breeder hen: shell quality *versus* oyster shell supplementation. Poultry Sci. 49: 814-816.
Woodard, A. E., H. Abplanalp and F. X. Ogasawara, 1961. Egg and semen production performance of turkeys under cage management. Poultry Sci. 40: 884-890.

Adaptation of the Domestic Chicken, *Gallus Domesticus*, to Continuous Feeding of Albumin Amylase Inhibitors from Wheat Flour as Gastro-resistant Microgranules

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ABSTRACT Albumin amylase inhibitors were extracted from wheat flour, precipitated by salting out the extract with ammonium sulphate, and enclosed in cellulose-coated microgranules resistant to the peptic action in the chicken gizzard. Continuous intake of gastro-resistant wheat albumins significantly ($P<0.01$) depressed chicken growth rate, whereas native wheat albumins did not show such an effect. After 4 weeks of treatment, treated chickens showed a growth rate identical to that of control chickens thus showing that an adaptation to the presence of wheat albumins in the diet had occurred. Treated chickens also showed pancreas hypertrophy and a number of histological changes in the pancreas indicating degenerative processes in progress. Moreover, in treated chickens the production of pancreatic amylase was markedly increased ($P<0.02$), whereas pancreatic protease activity was less affected. The data obtained suggest that the synthesis of pancreatic amylase in chicken is under some homeostatic control of α-amylase in the intestine.

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INTRODUCTION

WHEAT albumin is a highly heterogeneous protein fraction that can be extracted from wheat flour with water or dilute salt solutions and precipitated by salting out the clear supernatant between 0.4 and 1.8 M ammonium sulphate. The albumin fraction contains a very large number of protein components capable of inhibiting α-amylases from insect, mammalian, avian, and marine species (Shainkin and Birk, 1970; Silano *et al.*, 1973, 1975; Saunders and Lang, 1973; Bedetti *et al.*, 1974; Petrucci *et al.*, 1974) as well as a few protein components active in inhibiting trypsin (Shyamala and Lyman, 1964; Hochstrasser and Werle, 1969; Mikola and Kirsi, 1972; Camus and Laporte, 1973; Petrucci *et al.*, 1974). From a quantitative standpoint, wheat albumins that inhibit trypsin are negligible, whereas amylase inhibitors make up as much as 80% of the total albumin fraction from wheat flour.

In 1964, Applebaum showed that the addition of wheat flour albumins to a synthetic diet adversely affected development and greatly increased mortality of *Tenebrio molitor* L. larvae. This author attributed such effects to the *in vivo* inhibition of the insect amylase by wheat albumins. Recently, Puls and Keup (1973) have studied the influence of an amylase inhibitor preparation from wheat on blood glucose and insulin in human volunteers, rats, and dogs after ingestion of starch. They showed that hyperglycaemia and hyperinsulinaemia resulting from starch loading could be reduced dose-dependently by the addition of the inhibitor to the starch load. As similar effects were observed in diabetic and obese patients, these authors suggested that amylase protein inhibitors

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from wheat could possibly be deliberately employed as therapeutic agents. Further evidence of the specific interference of the amylase inhibitors from wheat with starch metabolism in rats has been recently reported by Saunders (1975) who showed that, under the experimental conditions adopted, the inclusion of the inhibitor into the diet caused a decrease in starch availability.

In spite of such large amount of work, no data concerning the ability of animal species to adapt to continuous feeding of amylase inhibitors from wheat are available. Moreover, no investigations on the physiological changes induced by repeated ingestion of the inhibitors have been reported. This paper deals with the effects induced in chickens by a continuous intake of a starch-rich diet supplemented with a purified albumin inhibitor preparation from wheat flour. Chickens were chosen for the feeding trial because *in vitro* experiments have shown (Silano *et al.*, 1975) that, among a number of mammalian and avian amylases tested, chicken α-amylase is the most effectively inhibited by wheat albumins. Moreover, as *in vitro* experiments have also shown (Petrucci *et al.*, 1976) a large inactivation of albumin amylase inhibitors after incubation with pepsin, our investigations have been carried out not only with native wheat albumins, but also with a cellulose-coated albumin preparation resistant to the peptic action in the chicken gizzard.

