Effect of Cobalt-60 Gamma-Irradiation on the Utilization of Energy, Protein, and Phosphorus from Wheat Bran by the Chicken

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ABSTRACT

The effect of insect deinfestation and extreme levels of cobalt-60 gamma-irradiation on several aspects of nutritional value was studied in wheat bran. Initial studies on metabolizable energy and amino acid composition showed no outstanding differences between treatments, and later permitted accurate isocaloric formulation and optimal supplementation of assay diets. Feeding the respective brans to chicks (1 to 3 weeks old) in semipurified diets as sole source of protein and phosphorus revealed significant improvements in growth and ratio of gain to feed consumed when amino acid supplementation was termed optimal and bran was irradiated. Also noted when bran was irradiated were significant improvements in net protein and P utilization. Although utilization of P was greater at the 5.0-Mrad treatment level than with 0.5 Mrad, this was not true for net protein utilization (NPU). On the basis of failure to demonstrate differences in growth, feed efficiency, or NPU between birds fed control and irradiated bran diets, when cystine was derived exclusively from the associated protein and the only limiting nutrient, it seemed improbable that this disulfide unit was measurably affected by the cobalt-60 treatment.

One way to extend the storage life of wheat is by preventing entomological infestation. An effective and economically practical means to accomplish this is through gamma-irradiation with appropriate radioactive isotopes (usually cesium-137 or cobalt-60). The accepted safe level of wheat irradiation at present is 0.02–0.05 Mrad (U.S. Department of Health, Education, and Welfare). As the term *rad* implies, energy is dissipated in the material (1 rad = 100 ergs of energy absorbed/g. of matter); thus, a definite possibility of an induced chemical and/or physical change exists. Hickman *et al.* (1) have already established that harmful or carcinogenic products were not encountered with wheat as a result of cobalt-60 gamma-irradiation (0.02–0.20 Mrad). Rats fed the treated grain for extended periods of time failed to demonstrate any effects on growth, reproductive performance, or progeny health. Similarly, there were no effects on various biochemical and physical parameters or milling characteristics with treatments with 0.01, 0.025, 0.05, 0.125, and 0.175 Mrad (2); however, germination was reduced with as little as 0.05 Mrad and viability was completely destroyed at the two highest levels. Although the breadmaking characteristics of the aforementioned wheats were unaffected, a scorched or burned odor was obvious when the loaves were removed from the oven, when radiation dosages were 0.125 and 0.175 Mrad. This undesirable aroma rapidly disappeared with cooling. Consequently, it would seem that the primary interests of health and economic considerations would not be affected by irradiation levels lethal to insects.

Ionizing radiation treatment of food or feedstuffs has frequently been shown to affect their nutritional value. Kennedy (3) has shown that Manitoba-grown whole wheat treated with cobalt-60 gamma rays to effect a 0.02-

Mrad dose caused no apparent change in the microbiological assay of nicotinic acid, thiamine, riboflavin, biotin, and total vitamin B_6; however, there was a slight decrease in pantothenic acid. Increasing the irradiation dosage to 0.2 Mrad caused losses of 12, 11, and 10% in nicotinic acid, pantothenic acid, and biotin, respectively. In other investigations with the same treatments, Kennedy (4) measured the "protein relative nutritional values (RNV)"; he used a turbidimetric microbiological technique with the proteolytic organism *Streptococcus zymogenes*. On the basis of the index of casein $= 100$, there was no loss of whole wheat RNV with a 0.2-Mrad treatment; however, a 6% reduction occurred when the dose was increased to 1.0 Mrad, which was not further affected by 5.0 Mrad. Irradiating a prepared wheat gluten was likewise without effect from a 0.02-Mrad dose, but larger amounts to the effect of 0.2, 1.0, and 5.0 Mrad caused RNV losses of 5, 7, and 26%, respectively. Supplementation of the cultures containing the aforementioned wheat glutens with methionine and lysine caused RNV to approach that observed for controls. In contrast, Metta and Johnson (5), from studies with rats, claimed there were no differences in protein biological value and digestibility of wheat gluten or corn protein irradiated with either 2.8 or 9.3 Mrad; and furthermore, the concentrations of lysine and arginine as analyzed by conventional techniques were unaltered. Destruction of the amino acids had previously been shown to be closely related to the level of irradiation with fresh frozen peas and lima beans (6). In general, there have been many reports on nutritional inactivation of amino acids by gamma-irradiation; however, cystine has been most often reported damaged (7,8).

