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The Biological Value of the Proteins of Maize and Maize Supplemented with Lysine and Tryptophane.

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In the continuation of our studies on the nutritive value of the different plant proteins, the proteins of maize were investigated by means of the Thomas-Mitchell method for determining the biological values of proteins.

As a feedingstuff maize occupies a unique position in this country in that it is the cheapest and most extensively grown animal feed, which provides both the nutritional elements now known to be seriously deficient in the natural pastures of the summer rainfall area during the winter seasons. Although maize is richest in carbohydrate and hence provides an excellent source of energy during winter supplementation, it has also been shown to provide sufficient protein for the maintenance requirements of animals when fed in the correct quantities. In maize, therefore, this country possesses a valuable animal feed which should be utilized to its fullest extent. It is, therefore, essential that a detailed knowledge about its nutritional qualities should be available. For this reason a special study has been undertaken to investigate the protein of maize in order to ascertain to what extent it can be utilized by the animal and which components limit its utilization so that, if possible, these deficiencies can be eliminatel or rectified in practice by scientific supplementation.

According to Osborne and Mendel (1914) zein the most important of the maize proteins is deficient in the essential amino acids lysine and tryptophane. Zein supplemented with tryptophane is capable of supplying the maintenance requirements of rats, but growth can only take place after further supplementation with lysine. A similar deficiency has also been established by Hogan (1917) for the proteins of the whole maize. In these experiments conducted by him the protein intakes were not equalized. Thus it is difficult to assess the true value of these results. Nevertheless, the reactions on growth, after supplementing with these amino acids, were sharp enough to justify the given interpretation. The results of Hogan do not conform with those of Mitchell and Smuts (1932). According to the latter workers no supplementation occurred when tryptophane alone was added and only a slight improvement after lysine supplementation. A marked improvement was, however, obtained by supplementing with lysine and tryptophane simultaneously.

Notwithstanding these amino acid deficiencies of maize proteins Contesco and Rowan (1936) succeeded in keeping 4½ months old pigs in excellent condition on a maize ration without the occurrence of any signs of ill-health. An average daily gain of 0·417 Kg. per head was obtained. It is well known that the different proteins of the maize kernel supplement each other. The protein contained in the embryo improves that of the rest. For the whole maize a biological value of 69 has been determined by Laporta and co-workers (1937), whilst the biological value of the proteins without the embryo is only 50. Similarly maize supplemented by a gluten preparation gives excellent growth with young pigs according to Hart and McCollum (1914).

The biological values of maize, with rats as experimental animals, are, as reported by Mitchell (1924) at 5 and 10 per cent. proteins levels, 72 and 60 respectively. With pigs on a 5 per cent. level Mitchell and Kick (1927) found a value of 54, whilst Gaucher and Popov (1936) obtained a slightly lower value of 49. Boas Fixsen and Jackson (1932) experimenting with rats found a biological value of 67 for whole yellow maize at a 7-8 per cent. protein level. An exceptionally high biological value of 98 has been established by Smuts (1939) for sheep on a maintenance ration.

Experimental.

Rats were used as experimental animals. All determinations were carried out according to the method of Mitchell (1924). Only one biological value was determined on a series of six rats. The nitrogen low period was conducted either prior to or after the protein period. Six to seven day periods were allowed on a nitrogen low ration to establish constant nitrogen excretion. For the protein periods at least 10 days were allowed. Collection periods were of seven day's duration. Fe_2O_3 was used as faecal markers. The urine was collected in acid and the daily faeces digested by the Kjeldahl method. The week's digests of faeces were made up to volume and aliquots distilled for nitrogen determination. The urine collected over the period was made up to a known volume and aliquots analysed for nitrogen. The rations were made up so as to contain approximately 8 per cent. protein. The percentage composition of the different rations is tabulated in Table I. Analyses were made of all rations after careful mixing. To prevent deterioration, the rations were stored in an ice chest.

Results.