MATERIALS AND METHODS

Preparation of the Amylase Inhibitors. The large amount of wheat albumins needed for the feeding trial was prepared with a large-scale extraction and purification procedure that involved the extraction of 50 Kg. lots of wheat flour with 150 l. of 0.15 M sodium chloride in a pilot plant and the salting out of the extracted albumins between 0.4 and 1.8 M ammonium sulphate. The extraction was carried out by suspending the wheat flour (*Triticum aestivum* var. Mentana) in the salt solution for 3 hr. under discontinuous mechanical mixing. The suspension was allowed to stand overnight and then the supernatant was transferred by a suction pump to a 300 l. tank cooled by running water.

Albumins were precipitated by salting out the extract with ammonium sulphate and collected by centrifuging twice at 15,000 r.p.m. with a Sharples centrifuge. The albumin precipitate was suspended in a small volume of water and the suspension was kept at 60° C. for 20 min. to destroy residual amylase activity. The suspension was dialyzed for 48 hours against distilled water at 4° C. and freeze-dried. The albumin yield was about 200 g. of protein/100 kg. of flour. This albumin preparation to be referred to as native wheat albumin (N.W.A) was characterized by gel filtration and gel electrophoresis by following the procedures described by Petrucci *et al.* (1974). Inhibition activity of N.W.A. toward chicken α-amylase and trypsin was tested according to Petrucci *et al.* (1974) whose definitions of amylase and trypsin inhibition units are also followed in this paper. For agglutination activity tests, chicken red blood cells were collected under sterile conditions and stored according to Kabat (1956). Standard amounts of cells were added to serial dilutions of wheat albumins in a V-shaped plate of a Takatsy's microtitrator (Cooke Engineering Co., Alexandria, U.S.A.). After 20 min., agglutination patterns were visually scored.

N.W.A. was powder-milled and further treated to prepare cellulose-coated gastro-resistant microgranules with the following composition: wheat albumin powder 56.9%, Eleema (cellulose) 25.8%, Cap (cellulose acetophthalate) 5.2%, Diffucril L (a mixture of polymetacrylate esters) 7.8%, Diffulac (highly purified Shellac) 2.2%, and talc 2.1%. The preparation technique of gastro-resistant microgranules, as well as the sources of the

reagents used, are reported in the Italian patent no. 929112. Microgranules containing talc instead of wheat albumins were also prepared and used as a control. Gastro-resistant wheat albumin microgranules, to be referred to as gastro-resistant wheat albumin (G.W.A.), did not release detectable amounts of protein when incubated at 37° C. for 2 hours at acidic pHs. However, at pH 7 or higher, 30% and 85% of the protein enclosed was released after 1 and 3 hours, respectively.

Feeding Trial. Male Warren chickens 1 day of age were kept in cages in a lighted room at the constant temperature of 22° C. ± 1° C. with free access to tap water and feed. The basal diet was composed of the following constituents per 100 g.: corn meal 60.0, heated soya bean meal 22.8, fish meal 6.0, milk powder 5.0, alfalfa 3.0, calcium phosphate ($CaHPO_4$) 1.1, calcium carbonate 1.0, sodium chloride 0.4g. The vitamin and methionine supplementation (0.7 g./100 g. of diet) had the following composition: vitamin A 1820 I.U., B_1 0.35 mg., B_2 0.63 mg., B_6 0.35 mg., B_{12} 1.54 mg., D_3 420 I.U., E 4.90 mg., K_3 0.21 mg., nicotinic acid 2.54 mg., choline chloride 52.5 mg., calcium pantothenate 1.75 mg., and methionine 28 mg. Moreover, in 100 g. of diet there were the following amounts of trace elements: cobalt 0.14 mg., copper 0.17 mg., iron 3.5 mg., iodine 0.14 mg., manganese 12.6 mg., zinc 5.6 mg. Four groups of 30 chickens each were fed from 1 to 21 days of age the basal diet with following additions: 3% talc microgranules (group A), 3% N.W.A. (group B), 0.3% G.W.A. (group C), and 3% G.W.A. (group D). In terms of wheat protein, chickens of group A did not receive wheat albumins, whereas birds of groups B, C, and D received amounts of wheat albumins corresponding to 0.8, 0.04, and 0.4%, respectively. As the basal diet contained about 20% crude protein, the contribution of wheat albumins to the protein content of the diet was negligible.

