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# Feeding combination of synthetic carotenoids improved performance, immune response and blood biochemical profiles in heat stressed broilers

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ABSTRACT

A biological study was conducted in newly hatched (200) broiler chicks (44.06 ± 2.18 g) divided into 5 treatments each consisting of 4 replicates with 10 birds in each, were fed with control (basal diet), $T_1$ (canthaxanthin (CAN) and apocarotenoid (APO) at 25 ppm each), $T_2$ (CAN 50 and APO 25 ppm), $T_3$ (CAN 25 and APO 50 ppm) and $T_4$ (50 ppm each). Birds were reared for 42 days under standard managemental conditions during hot-dry summer season with THI 84.66. Bi-weekly body weight (BW) and feed intake (FI) were recorded. Blood samples were collected and shank colour was measured at 28$^{th}$ and 42$^{nd}$ days of age. Cell mediated immune response and carcass characteristics were studied at 28 and 42 days of age respectively. Overall BW and body weight gain (BWG) were significantly higher in birds fed with combination of 25 ppm CAN and APO compared to other groups. BWG was significantly higher in $T_1$ than other treatment and control groups at finisher phase. Feed conversion efficiency (FCE) was significantly improved in $T_1$ and $T_2$ than all other groups. Dietary inclusion of synthetic carotenoids significantly increased the aspartate transaminase, phosphorus and total protein but reduced cholesterol and triglyceride level in blood plasma compared to unsupplemented dietary treatment group. Higher pigmentation was observed in $T_1$ than the other combinations of carotenoids. It could be concluded that feeding combination of synthetic carotenoids (CAN and APO) at 25 ppm each exhibited positive effect on performance, blood parameters and pigmentation in heat stressed broilers.

**Key words**: *Apo*-carotenoid, Broiler, Canthaxanthin, Immunity, Performance, Shank colour

Poultry industry is one the fastest growing industry in India; with the total poultry population of over 729.20 million, India ranks third in egg and fifth in meat production. About 5.23 and 2.50% of world's egg and meat production was contributed by India (DAHDF 2014–15). Colour of chickens greatly affects the purchasing behaviour of consumers. More than 750 carotenoids have been identified in nature (Britton *et al.* 2004). Since carotenoids are not produced by chickens, it must be supplied through the feed to maintain their normal pigmentation. Accumulated carotenoids may increase the quality of chicken by improving flavour, delaying oxidation and pigmenting bodies (Ali *et al*. 2016). Carotenoids are important sources of anti-oxidation and pigmentation (Higuera-Ciapara *et al.* 2006). Pigmentation by carotenoid is important in commercial poultry industry, as the extent of pigmentation in egg yolk and broiler meat defines the product acceptability among the consumers (Vanessa *et al.* 2011). Currently, a majority of the commercial carotenoids are synthesized *via* a chemical route (Ye *et al*. 2006, Luis Carlos *et al.* 2014). Carotenoids protect cellular biomolecules (protein, DNA and cell membranes) against oxidative stress injury. Carotenoids accumulated in egg yolk also transferred to embryonic tissues (Muller *et al*. 2012, Surai 2012). Most of the experiments on pigments have used extractions of plant origin which contained other compounds in addition to the desired pigments. Hence, in the present study, the effect of feeding a combination of relatively cheap synthetic canthaxanthin (CAN) and apocarotenoid (APO) pigments on broiler performance, biochemical attributes, immunity and carcass appearance was investigated.

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## MATERIALS AND METHODS

*Ethical approval:* The biological experiment was carried out as per the Institute Animal Ethics Committee's (IAEC) approved schedule (Permission no.: 452/01/ab/CPCSEA).

*Experimental design and dietary treatments:* Newly hatched CARIBRO-Vishal (200) broiler chicks were divided in 5 treatments. Each treatment consisted of 4 replicates with 10 birds in each. The birds were reared for 42 days under standard managemental practices. The birds were reared under high environmental temperature throughout the study period. The experiment groups were control without any supplementation of carotenoids, $T_1$ fed with 25 ppm CAN and APO each, $T_2$ fed with 50 ppm CAN and 25 ppm APO, $T_3$ fed with 25 ppm CAN and 50 ppm APO and $T_4$ fed with 50 ppm of both CAN and APO. The birds were fed with pre-starter, starter and finisher feeds as per ICAR (2013) nutrient recommendations to meet their nutritional requirements. The experimental feeds were assayed in duplicate (AOAC 1995).

