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Carcass traits and some blood stress parameters of summer stressed growing male rabbits of different breeds in response to boldenone undecylenate

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ABSTRACT

The present study was conducted to investigate the effects of boldenone undecylenate (BUL) on carcass traits and blood stress parameters in growing male rabbits. A total of 170 male rabbits comprising of three breeds namely New Zealand White (NZW; n=55), Californian (CAL; n=50), and Rex (RX; n=65) were taken for this study. The rabbits of each breed were divided into three groups *viz*., D0 (control), D1 provided with normal dose of BUL (at 4.4 mg/kg body weight), and D2 provided with BUL (at 8.8 mg/kg bwt). The rabbits were kept under temperature ranged from 28-32°C during the experimental period. Carcass traits of the rabbits were studied, and the blood parameters were measured by radioimmunoassay. Most of carcass traits, globulin and cholesterol levels in the serum were significantly improved for the injection of BUL at normal dose. Plasma corticosterone levels in normal dose injected rabbits were lowered by 27.21 and 15.25% as compared to controls and double dose, respectively. The effect of interaction between dose and breed was non-significant (*P*>0.05) on almost all carcass traits and blood parameters. In conclusion, BUL improves all carcass traits in male growing rabbits when injected with normal dose of BUL, with the exception of dressing-out%, and has a significant lowering effect on stress hormone (*i.e.*, corticosterone), and increasing effect on serum total protein, globulin and cholesterol.

Keywords

Boldenone undecylenate, Carcass, Dressing percentage, Globulin, Heat stress, Rabbit

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INTRODUCTION

Rabbits are sometimes used as a meat source for human, and contribute to family income, therefore play important roles in solving the problem of meat shortage and unemployment. Suitability of rabbit for subsistence agriculture comes from their amazing reproductive capabilities and ability to utilize fibrous portions of forage and agricultural by-products to produce high quality meat (McNitt et al., 2013). In Egypt, during summer season, the environmental temperature exceeds 40ºC especially when hot waves attack. Thus, it is difficult to maintain a comfortable temperature inside the rabbit house. As a result, rabbits undergo in stress condition, and their feed intake, growth rate and feed conversion are dramatically reduced. The producers become obligated to extend the fattening period which is not economical to them (El-Raffa, 2004). Boldenone undecylenate (BUL) can be used in this special situation of heat stress to promote growth not only to promote its anabolic activity but also to enhance its anticatabolic activity (Melloni et al., 1997; Thienpont et al., 1998). BUL is a synthetic androgenic steroid hormone having anabolic properties; thus, it can be used in meat producing animals for growth acceleration purpose and in athletics for enhancement of performance (Nielen et al., 2006).

The effect of anabolic steroids on carcass traits was investigated by Moran et al. (1991) who observed an improvement in pre-slaughter and hot carcass weight in heifers treated with trenbolone acetate. Similarly, Johnson et al. (1996) reported that carcass fat was unaffected by combined implant of trenbolone acetate and estradiol of feedlot steers. Nahed et al. (2010) reported that using of BUL in rabbit was not sufficient to confirm its use in rabbits although a considerable improvement of total weight gain, feed efficiency and feed conversion ratio were attained after application of BUL in male rabbit. However, there is no reported in detail study on the effect of BUL on all carcass traits and blood parameters. Therefore, we designed a factorial experiment to study the effect of different doses of BUL, breed and interactions between dose of BUL and breed on carcass traits and blood stress parameters of growing male rabbits under heat stress condition.

MATERIALS AND METHODS

Location and duration of the study: The present study was carried out at the Experimental Unit of the Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University, Egypt during summer (July and August) of 2014. Experimental procedures were conducted in accordance with the Zagazig University Animal Ethics Committee guidelines (ANWD-206).

