The tryptophan requirements of pullets in the early production stage

J.J. du Preez and J.S. Duckitt
Department of Poultry Science, University of Stellenbosch, Stellenbosch

A trial was conducted with 1 152 Amberlink laying pullets from the ages of 30 to 35 weeks to determine the tryptophan requirements. The eight treatments consisted of a series of diets made by dilution of a summit diet with a dilution mixture. All amino acids in the summit diet were calculated to be in excess of the requirements and tryptophan was calculated to be the most limiting amino acid but still in excess. The daily tryptophan requirement was estimated by means of the Reading model, and a response equation was derived. The coefficients of the equation represent the amounts of tryptophan required per individual bird for egg formation and for body maintenance, being 2,017 mg tryptophan/g egg output and 8,256 mg tryptophan/kg body mass respectively. From a multiple regression fit of a parabolic curve, the daily tryptophan requirement for a bird, weighing 2,10 kg, with a potential egg output of 48,7 g per bird per day, was estimated to be 166 mg per bird per day (1,51 g per kg diet at a consumption rate of 110 g per bird per day). This estimate is valid for an economic situation when the marginal cost of 1 kg tryptophan is 20 times the marginal value of 1 kg egg. Under the same price structure and potential egg output the estimated daily tryptophan requirement for a flock was found to be 150 mg per bird per day (1,36 tryptophan per kg diet for a consumption rate of 110 g per bird per day) when the Reading model was implemented.
S. Afr. J. Anim. Sci. 1984, 14: 70 – 74

'n Proef waarin 1 152 Amberlink-jonghenne vanaf die ouderdom van 30 tot 35 weke gebruik is, is uitgevoer om hulle triptofaan-behoeftes te bepaal. Die agt behandelings het bestaan uit 'n reeks diëte wat verkry is deur verdunning van 'n dieet met hoë digtheid t.o.v. aminosure met een van lae digtheid. Alle aminosure in die hoëdigtheid-dieet was sodanig dat die berekende waardes meer was as die normale behoefte van jonghenne en triptofaan was ook in oormaat, maar tog die mees beperkende aminosuur in verhouding tot die ander. Die daaglikse behoefte aan triptofaan is vasgestel deur van die sg. Readingmodel gebruik te maak. In die eerste plek word 'n responsiekurwe afgelei waarvan die hoeveelheid triptofaan benodig vir eiervorming en vir onderhoud die enigste twee komponente uitmaak. Die koëffisiënte van hierdie twee komponente weerspieël die doeltreffendheid van triptofaan-omsetting t.o.v. die twee funksies. Volgens die Readingmodel-oplossing word 2,017 mg triptofaan benodig per gram eier geproduseer en 8,256 mg triptofaan per kg liggaamsmassa as onderhoudsbehoeftes. 'n Vergelyking wat verkry is deur passing van 'n meervoudige regressie het 'n paraboliese vorm aangedui. Volgens die vergelyking van die parabool is die triptofaanbehoefte van 'n jonghen met 'n massa van 2,10 kg met 'n eieruitsetpotensiaal van 48,7 g eier per dag, vasgestel op 166 mg per hen per dag (1,51 g per kg dieet as die jonghenne 110 g voer per hen per dag inneem). Hierdie bepaling geld by 'n situasie waar 1 kg triptofaan 20 maal soveel kos as 1 kg geproduseerde eiers. As dieselfde ekonomiese situasie geneem word en vir dieselfde grootte henne met dieselfde eierproduksiepotensiaal, is die triptofaanbehoefte 150 mg per hen per dag (1,36 triptofaan per kg dieet as die henne 110 g per hen per dag inneem) met die Readingmodel-metode.
S.-Afr. Tydskr. Veek. 1984, 14: 70 – 74

Keywords: Tryptophan requirements, pullets, egg production

J.J. du Preez* and J.S. Duckitt
Department of Poultry Science, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

*To whom correspondence should be addressed
Received 27 June 1983

Introduction

Tryptophan is not only one of the essential amino acids for poultry but is also often one of the important economic constraints in least-cost diet formulation. The extent to which the final cost of the diet will be influenced, depends upon the requirement value. The latter value is normally set as a minimum level in the computer operation used to obtain a least-cost formula. At the moment a large discrepancy exists between the NRC (1971) recommended value of 1,1 g tryptophan per kg diet and the ARC (1975) recommendation of 1,7 g tryptophan per kg diet. Morris & Wethli (1978) published results which were in support of the latter recommendation. On the other hand trials to estimate the tryptophan requirement based on the composition of whole egg (Johnson & Fisher, 1958) support the lower value recommended by the NRC.

