

ANTIBIOTICS AND NUTRITION

Effect of Dietary Aureomycin and Different Levels of Protein on Several Phosphorus and Nitrogen Compounds in Hams

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A study has been made of the effect of three levels of protein (14.3, 17.6, and 20.9%) with and without Aureomycin, in the ration of swine, on the concentration of phosphorus (acid-soluble, lipid, nucleic acid, and phosphoprotein), nitrogen (protein, ammoniacal, nucleic acid, and phosphoprotein), and total solids in the hams. Statistical analysis of the data showed no significant differences in the acid-soluble and nucleic acid phosphorus, ammoniacal and nucleic acid nitrogen, and total solids values obtained for the six dietary groups. The lipid and phosphoprotein phosphorus and the protein and phosphoprotein nitrogen showed variances between the dietary groups at the 0.05% level of significance. The lipid phosphorus variation was due to interaction of the protein and the Aureomycin. The antibiotic increased the phosphoprotein phosphorus in the intermediate and high protein rations, but had no effect in the low protein ration. The higher levels of dietary protein resulted in an increase of the protein and phosphoprotein nitrogen, but the Aureomycin had essentially no influence on these two components of the hams.

THE WIDE USE OF ANTIBIOTICS suggests the need for studies of the chemistry of tissues from animals to provide data for comparison of the action of different antibiotics on different species and rations. Wallace *et al.* (5) studied the influence of Aureomycin on the protein requirement and carcass characteristics of swine fed three levels of protein, with and without the antibiotic. They found that weanling pigs fed 14.3% crude protein in dry lot with Aureomycin supplement made as effective weight gains as those fed levels of 17.6 and 20.9% protein, with and without, added Aureomycin. The dressing percentages averaged slightly higher for pigs that received the Aureomycin, and were significantly greater on the low and intermediate protein rations than on the high protein ration. The object of the present investigation was to evaluate the influence of the above rations on several phosphorus and nitrogen compounds in the hams from these swine. The literature does not reveal any such study of swine, although Mirone (2) reported on the influence of Aureomycin on the growth, moisture, fat, and nitrogen content of mice. Also, the present study presents values for the concentration of acid-soluble, lipid, nucleic acid, and phosphoprotein phosphorus, and ammoniacal, nucleic acid, and phosphoprotein nitrogen in hams which apparently have not previously been reported.

Experimental

Swine and Rations

The hams were obtained from the swine as reported by Wallace *et al.* (5). The swine, 42 purebred Duroc and 12 crossbred Duroc X Hampshire weanling gilts and barrows, were divided into six groups according to breed, litter, weight, and sex. All groups received 5% alfalfa meal, 0.5% bone meal, 1.0% limestone, 0.53% salt-trace mineral mix, and added B complex vitamins in their rations. Two groups were started on each of 14.3, 17.6, and 20.9% levels of corn-soybean protein. These levels will be referred to as low, intermediate, and high protein rations, respectively. One group of pigs from each of the protein levels was given 20 grams of Aureomycin per 100 pounds of feed. The levels of ground yellow corn and expeller soybean oil meal in the different protein rations were as follows:

Protein Level	Soybean Oil Meal		Protein, %
	Corn, %	%	
...	86	7	11.7
Low	78	15	14.3
Intermediate	69	24	17.6
High	61	32	20.9

The various groups averaged slightly more than 40 pounds when placed on the rations. When the average weight

reached 100 pounds, each lot was changed to the ration having the next lower concentration of protein. The low protein group was started on the 11.7% ration at this time.

Preparation Of Samples

When the swine were sacrificed, all groups had an average weight of 191 ± 4 pounds. The meat was removed from the bone of the hams, the skin discarded, and the remainder passed through a meat grinder for three times, with hand mixing between grinding to ensure homogeneity. Then, the ground mixture was stored at 32° to 33° F. until analyzed. The hams of seven swine from each of the six dietary groups were analyzed for the various chemical components.

Chemical Procedures

In order to separate the acid-soluble, phospholipid, nucleic acid, and phosphoprotein phosphorus, the procedure of Schneider (3) was employed. Phosphorus was determined directly on the acid extracts. The lipid, nucleic acid, and phosphoprotein phosphorus extracts were dried with magnesium nitrate (7), ashed at 600° C., and then, phosphorus was determined by the colorimetric phosphomolybdate procedure on dilute acid solutions of the ash. The nucleic acids were not separated into ribose and desoxyribonucleic acids, nor were pentose evaluations made; only the phosphorus was determined in the nucleic acid extract. Aliquots of nu-

Table I. Concentrations of Several Phosphorus and Nitrogen Compounds and Total Solids of Hams

(Swine fed varying levels of protein, with and without Aureomycin)

