

Original Article

Effect of stocking density on growth performance, some blood parameters and carcass traits in purebred Californian and crossbred rabbits

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ABSTRACT

Objective: The main objective of this study was to study the effect of stocking density in two genetic groups of rabbits (purebred Californian (CAL × CAL) and Californian × Rex (CAL × RX) crossbred rabbits) on growth performance, some blood hematological, biochemical and immunological parameters and carcass traits.

Materials and methods: A total of 120 weaned rabbits were randomly assigned to a completely randomized design with a 2 × 3 factorial arrangement of treatments (two genetic groups; 60 of each CAL × CAL and CAL × RX and three stocking densities; 8, 12 and 20 rabbits/m²; the number of rabbits under each stocking density was 24, 36 and 60; respectively) and 6 replicates.

Results: The effects of genetic group × stocking density interactions were significant ($P < 0.05$) on most of growth performance traits, blood biochemical parameters and phagocytic activity, whereas the effects were non-significant on majority of blood hematological parameters and carcass traits. CAL × CAL rabbits stocked at 20 rabbits/m² had the lowest final body weights and total average daily gains, but had the highest feed to gain ratio. CAL × CAL rabbits stocked at 20 rabbits/m² had the lowest total protein and the highest glucose, corticosterone, liver function tests, and total antioxidant capacity (TAC).

Conclusion: CAL × CAL rabbits stocked at 20 rabbits/m² recorded higher liver and kidney function tests, glucose, TAC, corticosterone levels and lower phagocytic activity which refers to the response of this genetic group to the stress of this higher stocking density and subsequently lower growth performance was observed in these rabbits.

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KEYWORDS

Californian; Carcass; Crossbred; Purebred; Total protein

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INTRODUCTION

The rabbits are characterized on the rest of the livestock in converting fibrous low quality vegetables to a high quality meat for the human consumption, and this is the primary goal of raising livestock, therefore and without doubt it is possible for rabbits to occupy an important position as a meat animal among livestock in many countries, especially in developing countries where there is an imbalance between human population density and high quality grains and plant protein sources ([McNitt et al., 2013](#)).

Differences among genetic groups for carcass traits have been reported in previous scientific papers ([Metzger et al., 2006](#)) as well as blood parameters ([Abdel-Azeem et al., 2010](#)). However, these differences not exist for carcass traits ([Maj et al., 2009](#)) and blood parameters ([Al-Dobaib et al., 2007](#); [Abdel-Hamid and Farahat, 2015](#)).

The stocking density or the cage's floor space available for each rabbit is one of the most important factors affecting well being and thereby has a great impact on production due to its influence on freedom of movement and comfort ([Szendrő et al., 2009](#)) and finally the profitability from the rabbit operation ([Vanhonacker et al., 2009](#)). Many researchers have investigated the effect of stocking density on blood parameters ([Onbasilar and Onbasilar, 2007](#); [Kalaba, 2012](#)) and carcass merits ([Villalobos et al., 2008](#); [Lazzaroni et al., 2009](#); [Paci et al., 2013](#); [Xiccato et al., 2013](#); [Volek et al., 2014](#)).

[Maertens and De Groote \(1984\)](#) have documented a cage density of 40 Kg/m² (by weight of rabbits per m²) or 16 rabbits of 2.5 Kg/m² (by number of rabbits per m²) has been considered the maximum to avoid the negative impact on production and is still commonly used on the commercial scale ([EFSA, 2005](#)).

The intensive rabbit production is dependent on high stocking density which may give rise to a stress condition and consequently results in a deterioration of growth performance and immunosuppression, which reflected negatively on profitability. So, examining different genotypes of rabbits for growth performance, health and immunity parameters under stress condition of high stocking density is outstanding. Therefore, this experiment was conducted to study the effect of raising two genetic groups (CAL × CAL and CAL × RX) under different stocking densities on some blood hematological, biochemical and immunological parameters, and carcass traits of growing rabbits, which to our knowledge has not been studied.

MATERIALS AND METHODS

Ethical approval: Guidelines for Animal Ethics Committee of the University of Zagazig have been followed during the conduct of this experience (ANWD-206).

Experimental animals: The present experiment has been conducted in the rabbit farm of the Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt in the period from December 2016 to March 2017. A total of 120 CAL × CAL purebred and CAL × RX crossbred rabbits were randomly assigned to a completely randomized design with a 2 × 3 factorial arrangement of treatments (two genetic groups; 60 of each CAL × CAL and CAL × RX and three stocking densities; 8, 12 and 20 rabbits/m²; the number of rabbits under each stocking density was 24, 36 and 60, respectively) and 6 replicates.

Rabbits were weaned at 4 weeks of age, ear tagged and were of the male sex only with average initial weight (529.28±4.52 and 521.73±4.52 gm (mean±standard error), for CAL × CAL and CAL × RX, respectively).

Animal were reared in an open sided house, where there were a means of protection against sun and predators in flat-deck cages measuring 500 × 100 × 500 mm (0.5 m²). Each cage was provided with a one automatic drip nipple drinker and a one hopper feeder (30 cm available). Rabbits were housed at density of 8, 12 and 20 rabbits/m² (4, 6 and 10 rabbits/cage, respectively).

Rabbits were supplied with a commercial pelleted diet (17.5% crude protein, 14-16% crude fibers and 2300-2500 kcal/Kg diet digestible energy) and water for *ad-libitum* consumption. The day light was maintained at a 14 h inside the house during the whole experimental period. The temperature inside the house was maintained as possible between 18-24°C throughout the whole period of the experiment.

Growth performance traits: From 4 to 12 weeks of age, individual body weights, and feed intake per cage and registered weekly and average daily weight gains and feed to gain ratios were calculated.