The ingredient and chemical compositions of the experimental feeds are shown in Table 1. Biweekly body weight, feed intake was recorded and feed conversion efficiency was calculated. The mortality was recorded as and when occurred. The 24 h cell mediated immune (CMI) response against the intradermal injection phyto-haemagglutin (PHAP) in birds toe web was measured by using (Spherometer/ Vernier Caliper) at 28 d of feeding. The extent of pigmentation in broilers was assessed by measuring the shank colour using broiler skin colour fan (DSM®).

*Biological sample collection and analysis:* Blood samples were collected at 28th and 42nd day of feeding experiment. Six birds per treatment were randomly selected and sacrificed. At the end of 42 days of feeding experiment, six birds (three males and three females per replicate) were sacrificed and various carcass characteristics were studied.

Table 1. Ingredient and chemical composition of the basal diet

| Ingredient (%) | Pre-starter | Starter | Finisher |
|---|---|---|---|
| Maize | 54.60 | 54.20 | 57.62 |
| Soybean (46% CP) | 39.58 | 37.80 | 32.58 |
| Rice bran oil | 02.12 | 04.24 | 05.86 |
| Calcite | 01.54 | 01.52 | 01.73 |
| Di-calcium phosphate | 0.90 | 0.95 | 1.10 |
| Salt | 0.18 | 0.18 | 0.18 |
| Lysine | 0.30 | 0.15 | 0.17 |
| Methionine | 0.30 | 0.28 | 0.27 |
| Phytase | 0.015 | 0.15 | 0.15 |
| B-complex vitamins[1] | 0.015 | 0.15 | 0.15 |
| Vitamin $AD_3EK$[2] | 0.014 | 0.14 | 0.14 |
| Coccidiostat[3] | 0.01 | 0.01 | 0.01 |
| Toxin binder | 0.05 | 0.05 | 0.05 |
| Total | 100 | 100 | 100 |
| *Nutrient composition (%)* | | | |
| Crude protein | 22.65 | 21.65 | 19.70 |
| ME (kcal/kg) | 3000 | 3125 | 3250 |
| Calcium | 0.96 | 0.95 | 0.90 |
| Available phosphorus* | 0.45 | 0.46 | 0.46 |
| Lysine* | 1.42 | 1.25 | 1.14 |
| Methionine* | 0.62 | 0.59 | 0.55 |

*Calculated values.[1]Addition of 0.1% mineral mixture contained manganese, 91; zinc, 91; iron, 85; iodine, 1.82; copper, 30.24; cobalt, 0.365. [2]Vitamin premix contained vitamin A,16500 IU; $B_2$,13 mg; $D_3$, 3200 IU; vitamin K, 2 mg; thiamine, 5 mg; pyridoxine, 8 mg; niacin, 320 mg; cyanocobalamine, 0.05 mg; vitamin E, 95 mg; calcium D pantothenate, 27.5 mg; folic acid, 14 mg; calcium, 30.1 mg. [3]Coccidiostat supplied 125 mg of Di-nitro-ortho-toluamide.

*Biochemical profile:* Separated serum was subjected to blood biochemical test, viz. serum total protein, total cholesterol, triglycerides, calcium, phosphorus and aspartate transaminase (AST) by using standard commercial kits (Cogent, SPAN diagnostic Ltd, India) (Kumar and Krupakaran 2014).

*Statistical analysis:* Data emanated from different treatments were analyzed for statistical significance using completely randomized design (CRD) by following standard methods (Snedecor and Cochran 1989). Variables having unequal observations were analysed following least square design method and the Tukey's test.

