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The Effect of Different Levels of Tomato Pomace with or without Multi–Enzyme on Performance and Egg Traits of Laying Hens

Research Article

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ABSTRACT

This experiment was designed to evaluate the effects of different levels of tomato pomace (DTP) with different multi enzymes on the performance and egg quality of laying hens at the first phase of production. In this experiment 432 hens were used in a completely randomized design in a factorial arrangement of 4×4, with four levels of tomato pomace (0, 4, 8 and 12%) and different enzymes (without enzyme, Natozyme, Rovabio and Kemin) with three replicates of 12 laying hens in each replication. These rations were fed for a 12-wk period by hens (Hy-Line W-36) beginning at 33 wk of age. The results showed that the inclusion of DTP significantly affected egg weight (g), egg production (%) and difference between means of treatments were significant ($P<0.05$). Egg mass (g), feed intake (g), feed conversion ratio (kg/kg) and egg quality traits (specific gravity, yolk Index, Eggshell thickness (mm) shell weight (g), Haugh unit) were not affected with DTP and different enzymes. There were no significant interactions between the DTP and enzyme supplementation on egg production and egg quality. There was a significant improvement in the egg yolk color in treatments containing DTP. The inclusion of DTP, resulted in a decrease of cholesterol in the serum ($P<0.05$). Moreover, the inclusion of DTP had no effect on yolk cholesterol. A suggested maximum inclusion level of 8% TP, based on these data can be used in commercial diets, but it is speculated that the incorporation of multi enzyme was not effective on improvement of DTP effects.

KEY WORDS enzyme, laying hens, performance, tomato pomace.

INTRODUCTION

Tomato pomace is a by-product of tomatoes processed into various products and consists of peels, cores, culls, trimmings, seeds, liquor, and unprocessed green tomatoes picked by harvest machinery. Tomato pomace is known for its value as a protein source in poultry diets but can have a medium degree of variability in its nutritional content. This variability is found not only in nutrients content such as protein and amino acid composition but also among the anti-nutritional factors in poultry such as phytic acid and non-starch polysaccharides.

Iran produces up to 4.2×10^6 tones of fresh tomatoes annually (FAO, 2006), most of which are used to process in tomato cannery factories, producing a considerable amount of wet tomato pomace as a by-product. Using tomato pomace to formulate more economic diets showed proper performance results in laying hens (Persia *et al.* 2003). Dried tomato pomace was fed to laying hens at an inclusion rate of 12%, which resulted in similar egg production, feed efficiency, egg weight and shell thickness compared with the hens fed upon a corn-soybean meal based diet (Dotas *et al.* 1999). Yannakopoulos *et al.* (1992) reported that the inclusion of 8 or 15% tomato meal in diets of laying hens had no negative effects on hen weight, egg number, shell quality, egg shape index, feed consumption and mortality, and yolk color score was significantly increased.

Supplementing diets with endo-acting polysaccharide hydrolases can decrease the degree of polymerization of the recalcitrant NSP, leading to a considerable reduction in digesta viscosities (Bedford and Classen, 1992). In addition, the products of cellulase and xylanase activities are more prone to fermentation by the microbial organisms that colonize the last compartments of the gastrointestinal tract, and consequently more energy is absorbed from the hydrolysis of NSP (Bedford and Apajalahti, 2001). Finally, breakdown of plant cell wall polysaccharides improves the access of the digestive biocatalysts to the endosperm contents that were, otherwise trapped (Chesson, 1993). The various effects of enzyme supplementation in the digestive process are usually reflected by a considerable improvement of growth and feed conversion rates by poultry (Hesselman and Aman, 1986; Cambell *et al.* 1989; Pettersson and Aman, 1989; Choct *et al.* 1996), although, the mechanism of action of feed enzymes is not fully understood. It should be remembered that all multi enzymes products are not equal. Multi enzymes differ in the source from which they are derived. They may differ in kinds of enzymes, activity and characteristics such as optimum pH, thermo stability, and ability to resist hydrolysis within the digestive tract. For example, any difference in these characteristics will affect the ability of the enzymes to function effectively and consistently within the digestive tract (Onyango *et al.* 2005). Therefore, commercial multi enzymes must be tested in vivo to ensure efficacy before they are introduced to the mono gastric feed market.

