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EFFECT OF AMBIENT TEMPERATURE AND SOME DIETARY SUPPLEMENTATIONS ON SOME PHYSIOLOGICAL TRAITS OF LAYING JAPANESE QUAIL

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ABSTRACT

A total number of 360 mature Japanese quail, 3 months of age (240 females and 120 males), were used to investigate the effect of ambient temperature and dietary chromium (Cr), as chromium picolinat and/or acetylsalicylic acid (ASA) on some physiological traits of laying Japanese quail. Chicks were assigned to 2 x 5 factorial design, with two levels of ambient temperature (22-24°C and 32-34°C) and five dietary treatments (control, 0.00%; 500 µg Cr / kg diet; 1000 µg Cr / kg diet; 0.05 % ASA and 0.1 % ASA). The experimental period lasted for two months. Obtained results showed that high ambient temperature significantly increased rectal temperature ($P \leq 0.01$) and respiration rate ($P \leq 0.05$ or 0.01) through the first and the second month of the experiment. Packed cell volume (PCV), hemoglobin value (Hb), and some plasma measurements (globulin, total lipids, T_3 and T_3/T_4 ratio) were significantly ($P \leq 0.05$ or 0.01) decreased under heat stress conditions. While, H/L and A/G ratios; plasma levels of total proteins, albumin, cholesterol, glucose, AST (aspartate aminotransferase) and ALT (alanine aminotransferase) were significantly ($P \leq 0.01$) increased in heat stressed quail.

Rectal temperature and respiration rate are reduced through the first and the second month of the experiment due to the effect of dietary chromium and ASA, but this reduction was only significant ($P \leq 0.05$ or 0.01) for ASA. While, Hemoglobin (Hb) value increased significantly ($P \leq 0.01$) due to the effect of dietary chromium. All dietary treatments significantly ($P \leq 0.01$) increased PCV and plasma levels of AST, ALT, T_3, T_4 and T_3/T_4 ratio and significantly ($P \leq 0.01$) decreased H/L ratio and cholesterol level. Quail group treated with 1000 µg Cr showed a significant increase ($P \leq 0.01$) in total proteins and globulin and a significant ($P \leq 0.01$) decrease in A/G ratio, as compared with un-treated quails (control). Otherwise, glucose concentration significantly ($P \leq 0.01$) reduced only in group treated with 0.1% ASA.

It is concluded that high ambient temperature causes deleterious effects on physiological traits of Japanese quail. These effects could be alleviated by chromium supplementation at levels of 500 and 1000 µg/kg diet and by aspirin supplementation at levels of 0.05 and 0.1%.

INTRODUCTION

Japanese quail is considered a good and economical source for animal protein production because of its small body size, early sexual maturity, high rate of production, short generation interval, low maintenance cost, high resistance to many common poultry diseases and more successful than domestic fowl in sustaining growth rate under high ambient temperature (Baumgartenr, 1994). However these biological advantages are affected by several factors such as strain, sex, age, stocking density, photoperiod and environmental temperature. Since, high environmental temperature lead to a wide range of deleterious changes in behavioral, biochemical, physiological and molecular adjustments in poultry (Etches *et al.,* 1995). The thermo-neutral zone for Japanese quail ranged between 18 and 20ºC as reported by Ensminger *et al*. (1990). When environmental temperature goes above this range, a wide range of changes was noted in rectal temperature, respiration rate, several hematological parameters and many blood constituents i.e. liver enzymes and thyroid hormones (Altan *et al.,* 2000). Therefore, researches conducted to alleviate these deleterious effects of heat stress are very important. Several manipulations such as chromium and acetyl salicylic acid (ASA) were served for this purpose by several investigators. Many investigators recorded beneficial effects of chromium on several physiological traits (Sands and Smith, 2002), either under high or milieu ambient temperature. Whereas others did not showed these beneficial effects (Lien *et al*., 2004 and Uyanik *et al*., 2005). Since, chromium is involved in metabolism of carbohydrates, lipids, proteins and nucleic acids (McCarty, 1991), it is recognized as a glucose tolerance factor (GTF) (Steele and Rosebrough, 1981), and has been shown to stimulate insulin activity (Evan, 1989). Insulin metabolism influences lipids peroxidation (Gallaher *et al*., 1993). therefore, chromium is postulated to function as antioxidant (Preuss *et al*., 1997). Acetylsalicylic acid (ASA), the active ingredient of aspirin, is well known as an antipyretic drug (Weissmann, 1991). Aspirin inhibits prostaglandins synthesis, hence, it may "resets the hypothalamic thermostat". Acetyl salicylic acid supplementation to birds diet improved many physiological traits under heat stress conditions (Abou El-Soud *et al*., 2006 and Abdel-Fattah, 2006). While, other investigators did not record this improvement (McDaniel and Parker, 2004).

