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Intravenous glucose tolerance test in Baluchi ewe lambs receiving dietary supplemental selenium-methionine and chromium-methionine

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ABSTRACT

The impact of dietary supplementation of 1.5 mg of Selenium/kg of diet as Selenium-methionine (Se-Met) and 0.8 mg of Chromium/kg of diet as Chromium-methionine (Cr-Met) and their combination (Se-Cr-Met) on glucose and insulin responses during intravenous glucose tolerance test (IVGTT) was examined on 24 Baluchi ewe lambs (18-20 weeks of age) with 6 replicates per treatment. Blood samples were collected before (time 0) and 2, 10, 20, 30, 45, 60, 90, 120 and 180 min after IVGTT. The supplementation of Se- and/or Cr-Met resulted in a lower peak glucose concentration at 2 minutes and a diminished concentration at 180 minutes following the infusion ($P < 0.05$). Additionally, lambs receiving Cr-Met and Se-Cr-Met exhibited lower serum glucose levels at 120 minutes in comparison to the control group ($P \leq 0.05$). Furthermore, animals consuming diets supplemented with Se-Met and/or Cr-Met showed a reduced area under the curve (AUC) for glucose and insulin at 180 minutes following the IVGTT ($P < 0.05$). The quantity of circulating malondialdehyde, which serves as an indicator of oxidative stress, was reduced in lambs supplemented with Se-Met and/or Cr-Met ($P < 0.05$). It can be concluded that the supplementation of organic Se-Met and/or Cr-Met enhanced glucose clearance and insulin sensitivity in growing Baluchi ewe lambs, with these effects possibly mediated by a decrease in systemic oxidative stress.

Keywords: Chromium, Ewe lamb, Glucose tolerance test, Insulin, Selenium

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Introduction

Insulin, produced by the pancreatic beta cells, is the hormone that plays a crucial role in reducing blood glucose levels and promoting energy storage. It aids in the synthesis and storage of glycogen, lipids, and proteins (Rahman et al., 2021; Qiao et al., 2024). Research indicated that insulin sensitivity diminishes with maturation and aging in both animals and humans; however, the timing and degree of compensatory

hyperinsulinemia, along with its effects on glucose tolerance, remain inadequately understood (Gatford et al., 2004). Additionally, insulin resistance (IR) may impair weight gain in developing animals. Insulin facilitates cellular glucose uptake, stimulates glycolysis, and encourages the synthesis of hepatic and muscle glycogen, adipose triglycerides, and skeletal muscle protein, while also inhibiting their degradation (Baumgard et al., 2015). Furthermore, a decline in insulin sensitivity is observed during the first year of life in sheep (Clarke et al., 2000).

Selenium (Se), recognized as an essential micronutrient, exerts its beneficial impacts through selenoproteins such as glutathione peroxidase (GPX1), which acts as an inhibitor of reactive oxygen species (Vonnahme et al., 2010, Mousaie, 2021). Ezaki (1990) illustrated that Se induced various insulin-like effects in rat adipocytes, including the stimulation of glucose transport activity. McNeill (1991) built upon Ezaki's findings, demonstrating that selenium functioned as an insulin-mimetic agent in vivo. A study highlighted the positive effects of antioxidants, including Se, on glucose tolerance and insulin sensitivity in dairy cows (Abuelo et al., 2016). Nevertheless, limited information exists regarding the effects of dietary Se supplementation on glucose tolerance in ruminant animals, particularly in sheep. Similarly, chromium (Cr) intake is associated with diverse metabolic functions in both humans and livestock. The trivalent form of chromium (Cr^{+3}), which is a component of low-molecular-weight chromium-binding substance (LMWCr) or *chromodulin*, is crucial for metabolism as it enhances glucose tolerance by amplifying insulin action and is involved in the metabolism of proteins, lipids, and carbohydrates (Vincent, 2007). While the organic source of Cr as chromium-methionine (Cr-Met) has been reported to improve the growth performance of Baluchi lambs (Mousaie et al., 2014), there exists contradictory evidence concerning the glucose-reducing effects of Cr supplements in ruminant animals. The glucose tolerance test provides a more accurate assessment of an animal's insulin status compared to basal insulin and glucose levels. Moreover, to the authors' knowledge, there is a lack of research examining the impacts of Se and Cr administration on the intravenous glucose tolerance test (IVGTT) in sheep particularly ewe lambs. The aim of this study was to evaluate the effects of dietary organic selenium and chromium supplementation on the insulin sensitivity of growing ewe lambs using IVGTT, representing the first investigation of its kind in this sheep breed.