The nitrogen metabolism data as well as the calculation of the biological values are given in Table II. The standardizing periods on the nitrogen low ration preceded the protein feeding periods in the three cases of yellow maize supplemented with lysine and tryptophane separately and yellow maize supplemented with lysine and tryptophane simultaneously. For the yellow and white maize periods the nitrogen low period was conducted after the protein periods.

The calculation of the biological values is based on the principles as expounded by Mitchell (1924) namely that the metabolic faecal nitrogen is proportional to the dry matter intake and that the endogenous urinary nitrogen is proportional to the body weight.

TABLE I.

Percentage Composition of the Rations.

Ingredients.	N. Low.	Whole White Maize.	Whole Yellow Maize.	Whole Yellow Maize + Lysine.	Whole Yellow Maize + Tryptophane.	Whole Yellow Maize + Lysine + Tryptophane.
Whole white maize meal........	—	81·6	—	—	—	—
Whole yellow maize meal.......	—	—	84·2	82·5	82·8	81·1
d-Lysine-di-hydrochloride.......	—	—	—	0·2	—	0·2
Tryptophane..................	—	—	—	—	0·15	0·15
Butter fat (1)..................	8·0	8·0	8·0	8·0	8·0	8·0
Cod liver oil..................	2·0	2·0	2·0	2·0	2·0	2·0
Sucrose......................	10·0	3·4	0·8	2·3	2·05	3·55
Harris' Vitamin B complex (2)..	2·0	2·0	2·0	2·0	2·0	2·0
Salt mixture (Hubbel etc.) (3)...	2·0	2·0	2·0	2·0	2·0	2·0
Whole egg white (4)...........	3·8	—	—	—	—	—
NaCl........................	1·0	1·0	1·0	1·0	1·0	1·0
Agar........................	2·0	—	—	—	—	—
Starch (dextrinized)...........	69·2		—	—	—	—
TOTAL..............	100·0	100·0	100·0	100·0	100·0	100·0
PERCENTAGE N......	0·67	1·44	1·44	1·59	1·51	1·49

(1) The butter fat has been filtered through a course filter paper to remove any casein.

(2) The Harris' Vitamin B complex is a preparation of "The Harris Laboratories", Tuckahoe, New York.

(3) A new salt mixture described by Hubbel, R., Mendel, J. B. and Wakeman, A. J. (1937) *J. Nutr.* Vol. 14, pp. 273–285.

(4) The whole egg white has been dried on a waterbath and extracted with ether.

As can be seen from these tables a significant difference is manifested between the biological values of whole white maize and whole yellow maize, the values being $76 \pm 1{\cdot}91$ and $67 \pm 0{\cdot}98$. The difference of $10 \pm 2{\cdot}15$ which gives a t value for $N \pm 10$ of $4{\cdot}66$ denotes a significant difference at $p \pm 0{\cdot}001$. No significant difference is manifested between yellow maize and yellow maize supplemented separately with lysine and tryptophane. The biological values for the supplementations are $70 \pm 2{\cdot}63$ and $66 \pm 0{\cdot}75$ respectively. The differences are $3 \pm 2{\cdot}63$ and $1 \pm 1{\cdot}23$, which gives t values for $n = 10$ of $1{\cdot}14$ and $0{\cdot}81$ respectively. These t values denote that the differences are insignificant. The simultaneous supplementation with the amino acids, however, enhanced the biological value to $81 \pm 0{\cdot}61$, which means a difference of $14 \pm 1{\cdot}15$ and therefore a t value for $n = 10$ of $12{\cdot}15$ making this result highly significant at $p = 0{\cdot}001$.

TABLE II.

Nitrogen Metabolism Data and the Calculation of the Biological Value.

