Heat-Processing of Wheat Germ Meal and Its Effect on Utilization and Protein Quality for the Growing Chick: Toasting and Autoclaving[1]

E. T. MORAN, Jr., and **J. D. SUMMERS,** Department of Poultry Science, University of Guelph, Guelph, Ontario, Canada; and **E. J. BASS,** International Milling Co., Minneapolis, Minnesota

ABSTRACT

Raw wheat germ meal was autoclaved for 20, 45, and 90 min. at 121°C., or toasted. Hemagglutination and antitrypsin activities were both very high for the raw and negligible for the toasted and autoclaved meals. The respective meals were used as sole sources of dietary protein (15 or 20%) in semipurified rations calculated to be isonitrogenous and isocaloric. Net protein utilization (NPU) values were obtained for the chick (7 to 21 days of age) with the 15% protein level. Toasting significantly improved NPU; the 20-min. autoclave treatment was without apparent effect. Further autoclaving progressively reduced NPU. Growth experiments employing the 20% protein level showed that cystine was the only amino acid limiting for the chick when the meals were raw, toasted, or autoclaved for 20 min. Pressure processing beyond 20 min. resulted in destruction of lysine, cystine, and arginine to the point where in the 90-min. autoclaved meal they became first through third limiting, respectively, and seriously impaired chick growth and feed utilization. Both energy (corn oil) and further amino acid supplementation of the 90-min. autoclaved meal were ineffective in improving chick growth and feed utilization comparably to those observed with the lesser processed meals.

Because of its relatively high protein content, abundance of vitamins, and ready availability, wheat germ meal has potential as a valuable and economic human feedstuff. To this end, another valuable asset of wheat germ is its present acceptability as a food. Wheat germ meal is sold throughout North America, in bulk as a feed ingredient and in major chain stores as a food for human consumption. Nevertheless, its biological advantages and disadvantages are relatively unknown. Studies on the growing chick may provide an insight into the nutritive value of wheat germ.

In studying the nutritive value of raw germ meal for the chick, Creek *et al.* (1) found a thermolabile factor which impaired digestion and/or utilization; autoclaving significantly improved growth and feed efficiency. Employing an extraction procedure previously used for raw soy and navy beans (2), Creek and Vasaitis (3) removed a water-soluble fraction from the raw germ that inhibited *in vitro* pancreatin digestion of casein; autoclaving inactivated the fraction. Further investigations by Creek *et al.* (4) revealed that autoclaving whole raw wheat germ also significantly increased nitrogen retention and fat utilization by the chick. Parrish and Bolt (5) confirmed the previously observed growth depression and increased fecal fat; however, they contended that the adverse effects of raw meal were physical in origin. The raw germ meal apparently formed a paste on the beaks of chicks that resulted in reduced feed consumption; the increased fecal fat was attributed to cleaning of beaks on the wire floors and contamination of the feces. In a reinvestigation with precautionary procedures, Attia and Creek (6) re-

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affirmed their original work and further reported pancreatic hypertrophy and detection of a hemagglutinin factor; again, autoclaving alleviated all detrimental properties of the meal.

Heat-processing of a raw vegetable feedstuff is not an unusual requirement to facilitate animal utilization. Heating of raw soybean meal is known to correct the depressed fat absorption by chicks (7). Similar processing of raw soybeans increased meal digestibility from 64 to 88% with the cockerel (8), and from 54 to 89% for the laying hen (9). Turkey poults are also known to realize a greater biological value from soybeans after heat-treatment (10). These observations of increased utilization by heating are not surprising if we consider that native proteins, because of their ordered and stabilized structure, are generally more resistant to enzymatic attack than are denatured ones (11).

Heat-processing may not only improve the value of feedstuffs but, if excessive, may reduce their effectiveness. The browning or Maillard reaction is known to occur between various free sugars and the free amino, quanido, and imidazole groups of proteins, resulting in nutritionally unavailable complexes (12,13,14). Temperature (15,16), moisture (17,18,19,20), duration (21), possibly reducing chemicals, e.g., sulfites (22), and availability of free sugars are the prime factors among the many considerations which can affect the rate of reaction. Soybean meal can be autoclaved at 120°C. for up to 60 min. without serious reduction in utilization or chick growth (23), but dried egg white, with even greater quantities of reactive groups, is severely damaged by autoclaving at 121°C. for 10 min. (24).

Lysine is the amino acid primarily affected by the browning reaction regardless of protein source (25,26,27,28,29,30). Lysine is also the most limiting amino acid for low-income humans (31), not to mention monogastrics of economic importance. Wheat germ protein is high in lysine (about 5.5% (31)) relative to other proteins of vegetable origin. Consequently, processing of the germ must be sufficient to ensure utilization of its inherent nutrients, but not so extensive as to detract from its prime asset, lysine.