Apparently carbohydrate can also be affected by high-energy irradiation. Linko and Milner (9) found the maltose values of irradiated wheat to be substantially increased with gamma treatments above 0.5 Mrad. The absolute concentration increase of this disaccharide was explained in terms of starch-granule rupture and subsequent endogenous amylase action, which in turn caused starch gelatinization viscosities to decrease. This hypothesis is open to question on consideration of recent irradiation experiments by Phillips *et al.* (10,11,12) with purified carbohydrates. Both alpha and beta D-glucose were degraded by cobalt-60 gamma-irradiation to several products, among which gluconic and glucuronic acids have been identified. With the more complex cyclohepta-amylase, rupture of the cyclic alpha-1,4-glucosidic system occurs with evolution of gluconic acid, maltohexose, hydrogen, carbon dioxide, and carbon monoxide. In aqueous solutions the products are more like those expected from acid hydrolysis.

Although ionizing radiation has been shown not to affect the milling and breadmaking properties of wheat treated with deinfestation doses, possible alterations in the utilization of energy and/or protein by the monogastric have not been extensively studied. Wheat bran offers two distinct advantages as a medium to determine possible radiation effects: 1) it contains large quantities of indigestible carbohydrate which, as a consequence, should be able by metabolizable energy alterations to indicate if there was radiation-induced glucosidic bond hydrolysis of any magnitude; and 2) the limiting amounts of methionine and cystine will, through chick performance experiments,

permit any destruction of these protein units to be determined. Because there are relatively large concentrations of phytin phosphorus associated with bran which is of questionable availability, an investigation on the utilization of this macromineral with irradiation was also performed.

EXPERIMENTAL MATERIAL

All wheat bran used in the present series of experiments was from a single lot which had been thoroughly blended and mixed[1]. The homogeneous bran was divided into three separate and equal parts, then respectively treated: control plus irradiation at 0.5- and 5.0-Mrad levels. The lower 0.5-Mrad dose was chosen because it is the maximum that one would ever expect to attain for entomological control purposes. Irradiation with the extreme of 5.0 Mrad was intended to cause definite nutritional alterations and to accentuate any effects demonstrated with the lower level. Type and source of radiation used in all cases were the gamma emissions from cobalt-60[2]. The experimental brans were stored in 10-lb. polyethylene-cardboard box units at approximately 0°C. until use.

SPECIFIC EXPERIMENTAL PROCEDURE AND RESULTS

Experiment 1

Experiment 1 was initiated to determine if the metabolizable energy of the bran was affected by irradiation. As mentioned earlier, extensive glycosidic bond rupture could improve the metabolizable energy by increasing the digestibility of the large amounts of fiber.

To determine metabolizable energy, 20 adult White Leghorn roosters were employed. These animals were maintained in individual cages which permitted *ad lib.* access to feed and water and excreta were quantitatively collected. The birds were divided into four experimental groups of five: one group was offered the basal described in Table I; the second through fourth

TABLE I

COMPOSITION OF BASAL DIET USED IN DETERMINATION OF METABOLIZABLE ENERGY[a]

INGREDIENT	%
Corn meal	65.75
Soybean meal (50% protein)	30.00
Dicalcium phosphate (21% Ca, 20% P)	2.20
Limestone	1.00
Iodized salt (0.015% KI)	0.30
Vitamin mix[b]	0.50
Mineral mix[c]	0.25
Total	100.00

[a] Calculated analysis: Protein (N × 6.25), 20.80%; Ca, 0.94%; P, 0.80%; ME, 2.88 kcal./g.
[b] Supplies the following per kg. of complete diet: vitamin A, 6,606 IU; vitamin D₃, 1,321 ICU; riboflavin, 6.6 mg.; pantothenic acid, 8.8 mg.; niacin, 26.4 mg.; choline, 220 mg.; folic acid, 1.3 mg.; and vitamin B₁₂, 0.013 mg.
[c] Supplies the following per kg. of complete diet: manganese, 52.4 mg.; zinc, 50.2 mg.; copper, 7.9 mg.; and iron, 20.3 mg.

received rations containing a 50:50 mixture of the basal with the variously treated wheat brans. After an initial 3-day adjustment period, feed consumption was determined, and total excreta were collected daily for a subsequent 4 days. Excreta were frozen (–20°C.), lyophilized, weighed, ground, and mixed; representative samples were saved for analysis. Gross energy of both feed and excrement was determined by use of a Parr adiabatic bomb calorimeter, according to established procedure[3].

