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The assessment of zeolite addition to diets with or without urea on some productive and physiological indicators in Awassi lambs

Mohamed Alrez^{1*} , Adel Jammoul¹, Walid Al-Rahmoun¹ and Yaser Al-Omar²

Abstract

Background Interest is growing in the search for alternatives to traditional feed additives, so this study aimed to investigate the effect of adding zeolite to the concentrate diets of Awassi lambs with or without urea on growth performance, nutrient digestion, and health status. A total of 45 Awassi lambs similar in weight (24 ± 2 kg) and age (3 months \pm 4 days) were divided into three groups: the first group (G1) fed on a concentrate diet supplemented with 1% urea; the second group (G2) fed on the concentrate diet supplemented with 1% urea and 3% zeolite, and the third group (G3) fed on a urea-free diet supplemented with 3% zeolite.

Results The results showed a significant increase ($P \leq 0.05$) in final live weight, daily and total weight gain for group G2 compared to groups G1 and G3, with a significant superiority ($P \leq 0.05$) of group G3 over the control group G1. Significant improvement ($P < 0.05$) was also observed in feed consumption and feed conversion ratio in diets supplemented with zeolite for groups G2 and G3 compared to the control group G1. Additionally, there was a significant increase ($P \leq 0.05$) in nutrient digestion and nutritional value in the diet of group G2 containing urea with added zeolite compared to groups G1 and G3. Zeolite did not affect the levels of glucose, triglycerides, cholesterol, and albumin in the blood, but a significant increase ($P \leq 0.05$) in total blood protein level and a significant decrease ($P \leq 0.05$) in blood urea level were noted for groups G2 and G3 compared to the control group G1.

Conclusions Adding zeolite to urea-containing concentrate diets improved growth rates and nutrient digestion, of Awassi lambs. In diets without urea, zeolite improved growth rates without affecting nutrient digestion coefficients, in both types of diets, zeolite increased total protein levels and decreased blood urea levels, with all physiological indicators in lambs blood remaining within normal limits.

Keywords Zeolite, Urea, Growth, Nutrient digestion, Physiological indicators, Lambs

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Background

Dietary supplements are substances that are not found in feed sources, but are added to animal feed in small quantities during mixing or different manufacturing stages to meet the requirements of good nutrition [1]. Zeolite is one of the feed additives used, as it is one of the clay minerals, composed of crystals of alkaline aluminum silicates, as the pores of the homogeneous shape and size of zeolite crystals give important effects and properties to this material, as zeolite has three main properties, namely ion exchange, which is its main industrial application [2],

Adsorption of zeolite with its ability to absorb organic and mineral particles and its catalytic properties [3], due to its characteristics and chemical composition, it has been used in various fields, including animal nutrition, where its addition to animal feed has played an important role in improving feed utilization, increasing its nutritional value, and improving production [4]. Ruminants are characterized by their high ability to utilize non-protein nitrogen compounds in feed, including urea, especially those with high production, to meet the animals' protein needs for sustainability and production [5]. Where the rumen microorganisms hydrolyze urea to ammonia ($\text{NH}_3\text{-N}$) using the urease enzyme, and then convert $\text{NH}_3\text{-N}$ to microbial protein, and work to increase the numbers of these rumen microorganisms, improving the digestion of feed compounds and the amount of microbial protein passing into the small intestine [4]. The specific factor for benefiting from urea is related to the rate of $\text{NH}_3\text{-N}$ release inside the rumen, and when the level and concentration of urea administration to the animal are not regulated, the $\text{NH}_3\text{-N}$ level in the body may reach toxic levels [6]. Zeolite, thanks to its properties, regulates the concentration of $\text{NH}_3\text{-N}$ and the rumen environment [7], as it acts as a reservoir for $\text{NH}_3\text{-N}$ ions resulting from rapidly decomposing nitrogen sources, increasing in size when exposed to moisture, thereby increasing the surface area that absorbs $\text{NH}_3\text{-N}$ resulting from urea breakdown and regulating its release according to $\text{NH}_3\text{-N}$ concentration inside the rumen, reducing $\text{NH}_3\text{-N}$ concentration when it is high in the rumen and gradually releasing it when its level decreases [8]. This improves animal performance by promoting growth, improving feed conversion ratio and nutrient digestion in sheep [9], Increasing milk production and improving its quality [10]. It also maintains the natural values of the blood picture [11], and the level of liver enzyme activity in the blood [12], while adding 2% of zeolite to the lambs' diet led to a significant increase in total protein concentration and a decrease in blood calcium and phosphorus, but within normal limits [9]. Its use also led to a significant decrease in urea and triglycerides in the blood [10]. Due to the importance of using nutritional supplements to achieve the best possible benefit from