Because of the previously mentioned detrimental properties of wheat germ meal and their alleviation by autoclaving, a series of experiments was initiated to investigate the effect of various heat-processing procedures on germ utilization and on protein quality for the chick.

MATERIALS AND GENERAL METHODS

Processing of Wheat Germ

Raw, high-purity wheat germ meal was autoclaved at 121°C. (15 p.s.i.) for 20, 45, and 90 min. in approximately 70-lb. lots. Each lot was placed to a depth of 2 in. in specially constructed wire-bottomed autoclave trays. After completion, the hot germ was broken up by hand and placed on racks of a Proctor-Swartz oven to a depth of 1.5–2 in. In the oven, the germ was dried at 50°C. with continuous air circulation for 3 hr.; every 30 min. during the drying cycle the meal was stirred manually. Sifting through a No. 8 wire screen and blending in a large Strong-Scott mixer yielded uniform end products which were stored in polyethylene-multiwall-burlap units of 70 lb. at –1°C. until used.

A different sample of wheat germ meal was toasted by the "Kretschmer process," a 45-min. treatment in a rotary drum dryer at 121°C. The drying chamber is sealed, so that the germ dries essentially under its own vapor pressure. Sufficient moisture is pumped from the system to dry the germ from an initial moisture content of 12–13% to a final 5%.

Analyses of Wheat Germ

Standard procedures (32) were used to determine moisture, ash, protein, fat, and fiber (Table I).

TABLE I

PROXIMATE ANALYSIS OF THE EXPERIMENTAL WHEAT GERM MEAL SAMPLES WITH THEIR HEMAGGLUTININ AND ANTITRYPSIN ACTIVITIES[a]

WHEAT GERM SAMPLE	MOISTURE	ASH	PROTEIN[b]	FAT	FIBER	HEMAGGL. ACTIVITY[c]		ANTITRYPSIN ACTIVITY
						Regular	Papain	
	%	%	%	%	%			
Raw	13.8	4.41	29.0	10.3	2.1	2,560	5,120	very high
Toasted	5.4	4.85	30.5	9.6	2.2	0	20	trace
Autoclaved								
20 min.	7.4	4.81	28.6	9.5	2.5	0	320	0
45 min.	6.0	3.88	28.7	9.8	2.3	0	160	0
90 min.	7.5	4.78	29.0	9.7	2.6	0	160	0

[a] All data have been adjusted to 14% moisture basis.
[b] Kjeldahl N $\times$ 6.25.
[c] Expressed as dilution necessary to attain zero activity.

The hemagglutinin assays were carried out according to the method of Liener and Hill (33), both with and without papain-sensitized chicken red blood cells and as modified by Attia and Creek (6) for wheat germ meal. Antitrypsin activities were determined by the delayed gelation technique described by Learmonth and Wood (34) with wheat flour.

Net protein utilization (NPU) was termed the difference in total carcass protein between those chicks fed the N-free and the experimental rations divided by the protein consumed, quantity $\times$ 100. NPU takes into consideration the maintenance requirements in addition to growth necessities. Composition of the N-free diet was the same as that of the experimental ration, with the exception that glucose monohydrate replaced the dietary protein source. Protein was determined by the standard Kjeldahl procedure (N $\times$ 6.25) on representative aliquots of diet or lyophilized whole carcass.

Experimental Design

To obtain maximal effects, the protein of all experimental rations was supplied entirely by either wheat germ meal or soybean; the remainder of the diet supplied purified carbohydrates and fat, as well as adequate vitamins and minerals (Table II). The differential levels of protein in the experimental meals, due to moisture content variations, were compensated for in the diets at the expense of glucose monohydrate.

Single-comb White Leghorn chicks were raised from hatching to 1 week of age on a standard starting ration. The birds were then individually weighed and placed in respective weight groups at 5-g. intervals on either side of the total group mean. Random distribution of each weight group over the en-

TABLE II
COMPOSITION OF EXPERIMENTAL RATIONS

INGREDIENT	WHEAT GERM MEAL		SOYBEAN		
			Isolated[a]		Meal, 20% Protein
	15% Protein	20% Protein	15% Protein	20% Protein	
	%	%	%	%	%
Wheat germ meal[b]	47.00	63.8	....	....	....
Isolated soybean	....	....	18.10	24.24	....
Soybean meal (44% protein)	....	....	....	....	45.45
Starch[c]	15.00	15.00	15.00	15.00	15.00
Glucose monohydrate[d]	30.95	14.15	58.60	52.61	25.30
Corn oil	2.00	2.00	2.00	2.00	8.10
Choline chloride	0.20	0.20	0.20	0.20	0.20
Macro mineral mix[e]	4.25	4.25	4.25	4.25	4.25
Micro mineral mix[f]	0.40	0.40	0.40	0.40	0.40
Vitamin mix[f]	0.20	0.20	0.20	0.20	0.20
DL-methionine	....	....	0.25	0.20	0.20
Glycine	....	....	1.00	0.90	0.90

