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EVALUATION OF PEPPER SPENT AS AN EGG YOLK COLOURING AGENT IN THE DIET OF WHITE LEGHORN LAYERS

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ABSTRACT: The egg yolk pigmenting properties of increasing levels (0, 1, 2, 3, 4 and 5%) of pepper spent in the diet of White Leghorn layers was evaluated. Six months old two hundred and forty layers were divided randomly into six groups of 40 layers. Each group was further divided into two replicate groups of 20 layers and assigned to a pen with deep litter. The six treatment diets were assigned to two of the pens at random. Evaluation was based on egg yolk colour intensity measured based on Roche colour fan scores, potassium dichromate grades and consumer preference scores on raw, boiled and fried egg samples. Egg production, feed consumption, feed/dozen eggs, fertility and hatchability of eggs, albumin quality and egg weight were also measured. Significant ($p<0.01$) differences in Roche colour fan and potassium dichromate scores were obtained. Highest scores for the Roche colour fan (8.63) and potassium dichromate (8.09) grades were attained at the 5% level of pepper spent inclusion, while the lowest values were obtained for the control (0% inclusion). There were also significant ($p<0.05$) differences in consumer preference scores for boiled eggs. The highest scores (3.51) were given for eggs from birds on the 5% pepper spent diet. No differences ($p>0.05$) in the preference scores on the raw and fried eggs were observed with all ranging between good and very good. No significant differences ($p>0.05$) in egg production, feed consumption, feed conversion efficiency, feed cost per dozen eggs, fertility and hatchability of eggs, albumin quality and egg weight were noted. Based on most of the measurements used and maintaining consumer acceptability of the resulting egg products, the closest yolk colour to that of local eggs relished by local consumers was produced by inclusion of 4% pepper spent in the diet. This level of inclusion can, thus, be recommended.

Key words/phrases: Pepper spent, yolk colour, yolk-colouring agent

INTRODUCTION

Poultry production in Ethiopia is dominated by the backyard system of production characterized by birds left to scavenge with no serious attention given in terms of provision of supplements, shelter, water and health care. Intensive commercial poultry management based on specialized breeds (egg or meat) has been markedly increasing in recent years (Alemu Yami and Tadelle Dessie, 1997). Ethiopian consumers have a strong preference for eggs with deep yolk colour, and are willing to pay much higher prices for the very small sized eggs with deep yellow yolk colour from the scavenging local chicken. Commercial producers use imported purified or synthetic pigments in order to satisfy the consumer preference for the deep yellow egg yolk colour, but at extremely high cost (Alemu Yami and Tadelle Dessie, 1997). If this system is to flourish, it has to produce at a low cost commensurate with the low buying power of the population yet provide products that meet local consumer preferences. This situation calls for investigations of egg yolk colouring agents from locally available resources. Pepper spent, a by-product from the Ethiopian Spice Extraction Factory (ESEF), could be a good source of egg yolk pigmentation agent. Some 2000 tons of pepper spent is being produced annually. Pepper (*Capsicum annum L.*) is extracted for the production of oleoresin and capsicum using solvent extraction procedures. Oleoresin is used as a colorant in the food industry and capsicum is used in the manufacture of medicaments. After extraction, the remaining dried and ground flesh (pepper spent) is left as a by-product. Small-scale poultry producers can use the by-product to feed egg-laying hens. In this regard, it was shown in an earlier preliminary observation at the Debre Zeit Agricultural Research Centre (unpublished) that this product can be used as yolk colouring agent. However, the right level of inclusion of pepper spent that produces desirable egg yolk colour without affecting performance has not been determined.

Therefore, the objective of this investigation was to determine the level of pepper spent in the diet of White Leghorn layers that produces desirable egg yolk colour without affecting egg production, fertility or hatchability.

MATERIALS AND METHODS

Experimental rations

Six diets were formulated so that pepper spent replaced 0, 1, 2, 3, 4 and 5% dried alfalfa leaf meal (Table 1). The control diet with 5% alfalfa was based on earlier reports that inclusion of alfalfa at this level produced acceptable yolk colour (DZARC, 1984).

Chemical analyses

Feed samples (ingredients and diets) were analysed for dry matter, crude protein, ash and ether extract according to the standard procedures of AOAC (1975). Acid and neutral detergent fibbers were determined according to Goering and Van Soest (1970). Metabolizable energy was calculated according to the NRC (1971).