Analysis	Dietary Groups						Av. std. dev. ^a
	Low Protein		Intermediate Protein		High Protein		
	Without Aureomycin	With Aureomycin	Without Aureomycin	With Aureomycin	Without Aureomycin	With Aureomycin	
Milligrams per 100 grams of wet weight							
Acid-soluble P	73.7	78.7	81.3	85.3	79.3	79.6	14.40
Lipid P	13.7	13.6	11.9	15.9	14.6	13.7	2.62
Nucleic acid P	5.67	5.31	4.16	5.56	5.40	4.59	1.56
Phosphoprotein P	11.8	11.8	6.3	15.1	8.30	9.30	4.38
Per cent of wet weight							
Protein	13.6	13.3	12.6	13.8	14.8	14.4	1.30
Nucleic acid N	0.37	0.39	0.37	0.37	0.34	0.29	0.14
Phosphoprotein N	1.33	1.05	1.27	1.33	1.41	1.55	0.20
Ammonia N	0.021	0.016	0.021	0.020	0.020	0.027	0.005
Total solids	52.3	55.0	51.5	49.9	47.7	50.9	4.92

^a Average of standard deviations obtained in analysis of each dietary group.

cleic acid and phosphoprotein fractions were determined for the nitrogen present by the Kjeldahl method (7). Total protein and ammoniacal nitrogen were also determined by recommended Kjeldahl procedures (7). Total solids were evaluated by heating samples in a vacuum oven at 60° C. overnight or to constant weight (7).

Results and Discussion

The concentrations of the various chemical components of the hams are shown in Table I, and a summary of the analysis of variance on these data is presented in Table II. The variance calculations were made according to Snedecor's recommendations (4) for equal subclass members with two or more criteria of classification. The acid-soluble, lipid, nucleic acid and phosphoprotein phosphorus values are expressed as milligrams of phosphorus per 100 grams of wet weight; whereas, the nitrogen compounds and total solids are expressed as percentages of the wet weight.

The acid-soluble, lipid, nucleic acid, and phosphoprotein phosphorus made up approximately 73.1, 12.7, 4.7, and 9.5% of the total phosphorus, respectively. No significant dietary influence on the acid-soluble and nucleic acid phosphorus values was observed. The lipid

phosphorus was not influenced by either protein level or Aureomycin alone, but an interaction of these two dietary components showed a variance at the 0.05% level of significance. Variances were significant at the 0.05% level in the case of the phosphoprotein phosphorus for both the dietary groups and the Aureomycin in the ration; the Aureomycin was effective in increasing the values only with the intermediate and high protein groups.

The percentage of protein in the hams ranged from 12.6 for the intermediate protein group without Aureomycin to 14.8% for the high protein group without Aureomycin. Statistically, the variances were significant at the 0.05% level for both dietary groups and the amount of protein in the ration. No effect of the Aureomycin was observed. The higher concentrations of protein in the hams of the swine receiving the high protein rations suggests that either the animals were able to store the dietary protein as such in the hams or that the high dietary level has a sparing action on the body stores of this component. Phosphoprotein nitrogen tended to parallel the corresponding protein concentrations found in the hams; the highest values were found in the hams of those dietary groups receiving the most protein in the ration. Statistically, the variance was significant at

the 0.05% level for both dietary groups and for level of dietary protein.

The range of nucleic acid nitrogen was from 0.29% in the group receiving high protein plus Aureomycin to 0.39% in the corresponding group receiving the low protein diet. These differences were not significant. The high nitrogen compared to the phosphorus concentration in the nucleic acid fractions is probably due to the presence of nitrogenous compounds other than nucleic acids in this extract. Schneider (3) found that nucleic acid extracts from rat livers contained a greater proportion of nitrogen to phosphorus than expected, and suggested that it was due to a greater amount of nitrogen's being present in nucleic acids than postulated, or that other compounds were also present. Schneider (3) also obtained higher nucleic acid phosphorus values and lower nitrogen values in rat livers than the corresponding values found in the present investigation with swine hams.

Ammonia nitrogen was determined in hams of only three swine from each of the six groups, and values ranged from 0.016 to 0.027% between the low protein dietary group receiving Aureomycin and the high protein group receiving Aureomycin. These differences were not significant.

Values for total solids ranged from

Table II. Summary of Analysis of Variance

Source	D.F.	Acid-soluble P M.S.	Lipid P M.S.	Nucleic Acid P M.S.	Phosphoprotein P M.S.	Protein M.S.	Nucleic Acid N M.S.	Phosphoprotein N M.S.	Ammonia N (10 ⁻⁴) M.S.	Total Solids M.S.
Groups	5	99.7	12.5	2.52	68.6 ^a	4.32 ^a	0.010	0.194 ^a	0.37	41.5
Protein	2	176.9	0.85	1.57	32.1	8.03 ^a	0.020	0.300 ^a	0.37	69.4
Aureomycin	1	102.5	10.0	0.06	111.4 ^a	0.16	0.001	0.010	0.01	20.3
Prot. X Aureo	2	21.1	25.5 ^a	4.70	83.7	2.69	0.005	0.180	0.55	24.3
Within ^b	36	137.6	7.63	2.67	26.6	1.71	0.023	0.072	0.26	25.3

^a Denotes significance at the 0.05% level.

