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Original article

Effects of boldenone undecylenate on growth performance, maintenance behaviour, reproductive hormones and carcass traits of growing rabbits

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Abstract

The present study was done to evaluate the effect of boldenone undecylenate (BOL) on growth performance, maintenance behaviour, reproductive hormones and carcass traits of male rabbits. Sixty apparently healthy New Zealand White male rabbits, 5 weeks of age, were allotted to 3 equal groups. Each group was subdivided into 5 replicates, where the first group is control. The second group (B₁) comprised rabbits that received 2 intramuscular injections of BOL (5 mg/kg) with 3 week intervals (9 and 12 weeks of age), while the third group (B₂) included rabbits that received 3 intramuscular injections of BOL (5 mg/kg) with 2 week intervals (8, 10 and 12 weeks of age). The end of the trial was after 4 weeks from the last injection (16 weeks of age). The results revealed that the treated groups had a significant increase in total body weight, daily gain and feed efficiency, with a significant decrease in feed conversion ratio (FCR). Ingestive, locomotion and grooming behaviors were significantly higher in treated groups. Lateral pasture and exploratory behaviors were significantly higher in the control group. Administration of BOL resulted in a significant increase in dressing % and a significant decrease in testes %. Groups treated with BOL had a significantly ($P < 0.05$) decreased serum testosterone level, simultaneously with a significantly increased estradiol level. The results indicate that BOL improves performance and carcass traits. Furthermore, there are hormonal-behavioral correlations through enhancement of ingestive and locomotion behaviors of treated animals.

Key words: boldenone, performance, behaviour, carcass traits, rabbit

Introduction

In recent years, domestic rabbits have been identified as an economy livestock for solving the problem of meat shortage in high human population developing countries including Egypt. Rabbit meat has a very good nutritive value, being comparatively high in pro-

tein, low in fat, calories and sodium, and so could bridge the wide gap in dietary protein intake (Adeyinka et al. 2007). Anabolic steroids are a class of steroid hormones based on the androgen testosterone and are recognized for their effects on building up muscle and are used as performance enhancing drugs (Thienpont et al. 1998). However, there is insufficient

available information for their use in these animals. Recently, boldenone undecylenate (androgenic steroid) has been used in improvement of growth and food conversion in food-producing animals. It is also well known for increasing vascularity in preparation for body building contests. It might also play an important role not only in controlling normal testicular development but also in maintaining normal testicular function and spermatogenesis (Tousson et al. 2012). Anabolic steroids increase muscle size by the promotion of positive nitrogen balance by stimulating protein production and decreasing destruction (Guan et al. 2010).

Thus, hormonal-behavioural correlations can be due to hormonal effects on behaviour, but certain behaviours (physical exercise, stress, sexual behaviour, and nutrition) are also known to influence hormone levels as well (Christiansen 2004). Salas-Ramirez et al. (2010) indicated that the brain is exquisitely sensitive to testicular steroids and endogenous testosterone has a role in normal development of the male brain, which has both short-term and long-term consequences on behaviour. Moreover, several studies reported that gonadal steroid hormones influence adolescent brain development by both organizing and activating neural circuits underlying social behaviour (Schulz and Sisk 2006). Khalil et al. (2014) found that male rabbits treated with boldenone undecylenate showed significant increases in both sexual and aggressive behaviours, whereas, a BOL-withdrawal group showed significantly lower levels of sexual behavior than the control group. The potential behavioral effects of AAS abuse in human populations have received prominent coverage (Kuhn 2002). However, less attention has been paid to the behavioral effects using animal models. Indeed, the effects of AAS on reproductive capacity have been hardly studied in human as well as in many animal species (Thabet et al. 2010). Therefore, the aim of the present study was to investigate the effect of boldenone undecylenate on growth performance, some maintenance behaviour, serum reproductive hormones and carcass traits in male rabbits.

