The Effects of Stanozolol and Nandrolone Decanoate Hormones on Erythropoietin and Testosterone Serum Concentrations in Dogs

Bahman Mosallanejad 1*, Saad Gooraninejad 1, Anahita Rezaie 2, Seyyed Reza Fatemi Tabatabaei 3, Hadi Imani Rastabi 1, Saman Salmani 1

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Abstract

BACKGROUND: Stanozolol and Nandrolone decanoate are the most commonly used hormones in canine medicine. They are mainly administered to strengthen the muscle, gain weight, treatment of anemia and stimulate the appetite. One of the effects of hormones is to enhance the erythropoietin production and eventually an increase in the number of RBCs.

OBJECTIVES: Prolonged usage of hormones may be relevant to the liver and renal disorders; therefore, the present survey was aimed to evaluate the effects of stanozolol and nandrolone decanoate on liver and kidney indices, erythropoietin and testosterone serum concentrations and hematocrit changes in healthy dogs.

METHODS: Sixteen dogs were randomly categorized in two groups of A (stanozolol) and B (nandrolone decanoate). Each group was divided into two subgroups (A1, A2 and B1, B2). The second testicle was removed on day 28 in the first subgroups (A1 and B1), and day 42 in the second subgroups (A2 and B2). The first testicle was removed at time zero. Stanozolol (50 mg per dog) was administered to all dogs of group A as IM once a week for six weeks. Group B was similar to group A with the difference that nandrolone decanoate was injected (1 mg/kg) instead of stanozolol. Blood samples were collected on days 0, 3, 14, 28 and 42.

RESULTS: Erythropoietin and testosterone concentrations were virtually increased in both groups A ($P<0.05$) and B ($P<0.05$). The effect of stanozolol on erythropoietin (subgroup A1) ($11.35\pm1.31$ ng/mL) was significantly higher than nandrolone decanoate (subgroup B1) ($8.02\pm0.55$ ng/mL) ($P<0.05$); nevertheless, the changes in testosterone levels, was not significant between groups A and B ($P>0.05$). The liver enzymes of ALP, ALT and AST were increased more significantly in group A than group B ($P<0.05$).

CONCLUSIONS: Erythropoietin and testosterone levels were virtually increased in both groups A and B; however, stanozolol had more significant effect than nandrolone decanoate in increment of erythropoietin; nevertheless, it had more side effects on liver indices. It is suggested that nandrolone decanoate to be administered for the therapeutic goals.

KEYWORDS: Dog, Erythropoietin, Nandrolone decanoate, Stanozolol, Testosterone

Correspondence: Mosallanejad, B, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran Tel: +98 (061) 333330010-19, Fax: +98 (0613) 33360807, Email: bmosallanejad@scu.ac.ir

Received: 2021-02-09
Accepted: 2021-04-26

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How to Cite This Article
Introduction

Anabolic and androgenic steroids (AASs) are a major class of lipophilic hormones. Testosterone is responsible for different physical characteristics specific to males. About 95% of testosterone is secreted by Leydig cells. As a result of weight gain, significant physiological changes occur in the animal body, including the concentration of sex hormones. Body weight increases with enhancement of age and significantly with castration in dogs. Castration can cause a meaningful reduction in testosterone serum concentration (Bachman et al., 2014; Mhillaj et al., 2015).

Stanozolol and nandrolone decanoate are the two most commonly used drugs in dogs. They are mainly administered to strengthen the muscles, stimulate the appetite, and treatment of anemia in animals such as dogs and horses. The dosage is 2 to 4 mg per dog twice daily in large breeds, or 50 mg of the injectable form is given intramuscularly (IM) once weekly. Stanozolol has been used to treat anemia in chronic diseases; nevertheless, it may cause unwanted side effects. Some 17 α-alkylated anabolic steroids such as stanozolol are related to hepatotoxicity (Bond et al., 2016). Nandrolone decanoate is indicated for the treatment of anemia in animals with renal failure and also has been shown to increase hemoglobin level. The actual anabolic steroids have an extensive margin of safety, remarkable activity, easiness of administration and similarity with other compounds (Pomara et al., 2016; Ozcagli et al., 2018).

