# EFFECT OF LINSEED OIL MEAL AND ARSENICALS ON SELENIUM POISONING IN THE RAT[1]

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Two organic arsenicals, arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid, have been found to give partial protection against selenium poisoning in rats (1). Similar results have also been observed in hogs (3). Moxon (3) found that linseed oil meal gave good protection against selenium poisoning in rats and dogs when incorporated into the ration at a level of 25 per cent. This study with rats was undertaken to determine whether or not linseed oil meal incorporated into the ration at lower levels would add to the protective effect of the organic arsenicals, thus giving more complete protection. Since the organic arsenicals are now being used as growth stimulators for poultry and, to some extent, for hogs, their inclusion in hog rations along with linseed oil meal at levels normally used for protein supplementation appeared to give promise as a practical control measure for selenium poisoning.

## EXPERIMENTAL

Sixteen groups of 6 male albino Sprague-Dawley rats were used in this study. They were housed in individual cages and allowed feed and water ad libitum. The rats were placed on experiment when they averaged about 80 grams in weight, and were fed for five weeks. Rats that died during the experiment were autopsied and liver size and damage was determined. At five weeks, all rats in certain groups were killed and their livers were weighed, observed for damage, and immediately placed in a wet digestion mixture used for selenium determination in the method of Klein (4). In order to provide enough material for analysis, three livers were combined for each determination.

The basal ration used in these studies is shown in Table I.

**TABLE I**
**BASAL RATION**

| | |
|---|---|
| Corn | 79.9% |
| Casein (vitamin free) | 12.0 |
| Brewers yeast | 2.0 |
| Salts[1] | 3.0 |
| Animal Protein Factor | 0.1 |
| Wesson Oil | 3.0 |
| Fish Liver Oil | Orally, once a week |

[1]Phillips and Hart, Salts IV (5)

[1]Approved for publication by the Director of the South Dakota Agricultural Experiment Station as paper **318** of the Journal Series.



Selenium was added to the various rations by sprinkling a solution of sodium selenite over the ration which had been spread thin, allowing it to dry, and mixing well. All rations to which selenite was added contained 10 parts per million of selenium. The arsenicals were added in a similar manner. Sodium para-aminophenylarsonate (Abbott Laboratories) was added, in those rations where it was used, to give a concentration equal to 0.01 per cent of arsanilic acid, while 3-nitro-4-hydroxyphenylarsonic acid (Dr. Salsbury's Laboratories), where it was used, was added to give a concentration of 0.005 per cent.

In the rations where linseed oil meal (LOM) was used, it was added at the expense of corn. The product used was an unheated linseed oil meal which had been hexane extracted. No attempt was made to equalize protein contents of the rations.

## RESULTS AND DISCUSSION

The description of Groups and the results of the study are shown in Table II. The data for groups I through IV show that the substitution of LOM for corn decreases the growth of the rats to some extent. One rat on the 20 per cent LOM ration (Group IV) began to lose weight after two weeks on experiment, became edematous, and died. It is not known if this was the result of the high level of LOM in the diet or not. One rat in Group XI also died with edema and without any other apparent symptoms of selenium poisoning. In this case the edema may have been the result of selenium damage, but a LOM effect is not ruled out.

The rats on the seleniferous ration (Group V) exhibited a slow rate of gain, a high mortality rate, and extensive liver damage. The inclusion of 5 per cent LOM (Group VI) had no beneficial effect, since rate of gain in weight and liver damage was about the same and mortality was increased. The inclusion of 10 per cent LOM (Group VII) in the ration gave a slightly increased rate of gain over the rats in Group V, but otherwise no protective effect was indicated. However, at the 20 per cent level (Group VIII) LOM gave excellent protection against liver damage and death, and also a fairly good rate of gain in weight.

Arsenicals alone (Groups IX and XIII) gave partial protection against selenium poisoning, as evidenced by fairly good growth and liver size responses and only mild liver damage. This is in agreement with previous findings (1). Supplementation of the arsenical diets with LOM in various amounts showed that high levels (10-20 per cent) are essential to enhancing protection over arsenicals alone. Even at the 20 per cent level, only a minor and not statistically significant increase in growth was obtained. Since the livers were not examined in animals receiving the 5 and 10 per cent levels, it is not known whether levels of LOM which give no liver protection when used alone (5 and 10 per cent) would give added protection in the presence of arsenicals.

