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Full Length Research Paper

Egg yolk cholesterol lowering effects of garlic and tea

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This study was conducted to evaluate the effect of garlic and tea on the performance, egg traits and laying parameters of laying hens. Five groups of black leghorn hens, Yafa breed (five birds per group) and aged 21 weeks were used for this experiment. Each group was fed basal diet (layers mash) supplemented with garlic at 1% garlic powder (group 1), 2% garlic powder (group 2), 1% black tea (group 3), 2% back tea (group 4) and a combination of 2% garlic and 2% tea (making 4% supplementation, group 5). Feeding was done for 4 weeks after a one week acclimatization period on test and control feeds. The effects of supplementation on the number and weight of eggs layed, the weight of hens and the weight of egg yolk were determined. Also determined were the total triglycerides, HDL-, LDL- and total cholesterol content of egg yolk. Feeding of hens for 4 weeks with test and control diets resulted in non-significant changes ($P > 0.05$) in the weights of birds, egg and egg yolk. All the garlic supplemented feeds resulted in significant reductions ($P < 0.05$) of total cholesterol, total triglyceride, LDL- and HDL-cholesterol. With exception of the 1% tea supplemented diet, the other tea supplemented diet resulted in significant reductions in the egg yolk concentration of the cholesterols tested. 1% tea supplementation had no significant effect on LDL-cholesterol concentration of egg yolk ($P > 0.05$). The combination of garlic and tea resulted in significant reductions of total- LDL- and HDL-cholesterol ($P < 0.05$) but not total triglycerides ($P > 0.05$). The control diets had in most cases non-significant effects on the lipid parameters tested. The results show that garlic and tea have great potential when low cholesterol egg is desired.

Key words: Garlic, black tea, cholesterol, triglycerides, egg yolk.

INTRODUCTION

The effect of dietary cholesterol and saturated fat in raising plasma cholesterol levels has been identified and verified in numerous metabolic studies. Some cross section population studies have established a correlation between dietary cholesterol and plasma cholesterol levels in humans (Schonfeld et al., 1982). Earlier studies (Grande et al., 1965) suggested that the P/S ratio did not affect the changes in plasma cholesterol in response to dietary cholesterol. However, most studies agree that the fat composition of the diet influences the plasma cholesterol changes in response to cholesterol consumption. Recent studies have focused on the effect of dietary lipids on plasma lipoproteins and apoproteins (Schonfeld et al., 1982). These studies have generally shown that dietary cholesterol increases LDL-cholesterol but either is without effect (McMurry et al., 1982) or increases (Schonfeld et al., 1982) HDL-cholesterol. Most investigators have observed a large inter-individual variability in response to cholesterol- and saturated fat-containing diets (Jacobs et al., 1983).

Animal studies have suggested that garlic supplemented diets may inhibit the synthesis of cholesterol and fatty acids in the liver (Yeh and Liu, 2001). Warshafsky et al. (1993) suggested that a cholesterol-lowering effect of ~ 9% (0.59 mmol/L) could be achieved by a daily consumption of 1.5 – 3 g of fresh garlic for 2 – 6 months. Allicin, which causes the characteristic garlic odour, is believed to be the active lipid-lowering compound in garlic. Also, flavonoids from green and black tea, when added directly to isolated LDL, protect against lipid peroxidation induced by free radicals, copper ions and cells (Ishikawa et al., 1997). The effect of tea consumption on the development or regression of atherosclerotic lesions in humans has not been directly examined (Riemersma et al., 2001).

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Abbreviations: HDL, High density lipoprotein; LDL, low density lipoprotein.

According to Guéye (2003), egg consumption in Africa is estimated to be around 2.1 kg/person/year. The egg contributes 46% of the daily cholesterol intake of the American diet (McGill, 1979) and several previous studies have used eggs as cholesterol source in human nutritional experiments (Porter et al., 1977). Egg consumption was associated with increases in LDL-cholesterol and with small or negligible changes in HDL-cholesterol (Sacks et al., 1984).

Several studies showed that plasma cholesterol lowering drugs, such as statins, were able to reduce significantly the cholesterol concentration of egg yolk (Elkin et al., 1999). The present study was therefore conducted to evaluate the effect of garlic and tea on the performance, egg traits and egg yolk cholesterol concentration of laying hens.

