Studies with Arsanilic Acid and Related Compounds

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Organic arsenicals are widely used as growth promotants and disease-control agents in feeds. Data on differences between the action of arsonic acids and antibiotics were sought, as well as differences between arsenicals themselves. There appears to be no good rationale why some arsenicals promote growth and others do not. Arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid, both arsonic acids but differing in potency and in toxicity, have been most widely studied. Toxicity of arsenicals is a direct consequence of the arsenic which reaches vital tissues. Arsanilic acid, which is well tolerated in chickens, turkeys, swine, and dogs, gives low tissue arsenic levels. Arsanilic acid did not interfere with reproduction over three generations in rats. Unlike antibiotics, arsonic acids do not lessen the need for vitamin B_{12}. Organic arsenicals provide important aids to animal production distinct from the antibiotics. That they can be safely used, with adequate controls and proper precautions, has been demonstrated.

Beneficial effects of feeding inorganic arsenic to chickens, pigs, and rabbits were described by Gies in 1877 (*29*). He dramatized the historical role of arsenic as a tonic on the one hand and a poison on the other. Therapeutic use of arsenic was described a century earlier by Fowler (*25*), but its empirical use goes back at least to the time of Galen. Interest in arsenicals for medical use culminated in discovery of the trypanocidal activity of sodium arsanilate (atoxyl) and finally in the synthesis and screening of thousands of organic arsenicals of varying parasiticidal value. This phase is well told elsewhere (*1*, *57*, *65*). Probably more work has been done to correlate the chemical structure and parasiticidal action of organic arsenicals than for any other group of chemicals (*19*, *21*, *70*).

Discovery of the positive effects of antibiotics and arsonic acids on animal production may go down as one of the great advances in practical nutrition, but it is still too early to place these advances in proper historical perspective. An attempt is made here to review general results of work to date with organic arsenicals as related to animal production. Data are presented on the safety and tolerance of representative compounds.

Disease Control in Poultry

In general, the arsenicals tested in poultry diseases, until recently, were those developed for use in human medicine, including tryparsamide, neoarsphenamine, and Mapharsen. As early as 1907 atoxyl (*69*) was used to control spirochetosis in hens. Many of the compounds were tested by injection, an impractical method, and the effects were inconclusive. Nevertheless they showed the intrinsic value of arsenicals for control of some diseases of poultry.

Of 20 arsenicals tested as coccidiostats by Goble (*30*), the most promising were reported to be found in the phenylarsonic (benzenearsonic) group. Mapharsen, interestingly enough, was reported to be ineffective. It is now clear, however, that certain phenylarsenoxides are more potent coccidiostats for chickens than the corresponding arsonic acids. Studies on control of turkey coccidiosis by Morehouse (*49*) showed a wide difference in both the efficacy and toxicity of different arsonic acids, depending on the substitution in the benzene ring. Both workers reported high activity and high toxicity for 4-chlorophenylarsonic acid and for 3-nitro-4-hydroxyphenylarsonic acid and low activity and low toxicity for arsanilic acid. Subsequent work by F. C. Goble and Russell Krueger in this laboratory indicates that arsanilic acid is effective as a coccidiostat in its upper range of tolerance for chickens—i.e., 0.05 to 0.1% of the diet.

The chemotherapy of blackhead in turkeys with arsenicals has been reviewed (*5*, *51*). McGuire and Morehouse (*45*) in seeking an effective drug, which could be fed continuously, arrived at 4-nitrophenylarsonic acid as an arsenical of choice. Barger and Card (*5*) also reviewed the use of organic arsenicals for treatment of spirochetosis, a disease more commonly found in Europe and other parts of the world than in the United States. Marcos and coworkers (*46*) reported atoxyl and myosalvarsan to be specifics for control of this disease, whereas sulfonamides or pencillin appeared ineffective.

Effects on Growth and Appearance in Poultry

In 1946 Moore and coworkers (*48*) first indicated the growth-stimulating effect of streptomycin and succinylsulfathiazole in chicks, the same year in which Morehouse and Mayfield (*52*) briefly mentioned the growth-promoting effects of 3-nitro-4-hydroxyphenylarsonic acid and 4-hydroxyphenylarsonic acid. The growth response to 3-nitro-4-hydroxyphenylarsonic acid was followed up in 1949 in an extensive study by Morehouse (*50*). The antibiotic growth effect was spotlighted in this same year, as reviewed by Stokstad (*66*). Bird, Groschke, and Rubin (*8*), also in 1949, fully confirmed the growth-stimulatory effect of 3-nitro-4-hydroxyphenylarsonic acid for chicks. They extended the series of active arsonic acids to include phenylarsonic acid. Thus it was clear before 1950 that many compounds, related only by their capacity to inhibit microbial growth, were all capable of stimulating growth of chickens.

Direct comparison of the growth-promoting effects of 3-nitro-4-hydroxyphenylarsonic acid with that of streptomycin and Aureomycin was next made by Stokstad and Jukes (*67*). The data presented indicate that all three compounds stimulated chick growth about equally. McGinnis and coworkers (*44*) noted a similar comparison in turkey poults. Scott and Glista (*62*) in a study with chicks reported only a slight stimulation during the first few weeks with either Aureomycin or 3-nitro-4-hydroxyphenylarsonic acid. They raised a question as to the significance of the response and its relation to the completeness of the diet. Recently Anderson, Cunningham, and Slinger (*2*) reported highly significant growth responses in turkey poults with magnesium 4-hydroxyphenyl arsonate and 3-nitro-4-hydroxyphenylarsonic acid alone or in combination with Terramycin. Elam, Jacobs, Tidwell, Gee, and Couch (*22*) likewise reported gains over control chicks for arsanilic acid, sodium arsanilate, procaine penicillin, bacitracin, Aureomycin, and Terramycin.

Commercial evaluation of antibiotics in poultry feeds rapidly led to their widespread adoption. Similarly the arsonic acids came into use largely on the basis of commercial experience following extensive field trials. Bird (*24*) summarizes the situation briefly as follows: "Derivatives of phenylarsonic acid were first used in poultry feed as antiparasitic drugs and later as growth stimulants, but they are being used now mainly for improvement of feathering and increase of pigmentation of shanks, skin, comb, and wattles. Their effect on appearance is quite consistent. Their effect on growth when they are added to a diet already containing an antibiotic is inconsistent. Sometimes there is a growth stimulation in addition to that provided by the antibiotic and sometimes there is not." Further evidence for the oft-cited effect of arsonic acids on pigmentation is presented in a preliminary report by Couch (*17*).