The diet dilution technique which Fisher & Morris (1970) used for measuring the response of laying hens to methionine intake and later used for response to dietary lysine (Pilbrow & Morris, 1974) has become an established procedure. The advantages of working with an appropriate dose response curve from which the requirement of different flocks for the specific nutrient can be determined under any set of economic conditions were outlined by Fisher (1976) and the model describing the response curve was published by Fisher, Morris & Jennings (1973). Although the requirement values mentioned above are expressed as grams per kilogram diet, the method referred to here relates the response to the daily quantity of amino acid consumed rather than to the concentration in the diet. The latter can be calculated according to the daily consumption of the birds at a particular phase of production. Since not only phase of production and environmental factors such as temperature, but also dietary factors such as energy concentration of the diet influence food consumption, the daily amount of amino acid required seems to be a logical way of expressing requirements. This was specifically acknowledged by Pilbrow & Morris (1974) when reporting on the lysine requirement for egg production and by D'Mello (1976) when reporting on amino acid requirements of growing birds. ARC (1975) used both methods to express requirements for layers.

In the trial reported here the tryptophan requirement of Amberlink laying birds was determined. The diet dilution technique was used, followed by analysis of the data according to the method described by Fisher *et al.* (1973). Recently it has become a more acceptable practice to use available amino acid values for diet formulation and therefore it was decided to use available tryptophan as a basis for requirement evaluation in the work reported here.

Materials and Methods

Experiment

The experiment was conducted with 1 152 Amberlink pullets 30 weeks old, divided into 48 groups of 24 birds. Each group was made up of one row of a two-tier battery and one bird was housed per cage (250 × 400 × 400 mm). Treatments were allocated randomly in the house so that 120 birds (five replicate groups) received the same dietary treatment except for treatments 9 and 10 which could be offered to only 96 birds (four replicate groups). Food was continuously available but water was given at four-hourly intervals for an hour at a time throughout the day. Food consumption, egg numbers and egg mass were recorded. The latter was only measured for two days in seven in a fixed pattern; skip two days measure two days, skip three days *etc.* The body mass of individual birds was measured at the beginning and end of the trial. Recording of the parameters started only after the treatment effects on the egg output stabilized (Nine days in this case) and continued for 21 days.

Experimental diets

The experimental diets were obtained by mixing proportions of the summit and dilution (basal) diets as indicated in Table 1. The composition as well as the amino acid content of the summit and dilution diets is shown in Table 2. Owing to the fact that the calculated isoleucine content of the summit diet expressed as a multiple of the birds' requirement was most limiting, apart from tryptophan, a small preliminary trial was done in which it was demonstrated that isoleucine was not limiting and in the same trial it was shown that the diluted summit diet responded positively to the addition of L-tryptophan.

Table 1 Proportions of summit and basal (dilution) diets used to construct experimental diets

Diet code	Calculated dietary tryptophan g/kg	Mixes	
		Summit	Basal
1	1,93	600	–
2	1,75	516	84
3	1,56	427	173
4	1,38	342	258
5	1,20	258	342
6	1,02	173	427
7	0,83	84	516
8	0,65	–	600

The summit diet was diluted with maize starch to reduce the crude protein to 80 g/kg diet but leaving the other nutrients unaltered. The diluted diet supported an egg output of 18,44 ± 2,67 g/hen/day. Supplementation of this diet with 0,95 g tryptophan/kg increased egg output to 24,7 ± 3,0 g/hen/day. Supplementation of the diet with 1,0 g isoleucine/kg decreased egg output to 16,22 ± 2,58 g/hen/day. Rhode Island Red birds, 50 weeks old and 18 randomly selected per treatment, were used after a depletion period of one week.