In view of the findings just discussed, it appears that LOM, either in the presence or absence of arsenicals, may not prove a practical help in

**TABLE II**
**DESCRIPTION OF GROUPS AND SUMMARY OF RESULTS**

| Group | Addition to basal ration: Se p.p.m. | LOM % | Arsanilic acid % | 3-nitro-hydroxy-phenylarsonic acid % | Average initial weight Grams | Average final weight Grams | Number Of Survivors[4] | Liver conditions[2] | | Se content of liver p.p.m. |
|---|---|---|---|---|---|---|---|---|---|---|
| I | -- | 0 | --- | ----- | 80 | 307 | 6 | | | |
| II | -- | 5 | --- | ----- | 80 | 289 | 6 | | | |
| III | -- | 10 | --- | ----- | 80 | 286 | 6 | | | |
| IV | -- | 20 | --- | ----- | 81 | 294 | 5 | | | |
| V | 10 | 0 | --- | ----- | 81 | 197 | 3 | +++ | (3.8) | 1.87[3] |
| VI | 10 | 5 | --- | ----- | 80 | 199 | 1 | +++ | (4.0) | |
| VII | 10 | 10 | --- | ----- | 81 | 214 | 3 | +++ | (3.8) | |
| VIII | 10 | 20 | --- | ----- | 81 | 260 | 6 | ----- | (4.9) | 2.48 |
| IX | 10 | 0 | 0.01 | ----- | 81 | 253[4] | 6 | + | (4.8)[4] | 2.94 |
| X | 10 | 5 | 0.01 | ----- | 81 | 243 | 6 | | | |
| XI | 10 | 10 | 0.01 | ----- | 81 | 278 | 5 | | | |
| XII | 10 | 20 | 0.01 | ----- | 80 | 275[4] | 6 | ----- | (5.3)[4] | 2.91 |
| XIII | 10 | 0 | --- | 0.005 | 80 | 261[4] | 6 | + | (4.8)[4] | 2.91 |
| XIV | 10 | 5 | --- | 0.005 | 81 | 263 | 6 | | | |
| XV | 10 | 10 | --- | 0.005 | 81 | 259 | 6 | | | |
| XVI | 10 | 20 | --- | 0.005 | 81 | 276[4] | 6 | ----- | (5.3)[4] | 3.33 |

[1]Six rats were started on experiment in each group.
[2]Figures in parentheses represent the average of the ratios of liver weight in grams to 100 grams of body weight. The degree of liver damage as determined by observation is also indicated as follows:
+++ Severe atrophy, necrosis and cirrhosis. + Slightly cirrhotic in some rats. ----- No obvious damage.
[3]Analysis on livers of 3 survivors only.
[4]By statistical analysis (6):
Weight difference between Groups IX and XII was not significant at the 5% level.
Weight difference between Groups XIII and XVI was not significant at the 5% level.
Difference between ratios of liver wt./100 g. body weight for Groups IX x XII was significant at the 1% level; for Groups XIII and XVI was not significant at the 5% level.



controlling selenium poisoning in livestock because of the high level at which it must be used. However, the data for groups IX, XIII and XII, XVI show quite clearly that the effects of the arsenicals and LOM are not antagonistic; should the LOM protective factor be identified this may become important.

Halverson **et al.** (7) attempted to compare the hull and embryo of flax seed as to their protective effects against selenium poisoning in rats. They used 15 per cent of fat-free hull or embryo, and although the embryo seemed to protect best, the results were variable. The data here may explain the variability. Since at a 10 per cent LOM level protection was essentially lacking, while at 20 per cent it was good, a level of 15 per cent could be expected to be perhaps too low for consistent protection.

Klug **et al.** (8) found that the livers of rats on a seleniferous diet were as high in selenium when the rats were protected against selenium toxicity with sodium arsenite as when they were not. They concluded that the arsenic did not protect by preventing deposition of the selenium in the tissues. The results of liver selenium analysis in this study indicate that the same is true, for this particular organ at least, in the case of organic arsenicals and LOM. In fact, protection resulted in an increased selenium content.

## SUMMARY

Linseed oil meal (LOM) included in the ration at a level of 20 per cent gave good, although not complete, protection in rats against chronic selenium poisoning by 10 p.p.m. of selenium as the selenite. LOM at 5 and 10 per cent levels did not.

Both arsenilic acid and 3-nitro-4-hydroxyphenylarsonic acid gave good, although not complete, protection against the same level of selenium. No increased protection was afforded by adding 5 per cent of LOM to the arsenical fortified rations. LOM at 20 per cent gave some additional response with both arsenicals. At the 10 per cent level LOM did appear to further protect the arsonilic acid-fed group but not those fed the 3-nitro-4-hydroxyphenylarsonic acid.

Neither the organic arsenicals, the LOM, or mixtures of the two caused any reduction of selenium content of the livers.

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