Carcass traits: All the slaughter procedures were carried out in accordance with the World Rabbit Science Association recommendation as described by [Blasco and Ouhayoun \(1996\)](#). The slaughter was done at the end of experimental period (12 weeks of age). Exactly half the

number of rabbits in each cage were chosen at random (Two, three and five rabbits from each cage of 4, 6 and 10 rabbits/cage stocking density groups, respectively), so that 30 rabbits of each genetic group and 12, 18 and 30 rabbits of 4, 6 and 10 rabbits/cage stocking density groups, respectively have been slaughtered. Before slaughtering, the rabbits were weighed, electrically stunned then the jugular veins were severed and the rabbits were left to bleed fully. Skin including distal legs and tail, full stomach, full intestine, and urogenital tract with empty bladder were detached and weighed. The remaining is the hot carcass which was weighed and chilled in a ventilated room at 4°C for 24 h. After the expiry of the chilling period, the chilled carcasses were converted to reference ones by separation of head, thoracic viscera, esophagus, trachea, thymus gland, and kidney free of perirenal fat. Thereafter the carcass cuts were obtained by cutting between the 7th and 8th thoracic vertebra, and between the 6th and 7th lumbar vertebra to free the forepart, loin and hind part.

Blood samples: Blood samples have been taken during the cutting of the jugular vein during slaughtering, of each rabbit two samples had been taken in two heparinized test tubes, one was used directly for measuring red blood cell count (RBC) (Coles, 1986), white blood cell count (WBC) (Cline and Hutton, 1983), hemoglobin concentration (Hb) (Singh, 1983), packed cell volume (PCV) (Wintrobe, 1965), differential leucocytes count (Lucky, 1977) in the whole blood. According to the method of Kawahara et al. (1991), the phagocytic activity was determined and phagocytic index was expressed as the number of phagocytized organisms divided by the number of phagocytic cells.

The other tube was used for separation of serum which stored at -20°C until the analysis date. Parameters that have been measured in serum were: Total protein, albumin, triglycerides, cholesterol, glucose, lactate, lactate dehydrogenase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, urea and creatinine using commercial kits (Diamond Diagnostics, Halliston, MA, USA), and corticosterone hormone using a radioimmunoassay (RIA) (Sainio et al., 1988), whereas the globulin was recorded by subtracting albumin from total protein and albumin/globulin ratio was calculated. The automatic biochemistry analyzer (HITACHI 747, Japan) was used to determine the concentration of serum IgG. Depending on the method that was described by Koracevic et al. (2001), the TAC was determined.

Statistical analysis: A general linear model (GLM) procedure of SAS (SAS, 2008) has been utilized in the analysis of data concerning blood hematological,

biochemical and immunological parameters as well as carcass traits. Genetic group, stocking density and the genetic group × stocking density interaction were assigned to the model as fixed effects, beside the random error effect. Slaughter weight was incorporated into the model as a covariate during the analysis of data regarding the carcass traits. The treatment means were compared using a Duncan Multiple Range Test (Duncan, 1955) at $P < 0.05$.

RESULTS

Growth performance: Genetic group × stocking density effect was significant on most of growth performance traits (final body weight, $P=0.002$; total average daily gain, $P=0.042$; feed to gain ratio, $P=0.044$) (Table 1).

CAL × RX rabbit stocked at 8 rabbits/m² had the highest final body weights and total average daily gain, whilst CAL × CAL rabbits stocked at 20 rabbits/m² were the lowest in these two traits, but recorded the highest feed to gain ratio. CAL × CAL rabbits stocked at 12 rabbits/m² had the lowest feed to gain ratio (Table 1).

Blood hematological parameters: The effects of genetic group × stocking density interactions were non-significant ($P > 0.05$) on all blood hematological parameters except for Hb concentration ($P=0.021$), lymphocytes ($P=0.005$) and eosinophiles ($P=0.034$) (Table 2).

There were significant differences ($P < 0.05$) had been detected among stocking density groups for all blood hematological parameters except for monocytes, basophiles and WBC cont. Rabbits stocked at 20 rabbits/m² had the lowest significant RBC ($P=0.018$), PCV ($P < 0.001$), neutrophils ($P=0.004$) and heterophils/lymphocytes ($P < 0.001$), in contrast, they the highest lymphocytes ($P < 0.001$) (Table 2). CAL × RX rabbits had significantly higher ($P=0.011$) heterophils/lymphocytes than CAL × CAL rabbits (Table 2).

Blood biochemical parameters: The effects of genetic group × stocking density interactions were significant ($P < 0.05$) on the majority of blood biochemical parameters. CAL × CAL rabbits stocked at 20 rabbits/m² had the lowest total protein ($P=0.022$) and the highest glucose ($P < 0.001$), corticosterone ($P < 0.001$), ALT ($P=0.003$), AST ($P=0.010$), lactate dehydrogenase ($P=0.001$), urea ($P=0.044$) and TAC ($P=0.001$). CAL × RX rabbits had the highest creatinine ($P=0.013$) (Table 3).

Table 1. Effects of genetic group, stocking density, and genetic group × stocking density interactions on growth performance traits

Variable	Stocking density						RMSE	P-value		
	8 rabbits/m ²		12 rabbits/m ²		20 rabbits/m ²			GG	SD	GG×SD
	CAL×CAL	CAL×RX	CAL×CAL	CAL×RX	CAL×CAL	CAL×RX				
Rabbits, no.	12	12	18	18	30	30				
Initial weight, gm	532.50	516.25	535.00	519.72	520.33	529.16	32.68	0.239	0.916	0.126
Final weight, gm	2390 ^{ab}	2425 ^a	2379 ^{ab}	2364 ^b	2084 ^d	2191 ^c	82.43	0.01	<0.001	0.002
Total average daily gain, gm/d	33.16 ^a	34.08 ^a	32.92 ^a	32.92 ^a	27.91 ^c	29.67 ^b	1.63	0.006	<0.001	0.042
Cages, no.	3	3	3	3	3	3				
Total feed intake, gm/d/rabbit	149.61	159.16	145.44	151.66	132.66	139.12	34.26	0.028	0.197	0.965
Feed to gain ratio	4.51 ^{bc}	4.67 ^{ab}	4.42 ^c	4.61 ^{abc}	4.77 ^a	4.70 ^{ab}	0.26	0.078	0.001	0.044

Means within the same row having no or common superscript letters are not significantly different ($P>0.05$), CAL × CAL= male Californian × female Californian, CAL × RX= male Californian × female Rex, RMSE= root mean square error, GG= rabbit genetic group, SD= stocking density, GG × SD= genetic group × stocking density interaction.

Table 2: Effects of genetic group, stocking density and genetic group × stocking density interactions on some blood hematological parameters of rabbits at 12 weeks of age.