Materials and Methods

Experimental animals and management

A total of sixty apparently healthy growing New Zealand White male rabbits, 5 weeks of age, were kept in battery cages 75 x 50 x 30 cm (L X W X H). The rabbits were vaccinated using a prophylactic dose of viral hemorrhagic septicemia vaccine. Anticoccidial drugs and ivermectine were also used as prophylactic treatment against coccidiosis and scabies, respectively.

The beginning of the trial was after one week for adaptation „at 6 weeks”, where the rabbits averaged 750 ± 14 g live body weight (LBW). They were allotted to 3 equal groups (20 rabbits / group). Each group was subdivided into 5 replicates reared in cages of equal size (4 rabbits / cage) with identical rearing programs under similar environmental conditions. The control group included rabbits that were injected intramuscularly with sesame oil (0.25 ml/kg body weight). The second group (B₁) included rabbits that received 2 intramuscular injections of boldenone (5 mg/kg body weight) at 9 and 12 weeks of age. The third group (B₂) included animals that received 3 intramuscular injections of boldenone (5 mg/kg body weight) at 8, 10, and 12 weeks of age. The experimental diet was commercial pelleted standard rabbit ration (CP 19 %, CF 16.15%, EE 2.5% and DE 2500 kcal/kg) ad-libitum and analyzed according to standard procedures of the AOAC (2002). The Experimental diet was offered twice daily at 8 am and 4 pm, and fresh clean drinking tap water was supplied ad-libitum. At the end of the trial (16 weeks), a total of fifteen rabbits from each group were used for collecting blood to measure hormonal assays and for slaughtering to measure carcass traits throughout 4 weeks from the last injection.

Chemicals and reagent

Equi-gan® vials were purchased from Laboratorios Tornel, Co. S.A. Mexico. Each vial contained an oily solution consisting of synthetic testosterone (50 mg boldenone undecylenate „BOL” / ml vehicle). All chemicals, reagents and stains were of analytical grade, obtained from El-Gomhoria and Sigma companies.

Growth performance parameters

The bucks were weighed weekly before morning feeding. Average feed intake (g) was recorded daily with collection and weighing of residues. Total weight gain (g) was determined as the difference between the weights of rabbits at the beginning and end of the trial. Feed conversion ratio (FCR) was carried out by dividing the total feed intake (g) by the total body weight gain (g). Feed efficiency was carried out by dividing the average daily body weight gain (g) by the average daily feed intake.

Behavioural observation

Behavioural observation was performed according to Paul and Patrick (2007). During the trial, the obser-

vations were carried out for 3 hrs every week for each group by direct observation, where the rabbits were observed three times (20 minutes per time) daily, at early morning (7 a.m. to 8 a.m.), early afternoon (12 p.m. to 13 p.m.) and late afternoon (16 p.m. to 17 p.m.) on three alternate days (Abdelfattah et al. 2013). The observed behavioral patterns (duration and frequency), as mentioned by Dusanka et al. (2008; 2011), were as follows:

I – Ingestive behaviour, includes feeding, drinking and caecotrophy.

Caecotrophy: rabbits bowed down, pushed their head between their hind legs and ingested caecotrophs directly from the anus, then raised their head and chewed for a few moments.

II – Resting behaviour: we distinguished 3 variations according to body posture:

Abdominal posture: resting with belly on the ground, hind limbs tucked under the body and forelimbs either tucked beneath the body or stretched forward and the head in upright position.

Abdominal-Lateral position: as abdominal posture but the hind limbs more or less stretched away from the body.

Lateral position: resting on the side with all legs stretching away from the body head in lying position.

III – Locomotion behaviour: is any voluntary change of body position, such as walking and circling, where it might be seeking attention or wanting to play.

IV – Exploratory behaviour: we distinguished:

Rearing up: sitting on the hind legs with the body in an upright position.

Sniffing: rabbits sniffed the air or the cage itself (wall, floor, feeder etc.).