Erythropoietin is a synthetic form of the naturally hormone produced in the kidney. It is used to treat anemia in companion animals that is occurred secondary to the chronic renal failure. If the animal becomes more anemic during the treatment, a resistance associated with the immune system and discontinued administration of the hormone should be considered (Ettinger et al., 2017; Torres et al., 2017). In some species, the immune system may react to the hormones, making the animal body resistant to their useful effects. For this reason, these drugs must be used with caution (Randolph et al., 2004). Since the hormones circulate in low quantities in blood, measurement of these compounds requires sensitive assays, usually in a competitive form. Normal concentrations of hormones can vary significantly between species. The reference range of erythropoietin and the testosterone concentration were reported between 1.3-13.4 ng/mL and 0.5-9 ng/mL, respectively in dogs, measured by RIA (Krawski et al., 2014).

There are several reports regarding the adverse effects of anabolic steroids on the organ systems including testes and liver in some species. Anabolic and androgenic steroids (AASs) have harmful effects on the testicular functions, during onset of puberty (Mohd Mutalip et al., 2013). The administration of stanozolol in cats has caused increase in the hepatic enzymes and hepatotoxicity (Harkin et al., 2000). In the recent years, the use of these hormones has grown significantly; however, it has been associated with different results. The present study was accomplished in the completion of previous researches. Prolonged usage of hormones may be related to the liver and kidney complications. The initial hypothesis was that steroid hormones would increase hematopoiesis levels in the body and improve the animal's condition by increasing the erythropoietin and testosterone levels; therefore, the main objective of this experiment was to consider the effects of stanozolol and nandrolone decanoate hormones on erythropoietin and testosterone serum concentrations, liver and kidney function indices, and hematocrit and body weight changes in clinically healthy dogs. The results of this survey can also be helpful in the cases of cryptorchidism.

Materials and Methods

Animals

The experiments were performed on adult male dogs (n=16) with age 1.5-2 years old, mixed breed and weight of 18.25-23.75 kg. The dogs were kept in separate cages, with controlled temperature (25±3°C) and 12-h light/12-h dark cycles, in Ahvaz district with warm and humid climate conditions. They were clinically healthy and fed with the standard meat diet (chicken). Water was provided ad libitum. This survey was approved by the Animal Care and Research Committee of Shahid Chamran University of Ahvaz. It was conducted based on the Guidelines for Animal Care and Use (Ethical code: 9338404).
Experimental Design

Sixteen dogs were randomly selected and categorized in two equal groups of A (stanozolol) and B (nandrolone decanoate). There were eight dogs in each group. Stanozolol (Winstrol depot, 50 mg/mL injection, Lowa, USA) was administered 50 mg per dog as IM to all dogs of group A, once a week and for six weeks. Group B was similar to group A, with the difference that nandrolone decanoate (Deca Durabolin, 25 mg/mL injection, Tehran, Iran) was administered 1 mg/kg instead of stanozolol (Ettinger et al., 2017). The first testicle of all dogs was removed on the first day of the challenge by surgery. Furthermore, each group was divided into two subgroups (A1, A2 and B1, B2). The difference between subgroups was that in the first subgroups (A1 and B1), the second testicle was removed on day 28 and in the second subgroups (A2 and B2) it was removed on day 42.

Collection of Samples

Blood samples were collected from the cephalic vein or external saphenous, five times on days 0, 3, 14, 28 and 42. The blood collection on day 42 was performed 12 h after removing the second testicle. The reason of the removal of testicles was to observe the changes in serum testosterone concentration, following testicles surgery. Daily body weight changes was recorded before choosing a treatment and continued for six weeks. The dosages of stanozolol and nandrolone decanoate hormones were chosen based on their application in the large breeds (Ettinger et al., 2017). Erythropoietin (IBL Co., Hamburg, Germany) and testosterone (Atieh Perfect Diagnostic Co., Iran) serum concentrations were measured using ELISA reader (Tisa Teb Novin Azma, Tehran, Iran) in a specific wavelength, selected by an optical filter. Biochemical analysis was conducted for the measurement of ALT, AST, ALP, total and direct bilirubin, BUN and serum creatinine in an automated chemical analyzer (BT 3000 Plus, Biotechnica, Milan, Italy) and using diagnostic kits (Pars Azmoon Co., Tehran, Iran). Normal values were referred to the sources for biochemical profiles (Ettinger et al., 2017).

