EFFECT OF RONNEL ON GROWTH, RUMINAL CONSTITUENTS, AND RATION DIGESTIBILITY OF BEEF STEERS

by

STEVEN DUDLEY PLEGGE

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Major Professor

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INTRODUCTION

Ronnel [O,O-dimethyl-O-(2,4,5-trichlorophenyl) phosphorothioate] is an organophosphate cleared for use in beef and nonlactating dairy cattle as an oral systemic for control of lice and heel fly larva and as an oral larvicide for horn fly control. Since its introduction in 1956, a majority of the research with the compound has centered on its ability to control parasites. In recent years, interest has focused on possible growth promoting abilities of the compound. Wooden and Algeo (1977), Matshishuma (1978), Riley and Ware (1977), and Thomas (1978) have shown that ronnel will increase consumption, improve daily gain, and improve feed efficiency when fed to finishing cattle. All these studies used cattle which had been treated for internal and external parasites. Data on the effect of ronnel on performance of grazing cattle is not available in studies where parasites have been controlled in all treatment groups.

Data are also limited on the effect of ronnel on ration digestibility. Rumsey *et al*., (1975) reported no difference ($P>.05$) in digestibility when ronnel was added to a 70% concentrate diet. Information on the effect of ronnel on digestibility of a high roughage ration needs to be obtained in order to better understand the compound's mode of action in grazing cattle.

Research is needed to determine the subsequent feedlot performance of steers that received ronnel prior to being fed a finishing ration. If steers that received ronnel while on grass and prior to being placed on a finishing ration respond differently than steers never exposed to the compound, perhaps the response to the compound is related to the amount or length of time the compound was fed. These unanswered questions about ronnel's activity prompted this study to be undertaken.

REVIEW OF LITERATURE

Ronnel is an organophosphate cleared for use in beef and non-lactating dairy cattle as an oral systemic for control of lice and heel fly larva and as an oral larvacide for control of horn flies. Ronnel was introduced to the market in 1956. The chemical name of the compound is [0,0-dimethyl,0-(2,4,5-trichlorophenyl) phosphorothioate] . It is a member of the organophosphate family indicating that it contains both carbon and phosphorous in its chemical structure. Organophosphates have been widely used in animal agriculture for control of internal and external parasites. The primary means by which organophosphates control parasites is through the anticholinesterase activity displayed by the organophosphate or its metabolites on the parasite. In the case of ronnel, the amount necessary for parasite control is far below the amount at which the animal begins to experience toxic symptoms. The levels of ronnel approved for use by the Food and Drug Administration for grub control are 8 mg/kg of body weight for 14 days and 18 mg/kg of body weight for 7 days (Feed Additive Compendium, 1979). Radeleff and Woodard in 1957 gave a one time dose to yearling cattle and mature cows with graded levels of ronnel from 0 up to 400 mg/kg of body weight. They observed mild toxicity symptoms at 125 mg/kg and above. However, they were unable to produce a fatality among the test animals. The symptoms associated with excess ronnel are depression, muscular weakness, incoordination, prostration, and usually diarrhea. Unlike most organophosphates ronnel does not cause a rapid decrease in cholinesterase activity but rather a gradual decline which peaks at about 6-8 days. Ronnel should not be used in conjunction with other cholinesterase depressing drugs (Feed Additive Compendium, 1979).

Plapp and Casida (1958) administered phosphorus-32-labeled ronnel to a lactating cow in order to establish the metabolic fate of the compound. They recovered 7% of the label as ronnel or its metabolites in the feces while recovering 49% of the label in the urine. The authors noted that urine recovery was incomplete and possibly more label was voided in the urine than detected. The metabolites of ronnel that are present in the urine and feces are the result of phosphorothioate oxidation and hydrolysis of the phosphorus-oxygen-phenyl bond. Under _in vitro_ conditions the authors were able to establish that incubation of ronnel with rumen fluid would produce phenyl phosphoric acid and dimethyl phosphoric acid as a result of ronnel breakdown. The metabolic significance of these acids is not known. The extent of ronnel degradation was not determined under _in vivo_ conditions and the primary absorption site of ronnel or its metabolites is unknown. Rumsey _et al._, (1975) reported that ronnel when fed at the rates of 4.5 mg/kg of body weight to steers receiving a 70 % concentrate ration increased ($P < .01$) molar percent acetic acid. A depression in molar percent butyric acid was observed but was not significant. In 1976 Rumsey fed steers a similar diet and in this study the ronnel steers had an increased molar percent acetic acid and a decreased molar percent butyric acid resulting in a higher acetate to butyrate ratio ($P < .05$) for the ronnel supplemented steers. In both studies, total VFA concentration was not affected by ronnel.