nutrition rations, improve nutrient digestion processes, and increase weight gain in sheep, and the use of urea as a main source of nitrogen used in producing microbial protein in the rumen, and the risks resulting from their use, the adoption of any nutritional supplements is linked to maintaining the physiological and health status, and stabilizing them within their natural limits, and enhancing them in line with improving the animal's productive performance. Therefore, the research aimed to study the effect of adding zeolite to the diet of Awassi lambs containing urea, on growth, digestion of nutrients, and some physiological indicators in the blood.

Materials and methods

Animals, treatments and experimental design

The study was conducted using 45 male Awassi lambs, similar of age (3 months \pm 4 days) and weight (24 \pm 2 kg, mean \pm standard deviation), from a private farm for raising sheep in the south of Hama city. Before starting the experiment, the lambs were fed a basal concentrate diet consisting of barley, bran, urea at a rate of 1%, in addition to salt, vitamins, mineral salts, calcium carbonate, and an antifungal for 15-day as an adaptation period. The basal diet was formulated to meet the nutritional needs of the lambs according to guidelines of the national research council (NRC, [13]). During the adaptation period, 5–10% of each meal of the control diet was gradually replaced daily with the treatment concentrate diets until the lambs were completely converted to the new experimental diet. The experimental period lasted for 3 months where lambs were divided using a completely randomized design to the following three treatment concentrate diets in = 15 lamb /treatment: The first group (G1) fed on a concentrate diet supplemented with 1% urea; the second group (G2) fed on the concentrate diet supplemented with 1% urea and 3% zeolite, and the third group (G3) fed on a urea-free diet supplemented with 3% zeolite. The ingredients and chemical composition of the experimental diets were shown in Table 1. The experimental urea feed additive produced by Hebei Zhongchang Fertilizer Co., Ltd (Hebei, China) in granular form with 99% purity was used. The natural zeolite used was obtained from Arab Zeolite and Natural Fertilizers (Syria, Damascus) with the chemical composition and physical properties shown in Table 2.

Zeolite and urea were added in precise proportions and mixed with the rest of the feed components to achieve homogeneity by distributing the additions throughout the feed. All lambs had free access to fresh water and wheat straw, while the experimental concentrate diets were provided twice a day at 7 a.m. and 4 p.m.

During the experimental period, all lambs were placed in sheds that meet the requirements of health care, numbered for ease of monitoring and recording results,

Table 1 Ingredients and chemical composition of experimental diets

Items	Experimental concentrate diets %			
	G1	G2	G3	
Ingredients				
Barley	68	68	64	
Bran	27	27	14	
Urea	1	1	0	
Decorticated cottonseed	0	0	18	
Salt	1	1	1	
Calcium carbonate	1	1	1	
Vitamins and minerals ¹	1.75	1.75	1.75	
Antifungal ²	0.25	0.25	0.25	
Chemical composition			Wheat straw	
Dry Matter	88.46	88.46	89.13	91
Organic Matter	96	96	95.85	93.3
Crude Protein	14.65	14.65	15.23	4.2
Ether Extract	3.55	3.55	3.09	1.4
Crude Fiber	6.57	6.57	7.39	41.5
Ash	4	4	4.15	6.7
Nitrogen Free Extract	69.1	69.1	66.23	46.2
Metabolic Energy Kcal/Kg	2480	2480	2576	997

G1: Control group: feed containing urea without additives. G2: A feed containing urea with zeolite

G3: Feed ration without urea and with zeolite

¹ Each kg contained: vitamin A (2000000 IU), vitamin D3 (500000 IU), vitamin E (2500 mg), iron sulphate (5000 mg), manganese sulphate (1000 mg), copper sulphate (3000 mg), potassium iodide (100 mg), zinc sulphate (5000 mg), cobalt sulphate (50 mg), sliant sodium (100 mg), sodium chloride (25000 mg)