However, DTP is usually used at very low levels in poultry nutrition, especially due to its high fiber and low energy contents. Several attempts have been made to increase the nutritional value of DTP by adding enzymes, either before or after processing (Squires *et al.* 1992). Tomato pomace could be a valuable feed ingredient for poultry if the effects of its anti nutritional agents could be declined. There are several commercial multi-enzyme products in market. Although, tomato by-products have been evaluated in poultry production to a limited extent, little work has been carried out to determine the effects of various commercial Multi enzymes and varying levels of tomato pomace on layer hen performance and egg traits.

MATERIALS AND METHODS

Experimental Design and Diets

Tomato pomace was obtained from a commercial processor (Urmia, Iran). Then it was dried up at 65 ºC to achieve 90% dry matter content, and grounded by a Hammermill (Table 1).

Table 1 Chemical composition of tomato pomace* (mean±SD)

Ingredient	Mean (%)
Dry matter	90±0.5
Crude protein	19.68±0.35
Crude fiber	29.75±0.54
Ether extract	8.1±0.13
Ash	4.72±0.21
Nitrogen free extract	27.75±0.72
Calcium	0.42±0.03
Phosphorus	0.31±0.013
MEn (Mcal/kg)	1730±55

*Tomato pomace analyzed according to AOAC(1990) (n=4).
Metabolizable value was taken from NRC (1971).

In this experiment, four rations were formulated to produce various levels of tomato pomace (0, 4, 8 and 12%). Each ration was supplemented with or without multi-enzyme (Kemin, Natozyme and Rovabio) and fed to layer hens (Hy-Line W-36) in a factorial arrangement with a completely randomized design. The first factor was tomato pomace with 4 levels (0, 4, 8, and 12%) and second factor was enzyme with 4 levels (control, Kemin, Natozyme and Rovabio) resulting in a total of 16 experimental treatments (Table 2).

Table 2 Enzyme supplementation used in layer maintained for 12 weeks*

Enzyme Inclusion	% of diet
Diet 1, 2, 3 and 4: No enzyme	0
Diet 5, 6, 7 and 8:+Kemin	0.05
Diet 9, 10, 11 and 12:+Natozyme	0.05
Diet 13, 14, 15 and 16:+Rovabio	0.05

*All diets in a row showing in Table 2 including 0, 4, 8 and 12% DTP,respectively supplemented with three of multi-enzymes or without enzyme.

Each treatment was assigned to 9 replicate cages with 4 hens per cage.

The composition of experimental diets and calculated analysis are outlined in Table 3. The control diet was formulated to meet nutrient requirements recommended by the Hy-line W-36 management guide (Anonymous, 1998). Diets were formulated to be isocaloric, and isonitrogenous and equal calculated essential amino acids, calcium, and phosphorus. Hens were fed the experimental diet for a 12 wk period beginning at 33 wk of age and continuing to 45 wk of age.

The supplements used in this experiment, Natozyme, Rovabio and Kemin, were microbial multi enzymes and was incorporated into the feed formulations at a rate of 0.05%. The components of these enzymes are shown in Table 4. Rearing program was accomplished accordance to Hyline manual recommendations. Hens were exposed to a daily lighting schedule of 16 lx:8 d. All birds were kept under the same environmental conditions throughout the experimental period. Diets were presented in mash form and provided daily according to expected intake and hens had free access to water.

Measurements

Egg production (EP) was determined each day and was calculated on a hen day basis. Hens were given ad-libitum access to 105 g of feed per hen each day; unconsumed feed was measured each morning. Feed conversion (FC) was calculated as the ratio of grams of feed to grams of egg produced per hen-day.

