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Effect of Omega-3 Enriched Layer Rations on Egg Quality

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Abstract: Human consumption of omega-3 Polyunsaturated Fatty Acids (n-3 PUFA) has increased due to reported health benefits. Despite the benefits of n-3 PUFA eggs, the addition of omega-3 enriched feedstuffs like flaxseed and fish oil may reduce egg quality. A standard n-PUFA diet containing flaxseed and fish oil (Diet A), a standard n-PUFA diet supplemented with vitamin B6 (Diet B), a standard n-PUFA diet without fish oil (Diet C) and a conventional milo ration (Diet D) were fed to four separate groups of laying hens to evaluate the effect of these diets on egg quality. Three individual shipments of eggs collected from hens fed these diets were received from a commercial layer operation, where these hens were reared. The eggs were stored for three weeks in a refrigerator to simulate consumer storage conditions. The eggs were evaluated for quality by determining whole egg weight, yolk weight, albumen thickness and Vitelline Membrane Strength (VMS). Significant increases in egg weights were observed in Diet B samples in the first egg shipment and Diets B, C and D in the third egg shipment. Yolk weights were significantly increased with Diet B samples from the third collection. No differences were observed in albumen thickness in any of the three shipments of eggs. Numerical increases in VMS were observed in Diets B, C and D in each of the three shipments, but only the third shipment had significant differences with these treatments. These data suggests that the addition of flaxseed and fish oil may negatively affect egg quality. The addition of vitamin B6 or the removal of fish oil to a standard n-PUFA diet may be comparable to a conventional layer ration's effect on egg quality.

Key words: Flaxseed, fish oil, omega-3 fatty acid, egg quality

INTRODUCTION

Flaxseed and fish oil contain high levels of omega-3 Polyunsaturated Fatty Acids (n-3 PUFA) and these compounds have been reported to reduce the incidence of heart disease and cancer in humans (Pandalai *et al.*, 1996; Temple, 1996; Rose, 1997). Flaxseed and fish oil are also added to laying hen rations to increase levels of n-3 PUFA in table eggs to appeal to the consumer's desire to increase their consumption of n-3 PUFA in a more palatable form. Although the n-3 PUFA content of the yolks was significantly enhanced, previous research indicates reduced yolk and egg weights collected from hens fed standard omega-3 rations containing flaxseed and fish oil (Hargis and Van Elswyk, 1993; Scheideler and Froning, 1996; Van Elswyk, 1997).

Vitamin B6 antagonism: Klosterman *et al.* (1974) identified linatine as a naturally occurring dipeptide of 1-amino-D-proline and glutamic acid found in flaxseed. Pyridoxine, or vitamin B6, is inhibited when the 1-amino-D-proline reacts with pyridoxal phosphate to form a hydrazone. Because B6 is a necessary cofactor for amino acid metabolism, antagonism of the vitamin may lead to decreased embryonic protein synthesis. The proteins of the vitelline membrane are directly related to the function of the membrane, while the strength of the vitelline membrane is critical in maintaining the functionality of the albumen proteins (St. John and Flor, 1931; Kelley, 2003). The strength of the vitelline membrane, particularly its ability to withstand breaking, is a key factor in producing good quality egg albumen (Kirunda and McKee, 2000). The albumen of a chicken egg consists of approximately 40 different proteins which function in the egg to maintain homeostasis and transfer nutrients to the developing embryo (Gilbert, 1971). The observed reduction in egg quality from decreased Vitelline Membrane Strength (VMS) and albumen thickness may be explained by the vitamin antagonism caused by linatine in the flaxseed.

Fatty liver syndrome: When hepatic lipogenesis exceeds capacity for Very Low Density Lipoprotein (VLDL) secretion, triacylglycerides are stored in the liver above normal levels, causing fatty liver (steatosis) (Hansen and Walzem, 1993; Hermier, 1997). Fatty Liver (FLS) symptoms include hepatic scarring, hardening and an overall decrease in liver function. The condition has been associated with decreased production and

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increased mortality in layer flocks (Hermier, 1997). Hansen and Walzem (1993) reported that a fatty liver may indicate the early stages of a more acute metabolic disorder known as Fatty Liver Hemorrhagic Syndrome (FLHS). Both Hargis and Van Elswyk reported that lipid accumulation occurs in the liver with as little as 3% dietary fish oil, which is a factor in layer flocks predisposed to FLS (Hargis *et al*., 1991; Hargis and Van Elswyk, 1993; Van Elswyk, 1997). Bean and Leeson (2003) found that hens fed flaxseed had a higher incidence of liver hemorrhage as well as smaller percentage yolk compared to control birds, but found that flaxseed had no effect on albumen height or whole egg weight (2003).

