Safety range of Boldenone Undecylenate injection in beef bulls

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To evaluate the effect of Boldenone Undecylenate (BOL) injection, on growth performance, some blood parameters and concentration of residual testosterone in raw, fried, grilled and boiled meat, twelve beef bulls were divided randomly into three equal groups (4 animals each group). Group 1 (G1) was injected intramuscularly (im) with saline as control group. G2 and G3 were injected im with BOL 1 ml/45 kg and 1 ml/90 kg BW, respectively. BOL was given three times with three weeks interval. Blood samples were collected at day 0, 60th and 120th for assays of total protein, cholesterol, testosterone and estradiol concentrations. Final body weight, total body gain, daily gain and feed efficiency were significantly (P<0.05) increased in G2 in comparison with G1 and G3. In addition, total protein, cholesterol, testosterone and estradiol concentrations were significantly (P<0.05) higher in G2 than G1 and G3 at the 60th and 120th days of experiment. The average pre-slaughter body weight, dressing percentage, and testes, eye muscle and bone weights as well as protein concentration in meat were significantly higher in G2 than those of G1 and G3. The concentrations of testosterone in the raw and cocked (using different cooking methods) were higher in G2 and G3 than those of G1, but the residual concentrations were safe for the human consumers. Boldenone undecylenate injection (1 ml/45kg or 1 ml/90 kg) in fattening bull improved growth performance without adverse effect on the residual concentrations of testosterone in meat.

Keywords: Boldenone Undecylenate, beef bulls, growth performance, blood parameters, meat residues, meat cooking effect

INTRODUCTION

In many parts of the world, meat is a considerable portion of diet. However there is a shortage of animal protein especially in the third world countries. Therefore, recent interest has grown to search some of growth promoter, which can enhance the meat production from animals. Anabolic steroids and other types of androgenic growth promoters such as boldenone undecylenate, 1, 4-androstadiene-17beta-ol-3-one, (BOL) is a derivative of the testosterone, which exhibits strong anabolic and moderately androgenic properties that improves the growth rate and feed conversion of animals. BOL and its derivatives were used to improve the growth rate and feed conversion in animals (Kuhn, 2002; Cannizzo et al., 2007; Soma et al., 2007; Kicman, 2008 and Guan et al., 2010). Also, BOL is used as
a growth promoter for beef cattle in the United States (Yesalis et al., 1993).

For the past two decades, BOL anabolic steroid is forbidden for meat production in animals worldwide (Yesalis et al., 1993) but still used in some countries in European Union (Kuhn, 2002; Cannizzo et al., 2007; Soma et al., 2007; Kicman, 2008; Guan, et. al., 2010 and Stephany, 2010). The adverse effects associated with misuse of BOL include disturbances of endocrine functions leading to disorders in physiological processes and immune function of human and animal body. Moreover, alterations of sebaceous glands and skin appendages, haemostatic and urogenital tract, cardiovascular, hepatic dysfunctions as well as and cancers were predisposed by misuse of anabolic hormones (Maravelias et al., 2005 and Oda & El-Ashmawy, 2012). These systemic disorders occurred by direct or indirect administration of androgen through accumulative effect in meat animals consumers (Hall & Hall, 2005). In light of the carcinogenic potential of anabolic steroids residues and obvious human health risks, several recent reports implicated them as a consequence for induction of hepatocellular adenoma and carcinoma (Socas et al., 2005; Cannizzo et al., 2007; Soma et al., 2007; Gabr, et. al., 2009; Hussein and Khalil, 2013). Effect of BOL on animal health is controversial. Thus, there are variations in the literatures for evaluations the positive or negative effect of BOL injection in animal production (Cannizzo et al., 2007; Gabr et al., 2009). Therefore, the present study was carried out to investigate the possible effect of BOL injection in beef bull as growth promoter on growth performance, some physiological responses, reproductive performance, protein level in meat and residual testosterone levels in raw meat and in meat after using different cooking methods (grilling, frying and boiling).