After 21 days of treatment the birds of group D were divided into three groups of 10 chickens each. One of these was transferred to the control diet, the second to a diet containing 6% G.W.A. and the last one was continued on the diet with 3% G.W.A. till the end of the whole experiment that lasted 31 days. Chickens were individually weighed two times a week.

Biochemical and Histological Investigations. At the end of the experiment, five chickens of each group were sacrified after intracardiac bleeding. Blood was collected and allowed to clot. The supernatant serum was collected by centrifuging at 10,000 *g* and used for amylase assay with no further treatment. The pancreas and the carcass were weighed and small samples of pancreatic tissue were submitted to histological examination after immediate fixation in Bouin's solution and conventional staining with haematoxylin-eosin, Van Gieson and Mallory stains. The remaining pancreas was homogenized with distilled water (1:10 w./v.) in a Potter homogenizer and then the homogenate was centrifuged at 45,000 *g*. The clear supernatant was used for amylase and protease assays. Amylase activity was determined in a 0.03 M acetate/barbiturate buffer (pH 7.6) as described by Silano *et al.* (1975) and protease activity was measured by following the Anson's method (1939) after enterokinase activation of zymogens according to Lepkovsky *et al.* (1965).

Analysis of Data. Significance of differences of data was analyzed by using Student's *t*-test.

RESULTS

Wheat Albumins. The albumin preparation obtained from wheat flour with the large-scale extraction and purification procedure described in the experimental section exhibited a specific inhibitory activity toward chicken