^b The within degrees of freedom were 19 less for the ammonia nitrogen values.

47.7% in the high protein dietary lot to 55.0% in the low protein group receiving Aureomycin. From these data the high-protein dietary levels appear to decrease the total solids content of hams; however, the variance mean squares were not significant, but appreciable. The observation that the hams having the greatest protein content also have the greatest water (volatile matter) content is in agreement with the knowledge that muscle contains more water than nonmuscular tissues, such as fat.

Conclusions

The data present concentrations of several phosphorus and nitrogen compounds in hams that apparently have not previously been reported. The values obtained show that an appreciable range of protein in the ration, with or without Aureomycin, does not affect either the concentrations of acid soluble and nucleic acid phosphorus, or nucleic

acid and ammoniacal nitrogen and total solids, at statistical levels of significance. The interaction effect of protein and Aureomycin on the lipid phosphorus may be associated with the fact that the swine receiving the antibiotic had greater back fat thickness (5). The dietary protein and Aureomycin effects on the phosphoprotein phosphorus and nitrogen suggest that phosphoprotein metabolism is a determining factor in the amount of total protein found in the hams. In general, the level of dietary protein as indicated by the data has a greater influence on nitrogen compounds than the antibiotic.

Acknowledgment

The writers wish to thank the Lederle Laboratories for supplying the Aureomycin and B complex vitamins used in the rations, and the U.S. Public Health Commission for the grant-in-aid financial assistance during the study.

The authors also wish to acknowledge the technical assistance in this study of Mike Milicevic, Dave O'Connor, Robert Johnson, and Jon Herring.

Literature Cited

- (1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 7th ed., 1950.
- (2) Mirone, Lenora., *J. Agr. Food Chem.*, **1**, 519 (1953).
- (3) Schneider, Walter C., *J. Biol. Chem.*, **161**, 293 (1945).
- (4) Snedecor, George W., "Statistical Methods Applied to Experiments in Agriculture and Biology," Ames, Iowa, Collegiate Press, Inc., 1946.
- (5) Wallace, H. D., Milicevic, M., Pearson, A. M., Cunha, T. J., and Koger, M., *J. Animal Sci.*, **13**, 177 (1954).

Received for review April 29, 1954. Accepted July 22, 1954. Authorized for publication as Paper 270, journal series, Florida Agricultural Experiment Station.

ATMOSPHERIC FLUORINE

Fluorine Acquired by Forage Cultures in Outdoor And Washed Atmospheres at Columbia, Tenn.

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Multiple pot cultures of red clover and of rye grass were grown 6 weeks at Knoxville, without any additive, in normal atmosphere on soil low in fluorine content, and then were transported to Columbia, where the atmosphere was purported to have higher occurrences of fluorides. After 21 days, the fluorine contents of the plants grown outdoors and in the washed atmosphere of an adjacent closed chamber were compared to the fluorine content of check plants that were retained at Knoxville. Sudan grass cultures were used in similar comparisons in two subsequent experiments. In all three experiments the transported plants acquired substantial increases in fluorine contents during their growth outdoors at Columbia, in comparisons with the check plants that were kept outdoors at Knoxville. The removal of the fluorine content of the Columbia atmosphere was reflected in the relatively low content of fluorine in the transported plants that were grown in the washed atmosphere of the nearby chamber. When forage cultures were grown in an atmosphere contaminated with ionized fluorides, the plants acquired more fluorine than they acquired in the washed atmosphere and the amounts derived from the atmosphere exceeded the uptakes of fluorine from the soil. However, the results do not demonstrate that the increases in fluorine content acquired by the plants grown outdoors were due solely to ionized fluorides.

FLUORINE OCCURRENCES IN THE atmosphere are minute at points distant from industrial operations. Hence, under normal atmospheric conditions, field crops derive their fluorine content from the mineral fluorides that occur discretely in most soils, and according to soil pH

and abilities of particular plant roots to effect uptake of fluorine. However, abnormal occurrences of fluorine may occur as particulates in localities where rock phosphate is mined and processed, and as gaseous phases in those locales where fluorine emissions come from phos-

phate, phosphorus, and other manufacturing operations (1, 3). Such occurrences have been determined through 3-year analyses of a succession of replicated exposures of Spanish moss at different points in Tennessee (5).

Of particular concern are contentions