²Contains: Calcium propionate, Benzoic acid, Calcium formate, Citric acid, Calcium lactate, Plant essential oil extracts

Table 2 Chemical composition and physical properties of the natural form of zeolite

Item	Zeolite characteristics
Chemical properties	
SiO ₂	65–72%
AL ₂ O ₃	10–12%
Na ₂ O	0.3–0.65%
K ₂ O	2.3–3.5%
Fe ₂ O ₃	0.8–1.9%
MgO	0.9–1.2%
Ca O	2.5–5.1%
Physical properties	
Appearance	White to pale green powder
Porosity	27–31%
Specific gravity	2100–2350 kg/m ³
Volume density	1650–1775 kg/m ³

vaccinated preventively, and given internal and external parasite control before starting the experiment.

Growth performance

The growth performance was recorded during the period from the beginning of March until the end of June 2022, where the initial weight of the lambs was taken at the beginning of the experiment, and at the end of the

experiment the final weights of the lambs were taken. During the experimental period, the amounts of feed offered and orts were recorded daily, to calculate the daily dry matter intake (DMI) by the difference between the offered and orts. The feed conversion ratio as was also calculated as follows:

Feed conversion ratio = amount of feed consumed (g) / rate of weight gain (g).

Blood analysis

Blood samples were collected from the jugular vein of lambs by sterile medical syringes on the last day of the growth experiment, and placed in tubes that do not contain anticoagulants, then transferred to the laboratory in a container containing ice cubes to avoid blood hemolysis, then the tubes were placed in the refrigerator in a slightly tilted position at a temperature of 4 °C for 24 h. The serum was separated from the rest of the blood components by centrifugation (KL04A-II, Hunan Kaida Scientific Instrument Co., Ltd. Hunan - China) at a speed of 3500 rpm for 15 min and the resulting serum was withdrawn using a sterile medical syringe and placed in clean and sterile test tubes and stored in the freezer at a temperature of (-16) to (-20) °C until analysis. Total protein, glucose, cholesterol, triglycerides, urea and albumin were measured using 10 µl of blood serum with 1 ml of diagnostic calibration kit (Tulip Diagnostics, Gitanjali-India) using a Biochemistry Analyzer from Biosystems, according to the wavelengths and temperatures of each indicator [9, 15].

Digestion experiment

After the fattening experiment was completed, on the first day of July, the digestion experiment began. 12 male Awassi lambs were selected for use in the fattening experiment, with an average age of (6 months ± 4 days) and a weight of (44.5 ± 3 kg). were selected for easy collection of dung and urine. They were divided into three groups, each group containing 4 male lambs, and placed in metabolic cages measuring (1.2 × 0.75) meter. Bags dedicated to collecting dung and urine were attached with special ties, after each meal provided in order to calculate the digestibility coefficients of dry matter, crude protein, ether extract, crude fiber, organic matter, and total digestible nutrients (TDN). The offered and refusals of the diets manure and urine samples were collected and recorded daily and for a 10-day period to calculate the digestibility coefficients for dry and organic matter, crude protein, crude fat, and crude fiber [14]. Chemical analyzes of the feed, urine and feces were conducted following the methods described in reference [14].

Table 3 The effect of zeolite addition to concentrate diets on growth performance of lambs

Growth performance	Experimental groups (Mean ± SD)			P-value
	G1	G2	G3	
Initial weight (kg)	24.52 ± 0.45	24.44 ± 0.21	24.66 ± 0.14	-
Final weight (kg)	42.47 ± 2.04 ^c	48.24 ± 1.53 ^a	45.74 ± 1.34 ^b	0.03
Total weight gain (kg)	17.95 ± 6.47 ^c	23.8 ± 8.58 ^a	21.08 ± 7.59 ^b	0.02
Average daily gain (g)	199 ± 6.32 ^c	264.33 ± 9.99 ^a	234.17 ± 13.44 ^b	0.04
Feed intake (g/d)	983 ± 52 ^b	1213 ± 72 ^a	1172 ± 65 ^a	0.03
Feed conversion ratio	5.90 ± 0.28 ^a	4.32 ± 0.48 ^b	4.74 ± 0.40 ^b	0.01

