A OF LEUCAENA LEUCOCEPHALA AND GRASS MEALS AS SOURCES OF YOLK PIGMENTS IN DIETS FOR LAYING HENS[1]

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Forty eight laying hens were used in a 6 x 8 randomised block design to compare the efficiency of Leucaena leaf meal and grass meal as sources of yolk pigments. Leaf meals were added to a low pigment diet (LP) to supply 10 or 20 mg dihydroxyxanthophyll (DHX) / kg. Leucaena from Malawi was added to supply both 10mg (L10) and 20mg DHX/kg (L20), that from Bangkok was added to supply 10mg DHX/kg (B10) and the grass to supply 20mg DHX/kg. A fifth diet (L20c) was the same as the L20 diet except that coconut oil replaced the groundnut oil in the basal diet.

There were no significant differences between treatments with respect to egg production, egg yield, mean daily food intake and liveweight change during the 28-day experimental period.

After 7 days, significant differences ($P<0.001$) in Roche fan score (RFS) and Beta- carotene equivalent (BCE) values were evident between the 3 DHX levels (0, 10 and 20 DHX/kg). Further changes during the second week were followed by stabilisation in yolk colour during the last two weeks.

No differences were detected within DHX level for visual yolk colour measurement (RFS) except that on day 28 the RFS for the L20c diet was detected as significantly greater ($P <0.05$) than than the other two 20mg DHX treatments (L20 and G20).

BCE measurements gave significant differences ($P<0.001$) within the 20mg DHX treatments (L20 vs L20c +G20) from day 7 onwards. In addition on day 28 the BCE values for diets L20c and G20 were detected as significantly different ($P<0.001$).

The absence of any deleterious effects on egg production in this short term study suggests that at inclusion rates of 10-25 g/kg leucaena is an effective yolk pigmenter.

Key words: Laying hens, leucaena meal, grassmeal, yolk pigmentation, saturated and unsaturated fats

The leguminous shrub Leucaena leucocephala, widely used as a protein supplement in ruminant and non-ruminant diets has, when used as a poultry feed, another attribute which has yet received very little attention: its high carotenoid content produces highly pigmented broilers and egg yolks.

The purpose of this work was to compare the efficiency of leucaena leaf meal and grass meal as sources of yolk pigment. The effect of dietary fat on yolk pigmentation was also investigated.

Materials and Methods

Animals: Forty eight "Ross Tint" hens (White Leghorn x Rhode Island Red type) in their first year of lay were used in the experiment. From hatching, the chicks had 22 h light/day, reduced to 8 h/day by the third week of age and held constant until point of lay at 18 weeks. At this time the light hours were increased by 20 minutes/week to reach a level of 13 h/day. After 4 months in lay, during which time they were used for

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other yolk colour investigations, they were transferred to the Centre for Tropical Veterinary Medicine (CTVM), University of Edinburgh, where they were housed in individual cages, in two non-lightproof rooms , receiving a constant 19 h light/day. Food and water were available at all times. During the pre-experimental period the birds received a standard layers' ration, in order to eliminate any differences in yolk colour due to previous experimental treatments. On two occasions during this period a random sample of 16 eggs were broken out and compared using the Roche colour fan (Strieff 1970). The pre-experimental period ended after 13 days, when, on the basis of the second comparison, it was decided that yolk colour was sufficiently uniform to start the 28-day experimental period.

Hens were allocated to individual cages at random. A randomized block design was used such that each of the 6 diets was represented in each of the 4 rows of cages in each of the 2 rooms.

Experimental diets: The six diets were mixed five days before the start of the experiment and were fed in meal form (Table 1). A wheat, barley, soya bean meal and

Table 1:
Composition of the experimental diets (g/kg)

	LP	L10	L20	B10	G20	L20c
Wheat	533.5	521.3	509.1	516.5	483.9	509.1
Barley	181.0	181.0	181.0	181.0	181.0	181.0
Soyabean meal	80.0	80.0	80.0	80.0	80.0	80.0
Fishmeal meal	80.0	80.0	80.0	80.0	80.0	80.0
Groundnut oil	30.0	30.0	30.0	30.0	30.0	-
Calcium Carbonate	70.0	70.0	70.0	70.0	70.0	70.0
Sodium Chloride *	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium Phosphate	20.0	20.0	20.0	20.0	20.0	20.0
Mineral and vitamin mix	2.4	2.4	2.4	2.4	2.4	2.4
DL-methionine *	1.0	1.0	1.0	1.0	1.0	1.0
Leucaena (Malawi)	-	12.2	24.4	-	-	24.4
Leucaena (Bangkok)	-	-	-	16.1	-	-
Grass meal (CTVM)	-	-	-		49.6	-
Cocoput oil	-	-	-	-	-	30.0
BHT *	0.1	0.1	0.1	0.1	0.1	0.1
	1000.0	1000.0	1000.0	999.1**	1000.0	1000.0

* Added as a premix
** at mixing sufficient Bangkok leucaena was available to add 16.1 g/kg in place of 17.0 g/kg

fishmeal diet served as the low pigment ration (LP), and variations to this diet were made by the addition of either dried leucaena or grass meal, at the expense of wheat. The leucaena came from Malawi (cv Cunningham) or Bangkok (cv unknown). The grass meal was prepared from grass cut at the CTVM.

