# COMMUNICATION

# SOYBEANS

# Failure of Antibiotics to Stimulate the Growth of Rats Fed Digested Raw Soybean Meal

Dietary antibiotics counteract the growth depression resulting from feeding raw soybeans to rats. The hypothesis is presented that the antibiotics exert a beneficial effect by suppressing bacterial protease production in the intestinal tract, the bacterial proteases being active in

Antibiotic supplementation stimulates the growth of rats fed a raw soybean meal ration, so that their growth rate is comparable to animals fed a heated soybean meal ration (Borchers et al., 1957); without antibiotics, growth is poor on the raw soybean ration. The antibiotic has been postulated to exert its effect by preventing the liberation of the soybean growth inhibitor from the raw soybean meal (Borchers, 1965). On the other hand, papain digestion of raw soybean meal prior to feeding results in growth rates less than on the original raw meal (Borchers, 1963). This latter effect has been postulated to result from a greater liberation of the soybean growth inhibitor from a bound form in the raw meal by pretreatment with proteolytic enzyme. If the proteolytic treatment of raw soybean meal liberates the soybean growth inhibitor, antibiotic supplementation of the digested raw soybean meal rations should have no effect. The following report describes a test of such a hypothesis.

#### Experimental

Raw soybean meal was obtained from a commercial source and was prepared by solvent extraction with a minimum of heat treatment according to the manufacturer's statement. This raw meal was digested by suspending 250 grams of meal in 2.5 liters of water at liberating the soybean growth inhibitor from a bound form present in the raw soybeans. When the raw soybean was digested prior to feeding, the dietary antibiotics had no beneficial effect, since the soybean growth inhibitor had already been liberated by papain digestion.

37° C. plus 12.5 grams of crude papain or 0.5 gram of crystalline subtilisin and incubating at 37° for 16 hours with gentle stirring. The digest was dried in an air blast below 50° and incorporated into rations prepared as described (Borchers et al., 1957). The rations were fed to weanling rats of the Holtzman strain for a 20-day period. Each feeding experiment consisted of four females and four males fed the heated, but not digested, soybean ration and a similar group paired as to litter, sex, and initial weight and fed the raw or digested raw soybean meal ration. The animals were housed at  $30^{\circ}$  C. with free access to food and water.

# Results

To illustrate the usual growth depression of raw soybean meal and the beneficial effect of dietary antibiotics, experiments 1 and 2 are presented in Table I. Experiment 3 shows the detrimental effect of papain digestion on the growth rate when compared with untreated raw soybean in experiment 1. However, when an antibiotic supplement was added to a papain-digested raw soybean meal ration (experiment 4) no significant improvement was noted as in experiment 2. This is in keeping with the hypothesis that the soybean growth inhibitor had already been liberated by the proteolytic action

Table I. Growth of Weanling Rats Fed Digested Soybean Meal with or without Antibiotic Supplement

	Enzyme <sup>e</sup> for Digestion	Antibiotic <sup>b</sup> Supplement	Growth		
Expt. No.			Control,° g./day ±SE	Exptl., g./day ±SE	Expt./control,
1	None	None	$4.71 \pm 0.21$	$3.83\pm0.17$	81
2	None	Added	$4.40 \pm 0.21$	$4.22 \pm 0.22$	96
3	Papain	None	$3.99 \pm 0.19$	$2.46 \pm 0.28$	62
4	Papain	Added	$4.02 \pm 0.19$	$2.71~\pm~0.19$	67
5	Subtilisin	None	$4.25\pm0.16$	$0.04 \pm 0.08$	1
6	Subtilisin	Added	$4.63 \pm 0.22$	$0.39\pm0.27$	8

<sup>a</sup> Enzymes used for digestion were crystalline subtilisin or crude papain. See text for details of preparation of digested soybean meal.
<sup>b</sup> Antibiotic supplement was 0.1% of procaine penicillin plus 0.1% of streptomycin sulfate.
<sup>c</sup> Control ration contained soybean meal autoclaved at 15-lb. steam pressure for 20 minutes but not digested.

and, hence, antibiotics could not prevent such liberation.

To illustrate further the effect of digestion of raw soybean meal on growth depression, experiment 5 of Table I reports the results of feeding subtilisin-digested raw soybean meal. With this preparation, growth was completely suppressed; the rats merely maintained their initial weight. However, all animals survived the feeding period. These results illustrate the greater effectiveness of a bacterial protease in liberating the soybean growth inhibitor over that of papain (experiment 3). Again, an antibiotic supplement was ineffective when added to the subtilisin-digested raw soybean meal (experiment 6) as was the case when added to the papaindigested soybean meal in experiment 4.

### Discussion

In a recent report, Goldberg and Guggenheim (1964) expressed the view that antibiotics exerted a beneficial effect in raw soybean-fed animals via a direct action of the antibiotics on the pancreas. Barnes et al. (1965) found that the beneficial effect of dietary penicillin with raw soybean rations was abolished when coprophagy was completely prevented. This observation suggests that some constituent of the fecal material was involved in the nutritional interaction of raw soybeans with antibiotics. More recently, Barnes and Kwong (1965) have presented a rather involved hypothesis for the antibiotic action. These authors feel that the antibiotic reduced bacterial proteases in the intestinal tract, thereby reducing the proteolytic digestion of pancreatic secretions so that more cystine reaches the large intestine. Since more cystine would be lost by this means, it is difficult to see how the dietary antibiotic exerts a beneficial effect.

The hypothesis that the antibiotics reduced bacterial proteases in the intestinal tract and, thereby, prevented the liberation of the soybean growth inhibitor from a bound form has the advantages of simplicity and consistency with the experimental data—that is, greater growth depression after proteolytic digestion and failure of antibiotics to exert an effect after proteolytic digestion.

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