Materials and methods

Animals and experimental treatments

This experiment was carried out at the Research Station of the Department of Animal Science, Ferdowsi University of Mashhad, Iran. Animals were managed in accordance with the protocols established by the Iranian Council of Animal Care (1995). A total of four experimental diets were randomly allocated to 24 nulliparous fat-tailed Baluchi ewe lambs, aged 18-20 weeks and with an average live weight of 24.2 ± 0.4 kg, utilizing a completely randomized design with six replications for each experimental group. The experimental period spanned 11 weeks during the summer months.

Following a 2-week acclimatization phase to a control diet and the experimental conditions, the lambs were randomly assigned to one of four dietary treatments: 1) Basal diet (Control group); 2) Basal diet supplemented with 1.5 mg of Selenium (Se) per kg of diet in the form of selenium-methionine (Se-Met; Availa®Se 1000, containing a minimum concentration of 1000 mg of Se per kg from a selenium-L-methionine complex, Zinpro Corporation, USA); 3) Basal diet supplemented with 0.8 mg of Chromium (Cr) per kg of diet in the form of chromium-methionine (Cr-Met; Availa®Cr 1000, which includes a minimum concentration of 1000 mg/kg of Cr from a chromium-L-methionine complex, Zinpro Corporation, USA); and 4) Basal diet supplemented with both 1.5 mg of Se, as Se-Met and 0.8 mg of Cr as Cr-Met per kg of dry matter (Se-Cr-Met). The composition of diet ingredients and nutrients is presented in Table 1.

Table 1. Ingredients and chemical composition of the experimental diet

Ingredient	g /kg DM
Alfalfa hay mid-bloom	100
Corn silage	100
Wheat straw	50
Barely grain	300
Soybean meal	90
Extruded linseed	80
Wheat bran	150
Beet pulp-shreds	115
Mineral-vitamin mixture [a]	10
Salt	5
Chemical composition	
Crude protein	157
Ether extract	50
Neutral detergent fiber (NDF)	360
Ash	70
Calcium	8.5
Phosphorous	5.9
Metabolizable energy (Mcal/kg) [b]	2.57

[a] Mineral and vitamins g/kg supplement: Se, 0.01g (Na2Seo3); Mn, 2 g; Zn, 3 g; Co, 0.1g; I, 0.1g; Ca, 195g; P, 90g; Mg, 20g; Fe, 3g; Antioxidant, 0.4g; Vit. A, 500,000 IU; Vit. D3, 100,000; Vit. E 0.1 g. [b] Calculated with Small Ruminant Nutrition System (SRNS) software.

Intravenous glucose tolerance test

An intravenous glucose tolerance test (IVGTT) was conducted at the end of the experiment. All ewe lambs underwent an overnight fasting period, during which they were weighed. Jugular veins were catheterized using Certofix trio 720 catheters (14-gauge, 20 cm length, B-Braun, Melsungen, Germany) under local anesthesia and in accordance with aseptic procedures. The animals were permitted a recovery period of 24 hours' post-catheterization to minimize the potential influence of stress hormones on blood glucose and insulin levels. The animals were provided with unrestricted access to water and feed throughout the recovery period. Then, the animals underwent an overnight fasting prior to the IVGTT. Subsequently, a bolus dose of glucose (dextrose solution; Merck Co., Germany) was administered (50% wt/vol; 0.50 g of glucose/kg of body weight), following the methodology outlined by Kitchalong et al. (1995).