The work of Plapp and Casida (1958), Rumsey _et al._, (1975), and

Rumsey (1976) indicate that ronnel has some ruminal activity but any ability to improve performance by alteration of rumen VFA levels is not likely since acetic acid is the least energy efficient VFA produced in the rumen.

Rumsey et al., (1975) reported that steers fed ronnel did not differ from control steers in ration digestibility or nitrogen retention when fed a 70% concentrate diet. However, the compound may influence intestinal absorption since steers fed ronnel for 90 days had higher ($P < .01$) essential and nonessential plasma amino acids. Intake differences existed between the ronnel and control steers and the increase in plasma amino acids may have been the result of increased protein reaching the abomasum, although proteolysis in the rumen does not appear to be affected since ronnel did not alter rumen ammonia or rumen pH in this study. Under similar conditions Rumsey (1976) again found that ronnel did not alter rumen ammonia or pH. No attempt has been made to determine if ronnel enhances ruminal escape of protein or if the microbial population is altered by ronnel feeding resulting in a more desirable amino acid profile in the lower gut.

Most of the work done with ronnel has been to evaluate its ability to control parasites and not to determine if it possess growth promoting qualities. Raun and Herrick (1960) conducted a 162 day finishing trial with steers in which ronnel was fed at 25 mg/kg/day for 6 days compared to untreated control steers. Steers on both treatments gained at the same rate. However, grub infestation was low (3.25 grubs/steer) in the control group and ronnel was fed for only a short time instead of continuously.

In three feedlot trials, Thurber and Peterson (1960) orally dosed both steers and heifers with ronnel at 100 mg/kg of body weight. Ronnel treated cattle showed a slight improvement in daily gain, however it was not significant. Grub infestation in this study was moderate with the number of grubs per animal ranging from 15 to 27, so that controlling the grub population may have improved daily gain. In contrast to this work, Raun and Herrick (1957) found that steers dosed with 110 mg ronnel/kg of body weight gained 6.4% faster and were 5.7% more efficient than steers receiving no ronnel. Grub infestation in this trial averaged 2.2 grubs/steer.

Rogoff and Kohler (1959) in a series of experiments fed ronnel in a free choice mineral mixture to 400 pound calves receiving brome hay free choice and 4 pounds of grain daily. In trial 1, calves receiving an average of 9 mg ronnel daily for 66 days gained less than the controls (1.24 lbs. vs 1.56 lbs.). In trial 2, ronnel was fed at 8, 2, and 0 mg/head/day for 66 days. Gain was depressed by feeding 8 mg/day (1.47 lbs. vs 1.62 lbs. for the controls). Calves receiving 2 mg/day ronnel gained 1.65 pounds. In a third trial which lasted 59 days calves were fed the following levels of ronnel: 13 and 7 mg/head/day the first 28 days only and 10, 5, and 0 mg/head/day the entire 59 days. Daily gains for the treatments respectively were .84, 1.30, 1.12, 1.37, 1.21 lbs. Calves receiving 5 mg ronnel for 59 days gained faster than the controls although the difference was not significant. Feeding 10 mg ronnel for 59 days decreased daily gain. The response to ronnel appears to be dosage related indicating that animals may not have the ability to metabolize the compound at the higher dose levels. However toxicity symptoms were not evident in any ronnel treated calves. In these studies, ronnel treated calves contained fewer grubs than the controls so it is difficult to distinguish between a grub control response and possible growth promoting abilities of the compound.

The growth promoting ability of ronnel became evident in recent feedlot studies where parasites were controlled in all treatment groups. Riley and Ware (1977) fed graded levels of ronnel to finishing steers in a 139 day feedlot trial and found that ronnel added at 64 grams per ton of air dry feed increased daily gain 7.9%, improved feed efficiency 6.3%, and increased dry matter consumption 2.5%. These differences were not significant ($P > .05$). In this study steers fed ronnel at 128 grams per ton of air dry feed had lower daily gains and were less efficient than the control steers. No differences were found in carcass traits between treatments.