Statistical Analysis

All data were expressed as mean ± SE in different groups (A and B). The statistical comparisons were done among groups using repeated measures one way analysis of variance (ANOVA) and followed by Duncan’s post-hoc test. The statistical analyses were performed using SPSS (Version 16.0; SPSS Inc., IL, Chicago, USA). Differences were considered significant when P-value ≤ 0.05.

Results

The results showed that erythropoietin and testosterone serum concentrations were virtually increased in both groups A (P<0.05) and B (P<0.05). The levels of hormones were increased with continuous injections. The changing processes were started between days 3 and 14, and the difference was absolutely significant on day 14 (Table 1).

<table>
<thead>
<tr>
<th>Days</th>
<th>Zero</th>
<th>3</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stanozolol (subgroup A1)</td>
<td>A1.87±0.92a</td>
<td>A2.45±0.47a</td>
<td>A5.42±1.38b</td>
<td>A8.75±1.33c</td>
<td>A11.35±1.31d</td>
</tr>
<tr>
<td>Stanozolol (subgroup A2)</td>
<td>A1.92±0.80a</td>
<td>A2.67±0.97a</td>
<td>A4.55±0.60b</td>
<td>A7.17±0.35c</td>
<td>A8.80±0.18c</td>
</tr>
<tr>
<td>Nandrolone decanoate (subgroup B1)</td>
<td>A2.00±0.46a</td>
<td>A2.62±0.48a</td>
<td>A5.50±0.48b</td>
<td>A7.47±1/06c</td>
<td>A8.02±0.55c</td>
</tr>
<tr>
<td>Nandrolone decanoate (subgroup B2)</td>
<td>A1.75±0.71a</td>
<td>A2.45±0.31a</td>
<td>A4.37±0.80b</td>
<td>A7.45±0.42c</td>
<td>A8.45±0.56c</td>
</tr>
</tbody>
</table>

*Different lower-case letters show significant differences within groups over time (P<0.05).

*Different higher-case letters show significant differences between groups (P<0.05).

Stanozolol (50 mg per dog) was administered to all dogs of group A. Group B was similar to group A, with the difference that, nandrolone decanoate was injected with dosage 1 mg/kg. Each group was divided in two subgroups (A1, A2 and B1, B2). The difference between two subgroups was that in the first subgroups (A1 and B1), the second testicle was removed on
The effect of stanozolol on erythropoietin concentration (subgroup A1) (11.35±1.31 ng/mL) was significantly higher than nandrolone decanoate (subgroup B1) (8.02±0.55 ng/mL) on day 42 (P<0.05); nevertheless, the changes on testosterone concentration was not significant between groups A (subgroups A1 and A2) and B (subgroups B1 and B2) (P>0.05) (Table 2). As shown in Table 2, following the removal of testicles on day 28, the values of testosterone concentration were decreased in subgroups A1 (2.02±0.35 ng/mL) and B1 (1.77±1.03 ng/mL) on day forty-two. The levels of testosterone concentration on day 42 were obtained 8.80±0.18 ng/mL and 6.25±0.23 ng/mL in subgroup A2 and B2, respectively. The results showed that liver enzymes of ALP (226.88±66.36 IU/L), ALT (235.38±32.14 IU/L) and AST (143.13±8.28 IU/L) were increased significantly on day 42 in the group A (P<0.05), suggesting that stanozolol has a hepatotoxicity effect. The values for ALP, ALT and AST enzymes were in normal range (66.88±17.41, 48.0±11.5 and 47.4±50.53 IU/L), respectively in group B (Table 3).

