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Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

# Effects of natural and synthetic lycopene dietary supplementation on selected biochemical traits and antioxidant status of Japanese quail (*Coturnix coturnix japonica*)

**Rabia Jaddoa Abbas[1]*, Issa Abdullah Thamir AL-Jrrah[2]**

[1] Department of Animal Production t., College of Agriculture, University of Basra, Iraq

[2] Ministry of Agriculture, Directorate of Basra Agriculture, Basra, Iraq

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**The present study was undertaken to investigate the effects of natural and synthetic sources of lycopene on selected biochemical traits and the antioxidant status of Japanese quail. A total of 420 one–day-old Japanese quails were randomly allocated to seven treatments (n=60), with 3 replicates per treatment and 20 birds per replicate. The first group was fed a basal diet (with no supplementation) and served as the control. The second and third groups were fed the basal diet supplemented with tomato (*Lycopersicon esculentum*) fruit powder (TOM) (17, 34 g/kg) to supply 50 and 100 (mg/kg) lycopene (LY), respectively. The fourth and fifth groups were fed the basal diet supplemented with red bell pepper (*Capsicum annuum* L.) fruit powder (RBP) (16.23, 32.46 g/kg) to supply 50 and 100 (mg/kg) lycopene. The sixth and seventh groups were fed the basal diet supplemented with 50 and 100 (mg/kg) pur**e lycopene powder, respectively. Results showed that the serum biochemical indices in quail chicks fed TOM and RBP fruit powder showed a significant increase (**$p \leq 0.05$**) in glucose (in T2, T3 and T4) and **high density lipoprotein cholesterol (HDL-c) levels** in the supplement treatments **as compared to the control and T2. Serum total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) levels were decreased significantly in all the treatments compared to the control. Additionally, the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as well as malondialdehyde acid (MDA) concentration were decreased (**$p \leq 0.05$**) in all the treatments compared to the control. Dietary TOM and RBP** fruit powder **as well as pure lycopene raised serum glutathione peroxides (GSH-PX) and superoxide dismutase (SOD) activity in quails at 49 days of age. It could**

*Corresponding authors: rj.abbas@yahoo.com

**be concluded that TOM and RBP** fruit powder as well as **pure LY at 34, 32.46 (g/kg) and 50 (mg/kg), respectively, have beneficial effects on selected biochemical indices and serum antioxidant enzyme activity. The best effects were provided by the application of 32.46 (g/kg) RBP** fruit powder as a **natural source of lycopene in the diet of Japanese quail chicks.**