**MATERIALS AND METHODS**

**Experimental design:**

The present study was carried out at the Experimental Research Farm of Animal Production Department, Faculty of Agriculture, Sohag University. Twelve beef bulls (266.88-269.70 ± 2.61 kg average body weight (BW)) were used in this study and divided randomly into three groups (4 animals each). Group 1 (G1) was injected intramuscularly (im) with 1.0 ml/45 kg BW of saline solution and served as control. Groups 2 & 3 (G2 & G3) were injected with BOL (1 ml/45 kg BW) and (1 ml/90 kg BW), respectively. BOL was given three times with three weeks interval. The animals were kept separately in pens and fed individually on concentrate fed mixture, hay and wheat straw to cover the requirements for average body weight and total and daily gains of beef according to NRC (2000) requirements. Concentrate feed mixture composed of white corn 50%; wheat bran 30%; soybean meal 17%; limestone 2%; sodium chloride 0.5% and premix 0.5%. Fresh water was available ad libitum. Experimental period lasted 120 days. Body weights (BW) of animals were recorded at the beginning of the experiments and monthly thereafter, to study the effect of BOL injection on total and daily gains. Values of total and daily feed intakes as dry matter were calculated. Feed efficiency (gain divided on feed intake) was calculated during the experimental period.

**Blood analysis:**

Blood samples were collected from each animal at the beginning of the experiment (0 day), after two months (60 days) and four months (120 days). Serum samples were obtained by centrifugation for 10 minutes at 3000 r.p.m, and then stored at -20 °C for biochemical analysis. Serum samples were assayed for determination of testosterone and estradiol hormones concentrations using radioimmunoassay (RIA) technique (Garibaldi et al., 1993; Holownia et al., 1993). Cholesterol concentrations were determined using appropriate commercial kits (Biodiagnostics, Egypt, Synthe Chol ®) according to (Allain et al., 1974). Total protein was determined according to Doumas and Biggs (1972).

**Meat analysis:**

From twelve bulls slaughtered, eye muscle samples transferred in sanitary food bags on ice box to the central laboratory, Faculty of Veterinary Medicine, New Valley University for cooking by grilling, frying and boiling to estimate their effect on the residual testosterone concentrations in muscle samples and study the effect of different cooking methods on its concentrations. Testosterone residue was estimated using special MaxSignal® Boldenone ELISA Test Kit (bioscientific, PerkinElmer Company, Texas, USA)

**Carcass parameters:**

At the end of the experimental period, animals were slaughtered after 12h fasting with free water supply. Body weight pre slaughter was
recorded. Carcass, eye muscle, bone and testes were weighted. Also, dressing % was calculated. Protein concentrations in eye muscle samples were determined by Kjeldahl method.

Statistical analysis:
Data were analyzed using General Linear Model (GLM) procedure of SAS (SAS, 1998) according to the following model: \( Y_{ij} = \mu + Ti + Eij \)
Where: \( \mu = \) Mean, \( Ti = \) Effect of treatment and \( Eij= \) Standard error
Duncan's multiple range tests, Duncan (1955) was used to compare between means of the control and treated groups.

RESULTS AND DISCUSSION

Growth performance:
The effect of BOL injection on some productive performance is presented in Table (1). The average of the initial live body weights of G1, G2 and G3 were 269.70, 268.45 and 266.88 kg, respectively, however, The average of final body weights were 419.48 ± 8.13, 450.36 ± 8.13 and 431.33 ± 8.13, respectively. The average body weights recorded a non-significant difference at the beginning of the experiment, but it significantly (\( P<0.05 \)) increased in G2 in comparison with G1 and G3 at the end of experiment. The increase of BW was 7.27 % and 2.82 % in G2 and G3, respectively compared to the G1 at the end of the experiment. The total and daily body gains significantly (\( P<0.05 \)) increased in G2 in comparison with G1 and G3. The total and daily gains were the highest in G2, followed by G3 and then G1. The present results are agreement with (EC, 1996; Nasrollah and Shahidi, 2001 and EC, 2010) in sheep. They found that BOL injection led to positive effect on growth performance.