G1: Control group: feed containing urea without additives. G2: A feed containing urea with zeolite

G3: Feed ration without urea and with zeolite

Different letters a, b, c within the same line indicates significant differences between groups at a level of ($P \leq 0.05$)

Table 4 The effect of zeolite addition to the diets on some blood indicators in lambs

Characteristics	Experimental groups (Mean ± SD)			P-value
	G1	G2	G3	
Glucose mg/dL	66.42 ± 2.70 ^{ns}	67.20 ± 3.86 ^{ns}	65.05 ± 1.40 ^{ns}	0.07
Triglycerides mg/dL	52.13 ± 3.00 ^{ns}	51.94 ± 3.42 ^{ns}	52.01 ± 3.07 ^{ns}	0.16
Cholesterol mg/dL	69.23 ± 3.47 ^{ns}	69.03 ± 4.12 ^{ns}	70.03 ± 5.99 ^{ns}	0.12
Albumin g/dL	3.32 ± 0.42 ^{ns}	3.34 ± 0.25 ^{ns}	3.37 ± 0.29 ^{ns}	0.06
Total Protein g/dL	6.46 ± 0.32 ^b	7.45 ± 0.30 ^a	7.31 ± 0.25 ^a	0.04
Urea mg/dL	19.13 ± 1.96 ^b	12.23 ± 1.45 ^a	12.47 ± 1.35 ^a	0.02

G1: Control group: feed containing urea without additives. G2: A feed containing urea with zeolite

G3: Feed ration without urea and with zeolite

ns indicates that there are no significant differences between the means of the groups within one line at the level ($P \leq 0.05$). Different letters a, b within the same line indicates significant differences between groups at a level of ($P \leq 0.05$)

Statistical analysis

ALL data were analyzed statistically using SPSS 26 software through One Way ANOVA at a significance level of 5%, and performed the Duncan multiple range test to assess the significance of differences between the means [16].

The mathematical model was as follows: $Y_{ij} = \mu + T_i + e_{ij}$.

Where:

Y_{ij} = Individual observation.

μ = The overall mean for the trial under consideration.

T_i = The effect of the i^{th} treatment.

e_{ij} = Random residual error.

Results

Growth and feed intake

The results in the Table 3 indicated a significant increase ($P < 0.05$) for group G2 in terms of final live weight, daily weight gain, and total weight gain of the lambs, compared to the group G1 and group G3. The average final live weights for the three groups were 42.47, 48.24, and 45.74 kg respectively for G1, G2 and G3, and the daily weight gain was 199, 264.33, and 234.17 g respectively, and the total weight gain was 17.95, 23.8 and 21.08 kg for G1, G2 and G3, respectively.

The results also indicate a significant increase ($P < 0.05$) in the amount of concentrated feed consumed by the lambs in groups G2 and G3, with averages of 1213 and 1172 g/day respectively, compared to the control group

G1 with an average of 983 g/d. A significant improvement ($P < 0.05$) was also observed in the feed conversion efficiency by adding zeolite to the lamb diets in groups G2 and G3 compared to the control group G1. The feed conversion efficiency values for G1, G2 and G3 groups were 5.90, 3.24, and 4.74 g per g of feed, respectively.

Lamb blood indicators

The results in Table 4 showed that there was no significant ($P > 0.05$) effect of adding zeolite to the lambs' diets on the levels of glucose, triglycerides, cholesterol, and albumin in the blood, as the glucose levels reached 66.42, 67.20, and 65.05 mg/dL in the blood of the lambs of the experimental groups G1, G2, and G3, respectively, The triglyceride level reached 52.13, 51.94, and 52.01 mg/dL, respectively, and the cholesterol level reached 69.23, 69.03, and 70.03 mg/dL, respectively, The albumin level in the blood of lambs reached 3.32 and 3.34 and 3.37 g/dL, respectively. The results also showed a significant increase ($P < 0.05$) in the level of total protein in the blood of lambs of groups G2 and G3, where it reached 7.45 and 7.31 g/dL, respectively, compared to the control group G1, where the rate reached 6.46 g/dL. The results also showed a significant decrease ($P < 0.05$) in the level of urea in the blood of lambs from groups G2 and G3 whose diets were supplemented with zeolite, reaching 12.23 and 12.47 g/dL, respectively, compared to the control group G1 at a level of 19.13 g/dL.

