Role of Microbial Fermentation in Improvement of Barley by Water Treatment.*† (25458)

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Fry *et al.*(1) showed that the nutritional value of barley for chicks was improved by a simple water treatment. Similar results were obtained by other workers(2,3,4). Since studies on the mechanism(s) involved in improvement were not reported, experiments were conducted to investigate the role of microbial action.

Methods. Microbial counts on samples of ground barley treated in different ways were made to correlate improvement in nutritional value with microbial growth. Two treated samples were taken from the drying oven in sterile 250 ml Erlenmeyer flasks containing glass beads. The flasks were shaken 2 hours to grind large aggregates to fine particles. Serial dilutions were made of weighed samples and bacterial counts determined, using tryptone-glucose extract agar and incubating 41 hours at 37°C. No attempt was made to enumerate fungi. Since Kent-Jones and Amos(5) found *B. subtilis* and *B. mesentericus* common inhabitants of wheat flour, *B. subtilis* (ATCC 9943) and an unidentified, spore-forming, Gram positive rod isolated from our feed preparation area, were used to inoculate barley in 2 experiments. Treatments involving addition of water to grain consisted of adding 1¼ parts liquid (50°C) to 1 part coarsely ground grain, mixing, spreading approximately ½″ deep on trays, and drying in a forced draft oven for 24 hours at 70°C. The treated grain was reground through a ⅛″ screen and mixed in the diets. Wet barley was autoclaved in trays (Tables I and III), or metal 30 lb egg cans (Table II) to facilitate sterilization. Hot sterilized barley in cans was inoculated with culture material, aseptically mixed, sealed, stood 4 hours at room temperature, spread on trays, and placed in drying oven. Two 20-hr nutrient agar cultures were swirled separately for 2 minutes with 50 ml 0.5% peptone water each; both were added to barley as the inoculum. Barley autoclaved in trays was removed and dried immediately in the oven. The large inoculum added directly to dry barley diets was 2 separately grown 200 ml, 36-hr nutrient broth cultures; the small inoculum was one 100 ml culture. Fungal enzyme‡ (fungal amylase) was used as positive control, since our previous work has shown it improves the nutritional value of dry barley diets(6). Day-old New Hampshire Chicks of both sexes were randomly distributed into groups of 10 birds each,

TABLE I. Effect of Various Treatments of Barley on Microbial Count and Chick Growth.

Diet	Avg wt 2 wk (g)	Organism $\times 10^6$/g
Barley	90	2
Sterilized* barley	116	5
Water-treated barley	122	94
Sterilized*(dried), water-treated	122	154
" inoc. fungal amylase (4.4 g/kilo)	126	177

* Autoclaved 10 p.s.i., ½ hr.

TABLE II. Effect of Bacterial Additions to Sterile Barley on Chick Growth and Feed Efficiency.

Barley treatment	Drying temp. (°C)	Avg wt 2 wk (g)§	Feed gain
None		84	2.24
Sterilized, inoculated*‡	95	84	2.13
" ‡‖	70	93	2.05
" ‡‖ " †	70	112	1.74
" ‡‖ " *	70	113	1.78
Fungal amylase (4.4 g/kilo)		108	1.85
Water-treatment	70	110	1.71

* Unidentified organism.
† *B. subtilis* (ATCC-9943).
‡ No standing period.
§ Treatments within a common bracket are not significantly different from each other. Treatments without a common bracket are statistically significant at the ($P < .01$) level.
‖ Autoclaved 15 p.s.i., ¾ hr.

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† Portions of these data presented at 48th Annual Meeting Poultry Sci. Assn., Iowa State University, Ames.

‡ Merck Sharp and Dohme, Rahway, N. J.

TABLE III. Effect of Adding Whole Bacterial Cultures to Dry Barley Diets and Autoclaving Water-Treated Barley on Chick Growth and Feed Efficiency.

Barley treatment or addition	Avg wt 2 wk (g)*	Feed gain
400 ml media	108	2.18
None	109	2.05
100 ml culture	116	1.98
400 " "	125	1.96
Fungal amylase (4.4 g/k), 400 ml culture	130	1.88
Water-treated barley	131	1.70
Fungal amylase (4.4 g/k)	134	1.78
Water-treated (dried), sterilized (125% H_2O),† dried 95°C	134	1.76
Water-treated (dried), sterilized (25% H_2O),† air dried	135	1.79
Water-treated, 400 ml culture	137	1.74

* Treatments within a common bracket are not significantly different from each other. Treatments without a common bracket are statistically significant at the ($P < .05$) level.

† Autoclaved 15 p.s.i., ½ hr. Dry water-treated barley contains 4% moisture.