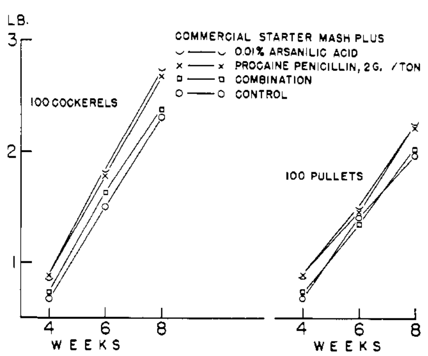


Figure 1. Growth rate of White Rock cockerels and pullets raised in floor pens with an arsonic acid and an antibiotic added alone and in combination to a commercial starter mash

330 White Rocks per group

Combs and Laurent (*15*) describe an experiment conducted under field conditions, wherein a combination of Aureomycin with 3-nitro-4-hydroxyphenylarsonic acid was more effective than either compound alone in reducing mortality and increasing growth and feed efficiency. The results of their study led these authors to conclude that such combinations are economically sound, particularly where disease outbreaks are considered likely. Similarly, Combs and coworkers (*16*) reported that arsanilic acid improved growth and food utilization in the presence of penicillin. A noteworthy facet of this study was that arsanilic acid appeared effective only when sources of the unidentified "fish factors" were present in the diet. Romoser (*59*) estimated a high economic return from arsanilic acid in broiler feed.

The authors' experiments with arsanilic acid conducted under farm conditions have shown variable results to date. Figure 1 shows the weight data for four groups of 330 White Rock chicks to 8 weeks, comparing the effect of arsanilic acid and penicillin alone and in combination. The basal diet was a commerical growing mash minus the regular antibiotic supplement. There were no real differences in feed conversion, appearance, or mortality between groups. Both cockerels and pullets showed a better response to the individual drugs than to the combination. A similar experiment was conducted a few weeks later in the same floor pens, using 200-day-old Broad Breasted Bronze poults per group. The arsanilic acid and procaine penicillin supplements were added by the feed manufacturer to regular commercial turkey starter ration ordinarily used on this farm. Results to 7 weeks and 3 days are shown in Figure 2. Here combined drugs produced both greater growth and feed efficiency than either drug alone.

Specificity of Arsenicals

The relative simplicity of the assays for treponemicidal activity and toxicity afforded Doak and Eagle (*19*) an opportunity to determine the therapeutic index of a large series of arsenicals. Review of their work shows the profound effects of substituent groups. Mapharsen was found to have only 42% of the treponemicidal activity of phenylarsenoxide, but was only 7 to 9% as toxic. Therapeutic acceptance of Mapharsen depended not so much on its unusual activity as on its relative nontoxicity (68). Eagle and coworkers (*20*, *36*) treated this phase of arsenical research in detail. Of the arsenicals studied thus far, only 3-nitro-4-hydroxyphenylarsonic acid, arsanilic acid, 4-hydroxyphenylarsonic acid, and phenylarsonic acid appear to be effective as growth stimulants. Comparison of arsonic acids, one with the other, for growth stimulation depends, however, on optimum level for each in the diet. On the basis of 4-week weights Morehouse (*50*) concluded that the optimum feeding level for 3-nitro-4-hydroxyphenylarsonic acid is 0.009%. On the basis of 8-week tests, on the other hand, the data of Bird and coworkers (*8*) suggested that 45 grams per ton, 0.005% of the feed, is about optimal and that 0.01% closely approaches the level of growth inhibition. The optimum feeding level for arsanilic acid for chickens has not been exactly determined, but appears to be 90 grams per ton, or more. The range between 0.01 and about 0.07%, where inhibition is first seen, requires exploration. The 0.02% level was at least as effective as 0.01% arsanilic acid in early collaborative field tests. The level first recommended, 60 grams per ton, was found suboptimal in large scale field trials by feed manufacturers and was increased to 90 grams per ton, a level easily consistent with safety (*27*).

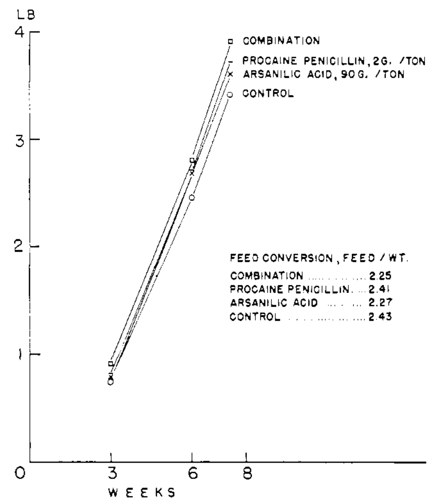


Figure 2. Growth rate of Broad Breasted Bronze turkeys

To 7 weeks and 3 days of age in same floor pens and with same supplements as used for chick experiment shown in Figure 1. Supplements made to commercial turkey starter. 200 turkeys per group

Compounds which have shown dubious growth-promoting activity include 3-nitrophenylarsonic acid, tested by Bird and coworkers (*8*), and 3-amino-4-hydroxyphenylarsonic acid and 3-acetylamino-4-hydroxyphenylarsonic acid, reported by Anderson and coworkers (*2*). A sample of 3-methyl-4-aminophenylarsonic acid, tested by Bird (*7*) proved inactive. All these compounds were tested at only one concentration, in the range 0.005 to 0.007% of the diet. It is possible that they would show activity at higher concentration. Experience is too limited to draw conclusions as to the nature of the groupings which may or may not make for growth-promoting activity of organic arsenicals.

An interesting aspect of the arsenical growth stimulant work is the part played by arsenic itself. The biological role of arsenic both as a tonic and as a toxin is reviewed in part elsewhere (*27*). As cited by Bird and coworkers (*8*), Tangle reported that the growth of chickens was stimulated by feeding cuprisulfarsenite to supply 35 γ of arsenic per chick per day. No such stimulation was observed by the Beltsville group with sodium arsenate under conditions wherein various phenylarsonic acids gave a response. On the other hand, Carlson and coworkers (*11*) recently reported a growth response in chicks to sodium arsenite and an even greater response to arsonic acids.