**KEY WORDS: biochemical traits / Japanese quail / lycopene / red bell pepper / Tomato**

Fruits and vegetables are among the most important sources of phytochemical compounds in the human diet. A diet rich in fruits and vegetables is well established for its effectiveness in promoting human health, especially regulating body weight [Estruch *et al*. 2013, Mozaffarian 2016]. Fruits and vegetables are significant sources of carbohydrates, dietary fibre, minerals and micronutrients, including carotenoids, polyphenols and antioxidants [Yahia 2009, Septembre-Malaterre *et al*. 2018]. It is evident that natural antioxidants are extremely important for maintaining an individual's health. On the other hand, natural products can also be fed to animals to improve the quality of animal products [Yeung *et al*., 2019a]. According to Leite *et al*. [2011], a diet enriched in fruits and vegetables has a positive impact on several chronic conditions, such as obesity, diabetes, cancer, cardiovascular and neurodegenerative diseases, in addition to other beneficial effects of natural products [Yeung *et al*. 2019b]. Natural plant supplements in poultry diets can be used to enhance antioxidant defense mechanisms and reduce the intensity of oxidation processes, which negatively affect the quality of poultry products [Ognik *et al*. 2016]. The decreased peroxidation process related to malondialdehyde levels in the saddle and leg of the Polish Merino is crucial in the context of animal health and meat quality [Lipinski *et al*. 2019]. A previous study [Lipinski *et al*., 2017] pointed out the positive role of plant by-products linked with antioxidative protection. In this regard, Tawfeek *et al*. [2014] concluded that the use of antioxidant compounds in feeding of broilers can improve immune function and reduce deleterious effects of reactive oxygen species (ROS) on cells. Lycopene is a carotenoid present in vegetables and ripe fruit and has been proved to be the most potent antioxidant among various common carotenoids [Sun *et al*., 2014]. It is found mainly in tomatoes and other red fruits and vegetables [Mendelová *et al*. 2013], with tomatoes and their products being key sources of lycopene [Arain *et al*. 2018]. According to Abdullah *et al.* [2019], the Jordanian vine tomato dried waste has a substantial amount of lycopene with the oxidative free radical scavenging activity being 2-fold greater than that of ascorbic acid. Palozza *et al*. [2012] in their study reported that lycopene scavenging capacity was two times greater than that of α-tocopherol and ten times higher than that of β-carotene. Previous studies focused on carotenoids, which are fat-soluble antioxidants found in peppers in view of their antioxidant properties [Rao and Rao 2007] as well as provitamin A activity, antioxidant action, immune modulation and involvement in cell signaling [Tundis *et al*. 2011]. Additionally, the role of lycopene in maintaining the oxidative balance in the host's body is related to its various activities, such as scavenging reactive oxygen species (ROS), its role in regulating the production of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), ctalase (CAT), glutathione S-transferase (GST) and quinone reductase through the activation of the so-called antioxidant response element (ARE) [Martinez *et al*. 2008, Van Breemen and Pajkovic 2008, Sahin *et al*. 2011, 2013]. Additionally, it has been shown to activate antioxidant enzymes and nuclear transcription factor systems in heat-stressed broilers [Sahin *et al*. 2016]. The dietary intake of tomato lycopene has also been found to have a beneficial effect against oxidative stress (OS) in patients suffering from OS [Sarkar *et al*. 2012]. Several studies reported the health promoting and antioxidant potential of lycopene. In this regard, Sahin *et al*. [2008] showed that lycopene-rich tomato powder significantly improved feed consumption, body weight gain and decreased the concentration of malondialdehyde (MDA) in muscles, liver and serum of Japanese quails reared under heat stress. In turn, Selim *et al*. [2013] reported that dietary inclusion of lycopene-enriched tomato by-products at a level of 1% in the feed of broilers reared under heat stress enhanced total antioxidant capacity and lowered MDA levels. Recently, Mezbani *et al*. [2019] reported that lycopene (100 mg/kg) improved performance and antioxidant status of broilers. Therefore, the aim of this study was to investigate the effect of different sources of lycopene on antioxidant enzyme activity and blood lipid profile in Japanese quails.

## Material and methods

The experiment was conducted in the Quail Farm, the Department of Animal Production, College of Agriculture, University of Basra during the period from 19/3/2018 to 7/5/2018.

### Animal Husbandry and Treatments

A total of 420 one-day-old , unsexed Japanese quails were randomly allocated to seven treatments (n=60), with three replicates per treatment and 20 chicks per replicate according to the complete random design. All chicks were reared in cages (replicates) of 60 × 70 × 60 cm. The chicks were allowed free access to feed and water and they were fed the basal diet formulated to meet the nutrient requirements of the Japanese quail. The following seven dietary treatments were prepared by adding various levels of dried tomato (*Lycopersicon esculentum*) fruit powder (TOM) and red bell pepper (*Capsicum annuum* L.) fruit powder (RBP) to the basal diet as sources of natural lycopene, the content of which was previously estimated from lycopene (Tab. 2).

The experimental diets were designed as follows:

T1: Diet without any lycopene sources (control diet)
T2: Diet supplemented with 17 g/kg TOM (to supply 50 mg/kg lycopene);
T3: Diet supplemented with 34 g/kg TOM (to supply 100 mg/kg lycopene);
T4: Diet supplemented with 16.23 g/kg RBP (to supply 50 mg/kg lycopene);
T5: Diet supplemented with 32.46 g/kg RBP (to supply 100 mg/kg lycopene);
T6: Diet supplemented with 50 g/kg synthetic lycopene;
T7: Diet supplemented with 100 g/kg synthetic lycopene.