The aim of this work was to estimate some physiological changes of Japanese quails as affected by the ambient temperature (22-24 or 32-34°C) and dietary treatment with chromium and/or acetyl salicylic acid from 3 to 5 months of age.

MATERIALS AND METHODS

The present study was carried out in Quail Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The experiment started from January 2006 for two months.

A total number of 360 mature Japanese quail, 3 months old, (240 females and 120 males) were assigned, to 2 x 5 factorial design arrangement to 10 treatment group, 36 birds each, then subdivided into 4 replicates each contained 9 birds (3 male + 6 female). Replicates had nearly equal average live body weight for each male and female. The main two groups of birds were kept at either 22-24°C or at 32-34°C. The birds, at both ambient temperature groups, were fed the basal diet (control) or the basal diet supplemented with either chromium picolinate (Cr) at levels of 500 or 1000 µg / kg diet and ASA at levels of 0.05 or 0.1%. All birds were kept under the same managerial conditions, and were exposed to 16 hours of light per day was provided. Birds were fed *ad-libitum* and the fresh water was available along the time. The composition and calculated chemical analysis of basal diet is shown in Table 1.

Rectal temperature (°C) and respiration rate were measured monthly. Rectal temperature was measured using a thermocouple thermometer inserted 1-1.5 cm into the cloaca for few minutes until a fixed reading was obtained. Respiration rate was measured by counting the chest movement for one minute with the aid of a stop-watch.

Table 1. Composition and chemical analysis of the basal diet

Ingredient	(%)
Yellow corn	60.05
Soybean meal (44%)	25.00
Corn gluten meal (60%)	5.70
Bone meal	3.30
Limestone	3.80
Vit. & Min. premix(1)	0.25
NaCl	0.20
Dl- Methionine	0.05
L-Lysine Hcl	0.15
Cotton seed oil	1.50
Calculated analysis(2)	
Crude protein %	20.03
ME (kcal/kg)	2922
Crude fiber %	3.22
Crude fat %	2.62
Calcium %	2.51
Available phosphorus %	0.55
Lysine %	1.08
Methionine + Cystine %	0.77

(1)Layer Vit. & Min. premix: Each 2.5 kg of vitamins and minerals premix (commercial source pfiezer Co.): consist of Vit. A 12 MIU, Vit E 15 KIU, Vit. D_3 4 MIU, Vit. B1 1g, Vit B2 8 g, Vit B6 2 g, Vit B12 10 mg, Pantothonic acid 10.87 g, Niacin30 g, Folic acid 1 g, Biotin 150 mg, Copper 5 g, Iron 15 g, manganese 70 g, Iodine 0.5 g, Selenium 0.15 g, Zinc 60 g and antioxidant 10g. (2) Calculated according to NRC (1994).

At the end of the experiment, blood samples were collected, into heparinized clean tubes from 4 birds (2 males and 2 females) randomly taken from each treatment. Fresh blood samples were used to determine hemoglobin level (Hb) and Packed cell volume (PCV%). Blood smears were also done, stained with Wright's stain procedure and used to calculate the number of Lymphocytes (L) and Heterophiles (H) in 100 white blood cells, then H / L ratio was calculated. The remainder blood samples were Centrifuged (4000 rpm) for ten minutes and the plasma samples were stored frozen at -20°C until used for analysis. Plasma total proteins (g/dl), albumin (g/dl), total lipids (g/L), cholesterol (mg/dl), glucose (mg/dl), AST (U/L) and ALT (U/L) concentrations were determined by using available commercial kits as described by the manufacture companies (spectrum, Diagnostics, Egypt, Co. for Biotechnology, S. A. E.). Thyroid gland hormones, T_3 (ng/ml) and T_4 (ng/ml), were determined by RIA technique as described by Akiba *et al*. (1982).