Table 1. Ingredient and chemical composition of experimental diets.

	Treatments					
	1	2	3	4	5	6
Ingredient composition (%)						
Ground Alfalfa	5	4	3	2	1	0
Pepper spent[1]	0	1	2	3	4	5
Ground white corn	37	37	37	37	37	37
Wheat short	9	9	9	9	9	9
Noug meal	32	32	32	32	32	32
Meat and Bone meal	12	12	12	12	12	12
Vitamin premix[2]	2	2	2	2	2	2
Limestone	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition (% of DM) of test diets						
Dry matter (as fed)	90.34	90.00	90.37	90.41	90.30	90.71
Crude Protein	21.31	20.50	22.56	21.50	21.63	22.13
Ether Extract	7.57	8.29	8.26	7.47	8.88	8.87
ADF	10.73	1.12	12.56	11.66	13.27	15.24
NDF	27.23	28.83	28.20	27.76	28.33	27.53
ME (Mcal/kg)[3]	3.03	2.98	2.99	3.01	2.99	3.01

1 13.3% CP; 38.3% NDF; 2.7 Mcal/kg ME
2 Commercial premix that supplied per kg of feed: Vit. A=9,000 IU; Vit. D=2,000 IU; Vit. E =15 IU; Fe =25 mg; Cu=7.5 mg; Zn=50 mg; Mn=75; Co =0.2 mg; I=0.76 mg; Se=0.14 mg.
3 Calculated according to NRC (1971)

Birds and their management

Two hundred and forty White Leghorn layers, 6 months of age, were used. The birds were randomly distributed into six groups of 40 layers. Each group was further divided into two replicates of 20 layers and randomly assigned to one of the experimental diets. The birds were raised on deep litter with barley straw as litter material. Two cocks were also assigned to each pen. A 6-month data collection period commenced after 2 weeks of adaptation. The birds were fed and watered *ad libitum* in groups.

Measurements

Feed offered and refused was recorded every other day. Eggs were collected twice at 11:00 and 16:30 hours each day. Feed conversion efficiency was calculated as the weight of feed (kg) consumed to produce a dozen of eggs. Individual egg weights were taken every 15 days. Four of the weighed eggs from each replicate were randomly selected, labelled, broken out on a flat tray and the height of the thick albumen measured using a tripod micrometer and the average Haugh unit value computed using the formula of Haugh (1937). At the same time, thickness of the shell of each broken egg was measured at three sites namely, two from the equatorial side and one from the top end using a micrometer gauge. At the end of the experiment, an equal number of eggs from each replicate were randomly selected and incubated. The incubated eggs were candled on the 18th day to determine the proportion of fertile eggs. Hatchability was determined at the end as a percentage of chicks hatched from each replicate to the number of eggs set and as a percentage of fertile eggs.

Yolk colour intensity was measured by scoring using the Roche colour fan scale and potassium dichromate grading (Bornstein and Bartov, 1966). An egg each from the morning and afternoon collections of each replicate was taken and egg yolk colour scoring was conducted on the same day. Twelve local eggs purchased from a nearby market and twelve eggs from intensively managed chicken that received no colouring material in the diet were also taken for comparison. The eggs were individually broken out on a white flat dish and the yolk was separated from the white. The yolk was then thoroughly mixed. A droplet was placed on white paper and compared with the different colours in the Roche colour fan until a leaf with a matching colour was identified. The score on the corresponding leaf was recorded.

For the potassium dichromate grading, potassium dichromate solutions of progressively increasing concentrations (12 grades ranging from 1 for very light yellow to 12 very deep yellow) were prepared as described by Bornstein and Bartov (1966). Yolk colour grade was estimated by matching the dichromate standard colour solutions with the yolks placed on white paper.

For yolk colour evaluation by visual judgement, 40 people gave scores on a 5-point scale (1= not attractive to 5 = excellent) for raw, boiled and fried eggs from all treatment groups.

