Broiler Pigmentation Using Pigment Concentrate Extracted From Legumes Leaves

I.W.R. Susana, B. Tangendjaja and E. Wina

Research Institute for Animal Production, P.O. Box 221 Bogor 16002 INDONESIA

ABSTRACT: Legume leaves contain high amount of carotenoids which potential for pigmentation. Previous experiment had developed a technology to extract the pigment from legume leaves (*Gliricidia sepium, Leucaena leucocephala, Calliandra calothyrsus* and *Sesbania grandiflora*.) and bound to a carrier. The purpose of this experiment is to prove that this product (pigment concentrate = PC) can be used as a pigmentor for broiler skin. The experiment was carried out using 216 DOC strain "Indian River" placed in 27 wired cages which had 8 chicks in each. The design of experiment was Completely Randomized Design with 9 types of diets containing 0.3 and 0.6% of PC from 4 types of legume leaves and ba sal diet (without PC). Parameters measured were body weight, feed consumption, feed conversion and shank colour. Analysis of carotenoids was also carried out on PC, feed and liver. The results showed that there were no significant difference ($P > 0.05$) among treatments on body weight gain(823 -898 grams), feed consumption (1247 - 1442 grams) and feed conversion(1.46 -1.66). While the shank colour was significantly different ($P < 0.05$) among treatments. The highest score was found using 0.6% PC from Calliandra leaves (8.67) followed by Sesbania (8.50), Leucaena(7.58), and Gliricidia(7.25). The xanthophyll content in the liver was very low with the highest value was that fed with diet containing PC from Calliandra leaves. In conclusion, pigment concentrate extracted from Calliandra leaves was the best pigmentor and caused no negative effect to the performance of birds.

Key Words: Pigmentation, Pigment Concentrate, Legume

Introduction

Pigmentation of poultry product which is bright yellow on both raw or processed egg yolk, skin or shank is visible identified by most consumers. Chemical compound related with pigmentation is caused by accumulation of carotenoids. Carotenoids is divided into two subgroups, the carotenes and xanthophylls. The carotenes are completely hydrocarbon as in the common member is ß-carotene where in poultry is almost completely converted to vitamin A or is otherwise metabolized. Therefore, ß-carotene does not contribute to the pigmentation of the poultry product. The xanthophylls or oxy-carotenoids carry one or more oxygen in the molecules are accumulated in poultry product. Since the poultry cannot synthesize carotenoids *de novo* the source of pigmentation is dietary. Natural pigments especially from green plants are becoming increasingly popular as sources of pigments in the diets for chickens. They reduce potential health hazard to humans which is linked to use of synthetic pigments in rations for poultry.

Previous experiment (Tangendjaja et al. unpublished) showed that carotenoids content of plant or plant products is high and leguminoses such as *Gliricidia sepium, Leucaena leucocephala, Calliandra calothyrsus* and *Sesbania grandiflora* etc. have high amount of both carotenes and xanthophylls. These compounds are very unstable, easily oxidised during storage (decreased 60% in one month) and loss their pigment activity. The technology for separating carotenoids from the leaves matrix had been established (Susana et al. 1992) and many research to maintain the stability of pigment have been done and reported (Fletcher et al. 1985, Layug et al. 1993). The product is called pigment concenctrate (PC), green powder that contains up to 1000 ppm of both carotenes and xanthophylls and used as feed additive. The inclusion of PC in layer ration increased egg yolk pigmentation (Susana et al. 1994). This paper discussed the utilization of PC as broiler pigmentation.

Materials and Methods

Pigment concentrate was prepared as previously described (Susana et al. 1992). Legumes leaves (*Gliricidia sepium, Leucaena leucocephala, Calliandra calothyrsus* and *Sesbania grandiflora* , were collected from the research station of RIAP - Ciawi, Bogor. After blanching in boiling water, the leaves were dried and ground. The pigment was extracted with hexane-acetone (1:1), after filtration, the filtrate was evaporated until almost dry and bound to a carrier (fine rice hull). Evaporation was continued until the solvent smell dissappeared.