Blood samples collection and analysis

Blood samples for the analysis of glucose and insulin were collected at baseline and at 2, 10, 20, 30, 45, 60, 90, 120, and 180 minutes following the infusion. Catheters were thoroughly flushed with saline after the glucose infusion and each blood sampling. Blood samples were centrifuged at 3000 × g for 10 minutes, and the serum was stored at -80°C until further analysis. Glucose concentration was determined using the glucose oxidase/peroxidase method (Pars Azmoon Co., Tehran, Iran), while insulin levels were measured using an ELISA kit (Monobind Inc., Lake Forest, CA, USA). The glucose and insulin responses to the glucose tolerance test were assessed by calculating the areas under the curve (AUC) for the periods of 0 to 60, 0 to 90, 0 to 120, and 0 to 180 minutes' post-infusion. Additionally, the concentrations of malondialdehyde (MDA) as thiobarbituric acid-reactive substances and the total antioxidant capacity of serum

were evaluated using the ferric reducing antioxidant power (FRAP) assay, following the protocol established in a prior study (Mousaie et al., 2017).

Statistical analysis

Data were analyzed using a mixed model that incorporated fixed effects for treatment, time of sampling, and the interaction between treatment and time, as well as the random effect of the animal within each treatment group (SAS 9.2). The body weight of the lambs prior to the IVGTT was included as a covariate in the models to enhance the precision of the analysis. The AUC for glucose and insulin during the IVGTT was calculated utilizing the trapezoidal method alongside actual concentration values, after excluding the baseline concentration. Basal glucose and insulin concentrations, measured immediately before the IVGTT, were derived by averaging values from samples collected 10 and 0 minutes prior to the test.

$$AUC = (t_b\text{-}t_a) \times ([t_a + t_b]/2).$$

Where $[t_a]$ is the concentration of metabolite at time a (t_a), and $[t_b]$ represents the concentration of the metabolite at time point b (t_b). To account for the differing correlations of repeated measures within the same subject, mixed models with various covariance structures (e.g., compound symmetry, heterogeneous compound symmetry, autoregressive (1), heterogeneous autoregressive (1), and unstructured) were evaluated to identify the structure yielding the best fit criteria. The autoregressive (1) model was determined to be the most suitable option. The data are presented as least square means ± standard error (SE). Statistical significance was established at $P \leq 0.05$, while trends were noted at $P \leq 0.10$.

Results

Blood glucose and insulin concentrations

Based on the findings of this study, no significant differences were observed among treatments in serum glucose levels at time 0 (prior to the glucose administration). However, the average serum glucose levels during the IVGTT for the Se-Met (254.0 mg/dl), Cr-Met (254.2 mg/dl), and Se-Cr-Met (255.0 mg/dl) groups were lower than that of the control group, which had an average of 284.0 mg/dl ($P < 0.05$, Figure 1). After the glucose infusion, serum glucose concentrations increased, with the Se-Met and/or Cr-Met groups exhibited significantly lower quantities than the control lambs at both 2 minutes (the peak of glucose concentration) and 180 minutes following the IVGTT ($P < 0.05$). At the 120-minute mark, the Cr-Met ($P = 0.045$) and Se-Cr-Met ($P = 0.04$) groups presented lower serum glucose levels compared to the control, while the Se-Met animals demonstrated a tendency toward reduced serum glucose concentration ($P = 0.06$). The data related to the AUC for glucose are detailed in Table 2. Lambs receiving the Se-Cr-Met supplementation had a lower AUC at the 120-minute interval ($P = 0.02$), while all lambs given Se-Met and/or Cr-Met supplemented diets exhibited a reduced AUC of glucose at the 180-minute point in comparison to the control ($P < 0.05$). Serum insulin concentrations, as illustrated in Figure 2, did not show significant differences among the experimental groups. However, lambs received diets supplemented with Se-Met ($P = 0.02$) and Cr-Met ($P = 0.02$) had a lower AUC of insulin at the 180-minute mark following the IVGTT, as indicated in Table 2.

Blood antioxidant biomarkers

Serum MDA concentrations (mean ± SEM) for the control, Se-Met, Cr-Met, and Se-Cr-Met groups were recorded at 7.68 ± 0.260, 5.80 ± 0.260, 5.48 ± 0.260, and 5.52 ± 0.260 nmol/ml, respectively. The MDA levels in lambs fed Se- and/or Cr-Met supplements were significantly lower than those observed in the control group ($P < 0.05$). Total antioxidant capacity, as measured by FRAP values, was recorded at 110 ± 14.37, 147 ± 14.37, 127 ± 14.37, and 145 ± 14.37 µmol/ml for the control, Se-Met, Cr-Met, and Se-Cr-Met groups, respectively. Despite the elevated FRAP values observed in the Se- and/or Cr-Met groups, no significant differences were noted between the serum FRAP values of the treated animals and those of the control group.