The matrix for the ingredients with which the linear least-cost formulation was performed, included calculated available amino acid values for all commodities with the exception of the available tryptophan values (Table 2) which were determined by the slope ratio bioassay method described by Du Preez & Hayes (1981). Total tryptophan values were not determined and available tryptophan values of diet mixtures were not verified by bioassay.

Table 2 Summit and basal diets formulated to construct the experimental diets. (Composition in g/100 g)

Ingredients	Available tryptophan g/100 g	Summit diet	Basal diet
Yellow maize meal	0,080	35,58	24,04
Groundnut oil cake meal	0,406(± ,007)***	10,30	1,43
Fish meal	0,742(±,006)	9,79	
Maize starch	0	7,64	20,00
Maize gluten meal (± 56% protein)	0,266(±,023)	19,00	14,41
Oat husks	0	7,55	12,86
Salt		0,13	0,40
D L-Methionine		0,15	0,15
L-Lysine monohydrochloride		0,57	0,30
Limestone flour		7,59	8,06
Dicalcium phosphate		1,28	2,64
Sucrose			15,00
Vitamins & trace minerals**		0,20	0,20
Vitamin B_{12} (g/100 g mix)		–	0,0002
Maize oil			0,50
Amino acid contents expressed as multiple of requirement*			
Lysine		2,40	0,88
Methionine		2,66	1,50
Methionine + cystine		2,50	1,34
Arginine		2,40	0,80
Threonine		2,39	1,13
Histidine		2,40	
Isoleucine		1,98	0,93
Leucine		3,77	2,16
Phenylalanine + tyrosine		3,13	1,63
Valine		2,22	1,04
Tryptophan		1,60	0,54

* according to Johnson & Fisher (1958)
** to conform to ARC standards (Metabolizable energy 11,5 MJ/kg)
*** figures in brackets indicate standard error (n = 5)

Statistical analysis and calculations

The data were subjected to a one-way analysis of variance on egg output (number of eggs/100 birds/d; g/bird/d), food intake (g/bird/d), tryptophan intake (mg/bird), change in body mass (g/bird/21 d), mean body mass (kg/bird). For determination of the input that would achieve optimum economic response, two approaches were followed. The first approach was taken from the illustration by Fisher (1967) in which the assumption is made that a parabolic curve would fit the data. The calculation then proceeded as follows:

Response curve represented by equation:

$$y = f(x)$$

when y = mass of egg produced/hen/d and x = mass of nutrient fed to produce y assuming all other factors are such that response y is not affected.

Assume the following function fits the data best:

$$y = a + bx + cx^2 \text{ (parabola)} \quad \ldots\ldots 1$$

Profit is revenue minus cost

$$z = py - kx \quad (2)$$
$$= p\,f(x) - kx \quad (3)$$

when p = marginal value of eggs per unit mass; k = marginal cost of tryptophan per unit mass,

Substitute 1 into 3:

$$z = p(a + bx + cx^2) - kx \quad (4)$$

To maximize:

$$\frac{dz}{dx} = pb + 2\,pcx - k$$

$$\frac{d^2z}{dx^2} = 2\,pc$$

$c < 0$ for maximizing z and also
$b > 0$

then $pb + 2\,pcx - k = 0$

$$x = \frac{k - pb}{2\,pc}$$

$$= \frac{k/p - b}{2\,c}$$

If $k/p = F$ (cost ratio)

then $x' = \frac{F - b}{2\,c}$

The 40 individual data points were used in fitting the curve. The second approach was to fit a curve and determine the coefficients of the response function according to the procedure described by Fisher *et al.* (1973) and subsequently determine the appropriate input of tryptophan that would yield an optimum economic result.

