or oral KCl boluses.

2. Lower ($P<.001$) levels of plasma Mg were observed in cows on the high K pastures.

3. Plasma glucose levels increased ($P<.001$) during the first week cows were on the experimental pastures, but were not affected by either treatment.

4. Insulin levels also increased ($P<.01$) during the first week of sampling. These increases were greater ($P<.001$) in cows on the high-K pasture, but were not affected by oral KCl dosing.

TABLE 1. Mean plasma Mg, glucose and insulin levels of lactating cows grazing control or high-K pastures with and without KCl bolusing.

Day on pasture	Control pasture		High-K pasture	
	No KCl	With KCl	No KCl	With KCl
	----------Plasma Mg (mg/dl)----------			
0	2.13	1.89	2.03	1.96
3	1.80	1.81	1.80	1.85
5	2.20	2.11	1.79	1.83
7	2.12	2.01	1.71	1.66
9	2.21	2.16	1.74	1.61
11	2.24	2.20	1.62	1.64
13	2.27	2.13	1.43	1.38
	----------Plasma Glucose (mg/dl)----------			
0	57.03	58.66	58.37	57.32
3	64.78	60.67	60.66	61.34
5	63.88	64.38	67.26	64.53
7	62.05	61.50	60.94	59.14
9	61.44	61.30	62.42	62.21
11	61.48	59.90	61.50	60.03
13	58.58	59.71	61.61	58.64
	----------Plasma Insulin (μU/ml)----------			
0	11.40	11.06	10.96	10.11
3	14.35	13.06	14.93	14.42
5	13.70	13.57	16.23	17.93
7	14.12	12.55	14.35	13.66
9	10.77	11.64	13.47	13.31
11	12.00	11.86	14.38	13.68
13	10.92	11.74	16.40	13.18

RESPONSE TO SELECTION FOR MILK YIELD IN JERSEYS

B. R. Bell, D. O. Richardson,
E. D. Moore and J. R. Owen

First lactations of 625 Jerseys calving between 1970 and 1982 were used to evaluate the production response in a selection experiment at the Dairy Experiment Station (DES). In 1967, cows were assigned randomly within sire on a 3:1 ratio to selection or control lines, respectively. The selection line of 120 cows and associated young stock were bred to the highest A.I. sires available. Sires were selected on the basis of their Predicted Difference for milk (PDM) and were required to have at least 60% repeatability. Once selected, sires were used for four consecutive years with a new bull added to the group each year. Exceptions were three of the initial sires and certain bulls with limited availability. Culling of females in the selection line was based on milk yield.

The control line of 40 cows and associated young stock were mated to 20 young sires which were randomly selected from A.I. sampling programs at the time the project was initiated. Five young sires bred at DES with similar pedigree indexes were used also. Culling of females beyond involuntary losses was at random. Production traits included were 305 day, 2x, mature equivalent milk yield, 4% fat-corrected milk, and fat percentage. First lactation performance records were available for 446 daughters of 15 selection bulls and 167 daughters of 21 different control line sires. A comparison of the average PDs of bulls used in the two lines are presented in Table 1.

Least squares means by line are presented in Table 2. Selection cows produced significantly more ($P<.001$) milk, fat, and fat-corrected milk. However, fat percentage decreased significantly ($P<.05$).

Regressions of milk, fat, and fat percentage on PDs for the respective traits are shown in Table 3. The regression of milk yield of a daughter on the PD milk of her sire was greater than the expected value of 1.0 ($P<.01$). This finding agrees with evidence from other studies. Results indicated that under high management conditions, dairymen might expect larger increases in production by using genetically superior bulls than their PDs would indicate. However, regressions of fat yield and fat percentages of a bull's progeny on his PD were less than 1.0.

Table 1. Comparison of Latest Proofs of Selection and Control Sires

	Selection n = 15	Control n = 21
Avg. Predicted Difference (PD)		
PD milk (kg)	525	49
PD fat (kg)	17	-1
Weighted avg. PD		
PD milk (kg)	479	53
PD fat (kg)	17	1

Table 2. Least Squares Means of Mature Equivalent Yield Traits by Line[a]

	n	Milk (kg)	Fat (kg)	4% Fat corrected milk (kg)	Fat %
Selection	446	6226 ± 143	288 ± 6	6821 ± 141	4.68
Control	167	5472 ± 122	267 ± 5	6189 ± 121	4.87
Difference		754**	21**	632**	-.19*

[a]Model Y = year - season + line + sire (line) + generation (line)

**P≤.01, *P≤.05

Table 3. Regressions of Yield on Predicted Differences (PD)

	PD milk	PD fat	PD %
Entire data set	1.89 ± .17	1.44 ± .23	.64 ± .12
Selection only	2.28 ± .51	0.65 ± .30	.62 ± .10

EVALUATION OF EFFECTS OF VARIOUS CALVING INTERVALS AND FEMALE REPLACEMENT POLICIES ON THE PERFORMANCE OF A DAIRY HERD

B. R. Bell, D. O. Richardson, H. H. Dowlen and J. R. Owen

Maximizing profit is the objective of most dairy breeders. Production and reproduction are major contributors to profitability. A calving interval of 12 to 13 mo. is thought to be ideal. However, this interval may not be optimum for maximization of profits from high yielding cows.

In addition to maximizing profit, genetic improvement of a dairy herd is also an objective of most dairymen. The use of A.I. creates the possibility of an annual increase in the average genetic merit of replacement heifers. Therefore, increasing the number of replacements entering the milking herd should lead to an increase in the average genetic merit of the herd. While higher replacement rates will lead to an increase in genetic merit, it may not necessarily lead to increased profit since a higher proportion of the herd is in first lactation and not producing at mature yields.

The objectives of this experiment are: (1) to evaluate the interrelationships of calving interval and profitability utilizing high yielding cows; and (2) to evaluate the effect of female replacement policies and calving intervals on the interpretation of traditional expressions of production and breeding ability.

In 1982 the DES herd was divided into 6 groups of 25 cows. These groups were randomly assigned to a 2 x 3 factorially designed treatment regime that includes three reproductive management systems and two replacement programs. The three reproductive management systems vary only in the post-partum waiting period prior to rebreeding. The three groups are 35, 80, and 125 days post partum before initiation of breeding. The two replacement programs consist of replacement rates of .24 and .36.

Raw means for production and reproductive traits are presented in Tables 7 and 8, respectively.

Table 1. Means of Production Traits for the Treatment Groups[a]

Breeding Regimes	Replacement Regimes					
	.24			.36		
	n	Milk (kg)	Fat (kg)	n	Milk (kg)	Fat (kg)
Bred at 1st heat after 35 days post-partum	19	7705	343	19	7564	328
Bred at 1st heat after 80 days post-partum	23	7516	338	22	7573	325
Bred at 1st heat after 125 days post-partum	23	7895	340	20	7694	336

[a]Production traits are expressed on mature equivalent basis.

Table 2. Means of Reproductive Traits for Treatment Groups

Treatment	Reproductive Traits				
	Avg days to 1st breeding	Projected calving interval	Avg days open	Breeding per conception	1st breeding conception rate (%)
35 days					
Repl. rate (.24)	68	12.6	103	2.9	38
Repl. rate (.36)	54	12.1	88	2.4	47
80 days					
Repl. rate (.24)	95	13.5	130	2.5	50
Repl. rate (.36)	91	13.0	117	1.9	67
125 days					
Repl. rate (.24)	132	14.3	154	2.3	65
Repl. rate (.36)	136	14.0	144	1.1	88

THE EFFECT OF A LONG HALF-LIFE, HIGH-ACTIVITY PROSTAGLANDIN ANALOGUE, FENPROSTALENE, ON UTERINE MOTILITY IN THE BOVINE

W. H. Byrd, F. M. Hopkins, H. Eiler and W. A. Lyke

Retained fetal membranes are a costly postpartum disorder resulting from a diverse range of predisposing factors such as vitamin and mineral deficiencies, dystocia, heat stress, and high lactational demands. The effectiveness of prostaglandin $F_{2\alpha}$ in the treatment of bovine pyometra, higher than normal levels of plasma progesterone in cows retaining, and lower than normal cotyledonary $PFG_{2\alpha}$ concentrations in cows suffering from retained fetal membranes indicate a possible use of $PFG_{2\alpha}$ in the treatment of this problem. Although other researchers have found the postpartum uterus to be refractory to intramuscular injection of $PFG_{2\alpha}$, the recent development of a synthetic analogue possessing an 18-23 hour half-life and 25 times the leutolytic activity of $PFG_{2\alpha}$ could provide an effective means of retained fetal membrane treatment. The purpose of this study was to determine the effect of fenprostalene on the uterokinetic response in the postpartum and cycling bovine in vivo.

Uterine motility was measured in six open Holstein cows and eight postpartum mixed breed beef cows by the intrauterine balloon technique. Motility was measured on two consecutive days, beginning within 24 hours after calving in the postpartum group. Approximate stage of estrous cycle was determined in all open cows prior to each experiment by rectal palpation. Baseline motility was recorded for one hour in all cows on each experimental day. On day one, fenprostalene (1 mg, 1M) was injected and motility was recorded for two hours, followed by fenprostalene (1 mg, IV) and oxytocin (40 units, IV), each recorded for one hour. After the baseline period on day two, oxytocin (100 units 1M) was injected and after a two hour recording period fenprostalene (1 mg, IV) and oxytocin (40 units IV) were again administered and motility recorded for one hour per injection. Area under the curve (mm^2) was calculated by five minute time intervals for comparisons.

1. Although statistical inferences have not been determined, raw means indicate a much larger response of the uterus to oxytocin than fenprostalene although each injection resulted in an increase over baseline (Figure 1).

2. Open cows exhibited increased motility over postpartum cows as did one cow which retained over those cows with normal membrane expulsion (Figure 2).

3. Baseline motility was increased on day two as compared with day one with respective mean areas of 1780.23 ± 112.66 mm^2, 1414.68 ± 135.34 mm^2 for the open cows, and 563.39 ± 37.43 vs 547.85 ± 36.84 mm^2 for the postpartum cows (Figure 2).

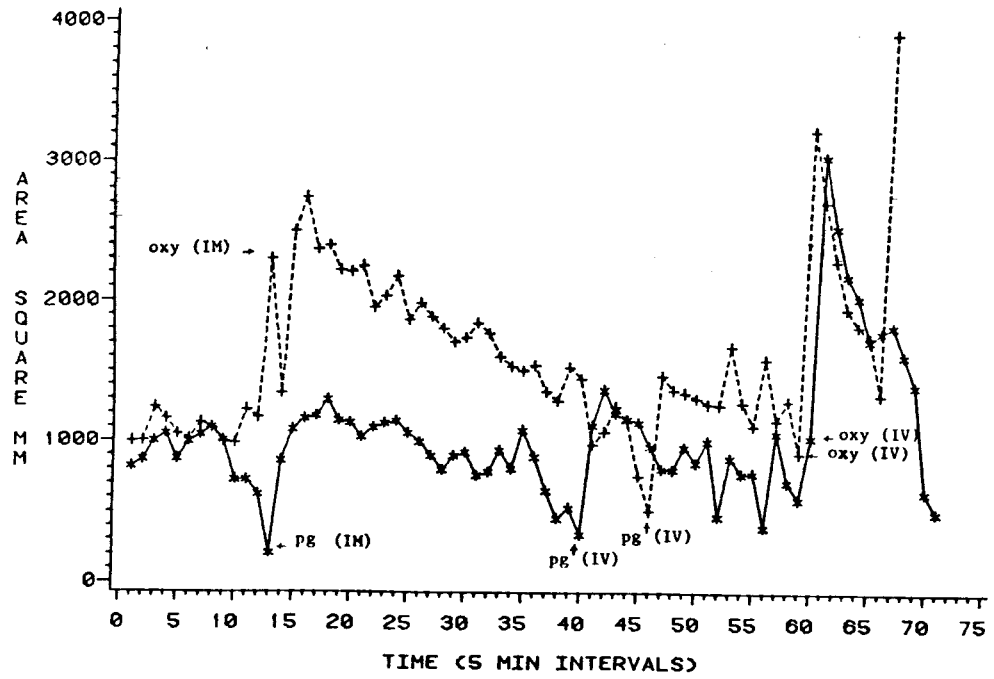


Figure 1. Comparison of raw means for day one and day two. (All cows: n = 14).
Day one = Solid Line.
Day two = Dotted Line.

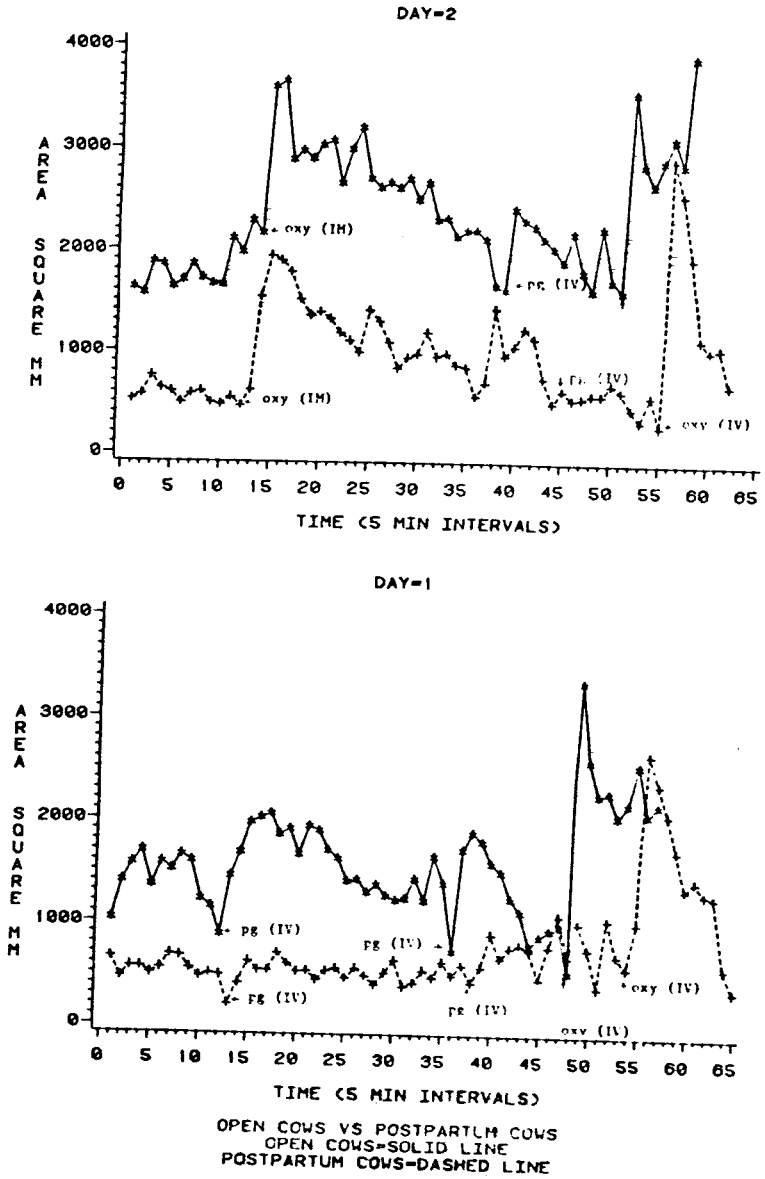


Figure 2. Comparison of raw means for open vs postpartum cows. By experimental day.
Open cows n = 6
Postpartum cows, n = 8

HYPOMAGNESEMIA IN YOUNG CALVES AND EFFECTS OF MAGNESIUM REPLETION ON PLASMA AND CSF MINERAL LEVELS

K. A. McKim, M. C. Bell, J. K. Miller, C. K. Reynolds, M. H. Sims and N. Ramsey

Hypomagnesemia, a metabolic disorder that occurs in ruminants which generally appears within the first two weeks after cattle have been put on lush spring pasture, has been associated with low magnesium (Mg) levels on plasma and cerebrospinal fluid (CSF). The objectives of this study was to produce hypomagnesemia in calves and determine the effects of excess potassium (K) on Mg metabolism. An additional objective was to compare an oral repletion of 30% $MgCl_2 \cdot 6H_2O$ solution on plasma and CSF Mg, Calcium (Ca) and K levels.

