



Original Article

## Short-Term Incorporation of Tomato Pomace and Invert Sugar in Diets of One- and Two-Year-Old Layers

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### ABSTRACT

By-products - tomato pomace (TP) and powdered invert sugar (IS) - were substituted at low concentration in commercial Layena<sup>®</sup> crumble (L) diets of second and third cycle laying hens to simulate feeding of backyard layers for two wk. to determine the effect on several production measurements. In Experiment I, one-yr.-old layers were fed L as the control, LTP (90.0% L plus 10.0% *ground* TP) or LTPIS (90.0% LTP plus 10% *powdered* IS). Feed consumption was similar ( $P \leq 0.05$ ) across diets and hens fed L maintained BW for two wk. in contrast to those fed LTP and LTPIS. Hen-d. production for L, LTP and LTPIS was similar ( $P \leq 0.05$ ) while yolk color for eggs from hens fed TP was significantly ( $P \leq 0.05$ ) darker than that of eggs from hens fed L. Egg shell thickness values for all eggs in Experiment I were similar ( $P \leq 0.05$ ). To compare the *form* of diets containing TP, in Experiment II, two-year-old hens were fed LTP (90.0% L plus 10.0% *ground* TP) as the control or diets that were pelleted, then crumbled (LTPp or LTPISp). Hens fed LTP did not maintain BW for two wk. while those fed LTPp had BW equal ( $P \leq 0.05$ ) to that of their initial BW by the end of wk 2. Hens fed LTPISp maintained BW for 2wk. Pellet formation caused statistically ( $P \leq 0.05$ ) lower yolk color compared to that from eggs of hens fed LTP. Age and strain of hens produced low hen-d egg production of 20 to 30%, likely obfuscating the effect of diet. Egg shell thickness values for eggs from hens given LTPp met the standard (33 mm) while that (32 mm) for eggs from hens administered LTP and LTPISp did not. Older layers will need excess supplemental calcium when fed pellet-crumble diets with TP at 10.0%. Pellet formation will add extra cost to backyard production and negatively affect consumer preference for darker yolks.

**Keywords:** Production measurements, tomato pomace, invert sugar, pellet-crumble diets, Second- and third-cycle layers.

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## INTRODUCTION

In the United States, urban areas are developing ordinances for small holdings of domestic animals, such as poultry, in

municipalities where they were disallowed previously (USDA, 2014). Thus, more consumers are feeding laying hens in backyards

for one or more reasons - food source, gardening partners, pets and economic gain (Elkhoraibi *et al.*, 2014). As well, backyard laying hens are found in many poor countries; it was estimated that in many Africa countries, 80% of the poultry population was produced in traditional production systems (Gueye, 1998).

Feed costs are 69% to 70% of production expenditure (Mireles, 2013); this is vitally important when considering production for low income backyard producers in developing and developed countries. According to Ibarburu and Bell (2013), feed cost for layers in the US doubled from 1999 to 2012, moving from \$6.00 to nearly \$14.00 for 45.45 kg. Yegani (2007) noted that corn/soy feed prices may continue to rise worldwide due to diverting of corn into ethanol (and soy into biofuel), an increased demand for emerging economies and problems of harvesting associated with climate change. To offset the need for whole/cracked corn or soy meal for backyard production, by-products from processing of fruits, grains (including corn and soy), nuts, seeds and vegetables are under investigation as feed ingredients in diets of backyard laying hens or in small-scale production.

Another significant benefit from use of horticultural by-products as feed ingredients for laying hens is reduced deposition in landfills from approximately 40% loss of material worldwide as it moves along the supply chain from production to consumption (Gustavasson, 2001).

**Table 1: Selected nutrient content of tomato pomace**

Analysis	Tomato Pomace
AME <sub>n</sub>	(8.22MJ/kg)
Protein (%)	19.0 – 26.9
Crude fat (%)	11.9
Crude fiber (%)	26.3 – 59.0
Moisture (%)	5.1
Ash (%)	3.5 - 3.9
Sodium (%)	1.79

AME<sub>n</sub>, gross energy of feed with corrections for gross energy of the excreta after correcting for retention of nitrogen by the body. King and Zeidler, 2004; Del Vaile *et al.*, 2006; Mansoori *et al.*, 2007; Assi and King, 2008; Murzaei - Abbeddou *et al.*, 2011; Aghsaghali *et al.*, 2011.

For example, Ghanaian food loss includes by-products from production of cassava, cereals (maize, millet and rice), cocoa, plantain, potatoes, oil palms, shea nuts, yams and other crops from rural and urban agricultural areas. These by-products in Ghana and other

developing countries are not only deposited in landfills but are discarded in municipalities as waste in ways that cannot be sustained (Akowuah, 2011).

When managing fruit and vegetable waste, disposal in landfills is the seventh and last method listed after all others by the University of Georgia's Extension Service (2013). Cons for disposal include cost of disposal and transportation, methane development, public complaints and adverse news coverage due to loss of leachate during transportation.

