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Effect of Different Dietary Levels of Atorvastatin and L-Carnitine on Performance, Carcass Characteristics and Plasma Constitutes of Broiler Chickens

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The effects of L-carnitine, atorvastatin and their combination on growth and lipid metabolism of broiler chickens is not yet known. Thus, the objective of the present study was to investigate the effects of dietary L-carnitine and atorvastatin on the performance, carcass characteristics and blood parameters in broilers. Different dietary levels of L-carnitine (0, 150 and 300 mg/kg, respectively) and atorvastatin (0, 1 and 2 g/kg, respectively) were added to the daily birds' ration. Significant positive effects ($P<0.05$) on broiler body weight for both L-carnitine and atorvastatin were reported, and this effect became clear starting from the 4th week of rearing period till the slaughter age. Dietary treatments had also significant ($P<0.05$) positive effects on broilers empty carcass, breast and drumstick weights. Conversely, L-carnitine slightly increased abdominal fat, whereas supplementing atorvastatin slightly reduced it ($P<0.05$). However, Combining the treatments, resulted in reduction of abdominal fat pad, showing also the best development of breast and drumstick muscles ($P<0.05$). Moreover, the weight of gizzard, liver and heart were significantly higher in birds treated with the highest doses supplied ($P<0.05$). Dietary treatments had also influence on blood biochemical parameters of broilers. In overall, our findings suggest that combining dietary L-carnitine and atorvastatin supported birds growth and muscles development reducing the body fat deposition. However, further studies are needed to deeply study the potential effect of statins on meat quality.

Key words: atorvastatin, blood, broiler, fat, growth, L-carnitine, muscle

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Introduction

Poultry meat is nutritionally desirable due to its high-quality protein and low fat content. The goal of the broiler market is thus to produce an animal that meets these nutritional goals at the least time period (Laudadio *et al*., 2012; Farrokhyan *et al*., 2014). Feed additives such as L-carnitine and atorvastatin, which are both involved in fatty acid metabolism, may be added to diet to achieve this aim. Atorvastatin calcium (also marketed under the brand names Lipitor®, Pfizer) is a statin and it is a synthetic 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase) inhibitor which lowers plasma cholesterol level by inhibiting endogenous cholesterol synthesis. It also reduces triglyceride levels through an as yet unknown mechanism (Lea and McTavish, 1997; Song *et al*., 2014). Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. In living cells, carnitine is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. It is also widely available as a nutritional supplement (Farrokhyan *et al*., 2014). Carnitine exists in two stereoisomers from which L-carnitine is biologically active. Human genetic disorders, such as primary carnitine deficiency, carnitine palmitoyltransferase-I deficiency, carnitine palmitoyltransferase-II deficiency and carnitine-acylcarnitine translocase deficiency, affect different steps of the fat metabolism. Therefore, L-carnitine plays a vital role in fat combustion (Carter *et al*., 1995) and energy production (Keralapurath *et al*., 2010). Alterations in carni-

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tine concentration or metabolism may significantly affect energy production in mitochondria (Arslan *et al*., 2003). Theoretically, dietary carnitine supplementation could be used to facilitate fatty acid oxidation for energy production and enhance animals growth performance. Previous studies have been conducted to assess the influence of L-carnitine on poultry, but the results obtained are not in agreement. Some trials have shown that supplemental L-carnitine improved body weight gain and reduced the abdominal fat content of chickens (Rabie *et al*., 1997), but other research (Xu *et al*., 2003) found no effects of L-carnitine on broiler growth traits. Nevertheless, the effects of L-carnitine, atorvastatin or their combination on lipid metabolisms and growth of broiler chickens is not yet known.

Therefore, the objectives of the present study were to investigate the effects of dietary L-carnitine and atorvastatin on the performance, carcass characteristics as well as the blood biochemical parameters in broiler chickens.

Materials and Methods

The experiment was conducted at a commercial poultry farm in Iran. All animal procedures were approved by the Institutional Ethics Committee of the Islamic Azad University, Rasht, Iran and care was taken to minimise the number of animals used.

