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Effect of dietary simvastatin and L-carnitine supplementation on blood biochemical parameters, carcass characteristics and growth of broiler chickens

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ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi efek dari simvastatin (SIM) dan L-carnitine (LC) serta interaksinya, pada penampilan sifat produksi produktif dan kualitas karkas broiler. Untuk tujuan ini, percobaan pakan dilakukan, berdasarkan racangan faktorial 3 × 3, menggunakan SIM pada tingkat 0, 1 atau 2 g / kg dan LC pada tingkat 0, 150 atau 300 mg / kg dalam pakan dasar. Sembilan perlakuan adalah: T1 (kontrol-diet, 0/0), T2 (0/150), T3 (0/300), T4 (1/0), T5 (1/150), T6 (1/300), T7 (2/0), T8 (2/150), dan T9 (2/300). Asupan pakan lebih tinggi pada T2 (4716 g), T7 (4722 g) dan T9 (4698 g) daripada di T1 (4545 g; P <0,05) mempertimbangkan fase pertumbuhan terakhir (35-42 hari) atau seluruh produksi 42 hari. Peningkatan efisiensi pakan juga diperoleh pada T8 (1,64) dan T9 (1,67) dibandingkan dengan T1 (1,77; P <0,05), dan ini terutama disebabkan oleh interaksi SIM × LC (P <0,05). LC mempengaruhi secara positif (P <0.05) karkas, dada, *drumstick* dan lemak bagian perut, serta tingkat trigliserida plasma. Kesimpulan penelitian ini adalah kombinasi aditif SIM dan LC memiliki pengaruh positif terhadap kinerja pertumbuhan dan sifat karkas ayam broiler.

Kata kunci: broiler, karkas, pertumbuhan, simvastatin , metabolism lemak

ABSTRACT

The aim of this study was to evaluate the effect of simvastatin (SIM) and L-carnitine (LC) additives and their interactions, on productive performance and carcass quality of broilers. For this purpose, a feeding trial was conducted, under 3×3 factorial design, using SIM at 0, 1 or 2 g/kg level and LC at 0, 150 or 300 mg/kg level in a basal-diet and originating a total of nine treatments: T1 (control-diet, 0/0), T2 (0/150), T3 (0/300), T4 (1/0), T5(1/150), T6 (1/300), T7 (2/0), T8 (2/150), and T9 (2/300). Feed intake was higher in T2 (4716 g), T7 (4722 g) and T9 (4698 g) than in T1 (4545 g; P<0.05) considering the last growing phase (35-42 days) or whole 42-day production cycle. An improvement of feed efficiency was also observed in T8 (1.64) and T9 (1.67) when compared to T1 (1.77; $P<0.05$), and these were mainly due to SIM × LC interactions ($P<0.05$). The LC influenced positively ($P<0.05$) the weight of eviscerated carcass, breast, drumsticks and abdominal fat, as well as plasma triglycerides level. This findings suggests that the combination of SIM and LC additives have a positive influence on growth performance and carcass traits of broiler chickens.

Keywords: broiler, carcass, growth, simvastatin, lipid metabolism

INTRODUCTION

Simvastatin (SIM) is a hydroxy-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitor which can induce a reduction of (very) low-density lipoprotein (LDL) cholesterol as well triglycerides and apolipoprotein B and commonly already used in humans (Pedersen and Tobert, 2004; Fox *et al.*, 2007) and also in animals (Laniesse and Beaufrere, 2014).

In poultry, during the last five decades, SIM and other statins (e.g., lovastatin and pravastatin) were evaluated in order to produce eggs with low cholesterol levels (Kim *et al.*, 2004; Elkin, 1999, 2007; Kumar *et al.*, 2012; Botelho *et al.*, 2015) or even as therapeutic in avian pets (e.g., parrots) regarding atherosclerosis disease (Laniesse and Beaufrere, 2014). The reduction of egg cholesterol can be obtained by supplementing hens' diet with 0.03 or 0.06% SIM (Kim *et al.*, 2004). Jasiecka *et al.* (2013) suggested that higher daily doses are necessary to obtain effective cholesterol reduction in poultry compared to other species mammals (e.g., pig). However, limited studies regarding the effects of different SIM doses on broiler production performance and traits were found.

