The Effect of Apple Pulp and Multi Enzyme on Performance and Blood Parameters in Native Laying Hens

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Received: 1 August 2013
Accepted: 21 November 2013

ABSTRACT

This experiment was conducted to evaluate the effects of different levels of apple pulp and multi-enzyme on performance, egg traits and blood metabolites of native laying hens. In this experiment, 240 Azerbaijan province native laying hens aged from 28 to 40 weeks old were used in eight treatments and three replicates (10 birds per replicate) in a (4×2) factorial arrangement in a completely randomized design. The hens were fed apple pulp at 0, 5, 10 and 15% with two levels (0 and 0.05%) of multi-enzyme. The results showed that apple pulp or multi-enzyme and their combination significantly affected the performance, egg traits, blood biochemical parameters and immunity cells of native laying hens (p<0.05). Multi-enzyme improved the amounts of egg weight, mass and production percentage. Inclusion apple pulp at 10% did not show any adverse effects on performance compared to the control. However, at 15%, egg weight and egg production percentage were reduced and the feed conversion ratio was increased. In the interaction effects, 10% of apple pulp with multi-enzyme showed positive effects compared to other treatments. Multi-enzyme increased the percentage of albumin, while it decreased the egg yolk percentage and egg specific gravity. In the combined treatments, albumin percentage was increased when apple pulp was used at 10%. On the other hand, inclusion of multi-enzyme in diets significantly reduced serum total protein and glucose, while by inclusion 10% resulted in a decrease in uric acid and an increase in blood glucose. The combined treatment showed the best effect on blood uric acid. In 10% of apple pulp group, the amount of heterophile and heterophile to lymphocyte ratio was increased. In conclusion, inclusion of 10% apple pulp and 0.05% multi-enzyme improved laying hens’ performance, egg traits and blood parameters with no adverse effects.

Keywords: Native laying hens, Apple pulp, Egg traits, Performance

INTRODUCTION

Iran has diverse agro-climatic conditions that have enabled the country to produce a wide variety of horticultural crops. Amongst these crops, apple occupies a prominent position in Iran. Apple processing industries are one of the major industries of Iran. Apple pulp is residue after
juice extraction containing peel, seeds and the remaining solid parts that represents about 25-35% of the weight of the fresh apple processed. It is a waste and its disposal is a major environmental problem but being a precious resource, its utilization is a challenge and opportunity to the scientists and technologists (Zafar et al., 2005).

Apple pulp is a rich source of pectin besides other nutrients like carbohydrates, dietary fibers, minerals and vitamin C (Fanimo et al. 2003). Using of apple pulp in animal diets showed different results. In rabbits, addition of 30% apple pulp in the diets improved their performance (Fanimo et al. 2003). Inclusion of 15% apple pulp with 10% sugar beet molasses did not have any adverse effects on broilers performance (Ahmad, 2004). It was shown that in broilers, replacement with 20% of diet corn with apple waste is possible without any decrease on their performance (Zafar et al., 2005). As apple pulp contain major amounts of pectin and crude fiber, it is thought that adding multi-enzymes into diets can have improving effects of efficacy of apple pulp. Replacement with 15% and 20% of broilers ration corn by multi-enzyme in diets was possible, but in absence of multi-enzyme, it reduced the broilers performance (Matoo et al. 2001). In rats, using apple pulp for 4 weeks significantly reduced their blood triglyceride and cholesterol level (Khayat Nouri and Rezapour, 2011).

