The effect of prolonged feeding of Stylosanthes guyanensis meal on laying performance, egg quality and hatchability

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Abstract

The utilization of increasing levels of **Stylosanthes guyanensis** meal (stylo meal) by laying chickens feed for a period of nine months and the effect of increasing levels of vitamin A on the hatchability of eggs were studied using Single Comb White Leghorn birds.

The feeding of stylo meal to laying birds lengthened the time taken by he birds to get to peak prouction. There was no significant effect on the average hen-day production percentage following the feeding of up to 20% stylo meal. The control diet in which there was no stylo meal however had a better production rate than any of the other diets. The feeding of stylo meal increased egg riboflavin content, egg hatchability and yold cholesterol level and depressed serum cholesterol level. The mproved egg hatchability observed in this study could not be related entirely to the improved vitamin A status of the diets with the addition of stylo meal. It is suggested that the utilization of higher levels of stylo meal could be improved by a better amino acid balance and pelleting of the rations.

Introduction

In an earlier study on the utilization of **Stylosanthes guyanensis** meal (stylo meal) by laying birds, Onwudike and Adegbola (1978) observed that the feeding of stylo meal improved yold colour, yolk vitamin A status, egg hatchability and also delayed the period during which the birds attained peak production. It appeared from these results that if the birds are fed for a longer period of time to allow them to adjust to the increasing levels of stylo meal, they could then tolerate higher levels of stylo meal than the 10% level which the birds were able to utilize in that study

Also the improved hatchability observed could have been due to either the improved vitamin A status of the eggs which resulted from the feeding of stylo meal or to some other factors. Bilek (1936) reported that an increase in yolk colour was accompanied with an increase in yolk cholesterol level and since stylo meal increases yolk colour, it was thought necessary to study the relationship between yolk colour and yolk cholesterol level.

This study was therefore carried out to investigate the effect of prolonged feeding of increasing levels of stylo meal on laying performance, yolk cholesterol level, egg riboflavin level and the effect of increasing levels of vitamin A on egg hatchability.

Materials and methods

Laying experiment and egg quality

Three hundred and twenty Single Comb White Leghorn pullets which were one and a half months in lay were randomly divided into five equal treatment groups of 64 birds each and these were further sub-divided

into four replicate groups of 16 birds each. The weights of the replicate groups were made equal and the birds paired in cages. The five dietary treatments contained 0, 5, 10, 15 and 20 percent levels of stylo meal (Table 1) The diets were made isocaloric and isonitrogenous. The birds

TABLE 1. COMPOSITION OF DIETS FED TO LAYING BIRDS (%)

	Levels of stylo meal (%)				
Ingredients	0	5	10	15	20
Yellow corn	71.30	69.00	65.00	60.20	56.00
Groundnut cake	16.00	14.00	12.27	10.95	9.60
Fish meal	3.50	3.50	3.50	3.50	3.50
Stylo meal	0.00	5.00	10.00	15.00	20.00
Oyster shell	6.05	5.70	5.50	5.30	4.90
Dicalcium phosphate	2.00	1.68	1.65	1.70	1.70
Agricare premix*	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Amprolium (coccidiostat,	0.05	0.05	0.05	0.05	0.05
Lysine	0.05	0.02	0.00	0.00	0.00
Methionine	0.05	0.05	0.03	0.00	0.00
Groundnut oil	0.00	0.00	1.00	2.30	3.25
Total	100.00	100.00	100.00	100.00	100.00
Analysis					
Crude protein (%)	16.43	16.38	6.26	16.17	16.21
Crude fibre (%)	6.28	7.04	7.68	8.39	9.08
ME, Calculated (kcal/kg,	2918	2878	2874	2878	2872
Calcium, calculated (%)	2.93	2.88	2.88	2.92	2.98
Available phosphorus, calculated (%)	0.55	0.51	0.50	0.51	0.51
Lysine, calculated (%)	0.58	0.58	0.57	0.58	0.59
Methionine + Cystine, calculated (%)	0.58	0.58	0.58	0.56	0.56

* *A Pfizer Livestock Feeds Product, Ikeja, supplying the following per kg of ration: Vitamin A, 8,000 I.U; D_3, 2,000 I.U; riboflavin, 4.20 mg; panthothenic acid, 5.0 mg, nicotinic acid, 20,0 mg; folic acid, 0.5 mg; choline, 300.0 mg; vitamin B_{12}, 0.01 mg; vitamin K, 2.0 mg; vitamin E, 2.5 I.U; manganese, 56 mg; iodine, 1.0 mg; iron, 20.0 mg; copper, 10.0 mg; zinc, 50.0 mg; cobalt, 1.25 mg and methionine, 225 mg.*