The difference in energy consumed and excreted is termed the metabolizable energy (ME). The basal ration, known to be adequate in all nutrients, was fed alone so that this half of the ME contribution of the mixed feed could be calculated. Assuming that the addition of wheat bran did not influence utilization of energy in the basal fraction of the mixed diet, the contribution of bran to the ME of the mixed diet could be calculated, and hence, the ME of the bran samples themselves. Corrections for nitrogen retained as suggested by Hill and Anderson (13) were not made, because the roosters used were in nitrogen equilibrium.

TABLE II

EFFECT OF ^{60}CO γ-IRRADIATION ON METABOLIZABLE ENERGY OF WHEAT BRAN

	METABOLIZABLE ENERGY	
DIET	Whole Diet[a]	Bran[h]
	kcal./g.	kcal./g.
Basal	2.92 ± 0.13	....
50% Basal + 50% control bran	2.21 ± 0.11	1.50
50% Basal + 50% 0.5 Mrad bran	2.19 ± 0.03	1.46
50% Basal + 50% 5.0 Mrad bran	2.22 ± 0.05	1.52

[a] An average from five birds each replicated four times ± standard deviation.

[h] $2[(ME \text{ of whole diet}) - \dfrac{(ME \text{ of basal})}{2}]$

Results from the ME determinations are shown in Table II. Considering the standard deviation of the ME values for the whole diet, the small differences between control and experimental brans cannot be considered significant. Thus, irradiation, at least with the 0.5- and 5.0-Mrad levels and under the present experimental circumstances, had no effect on the ME of wheat bran. These results, however, should not be interpreted to mean that irradiation-induced hydrolysis did not occur; on the contrary, it is possible that glycosidic bond cleavage could have proceeded to disaccharide cellobiose units without an increase in ME. However, if the process is random and of any magnitude, some free glucose should have been released and measured.

Experiment 2

To adequately assess any protein quality changes due to irradiation and also optimally supplement experimental chick diets, it was necessary to conduct amino acid analyses and determine if any gross alterations had occurred. Analyses were performed on single representative samples from each of the treated brans with a Technicon amino acid analyzer, according

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[3] Parr Instrument Co., Moline, Ill.

TABLE III
AMINO ACID COMPOSITION OF EXPERIMENTAL WHEAT BRANS

AMINO ACID	WHEAT BRAN (PERCENT OF PROTEIN)				
	Control[a]	0.5 Mrad[a]	5.0 Mrad[a]	Average	Waggle et al.[b]
	%	%	%	%	%
Aspartic acid	7.4	8.8	8.2	8.1	8.2
Threonine	3.6	3.7	3.9	3.7	2.9
Serine	4.9	5.2	5.2	5.1	4.8
Glutamic acid	25.1	26.2	24.9	25.4	20.6
Proline	7.7	8.2	7.5	7.8	6.3
Glycine	6.8	7.3	6.9	7.0	6.5
Alanine	5.2	5.5	5.6	5.4	5.5
Cystine[c]	1.8	1.7	2.2	1.9	2.7
Valine	4.8	5.1	4.9	4.9	5.0
Methionine	1.4	1.5	1.3	1.4	1.6
Isoleucine	3.3	3.3	3.3	3.3	3.4
Leucine	6.9	6.7	7.1	6.9	6.6
Tyrosine	3.5	3.3	3.7	3.5	3.2
Phenylalanine	4.5	4.6	4.6	4.6	4.2
Lysine	4.4	4.3	4.1	4.3	4.3
Histidine	3.3	3.1	3.1	3.2	3.1
Arginine	8.3	8.1	8.7	8.4	7.9

[a] Single sample values of the respectively treated brans.
[b] An average of nine different wheats, according to Waggle et al. (15).
[c] Note that the cystine values of the experimental brans have not been corrected for destruction during sample hydrolyses, whereas that from Waggle et al. (15) was determined as cysteic acid in a preoxidized sample.

to established procedure[4]. Samples were hydrolyzed with $6N$ HCl at 104°C. for a 24-hr. period as suggested by Lewis (14), and no attempts were made to correct for possible destruction of cystine. All data were expressed as g. amino acid/16 g. nitrogen. The analyses (Table III) reveal little, if any, consistent difference in amino acid composition with irradiation. In general, the values obtained demonstrate reasonably good agreement with those averaged from Waggle et al. (15).

