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Study of the relationship between the weights of Iraqi lambs and some of their reproductive indicators fed a diet containing Cr-methionine, Zn-methionine, and their mixture

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Abstract

This experiment was conducted in the field of lambs affiliated to Tikrit University/College of Agriculture/Department of Animal Production for the period from 25/10/2021 to 3/1/2022, preceded by a 14-day preparatory period for lambs to acclimatize to concentrate feed. In the study, 16 Awassi lambs, aged 4-5 months, with an average initial weight of (25.350 ± 2.25) kg, were used. The lambs were divided into 4 treatments (4 lambs per treatment) and they were randomly distributed to the groups as follows: the first was a control diet, and the second was an addition (1 gm per 100 kg live weight) of Cr-Met, and the third was the addition of (0.33 gm per head) of Zn-Met, and the fourth was the addition of (1 g of Cr-Met + 0.33 gm of Zn-Met), in addition to salts, vitamins and minerals for all treatments with hay throughout the days of the experiment. This study examines how chromium methionine and zinc methionine affect sexual maturity, weight, and hormones in Awassi sheep. On day 35, control lambs showed insulin's positive and cortisol's negative correlations with weight. Treatment 2 (Cr-Met) highlighted insulin's strong negative correlation and TSH's positive effect on weight. Treatment 3 (Zn-Met) showed moderate correlations with hormones affecting weight. Treatment 4 (Cr-Met + Zn-Met) indicated complex interactions, notably cortisol and thyroid hormones' strong negative impacts on weight. On day 70, insulin and cortisol showed opposing effects on weight, with insulin negatively and cortisol positively correlated. TSH and thyroid hormones (T_4 , T_3) also influenced weight differently. Chromium and zinc supplementation altered these effects, improving metabolic balance but not entirely counteracting hormonal impacts.

Keywords: Insulin, cortisol, chromium methionine (Cr-Met), zinc methionine (Zn-Met), weight

Introduction

Minerals are essential nutrients in ruminant diets, classified into major (macro) minerals and trace (micro) minerals. Major minerals are measured in grams, whereas trace minerals, including chromium (Cr) and zinc (Zn), are measured in grams or micrograms and are considered among the rare elements. These trace minerals are crucial for improving animal production, health, and disease prevention by stimulating the immune system. Insufficient mineral intake can lead to reduced growth, vitality, and fertility. The bioavailability of trace elements like chromium and zinc can be influenced by factors such as nutrient interactions, chemical form, solubility, and rumen pH.

Chromium is widely distributed in the environment and is the 21st most abundant element in the earth's crust. It exists in both organic and inorganic forms, with trivalent chromium (Cr (III)) being more stable and biologically active than hexavalent chromium (Cr (VI)). Cr (III) is known for its antioxidant properties and enhances insulin activity through its involvement in glucose tolerance factor (GTF), a complex assumed to include Cr (III) and amino acids such as nicotine, glutamic acid, glycine, and cysteine (Anderson, 1997) ^[1].

Zinc is the 23rd most abundant element in the earth's crust,

making it rarer than many other minerals, and is essential for numerous biological functions. It ranks as the second most abundant trace mineral in the body, following iron, and is vital for enzyme function across various biological processes.

Studies have demonstrated that chromium methionine (Cr-Met) and zinc methionine (Zn-Met) supplementation can impact various physiological aspects in animals. For instance, Cr-Met has been shown to enhance insulin sensitivity and improve metabolic responses, which may influence growth and reproductive development (Anderson, 1997) ^[1]. Similarly, Zn-Met plays a crucial role in hormonal regulation and growth, with evidence suggesting its effects on sexual maturity and metabolic balance (Smith *et al.*, 2014) ^[19]. The interaction of these minerals with hormones such as insulin, cortisol, thyroid-stimulating hormone (TSH), thyroxine (T_4), and triiodothyronine (T_3) can influence weight gain, reproductive efficiency, and overall health (Yen, 2001; Boelaert & Franklyn, 2005; Walker, 2007) ^[22, 2, 21].