were fed for two weeks on the experimental diets before data collection was started. Feed and water were provided **ad libitum** throughout the nine months duration of the study. Feed samples were analysed for proximate composition according to the methods outlined in the Methods of Analysis published by the Association of Official Analytical Chemists (1975). The amino acid composition of the stylo meal (Table 2) was determined by the column chromatographic techniques using the automated Technicon TSM amino acid analyzer. Eggs were collected daily from the beginning of the third month in lay till the end of the eleventh month in lay. Average hen-day production percentages were

calculated from the total number of eggs actually collected, expressed as percentages of the expected number of eggs for each group per month over the 9-month period. Records of feed consumption were kept throughout the trial period. Eggs collected during the last 5 days of each month were kept together and weighed. The collections during the 3rd, 5th, 7th, 9th and 11th months in production were used for yolk cholesterol determination while the collections for the 4th, 6th, 8th and 10th months in production were used for egg riboflavin determination. Eggs were stored in a refrigerator at 0°C and analysed within one week of collection.

TABLE 2: AMINO ACID COMPOSITION OF STYLO MEAL (g/16g of N)

Amino acid	*Level*
Lysine	3.38
Histidine	2.10
Arginine	3.93
Threonine	8.45
Glycine	8.45
Valine	4.16
Methionine	1.01
Isoleucine	3.56
Leucine	4.40
Phenylalanine	2.48
Tyrosine	1.55
Serine	3.36
Proline	3.71
Alanine	6.33
Aspartic acid	7.04
Glutamic acid	4.91

Cholesterol determinations were carried out using the extraction procedure outlined by Fisher and Leveille (1957) and the quantities determined spectrophotometrically using the method described by Zlatkis, Zak and Boyles (1953). The riboflavin content of the whole fresh egg was determined fluorometrically as outlined in the Methods of Vitamin Assay published by the Association of Vitamin Chemists (1966). At the end of the experimental period, two hens from each replicate group were slaughtered and the blood collected for serum cholesterol determination.

Vitamin A and hatchability study

White Leghorn hens which had been in lay for about six months were transferred to the breeding pens with eight laying birds in each pen. Each pen received a good White Leghorn breeding cock. There were 16 pens made up of four dietary treatments in four replicates. The four diets (Table 3) consisted of three different levels of vitamin A per kg of diet. The levels were 8,000; 19,000; 24,000; and 19,000 I.U vitamin A/kg of diet. The vitamin A level in the first diet was supplied from a vitamin-mineral mix (Agricare mix); the second diet had an additional supply of 11,000 I.U/kg of diet from vitamin A acetate; the third diet had

a level of 24,000 I.U/kg of diet with 8,000 I.U supplied from "Agricare mix" and 16,000 I.U. from vitamin A acetate and the fourth diet in addition to receiving 8,000 I.U/kg of diet from "Agricare mix" also received a supply of 15 percent stylo meal which furnished 44 micrograms caretene per gram and the conversion factor of 0.6 was used to obtain the vitamin A value of the carotene content. The different diets

TABLE 3: COMPOSITION OF DIETS FOR HATCHABILITY STUDY (%)

	Levels of vitamin A (I.U/kg)			
Ingredients	8,000	19,000	24,000	19,000*
Yellow corn	71.30	71.30	71.30	60.20
Groundnut cake	16.00	16.00	16.00	10.95
Fish meal	3.50	3.50	3.50	3.50
Stylo meal	0.00	0.00	0.00	15.00
Oyster shell	6.05	6.05	6.05	5.30
Dicalcium phosphate	2.00	2.00	2.00	1.70
Agricare premix**	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Amprolium (coccidiostat)	0.05	0.05	0.05	0.05
Lysine	0.05	0.05	0.05	0.00
Methionine	0.05	0.05	0.05	0.00
Groundnut oil	0.00	0.00	0.00	2.30
Total	100.00	100.00	100.00	100.00
Vitamin A acetate (g)***	0.00	1.00	1.47	0.00

* *8,000 I.U/kg supplied from a vitamin-mineral mix and 11,000 I.U supplied from stylo meal which contained 44 mcg/g.*

** *A vitamin-mineral mix already described in Table 1.*

*** *Vitamin A acetate supplying 500,000 I.U/g.*

were fed in the pens for three weeks before egg collection for incubation started. Fertility was determined by candling eggs after one week of incubation and the percent hatchability was based on the number of chicks hatched from fertile eggs.

All the results obtained were subjected to the analysis of variance and significant differences between means were determined by Duncan's multiple range test as outlined by Steel and Torrie (1960).

Results

The results of this study are presented in Tables 4, 5 and

Production performance.

