Antioxidant, cholesterol reducing, immunomodulating and other health promoting properties of herbal enriched designer eggs

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Summary

Standard Designer Eggs (SDE) were produced by feeding hens with feeds rich in N3 Fatty acids, Vitamin E, Selenium and Carotenoid pigments. Along with the SDE, 5 different varieties of Herbal Enriched Designer Eggs (HEDE), were produced by incorporating in hens' diet 5g/Kg of Garlic pearls, Fenugreek seeds, Bay leaves or Basil leaves or 1g/Kg Spirulina. The antioxidant, cholesterol lowering, immunomodulating and other health promoting properties of the eggs and the immune status in the birds were estimated.

All the herbs, especially the Bay leaves had increased the antioxidant property of the eggs. The yolk cholesterol level was significantly reduced in the SDE as well as in the HEDE, especially due to the Basil leaves and Garlic supplementation. The immune status of the hens as well as the Immunoglobulin levels in the eggs were increased due to all treatments, especially by Fenugreek and Basil leaves. Except Garlic, other treatments produced consumer acceptable eggs. The N3 PUFA levels in all the 6 SDE were significantly increased, with proportionate reduction in the saturated FA level; resulting in favourable N3/N6 ratios. Consumption of SDE and HEDE produced healthy changes in the serum lipid profile in human volunteers.

Introduction

Several Designer Eggs (DE) like N3 PUFA, Vitamin E, Selenium, Lutein, Folic acid and Iron rich eggs were developed earlier by many workers (Jiang and Sim, 1993; Farrell, 1998 and Leeson, 2004). In India Narahari *et al*. (2004) has developed HEDE, which are not only rich in N3 PUFA, Vitamin E, Selenium and Carotenoids; but also rich in herbal active principles like Allicin, Euginol, Natural antioxidants, Quercitin, Murrayanol and others. This experiment was conducted to further enrich the SDE with herbal active principles, by supplementing selective herbs in the hens' feed.

Materials and methods

A biological study of 12 weeks duration was carried out using 280 commercial layers (BV 300) 30 weeks of age in cages. They were randomly divided into 28 groups of 10 hens each. Four replicates were randomly allotted to each of the 7 dietary treatments namely,

T1	:	Control fed with regular layer mash
T2	:	Standard Designer Feed (SDF): supplemented with flaxseed, fish oil, vitamin E, Selenium and carotenoids
T3	:	SDF + 5g/Kg Garlic pearls
T4	:	SDF + 5g/Kg Fenugreek seeds
T5	:	SDF + 5g/Kg Bay / Curry leaves (*Murrya Koeingii*)
T6	:	SDF + 5g/Kg Basil leaves (*Oscimum sanctum*)
T7	:	SDF + 1g/Kg Spirulina

The hens were fed *ad libitum* with respective feeds from 31-42 weeks of age.

In order to boost the immunity levels in all the birds including the control, all the birds were dewormed with Levamisole at 30 weeks of age, followed by vaccination against New castle Disease (ND).

The eggs were collected for various assay procedures form 34 weeks of age. The blood samples were collected from 2 hens from each replicate, the serum was separated for estimation of Haemagglutination Inhibition (HI) titre levels as the per the techniques of Allan and Gough (1974). The serum samples were also utilized for estimation of ELISA titre for ND according to the procedure of OIE (2000). The ImmunoglobulinY (IgY) level in the yolk was assayed according to the technique of Polson *et al.* (1980).

During 35th week the blood samples were collected from another 2 birds from each replicate the serum was separated and analyzed for their lipid profile consisting of Triglycerides (TG), Total Cholesterol (TC), HDL Cholesterol (HDLC) LDL Cholesterol (LDLC), VLDL Cholesterol (CLDLC), by adopting the procedures of Wybenga *et al.,* (1970).

Six eggs from each replicate were utilized for estimation of their yolk cholesterol levels, following the method of Washburn and Nix (1974).The yolk vitamin E, egg selenium and their fatty acid composition of yolk lipids were estimated following the techniques of Abdollahi *et al.,* (1993), Canter and Tarino (1982) and Wang *et al.,* (2000), respectively.

The Iodine value of the experimental egg yolk lipids were determined according to the Official Methods of Analysis (AOAC, 1995).

In order to study the anti-oxidant properties of the eggs, six eggs from each replicate were stored at room temperature for 2 weeks. Later, the eggs were assayed for their peroxide value, TBA value and Free Fatty Acid (FFA) levels following the procedure of AOAC (1995) and Tarladgis *et al.* (1960). The Ferric Reducing/ Antioxidant Power (FRAP) values of the eggs were determined according to the procedure of Benzie and Strain (1996).