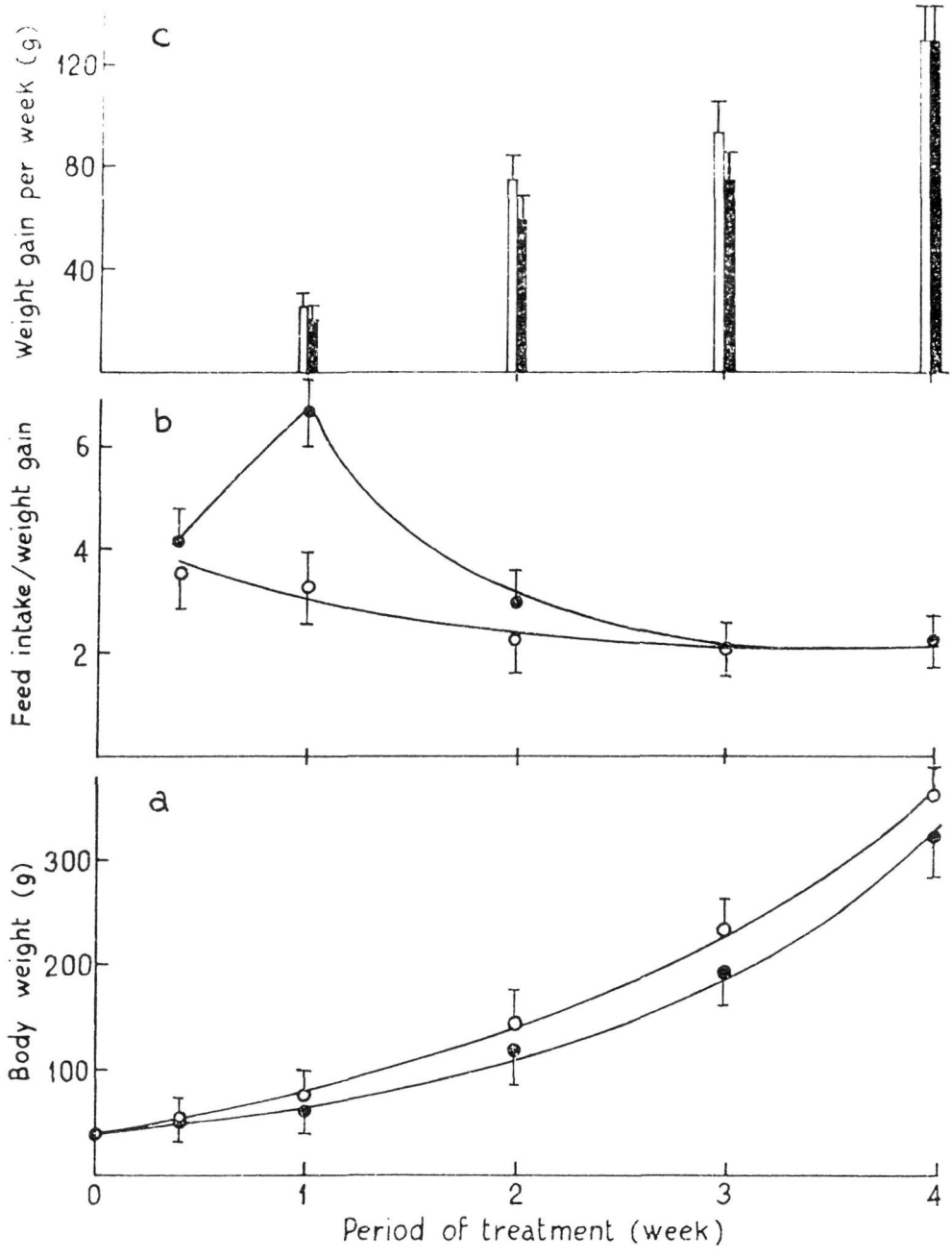

FIG. 1. Effect of gastro-resistant wheat albumins upon chicken growth and feed utilization. ○, control chickens; ●, chickens fed 3% G.W.A.

α-amylase of 2.0 I.U./μg. of albumin and toward trypsin of about 0.2 I.U./μg. When analyzed by gel filtration and electrophoresis, the albumin preparation exhibited amylase and trypsin inhibition patterns identical to those previously reported by Petrucci *et al.* (1974). This albumin preparation also showed an extremely low agglutinating activity toward chicken red blood cells. The minimal agglutinating concentration was 12 mg./ml. and the agglutination was inhibited by ovomucin added at 10 mg./ml. concentration. Such findings are consistent with the presence in this preparation of a wheat germ agglutinin contamination lower than 0.1% (Burger and Goldberg, 1967; Schnebli and Bachi, 1975).

Chicken Growth and Feed Uptake. As shown in Fig. 1a and c, when fed as gastro-resistant microgranules at 3% level (corresponding to 0.4% flour protein) since one day of age, wheat albumins caused a small, but significant ($P < 0.01$), retarding effect upon chicken growth. Moreover, as shown

