IJAS Iranian Journal of Applied Animal Science

The Effects of Different Levels of Tomato Pomace and Processing Methods on Production Performance and Blood Metabolites of Native Laying Hens

Research Article

A. Jalalinasab[1], A. Nobakht[2*] and S. Razzaghzadeh[1]

[1] Agriculture Jihad Organization of Urmia, Urmia, Iran
[2] Department of Animal Science, Maragheh Branch, Islamic Azad University, Maragheh, Iran

Received on: 12 Jul 2013
Revised on: 11 Aug 2013
Accepted on: 15 Aug 2013
Online Published on: Jun 2014

*Correspondence E-mail: anobakht20@iau-maragheh.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

In this experiment 180 Azerbaijan province native laying hens from 28 to 40 weeks were separated in 6 treatments and 3 replicates (10 birds per replicate) as a (2 × 3) factorial arrangement of treatments in a completely randomized design. Treatments included two levels of dried tomato pomace (DTP) (8 and 16%) and 3 types of using methods (unprocessed, alkali and acid processed). The results showed that different levels of DTP and processing methods significantly affected the performance, egg traits and blood parameters of native laying hens ($P<0.05$). Using 16% of DTP in contrary with 8%, without having any other adverse effects on feed conversion ratio and other production parameters, only reduced the eggs weight. In interaction between the levels of DTP and processing methods, alkali processing, improved the adverse effects of high level of DTP on eggs weight, while acid processing could not show this effect. DTP processing and levels, improved the egg yolk color index. Processing with acid increased the values of triglyceride and albumin in the hens' blood. In 16% of DTP, more than triglyceride and albumin, the level of total protein in contrary with 8% increased. Interaction between DTP and processing methods showed that with 16% of DTP in alkali treating, the values of triglyceride and albumin in blood reduce, while the total protein increased. In acid treating, the percentages of heterophile and heterophile to lymphocyte ratio reduced, while the lymphocyte percentage increased. The overall results showed that in native laying hens, using dried tomato pomace up to 16% of their diets, not only did not have any adverse effects on their performance, egg traits and blood parameters, but also increases some of them, that these beneficial effects improved by alkali and acid processing methods.

KEY WORDS egg traits, native laying hens, performance, tomato pomace.

INTRODUCTION

Tomato (*Lycopersicon ensculentum*) is one of the most popular vegetable used as a salad in food preparations and also commercially in form of juice, soup, puree, ketchup or paste. During commercial processing of tomato, large amount of waste are produced at various stages**.** From 1000 kg of fresh tomatoes, 100 to 300 kg of wet tomato pulp is produced and can be sold as animal feedstuffs. Its nutritional value is highly dependant on the tomato cultivars, growing conditions, degree of drying and processing method (Persia *et al.* 2003). Wet tomato pulp can be further dried to approximately 900 g/kg DM and, because of its chemical composition, which can use in poultry industry. A number of studies have determined the feasibility of feeding tomato waste in poultry. Literature review demonstrated that inclusion of dried tomato pomace (DTP) in laying hens diet resulted in similar performance with hens fed with corn soybean meal diet (Dotas *et al.* 1999) or increased egg production (EP) and egg mass (EM) at the levels up to 100 g/kg in diets of laying hens (Jafari *et al.* 2006). However, the inclusion of DTP at a higher level (150 g/kg of diet) decreased EP and EM and increased feed conversion ratio (FCR) (Jafari *et al.* 2006). Studies by Nobakht and Safamehr (2007) indicated that in laying hens feeding of DTP, increased feed intake (FI), EP, egg weight (EW), EM and eggshell weight (EST), whereas serum and egg yolk cholesterol content were not significantly affected. Some researchers found that supplementing laying hen diet with DTP did not influence the performance parameters but increased the yolk color (Mansoori *et al.* 2008). However, the effects of adding DTP in diet on laying hens performance and egg quality have been well described (Yannakopoulos *et al.* 1992; Mlodowski and Kuchta, 1998; Dotas *et al.* 1999; Jafari *et al.* 2006; Nobakht and Safamehr, 2007; Mansoori *et al.* 2008), but the studies available on the effects of DTP on serum metabolites of laying hens is limited and inconsistent. Feeding DTP to laying hens had no effect on plasma cholesterol and low-density lipoproteins (LDL) (Nobakht and Safamehr, 2007), serum albumin, globulin, glucose and triglyceride contents (Rahmatnejad *et al.* 2009). Also, feeding DTP at a rate of 24% of broiler chickens increased total serum protein, and levels of 16 and 24% cause a significant increase in the mean values of high-density lipoproteins (HDL) and a decrease in serum cholesterol and LDL (Rahmatnejad *et al.* 2009). Lucopene, folate, vitamin C, vitamin A, phenolics and flavonoids are beneficial compounds found in the tomatoes (Sahin *et al.* 2008). Lycopene is a major red carotenoid present in tomatoes and exhibits cholesterol lowering effect in human beings (Gerster, 1997). Also, tomato's pectin increases the activity of plasma lecithin cholesterol acyl transferase and significantly decreases the concentration of serum cholesterol in rats.