MATERIALS AND METHODS

Preparation of layer's mash

The layer's mash used in this study was prepared at the Hephzibah Integrated Farms, Irrua, in mid-western Nigeria. Its composition is as shown in Table 1.

Table 1. Composition of layer's mash.

Components	Quantity (kg)	Percent content (%)
Corn meal	57	53.6
Soya meal	13	12.2
Fish meal	9	8.5
Bone meal	2.2	2.1
Wheat offal	6.3	5.9
Groundnut cake	10.0	9.4
Methionine	0.1	0.1
Lysine	0.08	0.1
Premix	0.25	0.2
Salt	0.25	0.2
Limestone	8.2	7.7

Preparation of garlic powder and tea and test feed

Garlic was purchased from a local market in mid-western Nigeria. It was initially cut into smaller pieces and then sun-dried for about two days. After sun drying, the garlic was milled into a powder. This was stored in closed containers pending usage. The black tea (LIPTON™ tea brand), packaged in 2 g portions, was purchased from a local supermarket in mid-western Nigeria. The tea powder was removed from its packaging material and placed in a bowl just before weighing to compound the test feed. The test feed was prepared by weighing out 90 and 180 g of garlic powder and making up to 9,000 g with layer's mash to obtain the 1 and 2% supplementations, respectively. Tea was administered brewed in tap water as 1 and 2% tea powder in water (w/v). This was used in place of water for the tea supplementation group.

Preparation of poultry

The Ambrose Alli University Poultry was used for this study. The experimental poultry cages constructed with compartments for housing a single bird were used. Each compartment had dimensions 35 x 20 x 37 cm for length, breadth and height, respectively. The cages and the poultry house were first of all disinfected. Two troughs each were placed in the cage compartment for feed and water/tea, respectively.

Administration of feeds

The black leghorn hens, Yafa breed and aged 21 weeks, which were already laying eggs, were used for this experiment. A total of six experimental groups of five birds each were used. They were all allowed to acclimatize on normal layer's mash for one week before test feeding was commenced. Each bird was given 100 g of feed per day and water/tea was administered *ad libitum*. One group (control) received layer's mash and water while the other five groups (test) received supplemented feeds. Feeds were supplemented with garlic powder at 1% level (group 1), 2% level (group 2), 1% black tea (group 3), 2% back tea (group 4) and a combination of 2% garlic and 2% tea (making 4% supplementation, group 5).

Data collection

The quantity of feed left after each day was collected and weighed. The number of eggs layed was also noted on daily basis. The weight of the birds and weight of eggs were recorded on weekly basis. The egg used analyses was collected one per hen on day 0, 7, 14, 21 and 28.

Preparation of egg yolk for analyses

The eggs used for analyses were prepared according to the procedure described by Elkin et al. (1999). The eggs were first of all hard-cooked, allowed to cool, after which the weight of the boiled egg was noted. The egg shell was peeled off and also weighed followed by the careful removal of the egg white (albumen). The yolks were separated, weighed and crumbled. A 1 g sample of each yolk was homogenized with 15 ml of chloroform-methanol 2:1 (v/v), thoroughly mixed and filtered. Egg homogenate filtrates were designated egg yolk samples.

Analyses

Total cholesterol, HDL-cholesterol, total triglycerides concentrations of egg yolk were determined using the respective RANDOX® cholesterol assay kit. The kit contained cholesterol assay reagent and standard cholesterol solution, used for calibration curve.

Total cholesterol

10 µl of egg yolk sample, 10 and 10 µl of deionized water were pipetted into their respective test tubes. This was followed by 1000 µl of total cholesterol assay reagent. The test tubes were incubated for 15 min at 37°C after which absorbance of sample was read 500 nm against the reagent blank. The various cholesterol concentrations recorded as mg/g of egg yolk was computed from values obtained from the various cholesterol standard curves.

HDL-Cholesterol

The HDL-cholesterol assay kit (RANDOX®) contained a cholesterol

precipitant. 500 µl of egg yolk sample was pipetted into a test tube and 1000 µl of the precipitant was added, mixed and then centrifuged for 15 min. After centrifuging, 100 µl supernatant, and 100 µl of deionised water were pipetted into their respective test tubes. This was followed by the addition of 1000 µl of HDL-cholesterol reagent. The test tubes were incubated for 15 min at 37°C after which absorbance of sample and standard, read at 500 nm, as well as the computation of HDL-cholesterol concentration of egg yolk was done as stated for total cholesterol.