Feeding trial.

The experiment was carried out using 216 DOC strain "Indian River" and vaccinated (New Castle Disease) by eyedrops. The birds were allocated randomly for 9 treatments and 3 replicates with 8 birds in each wired cage. The design of experiment was Completely Randomized Design with 9 treatments of diets as followed: I = basal diet using white sorghum as control, II, III, IV and V = Basal diet + 0.3 % *Gliricidia sepium* , *Leucaena leucocephala, Calliandra calothyrsus and Sesbania grandiflora* PCs respectively; treatmens VI, VII VIII and IX = basal diet + 0.6% *Gliricidia sepium, Leucaena leucocephala, Calliandra calothyrsus and Sesbania grandiflora* PCs. The composition of the basal diet is shown in Table 1. Parameters measured were body weight, feed consumption, feed conversion and shank colour after 4 weeks.

Chemical analysis

Total carotenes and xanthophylls in the Pigment Concentrate, feed and liver were analysed as described in AOAC (1984) and modified by Wina (1993). Weighed out 0.5 g ; 2 g and 10 g samples of PC, diets and fresh liver respectively and extracted with hexane-acetone-ethanol- toluen (10:7:6:7) for at least 16 hours. After saponification with KOH-methanol, the supernatant was separated with MgO-hyflo supercell column and eluted with hexane - acetone (9:1) for total carotenes and hexane-acetone-methanol (8:1:1) for total xanthophylls fraction. Absorptions were measured at 436 nm and 474 nm by Hitachi U-2000 Spectrophotometer for total carotenes and xanthophylls, respectively.

Shank colour

Shank pigmentation refers to the colour of the broiler leg and foot (Bird, 1994). After 4 weeks, one bird in every cage was killed for shank colour test . Scoring was done through visual observation by 16 peoples. Score 5 was the same as control, score above 5 meant that the degree of yellow colouration increased and *vice versa*. The results was analysed by Completely Randomized Design and the significant difference was tested by Least Significant Difference (P<0.05).

Table 1. Formulation and diets composition

Ingredients	%
Sorghum	50.00
Soybean meal	18.30
Rice bran	10.00
Fish meal	8.00
Peanut press-cake	5.00
Meat-bone meal	3.66
Coconut oil	4.00
Methionine	0.25
Lysine	0.21
Premix *	
Chemical Composition (calculated)	
ME (Kkal/kg)	3000.00
Crude Protein (%)	21.50
Methionine (%)	0.60
Methionine + Cystine (%)	0.91
Lysine (%)	1.18
Calcium (%)	0.90
Avaiable Phosphor (%)	0.50

[a] In 1000 kg diet contained 3 kg salt, 1 kg Choline Chloride, 12,000 IU vitamin A, 2.000 IU vitamin D 3, 16 mg vitamin E, 6 mg vitamin K, 2.7 mg vitamin B, 10.5 mg vitamin B 2, 2 mg vitamin B 6, 1.6 μg vitamin B 12, 12 mg Ca- Pantotenat, 30 mg Niacin, 0.25 μg folic acid, 0.4 μg biotin, 40,3 mg Fe, 102 mg Mn, 3.5 mg Cu, 0.36 mg I, 0.16 mg Co, 69.2 mg Zn, 0.2 mg Se, 0.56 g $CaCO_3$, 90 mg salinomycin and 20 mg Virginiamycin.

Results and Discussion

From the previous report (Susana *et al.* 1994), pigment concentrate from *Calliandra calothyrsus* contained the highest concentration of carotenoids followed by *Leucaena leucocephala*, *Sesbania grandiflora* and *Gliricidia sepium*. Therefore, when mixed PC in the diets, the highest carotenoids was in the diet containing PC of *Calliandra calothyrsus* (0.6 %) The total carotenes and xanthophylls in the diets and liver are shown in Table 2.

