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Changes in the plasma concentrations of free amino acids in relation to egg formation in the hen

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1. The changes occurring in the free amino acids of the plasma of laying hens in relation to egg formation have been investigated in fed and starved hens in three experiments, each with eight birds.

2. The mean concentrations of most amino acids and of the totals were higher at night than in the morning.

3. In general, egg formation was associated with increases in the concentrations of non-essential and decreases in the concentrations of essential amino acids. Cystine and glutamic acid tended to behave like the essential amino acids.

4. After 40 h starvation seven amino acids, particularly serine, histidine and lysine, increased in concentration and only three, proline, ornithine and arginine, decreased significantly.

5. When eight cocks were injected with oestrogen most of the amino acids increased in concentration. The essential amino acids (with the exception of phenylalanine), serine, proline, cystine and ornithine showed the greatest increases.

6. The results are discussed in relation to the possibility that the voluntary food intake of hens may be influenced by changes in the plasma levels of one or more essential amino acids associated with the synthesis of egg albumen. Arginine appeared to be the only amino acid that might possibly fulfil this role.

7. It was concluded that investigations of changes in the free amino acids of hen plasma are unlikely to provide a useful approach to a study of the amino acid requirements or the nutritive value of particular proteins for egg production.

Laying hens consume approximately 25% more food on egg-forming than on non-egg-forming days when given a normal layers' diet (Morris & Taylor, 1967) but the mechanism by which this short-term control of appetite is exercised is not fully understood. One possibility we have considered is that the plasma concentration of one or more of the essential amino acids is elevated on non-egg-forming days, when the synthesis of egg albumen may be assumed to be minimal, and that the appetite-controlling centres in the brain are sensitive to these changes. The object of the present experiment was to investigate the changes that occur in the plasma free amino acids of hens during the egg cycle to determine whether or not there is any basis for such a mechanism of appetite control. The effects of oestrogen-induced synthesis of yolk protein on the plasma amino acids of cocks were also studied, in order to separate the effects of yolk protein synthesis from those of egg albumen synthesis.

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EXPERIMENTAL

Birds and their management

All birds used in the main experiments were medium hybrids derived from the Thornber '404' strain. The hens had been in lay 3–7 months and the cocks were 14 weeks of age at the start of the experiment. They were kept in separate metabolism cages and given food and water *ad lib.*, except in Expt 2 in which food was withheld. The diet was a conventional layers' diet made up in a pelleted form (Bolton, 1967). They were housed in a windowless room and artificial light was provided for 14 h daily from 05.30–19.30 hours.

Bleeding procedure

In Expts 1, 2 and 4 blood samples were taken in pairs in the morning (09.00–10.00 hours) and at night (23.00–24.00 hours), but in Expt 3 samples were taken in the morning only. Blood (about 5 ml) was taken from the wing vein into a heparinized syringe and the plasma was immediately separated by centrifugation. Altogether, four samples of blood were taken from each bird.

Analysis of plasma

Two volumes of plasma were deproteinized with one volume of sulphosalicylic acid (7·5%, w/v) containing the norleucine standard (12 mg/100 ml) and, after mixing, the tubes were allowed to stand for 1 h before centrifuging. The clear supernatant liquid was stored at −20° until required. The maximum time that any sample was stored before analysis was 6 months, and separate tests showed that no losses of amino acids were suffered during this period. On thawing the samples, a fine precipitate was observed and this was removed by centrifugation before portions were taken for analysis with an automatic amino acid analyser (Technicon Instruments Co. Ltd, Chertsey, Surrey). For Expts 1, 2 and 4 a model NC-2 analyser was used and for Expt 3 model TSM. Portions of 1 ml plasma filtrate were used with the former and of 0·5 ml (on each column) with the latter. In Expt 1, 2:4:6-trinitrobenzene sulphonic acid was used for colour development (Waring & Bolton, 1967), so that no values for proline were obtained, but in all subsequent experiments ninhydrin was employed. Tryptophan, which was present in only small amounts, was not well resolved and no results are given for this acid. Glutamine was resolved only with the TSM instrument. Samples were normally analysed once only, since replicate determinations were known by experience to show good agreement. However, analyses were repeated whenever there was any doubt about the first analysis, as for example, when some of the peaks were poorly resolved.