Disease Control and Growth Stimulation

Swine Investigations on the effect of 3-nitro-4-hydroxy-phenylarsonic acid on the growth of swine were first reported by Carpenter (*13*). Despite evidence of toxicity, the arsenical pigs were reported to appear more thrifty and to have smoother and glossier hair coats than the controls. The prevalence of dysentery in the test herd was described by Carpenter and Larson (*14*) in a subsequent paper. It appears likely that the positive effects of the arsonic acid were in part associated with control of this disease. Becker and coworkers (*6*) and Wallace and coworkers (*71*) reported variable results with this same compound. In a full-term experiment Patrias (*24*) reported favorable results and no toxicity from arsanilic acid at 0.01 and 0.02% of the diet. Experiment station reports from Minnesota (*32–34*) and Nebraska (*47*) describe the early growth response to arsanilic acid in pigs in the range 0.0033 to 0.01% of the diet. Schendel and Johnson (*61*) observed a marked growth stimulation in baby pigs fed "synthetic milk" to 8 weeks with arsanilic acid at 0.01% of the milk solids. Death occurred among some of the supplemented pigs, but the causes are not clear.

Arsonic acids appear to have unusual promise for control of swine dysentery (*9*, *39*, *60*). Combinations of safe levels of arsonic acids with antibiotics appear more effective and less costly than therapeutic levels of antibiotics alone. No pathology was seen in this laboratory in liver and kidney sections from pigs fed arsanilic acid at 0.02% of the diet for $5^1/_2$ months. Some hypercalcification of the bone was apparent. This effect is apparently similar to that described by Gies (*29*) and was readily seen in the leg bones following silver nitrate staining. The authors have not found any change in the bone of chicks fed 0.1% arsanilic acid for 12 weeks or in rats fed arsanilic acid at 0.02% through several generations. The arsenic tissue levels found in pigs were higher than those in chickens fed 0.01% arsanilic acid. Averages for arsenic trioxide in liver and muscle of the pigs were 4.2 and 0.57 p.p.m., respectively, whereas the corresponding averages for chickens were consistently about 1.4 and 0.3 p.p.m. This is further evidence of the difference in metabolism of arsonic acids by different species.

Ruminants An antibioticlike effect of arsanilic acid in decreasing scours and increasing growth in calves to 4 weeks was suggested by the report of Graf and Holdaway (*31*). The effect was said to be better at the levels of 60 and 120 grams per feed ton than at the 240-gram level. Leighton (*41*) further indicates that the choice of level may be important. In one phase of the Texas study (*78*) five unthrifty calves appeared to respond both in rate of gain and in general appearance to a daily supplement of 15 mg. of sodium arsanilate or dodecylamine *p*-chlorophenylarsonate. No response was noted in a calf which received 30 mg. of sodium arsanilate per 100-pound weight for 45 days. Furthermore, a calf which received 60 mg. of sodium arsanilate per 100-pound weight made no gain and went off feed in 25 days. These data again suggest the critical nature of the feeding level.

Studies in growing fattening lambs by Bucy, Garrigus, Forbes, and Hale (*10*) failed to show any toxicity for potassium arsenite, arsanilic acid, or 3-nitro-4-hydroxyphenylarsonic acid at levels up to 0.024% of the latter compound, or the arsenic equivalent of the other two. No consistent effects were apparent from these experiments.

Mode of Action

Similarity in the action of antibiotics and arsonic acids is seen in the papers of Anderson and coworkers (*2*) and Elam and coworkers (*22*). Both groups reported fairly comparable changes for both types of compound in the intestinal flora of chickens. The Ontario group observed increases in *lactobacilli* counts and a fall in pH of cecal contents, which accompanied the weight increase of turkey poults. The group at Texas A. & M. reported a marked drop in total *Clostridia* per gram of chick feces, a change which appeared to reflect the ability of arsanilic acid and various antibiotics to stimulate growth.

Table I. Effect of Penicillin and Arsanilic Acid in Vitamin B_{12}–Deficient Chicks

Supplement to B_{12}–Deficient Diet[a]	Av. 4-Weeks Wt. Gain[b], Ratio to Controls, %
None	100
Vitamin B_{12}, 12 γ/kg.	120
Arsanilic acid, 45 mg./kg.	95
K penicillin, 2.2 mg./kg.	114
B_{12} + K penicillin + arsanilic acid	128

[a] Diet C of Bird, Groschke, and Rubin (*8*).
[b] 12 White Leghorns per group.

Sieburth and coworkers (*64*) had earlier implicated *Clostridium perfringens* as a possible inhibitor of animal growth, when they found that antibiotics greatly depressed the numbers of this organism in the intestinal tract of turkey poults. Larson and Carpenter (*40*) in work with pigs did not observe a correlation between growth and *Clostridia* count, even though their data suggest that this count is altered by feeding antibiotics. Their study included 3-nitro-4-hydroxyphenylarsonic acid at two different feeding levels. The lower level of this compound failed to stimulate growth and the *C. perfringens* count for this group did not differ from that of the controls. Clearly, much work is needed to develop these leads which implicate specific bacterial groups. Standardization of techniques will prove increasingly important in interpretation of data between laboratories. Similarity in action of various antibiotics and arsanilic acid to increase blood calcium levels in chickens was reported recently by Shaffner (*63*).

Table II. Effect of Penicillin and Arsonic Acid on Growth of Vitamin B_{12}–Depleted Rats

Supplement per Kg. Diet[a]	Av. 3-Week Weight Gain[b], Grams
None	27
Vitamin B_{12}, 2.5 γ	45
Vitamin B_{12}, 5.0 γ	53
Procaine penicillin	
2 mg.	46
10 mg.	49
50 mg.	55
3-Nitro-4-hydroxy-phenylarsonic acid, 50 mg.	29

[a] Vitamin B_{12}–deficient diet (*28*).
[b] 15 female rats per group.

Lih and Baumann (*43*) established the very significant sparing effect of antibiotics for B-complex vitamins in rats, an effect not shared by 3-nitro-4-hydroxyphenylarsonic acid. Stokstad (*66*) has reviewed this feature of antibiotic action. The authors were interested to explore this difference as applied to arsanilic acid and directed their work particularly to vitamin B_{12} and pantothenic acid.

In studies with chicks an appropriate vitamin B_{12}–deficient diet and day-old cockerels hatched from hens on a B_{12}–low diet were used. Table I shows the ratio of the average weights at 4 weeks for the supplemented groups as compared with the controls. Arsanilic acid had no effect in absence of vitamin B_{12}, whereas potassium penicillin gave a 14% response over the controls. The combination of all three drugs gave a still greater response.

The ability of antibiotics to spare vitamin B_{12} was an important feature of studies on the assay of this vitamin in rats (*28*). Table II shows average weight gain at two critical levels of vitamin B_{12}. Three levels of procaine penicillin gave responses equal to these low levels of vitamin B_{12} in this experiment. In other experiments penicillin has not spared vitamin B_{12} so completely. The arsonic acid used in this experiment, 3-

nitro-4-hydroxyphenylarsonic acid, had no apparent effect on the growth rate of the vitamin B_{12}–deficient rats.