This study aims to explore the effects of Cr-Met and Zn-Met supplementation on the biochemical characteristics of Awassi sheep, focusing on their influence on sexual puberty and the relationship between weight, mineral levels, and key

hormones including insulin, cortisol, TSH, T₄, and T₃.

Materials and Methods

The number of experimental animals was 16 Awassi lambs with an average weight of (25.3502.25±) kg purchased from the local markets in Salah El-Din Governorate, their ages ranged from 4-5 months. For the purpose of stabilizing the initial weight using an electronic field balance, it was randomly distributed to four groups (4 animals/group), as the average weights of those groups were close (25.37±2.25, 25.22±1.35, 25.27±1.3 and 25.27±1.1) kg for the first, second and third groups. And fourth in a row.

Experience Design

The experimental transactions were divided into four treatments: The first group (control treatment). The second group added (1 g per 100 kg live weight) of Cr-Met (added according to the recommendation of the product manufacturer (Avalia Cr1000), the American company Zinpro).

The third group added (0.33 g per head) of Zn-Met (added

according to the recommendation of the manufacturer of the product (Avalia Zn 120) the American company Zinpro). The fourth group added (1 g of Cr-Met + 0.33 g of Zn-Met).

Feeding System

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% coarse feed) to meet their nutritional needs and achieve a weight gain of 120 g/day/animal according to (NRC, 1985). The components of the feed were mixed well, and placed in plastic bags of 50 kg, then the amount of concentrated feed was 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, where the amount of feed was given in the form of Two meals per day at eight in the morning and four in the evening for the duration of the experiment of (70) days. The proportions of the components of the concentrated feed and its chemical composition were as follows:

Table 1: The proportions of the components of the concentrated feed and its chemical composition

Feed material	% in the ration	Chemical composition	%
Crushed barley wheat bran soybean meal	60	Dry matter	89.90
		Organic matter	85.45
	26	Crude protein	15.60
		Crude fiber	7.40
Salt limestone	12	Crude ether extract	2.19
	1	Nitrogen-free extract	60.35
	0.5	Ash	4.36
Mixed vitamins and minerals	0.5	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)

Serum Zn concentrations were measured with a commercially available Rel Assay zinc measurement kit (Gaziantep, Turkey) using a fully automatic photometric method (Abbott ARCHITECT ci16200 clinical chemistry analyzer). The measurement of Zn levels is based on the principle that under alkaline conditions, the red-orange color of 5-Br-PAPS changes from the Zn contained in the sample to light pink. The change in absorbance at 548 nm is proportional to the total Zn level in the sample. The intra-assay coefficient of variation for Zn is 2.32%. The inter-assay coefficient of variation for Zn is 3.54%. The assay is calibrated with Zn sulfate dissolved in deionized water. Serum Cu concentrations were measured with a commercially available Rel Assay copper measurement kit (Gaziantep, Turkey) using a fully automatic photometric method (Abbott ARCHITECT ci16200 clinical chemistry analyzer). The measurement of Cu levels is based on the principle that 3, 5-DiBr-PAESA changes the red-orange color of the Cu in the sample to violet- blue under acidic conditions. The change in absorbance at 572 nm is proportional to the total Cu level in the sample. The intra-assay coefficient of variation for Cu is 1.85%. The inter-assay coefficient of variation for Cu is 2.74%. The assay is calibrated with copper sulfate dissolved in deionized water.

Results and Discussion

Table 2 shows serum iron levels in lambs under different feeding conditions over 35 days. The control group had stable iron levels (229.00 µg/dL). The chromium group showed a significant increase (324.33 µg/dL), and the zinc group had similar levels (325.00 µg/dL) but with less variation. The combination group had the highest levels (348.67 µg/dL), indicating a synergistic effect. Studies have shown that chromium and zinc supplements can significantly impact iron levels in animals. For instance, high chromium doses affect iron status in Wistar rats (Krol *et al.*, 2018) ^[9], and different forms of supplements influence mineral bioavailability and retention (Dobrzanski *et al.*, 2008; Ma *et al.*, 2014) ^[3, 14].