[a] C-1 Assay protein, Archer, Daniels, Midland Co., Cincinnati, Ohio.
[b] Adjustments were made at the expense of glucose monohydrate to ensure isonitrogenous conditions with each experimental meal.
[c] "Ground corn starch," St. Lawrence Starch Co., Port Credit, Ontario.
[d] "Clintose," Clinton Corn Products Co., Clinton, Iowa.
[e] Supplies the following percentage of minerals: dicalcium phosphate (21% Ca, 20% P), 2.5; limestone (37% Ca), 1.00; sodium chloride, 0.40; and, potassium chloride, 0.35.
[f] Calculated to supply the complete requirement for vitamins and minerals; see Moran *et al.* (55).

tire experimental area considerably reduced "between-pen" (or replicate) variation. Electrically heated brooder batteries with raised wire floors housed the birds and permitted *ad libitum* feed and water. After a 2-week experimental period (3 weeks of age) each pen of chicks was weighed and total feed consumption determined.

The data were analyzed by the procedure for a completely randomized design (35). Duncan's Multiple Range Test permitted individual treatment comparisons at the 5% level of significance (36). The lower-case letters after the experimentally derived figure in the respective tables indicate statistical confidence; those treatments without a common letter are significantly different from each other; those with a common letter are not.

SPECIFIC EXPERIMENTAL PROCEDURE AND RESULTS

Experiment 1

Experiment 1 was designed to determine the NPU of raw and experimentally heat-treated meals by the chick.

For NPU determination, the 15% protein rations described in Table II were used in all cases. At this low protein level, all essential amino acids except threonine and histidine were calculated to be deficient.[2] Consequently any destruction of amino acids by processing should be revealed in alteration of NPU value. Each ration was fed to four replicate pens of chicks (10 per pen) for a 2-week experimental period. At termination, each pen of birds was sacrificed and all carcasses per replicate pooled for nitrogen determination.

[2] Based on the amino acid requirements of the growing chick established by Dean and Scott (37) and the value for "wheat germ" (Table IV) by Block and Bolling (31).

TABLE III

GROWTH, FEED EFFICIENCY, AND NET PROTEIN UTILIZATION BY CHICKS FED VARIOUS HEAT-PROCESSED WHEAT GERM MEALS AT 15% PROTEIN DIETARY LEVEL

TREATMENT	3-WEEK WEIGHT[a]	GAIN / Feed Consumed	NET PROTEIN UTILIZATION
	g.		
Isolated soybean protein	159f[b]	0.42	59.4e[b]
N-free	47a	....	....
Raw wheat germ	135e	0.35	47.7c
Toasted	154f	0.38	53.0d
Autoclaved			
20 min.	127d	0.33	48.4c
45 min.	118c	0.29	43.9b
90 min.	83b	0.13	32.9a

[a] Average 1-week starting weight was 67 g. for each treatment. The 3-week weight represents an average of four pens of ten chicks per pen.

[b] Duncan's Multiple Range Test (36) at 5% level of significance. Note that NPU data were analyzed after transformation to angles (59).

The results of Experiment 1 are shown in Table III. Toasting significantly improved NPU of wheat germ meal; autoclaving for 20 min. was without apparent effect. Further pressure processing beyond 20 min. progressively depressed NPU below that observed with raw meal. The NPU of isolated soybean can be compared with that of heat-treated germ meals, because protein level was equivalent and caloric density differences were not expected to be excessive (39). The significantly higher NPU of isolated soy is probably due to its supplementation with glycine and first limiting methionine. The observation of equivalent growth between chicks fed isolated soy and toasted wheat germ should indicate comparable protein availability and quality.

Growth and feed efficiency of birds fed the respective germ meals followed the same trend as NPU, with the exception of chicks fed the 20-min.-autoclaved meal. NPU values of raw and 20-min. autoclaved meals indicate at least equivalent nitrogen utilization. However, growth of chicks fed the 20-min. heat-treated meal was significantly inferior to that with raw meal. Destruction of the calculated first limiting amino acid, methionine, directly or indirectly by cystine inactivation, could account for the growth differences.

The color of wheat germ meals varied from a light yellow for the raw, golden for the roasted and 20-min. autoclave treatment, to a dark brown for the 90-min. autoclaved meal. The feces of chicks receiving the meals so treated were correspondingly colored. The feces of birds receiving the 90-min. treated meal were especially black and gummy. Those relative gradations of color support the general opinion that products formed by the browning reaction are not digestible.