N—LOW PERIOD.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N. Per gm. Food.	Metabolic N. Per Day.	Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N. Per 100 gm. Wt.	Endogenous N. Per Day.	Food in Urine.	Retained N.	N Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.			
1	179	185	182	14·1	—	49·6	3·52			—	—	23·6	13·0	—	—			—	—
2	210	210	210	12·3		36·2	2·94	—	—	—	23·6	13·0		—		—	—	—	—
3	157	155	156	10·7	—	34·4	3·21		—	—	24·0	15·4	—		—	—	—	—	—
4	170	174	172	11·1		36·4	3·28	—	—	—	20·8	12·1		—			—	—	
5	149	150	150	11·9	—	37·8	3·18	—	—	—	24·8	16·5	—		—	—	—	—	—
6	164	160	162	12·1	—	41·4	3·42	—	—		27·6	17·0	—	—				—	—
	WHOLE WHITE MAIZE PERIOD (1·44 PER CENT. N.)																		
1	168	190	179	16·7	240·5	56·0	3·52	58·8	− 2·8	240·5	71·6	13·0	23·3	48·3	192·2	+112·9	77	100	80
2	210	220	215	17·4	250·6	41·2	2·94	51·2	10·0	250·6	107·6	17·0	36·6	71·0	179·6	+101·8	84	100	72
3	140	158	149	15·4	221·8	38·0	3·21	49·4	−11·4	221·8	72·8	15·4	22·9	49·9	171·9	±111·0	83	100	78
4	157	175	166	14·6	210·2	40·0	3·28	47·9	− 7·9	210·2	79·2	12·1	20·1	59·1	151·1	+ 91·0	81	100	72
5	142	150	146	12·1	174·2	30·4	3·18	38·5	− 8·1	174·2	53·2	16·5	24·1	29·1	145·1	+ 90·6	83	100	83
6	150	164	157	15·3	220·3	48·0	3·42	52·3	− 4·3	220·3	87·2	17·0	26·7	60·5	159·8	+ 85·1	78	100	73
																	81	100	76
	N-LOW PERIOD.																		
13	218	216	217	14·3	—	49·8	3·48	—	—	—	30·8	14·2	—	—	—	—	—	—	—
14	156	160	158	12·9	—	44·2	3·43	—	—	—	21·2	13·4	—	—	—	—	—	—	—
15	181	188	185	14·6	—	48·4	3·32	—	—	—	30·0	16·2	—			—	—	—	—
16	188	190	189	12·6	—	44·0	3·49	—	—	—	30·4	16·1	—	—	—	—	—	—	—
17	198	196	197	13·3	—	41·4	3·11	—	—	—	28·8	14·6	—	—	—	—	—	—	—
18	147	149	148	12·4	—	45·6	3·68	—	—	—	32·4	21·9	—	—		—	—	—	—
	— WHOLE YELLOW MAIZE PERIOD (1·44 PER CENT. N.)																		
13	200	216	208	18·1	260·6	49·6	3·48	63·0	−13·4	260·6	114·0	14·2	29·5	84·5	176·1	± 97·0	81	100	68
14	142	164	153	17·0	244·8	44·0	3·43	58·3	−14·3	244·8	104·8	13·4	20·5	84·3	160·5	± 96·0	82	100	66
15	170	194	182	18·1	260·6	38·4	3·32	60·1	−21·7	260·6	112·0	16·2	29·5	82·5	178·1	±110·2	85	100	68
16	184	198	191	14·2	204·5	36·0	3·49	49·6	13·6	204·5	106·4	16·1	30·8	75·6	128·9	± 62·1	82	100	63
17	185	201	193	18·1	260·6	40·8	3·11	56·3	−15·5	260·6	107·2	14·6	28·2	79·0	181·6	±112·6	84	100	70
18	140	158	149	15·9	229·0	42·4	3·68	58·5	−16·1	229·0	106·0	21·9	32·6	73·4	155·6	±80·6	81	100	68
																	83	100	67

TABLE II (*a*).