Hematocrit was increased in both groups, but these changes were more significant in group A (42.02±5.96) than group B (34.47±5.39) (P<0.05). The initial weights of the dogs were 20.10±1.60 and 20.31±1.57 kg in groups A and B, respectively. The assessment of body weight showed a significant difference between both groups during the challenge (22.18±2.20 vs 20.99±1.65 kg) (P<0.05). Using hormones did not cause any side effect on kidney indices. In group A, the means of BUN and serum creatinine were 19.75±0.28 mg/dL, respectively in group B (Table 3). All dogs were healthy during the treatment and had no symptoms such as anorexia, vomiting, jaundice and polyuria or polydipsia. The results are summarized in Tables 1-3.
**Discussion**

The obtained results of the present survey showed that stanozolol had the most additive effects on the erythropoietin concentration, hematocrit and body weight in subgroup A1. The difference in the raise of erythropoietin level was significant between subgroups A1, A2 and subgroups B1, B2, showing that stanozolol had more potency than nandrolone decanoate (Table 1). In our research, duration of treatment was six weeks and the hormones were administered once a week. By removing the first testicle on day zero, testosterone concentration was decreased on day three in all groups, but in the following, this process started to increase on day 14, possibly due to testosterone injections (Table 2). The removal of testicles (as the main source of testosterone secretion) was done for better understanding of the hormone changes at different times.

Hormonal injection plays an important role as an exogenous source of testosterone. The values of testosterone concentrations were decreased in subgroups A1 and B1 on day 42. The major source of testosterone secretion was discontinued from Leydig cells, by removal of the testicles on day 28. Although a decrease in testosterone concentration occurred on day 22, they never reached less than zero time. The possible reason is the injection of stanozolol and nandrolone decanoate hormones on intermittent days (Table 2). Our previous study has confirmed these findings in cats. In this survey that was done on ten male cats, both hormones had effective functions in the increment of erythropoietin, testosterone, and body weight, but stanozolol was more effective than nandrolone decanoate (Mosallanejad et al., 2018).

Hepatotoxicity is the most common serious adverse effect associated with AASs. Stanozolol administration has shown to increase the hepatic enzymes and hepatotoxicity in cats with chronic kidney failure consistently (Harkin et al., 2000). In the present study, ALP, ALT and AST liver enzymes were increased significantly in group A, suggesting that stanozolol has a narrow margin of safety and is hepatotoxic in dogs, of course, these effects are probably reversible after discontinuation of hormones. The levels of liver enzymes were in normal range in group B. This hormone is not \(c\)-17 \(\alpha\)-alkylated and it is not known to have hepatotoxic effects (Ozcagli et al., 2018).

The AASs have been modified to improve their anabolic rather than androgenic activities (Carrero et al., 2012). The anabolic steroids should be used properly to obtain suitable clinical response. Many cases of hormone-induced hepatopathy are mild and present with ambiguous symptoms of anorexia, lethargy, vomiting or jaundice. Hepatotoxicity of hormones varies significantly between different species. The known factors to be hepatotoxic in other animals cannot be assumed to be hepatotoxic in dogs as well. In addition, some agents may cause hepatic damage in dogs but not in other species (Maddison et al., 2002). In the present study, all dogs were clinically healthy during the challenge and had no sign of anorexia, vomiting, jaundice, polyuria or polydipsia or renal diseases. Monitoring the patients by measurement of different biochemical profiles is very important.

It has been assumed that the action mechanism of the hepatotoxicity is due to an immunologic response to active metabolites. This hormone should be used with caution in the affected animals with heart, liver or renal diseases. Prolonged usage of

### Table 1: Comparison of hormone concentrations between subgroups

<table>
<thead>
<tr>
<th>Nandrolone decanoate (B)</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.25±16.00</td>
<td>51.25±27.45</td>
<td></td>
</tr>
<tr>
<td>43.50±6.16</td>
<td>52.88±19.31</td>
<td></td>
</tr>
<tr>
<td>43.50±6.16</td>
<td>52.88±19.31</td>
<td></td>
</tr>
<tr>
<td>47.50±4.53</td>
<td>51.75±11.56</td>
<td></td>
</tr>
<tr>
<td>47.50±4.53</td>
<td>51.75±11.56</td>
<td></td>
</tr>
</tbody>
</table>

*Different lower-case letters show significant differences within groups (\(P<0.05\)).

*Different higher-case letters show significant differences between groups (\(P<0.05\)).

Stanozolol (50 mg per dog) was administered to all dogs of group A. Group B was similar to group A, with the difference that, nandrolone decanoate was injected with dosage 1 mg/kg.
Stanozolol has been associated with liver damages (Maddison et al., 2002).