Analysis of variance for data was accomplished using SAS General Liner Models Procedure (SAS Institute, 1996). The statistical fixed model used was:

$$Y_{ijk} = \mu + H_i + T_j + HT_{ij} + e_{ijk}$$

Where: Y_{ijk} = observation on the ijk^{th} trait, μ = Overall mean, H_i = fixed effect of i^{th} heat (i : 22-24°C and 32-34°C), T_j = fixed effect of j^{th} treatment (j : control (un-treated); 500 or 1000 µg chromium / kg diet and 0.05 or 0.1% ASA), HT_{ij} = interaction effect of heat degrees and treatment types (ij: 1: 2……….and 10), e_{ijk} = The random error assumed to be independently randomly distributed. Duncan's new multiple range test (Duncan, 1955) was used to test the differences among the means.

RESULTS AND DISCUSSION

Rectal temperature and respiration rate:

From results presented in Table 2. it is clear that high ambient temperature significantly increased rectal temperature (P≤0.01) and respiration rate (P≤0.05 or 0.01) during the two months of the experiment. In this respect, Sturkie (1986) demonstrated that respiration rate increased in hot whether to dissipate the excessive heat via evaporative cooling from respiratory passages. These results are in agreement with those obtained by Durgun and Kestin (1998) and Altan *et al* (2000).

Table 2. Average values (x ± SE) of rectal temperature (°C) and respiration rate (frequency per minute) of Japanese quails as affected by ambient temperature, different dietary treatments and their interaction.

Means in the same column within each classification bearing different letters are significantly (P ≤ 0.05) different. **(P ≤ 0.01), * (P ≤ 0.05) and NS = not significant.

Rectal temperature and respiration rate were reduced through all experimental periods due to the effect of dietary chromium and ASA, but this reduction was only significant (P≤0.05 or 0.01) for ASA, as compared with the control un-treated group. The reduction in rectal temperature and respiration rate resulted from dietary ASA supplementation may be due to the known action of ASA as antipyretic drug. It could inhibit the prostaglandins synthesis and reset the hypothalamic thermostat (Abou El-Soud *et al*., 2006). The present findings confirmed those obtained in Japanese quail by Abou El-Soud *et al*. (2006) and Abdel-Fattah (2006). On the other hand, McDaniel and Parker (2004) reported that dietary supplementation of ASA failed to decrease the rectal temperature of heat stressed roosters.

The interaction effect between ambient temperature and dietary treatment on rectal temperature and respiration rate was insignificant during the two months of the experiment.

Hematological parameters:

As shown in Table 3, high ambient temperature significantly decreased hemoglobin value (Hb) (P≤0.01), packed cell volume (PCV) (P≤0.05) and significantly (P≤0.01) increased H/L ratio in quail blood, as compared with the control group. Results in the present study are nearly similar to those reported by Zhou *et al* (1999). The reduction in Hb and PCV, under heat stress, may be due to the high water retention by birds and/or due to the modulation of the supply of oxygen to accommodate changes in heat production as reported by Yahav*. et al.* (1997). Higher H/L ratio in heat stressed quail than unheated quail may be due to the high level of corticosterone that causes the release of heterophils (Aengwanich and Chinrasri, 2003).

Dietary chromium supplementation significantly (P≤0.01) increased hemoglobin value and packed cell volume, as compared with the control group. This may be due to the role of chromium in stabilizing red blood cells against cellular changes caused by peroxidations Linder (1991).While chromium supplementation significantly (P≤0.01) decreased H/L ratio, only at level of 1000 µg/kg diet. Acetyl salicylic acid supplementation had no significant effects on hemoglobin value, while, PCV significantly (P≤0.01) increased, only, at level of 0.1%. H/L ratios were significantly (P≤0.01) lower at the two levels of ASA than in the un-supplemented group. These beneficial effects of chromium and ASA on H/L ratio may be due to their role in reducing corticosterone releasing (Sahin *et al.,* 2003 and Abou El-Soud *et al*. (2006). Since, corticosterone has a positive effect on heterophils releasing (Aengwanich and Chinrasri, 2003). Similar findings on H/L ratio were obtained by Uyanik *et al*. (2002) for chromium effect and by Abou El-Soud *et al*. (2006) for ASA effect. While, Trash *et al*. (2000) noted in-significant effects of dietary ASA on the hematological parameters (red blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration) in broilers chickens.