Experimental animals and management: The source of experimental animals was the San-El-Hagar Agricultural Company Farm, Sharkia Governorate, Egypt. A total of 170 weaned male rabbits of three breeds (55 NZW, 50 CAL and 65 RX) were randomly assigned to a completely randomized design with a 3 × 3 factorial arrangement of treatments (three breeds: NZW, CAL and RX; and three doses: control, normal dose, and double dose). The rabbits were weaned at 30 days of age, and the mean weaning weight was 524±9.4 g, ear tagged, and transferred to fattening cages (40 cm × 50 cm × 50 cm) to complete their growing period of 1.5 months. The cages were provided with a nipple system for watering and manual trough feeder. The rabbits were fed on commercial pellets (17.5% crude protein, 14-16% crude fibre and 2300-2500 kcal/kg diet digestible energy). The feed and the water were offered *ad libitum*. All the animals were grown under identical environmental and feeding conditions as well as the same stocking density. A 12L:12D photoperiod (from 0900 to 2100 h) was used throughout the experimental period. The temperature inside the house was maintained at a range of 28-32ºC. The animals were exposed to some attacks of hot waves during the experimental periods where the temperature jumped over 40ºC.

BUL injection: Males of each breed were divided into three groups; D0 (control), D1, and D2. The rabbits of the control group were injected with sesame oil dosed at 0.25 mL/kg bwt; D1 or normal recommended dose was injected with 4.4 mg/kg bwt BUL (Equi-gan®; Lab Tornel, Co., Mexico); and D2 or double dose was injected with 8.8 mg/kg bwt BUL. The doses of BUL were calculated according to Paget and Barnes (1964). All rabbits were injected intramuscularly at day 40 of age, and repeated once at day 47 of age. The animals were slaughtered at day 72 of age so that there was a withdrawal time of 20 days, as recommended by the manufacturer.

Carcass traits: Ten rabbits from each group were selected on the basis of average ±0.3 kg bwt. Selection within the average ±0.3 kg body weight category was random, as described by Metzger et al. (2006). The rabbits were slaughtered in two consecutive days, 45 rabbits per day, without fasting at 72 days of age. The number of rabbits slaughtered each day was balanced according to the dose of BUL and breed. Just prior of slaughtering, the rabbits were weighed, and the jugular veins were severed, and the rabbits were lifted for complete bleeding. After complete bleeding, the carcass was weighed again, and the difference between the two weights was considered as the blood weight. Slaughtered rabbits were skinned, and skin weight was recorded, and then eviscerated. The stomach, intestine, liver, heart with lung, periscapular and perirenal fat were separated and weighed. The remaining part was considered as hot carcass. Dressing-out% was calculated as (hot carcass weight divided by live weight × 100). Slaughtering and dissection were carried out according to World Rabbit Science Association (WRSA) recommendations (Blasco and Ouhayoun, 1996). Hot carcasses were chilled at 4ºC for 24 h. After chilling, the carcasses were dissected into three anatomical parts (between the 7th and 8th thoracic vertebrae, and between the 6th and 7th lumbar vertebrae) and head was separated. Then head, fore part, intermediate part and hind part were weighed.

Blood parameters: Blood samples were collected while the animals were slaughtered. The samples were taken in two test tubes, one with anticoagulant for measuring plasma corticosterone levels and the other one without using anticoagulant to separate the serum for measuring serum total proteins, albumin, cholesterol and triglyceride levels. Plasma and serum samples were stored at -20°C until assayed. Serum total proteins, albumin, cholesterol and triglyceride levels were measured using commercial kits. Radioimmunoassay (RIA) technique was used to measure plasma corticosterone levels commercial kits (Gamma-B ^{125}I Corticosterone, Code AA-13 F1) for IDS double antibody RIA technique, with a Berthold LB211 gamma counter. Five samples from each treatment were assayed.

Statistical analysis: General Linear Model (GLM) procedures of SAS (2008) were used to determine the effects of BUL and breed on the carcass traits and blood parameters using the following model:

$$Y_{ijk} = U + D_i + B_j + (DB)_{ij} + E_{ijk}$$

Where: Y_{ijk}: An observation of each trait. U: The overall mean. D_i: effect of dose of BUL (i = D0, D1 and D2, i.e. control, normal dose and double dose). B_j: effect of breed (j = 1, 2, and 3, *i.e.*, NZW, CAL and RX). $(DB)_{ij}$: effect due to interaction between dose and breed. E_{ijk}: random deviation due to unexplained source. Differences among means were compared statistically using Duncan's multiple range tests (Sokal and Rohlf, 1969). For carcass traits, day of slaughter and slaughter weight were used as a covariate.