Experiment 2

Experiment 2 was carried out to verify the amino acid deficiencies calculated for 20% protein wheat germ meal rations and to determine whether these inadequacies are altered by processing.

According to Table IV, five amino acids were calculated to be deficient for the chick. These, in order of limitation, are methionine, glycine, cystine,

TABLE IV

CALCULATED ESSENTIAL AMINO ACID CONTENT OF THE 20%-PROTEIN, SEMIPURIFIED WHEAT GERM MEAL RATIONS

	WHEAT GERM MEAL[a]		REQUIREMENT[b]		
AMINO ACID	Percent of Protein	Percent of Diet	Percent of Diet	Percent Supplied	LIMITATION ORDER
	%	%	%	%	
Arginine	6.0	1.20	1.10	109	
Histidine	2.5	0.50	0.30	167	
Lysine	5.5	1.10	1.10	100	
Tyrosine	3.8	0.76	0.63	121	
Tryptophan	1.0	0.20	0.225	89	5
Phenylalanine	3.0	0.60	0.68	88	4
Cystine	1.4	0.28	0.35	80	3
Methionine	1.3	0.26	0.45[c]	58	1
Threonine	6.3	1.26	0.65	194	
Leucine	6.7	1.34	1.20	112	
Isoleucine	4.5	0.90	0.80	113	
Valine	4.3	0.86	0.82	105	
Glycine[d]	5.7	1.14	1.60	71	2

[a]Based on figures of Block and Bolling (31).

[b]As established by Dean and Scott (37).

[c]Reduced by the safety factor from 0.55 to 0.45.

[d]For defatted wheat germ meal (Block and Weiss, 38).

phenylalanine, and tryptophan. With the exception of glycine, the quantity of deficient amino acid supplemented approximated the difference between that calculated to be present in the diet and that termed optimal. Because of the variability in glycine requirement and its ability to possibly increase feed utilization, an additional 1% glycine above the requirement was used (40). Direct cystine supplementation was avoided by inclusion of additional methionine.[3]

Regardless of processing, glycine was without effect in evoking either a growth or feed utilization response (Table V). Methionine supplementation significantly improved chick growth with all experimental meals except that autoclaved 90 min. Further amino acid additions of tryptophan and phenylalanine were ineffective in stimulating gain above that observed with methionine, and were without benefit for those chicks fed the 90-min. autoclaved germ. The soybean meal ration of near equal energy density and supplemented with methionine and glycine compared favorably with the corresponding toasted and 20-min. autoclaved wheat germ diets.

The greater the extent of heat-treatment, the poorer the observed chick growth and feed efficiency. Regardless of amino acid supplements, the toasted and 20-min. autoclaved wheat germ meals supported the best gains and feed efficiencies; the raw and 45-min. autoclaved meals were consistently inferior. Autoclaving 90 min. significantly reduced growth and feed utilization below that observed for any other treatment.

Experiment 3

Experiment 3 was designed to determine whether sulfur amino acids were being destroyed by the heat-processing and if deficiencies of lysine and/

[3]The SCWL chick readily converts methionine to cystine to meet demands for maximal growth; however, cystine can only spare methionine to a limited degree (41,42).

TABLE V

GROWTH AND FEED EFFICIENCY OF CHICKS FED RAW AND HEAT-TREATED WHEAT GERM MEAL RATIONS SUPPLEMENTED WITH AMINO ACIDS CALCULATED TO BE DEFICIENT (20% Protein level)

			WHEAT GERM				
					Autoclaved (121°C., 15 p.s.i.)		
SUPPLEMENT	3-WEEK[a] CRITERIA	SOYBEAN MEAL (44% PROTEIN)	Raw Meal	Toasted Meal	20 Min.	45 Min.	90 Min.
Basal	wt., g.	209 ab[b]	164 ghi	178 efg	172 fgh	152 ij	98 k
	G/F[c]	0.59	0.44	0.50	0.45	0.40	0.23
Glycine	wt., g.	....	157 hi	186 cdef	171 fgh	140 j	91 k
	G/F	....	0.44	0.51	0.45	0.40	0.22
Gly. + methionine	wt., g.	....	181 defg	217 a	203 abcd	190 cedf	104 k
	G/F	....	0.53	0.63	0.59	0.53	0.24
Gly. + meth. + trypt.	wt., g.	....	185 cedf	205 abc	207 abc	188 cedf	98 k
	G/F	....	0.57	0.61	0.58	0.52	0.26
Gly. + meth. + trypt. + phenylalanine[d]	wt., g.	....	175 fgh	210 ab	198 abcde	193 bcdef	105 k
	G/F	....	0.54	0.63	0.60	0.51	0.29

[a] Average 1-week starting weight was 65 g. for each treatment. The 3-week weight represents an average of three pens of ten chicks per pen.