The L-carnitine (LC; β-hydroxy γ-trimethylamino-butyrate) is ~~a~~ an endogenous zwitterionic amine which promote long-chain fatty acids into the mitochondria for beta-oxidation (Borum, 1983) and consequently it decreases ~~it~~ the availability for adipocyte storage. It is synthesized in body from L-lysine and L-methionine amino acids as substrate (Pekala *et al.*, 2012; Tufarelli *et al.*, 2018; Hussein *et al.*, 2019). The positive effects of single or combined LC dietary supplementation on productive performance and lipid metabolism in poultry were well documented ~~in~~ by using different diet and under different environmental conditions (Rabie *et al.*, 1997a,b; Buyse *et al.*, 2001; Xu *et al.*, 2003; Azadmanesh and Jahanian, 2014; Jia *et al.*, 2014; Murali *et al.*, 2015; Awad *et al.*, 2016; Rehman *et al.*, 2017), but not in combination with SIM as additive.

Therefore, the present study aimed to evaluate the effects of SIM and LC additives on broiler chicken's performance and carcass traits as well on plasma biochemical profile.

MATERIALS AND METHODS

Animals and Treatments

A total of 270 one-day old male Ross 308 chicks were individually weighed (44.0±0.8 g) and randomly assigned to nine groups, each with three replicates of ten birds, under 3×3 factorial design. Each replicate was housed in a floor pen (1.0 × 1.2 m), where room temperature was maintained at 32°C for the first week and gradually reduced by 2°C every week until 22°C. Room temperature was monitored by three thermometers, which were placed in the middle and at the two ends of the broiler house. Light regime was regulated as follows: 24 h light (1st day), 23 h light and 1 h dark (2^{nd}-6^{th} day), 16 h light and 8 h dark (7-21^{th} day), 18 h light and 6 h dark (22-28^{th} day), and 23 h light and 1 h dark (29-42^{th} day). The birds were vaccinated against Bronchitis (1 and 14 days of age), Newcastle disease (1, 8, 19, and 30 days of age), Gumboro (8, 16 and 23 days of age) and Influenza (8 days of age) following standard vaccination program.

Feed and water were provided *ad libitum* in chute feeders and conical drinkers, apart from the 1^{st} week when feeder trays were used. Table 1 shows the ingredients and the composition of the starter, grower and finisher diets used in the trial. Diet (14-42 days of age) supplemented either with SIM at 0.0, 1.0, and 2.0 g/kg, or LC at 0, 150 and 300 mg/kg, or both from 15^{th}-42^{nd} days of age as: T1 (Treatment 1; control-diet) without SIM and LC; T2, SIM (0 g/kg) and LC (150 mg/kg); T3, SIM (0 g/kg) and LC (300 mg/kg); T4, SIM (1.0 g/kg) and LC (0 mg/kg); T5, SIM (1.0 g/kg) and LC (150 mg/kg); T6, SIM (1.0 g/kg) and LC (300 mg/kg); T7, SIM (2.0 g/kg) and LC (0 mg/kg);

Table 1. Ingredients and Nutrient Analysis of Diets Fed to Broilers

Ingredient (g/kg as fed)	Starter	Grower	Finisher
Corn	558.5	624.0	630.0
Soybean meal (44% CP)	375.0	320.0	302.0
Soybean oil	20.0	20.0	20.0
Monocalcium phosphate	17.0	11.0	13.0
Calcium carbonate	11.3	13.0	9.0
Mineral Premix[1]	3.0	2.5	3.0
Vitamin Premix[2]	3.0	2.5	3.0
Vitamin K_3	1.0	1.0	1.0
Vitamin E	1.0	1.0	0.5
DL-Methionine	3.3	1.8	1.7
Lysine-Hydro-Chloride	2.2	1.0	1.0
Salt	1.9	2.0	2.5
Anticoccidial	0.5	0.5	0.5
Sodium bicarbonate	2.5	1.5	1.5
Multi-enzyme premix	0.35	0.35	0.35
Phytase	0.1	0.1	0.1
Nutrient analysis			
ME (kcal/kg)	2,930	3,050	3,100
Crude protein (%)	23.0	21.5	20.0
Crude fiber (%)	3.0	3.0	2.9
Calcium (%)	1.0	0.86	0.8
Available Phosphorus (%)	0.5	0.43	0.4
Sodium (%)	0.16	0.16	0.16
Lysine (%)	1.28	1.1	1.0
Methionine (%)	0.58	0.45	0.45
Linoleic acid (%)	1.0	1.0	1.0
Potassium (%)	0.8	0.8	0.8
Methionine + Cysteine (%)	0.93	0.77	0.75
Chlorine (%)	0.16	0.15	0.15