Table 3. Average values (x ± SE) of Hemoglobin value (mg/100ml), packed cell volume (PCV) and heterophiles/lymphocytes ratio (H/L ratio) of Japanese quails as affected by ambient temperature, different dietary treatments and their interaction.

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different. **($P \leq 0.01$), * ($P \leq 0.05$) and NS = not significant.

Interaction effects between ambient temperature and dietary treatments were only significant (P≤0.01) for hemoglobin values, since quails reared under thermo-neutral condition and supplemented with 1000 µg Cr/kg diet had the highest value of hemoglobin. The lowest value of hemoglobin was recorded in quails reared under heat stress condition and received the control diet. Packed cell volume and H/L ratio were not significantly affected by the interaction. However, the lowest value of packed cell volume and the highest ratio of H/L were numerically noted in quails reared under heat stress condition and received the control diet.

Blood proteins, lipids and glucose:

High ambient temperature significantly (P≤0.01) increased plasma measurements of total proteins, albumin, A/G ratio, cholesterol and glucose, as compared with thermo-neutral condition (Table 4). plasma concentrations of globulin and total lipids significantly (P≤0.01) decreased in quail reared under high ambient temperature. The high levels of total proteins, albumin, cholesterol and glucose in this study may be due to greater dehydration and/or increased protein catabolism associated with corticosteroid releasing under heat stress conditions (Abdel-Fattah, 2006). These results are fairly similar to those noted by Tollba and Hassan (2003) and Abdel-Fattah (2006). While, these results are disagreement with those noted by Zhou *et al* (1999), Zulkifli *et al* (1999) and Abdel-Fattah (2006).

Results in Table 4. indicate that, quail group treated with 1000 µg Cr showed a significant increase (P≤ 0.01) in total proteins and globulin and a significant (P≤ 0.01) decrease in A/g ratio, as compared with un-treated quails (control). This improvement may be due to the increasing of amino acids synthesis in the liver via insulin which enhance the incorporation of several amino acids into protein (Moonsie- Shageer and Mowate*,* 1993). Plasma concentration of cholesterol significantly (P≤0.01) decreased in all supplemented groups as compared with un-supplemented ones.

This may attribute to the role of supplementations in reducing the secretion of corticosterone that has a catabolic effect (Muller *et al.,* 1988 and Sahin *et al*., 2003). Acetyl Salicylic acid in quail diet at level of 0.1% significantly (P≤0.01) reduced plasma concentrations of glucose, as compared with other groups. Results concerning effects of chromium supplementation are partially in agreement with those obtained by Sands and Smith (2002), Uyanik *et al*., (2002), Sahin *et al* (2003) and Lien *et al*. (2004). Other authors disagree with the present results (Sahin *et al,* 2002 and Uyanik *et al*., 2005). Results obtained in respect to the effect of ASA supplementation on plasma globulin, total lipids and glucose levels are in agreement with Abdel-Fattah (2006), who showed that dietary ASA supplementation at level of 0.05% decreased levels of total proteins, albumin, total lipids, cholesterol and A/G ratio in heat stressed quails.

There are significant differences in plasma total proteins (P≤0.05), globulin (P≤0.01), A/G ratio (P≤0.01) and glucose (P≤0.01) due to the interaction effect (Table 4). Since the highest level of total protein in plasma was recorded in quail reared under thermo-neutral condition and received diet containing 1000 µg Cr/kg diet, while the lowest level was recorded in quail reared under thermo-neutral condition and received diet containing 0.1% ASA. The highest level of globulin in plasma was noted in groups supplemented with chromium under thermo-neutral conditions. Quail birds reared under heat stress conditions and supplemented with dietary 0.05% ASA had the highest A/G ratio. Birds reared under thermo-neutral conditions and supplemented with chromium had the lowest A/G ratio. Plasma concentrations of glucose were at the lowest level in quail reared under heat stress condition and received 0.1% dietary ASA.