Eggs were saved 1 d each week to measure egg weight. Egg mass was calculated as a factor of egg weight and hen-day EP. Three eggs were randomly chosen in each replicate from the eggs laid during the three consecutive days at every 28-day period to determine eggshell weight, specific gravity (Densitometer, Mettler-Toledo, Iso-14001, Switzerland), and shell thickness. The shell thickness was measured at three different locations (middle, broad and narrow end) using a micrometer gauge (Mitutoyo code, 7027), and the mean value was calculated. During each 28 day period, three eggs were randomly selected from each replicate for two consecutive days to determine the Haugh unit. The Haugh unit was calculated using the following formula:

$$\text{Haugh unit} = 100 \log H_A + 7.57 - 1.7W_E^{\,0.37}$$

Where, H_A is albumen height and W_E is egg weight (Doyon *et al.* 1986).

Yolk color was measured biweekly on one egg per cage (20 eggs/treatment) using a Roche color fan (Vuilleumier, 1969). Blood samples were collected from the wing vein of all the birds at the end of the experiment, serum was separated and used for biochemical assays. Serum cholesterol was measured using commercial kits on an auto-analyzer (Technicon RA-1000). Yolk cholesterol was extracted by the method of Folch *et al.* (1956) as modified by Washburn and Nix (1974) from 2 eggs from each replicate. Yolk cholesterol was estimated by the colorimetric Libermann-Burchard method.

Statistical analysis

Data were analyzed by the General Linear models procedure of SAS Institute (2001). Means for treatments showing significant differences in the analysis of variance were compared using Duncan's multiple range tests. All statements of significance are based on the probability level of 0.05. The general linear model (GLM) was used to determine the main effects of factors and any possible interactions between factors.

RESULTS AND DISCUSSION

The primary goal of this research was to determine the optimal concentrations of dried tomato Pomace (DTP) in laying hen diets and monitor the effects of commercial sources of multi-enzymes in diets containing DTP. The results of performance traits are presented in Table 5. The improvement in egg production observed in diets containing 4 and 8% of DTP in comparison to control diet (P<0.05). Enzyme supplementation and interactions of DTP and enzymes did not affect the egg production. One possible explanation for this observation might be the variable concentrations of NSP in by-products originated from different sources. It seems that another factor affecting production is the presence of polyunsaturated fatty acids and antioxidant properties of DTP (ideal source of vitamin E, A and lycopene). The reason of decline in egg production as a result of increasing DTP was related to the value of fiber in these diets and consequently reduction in digestibility of nutrients. In Comparison with our finding in other studies (Nobakht and Safamehr, 2007; Petrenko *et al.* 1984; Tomezynki and Soska, 1976), the inclusion of DTP increased egg production (P<0.05). In contrast, Dotas *et al.* (1999) and Yannakopoulos *et al.* (1992) found a similar result in egg production when DTP was included by 15% in diets. The reason of this discrepancy in results could primarily be due to different tomato cultivars, growing conditions, value of crude fiber, and processing methods (i.e. the amount of seeds, pulp, and skins, pulp and leaves in wet pomace in the waste by-product), or age of hens and the composition of basal diet (Persia *et al.* 2003; King and Zeidler, 2004). Moreover, it is also possible that the effects of enzyme supplementation in poultry nutrition are less evident in older birds, which is the case when exploiting laying hens for egg production. The data presented here suggests that exogenous enzymes were unable to reduce the anti nutritive properties associated with the incorporation of DTP in laying hen diets. A similar effect has been reported for diets containing alfalfa in broiler chicks diets (Ponte *et al.* 2004). Compared with control group, egg production increased by inclusion of 4 and 8% of DTP in diets, while the difference between 12% with other treatments were not significant. These results did not confirm the previous research in laying hens (Yannakopoulos *et al.* 1992) where 15% DTP showed no significant reduction in hen weight gain, number of eggs laid, feed consumption, feed efficiency, and mean egg weight. Another study (Petrenko and Banina, 1984) has shown that tomato pulp at an inclusion rate of 5% resulted in increase in egg production (P<0.05). There was no effect of enzyme supplementation on egg production that is in ag-

Table 3 Composition of laying hen diets (as fed basis)