Results and Discussion

A summary of the results of the trial is given in Table 3. A response curve shown in Figure 1 could be constructed from the results. The calculated values for adequacy of amino acids (Table 2) show that tryptophan is assumed to be the first limiting amino acid in the summit diet. In the preliminary trial the assumption was tested and a trend could be demonstrated. The object of including treatments 9 and 10 was to verify tryptophan response in both the summit and basal diets. It was successful only for the basal diet as indicated by significant differences between mean egg output of treatments 8 and 10 in Table 3. Owing to the pronounced plateau the mean egg output of treatments 2, 4 and 9 are not statistically different. The mean egg output of diet 4 was 48,48 ± 0,75 and by supplementing diet 4 with 0,37 g tryptophan/kg diet the mean egg mass output increased to 49,25 ± 0,56 which indicates a positive tendency.

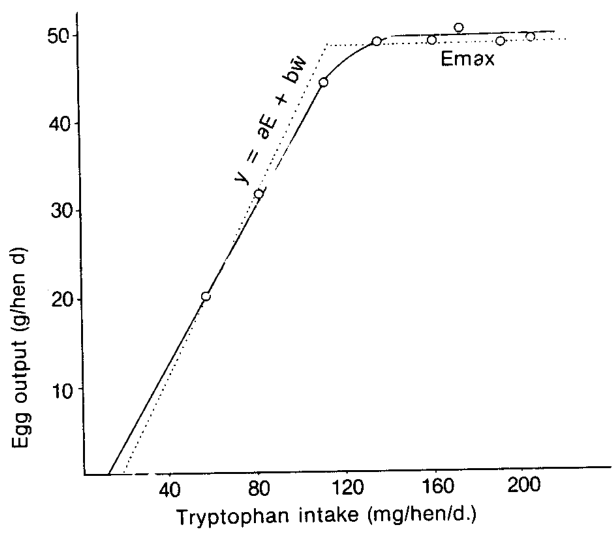

Figure 1 Egg output of Amberlink laying pullets as a function of tryptophan intake . . . . . Reading Model fit (response of individual bird) •——• Data prints merge (flock response)

A parabolic curve was fitted to the data using a multiple linear regression procedure. A highly significant regression was obtained explaining 95 % of the total variation by the equation

$$y = -16{,}8 + 784\,x - 2295\,x^2$$

where y represents tryptophan input (g/hen/d) and x represents

Table 3 Summary of the results of a 21 day trial to determine egg output response of young laying Amberlink pullets on different tryptophan intake levels

Treatment	Calculated dietary tryptophan*** g/kg	Egg production for period eggs/100 birds/d	Egg production g/bird/d	Food intake g/bird/d	Tryptophan intake mg/bird/d	Change in body mass for 21 days g/bird	Mean body mass/bird in kg $\frac{WI + WT}{2}$
1	1,93	89,8 a**	48,50(±1,77)* a	106 ab (±5,16)	204 a (± 10)	82 ab	2,121(±,026) a
2	1,75	88,8 ab	48,13(±2,10) a	109 ab (±1,16)	191 a (± 2)	58 ab	2,126(±,026) a
3	1,56	91,2 a	49,46(±1,79) a	110 a (±4,92)	172 b (± 7,7)	48 ab	2,092(±,029) ab
4	1,38	89,9 a	48,48(±1,64) a	116 a (±8,80)	160 b (±12,1)	117 b	2,087(±,035) ab
5	1,20	89,1 ab	48,34(±1,91) a	113 a (±4,62)	136 c (± 5,5)	104 ab	2,095(±,027) ab
6	1,02	82,1 bc	43,85(±2,99) b	109 ab (±4,21)	111 d (± 4,3)	−8 a	2,021(±,051) ab
7	0,83	60,4 d	31,24(±1,77) c	98 bc (±3,07)	82 e (± 2,5)	36 ab	1,921(±,036) cd
8	0,65	40,9 e	20,06(±2,10) d	87 c (±6,06)	57 f (± 3,9)	92 ab	1,823(±,085) d
9	1,75 (1,38+0,37)	90,4 a	49,25(±1,23) a	114 a (±7,86)	199 a (± 1,3)	86 ab	2,099(±,114) ab
10	1,02 (0,65+0,37)	75,8 c	39,49(±1,83) b	110 ab (±4,51)	112 d (± 4,5)	84 ab	1,984(±,036) bc

* Standard deviation (five replicates).
** Same alphabetic postscripts indicate that means do not differ significantly ($P \leq 0{,}05$) from one another.
*** Tryptophan of dietary ingredients bioassayed before formulation for available tryptophan (method: du Preez & Hayes, 1980).

egg output (g/hen/d). In a price structure where marginal cost of 1 kg of tryptophan is 20 times the marginal value of 1 kg of egg, the most economical input level of tryptophan would be 166 mg/d (1,51 g/kg diet at a consumption rate of 110 g/hen/d) according to this analysis.

