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Account of Dr. Lyman's Research on Gossypol and Its Significance and Impact on the Cottonseed Industry[1]

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Abstract

Carl M. Lyman and his associates made highly significant contributions to the development of basic and applied knowledge of gossypol and gossypol-like pigments and their relationship to the utilization of cottonseed protein. Included among their studies were investigations of physiological activity, effect upon protein quality for monogastric animal nutrition, elimination and inactivation, biosynthesis of gossypol in the cotton root, isolation and characterization of a previously unidentified gossypol-like compound, and ration formulation procedures for efficient feeding of both glanded and glandless cottonseed protein.

Introduction

The opportunity to present this paper carries with it both a privileged honor and a sense of deep personal emotional involvement. Carl M. Lyman was one of the most capable and dedicated scientists I have known. As a man, his personal and scientific principles were beyond question, his love and concern for people were great, and his understanding and leadership were superb. His brilliance of mind and his conviction that his science was worthwhile only as it contributed to the welfare of others were contagious and became a part of those who knew him. The advances he achieved in the development of scientific knowledge shall always be prized by the scientific community; the contributions he made to the welfare of man shall be beneficial long beyond personal memories because they have become living, growing entities.

The cottonseed industry has cause to be especially grateful to Dr. Lyman because he chose to work with it, understood its nature, and believed in its worth and the value of its products. To it he was always a friend, a ready counselor and an inspiring leader.

This Memorial Symposium relates to gossypol, a problem of the cottonseed industry. Dr. Lyman was one of many who contributed toward the solution of that problem. Because of his achievements, and those of others, gossypol need no longer be a severe problem to the cottonseed industry and those it attempts to serve.

Review

It would be unwise to attempt, in this brief paper, a complete review of Carl Lyman's work with gossypol. Rather, the paper emphasizes the significance of his studies of (a) the physiological activity of gossypol and gossypol-like compounds, (b) the relationship of gossypol to protein quality, (c) analytical procedures, (d) the solution of gossypol limitations, and (e) fundamental investigations which become the foundation for applied studies. Finally, it discusses his participation in the application of his research.

Early in his gossypol research Dr. Lyman found the need to measure more precisely the physiologically active gossypol in cottonseed meal, and he published a colorimetric method in 1943 (1).

Because unbound gossypol is physiologically active when fed to monogastric animals, Dr. Lyman and his associates sought to determine the tolerance levels of various species and ages of these animals. Couch et al. (2) fed New Hampshire chicks 0.02% to 0.25% free gossypol from isolated pigment glands in a semi-purified diet containing 50% of a low free gossypol cottonseed meal for six weeks. Mortality, gain rate and feed efficiency were not affected at dietary free gossypol levels as high as 0.06%. When DL-lysine

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monohydrochloride was added at a level of 1.0% of the diet, growth rate was improved, but mortality at the higher free gossypol levels was not affected. Hale and Lyman (3) reported that pigs fed a 15% protein ration composed of sorghum grain, cottonseed meal and soybean meal did not exhibit toxicity with dietary levels of free gossypol as high as 0.01%, and, at low free gossypol levels, rate of gain was equal on the half cottonseed meal-half soybean meal ration to that of the ration containing all soybean meal. At free gossypol levels of 0.015% or more toxicity symptoms developed when the ration protein level was restricted to 15%. However, when the protein level was increased to 30% no toxicity occurred even when the dietary free gossypol level was 0.03%. Thus, it was demonstrated that the dietary tolerance level for growing and fattening swine is in excess of 0.01%.

Lyman and Hale (4) demonstrated that with a dietary level of 0.02% free gossypol high rate of gain and freedom from toxicity were maintained by including 400 ppm of iron salts in the ration. These studies have contributed to the increased use of high free gossypol solvent extracted cottonseed meals in swine rations without danger of toxicity.

Much of Lyman's work was directed toward bound or total gossypol content of cottonseed meal fed monogastric animals (5–7). He and his associates reported (8) that in a group of prepressed solvent extracted cottonseed meals there was a negative correlation coefficient of 0.899 between chick growth rate and total gossypol. In a study (6) of free lysine ϵ-amino groups (EAF lysine) he and his associates observed that the reduction in these free amino groups by reaction with carbohydrate does not have quantitatively the same effect in reducing protein quality as that caused by reaction with gossypol. Lyman et al. (5) found that when gossypol was reacted with purified cottonseed protein, in vitro digestion with either trypsin or pepsin was poor while formation of a protein-carbohydrate complex reduced amino nitrogen liberation by trypsin but not by pepsin. Baliga and Lyman (7) selected a cottonseed meal containing the high level of 1.32% total gossypol and treated it with aniline, acetone and ethyl ether to reduce the total gossypol to 0.49%. This treatment increased nitrogen solubility from 61.0% to 77.0%, lysine availability (microbiological assay of feed and feces of rats) from 54.9% to 70.4%, rat protein-repletion test gain from 25.8 to 45.8 g, and four-week chick gains from 121.7 to 222.4 g. They concluded that the results of the study are in accord with the concept that inactivation of gossypol during processing is accomplished by formation of an insoluble, inert gossypol-protein complex which results not only in rendering the gossypol harmless but also in loss of a part of the protein. Lyman hypothesized that free lysine failed to prevent physiological activity of free gossypol following ingestion because the complex of gossypol-free lysine was soluble in contrast to the insolubility of the gossypol-protein complex. The significance of the loss incurred by binding gossypol to protein was further elucidated by Lyman et al. (5). Reactions of gossypol with purified cottonseed protein to produce a total gossypol content of 2.0% reduced lysine with free ϵ-amino groups by more than 28%. The ratio of moles of bound lysine to moles of bound gossypol was 1:1.49. This molar relationship remained almost constant with bound gossypol levels of 2.0%, 2.5% and 3.5%. Reaction of gossypol with bovine plasma albumin proceeded to levels as high as 11.4% under the same conditions. The authors suggested that the lower order of reaction with purified cottonseed protein might be caused by the complex becoming insoluble and precipitating after a small amount of gossypol had reacted with the protein. This appears to be in agreement with our observations in a commercial mill in which glanded meal containing about 1.0% total gossypol showed a reduction of more than 13% in EAF lysine from the value of glandless meal processed under comparable conditions.