RESULTS AND DISCUSSION

Carcass traits

The effects of dose of BUL, breed and their interaction on the carcass traits in growing male rabbits are presented in **Table 1**. The effect of dose was found highly significant ($p \leq 0.01$) on all carcass traits with the exception of kidney, heart with lungs, mid part weight and dressing-out%. However, non-significant ($P > 0.05$) effect of breed on most carcass traits was detected. All the carcass traits were improved after injection of male rabbit with normal dose (at 4.4 mg/kg bwt) of BUL, except dressing-out%, which might be due to negative phenotypic correlation between blood, stomach, intestine and liver weights with dressing-out%, as the greater the weights of these organs, the reduction of the dressing-out% will be come out (Ayyat et al., 1994). On the other hand, interactions between dose and breed revealed significant variations ($P < 0.05$) among treatments where the CAL males injected with normal dose (at 4.4 mg/kg bwt BUL) depicted the highest (60.12±0.44%) dressing-out% **(Table 2)**. The improvement of dressing-out% is important for the processing industry where the income depended on kilograms of carcasses marketed (Zeferino et al., 2013).

Effect of dose × breed interactions was non-significant on all carcass traits with the exception of dressing-out% and intestine weight. Control CAL males occupied the top position (205±8.13 g) for intestine weight, while control NZW males (160±5.72 g) being the inferior **(Table 2)**. Slaughter weight was improved by 11.29 and 6.93% ($P < 0.001$) in males injected with normal dose (at 4.4 mg/kg bwt) as compared to controls and those injected with double dose (at 8.8 mg/kg bwt). Males injected with normal dose showed a great improvement ($P < 0.001$) in blood by 26.79 and 24.73% as compared to those of controls and double dose, respectively. Also, increasing liver weight by 17.14 and 22.03% was observed as compared to those of controls and double dose, respectively. Indeed, normal dose increased hot carcass weight by 10.19 and 6.32%, fore part weight by 16.41 and 5.51%, and hind part weight by 10.46 and 3.81%, as compared to those of control and double dose, respectively. Concurrently, normal dose increased periscapular fat weight by 1.15 and 1.09 g and perirenal fat weight by 2.91 and 2.20 g over control and double dose, respectively.

Regarding slaughter weight, concurrent results are depicted by Tousson et al. (2012) who recorded that adult male NZW rabbits that received 3 intramuscular injections of 5 mg/kg bwt BUL showed the highest final body weight than other groups. Also, Abdel-Hamid (2014) found that dose of BUL significantly affected final weight at day 72 of age when treated with normal dose, showing the highest value as 1716±20.20 g. Symeon et al. (2010) reinforced the present results, and recorded that caponization had a depressive effect on final body weights at 10 weeks of age as compared to intact males ($p < 0.05$) which indicated the important anabolic effect of sex hormones. However, contradictory results were recorded by Oda and El-Ashmawy (2012) and Khalil et al. (2014) who found that BUL injection had a non-significant effect on body weights in mature male NZW.

The improvement of hind part weight in normal dose treated males was reinforced by the finding of Symeon et al. (2010) who reported that capons exhibited lighter drumstick weight than intact males which reflected that male sex hormones improved carcass retail cut. Increasing organs weights after treatment with BUL was in agreement with previous authors (Chi, 2004; Chen et al., 2010a) who reported that heart and the relative heart weight reduced in capons than intact males and increased in all androgen implanted capon of Single Comb White Leghorn chickens. Cardiac muscle contains androgen receptors upon it,

Table 1. The effects of dose of boldenone undecylenate (BUL), breed and their interactions on carcass traits of growing male rabbits at 72 days of age. Slaughter weight was used as covariate.