Three separate trials were conducted in which a total of 19 Holstein bull calves were utilized. The calves were randomly assigned to one of four semi-purified diets which consisted of: (1) Mg deficient, (2) adequate in Mg and K, (3) Mg deficient plus excess K, and (4) adequate in Mg and excess K. Immediately following the collection of balance data, calves were orally repleted with 30% $MgCl_2 \cdot 6H_2O$ solution. Plasma and CSF samples obtained at 0, 5, 15, 30, 60, 120 and 180 minutes were analyzed for Mg, Ca and K.

1. Calves fed the Mg deficient diet had plasma Mg elevated ($P<.05$) from a baseline value of .44 mg of Mg/dl to a 120-minute value of .60 and 180-minute level of .62 mg/dl of Mg dosed with 50 ml of 30% $MgCl_2 \cdot 6H_2O$ (Figures 1 and 2).

2. Oral Mg solutions of 50 ml of 30% $MgCl_2 \cdot 6H_2O$ had no effect on plasma Mg in calves on other treatments.

3. Of the 6 calves exhibiting tetany, 4 were successfully orally repleted; however, calves orally repleted with 50 ml of 30% $MgCl_2 \cdot 6H_2O$ solution had increases in plasma and CSF Mg minimal to the increases from rectal infusions done previously.

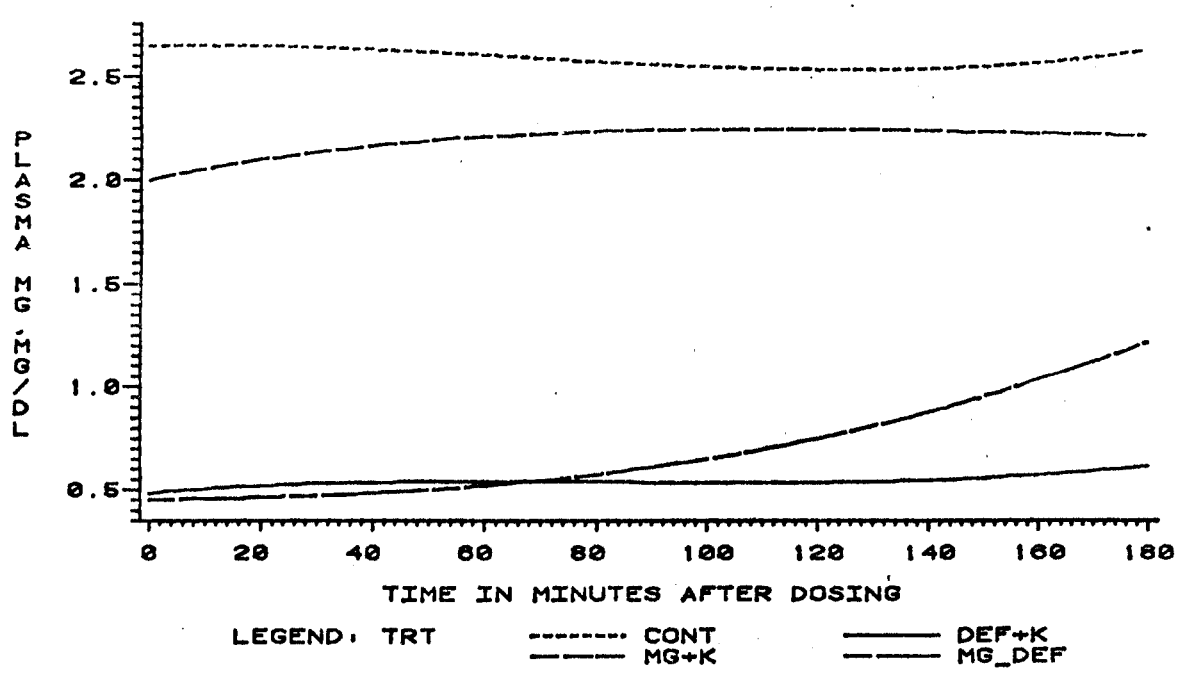


Figure 1. Regression curves for plasma Mg in hypomagnesemic or normomagnesemic calves fed normal or excess K after oral dosing with 50 ml of 30% $MgCl_2 \cdot 6H_2O$.

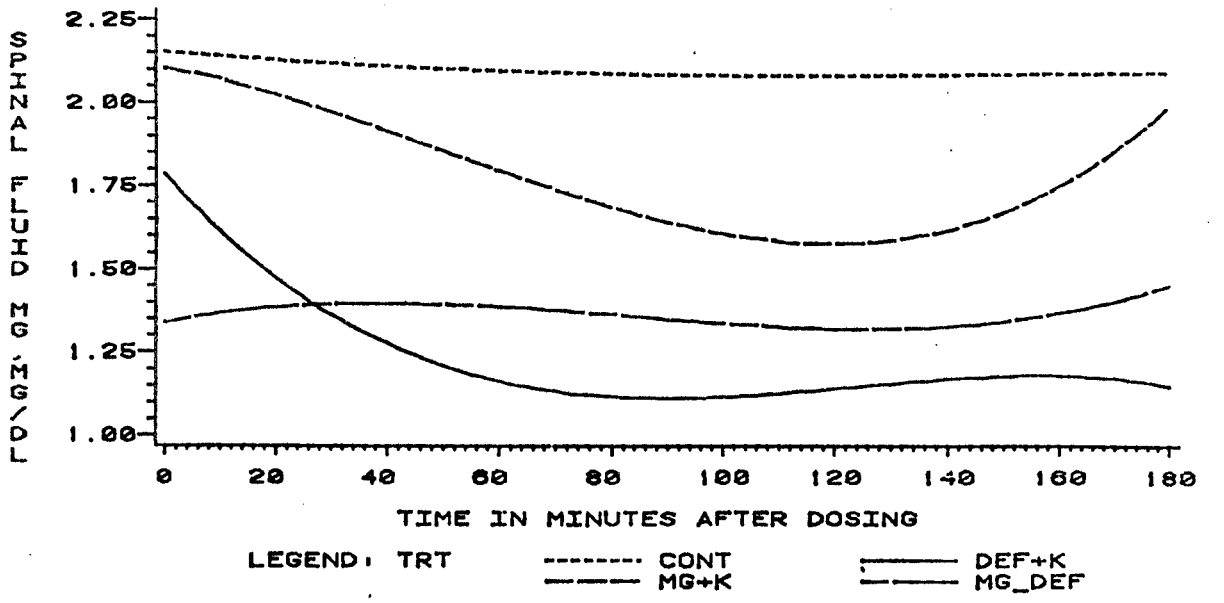


Figure 2. Regression curves for CSF Mg in hypomagnesemic or normomagnesemic calves fed normal or excess K after oral dosing with 50 ml of 30% $MgCl_2 \cdot 6H_2O$.

CORRELATED RESPONSE IN FEED EFFICIENCY ACCOMPANYING SELECTION FOR MILK YIELD IN JERSEYS

C. D. Smothers, B. R. Bell, D. O. Richardson and J. R. Owen

Feed efficiency were collected on 350 first lactation Jerseys over 21 trials from 1971-82. Feed intake and production data were recorded for a 28-day period on individual cows during mid to late lactation. Bodyweight changes were recorded on each individual during the test periods.

Forages used throughout the experiment included alfalfa-orchardgrass hay and corn silage fed ad. lib. Grain was fed according to production of the individual. Feed samples were taken and compiled by trial. These samples were subjected to analysis to derive estimated net energy (ENE) values. The 28-day milk productions were converted to a 4% fat-corrected-milk basis. Our estimate of feed efficiency (FE) included T_ENE, which is a composite of the estimated total energy consumed over the 28-day period, and is defined in this report as the ratio fat-corrected-milk/total ENE in feed consumed.

Least squares means for T_ENE, FCM and feed efficiency (FE) adjusted for all fixed effects and bodyweight change, are presented in Table 1. The selection line had significantly higher milk yields ($P<.001$), but was also significantly higher in total estimated net energy consumed ($P<.001$). There was no apparent correlated response in FE with selection for yield. These results differ from those published in similar studies. A possible explanation is that selection for yield may not increase efficiency of feed utilization but may lead to more efficient catabolism of body tissues. Since the data were collected on cows in mid- to late lactation, differences may be due to the energy balance status of the cows. Failure to detect a correlated response in FE may be due to a high percentage of being in a positive energy balance.

Table 1. Least Squares Means for T_ENE, FCM and FE[a]

Line	n	T_ENE(therms)	FCM (kg)	FE
Selection	231	604.317**	2339.37**	.606
Control	119	553.075	2116.86	.602

[a]Model Y = trial + b(body-weight change) + stage of lactation + line

**(P<.001)

STEM-SPERMATOGONIAL SURVIVAL AND INCIDENCE OF RECIPROCAL TRANSLOCATIONS IN THE γ-IRRADIATED BOAR

B. H. Erickson and P. G. Martin

Variables characterizing the irradiation response of the spermatogonial stem cell have been shown experimentally and mathematically to greatly influence the incidence of reciprocal translocations. And, by extension, it has been concluded that interspecific variation in stem-cell kinetics would materially affect the reliability of interspecific extrapolation.

To extend our knowledge of the magnitude of species difference and the probable basis for its cause, we have assayed incidences of reciprocal chromosomal translocations and survival in the spermatogonial stem cells of the γ-irradiated boar.

Testes of Yorkshire boars 7 to 9 months old were irradiated with varying doses of ^{60}Co γ-radiation in a multipoint field at a dose rate of 12R/min. Three boars were irradiated at each of the doses shown in Table 11 and unilateral castrations were effected at 3 days and 12 weeks after irradiation. Stem-cell survival was assessed in day-3 tissue and incidence of chromosomal abnormalities was determined in testes excised 12 weeks after irradiation.

1. Stem-cell survival was markedly affected by 100 rad (51% of control) and reduced to 34% of control by 400 rad (Table 1).

2. Production of differentiating spermatogonia was all but completely interrupted by 200 rad and spermatogonial renewal was incomplete at 12 weeks.

3. From the state of the seminiferous epithelium at 12 weeks, estimates of the percent of permanent impairment of sperm-producing capacity ranged from 20±6 (100 rad) to 67±10 (400 rad).

4. Incidence of translocations peaked at 200 rad and number occurring at 100 and 400 rad was similar (Table 2).

5. Kinetics of spermatogonial renewal in the boar were found to differ considerably from that of the rodent and this probably accounts for the boar's comparatively high sensitivity to irradiated-induced stem-cell killing and low sensitivity to translocation.

6. Hence, the boar's fertility is more seriously threatened by irradiation than is the genetic health of his sperm.

Table 1. Effect of ^{60}Co radiation on spermatogonial survival in the boar

Dose (rads)	Spermatogonia/100 Sertoli cells							
			A_1			Stem		
	Total	% of Control	Number	%	% of Control	Number	%	% of Control
0	37.7 ±2.0	--	29.8 ±1.8	79	--	7.9 ±0.4	21	--
100	9.4 ±0.7	24.9	5.4 ±0.5	57	18	4.0 ±0.2	43	51
200	3.6 ±0.3	9.5	0.7 ±0.1	21	2	2.9 ±0.3	81	37
400	3.1 ±0.1	8.2	0.4 ±0.5	7	1	2.7 ±0.1	87	34

Table 2. Incidence of reciprocal chromosomal translocations[a] in the γ-irradiated boar

Dose (rads)	Cells evaluated (number)	Translocations, number			
		Rings	Chains	Total	% affected
0	300	0	0	0	0
100	593	6	0	6	1.0
200	230	3	1	4	1.7
400	141	2	0	2	1.4

[a]Evaluated in diakinesis-metaphase spermatocytes.

REPRODUCTIVE AND GENETIC EFFECTS OF CONTINUOUS PRENATAL IRRADIATION IN THE PIG

B. H. Erickson and P. G. Martin

In the apparent absence of other effects, germ cells are completely eliminated when the pig is continuously irradiated during the prenatal period. Sterility followed a dose rate of 3 rad/day, and at the lowest rate tested (0.25 rad/day) stem-germ-cell survival was reduced to approximately 40 and 60% of control in the male and female, respectively. This study was conducted to determine whether sensitivity to killing was also marked by high sensitivity to mutation and, how a germ-cell population depleted prenatally affects reproductive performance. Germ cell populations were reduced by continuously irradiating sows at dose-rates of either 0.25 or 1.0 rad/day for the first 108 days of gestation. The prenatally irradiated boars were tested for sperm producing ability, sperm abnormalities, dominant lethality, reciprocal translocations and fertility. Prenatally irradiated females were allowed to bear and nuture one litter and tested for dominant lethality in a second litter. Germ cell survival and follicular development were assessed in serially sectioned ovaries.

Results and Conclusions:

1. Sperm production was not significantly affected in the 0.25 rad boars. Boars irradiated with 1.0 rad per day produced sperm at only 17% of the control level.

2. Incidence of defective sperm was 2.3, 4.9% and 11.1% in the 0.0, 0.25 and 1.0 groups respectively. Four of the 1.0 rad boars were infertile, but prenatal irradiation apparently caused neither dominant lethality nor reciprocal translocations.

3. Number of oocytes was reduced to 66+7% of control in the 0.25 rad gilts, but reproductive performance was unaffected and no dominant lethality was observed. Only 7±1% of the oocytes survived in the 1.0 rad group.

4. Reproductive performance was normal for the first litter, but 4 of the 23 sows tested were infertile at the second litter and a significant incidence of dominant lethality was observed, Table 1.

5. The dominant lethality seen in the second litter could either be a direct irradiation effect or an effect that may be the normal consequence of oocyte depletion.

6. Diminished reproductive capacity rather than mutation appears to be the major consequence of continuous prenatal irradiation in the pig.

Table 1. Effect of continuous prenatal irradiation on the reproductive performance of the pig

Pregnancy I				Pregnancy II				
		Piglets/gilts			Fetuses/sow		Corpora lutea	
Dose rate (rad/day)	Gilts bred (No.)	No. alive	No. dead	Sows bred (No.)	No. alive	No. dead	No./ sow	No./live fetus
0	24 (2)[a]	7.9 ±0.6	2.5 ±0.5	24 (2)[a]	11.6 ±0.6	0.62 ±0.2	15.1 ±0.6	1.30
0.25	24 (3)[a]	7.8 ±0.5	2.6 ±0.5	24 (2)[a]	11.5 ±0.6	0.38 ±0.1	13.9 ±0.4	1.21
1.0	24 (2)[a]	7.7 ±0.4	1.5 ±0.1	23 (4)[a]	8.4 ±0.8[b]	0.52 ±0.2	16.5 ±0.8	1.96[b]

[a]Number of animals infertile.

[b]Difference from the other two treatments is significant ($P<0.01$).

HYDROCORTISONE ACETATE OR ACTH ADMINISTRATION DURING DIESTRUS IN GILTS: I. EFFECTS ON PERIPHERAL PLASMA CORTISOL AND PROGESTERONE LEVELS

H. G. Kattesh, J. R. Hembree and T. T. Chen

Elevated temperatures during the first two weeks of gestation has been noted to increase embryonic mortality in swine. Likewise, elevation of plasma glucocorticoid levels as a result of adrenocorticotropin (ACTH) or hydocortisone acetate (HA) administration, has been shown to interrupt pregnancy in rats and to increase embryonic loss in sheep and mice. In those studies using sheep it was suggested that elevated glucocorticoid levels created an aberrant uterine environment which was non-conducive for embryo maintenance. The hormone progesterone, produced by the corpus luteum (CL), is essential for maintaining pregnancy as well as in regulating the secretion of uterine proteins necessary for establishing a proper uterine environment for maintaining embryos. Therefore, altered progesterone production by the CL as a result of exogenous ACTH or HA might suggest a means by which these stress related factors adversely affect embryonic survival in swine. It was the objective of this experiment to examine peripherally measured plasma progesterone and cortisol levels of gilts during the luteal phase of the estrous cycle in response to exogenous ACTH or HA.