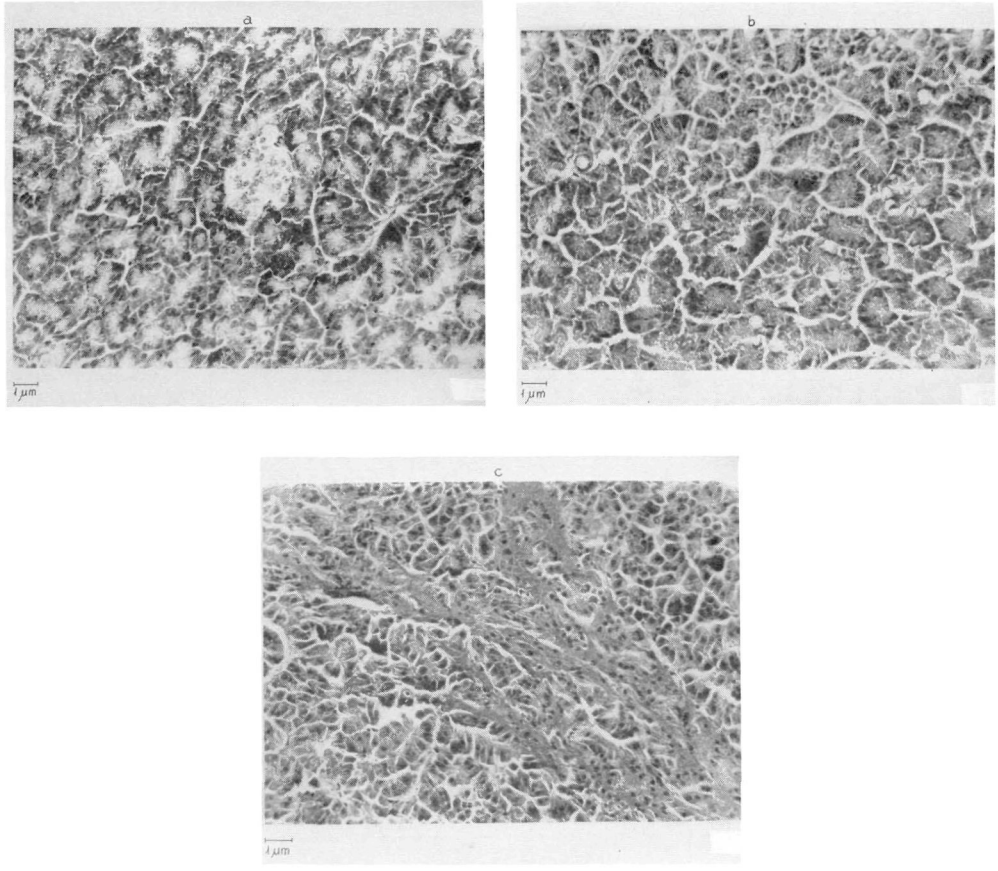

FIG. 2. Microphotographs of the pancreas of control chickens and of chickens fed gastro-resistant wheat albumins.

a. control chickens; b. chickens fed 3% G.W.A. for 4 weeks; c. chickens fed 3% G.W.A. for 3 weeks and 6% G.W.A. for 1 week. × 125.

in Fig. 1b, during the first part of the experiment the treated chickens ingested a larger amount of feed thus showing that G.W.A. also increased chicken appetite. It took about 4 weeks for birds to overcome the presence of G.W.A. in the diet. After 4 weeks of treatment, in fact, a difference was no longer detectable between treated and control birds as far as weekly weight gain (Fig. 1c) and conversion factor (Fig. 1b) are concerned. The adaptation of the birds to the continuous intake of G.W.A. was confirmed by the fact that body weights of chickens fed 3% G.W.A. for 3 weeks and then transferred for 1 week to a diet containing no wheat albumins or 6% G.W.A. were identical to that exhibited by chickens kept for 4 weeks on the 3% G.W.A. diet (Fig. 1a).

Chickens treated with 0.3% G.W.A. or with 3% N.W.A. did not show any significant difference in growth/feed consumption ratio as compared to control chickens throughout the whole experiment.