Variable	[1]DD			[2]BB			*p*-value		
	D0	D1	D2	NZW	CAL	RX	DD	BB	DD × BB
Slaughter weight	1620±18.73[c]	1803±18.73[a]	1686±18.73[b]	1692±18.73	1686±18.73	1733±18.73	<0.001	0.163	0.790
Blood weight	44.03±1.67[c]	55.83±1.67[a]	47.66±1.67[b]	48.70±1.67	49.00±1.67	49.83±1.67	<0.001	0.884	0.759
Skin weight	271.16±5.10[b]	293.66±5.10[a]	293.00±5.10[a]	286.66±5.10	291.00±5.10	280.16±5.10	0.003	0.325	0.082
Stomach weight	88.67±2.87[b]	100.40±2.87[a]	86.17±2.87[c]	93.57±2.87[a]	84.83±2.87[b]	96.83±2.87[a]	0.001	0.010	0.191
Kidney weight	11.50±0.42	12.42±0.42	11.70±0.42	11.27±0.42[c]	11.52±0.42[b]	12.82±0.42[a]	0.282	0.025	0.094
[3]H&L weight	16.84±0.57	18.80±0.57	17.44±0.57	17.95±0.57	17.08±0.57	18.05±0.57	0.054	0.431	0.516
Liver weight	49.73±2.13[c]	62.03±2.13[a]	50.83±2.13[b]	56.33±2.13[a]	49.10±2.13[b]	57.16±2.13[a]	<0.001	0.016	0.236
Intestine weight	168.83±3.31[c]	187.93±3.31[a]	177.83±3.31[b]	179.33±3.31[a]	168.26±3.31[b]	187.00±3.31[a]	0.001	0.001	0.002
[4]HCW	961±12.23[c]	1059±12.23[a]	996±12.23[b]	990±12.23	1010±12.23	1017±12.23	<0.001	0.284	0.797
[5]Dressing-out%	59.33±0.28	58.71±0.28	59.07±0.28	58.59±0.28[b]	59.86±0.28[a]	58.66±0.28[b]	0.309	0.003	0.045
[6]PSF weight	3.73±0.15[b]	4.88±0.15[a]	3.79±0.15[b]	3.81±0.15[b]	4.50±0.15[a]	4.09±0.15[ab]	<0.001	0.009	0.751
[7]PRF weight	18.93±0.68[b]	21.84±0.68[a]	19.64±0.68[b]	20.50±0.68	19.32±0.68	20.59±0.68	0.010	0.349	0.928
Head weight	111.43±1.55[b]	121.00±1.55[a]	115.16±1.55[b]	116.33±1.55	113.60±1.55	117.66±1.55	<0.001	0.174	0.972
Fore part weight	312.11±8.77[b]	363.33±8.77[a]	344.33±8.77[a]	329.61±8.77	342.50±8.77	347.66±8.77	<0.001	0.339	0.528
Mid part weight	196.10±7.20	204.16±7.20	190.76±7.20	187.16±7.20	197.66±7.20	206.20±7.20	0.420	0.180	0.129
Hind part weight	333.00±4.52[c]	367.83±4.52[a]	354.33±4.52[b]	344.83±4.52	354.33±4.52	356.00±4.52	<0.001	0.176	0.550

[1]DD, dose of boldenone undecylenate; D0, control; D1, normal dose (4.4 mg/kg bwt); D2, double dose (8.8 mg/kg bwt). [2]BB, breed; NZW, New Zealand White; CAL, Californian; RX, Rex. [3]H&L, heart and lung; [4]HCW, hot carcass weight; [5]dressing-out%, expressed as HCW as a proportion of slaughter weight; [6]PSF, periscapular fat; [7]PRF, perirenal fat.
Means within the same row not sharing the same letter within each category (dose, breed) were significantly different ($P<0.05$).

Table 2. The Effect of interaction between dose of boldenone undecylenate (BUL) and breed of growing male rabbits on traits showed significant *p*-value.

[1]DD	[2]BB	Intestine weight	Dressing%	Albumin (g/dL)	Globulin (g/dL)	Albumin / Globulin
D0	NZW	160±5.72[d]	59.76±0.71[a]	3.87±0.042[a]	1.31±0.099[d]	2.96±0.074[a]
	CAL	205±8.13[a]	57.33±0.30[b]	3.48±0.042[b]	2.22±0.099[b]	1.58±0.074[c]
	RX	173±6.20[bcd]	58.69±0.50[ab]	3.44±0.042[b]	2.50±0.099[ab]	1.38±0.074[cd]
D1	NZW	162±7.38[d]	59.55±0.38[a]	3.46±0.042[b]	1.72±0.099[c]	2.01±0.074[b]
	CAL	172±5.39[cd]	60.12±0.44[a]	3.30±0.042[cd]	2.23±0.099[b]	1.48±0.074[cd]
	RX	170±5.42[cd]	59.90±0.67[a]	3.25±0.042[d]	2.46±0.099[ab]	1.33±0.074[d]
D2	NZW	184±3.92[bc]	58.70±0.49[ab]	3.48±0.042[b]	1.73±0.099[c]	2.01±0.074[b]
	CAL	186±2.36[bc]	58.68±0.33[ab]	3.44±0.042[b]	2.61±0.099[a]	1.35±0.074[cd]
	RX	190±4.96[ab]	58.61±0.46[ab]	3.42±0.042[bc]	2.48±0.099[ab]	1.38±0.074[cd]
***P*-value**		0.002	0.045	0.001	0.037	<0.001