The paper of Cater and Lyman, of this Symposium, further clarifies the gossypol-protein reaction.

We can conclude from the observations of Lyman and others that significant improvement in the usefulness of cottonseed meal may be achieved by the type of seed and processing which reduced total gossypol content and maintains high levels of available lysine. This principle became almost a crusade on the part of Dr. Lyman and drastically affected the activities of the cottonseed industry in the development of glandless cottonseed and the processing of meal with high protein availability for poultry and swine.

However, Dr. Lyman recognized the value of and worked toward the practical use of cottonseed meals as commonly produced from glanded cottonseed. Hale and Lyman (9) fed 42-pound pigs a sorghum grain based ration containing either a very low protein quality cottonseed meal (18.2% nitrogen solubility and 1.15% total gossypol) or a medium quality cottonseed meal (57.9% nitrogen solubility and 0.84% total gossypol). The low quality meal and the medium quality meal supported daily gains of 0.34 and 1.07, and feed efficiency of 0.170 and 0.270 of a pound of gain per pound of feed, respectively. When 0.31% L-lysine was added gains were equal at 1.66 and 1.61 lb. daily, respectively, as were feed efficiencies at 0.291 and 0.286. Thus, they provided the foundation for the development of practical, nutritionally balanced rations using all types of cottonseed meals as a part of the ration.

Dr. Lyman contributed materially to the development of analytical procedures for chemical determination of protein quality in cottonseed meal to be fed to monogastric animals. Lyman et al. (8) proposed solubility of nitrogen in 0.02 N NaOH as a guide to the availability of protein. Lyman and co-workers (10) studied the application of this procedure to a large number of commercially processed prepress solvent meals and found a positive correlation coefficient of 0.829 between the chemical index and chick growth rate values. The method has been widely used commercially and has provided a part of the foundation for increased use of cottonseed meal in poultry and swine feeds. Like other workers, Dr. Lyman sought a definitive method for the measurement of protein availability based upon unreacted lysine. Procedures based on free ϵ-amino lysine groups (EAF lysine) were published by him and his co-workers (6,11) and he continued to work toward improvement of the procedures until his death. EAF lysine is generally considered a more precise measurement of protein availability and has been used extensively in research investigation. However, it has not been widely used commercially for quality control because the complex laboratory procedure is more costly than is nitrogen solubility which is generally acceptable for such use.

While free gossypol determination has been generally accepted as being representative of physiologically active gossypol, most workers have recognized that it is an arbitrary determination and observations have been made that the free gossypol in cottonseed meal does not have the same order of toxicity as does

the pigments determined as gossypol in pigment glands. This, in part, stimulated Dr. Lyman and his associates to investigate other pigments contained in the glands, resulting in the isolation of a compound they named gossyverdurin (12,13). The compound has been characterized and its acute toxicity determined (13). It was reported that pigment glands, gossypol, gossypurpurin and gossyverdurin have rat LD-50 values of 1.12, 2.57, 6.68, and 0.66 g/kg body weight, respectively. The pathology was described.

Significance of Investigations

In consideration of the magnitude of the problem which gossypol in cottonseed presented and the large number of research workers who have investigated it, there is much difficulty in assessing the relative contributions of the major workers. However, it is apparent that Dr. Lyman is unsurpassed in his achievements. His investigations have been very broad in scope. The cottonseed industry and the consumers of its products have benefited to a marked degree from practical application of the results of both his basic and applied studies.

Perhaps the best measurement of the contributions of gossypol research to the utilization of cottonseed protein can be made by a comparison of the status of the product during Dr. Lyman's early work with that of today. Cottonseed meal has been moved from the position of a protein supplement for ruminants to that of a universal protein source. Our best estimates of present consumption suggest that approximately one third of the present production is used in monogastric animal feeds. This contrasts sharply with the insignificant amounts traditionally used for this purpose.

It is apparent that cottonseed processing mills now know how to mill glanded cottonseed in such a manner that cottonseed meal will have maximum value in swine and poultry rations. Equally important is the fact that knowledge is now adequate for the optimum use of all types of cottonseed meal in monogastric animal feeds. It appears that the commodity is now at the threshold of greatly expanded utilization.

Dr. Lyman made outstanding contributions toward the development of glandless cottonseed by elucidation of the gossypol problem and by his stimulation of glandless cottonseed breeding, as well as his study of the characteristics of gossypol-free cottonseed protein.

He was a tremendous force in the stimulation of studies which promise to make cottonseed protein an important component of human food products.

Research has given the cottonseed industry the opportunity to move forward in a productive way. This opportunity has, to a very important degree, been made possible by the investigations, the friendly counsel, and the encouragement of Carl M. Lyman.

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