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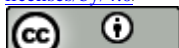
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Tikrit Journal for Agricultural Sciences (TJAS)

**Effect of adding Chromium-Methionine and Zinc-
Methionine on production performance and some hormones
in the blood of Awassi lambs.**

ABSTRACT

This study was carried out in the sheep field of the Department of Animal Production / College of Agriculture - Tikrit University for a period from 25/10/2021 to 2/1/2022 (70 days), preceded by a 14-day preliminary period to acclimatize the lamb to concentrated feed. In the sixteenth month, Awassi lambs aged 4-5 months were used from local trade, the initial weight was adjusted (25.350 ± 2.25) kg, the experiment was divided into two periods (0-35 days, 36-70 days) respectively .

Study The results of this study are as follows:

There are significant differences in efficiency for the second, third fourth and treatments ($P \leq 0.05$) on the first treatment. food conversion. It was in those qualities.

Adding a mixture of (Cr-Met and Zn-Met) led to a significant ($P \leq 0.05$) increase in insulin concentration over the rest of the treatments, while the first treatment recorded a significant ($P \leq 0.05$) increase in cortisol concentration in lambs' blood over the rest of the treatments. Significantly ($P \leq 0.05$) the two treatments (T2 and T3) over the rest of the treatments in the concentration of thyroxine (TSH), and the addition of Cr-Met recorded a significant increase ($P \leq 0.05$) over the rest of the treatments in the concentration of thyroxine (T4). It was noticed a significant decrease ($P \leq 0.05$) in the treatments (T2, T3, T4) compared to the control treatment (T1) in the concentration of threonine (T3). The length of the treatment period and the interaction between the periods and the treatments had a significant effect on these characteristics.

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INTRODUCTION

The scarcity of minerals in feed greatly affects the health and productive performance of animals, although there are sufficient organic and inorganic nutrients, the requirement for trace minerals of rare than 100 ppm in the diet, none of which exceeds a concentration of 50 ppm per million in the whole body constitutes a basic and necessary need to sustain growth and reach the final weight (Mir et al., 2020).

Chromium (Cr) is a trace element that can be considered a metabolic mediator with antioxidant properties (Domínguez-Vara, et al. 2009). It is noteworthy that chromium has a role in increasing glucose clearance and a positive effect on insulin sensitivity (Vincent, 2018). Chromium supplementation with methionine can improve feed utilization efficiency, glucose catabolism, and increase serum insulin concentration in calves, however, it is widely known that this supplementation has no negative effect on growth performance (Ohh and Lee, 2005).

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Zinc (Zn) is one of the most studied minerals or trace elements in the twenty-first century, as it participates in practically every function of the body. Zinc methionine is often promoted as a dietary supplement that can benefit growth, reduce damage from stress, immune response, nervous system health, and reproduction. Zinc methionine supplementation may be beneficial, because methionine is a sulfur amino acid and zinc positively enhances the benefit of medical treatments offered (Blowey, 2005).

From the foregoing, this study was designed to show the effect of Chromium-Methionine (Cr-Met) and Zinc-Methionine (Zn-Met) on fattening Awassi lambs by using some blood and productivity criteria to reach the final weight.

Materials and methods:

At the beginning of the experiment, the lambs were weighed after being removed from the feed for 12 hours and before providing morning feed using a scale to represent the initial weight, then the lambs were distributed among the treatments randomly within the collective cages. The first group (control treatment) (T1). The second group (T2) added (1 g per 100 kg live weight) of Cr-Met (added according to the recommendation of the product manufacturer (Availa Cr1000), the American company Zinpro). The third group (T3) added (0.33 g per head) of Zn-Met (added according to the recommendation of the manufacturer of the product (Availa Zn 120) the American company Zinpro). The fourth group (T4) added (1 g of Cr-Met + 0.33 g of Zn-Met).