Using the Reading model and the same price structure the optimal economic output is achieved at a tryptophan input of 150 mg/d (1,36 g tryptophan per kg diet). The equation according to the Reading model procedure was

$$\bar{y} = 8,26\ \bar{W} + 2,017\ E$$

where y represents the tryptophan input (mg/hen/d). $\bar{W}$ represents mean body mass (kg/hen/21 d) and E represents egg output (g/hen/d) for an individual bird. The mean body mass in the study was 2,10 kg per bird and the maximum response (E_{max}) was 48,74. In the analysis of variance the residual mean square (five degrees of freedom when three degrees of freedom are used in fitting the curve) was compared with the mean square value within treatment groups and the resulting F ratio of less than one indicated a valid fit. Estimates of variance and covariance to obtain the response curve using the model were 9,8 g and 0,21 kg respectively, representing standard deviations of egg output and body mass. It was assumed that $rEW = 0$. The minimum theoretical value for the coefficient of E in the equation was given by Morris & Wethli (1978) as 2,18 mg/g egg mass. The estimate of the coefficient in this experiment is lower than the theoretical value and considerably lower than the pooled value of 2,25 for two experiments including three breeds of birds in their experiment. The Amberlink bird in our trial corresponded to the Arbor Acre (2,09 mg/g) in this respect while the Warren birds were yet lower with 1,9 mg/g egg mass output. The coefficient of W in our experiment is slightly lower than the pooled value of 10,25 mg tryptophan per kg body mass given by the latter authors.

Table 4 Optimum tryptophan intakes (mg/hen/d) for young laying pullets* at different rates of egg output and different mean body mass

Potential egg output of flock (g/hen/d)	Mean body mass (kg)	Cost ratio** (k) 0,015	0,020	0,025
	1,9	146	143	141
46	2,1	147	145	143
	2,3	149	146	144
	1,9	151	149	147
49	2,1	153	151	149
	2,3	155	152	150
	1,9	158	155	153
52	2,1	159	157	155
	2,3	161	158	156

*It is assumed that tryptophan requirements are 2,017 mg/g egg output and 8,26 mg/kg body mass and standard deviations of 9,8 g/hen/d for egg output and 0,21 kg for body mass.

$$**k = \frac{\text{marginal cost of 1 mg tryptophan input}}{\text{marginal value of 1 g egg output}}$$

The birds used in the trial reported here were about 30 weeks old and the duration of the data collection was only three weeks subsequent to a depletion period of just over one week. From formulae developed earlier e.g. by Thomas (1967) and Combs (1960), it was assumed that daily amino acid requirement is dependent on a simple linear relationship of egg output, plus body mass, plus change in body mass and the latter term normally made a small contribution to the total requirement of an individual hen. Hence the short depletion period was thought to be justifiable in our experiment. In addition the body mass component in the Reading model as we used it takes into account the body mass at the start and the end of the data collection period and the assumption was made that the effect of body mass change could be ignored.

There is no doubt, however, that the method of analysis used in the Reading model requires that stability is reached in the input-output relationships (Morris & Wethli, 1978; Morris, 1981). The model does not allow a change in body mass because these changes probably represent changes in body protein of the animal. The amounts of amino acid involved would not be available to support egg output, leading to an underestimate of coefficient a. In the trial reported here the birds had not reached mature body mass and it can be seen from the figures in Table 5 that birds on most of the treatments gained body mass after an initial loss in mass. The ideal is to discard the data for the period during which stability has not been achieved. It was decided to recalculate the output values in such a way that the egg output and change in body mass constitutes a combined output value. In doing this the assumption is made that the tryptophan content of egg and live body mass is approximately the same. The outcome of this procedure after fitting a curve to the altered data using the Reading model was that the coefficients changed slightly. The coefficients obtained for a and b were 2,16 and 3,2 respectively.