The fruits of tomato (*Lycopersicon esculentum*) and red bell pepper (*Capsicum annuum* L.) used in this study were provided by local producers. Fruits of both tomato and red peppers were dried in the shade, ground into a fine powder using an electric dry mill and then powders were stored in black plastic bags at room temperature

**Table1.** Ingredients and nutrient composition of quail starter and grower diets

| Ingredient (%) | Starter diet 1-21 day | Grower diet 22-49 day |
|---|---|---|
| Yellow corn | 50.00 | 56.00 |
| Wheat | 8.50 | 8.50 |
| Soybean meal (44%) | 33.00 | 28.00 |
| Protein concentrates[1] (44%) | 6.00 | 5.00 |
| Vegetable oil | 1.00 | 1.00 |
| Dicalcium phosphate | 0.25 | 0.25 |
| Limestone | 0.75 | 0.75 |
| Premix | 0.25 | 0.25 |
| Sodium chloride | 0.25 | 0.25 |
| Total | 100 | 100 |
| Calculated composition[2] | | |
| Metabolisable energy (Kcal /Kg) | 2934 | 2995 |
| Crude protein (%) | 22.60 | 20.47 |
| Crude fat (%) | 3.10 | 3.08 |
| Crude fiber (%) | 3.61 | 3.53 |
| Calcium (%) | 0.74 | 0.68 |
| Available phosphorus (%) | 0.35 | 0.32 |
| Lysine (%) | 1.16 | 1.07 |
| Methionine (%) | 0.42 | 0.39 |
| Methionine + Cysteine (%) | 0.77 | 0.73 |
| Calorie:protein ratio | 129.82 | 146.31 |

[1]Protein concentrate used from Al-Hayat Company, Jordanian Origin, to provide the following per kg of diet: 44% protein, 2800 kcal/kg ME, 12% fat, 25% ash, 5% calcium, 2.9% phosphorus, 2.55% methionine + Cysteine, 2.8% lysine.
[2]Was calculated according to the chemical composition of feedstuff contained in NRC [1994].

**Table 2.** Proximate analysis (% on dry weight basis) of tomato fruit and red bell pepper powder

| Component (%) | Tomato fruit powder | Red bell pepper |
|---|---|---|
| Dry matter | 85.08 | 91.52 |
| Crude protein | 14.53 | 13.13 |
| Crude fat | 3.71 | 11.47 |
| Ash content | 8.36 | 6.72 |
| Crude fiber | 10.60 | 23.90 |
| Available carbohydrate | 62.80 | 44.78 |
| Organic matter | 91.64 | 93.28 |
| Metabolised energy (Kcal/Kg)* | 2793.86 | 2729.04 |
| Lycopene (mg/100g) | 293.58 | 307.99 |

*ME was calculated according to Lodhi *et al*. [1976].

(25°C). With the beginning of the experiment, fruit powder was added to the basal diet in various levels. Pure lycopene powder used in this experiment was purchased from Shaanxi Jintai Biological Engineering Company (China). The components and the chemical composition of the basal diet is shown in Table 1. The chemical analysis of TOM and RBP fruit powders was carried out according to AOAC [2006], while the amount of lycopene was estimated according to the method of [Markovic *et al.* 2006] (Tab. 2). Quail chicks were fed a starter diet until 21 days of age, followed by a finisher diet from 22 to 49 days. All chicks were kept under uniform management conditions throughout the experiment period of 49 days.

#### Evaluation of biochemical parameters

At 49 days of age three birds were slaughtered and blood samples (5 ml) were taken from each treatment (chick/replicate) randomly. **B**lood samples were collected and allowed to clot at room temperature (25°C) for 1 h prior to serum collection. The serum was separated by centrifugation and stored at -20 C° for further analysis. Blood serum glucose, cholesterol and triglyceride concentrations were determined according to the methods of Tietz [1999] using commercial kits (Biolabo AS, France). The concentration of high density lipoprotein cholesterol (HDL-c) in the serum was estimated according to [Warnick and Wood 1995].The low density lipoprotein cholesterol (LDL-c) was estimated as the difference between total cholesterol and high density lipoprotein with triglyceride content divided by five following the equations described by [Friedewald *et al.* 1972, Wilson 1998]. The activities of serum liver enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were measured using diagnostic kits (QCA, Amposata, Spain). Activities of antioxidant enzymes: glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and catalase (CAT), were measured according to the method described by Wheeler *et al.* [1990], while the level of malonyldialdehyde acid (MDA) was estimated according to the method of Buege and Aust [1978].