The real incidences of hormone-induced hepatic diseases are unknown in dogs. Clinical signs and laboratory test results are non-specific and do not differentiate hormone-induced diseases from other causes of hepatic diseases. During a prolonged therapy duration, the measurement of serum enzyme concentrations is recommended to allow dosage regulation if necessary (Maddison et al., 2002). The most consistent findings are increase in ALT and AST activities. Serum lactate dehydrogenase (LDH) and ALP enzymes may be increased (Hsu, 2008). In the present study, ALP, ALT and AST liver enzymes were increased significantly in stanozolol group. Our results were similar to the findings of some researches that reported the anabolic steroid-induced hepatotoxicity. These values were normal in nandro- lone decanoate group. Intramuscular injection of stanozolol had an adverse effect on the liver tissue. This hormone has a group of c-17 α-alkylated (Kahn and Line, 2008).

It was specified that stanozolol had hepatotoxicity effects in cats. The serum ALT concentration was increased in 14 out of 18 cats after administration of stanozolol, but serum ALP level was mildly increased in only three cats. The hepatic enzymes were placed in normal range after discontinuation of the drug (Harkin et al., 2000). Successful treatment of stanozolol-induced hepatotoxicity with silymarin was reported in a bitch. Using stanozolol caused an increase in serum enzyme activities of ALT, AST, ALP, LDH, total and direct bilirubin in the studied dogs. Administration of silymarin improved clinical condition and serum enzyme levels returned to the normal range (Mosallanejad et al., 2011).

Stanozolol has more anabolic than androgenic efficacies (Carrero et al., 2012). It was reported that stanozolol had positive effects on body condition score (BCS) in dogs with chronic kidney failure (Harkin et al., 2000). The administration of stanozolol increased antioxidant capacity in skeletal muscles from sedentary rats. A possible reason of distinct results was administration of stanozolol with high dosage (Delgado et al., 2010). Supraphysiological dosage of nandro- lone decanoate may negatively affect the number of Leydig cells, and testosterone level in immature rats (Jannatifi et al., 2015).

Some factors such as age, gender, genetic agents, malnutrition (protein deficiency), and simultaneous use of other drugs can influence on the severity of hormone-induced hepatic disorders. The metabolism of hormones may decrease in younger dogs due to lower hepatic enzyme concentrations. Older dogs have more concurrent diseases that influence on hormone metabolism (Kahn and Line, 2008). It was stated that both injectable and oral stanozolol cause a significant increase in nitrogen retention. The response to intramuscular administration was significantly more than oral regimen. It was proposed that the action of stanozolol may be beneficial in dogs under stress of surgery and chronic disorders (Olson et al., 2000). Some researchers focused on the effects of boldenone consumption and resistance exercise on hepatocyte damages in rats. The administration of hormone had a marked harmful influence on the liver tissue, even with low dose. Boldenone increased nitrogen retention and protein synthesis. This hormone stimulated the release of erythropoietin in the kidneys and reduced protein destruction. Boldenone also produced retention of water, nitrogen, sodium, potassium and calcium ions (Matinhomae et al., 2014). On the other hand, it is stated that the administration of anabolic androgenic steroids may cause problems in the genital system. They disturb the regular endogenous production of testosterone and gonadotropins that may persist for months after drug discontinuation (Ettinger et al., 2017).

The harmful effects of anabolic steroids can be associated with the pharmacologic action of steroids. The increased spread of some tumors has been reported in human (Maddison et al., 2002). Some 17 α-alkylated oral anabolic steroids (such as oxymetholone and stanozolol) are related to hepatotoxicity. Although anabolic steroids have specified therapeutic applications, they are administered to stimulate the growth and development of muscles. When they are used in excess or abused, many negative side effects may be appeared such as infertility, and increased risk of cardiovascular, liver, and kidney diseases (Maddison et al., 2002).