The daily feed intake revealed a non-significant difference between groups (G1, G2 and G3). But, the feed efficiency (FE) was improved significantly (\( P<0.05 \)) in G2 in comparison with those of G1 and G3. The improvement of FE in G2 and G3 is due to the significant increases of daily gains in G2 and G3 compared to G1 associated with a non-significant difference in daily feed intake between groups. The present results are agreement with (Gabr et al., 2009; El-Moghzay, et. al., 2012 and Tousson, et. al., 2013).

Blood analysis:
The effect of boldenone undecylenate injection on blood serum total protein, cholesterol, testosterone and estradiol levels is presented in Table (2). The total protein was not significantly changed in the treated groups as well as during all time points of the experiment. at the beginning of the experiment, there was no significant changes in levels of cholesterol, testosterone and estradiol in the treated groups. In contrast, they significantly increased (\( P<0.05 \)) at the 60th and 120th days post-treatment. Moreover, they were significant (\( P<0.05 \)) higher in group 2 than those of group 1 and 3. BOL injection in lambs led to increase cholesterol and testosterone concentrations in blood (Gabr, et al., 2009). The concentrations of cholesterol, testosterone and estradiol were significantly (\( p<0.05 \)) increased with advancing in age. Cholesterol is considered as precursor of building testosterone and estradiol hormones (Shahidi, 2001 and Hafez & Hafez, 2013). A significant increase of serum cholesterol, testosterone and estradiol concentrations is correlated to age. In growing buffalo heifers, plasma cholesterol increased significantly from 71.33 mg/100ml at 6 months of age to 90.86 mg/100 ml at 12 months of age (El-Ashry et al., 1994). Concentration of testosterone increased with the advancement of bull age attaining a value of (1.4 ng/ml) at 12 months of age and of (3.98 ng/ml) in adult buffalo bulls (Agarwal et al., 1983) between treatments or between different ages. But, generally concentrations of total protein in G2 recorded more value compared to G1 and G3. The present results are agreement with Gabr et al. (2009). They found that the injection of BOL led to an increase in total protein in lambs. Also, there are increase in the concentrations of total protein with advancing of age. Values of cholesterol, testosterone and estradiol concentrations were not significant at the beginning of the experimental (Table, 2), while the values were differed significantly (\( P<0.05 \)) at 60 and 120 days. The previse parameters were higher (\( p<0.05 \)) in G2 compared to G1 and G3.In the previse study by Gabr et al., (2009) illustrated that In addition, data indicated that the concentrations of cholesterol, testosterone and estradiol values were significantly (\( p<0.05 \)) increasing with advancing of age. Cholesterol concedes as precursor of building testosterone and estradiol hormones (Hafez, 1987). Concerning the effect of age (regardless of treatments) on serum of cholesterol, testosterone and estradiol concentrations, data in Table (2) illustrated that there are significantly (\( P<0.05 \)) increase with advancing ages. El-Shahed, (1993) studied the effect of age on serum cholesterol concentration

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in Buffalo bulls. He found that age play an important role in increasing level of blood cholesterol.

Table (1): Effect of boldenone undecylenate injection on some productive performance of beef bulls (LSM ± SE)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatments</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (Kg)</td>
<td>269.70 ± 2.61</td>
<td>268.45 ± 2.61</td>
<td>266.88 ± 2.61</td>
<td></td>
</tr>
<tr>
<td>Final body weight (Kg)</td>
<td>419.48 ±8.13</td>
<td>450.36 ± 8.13</td>
<td>431.33 ± 8.13</td>
<td></td>
</tr>
<tr>
<td>Total gain (Kg)</td>
<td>149.78 ±6.37</td>
<td>181.91 ± 6.37</td>
<td>164.45 ± 6.37</td>
<td></td>
</tr>
<tr>
<td>Daily gain (Kg)</td>
<td>1.25 ± 0.06</td>
<td>1.52 ± 0.06</td>
<td>1.37 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Daily feed intake (Kg)</td>
<td>8.52 ± 0.23</td>
<td>8.63 ± 0.23</td>
<td>8.83 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>Feed efficiency (Kg)</td>
<td>0.15 ± 0.001</td>
<td>0.18 ± 0.001</td>
<td>0.16 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