Experiment 3

Experiment 3 was designed to determine whether the nutritional value of the protein and phosphorus from the experimental brans was altered from that of the control and if the treatment reduced the effective cystine concentration. Eldjarn and Pihl (16) have contended that the cystine disulfide linkage, essential to the tertiary structure of most proteins, is particularly sensitive to ionizing radiation. Phosphorus in grains is known to be upward of 90% in the form of phytic acid (17); however, the extent of utilization from this organic form is highly variable owing to numerous dietary factors, e.g., level of inorganic calcium and/or phosphorus (18,19), amount of vitamin D_3 (20), associated cation (21), etc. Ionizing radiation could conceivably affect the ester linkage of this inositol hexaphosphate, and hence, availability of the large quantities of this valuable macromineral.

The respective experimental brans supplied all the dietary protein and phosphorus (Table IV). As a positive control, soybean meal with added

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[4] Technicon Corp., Chauncey, N. Y.

TABLE IV
COMPOSITION OF EXPERIMENTAL DIETS

INGREDIENT	N AND P-FREE	SOYBEAN MEAL	WHEAT BRAN
	%	%	%
Soybean meal	....	22.50	....
Bran	....	....	75.00
Corn oil[a]	....	....	18.00
Limestone	2.40	2.40	2.40
KH_2PO_4	....	2.65	....
NaCl	0.35	0.35	0.35
Micro minerals[b]	0.40	0.40	0.40
Vitamin mix[c]	0.20	0.20	0.20
Choline chloride	0.20	0.20	0.20
Glucose monohydrate[d]	(to 100%)	(to 100%)	(to 100%)
Protein (N × 6.25), %	0.00	12.85	13.00
Ca, %	1.64	1.64	1.56
P, %	0.00	0.62	1.01
Calc. ME, kcal./kg.[e]	3,240	2,447	2,447

[a] Refined corn oil, St. Lawrence Starch Co., Port Credit, Ontario.

[b] Supplies the following in g./kg. of complete diet: $CoCl_2.6H_2O$, 0.02; $MnSO_4$, 2.5; $FeSO_4$, 0.1; $MgSO_4.H_2O$, 0.2; KI, 0.006; $CuSO_4$, 0.012; $ZnCO_3$, 0.2; $Na_2MoO_4.2H_2O$, 0.01.

[c] Supplies the following per kg. of complete diet: thiamine.HCl, 20 mg.; riboflavin, 12 mg.; Ca pantothenate, 20 mg.; pyridoxine.HCl, 6 mg.; biotin, 0.6 mg.; menadione, 4 mg.; vitamin B_{12}, 0.02 mg.; ascorbic acid, 150 mg.; niacin, 60 mg.; folic acid, 3 mg.; vitamin A, 5,000 IU; vitamin D_3, 600 ICU; vitamin E, 7.5 IU (alpha-tocopherol acetate); ethoxyquin, 250 mg.

[d] "Clintose," Clinton Corn Products Co., Clinton, Iowa.

[e] Based on the following in kcal./g.; soybean meal, 2.53; bran, 1.49; corn oil, 8.81; glucose mono-hydrate, 3.24.

monobasic potassium phosphate was included in the design for purposes of comparing growth and feed efficiency. The remainder of the diet was composed of glucose monohydrate and/or refined corn oil, along with vitamins and minerals necessary to satisfy the complete requirements. All rations, with the exception of the diet without nitrogen and phosphorus, were calculated to be isocaloric.

Essential amino acids were added for two purposes: 1) to obtain levels with the potential to promote near-optimal growth, and 2) to facilitate determining if, and the extent to which, cystine was destroyed. The amino acids and levels of inclusion (Table V) were dependent upon the average analysis (Table III) and the requirements for maximal growth according to Dean and Scott (22). Supplementation of the soybean meal diet entailed the same procedure, except that amino acid values of the meal were based on those of Block and Bolling (23). To ascertain whether the nutritionally active cystine concentration had been affected by irradiation, this amino acid was omitted from the complete supplementing mixture. Under terms of adequate absolute methionine and a cystine level which was submarginal and solely dependent upon the bran, any alterations of this first-limiting protein unit due to irradiation should be revealed in decreases in growth and feed efficiency below that observed with birds fed the control diet. This technique of demonstrating cystine destruction had previously been shown to be effective with wheat germ meal that had been excessively autoclaved (24).