Seventeen normal cycling gilts were allotted to one of three treatment groups as follows: control (N = 5), receiving 2 ml sesame oil injected intramuscularly (IM) and 2 ml 14% gelatin injected subcutaneously (SQ); HA treated (N = 6), receiving 2 mg HA in sesame oil/kg body weight (IM); and ACTH treated (N = 6), receiving 1 I.U. ACTH in 14% gelatin/kg body weight (SQ). Injections were administered twice daily (0800 and 2000 hr) on days 2-7 (P1; N = 8) or days 8-13 (P2; N = 9) of the estrous cycle (day 0 = estrus). A daily blood sample (20 ml) was taken via jugular puncture from all gilts for radioimmunoassay of plasma cortisol and progesterone concentrations.

1. Cortisol levels for control animals were randomly scattered over both six day bleeding periods. Regression analysis of plasma cortisol over day of cycle, taken to the third order, was not significant ($P>.05$) for either period. For both P1 and P2 plasma cortisol increased ($P<.01$) following HA and decreased ($P<.05$) following ACTH, in a linear fashion, compared to the controls (Figure 1).

2. Plasma progesterone profiles were similar ($P>.10$) for all treatment groups measured during P1. During P2, progesterone increased ($P<.05$) in a linear fashion following HA or ACTH in contrast to that measured for the controls (Figure 2).

3. The results of this experiment indicate that peripherally measured plasma progesterone can be altered by exogenous ACTH or HA when given during mid to late diestrus in gilts.

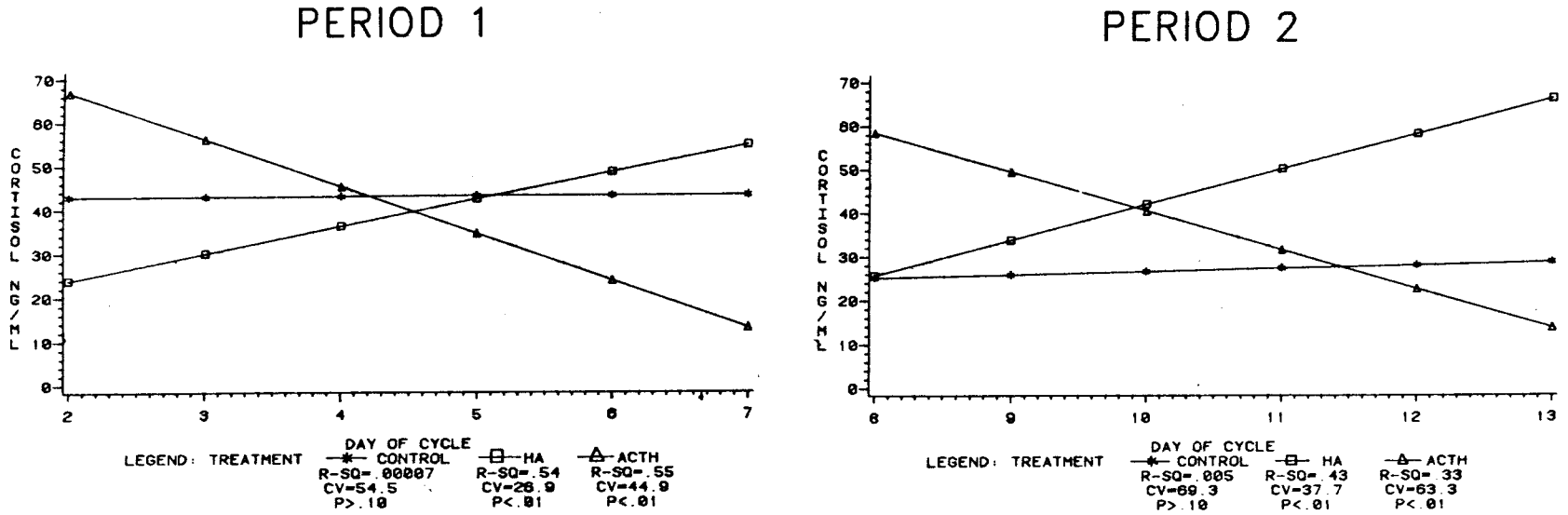


Figure 1. Plasma cortisol patterns of control, HA and ACTH treated gilts during period 1 (days 2-7) and period 2 (days 8-13) of the estrous cycle.

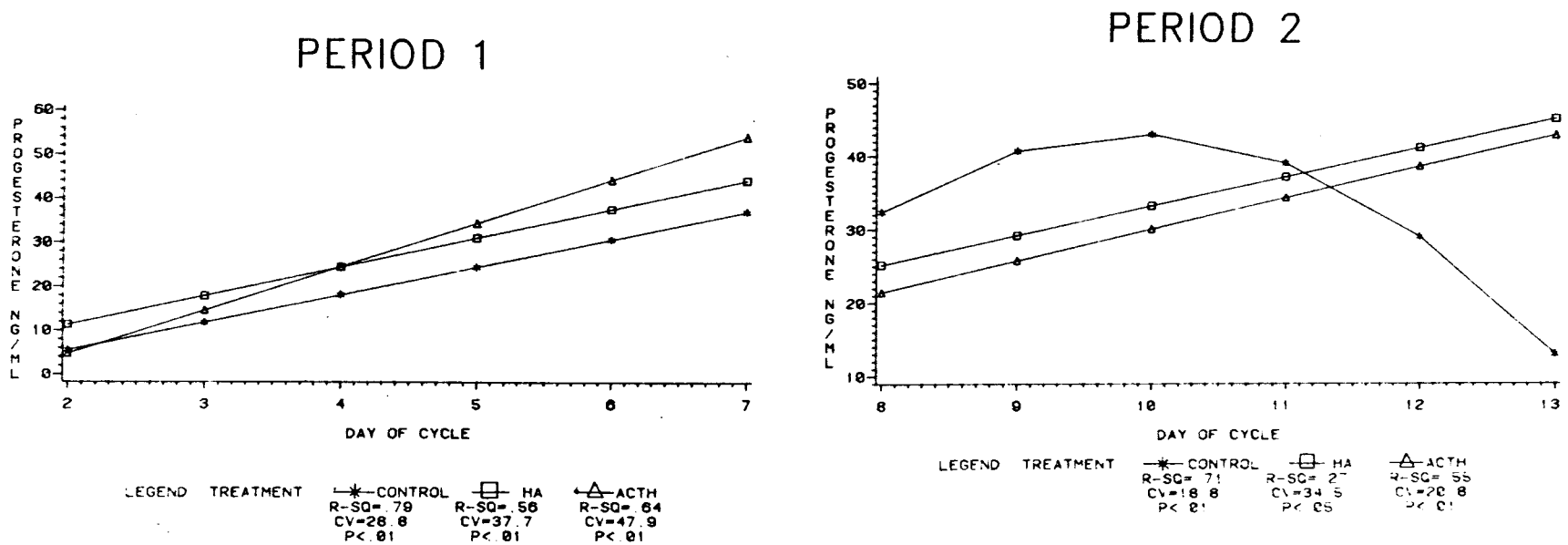


Figure 2. Plasma progesterone patterns of control, HA and ACTH treated gilts during period 1 (days 2-7) and period 2 (days 8-13) of the estrous cycle.

24-HOUR PERIPHERAL PLASMA CORTISOL LEVELS IN GILTS FOLLOWING ONCE VS TWICE DAILY INJECTION WITH ACTH OR HYDROCORTISONE ACETATE

H. G. Kattesh, J. R. Hembree and T. W. Parrott

In a previous study conducted in our laboratory it was reported that administration of a long acting adrenocorticotropin (ACTH) preparation (Corticotropin Zinc, Organon Pharmaceuticals, West Orange, NJ) at a dosage of 80 units will elicit a significant adrenal glucocorticoid response in a 150 kg gilt. The result was a two-fold increase in plasma glucocorticoid levels which peaked 4 to 6 hours post-ACTH injection. Although the effectiveness of this hormone preparation as a suitable adrenal stimulant is apparent, its application in extensive studies using swine is limited due to its high cost. The present experiment was conducted to determine the relative effectiveness of laboratory prepared solutions of ACTH (ACTH, grade V porcine, Sigma Chemical Co., St. Louis, MO) dissolved in gelatin and hydrocortisone acetate (HA; hydrocortisone 21-acetate, Sigma Chemical Co., St. Louis, MO) dissolved in sesame oil, administered either once or twice, in elevating circulating plasma cortisol levels in gilts measured over a 24-hour period.

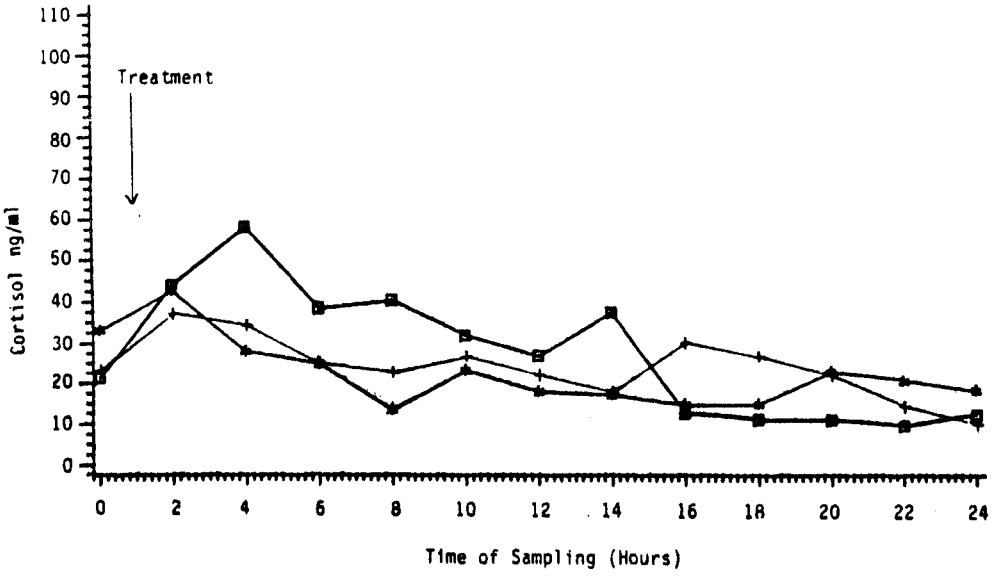
Four eight-month-old gilts were each surgically fitted with an indwelling jugular cannula between days 5 and 8 of the estrous cycle and maintained throughout the treatment period in individual crates. Treatments consisted of each animal receiving the following: control (2 ml sesame oil IM and 2 ml gelatin SQ) on day 10 of the estrous cycle; HA (2 mg HA/kg body weight IM) on day 12; and ACTH (1 IU ACTH/kg body weight SQ) on day 14. The four gilts were randomly allotted in pairs; one pair received a single injection and the other pair received a second injection of the prescribed treatments 12 hours after the initial injection. Blood samples (10 ml) were collected via the cannula immediately prior to each treatment and at two-hour intervals for 24 hours. Plasma was received from each sample and stored at -20C until analyzed for cortisol concentration using radioimmunoassay.

1. Plasma cortisol levels in control, HA and ACTH treated gilts measured over a 24-hour period are depicted in Figure 1.

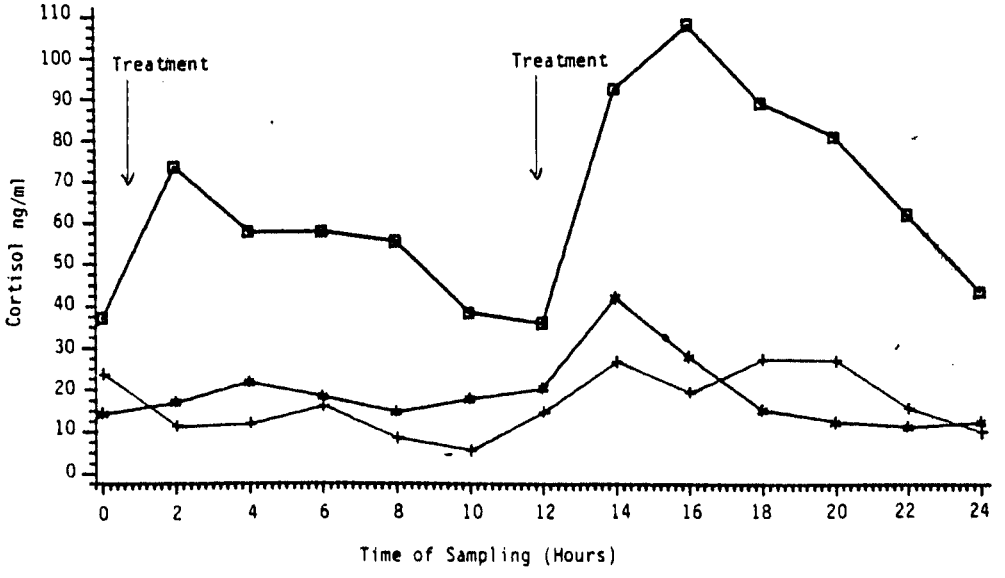
2. No significant ($P>.10$) difference in mean total area ($x \pm SEM$) under the curve was noted in response to a single injection of HA (2871.6 ± 59.4 mm^2) or ACTH (3598.1 ± 639.1 mm^2), or two injections of HA (2572.5 ± 932.6 mm^2) when compared to values determined for once injected (3157.2 ± 289.0 mm^2) or twice injected (2273.4 ± 250.2 mm^2) control animals.

3. Two injections of ACTH significantly ($P<.05$) elevated the cortisol response curve (7854.4 ± 108.9 mm^2) above that of the twice injected controls.

4. The results obtained in this experiment indicate that twice daily injection of ACTH results in a more sustained elevation in plasma cortisol levels over 24 hrs when compared to control treated animals. HA administration did not significantly elevate plasma cortisol levels over the 24 hr period. However, the approximate two-fold increase in cortisol following the second injection of HA suggests that there was some response to the second injection.



A. Single Injected



B. Twice Injected

Figure 1. Plasma cortisol concentration of gilts receiving control (+), hydrocortisone acetate (*) or adrenocorticotropin (□) treatment either once (A) or twice (B) over a 24 hour period.

HYDROCORTISONE ACETATE OR ACTH ADMINISTRATION DURING DIESTRUS IN GILTS: II. EFFECTS ON TOTAL UTERINE PROTEINS AND CORPORA LUTEA LH/hCH BINDING CHARACTERISTICS

J. R. Hembree, H. G. Kattesh and T. T. Chen

In the porcine it is well documented that progesterone exerts an essential role in regulating both qualitative and quantitative aspects of uterine protein secretion which are, in turn, necessary for maintaining a favorable uterine environment for embryos. Alteration of normal progesterone secretion by the corpus luteum (CL), following adrenocorticotropin (ACTH) or hydrocortisone acetate (HA) administration, could adversely affect the amount and/or kinds of proteins produced by the uterine endometrium and ultimately make the uterine environment less favorable for embryo development. Ultimately the regulation of ovarian function occurs at the cellular level. Gonadotrophins have been well documented as the primary regulators of gonadal function which exert their effect by binding to specific, high affinity receptors on the target cell surface. It was the purpose of this study to examine the effects of administering ACTH or HA to the normal cycling gilt during either early to mid- or mid-to late diestrus on total uterine proteins and binding capacity and binding affinity of LH/hCG to the CL.

Seventeen normal cycling gilts were allotted to one of three treatment groups as described in the previous study (control, HA treated and ACTH treated). Six additional gilts of similar age and weight (3 each in P1 and P2) were maintained as non-bled, non-treated controls. On either day 8 (P1) or day 14 (P2) gilts were anesthetized with halothane gas and their reproductive tract exposed by mid-ventral laparotomy. Each uterine horn was flushed with 20 ml .33% hypotonic saline solution, the fluid recovered and stored at -20C until analyzed for total proteins. After the uterine flushings were collected, the ovaries were removed, weighed, number of corpora lutea (CL's) counted and individual CL excised. The CL's were then frozen using dry ice and acetone and stored at -70C until analyzed for luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor characteristics.

1. There was no difference ($P>.10$) between the bled and non-bled control gilts for any of the parameters measured within P1 or P2 (Table 1). Therefore, all data from the two control groups were pooled and the pooled estimates were used in making treatment comparisons.

2. Within P1 (day 8) total uterine proteins, LH/hCG binding capacity and binding affinity of HA or ACTH treated gilts were not different ($P>.10$) from that of the pooled controls.

3. For P2 (day 14) total uterine proteins of ACTH treated gilts was lower ($P<.05$) than that of the controls. CL LH/hCG binding affinity for HA and ACTH treated gilts tended ($P<.10$) to be lower than that of the controls.