The dried leaf materials were finely ground and analysed for dihydroxyxanthophyll (DHX) content (Quackenbush, Dyer and Smallidge 1970).

Additions were made to the low pigment diet to supply either 10 mg or 20 mg DHX/kg diet. The leucaena from Malawi was added to supply both 10 mg (L10) and 20 mg DHX/kg (L20), that from Bangkok was added to supply 10 mg DHX/kg (B10) and the grass to supply 20 mg DHX/kg (G20). The fifth diet was the same as the L20 diet except that coconut oil was melted and added in place of the groundnut oil (L20c).

After mixing,a sample of each diet was taken and used at the end of the experiment for DHX determination (Table 2). This determination showed a much lower DHX content of the G20 diet (12.6 instead of 20 mg DHX/kg).

Table 2:

Dihydroxyxanthophyll (DHX) content of the experimental diets as determined at the end of the experiment

Diet	DHX content mg/kg
LP Low pigment	0.56
L10 Leucaena 1.22%	9.30
L20 Leucaena 2.44%	21.00
B10 Bangkok leucaena 1.61%	8.83
G20 Grassmeal 4.96%	12.66*
L20c Leucaena 2.44% & Coconut oil 3%	20.45

* see discussion

Reanalysis of the grass meal used indicated that only part of this discrepancy could be accounted for by a deterioration of the pigments during the experimental period.

Measurements : Eggs were collected daily and weighed. The first laid each week by each hen was examined for pigmentation. The yolk was separated from the albumen, placed in a petri dish and weighed. It was then colour scored against a white background using the Roche colour fan (Streiff 1970).

The yolks were then broken and homogenised and a second Roche fan score recorded. A 2.5 g sample of the fresh yolk was then subjected to a modification of the AOAC beta-carotene equivalent (BCE) method (Forsythe 1958). The same pigment extraction procedure was followed; however, the extinction of the yolk pigments was compared with a working solution of Sudan 1 * in place of a standard curve derived from the absorbance (or transmittance) of standard beta-carotene solutions. The primary reason for using Sudan 1 as an indirect standard is that solutions of beta-carotene are subject to rapid deterioration. The beta-carotene concentration was thus computed assuming that the Sudan 1 working solution has the same absorbance as 2.35 mg carotene/litre at 436 nm (AOAC 1970).

Individual feed intakes were measured weekly.

Xanthophyll utilisation quotients were calculated for each diet relating output (measured in terms of beta-carotene equivalents) to input (measured in terms of DHX). Pigment output for days 14, 21 and 28 was computed from beta-carotene (BCE) concentration (μ g/g yolk) and yolk weight. This was related to pigment intake per egg laid, calculated from the feed conversion ratio (kg feed/egg) over the previous 7 days and the DHX content of the diet (mg/kg) as determined at the end of the experiment (Table 2).

* 0.04 millimolar 1-(phenlazo)-2naphthol dissolved in a 1:1 mixture of acetone and isopropanol

The liveweight of the birds was recorded at the beginning and end of the experimental period.

Statistical analysis : The Roche fan values obtained for broken yolks were compared using the chi-square test (Snedecor and Cochran 1967).

Egg production data were analysed using the covariance technique.

Results

The overall means for egg production and egg yield during the experimental period were 0.888 ± 0.024 egg/hen/day and 56.72 ± SE 1.82 g/hen/day. No significant effect of treatments was detected for either parameter, although a long-term trial would be advisable for confirmation.

The overall 1 mean food intake was 127.3 ± 9.32g/hen/day, with no significant differences between treatments.

Liveweight change during the experimental period was variable (overall mean ± 92 g ± SE 30 g).

Yolk colour: 1. Visual assessment. Roche colour fan scores of whole and broken yolks showed similar trends. The effect of the experimental diets on yolk colour was evident on the 7th day, however further changes during the 2nd week were followed by a stabilisation in yolk colour during the last two weeks (Figure 1).

A significant difference ($p < 0.001$) was detected in the Roche fan scores between the 3 DHX levels from after the first week.

There were no significant differences within the DHX levels except that on day 28 the Roche fan score for the coconut oil treatment (L20c) was detected as significantly greater ($P < 0.05$) than the other two 20 mg treatments (G20 and L20).