Biochemical and Histological Investigations. Chickens fed 3% G.W.A. for 4 weeks showed, as compared to control birds, significatively ($P < 0.05$) higher pancreatic amylase activity (335 A.U./mg. fresh pancreas against 196 A.U./mg. fresh pancreas), but identical serum amylase activity (24 A.U./ml. of serum). Some histological differences consisting of somewhat enlarged cells, granular cytoplasm, and a number of vacuoles were evident (Fig. 2a and 2b) between the pancreas of treated and control chickens, but relative pancreas weights of the two avian groups were identical (0.41% of total body weight). No significant difference between pancreatic protease activity of treated and control birds could be detected.

Pancreatic amylase activity of chickens fed 3% G.W.A. for 3 weeks and 6% G.W.A. for 1 week was equal to 396 A.U./mg. fresh pancreas with a 96% increase ($P < 0.02$) over that of control birds and 18% increase ($P < 0.05$) over that of birds fed 3% G.W.A. for 4 weeks. Also serum amylase and pancreatic protease activities increased upon such treatment with the inhibitor; the increments were equal to 48 and 46%, respectively, which were statistically significant ($P < 0.05$). Moreover, pancreas weight of treated birds was equal to 0.58% of total body weight which was 41% greater ($P < 0.001$) than the relative pancreas weight of control birds. Histological examination of the enlarged pancreas revealed (Fig. 2c) that pancreatic enlargement was the result of an increase of cell size rather than the number of cells. Degenerative phenomena were also detectable in the hypertrophic pancreas. A number of exocrine cells were transformed into an amorphous basophilic mass. Moreover, the number of cell nuclei decreased, whereas the amount of connective stromatic tissue increased.

DISCUSSION

The data reported show that the continuous intake of gastro-resistant wheat albumins depresses growth rate and causes hypertrophy of the pancreas in chickens fed *ad libitum* with a diet containing a large amount of starch. Similar effects have been observed in chickens (Chernick *et al.*, 1948; Applegarth *et al.*, 1964; Nitsan and Alumont, 1964; Nitsan and Bondi, 1965; Lepkovsky *et al.*, 1965; Garlich and Nesheim, 1966) and in rats (Haines and Lyman, 1961; Konijn and Guggenheim, 1967; Pope *et al.*, 1975) upon feeding raw soya beans or purified soya bean trypsin inhibitor. Along with these effects, gastro-resistant wheat albumins caused a marked increase in the production of pancreatic α-amylase and a minor increase of pancreatic proteases. On the contrary, feeding raw soya beans or purified soya trypsin inhibitor caused an increased production of pancreatic proteases (Chernick *et al.*, 1948; Applegarth *et al.*, 1964; Nitsan and Alumont, 1964; Lepkovsky *et al.*, 1965) and a decreased produc-

tion of pancreatic amylase (Konijn *et al.*, 1969). The modifications induced in chickens by wheat albumins appear to be adaptive responses elicited by the ingestion of the very active amylase inhibitors that make up the major part of wheat albumins. Moreover, our findings suggest that the synthesis of pancreatic amylase is under some homeostatic control of α-amylase in the intestine. Whether the control mechanism from the intestinal lumen to the pancreas, causing the pancreas to respond to the decrease of amylase in the contents of the intestine so as to restore the level to normal, is a negative feedback, as that exercised by protease (Lyman *et al.*, 1974), cannot be established at the moment.

Native wheat albumins did not show the depressive effect upon chicken growth displayed by gastro-resistant albumins thus showing that gastric digestion in chicken is very effective in inactivating albumin amylase inhibitors.

In relation to possible therapeutic uses of wheat albumin inhibitors, the changes observed in the pancreas as well as the chicken adaptation to the continuous intake of wheat albumins cast doubt upon the desirability of using oral administration of amylase inhibitors to control postprandial hyperglycaemia in man. In any case, our data show that gastro-resistant preparations can be successfully used to increase *in vivo* effectiveness of wheat amylase inhibitors.