[1]Calcium Pantothenate 4 mg/g; Niacin 15 mg/g; Vitamin B_6 13 mg/g; Cu 3 mg/g; Zn 15 mg/g; Mn 20 mg/g; Fe 10 mg/g; K 0.3 mg/g; [2] Vitamin A 5000 IU/g; Vitamin D_3 500 IU/g; Vitamin E 3 mg/g; Vitamin K_3 1.5 mg/g; Vitamin B_2 1 mg/g.

T8, SIM (2.0 g/kg) and LC (150 mg/kg); and T9, SIM (2.0 g/kg) and LC (300 mg/kg). Feed intake (FI) and body weight (BW) were measured at the age of 21, 28, 35 and 42 days of age. Feed conversion ratio (FCR) was calculated as FI divided by BW gain (BWG) for each replicate (Alimohammadi-Saraei *et al.*, 2016). At 42 days of age, five chicks per replicate were selected and sacrificed. Feet were separated from the carcass in the tibio-tarsal joint. Abdominal fat, organs and carcass components were removed and weighed.

Blood samples were also collected from the same subjects for analyses of hematological parameters based on Nahavandinejad *et al.* (2014). A blood sample of 5 mL was collected from the ulnar vein of birds at the age of 42 days and put in a tube containing anticoagulant EDTA. Samples were further centrifuged at 2000 $g \times 20$ min, and blood plasma was stored at -20°C for further analyses. Collection of blood was performed early in the morning to minimize the circadian variations in the examined plasma parameters. Feed was also removed for a period of 4 h before blood sampling.

Total plasma cholesterol and triglyceride levels were determined by using the respective enzymatic methods (TeifAzmoon Pars, Co., Tehran, Iran) according to Schmid and Von Forstner (1986). Cholesterol fractions (HDL and LDL) were measured directly by HDL-C and LDL-C diagnostic kits (TeifAzmoon Pars Co, Tehran, Iran). The colorimetric index of cholesterol was also determined using the cholesterol oxidase procedure (Schmid and Von Forstner, 1986). Plasma glucose levels were measured using a glucose oxidase kit based on the oxidase-peroxidase procedure (TeifAzmoon Pars, Co., Tehran, Iran) as described by Trinder (1969) and Barham and Trinder (1972). Finally, alkaline phosphatase was evaluated based on Bessey *et al.* (1946).

Statistical Analysis

Data were analyzed by ANOVA, considering three SIM treatments (0.0, 1.0, and 2.0 g/kg) and three LC treatments (0, 150, and 300 mg/kg), using a two-way procedure of SPSS (1997). The means were compared by using Duncan's Multiple Range test and the results were considered significantly different at $P<0.05$.

RESULTS

Regarding the whole broiler 42-days production cycle, a significant increase of FI was observed (Table 2). No significant effect of

Table 2. Effect of Dietary Ttreatments on Feed Consumption (g) of Broilers

Item		14-21 DOA	22-28 DOA	29-35 DOA	35-42 DOA	1-42 DOA
	0.0	697.8	909.2	1238.2	1310.5	4641.8
Simvastatin (SIM, g/kg)	1.0	720.5	903.6	1242.5	1294.1	4643.9
	2.0	681.4	892.2	1284.8	1322.4	4667.0
	0	704.7	875.7^{a}	1259.8	1302.2	4628.5
L-Carnitine (LC, mg/kg)	150	690.2	936.6^{b}	1253.1	1287.7	4653.8
	300	704.8	892.5^{ab}	1252.8	1284.1	4670.8
SIM × LC		NS	<0.05	NS	<0.05	<0.05
SIM (0) - LC (0)		705.5	906.3^{ab}	1194.7	1253.0^{a}	4545.0^{b}
SIM (0) - LC (150)		694.0	943.3^{ab}	1253.7	1338.7^{ab}	4715.7^{a}
SIM (0) - LC (300)		694.7	878.0^{abc}	1266.3	1340.0^{ab}	4665.2^{ab}
SIM (1.0) - LC (0)		739.3	807.0^{c}	1283.0	1303.3^{ab}	4617.7^{ab}
SIM (1.0) - LC (150)		704.3	967.3^{a}	1225.7	1280.3^{ab}	4663.2^{ab}
SIM (1.0) - LC (300)		718.0	936.7^{ab}	1219.0	1289.7^{ab}	4649.9^{ab}
SIM (2.0) - LC (0)		670.0	914.0^{ab}	1301.7	1350.3^{ab}	4722.0^{a}
SIM (2.0) - LC (150)		672.3	899.3^{ab}	1279.0	1244.3^{b}	4581.0^{b}
SIM (2.0) - LC (300)		702.0	863.3^{bc}	1274.0	1372.7^{a}	4698.0^{a}