[1]DD, dose of boldenone undecylenate; D0, control; D1, normal dose (4.4 mg/kg bwt); D2, double dose (8.8 mg/kg bwt). [2]BB, breed; NZW, New Zealand White; CAL, Californian; RX, Rex.
Means within the same column not sharing the same letter were significantly different ($P<0.05$).

Table 3. The effects of dose of boldenone undecylenate (BUL), breed and their interactions on some blood stress parameters of growing male rabbits at 72 days of age.

Variable	[1]DD			[2]BB			*p*-value		
	D0	D1	D2	NZW	CAL	RX	DD	BB	DD × BB
Total protein (g/dL)	5.19±0.053[b]	5.77±0.053[a]	5.85±0.053[a]	5.61±0.053[a]	5.48±0.053[b]	5.72±0.053[a]	<0.001	0.009	0.057
Albumin (g/dL)	3.60±0.024[a]	3.41±0.024[b]	3.37±0.024[b]	3.60±0.024[a]	3.34±0.024[c]	3.45±0.024[b]	<0.001	<0.001	0.001
Globulin (g/dL)	1.58±0.057[b]	2.36±0.057[a]	2.48±0.057[a]	2.01±0.057[b]	2.14±0.057[ab]	2.27±0.057[a]	<0.001	0.009	0.037
Albumin / Globulin	2.33±0.043[a]	1.47±0.043[b]	1.36±0.043[b]	1.97±0.043[a]	1.61±0.043[b]	1.58±0.043[b]	<0.001	<0.001	<0.001
Triglyceride (mg/dL)	16.73±0.022	16.74±0.022	16.77±0.022	16.76±0.022	16.74±0.022	16.74±0.022	0.468	0.624	0.827
Cholesterol (mmol/L)	0.87±0.004[c]	0.96±0.004[a]	0.91±0.004[b]	0.92±0.004[a]	0.90±0.004[b]	0.92±0.004[a]	<0.001	0.049	0.164
Corticosterone (nmol/L)	4.98±0.029[a]	3.61±0.029[c]	4.26±0.029[b]	4.30±0.029	4.27±0.029	4.28±0.029	<0.001	0.640	0.187

[1]DD, dose of boldenone undecylenate; D0, control; D1, normal dose (4.4 mg/kg bwt); D2, double dose (8.8 mg/kg bwt). [2]BB, breed; NZW, New Zealand White; CAL, Californian; RX, Rex.
Means within the same row not sharing the same letter within each category (dose, breed) were significantly different ($P<0.05$).

testosterone act to increase anabolic activity. Mašek et al. (2013) found that heart weight and liver weight were similar between sham and castrated birds, indicating that sex hormones have no effect on heart or liver weights. The results of the present study disagreed with the findings of Chen et al. (2009) who demonstrated that exogenous androgen implantations depress abdominal fat deposition and reduce fat accumulation in the intestine. However, Chen et al. (2010b) reported a high accumulation of abdominal fat in birds implanted with 19-Nortesto-sterone (19-NORT) implants. Among the retail cuts of carcass, if fore part and hind part added together they will represent about 70.14, 69.04, and 67.13% of the hot carcass weight (including head) of double, normal dose injected rabbits and controls, respecttively, suggesting that they are the most sites for lean tissue deposition especially in double dose injected rabbits. These finding were harmonized with those of Oliveira et al. (2004) who indicated that thigh is the highest muscle deposition site in rabbit body.