## RESULTS AND DISCUSSION

The overall body weight showed significant (P<0.01) difference among the treatment groups (Table 2). Highest overall body weight was observed in birds fed with $T_1$ and lowest in birds fed in $T_2$. Likewise, overall body weight gain was significantly (P<0.01) high in $T_1$ and lowest body weight gain was observed in $T_2$ treatment group. However, no significant difference was observed in body weight and body weight gain during pre-starter and starter phases. Similar to the present findings, a study by European Food Safety Authority (2014) and Rosa *et al.* (2012) observed no significant difference in body weight or body weight gain during 0–6 weeks feeding period (60 ppm CAN). Inborr and Waldenstedt (2003) observed no significant difference in broiler growth performance due to supplementation of astaxanthin rich algae meal in different doses (7, 36 or 179 ppm).Yang *et al*. (2006) found feeding astaxanthin (0.7, 0.9, 0.11 and 0.13 ppm) to laying hens, had no significant effect on growth performance. Feeding Carophyll Red (6 ppm), which contain CAN (15%) to broiler breeders showed no significant changes in body weight (Zhang *et al*. 2011). Similarly, feeding 25 ppm CAN to broilers showed no significant difference in body weight (Kardas *et al*. 2016). However, in the present study, higher body weight gain and final body weight was observed due to supplementation of synthetic carotenoids which might be due to alleviation of heat stress effects through its anti-oxidant property (Rober *et al*. 2007, Lucas *et al*. 2014, Ali *et al*. 2016). The effect of heat stress will be more during the finisher phase where the thermo-neutral zone is low, hence, the significant results could be observed. During finisher growth phase, the birds experience higher heat stress due to heavy generation of reactive oxygen species as a result of higher metabolic rate (Metcalfe and Alonso-Alvarez 2010). High ambient temperature alters the metabolic activities and reduces feed consumption in birds; supplementation of anti-oxidants (ascorbic acid) increases feed intake and in turn growth rate. In this regard, being a lipophilic anti-oxidant, the supplemented carotenoids might have reduced the oxidative stress in broilers during this phase which could have resulted in significantly higher production performance (Alonso-Álvarez and Velando 2012). The analysis of data on feed intake did not show significant difference among the

Table 2. Body weight and body weight gain in broilers fed with combination of synthetic carotenoids

| Group | Hatch weight | Body weight (g) | | | Body weight gain (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Pre-starter | Starter | Finisher | Pre-starter | Starter | Finisher | Overall |
| Control | 43.88± 0.51 | 385.83± 4.80 | 826.05± 16.33 | 1649.66$^{b}$± 32.29 | 341.95± 4.67 | 440.22± 15.25 | 823.61$^{b}$± 23.50 | 1605.78$^{b}$± 41.04 |
| T1 | 46.33± 14.70 | 379.92± 7.28 | 846.43± 21.01 | 1724.95$^{a}$± 35.86 | 333.59± 16.20 | 466.51± 14.10 | 878.52$^{a}$± 21.05 | 1678.62$^{a}$± 37.21 |
| T2 | 44.63± 1.21 | 371.84± 7.00 | 798.16± 24.80 | 1617.19$^{b}$± 60.44 | 327.21± 6.68 | 426.32± 12.81 | 819.03$^{b}$± 27.64 | 1572.56$^{b}$± 38.47 |
| T3 | 43.72± 0.70 | 363.33± 4.48 | 794.33± 14.58 | 1643.76$^{b}$± 48.58 | 319.61± 4.60 | 431.00± 17.04 | 849.43$^{b}$± 16.03 | 1600.04$^{b}$± 41.75 |
| T4 | 42.75± 0.72 | 376.42± 5.75 | 845.50± 15.89 | 1654.31$^{b}$± 56.12 | 333.67± 5.82 | 469.08± 12.62 | 808.81$^{b}$± 29.55 | 1611.56$^{b}$± 31.38 |
| P-value | 0.300 | 0.066 | 0.169 | 0.051 | 0.172 | 0.331 | 0.015 | 0.012 |

$^{a,b,c}$Means within column bearing different superscript differ significantly (P<0.05).