Variable	Genetic group (GG)		Stocking density (SD)			RMSE	P-value		
	CAL × CAL		8 rabbits/m ²	12 rabbits/m ²	20 rabbits/m ²		GG	SD	GG×SD
	CAL × RX								
Rabbits, No.	30	30	12	18	30	-	-	-	-
RBC (×10 ⁶ /mm ³)	4.574	4.779	4.908 ^a	4.855 ^a	4.265 ^b	0.65	0.266	0.018	0.314
Hb (gm/dL)	11.886	12.150	11.549	12.277	12.228	1.26	0.455	0.242	0.021
PCV (%)	35.115 ^B	35.926 ^A	38.160 ^a	35.361 ^b	33.040 ^c	0.88	0.002	< 0.001	0.167
WBC (×10 ⁹ /L)	8.239	8.761	8.349	8.876	8.274	1.68	0.268	0.475	0.109
Neutrophils%	37.304	38.716	40.578 ^a	39.869 ^a	33.5833 ^b	5.78	0.382	0.004	0.813
Lymphocytes (%)	42.235	41.748	36.658 ^c	41.835 ^b	47.483 ^a	4.22	0.678	< 0.001	0.005
Monocytes (×10 ³ /mm ³)	4.511	4.451	4.492	4.472	4.480	0.30	0.472	0.985	0.682
Basophils (×10 ³ /mm ³)	0.923	0.920	0.920	0.919	0.926	0.03	0.626	0.689	0.809
Eosinophils (×10 ³ /mm ³)	8.188	8.379	8.130	8.379	8.342	0.38	0.079	0.191	0.034
Heterophils/Lymphocytes	73.812 ^B	75.088 ^A	76.133 ^a	76.083 ^a	71.133 ^b	1.74	0.011	< 0.001	0.381

Means within the same row and within each category (genetic group and stocking density) that have no or common superscript letters (upper case letters for the genetic group and lower case letters for stocking density) are not significantly different ($P>0.05$), CAL × CAL= male Californian × female Californian, CAL × RX= male Californian × female Rex, RMSE= root mean square error, GG × SD= Genetic group × stocking density interaction, RBC= red blood cell count, WBC= white blood cell count, PCV= packed cell volume, Hb= hemoglobin concentration.

Table 3. Effects of genetic group × stocking density interaction on some blood biochemical parameters of rabbits at 12 weeks of age

Variable	Stocking density						RMSE	P-value		
	8 rabbits/m ²		12 rabbits/m ²		20 rabbits/m ²			GG	SD	GG×SD
	CAL×CAL	CAL×RX	CAL×CAL	CAL×RX	CAL×CAL	CAL×RX				
Rabbits, No.	6	6	9	9	15	15	-	-	-	-
Total protein (gm/dL)	5.843 ^{bc}	6.218 ^a	6.083 ^{ab}	5.922 ^{abc}	5.683 ^c	5.867 ^{bc}	0.31	0.134	0.060	0.022
Albumin (gm/dL)	4.135	3.898	3.744	3.638	3.827	3.743	1.09	0.001	<0.001	0.307
Globulin (gm/dL)	1.548	1.968	2.339	2.284	2.016	2.475	0.35	0.008	<0.001	0.053
Albumin/Globulin ratio	2.820	2.106	1.630	1.623	1.966	1.536	0.40	0.001	<0.001	0.055
Cholesterol (mmol/L)	0.898	0.926	0.897	0.915	0.944	0.921	0.04	0.579	0.181	0.221
Triglycerides (mg/dL)	16.858	16.885	16.832	16.876	16.794	16.916	0.13	0.099	0.928	0.488
Glucose mmol/L	6.282 ^c	6.513 ^c	6.650 ^{bc}	7.113 ^b	7.923 ^a	6.707 ^{bc}	0.56	0.022	<0.001	<0.001
Corticosterone (nmol/L)	3.730 ^c	3.637 ^c	4.100 ^b	3.571 ^c	4.802 ^a	4.325 ^b	0.29	0.555	<0.001	<0.001
ALT (IU/L)	60.167 ^d	62.598 ^{cd}	64.222 ^c	62.137 ^{cd}	72.261 ^a	67.401 ^b	2.96	0.072	<0.001	0.003
AST (IU/L)	50.667 ^c	52.432 ^c	54.000 ^{bc}	52.137 ^c	61.795 ^a	56.601 ^b	3.30	0.059	<0.001	0.010
Lactate dehydrogenase (IU/L)	307.940 ^d	310.335 ^b	308.663 ^{cd}	309.539 ^{bc}	314.543 ^a	313.755 ^a	1.18	0.015	<0.001	0.001
Lactate (mmol/l)	7.208	7.348	7.377	7.744	9.943	10.086	0.56	0.174	<0.001	0.782
Calcium (mg/dL)	11.097	10.952	10.931	10.966	13.002	13.003	0.57	0.821	<0.001	0.909
Urea (mg/dL)	19.040 ^c	20.247 ^b	18.960 ^c	19.408 ^c	24.716 ^a	24.486 ^a	0.83	0.046	<0.001	0.044
Creatinine (mg/dL)	0.657 ^b	0.682 ^a	0.635 ^c	0.648 ^{bc}	0.661 ^b	0.614 ^d	0.00	0.031	<0.001	0.013
TAC (µm/l)	460.46 ^b	457.35 ^{bc}	453.94 ^c	462.41 ^b	472.90 ^a	469.01 ^a	5.50	0.751	0.001	0.001

Means within the same row having no or common superscript letters are not significantly different ($P>0.05$), CAL × CAL= male Californian × female Californian, CAL × RX= male Californian × female Rex, RMSE= root mean square error, GG= Genetic group, SD= stocking density, GG × SD= genetic group × stocking density interaction, ALT= alanine aminotransferase, AST= aspartate aminotransferase, TAC= total antioxidant capacity.

Blood immunological parameters: The effect of genetic group × stocking density interactions was significant ($P<0.05$) on phagocytic activity and non-significant ($P>0.05$) on phagocytic index and IgG concentration (Table 4). Rabbits stocked at 20 rabbits/m² had the lowest phagocytic activity and

phagocytic index ($P<0.001$) compared with those stocked at 8 and 12 rabbits/m² (Table 4).