### Tomato Pomace (TP) Availability and Nutrient Value

Recently, production and use of by-products from horticultural sources in feed of monogastric animals was discussed by King (2013, Figure 1). In several parts of the world, one by-product of interest is tomato pomace (TP as peels, seeds, juice, stems, liquor and green tomatoes) from processing of tomatoes. As the world leader in production of processing tomatoes, California produces about 96% of those grown in the US. Additionally, in California, backyard production of laying hens is popular and growing for reason mentioned above (Elkhoraibi *et al.*, 2014) and the need for alternative feedstuffs will grow as well.

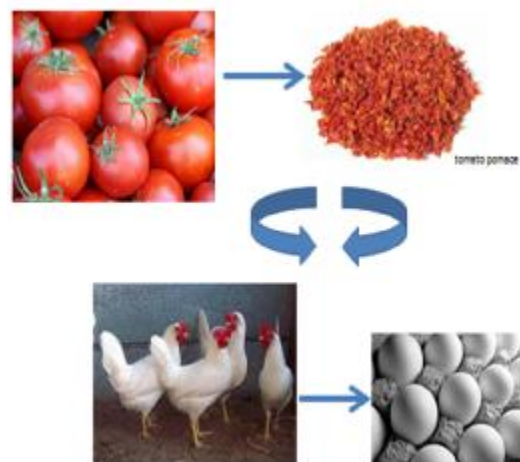


Figure 1: Many fruit and vegetable by-products, like tomato pomace, that are produced in California (US) and elsewhere can be further processed for inclusion in animal feed.

Two developing countries - Egypt and India - are among the ten countries that produce 65% of processed tomatoes and concomitant TP (Branthôme, 2010). Tunisia produces processed tomatoes in Africa and the Mediterranean

(Republic of Tunisia, 2011). Nigeria is ready for increased tomato production and processing also (IITA, 2011). Additionally, tomato production and processing are underway in other African countries, Southeast Asian countries (Vietnam, Cambodia, and Laos), Mexico, Central America and South America (Branthôme, 2010; AVRDC, 2011). As mentioned above, these areas of the world also have extensive backyard poultry production where use of TP could be useful to lower overall cost for production of nutritious eggs for families (especially children) and to enhance family income.

As shown in Table 1, TP from tomato processing contains high protein, fiber and minerals. It also contains fatty acids as well as  $\alpha$ -tocopherol (an antioxidant) and lycopene (an antioxidant and coloring agent) (King and Zeidler, 2004). Wet TP in combination with wheat is ensiled and fed to ruminants to improve growth performance (Denek and Can, 2006; Abdollahzadeh *et al.*, 2010) and can be fed in grower and finisher diets of pigs (Wadhwa and Bakshi, 2013). Due to (1) high production of tomatoes and TP and (2) the nutritional quality of the by-product, research on TP continues to focus on its inclusion as a feed ingredient in diets of laying hens.

### **Incorporation of TP in Experimental Diets of Laying Hens**

Research on use of TP in laying hens has focused on its use for commercial layers. Elliott *et al.*, (1981) fed several levels of TP to laying hens to determine its effects on egg production measurements, cholesterol content of egg yolk and serum cholesterol content in humans. While yolk cholesterol and serum levels were not affected, researchers noted that TP at 10% inclusion could be an alternative feedstuff in laying hen diets without adversely affecting hen performance and egg quality. Yannakopoulos *et al.*, (1992) revealed that TP at 8.0 to 15.0% in diets did not negatively affect egg number, shell quality, egg shape index, BW, feed consumption and mortality. Yolk color from hens fed TP was significantly darker than that from hens fed the control. Mansoori *et al.*, (2008a) fed 54-wk-old laying hens several diets for nine weeks and reported that TP had no effect on the weight ratio of shell: egg or shell thickness, but it increased the yolk color score as compared to that of hens fed diets containing wheat. Overall,

these investigators concluded that at 4.5% of feed, TP not only produced a significantly darker egg yolk color, but also had egg quality and layer performance measurements comparable to those from hens fed wheat bran.

While other investigators agreed with those above and reported no adverse effects of dried TP in diets of commercial layers at 10–20% for egg production, BW (Calislar and Uygur, 2010) and overall egg quality (Salajegheh *et al.*, 2012), research has not focused on the use of TP for use in backyard production. Nor has diarrhea (observed as greater fecal spread, cone height and excess fluid), been generally discussed as an adverse effects. Diarrhea was observed for layers when feeding TP from processing tomato varieties grown in the Central Valley of California (unpublished data) and is discussed below.

In an earlier study, visual observations suggested that palatability and high quantities (and types) of fiber not digested by non-ruminants (monogastrics) such as laying hens and unfamiliarity with the diet contributed to reduced consumption (Appleby *et al.*, 2004; Patwardhan *et al.*, 2011). Other investigators suggested that any BW differences caused by addition of TP may be due to the form (*mash or pellet-crumble*) of the diet (Jafarnejad *et al.*, 2011). Moreover, there may be a need for additional nutrients in diets (calcium and protein) due to loss caused by diarrhea.