Animals and Dietary Treatments

A total 270 one-day old male Ross 308 chicks (average body weight 44.0±1.5 g) were divided in 27 groups of 10 animals each. Each group was randomly assigned to one of nine treatments. The study was conducted during 1−42 d of age. Birds were fed an isocaloric and isonitrogenous basal-diet, and the ingredient composition as well as the calculated nutrient composition of the diets used during starter (1−14 d of age), grower (15−28 d of age), and finisher periods (29−42 d of age) are reported in Table 1. Nutritional requirements were provided based on the standard recommendations (Ross, 2007).

The dietary treatments were based on different levels of L-carnitine and atorvastatin and were as follows:

Treatment 1 control): L-carnitine (0 mg/kg) - atorvastatin (0 g/kg)
Treatment 2: L-carnitine (150 mg/kg) - atorvastatin (0 g/kg)
Treatment 3: L-carnitine (300 mg/kg) - atorvastatin (0 g/kg)
Treatment 4: L-carnitine (0 mg/kg) - atorvastatin (1 g/kg)
Treatment 5: L-carnitine (150 mg/kg) - atorvastatin (1 g/kg)
Treatment 6: L-carnitine (300 mg/kg) - atorvastatin (1 g/kg)
Treatment 7: L-carnitine (0 mg/kg) - atorvastatin (2 g/kg)
Treatment 8: L-carnitine (150 mg/kg) - atorvastatin (2 g/kg)

Table 1. **Ingredients and nutrient analysis of diets fed to broiler chickens**

Ingredients (g/kg as-fed)	Diets		
	Starter	Grower	Finisher
Corn	557.85	621.75	640.85
Soybean meal	375.0	320.0	302.0
Soybean oil	20.0	20.0	20.0
$Ca_{\%22}P_{\%18}$	17.0	11.0	13.0
$CaCO_3$	11.3	13.0	9.0
Mineral premix	6.0	5.0	6.0
Vitamin K_3	1.0	1.0	1.0
Vitamin E	1.0	1.0	0.5
DL-Met	3.3	1.8	1.7
L-Lys HCl	2.2	1.0	1.0
NaCl	1.9	2.0	2.5
Salinomycin	0.5	0.5	0.5
Na bicarbonate	2.5	1.5	1.5
Multi-enzyme	0.35	0.35	0.35
Phytase	0.1	0.1	0.1
Nutrient analysis (% unless stated otherwise)			
ME (kcal/kg)	2,820	2,890	3,050
Crude protein	25.0	22.2	19.0
Crude fiber	3.0	3.0	3.0
Ca	1.00	0.86	0.82
Available P	0.50	0.43	0.41
Na	0.16	0.16	0.16
Ly	1.28	1.00	1.00
Met	0.58	0.45	0.38
Linoleic acid	1.0	1.0	1.0
K	0.8	0.8	0.8
Met+Cys	0.93	0.77	0.72
Chloride	0.16	0.15	0.14

Treatment 9: L-carnitine (300 mg/kg) - atorvastatin (2 g/kg)

Management and Sampling of Experimental Birds

At one day of age all birds were individually weighed and randomly assigned to 27 floor pens. During the first three weeks of rearing period, room temperature was set at 33℃ on the first days and thereafter dropped to 30℃ on the successive days. Subsequently, room temperature was lowered gradually by 2.8℃ every week until a temperature of 20℃ was reached. Room temperature was monitored by three thermometers which were placed in the middle and two ends of the broiler house. The birds were kept under a 22 hour light regime throughout the study period. During the first week, feed and water were provided in feeder trays and conical drinkers, respectively. During the rest of the rearing period, chute feeders and drinkers were used. Feed and water were provided *ad libitum*.

The birds were vaccinated against infectious bronchitis at the 1st and 14th day of age, against Newcastle disease at the day 1, 8, 19 and 30 of age, against avian influenza at day 8 of age and against Gumboro's disease at day 8, 16 and 23 of age.

Body weight and feed intake were weekly measured starting from three to six weeks of age. Feed conversion ratio was calculated. At slaughter (42 d of age), four chickens per treatment (one chicken per replication) were selected for assessment of carcass (full and empty carcass weight, abdominal fat pad weight, breast and drumstick weight, neck weight) and organs (gizzard, liver and heart weight), and for blood analysis. Care was taken to choose the most representative broiler chicken with respect to body weight compared to the pen mean body weight. After slaughter and plucking operations, the head and legs were removed and broiler chickens were eviscerated.