Native laying hens in contrast with commercial laying hens have low level of egg production and developed digestive tract; it thought that they can tolerate major amounts of apple pulp in their diets. In the present study the possibility of using apple pulp and the efficacy of multi-enzyme on apple pulp in Azerbaijan native laying hens were evaluated

**MATERIALS AND METHODS**

**Birds, Housing and Experimental Design**

Two hundred and forty Azerbaijan provinces native laying hens from 28 to 40 weeks old were used in factorial arrangement (4 × 2) in completely randomized design with 8 treatments and 3 replicates of 10 birds. The added amounts of apple pulp were 0, 5, 10 and 15% accompany with 2 levels (0 and 0.05%) of multi-enzyme. Sufficient amount of apple pulp was supplied from apple juice factory, after drying, the composition of dried apple pulp was determined according to AOAC (2002). After fine milling; the pulp was mixed with other diets ingredients (Table 1). The multi-enzyme was Safyzym that contained ß-glucanase and xylanase enzymes. The diets were formulated to meet the requirements of birds established by the NRC (1994) for semi-heavy broiler breeder hens (Table 2). 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. Mortality was recorded if it occurred.

**Performance and Egg Traits**

Feed intake, feed conversion, egg production percentage, egg mass and egg weight were determined weekly. 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 shell (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 final thickness of 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).

**Table 1.** The chemical composition of apple pulp (100% dry matter basis)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Metabolizable energy (Kcal/kg)</th>
<th>Crude protein (%)</th>
<th>Crude fiber (%)</th>
<th>Calcium (%)</th>
<th>Phosphorus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2340</td>
<td>6.40</td>
<td>23.00</td>
<td>0.56</td>
<td>0.28</td>
</tr>
</tbody>
</table>

**Table 2.** The composition of basic diets

<table>
<thead>
<tr>
<th>Feed ingredients (%)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>47</td>
<td>44</td>
<td>39.25</td>
<td>35</td>
</tr>
<tr>
<td>Wheat</td>
<td>21</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>21</td>
<td>20</td>
<td>19.80</td>
<td>19</td>
</tr>
<tr>
<td>Apple pulp</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>8.30</td>
<td>8.30</td>
<td>8.30</td>
<td>8.30</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.40</td>
<td>1.40</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>Salt</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine Hydrochloride</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin A, D, E, K, C</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix¹</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral premix²</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated composition</th>
<th>Metabolisable energy (Kcal/kg)</th>
<th>Crude protein (%)</th>
<th>Calcium (%)</th>
<th>Available phosphorus (%)</th>
<th>Sodium (%)</th>
<th>Crude fiber (%)</th>
<th>Lysine (%)</th>
<th>Methionine (%)</th>
<th>Methionine + Cysteine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2670</td>
<td>15.50</td>
<td>3.61</td>
<td>0.31</td>
<td>0.40</td>
<td>2.93</td>
<td>0.93</td>
<td>0.36</td>
<td>0.66</td>
</tr>
</tbody>
</table>

¹Vitamin premix per kg of diet: vitamin A (retinol), 8500000 IU; vitamin D₃ (cholecalciferol), 2500000 IU; vitamin E (tocolperyl acetate), 11000 IU; vitamin k₃, 2200 mg; thiamine, 1477 mg; riboflavin, 4000 mg; panthothenic acid, 7840 mg; pyridoxine, 7840 mg; cyanocobalamin, 10 mg; folic acid, 110 mg; choline chloride, 400000 mg.
²Mineral premix per kg of diet: Fe (FeSO₄·7H₂O, 20.09% Fe), 75000 mg; Mn (MnSO₄·H₂O, 32.49% Mn), 74.4 mg; Zn (ZnO, 80.35% Zn), 64.675 mg; Cu (CuSO₄·5H₂O), 6000 mg; I (KI, 58% I), 867 mg; Se (Na₂SeO₃, 45.56% Se), 200 mg.
* In diets contained multi enzyme the amounts of multi enzyme (saphizyme) were 0.05%.
Blood Biochemical and Immunity Parameters

At the end of the experiment, two birds from each replicate were randomly chosen for blood collection and approximately 5 ml blood samples were collected from the brachial vein. One ml of collected blood was transferred to 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) were 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 completely randomized design using the General Linear Model procedures of SAS Institute (2005). Means were compared using the Duncan multiple range test. Statements of statistical significance are based on p<0.05.

