

Synergistic Effect of Raw *Tribulus terrestris* With Vitamin E on Some Biochemical and Physiological Parameters in local Male Rabbits

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Abstract

This study was designed to investigate the synergistic effect of adding *Tribulus terrestris* with vitamin E to the feeding of domestic male rabbits on some biochemical and physiological traits. Where the experiment was conducted in the animal house at the Faculty of Veterinary Medicine, University of Baghdad, for 60 days from January until the end of February 2022. The experiment was carried out using 28 domestic rabbits 3 to 4 months of age, were divided into four groups, all animals were fed the same traditional feed mixture - the first control group T1, the 2nd group T2 received (*Tribulus terrestris*) , 3rd group T3 received *Tribulus terrestris* with vitamin E and 4th group T4 only received vitamin E. The dose of *Tribulus terrestris* was 1 g /animal /daily while the dose of vitamin E was 60 mg/ animal/daily for 8 weeks. The results are shown the addition *Tribulus terrestris* with or without vitamin E increased significantly ($P<0.05$) in hemoglobin levels also hematocrit while non-significant differences in leukocytes, erythrocytes count and glucose concentration, while that the total protein, albumin and globulin increased significantly ($P<0.05$) in rabbits as compare with control group. From results concluded that the *Tribulus terrestris* plant and vitamin E have a positive effect on some blood traits and serum proteins.

Keywords: Hemoglobin Rabbits , *Tribulus terrestris*, vitamin E, Blood parameters.

التأثير التآزري لنبات الكطب و فيتامين هـ في بعض المعايير البيوكيميائية والفيسيولوجية في ذكور الأرانب المحلية

الخلاصة

صممت هذه الدراسة لمعرفة التأثير التآزري لإضافة نبات الكطب الخام و فيتامين هـ في تغذية ذكور الأرانب المحلية على بعض الصفات البيوكيميائية والفيسيولوجية. أجريت التجربة في البيت الحيواني في كلية الطب البيطري. جامعة بغداد. لمدة 60 يومًا من يناير حتى نهاية فبراير 2022. تم استخدام 28 أرنبًا محليًا تتراوح أعمارهم بين 3 إلى 4 أشهر ، وقسمت إلى أربع مجاميع متساوية ، تم تغذية جميع الحيوانات بنفس خليط العلف التقليدي - المجموعة الأولى T1 مجموعة سيطرة ، المجموعة الثانية T2 اعطيت نبات الكطب الخام ، المجموعة الثالثة T3 اعطية نبات الكطب الخام مع فيتامين هـ ، بينما المجموعة الرابعة T4 اعطيت فيتامين هـ فقط. كانت جرعة نبات الكطب الخام 1 غم / حيوان / يوميًا بينما كانت جرعة فيتامين هـ 60 ملغم / حيوان / يوميًا لمدة 8 أسابيع. أظهرت النتائج أن إضافة نبات الكطب الخام مع أو بدون فيتامين هـ زادت معنويًا ($P<0.05$) من مستويات الهيموجلوبين وكذلك الهيماتوكريت بينما لم تكن معنوية في اعداد كريات الدم البيضاء و الحمراء و ايضا الكلوكوز ، فيحين حصلت زيادة معنوية ($P<0.05$) للبروتين الكلي والألبومين والكلوبيولين مقارنة بمجموعة السيطرة . نستنتج من هذه الدراسة أن نبات الكطب و فيتامين هـ لهما تأثير إيجابي في بعض صفات الدم وبروتينات المصل.

Introduction:

The antibiotics has resulted in negative impact on animal health such as bacterial antibiotic resistance and tolerance, as well as residual drugs in animal products. This situation represents great future danger to healthy of human (1,2). Medicinal plants can be used as a dietary supplement and play a role in supporting the performance and health of animals. The beneficial effect of the content of these herbs may include, An effective role in nutrition, such as stimulating appetite, eating feed, and stimulating immune system, antibacterial, antioxidants and antihelminths. In practical application they have great potential seen (3,4).