Statistical analyses

Analysis of variance (Gomez and Gomez, 1984) was used to test treatment effects on production parameters and egg quality parameters except yolk colour scores. When the ANOVA revealed the existence of significant differences among treatment means, Duncan's (1955) multiple range test was used to test and locate means that were significantly different from the rest. For the yolk colour score, the data were tested for normal distribution according to the Wilk-shapiro procedures (SAS, 1987). The data were skewed and, thus, subjected to non-parametric analysis using the Friedman Chi-square test to detect the degree of variation among the treatments. When the analysis of the Friedman test revealed the existence of significant variation, a set of sign tests were used to perform multiple comparison of means (SPSS, 1996). Regression analysis was also performed to determine the degree of relationship of pepper spent in the diet to Roche colour fan and potassium dichromate scores.

RESULT

Chemical composition of experimental diets

The ingredient and chemical compositions of the experimental diets are given in Table 1. Chemical compositions of all the treatment diets including the control diet were similar since they were based on the same types and proportions of ingredients were used except the substitution of alfalfa in the control diet with increasing proportions of pepper spent. The substitution did not seem to have made a big difference in the compositions of the determined nutrients.

Performance of birds

The mean percentage rate of lay on a hen day basis, daily feed consumption, feed/dozen of eggs and feed cost per dozen eggs for layers fed on the 6 treatment diets are given in Table 2. There were no differences among treatments (P>0.05) in laying performance. Daily feed consumption and feed efficiency (*i.e.*, feed/dozen of eggs) were not affected (p>0.05) by dietary level of pepper spent.

Table 2. Effect of increasing levels of pepper spent on average rate of lay (hen-day production), daily feed intake, feed / dozen eggs and feed cost/ dozen eggs.

Treatments	% lay (hen-day)	Feed intake (g/hen/day)	Feed/dozen eggs[1]	Feed cost (Birr)[2]/ dozen eggs
Control	52.57	110.94	2.92	2.36
1% pepper spent	56.82	112.66	2.77	2.21
2% pepper spent	60.38	113.05	2.64	2.12
3% pepper spent	54.44	112.65	2.89	2.34
4% pepper spent	54.28	111.96	2.93	2.31
5% pepper spent	55.56	113.11	3.00	2.29
SE	1.76	0.70	0.095	0.064
Significance	NS	NS	NS	NS

[1]Calculated as weight of feed (kg) consumed to produce a dozen of eggs.
[2]1 Birr = 0.15 US cents; NS = Not significant (p> 0.05)

Fertility, hatchability and albumen height of eggs

Fertility, hatchability, weight and Albumen height (Haugh units) of eggs are shown in Table 3. The treatments did not influence (p>0.05) fertility, hatchability (as % of total eggs set and % of fertile eggs), egg weight or albumen height (Haugh units).

Table 3. Effect of increasing levels of pepper spent in the diet on fertility, hatchability, egg weight and Haugh units.

Treatments	Apparent Fertility (%)	Hatchability Egg set (%)	Hatchability Fertile eggs (%)	Egg weight(g)	Haugh Units
Control	94.00	83.37	88.86	58.26	85.65
1% pepper spent	92.88	82.93	89.08	55.97	90.40
2% pepper spent	96.54	83.91	86.81	56.08	85.68
3% pepper spent	96.08	76.57	79.74	54.81	87.62
4% pepper spent	96.11	86.43	89.91	54.75	86.06
5% pepper spent	90.14	76.25	83.26	56.24	85.48
SE	2.34	2.84	2.87	0.94	2.59
Significance	NS	NS	NS	NS	NS

NS = Not significant (p> 0.05).

Egg yolk colour scores (Roche colour fan scores, potassium dichromate grades and visual preference scores) are presented in Table 4. There were differences ($p < 0.05$) among treatments in Roche colour fan scores. The score was lowest ($p<0.05$) for the control and greater ($p < 0.05$) for the diets with 4 and 5% compared with 1 or 2% pepper spent. Regression analysis showed a significant linear relationship between pepper spent level in the diet and Roche colour fan ($p<0.003$) and potassium dichromate ($p<0.01$) scores.

Table 4. Effect of increasing levels of pepper spent in the diet on mean scores for Roche colour fan, potassium dichromate grade and visual preference.