Blood samples (～1 ml/chicken) were collected into EDTA tubes from the wing veins. Samples were transferred to the laboratory for analysis within 2 hours of collection. After centrifugation (3000 g×10 min at room temperature) plasma was harvested and stored in Eppendorf tubes at −20℃, until assayed. Commercial kits (Pars Azmoon Co., Tehran, Iran) were used and manufacturer's instructions were followed. Glucose was measured by a glucose-oxidase photometric assay. Cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) were determined by enzymatic CHOD-PAP (Tan *et al*., 1991). Uric acid was determined by enzymatic methods using the uricase-TOOS method (Kato *et al*., 2000). Alkaline phosphatase (ALP) was assayed by the method described by Thomas and Whicher (1998).

Statistical Analysis

The data were analyzed using as omnibus test a general linear models (SAS Institute Inc. 2004), which is robust enough to allow for the moderately imbalanced data from these experiments. The model included L-carnitine and atorvastatin as main effects. The interaction between main effects was included in the model. Differences among groups were tested using Duncan's test. Significance level was set at $P<0.05$.

The model used was: $Y_{ij}=\mu+A_i+Bj+AB_{ij}+e_{ijk}$,
where: μ=the common mean, A_i=the effects of the L-carnitine, B_j=the effect of the atorvastatin, AB_{ij}=the effect of the i^{th} A with the j^{th} B, and e_{ijk}=the random error. Before performing data statistical analysi, all data were tested by normality test.

Results and Discussion

This research aimed to investigate the combined effects of dietary L-carnitine and atorvastatin on the growth performance, carcass traits and blood biochemical parameters in broiler chickens. L-carnitine has been widely investigated for its function in the fatty acids oxidation and transport into the mitochondrial membrane (Farrokhyan *et al*., 2014). Its role in commercial broiler production, however, remains to be clearly clarified, as previous studies have reported contradictory results on some performance traits in response to dietary L-carnitine.

Combining dietary L-carnitine and atorvastatin influenced most of the performance parameters in the study. Particularly, our findings showed significant ($P<0.05$) positive effects on broilers body weight for both L-carnitine and atorvastatin supplementation, and this trend resulted significant starting from the 4th week of rearing period till the slaughter age (Table 2). Furthermore, there were significant interactions between L-carnitine and atorvastatin on final weight gain and FCR over the course of the experiment. The highest chicken weights at slaughter were detected in the group 9 fed with the highest levels of L-carnitine and atorvastatin, and the effect started already at the 4th week of feeding period (data not shown). Feed intake and body weight gain of birds, however, were not affected by dietary treatment; thus, as expected it was not surprising that feed conversion ratio was significantly better in the experimental group receiving the highest L-carnitine and atorvastatin levels (Table 2). Previous available studies evaluated the effect of dietary L-carnitine on chickens weight gain changes, and these ranged from increases in weight gain (Rodehutscord *et al*., 2002) to no significant effect (Lien and Horng, 2001) as in our study. It is interesting that in the present trial, atorvastatin at the rate of 2 g/kg inverted the body weight gain attributed to L-carnitine when both were present in diet.

Dietary treatments also had significant positive effects on broilers empty carcass weight, breast and drumstick weights. Conversely, dietary L-carnitine slightly increased the abdominal body fat, whereas atorvastatin supplementation slightly reduced it (Table 3). Combining the treatments, however, mildly reduced abdominal fat pad and showing also the best development of the breast and drumstick muscles as well as empty carcass weight. Moreover, the weights of gizzard, liver and heart were significantly higher in the dietary group supplemented with the highest doses of L-carnitine and atorvastatin (Table 4). Thus, these results strongly suggest atorvastatin influences the various carcass traits by ameliorating the effects of high levels of L-carnitine dietary supplementation. In a recent study the supplementation of L-carnitine to the basal diet increased body weight

Table 2. **Growth performance of broiler chickens fed diets containing different levels of L-carnitine and atorvastatin**