Table 2: Effects of nutritional supplements on serum iron, copper, and zinc levels in lambs over a 35-day period

Treatments	Iron	Coper	Zinc
T1: Control	B229.00±2.08	B63.00±1.53	82.67±1.20
T2: Cr	AB324.33±10.17	A71.67±2.73	85.00±2.52
T3: Zn	AB325.00±1.73	AB66.67±1.20	82.67±0.88
T4: Cr + Zn	A348.67±2.40	A70.00±0.58	85.33±2.33

Different letters in the table indicate significant differences at the level 0.05.

Copper levels in the serum of lambs under different

nutritional treatments over a 35-day period, during which the animals had not yet reached sexual maturity. The control group, which received no additional supplements, showed average copper levels of 63.00 µg/dL. The chromium group exhibited a significant increase in copper to 71.67 µg/dL, indicating chromium's positive effect on copper accumulation. The zinc group had lower copper levels at 66.67 µg/dL, suggesting a different effect compared to chromium. The combined chromium and zinc group showed copper levels of 70.00 µg/dL, indicating a synergistic effect. Studies show that chromium supplementation increases copper levels (Lichtenstein *et al.*, 2012) ^[13], while zinc supplementation may decrease them (Ellis *et al.*, 2015) ^[4]. Patel *et al.* (2018) ^[16] highlighted the synergistic effect of chromium and zinc on copper metabolism, emphasizing the need for balanced nutrient intake.

Represent zinc levels in the serum of lambs subjected to different nutritional treatments over a 35-day period, during which the animals had not yet reached sexual maturity. The control group, which did not receive additional supplements, had average zinc levels of 82.67 µg/dL with a standard deviation of ±1.20. The chromium group showed a significant increase in zinc levels to 85.00 µg/dL, with a standard deviation of ±2.52, indicating chromium's positive effect on zinc accumulation. In contrast, the zinc group had levels similar to the control (82.67 µg/dL, ±0.88), suggesting a different effect compared to chromium. The combined chromium and zinc group had the highest levels at 85.33 µg/dL with a standard deviation of ±2.33, indicating a synergistic effect. Studies support these findings, with Johnson *et al.* (2014) ^[7] showing that chromium supplementation increases zinc levels in rats. Lee *et al.* (2017) ^[12] found that zinc and chromium together affect serum zinc levels in sheep, and Smith *et al.* (2019) ^[20] demonstrated that the combination of these elements enhances mineral metabolism in livestock. These studies underscore the importance of balancing chromium and zinc for optimal zinc levels in the body.

Table 3: Effects of nutritional supplements on serum iron, copper, and zinc levels in lambs over a 70-day period

Treatments	Iron	Copper	Zinc
T ₁ : Control	AB229.33±1.20	A64.67±0.88	A79.67±1.45
T ₂ : Cr	AB233.00±1.53	A62.33±0.88	B62.33±2.40
T ₃ : Zn	B169.33±9.68	A64.00±0.58	B62.00±2.65
T ₄ : Cr + Zn	A250.67±0.88	B57.67±0.88	B57.33±1.20

Different letters in the table indicate significant differences at the level 0.05.

Iron Levels: The control group exhibited stable iron levels with a mean of 229.33 µg/dL and minimal variation (±1.20). Supplementation with chromium resulted in a slight increase in iron levels (233.00 µg/dL, ±1.53), while zinc alone led to a notable decrease (169.33 µg/dL, ±9.68). The combination of chromium and zinc yielded the highest iron levels (250.67 µg/dL, ±0.88), demonstrating a synergistic effect. This suggests that chromium enhances iron levels more effectively when combined with zinc, possibly due to improved absorption or metabolic interactions. Studies support these findings, as Krol *et al.* (2018) ^[9] observed significant effects of chromium on iron status, and Dobrzanski *et al.* (2008) ^[3] and Ma *et al.* (2014) ^[14]

highlighted how different supplement forms influence mineral bioavailability and retention.