Hen experiments

In preliminary experiments samples of blood were taken from twelve laying Brown Leghorn hens, some of which were engaged in egg formation while others were not, and it was found that the variations between birds were so great that significant differences in the concentrations of individual amino acids in relation to egg formation were not obtained. In two birds, for example, threonine accounted for 25% of the total free amino acids, compared with 10–12% for the majority. In subsequent experiments, therefore, the same birds were bled on both egg-forming and non-egg-forming days but different birds were used in each experiment. A day was designated 'egg-forming' if an egg was present in the shell gland at the evening bleeding. On a 'non-egg-forming' day the bird laid the last egg of a sequence one afternoon and the shell-gland was empty at the time of the night bleeding and when the bird was bled again the following morning. The time that had elapsed since the end of secretion of egg albumen was estimated to have been 4–10 h at the night bleeding on egg-forming days and 28–30 h on non-egg-forming days. The corresponding times for the morning bleedings were 14–20 h and 38–40 h, respectively.

Expt 1. Eight hens were fed *ad lib.* and bled at the standard times on both egg-forming and non-egg-forming days.

Expt 2. This experiment was designed to eliminate the effects of amino acids of dietary origin on the changes in the plasma concentrations of amino acids associated with egg formation, and in order to achieve this object eight birds were fasted completely for a period of 40 h. Food was removed at 17.00 hours one day and the first (night) bleeding was carried out approximately 30 h and the second 40 h later.

Expt 3. In comparing the results of Expts 1 and 2 it was not possible to distinguish differences due to the individuality of the birds from those due to starvation, since each experiment was carried out on a different group of birds. In Expt 3 the same eight birds were bled (mornings only) while fed normally and after 40 h starvation, on both egg-forming days and non-egg-forming days, so that the effects of starvation could be separated from those due to egg formation.

Cock experiment

Expt 4. The object of this experiment was to study the effects of yolk protein synthesis on the free amino acids of the plasma by comparing their concentrations before and after oestrogen treatment. Eight cocks (mean live weight 2·0 kg) were given four intramuscular injections each of 1 mg oestradiol dipropionate (Ovocyclin P; Ciba Laboratories Ltd, Horsham) on alternate days. They were bled at the standard times on the day before the first injection and on the day after the final injection.

Statistical treatment of results

Values both for the actual concentrations of each amino acid and for the relative concentrations (values expressed as a percentage of the total amino acids) were analysed by analysis of variance.

RESULTS

Mean values for each amino acid at each bleeding are shown in Tables 1–4 for Expts 1–4 respectively, together with the results of the statistical analyses.

Expt 1. The mean concentrations of all amino acids were higher at night than in the morning and in almost all instances the differences were statistically significant. When the concentrations were calculated as percentages of the totals the only amino acids showing significant differences between the night and morning samples were methionine and phenylalanine, both of which were lower at night. In general, the essential amino acids tended to be lower and the non-essential ones higher during egg formation than in the absence of egg formation, but leucine and histidine showed no changes in relation to egg formation. The differences were accentuated when comparisons were made on a relative basis, particularly for glutamic acid, glycine and alanine, all of which were significantly elevated during egg formation. Among the essential amino acids, threonine, valine and isoleucine showed the greatest differences in relation to egg formation. Cystine behaved in the same manner as the essential amino acids and was significantly depressed during egg formation, but methionine showed no consistent changes. The concentration of arginine, an essential amino acid for birds, was lower at the morning bleeding when egg formation was in progress but higher at night, compared with the non-egg-forming values, but these differences were not significant.

Table 1. *Expt* 1. *Mean values for the plasma amino acid concentrations* (*μmoles/l*) *of eight fed hens in the morning* (09.00 *hours*) *and at night* (23.00 *hours*) *on egg-forming* (*E*) *and non-egg-forming* (*N*) *days, together with the results of the statistical treatment* (*by analysis of variance*)