Means within each column with no common superscript differ significantly at $P < 0.05$; NS: Not significant; DOA: days of age treatments (P>0.05) was observed on BWG among groups (Table 3), however the overall FCR improved (P<0.05) for T8 and T9 groups when compared with T1 (1.77) (Table 4). The different results were mainly due to significant SIM×LC interactions (P<0.05) on FI, BWG and FCR. The diet containing 300 mg/kg LC improved (P<0.05) the weight of eviscerated carcass, breast, drumsticks and decreased (P<0.05) the weight of abdominal fat when compared with diets supplemented with 150 mg/kg LC or without LC supplementation (Table 5). However, no SIM×LC significant interactions (P>0.05) were observed. In overall, nor SIM neither LC influenced (P>0.05) the plasma biochemical profile with the exception of LC supplementation which decreased (P<0.05) the plasma triglycerides level.

DISCUSSION

The main findings of the present study were the positive improvement of T9 on FI and FCR and a significant reduction of abdominal fat (about 46%; 10.6 g/carcass), as well plasma triglycerides concentration (~52%; 66.88 mg/kg) when LC was added to 300 mg/kg level and regarding whole 42-day production cycle. No significant effects of 0.03 or 0.06% simvastatin, lovastatin and pravastatin on FI and FCR was observed by Kim *et al.* (2004) in laying hens. However, a low FI, without influence in feed efficiency, was observed by the same researchers when 0.06% lovastatin was added to diet. In a similar way, no significant effects of 25 to 100 mg/kg or even 900 mg/kg LC supplementation (alone) of broiler diets on FI, BWG or FCR were observed by Xu *et al.* (2003) and Murali *et al.* (2015), respectively. However, Parsaeimehr *et al.* (2014) observed a positive effect of 300 mg/kg LC plus 5 or 6% animal fat on all these indexes during the last growing phase (4-6 week) or whole 42-day cycle. In agreement with these studies, we

Table 3. Effect of Dietary Treatments on Body Weight Gain (g) of Broilers

Item		14-21 DOA	22-28 DOA	29-35 DOA	35-42 DOA	1-42 DOA
	0.0	494.6	548.5	557.6	662.3	2637.9
Simvastatin (SIM, g/kg)	1.0	444.8	559.6	577.0	647.6	2660.1
	2.0	393.4	619.3	611.7	743.3	2739.4
	0	394.5	536.7	593.22	618.1	2605.3
L-Carnitine (LC, mg/kg)	150	490.2	568.5	574.3	717.3	2724.4
	300	501.7	622.3	578.8	717.8	2707.6
SIM × LC		<0.05	<0.05	NS	<0.05	NS
SIM (0) - LC (0)		505.5^{a}	534.3^{ab}	557.3	596.0^{c}	2571.9
SIM (0) - LC (150)		485.7^{ab}	574.0^{ab}	536.3	701.7^{abc}	2671.6
SIM (0) - LC (300)		493.3^{a}	537.3^{ab}	539.3	729.3^{abc}	2670.2
SIM (1.0) - LC (0)		476.7^{ab}	511.3^{b}	538.7	687.7^{abc}	2593.2
SIM (1.0) - LC (150)		502.7^{a}	601.0^{ab}	603.6	616.0^{bc}	2702.2
SIM (1.0) - LC (300)		516.0^{a}	566.7^{ab}	588.7	639.3^{abc}	2684.9
SIM (2.0) - LC (0)		202.0^{b}	821.3^{a}	643.7	610.7^{bc}	2684.9
SIM (2.0) - LC (150)		482.3^{a}	530.7^{a}	583.0	836.0^{a}	2799.6
SIM (2.0) - LC (300)		496.0^{a}	506.0^{b}	608.7	783.3^{ab}	2767.9
SEM		9.22	10.09	10.83	11.74	25.73

Means within each column with no common superscript differ significantly at P < 0.05; NS: Not significant; DOA: days of age.