V – Comfort behaviour: this includes grooming and stretching.

Blood sampling and hormonal assays

Blood samples were collected from each animal from the ear vein at 30 days from the last injection of all groups in clean sterilized tubes. Serum samples were separated and preserved for subsequent hormonal assay. Serum testosterone and estradiol were determined using electrochemiluminescence immunoassay (ECLIA) kits (Thienpont et al. 1998). It had been measured by Roche Elecsys 2010 (Hitachi Ltd, Boehringer, Mannheim, Tokyo, Japan) using associated reagents, such as Elecsys testosterone II reagent kits, Cat. No. 05200067190-100 test.

Carcass traits

At the end of the trial, a total of fifteen rabbits were slaughtered randomly from each group and internal organs (testes, liver, kidneys, heart, spleen, intestine and lung) were weighed separately. The dressing percentage was calculated as dressed eviscerated carcass weight without the giblets (liver, kidneys and heart) to the live body weight. Percentages of internal organs were relative to carcass weight.

Statistical analysis

All statistical procedures were performed using the SAS statistical system Package V9.2 (SAS, 2002). Data were analyzed using repeated measure analysis of variance (ANOVA) with the general linear models (GLM) procedure, after verifying normality using the Kolmogorov-Smirnov test. The mixed model analyses included the fixed effects of the treated groups (control, B₁ and B₂) and the random effects of replicates and males nested within the group. The comparison of means was carried out using Duncan's multiple range tests.

Results

Growth performance

The effect of boldenone on the overall performance of male rabbits is shown in Table 1. The results revealed that boldenone injection in male rabbits resulted in a significant ($p < 0.05$) increase in total final BW, final BW gain, average daily gain and feed efficiency with a significant ($p < 0.05$) decrease in the total FCR for groups (B₁) and (B₂) compared with the control. The average daily feed intake was not significantly ($p > 0.05$) different among groups. The best results were for group (B₂) which includes rabbits which received 3 intramuscular injections biweekly, then group (B₁) which includes rabbits which received 2 intramuscular injections with 3 weeks intervals, compared with the control group.

Behavioural measurement

There are correlations between behaviour and growth promoter, through recording the following behaviour: ingestive, resting, locomotion, exploratory and comfort behaviors, as shown in Table 2, ingestive,

Table 1. Effect of boldenone undecylenate on growth performance parameters of male rabbits.

Parameters	Experimental groups		
	Control	B ₁	B ₂
Initial BW at 6 week (g)	752 ± 3.71	754 ± 4.00	753 ± 3.58
Final BW at 16 week (g)	2447 ± 18.9 ^c	2709 ± 30.5 ^b	2838 ± 28.4 ^a
Total BW gain (g)	1695 ± 15.6 ^c	1954 ± 26.8 ^b	2084 ± 24.9 ^a
ADG (g)	24.2 ± 0.22 ^c	27.9 ± 0.38 ^b	29.8 ± 0.35 ^a
Total feed intake (g)	5543 ± 21.3	5566 ± 9.1	85569 ± 8.55
Daily feed intake (g)	79.2 ± 0.30	79.5 ± 0.13	79.5 ± 0.12
FCR	3.27 ± 0.02 ^a	2.85 ± 0.03 ^b	2.67 ± 0.02 ^c
Feed efficiency	0.30 ± 0.002 ^c	0.35 ± 0.004 ^b	0.39 ± 0.005 ^a

Control: injected intramuscularly with sesame oil (0.25 ml/kg body weight).

B₁: received 2 intramuscular injections of boldenone (5 mg/kg body weight) at 9 and 12 weeks.

B₂: received 3 intramuscular injections of boldenone (5 mg/kg body weight) at 8, 10, and 12 weeks.

BW: body weight; ADG: average daily gain; FCR: feed conversion ratio.

^{abc} Means in the same row with different superscripts are significantly different at (p<0.05).