Tribulus terrestris (TT) is a common herbal plant with different categories that grows in many countries of the world (5). TT, an annual herb with a prostrate hairy structure and a tolerance to drought that grows in the summer and is also known as puncture vine, caltrop, and "Qutub" in Arabic, is a member of the Zygophyllaceae family (6,7). It can be found across the world's tropics and subtropics (8,9), as well as in many of Iraq's regions (Baghdad, Sulaimaniya, Mousil, Rutba, and Habbania, as well as the Al-Najaf desert) (8,9).

One of the most commonly utilized aphrodisiacs today is *Tribulus terrestris*. It is renowned for its capacity to treat impotence, sexual dysfunction, and hormonal imbalance in animals. It has grown in popularity and is currently one of the most researched medicinal

herbs due to the number of its active components that may be utilized to treat sexual disorders in the plant (5). The effects, mechanisms of action, and applications of *Tribulus terrestris*, a plant that has been used as a folk cure for thousands of years, still need to be well understood. Physiology and general health are also affected (10).

Vitamin E supplementation, either dietary or oral, is critical for lowering cholesterol levels and boosting antioxidant status in animal, reducing lipid oxidation in dairy, egg, and muscle products is also important. Consumers can benefit nutritionally from vitamin E-enriched animal products' bioavailability, particularly in resource-constrained places where vitamin E shortage puts some cellular functions at risk. The effects of vitamin E supplementation as an antioxidant on livestock performance and animal products must therefore be the primary focus of research (11). Growth, haematology, and the serum biochemical profile of rabbit bucks can all be noticeably improved by including vitamin E in their diet feed in a different amount (12).

Many researches find that medicinal plants and their essential oils or components in general have a positive effect on public health as well as the productive performance of the organism. It was found that adding many of them to the feed mixture improved the final weight of the experimental animals, the daily weight increase and the feed conversion factor (13,14). Therefore, in view of the important role that *Tribulus terrestris* plays in the health of animals, it also has

medicinal properties and is widely spread in Iraqi farms and the importance of food additives in improving the performance of animals and the importance of vit. E in health of animals. The objective of this experiment was to investigate in the effect of *Tribulus terrestris* with the vitamin E on some physiochemical properties in local male rabbits. The results

Material and methods:

Experimental animals and management:

Twenty-eight rabbits at the age of 3 to 4 months at first, a body weight range 0.820 - 0.868 kg was used. Clinical tests were conducted on all animals to ensure that they are free from any abnormal condition. Individual cages to house rabbits (50 cm x 50 cm x 40 cm) were used in this study, which lasted for 60 days from January until end of February 2022. This was preceded by a two-week as adaptation period, daily feeding of ready-made forage, with adding *Tribulus terrestris* 1gr and vitamin E 60 mg/animal, as powders, and water adlib.

Experimental design and treatments:

The rabbits that were used in this experiment were distributed as follows: First group (T1). was daily fed on ration and kept as control group. Second group (T2).was daily fed on the same ration with the addition the *Tribulus Terrestris* 1g in each animal /daily. Third group(T3). was daily fed on the same ration with the addition the *Tribulus Terrestris* 1g with Vit. E.(oraly) 60 mg in the daily ration of each animal. Fourth

group(T4). was daily fed on the same ration, with the addition of the Vit. E. 60 mg in the daily ration of each animal.

Parameters of study:

Using disposable, sterile syringes, blood samples were collected from the heart (heart puncture). It was sanitized in the area. Three milliliters of blood were drawn, divided into two parts, and one milliliter of the first component was retained in specialized tubes with anticoagulants called EDTA to determine hemoglobin levels. PCV, total WBCc count and RBCc, blood glucose, The remaining portion of the blood samples (2 ml), which was devoid of anticoagulants, was kept in a sterile tube, to estimate concentration of total protein ,albumin and globulin, and testosterone hormone, were estimated . Sera separation samples of all animals were done by using centrifuge (3000 rpm) for 5 minutes and preserved in freezing at (-16 °C - 20 ° C) for later use, that's done at the Meat Hygiene Laboratory, in public health of College of Veterinary Medicine. Hemoglobin concentration(Hb) according to (15), packed cell volume (PCV) was calculated using the spectrophotometric method, while (15) calculated PCV using microhematocrits. using the method of estimation, the total number of white and red blood cells was (15), The blood sugar level was calculated using (16). Albumin content was determined in accordance with (17), Globulin concentration was determined in accordance with (18), and Total protein concentration was tested

using biuret technique (19).