Table 4. Effect of Dietary Treatments on Feed Conversion Ratio (g/g) of Broilers

Item		14-21 DOA	22-28 DOA	29-35 DOA	35-42 DOA	1-42 DOA
	0.0	1.42	1.67	2.05	2.25	1.70
Simvastatin (SIM, g/kg)	1.0	1.46	1.62	2.03	2.19	1.74
	2.0	1.01	1.58	1.85	2.11	1.76
	0	1.50	1.53	2.05	2.16	1.77
L-Carnitine (LC, mg/kg)	150	1.40	1.65	1.86	2.20	1.71
	300	1.40	1.67	1.89	2.18	1.72
SIM × LC		NS	NS	NS	<0.05	<0.05
SIM (0) - LC (0)		1.40	1.70	2.01	2.34^{a}	1.77^{a}
SIM (0) - LC (150)		1.44	1.65	1.94	2.33^{a}	1.77^{a}
SIM (0) - LC (300)		1.41	1.65	1.86	2.36^{a}	1.75^{a}
SIM (1.0) - LC (0)		1.56	1.58	1.91	2.43^{a}	1.79^{a}
SIM (1.0) - LC (150)		1.40	1.61	2.03	2.15^{bc}	1.73^{a}
SIM (1.0) - LC (300)		1.40	1.65	2.09	2.15^{bc}	1.73^{a}
SIM (2.0) - LC (0)		1.31	1.32	2.02	2.23^{b}	1.78^{a}
SIM (2.0) - LC (150)		1.39	1.70	1.89	2.20^{b}	1.64^{b}
SIM (2.0) - LC (300)		1.42	1.71	1.81	2.10^{c}	1.67^{b}
SEM		0.10	0.13	0.14	0.16	0.15

* Means within each column with no common superscript differ significantly at P < 0.05; NS: Not significant; DOA: days of age

observed that the SIM or LC alone added to diet did not influence BWG and FCR. However, a higher FI was observed in T2 (LC alone), T7 (SIM alone) or T9 than in T1 (control group). A significant improvements of growth traits were observed during the last growing phase (35-42 days of age) or the whole 42-day cycle, when high levels of both additives were supplemented together.

Fathi and Farahzadi (2014) suggested that the fat metabolism (burning) exchange, which can decrease calorie requirements and a potential intestinal absorption improvement caused by LC, can justify the FCR improvement. The influence of LC on fat metabolism exchange was also confirmed in our study by a significant decrease of plasma triglycerides concentration. The SIM additive can have a similar effect at high dose (2 g/kg). A positive SIM×LC interaction was suggested by our results when both additives were added at highest levels on diet.

The positive effect of LC on eviscerated carcass, breast and drumsticks weighs observed in our study was in agreement with previous reports. Awad *et al.* (2016) observed an improvement of the percentage of eviscerated carcass and total edible parts adding LC at 150 to 600 mg/kg in ducks' basal diet. This improvement on eviscerated carcass and/or different edible parts was also reported in broiler by Hrnčár *et al.* (2015) using 1 ml LC per 1.2 l of drinking water, Celik and Oztürkcan (2003) using 50 mg/kg LC in diet under different hair temperatures or by Rabie MH and Szilágyi (1998) also using 50 mg/kg LC in diet.

CONCLUSION

This results support the available literature that the supplementation of 2 g/kg SIM plus 300

Table 5. Effect of Dietary Treatments on Carcass Components (g) in Broilers at 42 Days of Age