Blood samples were again collected during 36th week from 2 different hens from each replicate for estimation of the RBC, WBC, Hb level and WBC differential count, following the techniques of Schultze and Elvehjem (1934) and Tuisselmann (1939).

The organoleptic acceptability of the eggs from different treatments was assessed by 10 trained taste panellists during the 6th week of the study. Hard-boiled eggs from each treatment were served to the panellists to grade the eggs on a 4 point Hedonic scale for their flavour, colour, texture, taste and overall acceptability. Number 1 indicates least acceptability and number 4 indicate the highest acceptability.

In order to find out the effect of consumption of regular eggs, DE and HEDE on the serum lipid profile in human volunteers. 48 male volunteers, in the age group of 30-60 years, who ware not having any history of any chronic diseases, were selected. They were randomly allotted to the 4 treatments with 12 volunteers for each treatment.

The 1st group of volunteers were given a Placebo containing 15g of peanuts supplying the same energy and lipids as in one egg, but without any cholesterol, for a period of 1 month, followed by 30g of peanuts/ day for the next 1 month. The group 2 volunteers were given 1 regular egg/ day for 1 month followed by 2 regular eggs during the next month. The group 3 volunteers were given SDE for 1st month and 2 egg during the 2nd month. The group 4 volunteers were given the HEDE (mixed eggs from treatments 3-7) as above.

All the Volunteers were advised to take their normal daily diet, but without any egg or high cholesterol and high lipid foods, during the 2 months experimental period. Their blood samples were collected at the start and end of the experiment, for estimation of their serum lipid profile according to the procedure of Wybenga *et al.* (1970).

All the data collected were subjected to ANOVA for significance according to the procedure of Snedecor and Cochran (1989). The significance was tested using Duncans' multiple range test (Duncan, 1955).

Results and discussion

The Iodine value and the antioxidant properties of the HEDE, expressed as Peroxide Value, TBA value, % FFA and FRAP value are summarised in Table 1. The Iodine values showed significant differences ($P<0.05$) between the regular and SDE or HEDE. Higher levels of unsaturated fatty acids in all the 6 DE had resulted in higher Iodine values than the regular eggs. Michealraj (2004) and Narahari *et al.* (2004) also reported higher Iodine values for eggs enriched with N3 PUFA.

The peroxide value, TBA value and per cent Free Fatty Acids (FFA) also showed significantly higher values for certain HEDE than the regular egg. Higher levels of N3 PUFA might be responsible for this enhancement. However, Bay leaf enriched eggs was able to reduce these values comparable to that

of regular eggs, in spite of higher N3 PUFA. This indicates better antioxidant property of the Bay leaves.

The FRAP value of the eggs, which is the direct estimate of the antioxidant property of any substance suggested that the Bay Leaves enriched eggs had the highest antioxidant property. Garlic and Basil leaves supplemented eggs also possessed better antioxidant properties. In fact, all HEDE had better antioxidant properties than SDE or regular eggs. The active principles Murrayanol, Mahanibine and Mahanine present in Bay leaves might have been incorporated in the egg and enhanced the antioxidant property of these eggs. The FRAP value observed in HEDE were comparable with the FRAP values of apricot, mango and peach reported by Guo *et al.* (2003).

Similarly, the Allicin and Ajoene in Garlic and Eugenol in Basil leaves might be responsible for these enhanced antioxidant properties of these eggs. Similarly observations were made earlier by Ryu *et al.* (2001), Tachibana *et al.* (2003), Michealraj (2004) and Narahari *et al.* (2004).

The yolk cholesterol and serum cholesterol levels in hens as influenced by dietary treatments are reported in Table 2. The yolk cholesterol levels were significantly reduced ($P<0.01$) in SDE and all HEDE. Among the HEDE, Basil leaves and Garlic were found to be more effective in reducing the yolk cholesterol levels. Chowdhury *et al.* (2002) and Michealraj (2004) reported earlier the powerful hypocholestermic effects of Garlic. Kirubakaran (2003) and Narahari *et al.* (2004) reported the cholesterol lowering property of the Basil leaves.

The serum lipid profile in the hens were also favourably altered by the designer feeds and herbs. Among the herbs tried, Garlic and Basil leaves were very effective in reducing the Triglycerides, Total Cholesterol, LDLC and VLDLC with proportionate increase in the good HDLC. Other herbs were also effective in increasing the HDLC, compared to SDF. Sujatha (2002), Kirubakaran (2003) and Narahari *et al.* (2004) reported favourable serum lipid altering effects of designer feeds.