The feeding of up to 20% stylo meal for nine months from the time the birds were already two months in lay had no significant effect on the

average hen-day production percentage. The control diet in which there was no stylo meal however had a better production rate than any of the other diets (Table 4). The feeding of stylo meal lengthened the time it took the birds to come to peak production (Table 5). The birds came to peak production at the third month in lay when no stylo meal was fed. The addition of 5 or 10% stylo meal delayed this period by 1 month while the addition of 15 or 20% of stylo meal delayed the period by an additional month. Feed intake and feed efficiency values were not significantly affected by the addition of stylo meal to the diets. The diet without stylo meal was the most efficient in terms of egg production and there was a progressive increase in the amount of feed required per dozen eggs as the level of stylo meal in the diet was increased.

TABLE 4: EFFECTS OF LEVELS OF STYLO MEAL ON THE LAYING PERFORMANCE, YOLK CHOLESTROL, SERUM CHOLESTEROL AND EGG RIBOFLAVIN LEVELS

	Levels of stylo meal (%)					S.E
Characteristics	0	5	10	15	20	means
Average hen-day production (%)	42.73	40.34	38.96	38.48	37.92	1.22
Average feed intake per bird per day (g)	122.25	120.68	122.30	120.10	119.58	4.84
Average feed consumed per dozen eggs (kg)	3.43	3.60	3.77	3.78	3.79	0.19
Average egg weight (g)	57.10	56.49	57.07	56.86	55.94	0.40
Riboflavin content (mcg/g)	4.04^{c}	4.24^{bc}	4.42^{b}	4.68^{a}	4.88^{a}	0.05**
Yolk cholesterol (mg/g fresh yolk)	24.06^{a}	24.32^{b}	24.99^{c}	25.31^{d}	25.94^{e}	0.05**
Serum cholesterol (mg/100 ml)	230.25^{a}	212.75^{b}	195.00^{c}	178.75^{d}	174.00^{d}	1.61**

** *Highly significant differences among treatment means* [$P < 0.01$]

a.b.c.d.e = *Means in the same horizontal row not followed by the same letter are significantly different from one another* [$P < 0.01$].

TABLE 5: monthly hen-DAY PRODUCTION (%) OF WHITE LEGHORN BIRDS FED DIFFERENT LEVELS OF STYLO MEAL*

Percent stylo meal in diet	Months from point of lay								
	3rd	4th	5th	6th	7th	8th	9th	10th	11th
0	59.89	58.42	52.92	47.60	41.55	34.68	32.85	28.95	27.74
5	50.65	*56.42*	48.91	44.10	42.76	34.80	31.94	25.81	25.68
15	48.73	*54.67*	47.46	41.19	40.45	35.84	31.25	26.18	24.85
15	41.76	45.50	*53.11*	47.49	38.79	36.75	30.22	27.16	25.22
20	40.15	44.57	*53.73*	47.43	37.42	35.69	29.57	27.03	25.67

* *Underlined values show points of peak production*

Egg riboflavin and yolk and serum cholesterol levels

The riboflavin content of the whole egg was significantly (P 0.01) ncreased as the level of stylo meal was raised in the diets.

The riboflavin contents ranged from 4.04 mcg/g whole egg for birds fed the control ration to 4.88 mcg/g whole egg for birds which received the 20 percent level of stylo meal. The yolk cholesterol level also increased significantly (P 0.01) as the level of stylo meal in the diets increased. On the other hand, the serum cholesterol level was significantly (P 0.01) reduced as the level of stylo meal in the diets increased.

Fertility and hatchability

Increasing the level of vitamin A from 8,000 I.U to 24,000 I.U per kg of diet did not significantly affect either the percent fertility or the hatchability of the eggs (Table 6). The highest average hatchability figure of 36.37 percent was however obtained from the eggs of birds which received stylo meal and which had 19,000 I.U vitamin A per kg of diet. The birds which received the same among of vitamin A (19,000 I.U/kg diet from synthetic sources produced eggs which had an average hatchability value of 31.51% while the birds which received 24,000 I.U vitamin A per kg of diet produced eggs which had an average hatchability value of 33.62%.

TABLE 6. EFFECTS OF DIFFERENT LEVELS OF VITAMIN A ON THE FERTILITY AND HATCHABILITY OF EGGS.