Tomato pomace contains higher level of crude fiber, as the major amount of fiber in laying hens diets, have adverse effects on their performance. Several attempts such as adding enzymes, processing with physical and chemical agents have been made to increase the nutritional value of DTP (Safamehr *et al.* 2011). It was shown that using urea processed DTP increase the performance of laying hens (Jafari *et al.* 2006). In processing with heat, water, alkali and acid agents, using 20% of DTP in broiler diets without having any adverse effects is possible (Squires *et al.* 1992).

Feeding native hens with unconventional and low price ingredients is recommended. By using theses kinds of feeds in Native hen diets, the production cost and environmental problems could be reduced. In the current experiment the effectiveness of tomato pomace levels and processing methods on performance and blood biochemical constitute of laying hens were investigated.

MATERIALS AND METHODS

Birds and experimental design

In this experiment 180 Azerbaijan province native laying hens from 28 to 40 weeks of age in 6 treatments and 3 replicates (10 birds per replicate) as a (2 × 3) factorial arrangement of treatments were used in completely randomized design. Treatments included two levels of (DTP) (8 and 16%) and 3 kinds of using methods (unprocessed, alkali and acid processed). Wet tomato pomace was supplied from tomato ketchup factory, after drying, enough amounts of DTP treated with 0.0487 *M* of NaOH as alkali agent and 0.2% of chloride acid as acid agent. The compositions of different forms of DTP were determined according to AOAC (2002). The compositions of different forms of DTP were similar to NRC measures, so NRC dates were used in rations formulation.

Diets preparation

The diets were formulated to meet the requirements of birds established by the NRC (1994) for semi heavy broiler breeder hens (Table 1). The lighting program for laying hens during the experimental period was 16 hours light and 8 hours darkness. Environmental temperature was controlled and was about 18 ˚C. Feed intake, feed conversion, egg production percentage, egg mass and egg weight were determined weekly. Mortality was recorded if it occurred. The collected eggs were classified as normal or damaged; the latter including fully cracked eggs (an egg with broken shell and destroyed membrane), hair cracked eggs (an egg with broken shell but intact membrane) and eggs without shells (an egg without shell but with intact membrane). For measuring the egg traits, at the end of the experiment, 3 eggs were collected from each replicate. Egg specific gravity was determined by placing them in salty water. Egg shells were cleaned and maintained at environmental temperature for 48 h until were dried, and then they were weighed. Then, their average was considered as the final thickness of an egg shell for each experimental unit. Color index of the yolk (Roche color index), yolk index, egg albumin index, Haugh units were also determined (Farkhoy *et al.* 1994).

Blood biochemical and immunity parameters

At the end of the experiment, 2 birds from each replicate were randomly chosen for blood collection and approximately 5 mL blood samples were collected from the brachial vein.