G1=Control; G2 = Injection with BOL (1 ml /45 kg BW); G3 = Injection with BOL (1 ml /90 kg BW).

This increase with advancing age is significant and the correlation between age and cholesterol concentration showed strong positive relationship (0.60). In addition, El-Ashry, (1994) in growing Buffalo heifers, found that plasma cholesterol increased significantly from 71.33 mg/100ml at 6 months of age to 90.86 mg/100 ml at 12 months.
of age. Similar results were reported by Abu-Elawa (1995) in Friesian and Buffalo calves. He found that cholesterol level increased in both breeds with advanced of age from 9 to 18 months. In addition, Agarwal et al., (1983) reported that the concentration of testosterone increased with the advancement of bull age attaining a value of about 1.4 ng/ml at 12 months of age and the concentration rose to 3.98 ng/ml in adult Buffalo bulls. Similar results obtained by Mokhless and Ibrahim, (1990) in Egyptian Buffalo. They found that testosterone concentration increased gradually with the progress of age from 12 to 24 months. Also, the obtained results by Abu-Elawa (1995) in Friesian and Buffalo calves indicated that a gradual significant increase in plasma testosterone concentrations with advancing of age from 9 to 18 months. Similar, observation reported by Kassab (2007) in sheep.

Some carcass parameters:

The effect of BOL injection on some carcass parameters is illustrated in Table (3). Body weight pre slaughter, carcass weight, percentage of dressing increased significantly (P<0.05) in G2 when compared to G1 and G3. Weight (kg) of eye muscles, bones and testes were non-significant higher in G2 than those of G1 and G3. The increase of the previous parameters may be due to pre slaughter body weight of bulls in G2 higher than G1 and G3.. The increase of the previous parameters may be due to pre slaughter body weight of bulls in G2 higher than G1 and G3. Thus, injection of BOL led to improvement in the live body weight of bulls and increasing the growth of different body organs including the testes. A significant positive correlation was observed between live body weight and testicular measurements in sheep (EC, 1996; Hamdon, 2005; Kassab, 2007; EC, 2010 and Saleh, 2013). Also, in both Buffalo and cattle Abo-Elawa, (1995) reported similar results. The improvement in the testes weight may be attributed to increasing testosterone hormone concentration in blood (El-Barody et al., 2010).

Protein content in meat:

The effect of BOL injection on percentage of protein in bulls' meat samples of treated groups is illustrated in Fig. (1). The average of protein concentration (%) in meat ranged between 17.93 to 18.14%. No significant difference in protein percentage in meat was observed between groups. Increasing the concentration of protein % in meat samples may be due to that BOL increases muscle size due to promotion of positive nitrogen balance by stimulating protein production and reducing protein destruction. Moreover, it produces retention of body water, nitrogen, sodium, potassium and calcium ions (Forbes, 1985; Mooradian et al., 1987 and EC, 2010).

Level of testosterone meat:

The mean residual testosterone concentrations in raw and cocked (using different cooking methods) in meat samples was significantly (P<0.05) increased in G2 and G3 when compared to G1 (Table 4). The higher values recorded in G2 followed by G3, while the lowest values recorded in control group (G1). Generally, residual testosterone concentration ranged between 0.013 to 0.022 µg/kg. Grilling of meat led to more reduction of testosterone concentrations in different treatments followed by frying and then boiling. According to FAO/WHO (2004) the maximum limit of testosterone in meat should not exceed 0.100 µg/kg, while the Egyptian Organization for Standardization and Quality Control (2008) recommended not more than (0.05 µg/kg) of testosterone in meat in daily human food. Thus, the present concentration of testosterone in meat in control as well as different treatment groups was within the local and international permissible limits and meat was safe for the human consumers.