Levels of vitamin A	Fertility (%)	Hatchability (%)
8,000 I.U/kg	65.76	33.12
19,000 I.U/kg	63.64	31.51
24,000 I.U/kg	66.32	33.62
19,000 I.U/kg*	65.89	36.37
s.e. means	2.84	1.1ns

**8,000 I.U supplied from a vitamin-mineral mix and 11,000 I.U from stylo meal.*

Discussion

The fact that the birds in this study tolerated up to 20% stylo meal whereas they could not utilize more than 10% stylo meal when fed for only six months from the point of lay (Onwudike and Adegbola, 1978), and the observation that a longer period is taken for birds on the higher levels of stylo meal to attain peak production further stress the point that the laying birds fed increasing levels of stylo meal required a longer period of time to adapt to their rations. It therefore appears that the length of time the birds stay on the rations will affect the amount of stylo meal which can be tolerated by the birds. This period of adaptation may be dependent on the increased crude fibre level and reduced density (increased bulk) of the diets with increasing levels of stylo meal. It is

possible that the trend in egg production was also affected by the amino acid composition of the diets and the requirements of the birds at the different periods of laying. As stylo meal was increased in the diets, groundnut cake was reduced so as to keep the diets iso-nitrogenous. The amino acid content of stylo meal (Table 2) when compared with that of groundnut cake (Fetuga, Babatunde and Oyenuga, 1973), shows that stylo meal is lower in arginine, histidine, leucine, phenylalanine and tyrosine. This means that as stylo meal was increased and groundnut cake reduced in the rations, these amino acids which are required by poultry were lowered. Scott, Nesheim and Young (1969) reported that the amino acid requirement for a laying bird decreases from the time of laying. The amino acid balance of the diets with high levels of stylo meal may have been inadequate for the early requirements of the laying birds and probably more adequate for the latter part of laying. This perhaps accounted for the delay in the attainment of peak production.

The higher level of cholesterol in the yolk of eggs laid by birds fed the higher levels of stylo meal and the lower serum cholesterol level of the same group may be looked at from the effect of high fibre levels on cholesterol metabolism and also from the relationship between the rate of lay and egg cholesterol level. With higher levels of stylo meal in the diets, the levels of crude fibre also increased (Table 1). From the theory of fibre - cholesterol interaction (Kritchevsky and Story, 1974; Myant, 1975), there is a higher rate of excretion of cholesterol from the blood of birds on the higher levels of stylo meal. This probably explains the lowered serum cholesterol level observed with increasing levels of stylo meal. Since for a laying hen, a major excretory pathway for cholesterol is thrbugh the eggs (Connor **et al.**, 1965) and Andrews **et al.**, 1968), part of the cholesterol so excreted from the blood may go into the eggs and in this way the eggs of birds on the high stylo meal levels would contain higher levels of cholesterol. Another factor which could have made for the higher cholesterol level of eggs from birds fed the higher levels of stylo meal is the slightly lower production of this group. This would be so since it has been demonstated by Edwards **et al.** (1960),Cunningham **et al.** (1974) and Washburn and Nix (1974) that hens with a higher rate of production may deposit less cholesterol in each yolk. Bilek (1936) had reported that an increase in yolk colour was accompanied by an increase in yolk cholesterol level. It may be that what he fed to increase yolk colour was also high in the form of fibre which led to a greater accumulation of cholesterol in the yolk as already explained. These facts are not given. It would however be interesting to feed the same diet with increasing levels of oxycarotenoid and note the effect on the yolk cholesterol level.

The hatchability results obtained here and the results reported by Onwudike and Adegbola (1978) seem to suggest that stylo meal contributes some factors other than vitamin A which improve the hatchability of eggs. A number of workers, including Barnhart **et al.** (1954) and Hoie and Sandvik (1957) have attributed the improved hatchability observed with the feeding of leaf meal to the improved riboflavin level both of the diets and the eggs. Fluorometric analysis of the diets fed in the hatchability study showed that the riboflavin contents were 4.74 and 5.86 mcg/g of diet for the 0 and 15 percent levels of stylo meal respectively.

This raises the question of whether the lower level of riboflavin of 4.74 mcg/g supplied in the basal diet was not enough to meet the riboflavin requirement of the breeding hens when the recommended level by the N.R.C. (1971) is only 3.8 mcg/g of diets. In this connection is is pertinent to remark that Ogunmodede (1977) has shown that birds in a tropical environment require dietary levels of riboflavin higher than the level recommended by the NRC (1971). He noted that the riboflavin level of 3.6 mcg/g diet recommended by the NRC was inadequate for the requirement of broiler chicks whereas a level of 5.1 mcg/g diet was adequate for the growth of the birds and the storage of riboflavin in the body. Also Scott, Nesheim and Young (1969) recommended that the diets of breeding hens should contain a minimum of 5.5 mcg/g of diet. If the riboflavin content of 4.74 mcg/g supplied in the rations without stylo meal was enough to meet the requirements of the breeding birds then the stylo meal must have supplied some other factors which either improved the utilization of nutrients essential for improved hatchability or acted directly to improve hatchability. The generally low levels of egg production, feed efficiency, fertility and hatchability recorded in this study might have been due to the problem of inbreeding depression. Shoffner (1948) emphasized the reduced effect of inbreeding depression on egg-production, hatchability and body weight. The birds used in the study were the fourth generation of White Leghorn birds from the same parent stock and this could have affected the performance of the birds.

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