ACKNOWLEDGEMENTS

We are gratefully indebted to Eurand S.p.a, Milan, Italy, for preparing gastro-resistant microgranules enclosing wheat albumins. We wish to thank Mr. A. Galli for his skillful assistance in the large-scale extraction and purification of wheat albumins, Mr. M. Biancini for his assistance in the feeding trial, Dr. E. Palliola for histological examinations, and Dr. M. Minetti for agglutination tests.

REFERENCES

Anson, M. L., 1939. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J. Gen. Physiol. 22: 79-89.

Applebaum, S. W., 1964. The action pattern and physiological role of *Tenebrio molitor* larval amylase. J. Ins. Physiol. 10: 897-905.

Applegarth, A., F. Furuta and S. Lepkovsky, 1964. Morphologic responses, gross and microscopic, of the pancreas of chickens on raw and heated soybean diets. Poultry Sci. 43: 733-739.

Bedetti, C., A. Bozzini, V. Silano and L. Vittozzi, 1974. Amylase protein inhibitors and the role of Aegilops species in polyploid wheat speciation. Biochim. Biophys. Acta, 362: 299-307.

Burger, M. M., and A. R. Goldberg, 1967. Identification of a tumor-specific determinant on neoplastic cell surfaces. Proc. Natl. Acad. Sci. U.S. 57: 359-366.

Camus, M. C., and J. C. Laporte, 1973. Teneurs en inhibiteurs trypsiques des divers blés, durs et tendres. Ann. Technol. Agric. 22: 111-120.

Chernick, S. S., S. Lepkovsky and I. L. Chaikoff, 1948. A dietary factor regulating the enzyme content of the pancreas: changes induced in size and proteolytic activity of raw soy-bean meal. Am. J. Physiol. 155: 33-41.

Garlich, J. D., and M. Nesheim, 1966. Relationship of fractions of soybeans and a crystalline soybean inhibitor to the effects of feeding unheated soybean meal to chicks. J. Nutr. 88: 100-110.

Haines, P. C., and R. L. Lyman, 1961. Relationship of pancreatic enzyme secretion to growth inhibition in rats fed soybean trypsin inhibitor. J. Nutr. 74: 445-452.

Hochstrasser, K., and E. Werle, 1969. Uberpflanzliche proteinaseinhibitoren, reindarstellung der trypsininhibitoren aus kaimen von weizen und raggensamen, lokalisierung der aktiven zentren. Hoppe-Seyler's Z. Physiol. Chem. 350: 249-254.

Kabat, E. A., 1956. Blood Group Substances. Academic Press Inc., New York, pp. 92.

Konijn, A. M., and K. Guggenheim, 1967. Effect of raw soybean flour on the composition of rat pancreas. Proc. Soc. Exp. Biol. Med. 126: 65-67.

Konijn, A. M., K. Guggenheim and Y. Birk, 1969. Amylase synthesis in pancreas of rats fed soybean flour. J. Nutr. 97: 265-269.

Lepkovsky, S., F. Furuta, T. Koike, N. Hasegawa, M. K. Dimick, K. Krause and F. J. Barnes, 1965. The effect of raw soya beans upon the digestion of proteins and upon the function of the pancreas of intact chickens and of chickens with ileostomies. Br. J. Nutr. 19: 41-56.

Lyman, R. L., B. A. Olds and G. M. Green, 1974. Chymotrypsinogen in the intestine of rats fed soybean trypsin inhibitor and its inability to suppress pancreatic enzyme secretions. J. Nutr. 104: 105-110.

Mikola, J., and M. Kirsi, 1972. Differences between endospermal and embrional trypsin inhibitors in barley, wheat and rye. Acta Chem. Scand. 26: 787-795.

Nitsan, J., and E. Alumont, 1964. Overcoming the inhibition of intestinal proteolytic activity caused by raw soybean in chicks of different ages. J. Nutr. 84: 179-184.