Table 2. Effect of boldenone undecylenate on some maintenance behaviour of male rabbits.

Behavioural Patterns	Experimental groups		
	Control	B ₁	B ₂
Ingestive behaviour			
Feeding time (Minutes / 3 hrs)	7.25 ± 0.16 ^b	9.02 ± 0.36 ^a	3.96 ± 0.14 ^c
Drinking time (Minutes / 3 hrs)	2.11 ± 0.36 ^b	3.12 ± 0.33 ^a	0.872 ± 0.09 ^c
Caecotrophy frequency (Frequencies / 3 hrs)	0.12 ± 0.12 ^b	1.25 ± 0.55 ^a	0.87 ± 0.22 ^{ab}
Resting behaviour (Minutes / 3 hrs)			
Abdominal posture	26.37 ± 1.46	28.06 ± 2.67	26.25 ± 0.58
Abdominal – lateral posture	13.90 ± 3.91	8.38 ± 1.31	12.16 ± 1.07
lateral posture	7.86 ± 1.95 ^a	1.62 ± 0.58 ^b	6.94 ± 2.44 ^{ab}
Locomotion behaviour			
Walk time (Minutes / 3 hrs)	0.810 ± 0.13 ^b	1.75 ± 0.22 ^a	0.749 ± 0.12 ^b
Circling frequency (Frequencies / 3 hrs)	0.75 ± 0.31 ^c	3.25 ± 0.36 ^a	2.00 ± 0.37 ^b
Exploratory behaviour (Minutes / 3 hrs)			
Rearing up time	6.20 ± 0.36 ^a	4.83 ± 1.03 ^{ab}	3.68 ± 0.48 ^b
Sniffing time	2.72 ± 0.47 ^a	0.88 ± 0.23 ^b	0.38 ± 0.11 ^b
Comfort behaviour			
Grooming time (Minutes / 3 hrs)	9.26 ± 0.70 ^b	10.12 ± 0.80 ^b	13.07 ± 1.18 ^a
Stretching frequency (Frequencies / 3 hrs)	0.75 ± 0.16	0.87 ± 0.31	1.00 ± 0.37

Control: injected intramuscularly with sesame oil (0.25 ml/kg body weight).

B₁: received 2 intramuscular injections of boldenone (5 mg/kg body weight) at 9 and 12 weeks.

B₂: received 3 intramuscular injections of boldenone (5 mg/kg body weight) at 8, 10, and 12 weeks.

^{abc} Means in the same row with different superscripts are significantly different at (p<0.05).

Table 3. Effect of boldenone undecylenate on reproductive hormones of male rabbits.

Parameters	Experimental groups		
	Control	B ₁	B ₂
Testosterone (ng/ml)	2.96 ± 0.08 ^a	1.73 ± 0.09 ^b	1.64 ± 0.10 ^b
Estradiol (ng/ml)	6.47 ± 0.33 ^c	10.24 ± 0.29 ^b	11.99 ± 0.32 ^a

Control: injected intramuscularly with sesame oil (0.25 ml/kg body weight).

B₁: received 2 intramuscular injections of boldenone (5 mg/kg body weight) at 9 and 12 weeks.

B₂: received 3 intramuscular injections of boldenone (5 mg/kg body weight) at 8, 10, and 12 weeks.

^{abc} Means in the same row with different superscripts are significantly different at (p<0.05).

Table 4. Effect of boldenone undecylenate on carcass traits of male rabbits.