### **Enhancing Palatability of Diets Containing TP**

Invert sugar (IS), a by-product of sugar refining, is said to enhance palatability of various agricultural by-products used to feed poultry and laying hens (John, 2013). IS, 70.0 to 74.0% of the sugar syrup, remains after separation and refinement of crystallized commercial sugar. The mixture of glucose and fructose in sugar syrup is called IS because it causes the opposite (inverted) rotation to a plane of polarized light compared to crystalline sucrose (Ophardt, 2003). Syrup at more than 10.0% of the feed formulation may cause handling difficulties; thus, *powdered* forms are used when higher concentrations for large scale operations are needed or for overall ease of use.

In the work reported here, we focused on substituting horticultural by-products, TP and IS, in diets of layers past their peak production

for *only two wk.* to simulate practices by backyard or small-scale producers to reduce the cost of feed when useful by-products may be free, inexpensive or available for a short period of time. Essentially, we investigated how production measurements are affected when consumers attempt to “stretch” their commercial feed for a short period of time. Of course, “stretched” diets would not be balanced to meet nutrient content of the control (commercial feed). We hypothesized that when fed in a commercial diet for two wk., these by-products would maintain production measurements of older laying hens. Thus, following recommendations for feeding of TP in low quantities, enhancing palatability with IS and the need to form pellets, alone and in combination with IS, in ground and in pellet crumble form – was to substituted in commercial diets of older laying hens without further balancing of diets. All diets met NRC requirements except for calcium. For the first in a series of studies, results for feed consumption, BW, mortality, egg production, egg shell thickness and yolk color over a 2-wk feeding period are reported.

## MATERIALS AND METHODS

### Feed Ingredients

Processed TP, obtained from Campbell Soup Company (Dixon, CA), was dried and ground according to procedures of Patwardhan *et al.*, (2011a). Powdered IS, chosen due to ease of use, was purchased from Robb Ross Foods, Inc. (Fresno, CA). Layena<sup>®</sup> crumble (L), a commercial feed, was purchased from Purina Mills, LLC, St. Louis, MO.

### Environmental Conditions

The Institutional Animal Care and Use Committee approved the protocol for Experiments I and II (IACUC, 2012). Laying hens were White Leghorn Inbred Crosses derived from line C-20 (University of CA, Davis, CA).

Available one-yr.-old (Experiment I) and two-year-old (Experiment II) hens were fed L since hatch. White Leghorn Inbred Crosses were chosen as appropriate models for backyard producers who often own layers for a period of time after the peak laying period in contrast to most commercial producers (Mench, 2014). Hens in individual commercial layer cages

(45.72 cm × 45.72 cm × 53.34 cm) experienced 16.0 h of light and 8.0 h of darkness within a 24.0 h cycle.

### Diet Composition and Design

For Experiment I, one-yr.-old hens received L as the control, LTP (90.0% L plus 10.0 % *ground* TP) or LTPIS (90.0% LTP plus 10.0% *powdered* IS) for two wk. For Experiment II, 2-yr-old layers were fed LTP (instead of L) as the control to compare *forms* of diets with TP. Thus, for two wk. in Experiment II, a diet containing *ground* TP was compared with LTPp or LTPISp where p denoted pelleting followed by crumbling. Pellets, formed by a pellet mill (Pellet Mill # SP6CL38782, California Pellet Mill, San Francisco, CA) were typically 4.6-4.8 mm in length and 0.5 cm in diameter. All diets and water were administered *ad libitum*.

To prevent nutritive deterioration, feed was stored in double plastic bags inside of dark plastic sealed containers under dry conditions at 7.2 °C – 12.8 °C to eliminate light, moisture and oxygen exposure (CFA, 2013; USDA, 2013) L, the base for all diets, contained an antioxidant (Assi and King, 2008; Ishida *et al.*, 2009). After making appropriate substitutions, diets were chemically analyzed for selected major components shown in Table 2 (UC Davis Laboratories, 2013) to determine if NRC (1994) requirements were met. Both Experiments were conducted as 3 diets x 2 blocks x 2 replications x 4 hens to provide 16 birds per treatment.

### Production Measurements

For both Experiments, feed consumption and BW were determined from recordings on the 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> d. Eggs per day were recorded. Hen-d egg production, egg shell thickness and yolk color were determined (Patwardhan *et al.*, (2011a). Egg shell thickness was measured by a micrometer (Serial No. 043162221, Murray Industrial Supply, Sacramento, CA) following procedures of Patwardhan *et al.*, (2011a). Egg yolk color, was scored by two individuals, blind to egg origin, using the Roche Yolk Color Fan (RYCF, DMS Nutritional Products, Basal, Switzerland), the most commonly used method for measuring yolk color. Variation on the fan ranges from 1 to 15, with 1 as light yellow and 15 as deep orange.