The vitamin B_{12}–sparing effect of antibiotics in deficient rats was checked using a well-known corn-soybean diet (*58*). As seen in Figure 3, Aureomycin hydrochloride appeared to have more than half the growth-promoting effect shown by an optimum level of vitamin B_{12}. Again an arsonic acid—in this case, arsanilic acid—had no effect.

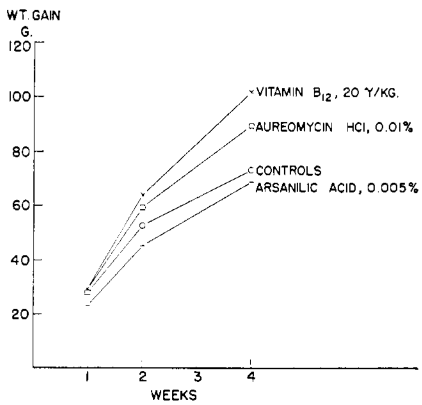


Figure 3. Growth stimulation in vitamin B_{12}–deficient rats by vitamin B_{12} or Aureomycin·HCl, but not by arsanilic acid

Corn-soybean, B_{12}-deficient ration (58)

The pantothenate-sparing effect of procaine penicillin and Aureomycin hydrochloride and the failure of sodium arsanilate along similar lines are shown in Figure 4. Two separate experiments were run, one involving the antibiotics, the other the arsonic acids. In each case groups of 10 male rats were placed on a standard-type pantothenate low diet at weaning. For positive control calcium-D(+)-pantothenate was fed at 0.001% of the diet, a suboptimum level. As shown, the negative control rats lost weight or gained very little. The two groups which received an antibiotic, on the other hand, showed appreciable gains.

The complete failure of arsonic acids, and equally clear-cut ability of antibiotics to spare vitamin B_{12} or pantothenate, represent an interesting difference between these two groups. The authors were interested then to determine whether representative compounds from both groups would have any effect on growth rate of stock colony rats raised on the regular stock breeding diet (*28*). Table III shows the average 6-week gains made by such groups. Additional vitamin B_{12} was made available to all groups, so that there would be no question of the adequacy of this vitamin for optimal growth. Both penicillin and Aureomycin stimulated growth rate, whereas all of the arsenicals tried appeared ineffective.

Effects of Arsonic Acids on Reproduction

As reviewed elsewhere (*27*), questions have arisen in the literature on the possible role of arsenic in reproductive processes. Reports by Morehouse (*24, 50*) indicate that 3-nitro-4-hydroxyphenylarsonic acid brought pullets into egg production earlier and that economic return was improved by feeding this compound from hatching through the first laying year.

The first concern with arsanilic acid was to establish the fact that it did not interfere with reproduction. Libby, Groschke, Evans, and Bandemer (*42*) reported the results of a study in which arsanilic acid appeared to have no unfavorable effects on egg production or hatchability when fed to pullets for 11 weeks at 0.01 to 0.02% of the diet. Accumulation of arsenic in eggs and tissues was well below allowable levels and was proportional to the amount in the ration (*23*).

In 1951 work was done to determine whether arsanilic acid would affect reproduction in stock colony rats when fed over three generations, and when fed repeatedly to the same mothers over three successive matings. The stock colony provided excellent subject material for such a test, as it has been inbred on essentially the same diet for 21 years.

Two levels of arsanilic acid were chosen for study, 60 and 180 grams per ton of stock diet. Weaning rats were raised on these diets to maturity. For the parent generation and for each successive generation eight females, selected at random, were caged with four males. The number of pups was reduced to eight soon after birth, consistent with regular colony procedure. A control group, representing the stock colony diet with no addition, was carried along with the test groups. The results for part of this study are shown in Table IV, which gives only the data for the control and the 0.02% arsanilic acid groups. Neither level showed any adverse effects. If anything, the litter size of the arsenical-fed groups was larger than the controls, but the data at hand do not warrant a conclusion on this point.

Table III. Difference in Growth Response of Abbott Stock Colony Rats to Antibiotics and Arsonic Acids

Supplement to Stock Diet[a]	6-Week Wt. Gain[b], % of Controls: Males	Females
None	100	100
Procaine penicillin, 0.01%	123	108
Aureomycin·HCl, 0.01%	119	115
Sodium arsanilate, 0.01%	100	96
Arsenosoaniline, 0.005%	94	100
3-Me-4-NH_2-phenylarsonic acid, 0.005%	94	99
Ethanolamine salt of *p*-chlorophenylarsonic acid, 0.005%	98	92

[a] 15 γ B_{12} per kg. added.
[b] 12 rats per group.

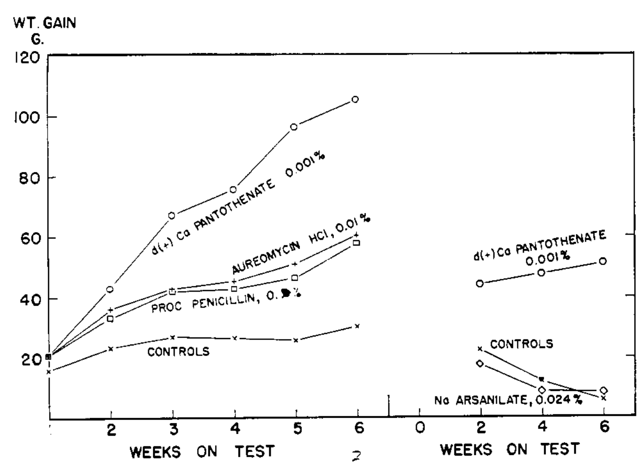
Wharton and Fritz (*72*) recently reported a trend toward greater weight gain and production of slightly heavier eggs in pullets fed 4-hydroxyphenylarsonic acid (0.005%), but indicated that differences were not statistically significant. There did appear to be a significant difference in the rate at which egg weight of the arsenical fed birds reached a maximum as compared with controls.

Factors Affecting Toxicity

The different arsonic acids differ markedly in toxicity. As might be expected, effectiveness against parasites, as well as general bacteriostatic power, parallels the toxicity to animals. Table V shows the approximate tolerance to single oral doses of various arsonic acids and of arsenosoaniline in several species. Table VI shows the relative tolerance in rats to continued feeding of different levels of some of these compounds.