Traits	Experimental groups		
	Control	B ₁	B ₂
Live BW (g)	2550 ± 11.45 ^c	2858 ± 31.81 ^b	2976 ± 53.90 ^a
Dressing %	56.2 ± 0.06 ^c	57.7 ± 0.10 ^b	58.8 ± 0.09 ^a
2 Testes %	0.39 ± 0.07 ^a	0.37 ± 0.002 ^b	0.36 ± 0.002 ^b
Liver %	4.04 ± 0.05	4.16 ± 0.02	4.21 ± 0.03
Kidney %	0.81 ± 0.03	0.78 ± 0.03	0.78 ± 0.04
Heart %	0.24 ± 0.01	0.23 ± 0.02	0.22 ± 0.01
Spleen %	0.080 ± 0.005	0.078 ± 0.003	0.076 ± 0.006
Intestine %	15.39 ± 0.45	15.50 ± 0.63	15.12 ± 0.58
Lung %	0.65 ± 0.03	0.63 ± 0.02	0.67 ± 0.05

Control: injected intramuscularly with sesame oil (0.25 ml/kg body weight).

B₁: received 2 intramuscular injections of boldenone (5 mg/kg body weight) at 9 and 12 weeks.

B₂: received 3 intramuscular injections of boldenone (5 mg/kg body weight) at 8, 10, and 12 weeks.

^{abc} Means in the same row with different superscripts are significantly different at (p<0.05).

locomotion and grooming behaviors were significantly higher in the treated groups than the control group, and stretching was higher in treated groups but the difference was not significant. Exploratory behaviour and lateral posture were higher in the control group, with significant differences. The abdominal-lateral and abdominal pastures have no significant differences among the groups.

Reproductive hormones

Rabbits treated with BOL had a significant (p<0.05) decrease in the serum testosterone levels, and a significant (p<0.05) increase in the serum estradiol levels as compared to the control group (Table 3). Group (B₂) had a significant (p<0.05) increase in serum estradiol compared with group (B₁) or control group, while the testosterone level for groups (B₁) and (B₂) was not significantly (p>0.05) different.

Carcass traits

Statistical analysis of data on carcass traits of male rabbits was presented in Table 4. Administration of boldenone had a significant (p<0.05) increase in dressing % and decrease in testes %, while the percentages of kidney, heart, spleen and lung did not significantly (p>0.05) differ among the groups. Group (B₂) had a significant (p<0.05) increase in dressing % in comparison with other groups. Testes % in relation to live body weight was significantly higher in the control group than BOL treated groups.

Discussion

The primary objective of this study was to evaluate the effects of boldenone undecylenate on growth performance, some maintenance behaviour, serum reproductive hormones and carcass traits in male rabbits. The growth performance improved in treated groups (B₁ and B₂) relative to the control group, which is consistent with previous reports (Thabet et al. 2010). The same observation for BOL was described by Tousson et al. (2012), who stated that the use of the anabolic steroid boldenone undecylenate (BOL) resulted in obvious improvement in the growth rate. This effect could be attributed to promotion of the body tissue building process by protein synthesis indirectly via stimulation of growth hormone, insulin like growth factor secretion, and animal appetite (Ferreira et al. 1998) or reduction of glucocorticoid receptor levels and sensitivity to endogenous glucocorticoids; therefore, the strong growth promoting potency is based not only on its anabolic activity as an antiglucocorticoid (Melloni et al. 1997, Thienpont et al. 1998). However, Tawfeek et al. (1994) reported that the growth performance of rabbits was not affected by testosterone injection.

Behavioural endocrinology is the study of the interaction between hormones and behaviour, where this interaction is bi-directional: hormones can affect behaviour and behaviour can alter hormone levels (Christiansen, 2004). Thus, the main results from the present study revealed that most behavioural patterns differed significantly among the groups of this experiment, which may be due to hormonal-behavioural correlation. Ingestive behaviour was significantly affected