2. Chemical measurements. After 7 days significant differences ($P < O.001$) in the BCE values (μg BCE/g yolk) became apparent (Figure 2) between the LP diet, the 10 mg DHX level treatments (L10 and B10), the L20 treatment and the other two 20 mg DHX treatments (G20 and L20c). No significant difference was detected within the 10 mg DHX treatments (L10 and B10) nor between the G20 and L20c treatments. This trend continued during weeks 2 and 3, however on the 28th day the BCE values for the G20 and L20c treatments diverged and the difference- was detected as significant ($P < 0.001$) (Figure 2).

The overall xanthophyll utilisation (XU) quotients are shown in Table 3.

Table 3:

Mean xanthopyll utilisation (XU) quotients for the six experimental diets

	Diet	Xanthophyll utilisation
L10	Leucaena 1.22%	0.29a
B10	Bangkok leucaena 1.61%	0.30a
L20	Malawi Leucaena 2.44%	0.23b
L20c	Malawi Leucaena 2.44% & coconut oil 3%	0.30a
G20	Grassmeal 4.96%	0.43c

a, b and c are significantly different ($P < 0.001$)

Figure 1:
Mean Roche for scores of the broken yolks from the six experimental diets: low pigment diet (•-•) Leucaena (Bangkok) 16.1g/kg (▲-▲); Leucaena (Malawi) 2.2g/kg (■-■) Leucaena (Malawi 24.4g/kg (○-○) Grass 49 -6 g/kg (△-△); Leuceana 124.4g/kg plus cocunut oil (□-□)

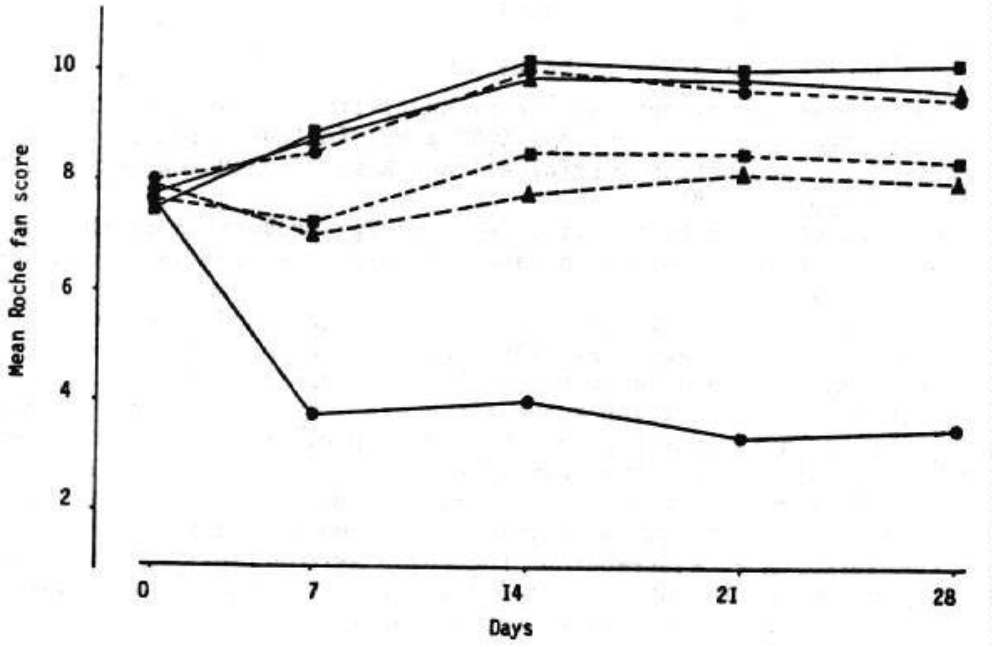
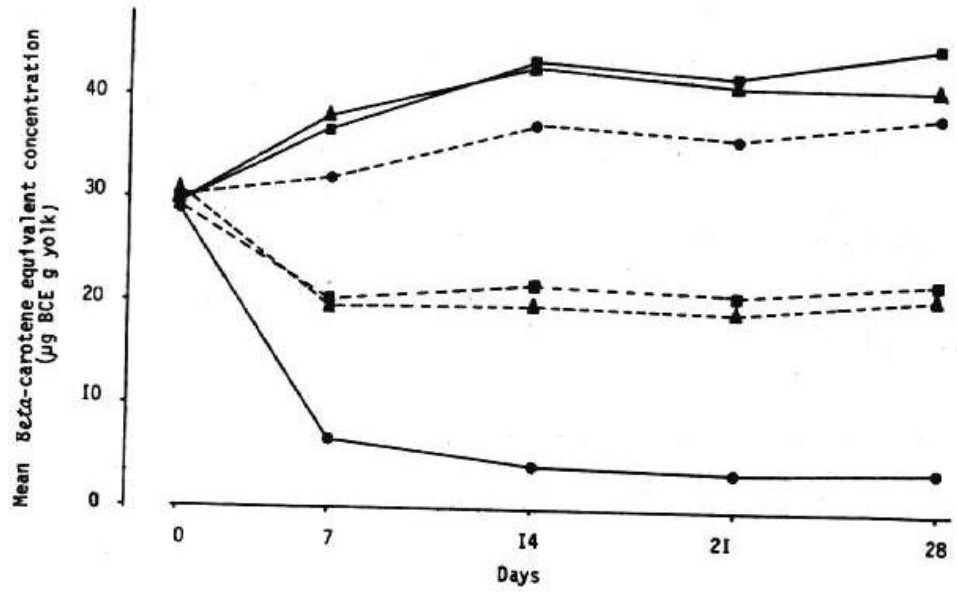