Histopathology:

The testis of different animals were examined, almost all the animals have mild degree of testicular edema and degeneration. Most of seminiferous tubules had all stages of spermatogenic cells and sperms in their lumen. However, focal areas of dispersed seminiferous tubules with interstitial edema and hyperemic blood vessels were observed. Depletion of spermatogenic cells leaving wide empty luminae was observed (Fig. 2a). The spermatogonia and primary spermatocytes had pyknotic nucleus and deeply acidophilic vacuolated cytoplasm. The spermatid cells revealed necrobiotic changes few spermatids metamorphosed to sperms (Fig. 2b). The degenerated spermatid cells appeared as eosinophilic dot bodies in lumen of s seminiferous tubules (Fig. 2c). Sometimes hemorrhages and edema were infiltrated in seminiferous tubules and interstitium (Fig. 2d). Efferent ductules have no prominent changes (Fig. 2e). Edema is characterized by deposition of eosinophilic material and congestion of blood vessels. The dense fibrous tissue of tunica albugina was dispersed with faint pink material (Fig 2f).
Table (4): Effect of boldenone undecylenate injection and cooking methods on testosterone concentration (µg/kg) in meat (Mean ±SE)

<table>
<thead>
<tr>
<th>Meat groups</th>
<th>Raw</th>
<th>Frying</th>
<th>Grilling</th>
<th>Boiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.018 ± 0.0002c</td>
<td>0.015 ± 0.0002c</td>
<td>0.013 ± 0.0003c</td>
<td>0.015 ± 0.0002c</td>
</tr>
<tr>
<td>G2</td>
<td>0.022 ± 0.0002a</td>
<td>0.018 ± 0.0003a</td>
<td>0.016 ± 0.0002a</td>
<td>0.019 ± 0.0003a</td>
</tr>
<tr>
<td>G3</td>
<td>0.020 ± 0.0003b</td>
<td>0.016 ± 0.0002b</td>
<td>0.015 ± 0.0003b</td>
<td>0.018 ± 0.0001b</td>
</tr>
</tbody>
</table>

a, b and c, values with the different superscripts in the same column differ significantly at P<0.05

Figure (1): Effect of boldenone undecylenate injection on percentage of protein in meat of treated groups.

G1=Control; G2 = Injection with BOL (1 ml /45 kg BW); G3 = Injection with BOL (1 ml /90 kg BW).
CONCLUSION
The injection of boldenone undecylenate at level of 1 ml/45kg or 1 ml/90 kg in fattening bull led to improvement growth performance and blood metabolites without adverse effect on the residual concentrations of testosterone in meat.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
The Animal Rights and Ethical Use Committee of New Valley University and South valley university have approved this study.

AUTHOR CONTRIBUTIONS
NTE: Corresponding author of the manuscript, study design, performed the meat analysis part of the study, write the paper, drafted and revised the manuscript and data analysis. MH: performed the histological part of study, write the histological part in the paper, drafted and revised the manuscript and data analysis.

AYK: collected the samples and injected it with the different doses of the tested drug, slaughtered the animals, estimated the animal production analysis parameters part of study, wrote the production analysis part in the paper, drafted and revised the manuscript and data analysis.

HAH: collected the samples and injected it with the different doses of the tested drug, slaughtered the animals, estimated the animal production analysis parameters part of study, wrote the production analysis part in the paper, drafted and revised the manuscript and data analysis.

All the authors shared laboratory examination and data analysis. All authors have read and approved the final manuscript.

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Figure 2: Histological examination of the testis of different animals
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REFERENCES


