

EFFECTS OF MACA (*LEPIDIUM MEYENII* WALP) POWDER ON SERUM INDICES AND METABOLIC RESPONSES IN RACEHORSES

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ABSTRACT

Eighteen racehorses, 6.5±1.17 years old, were divided into three groups. During 45 days, control group was fed basal diet, experimental groups M50 and M75 were fed basal diet plus 50 and 75 g/day maca extract, respectively. There were no significant differences between groups for initial and final body weight, average daily feed intake and blood hemogram values during the study. On day 45, while M75 had higher aspartate transaminase level than M50, gamma-glutamyl transpeptidase level in this group were higher than both control and M50 ($p<0.05$). M75 had lower Mg level than control ($p<0.037$). After feeding, M50 had higher adiponectin level than control and M75 at 30th min. ($p<0.05$). Insulin level in M50 and M75 were lower than control at 60th min., but it was higher in M50 than M75 at 120th min. ($p<0.003$). Thyroid stimulating hormone levels in M50 were lower than control at 60th min. before feeding and were higher than M75 at 15th min. after feeding ($p<0.05$). Triiodothyronine concentrations were higher in M75 than other groups ($p<0.001$). Similarly, M75 had higher thyroxine concentration than control and M50 during the study ($p>0.05$). The differences between groups were found statistically significant only at 60th min.

Key words: Horses, *Lepidium meyenii Walp*, Maca powder, Metabolic responses, Serum indices.

INTRODUCTION

Nutrition of the racehorses is a sophisticated and delicate subject. Racehorses require a good feeding program to reveal their genetic potential. When it was considered that all racehorses had a genetic potential, besides exercising regularly and well-formulated diets, they also need some additional applications to compete against each other. Therefore, the necessity for natural performance enhancing additives increased recently. Maca (*Lepidium meyenii Walp*) may play a role as natural alternative feed additive.

Maca is a tubular root plant of *Brassicaceae* family native to the high Andes of Peru. It has been using as a vegetable root and a medicinal herb for its physical and psychological effects since 2000 years ago. Fresh maca or its extract contains some important compounds such as tannins, saponins, isotiosionate, glucosinolates, macacene, macamide, fatty acids, steroid alcohols, amino acids, vitamins and minerals (Piacente *et al.*, 2002; Valentova and Ulrichova, 2003; Chung *et al.*, 2005). Maca tuber meal was found to have protein levels of 10-13% and highly hydrolysable carbohydrate levels of 59-68 % on a dry matter basis (Dini *et al.*, 1994; Canales *et al.*, 2000). Maca extracts are used as powder and capsule form for their enhancer effects on physical activity and fertility. Zheng *et al.*, (2002) observed that dietary maca extract increased physical activity and decreased the level

of lactic acid in mice during forced swimming test. Cicero *et al.*, (2001) reported that locomotor activity increased in rats fed maca depend on its dietary level. Similarly, in sportmen recruited from amateur cycling and triathlon clubs, the application of maca at 2000 mg/day increased performance and shortened their lap time (Stone *et al.*, 2009). There are many stress factors affecting the health and performance status of racehorses. Lopez-Fando *et al.*, (2004) reported that maca was capable of attenuating stress responses by decreasing changes of corticosterone and glucose levels, as well as reducing or abolishing stress-induced ulcers and homeostasis, and increases of adrenal gland weights in adult male Swiss mice. This beneficial effect of maca may play an advantageous role in the traditional nutrition of horses. It was also reported that there were no any toxic effect of maca on liver, testicle and other tissues in male rat (Gasco *et al.*, 2007). However, the positive effects of maca on hereditary defects in lipoprotein, antioxidant and glucose tolerance systems were detected in rats, and it was also suggested that this plant could be used as additive in the treatment and prevention of chronic illness characterized with atherogenic lipoprotein, hepatic steatosis, impaired antioxidant system and reduced glucose tolerance (Vecera *et al.*, 2007). These reports show that beneficial effects of maca on vital metabolic activities may provide important advantages for racehorses.

Although there were many studies on the effects of maca and almost all of these studies were carried out on human and laboratory animals such as mice and rat, there was no any published article about the use of maca in horses. Therefore, this study was carried out to investigate the effects of maca on serum indices and metabolic responses in racehorses.