#### Statistical analysis

All data were subjected to an ANOVA procedure of SPSS [2012]. Significant treatment means were separated by using the Least Significant Difference (L.S.D.) test [SPSS 2012]. A P-value of less than 0.05 was considered statistically significant.

### Results and discussion

#### Blood parameters

Table 3 shows the effects of dietary natural (TOM and RBP) and synthetic lycopene (Ly) supplementation on quail blood parameters at 49 days of age. Serum glucose was significantly ($p \leq 0.05$) increased in the T2, T3 and T4 dietary groups in comparison with the control and the other supplemented treatments. The serum lipid profile of quails showed significant reductions ($p=0.003$) in the levels of total cholesterol (TC) (groups

**Table 3.** Effects of dietary natural and synthetic lycopene supplementation on quail glucose and lipid profile levels at 49 days of age

| Dietary treatment | Parameter (mg/dl) | | | | | |
|---|---|---|---|---|---|---|
| | glucose | total cholesterol | triglycerides | HDL-c | LDL-c | VLDL-c |
| T1 | 144.57$^{b}$ | 128.20$^{a}$ | 229.97$^{c}$ | 28.52$^{b}$ | 45.81$^{a}$ | 45.99$^{a}$ |
| T2 | 161.23$^{a}$ | 123.40$^{ab}$ | 155.10$^{b}$ | 29.04$^{b}$ | 34.40$^{b}$ | 31.01$^{b}$ |
| T3 | 163.64$^{a}$ | 110.90$^{c}$ | 151.97$^{b}$ | 85.25$^{a}$ | 17.10$^{c}$ | 30.39$^{b}$ |
| T4 | 174.82$^{a}$ | 113.03$^{bc}$ | 156.00$^{b}$ | 85.50$^{a}$ | 15.30$^{c}$ | 31.19$^{b}$ |
| T5 | 159.23$^{ab}$ | 106.78$^{c}$ | 150.48$^{b}$ | 72.74$^{a}$ | 15.50$^{c}$ | 30.08$^{b}$ |
| T6 | 137.67$^{bc}$ | 106.16$^{c}$ | 103.40$^{c}$ | 83.57$^{a}$ | 34.38$^{b}$ | 17.74$^{c}$ |
| T7 | 137.68$^{bc}$ | 102.25$^{c}$ | 128.70$^{bc}$ | 76.12$^{a}$ | 33.92$^{b}$ | 25.74$^{c}$ |
| *P*< | 0.001 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 |
| SEM | 3.415 | 2.339 | 8.635 | 5.592 | 2.540 | 1.908 |

$^{abc}$Means in the same column with no common superscript are different significantly at p≤0.05. SEM – standard error of the mean. T1 – control; T2 and T3 – tomato fruit powder at 17 and 34 g/kg in basal diet; T4 and T5 red bell pepper at 16.23 and 32.46 g/kg in basal diet, which was equivalent to 50 and 100 mg /kg lycopene, respectively; T6 and T7 – lycopene powder at 50 and 100 mg/kg in basal diet, respectively.

T3-T7), *triglycerides (TG),* low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) in all the dietary groups compared with the control as a result of the inclusion of TOM, RBP and LY to quail diets. However, the level of high density lipoprotein cholesterol (HDL-c) was increased (p<0.001) in the supplemented treatments compared to the control and T2 (17 g/kg TOM) (Tab. 3).