treatment groups during all the three growth phases and overall feeding phase (Table 3). This was in hormony with findings of Yang *et al*. (2006), Ali *et al.* (2012) and Kardas *et al.* (2016). This was in accordance with findings of Cho *et al.* (2013) who found non-significant effect on feed intake when laying hens were fed with CAN at 0.11 and 0.21%. The feed efficiency (g feed/g gain) showed significant improvement during pre-starter (P<0.01) and overall (P<0.05) due to pigment supplementation (Table 3). During the pre-starter phase, the feed efficiency was better in control and other treatment groups than the T$_3$. Overall feed efficiency was better in T$_1$ and T$_2$ but higher rate of combination of carotenoids resulted in an adverse effect. However, the feed efficiency during starter and finisher phases remained similar among the treatments (P>0.05). Our present findings further strengthened the observations of EFSA (2014). European Food Safety Authority conducted a study trial in breeders fed with different dose level of canthaxanthin (6, 12, 24 and 60 ppm) and observed non-significant difference between dietary supplementation of synthetic pigment. Our results conflicted with the findings of Yang *et al*. (2006) and Zhang *et al.* (2011) who observed feeding different levels of astaxanthin (0.7, 0.9, 0.11 and 0.13 ppm) and canthaxanthin (6 ppm) did not influence the feed efficiency in laying hens and broiler breeder progenies respectively. The findings related to serum biochemical are present in Tables 4 and 5. The serum triglycerides level was significantly reduced due to supplementation of synthetic pigments at both 28 and 42d. This was in accordance with the findings of Ali *et al.* (2015), Agarwal and Rao (2000) and Sevcikova *et al*. (2008) who conducted

Table 3. Feed intake and feed efficiency in broilers fed with combination of synthetic carotenoids

| Treatment | Pre-starter | Starter | Finisher | Overall |
|---|---|---|---|---|
| | *Feed intake (g)* | | | |
| Control | 410±03* | 641±05 | 1606±06 | 2657±25 |
| T1 | 370±12 | 642±07 | 1597±09 | 2609±28 |
| T2 | 384±01 | 641±12 | 1523±15 | 2548±30 |
| T3 | 438±02 | 643±04 | 1596±05 | 2677±21 |
| T4 | 410±03 | 648±03 | 1553±04 | 2611±20 |
| P-value | 0.079 | 0.543 | 0.812 | 0.347 |
| | *Feed efficiency* | | | |
| Control | 1.19$^{b*}$±0.02 | 1.45±0.03 | 1.95±0.10 | 1.91$^{a}$±0.08 |
| T1 | 1.11$^{b}$±0.04 | 1.37±0.03 | 1.98±0.07 | 1.82$^{b}$±0.08 |
| T2 | 1.17$^{b}$±0.07 | 1.50±0.04 | 1.97±0.07 | 1.86$^{b}$±0.09 |
| T3 | 1.37$^{a}$±0.09 | 1.49±0.08 | 2.01±0.02 | 1.88$^{ab}$±0.05 |
| T4 | 1.23$^{b}$±0.04 | 1.39±0.05 | 1.98±0.01 | 1.92$^{a}$±0.07 |
| P-value | 0.005 | 0.464 | 0.136 | 0.036 |

$^{*,a,b,c}$Means within column bearing different superscript differ significantly (P<0.05).

Table 4. Serum total cholesterol, triglyceride and total protein levels in broilers fed with combination of synthetic carotenoids

| Treatment | Total cholesterol | | Triglycerides | | Total protein | |
|---|---|---|---|---|---|---|
| | D 28 | D 42 | D 28 | D 42 | D 28 | D 42 |
| Control | 164.73$^{b*}$±6.49 | 164.73$^{b}$±9.91 | 165.10$^{a}$±8.85 | 165.09$^{a}$±13.52 | 4.90$^{ab}$±0.07 | 4.90$^{b}$±0.10 |
| T1 | 202.73$^{ab}$±9.24 | 164.48$^{b}$±4.54 | 127.98$^{b}$±6.84 | 110.12$^{b}$±2.26 | 5.07$^{ab}$±0.16 | 4.93$^{b}$±0.31 |
| T2 | 178.47$^{ab}$±4.08 | 203.97$^{a}$±11.87 | 116.04$^{b}$±1.26 | 116.17$^{b}$±2.68 | 4.81$^{b}$±0.12 | 5.42$^{ab}$±0.20 |
| T3 | 190.96$^{ab}$±7.99 | 158.46 $^{b}$±10.05 | 115.10$^{b}$±1.42 | 112.78$^{b}$±2.20 | 5.41$^{a}$±0.21 | 5.80$^{a}$±0.18 |
| T4 | 210.50$^{a}$±15.40 | 172.54$^{b}$±10.74 | 127.28$^{b}$±5.24 | 103.51$^{b}$±2.28 | 4.94$^{ab}$±0.07 | 5.13$^{b}$±0.40 |
| P-value | 0.010 | 0.000 | 0.00 | 0.000 | 0.030 | 0.000 |

$^{*a,b,c}$Means within column bearing different superscript differ significantly (P<0.05).