The objectives of this study were to determine if the removal of flaxseed, removal of fish oil, or supplementation of a flaxseed/fish oil diet with vitamin B6 would improve egg quality.

MATERIALS AND METHODS

Animal care and treatments: All diets were formulated using a proprietary omega-3 diet based on the guidelines of Van Elswyk *et al.* (1997). Diet A represented a “standard n-3 PUFA” diet consisting of 8-10% flaxseed and 1-2% fish oil. Diet B consisted of the same standard n-3 PUFA diet supplemented with 10x the recommended level of vitamin B6 (pyridoxine). Diet C consisted of the standard n-3 PUFA diet with the fish oil removed and Diet D was a conventional milo ration. Four diets were fed to laying hens for 4 to 5 wks to achieve target nutrient levels prior to egg collection.

Table 1: Description of dietary treatment groups

Diet group	Diet fed
Diet A	Standard omega-3 ration (8-10% flaxseed and 1-2% fish oil)
Diet B	Standard omega-3 ration supplemented with 10x vitamin B6
Diet C	Standard omega-3 ration without fish oil
Diet D	Conventional milo ration

Sample collection: Birds 58 weeks of age were housed at a commercial layer facility for 6 weeks. The birds were cared for by the farm operator in accordance with company guidelines. Eggs were shipped and refrigerated for 3 wks at 4°C to simulate consumer storage conditions. There were 24 eggs per diet collected for each shipment, except in the last shipment where 48 eggs were collected and shipped per treatment due to the number of broken eggs in the first two shipments. The total sample size (n) for each shipment was n = 71 (shipment 1), n = 66 (shipment 2) and n = 145 (shipment 3). The process of collection, shipping, refrigeration and evaluation was done at three separate time points (shipment 1, shipment 2 and shipment 3) and sample sizes were slightly reduced due to accidental breakage.

Egg quality analysis: Parameters used to evaluate egg quality were: whole egg weight, yolk weight, Vitelline Membrane Strength (VMS) and albumen thickness. Whole egg and yolk weight were measured in grams using an electronic scale (Ohaus Champ, Fisher Scientific, Pittsburgh, PA). Vitelline membrane strength was quantified with an Instron machine, (Model 1011, Instron Corporation, Norwood, MA) which measured the force required to break the vitelline membrane (Strong, 1989). Albumen thickness was presented as Haugh units, which is an industry accepted method that uses a mathematical relationship between egg weight and albumen height (Haugh, 1973). After the eggs were stored for 3 wks, each egg was weighed and broken onto a level plate. The albumen height of the intact egg was measured. The albumen was removed by rolling the yolk on a wet paper towel. The intact yolk was placed in a 70 mm Pyrex crystallizing dish (Pyrex, Fisher Scientific, Pittsburgh, PA) with 70 mm filter paper lining the bottom to prevent rolling. The dish was placed on a tared scale and the yolk was weighed to the nearest 0.1 g (Kelley, 2003). After separating the yolk from the albumen, the Instron machine measured the force required to break the vitelline membrane. Rupture strength was taken using a compression anvil and Instron with the compression cell at 50 mm/min cross head speed, a 500 g load range and a 5 kg load cell. The rupture strength in kg was divided by the weight in grams of the yolk to give a final force calculation (Kelley, 2003).

Statistical software was used to analyze both the differences in the dietary treatments and time on egg quality (SPSS, Chicago, IL). An ANOVA procedure was used to analyze how each egg quality parameter was affected by dietary treatment for all shipments and then dietary treatment versus time. Means were significant at $p \leq 0.05$ and were separated using a Duncan’s multiple range test.

RESULTS AND DISCUSSION

Whole egg weight: There were no significant differences between shipment whole egg weights. Diets B and D significantly increased whole egg weights. Vitamin B6 supplementation in diet B may have had an impact on whole egg weight and countered any vitamin B6 antagonism that occurred in Diet A, which had decreased whole egg weight. The B6 supplementation yielded egg weights comparable to the conventional milo ration, which could indicate that there was vitamin antagonism occurring in the two diets with n-3 PUFA but without B6 supplementation. Diet C had significantly greater egg weights compared Diet A, which suggests the flaxseed diet without fish oil increased egg weights. Although there may be vitamin antagonism occurring from the use of flaxseed, the flaxseed alone increased egg weights compared to the flaxseed and fish oil combination diet.