Statistical analysis

The results obtained were analyzed by using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means $P < 0.05$ is considered statistically significant (20).

Results and Discussion

Blood parameters and biochemical:-

Blood hemoglobin (Hb) g/dl:-

The level of Hb in several rabbit groups increased steadily as they aged, while it remained within the normal range seen in (table 1), the treated groups showed significantly ($P < 0.05$) higher values than the control group at zero time. While, T2, T3 demonstrated significantly ($P < 0.05$) higher than other group in one and second month.

Table (1) Effect of *Tribulus terrestris* and vit.E in Hb g/dl of male rabbit (Means \pm SE).

Hb	Zero time	1 month	2 month
T1	A11.64 \pm 0.71 b	AB12.02 \pm 0.68 c	A12.26 \pm 0.63b
T 2	A13.82 \pm 0.44 a	A14.32 \pm 0.28a	A14.72 \pm 0.27a
T 3	A13.88 \pm 0.45 a	A13.68 \pm 0.54a b	A14.02 \pm 0.28a
T 4	A12.06 \pm 0.61 b	A12.54 \pm 0.34b c	A12.70 \pm 0.28a b
LS D	1.40		

The different small letter in the same column are significantly different ($P < 0.05$), a different capital letter in the same row are significantly different ($P < 0.05$)

Packed cell volume (PCV)%:-

Result of different groups illustrated significant ($P < 0.05$) differences in the means of PCV percentage (table 2), Shown no significant differences between months. While, among groups, the T2 and T3 that demonstrated significantly ($P < 0.05$) higher values than other groups T1 and T4 in one and second month period of experiment

Table (2) Effect of *Tribulus terrestris* and vit.E in the diet on blood packed cells volume (PCV %) of local male rabbit (means \pm SE).

PC V	Zero time	1 month	2 month
T1	A40.44 \pm 1.86 a	A36.68 \pm 2.00 b	A37.62 \pm 1.67c
T2	A41.68 \pm 1.19 a	A42.60 \pm 0.62 a	A44.56 \pm 1.08a
T3	A42.84 \pm 0.83 a	A42.22 \pm 1.32 a	A41.82 \pm 0.77a b
T4	A40.96 \pm 1.67 a	A37.38 \pm 1.32 b	A38.98 \pm 1.04b c
LSD	3.85		

The different small letter in the same column are significantly different ($P < 0.05$), a different capital letter in the same row are significantly different ($P < 0.05$)

Total Red blood cell count (RBCs):-

Results of different groups showed no significant in number of RBCs at all times period. While, observe the slightly and gradually increased in all groups with age progress, in values of RBCs count with no significant differences between groups. Illustrate that in table (3).

Table (3) Effect of *Tribulus terrestris* and vit.E in the diet on the total red blood cells counts (cells /mm³) of local male rabbit (means \pm SE).

RBC	Zero time	1 month	2 month
T1	5.69 \pm 0.18	5.98 \pm 0.23	6.47 \pm 0.19
T2	6.28 \pm 0.20	6.43 \pm 0.15	6.76 \pm 0.11
T3	6.15 \pm 0.07	6. 47 \pm 0.22	6.49 \pm 0.25
T4	6.34 \pm 0.29	6.14 \pm 0.31	6. 59 \pm 0.24
LSD	0.62		

All differences were not significant in mean values.

Total white blood cell count (WBCs):

Results of different groups showed no significant differences in number of total WBCc. Despite to slightly and gradually increased in all groups with age progress, and no significant differences found between groups (table 4).

Table (4) Effect of *Tribulus terrestris* and vit.E in the diet on the total white blood cells counts (cells /mm³) of local male rabbit (means \pm SE)

WBC	Zero time	1 month	2 month
T1	5.37 \pm 0.70	5.80 \pm 1.16	5.88 \pm 1.13
T2	4.57 \pm 0.47	4.50 \pm 0.36	5.21 \pm 0.49
T3	5.54 \pm 1.12	5.24 \pm 0.98	6.52 \pm 1.13
T4	5.00 \pm 0.71	4.71 \pm 0.91	5.01 \pm 0.96
LSD	2.50		

All differences were not significant in mean values.