The animals were fed for the duration of the experiment with two meals, morning and evening, at a rate of 3% of the live animal weight (70% concentrated feed + 30% Roaghges feed) to meet their nutritional needs and achieve a weight gain of 120 g/day/animal according to (NRC, 1985). Then the amount of concentrated feed is adjusted according to the weekly weight developments of the animals. The hay was offered freely in front of the animals, clean water and mineral salts briquettes were available throughout the day. The amount of feed was given in the form of two meals per day at eight in the morning and four in the evening throughout the duration of the experiment (70). This weighting process continued every week until the end of the experiment, in order to calculate the amount of feed actually consumed by each treatment. The proportions of the concentrated feed ingredients and its chemical composition were as follows:

Table (1): The proportions of the components of the concentrated feed

Feed material	% in the ration
crushed barley	60
wheat bran	26
soybean meal	12
salt	1
limestone	0.5
Mixed vitamins and minerals	0.5

(Palani et al., 2020)

Table (2): Chemical composition of the feed used

chemical composition	%
dry matter	89.90
Organic matter	85.54
Crude protein	15.60
Crude fiber	7.40
Crude ether extract	2.19
Nitrogen-free extract	60.35
ash	4.36
digested protein	12.17
Total digested nutrients	67.24
Representative energy, megajoules/kg dry matter	11.476

(Palani et al., 2020)

*Nitrogen-free extract = organic matter - (crude protein + crude fiber + ether extract)

On days 35 and 70 of the experiment, blood was drawn with a 10 mL Disposable syringe from the jugular vein of animals. 8 mL was placed in test tubes free of anticoagulant; It was left for 15

minutes at room temperature 25°C, then the serum was separated by placing the tubes in a centrifuge at 3000 rpm for 15 minutes, then the serum was divided into small samples and distributed in new and clean small plastic test tubes called Eppendorf tubes. Then it was kept at -18°C to maintain its enzymatic activity until laboratory analysis was performed. The TOSHO device model AIA360 manufactured by the Japanese company TOSOH BIOSCIENCE was used to analyze the concentrations of hormones (insulin, cortisol, thyrotropin TSH, thyroxine T4, threonine T3).

Statistical Analysis:

The statistical analysis of the data was carried out using the ready-made statistical analysis program SAS (2012) according to the design of a factorial experiment (4 * 2) and with a completely random two-way design, to show the effect of different treatments and their duration on the studied characteristics, and the significant differences between the means were tested using Duncan (1955) test, According to the following mathematical model:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ijk}$$

Since:

Y_{ijk} : the observed value k of transaction i and duration j .

μ : the general average of the adjective.

T_i : the effect of the treatment i

S_j : duration effect

TS_{ij} : the effect of the interaction between treatment and duration.

e_{ijk} : the naturally distributed random error with a mean equal to zero and the variance of $e^2\sigma$.

RESULTS AND DISCUSSION:

final weight

It is clear from Table (3) the effect of adding chromium methionine Cr-Met and zinc methionine Zn-Met on the final weight of Awassi lambs, where the second period 36-70 days (35.09) kg of fattening time outweighed the first period 0-35 days (29.87). kg significant at the level ($P \leq 0.05$), and by looking at the results, it turns out that the first, second, third and fourth treatments (32.39, 32.39, 33.20, 31.50), respectively, did not show any significant differences between the treatments, and the results of the interaction between, periods and treatments show that the treatment The second (29.58) and the fourth (29.12) decreased significantly at the level ($P \leq 0.05$) compared with the control group in the second period. The results agreed with the researcher Soumar et al. (2020), as he did not get significant differences between treatments in the final weight characteristic when different levels of Cr-Met and Zn-Met were used. The results also agreed with Sanchez-Mendoza et al. (2015) when Cr-Met was used. In feeding lambs, no significant differences were found between the treatments. While the results of this experiment differed with Alimohamady et al. (2019), where the results showed a significant significant improvement of the treatment in which Zn-Met was used compared with the control treatment, and these results also differed with Jafarpour et al. (2015) when Zn-Met was added at different levels to the ewes ration. A significant superiority was found between the treatments compared to the first treatment.

Total weight gain rate:

The results of the statistical analysis in Table (3) showed that there were no significant differences between the first and second periods (4.52, 3.85) kg, respectively, in terms of the rate of weight gain and total, and also the results did not show any significant differences between the treatments (4.85, 3.78, 4.58) , 3.53) kg, respectively, while the third treatment (5.77 kg) in the first period was significantly superior at ($P \leq 0.05$) over the second, third and fourth treatments (3.20, 3.40, 3.47) kg, respectively for the second period, and the first and second treatments did not record any Significant differences in the total weight gain. These results agreed with researchers Soumar et al (2020) and Sanchez-Mendoza et al (2015) when they used different levels of Cr-Met they found no significant differences between treatments. These results differed with Alimohamady et al. (2019) and Jafarpour et al. (2015), as they obtained significant differences between treatments when they used Zn-Met at different levels.