RESULTS

Performance

The effects of different levels of apple pulp and multi-enzyme on the performance of native laying hens are summarized in Table 3. Multi-enzyme, apple pulp and their interaction significantly affected the performance of native laying hens (p<0.05). In comparison with control group, using multi-enzyme increased the amounts of egg weight, egg mass and egg production percentage, while could not change the daily feed intake and feed conversion ratio. In contrast to control group, apple pulp addition until 10% did not have any adverse effects on laying hens performance, while in 15% inclusion, the egg weight, egg mass and egg production percentage reduced however feed conversion ratio increased. The best interaction was appeared using 10% of apple pulp and multi-enzyme compared to control group. In 15% using of apple pulp with or without multi-enzyme, the amounts of egg weight and egg mass significantly reduced, whereas the values for egg production percentage, daily feed intake and feed conversion ratio did not the significantly change.

Egg Traits

The effects of different levels of apple pulp, multi-enzyme and interaction between them on egg traits of native laying hens are shown in Table 4. Using multi-enzyme, apple pulp and interaction between them significantly affected the egg traits of native laying hens (p<0.05). Using multi-enzyme, the eggshell specific gravity and egg yolk percentage reduced, while the albumin percentage increased. In interaction effects, using 10% of apple pulp with no added enzyme improved the yolk color index and 10% of
apple pulp with multi-enzyme increased the albumin weight while reduced the egg yolk percentages.