Carcass traits: The effects of genetic group × stocking density interactions were non-significant ($P>0.05$) on all carcass traits except for slaughter weight ($P<0.001$),

Table 4. Effects of genetic group, stocking density, and genetic group × stocking density interactions on some blood immunological parameters

Variable	Genetic group (GG)		Stocking density (SD)			P-value			
	CAL×CAL	CAL×RX	8 rabbits/m ²	12 rabbits/m ²	20 rabbits/m ²	RMSE	GG	SD	GG × SD
Rabbits, No.	30	30	12	18	30	-	-	-	-
Phagocytic activity%	17.317	17.201	17.499 ^a	17.763 ^a	16.515 ^b	0.52	0.430	< 0.001	0.014
Phagocytic index%	1.562	1.557	1.574 ^a	1.595 ^a	1.509 ^b	0.05	0.731	< 0.001	0.236
IgG (IU/L)	1.615	1.644	1.647	1.628	1.614	0.08	0.234	0.517	0.074

Means within the same row and within the stocking density category having different superscript letters are significantly different ($P < 0.05$). CAL × CAL= male Californian × female Californian, CAL × RX= male Californian × female Rex, RMSE= root mean square error, GG × SD= Genetic group × stocking density interaction, IgG= Immunoglobulin G.

Table 5. Effects of genetic group and stocking density on carcass traits of rabbits were slaughtered at 12 weeks of age.

Variable	Genetic group (GG)		Stocking density (SD)			RMSE	P-value		
	CAL × CAL	CAL × RX	8 rabbits/m ²	12 rabbits/m ²	20 rabbits/m ²		GG	SD	GG × SD
Rabbits, No.	30	30	12	18	30	-	-	-	-
Slaughter weight, g	2284	2258	2387	2307	2119	56.03	0.098	< 0.001	< 0.001
Hot carcass, g	1450 ^b	1474 ^a	1465	1470	1450	36.17	0.022	0.536	0.674
Reference carcass, g	1185 ^b	1217 ^a	1207	1208	1188	37.05	0.004	0.604	0.147
Dressing-out, %	53.20 ^b	54.56 ^a	54.19	54.21	53.26	1.68	0.006	0.577	0.179
% of slaughter weight									
Skin	17.02	17.25	16.82	17.07	17.51	1.78	0.653	0.872	0.512
Full stomach	5.48	5.29	5.28	5.19	5.67	0.83	0.430	0.536	0.351
Full intestine	10.72 ^a	9.97 ^b	10.48	10.45	10.11	1.04	0.014	0.830	0.248
% of hot carcass weight									
Liver	4.91	4.46	4.45	4.82	4.79	1.00	0.123	0.664	0.111
Kidney	1.08	0.98	1.00	1.01	1.07	0.18	0.073	0.812	0.399
Head	10.59	10.47	10.67	10.51	10.41	0.67	0.530	0.839	0.280
% of reference carcass weight									
PSF & PRF	2.58	2.82	2.87	2.86	2.37	0.43	0.061	0.126	0.169
Hind part	38.58	38.06	38.33	37.90	38.73	1.43	0.203	0.403	0.707

Means in the same row within the genetic group having different superscript letters are significantly different ($P < 0.05$). CAL × CAL= male Californian × female Californian, CAL × RX= male Californian × female Rex, RMSE= root mean square error, GG × SD= genetic group × stocking density interaction, Reference carcass= chilled carcass minus head, liver, heart, lungs, esophagus, trachea, thymus gland, and kidney free of perirenal fat, Dressing-out percentage was calculated in relation to reference carcass weight, PSF= periscapular fat, PRF= Perirenal fat, Slaughter weight was incorporated into the model as covariates (2229 g).

Table 6. Effects of genetic group × stocking density interactions on some carcass traits of rabbits were slaughtered at 12 weeks of age

Variable	Stocking density						RMSE	P-value		
	8 rabbits/m ²		12 rabbits/m ²		20 rabbits/m ²			GG	SD	GG × SD
	CAL×CAL	CAL×RX	CAL×CAL	CAL×RX	CAL×CAL	CAL×RX				
Rabbits, no.	6	6	9	9	15	15				
Slaughter weight, g	2362 ^{ab}	2412 ^a	2316 ^b	2297 ^b	2064 ^d	2174 ^c	56.03	0.098	< 0.001	< 0.001
% of hot carcass weight										
Thoracic viscera	1.71 ^{ab}	1.34 ^b	1.54 ^{ab}	1.44 ^{ab}	1.71 ^{ab}	1.89 ^a	0.20	0.023	0.121	0.007
% of reference carcass weight										
Fore part	38.44 ^a	35.13 ^b	38.02 ^a	36.34 ^{ab}	36.95 ^{ab}	38.64 ^a	2.18	0.082	0.821	0.010
Loin	19.55 ^b	22.85 ^a	20.95 ^{ab}	22.11 ^a	21.35 ^a	20.29 ^{ab}	2.17	0.073	0.752	0.041

Means within the same row having different superscript letters are significantly different ($P < 0.05$). CAL × CAL= male Californian × female Californian, CAL × RX= male Californian × female Rex, RMSE= root mean square error, GG= Genetic group, SD= stocking density, GG × SD= genetic group × stocking density interaction.

thoracic viscera, ($P=0.007$), fore part ($P=0.010$) and loin ($P=0.041$) (Table 5-6).

CAL × RX rabbits stocked at 8 rabbits/m² had the highest slaughter weight and loin, whereas the lowest slaughter weight has been recorded in CAL × CAL rabbits stocked at 20 rabbits/m² (Table 6). CAL × RX rabbits registered higher significant hot carcass weights ($P=0.022$), reference carcass weight ($P=0.004$) and dressing-out percentage ($P=0.006$) than CAL × CAL rabbits. In contrast, CAL × CAL rabbits have higher significant ($P=0.014$) full intestine than CAL × RX rabbits (Table 5).