[b] Duncan's Multiple Range Test at 5% level of significance (36).

[c] Gain/feed consumed.

[d] Levels of supplemental amino acids when used were: glycine, 1.4; DL-methionine, 0.3; L-tryptophan, 0.05; DL-phenylalanine, 0.2% of diet.

or arginine were being induced by excessive autoclaving.

As in experiment 2, the 20% protein level was employed; both DL-methionine and L-cystine were supplemented in quantities to approximate the difference between calculated diet content and requirement. Lysine and arginine were added arbitrarily to supply one-half their requirement (0.6%).

In the presence of added cystine, the omission of methionine from the diets containing the raw, toasted, or 20-min. autoclaved wheat germ meals was without significant effect on chick growth (Table IV). Removal of cystine and half the supplemental methionine should have made sulfur amino acids suboptimal; however, there were still no significant differences in gain with those chicks fed either the fully supplemented or sulfur amino acid-low rations containing the aforementioned germ meals. Autoclaving 45 min. and removal of methionine or cystine with half the methionine caused significant growth depressions which were respectively more severe. The meal autoclaved 90 min. further accentuated the depressions and differences observed with autoclaving 45 min. Consequently, it would seem that the sulfur amino acid content of wheat germ meal was underestimated by calculation.

Inclusion or omission of lysine or arginine in the various heat-treated germ diets caused variable results (Table VI). Removal of lysine from the complete amino acid mixture caused no reduction in growth until the germ was autoclaved 90 min. Although arginine was calculated to be in excess of the requirement, its removal from the ration in the presence of added lysine and sulfur amino acids caused significant growth depressions with the raw, toasted, 20-min., and 90-min. autoclaved meals. Deletion of either

TABLE VI

EFFECT OF LYSINE, ARGININE, AND SULFUR AMINO ACID SUPPLEMENTATION ON GROWTH AND FEED EFFICIENCY OF CHICKS FED RAW AND HEAT-TREATED WHEAT GERM MEAL RATIONS
(20% Protein level)

			Wheat Germ				
					Autoclaved (121°C., 15 p.s.i.)		
Supplement	18-Day[a] Criteria	Soybean Meal (50% Protein)	Raw Meal	Toasted Meal	20 Min.	45 Min.	90 Min.
Basal	wt., g.	172 no[b]	146 ef	159 ijkl	157 ghijk	130 bc	90 a
	G/F[c]	0.50	0.45	0.48	0.46	0.38	0.18
+ EAA[d]	wt., g.	....	158 hijkl	174 nop	172 no	166 klmn	152 fghi
	G/F	....	0.51	0.55	0.54	0.52	0.44
w/o Methionine	wt., g.	....	149 efgh	171 mn	167 lmn	152 fghi	142 de
	G/F	....	0.47	0.53	0.52	0.47	0.39
w/o Cyst. and ½ Meth.[e]	wt., g.	....	148 efg	171	167	144 def	127 b
	G/F	....	0.45	0.53	0.51	0.43	0.33
w/o Lysine	wt., g.	....	159 ijkl	183 p	179 op	167 lmn	97 a
	G/F	....	0.52	0.59	0.56	0.50	0.24
w/o Arginine	wt., g.	....	137 cd	153 fghi	156 ghij	168 mno	142 de
	G/F	....	0.43	0.48	0.49	0.51	0.41

[a] Average 1 week starting weight was 69 g. for each treatment. The 18-day weight represents an average of three pens of 10 chicks per pen.

[b] Duncan's Multiple Range Test at the 5% level of significance (36).

[c] Gain/feed consumed.

[d] EAA = DL-methionine, 0.20; L-cystine, 0.10; L-lysine · HCl (99%), 0.6, and L-arginine · HCl, 0.60.

[e] EAA mixture without the cystine and half the supplemental methionine.

lysine or arginine from the rations containing the 45-min. autoclaved wheat germ had no effect on chick growth. Considering the total calculated dietary lysine level (1.7%), the variability in results may be explained in terms of a lysine-arginine interaction (43). Removal of supplemental arginine allowed expression of a lysine toxicity (44); detoxification was facilitated by extensive autoclaving. Thus, deficiencies of lysine or arginine were not truly encountered until the meal was autoclaved 90 min.

Experiment 4

Experiment 4 was performed in two parts: to determine 1) whether cystine and/or methionine was being nutritionally inactivated by the heating process, and 2) if further amino acid supplementation of the 90-min. (121°C.) autoclaved wheat germ meal ration would improve chick performance.