Treatment	Roche colour-fan scores [1]	Potassium dichromate scores [2]	Visual judgment		
			Raw	Boiled	Fried
Control	5.06[a]	4.23[a]	3.40	2.37[a]	3.39
1% pepper spent	6.67[b]	4.78[ab]	3.94	3.31[ab]	3.84
2% pepper spent	6.44[b]	5.22[bc]	4.22	3.11[ab]	3.58
3% pepper spent	7.13[bc]	4.96[ab]	3.77	3.46[ab]	3.42
4% pepper spent	8.38[c]	6.75[c]	4.14	3.31[ab]	3.61
5% pepper spent	8.63[c]	8.09[d]	3.43	3.51[b]	3.48
SE	1.32	1.46	0.35	0.42	0.17
Significance	**	**	NS	*	NS
Local eggs	8.00	7.00	3.8	3.2	4.0
No colouring agent [3]	3.5	2.5	2.31	2.14	1.58

* = Significant at P< 0.05; ** = significant at p< 0.01;NS = Not significant (p> 0.05)
a, b = Means in the same column with different superscripts are significantly different
[1] Roche colour-fan scores - 1-14 (pale yellow-deep yellow)
[2] Potassium dichromate scores 1-12 (pale yellow-deep yellow)
[3] Eggs from intensively managed birds that did not receive any colouring material in the diet.

Unlike the results in the Roche colour fan and potassium dichromate grading, the scores by the panel of testers for the visual judgement of yolk fan of raw and fried eggs did not vary among treatments ($p > 0.05$). All the egg samples were graded between good and very good. Scores for boiled eggs, however, varied ($p<0.05$) with treatment; the score (fair) for the control diet was less ($p<0.05$) than that for the 5% pepper spent level. The fan scores for the 4% pepper spent diet was closest to the scores for the local egg samples used as a check.

The relationship between Roche colour fan and potassium dichromate scores (Y) with level of pepper spent in the diet (X) was found to be linear. The relationship with Roche colour fan scores could be expressed by the equation:

$$Y = 5.36 + 0.676X \qquad (R^2 = 0.894,\ p < 0.003)$$

For the potassium dichromate scores, the relationship was:

$$Y = 3.89 + 0.713X \qquad (R^2 = 0.799,\ p < 0.01)$$

DISCUSSION

Inclusion of pepper spent up to 5% of the diet of layers was found to improve egg yolk coloration without affecting feed intake, feed/dozen eggs, egg production, egg weight, fertility, hatchability and internal quality of eggs (Haugh units). Carlson and Halverson (1964), and Kingan and Sullivan (1964) also reported that egg production was not affected by inclusion of upto 10% alfalfa meal as egg yolk colorant. Fletcher and Halloran (1983) noted that change in feed consumption could not be accounted for the treatment differences when Marigold extract and Paprika oleoresin were used as sources of egg yolk coloration at rates of inclusion similar to the present study. A depression of feed intake was expected as a result of the pungent characteristics of paper spent. Variations in rate of lay and feed consumption account for the greatest portion of the variations in feed utilization (Hirnick *et al.*, 1977). The non-significant variations in rate of lay and feed consumption, that are used for the calculation of feed conversion efficiency, have resulted in the non-significant differences in feed conversion efficiency.

The non-significant differences in fertility of eggs from layers receiving increasing levels of pepper spent was in agreement with the reports of Kingan and Sullivan (1964), who also used Alfalfa meal in their experimental diets. The non-significant changes in feed consumption may have been responsible for the absence of effects on egg weight.

All the diets with pepper spent resulted in higher Roche colour-fan scores than the control diet showing effectiveness of pepper spent as an egg yolk-colouring agent. The findings in this study are in agreement with those of Nelson and Baptist (1968), Chandra *et al.* (1978) and Fletscher and Halloran (1983), who reported that addition of Paprika extract in a layer diet increased the intensity of egg yolk fan as measured by the Roche colour fan score. Absence of significant differences in preference scores among the treatments for raw and fried egg samples suggests that even the lowest level of inclusion has produced acceptable egg yolk coloration which is in agreement with Bornstein and Bartov (1966). The preference score indicated that the 5% pepper spent diet had a significantly ($p<0.05$) higher score than the control. Overall, it can be concluded from this study that pepper spent inclusion at all the levels tested improved egg yolk coloration. Inclusion at 4% produced scores closest to that of local eggs relished by local consumers based on almost all the measurements used and maintaining consumer acceptability of the resulting egg products. This level of inclusion can, thus, be recommended.

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