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# Feeding Broccoli Floret Residues on Layers: II. Effects on Fatty Acid Deposition

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# Abstract

A study was conducted to determine the effects of feeding Dried Broccoli Florets (DBF) to layers on egg yolk fatty acid deposition. Seventy-two layers were randomly allotted to four dietary treatments (six cage replicates with three hens each) and fed diets containing 0, 4, 8, and 12% DBF for 56 days. Results showed that inclusion of DBF decreased (linear effect, P < 0.001) concentrations of Saturated Fatty Acids (SFA) and increased (linear effect, P < 0.001) concentration of palmitic acid decreased (linear effect, P < 0.001) while linoleic (quadratic effect, P = 0.003) and linolenic (linear effect, P < 0.001) acid concentrations increased as the level of DBF in the diet increased. It was concluded that feeding DBF at 12% of the diet increased PUFA concentrations and decreased those of SFA.

# Introduction

Broccoli (Brassica oleracea L. var. italica) is an important vegetable crop in Canada with an annual production of 32,000 tons. As with other vegetables, large amounts of broccoli wastes are generated during harvest, packaging and marketing. It has been estimated that about 40 to 50% of total broccoli produced is discarded during processing as a result of the high standards imposed by consumers and retailers, and due to consumer refusal at the retail level. Additional losses occur in the field, generating large quantities of florets, stems and leaves as crop residues. As broccoli production increases, there is a concomitant increase in the quantity of residues produced. These residues are often discarded into the environment where they pose major environmental concerns. There is growing interest in developing new feeds from waste vegetable such as broccoli by-products to replace conventional feeds. Recent research showed that broccoli residues such as broccoli leaves and stems and broccoli florets can be incorporated in layer diets to substitute conventional feeds such as soybean meal. Incorporation of dried broccoli leaves and stems up to 9% of the diets had no effect on egg production, but significantly improved egg quality with higher yolk xanthophyll and lower yolk cholesterol concentrations [1], In more recent study, [2] reported that feeding Dried Broccoli Florets (DBF) up to 12% of the diet had no negative effects on feed intake, egg production and feed efficiency and improve egg yolk color and a-tocopherol. Chemical analysis of different broccoli parts showed that florets contained higher CP (22.4%) but lower crude fiber (11.7%) concentrations than broccoli leaves and stems [3]. Broccoli florets are also a rich source of poly-unsaturated fatty acids which constitute 62% of the total fatty acids [3]. Inclusion of feeds rich in PUFA has been shown to increase n-3 PUFA deposition in egg yolks without compromising egg production [4-6]. To the best of our knowledge, no studies have investigated the effects of dried broccoli floret (DBF) residues on egg yolk fatty acid profile.

# **Materials and Methods**

#### Birds and housing

All animal procedures were approved by the Animal Care Committee of the Faculty of Agricultural and Environmental Sciences of McGill University. This study was part of larger study in evaluating the effects of feeding DBF on layer performance and total tract nutrient retention [2]. Preparation, processing and chemical composition were reported [2]. A Total of 72 (64-week-old) White Leghorn laying hens were weighed and placed in 24 cages (3 birds/cage) with six cage replicates. Each cage representing one replicate, was assigned to one of four experimental diets containing 0, 4, 8 and 12% DBF for 56 days. Dried broccoli florets partially replaced corn and soy bean meal (Table 2). All diets were formulated to be iso-caloric and iso-nitrogenous according to [7] and were offered in a mash form. Feed and water were provided ad libitum. Birds. Birds received equal daily lighting time (16L: 8D) at constant room temperature.

#### Sample collection

Feed intake was measured by-weekly. Two eggs from each cage replicates were collected at random, cracked, and yolks were separated from the whites. Samples of pooled egg yolks (2 eggs/cage replicates/ treatment) for each treatment were collected at week 2, 4, 6, and 8 of the experiment (n = 48). The yolk samples were frozen at -20 °C, freeze dried, and finely ground before fatty acid analysis. Methyl esters of fatty acids were prepared from yolk, feed, and DBF samples according to [8]. Acid composition of methyl esters was determined by gas chromatography as described by [6]

#### **Statistical Analysis**

Data were analyzed using the PROC MIXED procedure [9] with the following model:

 $Yijk = \mu + Ti + Cij + eijk$ 

Where:  $Y_{iik}$  = observation,  $\mu$  = overall mean,

 $T_i$  = fixed effect of i<sup>th</sup> treatment (i= 1, 2, 3 or 4),

 $C_{ij}$  = random effect of j<sup>th</sup> cage within i<sup>th</sup> treatment (j = 1, 2, 3, 4, 5 or 6),

 $e_{iik}$  = residual error (k = 1 or 2),

 $e_{iik} \sim N (0, \sigma 2e).$ 

The least significant difference method was used to identify statistically different means (P < 0.05). Orthogonal contrasts were used to test for linear and quadratic effects of adding DBF to the diet. The least square mean method was used to identify differences among treatment means and statistical differences were declared at p < 0.05.

