Effects of growth promoter Boldenone undecylenate on weaned male lambs

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Abstract

This study aimed to observe the effects of an anabolic androgenic synthetic commercial steroid (Boldenone, BOL) on the growth performance of prepubertal male lambs. Lambs were divided into three equal groups (n=4), the first group injected 5mg boldenone, the second group injected 2.5 mg and the third one injected olive oil served as control. All treated groups received 5 injections at three week interval. Blood samples and body weight were taken until the seventh week after last injection. Blood serum total proteins, albumin, urea, total cholesterol and high density lipoproteins (HDL), ALT and AST and creatinine were recorded in addition to some whole blood haemogram parameters. Testosterone, T_3 and T_4 were assayed. The results indicated a significant increase in body gain in treated groups, total proteins and haemoglonbin with a decrease in urea. An insignificant increase in testosterone was recorded in both treated groups. The study proved that boldenone improved the performance of male lambs and treated lambs reached puberty earlier than control. [Nature and Science. 2009;7(3):61-69]. (ISSN: 1545-0740).

Introduction

Anabolic androgenic steroids (AAS) is an official definition for all male sex steroid hormones, their synthetic derivatives and their active metabolites are synthetic derivatives of the male testosterone originally designed for therapeutic uses to provide enhanced anabolic potency with negligible androgenic effects (**Clark and Henderson, 2003)**. They are also used to enhance strength and endurance in canine, equine and human athletes through increasing muscle protein production **(Teale and Houghton, 1991; Schänzer & Donike, 1992; Schänzer, 1996).**

Boldenone (1,4-androstadiene-17beta-ol-3-one; BOL) and its precursor boldione (1,4-androstadiene-3-17dione; ADD) are used as anabolic steroids in livestock **(Cannizzo et al., 2007)**. This drug has been developed for veterinary use: with a low androgenic potency and a very long half-life and trace amounts can easily be detected for months after discontinued use (http://en.wikipedia.org/wiki/Boldenone). BOL increases muscle size due to promotion of positive nitrogen balance by stimulating protein production and reducing protein destruction, moreover it produces a retention of body water, nitrogen, sodium, potassium and calcium ions (**Forbes,1985 & Mooradian et al., 1987**). BOL improves growth and feed conversion in veal calves and therefore might be used illegally to achieve more efficient meat production (**Schilt et al., 1996; Arts et al., 1996; De Brabander et al., 2004 & Vanoosthuyze et al., 1994**). ADD is used by bodybuilders as a product with an even greater anabolic potency than BOL itself (**De Brabander et al., 2004&Geyer et al., 1987**). BOL is used as a growth promoter for beef cattle in the United States (**Sone et al. 2005).** BOL is used also for treatment of debilitation in cats **(Boebel and Ehrenford,1978)**

AAS have potent anabolic activity, increase muscle mass and aggression in animals **(Williams et al., 2000)**. In fillies, the age of first ovulation and the second breeding season was significantly delayed in those treated with the high dose (**Skelton et al., 1991)**. However, female rats treated with nandrolone decanoate showed estral acyclicity and there was destruction of follicular units and an absence of corpus luteum in the ovaries. In the uterus, the drug promoted morphological alterations, characterized by vacuolated epithelium and endometrial stroma fibrosis (**Gerez et al., 2005**) and have inhibitory effects on female hamster reproduction **(Triemstra and Wood (2004).**

Boldenone sulphate has provided direct evidence for the endogenous nature of boldenone in entire male horses (**Ho et al., 2004**). The abuse of boldenone has been reported in human, equine and greyhound dog sports (**Schänzer and Donike, 1992)**. In addition to the growth promoting effects, anabolic steroids have been shown to adversely affect the cardiovascular, hepatic, and endocrine systems **(Yesalis et al., 1993**). AAS administration will disturb the regular endogenous production of testosterone and gonadotrophins that may persist for months after drug withdrawal. Many other adverse effects associated with AAS misuse include disturbance of endocrine and immune function, alterations of sebaceous system and skin, changes of haemostatic system and urogenital tract (**Hartgens and Kuipers 2004).**

Plasma levels of testosterone do not permit detection of illegal treatments because plasma androgens always remained within the physiological range. Illegal treatment could be detected in blood samples when they were collected at least every 20 days (**Simontacchi et al., 2004).**

The objectives of the this study were to determine the effects of boldenone-17-undecylenate administration on body gain performance, whole blood and blood serum protein and lipid metabolites, liver and kidney function, testosterone and thyroid hormones of prepubertal weaned male lambs.