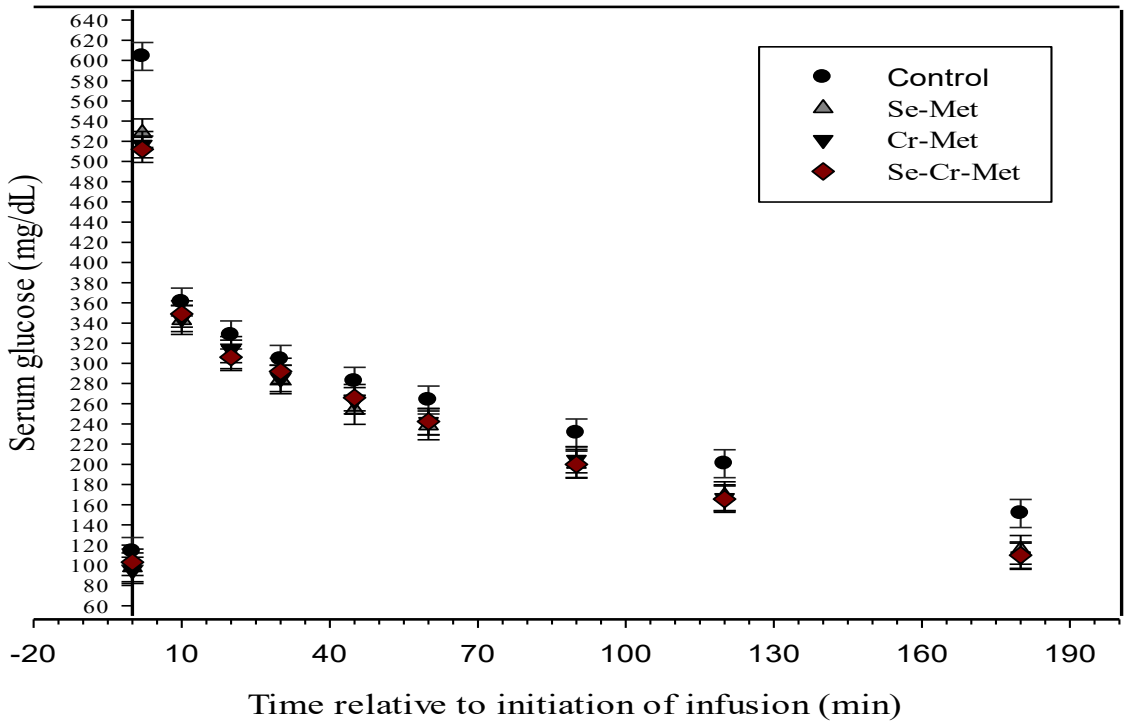

Figure. 1 Serum glucose concentration in response to an intravenous glucose infusion of ewe lambs fed Se-Met and/or Cr-Met supplements. n = 6 in each experimental group. Time 2: Se/Cr-Met vs Control $P < 0.05$; Time 120: Cr-Met & Se-Cr-Met vs. Control $P < 0.05$. Time 180: Se/Cr-Met vs Control $P < 0.05$