**Table 2: Selected nutrient content of diets**

Nutrient <sup>1</sup>	Diet <sup>2</sup>			Recommended <sup>3</sup> Value for Mineral Content in Egg Shells
	L	LTP	LTPIS	
Kcal/kg <sup>4</sup>	2,900	2,832	2,773	
Crude Protein (%)	18.30 <sup>a</sup> ±0.19	16.87 <sup>b</sup> ±0.57	16.28 <sup>b</sup> ±0.15	
Crude Fat (%)	3.89 <sup>a</sup> ±0.08	3.69 <sup>b</sup> ±0.09	3.19 <sup>c</sup> ±0.07	
Dry Matter (%)	93.18 <sup>b</sup> ±0.15	93.55 <sup>a</sup> ±0.13	93.65 <sup>a</sup> ±0.06	
Cellulose (%)	6.78 <sup>b</sup> ±0.58	7.95 <sup>a</sup> ±0.33	8.33 <sup>a</sup> ±0.43	
Hemicellulose (%)	10.56 <sup>b</sup> ±0.21	11.97 <sup>a</sup> ±0.56	11.58 <sup>a</sup> ±0.56	
Lignin (%)	2.48 <sup>c</sup> ±0.08	5.77 <sup>b</sup> ±0.14	7.08 <sup>a</sup> ±0.42	
Ash (%)	16.38 <sup>a</sup> ±0.97	12.00 <sup>b</sup> ±0.43	12.20 <sup>b</sup> ±0.48	
Calcium (%)	4.46 <sup>a</sup> ±0.35	2.98 <sup>b</sup> ±0.21	3.29 <sup>b</sup> ±0.11	3.25
Copper (ppm)	15.70 <sup>a</sup> ±1.36	14.65 <sup>a</sup> ±0.66	15.10 <sup>a</sup> ±1.77	
Iron (ppm)	223.40 <sup>a</sup> ±71.64	189.83 <sup>a</sup> ±10.07	202.25 <sup>a</sup> ±4.57	
Magnesium (%)	0.51 <sup>a</sup> ±0.01	0.45 <sup>b</sup> ±0.01	0.45 <sup>b</sup> ±0.004	
Manganese (ppm)	159.40 <sup>a</sup> ±7.54	156.00 <sup>a</sup> ±7.97	152.25 <sup>a</sup> ±4.99	20
Potassium (%)	0.99 <sup>a</sup> ±0.02	1.01 <sup>a</sup> ±0.01	0.95 <sup>b</sup> ±0.02	
Phosphorus (%)	0.63 <sup>a</sup> ±0.01	0.61 <sup>a</sup> ±0.01	0.59 <sup>b</sup> ±0.01	0.25
Sulfur (ppm)	2106.00 <sup>a</sup> ±42.78	1878.33 <sup>b</sup> ±30.61	1795.00 <sup>c</sup> ±25.17	
Zinc (ppm)	97.96 <sup>c</sup> ±5.12	221.92 <sup>b</sup> ±8.07	245.53 <sup>a</sup> ±3.61	35

<sup>1</sup>Mean of four to five samples,  $p \leq 0.05$ . Means with different superscripts are significantly different.

<sup>2</sup>Layena® crumble as the control (L), LTP (90.0% L plus 10.0% *ground* tomato pomace, TP) or LTPIS (90.0% LTP plus 10.0% *powdered* invert sugar, IS). All diets met or exceeded NRC (1994) requirements except for calcium.

<sup>3</sup>NRC 1994; Bell and Weaver. 2002.

<sup>4</sup>Calculated values.

## Statistical Analyses

Data from chemical analyses and each separate experiment were subjected to analysis of variance (SAS, 2004; Willits, 2013). Due to some abnormalities for older layers (outliers), data for BW were log transformed so that the ANOVA assumptions of normality and constant residual variance were valid. The impact of outliers was included in the analysis after Winsorizing, meaning that outliers were adjusted and repositioned so that they were consistent with a normal distribution. Thus, log transformed results for BW (log of mean weight) for each experiment are shown. Significance of all means was determined at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Use of Inbred Lines

Highly inbred lines can be models to explore the effect of a wide range of nutritional and environmental challenges. Such genetically uniform animals (homozygous for most traits) do not have the genetic variations that help buffer the response of the more variable outbred animals. While not a recognized popular backyard layer, the inbred layers are more likely

to be sensitive to subtle nutritional problems and give a clear response to adverse conditions such as novel feed components (Pisenti, 2014).

### Dietary Components and Welfare

As might be practiced in backyard production, second and third cycle laying hens were fed diets with ground TP for only a two wk period to simulate substitution of an alternative feed ingredients in a commercial diet (L) of layers at maintenance BW. In this type of substitution (without balancing all nutrients in the diet), it was expected and chemically determined that diets with TP contained less ( $P \leq 0.05$ ) nutrients (crude protein, fat, ash, calcium, potassium, phosphorus, and sulfur) than the control. However, except for calcium, all values of nutrients in diets with TP met or exceeded NRC (1994) requirements.