Copper Levels: The control groups had similar copper levels, ranging from 62.33 to 64.67 µg/dL, indicating a baseline status. However, the combination group showed a lower copper level (57.67 µg/dL), suggesting that combined chromium and zinc supplementation may interfere with copper absorption or metabolism. This is consistent with Lichtenstein *et al.* (2012) ^[13], who found that chromium supplementation can increase copper levels, while Ellis *et al.* (2015) ^[4] observed that high zinc levels may decrease copper levels. Patel *et al.* (2018) ^[17] also highlighted the complex interactions between these elements, emphasizing the need for balanced supplementation.

Zinc Levels: The control group had average zinc levels of 79.67 µg/dL. Supplementation with chromium or zinc alone led to significant reductions in zinc levels (62.33 µg/dL, ±2.40; 62.00 µg/dL, ±2.65), and the combination of both elements resulted in even lower levels (57.33 µg/dL, ±1.20). This reduction indicates that while chromium may enhance zinc absorption initially, excessive amounts or interactions with other elements could lead to decreased zinc levels. Johnson *et al.* (2014) ^[7] found that chromium supplementation can increase zinc levels, while Lee *et al.* (2017) ^[12] and Smith *et al.* (2019) ^[20] demonstrated that combining these supplements affects zinc metabolism and enhances overall mineral balance.

In summary, the results indicate that chromium and zinc supplementation can have significant, synergistic effects on iron levels but may adversely affect copper and zinc levels, reflecting complex interactions between these nutrients. Understanding these interactions is crucial for optimizing nutritional strategies in livestock.

Table 4: Analysis of correlation between weights and hormone levels in lambs on day 35 of the experiment

Treatments	Insulin	Cortisol	TSH	T ₄	T ₃
T ₁	1.000*	-1.000*	.986	-.877-	-.520-
T ₂	-.938-	.021	.947. ^a	.169	-.021-
T ₃	-.689-	.689	0.697	.959	-.450-
T ₄	.005	.827	-.450-	-.999*	-.935-

The given numbers represent the correlation coefficients between weights and levels of five hormones in lambs on day 35 of the experiment, before they have reached sexual maturity. In control a correlation value of 1.000 between insulin and weight indicates a perfect positive correlation, meaning that an increase in insulin levels is associated with a corresponding increase in the lambs' weight. In contrast, a correlation value of -1.000 between cortisol and weight represents a perfect negative correlation, suggesting that an increase in cortisol levels is associated with a corresponding decrease in weight. For thyroid-stimulating hormone (TSH), a correlation value of 0.986 reflects a strong positive correlation, indicating that higher TSH levels are strongly associated with an increase in lamb weight. On the other hand, a correlation value of -0.877 between thyroxine (T₄) and weight shows a strong negative correlation, meaning that an increase in T₄ levels is strongly associated with a decrease in lamb weight. Finally, a correlation value of -

0.520 between triiodothyronine (T_3) and weight represents a moderate negative correlation, suggesting that an increase in T_3 levels is moderately associated with a decrease in lamb weight.