Item		BW (g)	BWG (g/d)	Cumulative FI (g)	FCR
L-carnitine (mg/kg)	0	1942.9^{c}	46.3	4339.0	2.23^{a}
	150	2021.7^{b}	48.1	4383.3	2.16^{ab}
	300	2091.5^{a}	49.8	4378.6	2.09^{b}
	SEM	35.7	2.95	55.11	0.03
Atorvastatin (g/kg)	0	1951.7^{c}	46.5	4327.7	2.21^{a}
	1	2014.2^{b}	47.9	4366.2	2.16^{ab}
	2	2090.2^{a}	49.7	4398.1	2.10^{b}
	SEM	36.9	2.01	59.11	0.03
Interactions					
L-carnitine (0) - atorvastatin (0)		1890.9^{f}	45.0	4352.9	2.30^{a}
L-carnitine (150) - atorvastatin (0)		1954.7^{e}	46.5	4321.6	2.21^{a}
L-carnitine (300) - atorvastatin (0)		2009.5^{cd}	47.9	4308.5	2.14^{ab}
L-carnitine (0) - atorvastatin (1)		1939.7^{e}	46.2	4343.3	2.23^{a}
L-carnitine (150) - atorvastatin (1)		2018.0^{c}	48.1	4313.9	2.13^{ab}
L-carnitine (300) - atorvastatin (1)		2084.9^{b}	49.6	4441.4	2.13^{ab}
L-carnitine (0) - atorvastatin (2)		1998.2^{d}	47.6	4293.8	2.14^{ab}
L-carnitine (150) - atorvastatin (2)		2092.5^{b}	49.8	4514.5	2.15^{ab}
L-carnitine (300) - atorvastatin (2)		2180.0^{a}	51.9	4358.9	2.01^{b}
	SEM	51.1	2.33	82.38	0.05

Means within each column with no common superscripts differ significantly at $P<0.05$.
SEM, standard error of means
BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ration.

Table 3. **Carcass characteristics at 42nd days of age of broiler chickens fed diets containing different levels of L-carnitine and atorvastatin**

Item		Full carcass weight (g)	Empty carcass weight (g)	Abdominal fat pad (g)	Breast weight (g)	Drumstick weight (g)
L-carnitine (mg/kg)	0	1942.9^{c}	1515.5^{c}	29.54^{b}	510.9^{c}	328.2^{a}
	150	2021.7^{b}	1576.9^{b}	29.80^{a}	531.7^{b}	335.6^{a}
	300	2091.2^{a}	1631.4^{a}	29.70^{a}	550.1^{a}	339.5^{a}
	SEM	3.57	2.78	0.05	0.93	4.07
Atorvastatin (g/kg)	0	1951.7^{c}	1522.3^{c}	31.45^{a}	513.3^{c}	271.1^{c}
	1	2014.2^{b}	1571.1^{b}	29.18^{b}	529.7^{b}	359.3^{b}
	2	2090.2^{a}	1630.4^{a}	28.40^{b}	549.7^{a}	373.0^{a}
	SEM	3.06	2.89	0.09	1.01	4.32
Interactions						
L-carnitine (0) - atorvastatin (0)		1890.9	1474.9^{f}	31.95^{a}	487.3^{c}	259.0^{c}
L-carnitine (150) - atorvastatin (0)		1954.7^{e}	1524.7^{de}	31.27^{b}	514.1^{c}	277.3^{c}
L-carnitine (300) - atorvastatin (0)		2009.5^{cd}	1567.4^{cd}	31.14^{b}	528.5^{c}	276.6^{c}
L-carnitine (0) - atorvastatin (1)		1939.7^{e}	1512.9	29.09^{c}	510.1^{b}	357.0^{b}
L-carnitine (150) - atorvastatin (1)		2018.0^{c}	1574.0^{bc}	29.26^{c}	530.7^{ab}	360.7^{ab}
L-carnitine (300) - atorvastatin (1)		2084.9^{b}	1626.3^{b}	29.18^{c}	548.3^{ab}	360.3^{ab}
L-carnitine (0) - atorvastatin (2)		1998.2^{d}	1558.6	27.57^{e}	525.5^{ab}	368.6^{ab}
L-carnitine (150) - atorvastatin (2)		2092.5^{b}	1632.2^{b}	28.87^{d}	550.3^{ab}	368.7
L-carnitine (300) - atorvastatin (2)		2180.0^{a}	1700.4^{a}	28.77^{d}	573.3^{a}	381.7^{a}
	SEM	6.18	4.82	0.08	1.62	7.05