From the tables (1,2,3 and4) the total mean of (Hb) g/dl (PCV %) (RBCs) and (WBCs).The values were in the range of normal values reported by (21,22), But, the difference between the means of the all experimental groups were significant in (Hb) g/dl and (PCV %) and non-significant in (RBC) (WBC) were recorded in T2 and T3 as compared with the T4 and control groups . Hematological, markers could be utilized to recognize the probable nutritional effects on the health of farm animals (23, 24,25). (Hb) g/dl and (PCV %) were recorded a significant differences in T2 and T3 as compared with T4 and T1control groups, that's may be TT and TT with Vit E had apositive effect on the nutritional value and absorption of iron. Although some researchers have indicated that there is a more effect of TT in the diet of animals on amelioration of hematology. Confirmation,by Magouz *et al.*(26) The improved PCV and Hb values indicated that the animals are free of anemia and this is one of the factors that reflect the picture of healthy nutrition nutrition of rabbit fed vitamin E supplementation with TT.

Furthermore, the progressive increase in blood components such as Hb and PCV for all groups might be attributed to the rabbits being well managed and fed. the treatment groups showed a significant (P0.05) increase in age progression as compared to the control group, according to some researchers, vitamin E has a beneficial effect on increasing nutrient absorption through the intestine with increased feed intake as a result of an increase in appetite, which reflects

more metabolic activation in the animals. This effect may be related to the role of *tribulus terrestris* and vitamin E. Additionally, these feed additions have significant levels of crude protein and iron, which improve blood chemistry (27,28). While vitamin E plays an important role in hemoglobin synthesis and antioxidant protection, *tribulus terrestris* feed additives include a significant quantity of flavonoides, which promote vitamin E's action as an antioxidant against free radicals, as some researchers have pointed out (29,30,31). Numerous studies revealed an association between feed additives and packed cell volume, hemoglobin, and red blood cell count that was positive (12). That makes sense because dietary vitamin E is known to enhance erythropoietic factors, which enhances packed cell volume, hemoglobin, and red blood cell concentrations (32). This outcome is consistent with that of Abdel-Khalek *et al.*(33), who found that pregnant and lactating rabbit does receiving 20 mg of dietary vitamin E per kg DM had higher packed cell volume, hemoglobin, and red blood cells. However, In contrast to this conclusion, Shaibu (34) found no appreciable differences in packed cell volume, hemoglobin, or red blood cells in rabbit does receiving 40 mg of vitamin E per kg of diet. This may be accounted for due to differences in the sex of rabbits used in the two studies. Melillo (35); Jenkins (36) whom reported that some hematological parameters ex: red blood cells count and PCV values are influenced by stress, age, gender, season and genus in rabbits; where

anemia is defined as a PCV value below 30% and a decline in PCV that coincides with a rise in HGB (36). Additionally, it has been noticed that an increase in WBC counts in rabbits rarely reflects an infection; as a result, it typically varies according to varied stress factors and blood collection techniques (37,35,36). According to one study (38), the WBC levels of the rabbits housed alone were higher than those of the rabbits fed in groups. The slightly and gradually increasing WBCc of all rabbits with progress experimental periods, could be attributed to the development of all animals' immune systems as they grew older, and these findings were in agreement with those of other researchers who referred to the immune system's welldevelopment as they grew older and the presence of microorganism in the GIT (39,40). Therefore, the absence of significant differences in the total number of red and white blood cells in the results of this experiment indicates that the experimental animals were in good health during the course of the experiment in all groups. This confirms that the materials used in the experiment are free from toxicity and that the dose used of the plant that was consumed by the experimental animals was safe and had a positive effect.

Glucose:-

Results of all groups recorded fluctuated in blood glucose concentration show in (table 5). there are no significant differences between groups also with in periods in same group. But, the T2 during progress months of the experiment recorded less value in mean blood glucose

concentration in 2nd month as compared with zero time period .

Table (5) Effect of the *Tribulus terrestris* and vit. E in diet on blood glucose concentration (g /dl) of local male rabbit (means \pm SE).