Using the altered data and the same relationship between marginal cost of tryptophan and marginal value of egg output, it was concluded that the situation did not change financially and was in fact identical *viz.* an optimum input of 150 mg tryptophan per hen per day under these particular experimental conditions.

Table 5 Summary of body mass and body mass change of Amberlink pullets on different tryptophan input levels

Treatment	Mass loss* during adaptation period of 9 days (g/bird/d)	Body mass after adaptation period (g)	Change of body mass during 21 days of trial (g/bird/d)
1	2,2	2081	+ 3,9
2	0,3	2097	+ 2,7
3	3,5	2068	+ 2,3
4	8	2029	+ 5,6
5	6,3	2044	+ 5,0
6	8,3	2025	− 0,4
7	21,8	1903	+ 17
8	35,8	1777	+ 4,4
9	4,9	2056	+ 4,1
10	17,5	1942	+ 4,0

* Treatment groups were not weighed at onset of adaptation period but flock mean was assumed 2,1 kg per bird from a sample weighing.

An important distinction of the causal model (e.g. Reading model) as compared to the empirical models in that the coefficients obtained in the former model can be used to predict results (Fisher, 1980). Comparison of coefficients becomes an important aspect of the experimental results. The coefficient for E was 2,017 and was considered to be an underestimate in this experiment owing to changes in the body mass of the birds. The coefficient increased slightly to 2,166 when the body mass change was combined with the other output variable, namely, egg production. For the coefficient of W the result of the combined output was the opposite, a decrease from 8,256 to 3,892. Table 5 has been included to depict the changes in body mass of the birds during the 9-day adaptation period and the 21 days during which time the data were accumulated.

The assumption that tryptophan content of egg material is the same as that of body tissue *viz.* 0,18 g tryptophan/100 g, is well documented. The tryptophan content of poultry meat was summarized by Demby & Cunningham (1980) and the tryptophan content of egg output was calculated and published by Morris & Wethli (1978).

Daily changes in body mass (Table 5) are higher than normally seen in this type of experiment. Morris & Wethli (1978) reported changes of + 2,6 to − 1,2 g/bird/d and Pilbrow & Morris (1974) reported changes in the range of + 2,39 and − 3,17 g/bird/d. The larger changes in body mass that occurred in our experiment could arouse criticism of the experimental results reported in this paper.

The fact that a considerably higher tryptophan requirement value was estimated from the quadratic equation (parabola) than the value estimated from the Reading model under similar economic conditions, supports an earlier view held by Fisher & Morris (1970) that the former approach results in overestimated amino acid requirement levels. For most parabolic curves a premise is set *viz.* a situation described by Filmer (1974). The situation he describes occurs when a nutrient is added to a point beyond which E_{max} has been reached and a depressed output results owing to the second most limiting amino acid becoming the limiting one. It is not always possible to achieve in a formula the amino acid profile which has all amino acids elevated to similar multiple factors above the birds' requirement. Therefore it is conceivable that a situation as described by Filmer can arise. Obviously, owing to the theory underlying the diet dilution technique which was implemented in this study and as a result of no such tendency shown in Figure 1 ('bent stick' shape) or in the one way analysis of variance, the parabolic fit could have been discarded. Nevertheless the highly significant fit of the parabola also shown by the multiple correlation coefficient warranted inclusion of a tryptophan requirement estimate by that method.

Disadvantages of the quadratic fit were given by Fisher (1980). From the equation, calculations can be made for different economic situations but not for different flock (population) situations. Estimates of tryptophan requirements cannot be made of flocks that have body masses and/or egg outputs deviating from the means in the flock from which the equation was originally derived, without violating statistical confidence intervals.

It is concluded that the results from this experiment made a contribution to the material required for general prediction models in the field of commercial egg production. The estimated requirement value from the coefficients determined in the experiment lies between the value recommended by the NRC (1971) and ARC (1975).