Nitrogen Metabolism Data and the Calculation of the Biological Value.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N. Per gm. Food.	Metabolic N. Per Day.	Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N. Per 100 gm. Wt.	Endogenous N. Per Day.	Food in Urine.	Retained N.	N Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.			
	N-Low Period.																		
1	96	93	95	6·2	—	22·0	3·55	—	—	—	16·0	16·8	—	—	—	—	—	—	—
2	93	92	93	6·5	—	17·3	2·66	—	—	—	16·9	18·2	—	—	—	—	—	—	—
3	98	95	97	5·1	—	20·2	3·95	—	—	—	12·8	13·2	—	—	—	—	—	—	—
4	90	87	89	7·1	—	26·4	3·72	—	—	—	11·9	13·4	—	—	—	—	—	—	—
5	96	95	96	6·5	—	23·9	3·68	—	—	—	14·0	14·6	—	—	—	—	—	—	—
6	103	102	103	6·7	—	23·1	3·45	—	—	—	19·4	18·8	—	—	—	—	—	—	—
	Whole Yellow Maize + 0·25 Per Cent. d-lysine-di-hydrochloride Period (1·59 Per Cent. N.)																		
1	105	107	106	6·0	95·4	16·5	3·55	21·3	− 4·8	95·4	39·1	16·8	17·8	21·3	74·1	+ 39·8	83	100	78
2	90	100	95	6·1	97·0	16·9	2·66	16·2	0·7	96·3	47·4	18·2	17·3	30·1	66·2	+ 32·7	83	99	69
3	98	107	103	6·6	104·9	16·1	3·96	26·1	− 10·0	104·9	53·6	13·2	13·6	40·0	64·9	+ 35·2	85	100	62
4	99	104	102	7·3	116·1	16·9	3·72	27·2	− 10·3	116·1	44·5	13·4	13·7	30·8	85·3	+ 54·7	85	100	72
5	109	117	113	7·4	117·7	22·2	3·68	27·2	− 5·0	117·7	49·4	14·6	16·5	32·9	84·8	+ 46·1	81	100	72
6	90	98	94	7·1	112·9	17·3	3·45	24·5	− 7·2	112·9	58·9	18·8	17·7	41·2	71·7	+ 36·7	85	100	64
																	84	100	70
	N-Low Period.																		
13	123	118	121	7·9	—	34·8	4·41	—	—	—	18·4	15·2	—	—	—	—	—	—	—
14	112	113	113	7·5	—	27·2	3·63	—	—	—	13·6	12·0	—	—	—	—	—	—	—
15	115	104	110	6·7	—	27·2	4·06	—	—	—	26·6	24·2	—	—	—	—	—	—	—
16	112	109	111	6·9	—	28·0	4·06	—	—	—	17·6	15·9	—	—	—	—	—	—	—
17	108	108	108	7·5	—	29·6	3·95	—	—	—	18·4	17·0	—	—	—	—	—	—	—
18	112	114	113	7·5	—	29·2	3·89	—	—	—	14·4	12·7	—	—	—	—	—	—	—
	Whole Yellow Maize + 0·15 Per Cent. Tryptophane Period (1·51 Per Cent. N.)																		
13	131	142	137	11·4	172·1	32·0	4·41	50·3	18·3	172·1	81·5	15·2	20·8	60·7	111·4	+ 58·6	81	100	65
14	120	130	125	8·8	132·9	22·4	3·63	31·9	− 9·5	132·9	61·9	12·0	15·0	46·9	86·0	+ 48·6	83	100	65
15	112	125	119	10·2	154·0	25·6	4·06	41·4	− 15·8	154·0	78·6	24·2	28·8	49·8	104·2	+ 49·8	83	100	68
16	114	122	118	7·2	108·7	17·6	4·06	29·2	− 11·6	108·7	53·0	15·9	18·8	34·2	74·5	+ 38·1	84	100	69
17	111	123	117	8·1	122·3	18·0	3·95	32·0	− 14·0	122·3	62·2	17·0	19·9	42·3	80·0	+ 42·1	85	100	65
18	118	124	1·1	7·9	119·3	20·0	3·89	30·7	− 10·7	119·3	57·2	12·7	15·4	41·8	77·5	+ 42·1	83	100	65
																	83	100	66

Table II (*b*).