Figure 2:
Mean beta-carotene concentrations of yolk from the experimental diets: Low pigment diet (•-•) Leucaena (Bangkok) 16.1g/kg (▲-▲); Leucaena (Malawi) 2.2g/kg (■-■) Leucaena (Malawi 24.4g/kg (○-○) Grass 49 -6 g/kg (△-△); Leuceana 124.4g/kg plus cocunut oil (□-□)



Xanthophyll utilisation fell as the level of DHX in the diet increased from 10 mg to 20 mg/kg as shown by the highly significant difference between when diets L10 and L20 ($P < .001$). This difference disappeared, however, the coconut oil was added to the diet. The xanthophylls supplied by grass meal were used more efficiently than those supplied by leucaena ($P < 0.001$)

Discussion

The deleterious effects on the productivity of laying hens as a result of feeding high levels of leucaena (>50 g/kg) include: reduced food intake and reduced egg production (Springhall and Ross 1965; Mateo et al 1970; D'Mello and Taplin 1978).

The lack of any deleterious effects on egg production in this short term study suggests that at inclusion rates of 10-25 g/kg leucaena is an effective yolk pigmenter.

BCE values obtained in this experiment using a modification of the AOAC method (AOAC 1970) showed a similar relationship to Roche fan score, as obtained by Marnsich and Bauernfeind (1970) using natural feedstuffs.

In this study at a given Roche fan score leucaena pigments gave a lower value for BCE concentration than grass pigments. This is demonstrated by the fact that on day 0 the mean Roche fan score of diet L10 and B10 was 7.65, rising slightly during the experimental period to 8.19. In contrast the corresponding mean BCE value fell from 30~g/g yolk to 21.7 ~g/g yolk. Since the yolk pigments present on the day 0 were primarily due to grass meal in the pre-experimental layers' diet it seems that the BCE method "penalises" the yolk colour produced by leucaena. This suggestion is reinforced by the observation that there is no difference between diets L20 and G20 in terms of Roche fan score throughout the experiment, although G20 gave a significantly higher BCE concentration ($P < 0.001$) than the L20 treatment on days 7, 14 and 21.

It is suggested that this observation can be accounted for by the differences in the xanthophylls supplied by grass and leucaena (Table 4), such that at equal DHX levels

Table 4:
A comparison of the relative carotenoid fractions in leucaena and grass expressed as a percentage of total carotenoid

Leaf meal	Lutein	Zeaxanthin	Other carotenoids
Leucaena cv Cunningham	69	19	12*
Grass	80	10	10**

* Savory (1977)
** Streiff (1970)

(=lutein and zeaxanthin) the grass supplies substantially more lutein than leucaena due to their different lutein: zeaxanthin ratios (8:1 vs 3.6:1). Thus the differences in colour produced by either grass or leucaena are visually undetectable but differences in light absorbance of these two compounds at 436 nm would account for the penalisation of leucaena.

The current study indicates that coconut oil, when compared with groundnut oil increases transmission of pigments from the diet to the yolk, Abu-Serewa (1976) reports that both unsaturated fat (sunflower oil) and saturated animal tallow darken yolk colour; however, when the fats were mixed, yolk colour was deeper when the level of tallow was higher than that of sunflower oil and from this it was concluded that the magnitude of the increase was influenced by the fatty acid composition of the diet. This study supports this hypothesis and furthermore, indicates that it is the degree of saturation of the fat rather than its source that is important.

The insensitivity of the eye to the darker shades of yellow (Suede 1962) undoubtably explains the fact that while coconut oil increased yolk colour as measured by BCE concentration ($P< 0.001$) it had no effect on Roche fan score. This theory could be investigated in future work, by adding the coconut oil at lower levels of dietary xanthophyll, where an increase in yolk xanthophylls could be detected visually.

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