In a recently published paper (Morris & Blackburn, 1982) the subject of curve-fitting is thoroughly discussed and the shortcomings of the parabolic fitting is pointed out. The results presented in the present paper clearly demonstrate the overestimation of the tryptophan inclusion level obtained by a curve-fitting procedure compared to the Reading model approach. Under these circumstances and owing to the fact that the coefficients of the parabolic equation are meaningless, we conclude that the Reading model approach is to be preferred for estimating amino acid requirements of laying birds.

Acknowledgements

Dr Trevor Morris of Reading University contributed a great deal in stimulating us to commence a project which included the trial reported here and other similar trials. His valuable time spent on correspondence, consultation and analysing the data is highly appreciated.

Mr D. Page of the Faculty of Engineering was responsible for guidance in using his program to fit the parabolic function to the data and for making alterations to produce a suitable print-out.

We thank the Department of Agriculture for making it possible to complete this study by giving the necessary financial support.

References

AGRICULTURAL RESEARCH COUNCIL (1975). *The Nutrition Requirements of Farm Livestock — Poultry.* 2nd ed. London, Her Majesty's Stationery Office.

COMBS, G.F., (1960). *Proc. Md. Nutr. Conf. Fd. Mfrs.* p. 28 quoted by ARC (1975).

DEMBY, J.H. & CUNNINGHAM, F.E.N., (1980). Factors Effecting Composition of Chicken Meat. A Literature Review. *Worlds Poultry Sci. J.* 36: 25 – 67.

D,MELLO, J.P.F., (1976). Requirement of the young turkey for sulphur amino acids and threonine: comparison with other species. *British Poultry Science,* 17, 159.

DU PREEZ, J.J. & HAYES, J.P. (1981). Assaying feedstuffs for bioavailable tryptophan using maize gluten in the basal diet. *Agroanimalia* 13, 7 – 13.

FILMER, D.G. (1974). Factors influencing Food intake in practice — the laying Fowl. *Nutrition Conference for Feed Manufacturers.* Ed. H. Swan and D. Lewis, Butterworths, London, p. 165.

FISHER, C., (1967). Protein requirements for layers. *Protein Utilization by Poultry.* Ed. R.A. Morton and E.C. Amoroso, Oliver and Boyd, Edinburgh, p. 189.

FISHER, C. & MORRIS, T.R., (1970). The determination of the methionine requirements of laying pullets by a diet dilution technique. *British Poultry Science,* 11, 67.

FISHER, C., MORRIS, T.R. & JENNINGS, R.C., (1973). A model for the description and prediction of the response of laying hens to amino acid intake. *British Poultry Science,* 14, 469.

FISHER, C., (1976). Protein in the diets of the pullet and laying bird. *Protein Metabolism and Nutrition.* Ed. Cole, J.D.H.; Boorman, K.N.; Butterly, P.J.; Lewis, D.; Netke, R.J. and Swan, H.; Butterworths, London, p. 323.

FISHER, C., (1980). Protein deposition in Poultry. *Protein deposition in Animals.* Ed. Buttery, P.J. and Lindsay, D.B., Butterworths London p. 265.

JOHNSON, D. & FISHER, H., (1958). The amino acid requirement of laying hens 3. Minimal requirement levels of essential amino acids: technique and development of diet. *British Journal of Nutrition,* 12, 176.

MORRIS, T.R. & WETHLI, E., (1978). The tryptophan requirements of young laying pullets. *British Poultry Science,* 19, 455.

MORRIS, T.R. & BLACKBURN, HAZEL, A., (1982). The shape of the response curve relating protein intake to egg output for flocks of laying hens. *British Poultry Science,* 23, 405.

MORRIS, T.R., (1981). Private communication.

NATIONAL RESEARCH COUNCIL, (1971). *Nutrient Requirements of Poultry* 6th ed. National Academy of Science — National Research Council.

PILBROW, P.J. & MORRIS, T.R., (1974). Comparison of lysine requirements amongst eight stocks of laying fowl. *British Poultry Science,* 15, 51.

THOMAS, O.P., (1967). Feedstuffs 39 no. 1, 19 quoted by Agricultural Research Council (1975).