**Table 3.** The effects of feeding different levels of apple pulp and multi enzyme on the performance of native laying hens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Egg weight (g)</th>
<th>Egg production (%)</th>
<th>Egg mass (g/week)</th>
<th>Feed Intake (g in week)</th>
<th>Feed conversion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzyme levels (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No enzyme</td>
<td>52.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2882&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7393</td>
<td>2.59</td>
</tr>
<tr>
<td>0.05</td>
<td>53.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3140&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7613</td>
<td>2.44</td>
</tr>
<tr>
<td>SEM</td>
<td>0.762</td>
<td>1.123</td>
<td>64.18</td>
<td>116.74</td>
<td>0.065</td>
</tr>
<tr>
<td>P Value</td>
<td>0.070</td>
<td>0.027</td>
<td>0.012</td>
<td>0.201</td>
<td>0.118</td>
</tr>
<tr>
<td><strong>Apple pulp levels (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>53.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3114</td>
<td>7500</td>
<td>2.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>53.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3190</td>
<td>7435</td>
<td>2.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>53.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2970</td>
<td>7377</td>
<td>2.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>52.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2771</td>
<td>7700</td>
<td>2.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.356</td>
<td>0.047</td>
<td>90.77</td>
<td>1653.10</td>
<td>0.091</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.029</td>
<td>1.588</td>
<td>0.024</td>
<td>0.552</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Apple pulp × Enzyme (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No apple pulp × No enzyme</td>
<td>54.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.97</td>
<td>3210&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7300</td>
<td>2.29</td>
</tr>
<tr>
<td>No apple pulp × 0.05 enzyme</td>
<td>52.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>57.29</td>
<td>3018&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7700</td>
<td>2.56</td>
</tr>
<tr>
<td>5 apple pulp × No enzyme</td>
<td>50.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.51</td>
<td>2825&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7214</td>
<td>2.55</td>
</tr>
<tr>
<td>5 apple pulp × 0.05 enzyme</td>
<td>55.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.66</td>
<td>3555&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7657</td>
<td>2.15</td>
</tr>
<tr>
<td>10 apple pulp × No enzyme</td>
<td>53.11&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>55.12</td>
<td>2928&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7358</td>
<td>2.51</td>
</tr>
<tr>
<td>10 apple pulp × 0.05 enzyme</td>
<td>53.58&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>56.22</td>
<td>3012&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7396</td>
<td>2.46</td>
</tr>
<tr>
<td>15 apple pulp × No enzyme</td>
<td>52.32&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>49.03</td>
<td>2566&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7700</td>
<td>3.02</td>
</tr>
<tr>
<td>15 apple pulp × 0.05 enzyme</td>
<td>52.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>56.97</td>
<td>2977&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7700</td>
<td>2.60</td>
</tr>
<tr>