Average daily weight gain:

The results in Table (3) indicate that there were no significant differences between the first and second periods (129.19, 110.17) g, respectively, in the rate of daily weight gain, and the results also did not show any significant differences between the first, second, third and fourth treatments (138.56, 108.03, 131.06 101.06) gm, respectively, while the third treatment in the first period (164.99) gm was significantly superior at ($P \leq 0.05$) over the second, third and fourth treatments (91.42, 97.14, 99.28) gm, respectively for the second period, and the first and second treatments did not record any significant difference in daily weight gain. These results agreed with researchers Soumar et al (2020) and Sanchez-Mendoza et al (2015) when they used different levels of Cr-Met they found no significant differences between treatments. These results differed with Alimohamady et al. (2019) and Jafarpour et al. (2015), as they obtained significant differences between treatments when they used Zn-Met at different levels.

Feed consumed and feed conversion efficiency:

Through Table (3) it was noted that there were significant differences between the first and second periods in the amount of weekly consumed feed, where the second period increased (36.84) kg over the first period (31.36) kg. While there were no significant differences between the treatments in the amount of feed consumed (34.57, 34.01, 34.75, 33.07), respectively. Whereas, the first, second, and fourth treatments (31.21, 31.06, 30.58) kg, respectively, decreased significantly at the level ($P \leq 0.05$) for the first period compared with the control treatment (37.49) kg in the second period, and the second, third and fourth treatments were not recorded, respectively, in the second period, i.e. moral differences. These results agreed with Soumar et al. (2020) and Sanchez-Mendoza et al. (2015) in the amount of feed consumed in comparison with their respective treatments. While the results of this experiment differed with the researcher Jafarpour et al. (2015), as he obtained significant differences between the treatments in the amount of feed consumed.

Table (3): Effect of adding Cr-Met and Zinc-methionine Zn-Met on some weight characteristics, amount of feed consumed and feed conversion efficiency of Awassi lambs

		adjectives					
		final weight (kg)	total weight gain (kg)	daily weight gain (g)	Average weekly feed intake (kg)	feed conversion efficiency (kg feed/kg meat)	
Periods	35- 0day	± 29.87 0.44b	± 4.52 0.32a	± 129.19 9.16a	0.47 ± 31.36b	0.40 ± 7.33b	
	70-36day	± 35.09 0.76a *	± 3.85 0.40a	± 110.17 11.49a	0.80 ± 36.84a *	0.55 ± 10.31a *	
Treatments	T1	± 32.39 1.72a	0.68 ± 4.85 a	± 138.56 19.52a	1.81 ± 34.57a	0.60 ± 7.61b	
	T2	± 32.39 1.41a	± 3.78 0.34a	± 108.03 9.80a	1.48 ± 34.01a	0.94 ± 9.53a *	
	T3	± 33.10 1.02a	± 4.58 0.64a	± 131.06 18.44a	1.07 ± 34.75a	1.21 ± 8.70ab	
	T4	± 31.50 1.00a	± 3.53 0.16a	± 101.06 4.67a	1.05 ± 33.07a	0.43 ± 9.45a*	
Interaction	35day	T1	± 29.72 1.20c	± 4.35 0.33ab	± 124.28 9.44ab	1.26 ± 31.21c	0.37 ± 7.24c
		T2	± 29.58 1.03c	± 4.36 0.48ab	± 124.63 13.79ab	1.08 ± 31.06c	0.67 ± 7.33c
		T3	± 31.05 0.67bc	± 5.77 0.88a *	± 164.99 25.21a *	0.71 ± 32.60bc	1.04 ± 6.10c
		T4	± 29.12 0.58c	± 3.60 0.33b	± 102.85 9.54b	0.61 ± 30.58c	0.68 ± 8.67bc
	70day	T1	± 36.13 2.36a *	± 5.35 1.37ab	± 152.85 39.41ab	2.48 ± 37.94a *	1.20 ± 7.98bc
		T2	± 35.20 1.72ab	± 3.20 0.30b	± 91.42 8.65b	1.81 ± 36.95ab	0.70 ± 11.73a *
		T3	± 35.15 1.28ab	± 3.40 0.47b	± 97.14 13.55b	1.35 ± 36.90ab	1.12 ± 11.30a *
		T4	± 33.87 0.79ab	± 3.47 0.10b	± 99.28 2.94b	0.83 ± 35.56ab	0.13 ± ab