The wheat germ rations (20% protein) were supplemented with L-lysine· HCl (99%) and L-arginine · HCl to avoid deficiencies (0.4%). When added, only 0.1% DL-methionine was used; consequently, this amino acid should be marginal (about 0.36%), yet high enough to support optimal or near optimal growth. Cystine was added in quantities (0.30%) to nearly meet the requirement (0.35%), the rationale being that if cystine at best can only partially spare methionine (41,42), then any inactivation of this monosulfur amino acid would, if it is critical, be revealed by chick growth depression. Destruction of only cystine would not result in altered growth because of the supplemental plethora.

There were no differences between those birds receiving the complete

TABLE VII

EFFECT OF HEAT-TREATMENT OF WHEAT GERM MEAL ON THE SUPPLEMENTARY SULFUR AMINO ACID LEVELS NECESSARY FOR MAXIMAL GROWTH AND FEED EFFICIENCY (20% Protein)

Supplement	18-Day[a] Criteria	Soybean Meal (50% Protein)	Wheat Germ				
			Raw Meal	Toasted Meal	Autoclaved (121°C., 15 p.s.i.) 20 Min.	45 Min.	90 Min.
Basal	wt., g.	180 kl[b]	148 d	176 jk	158 fgh	133 c	93 a
	G/F[c]	0.52	0.46	0.52	0.47	0.42	0.20
+EAA[d]	wt., g.	....	163 fghi	194 m	188 lm	182 kl	163 fghi
	G/F	....	0.50	0.60	0.59	0.58	0.46
w/o Methionine	wt., g.	....	156 defg	188 lm	191 m	180 kl	164 fghi
	G/F	....	0.48	0.58	0.58	0.58	0.45
w/o Cystine	wt., g.	....	157	186 lm	174 jk	163 fghi	132 c
	G/F	....	0.48	0.57	0.52	0.49	0.36
w/o Meth. and cystine	wt., g.	....	147 de	170 ij	163 fghi	139 c	109 b
	G/F	....	0.46	0.51	0.46	0.40	0.27

[a] Average 1 week starting weight was 71 g. for each treatment. The 18-day weight represents an average of three pens of 10 chicks per pen.

[b] Duncan's Multiple Range Test at the 5% level of significance (36).

[c] Gain/feed consumed.

[d] EAA = L-lysine · HCl (99%), 0.4%; L-arginine · HCl, 0.4%; L-cystine, 0.3%; and DL-methionine, 0.1% of diet.

and methionine-devoid amino acid supplements (Table VII). Upon deletion of cystine from the amino acid mixture, only raw and toasted wheat germs promoted chick growth comparable to the respective fully supplemented groups; those groups receiving the 20-, 45-, and 90-min. autoclaved meals were significantly poorer. With one exception, removal of both methionine and cystine from the rations resulted in depressing growth to that of the basals. Birds fed the 90-min. autoclaved meal performed significantly better when lysine and arginine were added to the diet than those chicks fed the basal ration. Thus, it would seem that methionine is adequate for optimal growth and not destroyed by processing, whereas cystine is submarginal and sensitive to heat-treatment.

Prior experiments have shown that autoclaving wheat germ meal 90 min. resulted in deficiencies of sulfur amino acids, lysine and arginine; however, even with appropriate supplementation, chick performance was not fully recovered. On the assumption that 1) the browning reaction is responsible for the majority of the induced deficiencies, 2) the α-amino group of terminal protein units is as reactive as the lysine ε-amino group, and 3) because of the heterogeneity of germ proteins, the probability of each amino acid being a terminal one is approximately equal, amino acids previously marginal could be deficient after extensive processing.

To determine whether deficiencies induced by excessive processing could be overcome by further amino acid supplementation, 25% of the requirement for glycine, tryptophan, and valine[4] was arbitrarily added. Because of increased quantities supplied by the germ relative to optimal, only 10% each of

[4] The D isomer of the DL-valine supplement used was considered completely available, whereas D-isoleucine was not (45).

the requirement for leucine and isoleucine was used. Phenylalanine was not supplemented, because the present requirement (0.68) was considered excessive for the chick (46).

Further additions of glycine and tryptophan to 90-min. autoclaved meals already supplemented with sulfur amino acids, lysine and arginine, had no effect on chick growth, although there was a trend toward greater feed efficiency (Table VIII). Additional supplementation with valine resulted in significantly better chick growth than was observed without the three amino acid combinations. The depression of chick gain upon addition of leucine and its return to previous levels with isoleucine supplementation is indicative of the specific leucine-isoleucine-valine interaction (47,48) and shows no further benefit from amino acids.