Table 5. Blood biochemical profile (serum calcium, phosphorus and aspartate transaminase) of broilers fed with combination of synthetic carotenoids

| Treatment | Calcium | | Phosphorus | | AST | |
|---|---|---|---|---|---|---|
| | D 28 | D 42 | D 28 | D 42 | D 28 | D 42 |
| Control | 10.38[a]±0.57 | 10.13±0.57 | 5.93[b]±0.23 | 5.93[ab]±0.36 | 168.52[bc]±7.32 | 168.52[bc]±11.18 |
| T1 | 8.32[b]±0.83 | 9.72±0.83 | 7.42[a]±0.59 | 5.61[b]±0.64 | 122.22[d]±13.32 | 122.22[d]±20.35 |
| T2 | 8.36[b]±0.15 | 9.96±0.15 | 7.05[ab]±0.29 | 6.05[ab]±0.43 | 160.41[c]±3.38 | 160.41[c]±5.16 |
| T3 | 8.04[b]±0.14 | 9.64±0.14 | 6.80[ab]±0.15 | 5.10[b]±0.11 | 197.18[b]±10.37 | 197.18[b]±15.85 |
| T4 | 8.92[b]±0.59 | 9.32±0.59 | 6.54[ab]±0.11 | 7.46[a]±1.26 | 286.70[a]±4.96 | 286.70[a]±7.57 |
| P-value | 0.000 | 0.384 | 0.020 | 0.010 | 0.000 | 0.000 |

[a,b,c]Means within column bearing different superscript differ significantly (P<0.05).

Table 6. Cell mediated immune response against phyto-haemagglutinin-P and shank colour in broilers fed with combination of synthetic carotenoids

| Treatment | Cell mediated immune response | | Shank colour |
|---|---|---|---|
| | Absolute (mm) | Relative (%) | |
| Control | 0.18±0.04* | 116.95±4.72 | 104.00[c]±0.00 |
| T1 | 0.23±0.04 | 121.24±4.57 | 105.00[a]±0.00 |
| T2 | 0.22±0.03 | 119.42±2.55 | 104.33[bc]±0.17 |
| T3 | 0.30±0.04 | 127.50±4.17 | 104.67[ab]±0.17 |
| T4 | 0.26±0.03 | 124.31±3.20 | 104.67[ab]±0.17 |
| P-value | 0.234 | 0.339 | 0.000 |

[*,a,b,c]Means bearing different superscript within column differ significantly (P>0.05).

the present study but in contrast the total protein and triglyceride levels remained unaffected. Grcevic *et al.* (2016) also found that feeding leutin (level) in laying hens decreased circulating triglycerides levels. Ali *et al.* (2015), Englmaierova *et al.* (2011) and Barbara *et al.* (2009) reported that addition of lycopene showed increased total protein and reduced cholesterol level in broiler. Lee *et al.* (2010) stated that feeding carotenoids reduces serum cholesterol and triglyceride level in spent laying hens. Xanthophyll feeding can increase good cholesterol level (HDL-cholesterol) in laying spent hens. Polar carotenoid especially astaxanthin increases the good cholesterol; hence it could be improve the cardiovascular health in human subjects. The observations on serum phosphorus showed

Table 7. Effect of feeding combination of synthetic carotenoids on carcass characteristics (% body weight)