<td>SEM</td>
<td>0.424</td>
<td>0.246</td>
<td>128.37</td>
<td>233.49</td>
<td>0.129</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0001</td>
<td>0.104</td>
<td>0.014</td>
<td>0.687</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Means within a column with no common superscript are significantly different (p<0.05).
Table 4. The effects of feeding different levels of apple pulp and multi enzyme on egg traits of native laying hens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Egg specific gravity (mg/ml^3)</th>
<th>Egg color index (%)</th>
<th>Eggshell (%)</th>
<th>Albumin (%)</th>
<th>Egg yolk (%)</th>
<th>Haugh unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme levels (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No enzyme</td>
<td>1.082^a</td>
<td>3.262</td>
<td>9.541</td>
<td>59.817^b</td>
<td>30.491^a</td>
<td>86.388</td>
</tr>
<tr>
<td>0.05</td>
<td>1.077^b</td>
<td>3.262</td>
<td>9.661</td>
<td>62.421^a</td>
<td>28.202^b</td>
<td>83.670</td>
</tr>
<tr>
<td>SEM</td>
<td>0.002</td>
<td>0.126</td>
<td>0.325</td>
<td>0.660</td>
<td>0.625</td>
<td>1.739</td>
</tr>
<tr>
<td>P Value</td>
<td>0.099</td>
<td>0.996</td>
<td>0.798</td>
<td>0.013</td>
<td>0.020</td>
<td>0.285</td>
</tr>
<tr>
<td>Apple pulp levels (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.078</td>
<td>3.052</td>
<td>9.704</td>
<td>60.743</td>
<td>29.270</td>
<td>86.107^ab</td>
</tr>
<tr>
<td>5</td>
<td>1.083</td>
<td>3.303</td>
<td>10.453</td>
<td>59.885</td>
<td>29.648</td>
<td>89.920^b</td>
</tr>
<tr>
<td>10</td>
<td>1.082</td>
<td>3.442</td>
<td>8.505</td>
<td>61.907</td>
<td>29.572</td>
<td>78.888^b</td>
</tr>
<tr>
<td>15</td>
<td>1.075</td>
<td>3.252</td>
<td>9.742</td>
<td>61.942</td>
<td>28.897</td>
<td>85.202^ab</td>
</tr>
<tr>
<td>SEM</td>
<td>0.003</td>
<td>0.178</td>
<td>0.460</td>
<td>0.934</td>
<td>0.884</td>
<td>2.459</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.210</td>
<td>0.498</td>
<td>0.057</td>
<td>0.366</td>
<td>0.929</td>
<td>0.041</td>
</tr>
<tr>
<td>Apple pulp × Enzyme (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No apple pulp × No enzyme</td>
<td>1.083</td>
<td>2.710^b</td>
<td>10.078</td>
<td>59.183^bc</td>
<td>30.187^ab</td>
<td>87.637</td>
</tr>
<tr>
<td>No apple pulp × 0.05 enzyme</td>
<td>1.073</td>
<td>3.393^bc</td>
<td>9.333</td>
<td>62.303^ab</td>
<td>28.335^bc</td>
<td>84.577</td>
</tr>
<tr>
<td>5 apple pulp × No enzyme</td>
<td>1.089</td>
<td>3.140^ab</td>
<td>10.443</td>
<td>59.777^bc</td>
<td>29.760^ab</td>
<td>93.637</td>
</tr>
<tr>
<td>5 apple pulp × 0.05 enzyme</td>
<td>1.078</td>
<td>3.467^ab</td>
<td>10.463</td>
<td>59.993^bc</td>
<td>29.537^a</td>
<td>86.203</td>
</tr>
<tr>
<td>10 apple pulp × No enzyme</td>
<td>1.083</td>
<td>3.830^a</td>
<td>8.730</td>
<td>57.640^c</td>
<td>33.613^a</td>
<td>80.587</td>
</tr>
<tr>
<td>10 apple pulp × 0.05 enzyme</td>
<td>1.082</td>
<td>3.053^ab</td>
<td>8.280</td>
<td>66.173^a</td>
<td>25.530^c</td>
<td>77.190</td>
</tr>
<tr>
<td>15 apple pulp × No enzyme</td>
<td>1.075</td>
<td>3.367^ab</td>
<td>8.913</td>
<td>62.670^ab</td>
<td>28.403^bc</td>
<td>83.693</td>
</tr>
<tr>
<td>15 apple pulp × 0.05 enzyme</td>
<td>1.075</td>
<td>3.137^ab</td>
<td>10.570</td>
<td>61.213^bc</td>
<td>29.390^bc</td>
<td>86.710</td>
</tr>
<tr>
<td>SEM</td>
<td>0.004</td>
<td>0.251</td>
<td>0.651</td>
<td>1.321</td>
<td>1.250</td>
<td>3.478</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.536</td>
<td>0.050</td>
<td>0.291</td>
<td>0.009</td>
<td>0.011</td>
<td>0.536</td>
</tr>
</tbody>
</table>