Table 1 The composition of basic diets

	Tomato pomac (%)					
	Untreated		Alkali treated		Acid treated	
Feeds ingredients	8	16	8	16	8	16
Corn	45.00	43.00	45.00	43.00	45.00	43.00
Wheat	19.40	17.90	19.40	17.90	19.40	17.90
Soybean meal	17.00	12.50	17.00	12.50	17.00	12.50
Tomato pomace	8.00	16.00	8.00	16.00	8.00	16.00
Oyster shell	8.25	8.25	8.25	8.25	8.25	8.25
Dicalcium phosphate	1.40	1.40	1.40	1.40	1.40	1.40
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.05	0.05	0.05	0.05	0.05	0.05
Lysine hydrochloride	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin A, D, E, K, C	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Calculated composition						
Metabolisable energy (kcal/kg)	2670	2670	2670	2670	2670	2670
Crude protein (%)	15.50	15.50	15.50	15.50	15.50	15.50
Ca (%)	3.61	3.61	3.61	3.61	3.61	3.61
Available phosphorus (%)	0.31	0.31	0.31	0.31	0.31	0.31
Sodium (%)	0.40	0.40	0.40	0.40	0.40	0.40
Crude fiber (%)	2.93	2.93	3.99	2.93	2.93	2.93
Lysine (%)	0.93	0.93	0.93	0.93	0.93	0.93
Methionine (%)	0.36	0.36	0.36	0.36	0.36	0.36
Met + Cys (%)	0.66	0.66	0.66	0.66	0.66	0.66

[1] Vitamin premix per kg of diet: vitamin A (retinol): 8500000 IU; vitamin D_3 (cholecalciferol): 2500000 IU; vitamin E (tocopheryl acetate): 11000 IU; vitamin k_3: 2200 mg; Thiamine: 1477 mg; Riboflavin: 4000 mg; Panthothenic acid: 7840 mg; Pyridoxine: 7840 mg; Cyanocobalamin: 10 mg; Folic acid: 110 mg and Choline chloride: 400000 mg.
[2] Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 75000 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 74.4 mg; Zn (ZnO, 80.35% Zn): 64.675 mg; Cu ($CuSO_4.5H_2O$): 6000 mg; I (KI, 58% I): 867 mg and Se ($NaSeO_3$, 45.56% Se): 200 mg.

One mL of collected blood was transferred to ethylenediaminetetraacetic acid (EDTA) tubes in order to determine immunity parameters including red blood cells, hemoglobin, packed cell volume, white blood cells and lymphocytes (Gross and Siegel, 1983). The remaining 4 mL blood was centrifuged to obtain serum to determine the blood biochemical parameters including glucose, cholesterol, triglyceride, albumen, total protein, and uric acid. Kit package (Pars Azmoon Company; Tehran, Iran) was used to determine the blood biochemical parameters using Anision 300 auto analyzer system (Nazifi, 1997).

Statistical analysis

The data were subjected to one way analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2005). Means were compared using the Duncan multiple range test. Statements of statistical significance are based on P<0.05.

RESULTS AND DISCUSSION

Performance

The effects of different levels of DTP and processing methods on the performance of native laying hens are summarized in table 2.

Alkali and acid processing of DTP and different levels of DTP significantly affect the amount of egg weight in native laying hens (P<0.05).

Alkali processing, decreased the adverse effects of major amount of DTP in diets, so, the egg weight in group contained 16% alkali processed of DTP was similar with low level of DTP.

In acid processing, the heavy eggs were obtained with 8% of DTP. 16% of DTP in comparison with 8% reduced the egg weight. Treated and untreated DTP could not significantly affect the hens performance (P>0.05), but numerically improved it.

Egg traits

The effects of different levels of DTP and processing methods on the egg traits of native laying hens are shown in table 3.

Interaction between DTP levels and processing methods significantly affect the egg color index in native laying hens (P<0.05). Alkali and acid processing of DTP in contrary with unprocessed DTP, improved the egg color index, also yolk color in group that contained 16% of DTP was more than group contained 8% of DTP.