| Treatment | Abdominal fat | Giblet | Back | Wing | Thigh | Drumstick | Breast | Liver | Neck | Spleen | Bursa | Skin colour |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1.32*±0.02 | 7.96±0.07 | 15.21±0.25 | 8.83±0.05 | 8.99±0.19 | 10.22±0.50 | 13.72±0.11 | 2.44±0.08 | 4.46±0.15 | 0.20±0.01 | 0.22±0.00 | 101.00±0.00 |
| T1 | 1.25±0.07 | 9.14±0.51 | 15.90±0.34 | 9.38±0.20 | 9.31±0.23 | 9.25±0.05 | 12.85±0.52 | 2.59±0.18 | 4.52±0.13 | 0.20±0.01 | 0.26±0.02 | 101.30±0.00 |
| T2 | 1.39±0.08 | 9.91±0.87 | 16.22±0.12 | 8.97±0.04 | 9.75±0.18 | 9.17±0.13 | 12.84±0.30 | 2.91±0.07 | 4.54±0.09 | 0.17±0.03 | 0.25±0.05 | 102.00±0.00 |
| T3 | 1.62±0.12 | 9.20±0.08 | 15.04±0.26 | 9.20±0.12 | 9.79±0.12 | 9.30±0.08 | 13.31±0.40 | 2.63±0.05 | 4.85±0.16 | 0.19±0.05 | 0.17±0.03 | 102.00±0.00 |
| T4 | 1.07±0.15 | 8.48±0.12 | 15.46±0.32 | 9.15±0.19 | 9.70±0.32 | 9.57±0.10 | 14.26±0.25 | 2.62±0.08 | 4.74±0.15 | 0.08±0.02 | 0.22±0.04 | 102.00±0.00 |
| P-value | 0.342 | 0.152 | 0.321 | 0.452 | 0.233 | 0.218 | 0.316 | 0.551 | 0.226 | 0.238 | 0.421 | 0.798 |

*P>0.05.

feeding trials with broiler by addition of synthetic lycopene which reduced the serum triglycerides level. In the present study, during 28[th] d feeding, the total cholesterol content was significantly higher in $T_2$ than the other treatment groups. However, at d 42 higher total cholesterol content was observed in $T_2$ than all other groups. The total protein and aspartate transaminase was significantly (P<0.01) higher in $T_3$ and $T_4$ respectively at both day 28 and 42 d of feedings. In contrast, carotenoid lycopene inhibit triglycerides and cholesterol mechanized process in liver. According to EFSA (2014) reports, the feeding of CAN increased total cholesterol level, which reflected well in varying result in different age group in our study. Treatment $T_1$ and $T_4$ showed higher serum phosphorus levels during 28 and 42d respectively. The duration of supplementation increased the circulatory level of phosphorus at higher dose rate (50 ppm each). This finding was in agreement with Ali *et al.* (2012) who fed 20 ppm of commercial CAN to laying hens and reported significant (P<0.05) increase in serum phosphorus level compared to control group. Analysis of data on extent of pigmentation (shank colour), showed significant (P<0.05) improvement with the supplementation of combination of CAN and APO ($T_1$ group) (Table 6). In accordance with these findings, Hamelin (2013) reported that addition of 10 ppm of CAN significantly increased shank colour compared to 5 ppm in broilers. Addition of 25 ppm CAN significantly (P>0.01) improved skin pigmentation. Astaxanthin is responsible for orange and red colour in plants and animals. When CAN was added in maternal diet of broiler breeders, the offspring had significantly (P<0.01) higher yellow to orange-red colour of shank and skin pigmentation (Zhang *et al*. 2011). Data pertaining to carcass characteristics did not show any significant difference due to addition of synthetic carotenoids (Table 7). This finding was in harmony with finding of Ali *et al*. (2012) who reported that feeding 20 ppm of CAN to laying hens had non-significant effect on carcass characteristics. In contrast, feeding combination of CAN (0.005%), sodium sulphate (0.5%) and dried whey (0.5%) resulted in significantly (P>0.05) higher dressed carcass percentage (Ali *et al*. 2016). Analysis of data on cell mediated immune response, showed non-significant difference between synthetic carotenoid supplemented and unsupplemented treatment groups (Table 6). In contrast, Rajput *et al*. (2013) reported that feeding natural pigment showed significantly (P<0.05) higher antibody titre against ND in broiler chicken and increased B and T lymphocyte cell proliferation. Addition of 2 ppm of beta-carotene and canthaxanthin to rat diets, increased splenic B and T lymphocyte proliferation in response to mitogen stimulation.

The supplementation of synthetic carotenoids in combination @ 25 ppm improved the performance, blood biochemical characteristics, and shank colour of broilers under hot-dry summer season. Addition of synthetic carotenoids also increased shank, skin, meat and egg yolk colour as well as improved the birds health status.

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