Nutrient digestibility coefficient and nutritional value of the feed

Table 5 shows significant improvements ($P < 0.05$) were observed in the nutrient digestibility coefficients for lambs in group G2 compared to other groups. The nutrient digestibility coefficients for the G1, G2 and G3 groups were as follows: dry matter digestibility was 75.30%, 81.99%, and 75.38% respectively; organic matter digestibility was 76.81%, 81.58%, and 77.11%; crude protein digestibility was 76.52%, 81.01%, and 75.01%; and crude fiber digestibility was 57.82%, 60.30%, and 55.71%; The digestibility of crude fats was 82.03%, 86.81% and 82.66%, and the total digestible nutrients (TDN) reached 76.94%, 81.62%, and 76.69% for the three groups, respectively.

Discussion

The growth performance results highlight the positive effect of zeolite in improving growth and increasing the daily and total weight gain rate, especially when supplemented to urea-containing concentrate diets. These effects can be explained by the zeolite role in regulating the rumen environment, enhancing the digestion processes of nutrients, and utilizing the $\text{NH}_3\text{-N}$ resulting from the decomposition of urea in the rumen to synthesize microbial protein, thus improving growth. These results are consistent with [9], when zeolite was added at a rate of 1%, 2% and 3% to the diets of Awassi lambs, and consistent with [17], when zeolite was added at a rate of 4% to the diets of lambs, and contradict the results of [18], where no effect was observed of adding zeolite at a rate of 25, 50 and 75 g/day to the diets of lambs on growth and weight gain rate.

The results also showed an increase in feed consumption with the addition of zeolite, as zeolite improves the texture, taste, and utilization of the feed in the rumen, which is consistent with [19–22]. They observed an improvement in the amount of feed consumed when zeolite was added to it 1%, and 0.75%, 1.5%, 2.25%, 3% for cattle and 0.5% for lambs, which contradicts the results of [9], this may be due to the composition of the feed or the breeding conditions. The amount of feed consumed was

not affected when 1%, 2%, and 3% of zeolite was added to the diets of Awassi lambs. Zeolite added to groups G2 and G3 also improved the feed conversion efficiency, as it increased the utilization of $\text{NH}_3\text{-N}$ released in the rumen and improved the rumen environment to facilitate the activity of microorganisms in digesting nutrients. The effect of zeolite on the feed conversion coefficient of the animals of the experimental groups G2 and G3 is consistent with the studies conducted by Nikkha et al., [17]. When adding clay minerals to lamb rations at a rate of 3%, as well as with the results of [23], which reported an improvement in feed conversion efficiency when adding zeolite at a rate of 2 and 4% to lamb rations. In contrast, [9]. No effect was observed for adding zeolite to Awassi lamb diets on the feed conversion efficiency.

Blood physiological indicators are of diagnostic importance for the safety of the various body systems and their performance of their normal functions. through the results of our experiment no effects were observed for adding zeolite to the diets of the experimental groups on the levels of glucose, triglycerides, cholesterol, and albumin in the blood, compared to the control group. This is consistent with what was found by Toprak et al., [9], when adding different proportions of zeolite to the diets of Awassi lambs, and consistent with [24], where no significant effect was observed for adding zeolite to the diets of Awassi lambs on blood indicators, and somewhat consistent with [11], when adding zeolite at a rate of 1.25% and 2.5% to the diets of Holstein cows during the milk production stages. It also agrees with what was reached by El-Nile et al., [25] when using natural zeolite at a rate of 20 g/kg dry matter as a feed additive in the feed of dairy Shami goats, where no effect was observed on the level of cholesterol in the blood.