DISCUSSION

Growth performance: The impairment effect of high cage stocking density on growth performance is in accordance with the results have been published by Baiomy (2012) and Mousa-Balabel (2009), who reported a decrease in average daily gain and average daily feed intake per rabbit as the cage density increased, but they worked only on one rabbit genetic group (New Zealand White). The depressive effect that a high cage density did on growth performance might be attributed to the reduction in locomotion activities as the direct result of the low space available for each rabbit. Consequently, the

feed intake and body weight gain were decreased, leading to a reduction in the feed efficiency ([Mousa-Balabel, 2009](#)). Supportive results have been reported by [Trocino et al. \(2004\)](#). Confirmed results have been recorded by [Iyeghe-Erakpotobor and Olorunju \(2005\)](#), they observed that average daily gain and feed conversion ratio were improved in rabbits stocked at 6.7, 10 and 13.3 rabbits/m² than those stocked at 16.7 and 20 rabbits/m². In a study on California rabbits in Egypt, [Kalaba \(2012\)](#) detected that rabbits stocked at 0.063 m²/rabbit had the lowest final body weight at 12 weeks of age, body weight gains and feed intake at 4-12 weeks age interval. In contrast, they had the highest feed conversion ratio at the same age interval than those stocked at 0.25, 0.125 and 0.083 m²/rabbit. Rabbits stocked at 5 rabbits/m² had heavier final body weights and higher weight gain ($P<0.01$) than those stocked at 10 rabbits/m² ([Mbanya et al., 2004](#)). In a study on crossbred New Zealand, California, Butterfly, Dutch, and Satin rabbits, [Villalobos et al. \(2008\)](#) observed that for each unit increase in the cage density (rabbits/m²), there is an impairment in average daily gain and average daily feed intake by 0.31 ± 0.070 and 1.20 ± 0.25 gm, respectively ($P<0.001$).

Controversy results have been published in previous papers ([Szendrő et al., 2009](#); [Volek et al., 2014](#)), they reported that stocking density had a non-significant effect on growth traits. Other studies depended on weight of the rabbits per m² rather than the number of rabbits per m², reported that as the stocking density reached or exceeded 45 Kg/m² the productive performance of rabbits was badly affected ([Szendrő and Dalle Zotte, 2011](#)), in the present study the weight of the rabbits per m² is still under this weight although the stocking density affects the growth performance which might be attributed to genetic group \times stocking density interaction. [Onbasilar and Onbasilar \(2007\)](#) have been detected that rabbits stocked at 840 cm²/rabbit the growth performance was deteriorated compared with those stocked at 4200 and 1400 cm²/rabbit.

Blood hematological, biochemical and immunological parameters: The significant differences were observed between CAL \times CAL and CAL \times RX crossbred rabbits for some hematological parameters were in agreement with those reported by [Abdel-Azeem et al. \(2010\)](#), who found that Baladi Red rabbits had significantly higher RBC, Hb concentration and hematocrit than Baladi Red \times Chinchilla Giganta. The observed significant differences of globulin and albumin/globulin ratio were not consistent with those were documented by previous authors ([Al-Dobaib et al.,](#)

[2007](#); [Abdel-Hamid and Farahat, 2015](#)). A significant genetic group effect had been demonstrated for the automated white blood cell count ($P=0.01$). Indeed, crossbred rabbits recorded higher automated Hb ($P=0.03$), PCV ($P=0.03$) and RBC count ($P=0.01$) than New Zealand White rabbits. Total protein, albumin, globulin, Cholesterol, glucose, neutrophils-Segs and lymphocytes, all were not affected by genetic group ([Burnett et al., 2006](#)). Leukocytes, neutrophils and lymphocytes were significantly ($P<0.05$) affected by genetic group ([Abdel-Kafy et al., 2012](#)).

Globulin is considered as a part of total serum proteins and indicates the immunological status ([Ismail et al., 2002](#)), and there is an inverse relationship between the ratio of albumin to globulin and the immunoglobulin level, where it was found that the rise in the proportion of albumin to globulin accompanied by a decline in the immunoglobulin level. The albumin synthesis takes place in the liver, while globulin in the lymphatic tissues ([Jones and Bark, 1979](#)), thus the change in the albumin level is considered an evidence of a change in liver function ([Azoz and El-Kholy, 2005](#)).

The current results confirmed those reported by [Kalaba \(2012\)](#), they observed significant increase ($P<0.05$) in serum globulin level in rabbits stocked at 0.25 m²/rabbit compared with their counterparts stocked at 0.125, 0.083 and 0.063 m²/rabbit. Concurrent results were recorded by [Onbasilar and Onbasilar \(2007\)](#), they found stocking density up to 5 rabbits/cage did not influence the triglycerides levels. However, [Kalaba \(2012\)](#) reported that stocking density had a significant effect ($P<0.05$) on the triglycerides, as the level was only increased in rabbits stocked at 0.063 m²/rabbit, but his findings were contradictory with the present study, as he found that rabbits stocked at 0.063 m²/rabbit had the highest significant WBC count (16.08×10^3 mm⁻³, $P<0.05$) than those stocked at lower density and exceeded the normal range of WBC count in rabbit. [Yakubu et al. \(2008\)](#) found that stocking density had no effect on WBC count and lymphocytes.

CAL \times CAL rabbits stocked at 20 rabbits/m² had the lowest Hb concentration and lymphocytes, and these results confirmed those were recorded by [Kalaba \(2012\)](#). Supportive results were reported by [Yakubu et al. \(2008\)](#), they concluded that rabbits stocked at higher density depicted a decrease in RBC, Hb concentration, and PCV than those stocked at lower density. Total WBC count is rarely increased by the exposure of rabbit to illness, stress and the administration of cortical steroids ([Poljičak-Milas](#)

[et al., 2009](#)), but they more frequently altered differential WBC count as a consequences of their re-distribution ([Jenkins, 2006](#)).

Low total protein and high glucose and corticosterone levels that were detected in blood of CAL × CAL rabbits stocked at 20 rabbits/m² harmonized those of [Kalaba \(2012\)](#), these results suggested that this genetic group suffers more stress than CAL × RX when both stocked at 20 rabbits/m² and is reflected by lower growth performance and phagocytic activity and higher total antioxidant capacity that have been observed in CAL × CAL rabbits stocked at 20 rabbits/m². [Onbasilar and Onbasilar \(2007\)](#) detected that stocking density of 5 rabbits/cage had the highest significant ($P < 0.001$) plasma corticosterone and serum glucose levels compared with 1 and 3 rabbits/cage and there was no difference in serum cholesterol level of rabbits reared at 1, 3 and 5 rabbits/cage. [Kalaba \(2012\)](#) detected that plasma AST level was higher ($P < 0.05$) in rabbits stocked at 0.083 and 0.063 m²/rabbit than 0.25 and 0.125 m²/rabbit, whilst ALT did not differ significantly ($P > 0.05$).