Item		Carcass	Eviscerated carcass	Fat pad	Breast	Gizzard	Drumsticks	Liver	Heart
Simvastatin (SIM, g/kg)	0.0	2110.5	1729.4	17.2	666.1	64.4	536.6	67.7	15.5
	1.0	2118.8	1798.8	13.3	700.5	63.3	549.4	65.5	15.6
	2.0	2052.7	1732.2	16.1	671.1	62.2	537.1	68.3	13.9
L- Carnitine (LC, mg/kg)	0	2078.3	1769.4^{a}	19.4^{a}	681.6^{ab}	64.4	540.5^{ab}	65.0	15.0
	150	2048.3	1676.6^{b}	16.3^{ab}	639.4^{b}	60.0	522.1^{b}	66.1	14.3
	300	2155.5	1814.4^{a}	10.5^{b}	716.6^{a}	65.5	560.5^{a}	70.5	16.6
SIM × LC		NS	<0.05	<0.05	<0.05	NS	<0.05	<0.05	NS
SIM (0) - LC (0)		2071.7	1768.3^{abc}	20.0	703.3^{ab}	68.3	540.0^{ab}	58.3^{b}	16.1
SIM (0) - LC (150)		2136.7	1655.0^{c}	21.7	623.3^{b}	61.7	513.3^{b}	65.0^{ab}	15.9
SIM (0) - LC (300)		2123.3	1765.0^{abc}	10.0	671.7^{ab}	63.3	556.7^{ab}	80.0^{a}	16.7
SIM (1.0) - LC (0)		2108.3	1803.3^{ab}	10.0	678.3^{ab}	61.7	548.3^{ab}	65.0^{ab}	13.3
SIM (1.0) - LC (150)		2041.7	1720.0^{bc}	20.0	651.7^{b}	60.0	530.0^{ab}	68.3^{ab}	13.3
SIM (1.0) - LC (300)		2206.7	1873.3^{a}	10.0	771.7^{a}	68.3	570.0^{a}	63.3^{ab}	16.7
SIM (2.0) - LC (0)		2055.0	1736.7^{abc}	20.0	663.3^{ab}	63.3	523.3^{ab}	71.7^{ab}	16.7
SIM (2.0) - LC (150)		1966.7	1655.0^{c}	16.7	643.3^{b}	58.3	523.0^{ab}	65.0^{ab}	13.3
SIM (2.0) - LC (300)		2137.7	1805.0^{ab}	11.7	706.7^{ab}	65.0	555.0^{ab}	68.3^{ab}	16.7
SEM		24.25	18.31	1.94	7.65	2.31	8.89	3.15	0.98

Means within each column with no common superscript differ significantly at P < 0.05; NS: Not significant.

mg/kg LC in broiler basal diet have a positive effect on FI and FCR considering a whole 42-day production cycle. Moreover, studies are in progress to evaluate the dietary supplementation of simvastatin and L-carnitine on broiler meat quality.

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Table 6. Effect of Dietary Treatments on Blood Constitutes of Broilers (mg/dl)

Item		Glucose	Cholesterol	Triglycerides	ALP	HDL	LDL
	0.0	216.2	120.3	72.7	4124	72.6	123.6
Simvastatin (SIM, g/kg)	1.0	199.5	154.5	61.0	3131	70.1	102.4
	2.0	197.7	162.7	54.6	2996	83.2	102.3
	0	209.3	128.8	143.3^{a}	3918	81.2	130.0
L- Carnitine (LC, mg/kg)	150	199.8	203.3	78.1^{b}	3789	68.8	120.1
	300	204.2	135.3	66.8^{b}	2544	78.8	140.0
SIM × LC		NS	NS	<0.05	<0.05	NS	NS
SIM (0) - LC (0)		206.7	120.0	44.7^{bc}	5121^{a}	87.7	23.3
SIM (0) - LC (150)		217.3	119.3	85.0^{ab}	3963^{ab}	81.0	21.3
SIM (0) - LC (300)		229.0	122.5	96.5^{a}	2874^{ab}	79.0	27.5
SIM (1.0) - LC (0)		191.5	113.0	39.0^{c}	2751^{ab}	74.0	30.5
SIM (1.0) - LC (150)		194.7	125.0	81.0^{abc}	4116^{ab}	67.0	75.7
SIM (1.0) - LC (300)		209.7	166.7	55.7^{abc}	2204^{b}	78.3	77.0
SIM (2.0) - LC (0)		224.0	148.3	45.0^{bc}	3495^{ab}	89.0	36.3
SIM (2.0) - LC (150)		181.5	259.5	63.5^{ab}	3039^{ab}	77.7	65.0
SIM (2.0) - LC (300)		182.3	112.7	58.3^{b}	3282^{ab}	79.0	21.3
SEM		7.05	11.76	9.44	34.74	4.25	6.09

Means within each column with no common superscript differ significantly at $P < 0.05$; NS: Not significant.

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