4. These results suggest that uterine and luteal function can be altered in gilts during mid-to late diestrus following HA or ACTH administration.

Table 1. Number of corpora lutea (CL), weight of CL, total uterine proteins, and luteinizing hormone/human chorionic gonadotropin (LH/hCG) binding to porcine luteal receptors from gilts on Day 8 or 14 of the estrous cycle

Treatment	Day of estrous cycle	Number of gilts	Number of CL	Weight of CL (mg)	Total Uterine proteins (mg)	LH/hCG Receptor Characteristics	
						Binding affinity ($\times 10^{10} M^{-1}$)	Binding capacity ($\times 10^{-10} M/CL$)
Controls:							
Nonbled	8	3	14 ± 2[a]	985 ± 188	11.07 ± 1.59[c]	3.11 ± .42	1.36 ± 1.28
	14	3	17 ± 2	836 ± 188	24.71 ± 1.59[d]	2.06 ± .42	5.85 ± 1.28
Bled	8	2	17 ± 3	769 ± 230	12.73 ± 1.95[c]	2.61 ± .51	2.83 ± 1.57
	14	3	12 ± 2	1027 ± 230(n=2)[b]	20.97 ± 1.95(n=2)[d]	1.98 ± .51(n=2)	4.54 ± 1.57(n=2)
Pooled	8	5	15 ± 2	899 ± 141	11.73 ± 1.26[c]	2.91 ± .31	1.95 ± .96
	14	6	15 ± 2	912 ± 141	23.21 ± 1.26[d]	2.03 ± .31	5.33 ± .96
Hormone injected:							
Hydrocortisone Acetate	8	3	15 ± 2	796 ± 188	13.70 ± 1.59[c]	2.98 ± .42	2.06 ± 1.28
	14	3	13 ± 2	1012 ± 188	26.54 ± 1.59[d]	1.23 ± .42	5.27 ± 1.28
Adrenocorticotropin	8	3	13 ± 2	877 ± 188	13.30 ± 1.59[c,e]	2.48 ± .42	1.36 ± 1.57(n=2)
	14	3	12 ± 2	982 ± 188	17.87 ± 1.59[e]	1.39 ± .42	6.79 ± 1.28

[a]Least square mean ± standard error of the mean.

[b]n = number of gilts.

[c,d,e]Least square means within a column which have different letter superscripts are significantly different ($p<0.05$). Comparisons between days of estrous cycle occur only within treatments. Hormone treatments are compared only to the pooled control.

A COMPARISON OF THREE FARROW-FINISH PORK PRODUCTION SYSTEMS AT AMES PLANTATION*

E. R. Lidvall, M. C. Dixon, R. L. Wyatt, R. A. Reynolds and J. M. Anderson

A study has been under way for seven years at Ames Plantation comparing three systems of farrow-to-finish pork production. System I is a pasture system. Fifteen acres of orchardgrass-ladino clover pasture are utilized for spring farrowing and finishing of the pigs during the summer. An additional 18 acres is seeded to winter pasture for the production of fall farrowed pigs. Individual housing is utilized, pigs are farrowed in April and October, pigs are weaned at 8 weeks of age and one sow herd is maintained producing about 24 litters at each farrowing. System II is a modest investment system involving the use of a pen-type central farrowing house and finishing on pasture. Two sow herds are maintained; one herd farrowing in January and July and the other in April and October. Pigs are weaned at 7-8 weeks of age and are moved to three-acre lots for growing and finishing. Sufficient sows and/or gilts are maintained to insure about 24 litters at each farrowing. System III involves the use of a central environment control crate-type farrowing house, a pig nursery, and two partial slatted modified open front finishing barns. The farrowing house has a capacity of 24 farrowing crates, pigs are weaned at 4-5 weeks of age, sows are immediately rebred, pigs are moved to the total slatted environment control nursery for approximately 5-7 weeks, and are then moved to one of the two finishing barns. Sufficient sows and/or gilts are maintained to insure 24 litters at each farrowing. Sows are bred and gestated on pasture in Systems I and II. A confinement breeding and gestation barn has been in use for two years in System III. The breeding program involves 3 and 4-way crosses utilizing Duroc, Hampshire, Landrace and Yorkshire boars. The rations are mixed in a portable type mixer using grain, soybean meal and a vitamin-mineral pre-mix. A 7-year summary of the sow, litter and pig performance is presented in Table 1.

1. Pigs farrowed per litter, pig birth weight, pigs weaned per litter, percent weaned of those born alive, pigs marketed per litter and days to 230 lb market weight are generally similar for each system.

2. Conception rate continues to be slightly lower in System III, probably because of earlier weaning. Confinement breeding has not lowered conception rate in System III, however.

3. Feet and leg and other structural problems have not occurred in a higher frequency in System III after moving the breeding herd from pasture to confinement.

4. The pounds of feed required to produce a pound of live pork from the farm continues to favor System III because of greater production per sow/year and a more favorable feed efficiency during the growing and finishing phases of production.

*The University of Tennessee is the beneficiary of a perpetual trust under the terms of the will of the late Julia C. Ames.

Table 1. Seven-year average of sow, litter and pig performance of three swine production systems at Ames Plantation, years 1976-1982

	System		
	I	II[1]	III
Sow herds maintained	1	2	3.3[2]
Number sows/system	29.3	63.7	107.2
Number litters produced/yr	48	108	190
Conception rate, %	83	80	78
Litters produced/sow/yr	1.64	1.69	1.78
Pigs produced/sow/yr (weaned)	12.6	13.0	13.6
Pigs marketed/sow/yr	11.9	11.8	12.4
Pigs farrowed/litter	10.4	10.6	10.4
Pigs farrowed alive/litter	10.1	10.0	9.8
Pigs weaned/litter	7.6	7.7	7.7
Pigs weaned of born alive, %	75	77	78
Pig birth wt., lbs	3.4	3.4	3.2
Pig wean wt., lbs	35.0[3]	31.8[4]	18.3[5]
Final nursery wt., lbs	---	---	50.5
Average daily gain, nursery, lb[6]	---	---	0.70
Market wt., lbs	231	222	217
Days to 230 lb. mk. wt.	206	210	211
Lbs. of feed required to produce a lb. of live pork[7]	4.16	4.20	3.88

[1]Some adjustment made in production figures for unusual "predator losses" during some finishing periods in 1979, 1980 and 1982.

[2]Three sow herds maintained during years 1976-78, 1981-82; 4 herds 1979-80.

[3]Eight (8) week weaning.

[4]Seven to eight (7-8) week weaning.

[5]Four to five (4-5) week weaning.

[6]Days in nursery varied with weaning age.

[7]Includes breeding herd feed, creep feed, grower and finisher feed.

EFFECT OF REARING ENVIRONMENT ON SUBSEQUENT REPRODUCTIVE PERFORMANCE OF GILTS

F. B. Masincupp, H. G. Kattesh, M. C. Dixon,
R. L. Wyatt and E. R. Lidvall

Sixteen littermate pairs of crossbred gilts were allotted to either an outside (pasture) or inside (confinement) rearing environment to explore the possibility that rearing environment of replacement gilts affects reproductive performance. Gilts were selected from the finishing floor of System III, Ames Plantation Swine Unit, at about 230 pounds. One littermate was moved to an outside lot and the other littermate was moved to the breeding-gestation building, both groups for development, breeding, and gestation. All gilts were bred to farrow at approximately one year of age. All gilts were farrowed in a crate-type environment controlled farrowing house and pigs were weaned at four to five weeks of age. After the first farrowing, all sows were moved to the breeding-gestation building and kept in the same environment through two more parities.

1. Rearing environment had no significant effect on any of the parameters listed in Table 1.

2. Parity had a highly significant effect ($P<.01$) on sow farrowing and weaning weights.

3. Parity had a significant effect ($P<.05$) on pigs weaned, average birth weight.

4. Parity also affected adjusted 35-day weaning weights ($P<.10$).

5. Sows tended to lose less weight at farrowing as parity increased.

6. Gilts and sows that did not breed tended to weigh less than their contemporaries.

Table 1. Farrowing and weaning performance of outside vs inside reared gilts through three parities

Treatment*	T-1			T-2		
Parity	P-1	P-2	P-3	P-1	P-2	P-3
Total pigs born	9.4	10.7	11.4	10.6	11.8	10.7
Pigs born alive	8.4	10.1	10.8	10.1	10.9	10.4
Av. birth wt. (lbs)	3.4	3.0	3.4	3.3	2.8	3.4
Pigs weaned	7.2	9.1	8.8	6.9	7.9	9.1
Adjusted 35-day weaned wt. (lbs)	19.0	20.9	18.9	19.3	20.9	18.8
Sow farrowing wt. (lbs)	372.9	389.2	418.3	354.9	3965.9	421.2
Sow weaning wt. (lbs)	305.1	332.7	371.4	310.6	356.0	386.3
Number of sows	12	9	8	14	9	9

*Treatment 1 = outside rearing; Treatment 2 = inside rearing

EVALUATION OF TENNESSEE FEEDER PIG GRADES

F. B. Masincupp, E. D. Brooks, III,
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A total of 480 graded feeder pigs were utilized in this experiment including 90 U.S. No. 1, 90 U.S. No. 2 and 60 U.S. No. 3 pigs in each of two trials. The three grades were further divide into three body types as follows: type III - longer, larger framed pigs, type II - those considered average in length and height, and type I - shorter, more compact, earlier maturing pigs. Grades 1 and 2 utilized the three body types while grade 3 pigs were divided into body types I and II. Trial 1 began with pigs purchased in January and ended in May, 1983, and trial II was conducted from June to November, 1983.

The pigs were housed six per pen in a 40-pen, curtain walled, pit ventilated, partially slatted floor finishing unit. Each 6 x 10' pen was equipped with a nipple waterer and a two-hole self-feeder. Individual pig weights were recorded upon arrival at the station and subsequently at one, three, seven, ten and thirteen weeks on feed. Pigs were taken off test and moved to slaughter at a pen average weight of 230 lbs. Additional data collected included average backfat thickness at arrival, seven and thirteen weeks on feed and finally at a 230-lb. market weight. Pen average daily gain and feed efficiencies were calculated.

All pigs were photographed in a calibrated cage so that measurements of pig length and height could be used to further describe the grades and types of feeder pigs. Pigs were slaughtered at an average weight of 230 lbs. and carcass data was collected including muscling score, length, and backfat.

1. Preliminary analysis indicate that grades 1, 2 and 3 and types I and/or II within those grades are similar in ADG, FE, BF, and length (Table 1).

2. Grades 1 and 2 - type III pigs tended to gain slower, were less efficient feed converters, were fatter and slightly shorter than their type I and II counterparts.

3. Grade 3 - type III pigs were outperformed by all other grades and types of pigs in the study.

4. The unexpected good performance of grade 3 - type II pigs would suggest they were previously mismanaged, thus causing them to be graded as U.S. No. 3 feeders.

5. Physical measurements of feeder pigs are presently being evaluated.

Table 1. Performance of grades and types of feeder pigs[1]

Grade-type	ADG[2]	FE[3]	BF[4]	Length[5]
1-3	1.57	3.17	1.32	32.1
1-2	1.65	3.17	1.41	31.5
1-1	1.57	3.32	1.51	31.2
Grade avg.	1.60	3.22	1.41	31.6
2-3	1.68	3.15	1.43	32.3
2-2	1.63	3.20	1.43	31.8
2-1	1.54	3.35	1.53	31.1
Grade avg.	1.62	3.23	1.46	31.8
3-2	1.61	3.16	1.44	31.8
3-1	1.49	3.44	1.60	30.5
Grade avg.	1.55	3.30	1.52	31.2

[1]Unanalyzed simple means
[2]Average daily gain
[3]Feed efficiency
[4]Backfat
[5]Carcass length

THE INDUCTION OF CAGE LAYER FATIGUE IN LAYING HENS

H. V. Shirley

The increased confinement of laying hens in cages in recent years has given rise to the problem known as cage layer fatigue (CLF). The disease is characterized by the inability of the bird to stand and by marked fragility of the bones. Hens, on becoming paralyzed, die from lack of feed and water. The cause of CLF has not been determined. It may appear in certain flocks, but not in others, and the incidence within a flock may vary greatly. It is thought that the calcium-phosphorus ratio, high environmental temperature and inactivity due to confinement are contributing factors.

In a study designed to compare various methods of inducing molt in layers, an interesting pattern of CLF development and mortality was observed on certain diets and following certain handling procedures. The present study was designed to further investigate the effect of diet and temperature on incidence of CLF.

Two hundred forty S.C.W.L. hens laying at the rate of 85% were placed in four environmental rooms in 8" x 6" laying cages with 2 hens per cage. The following four treatments were imposed on these hens at 230 days of age for 7 days: (1) 3.5% dietary calcium and room temperature of 70°F, (2) 3.5% dietary calcium and room temperature of 90°F, (3) 1.0% dietary calcium and room temperature of 70°F, (4) 1.0% dietary calcium and room temperature of 90°F.

1. In groups 1 and 2 there was no significant change at 7 days compared with their initial values (table 1).

2. In groups 3 and 4, shell quality declined rapidly with all eggs being shell-less from day 3 to day 7. Almost all hens in groups 3 and 4 had ceased to lay by day 7.

3. In groups 4, 58% of the hens succumbed to CLF whereas no case of CLF was observed in the other 3 groups.

4. When hens from each group hyperventilated as a consequence of handling, 100% of the hens with CLF died within approximately 3 minutes of the initiation of handling.

5. The combination of low dietary calcium and high environmental temperature apparently resulted in hypocalcemia which led to impaired neuromuscular transmission and CLF. Forced hyperventilation and resultant alkalosis further reduced the plasma ionized calcium bringing about respiratory paralysis and death.

Table 1. Effect of dietary calcium levels, environmental temperatures and induced hyperventilation on the incidence of cage layer fatigue and mortality

Treatment	No. hens	7 days following initiation of treatment		
		Shell-less eggs	% hens with CLF	% mortality following forced hyperventilation
3.5% dietary Ca room temp.- 70°F	60	0	0	0
3.5% dietary Ca room temp.- 90°F	60	0	0	0
1.0% dietary Ca room temp.- 70°F	60	all**	0	0
1.0% dietary Ca room temp.- 90°F	60	all**	58**	100**

**Different from treatment 1 and 2 ($P<.01$).

EFFECTS OF β-HYDROXYBUTYRATE ON KETOGENESIS IN FASTED EWES

R. N. Heitmann

β-hydroxybutyrate (BOHB) infusions in fed sheep exhibited portal-drained visceral (PDV) and hepatic (HEP) autoregulatory effects for acetoacetate (ACAC) and PDV autoregulation of BOHB production. In addition HEP uptake of free fatty acids (FFA), the percursors of ketones, was decreased. However, additional prior work has demonstrated that autoregulation may be more important in physiological states of accelerated ketogenesis such as fasting.

The present study was conducted to determine the net fluxes of BOHB, ACAC, FFA, insulin and glucagon across the major splanchnic tissues. In addition to that work done previously on fed sheep, glucose— the more traditional energy metabolite — metabolism was measured and the fluxes across the lower muscle mass (RUMP) were calculated. The ewes were equipped with chronic indwelling catheters in the femoral artery, and portal, hepatic, mesenteric, femoral and jugular veins. BOHB was infused (0.4 g/kg$^{3/4}$/hr) into the jugular and paraminohipparate (1.5% at 0.764 ml/min) into the mesenteric vein in order to measure blood flow. Control and BOHB infusion samples were taken at 30-minute intervals from the artery and portal, hepatic and femoral veins.

1. Arterial concentrations and net fluxes of glucose, ACAC, BOHB, and FFA across the PDV, HEP, total splanchnic (TSP) and RUMP tissues are shown in Table 1.

2. Arterial concentrations of glucose were not effected by BOHB infusion. There was a net utilization of glucose by PDV which is typical of fasted ruminants. BOHB infusion decreased HEP and TSP production but also decreased RUMP utilization of glucose perhaps sparing glucose for central nervous system metabolism.

3. As in fed sheep, BOHB infusion increased PDV production but decreased HEP and TSP utilization of ACAC. However, the effects were 2 to 3 fold greater on the fasted ewes. There were no changes in ACAC metabolism across the RUMP.