-The absence of English letters means that there are no significant differences at the level ($P \leq 0.05$)

-The presence of English letters means that there are significant differences at the level ($P \leq 0.05$)

- T1: control
- T2: add (1 g per 100 kg live weight) of Cr-Met
- T3 : (0.33 g per head) of Zn-Met
- T4: add (1 g per 100 kg live weight) of Cr-Met and (0.33 g per head) of Zn-Met

The effect of adding chromium methionine Cr-Met and zinc methionine Zn-Met on the level of hormones:

As for the efficiency of feed conversion, it was shown from Table (2) that the second period (10.31) kg was significantly superior to the first period (7.33) kg in the comparison between them. Looking at the results of the statistical analysis in the table, it was found that there was a significant increase at the level ($P \leq 0.05$), where the second and fourth treatments (9.53, 9.45) kg, respectively, outperformed the first treatment (7.61) kg, and the third treatment did not record any significant differences. While the second and third treatments significantly (11.73, 11.30) kg, respectively, for the second period, with a level of ($P \leq 0.05$) in the interaction between treatments, over the first, second and third treatments (7.24, 7.33, 6.10) kg for the first period, respectively.

These results differed with researchers Soumar et al (2020) and Sanchez-Mendoza et al (2015) that there were no significant differences between treatments. The results of this experiment agreed with the results of the experiment of the researcher Jafarpour et al. (2015), as he obtained significant differences between treatments at the level ($P \leq 0.05$) in the characteristic of feed conversion efficiency when different levels of Zn-Met were used on ewes.

- Insulin hormone

It is clear from Table No. (4) the effect of adding chromium methionine Cr-Met and zinc methionine Zn-Met on the level of insulin in the blood of Awassi lambs, as the second period exceeded 70 days (8.75) microunits/ml of the fattening period over the first period of 35 days (6.16). microIs/ml significantly at the level ($P \leq 0.05$), while significant superiority was found in the comparison between treatments, as the fourth treatment (14.31) microIs/ml was superior to the first, second and third treatments (4.23, 6.53, 4.76) microIs/ml, respectively, at the level of ($P \leq 0.05$), and when the interaction between the durations and the treatments, it was noticed that the fourth treatment (15.90) microunits/ml for the second period was superior to all the treatments in the first and second periods at the level of ($P \leq 0.05$). These results are in agreement with those of Mousaie et al (2017) and Sung et al (2015). The reason for the superiority of insulin is attributed to the direct effect of Cr on insulin sensitivity in the blood (Vincent, 2018).

- Cortisol hormone

It was found from Table (4) that there was no significant difference between the first and second periods (0.83 and 0.77) $\mu\text{g/dL}$, respectively, in the level of cortisol, while a significant difference was found at the level ($P \leq 0.05$) between treatments in favor of the first treatment (1.54) $\mu\text{g/dL}$. deciliter on the second, third and fourth treatments (0.37, 0.33, 0.97) $\mu\text{g/dL}$, respectively, and significant differences were found in the interaction between the durations and treatments, as it was significantly superior at the level ($P \leq 0.05$) of the first treatment (2.16) $\mu\text{g/dL}$ for the second term over all treatments In the first and second periods, the level of cortisol hormone. These results are in agreement with the results of researcher Soumar et al. (2020) in his study on lambs, there was a significant and significant decrease in cortisol concentration at ($P < 0.05$) in the nutrient treatment on Zn-Met supplementation compared with control lambs. The reason could be that the use of Zn-Met supplementation in the diet of lambs led to a decrease in the level of cortisol in the blood of the lambs (Soumar et al., 2020).