					Statistical analysis			
	Egg-forming		Non-egg-forming					
Amino acid	09.00	23.00	09.00	23.00	SE†	E *v.* N	09.00 *v.* 23.00	Interaction
Aspartic acid	40	48	22	38	6	*	NS	NS
Threonine	462	604	625	790	43	***	**	NS
Serine	635	800	571	762	40	NS	***	NS
Glutamic acid	248	305	190	284	21	NS	**	NS
Glycine	514	639	513	562	23	NS	*	NS
Alanine	397	521	362	470	24	NS	***	NS
Valine	235	273	301	351	16	***	*	NS
Cystine	21	31	29	36	2	**	***	NS
Methionine	74	88	81	89	4	NS	*	NS
Isoleucine	92	109	116	136	6	**	*	NS
Leucine	225	269	227	268	12	NS	**	NS
Tyrosine	91	129	102	110	7	NS	***	NS
Phenylalanine	104	121	108	111	5	NS	NS	NS
Ornithine	57	72	66	86	7	NS	*	NS
Lysine	218	301	237	343	23	NS	***	NS
Histidine	176	226	175	223	10	NS	***	NS
Arginine	299	409	343	347	22	NS	*	*
Total	3888	4945	4068	5006	200	NS	***	NS

NS, not significant. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
† Based on pooled error variance.

Expt 2. As with the fed birds, the mean concentrations of total amino acids were significantly higher at night than in the morning, and this was true also of most individual amino acids (Table 2). Relative to the totals, serine, glutamic acid, glycine, alanine and phenylalanine were significantly lower at night, while proline, cystine, isoleucine, leucine, ornithine, lysine and arginine were significantly lower in the morning. The general difference in behaviour between the essential and non-essential amino acids was also the same as that observed in Expt 1. Glutamic acid, however, responded in the same manner as the essential amino acids in that it was significantly lower when egg formation was in progress than when it was not, and alanine showed only small changes. Differences in concentrations of serine and glycine were particularly large in relation to egg formation. Both tyrosine and phenylalanine behaved in the manner of non-essential amino acids when the samples were taken in the morning (in contrast to their behaviour in Expt 1) but the mean egg-forming and non-egg-forming values were very similar for the midnight samples. Compared with the mean values observed in Expt 1, the plasma arginine concentrations of the starved birds were much depressed, particularly during egg formation, whereas the lysine levels were greatly elevated.

Table 2. *Expt* 2. *Mean values for the plasma amino acid concentrations* (*μmoles/l*) *of eight starved hens in the morning* (09.00 *hours*) *and at night* (23.00 *hours*) *on egg-forming* (*E*) *and non-egg-forming* (*N*) *days, together with the results of the statistical treatment* (*by analysis of variance*)

	Egg-forming		Non-egg-forming		Statistical analysis			
Amino acid	09.00	23.00	09.00	23.00	SE†	E *v.* N	09.00 *v.* 23.00	Interaction
Aspartic acid	35	36	36	36	4	NS	NS	NS
Threonine	611	664	611	798	44	NS	*	NS
Serine	1102	1052	818	1008	33	***	*	**
Glutamic acid	150	132	173	183	10	***	NS	NS
Proline	186	237	169	249	15	NS	***	NS
Glycine	611	629	469	523	24	***	NS	NS
Alanine	463	500	476	511	24	NS	NS	NS
Valine	244	304	273	378	17	*	***	NS
Cystine	18	28	21	36	3	NS	***	NS
Methionine	86	102	80	105	3	NS	***	NS
Isoleucine	100	137	113	177	6	***	***	*
Leucine	183	266	199	365	13	**	***	NS
Tyrosine	143	162	88	169	8	**	***	**
Phenylalanine	103	107	88	104	4	*	*	NS
Ornithine	27	62	33	51	5	NS	***	NS
Lysine	516	726	576	830	50	NS	***	NS
Histidine	184	220	206	273	13	*	***	NS
Arginine	139	249	221	300	16	***	***	NS
Total	4901	5613	4650	6096	232	NS	***	*

NS, not significant. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
† Based on pooled error variance.

Expt 3. The general patterns of change in the amino acid concentrations in relation to egg formation were less marked than those observed in Expts 1 and 2. The mean concentrations of glutamic acid were lower during egg formation in both fed and starved birds, but the differences were not statistically significant.

The main object of this experiment was to determine the effects of starvation *per se*, and seven of the twenty acids studied, and the totals, were significantly increased during starvation (Table 3). Serine, histidine and lysine showed very large increases, but proline, ornithine and arginine concentrations were depressed ($P < 0{\cdot}001$). Tyrosine concentration was elevated during starvation only when egg formation was in progress.