Glucose	Zero time	1 month	2 month
T1	75.20 \pm 4.67	76.72 \pm 4.65	74.24 \pm 4.76
T2	A80.84 \pm 1.80	AB75.40 \pm 2.81	B67.84 \pm 2.86
T3	75.54 \pm 1.45	84.30 \pm 2.77	70.12 \pm 2.95
T4	76.84 \pm 5.39	78.86 \pm 2.51	68.54 \pm 2.14
LSD	9.85		

The different capital letter in the same row are significantly different (P<0.05)

Although there were no significant differences between the treated groups and the control group, the second group recorded a significant (P<0.05) decrease in blood sugar at the end of the experiment as compared with the start of the experiment as we seen in (table 5). This is due to the positive role of the plant in lowering the level of blood sugar, which is considered as insulin like hormone, according to some researchers indicated (41). While feeds rich in vitamin E helps to maintains blood sugar within the normal level (42). This is a confirmation of the results in this study that the vitamin E diet has maintained the normal level of blood sugar so that it was close to the control group throughout the experiment period. Other

research utilizing hyperglycemic mice discovered that consuming TT fruit extracts brought blood sugar down to a normal range (43). However, in an experiment employing TT fruit extracts on lab animals, a considerable drop in blood sugar was observed, and it was confirmed that these fruit extracts may have a hypoglycemic impact (44). According to Li *et al.* (45), saponins had a hypoglycemic impact by lowering the level of serum glucose. El-Tantawy and Hassanin (46) and Bonakdaram *et al.* (47) successfully treated various forms of diabetes with TT plant pieces, and the blood sugar returned to normal. El-Shaibany *et al.* (48) noted that rabbits were hyperglycemic after ingesting TT methanol extract.

Regarding to the vitamin E, observed in this study conditions the vit. E did not produce any significant effect on blood glucose levels, Despite its active role in maintaining the health of secretory cells from oxidative stress. Administration of Vit. E exhibited normal levels of blood glucose in this study, that's confirmed with researchers Verma and Singh (49). they findings indicate that vitamin E improves oxidative stress and hepatocellular function. Although insulin resistance also improves and can be works to maintain the normal level of blood sugar.

Total protein concentration:

The total protein was slightly and gradually increased with age progress up to the end of the experimental period for all groups in (table 4.6),

but the treated groups showed significantly ($P<0.05$) higher values in the end of periods than zero time, except the control group was not recorded significant differences. While the differences among groups showed in first and second month in treated groups recorded increased significantly ($P<0.05$) as compared with control group. (67.68 ± 0.76)(67.12 ± 2.22)(66.62 ± 1.07) (58.38 ± 2.66) respectively.

Table (6) Effect of *Tribulus terrestris* and vit.E in the diet on total serum protein concentration (g/dl) of local male rabbit (means \pm SE).

TP	Zero time	1 month	2 month
T1	A58.32 \pm 3.51 a	A54.70 \pm 3.11b	A58.38 \pm 2.66 b
T2	B60.10 \pm 2.62 a	B60.14 \pm 1.51ab	A67.12 \pm 2.22 a
T3	B59.68 \pm 1.19 a	A65.52 \pm 0.76a	A67.68 \pm 0.76 a
T4	B59.88 \pm 1.06 a	AB64.08 \pm 1.29 a	A66.62 \pm 1.07 a
LS D	5.80		

The different small letter in the same column are significantly different ($P<0.05$), a different capital letter in the same row are significantly different ($P<0.05$)

Globulin concentration:-

Results of different groups showed significant ($P<0.05$) differences in the means of globulin concentration in table (7) during zero, 1st and 2th experiment months with the progressive

experiment periods all groups demonstrated no significant differences with progress periods as compared with previous periods. Regarding the differences among groups within each month, the treated groups recorded significantly ($P<0.05$) higher in means of serum concentration than control group at 1th, 2th month.

Table (7) Effect of *Tribulus terrestris* and vit. E in the diet on serum globulin (g/dl) of local male rabbit (means \pm SE).