In the test depicted in Figure 5, arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid

Figure 4. Growth stimulation of rats on pantothenate-deficient diet by pantothenate, Aureomycin·HCl, or procaine penicillin, but not sodium arsanilate



were fed at recommended and ten times recommended feeding levels. This illustrates the marked difference in tolerance between these two compounds in the chick. Three White Leghorn cockerels used in this study were sacrificed at the end of the twelfth week of feeding. Liver and muscle samples were promptly frozen. Pooled samples from three birds were analyzed by J. B. Thompson of the Trace Metals Research Laboratory by an adaptation of the AOAC Gutzeit method. Separate liver and muscle samples from each of three birds were analyzed in this laboratory by a modification of the Kingsley-Schaffert method (*38*). Modification of this method dealt largely with an improved method for ashing samples described by Bandemer and Schaible (*4*) and applied to the determination of arsenic in eggs by Evans and Bandemer (*23*). As seen in Table VII, arsanilic acid appears to cause less deposition of arsenic in the liver than 3-nitro-4-hydroxyphenylarsonic acid, even when the latter is fed at half the level. This is true despite the fact that the latter compound contributes less than half as much arsenic to the diet.

Similarly, other arsenic derivatives, known to be less well tolerated than arsanilic acid, caused relatively high arsenic deposition values. Table VIII shows arsenic deposition values at 8 weeks for turkey poults fed arsanilic acid and dodecylamine *p*-chlorophenyl arsonate, both at 0.01% of the diet. As shown, the latter compound supplied only about half as much arsenic in the diet as arsanilic acid. The relatively high toxicity of *p*-chlorophenylarsonic acid to both host and parasites (*30*) is referred to earlier in this paper.

Hogan and Eagle (*36*) showed that the toxicity of organic arsenicals is directly related to the arsenic level bound in the tissues. Thus tryparsamide, phenylarsonic acid, and phenylarsenoxide injected in rabbits at their respective LD_{50} of 700, 16, and 0.8 mg. per kg. all yielded about the same tissue arsenic levels.

Carpenter (*12*) studied the arsenic blood levels of pigs fed 0.02% of three different arsenicals in the diet. In all cases the blood levels reached a maximum in 48 hours. The most toxic of the three compounds tested, 3-nitro-4-hydroxyphenyl arsonic acid, gave the lowest blood levels. Arsanilic acid was fed for 10 weeks with no symptoms of toxicity, but it produced higher arsenic blood levels than its more toxic analog, 4-nitrophenylarsonic acid. No attempt was made to determine whether the circulating arsenic was free or bound. How much is stored from any specific compound may be related to the rate of metabolism and excretion of the arsenical, or its degradation products. The fact that arsanilic acid, the least toxic compound, produces the highest blood levels of arsenic certainly suggests that it is metabolized differently from the other arsenicals studied. As indicated above, correlation with toxicity may be found in the relative rate of deposition of arsenic in the liver for the different arsenicals.

Carpenter reported also that arsenic appeared in the milk of sows fed arsanilic acid at the 0.02% level. Tissues of two pigs fed arsanilic acid, 0.04% of the diet from 100 to 200 pounds of body weight, were sent for analysis by S. W. Terrill of Illinois. Liver and muscle samples were found to contain 8.7 to 9.2 and 0.7 to 0.75 p.p.m. of arsenic trioxide, respectively. It was surprising, considering the high feeding level and the high liver arsenic levels, that no toxicity was seen in these pigs. It seems likely from the evidence at hand that older pigs can tolerate high feeding levels much better than very young pigs.

Tolerance studies on dodecylamine *p*-chlorophenyl arsonate also brought out the fact that this compound fed at or above its tolerance limit showed no growth inhibition during the first 4 weeks, but increasing inhibition thereafter (Figure 6). Arsanilic acid, 0.01%, was fed as a positive control in this test. The suggestion is clear that tolerance studies with arsenicals should be carried beyond 4 weeks, preferably to market weight.

Table V. Largest Single Oral Dose Tolerated in Different Species

	Rat[a], Mg./Kg.	Chicken[a], Mg./Kg.	Duck[b], Mg.
Phenylarsonic acid	10	35	
Arsenosoaniline	25	35	
Arsanilic acid	400	300–400	1000
Dodecylamine *p*-chlorophenyl arsonate	<100	100	
3-Nitro-4-hydroxyphenylarsonic acid	20	100	<100
4-Nitrophenylarsonic acid	75	<100	

[a] These doses resulted in no mortality or less than 10% mortality. Seventy-five 2.5- to 3-pound chickens and 106 young adult rats (100 to 150 grams) were used. From 9 to 18 chickens and 8 to 41 rats were used to establish levels for each compound.
[b] Approximate maximum tolerated single dose for 2.5-pound ducks estimated from data furnished by R. J. Karrasch and B. H. Barrows, Hales and Hunter Co.

Table VI. Effect of Concentration of Arsenical Added to Stock Ration on Growth Rate of Rats

Compound	Half Normal Growth at Diet Level Shown, % in Diet: Males	Females
Arsanilic acid	0.1–0.2	0.1–0.2
Arsenosoaniline	0.0125–0.025	0.0125–0.025
3-Nitro-4-hydroxyphenylarsonic acid	0.0125–0.025	0.0125–0.025

Dodecylamine *p*-chlorophenyl arsonate gave greater than half normal growth in males at 0.0125% of diet, but less than half normal in females.
4-Nitrophenylarsonic acid caused deaths in 1 to 4 weeks at 0.05% of diet, lowest level tested for this compound.

Arsanilic Acid Tolerance

In Turkeys An experiment was designed to test the tolerance of arsanilic acid in the diet of growing turkeys. The diet used was as follows: corn meal 42.5, ground wheat 5, pulverized oats 5, soybean meal (50% protein) 33, menhaden fish meal 12.5, limestone 1, dicalcium phosphate 0.5, salt 0.5, manganese sulfate 0.04, choline

Table IV. Effect of Arsanilic Acid at 0.02% of Regular Stock Colony Diet on Reproduction in Rats

	Controls			Arsanilic Acid, 0.02%		
Generation	Av. no. per litter	Av. litter wt., 21 days, g.	Survival 21 days, %	Av. no. per litter	Av. litter wt., 21 days, g.	Survival 21 days, %
Parent	...	199	85	...	229	85
F_1 generation						
1st mating	8.3	215	82	10.2	179	37
2nd mating	7.7	209	87	7.7	229	86
3rd mating	6	245	97	9.4	218	83
F_2 generation	6.3	199	74	7.5	222	73

chloride 0.05, betaine 0.2, niacin 0.0011, and riboflavin supplement (4 grams per pound) 0.05%. The diet contained also 4000 units of vitamin A, 1000 units of vitamin D, and 1 mg. of menadione per pound.