Data were analyzed by one-way ANOVA using the GLM procedure [9] with cages as experimental units. Least significant difference method was used to identify statistically different means (P < 0.05). Orthogonal contrasts were used to test for linear and quadratic effects of adding DBF to the diet.

#### Results

Linolenic acid (C18: 3n3) was the most abundant fatty acid followed by palmitic and oleic acid, respectively (Table 2). Dietary C18: 3n3 increased by 8.2, 24.5, and 61.2% as BDF increased by 4, 8, and 12%, respectively (Table 1).

Saturated (SFA) and mono-unsaturated (MUFA) fatty acids of egg yolk decreased (linear effect, P < 0.0001) with increasing dietary DBF (Table 5). The reductions in SFA and MUFA were mainly due to the declines in palmitic (quadratic effect, P = 0.0838) and oleic (linear effect, P < 0.0001) acids, respectively. In contrast, poly-unsaturated fatty acid (PUFA) concentrations in egg yolk increased (linear effect, P < 0.0001) as the level of dietary DBF increased.

Consequently, SFA: PUFA ratio decreased (linear effect, P < 0.0001) in yolks produced by layers fed DBF. As the level of dietary DBF increased, yolk linolenic acid content (quadratic effect, P = 0.011) with highest concentrations being achieved for layers fed 8 and 12% DBF diets. A similar increase in yolk linoleic (linear effect, P < 0.0001) content was also observed as the level of dietary DBF increased.

#### Discussion

Linolenic acid concentration of DBF constitutes 35.4% of the total fatty acids, which is consistent with the values reported for broccoli florets [9,10], The Fatty acid profile of egg yolk reflected the dietary fatty acid composition. The increase in PUFA and linolenic acid concentrations is likely due to the high levels of linolenic acid concentration in DBF-based diets (Table 1) and DBF (Table 2). Inclusion of DBF at 4, 8, and 12% of the diet were accompanied with significant increases (i.e. 23.3, 66.3, and 68.6%, respectively) in egg yolk deposition of linolenic acid when compared with the control diet. Feeding layers diets rich in linolenic acid such as cabbage residues [11], pasture [12], and flaxseed [4, 6] has been successfully used to increase the concentration of linolenic acid as well as other health promoting fatty acids in egg yolk. The lower egg yolk SFA and MUFA

concentrations produced by layers fed DBF diets can be attributed to their lower concentrations in DBF diets and \ or the inhibitory effects of PUFA. It is well documented that PUFA inhibit the activity of  $^{9}\Delta$  desaturase which is involved in MUFA synthesis [13, 14].

#### Conclusions

It was concluded that incorporation of DBF in layer diets reduced egg yolk concentrations of SFA and increased those of PUFA. Greater deposition of omega-3 fatty acids (e.g. C18: 3n3) can be achieved by 6 or 9% DBF.

Table 1. Ingredients and	chemical	composition	of dietary	treatments
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	Broccoli fi	Broccoli floret residue inclusion (%)				
	0.0	4.0	8.0	12.0		
Ingredients (%)						
Corn	53.68	51.64	49.61	47.57		
Soybean	31.47	29.10	26.73	24.37		
Dried broccoli floret residues	0.0	4.00	8.00	12.00		
Limestone	10.31	10.29	10.27	10.25		
Soybean oil	2.18	2.61	3.04	3.47		
Mono-calcium phosphate	1.25	1.22	1.20	1.17		
Mineral-vitamin mix1	0.50	0.50	0.50	0.50		
Salt	0.27	0.28	0.29	0.30		
Choline chloride	0.10	0.10	0.10	0.10		
Sodium carbonate	0.08	0.08	0.08	0.08		
Methionine	0.01	0.02	0.03	0.04		
Calculated analysis						
Metabolizable energy (kcal/ kg)	2775.00	2775.00	2775.00	2775.00		
Crude protein (%)	19.00	19.00	19.00	19.00		
Total lysine, (%)	1.10	1.10	1.10	1.10		
Total methionine (%)	0.30	0.30	0.30	0.30		
Total Ca (%)	4.30	4.30	4.30	3.00		
Total P (%)	0.60	0.60	0.60	0.60		
Analyzed fatty acids (% of fatty acids)						
C16: 0	13.4	12.4	12.5	12.5		
C16: 1	0.1	0.1	0.1	0.1		
C18: 0	2.5	2.5	2.6	3.0		
C18: 1	21.2	21.6	20.9	18.8		
C18: 2	54.9	54.9	54.1	53.6		
C18: 3	4.9	5.3	6.1	7.9		
C20: 4	0.2	0.7	0.7	0.9		
C22: 6	0.1	0.3	0.3	0.3		