Single-comb White Leghorn male chicks were fed a commercial start-

TABLE V
ESSENTIAL AMINO ACID COMPOSITION AND NECESSARY SUPPLEMENTATION OF EXPERIMENTAL SEMIPURIFIED WHEAT BRAN DIETS (13% PROTEIN)

Amino Acid	Diet[a]	Requirement[b]	Percent of Requirement	Supplementation and Form	Amino Acid	Diet[a]	Requirement[b]	Percent of Requirement	Supplementation and Form
	%	%	%	%		%	%	%	%
Arg	1.09	1.10	99	0.05 L-HCl	Meth	0.18	0.45	40	0.25 DL (98%)
Hist	0.42	0.30	140	....	Thre	0.48	0.65	74	0.40 DL[e]
Lys	0.56	1.10	51	0.60 L-HCl (95%)	Leu	0.90	1.20	75	0.30 L
Tyr	0.46	0.50[e]	92	....	Ileu	0.43	0.80	54	0.75 DL[e]
Tryp[d]	0.16	0.225	71	0.07 L	Val	0.64	0.82	78	0.30 DL[e]
Phe	0.60	0.50[e]	120	....	Gly	0.91	1.60	57	1.00
Cys	0.25	0.35	71	0.15 L					

[a] Based on the average values of Table III.
[b] Requirements according to Dean and Scott (22).
[c] According to Fisher et al. (31).
[d] Tryptophan, 1.6% of protein—Block and Bolling (23).
[e] The D form of valine was considered completely utilizable, whereas the D isomers of threonine isoleucine were not (Leveille and Fisher, 32).

ing diet for 7 days. The birds were then individually weighed and placed in respective weight groups at 5-g. intervals on either side of the total average. Each weight group was then randomly distributed over the entire experimental area until each pen contained 10 chicks. In this manner, estimated growth potential variation within pens was approximately equal, and average pen starting weight differences were negligible. For 14 subsequent days both water and experimental feed were given on an *ad lib.* basis. At termination, feed consumption and body weights were determined. The chicks in each pen were sacrificed, frozen, and saved for analysis.

Net protein and net phosphorus utilization are the amounts of these nutrients retained of that consumed after correction for maintenance requirements. These parameters were determined as total carcass difference between that observed for the experimental and N- and P-free groups, divided by the absolute consumption, and expressed as a percentage. Nitrogen, phosphorus, and calcium were all determined on wet digests of feed and carcass aliquots. Representative carcass samples were obtained from freeze-dried ground frozen birds pooled from each pen. The N and P concentrations were obtained with the Technicon autoanalyzer and the suggested sodium phenate and phosphomolybdate techniques, respectively[5]. Calcium was determined by EDTA titration with hydroxynaphthol blue[6] as indicator (25). Statistical procedures entailed use of the analysis of variance in accordance with a completely randomized design (26). Duncan's Multiple Range Test (27) permitted evaluation of individual treatment differences.

The results of experiment 3 are illustrated in Table VI. No significant growth differences were observed between any groups of chicks when they

[5] Technicon Corp., Chauncey, N. Y.
[6] Mallinckrodt Chemical Works, St. Louis, Mo.

TABLE VI

UTILIZATION BY THE GROWING CHICK OF WHEAT BRAN PROTEIN AND PHOSPHORUS
AFTER ^{60}CO γ-IRRADIATION[a]