Duplicate groups of 10-day-old, straight-run, Broad Breasted Bronze poults were raised in laboratory brooder cages for 4 weeks and shifted to large chicken batteries for the remaining 2 weeks of the test. Figure 7 shows the averaged results of the graded supplements of arsanilic acid which were added to the basal diet. The lowest level fed, 0.01%, which is the maximum level recommended for poultry feeding, gave a small growth response over the controls. The higher levels gave increasing inhibition of growth up to the 0.1% level, where all turkeys had died by the third week. There was close agreement throughout between the responses of the duplicate groups of turkeys. No evidence of toxicity, other than growth inhibition, was seen in the groups which received the 0.025 and 0.05% levels of arsanilic acid.

As a positive control in the above experiment, not shown in Figure 7, duplicate groups of 10 poults each received 4 grams of procaine penicillin per ton of feed. The average rate of gain for these groups was 9.6% above that of the controls. In addition, similar duplicate groups fed the basal diet supplemented with 0.0075% arsenosoaniline or 0.025% 4-nitrophenylarsonic acid averaged the same and 5% less in weight as compared with the control groups at 6 weeks of age.

Table IX shows the results of arsenic analyses carried out by the Evans-Bandemer method in this laboratory and by J. B. Thompson of the Trace Metals Research Laboratory (Gutzeit method) on liver and muscle samples from poults fed arsanilic acid or arsenosoaniline to 6 weeks of age. Deposition of arsenic is greater in poults than in chickens for arsanilic acid. This reflects the lesser tolerance to arsenicals shown by poults.

In Dogs C. M. McCay of Cornell called attention to the fact that certain poultry feed additives, notably nitrophenide, were recently found to be toxic to dogs. Although the arsonic acids were suspected at one stage of the Cornell investigation (*37*), they were apparently not involved in any of the cases reported. Following correspondence with McCay, a program was set up to determine tolerance to arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid, both in puppies and in adult dogs. Studies conducted by R. A. Rieker, of the Bioassay Division, showed first that about 10 mg. of arsanilic acid per kilogram of body weight daily is close to the maximum level which can be tolerated by dogs on continued feeding. Tolerance to 3-nitro-4-hydroxyphenylarsonic acid was clearly much less than that to arsanilic acid in these pilot studies.

In feed-tolerance studies four adult female dogs were fed a dry commercial dog food with 0.01% added arsanilic acid for 77 days. The level of arsanilic acid was then increased to 0.02% and fed for 107 days, still with no significant weight changes. The concentration of arsanilic acid was again doubled, to 0.04% in the feed, and fed for 28 days. Two of the four dogs lost 14 and 24% weight during this period, but the other two maintained weight throughout. All dogs were placed on the dog food alone at the end of this 28-day period. The two dogs which had lost weight on the 0.04% arsanilic acid level rapidly returned to normal weight. No symptoms of toxicity, other than the described weight loss, were apparent over the 7-month trial.

A litter of four puppies, 6 weeks old, was used to determine tolerance to arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid during growth. In this experiment good growth was seen in two of the puppies fed 0.01% arsanilic acid in a dry commercial dog food for 80 days. Doubling the arsanilic acid during the next 53 days did not depress growth rate. However, when the level was again doubled, to 0.04%, a weight plateau developed followed by weight loss and anorexia after 44 days. Following removal of the arsanilic acid, the dogs returned rapidly to normal, showing no evidence of irreparable damage over the next 90 days.

The two litter mate puppies fed 0.005% of 3-nitro-4-hydroxyphenylarsonic aid in a ground, dry commercial dog food for 83 days grew normally with no evidence of toxicity. When the level was doubled, to 0.01%, for the next 53 days growth continued, but at a reduced rate. Doubling the level again, to 0.02%, for the next 14 days led to marked weight loss and complete anorexia. The arsenical was withdrawn from the diet and both dogs gained weight rapidly for 14 days to peaks above those previously attained. Thereafter both dogs unaccountably lost appetite and declined in weight. One dog died after 18 days and 5.5-pound weight loss, the other after 34 days and 3.4-pound weight loss.

It would appear from these data that arsanilic acid is tolerated in growing dogs at least up to 0.02% of the diet, whereas tolerance to 3-nitro-4-hydroxyphenylarsonic acid appears to fall in the range 0.005 to 0.01%.

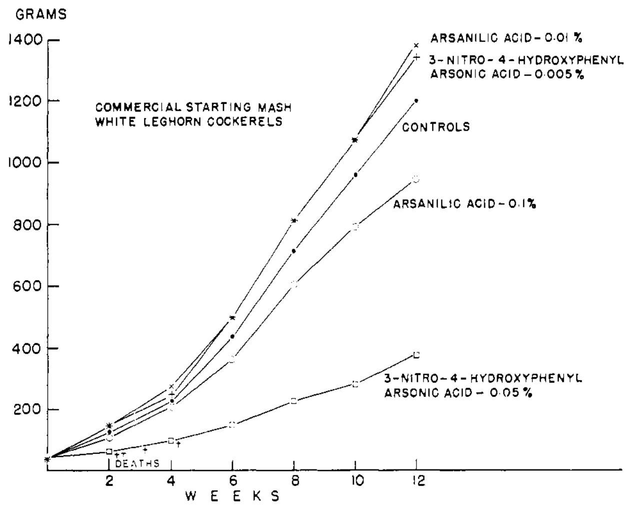


Figure 5. Growth stimulation in White Leghorn cockerels by two arsonic acids at recommended feeding levels

Inhibition at 10 times recommended feeding levels. Diet, standard commercial broiler mash. As obtained, it did not contain an antibiotic or other drug. Individual arsonic acids were thoroughly mixed into 50-pound portions of standard feed in Hobart mixer. Feed and water were offered ad libitum throughout. At 3 weeks of age birds were moved from brooder to battery cages

Table VII. Arsenic Deposition in Chicken Livers at Different Levels of Arsonic Acid Feeding

(Check analyses by two methods)

Feed Supplement	Arsenic Added to Feed, P.P.M. As_2O_3	As_2O_3 in Fresh Liver Samples, P.P.M. Trace Met. Res. Lab.[a], 3 pooled samples	Abbott av.[b] and range of 3
Arsanilic acid			
0.01%	45.5	1.5	1.2 (1.1–1.3)
0.1%	455	7.0	6.4 (4.2–8.4)
3-Nitro-4-hydroxyphenyl-arsonic acid			
0.005%	18.7	1.7	2.4 (2.0–2.8)
0.05%	187	5.0	7.5 (5.8–9.7)

[a] Gutzeit method.
[b] Kingsley-Shaffert method (*38*).