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Liver enzymes and thyroid hormones:

As shown in Table 5, plasma levels of AST and ALT were significantly (P≤0.01) increased due to high ambient temperature (32-34°C). The elevation of both enzymes is considered as a sensitive index of hepatic dysfunction. Plasma measurements of T_3 levels and T_3/T_4 ratio were significantly (P≤0.05) decreased under heat stress conditions. However, plasma levels of T_4 were not affected significantly by heat stress exposure (32-34°C) as compared with the control group. The present results indicate that only T_3 affected by high ambient temperature, since T3 is the principal metabolically active thyroid hormone in birds (Abdel-Fattah, 2006). Ringer (1975) concluded that the effect of heat stress on thyroid hormones in birds was through the nervous system of the bird. Results of T_3 and T_3/T_4 levels in the present study are in agreement with those reported by Liu *et al* (1998) and Tollba and Hassan (2003). In contrary, Bowen and Washburn (1985) found positive effects on T_4 and T_3 levels in broiler chickens exposed to heat stress (50°C for 1 hr at 5th day of age).

Chromium and ASA supplementations caused a significant (P≤0.01) increase in plasma concentrations of AST, T_3, T_4 and T_3/T_4 ratio, as compared with un-supplemented group, while, plasma level of ALT was significantly (P≤0.01) increased only with ASA supplementation at level of 0.1% (Table 5).

Similar results were obtained for chromium effect on T_3 and T_4 levels by Sahin *et al* (2002) and Sahin *et al*. (2003). The positive effect of chromium on T_3 and T_4 levels may be due to the reductive effect of chromium on concentration of corticosterone (Sahin *et al*., 2003) which have inversely relationship with T_3 concentration (Durgun and Kestin, 1998). Results concerning the effect of ASA on T_3 and T_4 levels are in agreement with those obtained by Abdel-Fattah (2006) who postulated that the decreasing effect of ASA supplementation on coticoserone concentration is via suppressing prostaglandins synthesis. On the other hand, Abou El-Soud *et al*. (2006) found that T_3 and T_4 did not affected in Japanese quails fed diets containing 0.025, 0.05 or 0.1% ASA under heat stress conditions except T_3 in birds treated with 0.1% dietary ASA which decreased significantly. However, results obtained concerning the effect of ASA on Levels of blood AST and ALT are in disagreement with those obtained with Abdel-Fattah (2006).

Interaction effect was only significant for AST (P≤0.05) and ALT (P≤0.01). Since the lowest levels of AST were recorded in the control group and in group fed diet supplemented with 500 µg Cr/kg under thermo-neutral conditions. The highest levels of AST were recorded in groups supplemented with 0.1% ASA either under thermo-neutral conditions or under heat stress conditions. The lowest levels of ALT, due to interaction effect, were recorded under thermo-neutral conditions in un-supplemented group and in group supplemented 500 µg Cr/kg diet. Whereas, the highest level was recorded in control group reared under heat stress condition.

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تأثير الحرارة المحيطة وبعض الإضافات الغذائية على بعض الصفات الفسيولوجية للسمان الياباني البياض

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لدراسة تأثير درجة الحرارة المحيطة بالسمان الياباني الناضج وتأثير الاضافات الغذائية من عنصر الكروم (في صورة بيكلونات الكروم) و/ أو حامض الأستيل سالسيلك على بعض الصفات الفسيولوجية للسمان الياباني البياض تم توزيع عدد ٣٦٠ سمانة ناضجة (٢٤٠ أنثى و ١٢٠ ذكر) عمر ٣ شهور طبقا للتصميم العاملي 2X5 ، حيث وضعت الطيور تحت مستويين من درجة الحرارة المحيطة (٢٢-٢٤ ºC و ٣٢-٣٤ ºC)، وتم معاملة الطيور تحت كل مستوى من مستويات الحرارة بخمسة معاملات غذائية (الكنترول (٠,٠٠%) ، ٥٠٠ ميكروجرام كروم ، ١٠٠٠ ميكروجرام كروم ، ٠,٠٥ % من حامض الأستيل ساليسيلك ، ٠,١ % من حامض الأستيل ساليسيلك) ، وقد استمرت التجربة لمدة شهرين.