Means within a column with no common superscript are significantly different (p<0.05).

Blood Biochemical Parameters

The effects of apple pulp, multi-enzyme and interaction between them on blood biochemical parameters of native laying hens are presented in Table 5. Apple pulp, multi-enzyme and interaction between them significantly affected the blood biochemical parameters in native laying hens (p<0.05). Using multi-enzyme significantly reduced the blood cholesterol, total protein and glucose in native laying hens. Addition of 10% apple pulp had the best effects in reducing uric acid and increasing blood glucose levels. The interaction results
revealed that, using all levels of apple pulp with or without multi-enzyme in contrast with control group, significantly reduced the amount of blood uric acid and the lowest amount of blood glucose was obtained using 15% of apple pulp and multi-enzyme.

Table 5. The effects of feeding different levels of apple pulp, multi enzyme and interaction between them on blood biochemical parameters of native laying hens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzyme levels (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No enzyme</td>
<td>195</td>
<td>176\textsuperscript{a}</td>
<td>8.22\textsuperscript{a}</td>
<td>9.03</td>
<td>176\textsuperscript{a}</td>
</tr>
<tr>
<td>0.05</td>
<td>219</td>
<td>150\textsuperscript{b}</td>
<td>5.86\textsuperscript{b}</td>
<td>8.81</td>
<td>150\textsuperscript{b}</td>
</tr>
<tr>
<td>SEM</td>
<td>31.26</td>
<td>7.89</td>
<td>0.147</td>
<td>0.445</td>
<td>3.25</td>
</tr>
<tr>
<td><strong>P Value</strong></td>
<td>0.591</td>
<td>0.038</td>
<td>0.007</td>
<td>0.740</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>Apple pulp levels (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>262</td>
<td>172</td>
<td>5.97</td>
<td>10.96\textsuperscript{a}</td>
<td>162\textsuperscript{ab}</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>163</td>
<td>5.68</td>
<td>7.84\textsuperscript{b}</td>
<td>173\textsuperscript{a}</td>
</tr>
<tr>
<td>10</td>
<td>198</td>
<td>148</td>
<td>5.29</td>
<td>7.77\textsuperscript{b}</td>
<td>169\textsuperscript{a}</td>
</tr>
<tr>
<td>15</td>
<td>243</td>
<td>171</td>
<td>5.22</td>
<td>9.11\textsuperscript{ab}</td>
<td>149\textsuperscript{b}</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>44.21</td>
<td>11.15</td>
<td>0.208</td>
<td>0.630</td>
<td>4.59</td>
</tr>
<tr>
<td><strong>P-Value</strong></td>
<td>0.175</td>
<td>0.695</td>
<td>0.071</td>
<td>0.008</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Apple pulp \times Enzyme (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No apple pulp \times No enzyme</td>
<td>259</td>
<td>178</td>
<td>5.35</td>
<td>15.57\textsuperscript{a}</td>
<td>166\textsuperscript{b}</td>
</tr>
<tr>
<td>No apple pulp \times 0.05 enzyme</td>
<td>265</td>
<td>166</td>
<td>6.58</td>
<td>6.16\textsuperscript{c}</td>
<td>158\textsuperscript{bc}</td>
</tr>
<tr>
<td>5 apple pulp \times No enzyme</td>
<td>116</td>
<td>183</td>
<td>5.36</td>
<td>6.88\textsuperscript{c}</td>
<td>201\textsuperscript{a}</td>
</tr>
<tr>
<td>5 apple pulp \times 0.05 enzyme</td>
<td>135</td>
<td>141</td>
<td>6.01</td>
<td>8.80\textsuperscript{c}</td>
<td>145\textsuperscript{cd}</td>
</tr>
<tr>
<td>10 apple pulp \times No enzyme</td>
<td>133</td>
<td>173</td>
<td>5.44</td>
<td>6.85\textsuperscript{c}</td>
<td>172\textsuperscript{b}</td>
</tr>
<tr>
<td>10 apple pulp \times 0.05 enzyme</td>
<td>263</td>
<td>122</td>
<td>5.13</td>
<td>8.70\textsuperscript{c}</td>
<td>166\textsuperscript{b}</td>
</tr>
<tr>
<td>15 apple pulp \times No enzyme</td>
<td>271</td>
<td>169</td>
<td>4.71</td>
<td>6.62\textsuperscript{c}</td>
<td>164\textsuperscript{bc}</td>
</tr>
<tr>
<td>15 apple pulp \times 0.05 enzyme</td>
<td>214</td>
<td>173</td>
<td>5.73</td>
<td>11.60\textsuperscript{b}</td>
<td>133\textsuperscript{d}</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>52.53</td>
<td>15.78</td>
<td>0.293</td>
<td>0.890</td>
<td>6.50</td>
</tr>
<tr>
<td><strong>P-Value</strong></td>
<td>0.528</td>
<td>0.290</td>
<td>0.083</td>
<td>0.009</td>
<td>0.004</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means within a column with no common superscript are significantly different (p<0.05).

**Blood Cells**

The effects of apple pulp, multi-enzyme and interaction between them on blood cells counts of native laying hens are presented in Table 6.

Different levels of apple pulp significantly affected blood cells in native laying hens (p<0.05). The highest amount of blood heterophile and high ratio of heterophile to lymphocyte
ratio were resulted using 10% of apple pulp. Different levels of apple pulp could not significantly affect the ratio of lymphocyte (p>0.05). Multi-enzyme and interaction between multi-enzyme and apple pulp did not have any significant effects on blood cells counts (p>0.05).