Measuring the level of total protein in the blood is one of the important indicators of the safety of liver and kidney functions and their performance of their normal functions, as an increase in the level of total protein in the blood was observed in the groups to which zeolite was added compared to the control group, but the level of total protein remained within its normal limits. This

Table 5 The effect of zeolite addition to diets on the nutrient's digestibility and the diet's nutritional value for lambs

Digestibility coefficient (%)	Experimental groups (Mean \pm SD)			P-value
	G1	G2	G3	
Dry Matter	75.30 \pm 5.3 ^b	81.99 \pm 1.5 ^a	75.38 \pm 3.72 ^b	0.03
Organic Matter	76.81 \pm 2.34 ^b	81.58 \pm 1.19 ^a	77.11 \pm 1.61 ^b	0.04
Crude Protein	76.52 \pm 1.9 ^b	81.01 \pm 1.73 ^a	75.01 \pm 2.65 ^b	0.02
Crude Fiber	57.82 \pm 1.06 ^b	60.30 \pm 1.82 ^a	55.71 \pm 1.55 ^b	0.04
Ether Extract	82.03 \pm 1.80 ^b	86.81 \pm 2.05 ^a	82.66 \pm 1.88 ^b	0.03
Total Digestible Nutrients	76.94 \pm 2.19 ^b	81.62 \pm 2.79 ^a	76.69 \pm 2.52 ^b	0.02

G1: Control group: feed containing urea without additives. G2: A feed containing urea with zeolite

G3: Feed ration without urea and with zeolite

Different letters a, b within the same line indicates significant differences between groups at a level of ($P \leq 0.05$)

may be due to the positive effect of zeolite in improving the coefficient of digestibility of crude protein and the utilization of $\text{NH}_3\text{-N}$ released in the rumen and its conversion into microbial protein absorbed from the lining of the rumen and intestine to be secreted into the blood. These results are consistent with [9], where a significant increase ($P < 0.05$) in the percentage of total protein in the blood was found when zeolite was added in different proportions to lambs' diets, and they conflict with the results of [24], where no effect was observed of adding zeolite to Awassi lambs' diets on the level of total protein in the blood.

The results also showed a significant decrease in the level of urea in the blood of lambs in the experimental groups whose diet contained zeolite compared to the control group, and this may be due to the role of zeolite in regulating the level of $\text{NH}_3\text{-N}$ in the rumen, and gradually excreting it when its level decreases, thus reducing its absorption and thus lowering its level in the blood. This is consistent with the results of [11] when zeolite was added at a rate of 1.25% and 2.5% to Holstein cows' diets during the final stages of milk production. While it contradicts the results of [12], where no effect was observed of adding zeolite on the level of urea in the blood of dairy cows.

The results in the current study show that group G2, that fed diet contained urea with the addition of zeolite, showed a significant improvement in the digestibility of nutrients and the nutritional value of the feed. This can be attributed to the fact that the addition of zeolite to the diets containing urea contributed to enhancing fermentation in the rumen, and the absorption of $\text{NH}_3\text{-N}$ resulting from the decomposition of urea in the rumen by the urease enzyme, and its gradual release as needed. This provides a continuous source for microbial protein synthesis by microorganisms in the rumen, and maintaining a suitable environment for their reproduction [26]. Urea is also a source of rapidly decomposing nitrogen for these microorganisms, this is consistent with what was found by Forouzani et al., [27], regarding the addition of zeolite at doses of 30 and 60 g/kg to lambs treated with urea fed on silage, and with [17], regarding the addition of zeolite to lamb diets, and partially consistent with the results of [12] on Holstein Friesian cow diets supplemented with zeolite at rates of 80 and 140 g/day.

Conclusions

It is concluded that the use of zeolite with urea in the feeding rations of Awassi lambs resulted in the best results in improving the standards of digestion of nutrients, in addition to the role of zeolite in improving growth rates, food conversion ratio, and feed consumption, while raising the level of total protein and reducing the level of urea in the blood, while keeping it within its normal limits in the blood.

Author contributions

Researcher Mohamed Alrez wrote the research, conducted the experiments, and prepared it for publication, while Dr. Adel Jammoul contributed to designing the experiment and reviewing the research. Dr. Walid Al-Rahmoun assisted in securing the experiment's requirements and facilitating the research procedures, and Dr. Yasser Al-Omar helped in conducting the statistical analysis of the results and tabulating them.

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Data availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request due to their sensitivity and are available upon request on the website.

Declarations

Ethics approval and consent to participate

Informed Consent was obtained from the owner of the animals to conduct experiments on them and publish their results, with a commitment to applying the best methods of veterinary care for the animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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