MATERIALS AND METHODS

Animals, diets and feeding: The procedures were approved by Istanbul University Ethic Committee (Protocol No: 64). The study was conducted at the Veliefendi Hippodrome of TJK (Turkish Jockey Club) in Istanbul. A total of 18 male racehorses 6.5 ± 1.17 years old were used in this study. Animals were divided into three groups of six horses each. Control group was fed basal diet (Table 1) and other two experimental groups were fed basal diet + 50 g maca/day (M50) and basal diet + 75 g maca/day (M75) during 45 days. The horses were fed 2 times a day at 08:00 and 18:00. Horses were exercised every morning between 04:00 and 06:00. Daily exercise programme was consisted of walking as a warm-up without a rider and trot and gallop with a rider for 2 km. Maca powder used in this study were imported by Dogaform Gıda Sanayii İth. İhr. Ltd. Sti. by the courtesy of Republic of Turkey Ministry of Food, Agriculture and Livestock. The characteristics of maca used in this were presented in Table 2.

Chemical, biochemical and statistical analysis: The chemical analyses of the feed samples were performed according to the methods of Association of Analytical Communities (1984). Digestible energy (DE) of basal diet was calculated according to the following formula reported by Harris (2001):

$$\text{DE, Mcal/kg} = 2118 + 12.18 (\text{CP}) - 9.37 (\text{ADF}) - 3.83 (\text{NDF-ADF}) + 47.18 (\text{EE}) + 20.35 (100 - \text{CP-EE-NDF-ash}) - 26.3 (\text{ash})$$

Horses were weighted individually at the beginning and at the end of the study. Body weight (BW) changes were calculated by the difference between weight on day 45 and initial. Amounts of offered feeds and refused feeds were recorded daily to determine average daily feed intake (ADFI).

To determine the potential effect of maca on the biochemical parameters, blood samples were collected by venipuncture from *V. jugularis* on day 0, 30 and 45 for the analysis of triglyceride, total cholesterol, glucose, aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), gamma-glutamyl transpeptidase (GGT), total protein (TP), albumin (ALB), globulin (GLOB), creatinine (CREA), blood urea nitrogen (BUN), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), chlor (Cl), lactic acid (LA). Hemogram

analyses were done only on samples collected on day 0 and 45. On day 30, serum ghrelin, adiponectin, leptin, insulin, thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) levels were also determined for hormonal activities at 60th min before feeding and 15th, 30th, 60th, 120th and 240th min after feeding.

Triglycerides, total cholesterol and glucose analysis were done at Thermo Scientific Helios UV-VIS spectrophotometer by using commercial kits (triglyceride/total cholesterol: Teco Dianostics, CA, USA; glucose: Medispec Diagnostics, USA) in Laboratory of Department of Biochemical, Faculty of Veterinary Medicine, Istanbul University. Hemogram analysis were performed by using Abott Cell Dyn 3500 Analyser, and other biochemical parameters (AST, ALP, LDH, CK, GGT, ALB, GLOB, CREA, BUN, Ca, Mg, P, Na, K, Cl, LA) were analysed by using Siemens Dimension Xpand at Biochemical Laboratory in Horse Hospital of TJK.

Hormones were determined by using Mquant Elisa Reader – KCjunior Software Package with ELISA kits (Ghrelin: Ghrelin Enzyme Immunoassay Kit, RayBiotech, Inc., Norcross, GA, USA; adiponectin: ADPN Elisa Kit, Shangha BlueGene Biotech Co. Ltd., China; leptin: Uscn Life Science Inc., China; Insulin/TSH/T3/T4: Demeditec Diagnostics GmbH, Kiel, Germany) in Laboratory of Department of Biochemical, Faculty of Veterinary Medicine, Istanbul University.

A one-way ANOVA was used to determine changes in body weight, feed intake, hemogram parameters, blood biochemical parameters and mineral concentrations. A two-way ANOVA for repeated measures was used to determine responses of ghrelin, adiponectin, leptin, insulin, TSH, T3 and T4 to time and diet, based on subset of the data from 60th min before to 240th min after feeding. Posthoc differences were determined by using a Tukey Multiple Range test (SPSS for Windows, Standard version 10.0, 1999; SPSS Inc., Headquarters, Chicago, IL, USA).