Table 6. The effects of feeding different levels of apple pulp, multi enzyme and interaction between them on blood cells values in native laying hens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Heterophil (%)</th>
<th>Lymphocyte (%)</th>
<th>Heterophil to lymphocyte ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme levels (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No enzyme</td>
<td>17.0</td>
<td>82.1</td>
<td>0.196</td>
</tr>
<tr>
<td>0.05</td>
<td>18.6</td>
<td>81.1</td>
<td>0.229</td>
</tr>
<tr>
<td>SEM</td>
<td>0.972</td>
<td>1.604</td>
<td>0.210</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.255</td>
<td>0.665</td>
<td>0.335</td>
</tr>
<tr>
<td>Apple pulp levels (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.0</td>
<td>0.128&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>18.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.7</td>
<td>0.235&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>24.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.7</td>
<td>0.287&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>16.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>83.0</td>
<td>0.195&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>1.375</td>
<td>2.268</td>
<td>0.030</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.002</td>
<td>0.091</td>
<td>0.013</td>
</tr>
<tr>
<td>Apple pulp × Enzyme (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No apple pulp × No enzyme</td>
<td>10.5</td>
<td>85.0</td>
<td>0.123</td>
</tr>
<tr>
<td>No apple pulp × 0.05 enzyme</td>
<td>14.0</td>
<td>87.0</td>
<td>0.135</td>
</tr>
<tr>
<td>5 apple pulp × No enzyme</td>
<td>16.5</td>
<td>81.5</td>
<td>0.202</td>
</tr>
<tr>
<td>5 apple pulp × 0.05 enzyme</td>
<td>21.0</td>
<td>79.5</td>
<td>0.269</td>
</tr>
<tr>
<td>10 apple pulp × No enzyme</td>
<td>24.0</td>
<td>76.0</td>
<td>0.301</td>
</tr>
<tr>
<td>10 apple pulp × 0.05 enzyme</td>
<td>24.0</td>
<td>76.0</td>
<td>0.315</td>
</tr>
<tr>
<td>15 apple pulp × No enzyme</td>
<td>17.0</td>
<td>82.5</td>
<td>0.206</td>
</tr>
<tr>
<td>15 apple pulp × 0.05 enzyme</td>
<td>15.5</td>
<td>83.5</td>
<td>0.185</td>
</tr>
<tr>
<td>SEM</td>
<td>1.945</td>
<td>3.208</td>
<td>0.043</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.729</td>
<td>0.746</td>
<td>0.720</td>
</tr>
</tbody>
</table>

Means within a column with no common superscript are significantly different (p<0.05).
DISCUSSION

Supplementing diets with endo-acting polysaccharide hydrolyses can decrease the degree of polymerization of the recalcitrant NSP, leading to the 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 which colonized at the last compartments of the gastrointestinal tract, and consequently more energy is absorbed from the hydrolysis of NSP, pectin and crude fibers (Bedford and Apajalahti, 2001). Finally, breakdown of plant cell wall polysaccharides improves the access of the digestive biocatalysts to the endosperm trapped contents (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 of poultries (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-enzyme products are not equal. Multi enzymes differ from the sources they derived from. They may differ in enzymes types, activity and some 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). In the present experiment, adding multi enzyme into native laying hens diets improved digestible nutrients (without increase the daily feed intake) and made major levels of essential elements available to support the production. Absorption of high amounts of nutrients into blood in contrast with control group, increased egg weight, egg mass and egg production percentage. Apple pulp is contained high level of crude fiber and pectin which can impair (especially in higher levels) the performance of hens (Zafar et al., 2005). Using enzymes by reducing the adverse effects of these substances increased the absorbed nutrients and enhanced performance (Matoo et al., 2001). Using apple pulp up to 10% of the diets in contrast with control diet did not have any adverse effects on laying hens performance, but at the 15% egg weight and egg production percentage reduced and feed conversion ratio increased. It was reported that, using more than 5% of apple waste in the diet of commercial laying hens has negative effects on their performance (Nobakht, 2013). Greater tolerance of native laying hens to higher levels of apple pulp in contrast with commercial laying hens may be related to their production levels and gastrointestinal ability. As previously mentioned, apple pulp contained major amounts of crude fiber and pectin which is more than their tolerance in the diets (15%), cause considerable fiber consumption by hens and impair their performance. Interaction of using multi enzyme with apple pulp reduced the adverse effects of high fiber and the best egg weight and egg mass were obtained by using 10% of apple pulp. The beneficial effects of exogenous enzymes in hydrolysis of NSP, pectin and crude fibers and releasing of essential nutrients have been reported by Bedford and Apajalahti (2001).