3 replicates/diet, and placed in electrically-heated batteries with raised wire floors. Continuous lighting was provided and feed and water supplied *ad lib*. A basal diet of following percentage composition was used: 63.5 barley, 25.5 dehulled soybean oil meal, 5 herring fish meal, 1.75 dicalcium phosphate, 1.15 limestone, .3 salt, .11 DL-methionine, .025 manganese sulfate, and a vitamin mix supplying 2650 I.U. Vit. A, 660 I.C.U. Vit. D, 11 I.U. Vit. E, 8.8 mg riboflavin, 10 mg pantothenic acid, 33.2 mg niacin, .66 mg folic acid, 884 mg choline chloride, and 22 mg procaine penicillin/kilo diet. Experimental data from Tables II and III were statistically analyzed, using Duncan's(7) Multiple F Test.

Results. Microbial counts markedly increased when barley was water-treated, sterilized (dried) and water-treated, or sterilized and supplemented (inoculated) with fungal amylase (Table I). Average weight of chicks fed diets containing these barley samples correlates positively with count data. The relatively large counts obtained would not be predicted since an oven temperature of 70°C was employed. Perhaps microbial growth occurred because evaporative loss of moisture from barley lowered the internal temperature of the wet grain to less than 70°C during the first few hours of drying. The sterilized barley was probably contaminated during transfer from autoclave to drying oven, since no precautions were taken to prevent contamination. The fungal amylase supplement added to sterilized barley was not a sterile product and greatly increased counts.

When an inoculum of peptone washed culture (100 ml — 2×10^8 organisms ml) containing either *B. subtilis* (ATCC 9943) or the unidentified organism was added to sterile barley and allowed to stand 4 hours prior to drying (Table II), chick growth and feed utilization were significantly ($P < .01$) improved. Improvement of barley by inoculation with either organism was comparable to that of water-treating barley or enzyme supplementation. Since inoculum was added to extremely hot barley, growth of microorganisms was possibly the result of germination of spores. Addition of the unknown organism to sterile (wet-autoclaved) barley which was subsequently dried at 95°C did not improve growth.

Possibly the organisms used contributed enzyme(s) to the barley which in some manner acted to improve its nutritional value. A proteolytic enzyme has been crystallized from cultures of *B. subtilis*(8) which is partially effective in improving growth of birds fed barley(9). This improvement may occur during water-treatment since the unknown organism was isolated from barley itself.

Addition of a small (100 ml—2×10^7 organisms/ml) culture of the unidentified organism directly to dry barley diet, improved growth and feed efficiency (Table III). A significant ($P < .05$) growth response, similar in magnitude to that obtained with dry fungal amylase, was obtained when a large (400 ml—2×10^7 organisms/ml) culture was added. When dry diets containing water-treated barley or fungal enzyme were inoculated with the unknown organism, no significant improvement in growth was obtained. Autoclaving previously water-treated and dried barley in the presence of 25 or 125% added moisture did not lower its nutritional value for chicks. The nutritional value was not reduced by autoclaving. Therefore, it appears

that barley was improved prior to incorporation in the diet and improvement did not depend on microbially produced enzymes acting in the digestive tract of the chick.

Only 100 ml of 20-hr peptone washed culture significantly ($P<.01$) improved the nutritional value of sterile barley in Exp. 2 (Table II). Thus, it is highly probable that microorganisms grew in wet barley, perhaps producing enzymes, which could have acted on the barley substrate during drying. Exp. 3 (Table III) showed that 400 ml of nutrient broth culture gave a significant ($P<.05$) growth response, presumably a result of enzymes produced during incubation of the stock cultures.

The data demonstrate that microbial fermentation is effective in improvement of barley by water-treatment. This, however, does not eliminate the possibility that enzymes endogenous to barley may also play a role in improving the nutritional value of this grain by water-treatment. Both phenomena could conceivably take place simultaneously.

Summary. Water-treated barley taken from drying oven contained many more bacteria than untreated barley, even though drying temperature was 70°C. The nutritional value of sterilized barley inoculated with Gram-positive rod isolated from dust, or with *B. subtilis* (ATCC 9943) and dried at 70°C was highly significantly improved. Large inoculations of the organism isolated and grown in nutrient broth elicited a significant growth response. This response was not as great as that obtained by water-treatment or addition of fungal enzyme to the feed. Autoclaving did not lower nutritional value of water-treated barley. This fact indicates that the change(s) responsible for improvement was effected prior to feeding and was not due to presence of an enzyme(s) produced during treatment process that later acts on barley in the digestive tract of the chick.

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Antibody Response to Influenza Vaccines Combined with Hexadecylamine. (25459)

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Certain lipids, including cholesterol, palmitic acid, stearic acid, and longer aliphatic amines, such as hexadecylamine (HDA) have been shown by Youngner and Noll to possess an affinity for several myxoviruses(1,2,3). Such virus-lipid complexes increase antigenicity, in an as yet unknown manner, to a level greater than that of equal amount of virus alone. This reaction is also notable in that large amounts of viral antigen can be adsorbed to a given quantity of HDA(3). These observations have an immediate bearing on the problem of enhanced potency of influenza vaccines. In an earlier study we reported preliminary observations on cholesterol containing vaccines(2). The following report describes the responses of man and animals to formalin-inactivated influenza viruses mixed with hexadecylamine, since, in comparative adsorption experiments, this compound ex-