RESULTS AND DISCUSSION

Body weights (initial and on day 45) and ADFIs were given in Table 3. There were no significant differences between groups for BW and ADFI during the study ($P > 0.05$). Brown-Douglas *et al.*, (2005) reported that the autumn-born foals were about 8 months old and the spring-born foals were about 11 months old when they entered puberty. When the age of horses in our study considered, phytoestrogenic and growth promotant effect of maca (Moreau *et al.*, 2002; Lee *et al.*, 2004) on BWs might have not seen because they had already completed their puberty periods. Also, no significant differences between groups for BWs and ADFIs can be interpreted as maca has no effect on the experimental groups.

The effects of maca powder on hemogram values were presented in Table 4. As is known, hemogram parameters are important indicators in terms of animal health and these parameters can be affected by feeding as well as increased sweating in hot and humid conditions, and exercise causing alterations in plasma volume (Snow, 1983). There were no significant effects of maca supplementation on blood hemogram parameters in this study. This result is in agreement with the study which reported that the administration of maca (*Lepidium peruvianum*) at levels of 0.75 and 7.5 g/kg during long-term (90 days) had no significant effect on morphology of blood in rats (Meissner *et al.*, 2006) This finding can be construed as a positive result of maca extract.

The effects of maca powder on serum AST, ALP, LDH, CK, GGT, TP, GLOB, ALB, CREA, BUN, LA, glucose, cholesterol and triglyceride were given on Table 5. On day 45, levels of AST were 333.00, 276.00, 349.17 IU/L and levels of GGT were 19.36, 15.00 and 48.00 IU/L in control group, M50 and M75 respectively. While group M75 had higher AST level than those of group M50, GGT level in this group were higher than both control and M50 ($p < 0.05$). The level of serum AST is an important marker in acute liver and muscle diseases, GGT level is a criteria considering in the diagnosis of diseases related kidneys, liver and pancreas and their high levels in horses are associated to the diseases of these organs (Bilal, 2004). It was reported that serum AST and GGT concentrations varied between 75-705 IU/L and 0-62 IU/L respectively in healthy horses (Bilal, 2004), and in this study, AST and GGT levels were between these reference values. However, there were no significant differences between groups for other biochemical parameters in the present study.

Insignificant differences between groups for glucose and triglyceride levels contradicts results reported by Meissner *et al.*, (2006) who observed increase in glucose level in rats received maca during long-term (90 days) but, agree with statistically insignificant differences in glucose and triglyceride levels in rats administered maca by the same researchers during short-term (28 days) at a level of 7.5 g/kg.

On the other hand, observed in our study consistency of glucose level in racehorses receiving maca contradicts results reported by Lopez-Fando *et al.*, (2004) who reported hypoglycemic effect of maca on stressed animals. Possible explanation of discrepancy in results in this study may be due to response of non-stressed animals used in our study.

The effects of maca powder on blood mineral levels were presented in Table 6. Except for serum Mg concentration, the administration of 50 or 75 g maca had no effect on the other mineral levels. At the end of the

study, group supplemented 75 g maca had lower Mg concentration than control (2.00 vs. 2.27 mg/dl) ($p < 0.037$). It was reported that the alterations in serum Mg levels were directly related to diet and normal levels varied between 1.4-2.3 mg/dl (Bilal and Bilal, 2003).

Hormone levels in groups before feeding and 0, 15, 30, 60, 120 and 240th min. after feeding were presented in Table 7. Adiponectin is secreted from adipose tissue and its concentrations in bloodstream are high (500-3000 $\mu\text{g/L}$) relative to other hormones (Berg *et al.*, 2002). Levels of adiponectin are inversely correlated with body fat percentage and it plays role in glucose regulation, fatty acid oxidation and insulin sensitivity (Yamauchi *et al.*, 2001). The production of adiponectin is induced by adipocyte differentiation and its secretion is stimulated by insulin. Adiponectin concentration in bloodstream is lower in obesity (Matsuzawa *et al.*, 1999). In this study, adiponectin concentration was higher ($p < 0.05$) in group supplemented 50 g/day maca at 30th min. after feeding than control and group supplemented 75 g/day maca.

Insulin is an anabolic hormone and its main metabolic function is the transition of glucose from blood to cells (Bilal and Bilal, 2003). In this study, insulin concentration in group M50 and M75 were lower ($p < 0.003$) than those of control at 60th min. after feeding. It was thought that this alteration was randomly realized because this was not appeared in another minutes.