The immunomodulating properties in the hens and their eggs as a result of feeding designer feeds and HE designer feeds are shown in Table 3. Both SDE and HEDE had significantly ($P<0.01$) higher IgY levels than regular eggs indicating that the designer feeds as well as the herbs tried, especially the Garlic, Fenugreek and Basil leaves were having powerful immunomodulating properties. In the hens also the immunity levels were boosted due to the designer feeds and herbs. Michealraj (2004) and Narahari *et al.* (2004) also reported higher immunomodulating status in eggs and hens due to designer feeds and herbs.

The consumer acceptability of the hard boiled designer eggs, except Garlic were comparable or better than regular eggs (Table 4). Garlic supplementation in hens' feed had incorporated the strong flavour of the garlic, which was not much accepted by the panellists. On the other hand, Bay leaves and spirulina supplemented eggs recorded better consumer acceptability due to richer yolk colour. Michealraj (2004) reported lesser consumer acceptance score for Garlic. However, Birrenkott *et al.* (2000) reported no differences in flavour of eggs from hens consuming dietary Garlic powder.

SDE and HEDE had significantly ($P<0.01$) higher levels of yolk carotenoids and Roche yolk colour values, due to more pigments present in these feeds. Bay leaves, Basil leaves and spirulina enriched eggs had high pigment levels than other eggs. Sujatha (2002), Kirubakaran (2003) and Michealraj (2004) observed darker yolks due to more carotenoids in the functional feeds.

The vitamin E and selenium levels were also higher in the SDE and HEDE due to supplementation of these nutrients in the hens' diet; witch concurs with the earlier findings of Kirubakaran (2003) and Michealraj (2004).

All the six designer feeds had increased the yolk N3 PUFA level highly significantly ($P<0.01$) at the expense SFA. This change also resulted in a favourable N3/N6 Fatty acid ratios in designer eggs. Ezhilvalavan (2003) and Narahari *et al.* (2004) noticed similar favourable changes in the designer eggs.

Consumption of SDE and HEDE for two months by human volunteers had favourably altered their serum lipid profile. The SDE and HEDE consumption had significantly reduced the serum TG, TC, LDLC and VLDLC and highly significantly ($P<0.01$) increased the good HDLC proportionately. Kirubakaran (2003) and Michealraj (2004) noticed significant favourable changes in serum lipid profile in humans due to designer egg consumption. Compared to SDE, the HEDE consumption was more effective in reducing the serum LDLC and increasing the HDLC, which might be due to the herbal active principles incorporated in the egg. Earlier works of Yeh and Liu (2001) and Narahari *el al.* (2004) confirmed the hypocholestremic property of Garlic and Basil leaves, respectively.

Conclusion

- HE designer feeds to hens had improved their immunity and overall health.
- The eggs from such hens had higher antioxidant properties, immunoglobulins, carotenoids, vitamin E, Selenium and N3 PUFA. These eggs had lower cholesterol and Saturated Fatty Acids.
- Consumption of such HEDE by human volunteers had produced favourable changes in their serum lipid profile.

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Table 1 Antioxidant properties of Herbal Enriched Designer Eggs.

Trait	*Regular Egg (Control)*	*Standard Designer Egg (SDE)*	*SDE + 0.5% Garlic*	*SDE + 0.5% Fenugreek seeds*	*SDE +0.5% Bay leaves*	*SDE + 0.5% Basil leaves*	*SDE + 0.1% Spirulina*
Iodine value*	$66.8^{a}\pm1.02$	$86.0^{b}\pm1.19$	$89.5^{b}\pm1.93$	$87.3^{b}\pm2.10$	$87.2^{b}\pm1.73$	$85.9^{b}\pm1.44$	$83.4^{b}\pm2.08$
Peroxide value (mEq O_2 /kg)*	$6.58^{a}\pm0.16$	$8.16^{b}\pm0.67$	$7.72^{b}\pm0.76$	$8.07^{a}\pm0.22$	$6.72^{a}\pm0.66$	$7.92^{b}\pm0.23$	$7.70^{b}\pm0.38$
T.B.A. value (mg MA/ kg)*	$0.28^{a}\pm0.04$	$0.34^{b}\pm0.06$	$0.32^{ab}\pm0.05$	$0.30^{ab}\pm0.05$	$0.27^{a}\pm0.07$	$0.31^{ab}\pm0.05$	$0.32^{ab}\pm0.06$
% FFA*	$2.68^{a}\pm0.08$	$4.01^{b}\pm0.34$	$3.87^{b}\pm0.17$	$3.71^{ab}\pm0.22$	$3.01^{ab}\pm0.19$	$3.28^{a}\pm0.11$	$3.55^{ab}\pm0.28$
FRAP Value (mmol/100g) **	$0.18^{a}\pm0.02$	$0.22^{ab}\pm0.03$	$0.34^{cd}\pm0.03$	$0.27^{b}\pm0.03$	$0.39^{d}\pm0.05$	$0.34^{cd}\pm0.04$	$0.30^{bc}\pm0.04$