Blood parameters

Effects of dose of BUL, breed and their interaction on some blood stress parameters are summarized in **Table 3**. Statistically highly significant differences (*P*<0.001) were recorded for dose effect on all blood parameters tested with the exception of triglyceride. Similarly, highly significant differences (*P*<0.01) were observed for breed effect on total protein, albumin, globulin and albumin/globulin ratio. In contrast, breed effect on triglyceride and plasma corticosterone level was found as non-significant (*P*>0.05). Indeed, significant differences were evidenced for interaction between dose and breed for albumin (*P*<0.05), Globulin (*P*<0.05) and albumin/globulin ratio (*P*<0.001). In fact, BUL increase the total protein and globulin in serum of rabbit especially with double dose. On the contrary, albumin and albumin/globulin ratio decreased where the controls depicted the highest values (3.60±0.024 and 2.33±0.043 g/dL, respectively). With respect to cholesterol, normal dose increased its level in the serum by 5.49% as compared to double dose and 10.34% over controls **(Table 3)**. Regarding interactions between dose and breed, control male NZW exhibited the highest level of albumin and albumin/globulin ratio (3.87±0.042 and 2.96±0.074 g/dL, respectively). On the other hand, male CAL injected with double dose depicted the highest globulin level (2.61±0.099 g/dL) in the serum **(Table 2)**. These findings were in agreement with those of previous report of Alm-Eldeen and Tousson (2012) who reported a significant increase in serum total protein and globulin, while serum albumin and albumin/globulin ratio were significantly reduced (*p*<0.05) after injections of BUL as compared to their corresponding levels in the control group of adult NZW rabbits. Similar results were reported by Gabr et al. (2009) but in weaned male lambs treated with BUL as a growth promoter. Concurrent results were depicted by Jockenhovel et al. (1999) who recorded significant increase in the serum total globulins in all treatments, while slight increase was observed in parenteral treatment modes of hypogonadal men.

Harmonious results were published previously by Chen et al. (2010b) who found that there was no difference for triacylglycerol level in blood of intact males or capons, and the later had significantly higher cholesterol level in blood as compared to intact males. Birds implanted with 19-Nortestosterone (19-NORT) implants showed higher cholesterol, and lower triglycerol levels than sham birds. Mašek et al. (2013) recorded that triglyceride and total cholesterol values were similar in both sham and castrated birds, indicating that sex hormones had no effect on blood cholesterol and triglyceride level. In contrast, some other authors found a significant increase in blood triglyceride (Mašek et al., 2010) and cholesterol (Shao et al., 2009).

Increasing serum total proteins and globulin had a stimulating effect on body weight gain which is assumed to be attributed to improve health and immune status of animal and to decrease protein catabolism. Since anabolism is a state in which nitrogen is differentially retained in lean body mass, either through stimulation of protein synthesis and/or decreased breakdown of protein anywhere in the body (Kuhn, 2002).

The effect of heat stress on total proteins and cholesterol levels in plasma of growing and adult rabbits of both sexes had been demonstrated by Ondruska et al. (2011) who found that heat stressed adult rabbit showed significantly (*P*<0.05) lower total proteins level; this might be due to higher water consumption leading to plasma dilution and thus lowering concentration of proteins (Okab et al., 2008), and/or transamination of amino acid and elevation of protein utilization (Habeeb et al., 1993), as well as inhibiting effect of cortisol which raised in serum of heat stressed rabbits on protein synthesis in tissues and its stimulating effect on protein catabolism (Okab and

El-Banna, 2003). Our investigation revealed that BUL caused significant increase in serum total proteins which could counteract the depressive effect of heat stress on serum total proteins level which might be attributable to its antiglucocorticoid effect (Thienpont et al., 1998).

The lower level of cholesterol in the serum of heat stressed rabbits had been reported by previous authors (Habeeb et al., 1996). In our study, we found that normal dose of BUL caused to increase level of cholesterol in the serum in male rabbit, which could be explained by anabolic steroids, that could affect expression of 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMGCR), the main enzyme involved in the cholesterol synthesis (Gårevik et al., 2012).

CONCLUSION

Growing male rabbits injected with normal recommended dose of boldenone undecylenate (BUL) show an improvement in all carcass traits with the exception of dressing-out%. The double and the normal recommended dose positively increase serum total protein and globulin, while normal recommended dose apparently increase serum cholesterol and decrease plasma corticosterone level. Therefore, BUL can be used during summer to minimize the negative effects of heat stress, but the withdrawal time must be followed to avoid human health hazard.

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