	% Tomato pomace			
Ingredient (%)	0	4	8	12
Corn	69.00	64.40	60.74	56.83
Soybean meal (44% cp)	21.29	20.50	19.47	18.52
Tomato pomace	-	4.00	8.00	12.00
Poultry fat	0.10	1.34	2.34	3.25
Bone meal	0.70	0.76	0.74	0.80
Oyster shell meal	7.16	7.12	7.10	7.05
Dicalcium phosphate	0.69	0.60	0.60	0.55
Salt	0.30	0.30	0.30	0.31
Vitamin and mineral premix	0.50	0.50	0.50	0.50
Dl-Methionine	0.09	0.10	0.12	0.13
l-lysine Hcl	0.12	0.08	0.04	0.01
Calculated composition				
AME_n (kcal/kg)	2800	2800	2800	2800
CP (%)	15.6	15.6	15.6	15.6
Calcium (%)	3.15	3.15	3.15	3.15
Available Phosphorus (%)	0.34	0.34	0.34	0.34
Methionin (%)	0.44	0.44	0.44	0.44
Methionin+Systein (%)	0.6	0.6	0.6	0.6
Lysine (%)	0.83	0.83	0.83	0.83
Sodium (%)	0.15	0.15	0.15	0.15
Chloride (%)	0.23	0.23	0.23	0.23

[1]Dry matter content 900 g/kg.[2]Premix supplied per kg of diet: 9000 IU vitamin A, 1.78 mg vitamin B_1, 6.6 mg vitamin B_2, 30 mg niacin, 10 mg pantothenic acid, 3 mg vitamin B_6, 0.15 mg biotin, 1500 mg choline, 0.015 mg vitamin B_{12}, 2000 IU vitamin D, 18 IU vitamin E, 2 mg vitamin K_3, 10 mg Cu, 0.99 mg I, 50 mg Fe, 100 mg Mn. 0.08 mg Se, 100 mg Zn.[3]All values were calculated from NRC (1994) values.

reement with Bedford and Schulze (1998), who found that enzyme supplementation to diets result in similar egg production in comparison to hens fed by a control diet. It is possible that the inherent complexity of tomato pomace plant cell wall affects the function of β-glucanases and β-1, 4-xylanases acting on more recalcitrant and complex polysaccharides, when compared with the usually targeted arabinoxylans and β- glucans in cereal based diets. Indeed, in cereal-based diets, the improvement of animal performance does not depend on the release of reducing sugars but rather on the reduction of the viscosity of the intestinal contents (Bedford and Classen, 1992; Pawlik *et al.* 1990).

Tomato pomace is introduced as palatable feed in poultry diets (Ammerman *et al.* 1965). Knoblich *et al.* (2005) reported that the use of DTP by-products enhance appetite in laying hens. The increase of feed intake in groups containing DTP is concurrent with these findings. It is possibly due to improvement in amino acid balance, increase in biological value of protein and much lysine in DTP that results in an improvement of egg production (Giesman, 1981). The highest feed intake observed in layers fed diets with 12% DTP. The feed intake was not affected by enzyme supplementation, regardless of the source. The interaction between enzymes and DTP on feed intake was significant ($P<0.05$). The increase in feed intake due to inclusion of DTP in diets probably is associated with adaptation response of birds to dilution of diet. This is in agreement with our earlier findings (Nobakht and Safamehr, 2007) and the results of other authors (Jafari and Pirmohammadi, 2006).

However, some authors have reported that the inclusion of DTP in diets did not affect feed intake (Dotas *et al.* 1999; Al-Betawi, 2005).

The dietary enzyme supplementation did not result in significant difference in egg production, which is in agreement with the studies of Bedford and Schulze (1998). In contrast, Scheideler *et al.* (2005) reported an increase in egg production following enzyme supplementation (Avizyme).

The effect of DTP and interaction of levels of DTP and Enzymes on egg weight was significant ($P<0.05$). Birds fed diets with 12% of DTP had the highest egg weight. The higher feed intake is correlated with increase in protein intake and also DTP is richer in lysine (Gisman *et al.* 1981),

Table 4 Components of different mixed enzymes

Enzymes	Kimin-K	Natozyme-N	Rovabio-R
Protease	*	*	
Amylase	*	*	
Cellulase	*	*	*
Pectenase		*	*
Glucanase	*	*	*
Xylanase		*	*
Hemicellulase		*	
Lipase	*	*	
Phytase		*	

* Represents the presence of enzyme.