[Qaid et al. \(2006\)](#) concluded that stocking density had a detrimental effect on performance and immune status of poultry from 1 to 14 days of age. Stocking density significantly increased the heterophilis and heterophilis to lymphocytes ratio and lymphocytes was significantly reduced ($P < 0.01$). In a previous study, there was no significant effect of stocking density on levels of plasma immune globulins ([Azzam and El-Gogary, 2015](#)). Similar results were recorded by [Tong et al. \(2012\)](#), but [Houshmand et al. \(2012\)](#) observed contrasting results.

Carcass traits: There is no doubt that rabbit producers prefer high weight at slaughter because it gives rise to an increase in income, which depends on the number of kilograms were sold in the market, whereas high slaughter yield is preferred by processing industry ([Zeferino et al., 2013](#)).

The significant effects of genetic group on carcass traits were consistent with that recorded by [Metzger et al. \(2006\)](#), who found that dressing percentage was lower in Hyplus hybrid by 0.5% than in purebred Pannon White as well as [Khalil and El-Zarie \(2012\)](#) have registered differences between genetic groups for majority of carcass traits.

The non-significant effect of genetic group on carcass traits have been reported in a previous study ([Abdel-Hamid and Farahat, 2015](#)), who worked on two genetic

groups (New Zealand White and New Zealand White × Rex). In parallel, [Maj et al. \(2009\)](#) found that majority of carcass traits were not affected by genetic group (New Zealand White, Californian, New Zealand White × Californian and the Californian × New Zealand White). It was found that there is difficulty in comparing carcass traits of the current study with those that have been registered previously because of differences in the age of slaughter, as well as methods of measuring these traits.

The current study was inconsistent with previous ones ([Ozimba and Lukefahr, 1991](#); [Hernández et al., 2006](#)). The purebred V-Line rabbits had significantly higher hot carcass weight and dressing, fur and viscera percentages relative to slaughter weight than crossbreds (V-Line × Saudi Gabali), but the head did not differ significantly ([Al-Dobaib et al., 2007](#)), they did not use slaughter weight as covariate in the model which may be the reason for these contrasting results, in addition, different genetic groups. Botucatu and New Zealand White × Botucatu rabbits showed equal performance with respect to commercial and reference carcass percentages relative to slaughter weight. However, significant differences were found between the two genetic groups for distal parts of legs, skin, thoracic viscera and forepart percentages relative to slaughter weight. Conversely, no differences were observed regarding the other carcass traits ([Zeferino et al., 2013](#)).

[Baiomy \(2012\)](#) compared the carcass traits in New Zealand White rabbits stocked at 6, 12, 18 and 24 rabbits/m², they concluded that stocking density did not influence carcass traits. In contrast, stocking density influenced slaughter and hot carcass weights, whereas dressing out percentage did not significantly differ ([Lambertini et al., 2001](#)). [Mousa-Balabel \(2009\)](#) detected that stocking density had a highly significant effect on slaughter weight of New Zealand White rabbits. In fact, rabbits stocked at 6, 8 and 10 rabbits/m² had the highest slaughter weight compared with the other groups. However, [Iyeghe-Erakpotobor and Olorunju \(2005\)](#) did not observe slaughter weight to be influenced by the stocking density. Californian rabbits stocked at 0.125 m²/rabbit had the highest slaughter weight at 12 weeks of age (2281 gm) and those stocked at 0.063 m²/rabbit had the lowest values (1958 gm) ([Kalaba, 2012](#)).

The effect of stocking density on hot carcass weight and dressing out percentage in the present study were consistent with those reported previously ([Villalobos et al., 2008](#); [Paci et al., 2013](#); [Xiccato et al., 2013](#); [Volek et al., 2014](#)), however, the effect of stocking density on fat

deposition in carcass was inconsistent with past authors (Villalobos et al., 2008; Lazzaroni et al., 2009).

The significant effect of stocking density on skin that was reported by Paci et al. (2013) was inconsistent with the current results. They detected that rabbits stocked at 16 rabbits/m² had the highest skin ($P < 0.01$) compared with those stocked at 5 and 2.5 rabbits/m².

The non-significant effect of stocking density on hind part was in disagreement with the results published previously (Pla, 2008), they observed an inverse relationship between stocking density and hind leg proportion. Gondret et al. (2009) reported that if growing rabbits were exposed to jumping exercise for five weeks, they would develop a higher hind part compared with those not jumping. Hence, rabbits stocked at a low density had a more space for jumping and will develop a higher hind part according to these findings.

CONCLUSION

The effects of genetic group × stocking density interactions were significant on most of growth performance traits, blood biochemical parameters and phagocytic activity, whereas the effects were non-significant on majority of blood hematological parameters and carcass traits. CAL × CAL rabbits stocked at 20 rabbits/m² recorded higher liver and kidney function tests, glucose, TAC, corticosterone levels and lower phagocytic activity which refers to the response of this genetic group to the stress of this higher stocking density and subsequently lower growth performance was observed in these rabbits.

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Nothing to disclose.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

AUTHORS' CONTRIBUTION

The manuscript is completely prepared by Tamer Mohamed Abdel-Hamid (experimental design, data collection, statistical analysis and English writing).