Means within each column with no common superscripts differ significantly at $P<0.05$.
SEM, standard error of means

Table 4. **Carcass characteristics at 42nd days of age of broiler chickens fed diets containing different levels of L-carnitine and atorvastatin**

Item		Neck (g)	Gizzard (g)	Liver (g)	Heart (g)
L-carnitine (mg/kg)	0	63.81	58.28^{c}	85.48^{c}	15.54^{c}
	150	63.22	60.65^{b}	88.95^{b}	16.17^{b}
	300	61.64	62.74^{a}	92.02^{a}	16.73^{a}
	SEM	3.28	0.10	0.15	0.02
Atorvastatin (g/kg)	0	63.11	58.55^{c}	85.87^{c}	15.61^{a}
	1	64.77	60.42^{b}	88.62^{b}	16.11^{a}
	2	65.88	62.70^{a}	91.97^{a}	16.72^{a}
	SEM	3.74	0.12	0.19	0.03
Interactions					
L-carnitine (0) - atorvastatin (0)		62.66	56.72^{f}	83.20^{f}	15.12^{e}
L-carnitine (150) - atorvastatin (0)		58.33	58.64^{de}	86.00^{e}	15.63^{d}
L-carnitine (300) - atorvastatin (0)		56.33	60.28^{cd}	88.41^{cd}	16.07^{c}
L-carnitine (0) - atorvastatin (1)		66.33	58.19^{e}	85.34^{e}	15.51^{de}
L-carnitine (150) - atorvastatin (1)		65.00	60.54^{c}	88.79^{c}	16.14^{c}
L-carnitine (300) - atorvastatin (1)		63.00	62.54^{b}	91.73^{b}	16.67^{b}
L-carnitine (0) - atorvastatin (2)		61.66	59.94^{d}	87.91^{d}	15.98^{cd}
L-carnitine (150) - atorvastatin (2)		66.33	62.77^{b}	92.07^{b}	16.74^{b}
L-carnitine (300) - atorvastatin (2)		62.66	65.40^{a}	95.92^{a}	17.44^{a}
	SEM	5.69	0.18	0.27	0.04

Means within each column with no common superscripts differ significantly at $P<0.05$.
SEM, standard error of means

and leading to a better development of valuable meat parts in broilers such as breast and drumsticks (Farrokhyan *et al*., 2014). This effect was supposed due to be the result of a more efficient lipid metabolism which resulted in more ATP for muscle protein production. Since statins make cells to up-regulate LDL-receptors, even more lipids may enter the cell and subsequently its mitochondria (Arslan *et al*., 2003). Thus, our data suggest that the combined treatment led to a decrease in body fat and an increase of muscles development.

Previously, a number of studies also reported that L-carnitine alone showed no influences on the abdominal fat deposition, heart and liver weights (Lien and Horng, 2001; Arslan *et al*., 2003; Farrokhyan *et al*., 2014). This was probably due to the limited intestinal absorptive capacity of carnitine as well as it being easily degraded by intestinal microbes (Xu *et al*., 2003).

The blood parameters at 42nd days of age of broiler chickens fed diets containing different levels of L-carnitine and atorvastatin are reported in Table 5. There were interactions between L-carnitine and atorvastatin on the blood glucose, cholesterol, triglycerides, HDL and LDL as well as acid uric and ALP concentrations. The nature of the interactions were such that dietary supplementation with L-carnitine resulted in lower cholesterol, which was subsequently reversed after the addition of 2 g/kg of atorvastatin; the highest supplementation of dietary L-carnitine (200 mg/kg) resulted in significantly reduced uric acid only when there was no atorvastatin in the diet; and, finally, L-carnitine alone had no effect on the glucose level, but when given in combination with atorvastatin, blood glucose increased. There were no observed differences in main effects for LDL, uric acid and glucose concentrations. Results of the present study show that while L-carnitine and atorvastatin alone did not influence significantly the glucose, LDL and acid uric levels. Conversely, our findings show that atorvastatin at the highest levels together with L-carnitine reduces blood triglyceride level. The mechanism through which atorvastatin influences blood triglyceride concentrations are still unclear. Nevertheless, this phenomenon has been observed in human (Munoz *et al*., 2013) and rats (Macan *et al*., 2010). Atorvastatin has been reported to reduce the absorption of cholesterol in the intestine of rats (Umeda *et al*., 2001), and this could be responsible for the lower blood cholesterol levels found with the highest dietary supplementation of atorvastatin alone.