Wooden and Algeo (1977) fed graded levels of ronnel to finishing steers for 195 days. Ronnel improved daily gain, feed efficiency, and dry matter consumption. When ronnel was fed at 64 grams per ton of air dry feed, gain improved 6.6%, feed efficiency improved 3.2%, and dry matter consumption increased 2.3%. Feeding ronnel at 128 grams per ton of air dry feed did not hinder performance in this trial. Carcass traits were not affected by ronnel feeding. Thomas (1978) and Matshishuma (1978) have shown similar results when ronnel was incorporated into the rations of feedlot cattle.

Rumsey _et al._, (1975) reported that ronnel fed steers receiving a 70% concentrate diet gained faster and consumed more dry matter than the controls. However, these increases were not significant. In contrast to his previous trial and recent feedlot trials, Rumsey (1976) reported no increase in feed consumption when steers received ronnel in a 70% concentrate diet but reported a 12% improvement in daily gain and feed efficiency.

In work with growing lambs Crookshank et al., (1972) found that ronnel added to the diet at 1000 ppm. depressed feed intake and daily gain, and resulted in poorer feed efficiency.

Literature pertaining to the effect of ronnel on animal performance is limited. More research is needed to better define the compound's mode of action and degree of response when fed continuously to grazing cattle. This study was undertaken to determine if an existing chemical compound presently cleared for parasite control had growth promoting properties which could be of value to the cattle industry.

EXPERIMENTAL PROCEDURE

Experiment 1

One hundred Hereford steers with a similar genetic background and purchased from one ranch were allotted on the basis of weight to a randomized complete block design. Four established Smooth bromegrass (*Bromus inermis* Leyss) pastures were subdivided into four equal paddocks. The pastures appeared to be of similar quality but an analytical estimation of quality was not performed. Pastures were used as statistical blocks to eliminate differences if the quality of the pastures varied. Stocking rate was one steer per .4 hectares. Three pastures contained 11.2 hectares and the other 6.4 hectares, thus allowing three replicates of 7 steers and one replicate of 4 per treatment. Treatments used in this study were 0, 480, 640, and 800 mg ronnel/head/day. Steers were group fed daily so that each animal received 1.2 kg ground yellow corn which was used as the carrier for the ronnel. Water and minerals were provided free choice. Flies were controlled over all treatments with dust bags containing a 1% Co-Ral dust. Five days prior to allottment the steers received an injectable anthelmintic, a pour on grubicide, and were implanted with Synovex S.

The trial lasted 100 days (April 17, 1978 to July 25, 1978) with treatment groups being rotated between paddocks within the same pasture at 45, 68, and 83 days so that all treatments grazed each paddock. No adaptation problems were encountered as all groups were consuming their respective daily allottment of ronnel within six days. At the conclusion of the trial, treatment groups were fed and caught inside portable corrals and transported to the research center weighing facility. Final individual weights were taken after being withheld 12 hours from feed and water. Time of final feeding was recorded and four hours later a rumen fluid sample was taken with a stomach tube. Rumen fluid pH was determined immediately using a Beckman pH meter and a combination electrode. Eighteen mls. of rumen fluid were preserved with 2 ml. of 6N HCL for determination of rumen ammonia (Conway, 1950) and volatile fatty acids. The volatile fatty acids were determined by centrifuging 10 mls. of the acidified rumen fluid sample for 30 minutes at 22,000 g; the supernant was decanted and 1 ul was injected into a gas chromatograph with a 1.83 m X .32 cm column packed with Chromosorb 101 (oven temperature 190 C, 35 ml/min N_2 carrier gas flow, flame ionization detector).

Data were analyzed by analysis of variance and mean differences were tested by Duncan's new multiple range test (Steel and Torrie, 1960).