The content of both total carotenes and xanthophylls in the basal diet is very low because white sorghum hardly contained any carotenoids. When PC was added at two inclusion levels to the basal, there was a proportional increase in concentration on both total carotenes and xanthophylls in diet. This concentration in the diets has fulfilled the recommendation of the level of carotenoids to achieve maximal intensity of colour (Marusich, 1981). In liver, the amount of total carotenes is higher than total xanthophylls. The highest level in the liver related to the highest content of carotenoids in the diet. It appeared that carotenes were more accumulated in the liver than xanthophylls. Xantophylls were more competent in mobilisation of pigment to broiler skin or shank (Marusich, 1981).

Table 2. Total carotenes and xanthophylls in the diets and liver of chicken fed with diets containing pigment concentrate

Treatments*		Diets		Liver	
		carotenes (ppm)	xanthophylls (ppm)	carotenes (ppm)	xanthophylls (ppm)
I	(basal diet)	1.51	0.61	0.184	0.025
II	(0.3 % Gli.)	9.05	7.15	0.686	0.165
III	(0.3 % Leu.)	11.07	9.12	0.951	0.341
IV	(0.3 % Cal.)	8.36	5.99	1.449	0.549
V	(0.3 % Ses.)	8.70	7.81	0.978	0.096
VI	(0.6 % Gli.)	26.24	26.85	1.393	0.388
VII	(0.6 % Leu.)	20.87	23.76	1.045	0.731
VIII	(0.6 % Cal.)	52.44	50.65	2.693	1.320
IX	(0.6 % Ses.)	21.09	23.03	1.511	0.479

* Gli = *Gliricidia sepium* Leu = *Leucaena leucocephala*, Cal = *Calliandra calothyrsus* Ses = *Sesbania grandiflora*

Table 3. Body weight gain, feed consumption and feed con version.

Treatments		Body weight gain (gram)	Feed Consumption (gram)	Feed Coonversion
I	(basal diet)	839	1393	1.66
II	(0.3 % Gli.)	824	1337	1.65
III	(0.3 % Leu.)	823	1287	1.57
IV	(0.3 % Cal.)	866	1247	1.46
V	(0.3 % Ses.)	842	1283	1.53
VI	(0.6 % Gli.)	863	1387	1.61
VII	(0.6 % Leu.)	898	1442	1.61
VIII	(0.6 % Cal.)	868	1349	1.55
IX	(0.6 % Ses.)	826	1290	1.56
	SEM	26.5	55.4	0.053

The results of body weight gain , feed consumption and feed conversion are presented in Table 3. Statistical analysis showed no significant difference (P > 0.05) among treatments on body weight, feed consumption and feed conversion. It shows that PC had no effect on broiler performance which indicated that PC contained no growth depression factor or toxicity for broiler rations. This has also been reported that utilization of PC in layer showed no negative effect on egg production (Susana et al. 1994). Whereas without pigment extraction, inclusion of leaves meal from legumes in the poultry rations caused growth depression when used more than 5% (Tangendjaja, 1987). Extraction of pigment from leaves matrix could left the toxic material unextracted in the leaves residue as it was soluble in water rather than non polar organic solvent.

Table 4. Shank colour score of chicken fed with diets contain pigment concentrate.

	Treatments[2]	Score[1]
I	(basal diet)	5.00[a]
II	(0.3 % Gli.)	6.00[b]
III	(0.3 % Leu.)	6.58[c]
IV	(0.3 % Cal.)	7.67[e]
V	(0.3 % Ses.)	6.50[c]
VI	(0.6 % Gli.)	7.25[d]
VII	(0.6 % Leu.)	7.58[e]
VIII	(0.6 % Cal.)	8.67[f]
IX	(0.6 % Ses.)	8.50[f]
	SEM	0.11

[1]Score was based on the difference of colour (score 5 = control, basal diets). Score above 5 mean that degrre of yellow colouration was increased.