Table 2 Serum and yolk cholesterol levels in hens fed with Herbal Enriched Designer Feeds.

Trait	*Regular Egg (Control)*	*Standard Designer Egg (SDE)*	*SDE + 0.5% Garlic*	*SDE + 0.5% Fenugreek seeds*	*SDE +0.5% Bay leaves*	*SDE + 0.5% Basil leaves*	*SDE + 0.1% Spirulina*
Serum TG (mg /dl)*	$737^{a}\pm5.8$	$720^{ab}\pm6.2$	$699^{b}\pm4.9$	$715^{ab}\pm2.9$	$716^{ab}\pm7.1$	$701^{b}\pm6.2$	$715^{ab}\pm3.9$
Serum TC (mg/dl)**	$154^{a}\pm1.2$	$135^{b}\pm1.1$	$128^{b}\pm1.0$	$130^{b}\pm1.1$	$133^{b}\pm1.3$	$130^{b}\pm1.2$	$138^{b}\pm1.3$
Serum LDLC (mg/dl)*	$27.0^{a}\pm0.12$	$24.6^{ab}\pm0.16$	$22.3^{b}\pm0.22$	$23.8^{b}\pm0.21$	$24.7^{ab}\pm0.28$	$23.9^{b}\pm0.18$	$23.8^{b}\pm0.16$
Serum VLDLC (mg/dl)**	$97.5^{a}\pm1.3$	$79.2^{b}\pm1.7$	$70.5^{b}\pm1.3$	$72.8^{b}\pm1.2$	$74.6^{b}\pm1.2$	$70.0^{b}\pm1.4$	$79.5^{b}\pm1.1$
Serum HDLC (mg/dl)*	$29.5^{a}\pm0.88$	$31.2^{ab}\pm0.92$	$35.2^{b}\pm1.01$	$33.3^{ab}\pm1.03$	$34.5^{b}\pm0.89$	$36.2^{b}\pm1.03$	$34.7^{b}\pm1.03$
Yolk cholesterol (mg/g)**	$12.38^{a}\pm0.37$	$11.60^{b}\pm0.48$	$9.62^{d}\pm0.26$	$10.63^{bc}\pm0.22$	$10.95^{bc}\pm0.17$	$9.48^{d}\pm0.23$	$9.90^{cd}\pm0.22$

Table 3 Immunomodulating properties of Herbal Enriched Designer Feeds and Eggs.

Trait	*Regular Egg (Control)*	*Standard Designer Egg (SDE)*	*SDE + 0.5% Garlic*	*SDE + 0.5% Fenugreek seeds*	*SDE +0.5% Bay leaves*	*SDE + 0.5% Basil leaves*	*SDE + 0.1% Spirulina*
Immunoglobulin–IgY** (mg/g yolk)	15.33^{a}±0.29	18.47^{b}±0.28	19.38^{b}±0.17	19.67^{b}±0.25	18.42^{b}±0.34	19.61^{b}±0.23	18.89^{b}±0.33
ELISA (log 2 values)*	2.77^{a}±0.05	2.98^{ab}±0.06	3.10^{b}±0.10	2.98^{ab}±0.07	2.96^{ab}±0.08	3.24^{b}±0.10	2.98^{ab}±0.12
HI titre (log 2 values)**	4.33^{a}±0.22	5.83^{b}±0.18	6.17^{b}±0.23	6.22^{b}±0.16	5.87^{b}±0.25	6.30^{b}±0.25	6.08^{b}±0.17
WBC ($X10^{3}$ /mm^{3})*	25.5^{a}±0.56	31.3^{b}±0.26	35.9^{c}±1.45	35.8^{c}±1.02	32.6^{b}±0.89	36.1^{c}±1.22	32.0^{b}±0.99
% Lymphocytes*	66.6^{a}±1.48	73.5^{b}±1.43	73.8^{b}±1.42	74.8^{b}±1.48	73.5^{b}±1.60	74.5^{b}±1.43	72.9^{b}±1.48
RBC (X 10^{6}/mm^{3})*	2.45^{a}±0.11	2.57^{ab}±0.07	2.95^{b}±0.10	2.82^{b}±0.12	2.64^{ab}±0.11	2.89^{b}±0.09	2.87^{b}±0.07
Haemoglobin (g%)*	9.13^{a}±0.21	9.41^{ab}±0.23	9.66^{b}±0.33	9.53^{b}±0.31	9.43^{ab}±0.28	9.60^{b}±0.22	9.45^{ab}±0.25