Expt 4. Before oestrogen treatment the plasma of the cocks did not show the typical differences between morning and night samples that were observed in the hens, and nine of the eighteen amino acids studied, and the totals, were lower at night than in the morning. Of these, serine, proline, glycine, alanine and ornithine showed the greatest differences. Only glutamic acid, tyrosine and lysine had substantially higher values at night than in the morning (Table 4).

Table 3. *Expt* 3. *Mean values for the plasma amino acid concentrations* (*μmoles/l*) *of eight hens when given food normally* (*F*) *and when starved* (*S*) *on egg-forming* (*E*) *and on non-egg-forming* (*N*) *days, together with the results of the statistical treatment* (*by analysis of variance*)

	Egg-forming		Non-egg-forming		Statistical analysis			
Amino acid	F	S	F	S	SE†	E *v.* N	F *v.* S	Interaction
Aspartic acid	55	54	44	44	4	*	NS	NS
Threonine	326	513	404	506	39	NS	**	NS
Serine	610	1107	639	842	42	*	***	**
Glutamic acid	401	382	416	408	17	NS	NS	NS
Proline	415	188	447	161	36	NS	***	NS
Glycine	460	554	431	422	21	**	NS	*
Alanine	343	376	340	420	22	NS	*	NS
Valine	188	218	241	229	21	NS	NS	NS
Cystine	25	26	34	23	3	NS	NS	NS
Methionine	52	66	62	62	3	NS	*	NS
Isoleucine	79	100	100	102	9	NS	NS	NS
Leucine	157	162	184	177	13	NS	NS	NS
Tyrosine	77	130	82	82	5	***	***	***
Phenylalanine	79	84	82	67	5	NS	NS	NS
Ornithine	73	27	65	30	6	NS	***	NS
Lysine	185	432	241	423	32	NS	***	NS
Histidine	104	130	114	160	10	*	**	NS
Arginine	230	139	242	210	10	***	***	**
Hydroxyproline	30	20	21	7	8	NS	NS	NS
Glutamine	251	250	254	280	50	NS	NS	NS
Total	4140	4958	4443	4655	228	NS	*	NS

NS, not significant. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

† Based on pooled error variance.

Oestrogen treatment raised the plasma concentrations of almost all the amino acids, particularly the night values. The main exceptions were the morning values, and sometimes the night values also, of aspartic and glutamic acids, alanine, tyrosine and phenylalanine.

The amino acids that showed the greatest elevations were the essential acids (with the exception of phenylalanine), serine, proline, cystine and ornithine.

DISCUSSION

As far as is known, there are no precise regulatory mechanisms whereby plasma amino acid levels are maintained, and their concentrations may vary greatly from time to time, therefore, according to their relative rates of movement into and out of the plasma. Entry into the blood is both from the digestive tract and from the tissues and removal is by tissue uptake and by urinary excretion.

The tissues act as a major reservoir of free amino acids. The concentration of total free amino acids in the liver of the fasting guinea-pig, for example, may be more than fifteen times and in the muscles at least five times that in the plasma (Christensen, Streicher & Elbinger, 1948). In addition, the total weight of the soft tissues is many times that of the blood and Harper & Rogers (1965), for example, calculated that the size of the free threonine pool in the muscle tissue of control rats was more than sixty times that of the plasma pool. It must also be remembered that the tissue free amino acid pool itself represents a very small proportion of the total amino acids in the body proteins. It is clear, then, that small percentage changes in the tissue concentration, due either to uptake or release of a particular acid, can result in large changes in the plasma concentration. These considerations make it difficult to interpret fully experimentally induced changes in plasma amino acid levels.