The administration of 50 g/day maca resulted in lower TSH concentrations than control at 60th min. before feeding and higher than group supplemented 75 g/day maca at 15th min. after feeding ($p < 0.05$). T3 concentrations at 0, 15, 30, 60, 120 and 240th min. after feeding were higher in group supplemented 75 g/day maca than other groups ($p < 0.001$). Similarly, group supplemented 75 g/day maca had higher T4 concentration than other groups during the study ($p > 0.05$). However these differences between groups were significant only at 60th min. after feeding. The concentrations of thyroid hormones in bloodstream are marker for the status of thyroid gland. It was reported that diets with high energy or protein and excess levels of Cu or Zn in diet were causes of the alterations in T3 and T4 concentrations without any symptom (Bilal and Bilal, 2003). Therefore, we thought that the differences in T3 and T4 concentrations in our study were not related to thyroid deficiency, however, these findings could be a result of dietary factors.

In conclusion, it was determined that 50 or 75 g/day maca supplementation had no negative effect or risk for health in horses. Although there were many studies in other species, further studies required to identify the effects and mechanism of maca in horses.

Table 1. Ingredients and nutritional content of basal diet (DM basis)

Ingredients (%)	
Alfalfa	60.00
Maize	3.00
Soybean Meal	2.20
Oat	34.00
Dicalciumphosphate	0.50
Vitamin-Mineral Premix ¹	0.10
Salt	0.20
Nutritional content (calculated)	
DM (%)	90.60
CP (%)	15.30
EE (%)	5.13
Ash (%)	4.68
CF (%)	22.55
NDF (%)	41.31
ADF (%)	28.65
DE (Mcal/kg)	2.79
Calcium (%)	0.76
Phosphorus (%)	0.30

¹Per kg: Vit A 1.500.000 IU, Vit D₃ 150.000 IU, Vit E 50.000 IU, Vit K₃ 175 mg, Vit B₁ 25.000 mg, Vit B₂ 1.000 mg, Vit B₆ 500 mg, Vit B₁₂ 10 mg, Vit C 37.500 mg, Niacin 10.000 mg, Folic acid 500 mg, Manganese 10.000 mg, Iron 10.000 mg, Zinc 10.000 mg, Copper 3.000 mg, Cobalt 20 mg, Iodine 30 mg, Selenium 40 mg, Magnesium 40.000 mg, Phosphorus 125.000 mg, Calcium 5.333 mg, Potassium 125.000 mg, Pantothenic acid 1.000 mg
DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; DE, digestible energy

Table 2. Characteristics of Maca powder used in the study

Plant Part Used	:	Root
Botanical Name	:	<i>Lepidium meyenii Walp</i>
Particle Size	:	60 Mesh
Composition	:	Organic Maca Root, 100%
Moisture	:	< 10%
Appearance	:	Homogenous powder
Color	:	Beige
Odor	:	Characteristic
Taste	:	Characteristic
Protein	:	13.72%
Carbonhydrate	:	69.80%
Fat	:	0.98%
Fiber	:	6.76%
Total Energy	:	342.9 Kcal/100 g

Table 3. The effect of Maca on body weight and feed intake in racehorses

Groups	Initial BW kg	Final BW kg	ADFI kg
Control	478.33±53.55	479.83±48.88	6.950±0.29
M50	478.83±58.36	479.83±56.75	7.000±0.24
M75	479.50±33.89	483.16±33.67	6.900±0.27

BW, live body weight; ADFI, average daily feed intake

Table 4. The effect of Maca on hemogram parameters in racehorses

Parameter	Period	Groups			SEM	p
		Control	M50	M75		
WBC, 10 ³ /μl	Initial	8.28	8.61	8.39	0.26	0.64
	Final	7.27	7.24	7.57		
NEU, %	Initial	4.67	4.98	4.89	0.16	0.48
	Final	4.33	4.34	4.75		
LYM, 10 ³ /μl	Initial	3.01	3.07	2.76	0.10	0.42
	Final	2.37	2.32	2.14		
MONO, 10 ³ /μl	Initial	0.38	0.41	0.54	0.03	0.08
	Final	0.41	0.45	0.53		
EOS, %	Initial	0.11	0.09	0.11	0.01	0.31
	Final	0.11	0.08	0.11		
BASO, %	Initial	0.07	0.06	0.08	0.01	0.11
	Final	0.05	0.05	0.04		
RBC, 10 ⁶ /μl	Initial	10.41	10.50	10.17	0.15	0.24
	Final	8.60	9.30	8.78		
HGB, g/dl	Initial	17.43	17.03	16.88	0.26	0.43
	Final	14.24	15.03	14.55		
HCT, %	Initial	52.98	51.80	51.80	0.80	0.45
	Final	43.88	45.75	44.58		
MCV, fl	Initial	50.79	49.31	50.91	0.50	0.17
	Final	51.02	49.18	50.55		
MCH, pg	Initial	16.71	16.20	16.57	0.18	0.30
	Final	16.54	16.20	16.57		
MCHC, g/dl	Initial	32.88	32.88	32.55	0.13	0.37
	Final	32.40	32.90	32.63		
RDW, fl	Initial	26.40	25.18	25.41	0.25	0.17
	Final	23.44	24.17	24.48		
PLT, 10 ³ /μl	Initial	122.63	128.52	135.33	10.39	0.66
	Final	118.27	131.65	133.53		