The actual mechanism of testosterone remains unclear in enhancement of hemoglobin and hematocrit; nevertheless, it has been announced that testosterone increases hemoglobin and hematocrit that is related to the stimulation of erythropoietin and reduction of...
ferritin and hepcidin levels (Bachman et al., 2014). In the present survey, hematocrit enhancement was 42.02±5.96 and 34.47±5.39 for the groups of stanozolol and nandrolone decanoate, respectively on day 42. As the erythrocyte lifespan varies from 110-120 days in dogs, in spite of the decrease in testosterone concentration in subgroups A1 and B1, hematocrit level was increased in both groups. On the other hand, the main hormone for the increase in red blood cells is erythropoietin which is largely produced in the kidneys (Etienne, 2020), thus, the hematocrit was still high in all groups (Table 1). The measurement of body weight showed a significant difference between groups A and B on day 42.

**Conclusion**

It can be concluded that stanozolol had more significant influence than nandrolone decanoate in raising the levels of erythropoietin, hematocrit and body weight in dogs, but it had more side effects as well. Therefore, nandrolone decanoate administration is suggested for the therapeutic goals in healthy dogs. Using anabolic steroids is not recommend in dogs for the long times more than six weeks. If stanozolol is administered in dogs, pre-treatment and post-treatment evaluation of liver function indices should be carefully followed up to ensure the possible toxic effects. Further researches are needed to detect more aspects of hepatotoxicity due to anabolic steroids in companion animals.

**Acknowledgments**

This study was financially supported by the Research Council of Veterinary Faculty, Shahid Chamran University of Ahvaz, Iran. I hereby declare all ethical standards have been respected in preparation of the submitted article. All procedures which might be associated with discomfort including castration, blood sampling and injections were performed by an experienced veterinarian.

**Ethical Approval**

I hereby declare all ethical standards have been respected in preparation of the submitted article. All procedures which might be associated with discomfort including castration, blood sampling and injections were performed by an experienced veterinarian.

**Funding/Support**

This study was funded by Shahid Chamran University of Ahvaz (grant number 9338404). There is no financial interest to disclose.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Effect of Stanozolol and Nandrolone on EPO and T

Mosallanejad B et al.


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چکیده
زمینه مطالعه: هورمون‌های استانوژول و ناندرولون دکانوآت، بیشتر در سگ‌ها کاربرد دارد. آنها عمدتاً جهت تقویت سرشت عضلانی، افزایش وزن و همچنین درمان‌های دیگر استفاده می‌شوند. هدف: استفاده طولانی مدت از هورمون‌های ممکن است با عوارض کبدی و کلیوی همراه بماند. بنابراین هدف از انجام تحقیق حاضر، رابطه اثرات استانوژول و ناندرولون دکانوآت در سگ‌ها بر نتایج نظارت و غلظت انتروپوئین و تستوسترون، در سگ‌های سالم بود.

روش کار: شانزده فلاده سگ به صورت تصادفی به دو گروه A (استانوژول) و B (ناندرولون دکانوآت) تقسیم شدند. هر گروه به دو زیر گروه تقسیم شدند. در اولین زیر گروه ها (A1 و B1)، در روز‌های ۱۵، ۲۵ و ۳۵ پذیرایی می‌شد. در دومین زیر گروه ها (A2 و B2) در روز‌های ۲، ۳ و ۴ انتروپوئین و تستوسترون مورد استفاده قرار گرفتند.

نتایج: نتایج نظارت و غلظت انتروپوئین و تستوسترون در هر دو گروه A و B با یکدیگر تفاوت معنی‌داری نداشتند. اثر معنی‌داری در گروه B، انتروپوئین در سطح ۰/۰۵، تستوسترون در سطح ۰/۰۱ و A1 انتروپوئین داشت. اثر معنی‌داری در گروه A، تستوسترون در سطح ۰/۰۱ و A2 تستوسترون در سطح ۰/۰۵ بود.

نتیجه گیری نهایی: منابع انتروپوئین و تستوسترون، در دو گروه A و B به صورت قابل توجهی افزایش پیدا کردند، اما انتروپوئین، اثر معنی‌دار در بیشترین نسبت به ناندرولون دکانوآت، در انتروپوئین داشت. با این وجود، اثرات جانبی بیشتر بر شاخه‌های کبدی، در جا گذاشته شد.

واژه‌های کلیدی: سگ، انتروپوئین، ناندرولون دکانوآت، استانوژول، تستوسترون.