Table 4. *Expt* 4. *Mean values for the plasma amino acid concentrations* (*μmoles/l*) *of eight cocks in the morning* (09.00 *hours*) *and at night* (23.00 *hours*) *before and after oestrogen treatment, together with the results of the statistical treatment* (*by analysis of variance*)

	Control		Oestrogen			Statistical analysis		
Amino acid	09.00	23.00	09.00	23.00	SE†	Control v. Oestrogen	09.00 v. 23.00	Inter-action
Aspartic acid	56	58	44	55	4	NS	NS	NS
Threonine	587	529	636	762	49	**	NS	NS
Serine	561	443	667	670	26	***	*	*
Glutamic acid	260	286	231	296	9	NS	***	NS
Proline	721	380	846	627	29	***	***	NS
Glycine	613	425	638	538	19	**	***	*
Alanine	604	462	524	562	21	NS	*	***
Valine	284	253	382	402	12	***	NS	*
Cystine	31	33	38	40	1	***	NS	NS
Methionine	67	72	73	95	2	***	***	**
Isoleucine	122	119	155	173	5	***	NS	*
Leucine	222	229	281	321	8	***	*	NS
Tyrosine	167	239	142	192	6	***	***	NS
Phenylalanine	111	112	98	108	3	*	NS	NS
Ornithine	66	37	91	88	5	***	***	NS
Lysine	442	531	492	724	36	**	***	NS
Histidine	166	180	160	217	5	**	***	***
Arginine	354	345	373	417	16	**	NS	NS
Total	5434	4733	5871	6287	171	***	NS	**

NS, not significant. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

† Based on pooled error variance.

In both fed and starved hens the mean concentrations of amino acids were higher in the blood samples taken at night than in the morning samples. It is unlikely that this was because the first samples to be taken were at night since the same phenomenon was observed in the preliminary experiments, in which the order of bleeding was reversed.

In the untreated cocks, however, there was no general elevation of the level of plasma amino acids at night, but oestrogen treatment increased the concentrations of most acids and established differentials between the night and morning levels similar to those observed in the laying hens. Whether or not the general increase in the plasma amino acid concentrations of laying hens at night, even when starved, is in response to an increase in the circulating level of oestrogens is a matter for speculation.

Such a generalized effect certainly suggests a hormonal influence, and another possibility is that the increase in the plasma amino acid concentrations at night is associated with a reduction in the plasma concentrations of insulin or growth hormone, or of both, resulting in a decrease in the tissue uptake of amino acids.

The significance of the differences between night and morning in the relative concentrations of the individual amino acids is not clear. In the fed birds, only methionine and phenylalanine showed differences, and concentrations of both were lower at night, while in the starved birds there was a marked tendency for the essential amino acids to be higher at night and for the non-essential ones to be higher in the morning.

In most species, it is virtually impossible to separate the effects of protein synthesis on the concentration of plasma free amino acids from dietary and hormonal effects, but in the laying hen it is possible by careful choice of sampling times to attempt to isolate the changes due to the synthesis of egg albumen proteins from changes brought about by other factors.

A basic assumption that was made in planning this investigation was that, in hens laying regularly, synthesis of yolk proteins by the liver is continuous and independent of the synthesis of egg albumen by the oviduct, except in so far as both processes are under the control of oestrogen. Egg albumen synthesis, too, is probably continuous, but it seems reasonable to expect that the rate of synthesis will be greater when the oviduct has recently secreted a large proportion of its stores, i.e. on egg-forming days, than when the stores are high because secretion has not recently occurred, i.e. on non-egg-forming days. It is assumed, therefore, that the differences in the plasma concentrations of free amino acids on egg-forming and non-egg-forming days reflect, primarily, differences in the rate of synthesis of egg-white proteins. These differences were found to be similar in both fed and starved hens, which lends support to this assumption.

The concentrations of the majority of the essential amino acids were elevated when egg-formation was not in progress, and if the appetite-controlling centres of the brain are sensitive to the plasma level of these acids a possible mechanism would be provided whereby the voluntary food intake is reduced on non-egg-forming days. However, all the essential amino acids, with the exception of arginine, increased in concentration during starvation also, which suggests that arginine itself could be the only possible candidate for the role of 'appetite-regulator'.

In an attempt to determine whether or not there was any relationship between the amino acid composition of albumen and the depression in the levels of essential amino acids in the plasma in relation to egg formation, samples of yolk and albumen were analysed and the albumen:yolk ratios were calculated for the essential acids and for cystine and tyrosine (Table 5). (Before it can be stated that a particular protein is 'rich' in particular amino acids a standard protein must be used for comparison and, for a laying hen, yolk protein appeared to be a good standard.) It will be seen that, relative to egg yolk, albumen contains excessive amounts of cystine, methionine, phenylalanine and valine, and virtually the same amount of isoleucine, and that it is not seriously deficient in any of the other amino acids. Of the most abundant acids only valine and isoleucine were significantly depressed during egg formation in both Expts 1 and 2, while cystine was depressed in Expt 1. Phenylalanine concentrations were elevated during egg formation in the morning when the birds were starved (Expts 2, 3), so that, taking all the essential amino acids together, there does not appear to be a very close relationship between the relative abundance of amino acids in albumen and the acids that suffered the greatest reduction in the plasma during egg formation.