- Thyroid Hormones

It is evident from Table (4) that there is a significant superiority at the level ($P \leq 0.05$) between the periods in the level of thyrotropin, as the second period (0.13) international units/ml outperformed the first period (0.11) international units/ml, and a significant superiority was found at the level of ($P \leq 0.05$) between treatments in favor of the second and third treatments (0.16, 0.16) IU/ml, respectively, on the first and fourth treatments (0.06, 0.10) IU/ml, respectively, and it was found that there was a significant difference at the level ($P \leq 0.05$) in the interaction between Durations and treatments, as the second treatment (0.18) IU/ml for the first period exceeded all

treatments for the first and second periods. Table (8) showed a significant superiority at the level ($P \leq 0.05$) between the periods in the level of thyroxine hormone T4, as the second period (6.66) $\mu\text{g/dL}$ was superior to the first period (4.90) $\mu\text{g/dL}$, and a significant superiority was observed at the level of ($P \leq 0.05$) between treatments in favor of the second treatment (7.74) $\mu\text{g/dL}$ over the first, third and fourth treatments (3.41, 7.43, 4.53) $\mu\text{g/dL}$, respectively, in the level of thyroxine hormone T4, while it was significantly superior at the level ($P \leq 0.05$) in The overlap between the durations and the treatments, as the third treatment (9.61) $\mu\text{g/dL}$ for the second period outperformed all the treatments for the first and second periods. It was found from Table (8) that there were significant differences at the level ($P \leq 0.05$) between the periods in the level of the threonine hormone, as the first period (8.09) ng/ml exceeded the second period (3.51) ng/ml. As for the comparison between the treatments, the table showed No. (8) significant superiority at the level ($P \leq 0.05$) in the first treatment (8.13) ng/ml over the second, third and fourth treatments (4.88, 8.83, 5.36) ng/ml, respectively, while a significant superiority was found in the interaction between the durations and treatments. In all treatments of the first period (first, second, third and fourth) (8.13, 8.06, 8.10, 8.07) ng/ml respectively, and the first treatment of the second period (8.13) ng/ml on the second, third and fourth treatments (1.70, 1.56, 2.66) ng/ml in a row for the second period. The decrease in the concentration of thyroid hormones may be due to the effects of stress on the hypothalamic-pituitary-adrenal (HPA) axis, reducing thyrotropin-releasing hormone that enables animals to reduce basal metabolism (Johnson, 1987). The reason for the increase in the T3 threonine hormone may be the activity of deiodinase enzymes, which work to convert T4 thyroxine into T3 threonine, and their activity is affected by the increase in zinc in the diet of lambs treated with zinc methionine (Soumar et al., 2020).

Table (4): Effect of adding Cr-Met and Zinc-methionine Zn-Met on the level of hormones

		adjectives					
		Insulin microunits/ml	cortisol $\mu\text{g/dL}$	TSH . Thyrotropin unit/ml	thyroxine T4 $\mu\text{g/dL}$	Threonine T3 ng/ml	
Periods	35day	1.15 \pm 6.16b	0.14 \pm 0.83a	0.02 \pm 0.11b	0.30 \pm 4.90b	0.01 \pm 8.09a *	
	70day	1.33 \pm 8.75a *	0.24 \pm 0.77a	0.01 \pm 0.13a *	0.84 \pm 6.66a *	0.81 \pm 3.51b	
Treatments	T1	0.07 \pm 4.23d	0.27 \pm 1.54a *	0.02 \pm 0.06c	0.09 \pm 3.41d	0.02 \pm 8.13a *	
	T2	0.66 \pm 4.76c	0.07 \pm 0.37c	0.01 \pm 0.16a *	0.69 \pm 7.74a *	1.42 \pm 4.88c	
	T3	0.95 \pm 6.53b	0.01 \pm 0.33c	0.01 \pm 0.16a *	0.97 \pm 7.43b	1.46 \pm 8.83c	
	T4	0.71 \pm 14.31a *	0.26 \pm 0.97b	0.01 \pm 0.10b	0.10 \pm 4.53c	1.20 \pm 5.36b	
Interaction	35 day	T1	0.08 \pm 4.23e	0.11 \pm 1.55b	0.01 \pm 0.02d	0.05 \pm 3.40g	0.03 \pm 8.13a *
		T2	0.17 \pm 3.30f	0.01 \pm 0.53d	0.01 \pm 0.18a *	0.11 \pm 6.20c	0.03 \pm 8.06a *
		T3	0.11 \pm 4.40e	0.01 \pm 0.92c	0.01 \pm 0.16ab	0.03 \pm 5.26d	0.05 \pm 8.10a *
		T4	0.12 \pm 12.73b	0.01 \pm 0.34ef	0.01 \pm 0.10c	0.12 \pm 4.73e	0.03 \pm 8.07a *
	70 day	T1	0.14 \pm 4.23e	0.01 \pm 2.16a *	0.01 \pm 0.12c	0.20 \pm 3.43g	0.03 \pm 8.13a *
		T2	0.08 \pm 6.23d	0.01 \pm 0.21f	0.01 \pm 0.15b	0.03 \pm 9.28b	0.20 \pm 1.70c
		T3	0.14 \pm 8.66c	0.01 \pm 0.31ef	0.01 \pm 0.16ab	0.01 \pm 9.61a *	0.01 \pm 1.56c
		T4	0.11 \pm 15.90a *	0.04 \pm 0.40de	0.01 \pm 0.10c	0.07 \pm 4.33f	0.01 \pm 2.66b