Interaction between DTP levels and processing methods did not have any significant effects on the egg traits in native laying hens (P>0.05).

Table 2 The effects of feeding different levels of DTP and treating methods on the performance of native laying hens

Treatments	Egg weight (g)	Egg production (%)	Egg mass (g)	Feed intake (g)	Feed conversion ratio
Treating method					
Untreated	53.375	55.212	29.469	90.400	3.068
Alkali treated	54.000	59.950	32.376	90.574	2.798
Acid treated	53.467	57.515	30.752	91.924	2.989
SEM	0.218	1.984	1.040	1.431	0.096
P-value	0.133	0.278	0.185	0.721	0.254
DTP level (%)					
8	54.107[a]	57.587	31.158	90.750	2.913
16	53.121[b]	57.531	30.557	91.183	2.984
SEM	0.178	1.620	0.848	1.175	0.078
P-value	0.002	0.981	0.631	0.799	0.460
DTP level × treating method (%)					
8% untreated DTP	54.357[a]	54.520	29.635	88.400	2.980
16% untreated DTP	52.393[b]	55.903	29.289	92.400	3.155
8% DTP × alkali treated	53.637[ab]	61.587	33.033	92.400	2.798
16% DTP × alkali treated	54.363[a]	58.313	31.701	88.748	2.800
8% DTP × acid treated	54.327[a]	56.653	30.778	91.448	2.971
16% DTP × acid treated	52.607[b]	58.377	30.710	92.400	3.009
SEM	0.308	2.806	1.149	3.035	0.136
P-value	0.002	0.621	0.898	0.209	0.800

DTP: dried tomato pomace.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 3 The effects of feeding different levels of DTP and treating methods on egg traits of native laying hens

Treatments	Yolk color index	Eggshell (%)	Albumin (%)	Yolk (%)	Hauah unit
Treating method					
Untreated	4.333[b]	9.960	59.853	30.195	82.428
Alkali treated	4.888[a]	9.522	61.048	29.407	85.007
Acid treated	4.943[a]	9.743	60.752	29.657	82.600
SEM	0.143	0.222	0.641	0.479	2.018
P-value	0.020	0.407	0.417	0.512	0.613
DTP level (%)					
8	4.184[b]	9.748	60.741	29.590	83.289
16	5.258[a]	9.736	60.361	29.916	83.401
SEM	0.117	0.182	0.523	0.391	1.648
P-value	0.001	0.963	0.617	0.567	0.962
DTP level × treating method (%)					
8% untreated DTP	3.777	9.943	60.007	30.070	82.177
16% untreated DTP	4.890	9.977	70.059	30.320	82.680
8% DTP × alkali treated	4.330	9.677	60.483	29.890	82.360
16% DTP × alkali treated	5.443	9.367	61.613	28.923	87.653
8% DTP × acid treated	4.447	9.863	59.770	28.810	85.330
16% DTP × acid treated	5.440	9.336	60.322	30.503	79.830
SEM	0.203	0.315	0.907	0.667	2.854
P-value	0.994	0.685	0.271	0.187	0.210

DTP: dried tomato pomace.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Blood biochemical parameters

The effects of different levels of DTP and processing methods on the blood biochemical parameters of native laying hens are summarized in table 4.

Processing methods, DTP levels and interaction between them significantly affect some of the blood biochemical parameters in native laying hens (P<0.05). In the interaction between DTP and processing methods, in alkali and acid treating of 16% of DTP, the values of blood albumin and total protein increased.

This effect on blood triglyceride level was seen in acid treated in contrary with unprocessed DTP, alkali treated caused the blood values of triglyceride and albumin reduce, while these values by acid processing increased. With 16% of DTP the amount of blood totals protein increase.

Blood cells

The effects of different levels of DTP and processing methods on the blood cells of native laying hens are shown in table 5.