4. As in fed sheep, BOHB infusion increased PDV utilization of BOHB. However, unlike fed sheep, BOHB infusion in fasted animals decreased HEP and TSP ketogenesis from BOHB dramatically by 50 and 60%, respectively. In addition, RUMP utilization of BOHB increased 2 fold, perhaps being used as an alternate energy fuel for glucose.

5. BOHB decreased arterial FFA concentration by 65%. Lipolysis in both PDV and RUMP adipose tissues was decreased by 70 and 80%, respectively, with BOHB infusion. Concomitantly, HEP and TSP utilization of FFA was decreased by 78 and 93%, respectively.

6. Autoregulation of ketogenesis is much more pronounced in fasted than fed ewes and may be due to both a decrease in PDV and RUMP lipolysis and a decrease in HEP and TSP utilization. However, insulin and glucagon fluxes and trials on diabetic treated and untreated animals must be included in order to determine the mechanism of action.

Table 1. Arterial concentrations and net fluxes of glucose (mM/hr), Acetoacetate (uM/min), β-hydroxybutyrate (uM/min), and free fatty acids (uM/min) in fasted sheep

	A	PDV	HEP	TSP	RUMP
Glucose (mg/dl)					
Control	48.92 ± 3.27	-3.74 ± 3.30	17.03 ± 6.93	13.29 ± 4.64	-1.06 ± 0.85
BOHB	49.05 ± 2.28	-5.00 ± 2.97	10.44 ± 4.87	5.44 ± 2.18	-0.36 ± 0.54
Acetoacetate (uM/1)					
Control	109.0 ± 8.77	-6.56 ± 15.09	123.4 ± 33.56	117.2 ± 24.76	-12.70 ± 2.97
BOHB	181.4 ± 24.37	91.30 ± 17.70	-175.6 ± 26.70	-84.32 ± 26.79	
β-hydroxybutyrate (uM/1)					
Control	1279 ± 58.49	-205.8 ± 81.47	1281.1 ± 137.8	1075.9 ± 143.4	-120.4 ± 25.69
BOHB	3170 ± 292.4	-255.5 ± 103.8	643.4 ± 111.9	387.9 ± 151.5	-245.6 ± 33.07
Free Fatty Acids (uM/1)					
Control	1100 ± 154	361.3 ± 80.4	-593.7 ± 113.3	-233.7 ± 89.5	78.4 ± 34.7
BOHB	391 ± 49	115.2 ± 68.8	-129.6 ± 69.2	-15.2 ± 113.4	15.6 ± 14.5

A = Arterial concentration
PDV = Portal-drained Viscera
HEP = Liver
TSP = Total Splanchnic

EFFECTS OF β-HYDROXYBUTYRATE ON KETOGENESIS IN NORMAL AND DIABETIC SHEEP

R. N. Heitmann and J. M. Fernendez

Intravenous pulse doses of β-hydroxybutyrate (BOHB) into fed and 1, 3 and 5 day fasted mature wethers caused a prolonged depression of plasma free fatty acids (FFA) and a transitory rise in plasma insulin. This would suggest that ketone bodies may have some auto-regulatory control over their own production rates. The extent of this auto-regulation may define the sheep's ability to resist pregnancy acetonemia and the lactating dairy cow's ability to resist ketosis.

In order to determine the target tissue(s) of BOHB and its mechanism of action, BOHB infusion ($0.4g/kg^{3/4}/hr$) trials were conducted on normal (5), diabetic insulin treated (DIT) (3), and diabetic untreated (DUT) (3) ewes. The animals were equipped with chronic, indwelling catheters in the femoral artery and portal, hepatic, mesenteric and femoral veins. BOHB was infused into the femoral and paraaminohippurate (1.5% at 0.76 ml/min) into the mesenteric vein in order to measure blood flow across the down stream tissues. Pre- and post-infusion samples were taken at 30-minute intervals from artery and portal and hepatic veins simultaneously.

1. The net rates of production (positive numbers) and utilization (negative numbers) for acetoacetate (ACAC), BOHB, FFA, and insulin across the portal-drained viscera (PDV), liver (HEP) and total splanchnic (TSP) tissues are shown in Table 1.

2. ACAC production by PDV increased with BOHB infusion in normal and DIT ewes but this was more than compensated for by an increase in HEP utilization and resulted in an overall decrease in TSP production. This effect was completely lost on DUT sheep.

3. PDV production of BOHB was switched to a state of utilization with BOHB infusion in normal and DIT sheep but not DUT ewes. No effects were noticed across the HEP and TSP.

4. HEP and TSP utilization of FFA, the precursor of ketogenesis, was decreased in all three treatments, but most dramatically so in the normal and DIT sheep.

5. Pancreatic (represented by PDV) production of insulin increased with infusion of BOHB in normal sheep but this was negated by increased HEP extraction resulting in no change in TSP production. Both diabetic treatments, of course, lost all significant fluxes of insulin.

6. Since normal and DIT sheep behaved similarly but most of the BOHB effects were lost on DUT sheep, it is suggested that the effects of BOHB infusion on ketones, FFA, and insulin may be mediated by an increased insulin receptor affinity and not by increased pancreatic production.

Table 1. Net production (+) or utilization (-) of ketones, free fatty acids, and insulin across the splanchnic tissues of fed sheep

	PDV	HEP	TSP
Acetoacetate (μM/min)			
Normal			
Control	24.7 ± 8.7	-35.8 ± 14.2	-11.1 ± 12.4
BOBH Infusion	67.9 ± 10.1[c]	-112.0 ± 12.6[c]	-44.2 ± 16.4
DIT			
Control	4.0 ± 5.7	12.5 ± 13.4	16.5 ± 11.1
BOBH Infusion	62.7 ± 16.9[c]	-177.2 ± 37.6[cd]	-114.5 ± 22.6[cd]
DUT			
Control	-9.4 ± 13.9[d]	71.7 ± 49.6[d]	62.3 ± 36.4[d]
BOHB Infusion	11.9 ± 5.5[d]	13.0 ± 8.2[d]	24.9 ± 7.9[d]
β-hydroxybutyrate (μM/min)			
Normal			
Control	137 ± 28	395 ± 51	532 ± 65
BOBH Infusion	-394 ± 94[c]	679 ± 127	285 ± 191
DIT			
Control	9 ± 26	749 ± 126	758 ± 113
BOBH Infusion	-278 ± 102	881 ± 100	602 ± 140
DUT			
Control	450 ± 471	1138 ± 788	1588 ± 859
BOHB Infusion	227 ± 435[d]	1445 ± 563	1672 ± 681[d]
Free Fatty Acids (mU/min)			
Normal			
Control	97 ± 33	-284 ± 31	-187 ± 50
BOBH Infusion	76 ± 19	-108 ± 19[c]	-33 ± 31[c]
DIT			
Control	3 ± 9[d]	-280 ± 49	-276 ± 50
BOBH Infusion	32 ± 10	-94 ± 22[c]	-62 ± 16[c]
DUT			
Control	43 ± 27	-485 ± 82[d]	-442 ± 81[d]
BOHB Infusion	-10 ± 43[c]	-319 ± 66[cd]	-329 ± 38[d]
Insulin (μM/min)			
Normal			
Control	24 ± 5.4	-16.7 ± 5.2	8.2 ± 4.4
BOBH Infusion	35.7 ± 5.2[c]	-26.6 ± 4.0[c]	9.2 ± 4.4
DIT			
Control	-0.9 ± 0.4[d]	-1.8 ± 1.0[d]	2.7 ± 0.8[d]
BOBH Infusion	-0.6 ± 0.3[d]	-2.8 ± 0.7[d]	-3.4 ± 0.7[d]
DUT			
Control	0.7 ± 0.7[d]	-1.5 ± 0.7[d]	-0.8 ± 0.6
BOHB Infusion	0.1 ± 0.6[d]	-0.2 ± 0.6[d]	-0.1 ± 0.6[d]

PDV = Portal-drained Viscera, HEP = hepatic, TSP = Total Splanchnic
c = Significantly different from Control, $P<.05$.
d = Significantly different from Normal, $P<.05$.

USE OF "EXOTIC BREEDS" OF SHEEP TO INCREASE LAMB PRODUCTION IN TENNESSEE

E. R. Lidvall and F. B. Masincupp

INTRODUCTION: The number of lambs born and reared per ewe per year is most important to Tennessee sheep producer profits. There are a number of breeds known for high lambing rates which have been imported into the United States and Canada in recent years. Three of these breeds were selected for their apparent desirable reproductive capabilities (multiple lambing rate and/or 'out-of-season' breeding). Two rams of each breed were crossed on the parent ewe flock (Knoxville Station) of predominately Hampshire breeding during 1976 and 1977 to produce F_1 crosses. The breeds utilized were:

Finnish Landrace: The Finnsheep breed was developed in Finland and has been bred for scores of generations with no intermixture of other blood. They are rather average in size, flat muscled, free of wool on the face and legs, and low yielding in regards to wool production. They are very prolific, averaging about 1.8 lambs for a yearling ewe and 2.65 lambs for and adult ewe.

Clun Forest: The Clun Forest is a European bred which has found favor in England and Wales. Only a few have been imported to the United States. They are a relatively large breed, have a typical mutton, are adequate in wool production and quality, and maintain a lambing rate well above 150%.

Barbados Blackbelly: The Blackbelly or "Barbs" were first recorded as imports from the West Indies to Texas in 1904. They tend to be dark in color, small in size and produce hair instead of wool. California observations report a lambing rate close to 3 lambs per ewe per year. They grow rather slowly and reach puberty at an early age.

All F_1 ewes were bred to Suffolk rams. Lambs were weaned at about 47 days of age and reared to a market weight of near 100 lbs. on slatted floors. Ewes had an opportunity to lamb three times in two years — January, September and May (8-month intervals). The ewes were first lambed at 16 months of age. Table 1 presents a summary of the ewe and lamb performance data for the study.

SUMMARY:

1. The Fin and Barbados ewes have averaged a 200% lamb crop/year reared to weaning; both far superior to the Clun Forest ewes.

2. The Finn ewes tend to "triplet" more often; however, both the Finn and Barbados ewes lamb a high percentage of strong twin lambs.

3. The Barbados ewes excel in the livability of their lambs, most likely because of greater heterosis in the cross.

4. Growth rate has been superior in the lambs from Clun ewes; however, a much higher percentage of these lambs are singles giving them an advantage in a slightly higher weaning weight as well as subsequent growth rate.

5. Lambs from Finn and Barbados ewes produced leaner carcasses.

6. Finn and Barbados crossed ewes tended to breed more readily 'out of season' and produced more lambs in September (April breeding).

Table 1. Use of "exotic breeds" of sheep to increase lamb production in Tennessee. Ewe and lamb performance data[1]

	Finnish Landrace	Clun Forest	Barbados Blackbelly
Ewes beginning project	14	19	18
Ewes lost	4	2	4
Ewes ending project	10	17	14
Av. ewes for project	11	18	16
Av. ewe lambing wt., lbs	156	165	139
Av. ewe wean wt., lbs[2]	139	152	121
Av. fleece wt., lbs	4.5	4.6	3.5
Total ewe lambings	66	91	99
Ewe lambings/yr	14.7	20.2	22.0
Prolificacy, % born	177	134	147
Total lambs born[3]	117	122	145
Lambs born/flock/yr	26.0	27.1	32.3
% lamb crop born/flock/yr	236	151	202
Total lambs weaned	104	111	142
Lambs weaned/flock/yr	23.1	24.7	31.6
% lamb crop weaned/flock/yr	210	137	197
Lbs. lamb weaned/ewe/yr	86	60	84
Total lambs marketed	94	96	125
Lambs marketed/flock/yr	20.9	21.3	27.2
% lamb crop marketed/flock/yr	190	119	174
Av. lamb birth wt., lbs	8.6	9.9	9.6
Av. lamb wean (47 da.) wt., lbs	41.3	43.4	42.1
ADG, birth-weaning, lb	.69	.75	.68
Av. lamb market wt., lbs.	96	98	93
ADG, wean-mk., lb[4]	.43	.45	.40
Birth-market, days	182	169	177
Carcass traits[5]:			
No. of lambs	24	21	33
Yield grade	3.4	4.0	3.2
Quality grade	Pr-	Pr-	Ch+

[1]These data not corrected for age of ewe and type of birth or rearing.
[2]Ewe weight at time lamb(s) weaned.
[3]All lambs are sired by Suffolk rams.
[4]Reared on slatted floors in confinement.
[5]About one-fourth of lambs slaughtered for detailed carcass data.

HYPOMAGNESEMIA AND HYPERTENSION IN PREGNANT OR LACTATING EWES

J. K. Miller, N. Ramsey, M. D. Schneider,
C. K. Reynolds and B. K. Al-Dabbagh

Conditions in early spring when clinical hypomagnesemia can be a problem in grazing ruminants include high forage potassium (K), reduced availability of magnesium (Mg) and the stresses of changing weather, parturition, and initiation of lactation. Medical evidence for a relationship between hypomagnesemia and certain peripheral vascular diseases including eclampsia and some forms of hypertension is convincing. Potassium, in addition to reducing absorption of Mg from the rumen, may also influence blood pressure but results have been inconsistent. Since hypertension may contribute to the grass tetany syndrome, the objective of this report was to determine effects of dietary Mg deficiency and K excess, singly or combined, on blood pressure of pregnant or lactating ewes.

Twelve Finnish cross ewes in the second month of pregnancy were initially fed wheat straw (0.08% Mg) and concentrate (0.24% Mg) consisting of 40% ground corn cobs, 18% corn starch, 15% dextrose, 12% corn gluten meal, 6% soybean protein isolate, 1% corn oil, minerals and vitamins. After 3 mo of gestation, the ewes were divided into four groups and the concentrate was modified to contain deficient (groups 1 and 3) or adequate (groups 2 and 4) Mg and adequate (groups 1 and 2) or excessive (groups 3 and 4) K. At weekly intervals blood plasma was analyzed for Mg and arterial blood pressure was measured using an automatic sphygmomanometer with a pediatric cuff on the animals forelimb.

1. Mean minimum plasma Mg concentrations remained at preliminary levels for 11 wk after diet changes in groups 2 and 4 but fell 59% in group 1 and 72% in group 3 (Table 1).

2. Average treatment effects on blood pressure were negligible during gestation or lactation but maximum systolic/diastolic pressures were elevated 22/12 to 23/20 mm Hg during gestation and 29/19 to 38/27 mm Hg during lactation when low Mg or excess K were fed.

3. In a comparison with other groups, ewes fed the adequate Mg-normal K diet (group 2) appeared calmer during blood sampling and blood pressure measurements and their lambs were stronger at birth and had a higher survival rate.

4. Greater clumping of platelets in peripheral blood obtained from ewes in groups 1 and 3 after parturition suggested that vasoactive substances released from hyperactive platelets may have contributed to elevated blood pressure in Mg-deficient ewes but not in ewes fed excess K alone.

TABLE 1. Survival of lambs, plasma magnesium and potassium, blood pressure and clumping of platelets in ewes fed diets deficient or adequate in magnesium.