REFERENCES

1. Abdel-Azeem AS, Abdel-Azim AM, Darwish AA, Omar EM. Haematological and biochemical observations in four pure breeds of rabbits and their crosses under Egyptian environmental conditions. *World Rabbit Science*. 2010; 18:103–110. <https://doi.org/10.4995/WRS.2010.18.13>
2. Abdel-Hamid TM, Farahat MH. Effect of dietary mannan-oligosaccharides on some blood biochemical, hematological parameters and carcass traits in purebred New Zealand White and crossbred rabbits. *Animal Production Science*. 2016; 56:2133–2139. <https://doi.org/10.1071/AN15032>
3. Abdel-Kafy EM, Hoda Shabaan MA, El-Sayed AFM, Azoz AAA, Abdel-Latif AM. Genotype and age effects on immunity traits, corticosterone and oxidative status in growing rabbits. In: Proceedings 10th World Rabbit Congress, Sharm El-Sheikh, Egypt. 2012; 107–111.
4. Al-Dobaib SN, Khalil MH, Hashad M, Al-Saef AM. Growth, carcass and caecal traits in V-Line and crossbred rabbits fed diets containing discarded dates. *World Rabbit Science*. 2007; 15:81–90. <https://doi.org/10.4995/wrs.2007.603>
5. Azoz AA, El-Kholy KH. Blood metabolites and hormones of V-line and Bouscat female rabbits under middle Egypt conditions. *Egyptian Journal of Rabbit Science*. 2005; 15:131–142.
6. Azzam MMM, El-Gogary MR. Effects of Dietary Threonine Levels and Stocking Density on the Performance, Metabolic Status and Immunity of Broiler Chickens, *Asian Journal of Animal and Veterinary Advances*. 2015; 10:215–225. <https://doi.org/10.3923/ajava.2015.215.225>
7. Baiomy AA. Growth and carcass traits of New Zealand White rabbits reared in upper Egypt as affected by cage density, *Egyptian Poultry Science Journal*. 2012; 32:475–481.
8. Blasco A, Ouhayoun J. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Science*. 1996; 4:93–99. <https://doi.org/10.4995/wrs.1996.278>
9. Burnett N, Mathura K, Metivier KS, Holder RB, Brown G, Campbell M. An investigation into hematological and serum chemistry parameters of rabbits in Trinidad. *World Rabbit Science*. 2006; 14:175–187. <https://doi.org/10.4995/wrs.2006.556>
10. Cline MJ, Hutton JJ. Hematology and oncology. In: Stein JH (eds). *Internal Medicine*, Boston, Little, Brown. 1983.
11. Coles EH. *Veterinary Clinical Pathology*, Fourth Ed. WB Saunders, Philadelphia, USA. 1986.
12. Duncan DB. The Multiple Range and F. Tests. *Biometrics*. 1955; 11:1–45. <https://doi.org/10.2307/3001478>
13. EFSA (European Food Safety Authority). The Impact of the current housing and husbandry

- systems on the health and welfare of farmed domestic rabbits. 2005; p. 137.
14. Gondret F, Hernandez P, Rémygnon H, Combes S. Skeletal muscle adaptations and biomechanical properties of tendons in response to jump exercise in rabbits. *Journal of Animal Science*. 2009; 87:544–553. <https://doi.org/10.2527/jas.2008-1286>
 15. Hernández P, Ariño B, Grimal A, Blasco A. Comparison of carcass and meat characteristics of three rabbit lines selected for litter size or growth rate. *Meat Science*. 2006; 73:645–50. <https://doi.org/10.1016/j.meatsci.2006.03.007>
 16. Houshmand M, Azhar K, Zulkifli I, Bejo MH, Kamyab A. Effects of prebiotic, protein level and stocking density on performance, immunity and stress indicators of broilers. *Poultry Science*. 2012; 91:393–401. <https://doi.org/10.3382/ps.2010-01050>
 17. Ismail AM, Abou-El-Ellaa MA, Sedki AA. Blood metabolites, growth and reproductive performance and immune responsiveness in growing and doe rabbits as affected by Acitrol treatments. *Egyptian Journal of Agriculture Research*. 2002; 80:1789–1808.
 18. Iyeghe-Erakpotobor GT, Olorunju SAS. Effect of stocking density on performance of growing rabbits in the semi-humid tropics. *Tropicultura*. 2005; 23:19–23.
 19. Jenkins JR. Clinical Pathology. In: Meredith A, Flecknell P. (eds). *BSAVA Manual of Rabbit Medicine and Surgery*, British Small Animal Veterinary Association, Gloucester. 2006; p. 45–48.
 20. Jones EA, Bark PD. Chemical diagnosis of disease. In: Brown SS, Mitchell FL, Young DS. (eds), Elsevier Biomed. Press, Amsterdam, Netherlands. 1979; p. 325–363.
 21. Kalaba ZM. Physiological response and stress indicators of Californian rabbits under intensive condition in Egypt. *Asian journal of Poultry Science*. 2012; 6:65–78. <https://doi.org/10.3923/ajpsaj.2012.65.78>
 22. Kawahara E, Ueda T, Nomura S. *In vitro* phagocytic activity of white spotted shark cells after injection with *Aeromonas salmonicida* extracellular products. *Gyobo Kenkyu*. 1991; 26:213–214. <https://doi.org/10.3147/jsfp.26.213>
 23. Khalil MH, El-Zarie MF. Genetic groups comparisons for growth, carcass, meat quality and blood parameters in program of synthesizing new lines of rabbits. In: 'Proceedings 10th World Rabbit Congress, Sharm El-Sheikh, Egypt. 2012; p. 241–249.
 24. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology*. 2001; 54:356–361. <http://dx.doi.org/10.1136/jcp.54.5.356>
 25. Lambertini L, Vignole G, Zaghini G. Alternative pen housing system for fattening rabbits: Effect of group density and litter. *World Rabbit Science*. 2001; 9:141–147. <https://doi.org/10.4995/wrs.2001.457>
 26. Lazzaroni C, Biagini D, Lussiana C. Different rearing systems for fattening rabbits: performance and carcass characteristics. *Meat Science*. 2009; 82:200–204. <https://doi.org/10.1016/j.meatsci.2009.01.011>
 27. Lucky Z. *Methods for the diagnosis of fish diseases*, Ameruno Publishing Co, PVT, LTd. New Delhi, Bombay, New York. 1977.
 28. Maertens L, De Groote G. Influence of the number of fryer rabbits per cage on their performances. *Journal of Applied Rabbit Research*. 1984; 7:151–155.
 29. Maj D, Bieniek J, Łapa P, Ina S. The effect of crossing New Zealand White with Californian rabbits on growth and slaughter traits. *Archiv Tierzucht*. 2009; 52:205–211. <https://doi.org/10.5194/aab-52-205-2009>
 30. Mbanya JN, Ndoping BN, Fomunyan RT, Noumbissi A, Mbomi ES, Fai EN, Teguaia A. The effect of stocking density and feeder types on the performance of growing rabbits under conditions prevailing in Cameroon. *World Rabbit Science*. 2004; 12:259–268. <https://doi.org/10.4995/wrs.2004.567>
 31. McNitt JI, Lukefahr SD, Cheeke PR, Patton NM. *Rabbit production*, Ninth ed. British Library, London, UK. 2013. <https://doi.org/10.4995/wrs.2004.567>
 32. Metzger SZ, Odermatt M, Szendrő Z, Mohaupt M, Romvári R, Makai A, Biró-Németh E, Radnai I, Sipos L. Comparison of carcass traits and meat quality of Hyplus hybrid purebred Pannon White rabbits and their crossbreds. *Archiv Tierzucht*. 2006; 49:389–399. <https://doi.org/10.5194/aab-49-389-2006>
 33. Mousa-Balabel TM. Effect of cage versus pen and stocking density on behavior and performance in rabbits. *Online Journal of Veterinary Research*. 2009; 13:48–55.
 34. Onbasilar E, Onbasilar I. Effect of cage density and sex on growth, food utilization and some stress parameters of young rabbits. *Scandinavian Journal of Laboratory Animal Science*. 2007; 34:189–195.
 35. Ozimba CE, Lukefahr SD. Comparison of rabbit breed types for post weaning litter growth, feed efficiency and survival performance traits. *Journal of Animal Science*. 1991; 69:3494–3500. <https://doi.org/10.2527/1991.6993494x>