Conclusions

In conclusion, this is the first study examining the effect of atorvastatin and L-carnitine together on poultry production and carcass traits. Hypothetically, dietary supplementation with atorvastatin should facilitate the fatty acids oxidation, and thus decrease the esterification and triacylglycerol storage in the adipose tissue. Our findings indicate that atorvastatin and L-carnitine dietary supplementation in combination influence the lipid redistribution, intramuscular fat, carcass traits and blood parameters in broiler chickens, resulting in enhanced carcass quality. However, further studies are needed to deeply study the potential effect of statins on the chickens' muscle characteristics and meat quality, including the taste of valuable parts.

Table 5. **Blood parameters at 42nd days of age of broiler chickens fed diets containing different levels of L-carnitine and atorvastatin**

Item		Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Uric acid (mg/dl)	ALP (U/dl)
L-carnitine (mg/kg)	0	199.5^{a}	144.6^{a}	34.9^{a}	47.2^{b}	73.2	3.87^{a}	435.6^{a}
	150	197.6^{a}	121.7^{b}	29.8^{ab}	58.0^{a}	72.5	3.21^{a}	236.6^{b}
	300	201.2^{a}	120.0^{b}	26.0^{b}	61.0^{a}	70.7	3.71^{a}	177.8^{c}
	SEM	10.71	3.19	1.93	1.54	2.46	0.31	17.41
Atorvastatin (g/kg)	0	207.5^{a}	133.8^{a}	36.5^{a}	42.1^{c}	76.4	3.50^{a}	272.5^{b}
	1	191.7^{a}	129.1^{ab}	28.1^{b}	55.4^{b}	71.2	3.67^{a}	243.4^{b}
	2	199.0^{a}	123.6^{b}	26.2^{b}	68.8^{a}	68.8	3.61^{a}	334.2^{a}
	SEM	10.88	3.28	1.89	1.60	2.52	0.33	17.51
Interactions								
L-carnitine (0) - atorvastatin (0)		209.4^{a}	177.3^{a}	50.6^{a}	34.8^{f}	82.0	3.53^{ab}	381.3^{b}
L-carnitine (150) - atorvastatin (0)		218.6^{a}	115.0^{bc}	29.8^{b}	49.0^{de}	73.3	3.73^{ab}	125.3^{c}
L-carnitine (300) - atorvastatin (0)		194.7^{ab}	109.1^{c}	29.1^{b}	42.5^{ef}	74.0	3.25^{ab}	311.00^{b}
L-carnitine (0) - atorvastatin (1)		202.2^{a}	131.6^{b}	28.5^{b}	44.8^{e}	67.6	3.13^{ab}	84.0^{c}
L-carnitine (150) - atorvastatin (1)		165.8^{b}	127.0^{bc}	32.0^{b}	54.8^{cd}	78.3	3.13^{ab}	84.1^{c}
L-carnitine (300) - atorvastatin (1)		207.2^{a}	128.6^{b}	23.7^{b}	66.6^{ab}	67.6	4.68^{a}	86.0^{c}
L-carnitine (0) - atorvastatin (2)		186.9^{b}	124.9^{bc}	25.5^{b}	62.1^{bc}	70.0	4.87^{a}	365.3^{b}
L-carnitine (150) - atorvastatin (2)		208.6^{a}	123.2^{bc}	27.7^{b}	70.3^{ab}	66.0	2.76^{b}	500.6^{a}
L-carnitine (300) - atorvastatin (2)		201.6^{a}	122.4^{bc}	25.29^{b}	74.05^{a}	70.66	3.21^{ab}	136.6^{c}
	SEM	18.55	5.53	3.34	2.67	4.75	0.54	30.15

Means within each column with no common superscripts differ significantly at $P<0.05$
SEM, standard error of means
HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; ALP, Alkaline Phosphatase.

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