Antidote to Selenium

The use of arsonic acids rather than sodium arsenite to counteract poisoning in farm animals by seleniferous grains offers many fascinating research problems. Initial work in rats by Hendrick, Klug, and Olson (*35*) with selenium at 10 p.p.m. of the diet indicates that arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid both offered partial protection at the levels used. Arsanilic acid proved effective at 0.025% of the diet on the basis of survival, liver protection, and maintenance of growth. The studies were started at dietary levels of the respective arsonic acids which provided 5 p.p.m. of arsenic in the diet, the level previously shown effective for sodium arsenite by Moxon (*53*). The 0.025% level of arsanilic acid contributed 86 p.p.m. of arsenic to the diet, and was the most effective level tested. The data indicate, on the other hand, that 3-nitro-4-hydroxyphenylarsonic acid was more effective than arsanilic acid when both were tested at 0.009% of the diet. This proved true, even though the former contains one fifth less arsenic. The fact that the 3-nitro compound makes for greater deposition of arsenic in tissues may account for this difference. A different metabolic lability of the two compounds and consequent difference in rate of release of arsenic appear as the most reasonable working hypothesis.

Table VIII. Arsenic Deposition in Turkeys on Arsenicals of Different Toxicity

(Check analyses by two methods)

Feed Supplement	Arsenic Added to Feed, P.P.M. As_2O_3	As_2O_3 in Fresh Tissue[a], P.P.M.	
		Liver	Muscle
None	. . .	0.05	0.04
Arsanilic acid, 0.01%	34.5	1.4 (1.0)[b]	0.43 (0.39)[b]
Dodecylamine *p*-chlorophenylarsonate, 0.01%	17.8	2.9 (1.4)	0.57 (0.32)

[a] Evans-Bandemer method (*23*).
[b] Values in parentheses by Gutzeit method (Trace Metals Research Lab).

The ability of arsonic acids to counteract selenium toxicity in chickens on practical-type rations is reported by Carlson, Guenther, Kohlmeyer, and Olson (*11*). In these studies arsanilic acid, 3-nitro-4-hydroxyphenylarsonic acid, and sodium arsenite were reported in both presence and absence of selenium as stimulants to growth. Growth promotion in both instances was greater for the arsonic acids than for inorganic arsenic. Arsanilic acid appeared to counteract selenium and to promote growth up to 0.04% of the diet.

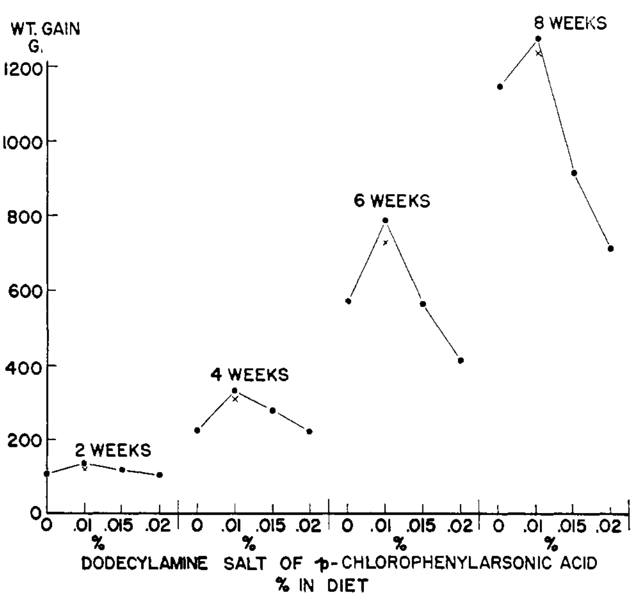
Relation to Other Chemical Additives to Foods

Frazer (*26*) in a recent excellent review of pharmacological aspects of chemicals in foods advances the view that, subject to careful experimental investigation and reasonable control, judicious addition of chemicals to foods is not deleterious to health and is valuable for conservation of the world's food supply. "The therapeutic dose of arsenic is of the order of 5 mg.; the toxic dose about 50 mg.; and a likely fatal dose about 500 mg. An ineffective level of dosage is 0.5 mg., and this is the same order of dosage that may result from the ingestion of arsenic in food within the limits of tolerance."

Arsenicals in feeds present two primary problems from the pharmacological viewpoint: the direct effect on the animals themselves and the long-term effect on populations consuming the tissues of animals fed arsenicals. The first problem is amenable to direct experiment and a great deal of experimental data should emerge rapidly along this line. The second problem can be appraised in part along with the already extensive medicolegal consideration of arsenical sprays. The present practice of limiting recommended feed levels of an arsenical so that no more than 3.5 p.p.m. of arsenic trioxide is found in tissue conforms with the legal limitation for residues on sprayed fruit. The compulsory stipulation requiring removal of the arsonic acid from the feed 5 days before slaughter provides an extra wide margin of safety to the consuming public.

Figure 6. Response of Broad Breasted Bronze male poults, 10 per group, to graded levels of dodecylamine p-chlorophenyl arsonate

Showing increasing inhibition with time at toxic levels. X denotes average weights of group which received 0.01% arsanilic acid as positive control



Our thinking on the use of arsanilic acid in feeds is guided partly by the recent official opinion regarding the safe use of chemical additives in foods. Two booklets (*54*, *55*) prepared by the food protection committee of the Food and Nutrition Board offer guiding principles, first, to safeguard food, and, second, to judge "safety" of chemicals in foods. The problems encountered with pesticides, most important of all food additives, carry over in large measure to the arsonic acids. In each case the additive offers protection against various types of injury to the developing food source. This protection is an aid to food production. Chemical agents which have a desired protective activity and good margins of safety are seen by Oser (*56*) to have a logical place. The potential hazards involved should be fully recognized and described by the manufacturer of the chemical and by the food manufacturer who undertakes the responsibility of using the chemical. Once in use, such compounds should remain under constant surveillance by official control organizations and trade associations. The Association of Official Agricultural Chemists is at present concerned with the development of official methods for the determination of arsonic acids in feeds. These methods provide the best tool for averting trouble at every point.

A simple, accurate, and rapid colorimetric method for the determination of arsanilic acid in feeds has been described (first action) (*3*). Arsanilic acid and sulfaquinoxaline both react to produce color by this test. It has been necessary, therefore, to work toward a method for differential determination of each compound in the same feed.

Prospective Research

Although arsonic acids did not lessen the need for vitamin B_{12} or pantothenic acid in these studies, recent work by Abbott, Bird, and Cravens at Wisconsin indicates that arsanilic acid acts like procaine penicillin in decreasing the need for thiamine in chicks. This finding, together with Shaffner's finding on the similar effect on calcium absorption, points again to the similarity in action of arsonic acids and antibiotics. The possibility that arsonic acids will be found to have a protein-sparing effect similar to that of antibiotics deserves exploration. The early finding that organic arsenicals decrease nitrogen excretion in humans (*27*) clearly supports the rationale for such studies.