Table 4 The effects of feeding different levels of DTP and treating methods on the blood biochemical parameters of native laying hens

Treatments	Triglyceride (g/dL)	Cholesterol (g/dL)	Albumin (mg/dL)	Total protein (mg/dL)	Glucose (g/dL)
Treating method					
Untreated	195.728[b]	145.768	2.303[c]	4.580	173.643
Alkali treated	160.635[b]	164.422	2.450[b]	5.308	130.657
Acid treated	278.493[a]	212.435	2.735[a]	5.295	156.762
SEM	24.473	19.210	0.076	0.232	11.016
P-value	0.015	0.077	0.005	0.076	0.061
DTP level (%)					
8	180.410[b]	155.225	2.307[b]	4.720[b]	155.360
16	242.828[a]	193.197	2.685[a]	5.402[a]	152.015
SEM	19.982	15.685	0.062	0.190	8.994
P-value	0.047	0.113	0.001	0.026	0.797
DTP level × treating method (%)					
8% untreated DTP	188.580[c]	101.985	1.805[c]	3.510[b]	182.320
16% untreated DTP	302.875[a]	189.550	2.800[a]	5.650[a]	164.965
8% DTP × alkali treated	239.605[ab]	184.075	2.585[b]	5.480[a]	138.859
16% DTP × alkali treated	181.665[c]	144.770	2.315[b]	5.135[a]	122.455
8% DTP × acid treated	213.040[ab]	179.600	2.530[b]	5.170[a]	144.900
16% DTP × acid treated	343.945[a]	245.270	2.940[b]	5.420[a]	168.625
SEM	34.610	27.167	0.107	0.328	15.579
P-value	0.001	0.081	0.001	0.007	0.354

DTP: dried tomato pomace.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 The effects of feeding different levels of DTP and treating methods on the values of blood cells in native laying hens

Treatments	Heterophil (%)	Lymphocyte (%)	Heterophil / lymphocyte
Treating method			
Untreated	18.500[b]	80.000[b]	0.231[b]
Alkali treated	22.000[a]	76.250[c]	0.288[a]
Acid treated	12.500[c]	85.500[a]	0.146[c]
SEM	1.599	1.622	1.612
P-value	0.004	0.006	0.005
DTP level (%)			
8	19.667[a]	78.833[a]	0.249
16	15.667[b]	82.333[a]	0.190
SEM	1.305	1.325	1.312
P-value	0.051	0.086	0.059
DTP level × treating method (%)			
8% untreated DTP	22.000[a]	77.500[a]	0.283[a]
16% untreated DTP	15.500[bc]	82.500[a]	0.188[b]
8% DTP × alkali treated	26.500[a]	71.000[b]	0.371[a]
16% DTP × alkali treated	17.500[b]	81.500[a]	0.214[a]
8% DTP × acid treated	10.500[c]	80.000[a]	0.131[c]
16% DTP × acid treated	14.500[bc]	83.000[a]	0.175[b]
SEM	2.261	2.294	2.278
P-value	0.030	0.017	0.021

DTP: dried tomato pomace.
SEM: standard error of the means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Processing methods, levels of DTP and interaction between them, significantly affect the values of blood cells in native laying hens (P<0.05). In contrary to the control group, acid processing caused the heterophile and lymphocyte percentages increase and the ratio of hetrophile tolymphocyte reduce. These kinds of results were obtained in the interaction between DTP levels and processing methods. So that the lowest percentage of heterophile, and the highest value of lymphocyte were observed in acid treated of DTP.

Tomato pomace using methods could not affect the production parameters of laying hens. These findings are in agreement with another experimental result (Safamehr *et al.* 2011).