<sup>1</sup>Composition of premix: Vitamin A 11,530 IU/kg; Vitamin D 2,400 IU/kg; Vitamin E 74.168 IU/kg; Cu 24mg/kg; Fe 200mg/kg; Mg 122mg/kg; Se 0.38mg/kg; Zn 131mg/kg; Co 0.46mg/kg; F 19mg/kg; I 0.80mg/kg.

Parameters	%
Fatty acids (% of fatty acids)	
C12: 0	0.2
C14: 0	5.4
C15: 0	0.3
C16: 0	22.8
C16: 1	3.6
C18: 0	2.1
C18: 1	17.6
C18: 2	15.1
C18: 3	35.4
C20: 0	0.5
C22: 0	10.3
C24: 0	0.5
Fatty acids (%)	1.8

Table 2. Chemical composition of broccoli floret residues

Table 3. Effects of broccoli floret residue inclusion on egg yolk fatty acid composition (% of fatty acids)<sup>1</sup>

		Broccoli floret residue inclusion (%)				Inclusion effect	
	0.0	4.0	8.0	12.0	SEM	L <sup>2</sup>	Q3
Saturated fatty acids							
C14: 0	0.26ª	0.26ª	0.24 <sup>b</sup>	0.24 <sup>b</sup>	0.005	0.005	< 0.001
C16: 0	27.21ª	26.65 <sup>b</sup>	26. <sup>06c</sup>	26.01°	0.143	<0.001	0.084
C18: 0	8.41	8.48	8.12	8.36	0.134	0.507	0.779
Mono-unsaturated fatty a	cids						
C16: 1	2.55	2.35	2.11	2.14	0.12	0.423	0.633
C18: 1	38.76ª	38.65ª	36.13 <sup>b</sup>	35.59 <sup>b</sup>	0.282	<0.001	0.412
Poly-unsaturated fatty act	ids			•		·	
C18: 2n-6	17.50 <sup>b</sup>	18.74 <sup>b</sup>	22.27ª	22.03ª	0.48	<0.001	0.138
C18: 3n-3	0.86°	1.06 <sup>b</sup>	1.43ª	1.45ª	0.033	< 0.001	0.011
C20: 2n-6	0.18	0.16	0.20	0.19	0.03	0.004	0.199
C20: 3n-6	0.18	0.17	0.19	0.19	0.004	0.154	0.536
C22: 1	1.89 <sup>ab</sup>	1.87 <sup>ab</sup>	1.80 <sup>b</sup>	2.00ª	0.046	0.216	0.020
C22: 6n3	1.07°	1.19 <sup>b</sup>	1.20 <sup>ab</sup>	1.28ª	0.024	< 0.001	0.411
C24: 1	0.11	0.10	0.11	0.11	0.002	0.247	0.046
SFA <sup>4</sup>	35.87ª	35.40ª	34.50 <sup>b</sup>	34.61 <sup>b</sup>	0.175	<0.001	0.190
MUFA <sup>5</sup>	43.20	42.86	40.04	39.73	0.294	<0.001	0.985
PUFA <sup>6</sup>	20.61 <sup>b</sup>	21.41 <sup>b</sup>	25.39ª	25.26ª	0.314	<0.001	0.165
SFA: PUFA	1.75ª	1.66ª	1.36 <sup>b</sup>	1.38 <sup>b</sup>	0.025	< 0.0001	0.0675

<sup>a-c</sup>Means in the same row with different superscripts are different.

<sup>1</sup>The values are means of 6 replicate cages

<sup>2</sup>L: Linear effect

<sup>3</sup>Q: Quadratic effect

<sup>4</sup>SFA<sup>3</sup>: Saturated fatty acids

<sup>5</sup>MUFA: Mono-unsaturated fatty acids

<sup>6</sup>PUFA: Poly-unsaturated fatty acids

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