Nitrogen Metabolism Data and the Calculation of the Biological Value.

N-Low Period.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food in Urine.	Retained N.	N Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.
							Per gm. Food.	Per Day.				Per 100 gm. Wt.	Per Day.						
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.			
19	113	109	111	9·4	—	36·0	3·83	—	—	—	12·8	11·5	—	—	—	—	—	—	—
20	112	112	112	7·7	—	28·4	3·69	—	—	—	16·7	14·9	—	—	—	—	—	—	—
21	103	99	101	7·2	—	28·4	3·94	—	—	—	19·2	19·0	—	—	—	—	—	—	—
22	101	100	101	8·6	—	32·8	3·81	—	—	—	13·6	13·5	—	—	—	—	—	—	—
23	97	95	96	6·8	—	28·4	4·18	—	—	—	12·8	13·3	—	—	—	—	—	—	—
24	95	90	93	6·5	—	26·0	4·00	—	—	—	14·0	15·1	—	—	—	—	—	—	—
Whole Yellow Maize + 0·2 Per Cent. d-lysine-di-hydrochloride + 0·15 Per Cent. Tryptophane Period (1·49 Per Cent. N.)																			
19	132	152	142	12·4	184·8	30·0	3·83	47·5	—17·5	184·8	55·0	11·5	16·3	38·7	146·1	+ 99·8	84	100	79
20	123	142	133	10·6	157·9	25·2	3·69	39·1	—13·9	157·9	51·5	14·9	19·8	31·7	126·2	+ 79·2	84	100	80
21	121	141	131	11·4	169·9	30·4	3·94	44·9	—14·5	169·9	57·4	19·0	24·9	32·5	137·4	+ 82·1	82	100	81
22	121	142	132	11·0	163·9	26·8	3·81	41·9	—15·1	163·9	46·6	13·5	17·8	28·8	135·1	+ 90·5	84	100	82
23	114	135	125	10·6	157·9	27·6	4·18	44·3	—16·7	157·9	49·4	13·3	16·6	32·8	125·1	+ 80·9	83	100	79
24	108	121	115	8·5	126·7	20·0	4·00	34·0	—14·0	126·7	40·0	15·1	17 4	22·6	104·1	+ 66·1	84	100	82
																	84	100	81

From the statistical analysis of these results it is clear that the protein of whole white maize is significantly superior to that of whole yellow maize. This difference was rather unexpected, and can at present only be explained by a constitutional difference in the protein moiety of different strains or varieties of maize. This point can, however, only be settled by direct experimentation on this aspect.

It is further also clear that the supplementation with lysine and tryptophane does not affect the utilization of the nitrogen contained in maize, while the simultaneous supplementation of lysine and tryptophane causes a marked increase in the nitrogen utilization of the proteins of yellow maize.

These results on the supplementary effect of the amino acids on the proteins of maize as measured by the nitrogen utilization are in accordance with the results obtained by Mitchell and Smuts (1932) with the paired feeding technique. It will be noted that in this study only a slight increase of $3 \pm 2 \cdot 63$ is obtained by the lysine supplementation. This small difference falls within the experimental error. This observation is, therefore, not in accordance with the findings of Mitchell and Smuts by means of the paired feeding test. It must, therefore, be assumed that the slight though insignificant increase in nitrogen utilization after the lysine addendum may in all probability become significant if the number of determinations is increased and hence verify the indications obtained that lysine supplementation only brings about a limited improvement in the utilization of the maize proteins.

Conclusions.

1. The biological values of whole white maize and whole yellow maize are $76 \pm 1 \cdot 91$ and $67 \pm 0 \cdot 98$ at approximately 8 per cent. protein level.

2. The proteins of white maize are significantly better than that of yellow maize.

3. Supplementation with lysine and tryptophane separately does not increase the nitrogen utilization of yellow maize to any marked extent.

4. Supplementation with lysine and tryptophane simultaneously markedly increases the nitrogen utilization of the yellow maize protein.

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