Based on the results of treatment 2, which included the addition of 1 g of chromium methionine (Cr-Met) per 100 kg of lamb weight, the relationships between hormone levels and weight show a mixed pattern. The strong negative correlation between insulin and weight shows that increased insulin is associated with a significant decrease in lamb weight, which may indicate opposing effects of insulin on growth in this case. In contrast, cortisol and triiodothyronine (T_3) show a slight and positive correlation with weight, indicating that their effect on weight is not significant. On the other hand, thyroid stimulating hormone (TSH) stands out as a strong positive factor associated with weight gain, indicating its important role in promoting growth. Finally, thyroxine (T_4) shows a weak negative correlation with weight, meaning that its effect on weight is weak and inconclusive. Overall, these results may indicate that the effect of Cr-Met supplementation affects the response of these hormones differently in controlling lamb weight. Based on the results of treatment 2, which included the addition of 1 g of chromium methionine (Cr-Met) per 100 kg of lamb weight, the strong negative correlation between insulin and weight may indicate that increased insulin levels, under the influence of chromium supplementation, lead to a decrease in weight. These results are consistent with some studies that have indicated that chromium may enhance insulin sensitivity and affect glucose metabolism, leading to improved energy utilization and reduced fat storage, as indicated by Anderson *et al.* (1997) ^[1] in a study that examined the effect of chromium on insulin sensitivity. As for thyroid stimulating hormone (TSH), the strong positive correlation with weight is consistent with studies that indicate that this hormone enhances thyroid activity and increases metabolic rate, which supports weight gain and growth, as demonstrated in Yen (2001) ^[22], which focused on thyroid function and its relationship to weight and growth. Regarding the negative association between thyroxine (T_4) and weight, the results support the findings of some studies that have shown that increased levels of thyroid hormones can increase metabolic rate and lead to weight loss. For example, the study by Boelaert & Franklyn (2005) ^[2] confirmed that high levels of T_4 can lead to increased energy expenditure and decreased weight. On the other hand, the small association between cortisol and weight suggests that its effect in this case was not significant, which is in line with studies such as Walker (2007) ^[21], which have argued that the effect of cortisol on weight may depend on multiple factors, such as stress status and glucocorticoid levels. Based on these data, it can be argued that the addition of Cr-Met supplements may have a complex effect on the regulation of metabolic hormones and weight in lambs, and that the hormonal response to such supplements requires further studies to understand their effects in a deeper way.

In the third treatment, where 0.33 g of Zn-Met was added per head, the correlation values represent the relationships between hormone levels and weights in lambs on day 35 of the experiment. A correlation value of -0.689 between insulin and weight indicates a moderate negative

correlation, meaning that an increase in insulin levels is associated with a moderate decrease in lamb weight. Conversely, a correlation value of 0.689 between cortisol and weight reflects a moderate positive correlation, suggesting that an increase in cortisol levels is associated with a moderate increase in lamb weight. The correlation value of 0.697 for thyroid-stimulating hormone (TSH) indicates a moderate positive correlation, meaning that higher TSH levels are moderately associated with an increase in lamb weight. Regarding thyroxine (T_4), the correlation value of -0.959 shows a strong negative correlation, meaning that an increase in T_4 levels is strongly associated with a decrease in lamb weight. Finally, a correlation value of -0.450 for triiodothyronine (T_3) indicates a moderate negative correlation, suggesting that an increase in T_3 levels is moderately associated with a decrease in lamb weight. In the third treatment, where 0.33 g of Zn-Met was added per lamb, the correlations observed between hormone levels and lamb weight provide insight into the physiological impacts of zinc supplementation. Zinc plays a critical role in various metabolic processes, including protein synthesis, immune function, and hormone regulation. Below is a scientific discussion of the results, supported by relevant studies. Insulin and Weight (Correlation: -0.689): The moderate negative correlation between insulin and weight suggests that higher insulin levels were associated with a decrease in lamb weight. This could be due to the role of zinc in improving insulin sensitivity and glucose metabolism. Studies have shown that zinc supplementation can enhance the function of insulin receptors, which might lead to increased glucose utilization and reduced fat accumulation, ultimately resulting in a decrease in weight. Cortisol and Weight (Correlation: 0.689): The moderate positive correlation between cortisol and weight suggests that higher cortisol levels were associated with increased lamb weight. Cortisol, a glucocorticoid hormone, is known to influence energy metabolism and fat distribution. Elevated cortisol levels can lead to increased lipogenesis (fat creation), particularly in response to stress. Although zinc has anti-inflammatory properties, its role in stress response might explain the observed relationship. TSH and Weight (Correlation: 0.697): The moderate positive correlation between TSH and weight indicates that higher TSH levels were associated with increased weight in the lambs. TSH stimulates the thyroid gland to produce thyroid hormones, which regulate metabolism. Zinc is essential for thyroid function, and deficiency in zinc can impair thyroid hormone production. Supplementation might have optimized thyroid function, thus supporting growth and weight gain, as suggested by research on zinc's role in thyroid health. Thyroxine (T_4) and Weight (Correlation: -0.959): The strong negative correlation between T_4 and weight indicates that higher thyroxine levels were strongly associated with a decrease in lamb weight. Thyroxine increases metabolic rate and energy expenditure, leading to weight loss. Zinc, which is crucial for proper thyroid function, may enhance the conversion of T_4 to the more active T_3 , thereby increasing metabolism and contributing to weight reduction. Triiodothyronine (T_3) and Weight (Correlation: -0.450): The moderate negative correlation between T_3 and weight indicates that higher T_3 levels were associated with a moderate decrease in lamb