Item	Treatment group			
	1	2	3	4
Dietary Mg (% of DM)	.03	.24	.04	.25
Dietary K (% of DM)	.70	.70	3.52	3.45
Plasma Mg (mg/dl)				
Preliminary	2.43	2.55	2.52	2.22
Mean during gestation	1.97	3.03	2.13	2.52
Mean minimum during gestation	1.65	2.47	1.95	2.29
Mean during lactation	1.43	3.14	1.31	2.36
Mean minimum during lactation	0.99	2.70	0.70	2.06
Systolic/diastolic pressure (mm Hg)				
Preliminary	124/74	124/74	110/63	116/73
Mean during gestation	127/72	125/71	109/65	131/84
Mean maximum during gestation	145/91	136/86	132/75	139/93
Mean during lactation	139/85	117/67	114/76	120/74
Mean maximum during lactation	153/93	130/76	148/100	149/101
Clumping of platelets after parturition (%)	18.22	7.92	21.51	5.64
Survival rate of lambs (%)	33	100	75	50

MORPHOMETRIC ANALYSIS OF MITOCHONDRIAL ALTERATIONS INDUCED BY SELECTED MONOAROMATICS

W. C. Cullen and T. W. Schultz

We tested the hypothesis that chemical compounds causing the uncoupling of oxidative phosphorylation produce a specific and quantifiable alteration in mitochondrial structure at sublethal concentrations and that the magnitude of this cellular response is related to chemical structure. Stereologic techniques were used to obtain precise, objective quantitative estimates of mitochondrial structural parameters in a cellular test system exposed to sublethal doses of the classic uncoupling agents 2,4 dinitrophenol (2,4 DNP) and arsenic. Since T. pyriformis is sensitive to 2,4 DNP and arsenic and has a structure and metabolism similar to metazoan cells, it provides a suitable test system. Other toxicants chemically related to 2,4 DNP and various arsenic compounds were also tested to determine which substituent(s) are required to effect the uncoupling response. These compounds include Na-arsenite, Na-arsenate, cacodylic acid, Na-cacodylate, monosodium methanearsonate, disodium methanearsonate, methanearsonate, monoammonium methanearsonate, nitrobenzene, 1,3 dinitrobenzene, 1,4 dinitrobenzene, phenol and 2,4,6 trinitrophenol.

The justifications of this study are two-fold: 1) Arsenicals, principally from herbicides and pesticides, and industrial organics, including phenol and other aromatic compounds, present a significant health and environmental threat. The stereological test system described here will provide a rapid and inexpensive means of quantitating sublethal, structural changes induced by these pollutants. The quantitative data generated can provide the means to compare and rank biological responses and this can be used in risk assessment analysis to aid in formulating regulatory policy. 2) The use of the ciliate T. pyriformis as a eukaryotic in vitro test system will provide an easily maintained and inexpensive alternative to whole animal testing.

Stereologic estimates have been made on the quantitative structural effects of the classic uncoupling agent 2,4 DNP and the structurally related compounds, NB and 1,4 dinitrophenol. The cells were exposed to the toxicants at the EC_{50} concentrations. The numerical results are shown in the table. These support the following conclusions:

1. Nitrobenzene (NB) and 2,4 dinitrophenal (2,4 DNP) cause significant declines in cell volume; 1,4 dinitrobenzene (1,4 DNB) does not.

2. The outer surface area of mitochondria per cell is increased by 2,4 DNP but mitochondrial volume is not. This suggests more but smaller mitochondria in these cells.

3. A decrease in mitochondrial volume but not surface area after treatment with 1,4 DNB suggests shrunken mitochondria.

4. Intramitochondrial alterations include increases in cristae volume and surface area coupled with a decrease in matrix volume with 2,4 DNP, decreases in cristae volume and surface area with NB and decreases in matrix and cristae volume with 1,4 DNB.

Insufficient data is currently available to identify possible correlations between chemical structure and mitochondrial structural alterations.

Table 1. Stereological estimates of mitochondrial parameters in T. pyriformis following treatment with monoaromatic toxicants[1]

Parameter	Control	2,4 DNP	NB	1,4 DNB
Cell volume[2]	2.414 (.217)	1.800 (.192)*	1.846 (.131)*	1.956 (.114)
Volume mitochondria[2]	0.203 (.012)	0.213 (.010)	0.177 (.010)	0.160 (.010)*
Surface area mito.[3]	2.230 (.130)	2.600 (.140)**	2.030 (.100)	1.937 (.114)
Volume mito. matrix[2]	0.087 (.002)	0.070 (.003)*	0.091 (.003)	0.066 (.003)*
Volume mito. cristae[2]	0.114 (.002)	0.139 (.003)**	0.082 (.003)*	0.082 (.002)*
Surface area cristae[3]	0.833 (.024)	1.115 (.056)**	0.677 (.016)*	0.764 (.028)

*Significant decrease ($p = 0.05$).
**Significant increase ($p = 0.05$).
[1]Table shows mean estimates per average cell (SE).
[2]Volume X $10^5 um^3$.
[3]Surface area X $10^5 um^2$.

TESTING OF HYPERADNENOCORTICISM IN THE DOG BY THE COMBINED DEXAMETHASONE SUPPRESSION/ACTH STIMULATION PROCEDURE

H. Eiler, J. W. Oliver and A. M. Legendre

Reactivity to test-ACTH is an indirect measurement of the mass of steroidogenic tissue available in the adrenal glands. Whereas the dexamethasone suppression test is the measurement of the efficiency of the inhibitory component of the feedback system. Both tests, ACTH-stimulation and dexamethasone-suppression, have been consolidated in our laboratory in a single "combined dexamethasone suppression/ACTH stimulation" procedure. The objective of the present work was to investigate the feasibility of the combined suppression/stimulation approach and to evaluate the cortisolemia response to the combined test of dogs clinically diagnosed as having canine Cushing's disease syndrome.

Four different groups of abnormal responses were identified in fifty-four dogs (Figure 1).

1. In group I (14.8%), the only abnormality was partial suppression with test-dexamethasone.

2. In group II (29.6%), two abnormalities were found: partial suppression with dexamethasone and hyperreactivity to test-ACTH.

3. In group III (48.1%), three abnormalities were found: baseline hypercortisolemia, partial suppression with dexamethasone and hyperreactivity to test-ACTH.

4. In group IV (7.4%), two abnormalities were found: baseline hypercortisolemia and partial suppression with dexamethasone (response to test-ACTH was normal).

5. The combined dexamethasone suppression/ACTH-stimulation test is a feasible, flexible and convenient procedure to be used in a clinical situation.

6. These results suggested that there may be several stages present in the negative feedback failure associate with Cushings disease syndrome in the dog (Figure 1).

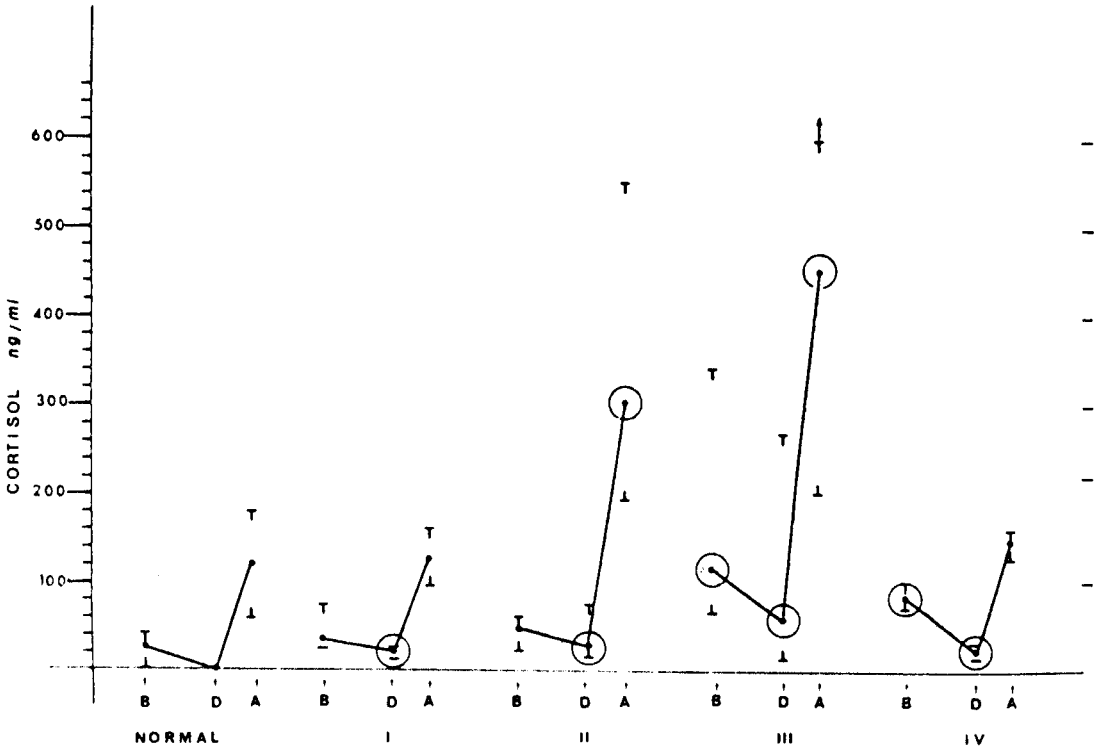

Figure 1

Cortisolemia response to the combined dexamethasone suppression/ACTH stimulation testing procedure of 54 dogs tentatively diagnosed as having Cushing's disease syndrome.

Dots are mean values, while dispersion bars indicate range. Encircled dots indicate abormal test result values. The letters B, D and A indicate baseline cortisolemia, the dexamethasone suppressed value and ACTH stimulated value, resectively.

Baseline cortisolemia values (mean $\pm$ standard deviation) were 24 $\pm$ 12, 35 $\pm$ 13, 46 $\pm$ 13, 115 $\pm$ 67, 82 $\pm$ 13 ng/ml for group normals and I-IV, respectively. Dexamethasone-suppressed cortisolemial values were < 10, 19 $\pm$ 4, 28 $\pm$ 20, 56 $\pm$ 54, 19 $\pm$ 10 ng/ml for group normals and I-IV, respectively. ACTH-stimulated cortisolemia values were 119 $\pm$ 52, 122 $\pm$ 32, 303 $\pm$ 112, 449 $\pm$ 129, 146 $\pm$ 30 ng/ml for group normals and I-IV, respectively.

"Normal" indicates the group of dogs that exhibited a normal response, while "I-IV" indicate the four different groups of dogs that exhibited abnormal responses.

Notice that in Groups I, II and III there are one, two and three abnormal test result values respectively.

EFFECTS OF A THROMBOCYTOPOIESIS-STIMULATING FACTOR ON MEGAKARYOCYTOPOIESIS OF MICE[1]

by
T.P. McDonald, G.D. Kalmaz, Marilyn Cottrell, Rose Clift

A thrombocytopoiesis-stimulating factor (TSF) will stimulate megakaryocytopoiesis and thrombocytopoiesis in mice; previously, TSF has been shown to increase the percentage of marrow small acetycholinesterase positive cells, will enhance megakaryocyte endomitosis, and will elevate the number of megakaryocyte colonies grown in vitro. TSF, therefore, should increase the total number and size of marrow megakaryocytes; however, studies to confirm this hypothesis have not been previously reported. In the present study, the marrow megakaryocyte concentration and diameter were determined in mice after they had received an injection of TSF. For controls, other mice were injected with saline, normal rabbit serum, or platelet specific antiserum.

Both TSF and rabbit anti-mouse platelet serum (RAMPS) caused significant ($P<0.0005$) increases in the number and diameter of marrow megakaryocytes 2-3 days after injection. Figure 1 shows a summary of the results of three separate experiments.

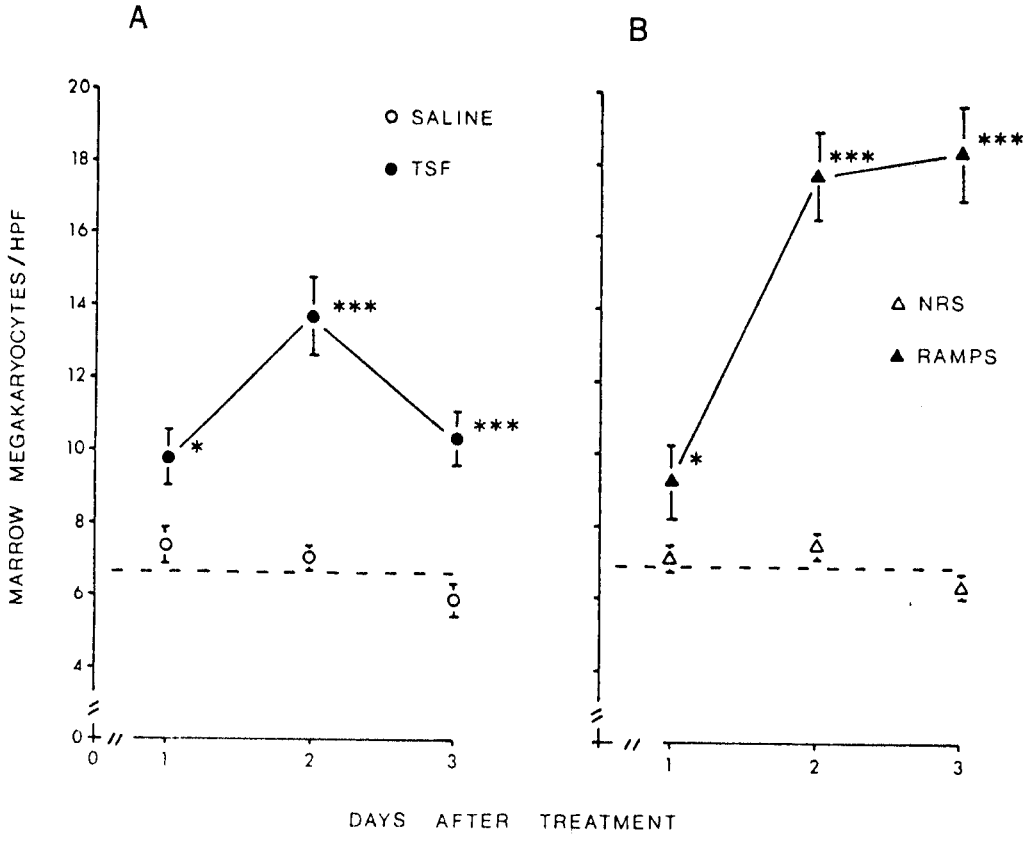

Figure 1
The effects of saline, a thrombocytopoiesis-stimulating factor (TSF), normal rabbit serum (NRS), rabbit anti mouse platelet serum (RAMPS) on the number of megakaryocytes per high powered field (HPF) with time. Vertical lines represent standard errors, horizontal dashed lines represent the average for controls. Each point is the average for 9 mice; values were significantly different from suitable controls: *$P<0.05$, *** $P<0.0005$.

[1]This work was supported by a grant from The National Heart, Lung, and Blood Institute, Grant No. HL 14637.

As compared to saline, the injection of TSF caused a significant increase of marrow megakaryocytes per high powered field on days 1-3 ($P<0.05$ on day 1 and $P<0.0005$ on days 2 and 3) with peak numbers being reached 2 days after TSF injection; decreasing values were observed at day 3 (Panel A, Figure 1). In Panel B the effects of normal rabbit serum and platelet specific anti serum on the numbers of marrow megakaryocytes are shown. In thrombocytopenic mice, a significant increase in the number of megakaryocytes was found by day 1 ($P<0.05$); the numbers increased markedly on days 2-3 ($P<0.0005$).

Figure 2 shows the relative sizes of megakaryocytes (expressed as micrometer diameter) from the same mice as presented in Figure 1. When compared to controls, highly significant increases in megakaryocyte diameters were observed after treatment with TSF and RAMPS on days 1-3 ($P<0.0005$).

After applying the multiple counting correction factor of Harker (J. of Clin. Invest. 47: 452-7, 1968), increases of 6 to 100 percent in the number of megakaryocytes were observed. Although the mice injected with RAMPS, to make them thrombocytopenic, showed greater increases in marrow megakaryocyte numbers and sizes than mice injected with TSF, the results demonstrate that TSF stimulated megakaryocytopoiesis.