Total triglyceride

The total triglyceride assay kit contained a buffer solution, enzyme reagent and standard triglyceride solution. The working enzyme reagent was constituted by adding 15 ml of buffer to the enzyme reagent. Triglyceride assay was performed as previously described except that test tubes were incubated for 15 min at room temperature.

LDL-Cholesterol

LDL-Cholesterol was estimated using the Friedewald equation (Friedewald, 1972), which is as follows:

$$\text{LDL-Cholesterol} = \frac{\text{Total cholesterol} - \text{Triglyceride} - \text{HDL-cholesterol}}{5}$$

Data analyses

Student's t-test was used to compare test results obtained after four weeks with results obtained at the start of experiment.

RESULTS

The results of the effects of test and control feeds of the weight of birds and some physical egg parameters are shown in Table 2. As stated earlier, test and control feeding was done for 4 weeks. It was found that supplementation at test levels did not significantly affect feed consumption and number of eggs layed ($P > 0.05$; data not shown). With the exception of the 1% garlic powder supplementation group, test diet resulted in non-significant changes ($P > 0.05$) in the weights of birds and egg (Table 2). 1% garlic powder supplementation resulted in 10% increase in average weight of bird ($P < 0.05$). Control diet resulted in 6% reduction in bird weight ($P > 0.05$). Only 1% supplementation resulted in slight increase in weight of egg yolk (+6%, $P > 0.05$). Only garlic powder supplementation had a positive effect on the weight of egg shell, while other test treatment resulted in non-significant reductions in weight of egg shell.

Table 3 shows the effect of feeds on the egg yolk concentration of total cholesterol, LDL-cholesterol, HDL-cholesterol and total triglyceride. Almost all the test treatment resulted in significant reductions ($P < 0.05$) in the egg yolk concentration of total cholesterol, LDL- and HDL-cholesterol and total triglyceride. The exception was the 1% tea supplemented diet, which resulted in a significant increase in LDL-cholesterol concentration in egg yolk. On the other hand control diet resulted in increases in total-cholesterol, LDL- and HDL-cholesterol. Similar patterns were observed for the various egg yolk cholesterols of eggs from hens which received 2% tea, 1 and 2% garlic supplementation as well as combination of 2% tea and 2% garlic powder.

DISCUSSION

The anti-cholesteromic agents found in garlic and in tea flavonoids could be responsible for the for the reduced cholesterol content of egg yolks. There are lots of published works on the clinical use of the hypocholesterolemic effect of garlic and tea in blood plasma. There is, however, scarcity of information on the effect of garlic and tea on egg yolk cholesterol. Yalcin et al. (2006) recently published a work on the effect of garlic supplementation on egg yolk total cholesterol. They also reported a significant reduction in total egg yolk cholesterol. They did not, however, report on LDL- and HDL-cholesterol.

The mechanisms underlying the possible lipid-lowering action of garlic are not well understood. Allicin, which causes the characteristic garlic odour, has been suggested to be one of the major sulphur-rich components in garlic that may contribute to its hypocholesterolemic effect. Animal studies have suggested that garlic supplemented diets may inhibit the synthesis of cholesterol and fatty acids in the liver (Yeh and Liu, 2001). Although the results from two earlier meta-analyses (Warshafsky et al., 1993) suggested a hypocholesterolemic effect of garlic, they did not provide strong evidence for the usefulness of garlic as a hypocholesterolemic agent because of the methodological shortcomings of many of the studies included. Not all the included trials provided information about the subjects' dietary intakes and body weights, or about the comparability of the smell or taste of the placebo and garlic preparations. In the meta-analysis performed by Warshafsky et al. (1993), only 5 randomized, placebo-controlled trials of the 28 identified trials were included. The reason for the lack of effect of garlic on the lipid and lipoprotein profile in recent well designed studies is unclear. It is possible that the allicin released from the garlic preparations, despite standardization, was not optimal (Zhang et al., 2001). Garlic preparations are often designed to release allicin enzymatically from alliin after consumption. It seems that the enzyme alliinase quickly denatures at a low gastric pH (Ackermann et al., 2001).