TREATMENT	3-WEEK[b] WEIGHT	G/F[c]	NPU	PHOSPHORUS Net Util-ization	PHOSPHORUS Reten-tion	CARCASS (MOISTURE-FREE) Ca:P	CARCASS (MOISTURE-FREE) Ca	CARCASS (MOISTURE-FREE) P
	g.		%	%	mg.		%	%
N and P-free	50e[d]	....	....	....	....	0.94a[d]	1.91 ± 0.01[e]	2.03 ± 0.02[e]
Soybean meal	96d	0.21d[d]	49.2d[d]	33.2e[d]	260ab[d]	1.02ab	1.77 ± 0.08	1.73 ± 0.02
+ EAA[f]	157a	0.40a	50.2d	37.6f	533e	1.11b	1.76 ± 0.13	1.59 ± 0.04
Control bran	117c	0.24c	36.0a	10.6a	232a	1.28c	1.83 ± 0.06	1.43 ± 0.04
+ EAA[g]	146b	0.37b	42.7bc	18.0c	378c	1.28c	1.94 ± 0.05	1.52 ± 0.06
No cystine[h]	147b	0.35b	41.7b	18.3c	386c	1.29c	1.96 ± 0.13	1.52 ± 0.06
0.5 Mrad bran	122c	0.26c	41.1b	13.6b	286c	1.28c	1.89 ± 0.20	1.48 ± 0.00
+ EAA[g]	160a	0.40a	44.3c	17.4c	400c	1.26c	1.83 ± 0.11	1.45 ± 0.03
No cystine	148b	0.36b	42.5bc	17.7c	378c	1.20c	1.77 ± 0.11	1.48 ± 0.04
5.0 Mrad bran	122c	0.26c	40.3b	13.4b	274b	1.26c	1.80 ± 0.08	1.43 ± 0.05
+ EAA[g]	157a	0.41a	45.2c	21.1d	451d	1.25c	2.03 ± 0.11	1.63 ± 0.06
No cystine	145b	0.35b	43.6bc	16.8c	367c	1.25c	1.85 ± 0.07	1.48 ± 0.06

[a] All values represent an average from four replicate groups of 10 chicks.
[b] Average 1-week starting weight was 70 g.
[c] Gain/feed consumed.
[d] Duncan's Multiple Range Test (27) at 5% level of significance. Those treatments without a common letter are significantly different from each other.
[e] Average of four determinations each on a pooled sample of 10 birds ± std. dev.
[f] 0.10% L-arginine.HCl, 0.30% L-lysine.HCl (95%), 0.30% L-cystine, 0.10% DL-methionine, 0.30% DL-threonine, 0.25% L-leucine, 0.20% DL-isoleucine, 0.20% DL-valine, and 1.00% glycine.
[g] Supplemented with the level and form as shown in Table V.
[h] The same amino acid mixture as described in footnote g, but without cystine.

were fed the basal rations containing the variously treated brans. When each ration was supplemented with the full complement of essential amino acids estimated to be necessary to meet requirements, a large improvement in growth was obtained in all cases. Those birds receiving the fully supplemented brans irradiated with 0.5 and 5.0 Mrad, though not different from each other, were significantly heavier than chicks fed a comparable control. When cystine was omitted from the amino acid mixture, gains were significantly reduced with the irradiated but not with the control bran diets; however, the absolute amount of growth obtained with each treatment was not different. In all cases, the ratio of gain to feed consumed exhibited the same patterns as observed for growth. Indicative that the birds fed the experimental brans were performing optimally, even though the diets appeared unduly bulky, is the near-equal growth and feed efficiency demonstrated by these chicks as compared with those receiving the supplemented isocaloric soybean meal ration. Consequently, it would seem that irradiation enhanced chick growth and feed utilization when more favorable nutritional conditions existed, whereas under terms of calculated equal but limiting cystine levels, irradiation was without apparent effect.

Contrary to observations on growth, differences in net protein and phosphorus utilization were evidenced without amino acid supplementation. In both cases the irradiation treatments were superior to that observed with the control bran. As expected, addition of essential amino acids improved nitrogen utilization in all cases; however, these NPU values were not signif-

icantly different from each other[7]. In every instance, removing cystine from the complete mixture caused reductions in protein efficiency which indicated that this sulfur amino acid was marginal; however, these decreases were not extensive enough to be statistically significant.

Both net phosphorus utilization and absolute retention significantly increased on addition of essential amino acids. When the supplementing mixture was complete, those increases were considerably more extensive with birds fed the bran irradiated with 5.0 Mrad than with those offered comparable diets containing either the control or the millfeed treated with 0.5 Mrad. Omitting cystine from the amino acid supplement caused a significant reduction in both net utilization and absolute retention with birds offered the bran with the higher level of irradiation; however, these decreases were not as apparent with the groups receiving the lower irradiation level and were completely absent with the control. The constancy of carcass Ca:P ratios from chicks fed all bran suggests that the observed utilization differences are not due to possible net changes in the manner by which the retained phosphorus was used. Waldroup et al. (21) have presented evidence to indicate that the phosphorus from calcium phytate and inorganic phosphate are used to different extents for growth and bone formation. The significantly larger absolute phosphorus retention and altered Ca:P ratios between birds of the same size fed bran and bran diets containing potassium phosphate suggest an altered net use after absorption.