weight. T_3 is the active form of thyroid hormone, and its role in increasing metabolic activity and energy expenditure is well-documented. Zinc's involvement in the conversion of T_4 to T_3 further supports this relationship, as higher T_3 levels can lead to enhanced fat burning and weight loss.

In the fourth treatment, where a combination of 1 g of Cr-Met and 0.33 g of Zn-Met was added to lambs before puberty, the correlations between hormone levels and lamb weight reveal several key relationships. Insulin and Weight (Correlation: 0.005): The negligible positive correlation suggests that insulin levels have virtually no impact on lamb weight. This minimal effect might indicate that the combined supplementation of Cr-Met and Zn-Met does not significantly influence insulin sensitivity or glucose metabolism at this stage of development. Cortisol and Weight (Correlation: -0.827): The strong negative correlation between cortisol and weight indicates that higher cortisol levels are strongly associated with a decrease in lamb weight. This could be attributed to the catabolic effects of cortisol, which promotes protein breakdown and fat redistribution under stress. Studies have shown that elevated cortisol can reduce weight by increasing energy expenditure and reducing appetite. TSH and Weight (Correlation: -0.450): The moderate negative correlation between TSH and weight suggests that increased TSH levels are moderately associated with a decrease in lamb weight. This may reflect the feedback mechanism where elevated TSH is a response to low thyroid hormone levels, which in turn could lead to slower metabolism and reduced weight gain. Thyroxine (T_4) and Weight (Correlation: -0.999): The perfect negative correlation between T_4 and weight indicates that an increase in T_4 levels is perfectly associated with a decrease in lamb weight. Thyroxine, which increases the basal metabolic rate, can significantly enhance energy consumption, leading to weight loss. This aligns with findings in metabolic studies showing that higher thyroid hormone levels can lead to significant weight reduction. Triiodothyronine (T_3) and Weight (Correlation: -0.935): The strong negative correlation between T_3 and weight further supports the role of thyroid hormones in metabolism. Increased T_3 levels enhance metabolic processes, promoting fat burning and weight loss, which is consistent with research on the effects of thyroid hormones on body weight. Overall, these results suggest that the combined Cr-Met and Zn-Met supplementation before puberty has complex effects on metabolic regulation, primarily through thyroid and stress hormones, which in turn significantly influence weight in lambs. Further studies are necessary to fully understand the mechanisms by which these supplements affect hormonal balance and growth.

Table 5: Analysis of correlation between weights and hormone levels in lambs on day 70 of the experiment

Treatments	Insulin	Cortisol	TSH	T_4	T_3
T ₁	-.596-	.982	-.971-	-.427-	-1.000-
T ₂	-.652-	-.401-	-.020-	.976	.221
T ₃	-.044-	-.799-	-.850-	-.945-	-.489-
T ₄	-.848-	-.030-	.360	.054	.167

These table (5) depict the correlations between animal weight and hormone levels on day 70 of the experiment, after the animals reached sexual maturity. A moderate

negative correlation of -0.596 between insulin and weight suggests that higher insulin levels are associated with lower body weight due to enhanced glucose utilization and reduced fat accumulation (Anderson *et al.*, 1997) ^[1]. Conversely, cortisol showed a very strong positive correlation of 0.982 with weight, indicating that increased cortisol levels are linked to greater body weight, likely due to fat accumulation (Walker, 2007) ^[21]. TSH exhibited a strong negative correlation of -0.971, implying that higher TSH levels correspond to lower weight, potentially due to reduced thyroid hormone levels affecting metabolism (Boelaert & Franklyn, 2005) ^[2]. T_4 had a weaker negative correlation of -0.427, suggesting a modest decrease in weight with increased T_4 levels (Larsen & Silva, 1981) ^[11]. Finally, T_3 showed a complete negative correlation of -1.000 with weight, highlighting its significant role in reducing body weight through enhanced metabolism (Yen, 2001) ^[22].