The results of this study indicated that supplementation with TOM and RBP fruit powders as well as LY at 34, 32.46 (g/kg) and 50 (mg/kg), respectively, to the quail diets improved serum concentrations of cholesterol, TG, LDL-c and VLDL-c by lowering the concentrations of these parameters in blood. These results support the research conducted by other authors, who reported significantly higher plasma glucose [Al-Janaby 2015] and lower cholesterol, LDL-c and VLDL-c concentrations [Sahin *et al*. 2006, Englmaierová *et al*. 2011, Palozza *et al.* 2012, Mulkalwar *et al*. 2012, Lee *et al*. 2016, Mezbani *et al*. 2019], triglycerides [Hosseini- Vashan *et al*. 2016] in the birds and rabbits that received tomato and tomato derivatives and lycopene in diets. With respect to HDL-c, Sun *et al*. [2015] collected data *in ovo* inclusion of lycopene in hatching eggs and showed that lycopene increases immune organ indices and serum high density lipoprotein cholesterol as well as regulates lipid metabolism in chicks. In contrast to the present study, Frederiksen *et al*. [2007] showed that dietary supplementation with an extract of lycopene rich tomatoes had no effect on cholesterol and triglyceride concentrations determined in the rabbit plasma. Similarly, in a crossover study Abete *et al*. [2013] reported that the daily consumption of 160 g of two tomato sauces (high-lycopene tomato sauce and commercial tomato sauce) did not change significantly such general biochemical variables as total cholesterol, LDL-c, HDL-c, triglycerides, insulin and glucose during both tomato sauce supplementation periods. The reduction in serum cholesterol levels in quails supplemented with TOM and RBP fruit powders and pure LY may be related to a decrease in cholesterol synthesis through the inhibition of the activity and expression of 3-hydroxy-3- methylglutaryl Coenzyme A (HMG-CoA) reductase, modulation of the LDL receptor, as well as the inhibition of acyl-coenzyme A: cholesterol acyltransferase (ACAT) activity [Palozza *et al.* 2012]. On the other hand, several studies indicated that red pepper could decrease total cholesterol, triglyceride and LDL levels and increase HDL levels. The hypolipidemic effect of red peppers may be related to several factors, including the activation of peroxisome proliferator-activated receptor α (PPARα) [Mueller *et al.* 2011], reduced intestinal absorption of cholesterol and elevation of cholesterol and bile acid excretion in the feces [Negulesco *et al*. 1987]. Similarly, the improvement of lipid serum parameters for supplementation groups has been associated with high concentrations of lycopene in the blood, which was confirmed by an increase in the concentration of antioxidant enzymes in this study.

This resulted in reducing oxidative stress by absorbing free oxygen and reducing oxidation of low-density lipoproteins (LDL), which act as cholesterol transporters, or improving the function of high density lipoproteins (HDL) [Iyawan 2018]. The most important mechanisms of lycopene acting as an antioxidant reduce LDL-c cholesterol levels through an increase in the activity of LDL-c receptors and in cholesterol levels of HDL and LDL-c oxidation resistance [Zhu *et al.* 2011, Di-Tomo *et al.* 2012].

**Antioxidant status variables**

Data in Table 4 showed that the activity of hepatic enzymes (ALT and AST) and MAD (an indicator of lipid peroxidation) concentration in blood decreased in the treated groups compared to the control, whereas the activity of antioxidant enzymes:

**Table 4.** Effects of dietary natural and synthetic lycopene supplementation on quail hepatic and antioxidant enzyme activity at 49 days of age

| Dietary treatment | Parameter | | | | | |
|---|---|---|---|---|---|---|
| | ALT (U/l) | AST (U/l) | GSH-PX (U/l) | OD (U/l) | Catalase (U/l) | MAD (μmol/l) |
| T1 | 255.00[a] | 243.33a | 21.72[a] | 2.62 | 2.41d | 0.30[d] |
| T2 | 170.00[b] | 182.00b | 10.72[c] | 3.63 | 5.29ab | 0.66[bc] |
| T3 | 185.00[b] | 201.00b | 11.27[cd] | 3.55 | 4.59b | 0.74[ab] |
| T4 | 184.33[b] | 185.67b | 12.27[cd]1.01 | 3.13 | 5.65a | 0.88[a] |
| T5 | 180.66[b] | 188.00b | 9.77[d] | 3.44 | 3.64c | 0.81[ab] |
| T6 | 176.00b | 194.33b | 14.16[bc] | 2.88 | 5.86a | 0.53[c] |
| T7 | 188.33[b] | 187.67b | 17.44[b] | 3.32 | 4.36bc | 0.61[bc] |
| SEM | 6.282 | 5.181 | 0.959 | 0.131 | 0.267 | 0.044 |
| P< | <0.001 | 0.003 | < 0.001 | 0.356 | <0.001 | <0.001 |