WBC, white blood cells; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelets; ns, non significant ($p>0.05$)

Table 5. The effect of Maca on blood biochemical parameters in racehorses

Parameter	Period	Groups			SEM	p
		Control	M50	M75		
AST, IU/L	Initial	329.66	331.70	324.5	6.85	0.05
	Day 30	350.50	346.00	363.33		
	Final	333.00 ^{ab}	276.00 ^b	349.17 ^a		
ALP, IU/L	Initial	150.50	139.83	139.50	3.09	0.59
	Day 30	157.50	153.33	166.83		
	Final	105.00	103.16	112.30		
LDH, IU/L	Initial	236.17	245.67	227.50	6.76	0.91
	Day 30	274.67	261.66	272.16		
	Final	272.83	288.80	274.81		
CK, IU/L	Initial	143.00	156.83	130.00	39.11	0.48
	Day 30	141.67	205.00	133.83		
	Final	160.17	117.83	176.80		
GGT, IU/L	Initial	24.30	24.00	27.70	1.84	0.01
	Day 30	29.33	27.16	48.00		
	Final	19.39 ^b	15.00 ^b	41.83 ^a		

Parameter	Period	Groups			SEM	p
		Control	M50	M75		
TP, g/dl	Initial	6.23	6.08	6.22	0.06	0.54
	Day 30	6.38	6.48	6.55		
	Final	5.73	6.03	6.05		
GLOB, g/dl	Initial	2.13	2.43	2.52	0.05	0.09
	Day 30	2.32	2.75	2.72		
	Final	2.30	2.70	2.68		
ALB, g/dl	Initial	4.10	3.65	3.70	0.03	0.24
	Day 30	4.06	3.73	3.83		
	Final	3.43	3.33	3.67		
CRE, mg/dl	Initial	1.27	1.33	1.32	0.02	0.58
	Day 30	1.35	1.37	1.33		
	Final	1.20	1.27	1.25		
BUN, mg/dl	Initial	12.33	11.50	15.33	0.34	0.06
	Day 30	13.50	11.83	12.80		
	Final	12.00	11.50	12.70		
LA, mmol/l	Initial	1.33	1.38	1.39	0.09	0.18
	Day 30	1.08	1.47	1.13		
	Final	1.56	2.27	2.05		
Glucose, mg/dl	Initial	79.94	97.39	96.05	2.98	0.09
	Day 30	89.19	103.81	98.44		
	Final	94.32	96.96	117.73		
Cholesterol, mg/dl	Initial	90.89	110.40	94.45	4.03	0.57
	Day 30	94.44	112.06	82.98		
	Final	91.02	91.79	85.45		
Triglyceride, mg/dl	Initial	19.00	26.68	21.75	1.75	0.39
	Day 30	27.79	39.66	44.18		
	Final	37.62	34.99	29.21		

AST, aspartate transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CK, creatine kinase; GGT, gamma-glutamyl transpeptidase; TP, total protein; ALB, albumin; GLOB, globulin; CREA, creatinine; BUN, blood urea nitrogen; LA, lactic acid.