DISCUSSION

Irradiation of wheat bran with cobalt-60 gamma rays has resulted in significantly better net protein and phosphorus utilization, as well as improved chick performance, when amino acid requirements were met. Because there were no observable ME alterations, increased digestibility of carbohydrate and/or protein as the reason for this improved feeding value is improbable. Absence of obvious changes in amino acid composition, particularly the first-limiting sulfur units, suggests that a protein quality change is not involved with this improvement. Under terms of an essentially complete organic dietary source of phosphorus in conjunction with a high calcium level (1.56% Ca determined), increased availability of phytic acid phosphate could explain the observed changes in growth and efficiency without alterations of amino acid composition or metabolizable energy. The primary observation which substantiates that there was improved phosphorus nutrition is the significant increase in net utilization and absolute retention with irradiation level when all amino acids were adequate and growth was unchanged. Supporting the contention of increased phosphorus availability is the recent study by Chung et al. (28) on the lipids of flour from gamma-irradiated wheat. These investigators find that there are considerable "polar phosphorus" increases in the water-saturated butanol extract. However, other explanations such as bacterial sterilization and inhibitor inactivation cannot be discounted.

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[7] It should be noted that no attempt was made to maintain isonitrogenous conditions between the respective basal and supplemented treatments; hence, care must be taken in comparing protein utilization values within a radiation dosage level.

The results obtained when cystine was the first and only limiting amino acid for growth suggest that the nutritionally active concentration of this disulfide amino acid was unaffected by irradiation. Under the aforementioned dietary circumstances, neither phosphorus (based on equal absolute retention) nor methionine (due to supplementation) was a critical factor; hence, any radiation effects on cystine activity should have had repercussions on chick growth and feed efficiency. The fact that omission of cystine from the complete supplement caused significant performance reductions with the birds fed the irradiated bran but not the control bran does not necessarily indicate that there were dietary concentration differences. On the contrary, greater growth with chicks fed the fully supplemented control bran diet most probably could be attained because of low phosphorus availability; thus a performance reduction on deletion of cystine could not be witnessed. The substantially lower retention and net utilization values of this macromineral with the control diets would tend to support this hypothesis.

According to Haissinsky (29), the products from the radiolysis of water are responsible for the oxidation of cysteine to cystine which, in turn, is ultimately degraded to the nutritionally inactive cysteic acid. The variability in reports on destruction of amino acids with irradiation could be due to different amounts of moisture in the treated material. The presence of radical or electron scavengers is known to greatly affect the rate of degradation in aqueous solutions (30). Thus, it should not be surprising to find the cystine concentration of this "dry" millfeed (about 10% moisture) relatively unaffected by gamma-irradiation.

Literature Cited

1. HICKMAN, J. R., McLEAN, D. L. A., and LEY, F. J. Rat-feeding studies on wheat treated with gamma irradiation. I. Reproduction. Food Cosmetics Toxicol. 2: 15–20 (1964).
2. FIFIELD, C. C., GOLUMBIC, C., and PEARSON, J. L. Effects of gamma-irradiation on the biochemical storage and breadmaking properties of wheat. Cereal Sci. Today 12: 253–257, 261–262 (1967).
3. KENNEDY, T. S. Studies on the nutritional value of foods treated with gamma radiation. I. Effects of some B-complex vitamins in egg and wheat. J. Sci. Food Agr. 16: 81–84 (1965).
4. KENNEDY, T. S. Studies on the nutritional value of foods treated with gamma radiation. II. Effects on the protein in source animal feeds, egg and wheat. J. Sci. Food Agr. 16: 433–437 (1965).
5. METTA, V. C., and JOHNSON, B. C. Biological value of gamma irradiated corn protein and wheat gluten. J. Agr. Food Chem. 7: 131–133 (1959).
6. TSIEN, W. S., and JOHNSON, B. C. The effect of radiation sterilization on the nutritive value of foods. IV. On the amino acid composition of garden peas and lima beans. J. Nutr. 68: 419–428 (1959).
7. JOHNSON, B. C., and METTA, V. C. The effect of radiation sterilization on the nutritive value of foods. I. Biological value of milk and beef proteins. J. Nutr. 59: 479–490 (1956).
8. SHEFFNER, A. L., ADACHI, R., and SPECTRO, H. The effect of irradiation processing upon the *in vitro* digestibility and nutritional qualities of proteins. Food Res. 22: 455–461 (1957).