Table 5. *Relative concentrations of the essential amino acids (and of some related acids) in egg proteins (residues per* 1000 *residues)*

Amino acid	Albumen	Yolk	Ratio, albumen : yolk
Cystine	14·2	8·9	1·60
Methionine	32·1	22·3	1·44
Phenylalanine	47·6	35·0	1·36
Valine	59·5	53·0	1·12
Isoleucine	40·2	41·0	0·98
Leucine	81·6	87·3	0·93
Histidine	19·3	21·2	0·91
Tyrosine	28·1	31·5	0·89
Threonine	51·7	58·8	0·88
Lysine	59·3	68·1	0·87
Arginine	42·0	52·3	0·80

The increases in the plasma concentrations of the non-essential amino acids during egg formation may be related to the abundance of these acids in the food and possibly in the free amino acid pools in the tissues, compared to egg albumen, which is particularly rich in essential amino acids.

In Expts 2 and 3 glutamic acid behaved as though it were an essential amino acid, and because of its central role in transamination reactions it is possible that it is in short supply during starvation and, in some individuals, when protein synthesis is occurring most intensely. Nutrition experiments with hens given protein-free diets have shown that birds cease to lay when glutamic acid is omitted from the diet, though they continue to eat (Johnson & Fisher, 1956), which suggests that this acid has a particularly important function in laying birds, and that it should be regarded as semi-essential in that requirements may, in certain circumstances, exceed the maximum rate of synthesis.

After 40 h starvation the plasma concentrations of most amino acids, with the notable exception of arginine, proline and ornithine, were elevated. Similar observations have been made in starved chicks by Hill & Olsen (1963), Charkey, Kano & Anderson (1954), Zimmerman & Scott (1967) and Boomgaardt & McDonald (1969), but in mammals it is generally found that starvation results in a decrease in total plasma amino acid concentrations, although there are marked differences in the behaviour of individual acids, particularly in relation to time of starvation. Thus, for example, Boomgaardt & McDonald (1969) observed a decrease in the concentration of branched-chain amino acids in the plasma of pigs after 24 h fasting, followed by an increase during the next 24 h of starvation. An increase in these acids had earlier been reported in six human subjects fasting for 48 h (Charkey, Kano & Hougham, 1955) and in rabbits after an 88 h fast (Block & Hubbard, 1962). Lysine concentrations, too, are normally observed to increase during starvation (e.g. Boomgaardt & McDonald, 1969 for the rat and pig) and this is usually attributed to the resistance of lysine to deamination.

Three amino acids in particular, proline, ornithine and arginine, were markedly depressed during starvation. The metabolism of these acids is closely linked and it is interesting to note that their levels in the plasma tended to rise and fall together. Thus, for example, they all rose under the influence of oestrogen (Table 4) and they all showed the same diurnal changes in concentration relative to the total amino acids during starvation (Expt 2).

Arginine is thought to be the sole source of ornithine in birds (Nesheim & Garlich, 1963; Tamir & Ratner, 1963), and it appears from these observations that it may also be a major source of proline. It is not known whether or not birds are able to synthesize proline from glutamic acid by the pathway present in mammalian tissues (Sallach, Koeppe & Rose, 1951).

Arginine was the only essential amino acid the concentration of which was depressed during starvation, which suggests that the tissue reserves of this acid must be small in relation to requirements. The well-established antagonism in birds between lysine and arginine (cf. Jones, 1964; O'Dell & Savage, 1966), the mechanism of which is not completely understood, may have contributed to the drastic fall in the plasma arginine concentration, for lysine was the essential amino acid that showed the greatest increase during starvation (Table 3).

Although the experiments were not designed to investigate the amino acid requirements of laying hens or the nutritive value of particular proteins for egg production, the results obtained do not encourage the belief that a study of the plasma concentrations of free amino acids in laying hens would be profitable in such investigations.

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