Globulin	Zero time	1 month	2 month
T1	A28.28 \pm 3.0 1a	A24.66 \pm 2.46 b	A25.90 \pm 1.7 2b
T2	A30.56 \pm 2.3 8a	A28.10 \pm 1.31 ab	A32.98 \pm 2.0 3a
T3	A28.84 \pm 0.8 4a	A31.30 \pm 0.47 a	A33.10 \pm 0.3 9a
T4	A30.94 \pm 0.9 2a	A31.00 \pm 0.98 a	A33.08 \pm 0.8 3a
LSD	4.73		

The different small letter in the same column are significantly different ($P<0.05$), a different capital letter in the same row are significantly different ($P<0.05$)

Albumin concentration:-

The results of different groups illustrated significantly ($P<0.05$) increasing in the means of serum albumin concentration (table 8) within group, during 2nd month period showed increasing in the means of serum albumin concentration in all experimental groups as

compared with other experiment periods. Concerning the differences among groups within each period, T3 and T4 recorded the significant increasing through 1 month period than other groups in serum albumin values, while no significant differences between groups at 2 month of experimental periods.

Table (8) Effect of *Tribulus terrestris* and vit. E in the diet on serum albumin (g/dl) of local male rabbit (means \pm SE).

Albumin	Zero time	1 month	2 month
T1	A30.04 \pm 1.0 6a	AB30.04 \pm 1.33 b	A32.48 \pm 1.2 4a
T2	B30.60 \pm 0.7 0a	AB32.04 \pm 0.97 ab	A34.14 \pm 1.0 0a
T3	B31.84 \pm 0.7 6a	AB34.22 \pm 0.58 a	A34.58 \pm 0.5 7a
T4	B31.29 \pm 1.2 7a	AB33.08 \pm 0.59 a	A33.94 \pm 0.4 4a
LSD	2.64		

The different small letter in the same column are significantly different ($P < 0.05$), a different capital letter in the same row are significantly different ($P < 0.05$)

The data in the tables (6,7 and 8) represented the means of total protein, Albumin, globulin, were in significant differences in their means of different groups in first half of experimental period. According to Almusawe and Jubouri(50) this is supported by experimental

data that shows that the onset was dispersed equally. They noted in their research that while the starting point or baseline was very identical across all studies, there were no significant changes in any parameter throughout the first phase of the investigation. The fact that total protein and its components were higher in all groups at the conclusion of the experiment than they were at the beginning may be because the experimental animals were in a stage of growth that is marked by an increase in metabolic rate. Baungarther and Pernthaner (51) reported similar findings. It's possible that an increase in metabolic rate with aging or the fact that the rabbits were developing in all groups contributed to the rise in total protein and its constituents. Additionally, Sahin et al. (52) and Shaheen, (53) reported that when animals are in the early stages of growth, feed additives have no discernible effects. On the other hand the significant ($P < 0.05$) differences in their means of total protein and its components in all treated group as compared with control group in end period of experimental could be due to the animals feeding with feed additives (*tribulus terrestris* or Vit. E) its have a direct or indirect role in the process of proteins metabolism, which improves the image of blood serum proteins. Furthermore, there was a significant increase in total protein and its components in the treated groups, this could be due to those animals affected by the feed additives used in this experiment, which have a positive factor that leads to increased feed intake and dietary protein absorption percentage, that's

agreement with AL- Shahat (28) and Mironova *et al.* (27) they found that feed additives play a key function in serum albumin and globulin with increased total protein availability. Many food additives can have appositive role in metabolism of nutrients, especially protein materials during improving the internal environment of the digestive system, which has a positive effect on digestion and absorption, and thus improving the image of blood serum protein (54). Regarding, the importance of vitamin E in the results of this experiment, the fact that feed additives contain vitamin E, which is considered an anti-oxidant agent and has a good effect on humeral immunity, this could explain the increase in globulin in the blood serum, which was indicated by the researcher (55). Although the researcher performed a study on vitamin E on blood biochemical profiles of rabbit bucks given various amounts of vitamin E, there were no significant differences in total protein, albumin, while globulin, differed significantly ($P < 0.05$) across treatments (12). This can be ascribed to dietary vitamin E, which is well known for regulating enzyme activity and serving as an important source of antioxidant in animals (56). In contrast, 200 mg of vitamin E per kilogram of food enhanced the oxidative stability of sperm (57). Vitamin E supplementation, according to Yousef *et al.* (58), lowers free radicals and improves the quality of rabbit meat.

Conclusions:

Conclude from this study that the *Tribulus terrestris* plant and vitamin E have a

positive improvement on some blood traits, serum proteins and globulin in local male rabbits.

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Conflict of interest

The authors have no conflict of interest.

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