Table 5 Performance of laying hens fed tomato pomace with or without enzyme supplementation*

Dietary treatment	Egg weight (g/egg)	Egg production (%)	Egg mass (g/hen/day)	Feed intake (g/hen/day)	FCR (g feed/g egg)
Tomato pomace (%)					
0	59.8^{d}	85.78^{b}	51.3^{b}	104.73^{b}	2.04^{b}
4	60.4^{c}	89.82^{a}	54.24^{a}	105.64^{bc}	1.95^{d}
8	61.02^{b}	89.0^{a}	54.3^{a}	108.84^{c}	2.004^{c}
12	61.91^{a}	87.48^{ab}	54.15^{a}	116.58^{a}	2.15^{a}
pooled SEM	0.143	0.857	0.471	0.665	0.008
P value	*	*	*	*	*
Enzyme (E)					
Without E	60.67	86.84	52.68	108.14	2.052
Kemin (K)	60.8	87.93	53.46	108.61	2.033
Natozyme (N)	60.83	88.78	53.99	109.24	2.033
Rovabio (R)	60.84	88.54	53.85	109.78	2.038
SEM	0.143	0.857	0.471	0.665	0.008
Interaction (E×DTP)					
WE×WDTP	59.67^{b}	84.22	50.24	103.75^{a}	2.064^{bc}
K×WDTP	59.78^{b}	85.84	51.31	104.42^{a}	2.035^{bcd}
N×WDTP	59.88^{b}	86.57	51.83	105.01^{a}	2.029^{cbe}
R×WDTP	59.88^{b}	86.51	51.8	105.34^{a}	2.036^{c-g}
WE×%4 DTP	60.42^{bcd}	88.08	53.21	104.4^{a}	1.96^{d-h}
K×%4 DTP	60.43^{bcd}	89.28	53.94	104.9^{a}	1.94^{fh}
N×%4 DTP	60.36^{bd}	91.11	54.93	105.7^{a}	1.93^{h}
R×%4 DTP	60.41^{bcd}	90.82	54.85	107.52^{a}	1.96^{d-h}
WE×%8 DTP	60.86^{abcd}	88.61	53.91	108.82^{a}	2.018^{c-f}
K×%8 DTP	61.09^{abcd}	88.43	54.13	108.82^{a}	2.01^{c-h}
N×%8 DTP	61.11^{abcd}	89.2	54.5	108.61^{a}	1.99^{c-h}
R×%8 DTP	61.01^{a-d}	89.76	54.75	109.41^{a}	1.998^{c-h}
WE×%12 DTP	61.73^{ad}	86.45	53.36	115.53^{b}	2.16^{a}
K×%12 DTP	61.9^{ac}	88.15	54.56	116.84^{b}	2.14^{ab}
N×%12 DTP	61.97^{a}	88.26	54.7	117.13^{b}	2.14^{abg}
R×% 12 DTP	62.04^{a}	87.07	53.99	116.61^{b}	2.16^{a}
SEM	0.286	1.71	0.943	1.02	.016
P Value	<0.05	NS	NS	<0.05	<0.05

*The means that have at least one common letter in each column, do not have significant difference (P>0.05).
NS= non significant (P>0.05).
WE: without enzyme.

Table 6 Egg quality of laying hens fed tomato pomace with or without enzyme supplementation

Dietary treatment	Yolk index	Eggshell weight(g)	Eggshell thickness (mm)	Haugh unit	Specific gravit (g)
Tomato pomace (DTP)					
0	40.60	6.30	0.469	102.12	1.086
4	41.63	6.40	0.462	103.03	1.087
8	41.06	6.50	0.49	102.86	1.087
12	41.54	6.34	0.46	102.77	1.085
SEM	0.512	0.20	0.009	0.60	0.001
Enzyme (E)					
Without E	41.48	6.33	0.462	102.50	1.085
Kemin	41.70	6.56	0.476	103.20	1.088
Natozyme	40.62	6.30	0.481	103.25	1.085
Rovabio	41.03	6.35	0.465	101.83	1.085
SEM	0.512	0.20	0.009	0.603	0.001
Interaction (E×DTP)	NS	NS	NS	NS	NS

NS= non significant (P>0.05).

which was reflected in higher egg weight. This finding is in agreement with the results of Nobakht and Safamehr (2007). In contrast, Jafari and Pirmohammadi (2006) reported that DTP inclusion up to 15% in corn-soybean based diets did not affect egg weight. There was no difference in egg weight among enzyme treatments, which is agreement with the results of Brense *et al.* (1993).