Although diets were isocaloric, total fiber (cellulose, hemicellulose and lignin) differed. Fiber in TP ranges from 26.0% to 59.0% (King and Zeidler, 2004; Del Vaille *et al.*, 2006; Mansoori *et al.*, 2007; Assi and King, 2008; Mirzaei-Aghsaghali *et al.*, 2011; Table 2.) For LTP and LTPIS, fiber content was 25.69% and 29.01%, respectively, while that for L was

19.82%, statistically less ( $P \leq 0.05$ ) than that of diets with TP (Table 2). This increase in lignin and other fibrous components in LTPIS, compared to LTP, was likely due to contamination associated with production of sugar from cane (Aguilar *et al.*, 2002). Excess fiber and its components in all diets containing TP possibly produced diarrhea. Characteristics of fiber - solubility, lignin content, and particle size - affect the need for dietary fiber (Mateos *et al.*, 2012) and may adversely affect fecal consistency. Major components of fiber are difficult to digest by non-ruminant animals such as broilers and laying hens due to the inability of enzymes to appreciably degrade the highly dispersed physical and chemical structure derived from the cross linking of lignin polymers with other polysaccharides (Figure 2; Yarris, 2009). Another possibility is that excess sodium in diets (from removal of tomato peels by sodium hydroxide solution during processing), contributed by TP (10%), caused increased water consumption, leading to diarrhea (Knoblich *et al.*, 2005). Excessive diarrhea could promote the spread of disease under husbandry practices where hens freely comingle as in backyard situations.

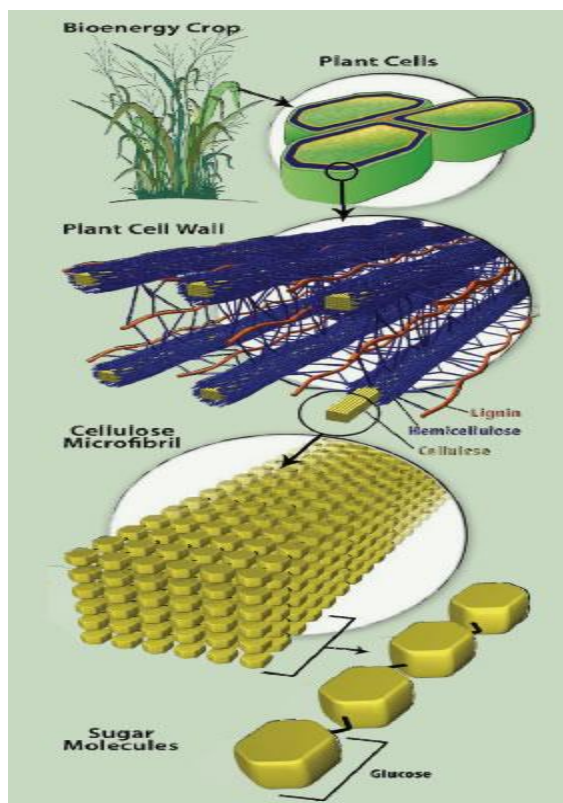


Figure 2: Complex association of cellulose, hemicelluloses and lignin (Yarris, 2013)

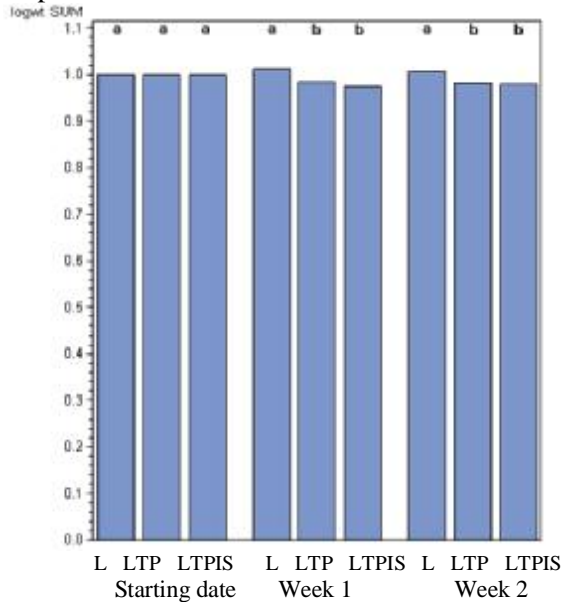
It has been suggested that loss of BW not exceed 15% of the original weight when birds are consuming experimental diets (Mench, 2010). Because TP can cause diarrhea and resultant weight loss of over 25% (Patwardhan *et al.*, 2011a) of the original weight when fed at concentrations higher than used in this study, BW was carefully monitored as a precaution. For Experiments I and II, weight loss for hens consuming experimental diets was never more than 15% of the original weight or of that for the control (L in Experiment I). Other effects for substituting TP in L without added nutrients are discussed below.

### Experiment I

**Mortality, Feed Consumption and Weight Gain.** Mortality was not observed. L, LTP and LTPIS had statistically similar feed consumption (data not shown). The overall mean feed consumption,  $0.114 \pm 0.064$  kg/hen/d, was in the range of that (100 g/hen/d) reported by Bell and Weaver (2002a) for 1-yr-old commercial hens. Because consumption was similar for all diets, it was concluded that IS did not enhance palatability over the two wk. period.