TABLE VIII
EFFECT OF FURTHER AMINO ACID SUPPLEMENTATION OF THE 90-MINUTE (121°C.) AUTOCLAVED WHEAT GERM MEAL RATIONS ON CHICK GROWTH AND FEED EFFICIENCY (20% Protein)

SUPPLEMENT	18-DAY WEIGHT[a]	GAIN / Feed Consumed
	g.	
Basal	93 a[b]	0.20
+ EAA[c]	156 defg	0.44
+ glycine	164 fghi	0.48
+ gly. + tryptophan	159 fgh	0.49
+ gly. + trypt. + valine	167 hij	0.50
+ gly. + trypt. + val. + leucine	155 def	0.47
+ gly. + trypt. + val. + leucine + isoleucine[d]	167 hij	0.50

[a]See corresponding footnote, Table VII.
[b]Duncan's Multiple Range Test (36). These values may be statistically compared with those in Table VII because they are all part of the same experiment.
[c]EAA = L-lysine · HCl (99%), 0.60; L-arginine · HCl, 0.40; L-cystine, 0.30; and DL-methionine, 0.2.
[d]When supplemented the following amino acids were added at the expense of glucose monohydrate: glycine, 0.40; L-tryptophan, 0.05; DL-valine, 0.20; L-leucine, 0.10; and DL-isoleucine, 0.25%.

Experiment 5

Other studies on the same heat-processed meals have shown that extensive decreases in metabolizable energy occurred when wheat germ was autoclaved 90 min.[5] In an attempt to fully recover chick performance and feed utilization lost by extensive processing, both amino acids and energy were supplemented.

The 20- and 90-min. autoclaved wheat germs were the only meals used (20% protein). Methionine, cystine, lysine, arginine, glycine, tryptophan, and valine were added to all rations at the expense of glucose and at the same levels described in experiment 3. Based on the ME of 3.03 kcal./g. for the 20-min. and 2.76 kcal./g. for the 90-min. autoclaved wheat germ meal, isocaloric conditions between the two identically composed diets (Table II) would be approached if 3% corn oil (about 8.8 kcal./g.) were substituted for an equal amount of glucose monohydrate (about 3.2 kcal./g.). Vantress × White Rock female day-old chicks were fed 4 days on a standard starting

[5]With the adult rooster the ME in kcal./g. for the raw, toasted, 20-, 45-, and 90-min. autoclaved meals were 2.97, 3.05, 3.03, 2.98, and 2.76, respectively (49).

ration, then wing-banded and individually weighed. The birds were then distributed over the experimental plot so that variation within pens was equal, and between pens, negligible. At 2 weeks of age the chicks were again weighed individually and feed consumption was determined.

The data for experiment 5 are shown in Table IX. With or without added

TABLE IX
EFFECT OF ENERGY SUPPLEMENTATION OF THE 90-MIN. (121°C.) AUTOCLAVED WHEAT GERM MEAL RATIONS ON CHICK PERFORMANCE AND FEED UTILIZATION
(20% Protein)

TREATMENT	2-WEEK WEIGHT[a]	GAIN / Feed Consumed
	g.	
20-min. autocl. wheat germ + EAA[b]	206 b	0.80
90-min. autocl. wheat germ + EAA	170 a	0.62
90-min. autocl. wheat germ + EAA + corn oil[c]	168 a	0.60

[a]Average 4-day starting weight was 60 g. for each treatment. The 2-week weight represents an average of three pens of eight chicks per pen.
[b]EAA = DL-methionine, 0.20; L-cystine, 0.30; L-lysine · HCl (99%), 0.60; L-arginine · HCl, 0.60; glycine, 0.40; L-tryptophan, 0.05; and, DL-valine, 0.20.
[c]Corn oil replaced 3% glucose monohydrate to make rations isocaloric.

corn oil, birds fed the 90-min. autoclaved wheat germ meal rations were significantly poorer than those birds offered the 20-min. autoclaved germ diet. Although it is not statistically verifiable, addition of energy in the form of corn oil tended to reduce both growth and feed utilization. The inability of both essential amino acids and energy to improve the extensively heated germ suggests that a large portion of the meal was irreparably damaged, and changes other than those related to amino acids and energy were involved.

After completion of our experimental work, an extensive study on the analyses of flour and millfeeds by Waggle *et al.* (50) appeared. Part of this study entailed amino acid analyses of germ from several types of wheat. Calculations of essential amino acids, based on a 20% protein dietary level and the average of reported values, are shown in Table X. These data essentially support the observations reported herein; namely, methionine was marginal and aromatic amino acids were adequate. However, contrary to our results with chick growth as an assay, cystine was calculated to be more than sufficient. This apparent discrepancy may be explained by considering both the possible partial oxidation of cystine during the milling process and heat-treatment and the method of determination. Performic acid treatment of the protein not only converts the cystine and cysteine to cysteic acid but also all intermediate oxidized forms; i.e., both nutritionally active and inactive forms are measured. Another discrepancy between diet content and chick growth studies existed with respect to isoleucine; however, these results can be explained in terms of a conservative estimate of requirement based on a diet containing high-energy purified amino acids (0.80%, Dean and Scott (37)) *vs.* a more realistic value determined with whole proteins (0.75% NRC, 0.65% France, and 0.50% ARC (51)).