Nitsan, Z., and A. Bondi, 1965. Comparison of nutritional effects induced in chicks, rats and mice by raw soyabean meal. Br. J. Nutr. 19: 177-187.

Petrucci, T., M. Tomasi, P. Cantagalli and V. Silano, 1974. Comparison of wheat albumin inhibitors of alpha-amylase and trypsin. Phytochemistry, 13: 2487-2495.

Petrucci, T., A. Rab, M. Tomasi and V. Silano, 1976. Further characterization studies of the 0.19 amylase protein inhibitor from the wheat kernel. Biochem. Biophys. Acta, 420: 288-297.

Pope, H. O., J. R. Pattern and A. L. Lawrence, 1975. The effect of raw soya bean on *in vitro* active and passive accumulation by rat small intestine. Br. J. Nutr. 33: 117-125.

Puls, W., and U. Keup, 1973. Influence of an alpha-amylase inhibitor on blood glucose, serum insulin and NEFA in starch loading tests in rats, dogs and man. Diabetologia, 9: 97-101.

Saunder, R. M., 1975. Alpha-amylase inhibitors in wheat and other cereals. Cereal Foods World, 20: 282-285.

Saunders, R. M., and J. A. Lang, 1973. Alpha-amylase inhibitors in *Triticum aestivum:* purification and physical-chemical properties. Phytochemistry, 12: 1237-1241.

Schnebli, H. P., and T. Bachi, 1975. Factors governing the agglutination reaction. Exptl. Cell. Res. 91: 175-183.

Shainkin, R., and Y. Birk, 1970. Alpha-amylase inhibitors from wheat: isolation and characterization. Biochim. Biophys. Acta, 221: 502-513.

Shyamala, G., and R. L. Lyman, 1964. The isolation and purification of a trypsin inhibitor from whole wheat flour. Can. J. Biochem. 42: 1825-1832.

Silano, V., M. Furia, L. Gianfreda, A. Macrì, R. Palescandolo, A. Rab, V. Scardi, E. Stella and F. Valfré, 1975. Inhibition of amylases from different origins by albumins from the wheat kernel. Biochim. Biophys. Acta, 391: 170-178.

Silano, V., F. Pocchiari and D. D. Kasarda, 1973. Physical characterization of alpha-amylase inhibitors from wheat. Biochim. Biophys. Acta, 317: 139-148.

NEWS AND NOTES

IOWA NOTES

Dr. Stanley L. Balloun, retired, on August 31, from Iowa State University, after 23 years of dedicated service. He is now living in Hot Springs, Arkansas.

Dr. Balloun graduated from Iowa State University in 1930 with a B.S. degree, majoring in agriculture. He taught vocational agriculture in Iowa and Illinois high schools during the '30s through the middle '40s. He then became Supervisor of the Alaska Experiment Station. In 1952 he received a Ph.D. degree from Iowa State, majoring in poultry nutrition, and joined Armour and Co. as Nutritionist. After a year he returned to Iowa State University as a staff member, responsible for research and teaching of poultry nutrition.

During his tenure at Iowa State, he authored or co-authored 85 research papers dealing with turkeys and chickens. He has also been the author of many publications aimed at the practical application of poultry nutrition. He has consulted on poultry nutrition problems in Mexico, the Caribbean, Central and South America, West and East Europe, the Middle East and Japan.

In 1965 Dr. Balloun was the recipient of the National Turkey Federation Research Award. He received the American Feed Manufacturers' Association Award, administered by the Poultry Science Association in 1974. The National Turkey Federation also presented an Award of Service to Dr. Balloun at their National Summer Meeting in 1976, for many years of service to the turkey industry.

PENNSYLVANIA NOTES

Four revised academic majors have been established in the College of Agriculture at the Pennsylvania State University, all of them leading to the Bachelor of Science degree. They involved revision of existing programs, so that it was possible to establish them without additional funding and within current budget allocations.

One major, poultry technology and management,

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