The lowest and highest feed conversion ratio observed in 4 and 12% of DTP, respectively, which was also significant (P<0.05) as compared to the control diet (without DTP). No significant difference in feed conversion ratio was observed among the enzyme treatment groups. Feeding the higher dietary fiber in 12% DTP could be explain the highest amount of feed conversion ratio, because authors reported that the main effect of dietary fiber is an increase in viscosity of digesta, high variability of feeding value, and amount of metabolisable energy in feeds (Bedford and clason, 1992). Nobakht and Safamehr (2007) reported that inclusion of DTP up to 7.5% in diets did not affect FCR during the second phase of egg production. However, some outhor reported that the inclusion of DTP in layer diets increased the FCR values concomitant with increase DTP in diets (Dotas *et al.* 1999; Petrenko and Banina. 1984). Um and Paik (1999) found no significant effect of phytase supplementation on FCR in laying hens. In contrast, Simons *et al.* (1992) found that adding 200 FTU/kg of phytase to diets without inorganic Phosphorus sources improved FCR (P<0.05). Birds fed diets with 4% DTP and Natozyme had significantly (P<0.05) lower FCR (1.93), which was higher in 12% DTP without enzyme supplementation. The effect of Natozyme containing phytase on FCR was not significant.

The egg mass was significantly different among treat ments containing DTP, 8% being highest and the control being lowest (Table 5). This variation was similar with average egg weights and egg production, which were higher than the control (without DTP).

The significant increase in egg mass by inclusion of DTP reported in other experiments (Nobakht and Safamehr, 2007). These results are somewhat different from the results of Dotas *et al.* (1999), who reported that adding DTP to diets had no effect on egg mass.

Enzyme supplementation did not significantly increase haugh unit for hens fed different sources of multi-enzymes. Haugh unit was not affected by dietary DTP level (Table 6). Egg specific gravity, shell thickness and also shell weight was not significantly affected by different sources of multi-enzyme and DTP level during this trail (Table 6). This result confirms that total calcium, phosphorus, and vitamin D_3 contents in all experimental groups are the same. And probably, their bio-availability was not affected by dietary DTP level. In the present experiment, the Yolk index was neither affected by the enzyme supplementation nor by the levels of DTP (Table 6). The yolk index mainly was affected by the age of hens, which is in agreement the finding of Dotas *et al.* (1999). Brense *et al.* (1993b) reported that multi-enzymes (0.2 g β-glucanase and 0.4 g pentosonase per kg) supplementation also did not affect egg weight and haugh unit. However, Aimonen and Uusi-Raauva (1991) supplemented laying hens with multi-enzymes (1 g/kg) for 6 months and reported an increase in yolk color, a decrease in shell thickness, and no changes in haugh unit. Other studies (Squires *et al.* 1992; Dotas *et al.* 1999) have shown that tomato pomace or waste can be fed safely to laying hens and broiler chicks. In this experiment, as the level of DTP increased, the yolk color positively increased (P<0.05)

Table 7 Eggshell quality, cholesterol in serum and yolk of laying hens fed tomato pomace with or without enzyme supplementation*

Dietary treatment	Roche color fan (%)	Cholesterol in serum (mg/DL)	Cholesterol in yolk (mg/g)
Tomato pomace (DTP)			
0	2.5[b]	237.41[a]	232.33
4	3.25[b]	194.25[ab]	243.66
8	4.5[a]	202[ab]	231.66
12	4.75[a]	174.25[b]	230
SEM	0.309	14.0	11.4
P value	<0.05	<0.05	NS
Enzyme (E)			
Without E	3.16	207.16	244.83
Kemin	4	174.83	221.66
Natozyme	3.91	212.16	246.66
Rovabio	3.91	213.75	231.5
SEM	0.309	14.006	11.4
Interaction (E×DTP)	NS	NS	NS

*The means that have at least one common letter in each column, do not have significant difference (P>0.05).
NS= non significant (P>0.05).