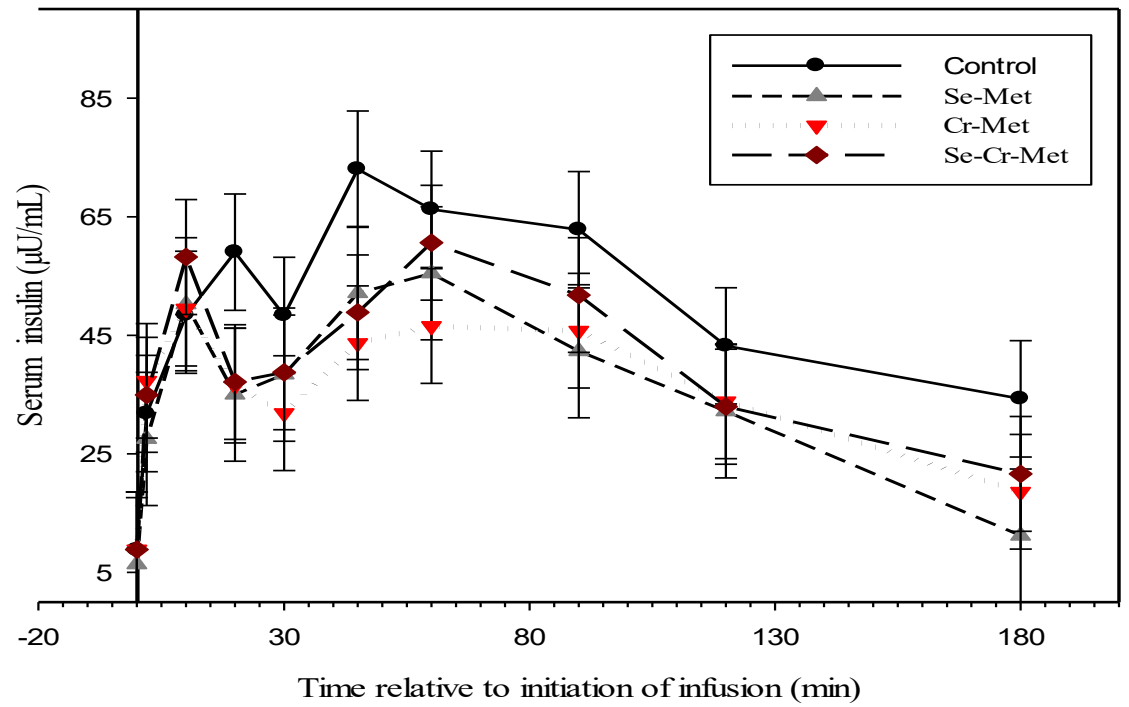

Figure. 2 Serum insulin concentration in response to an intravenous glucose infusion of ewe lambs fed Se-Met and/or Cr-Met supplements. n = 6 in each experimental group

Table 2 Effects of feeding Se-Met and/or Cr-Met supplements on serum glucose and insulin kinetics following an intravenous glucose infusion of Baluchi ewe lambs.

	Experimental group			
Area under the curve	Control	Se-Met	Cr-Met	Se-Cr-Met
Glucose				
AUC_{60}	13384	12998	12686	12310
AUC_{90}	17363	16643	16491	15775
AUC_{120}	20408 [a]	19238 [ab]	19290 [ab]	18168 [b]
AUC_{180}	24135 [a]	21870 [b]	21996 [b]	20155 [b]
SEM	848.7	882.2	771.6	776.9
Insulin				
AUC_{60}	1917	1319	1467	1690
AUC_{90}	3598	2597	2593	3112
AUC_{120}	4933	3523	3524	4120
AUC_{180}	6778 [a]	4443 [b]	4565 [b]	5229 [ab]
SEM	806.3	882.6	710.5	720.0

SEM: Standard error of means

n = 6 in each experimental group

AUC_{60} = area under the curve during the first 60 min of IVGTT [(mg/dL) × 60 min].

AUC_{90} = area under the curve during the 90 min of IVGTT [(mg/dL) × 90 min].

AUC_{120} = area under the curve during the 120 min of IVGTT [(mg/dL) × 120 min].

AUC_{180} = area under the curve during the 180 min of IVGTT [(mg/dL) × 180 min].

Different superscripts within a row indicate statistical significance at $P < 0.05$.

Discussion

A reduction in insulin sensitivity has been observed between 1 and 12 months of age in sheep, indicating that insulin sensitivity declines throughout the first year of life in lambs (Clarke et al., 2000). Glucose serves as the primary or exclusive source of cellular energy in various tissues, including red blood cells, immune cells, and the brain (Ravelo et al., 2025). The glucose tolerance test serves as an effective method for diagnosing glucose intolerance in both humans and animals. However, the establishment of catheters and the IVGTT pose challenges in sheep and goats. In this study, we opted to utilize a catheter designed for human cardiovascular surgery, despite its higher cost, to facilitate the IVGTT, thereby reducing the stress typically associated with catheterization. Dietary supplemental Se and Cr from organic sources showed favorable outcomes on glucose tolerance in ewe lambs. According to Ezaki's study (1990), Se enhances glucose tolerance in experimental animals. Vonnahme et al. (2010) demonstrated the positive effects of maternal high Se feeding in sheep on the insulin sensitivity of offspring. Nonetheless, research regarding the influence of Se on glucose and insulin dynamics in sheep remains limited. In the present investigation, serum MDA, a reliable index of lipid peroxidation (Mousaie, 2021), decreased in Se-supplemented lambs. Supporting these findings, Abuelo et