In animals fed chromium methionine, a moderate negative correlation of -0.652 between insulin and body weight indicates that increased insulin levels are associated with lower body weight, reflecting chromium methionine's potential to enhance insulin sensitivity and reduce fat storage (Anderson *et al.*, 1997) ^[1]. Cortisol showed a weak negative correlation of -0.401, suggesting that despite cortisol's usual association with weight gain, chromium may help reduce stress responses and improve metabolism (Walker, 2007) ^[21]. TSH had a very weak negative correlation of -0.020, indicating that chromium supplementation might stabilize thyroid hormone levels post-puberty, minimizing their impact on weight (Boelaert & Franklyn, 2005) ^[2]. Conversely, T_4 displayed a strong positive correlation of 0.976 with body weight, highlighting chromium methionine's role in boosting metabolism and growth (Larsen & Silva, 1981) ^[11]. T_3 showed a weak positive correlation of 0.221, reflecting a modest effect from improved hormonal balance (Yen, 2001) ^[22].

In animals fed zinc methionine, a weak negative correlation of -0.044 between insulin and body weight indicates that insulin levels have minimal impact on weight despite zinc supplementation. Conversely, cortisol showed a strong negative correlation of -0.799, suggesting higher cortisol levels are linked to significantly lower body weight; while zinc might reduce oxidative stress, its effect on cortisol's impact is limited (Walker, 2007) ^[21]. TSH had a strong negative correlation of -0.850, reflecting its role in metabolism regulation; despite zinc potentially improving metabolic efficiency, high TSH still correlates with reduced weight (Boelaert & Franklyn, 2005) ^[2]. T_4 displayed a very strong negative correlation of -0.945, indicating that higher T_4 levels significantly decrease weight by enhancing energy expenditure and fat burning (Larsen & Silva, 1981) ^[11]. T_3 showed a moderate negative correlation of -0.489, suggesting increased T_3 levels are linked to moderate weight reduction (Yen, 2001) ^[22]. Thus, while zinc methionine supports metabolism, it does not significantly offset the negative effects of cortisol and thyroid hormones on weight. In animals fed a combination of chromium methionine and zinc methionine, insulin levels exhibited a strong negative correlation of -0.848 with body weight, indicating that higher insulin levels are associated with significantly lower weight. This suggests chromium's role in enhancing insulin

sensitivity and reducing fat storage, especially in pubertal animals (Anderson, 1997) ^[1]. Conversely, cortisol had a weak negative correlation of -0.030, implying minimal impact on weight. Walker (2007) ^[21] suggested that chromium and zinc might moderate cortisol's effects by reducing oxidative stress. Thyroid-stimulating hormone (TSH) showed a weak positive correlation of 0.360, indicating a slight increase in weight with higher TSH levels, as TSH stimulates metabolism and zinc may support thyroid health (Boelaert & Franklyn, 2005) ^[2]. Both thyroxine (T₄) and triiodothyronine (T₃) had weak positive correlations of 0.054 and 0.167, respectively, suggesting minimal effects on weight despite their roles in metabolism (Larsen & Silva, 1981; Yen, 2001) ^[11, 22].

Overall, hormone levels significantly impact animal weight post-maturity. Insulin and cortisol show contrasting effects, with insulin correlating negatively and cortisol positively with weight. TSH and thyroid hormones (T₄ and T₃) also influence weight, though variably. Supplementing with chromium or zinc modifies these effects, enhancing metabolic balance but not fully counteracting hormonal impacts.

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