أدى ارتفاع درجة الحرارة المحيطة إلى ارتفاع معنوي في درجة حرارة المستقيم (P≤0.01) وفي معدل التنفس (P≤0.05 or 0.01) خلال الشهر الأول والثاني من التجربة ، و أدت أيضا إلى ارتفاع معنوي (P≤ 0.01) في نسب H/L و A/G وفي مستويات البلازما من البروتينات الكلية والالبيومين و الكوليسترول والجلوكوز و AST و ALT ، بينما انخفض الحجم الخلوي وقيمة الهيموجلوبين وبعض قياسات البلازما (الجلوبيولين ، الليبدات الكلية ، هرمون T_3 و نسبة هرمون T_3 إلى هرمون T_4) انخفاضا معنويا (P≤0.05 or 0.01) تحت تأثير الحرارة المرتفعة.

انخفض كلا من درجة حرارة المستقيم و معدل التنفس خلال الشهر الأول والثاني من التجربة تحت تأثير المعاملة بكلاً من الكروم وحامض الأستيل ساليسيلك و كان هذا الانخفاض معنويا (P≤0.05 or 0.01) تحت تأثير المعاملة بحامض الأستيل ساليسيلك فقط ، بينما أدت المعاملة بالكروم إلى ارتفاع معنوي (P≤ 0.01) في قيمة الهيموجلوبين ، وقد أدت كل المعاملات إلى ارتفاع معنوي (P≤ 0.01) في الحجم الخلوي وفي مستويات البلازما من AST و ALT و هرموني T_3 و T_4 و نسبة هرمون T_3 إلى هرمون T_4 ، بينما أدت إلى ارتفاع معنوي (P≤ 0.01) في نسبة H/L وفي مستوى البلازما من الكوليسترول ، في حين أن المعاملة بالكروم عند مستوى ١٠٠٠ ميكروجرام أدت إلى ارتفاع ملحوظ (P≤ 0.01) في مستوى البروتينات الكلية وفي مستوى الجلوبيولين و إلى انخفاض معنوي (P≤ 0.01) في نسبة A/G ، و أظهرت المعاملة بحامض الأستيل ساليسيلك عند مستوى ٠,١% انخفاض ملحوظ في تركيز الجلوكوز.

تأثير التداخل بين درجة الحرارة المحيطة و المعاملات الغذائية كان معنويا لقيم الهيموجلوبين (P≤ 0.01) ولمستويات البلازما من البروتينات الكلية (P≤ 0.5) و الجلوبيولين (P≤ 0.01) ونسبة A/G (P≤ 0.01) والجلوكوز (P≤ 0.01) و AST (P≤ 0.05) و ALT (P≤ 0.01).

من هذه الدراسة تتضح التأثيرات الضارة لارتفاع درجة الحرارة على معظم الصفات الفسيولوجية محل الدراسة في السمان الياباني والتي يمكن تخفيفها من خلال إضافة الكروم بمعدل ٥٠٠ و ١٠٠٠ ميكروجرام لكل كجم عليقة ومن خلال إضافة الأسبيرين بمعدل ٠,٠٥ و ٠,١ %.

Table 4. Average plasma levels (x ± SE) of total proteins (g/dl), albumin (g/dl), globulin (g/dl), lipids (mg/dl), cholesterol (mg/dl) and glucose (mg/dl); and albumin/globulin (A/G ratio) of Japanese quails as affected by ambient temperature, different dietary treatments and their interaction.