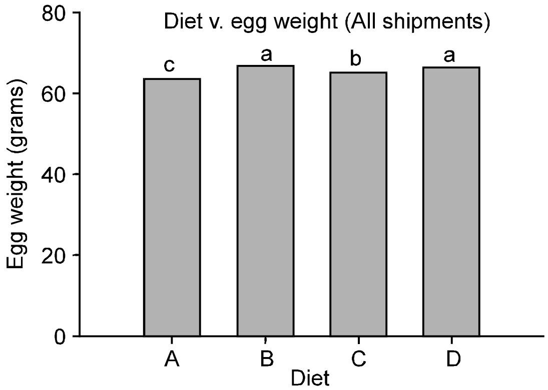

Fig. 1: Comparison of whole egg weights (grams) between dietary treatments for all shipments. [a-c]Means with different superscripts differ significantly ($p \leq 0.05$).

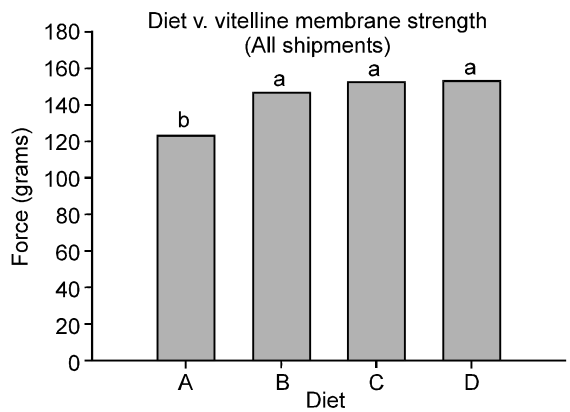

Fig. 2: Comparison of vitelline membrane strength (grams of force) between dietary treatments for all shipments.
[a-b]Means with different superscripts differ significantly ($p \leq 0.05$).

Yolk weight: Although depression of percentage yolk in response to dietary n-3 PUFA was noted by Scheideler and Froning, there were no significant differences between the shipments or diets in yolk weights in this study (1996).

Haugh units: Shipment 2 had the highest Haugh units, while shipment 3 had the lowest Haugh units (Table 2). We expected shipment 1 to have the thickest albumen, then for the albumen thickness to decrease over time. There were no significant differences in Haugh units between the diets, but there was a significant difference between shipments (Table 2). The lack of effect from the changes in diet on Haugh units is consistent with the findings of Jiang *et al.* (1991), who studied the effects of albumen thickness from feeding high amounts of linolenic acid. The data could indicate that diet, with or without additional n-3 PUFA, has little effect on albumen thickness, but age does have an effect.

Table 2: Comparison of measurements versus shipment or diet

Diet	Egg weight	Yolk weight	Force	Haugh units
A	63.56^{c}	18.37	123.31^{b}	70.18
B	66.60^{a}	18.93	146.70^{a}	70.62
C	64.99^{b}	18.40	152.69^{a}	70.54
D	66.40^{a}	18.75	153.52^{a}	69.82
Shipment				
1	64.77	18.49	150.59	71.70^{b}
2	65.49	18.58	154.74	74.98^{a}
3	65.93	18.75	138.45	67.42^{c}

[a-c]Means with different superscripts differ significantly ($p \leq 0.05$).

Vitelline membrane strength: There were no significant differences in the force required to break egg yolks between the shipments. Diet A eggs required significantly less force from the Instron machine, while the other diets were not different. The poorer performance of diet A eggs supports the previous conclusion that although there may be vitamin antagonism occurring from the use of flaxseed, diet C still yields better quality yolk strength and egg weights than diet A.

Conclusion and applications:

- The data suggests that the addition of flaxseed and fish oil in a laying hen ration can reduce vitelline membrane strength, egg weight and yolk weight.
- The addition of vitamin B6 to a flaxseed diet may counteract linatine antagonism, increasing VMS, egg weight and yolk weights.
- The application of these recommendations may significantly enhance omega-3 egg quality and extend the laying hen's production cycle, resulting in significant cost savings to the producer.

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