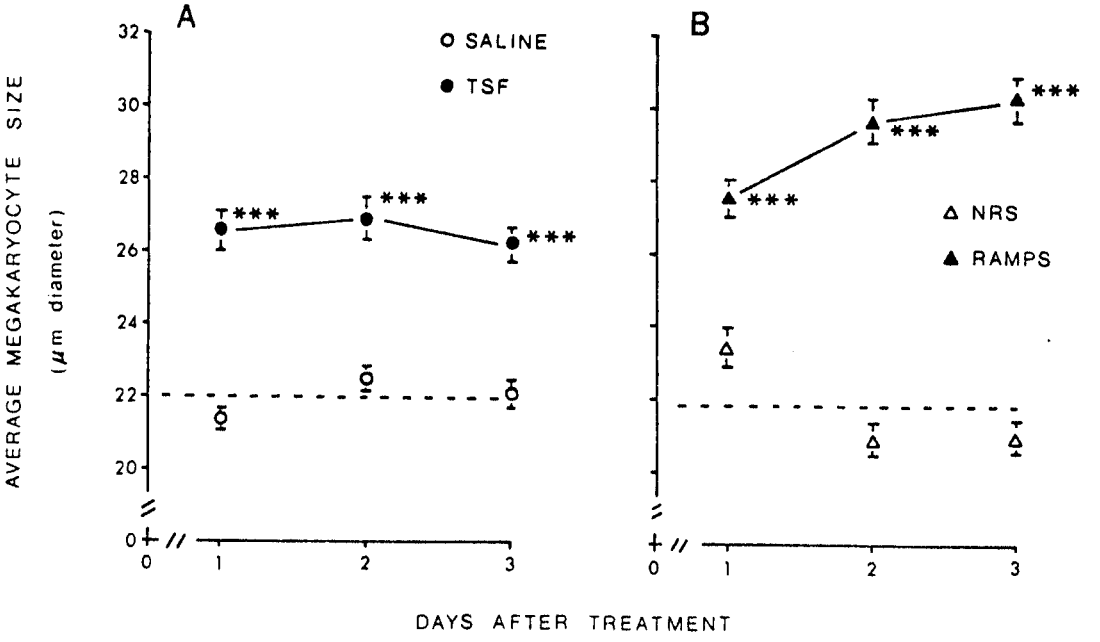

Figure 2:
Average diameter of marrow megakaryoctyes of the same mice as presented in Figure 1. The vertical lines represent standard errors, horizontal dashed lines represent the aveage for controls. Values were significantly different from suitable controls: ***$P<0.0005$.

THE SITE OF PRODUCTION OF THROMBOPOIETIN[1]

T. P. McDonald, G. D. Kalmaz, R. Clift and M. Cottrell

Previous investigations have suggested that the kidney is a site of production of a thrombocytopoiesis-stimulating factor (TSF or thrombopoietin). Moreover, human embryonic kidney cells in culture have been shown to produce TSF. In addition to studies in animals, other work showed that 50% of patients with chronic renal disease are thrombocytopenic. Further, thrombocytosis has been found in patients who have undergone renal transplantation. However, other workers have concluded that the kidney is not necessary for TSF production. In this study, platelet counts were only slightly increased in nephrectomized rats after injection with anti serum by day 3 (the time when rats usually die from uremia following nephrectomy). Since 4-5 days are required for platelet repopulation after an antiserum injection, the rats did not live long enough to establish conclusively the role of the kidneys in platelet production. This animal model is, therefore, unsuitable for such studies. However, an early cell in megakaryocytic series of rodents, i.e., the small acetylcholinesterase positive (SAChE +) cell, has been reported to increase in the marrow at 6-8 hours after induction of acute thrombocytopenia. The SAChE + cell appears to be controlled by the animals thrombopoietin titer. Therefore, in the studies to be reported, we tested the effects of unilateral and bilateral nephrectomy and ureter ligation on the production of SAChE + cells in the marrow of thrombocytopenic rats.

Figure 1 shows the percentages of SAChE + cells of rats following various treatments. When compared to suitable controls, injections of antiplatelet serum led to significant increases ($P<0.025$ - $P<0.005$) in percentages of SAChE + cells in the marrow of rats except in those rats subjected to bilateral nephrectomy. In addition, rats that were bilaterally nephrectomized but not given an anti platelet serum had decreased percentages of SAChE + cells ($P<0.005$) when compared to untreated control rats.

These findings indicate the importance of the kidney in the day-to-day production of thrombopoietin for the maintenance of SAChE + cells. Unilateral nephrectomy and ligation of the ureters had little effect on altering the percentages of SAChE + cells, suggesting that surgical stress and uremia do not interfere in the release and action of TSF. This study confirms previous work that the kidney is required for the production of thrombopoietin.

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[1]This work was supported by a grant from the National Heart, Lung, and Blood Institute, Grant No. HL 14637.

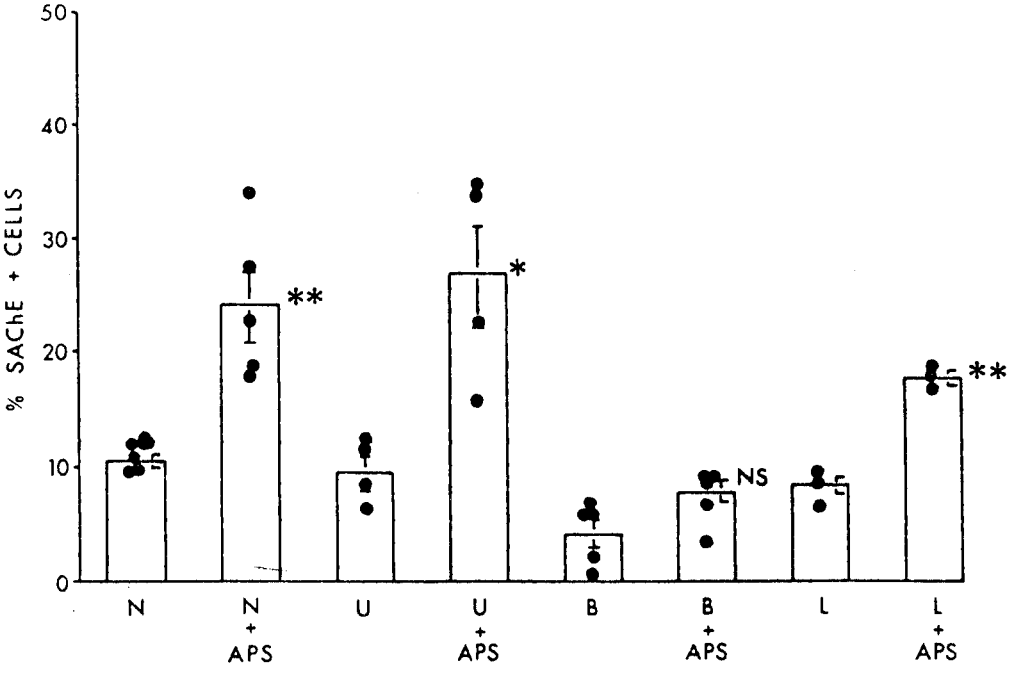

Figure 1

The effects of no treatment (N), unilateral nephrectomy (U), bilateral nephrectomy (B), or ureter ligation (L) with and without injection of platelet specific anti serum (APS) on the percentage of small acetycholinesterase positive cells in marrow of rats. Each point (O) is the average percentage SAChE + cells from 6 slides of about 1500 megakaryocytes from The bars represent the average for each treatment group and the vertical lines indicate the standard errors. NS, not significantly different from bilaterally nephrectomized rats; *P<0.025; **P<0.005 indicate significant changes from suitable control rats that were not injected with APS.

STRUCTURE-ACTIVITY CORRELATIONS OF SELECTED AZAARENES, AROMATIC AMINES, AND NITROAROMATICS

Becky A. Moulton and T. Wayne Schultz

Quantitative structure activity relationships (QSAR) are utilized as a powerful research tool that permits the ranking of chemicals according to correlations between molecular descriptors and biological activity. These molecular descriptors are of two basic types, with one type consisting of parameters that describe such physiochemical properties as electronic, hydrophobic and steric. The most widely used descriptor, the hydrophobic parameter, log 1-octanol/water partition coefficient, ($\log K_{OW}$), is found in this first category.

The second category of molecular descriptors used in the prediction of toxicity includes parameters involved with direct quantitation of molecular structure. Found in this category are parameters that utilize atom counts, molecular weight, molecular connectivity, molecular orbital theory and quantum mechanics. Of these descriptors, the most frequently used is molecular connectivity, which describes the topological characteristics of a compound using numerical indices. The $^1\chi^v$ index is the most common molecular connectivity descriptor and encodes information about the number and types of atoms. The extent of branching, cyclization and saturation in molecular compounds is also described by the $^1\chi^v$ index.

The free-living ciliate Tetrahymena pyriformis provides an excellent short-term screen with which to quantify biological activity in dose-response relationships. Since the number of Tetrahymena is proportional to optical density, biological activity or toxicity can be measured through population growth. In this investigation correlations between selected molecular descriptors, ($\log K_{OW}$, $^1\chi^v$, $(\log K_{OW})^2$), and toxicity are reported for 21 compounds which include azaarenes, primary aromatic amines and nitroaromatics.

The test compounds were dissolved in dimethyl sulfoxide (DMSO) and aliquots of these stock solutions were added to the culture medium. The test flasks were then inoculated with a culture of Tetrahymena and incubated for 60 hours. The concentrations which inhibits 50% of the growth (IGC_{50} value), was then determined and converted to mmol/L. The log of the inverse of this value, log BR (Biological Response), was subsequently used in correlation analyses to form structure-activity models.

Three of the test compounds did not elicit a response at saturation. Three linear relationships are suggested by the data for the molecular descriptors of interest and the biological activity for the remaining 18 test compounds. These QSAR models are represented by the following equations:

(1) $\log BR = 0.8255 (\log K_{OW}) - 1.6334$ $\quad r^2 = 0.869$

(2) $\log BR = 0.8152 (^1\chi^v) - 2.4505$ $\quad r^2 = 0.828$

(3) $\log BR = 0.1996 (\log K_{OW})^2 - 0.8995$ $\quad r^2 = 0.874$

All three of these descriptors are colinear ($P \geq 0.001$). The sequential sums of squares values for all combinations of two parameter regression models

using these descriptors reveal that log K_{ow} and $(\log K_{ow})^2$ significantly improve ($P < 0.05$) models which already include the $^1\chi^v$ parameter. Scrutiny of the r^2 values from equations 1-3 suggests that $(\log K_{ow})^2$ may be a better predictor of toxicity. However, the behavior of one compound (aniline), statistically an outlier in equation (1), biases the data suggesting a curvilinear relationship, $(\log K_{ow})^2$. Upon removal of aniline from the analysis, log K_{ow} becomes a better predictor of toxicity than $(\log K_{ow})^2$, thus making equation (1) an excellent predictive model.

Molecular connectivity indices such as $^1\chi^v$, while not describing physiochemical properties, correlate well with such properties, especially for structurally simple compounds. The strong colinearity suggested by this investigation between log K_{ow} and $^1\chi^v$ should become markedly reduced when more structurally complex molecules are tested. The substituent interactions of the more complex compounds should then be expressed better by log K_{ow} than $^1\chi^v$.

Previous log K_{ow} - dependent QSAR studies dealing with alkyl substituted mononitrogen, dinitrogen and multinitrogen heterocyclic aromatics have yielded similar models to that described by equation (1). Combination of these previous data with the present results yields the following prediction equation based on 49 molecules:

$$(4)\quad \log BR = 0.7230\ (\log K_{ow}) - 1.3459 \qquad r^2 = 0.903$$

Use of this model will facilitate study of the toxicity of structurally more complex compounds.

ELECTRON MICROSCOPIC ANALYSIS OF GROWTH PHASE DEPENDENT PHAGOCYTOSIS OF PASTEURELLA HEMOLYTICA BY BOVINE PULMONARY MACROPHAGES IN VITRO

T. W. Schultz, R. D. Walker and F. M. Hopkins

The bovine respiratory disease complex referred to as shipping fever pneumonia may occur when any of a variety of stress factors in conjunction with a primary viral infection predispose the lower respiratory tract to a more serious secondary bacterial infection. This disease complex is the most economically important disease problem in cattle. The bacterium Pasteurella hemolytica is the most important etiocogical agent associated with this disease process in the cattle industry. Yet, the interactions of this disease process, especially between P hemolytica and the bovine pulmonary defense system, are poorly understood. This has been compounded by the fact that the results of recent in vitro studies examining the ability of macrophages harvested from the lower respiratory tract to phagocytize P hemolytica are not in agreement.

To gain a better understanding of the interaction between P hemolytica and pulmonary macrophages, we induce resistance to P hemolytica in the bovine lung by pre-exposing calves to an aerosolized mist of P hemolytica 2 times at a 1-week interval. Pulmonary lavages were performed on the calves 72 hours prior to bacterial exposure, 4 days after each of the 2 exposures and 11 days after the declining growth phases of P hemolytica to determine if bovine pulmonary macrophages harvested from pre- and post-exposure lavage fluids are capable of phagocytizing P hemolytica.

The percent recovery of the lavage fluids ranged from 65 to 89%. Each lavage procedure yielded a high number of pulmonary macrophages ranging from 6.0×10^7 to 3.0×10^8 cells/lavage. Preaerosol lavages contained nearly 100% macrophages; whereas lavages performed 4 days following the firs aerosol exposure to P hemolytica contained approximately 17% neutrophils. The lavages performed 4 and 11 days after the second aerosol exposure contained approximately 30% and 25% neutrophils, respectively.

Transmission electron microscopic examination of 24-hour in vitro macrophages revealed cells characterized by numerous long, thin microvilli. Typically these cells contained a centrally placed, round or slightly ovoid leptochromatic nucleus with one or more nucleoli. The cytoplasm contained mitochrondria and scattered cisternae of rough endoplasmic reticulum. In addition, there was an extensive heterogenous population of lysosomes. The control macrophages from each lavage appeared to be essentially the same in ultrastructure.

Significant structural alterations were found in macrophages exposed to P hemolytica in the log growth phase. Most notable was a marked change in the morphology of the cytoplasmic processes which were short and blunt rather than long and thin as found in the controls. These macrophages also appeared more uniform in shape, being typically circular in section. In addition, several necrotic macrophages and much extracellular debris were observed in sections of these cell pellets. The phagocytic vesicles within the intact cells appeared to be filled with cellular debris. There was no evidence of P hemolytica being phagocytized; however, P hemolytica was seen extracellularly. There were no differences in cellular morphology or phagocytic capabilities seen between macrophages harvested before and after in vivo exposure to P hemolytica.

Structural alterations were also found in macrophages exposed to P hemolytica in the declining phase of growth. While most macrophages were circular in section with short, blunt cytoplasmic processes similar to those observed to log phase bacteria, there was greater structural variability between cells exposed to declining phase bacteria. In addition, some P hemolytica were phagocytized by pulmonary macrophages. However, fewer than 10% of the macrophages were seen to have phagocytized P hemolytica.

In an effort to assess phagocytic activity the yeast Saccharomyces cervisiae was used as a marker. The yeast was readily phagocytized in vitro. Approximately 30% of the observed macrophages engulfed one or more yeast cell. There were no observed differences in the percent of actively phagocytizing cells between the first three different lavages. Similarly, macrophages from the different lavage periods showed no structural differences, other than those associated with phagocytosis.

When P hemolytica was added to the macrophages and immediately decanted, S cerevisiae was phagocytized by approximately 10% of the macrophages. Under these experimental conditions there were variations in the morphology of the macrophages, ranging from the normal morphology as seen in the controls to the rounded cells with short, blunt, cytoplasmic processes. Macrophages exposed to P hemolytica in vivo showed no difference in cytotoxicity or their ability to phagocytize S cerevisiae when compared to pre-exposure macrophages.

After 30 minutes exposure to P hemolytica in vitro, none of the macrophages were seen to phagocytize S cerevisiae or P hemolytica. Instead, these macrophages were rounded with short, blunt cytoplasmic processes.

The major difference between reported in vitro results is the preparation of P hemolytica for phagocytic assays. Some investigators Walker used a 5-hour (log-phase) culture of P hemolytica that had been propagated on enriched artificial medial and passed through calves to maintain its virulence. Whereas, others used a 12-hour (declining phase) culture that has been propagated on non-supplemented bloodagar and passed in mice to maintain virulence. On the other hand, still others used an 18-hour culture propagated on unsupplemented blood agar.

The significance of these differences appears to be in the virulence factor(s) produced by P hemolytica. The production of a cytotoxin by P hemolytica is maximum during the logarithmic phase of growth peaking after 6 hours of incubation. Toxin production decreased in the stationary phase of growth and after 18 hours incubation is undetectable.

The data presented here demonstrates that P hemolytica can be phagocytized by pulmonary macrophages. However, the age of the culture contributes to its ability to resist phagocytosis. Phagocytosis of P hemolytica in the log phase of growth could not be demonstrated. Furthermore, cultures of this age were shown to be highly toxic to the macrophages. This toxicity also inhibited the phagocytosis of S cerevisiae, which had been readily phagocytized by pulmonary macrophages prior to their in vitro exposure to P hemolytica. Evidence of this inhibition of phagocytosis was present even in those cultures exposed to P hemolytica for less than 1 minute. On the other hand, P hemolytica, in a declining phase of growth, while toxic to some macrophages, was phagocytized.