Flavonoids from green and black tea, when added directly to isolated LDL, protect against lipid peroxidation induced by free radicals, copper ions and cells (Ishikawa et al., 1997). There is substantial evidence that oxidized LDL is central to early events leading to atherosclerosis

Table 2. Changes in weight of layers and some egg characteristics resulting from supplementation.

Weight change	0% Supplementation	1% Tea	2% Tea	1% Garlic powder	2% Garlic powder	2% Garlic and Tea*
Weight of Hens (kg)	1.91 ± 0.03 (-6%)**	1.89 ± 0.03 (+1%)	1.81 ± 0.11 (+3%)	1.97 ± 0.07 (+10%)	1.50 ± 0.07 (+6%)	1.41 ± 0.01 (+5%)
Weight of Egg (g)	63.25 ± 0.76 (+7%)	57.96 ± 1.08 (-4%)	56.07 ± 1.15 (-7%)	63.88 ± 4.06 (+7%)	63.90 ± 1.55 (+5%)	56.07 ± 1.15 (-7%)
Weight of Egg Yolk (g)	41.66 ± 5.70 (+8%)	15.21 ± 0.45 (-5%)	14.00 ± 0.36 (-6%)	16.03 ± 0.29 (+6%)	15.74 ± 0.85 (-3%)	14.00 ± 0.36 (-12%)
Weight of Egg Shell (g)	6.40 ± 0.13 (+17%)	5.49 ± 0.17 (-20%)	5.02 ± 0.22 (-23%)	6.57 ± 0.28 (+3%)	6.63 ± 0.12 (+18%)	5.02 ± 0.22 (-22%)

Results are means of at least 3 determinations ± SD.
*A total of 4% supplementation comprising 2% each of garlic powder and black tea.
**Values in brackets represent % change after 4 weeks of feed administration.

Table 3. Changes in cholesterol contents (mg/g) of egg yolk resulting from supplementation.

Cholesterol change	0% Supplementation	1% Tea	2% Tea	1% Garlic powder	2% Garlic powder	2 % Garlic and Tea*
Total Cholesterol	79.33 ± 6.96 (+ 28%)**	48.70 ± 4.86 (-29%)	17.94 ± 1.86 (-66%)	24.09 ± 6.14 (-70%)	22.97 ± 0.53 (-65%)	23.17 ± 0.68 (-64%)
LDL-Cholesterol	41.66 ± 3.43 (+2%)	16.96 ± 2.91 (+17%)	6.64 ± 0.77 (-66%)	13.28 ± 1.77 (-71%)	11.90 ± 4.23 (-69%)	11.93 ± 3.83 (-78%)
HDL-Cholesterol	37.41 ± 9.42 (+25%)	39.44 ± 5.64 (-35%)	9.06 ± 0.29 (-67%)	28.75 ± 1.67 (-27%)	15.16 ± 1.23 (-65%)	15.56 ± 1.67 (-64%)
Total Triglycerides	113.80 ± 23.84 (-12%)	122.86 ± 9.79 (-19%)	52.31 ± 4.88 (-46%)	102.27 ± 3.35 (-36%)	100.87 ± 8.68 (-6%)	104.46 ± 4.46 (-2%)

Results are means of at least 3 determinations ± SD.
*A total of 4% supplementation comprising 2% each of garlic powder and tea.
**Values in brackets represent % change after 4 weeks of feed administration.

(Riemersma et al., 2001). The effect of tea consumption on the development or regression of atherosclerotic lesions in humans has not been directly examined. A typical cup of tea (200 ml) contains 24 – 40 mg catechin, 8 – 15 mg flavonols, plus flavones, ~85 mg thearubigins and 7 – 15 mg of theaflavins, which together amount to 166 – 193 mg per cup (Riemersma et al., 2001). This makes tea one important source of phenols. Tea polyphenols, catechins and flavonols scavenge reactive oxygen species (Rice-Evans et al., 1996) and chelate transition metal ions in a structure dependent manner (Brown et al., 1998). Flavonoids found in tea scavenge NO and peroxynitrite produced from superoxide radical and NO (Pannala et al., 1997). The ways and means by which these events lead to a reduction in the levels of cholesterol in egg yolk should be the focus of further research. The results of this study, however, show that garlic and tea have great potential when low cholesterol egg is desired in the diet.

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