Experiment 2

In an attempt to estimate ronnel's effect on ration digestibility, six steers from each treatment in experiment 1 were used in a digestion study. Steers were fed the same level of ronnel they received during the grazing study. To simulate the conditions of experiment 1, chopped prairie hay was used in this trial fortified with 1.8 kg ground corn daily which supplied the ronnel. Nutrient composition of the ration components is shown in Table 1. The ration was fed at approximately 95% of *ad libitum*. Water and salt were provided free choice. Steers were placed in 1.5 X 7.3 meter solid concrete floored individual pens in a semi-confinement barn and fed their respective rations 14 days prior to the start of the digestion study. Rations were fed twice daily in equal amounts at 0600 and 1800 hours. Cromic oxide was used as an external marker and was fed 7 days prior and thoroughout the collection period. Five grams of technical grade chromic oxide were mixed with the grain at each feeding to ensure complete consumption of the marker. Fecal grab samples were taken at six hour intervals during the seven day collection period. Approximately 25 grams of each 1800 hour fecal sample were mixed with 100 ml of deionized distilled water and analyzed for pH using a Beckman pH meter with a combination electrode. Fecal samples were placed in a freezer within 30 minutes of collection. After all samples were collected they were dried in a forced air oven at 50 C, ground, and equal amounts composited. Proximate (AOAC, 1975) and starch (Macrae and Armstrong, 1968) analysis were performed on the feed and feces. Fecal samples were analyzed for chromium by atomic absorption on a perchloric acid digested sample.

Digestion coefficients were analyzed statistically as stated in experiment 1.

Experiment 3

Thirty steers from experiment 1 were used to evaluate the effect of ronnel on subsequent feedlot performance. The steers were maintained on their respective levels of ronnel for 21 days from the end of the grazing study to the initiation of this trial. For the 104 day (August 11, 1978 to November 23, 1978) feedlot study, 15 steers each from the 0 and 640 mg treatments were allotted to 3 replicates of 5 animals per treatment. Steers receiving 0 ronnel during the grazing study received a control ration (Table 2) while the ronnel fed steers received the same ration plus 80 grams ronnel per ton of dry matter.

Initial roughage content of the rations was 35% (dry matter basis) and was reduced 10% at 10 day intervals with the final ration shown in Table 2 being fed ad libitum for the balance of the trial. Treatment groups were randomly assigned to half concrete and half dirt 3.5 X 10.9 meter open pens with water provided free choice. Individual weights were obtained at 28 day intervals and rejected feed weighed back and discarded. Final individual weights were obtained after withholding feed and water for 18 hours.

Performance data were analyzed as stated in experiment 1.

RESULTS AND DISCUSSION

Daily gain for steers receiving ronnel while on Smooth bromegrass pasture is shown in Table 3. Steers receiving 640 mg ronnel daily gained faster ($P < .05$) than steers receiving 0 or 800 mg. Gain was depressed by feeding 800 mg ronnel, however this depression was not significant. Rumsey et al., (1975) reported that steers fed 4.5 mg ronnel/kg of body weight gained 15% faster than control steers when fed a 70% concentrate diet. In this trial the steers receiving 640 mg ronnel daily had an average intake of 2.5 mg ronnel/kg of body weight, while those receiving 800 mg had an average intake of 3.2 mg ronnel/kg. It appears that the animal's ability to utilize ronnel depends on the energy content of the diet since the steers in Rumsey's et al., (1975) trial were able to improve their performance when fed 4.5 mg ronnel/kg, while steers receiving 3.2 mg ronnel/kg in this grazing study gained 4.4% slower than the controls.

Rumen pH, ammonia, and the molar percentage of the VFA's are shown in Table 3. The only significant ronnel effect on rumen constituents was that the 480 mg treatment decreased ($P < .05$) molar percent butyric acid. Rumsey et al., (1975) reported an increase ($P < .01$) in the molar percent acetic acid and only a slight non-significant decrease in the molar percent butyric acid. The effect of ronnel on rumen volume and turnover is not known. The improvement in gain when either 480 or 640 mg ronnel were fed does not appear to be the result of alteration of rumen fermentation.

A possible explanation for the improved performance could be increased ration digestibility. Results from the digestion trial are shown in Table 4. Apparent digestion coefficients for dry matter, crude protein, crude fiber, ether extract, and starch were not affected (P>.05) when averaged across all levels of ronnel, however, ronnel improved all of the digestion coefficients except dry matter and crude protein at the 480 mg level. Rumsey et al., (1975) reported no differences (P>.05) in ration digestibility due to ronnel feeding, but there were virutally no differences in any digestion coefficient in contrast to this trial. The 800 mg treatment had the highest apparent digestibility, but this improvement in digestibility is difficult to relate back to the depressed performance noted in experiment 1. The depression in performance may have been caused by an excess of ronnel which altered nutrient uptake at the cellular level. Most of the compound is absorbed into the blood stream since only 7% of labeled ronnel or its metabolites were found in the feces of a lactating cow (Plapp and Casida, 1958).