* Significant (P<0.05), ** Highly significant (P<0.01)

Table 4 Consumers' acceptability and Health promoting components in the Herbal Enriched Designer Eggs.

Trait	*Regular Egg (Control)*	*Standard Designer Egg (SDE)*	*SDE + 0.5% Garlic*	*SDE + 0.5% Fenugreek seeds*	*SDE +0.5% Bay leaves*	*SDE + 0.5% Basil leaves*	*SDE + 0.1% Spirulina*
Consumers' acceptability @ *	3.20^{b}±0.21	3.56^{c}±0.22	2.67^{a}±0.21	3.18^{b}±0.33	3.68^{c}±0.28	3.58^{c}±0.29	3.70^{c}±0.34
Roche yolk colour value**	7.83^{a}±0.31	9.03^{b}±0.45	9.03^{b}±0.33	9.33^{b}±0.35	10.50^{c}±0.34	10.10^{bc}±0.42	11.12^{c}±0.40
Yolk carotenoids (mcg/g)**	48.00^{a}±0.81	64.01^{b}±1.02	64.83^{b}±1.10	68.67^{bc}±0.92	69.83^{bc}±0.72	70.20^{bc}±0.81	72.27^{c}±0.97
Yolk vitamin E (mcg /g)**	80.0^{a}±1.82	194.8^{b}±2.33	214.3^{c}±2.17	190.2^{b}±2.11	210.3^{c}±2.22	217.5^{c}±1.87	198.5^{b}±1.99
Egg selenium (ng/g)**	118.5^{a}±1.80	260.5^{b}±1.26	269.3^{b}±2.71	260.5^{b}±2.82	279.5^{bc}±2.71	284.7^{c}±2.85	278.2^{bc}±2.71
N -3 PUFA (g/100g TFA)**	0.28^{a}±0.07	10.38^{b}±0.21	12.71^{c}±0.22	10.91^{b}±0.18	11.38^{bc}±0.31	12.51^{c}±0.27	10.55^{b}±0.18
Saturated fatty acid (%)**	35.81^{a}±0.31	26.12^{b}±0.30	24.73^{c}±0.21	26.04^{b}±0.28	25.33^{bc}±0.27	24.82^{c}±0.25	25.97^{b}±0.28
N3/N6 ratio**	0.02^{a}±0.001	0.66^{b}±0.01	0.81^{c}±0.02	0.69^{b}±0.02	0.72^{bc}±0.02	0.80^{c}±0.03	0.67^{b}±0.02

@ 1 = Least acceptable, 4 = Most acceptable * Significant (P<0.05), ** Highly significant (P<0.01)

Table 5 Effect of consumption of Herbal Enriched (HEDE) Designer Eggs by human volunteers for 2 months on % change in their serum lipid profile.

% Change in Trait @	*Placebo Group*	*Regular Egg Consumption*	*SDE Consumption*	*HEDE Consumption*
Serum TG*	0.90^{a}±0.01	0.99^{a}±0.02	-7.10^{b}±0.07	-7.12^{b}±0.10
Serum TC*	0.52^{a}±0.02	0.83^{a} ±0.01	-6.00^{b}±0.05	-6.21^{b}±0.07
Serum HDLC **	2.07^{a}±0.09	2.17^{a}±0.10	6.92^{b}±0.29	12.64^{c}±0.32
Serum LDLC **	-2.01^{b}±0. 07	-1.07^{b}±0.10	-7.11^{c}±0.23	-12.30^{d}±0.42
Serum VLDLC*	-0.38^{b}±0.03	-0.60^{a}±0.11	-5.07^{c}±0.03	-7.10^{d}±0.28

@(-) = % decrease in the final value, compared to the initial value
No sign = % increase in the final value, compared to the initial value
* Significant (P<0.05), **Highly significant (P<0.01)