al. (2016) indicated that cows receiving antioxidant supplementation (vitamin E and Se) experienced improved insulin sensitivity by enhancing the antioxidant system. Regarding the effects of Cr on insulin sensitivity, Kitchalong et al. (1995) reported no change in glucose tolerance in lambs fed diets supplemented with Cr. Conversely, Kegley et al. (1997) observed a reduction in plasma glucose concentration following intravenous insulin infusion in calves that received chromium chloride or the chromium-nicotinic acid complex, aligning with our results. Emami et al. (2014) also documented a significant decrease in glucose AUC in goat kids after the inclusion of 1.5 mg of Cr/kg diet as chromium-methionine. The variation in studies' outcomes may be attributed to differences in animal breeds, physiological conditions, and the types and levels of minerals supplied. Insulin lowers circulating glucose levels by promoting its uptake in insulin-sensitive tissues, enhancing glycogen synthesis, and decreasing gluconeogenesis. The lower plasma glucose and insulin levels observed in chromium-supplemented lambs in this study suggest that less insulin was necessary to clear glucose from the bloodstream, indicating an increased tissue sensitivity to insulin (Emami et al., 2014). Consequently, the significantly lower peak of serum glucose at 2 minutes following glucose infusion may serve as an indicator of improved insulin responsiveness in lambs receiving dietary selenium-methionine and/or chromium-methionine supplements. A greater AUC would indicate higher peripheral glucose intolerance, while a lower insulin AUC would imply enhanced insulin efficiency or a diminished requirement for insulin to facilitate effective and timely peripheral glucose uptake (Hayirli et al., 2001). The concentration of MDA is commonly employed as a marker of lipid peroxidation. In accordance with the previous study (Mousaie et al., 2017), serum MDA levels were reduced through the administration of selenium-methionine and/or chromium-methionine, which may corroborate the hypothesis regarding the advantageous effects of decreased oxidative stress on insulin sensitivity in lambs supplemented with these compounds. The mechanisms that contribute to insulin resistance in ruminants remain inadequately elucidated. From a physiological standpoint, this condition bears some resemblance to human types I and II diabetes; however, a key distinction lies in the fact that ruminants exhibit lower glucose concentrations than humans. In the context of human type II diabetes, substantial evidence indicates that oxidative stress is instrumental in the onset and progression of insulin resistance, which can be significantly mitigated through the administration of antioxidant supplements (Chalmeh et al., 2021). The results of this study suggest a heightened insulin sensitivity, indicating that a reduced amount of insulin was required to remove glucose from the bloodstream of lambs fed with the Se- and Cr-supplemented diets.

Conclusion

The responses of glucose tolerance and insulin secretion to an intravenous glucose load in ewe lambs receiving diets supplemented with Se-Met and/or Cr-Met exhibited differences compared to those on a control diet. Specifically, animals on Se- and/or Cr-supplemented diets showed reduced serum glucose levels at 2- and 180-minutes' post-glucose infusion, along with a lower AUC for glucose and insulin at 180 minutes after the infusion. These findings suggest potential beneficial effects of these supplements on insulin sensitivity. Nonetheless, additional research is required to elucidate the precise mechanisms through which Se-Met and/or Cr-Met supplements influence insulin resistance in sheep.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Statement

The authors confirm their adherence to the ethical standards specified in the journal's author guidelines.

They also confirm that all procedures concerning animal care, experimental methods, and sampling were carried out in compliance with the regulations established by the Iranian Council of Animal Care (1995). Moreover, all research protocols, including the use of facilities and sampling procedures, received approval from the Department of Animal Science at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

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