Despite using multi enzyme, inclusion of 15% apple pulp into laying hens diets negatively impaired their performance and significantly reduced the production parameters. High level of apple pulp with increase the feed volume, amounts of dietary fiber and pectin, can impair the availability of essential nutrients for hens and therefore had adverse effects on their performance.
Base on the egg traits results (Table 4), in contrast with control group, egg specific gravity and egg yolk percentage reduced by using multi enzyme while egg albumin percentage increased. Egg specific gravity is an important index for evaluation of egg shell quality. Eggs with high specific gravity show that high amounts of calcium absorbed and deposited in eggshell and vice versa. Increase of the egg weight is mainly related to egg albumin, while the amounts of eggshell and egg yolk do not change (Farkhoy et al., 1997). So in the heavier eggs in contrast to smaller eggs, the eggshell thickness is thinner and they show low specific gravity. Decrease in the egg yolk percentage is the result of increase in the egg albumin percentage of the egg. Interaction of using 10% apple pulp with multi enzyme improved the egg color index and egg yolk percentage. Apple pulp has considerable amounts of beta carotene. Using multi enzyme accompany with apple pulp cause that the major amounts of carotenes release to blood and then positively changes the egg color after absorption. Increase the egg yolk color by using medicinal plants contained carotenes are also reported by other researchers (Nobakht and Mehmannavaz, 2009; Syidpiran et al., 2011). As the proportion of egg albumin do not vary significantly, the greatest percentage of egg yolk was obtained in this experimental group. The improving effects of commercial enzymes to overcome the inhibitory effects of some dietary contents such as crude fibers and increase the availability of nutrients and subsequent improvements in production traits have been previously reported by Bedford and Apajalahti. (2001). This report is in agreement with our obtained results about some of egg traits.

By using multi-enzyme, the performance of native hens increased due to the highest amounts of nutrients consumed to support the production parameters and for this reason the lowest levels of blood cholesterol and glucose were observed in diets contained multi-enzyme (Table 5). These results about the multi enzyme reducing effects on blood biochemical changes are not in agreement with the experiment of Safamehr et al. (2011). These differences in the results may be related to the type of multi-enzyme, diets ingredients, production level and hens breed. By using 5 and 10% of apple pulp the amounts of blood uric acid reduced, and glucose increased compared to control group. Apple pulp is a poor source of crude protein but contained considerable amounts of fruit sugar, so these results may be related to apple pulp composition. Increase in uric acid and decrease in blood glucose by using of 15% apple pulp may be related to adverse effects of high amounts of apple pulp consumption on nutrients digestion and absorption. In contrast with control group, the blood levels of uric acid and glucose reduced. These results may be related to positive effects of multi-enzyme on performance because the highest amounts of absorbed protein and glucose transferred into eggs, so the lowest amounts of them are present in blood. Changes in blood parameters of animals by using apple waste are reported by Khayat Nouri and Rezapour (2011).

Increase in the blood heterophile and heterophile to lymphocyte ratio (Table 6) can be the sign of weakened immune system (Sturkie, 1995). As by increasing in to levels of apple pulp, the performance of hens relatively increased, it seems that the highest levels of some important nutrients such as flavonoids, vitamin C and carotenoids were transferred into eggs. In the blood deficiency of these compositions the immunity level can be failed.
CONCLUSION

The overall results indicated that in native laying hens, using apple pulp up to 10% of diets, and 0.05% of multi-enzyme (Saphyzim) did not have any adverse effects on their performance, and blood parameters; however, apple pulp at a level of 15% showed adverse effects in these respects and not recommended.

REFERENCES


Nobakht A. 2013. The effects different levels of apple waste on egg performance of laying hens. Proceeding of the first conferences of using of wastes from agricultural urban and industrial sources in nutrition of domesticated animals. pp: 105.