36. Paci G, Preziuso G, D'Agata M, Russo C, Dalle Zotte A. Effect of stocking density and group size on growth performance, carcass traits and meat quality of outdoor reared rabbits. *Meat Science*. 2013; 93:162–166. <https://doi.org/10.1016/j.meatsci.2012.08.012>
37. Pla M. A comparison of the carcass traits and meat quality of conventionally and organically produced rabbits. *Livestock Production Science*. 2008; 115:1–12. <https://doi.org/10.1016/j.livsci.2007.06.001>
38. Poljičak-Milas N, Kardum-Skelin I, Vuđan M, Marenja TS, Ballarin-Perharić A, Milas Z. Blood cell count analyses and erythrocyte morphometry in New Zealand white rabbits. *Veterinary Archive*. 2009; 79:561–571.
39. Qaid M, Albatshan H, Shafey T, Hussein E, Abudabos AM. Effect of Stocking Density on the Performance and Immunity of 1- to 14-d- Old Broiler Chicks. *Revista Brasileira de Ciência Avícola*. 2016; 18:683–692. <http://dx.doi.org/10.1590/1806-9061-2016-0289>
40. Sainio EL, Lehtola T, Roininen P. Radioimmunoassay of total and free corticosterone in rat plasma: Measurement of the effect of different doses of corticosterone. *Steroids*. 1988; 51:609–622. [https://doi.org/10.1016/0039-128X\(88\)90056-6](https://doi.org/10.1016/0039-128X(88)90056-6)
41. SAS. *Statistics Analysis System user's Guide*, (Release 9.2), SAS Institute Inc., Cary, North Carolina, USA. 2008.
42. Singh K. Effect of heat stress on blood constituents in crossbred heifers. *Indian Journal of Animal Science*. 1983; 53:353–355.
43. Szendrő Z, Dalle Zotte A. Effect of housing conditions on production and behaviour of growing meat rabbits: A review. *Livestock Science*. 2011; 137:296–303. <https://doi.org/10.1016/j.livsci.2010.11.012>
44. Szendrő Zs, Princz Z, Romvári R, Locsmánci L, Szabó A, Bázár Gy, Radnai I, Biró-Németh E, Matics Zs, Nagy I. Effect of group size and stocking density on productive, carcass, meat quality and aggression traits of growing rabbits. *World Rabbit Science*. 2009; 7:153–162. <https://doi.org/10.4995/wrs.2009.655>
45. Tong HB, Lu J, Zou JM, Wang Q, Shi SR. Effects of stocking density on growth performance, carcass yield and immune status of a local chicken breed. *Poultry Science*. 2012; 91:667–673. <https://doi.org/10.3382/ps.2011-01597>
46. Trocino A, Xiccato G, Queaque PI, Sartori A. Group housing of weanling rabbits: effect of stocking density and cage floor on performance, welfare and meat quality. *World Rabbit Science*. 2004; 13:138–139.
47. Vanhonacker F, Verbeke W, Van Poucke E, Buijs S, Tuytens FAM. Societal concern related to stocking density, pen size and group size in farm animal production. *Livestock Science*. 2009; 123:16–22. <https://doi.org/10.1016/j.livsci.2008.09.023>
48. Villalobos O, Guillen O, Garcia J. Effect of cage density on growth and carcass performance of fattening rabbits under tropical heat stress conditions. *World Rabbit Science*. 2008; 16:89–97. <https://doi.org/10.4995/wrs.2008.631>
49. Volek Z, Chodova D, Tůmova E, Volkova L, Kudrnova E, Marounek M. The effect of stocking density on carcass traits, muscle fibre properties and meat quality in rabbits. *World Rabbit Science*. 2014; 22:41–49. <https://doi.org/10.4995/wrs.2014.1176>
50. Wintrobe PM. *Clinical hematology*, Lead and Febiger, Philadelphia, USA. 1965.
51. Xiccato G, Trocino A, Majolini D, Tazzoli M, Zuffellato A. Housing of growing rabbits in individual, bicellular and collective cages: Growth performance, carcass traits and meat quality. *Animal*. 2013; 7:627–632. <https://doi.org/10.1017/S175173111200198X>
52. Yakubu A, Adua MM, Adamude H. Welfare and hematological indices of weaner rabbits as affected by stocking density. In: *Proceeding 9th World Rabbit Congress*, Verona, Italy. 2008; 1269–1273.
53. Zeferino CP, Komiyama CM, Fernandes S, Sartori JR, Teixeira PSS, Moura ASAMT. Carcass and meat quality traits of rabbits under heat stress. *Animal*. 2013; 7:518–523. <https://doi.org/10.1017/S1751731112001838>