Fecal pH was not affected (P>.05) by ronnel as shown in Table 4. Fecal pH did not effectively predict starch digestion in this trial (r = -.297).

Results of the finishing trial are shown in Table 5. Steers fed ronnel had an average daily intake of 957 mg ronnel/head. Addition of ronnel did not affect (P>.05) daily gain, feed efficiency, or dry matter intake. These results disagree with the work of Wooden and Algeo (1977), Matshishuma (1978), Riley and Ware (1977), and Thomas (1978) who found that addition of 64 grams of ronnel per ton of 90% dry matter feed on the averaged increased gain 5.8% and improved feed efficiency 5.4%. However the longest time ronnel was fed in any of these trials was 195 days. In this study the steers received ronnel for 225 days and possibly their ability to metabolize the compound had been surpassed.

Many questions remain to be answered pertaining to the mode of action of ronnel. It does not appear to improve performance by ruminal alteration. Its main effect may be upon cellular uptake of nutrients. Optimum level of ronnel appears to be related to energy density of the diet.

TABLE 1. NUTRIENT COMPOSITION OF RATION COMPONENTS ON A DRY MATTER BASIS

	Prairie Hay (1-07-956)*	Yellow Corn (4-02-935)
Crude Protein, %	7.3	9.3
Crude Fiber, %	32.1	2.1
Ether Extract, %	3.2	3.4
Ash, %	8.9	1.6
Starch, %	7.5	77.0

* International Reference Number

TABLE 2. FEEDLOT RATION COMPOSITION

Ingredients	International Reference No.	% Dry Matter Basis
Yellow Corn	4-02-935	75
Sorghum Silage	1-04-372	15
Supplement[a]		
Soybean mean	5-04-600	5.59
Yellow corn	4-02-935	2.55
Limestone	6-02-632	1.01
Salt		.56
Animal fat	4-00-409	.15
Dyna-K		.11
Z-10 TM[b]		.03

[a] Supplement contained 30,000 I.U. Vitamin A/kg

[b] Trace mineral premix

TABLE 3. PERFORMANCE AND RUMINAL DATA OF STEERS GRAZING SMOOTH BROMEGRASS PASTURE FED GRADED LEVELS OF RONNEL

	mg Ronnel/head/day								
	0		480		640		800		Prob.
Grazing Data:									
No. of steers	25		25		25		25		
Initial wt., kg	207	(3.03)*	207	(2.57)	208	(2.39)	207	(2.40)	
Final wt., kg	299	(4.24)	303	(3.73)	307	(4.06)	295	(3.72)	
Avg. daily gain, kg	.91	(.04)[a,b]	.96	(.03)[b,c]	1.00	(.03)[c]	.87	(.03)[a]	.0095
Ruminal Fluid Data:									
pH	6.81	(.08)	6.77	(.09)	6.78	(.06)	6.74	(.09)	.92
Ammonia, mg N/100 ml	30.09	(4.55)	33.86	(3.79)	29.69	(4.44)	40.91	(5.01)	.19
Volatile Fatty Acids, gm/100 ml									
Acetate	65.48	(.66)	65.97	(.68)	66.40	(1.44)	64.33	(1.26)	.53
Propionate	17.11	(.50)	18.16	(.60)	16.46	(.96)	17.36	(.75)	.40
Isobutyrate	1.10	(.07)	1.06	(.08)	1.04	(.14)	1.05	(.08)	.96
Butyrate	14.46	(.38)[a]	12.98	(.56)[b]	13.85	(.38)[a,b]	15.24	(.55)[a]	.0088
Isovalerate	.91	(.05)	.82	(.04)	1.08	(.17)	1.07	(.05)	.13
Valerate	.93	(.07)	.96	(.09)	1.16	(.17)	.94	(.07)	.30

* ± Standard error of the mean (SEM).

a,b,c Means with different superscripts in the same row differ significantly ($P < .05$).