The mean starting BW of hens was 1.69 kg, numerically greater than weights for commercial layers (about 1.36 kg; Meunier and Latour, 2014) and that for noncommercial breeds (approximately 1.43 kg; UMN Ext., 2014). As shown in Figure 3, log transformation of data revealed that hens fed L maintained BW at 7 d (wk. 1) and 14 d (wk. 2). During the same periods, hens fed LTP (L plus 10% *ground* TP) and LTPIS (L plus 10% *ground* TP and 10% *powdered* IS) had statistically similar BW that was significantly ( $P \leq 0.05$ ) lower than that of their initial BW, presumably caused by lower quantities of nutrients in diets with TP due to diarrhea. As previously stated, there was significantly more fibrous material in the LTP and LTPIS diets than in the L diet as well as a higher quantify of sodium (not analyzed in this study) from processing. According to Mireles (2013), laying hens can digest more fiber than broilers; however, the quantities of fibrous component in TP, especially lignin, and excess sodium may not have been tolerated by one-yr-old laying hens. Additionally, reports of Mansoori *et al.*, (2008b) from two consecutive experiments with 15 adult laying-type cockerels (Hy-Line W36, 35-wk-old) revealed that dried

TP significantly increased ash, energy output, nitrogen and total dry matter in manure. Overall, these results and observations reveal that *ground* TP, as a high fiber and high protein/fat source, could not be substituted for even a very short period of time to maintain BW of laying hens compared to those fed L.



**Figure 3: Normalized geometric mean weight (log wt)<sup>1</sup> by diet and time for Experiment I.**

<sup>1</sup> Means with different superscripts are significantly different. BW for hens fed diets in wk 1 and 2 are compared to their BW at the starting date. 1-yr-old laying hens (1.69 kg/hen) were fed Layena<sup>®</sup> crumble as the control (L), LTP (90.0% L plus 10.0% *ground* tomato pomace, TP) or LTPIS (90.0% LTP plus 10.0% *powdered* invert sugar, IS). The experimental design was 3 diets x 2 blocks (pens) x 4 hens x 2 runs.

Another consideration to explain loss of BW by hens consuming diets with TP may be related to the *form* (*ground/mash* or *pellet-crumble*) of the diet instead of reduced nutrient content and further loss caused by diarrhea (Jafarnejad *et al.*, 2011; Mateos *et al.*, 2012). This observation is supported by that of Washlström *et al.*, (1999)

who showed that one line of laying hens had higher digestibility when fed a *pellet-crumbled* form of the diet compared to *mash*. Thus, TP and IS in diets with adequate NRC requirements may have maintained BW in older layers if the by-products were fed in *pellet-crumble* form. This hypothesis was tested in Experiment II, discussed below.

**Egg Production.** Egg production for a 1-yr-old flock is about 83.00% for White Leghorn commercial layers (Bell and Weaver, 2002b). Hen-d production for the laying hen line used in our study was similar ( $P \leq 0.05$ ) for L and LTP at 66.00 % and LTPIS at 62.50 %, but less than that for commercial layers. As seen in commercial breeds, hen-d egg production for various mature backyard flocks also begins to drop with age (UMN Ext., 2014).

**Yolk color.** Hens consuming LTP and LTPIS produced significantly ( $P \leq 0.05$ ) darker yolks when compared to those of hens receiving L alone (Table 3). The addition of IS in the LTPIS diet also significantly ( $P \leq 0.05$ ) diluted the color of yolks as compared to those from hens fed LTP. Presumably, the darker yolk color of eggs from diets with TP was due to the presence of the natural coloring agent, lycopene, up to 5,786 ug/100.0 g in some cultivars of tomatoes (Thompson *et al.*, 2000). Lycopene can be incorporated into yolk of laying hens more effectively at low rather than high dietary concentrations. Olsen *et al.*, (2008) reported that at a low dietary concentration of 0.07 g/kg, lycopene was incorporated at 45.0 g/kg and decreased to 6.0 g/kg at 10X the original concentration. Lycopene concentrations of egg yolks from hens fed diets in our study were not determined.

**Table 3: Yolk color and shell thickness from eggs of Single Comb White Leghorns Inbred Crosses fed various diets in Experiments I and II (EXP I<sup>1</sup> and II<sup>2</sup>)**

Measurements	Experimental Diets					
	EXP I			EXP II		
	L	LTP	LTPIS	LTP	LTPp	LTPISp
Egg yolk color <sup>3</sup>	7.29 <sup>c</sup> ±0.17	8.52 <sup>a</sup> ±0.17	8.10 <sup>b</sup> ±0.17	8.29 <sup>a</sup> ±0.17	5.61 <sup>b</sup> ±0.20	5.48 <sup>b</sup> ±0.17
Egg shell thickness	0.38±0.003	0.36±0.003	0.35±0.003	0.32±0.004	0.33±0.005	0.32±0.003

<sup>1</sup> 1-yr-old laying hens (1.69 kg/hen) were fed Layena<sup>®</sup> crumble as the control (L), LTP (90.0% L plus 10.0% *ground* tomato pomace, TP) or LTPIS (90.0% LTP plus 10.0% *powdered* invert sugar, IS). The experimental design was 3 diets x 2 blocks (pens) x 4 hens x 2 runs.