9. LINKO, P., and MILNER, M. Treatment of wheat with ionizing radiations. V. Effect of gamma radiation on some enzyme systems. Cereal Chem. 37: 223–227 (1960).
10. PHILLIPS, G. O., and BAUGH, P. J. Energy transport in carbohydrates. I. Molecular aggregation effects. J. Chem. Soc. (Section A) 1966: 370–377.
11. PHILLIPS, G. O., BAUGH, P. J., and LOFROTH, G. Energy transport in carbohydrates. II. Radiation decomposition of D-glucose. J. Chem. Soc. (Section A) 1966: 377–383.
12. PHILLIPS, G. O., and YOUNG, M. Energy transport in carbohydrates. III. Chemical effects of gamma radiation on the cycloamylolysis. J. Chem. Soc. (Section A) 1966: 383–387.
13. HILL, F. W., and ANDERSON, D. L. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64: 587–603 (1958).
14. LEWIS, O. A. M. Short ion-exchange column method for estimation of cystine and methionine. Nature 209: 1239–1240 (1966).
15. WAGGLE, D. H., LAMBERT, M. A., MILLER, G. D., FARRELL, E. P., and DEYOE, C. W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. Cereal Chem. 44: 48–60 (1967).
16. ELDJARN, L., and PIHL, A. In Mechanisms in radiobiology, vol. II. M. Errera and M. Forssberg (eds.). Academic Press: New York (1960).
17. COSGROVE, D. J. The chemistry and biochemistry of inositol polyphosphates. Rev. Pure Appl. Chem. 16: 209–224 (1966).
18. VAN DE POPULIERE, J. M., AMMERMAN, C. B., and HARMS, R. H. The relationship of calcium-phosphorus ratios to the utilization of plant and inorganic phosphorus by the chick. Poultry Sci. 40: 951–957 (1961).
19. TEMPERTON, H., and CASSIDY, J. Phosphorus requirements of poultry. II. The utilization of phytin phosphorus by the chick for growth and bone formation. Brit. J. Poultry Sci. 5: 81–86 (1964).
20. WALDROUP, P. W., AMMERMAN, C. B., and HARMS, R. H. The availability of phytic acid phosphorus for chicks. 3. Effect of calcium and vitamin D_3 levels on the utilization of calcium phytate. Poultry Sci. 43: 926–931 (1964).
21. WALDROUP, P. W., AMMERMAN, C. B., and HARMS, R. H. The availability of phytic acid phosphorus for chicks. 2. Comparison of phytin phosphorus sources. Poultry Sci. 43: 426–432 (1964).
22. DEAN, W. F., and SCOTT, H. M. The development of an amino acid reference diet for the early growth of chicks. Poultry Sci. 44: 803–808 (1965).
23. BLOCK, R. J., and BOLLING, D. The amino acid composition of proteins and foods. Charles C. Thomas: Springfield, Ill. (1951).
24. MORAN, E. T., JR., SUMMERS, J. D., and BASS, E. J. Heat-processing of wheat germ meal and its effect on utilization and protein quality for the growing chick: Toasting and autoclaving. Cereal Chem. 45: 304–318 (1968).
25. CATLEDGE, G., and BIGGS, H. G. A new indicator for the chelometric measurement of calcium in serum and urine. Clinical Chem. 11: 521–526 (1965).
26. FEDERER, W. T. Experimental design. Macmillan: New York (1955).
27. DUNCAN, D. B. Multiple range and multiple F tests. Biometrics 11: 1–42 (1955).
28. CHUNG, O., FINNEY, K. F., and POMERANZ, Y. Lipids in flour from gamma-irradiated wheat. J. Food Sci. 32: 315–317 (1967).
29. HAISSINSKY, M. Nuclear chemistry and its applications. Addison-Wesley: Reading, Mass. (1964).
30. PURDIE, J. W. Gamma radiolysis of cystine in aqueous solution. Dose-rate effects and a proposed mechanism. J. Am. Chem. Soc. 89: 226–230 (1967).
31. FISHER, H., JOHNSON, D., JR., and LEVEILLE, G. A. The phenylalanine and tyrosine requirement of the growing chick with special reference to the utilization of the D-isomer of phenylalanine. J. Nutr. 62: 349–355 (1957).
32. LEVEILLE, G. A., and FISHER, H. Amino acid requirements for maintenance in the adult rooster. III. The requirements for leucine, isoleucine, valine and threonine, with reference also to the utilization of the D-isomers of valine, threonine and isoleucine. J. Nutr. 70: 135–140 (1960).