-The absence of English letters means that there are no significant differences at the level ($P \leq 0.05$)

-The presence of English letters means that there are significant differences at the level ($P \leq 0.05$)

-T1: control

-T2: add (1 g per 100 kg live weight) of Cr-Met

-T3 : (0.33 g per head) of Zn-Met

-T4: add (1 g per 100 kg live weight) of Cr-Met and (0.33 g per head) of Zn-Met

CONCLUSIONS

The addition of (1 gm per 100 kg live weight) of Cr-Met and (0.33 gm per head) of Zn-Met and their mixture has no effect on the productive characteristics of sheep breeding, as this addition is considered a therapeutic addition, not productive or economic. Adding Cr-Met or Zn-Met or their mixture to the diet increases the concentration of the hormone insulin significantly. The addition of Zn-Met significantly reduces the level of cortisol hormone.

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تأثير إضافة الكروم-ميثيونين والزنك-ميثيونين في أداء الإنتاج وبعض الهرمونات في دم الحملان العواسي

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الخلاصة

تمت هذه الدراسة في حقل الأغنام التابع لقسم الإنتاج الحيواني / كلية الزراعة – جامعة تكريت للمدة من 2021/10/25 ولغاية 2022/1/3 (70 يوم) سبقتها مدة تمهيدية لمدة 14 يوم لتتأقلم الحملان على العلف المركز. أستعمل في التجربة ستة عشر حملاً عواسياً بعمر يتراوح 4-5 أشهر تم شراؤها من الأسواق المحلية، وبمعدل وزن ابتدائي (25.3502.25 ±) كغم، قسمت التجربة على مدتين (0-35 يوم ، 36-70 يوم) على التوالي. أشارت نتائج هذه الدراسة إلى ما يأتي :

لا توجد فروق معنوية ($P \leq 0.05$) للمعاملات مقارنة بالمعاملة الأولى (السيطرة) في الصفات (الوزن النهائي ، الزيادة الوزنية الكلية واليومية ، كمية العلف المستهلك) بينما كان هناك ارتفاع معنوي للمعاملات الثانية والثالثة والرابعة ($P \leq 0.05$) على المعاملة الأولى في كفاءة التحويل الغذائي . وكان لطول مدة المعاملة والتداخل اثرًا معنويًا في تلك الصفات.

إضافة خليط من (Cr-Met و Zn-Met) أدى إلى زيادة معنوية ($P \leq 0.05$) على بقية المعاملات في تركيز الانسولين ، بينما سجلت المعاملة الأولى ارتفاعاً معنوياً ($P \leq 0.05$) في تركيز الكورتيزول في دم الحملان على بقية المعاملات ، وارتفعت معنوياً ($P \leq 0.05$) المعاملتين (T2 و T3) على بقية المعاملات في تركيز الثايروترابين (TSH) ، وسجلت إضافة Cr-Met ارتفاعاً معنوياً ($P \leq 0.05$) على بقية المعاملات في تركيز الثايروكسين (T4) . ولوحظ انخفاضاً معنوياً ($P \leq 0.05$) بالمعاملات (T2 ، T3 ، T4) مقارنة بمعاملة السيطرة (T1) في تركيز الثيونين (T3) . وكان لطول مدة المعاملة والتداخل بين المدد والمعاملات اثرًا معنويًا في تلك الصفات.

الكلمات المفتاحية:

كروم ، زنك ، ميثيونين ، هرمون ، حملان .