Item	Total proteins (g/dl) x ± SE	Albumin (g/dl) x ± SE	Globulin (g/dl) x ± SE	A/G ratio x ± SE	Total lipids (mg/dl) x ± SE	Cholesterol (mg/dl) x ± SE	Glucose (mg/ dl) x ± SE
Ambient temperature(A.T)	**	**	**	**	**	**	**
22-24^{0} C	4.55 ± 0.16^{b}	2.56 ± 0.05^{b}	1.98 ± 0.14^{a}	1.39 ± 0.10^{b}	243.04 ± 6.32^{a}	129.46 ± 5.47^{b}	183.14 ± 13.07^{b}
32-34^{0} C	4.95 ± 0.22^{a}	3.36 ± 0.08^{a}	1.59 ± 0.09^{b}	2.23 ± 0.16^{a}	218.02 ± 4.37^{b}	154.49 ± 5.55^{a}	211.89 ± 5.15^{a}
Dietary treatments (D.T)	**	NS	**	**	NS	**	**
0.0 (control)	4.56 ± 0.19^{b}	2.94 ± 0.23	1.62 ± 0.10^{bc}	1.87 ± 023^{ab}	241.05 ± 5.66	176.95 ± 7.44^{a}	219.41 ± 4.52^{a}
Cr. 500 µg/ kg	4.74 ± 0.10^{b}	2.87 ± 0.21	1.87 ± 0.25^{b}	1.78 ± 0.36^{bc}	226.91 ± 13.60	128.37 ± 6.90^{cd}	214.36 ± 10.32^{a}
Cr. 1000 µg/ kg	5.33 ± 0.20^{a}	3.04 ± 0.21	2.28 ± 0.22^{a}	1.42 ± 0.22^{c}	227.52 ± 7.92	123.78 ± 8.80^{d}	215.88 ± 7.96^{a}
ASA 0.05%	4.67 ± 0.15^{b}	3.14 ± 0.23	1.52 ± 0.17^{c}	2.20 ± 0.34^{a}	223.88 ± 5.88	139.05 ± 4.77^{bc}	193.37 ± 6.67^{a}
ASA 0.1%	4.46 ± 0.28^{b}	2.81 ± 0.11	1.64 ± 0.18^{bc}	1.78 ± 0.13^{bc}	233.31 ± 14.80	141.74 ± 5.46^{b}	144.55 ± 23.86^{b}
A.T X D.T	*	NS	**	**	NS	NS	**
22-24^{0} C 0.0 (control)	4.22 ± 0.20^{de}	2.44 ± 0.12	1.77 ± 0.14^{bc}	1.39 ± 0.12^{de}	248.38 ± 4.23	162.52 ± 4.40	218.96 ± 4.56^{a}
Cr. 500 µg/ kg	4.85 ± 0.16^{abcd}	2.41 ± 0.10	2.44 ± 0.06^{a}	0.99 ± 0.06^{e}	244.20 ± 23.80	115.79 ± 5.89	208.64 ± 19.65^{a}
Cr.1000 µg/ kg	5.38 ± 0.29^{a}	2.69 ± 0.12	2.68 ± 0.21^{a}	1.01 ± 0.07^{e}	230.10 ± 11.60	105.83 ± 3.60	207.63 ± 14.70^{a}
ASA 0.05%	4.41 ± 0.15^{cde}	2.65 ± 0.14	1.76 ± 0.06^{bc}	1.50 ± 0.08^{de}	231.75 ± 9.85	130.35 ± 4.11	185.97 ± 12.60^{a}
ASA 0.1%	3.88 ± 0.16^{e}	2.61 ± 0.10	1.27 ± 0.07^{d}	2.06 ± 0.12^{c}	260.78 ± 16.19	132.81 ± 6.40	94.50 ± 1.29^{b}
32-34^{0} C 0.0 (control)	4.90 ± 0.18^{abcd}	3.43 ± 0.12	1.47 ± 0.09^{cd}	2.35 ± 0.14^{bc}	233.73 ± 9.40	191.38 ± 7.03	219.86 ± 9.03^{a}
Cr. 500 µg/ kg	4.62 ± 0.10^{bcd}	3.32 ± 0.09	1.30 ± 0.08^{d}	2.58 ± 0.20^{ab}	209.62 ± 7.50	140.95 ± 6.70	220.08 ± 10.70^{a}
Cr.1000 µg/ kg	5.28 ± 0.35^{ab}	3.39 ± 0.29	1.89 ± 0.20^{b}	1.83 ± 0.27^{cd}	224.93 ± 13.15	141.73 ± 7.35	224.13ح ± 5.76^{a}
ASA 0.05%	4.92 ± 0.13^{abc}	3.64 ± 0.12	1.28 ± 0.11^{d}	2.89 ± 0.32^{a}	216.01 ± 3.76	147.75 ± 4.64	200.78 ± 3.02^{a}
ASA 0.1%	5.03 ± 0.22^{abc}	3.01 ± 0.06	2.01 ± 0.14^{b}	1.50 ± 0.10^{de}	205.84 ± 8.85	150.66 ± 5.32	194.61 ± 18.42^{a}

Means in the same column within each classification bearing different letters are significantly (P ≤ 0.05) different. **(P ≤ 0.01), * (P ≤ 0.05) and NS = not significant.