(Table 7). This observation confirms that the yolks from eggs obtained from diets containing DTP were more deeply pigmented yellow. Dotas *et al.* (1999) and Yannakopoulos *et al.* (1992) reported that tomato by-products may have a positive effect on the yolk color in laying hens. In contrast, Garcia and Gonzales (1984) and Persia *et al.* (2003) have also reported no difference among control and DTP levels. Two studies conducted by Ammerman *et al.* (1965) showed a decrease in skin or shank pigmentation when tomato pulp was substituted for alfalfa meal at 3% in both laying hens and broiler diets. Tomato by-products contain considerable amounts of lutein, β-carotene, cis-β-carotene, and lycopene. A notable increase in yolk color of eggs, as a result of dietary inclusion of DTP in this experiment, was most likely due to the presence of appreciable amounts of carotenoid pigments, particularly lycopene, which is responsible for the red colour of DTP.

As shown in Table 7, there were significant differences in the cholesterol of serum. The inclusion of 12% DTP may be the result of decrease in cholesterol of serum compared to control diet ($P<0.05$). The group without DTP exhibited highest cholesterol in serum. Kavitha *et al.* (2004) reported that the DTP inclusion up to 15% without enzyme supplementation in broiler diets reduces the serum cholesterol.

The effects of crude fiber may be related to its capability to enhance fecal excretion of cholesterol and bile cholesterol (Moundres *et al.* 1997). Akiba and Matsomoto (1982) reported that the fat and cholesterol content of serum were less than in diets with higher fiber. The reason for our finding may be related to higher fiber in diet containing 12% of DTP. Our finding showed that high level of DTP decreased cholesterol, which is in agreement with the finding of Kavithe *et al.* (2004), that reported a reduction in serum and muscle cholesterol contents in broiler fed DTP up to 15% without enzyme supplementation. Also, in another study Meng *et al.* (1974) found that increasing dietary fiber level from 4.1 to 17.7% with cellulose caused a reduction in serum cholesterol and an increase in egg yolk cholesterol.

Although, the present study did not show an effect of DTP levels and enzyme supplementation on yolk cholesterol, Nakao *et al.* (1980) observed less yolk cholesterol in eggs derived from the layers fed diets containing 10% of alfalfa. Finally, the incorporation of DTP levels in the laying hen diets had no significant effects on the cholesterol content of the egg yolk (Table 7). These results do not agree with those of McNaughton (1978), who observed a significant reduction in egg yolk cholesterol by feeding alfalfa meal, although, the levels of incorporation were different from those used in this experiment. Also, Vargas and Naber (1984) studied the effect of increasing dietary fiber in rations of varying nutrient density on egg yolk cholesterol and demonstrated that yolk cholesterol tends to increase when laying pullets consume more than 387 kcal of ME/d or gain more than 100 g in body weight. Cholesterol can be directly obtained from diet, or it can be synthesized de-novo in animal cells from acetate groups of acetyl-coenzyme A. The percentage of cholesterol arising from de novo biosynthesis or from the diet basically depends on the levels of dietary cholesterol, because the de novo pathway is under feedback control by dietary cholesterol. Even when the levels of dietary cholesterol are very low, de novo biosynthesis will enable the production of the cholesterol re-

Iranian Journal of Applied Animal Science (2011) 1(1), 39-47

quired to supply the large variety of biological processes in which this molecule is involved. Bartov *et al.* (1971) concluded that the cholesterol content of egg correlated with level of diet energy. Therefore, the obtained results are due to the same energy level. The maximum inclusion level is suggested to be up to 8% for tomato pomace without enzyme supplementation in standard (commercial) laying hen diets, and enzyme supplementation did not have any significant effect on the studied traits.

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