by administration of BOL, where the frequency times of eating and drinking was significantly higher in group (B₁) than other groups. The frequency of caecotrophy in group (B₁) was significantly higher than the control group and not different in comparison to group (B₂). This may be due to promotion of body tissue building through protein synthesis indirectly via stimulation of growth hormone, insulin like growth factor secretion and animal appetite (Ferreira et al. 1998). The lateral posture was significantly higher in the control group than other groups, while there were no significant differences in other postures of resting behaviour among groups. Locomotion of rabbits represented in walking time and circling frequency was significantly higher in group (B₁) than other groups. These results may be attributed to an increase in the aggressiveness and activities of rabbits under the effect of BOL (Robert et al. 2000, Khalil et al. 2014). Rearing up time was significantly lower in group (B₂) than the other groups, but sniffing time was significantly higher in group (B₁) than the other groups. The differences in exploratory behaviour „rearing up and sniffing” may be due to the effect of anabolic steroids on social behaviour (Salas-Ramirez et al. 2008, 2010). Comfort behaviour was higher in group (B₂) than the other groups with a significant difference ($p < 0.05$) in grooming time (self and mutual), while the difference in frequency of stretching was not reach the significant ($P > 0.05$). Bjorn et al. (1980) found that sex hormones induce yawning behaviour. The behavioural responses to anabolic steroids depend on the chemical structure of the steroid administered, age, and duration of treatment (Salas-Ramirez et al. 2008).

The results of serum concentrations of testosterone and estadiol hormones agreed with Thabet et al. (2010), where boldenone undecylenate injection decreased serum testosterone and increased estadiol concentrations in rabbits. This may be due to BOL inducing a state of hypogonadotropic hypogonadism associated with a decrease of testosterone. The results of the present study were similar to Hall and Hall (2005), who stated that anabolic steroids application leads to decrease the serum testosterone in rabbits. The decrease in the serum testosterone concentration may be explained as local suppressive effects of excess androgens on the testes, leading to the suppression of testosterone production by the leydig cell (Hall and Hall 2005). However, the increase in serum estradiol concentration could be due to aromatization of BOL, as testosterone is the primary substrate for the synthesis of estradiol in males (Boyardjiev et al. 2000). Nevertheless, there was a significant ($p < 0.05$) increase in the concentration of plasma testosterone after anabolic-androgenic steroid injections in treated

groups compared with the control. These results support former reports (Gabr et al. 2009, Tousson et al. 2012), who mentioned that an increase of testosterone may be attributed to synthesis of substrate related to the primary male sex hormone.

The results of carcass traits are in agreement with the findings of Thabet et al. (2010) who reported a significant ($p < 0.05$) decrease in testes weight and testicular atrophy of BOL treated rabbits in comparison with the control group. Application of anabolic steroids leads to testicular derivatives (Hall and Hall 2005). This testicular atrophy (reduction in testes size in relation to carcass weight) could result from suppressed testosterone production (Cannizzo et al. 2007, Oda and El-Ashmawy 2012). On the other hand, Tawfeek et al. (1994) reported that the difference in the testicular weight between the control and injected groups of rabbits was not affected by testosterone injection. Our results show that the differences among the groups in the carcass weight, liver % and intestine % may be due to increased body weight. These results clearly show that the differences between treated groups (B₁ and B₂) in stimulating rabbit growth and other parameters may be a response to BOL administration in a dose dependent manner. The administration of BOL at 3 intramuscular injections biweekly in group (B₂) was the most favorable for growth and other studied parameters in comparison to administration of BOL at 2 intramuscular injections with 3 week intervals in group (B₁).

In conclusion, administration of BOL to male rabbits at a dose of (5 mg/kg body weight) had a positive and significant benefit on growth performance (feed efficiency, total and daily weight gain) and a significant ($P < 0.05$) increase in dressing %. Rabbits treated with BOL had a significant decrease in testes % and testosterone level, while estradiol was significantly ($P < 0.05$) increased in comparison with the control group. Furthermore, there are hormonal-behavioral correlations through enhancement of ingestive and locomotion behaviors of the treated animal. We can therefore conclude that BOL ameliorates growth performance and carcass traits, with hormonal-behavioral patterns enhancing the ingestive and locomotion behaviors. Further studies are required to confirm these adverse reproductive changes.

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