<sup>2</sup> 2-yr-old hens (1.76 kg/hen) were fed LTP (Layena<sup>®</sup> crumble, L plus 90.0% *ground* TP), LTPp (90.0% L plus 10.0% TP) in pellet-crumble form or LTPISp (90.0% LTP plus 10.0% IS) in pellet-crumble form. Pellet size was approximately 4.6 - 4.8 mm in length and 0.50 cm in diameter). The experimental design was 3 diets x 2 blocks (pens) x 4 hens x 2 runs.

<sup>3</sup> Means were compared within each experiment,  $p \leq 0.05$ . Means with different superscripts are significantly different

**Egg Shell Thickness.** There were no significant ( $P \leq 0.05$ ) differences among means for egg shell thickness in Experiments I. However, eggs from hens receiving L had numerically higher values than that of those from hens fed other diets (Table 3). Egg shell thickness is affected mainly by calcium, chloride, manganese, phosphorus, selenium, sodium and zinc content of feed (Bell and Weaver, 2002a). Chemical analysis of selected mineral content (calcium, manganese, phosphorus and zinc) for the L diet resulted in values that exceeded the recommended requirements for laying hens (NRC, 1994: Tables 2 and 3). High contents of these minerals in L most likely helped to produce excessive thickness of egg shells (Tables 2 and 3). Concomitantly, numerically lower values for shell thickness of eggs from hens fed diets with TP were likely due to the significantly ( $P \leq 0.05$ ) lower calcium that did not meet NRC requirements (Tables 2 and 3). The low quantity of calcium quantified for LTP is also of interest. A 10% reduction of calcium in L was offset by the addition of calcium in TP (0.31% - 0.42%, Mansoori *et al.*, 2008b; Abdollahzadeh *et al.*, 2010); however, the chemical analyses revealed greater than 10% reduction in the value of calcium for LTP. Clearly, more work is needed to clarify this issue.

Stadelman and Cotterill (1992) reported that eggs shell thickness values should be 0.33 mm in order to withstand the rigors of packing and shipping to market. While both diets with TP produced lower values than that of L, the values from hens fed all diets exceeded the standard of 0.33 mm. Thus, feeding 1-yr-old laying hens TP at 10.0% of L for two wk. would not be detrimental to shell thickness if eggs from the laying hen line used in this study were transported to market.

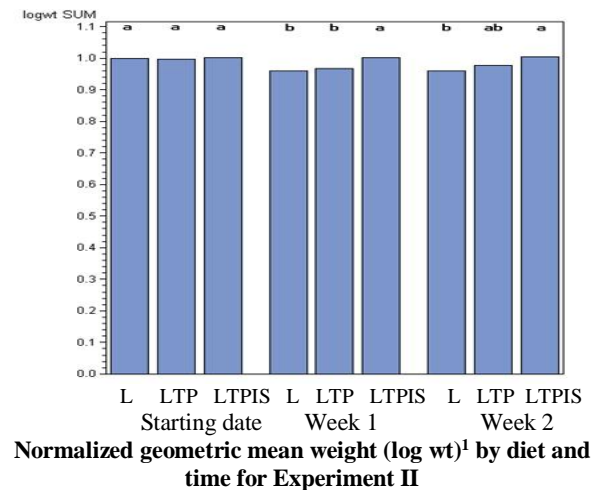
Overall, results showed that substitution of *ground* TP without and with *powdered* IS in L for two wk. reduced initial BW after two wk. However, feed consumption, hen-d egg production, yolk color and egg shell thickness were not adversely affected.

## Experiment II

**Mortality, Feed Consumption and Weight Gain.** Mortality was not observed. LTP, LTPp and LTPISp were fed to 2-yr-old hens in *pellet-crumble* form to compare the *forms* of diets

containing TP. Daily mean feed consumption for each hen was  $0.117 \pm 0.067$  kg, comparable to that reported by Bell and Weaver for 2-yr-old hens (Bell and Weaver, 2002a).

Initial BW of hens was 1.76 kg/hen, also comparable to that of 2-yr-old hens (Bell and Weaver, 2002a). As in Experiment I, Figure 4 indicates that hens fed LTP lost weight by wk 1 and did not regain it by wk 2. Hens fed LTPp also lost weight during wk 1 but had a BW equal ( $P \leq 0.05$ ) to their starting weight by wk 2 (Figure 4), likely indicating a 1 wk period of acclimation. Hens fed LTPISp had no significant change in BW at wk. 1 or wk. 2.



<sup>1</sup> Means with different superscripts are significantly different. BW for hens fed diets in wk1 and 2 are compared to their BW at the starting date. 2-yr-old hens (1.76 kg/hen) were fed LTP (Layena® crumble, L plus 90.0% ground TP), LTPp (90.0% L plus 10.0% TP) in pellet-crumble form or LTPISp (90.0% LTP plus 10.0% IS) in pellet-crumble form. Pellet size was approximately 4.6 - 4.8 mm in length and 0.50 cm in diameter). The experimental design was 3 diets x 2 blocks (pens) x 4 hens x 2 runs.