TABLE 4. APPARENT DIGESTION COEFFICIENTS AND FECAL pH OF STEERS FED GRADED LEVELS OF RONNEL

	mg/Ronnel/head/day								
	0		480		640		800		Prob.
Dry Matter	43.48	(3.70)*	41.34	(2.49)	45.45	(2.20)	49.55	(2.79)	.245
Crude Protein	34.40	(3.25)	27.91	(3.27)	35.11	(3.79)	39.42	(2.52)	.127
Crude Fiber	35.91	(4.78)	37.07	(3.10)	38.45	(2.82)	43.65	(3.42)	.462
Ether Extract	40.95	(4.10)	41.37	(2.05)	46.94	(2.55)	48.10	(2.51)	.216
Starch	90.37	(.90)	90.49	(.72)	92.35	(.71)	92.33	(.83)	.156
Fecal pH	6.63	(.03)	6.57	(.02)	6.62	(.09)	6.54	(.02)	.144

* ± SEM

TABLE 5. PERFORMANCE AND DRY MATTER INTAKE OF FEEDLOT STEERS FED RONNEL

	grams Ronnel/Ton of Dry Matter			
	0		80	
Initial wt., kg	315	(5.02)*	321	(2.76)
Final wt., kg	477	(5.56)	485	(5.59)
Avg. Daily Gain, kg	1.56	(.03)	1.57	(.05)
Avg. Dry Matter Intake, kg	10.76		10.85	
Feed/Gain Ratio	6.90		6.91	

* ± SEM

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The effect of ronnel on animal performance and rumen constituents of grazing steers, ration digestibility, and feedlot performance was evaluated. Ronnel [0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate] is an organophosphate cleared for use as an oral systemic for lice and heel fly larva and as an oral larvacide for horn fly control. Ronnel was supplied daily to 100 yearling steers (avg. initial wt. 207 kg) at four levels (0, 480, 640, and 800 mg/head). The steers grazed Smooth bromegrass (Bromus Inermis Leyss) pasture for 100 days. Internal and external parasites were controlled by approved methods. Average daily gain (ADG) for the 0, 480, 640, and 800 mg treatments, respectively, were .91; .96; 1.00; and .87 kg. ADG was improved ($P < .05$) by feeding 640 mg ronnel. The depression in gain at the 800 mg level was not significant. Rumen ammonia (mg/100 ml), rumen pH, molar percent acetate, propionate, and butyrate for the 0, 480, 640, and 800 mg treatments, respectively, were 30,09, 6.81, 65.48, 17.11, 14.46; 33.86, 6.77, 65.97, 18.16, 12.98; 29.69, 6.78, 66.40, 16.46, 13.85; and 40.91, 6.74, 64.33, 17.36, 15.24. Ronnel did not affect ($P > .05$) rumen ammonia, rumen pH, molar percent acetate or propionate. Molar percent butyrate was reduced ($P < .05$) by feeding 480 mg ronnel.

Ration digestibility was determined by assigning 6 steers from each grazing treatment to a chromic oxide digestion trial. Chromic oxide was fed 7 days prior and throughout the collection period. Fecal samples were taken every six hours for 7 days. Steers were fed chopped prairie hay supplemented with 1.8 kg ground yellow corn daily. Steers were continued on the same level of ronnel they received on pasture.

Apparent digestion coefficients for dry matter, crude protein, crude fiber, ether extract, and starch for the 0, 480, 640, and 800 mg treatments, respectively, were 43.48, 34.30, 35.91, 40.95, 90.37; 41.34, 27.91, 37.07, 41.37, 90.49; 45.45, 35.11, 38.45, 46.94, 92.35; and 49.55, 39.42, 43.65, 48.10, 92.33. Ronnel did not affect ($P > .05$) any of the digestion coefficients. Fecal pH was not a good indicator of starch digestibility in this trial ($r = -.297$).

Fifteen steers that received 0 and 640 mg ronnel while on pasture were placed on a finishing ration for 104 days to determine if ronnel would continue to improve performance under feedlot conditions. Steers fed 0 ronnel on pasture continued to receive 0 ronnel while those receiving 640 mg on pasture were fed 80 grams ronnel per ton of dry matter. ADG (kg), feed efficiency, and dry matter consumption (kg) for the 0 and 80 gram treatments, respectively, were 1.56, 6.90, 10.76; and 1.57, 6.91, 10.85. There were no differences ($P > .05$) between treatments.