TABLE X
CALCULATED ESSENTIAL AMINO ACID CONTENT OF THE 20% PROTEIN SEMIPURIFIED WHEAT GERM MEAL DIETS BASED ON MORE RECENT GERM PROTEIN ANALYSES

AMINO ACID	WHEAT GERM MEAL[a]		REQUIREMENT[b]		LIMITATION ORDER
	Percent of Protein	Percent of Diet	Percent of Diet	Percent Supplied	
	%	%	%	%	
Arginine	8.2	1.64	1.10	149	
Histidine	2.9	0.58	0.30	193	
Lysine	6.5	1.30	1.10	118	
Tyrosine	3.1	0.62	0.63	98	4
Phenylalanine	4.1	0.82	0.68	121	
Cystine	2.1	0.42	0.35	120	
Methionine	2.0	0.40	0.45	89	2
Threonine	4.2	0.84	0.65	129	
Leucine	6.9	1.38	1.20	115	
Isoleucine	3.6	0.73	0.80	91	3
Valine	5.0	1.00	0.82	122	
Glycine	6.3	1.26	1.60	79	1

[a]Based on an average of the amino acid analyses conducted by Waggle *et al.* (50).
[b]According to Dean and Scott (37).

DISCUSSION

The toasting procedure of processing wheat germ meal always proved nutritionally better than any autoclaving treatment. This superiority was particularly true when either protein was critical (15%) or sulfur amino acids low, or both. The observed differences in quality can be related back to one outstanding difference in processing—the presence of moisture. The quantity of moisture can greatly affect both the efficiency of denaturation and the rate of browning.

With denaturation of raw wheat germ protein, one would expect increased digestibility because of decreased resistance to enzymatic degradation and destruction of toxic factor(s). During denaturation, destruction of cystine by cross-link cleavage is likely, and consequently is an expense of the process. The resultant low moisture content of germ meal with toasting and its removal from the system upon evaporation are probably the reasons for its superiority over the 20-min. autoclaving treatment. It would appear that denaturation was sufficiently extensive to facilitate digestion, but not so complete as to detract from the meal by destroying its first limiting amino acid, i.e., hydrogen bond disruption but not cross-link cleavage. The cystine content of protein is known to be affected by heat-treatment (52) regardless of the presence of glucose or extent of browning (53). Almquist *et al.* (54) believe that this destruction is a necessary prerequisite in the availing of amino acids from soybeans and liken it to increased digestibility of keratins with cystine degradation (46,55,56). On the basis of results of experiments published herein, it is more likely that cystine destruction is an indication of denaturation in proteins containing low or moderate quantities of this disulfide amino acid and is not necessarily a desirable phenomenon.

Moisture content may also affect destruction of lysine and arginine, and hence, protein quality. The presence of moisture probably facilitates the browning reaction by acting as a carrier for the free sugar(s) and as a mode by which the reaction ensues. Because of the usual low concentrations of free monosaccharides *per se,* moisture could also facilitate availability of substrate(s) by entering directly into hydrolytic reactions with more complex carbohydrates such as starch and dextrin. Varying the moisture content of soybean flour before autoclaving has been shown to greatly influence the amount of lysine and arginine destroyed (19); however, too much water can reduce the extent of destruction (20). This reduction of browning with large quantities of water, apparently by preventing direct oxygen exposure, supports the view that the browning reaction is, in part, oxidative, as does inhibition by sulfites (57), and suggests another possible method of processing.

According to the present nutritional studies on toasted meal, wheat germ protein is at least equivalent to, and most likely (by virtue of a small but definite methionine advantage) better than soybean protein. However, it should be noted that this quality assessment is in relation to the growing chick, whose amino acid demands could and do vary extensively when compared with other species. Assuming a comparable relative amino acid composition of the keratin-free carcass, the human infant might be expected to better use wheat germ protein than the chick. The infant, unlike the chick, does not have to contend with extensive high-cystine-requiring keratin synthesis; and reduction in requirement for the first limiting amino acid would, in turn, allow a greater net protein utilization.

Addendum

After completion of this experimental work and manuscript, amino acid analyses of the respectively treated wheat germ meals in conjunction with protein utilization studies on rats were conducted by Miss Ellen Olsen of the Department of Nutrition. These analyses and experimentation results have been submitted to another journal for publication (58). In essence, her amino acid values substantiate the conclusions drawn by the investigations herein; namely, lysine and arginine are destroyed, the degree of destruction depending on heating extent, whereas methionine appears unaffected. Cystine was not determined.

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