Thus, LTP at 90.0% and IS at 10.0% in *pellet-crumble* form could be fed to the 2-yr-old inbred hens (previously fed L) for two wk. with no detrimental effect on initial BW. Results for LTP and LTPp fed to older hens in Experiment II seemed to confirm that of Jafarenejad *et al.*, (2011) who noted that a *pellet-crumble* diet produced significantly improved BW when compared to that for a mash diet containing the same ingredients. The result for LTPISp for 2-yr-old layers was also in contrast to that in Experiment I where LTPIS did not maintain two wk. BW of 1-yr-old hens, previously fed L. For Experiment I, we suggested that diarrhea may have increased nutrient loss. Results of Experiment II indicate that if nutrient loss



occurred during diarrhea for two wk., it was counteracted by greater digestibility due to the form of the diet and the inclusion of IS (Jafarnejad *et al.*, 2011; Mansoori *et al.*, 2008b; Sundu, 2005; Washlström *et al.*, 1999). Contrasting results of Experiments I and II created obvious gaps for further investigation. It remains to be determined if LTPp and LTPISp fed to hens - previously fed L- will maintain BW as well as L in an extended feeding trials for second and third cycle layers.

**Egg Production.** Hen-d egg production for 2-yr-old layers was low (20 to 30%) and not statistically different ( $P \leq 0.05$ ). A high number of soft shell eggs were observed for all diets including L; however, data for this measurement were not recorded. Results for all diets in the present study were undoubtedly due to the genetic line and further confounded by the age of hens as well as a need for more calcium as discussed below for egg shell thickness. Any differences that might have occurred due to diet were obfuscated by these confounding factors.

**Yolk Color.** Even though hen-d egg production was very low, enough eggs from hens fed each diet were collected to conduct a valid statistical comparison of egg yolk color scores. Egg yolks from eggs of hens fed LTPp and LTPISp were significantly ( $P \leq 0.05$ ) lighter in color when compared to that of eggs produced by hens fed LTP (Table 3). One possibility is that diets with added TP, thus added lycopene, had increased instability of lycopene when produced in pellet form. Pellets reached temperatures of up to 70 °C during processing (Hung, 2013). Kaur *et al.*, (2007) reported degradation of lycopene in TP at 50 °C. Presumably, heating and pressure during development of pellets caused destabilization of lycopene and other coloring agents in TP. Thus, using *pellet crumble* forms of diets with TP for weight maintenance will adversely affect desired darker yolk (unless other sources are added at greater levels) and incur cost as well as added time needed to form pellets.

**Egg Shell Thickness.** Even though hen-d egg production was very low, enough eggs from hens fed each diet were collected to conduct a valid statistical comparison of egg shell thickness values. Results showed that although calcium was reduced in all diets, LTPp produced shell thickness values that met the standard (0.33 mm) while that for LTP and LTPISp did not.

The genetic capacity of aging hens to deposit calcium in egg shells is reduced due to their diminished ability to mobilize it from bones to produce calcium carbonate (The Poultry Site, 2008). Thus, the form of diet and its effect on deposition of calcium in egg shell of older hens needs further study. These findings were similar to those reported by Patwardhan *et al.*, (2011 a, b) who indicated that third cycle laying hens fed TP (> 70% of the diet) during molt produced post molt eggs that had statistically lower values for egg shell thickness than that of the control even though adequate amounts of calcium were provided throughout the study. Hens also had a lower post molt bone density than the control. Undoubtedly, third cycle layers fed pellet-crumble diets with TP should receive adequate and likely excessive calcium.

Feeding of TP and IS in *pellet crumble* form to 2-yr-old hens for two wk. revealed that feed consumption, and BW were not affected. Pelleting adversely affected egg yolk color while hen-d egg production and egg shell thickness were confounded by the age of hens and lack of calcium.

## CONCLUSION

Overall, results for BW indicated that *pellet-crumble* was an acceptable mode of delivery for TP and IS for a two wk. period when fed to older laying hens previously fed L; however, yolk color was adversely affected by heat used during pellet formation indicating the need for stabilization of lycopene or additional lycopene and coloring agents from other sources. Otherwise, consumers using *pellet-crumble* diets with TP as an alternative feed ingredient will need to accept lighter yolk color. Third cycle layers fed diets with TP should receive excessive calcium to insure adequate egg shell thickness. Presently, a deterrent for use of California TP in *pellet-crumble* diets of laying hens is concomitant diarrhea.

Given the added initial cost of equipment for pellet formation and the loss of dietary lycopene that could affect preference and sale of eggs with lighter yolks than those from hens fed commercial feed alone, a cost analysis is needed to determine the overall profitability of using free available by-products like TP and IS in diets fed to older layers. As more by-products with reduced fiber are used in diets, formation of

*pellet-crumble* diets using horticultural by-products may become a new small business.

More research is needed to (1) compare LTPp and LTPISp to L when fed for a longer period to second and third cycle layers, (2) determine the usefulness of an additional protein source when feeding diets with TP, (3) clarify the usefulness of balanced *pellet-crumble* diets containing TP with and without reduced fiber for laying hens during growth and peak production and (4) assess the cause (fiber, excess sodium or both) of diarrhea in TP.

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