[abcd]Means in the same column with no common superscript are different significantly at p≤0.05. SEM – standard error of mean; AST – aspartate aminotransferase; ALT – alanine aminotransferase; GSH-PX – glutathione peroxides; SOD – superoxide dismutase; ATB – thiobarbituric acids; T1 – control; T2 and T3 – tomato fruit powder at 17 and 34 g/kg in basal diet; T4 and T5 – red bell pepper at 16.23 and 32.46 g/kg in basal diet; which was equivalent to 50 and 100 mg /kg lycopene, respectively; T6 and T7 – lycopene powder at 50 and 100 mg/kg in basal diet, respectively.

glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) increased significantly ($p \leq 0.05$), while that *of catalase* enzyme was not affected among the treatments, when TOM, RBP and pure lycopene were supplemented in the quail diets. The lower levels of liver enzymes (ALT and AST) indicate the better health of quail chicks. In the current study the decline in the concentrations of liver enzymes may be due to the anti-oxidative effect of lycopene from its natural and synthetic sources.

Similar results were recorded by Mezbani *et al.* [2019] in their study on broilers showing decreased serum activity alanine aminotransferase activity in lycopene supplemented groups (100 mg/kg) compared to the control**.** Our results revealed that lycopene from natural and synthetic sources increased GSH-PX and SOD activity compared with those in the control quails. These results indicated that tomato, red bell pepper and lycopene supplementation enhanced anti-oxidant enzymatic activity in the serum. Similar results have also been reported in quails and broilers (under heat stress conditions) [Sahin *et al.* 2011, 2016, Hosseini-Vashan *et al.* 2016]. Moreover, the results are consistent with the findings of Sun *et al.* [2014 ], who reported that breeding hens fed rice-soybean basal diets supplemented with different lycopene levels (0, 20, 40 and 80 mg/kg) showed significantly ($p<0.05$) increased serum SOD, glutathione peroxidase and GSH/GSSG levels. This increase may be due to the positive role of lycopene (from both natural and synthetic sources) in maintaining oxidative balance in birds through a variety of mechanisms, including serving as a free radical scavenger, inhibiting signaling pathways and activating host antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase [Arain *et al.* 2018]. On the other hand, lycopene, being a member of the carotenoid family and mainly found in tomato, is a strong antioxidant that prevents cell destruction caused by reactive oxygen species (ROS) [Mezbani *et al.* 2019]. According to Sahin *et al.* [2008], lycopene enriched tomato powder alleviated heat stress through a reduction of malondialdehyde (MDA) levels in the serum, liver as well as the leg and breast muscles in Japanese quails. Those authors attributed the decrease in oxidative stress to the effect of compounds found in tomato powder, which might have a role in diminishing the stress effects thanks to their antioxidant effect. Malondialdehyde is one of the most frequently used indicators of lipid peroxidation associated with oxidative stress [Sahin *et al*. 2008]. Similar results were reported recently [Mezbani *et al*. 2019], showing that supplementation of lycopene (100 mg/kg) significantly decreased serum MDA concentration in broilers. In turn, for the egg yolk MDA concentration Omri *et al.* [2019] reported that a diet supplemented with linseed (4.5%) plus a sweet red pepper and tomato mix (2%) caused no increase in egg yolk MDA concentration before and after 1-month storage at 4 C°. Contrary to the current findings, Sun *et al.* [2014] reported that the MDA level was not affected by lycopene in breeding hens.

## Conclusion

The supplementation of tomato and red bell pepper fruit powders as well as pure lycopene at 34, 32.46 (g/kg) and 50 (mg/kg), respectively, improves the lipid profile and the serum antioxidant status (GSH-PX and SOD), while it reduced the levels of liver enzymes and lipid peroxidation biomarker (MDA) in Japanese quails.

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