THE MITIGATION OF ACUTE TOXICITY OF COAL-DERIVED LIQUIDS BY HYDROTREATMENT

T. W. Schultz

Seventy-five percent of the United States' proven organic-based energy reserves are in the form of coal. The production of liquid hydrocarbons for industrial feed stocks from this coal appears to be inevitable. Since raw feed stocks derived from coal liquefaction processes contain significant quantities of polyaromatic hydrocarbons and heteroatom-containing aromatics, they typically require an upgrading to reduce the heteroatom and polyaromatic content if they are to be used as commercial sources. One of the more promising schemes for upgrading coal-derived liquids is hydrotreatment (HDT). Coal liquefaction materials representing 3 different technologies and 4 different severities of hydroprocessing were determined using population growth impairment of the ciliate Tetrahymena pyriformis as a bioassay.

Strain GL-C of Tetrahymena pyriformis was reared axenically at 28°C in a proteose-peptone based medium. The coal-derived liquids are described briefly in Table 1. In these studies HDT severity is defined as follows: low severity - HDT required to reduce N content to 50% of raw coal-derived liquids; intermediate severity - HDT required to reduce N content to 25% of raw coal-derived liquid; medium severity - HDT required to reduce N content to 500 mg/L; high severity - HDT required to bring N content to a level acceptable for refining into fuel products. Dimethylsulfoxide slurry stocks (20 mg/L) were prepared from each liquefaction material. Dimethylsulfoxide in concentrations as high as 0.75% does not affect Tetrahymena growth. Test cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of test solution. The test solutions formed a graded concentration series of test slurry in medium for each coal-derived liquid. Following 60-hr of exposure, test cultures were spectrohotometrically assayed for cell population density. A minimum of 3 replicates of each concentrations series was assayed. Each replicate was conducted using freshly prepared stock slurries.

Computer assisted regression analysis of percent control growth vs. concentrations was used to obtain the 60-hr IGC_{50} (inhibitor growth concentration) values and the 95% confidence intervals. In addition, computer assisted correlation analysis of major organic element and toxicity measured as log BR (i.e. 60-hr IGC_{50}^{-1}) was undertaken.

The percent distribution of the major elements in each coal-derived liquid and its log BR is presented in Table 1. In each technology suite toxicity is inversely related to degree of HDT. There is no significant difference ($p < 0.005$) between the toxicity of the unwashed and H_2O washed raw product from either technology I or III. A comparison of the weight distributions of the raw product from the three technologies shows a disparity in heteroatom content with technology I characterized by high O content and technology III characterized by a high S content. These differences in heteroatom content may reflect technology and/or feed stock differences. Water washing tends to increase H and O content probably because of residue water remaining after washing. A cursory examination of Table 1 reveals that H content increases with severity of HDT while heteroatom content and aromaticity concomitantly decrease. A comparison of variances of element weight percents as well as log BR shows N and O contents are highly correlated ($r^2 < 0.75$). In addition, H content appears to be a good indicator of acute toxicity.

Table 1. Distribution of major organic elements in selected coal-derived liquids

Technology	Sample description	Element weight %					log BR
		C	H	N	S	O	
I	Unwashed raw	86.58[a]	8.75[a]	0.95	0.041	3.68	-1.1511
	H_2O washed raw	85.88	8.88	0.98	0.032	3.68	-1.1550
	Low severity HDT	87.47	10.02	0.53	0.007	1.96	-1.3043
	Intermediate severity HDT	87.94	10.86	0.17	0.005	1.03	-1.3986
	Medium severity HDT	88.38	11.14	0.05	0.001[a]	0.43	-1.5725
II	Raw	88.44	10.51	0.10	0.02	0.93	-1.3727
	High severity HDT	87.23	12.77	<0.001	<0.001	0.006	-1.3004
III	Unwashed raw	88.04	9.27	0.53	0.13	2.04	-1.0927
	H_2O washed raw	88.04	9.39	0.57	0.13	1.85	-1.0597
	Low severity HDT	88.17	10.62	0.27	0.005	0.94	-1.3220
	Medium severity HDT	88.44	11.25	0.05	0.001	0.29	-1.6213
	High severity HDT	88.13	12.87	<0.001	<0.001	0.006	-2.3309

[a]Values estimated for correlation analysis.

THE DEVELOPMENT AND VALIDATION OF THE FROG EMBRYO TERATOGENESIS ASSAY: XENOPUS

T. W. Schultz

There is no question that a rapid and inexpensive screening tool is needed to assess potential teratogenicity. Although a number of relatively rapid bioassays, ranging from the invertebrates through lower vertebrates to mammals, as well as cell, organ and embryo culture systems, have been used to examine teratogenicity, none has been widely accepted for routine use. Among these is one which we submit as a valid model for preliminary assessment of potential teratogens. This model is referred to as FETAX (Frog Embryo Teratogenesis Assay: Xenopus). The assay has been standardized against known mammalian teratogens so that comparisons with materials with unknown teratogenic potential can be made. Such comparisons then permit one to rank unknowns and subsequently suggest priorities for further testing in higher order and/or mammalian assays.

One reason the amphibian embryo is attractive as an assay system is that it has been and remains a classical model for experimental embryological studies. Unlike some of the other teratogenesis screens that use cell or organ cultures, the frog embryo is an intact developing system which undergoes events (e.g. cleavage, gastrulation, organogenesis) comparable to those of other vertebrates, including mammals. Other attributes of the model which make it attractive are: (1) the assay is rapid. (2) It is relatively inexpensive. (3) Methodologically, the protocol is simple. (4) Large numbers of embryos (2,000 to 5,000) are routinely available for testing from a single spawning. (5) The developmental stages are easily observed and scored. (6) Dose levels of teratogens are easily controlled. (7) Pulsed exposures to specific developmental stages are easy, as are prolonged exposures beginning at specific stages. (8) FETAX allows direct exposure of the embryo to either the teratogen or its metabolites and has the potential for in vitro metabolic activation by microsomal (S9) preparations. Despite concern that the jelly coat surrounding the embryo may act as a permeability barrier, our experience has been that it does not. In any case, the jelly can be easily removed by chemical means. (9) The embryos are compatible with low concentrations of solubility vehicles including DMSO. (10) The embryos are functional organisms. They are motile at 96 hours so assessment of the functional state of the nervous and muscular systems can be made by simple observations of swimming ability. (11) Finally, endpoints are easy to recognize and score. We routinely use the following: mortality, anatomical deformities, growth, developmental stage attained, motility (behavior), and pigmentation.

The routine method employed is a static, 96-hour exposure of embryos obtained from a pair of Xenopus injected 8-10 hours earlier with gonadotropin to induce ovulation and amplexus. Healthy appearing, normally developing embryos, in the mid to late blastula stage of development are selected manually with the aid of a dissecting microscope. Groups of 25 embryos are placed in 10 ml of artificial pond water (salt solution) in 60 mm covered Pyrex dishes. Exposure is at room temperature (or at 25°C). At 96 hours the surviving embryos are scored for the degree of pigmentation and motility and fixed in 3% glutaraldehyde in 0.1 phosphate buffer (pH 7.3). They are then counted and the number of survivors and the number of abnormals recorded. Some embryos may be fixed and prepared for paraffin sectioning.

In order to standardize the FETAX assay a variety of compounds known to produce terata in several species of mammals were used. In general these compounds ranged from severe to milk mammalian teratogens (based on severity of terata produced and dose required). In most cases the FETAX ranking of these compounds indicated similar potencies on the amphibian embryos. Because of the necessity for solubilizing some of these compounds, carrier vehicles were used. Other parameters such as temperature and pH also required adjustment. Table 1, below presents the ranges within which these factors are used in FETAX.

Table 1. Effects of hepes buffer, DMSO, Temperature and pH

	Used in assay	LC_{50}	EC_{50}
Hepes buffer	1.5 mM	38.25 mM	26.61 mM
DMSO	<1.0%	1.96%	1.11%
Temperature	25°	30.20°	31.06°C
pH	7.0	5.84	6.45
		9.32	8.18

The magnitude or concentration of these factors routinely used under experimental conditions is well outside the ranges that would induce death of terata.

To date 40 compounds have been used for standardization. Interestingly, terata produced by these teratogens in mammals closely resemble those produced in FETAX. Additionally, their relative potencies in FETAX follows what has been demonstrated by others in mammals. One major area which needs to be addressed in the refinement of the assay is the development of an in vitro metabolic activation protocol using mammalian microsomal preparations.

TERATOGENICITY AND EMBRYOTOXICITY OF MONOSODIUM METHANEARSONATE HERBICIDE

T. W. Schultz

Arsenical compounds have been used in agriculture for more than a century and represent the single largest man-made addition of arsenic to the environment. Over the past few decades, inorganic arsenicals have been replaced for the most part by organic pesticides and organic arsenical herbicides. Included in the organic arsenicals used in agriculture today is monosodium methanearsonate (MSMA). First used as a selective herbicide for postemergence control of weedy grasses in turf, MSMA is presently applied extensively as a selective post-emergent herbicide for cotton and selected non-crop areas. While there is great variation in acute arsenic toxicity among animal species, MSMA is not highly toxic. However, it is possible that the herbicide could cause sub-lethal effects such as developmental abnormalities (teratogenicity). Thus, studies were undertaken to examine the embryotoxic and teratogenic effects of MSMA in the Frog Embryo Teratogenesis Assay: Xenopus laevis (FETAX) system.

Early to mid-blastula embryos of the african clawed-frog, Xenopus laevis (Daubin), were cultured under acute static conditions by placing 50 embryos each in 200 ml of test solution in 125 x 60 mm glass bowls. A minimum of three replicates of graded concentration series of Ansar (R) 529 HC (47.7% MSMA) was examined. At representative time points, the embryos were examined with the aid of a dissecting microscope. After 96-h of exposure, the relative motility and pigmentation were recorded, and the survivors were fixed in 3% glutaradlehyde. Selected embryos were fixed in Ammerman's fixative for histology. Data regarding mortality, number and types of malformations, stage of development and embryo length also were collected. In addition, acute toxicity of MSMA to newly metamorphosed frogs was assessed by testing five replicates of five animals each exposed to a four-step graded concentration series of MSMA for 96-h in 500 ml of test solution in 190 x 100 mm-glass bowls. The 96-h EC_{50} value (concentration which causes abnormalities in 50% of survivors) was determined by least-squares linear regression of the probit value of the percent affected (Y) vs. concentration MSMA (X). Similarly, the early embryo 96-h LC_{50} (concentration which kills 50% of the animals) was extrapolated and the newly metamorphosed frog 96-h LC_{50} was calculated.

Preliminary experiments have shown the LC_{50} value for embryos exposed at the mid-blastula stage for 96 h to be 225 mg/liter MSMA. The 96-h LC_{50} for newly metamorphosed frogs, an indication of adult toxicity, is 1132.40 mg/liter MSMA with a 95% confidence interval from 520.40 - 2464.10 mg/liter. Table 1 provides the combined concentration-dependent embryotoxicity and teratogenicity results of these studies. With all concentrations tested, 96-h mortality was never more than 20%. Abnormalities show a direct linear relationship to concentration (Table 1). The 96-h EC_{50} was calculated by regression analysis to be 45.81 mg/liter MSMA with the 95% confidence limits of 13.59 - 154.38 mg/liter. Table 2 provides a comparison between exposure concentrations, stage of development and length of embryos surviving 96 h of exposure. Analysis of variance of the embryo length reveals a significant difference at the 0.05 level between replicates as well as between concentrations. At the higher concentrations, this size reduction reflects retardation of developmental stage or rate of development. At the lower concentrations, development is

not affected, but there is a large standard deviation. Motility and pigmentation are affected very little, if any, by exposure to low concentrations (F 75 ppm) of MSMA. The concentration of 100 mg/liter MSMA, however, caused a marked reduction in motility and a severe reduction in pigmentation.

The types and severity of malformations are concentration-dependent. The 96-h control embryos have elongated, streamlined profiles and fully differentiated eyes. Embryos exposed to 25-50 mg/liter MSMA, while generally streamlined are dominated by optic cup formation, irregular lens development, and unusually small eyes. Similar but more severe optic malformations are observed at 75 and 100 ml/liter. In addition, abdominal edema and abnormal gut coiling appears at the higher concentrations. Histological analyses confirm and extend these gross anatomical findings.

Since almost any substance is teratogenic as a result of the right dose being administered at the proper time, a question as to whether MSMA is a real developmental hazard or simply a coeffective compound arises. Comparison of the teratogenic potential (EC_{50} of early embryos) and adult mortality (LC_{50} of froglets) shows MSMA to be a significant developmental hazard.

Table 1. The four-day toxic and teratogenic effects to Xenopus embryos exposed to various concentrations of MSMA

Concentration (mg/liter)	0	25	50	75	100
Number of test embryos	225	150	200	200	250
Percent survivors	95	93	87	80	81
Number of abnormal embryos	3	22	104	147	198

Table 2. The four-day stage of development and length of Xenopus embryos exposed to various concentrations of MSMA

Concentration (mg/liter)	0	25	50	75	100
Developmental stage[a]	47	47	46-45	44-43	43-41
Mean length (mm)	9.08	8.86	8.14	7.57	7.10
Standard deviation	0.88	1.43	1.00	0.59	0.44

[a]According to Nieuwkoop α Faber (1975).

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TENNESSEE ANIMAL SCIENCE RESEARCH FACILITIES

1. Tobacco Experiment Station - Greeneville - P. Hunter, Supt.
   Beef cattle - Approximately 150 brood cows in genetic and nutrition studies. Feeding facilities for steer and heifer silage feeding trials.

2. Knoxville Experiment Station - Knoxville and Alcoa - J. R. Hodges, III, Supt.
   Beef cattle - Three farms with a total of 525 brood cows used in genetic and nutritional studies. Feeding facilities for approximately 400 steers in feeding trials using silage and grain.
   Swine - Fifty sow operation for sow feeding and baby pig research. Duroc, Landrace, Yorkshire and Hampshire crosses.
   Sheep - Breeding flock of 45 ewes used in crossbreeding research to increase lamb production - limited amount of confinement feeding work.
   Dairy cattle - Mixed herd of 125 Holsteins and 25 Jerseys used in nutrition, physiology and management research with dry lot confinement.
   Poultry - Broiler and layer facilities for nutrition and physiology research. New facilities are being planned to improve and expand research capabilities.

3. Plateau Experiment Station - Crossville - R. D. Freeland, Supt.
   Beef cattle - 225 brood cows used in pasture management nutrition and genetic studies. Feeding facilities for 200 steers in nutrition experiments.
   Swine - Feeding facility for conducting research with 240 feeder pigs.

4. Dairy Experiment Station - Lewisburg - J. R. Owen, Supt.
   Jersey herd of 170 cows used in genetic, nutrition and waste management research.

5. Middle Tennessee Experiment Station - Spring Hill - J. W. High, Jr., Supt.
   Beef cattle - 100 brood cows used in pasture management studies. Facilities for feeding 135 steers in nutritional studies.
   Swine - Swine testing station.
   Dairy cattle - Herd of 110 Holstein cows used in genetic and nutritional studies.

6. Highland Rim Experiment Station - Springfield - L. M. Safley, Supt.
   Beef cattle - herd of 200 brood cows used in pasture management studies. Feeding facility for 150 steers in shipping fever research.

7. Ames Plantation - Grand Junction - J. M. Anderson, Supt.
   Beef cattle - 350 brood cows and 120 feeder animals used in pasture management research.
   Swine - Sow herds that produce approximately 2500 finished hogs annually in housing, breeding and nutritional research.

8. West Tennessee Experiment Station - Jackson - J. F. Brown, Supt.
   Dairy cattle - Jersey (100) and Holstein (40) used in nutrition and physiology research.
   Swine - Swine testing station.

9. Martin Experiment Station - Martin - H. A. Henderson, Supt.
   Beef cattle - 60 brood cows and 100 feeder steers used mainly in nutrition research.
   Dairy cattle - 35 Jerseys and 65 Holstein used mainly for nutrition research.
   Swine - 25 Yorkshire sows used in teaching and nutrition research.

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