They showed that the enzyme addition into of DTP contained diets could not affect the performance of commercial laying hens. While another experimental result indicated that DTP processing increase it efficacy in broilers. These differences in the results may be related to hens breed, production level, processing methods and DTP levels in hens diets. It thought that as the production values in native laying hens in contrary with commercial hens is low and they have a developed digestive tract, so they can tolerate high levels of DTP in their diets and processing methods could not improve the efficacy of these high fibrous feeds. In contrary with 8%, using 16% of DTP caused the amount of egg weight significantly reduced. As in contrary with 8%, 16% of DTP contained major amount of crude fiber, so with a high level of DTP, considerable amount of crude fiber consumed by hens. In this condition, usually before supplying sufficient amounts of nutrients, digestive tract physically impacted. Some nutrients such as amino and fatty acids have positive effects on egg weight, by using high level of DTP, maybe these nutrients could not supply enough amounts so caused the amount of egg weight reduce. In contrary to the results of the present experiment, it was reported that using DTP in laying hens diets not only did not have reduced effect on egg weight, but also increase it (Nobakht and Safamher. 2007; Safamehr and *et al.* 2011). DTP contains 13% of Lysine more than soybean meal (Dotas *et al.* 1999). Lysine has demining effects on egg weight, such as previous experiments by using DTP in commercial laying hen diets, the egg weight increased. The difference in the results of this experiment with reported results may be related to hens breed, production period and tomato levels. In interaction effects, treated (especially alkali treated) caused the major amounts of essential nutrients release and absorb into blood so the egg weight increased. The beneficial effects of DTP processing on laying hens and broiler performance were reported (Squires *et al.* 1992; Jafari *et al.* 2006). In another experiment reported that enzymatic processing of DTP could not change the production performance of commercial laying hens. These differences in the results may be related to processing methods, the levels of DTP and hens breed. As DTP is a rich source of carotenoids and vitamin A, using high level of DTP caused high amounts of these compounds supply for egg formation. Transferring them to egg yolk increased its color index. The result of this experiment is not in agreement with other research results (Nobakht and Safamher, 2007; Mansoori *et al.* 2008; Safamehr *et al.* 2011). These differences may be related to tomato pomace levels and production status of hens. In interaction effects, may be acid processing caused the high levels of nutrients release, by increasing the nutrients absorption, the levels of triglyceride, albumin and total protein increased. The improving effects of tomato pomace processing on nutrients availability were reported in other experiments (Squires *et al.* 1992; Jafari *et al.* 2006). On the base of blood biochemical results (Table 4), processing methods caused the high levels of nutrients release and absorb into blood. As in this experiment the production parameters in acid processing method in contrary with alkali treating is low, so the lowest amounts of them transferred into eggs and major amounts remain in body and increased the blood levels of triglyceride and albumin. Similar results were obtained in using 16% of tomato pomace.

The result of this experiment is in agreement with other experimental results (Nobakht and Safamher, 2007; Rahmatnejad *et al.* 2009). In the current study, the blood levels of cholesterol and glucose did not change. It may relate to diets composition, level of tomato pomace and production status of native hens. Lycopene is a major red carotenoid present in tomatoes and exhibits cholesterol lowering effect in human beings (Gerster, 1997). Also, tomato’s pectin increases the activity of plasma lecithin cholesterol acyl transferase and significantly decreases the concentration of serum cholesterol in rats.

Decrease in the percentage of heterophile, the ratio of hetrophile to lymphocyte and increase in the percent of blood lymphocyte (Table 5) can be a sign in improving of immune system (Sturkie 1995). As tomato pomace is a rich source of some immune modulating agents such as lucopene, floate, vitamin C, vitamin A, phenolics and flavonoids (Sahin *et al.* 2008). By acid treating of tomato pomace, maybe the blood levels of these compounds alleviate and increased immunity status. As the performance of hens in alkali treating of tomato pomace was high, so it thought that the major amounts of these substances has been transferred into eggs and could not effectively enhance the hen's immune function.

CONCLUSION

The overall results showed that in native laying hens using dried tomato pomace up to 16% of their diets, not only did not have any adverse effects on their performance, egg traits and blood parameters, but also increases some of them, that this improvement can be enhanced by using alkali and acid processing methods.

ACKNOWLEDGEMENT

The authors would like to thank Native hen Research Center of Agriculture Jihad Organization of Urmia for corporation in this study.

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