^{a, b} Means within a row with different superscripts are significantly different ($p < 0.05$); ns, non significant ($p > 0.05$)

Table 6. The effect of Maca on blood mineral concentrations in racehorses

Parameter	Period	Groups			SEM	p
		Control	M50	M75		
Ca, mg/dl	Initial	11.47	11.20	11.93	0.06	0.32
	Day 30	11.40	11.52	14.97		
	Final	11.83	12.17	15.12		
Mg, mg/dl	Initial	1.73	1.55	1.16	0.03	0.03
	Day 30	2.08	1.93	2.06		
	Final	2.27 ^a	2.10 ^{ab}	2.00 ^b		
P, mg/dl	Initial	3.62	2.98	3.12	0.07	0.24
	Day 30	3.05	3.23	3.43		
	Final	3.38	3.08	3.47		
Na, mmol/l	Initial	143.67	143.60	143.50	0.22	0.32
	Day 30	141.50	140.33	140.67		
	Final	136.00	134.67	135.50		
K, mmol/l	Initial	4.03	3.93	3.90	0.06	0.08
	Day 30	3.73	4.30	4.22		
	Final	3.93	4.43	4.32		
Cl, mmol/l	Initial	102.17	102.00	101.60	0.17	0.34
	Day 30	102.20	100.83	101.70		
	Final	99.67	97.66	98.70		

^{a, b} Means within a row with different superscripts are significantly different ($p < 0.05$); ns, non significant ($p > 0.05$)

Table 7. The effect of Maca on hormone concentrations in racehorses

Parameter	Group	Time (minute)						SEM	Effect of diet	Effect of time
		0	15	30	60	120	240			
Adiponectin, ng/ml	Control	6.25 ^d	8.04 ^c	23.45 ^{bB}	124.74 ^a	125.74 ^a	131.61 ^a	4.22	0.05	0.004
	M50	3.43 ^d	10.14 ^c	76.98 ^{bA}	124.21 ^a	106.88 ^a	126.50 ^a			
	M75	4.36 ^d	8.24 ^c	65.70 ^{bA}	107.34 ^a	134.60 ^a	141.11 ^a			
Leptin, ng/ml	Control	0.50	0.49	0.49	0.50	0.50	0.50	0.24	0.10	0.98
	M50	0.60	0.61	0.61	0.58	0.63	0.59			
	M75	0.72	0.62	0.58	0.57	0.60	0.48			
Ghrelin, pg/ml	Control	1341.83	1153.06	1000.00	1229.59	1454.08	1122.45	94.59	0.70	0.82
	M50	1155.10	1379.59	1665.31	1620.41	1183.67	1461.23			
	M75	1151.02	1093.88	1106.58	1244.90	1208.16	1081.38			
Insulin, µg/L	Control	0.02 ^c	0.18 ^b	0.21 ^a	0.39 ^{aA}	0.24 ^{aAB}	0.24 ^a	0.01	0.003	0.008
	M50	0.07 ^c	0.24 ^b	0.28 ^a	0.22 ^{aB}	0.30 ^{aA}	0.26 ^a			
	M75	0.04 ^c	0.18 ^b	0.21 ^a	0.15 ^{aB}	0.16 ^{aB}	0.16 ^a			
TSH, ng/ml	Control	0.91 ^{aA}	0.10 ^{bAB}	0.09 ^b	0.17 ^b	0.08 ^b	0.07 ^b	0.02	0.05	0.004
	M50	0.22 ^{aB}	0.18 ^{bA}	0.13 ^b	0.07 ^b	0.07 ^b	0.08 ^b			
	M75	0.49 ^{aAB}	0.08 ^{bB}	0.08 ^b	0.08 ^b	0.10 ^b	0.07 ^b			
T3, ng/ml	Control	1.72 ^{bB}	2.28 ^{abB}	2.50 ^{abB}	2.84 ^{aB}	2.21 ^{abB}	2.11 ^{bB}	0.44	0.001	0.005
	M50	3.17 ^{abB}	4.34 ^{aB}	3.77 ^{abB}	3.73 ^{abB}	4.28 ^{aB}	3.06 ^{bB}			
	M75	11.27 ^{aA}	9.54 ^{abA}	9.86 ^{abA}	9.91 ^{abA}	9.80 ^{abA}	6.60 ^{bA}			
T4, ng/ml	Control	14.10	22.64 ^b	32.17 ^{bB}	25.83	22.74 ^b	31.73	2.01	0.05	0.001
	M50	26.39	26.34 ^b	36.01 ^{bB}	33.42	33.85 ^b	27.45			
	M75	28.82	42.85 ^a	63.56 ^{aA}	45.56	45.44 ^a	35.62			

TSH, thyroid stimulating hormone; T3, triiodothyronine; T4, thyroxine

a, b, c, d Means within a row with different superscripts are significantly different depending on time (p<0.05)

A, B Means within a column with different superscripts are significantly different depending on diet (p<0.05)

ns, non significant (p>0.05)

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