Clarification of the inhibitor as well as the oligodynamic effects of arsenicals on various microorganisms invites attention. Either or both actions may explain the effective use of these compounds along certain lines. The fact that the arsenoso derivatives are effective coccidiostats, but ineffective for growth promotion, is a case in point. Effectiveness of arsonic acids at low levels for growth promotion and at higher levels for control of coccidiosis or dysentery almost certainly involves action on entirely different microbial groups. Growth of organisms which favor nutrition of the host may actually be stimulated by low levels of certain arsonic acids, whereas high levels have a clear-cut static effect on harmful parasites. Usually the latter verge on levels which are toxic not only to the parasites, but to the host as well.

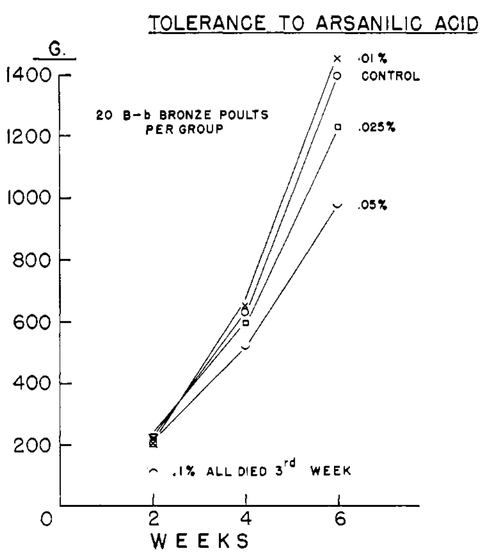


Figure 7. Response of Broad Breasted Bronze poults to various levels of arsanilic acid in diet

It may be hoped that general principles will emerge from studies of the bacteriology of these compounds which will help direct the further experimental work in animals. A prime field for investigation now is the control of certain diseases with strategic low-cost combinations of arsonic acids and antibiotics.

It is apparent from the work to date that metabolism differs for different arsenicals. The level of arsenic deposited in the liver appears to reflect the toxicity of the different compounds. Among the arsenicals studied and in various species, arsanilic acid appears to produce lowest tissue arsenic levels and to be least toxic. Arsanilic acid itself is less toxic than other commonly used medicated feed additives. Much more work is needed to achieve a better understanding of the metabolic fates of arsonic acids, as well as their comparative value for general feed use and for specific disease control.

Table IX. Arsenic Deposition in Turkeys

(6 weeks on diet)

Feed Supplement	As_2O_3 in Fresh Tissue[a], P.P.M. Liver	Muscle
Arsanilic acid		
0.01%	1.98 (1.1)[b]	1.0 (0.55)[b]
0.025%	4.18 (2.0)	1.16 (0.54)
0.05%	5.97 (3.0)	2.36 (0.65)
Arsenosoaniline, 0.0075%	2.57 (1.0)	...

[a] Evans-Bandemer method (*23*).
[b] Values in parentheses by Gutzeit method (Trace Metals Research Lab).

The possible nutrient role of arsenic itself poses one of the most intriguing problems of all. If arsenic in some form is shown to play a role in normal life processes, much of the onus attached to its reputation as a toxic substance will be removed.

The hypothesis that arsonic acids and antibiotics may act to further the incidence of the hemorrhagic syndrome in poultry by decreasing intestinal synthesis of vitamin K was not confirmed by experiments in this laboratory, which will be described in detail elsewhere. In essence, however, the data show that eight times the recommended level of arsanilic acid (720 grams per ton) added to a commercial broiler feed did not influence clotting time of chicks to 6 weeks. Furthermore, 450 grams per ton of arsanilic acid added to a vitamin K–low diet did not prolong clotting time over that of chicks on the basal diet alone. In each case the critically low level of 90 mg. of menadione sodium bisulfite per ton of feed sufficed to maintain normal clotting times. Finally, severe hemorrhage has been observed in large commercial flocks where no arsonic acid was used in the feed.

One may question the idea that sulfa drugs, antibiotics, and arsonic acids act similarly because they are all bacteriostatic agents. There is much evidence to suggest that there is an actual antagonism (vitamin-antivitamin) relationship between vitamin K and sulfa drugs. Sulfonamides at high levels cause a decrease in prothrombin and increase in clotting time of blood in various species. In addition, evidence strongly suggests that this effect of sulfa drugs is one of direct inhibition of liver function. In all experimental work to date vitamin K has protected against the specific effect of sulfa drugs to lower the concentration of prothrombin in the blood.

Conversely, the antibiotics and arsonic acids generally appear to spare nutrient requirements, rather than to increase them. It is difficult therefore to rationalize why vitamin K, the only nutrient clearly involved in this situation, should prove an exception. More work is needed to resolve the contradictory conclusions and the variance in viewpoints which have arisen with regard to the etiology of the hemorrhagic syndrome. As indicated above, the authors' findings fail to show any effect of arsanilic acid on vitamin K need, or on clotting time as such. A borderline content of vitamin K in many feeds, due to entirely different reasons, appears now to provide one plausible rationale for the sudden high incidence of the chick hemorrhagic condition. On the other hand, there is evidence that certain forms of field hemorrhagic disease did not respond to vitamin K.

Summary

Among the organic arsenicals, only the arsonic acids are recognized as growth stimulants for poultry and swine. The arsonic acids differ markedly in tolerance, in coccidiostatic power, and in power to promote growth. In general, toxicity appears to be related to the amount of arsenic deposited in tissue. Different animal species show widely different tolerance to various arsonic acids. Arsanilic acid is tolerated up to 0.1% of the diet for chicks and at least up to 0.02% of the diet for turkeys, swine, and dogs. The best level for calves is probably not above 0.003% of the diet. There was no interference with reproduction in rats over three generations when arsanilic acid was fed at 0.02% of the diet. Tolerance tests should be carried longer than 4 weeks to give a true picture.

Arsonic acids act like antibiotics in certain respects, unlike in others. One dissimilarity appears in the inability of arsonic acids to spare requirement for vitamin B_{12}. On the other hand, some nutrient requirements appear to be decreased by both compounds. Most of the antibiotic–arsonic acid benefits in animal production appear related to effects on the intestinal flora. We now picture the complementary effects of arsonic acids with antibiotics as due to more effective control of harmful organisms, but exact mechanisms are far from clear.

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