Table 5. Average plasma levels (x ± SE) of AST (U/L), ALT (U/L), T_3 (ng/ml) and T_4 (ng/ml); and T_3/T_4 of Japanese quails as affected by ambient temperature, different dietary treatments and their interaction.

Item	AST (U/L) x ± SE	ALT (U/L) x ± SE	T_3 (ng/ml) x ± SE	T_4 (ng/ml) x ± SE	T_3/T_4 ratio x ± SE
Ambient temperature (A.T)	**	**	*	NS	*
22-24^0 C	59.35 ± 3.62^b	14.95 ± 1.00^b	3.25 ± 0.21^a	16.26 ± 0.63	0.20 ± 0.008^a
32-34^0 C	74.15 ± 4.80^a	17.88 ± 0.72^a	2.85 ± 0.24^b	15.84 ± 0.81	0.18 ± 0.010^b
Dietary treatments (D.T)	**	**	**	**	**
0.0 (control)	44.89 ± 2.48^d	15.40 ± 2.40^{bc}	1.80 ± 0.18^c	12.39 ± 0.62^e	0.14 ± 0.012^c
Cr. 500 µg/ kg	56.92 ± 4.90^c	13.72 ± 1.17^c	3.52 ± 0.20^a	17.71 ± 0.52^b	0.20 ± 0.008^{ab}
Cr. 1000 µg/ kg	71.03 ± 6.07^b	15.88 ± 0.77^{bc}	3.73 ± 0.27^a	19.60 ± 0.47^a	0.19 ± 0.016^{ab}
ASA 0.05%	74.90 ± 5.13^b	17.32 ± 0.73^b	2.55 ± 0.17^b	14.31 ± 0.34^d	0.18 ± 0.012^b
ASA 0.1%	86.01 ± 3.02^a	19.73 ± 0.65^a	3.64 ± 0.15^a	16.23 ± 0.33^c	0.23 ± 0.012^a
A.T X D.T	*	**	NS	NS	NS
22-24^0 C 0.0 (control)	45.57 ± 3.50^d	10.30 ± 1.16^e	2.08 ± 0.13	13.53 ± 0.55	0.15 ± 0.012
Cr. 500 µg/ kg	46.61 ± 2.60^d	11.31 ± 0.72^e	3.59 ± 0.43	17.36 ± 0.91	0.21 ± 0.017
Cr. 1000 µg / kg	59.97 ± 3.90^c	16.95 ± 1.09^{bcd}	4.10 ± 0.20	19.67 ± 0.48	0.21 ± 0.006
ASA 0.05%	64.66 ± 4.40^c	16.83 ± 0.75^{bcd}	2.90 ± 0.16	14.42 ± 0.66	0.20 ± 0.012
ASA 0.1%	79.92 ± 2.07^b	19.35 ± 0.82^{abc}	3.59 ± 0.30	16.31 ± 0.35	0.22 ± 0.023
32-34^0 C 0.0 (control)	44.21 ± 4.25^d	20.51 ± 1.32^a	1.52 ± 0.27	11.24 ± 0.54	0.13 ± 0.017
Cr. 500 µg/ kg	67.24 ± 2.71^c	16.14 ± 0.76^{cd}	3.45 ± 0.12	18.07 ± 0.63	0.19 ± 0.003
Cr. 1000 µg/ kg	82.09 ± 6.81^{ab}	14.81 ± 0.82^d	3.36 ± 0.45	19.53 ± 0.94	0.17 ± 0.029
ASA 0.05%	85.15 ± 2.63^{ab}	17.81 ± 1.35^{abcd}	2.21 ± 0.07	14.21 ± 0.35	0.16 ± 0.006
ASA 0.1%	92.09 ± 2.16^a	20.12 ± 1.16^{ab}	3.70 ± 0.11	16.16 ± 0.64	0.23 ± 0.012

Means in the same column within each classification bearing different letters are significantly ($P \leq 0.05$) different. **($P \leq 0.01$), * ($P \leq 0.05$) and NS = not significant.