[2]Gli = Gliricidia sepium Leu = Leucaena leucocephala, Cal = Calliandra calothyrsus Ses= Sesbania grandiflora

The results of shank colour test is presented in Table 4. The shank colour tested was the colour that observed after 4 weeks of feeding. Colour has already already developed after 14 days of feeding. Whereas Bird, *in* Marusich and Bauerenfeind (1981) reported that excellent shank pigmentation was induce in a period of only 10 days by proper feeding. There was a significant difference among treatments (P< 0.05). The highest score was achieved by addition of 0.6% PC of *Calliandra calothyrsus*, and 0.6% PC *Sesbania grandiflora*,followed by 0.3% PC *Calliandra calothyrsus* and 0.6% PC *Leucaena leucocephala*. The results showed that eventhough the total xanthophylls content in the diet containing 0.6% PC of Calliandra was the highest compared to that containing Sesbania, the shank colour score of chicken received those two diets was similar. This might be due to the concentration of individual xanthophyll that responsible for shank colouration in those diets was the same. It was reported that zeaxanthin and cathaxanthin (oxy-carotenoids) are preferent deposited into the epidermis (Marusich et al, 1976). Another explaination that depth of colour as perceived by the eyes in broiler shank is not directly proportional to the pigment deposited as determined by colorimetry (Marusich, 1981).

In conclusion, pigment concentrate (PC) from legume leaves is potential for pigmentation of broiler products. Suplementation with 0.6% PC of *Calliandra calothyrsus* gave the highest intensity on the shank colouration and higher value of carotenoids in the liver. Suplementation of PC from legume leaves did not affect the performance of broiler.

Literature Cited

AOAC, 1984. Official Methods of Analysis of the Association Official Analytical Chemists. 14th Edition, William Byrd Inc. Richmond, Virginia. No. 43

Bird,J.N.,1994. Proceedings Feed Ingredients '94. The 1st International Conference. Singapore. 186.

Fletcher, D.L, C.M. Papa and F.X. Tirado. 1986. The Effect of Saponification on the broiler Coloring Capa-bility of Marigold Extracts. Poultry Science 65: 1708.

Layug, D.V., M. Ohshima, H.T. Ostrowski-Meissner and H. Yokota, 1993. The Effect of Antioxidants added to Processed Alfalfa on Loss of Carotenoids Pigment During the Recovery and Subsequent Storage of Leaf Extracts. Proceeding VII World Conference on Animal Production. Canada, 2, 81.

Marusich, W.L and J.C. Bauerenfeind, 1970. Oxycarotenoids in Poultry Pigmentation. Poultry Science 49, (6): 1569.

Marusich, W.L and J.C. Bauerenfeind, 1981 . Oxycarotenoids in Poultry feeds. *In* arotenoids as Colorants and Vitamin A Precursors. J.C. Bauernfeind (ed). Academic Press, 433.

Marusich W.L., F.F. Orginz, N. Camerllengon McCambley and M. Mitrovic. 1976. Zeaxanthin as a Broiler Pigmenter. Poultry Science 55(4): 1486.

Saylor, W.W. 1986. Evaluation of Mixed Natural Carotenoid Products an Xanthophyll Sources for Broiler Pigmentation. Poultry Science 65: 1112.

Susana I.W.R., E. Wina dan B. Tangendjaja. 1992. Pemanfaatan Pigment dari Sumber Alami: Teknologi Pembuatan Additif Pigment dan penyimpanannya. Prosiding : Agroindustri Peternakan di Pedesaan. Balitnak, Bogor, 569.

Susana, I.W.R, E. Wina, B. Tangendjaja. 1994. Potensi Konsentrat Pigmen sebagai Pengganti Sumber Pigmen Sintetik untuk Pewarnaan Kuning Telur. Prosiding : Seminar Nasional Sains dan Teknologi Peternakan , balai Penelitian Ternak, Bogor. 745.

Tangendjaja,B.; J.B. Lowry and A.J. Evans, 1987 Ilmu dan Nutritional value of leaf meal for broilers: Effect of Calliandra leaf meal and protein in ration. Ilmu dan Peternakan 3(2): 61.

Wina, E.and Susana I.W.R.. 1993. Analisis Kuantitatif Karotenoid dari Daun, Pakan Ayam, Telur dan Hati Ayam. Proceedings: Seminar Nasional Kimia dan Pembangunan, HKI, Bandung : 193.