Materials and Methods

Animals: Twelve weaned male lambs belonging to the research farm of Animal Reproduction Research Institute were divided into three equal groups. Animals in full dose group (n=4) received an intramuscular injection of 5 mg boldenone undecylenate while those of half dose group received 2.5 mg of boldenone undecylenate. Animals in control group (n=4) injected olive oil and served as control. Dosages were chosen according to literature (**Rosa Gastaldo et al., 2006**). Five injections were given at 3-week intervals for 15 weeks. All animals kept in the same yard under natural day light and temperature and fed the same nutrition .Water and blocks of salts were fed ad libitum

Blood sampling: Blood samples with and without anticoagulant were collected at each injection every 21 days and at the 7^{th} week after the fifth injection via jugular veinipuncture and serum was harvested then sera were stored at -20C° for clinical chemistry and hormonal assays.

Whole blood analysis: Differential leucocytic count was read by using Lishman's stain and the white blood cells was determined by method described by **Schalm (1986),** Determination of haemoglobin content was performed using method described by **Drabkin(1982).**

Clinical Chemistry: Total protein (g/dL), albumin(g/dL), total cholesterol(mg/dL), high density lipoproteins**(HDL)** and urea (mg/dl) were measured using diagnostic kit according to **Henery,(1968); Drupt,(1974); Watson,(1960)**; **Stein (1986)** and **Fawcett(1960)** respectively. The serum globulin was calculated by subtracting the value of albumin from the value of total protein according to **Doumas and Biggs (1972).** Serum creatinine was determined according to (**Bartles et al., 1972**), AST(GOT), and ALT (GPT) were determined according to **Reitman and Frankle (1957)**

Hormone profiles:

Total T_3 and T_4 assays were performed by radioimmunoassay RIA using Coat-A-Count kits (total $T_4$1 1081 and $T_3$1 501 for T_4 and T_3, respectively ; Diagnostic products Corp. Los Angles, CA) according to **Milter and Albyl, l985; Wrutniak et al., 1985**. Sensitivity of the assay was 0.25µg /dl and 7ng/dl for T_4 and T_3. Mean T_4 and T_3 intra-assay and inter-assay CVs were 3.9, 6.3 and 7.3,14.95 , respectively. Testosterone was assayed using the same RIA kit according to **Tietz (1994).** Sensitivity, intra and inter-assay coefficients of variation were 0.05ng/ml, 10 and 8.4, respectively.

Statistical analysis:

Data were subjected to statistical analysis using Statistical Package for Social Science **(SPSS 16, 2007).** The effect of treatments and injections were studied using split simple one-way ANOVA. The Duncan's multiple range tests was used in separating differences between significant means.

Results and discussion

Body gain: Lambs of both treated groups significantly ($P<0.05$) gained more body weight than control (Table 1). As well as, **Sinnett-Smith et al. (1983)** reported a significant increase in weight gain of lambs implanted with 80 mg of trenbolone acetate (TBA) which they attributed in part to a decrease in muscle protein degradation. Also, **Henricks et al. (1982)** reported an increase in growth rate of heifers administered 300 mg of TBA as an ear implant for 62 d. In ewe lambs, **DeHaan et al. (1987)** reported an increase in average daily gain (ADG) for ewes treated prenatally with testosterone propionate and rams treated prenatally with testosterone propionate grew at similar rates compared with controls rams but at a higher rate than prenatally treated ewes. In contrast, **Cannizzo et al., (2007**) detected no statistically relevant difference between different groups of veal calves treated with boldenone. Similarly, neither dosage of anabolic steroid nor duration of treatment had a significant effect on weight gain when compared to controls **(Howe and Morello, 1985)**. Moreover, average daily gain was not affected by trenbolone acetate (TBA) in rams or ewes (**Lough et al., 1993)**. Rams implanted with TBA gain BW faster than the control rams during the first 56 d **(Sillence et al. 1987**).

Total Proteins, albumin, globulin and urea: In this study, levels of total proteins in both treated groups were significantly higher when compared to those of controls (table 2) within all injections. Levels of total proteins significantly returned to pretreatment levels after last injection in only

full dose group but still high in half dose group till the end of the experiment. Albumin level was significantly low in both treated groups (table 2) at second injection but was high at fifth injection in both treated groups compared to control. In contrast to the present findings, pretreatment and post-treatment measurements of plasma albumin concentration did not indicate any beneficial effect of 0.5 mg/kg, 1.0 mg/kg, and 1.5 mg/kg of an anabolic steroid **(Finco et al., 1984).**

Globulin levels were high in both treated groups compared to control lambs (table 2) within all treatments. Within both treated groups, globulin levels were significantly higher than pretreatment levels even after last injection in only half dose group (Table 2). Similarly, **Jockenhovel et al., (1999)** found that serum total globulins were significantly increased in all treatments whereas the parenteral treatment modes showed a lower increase of total globulins. Urea levels decreased significantly in full dose group after first injection and half dose group after second injection of male lambs compared to control lambs indicating the decrease in protein breakdown (Table 2).In agreement with our results, estradiol 17β increased nitrogen retention and decreased blood urea nitrogen concentrations (**Cecava and Hancock,1974&Istasse et al.,1988)**.Similar to the presented results, animals in the treatment groups converted food to live-weight gain more efficiently faster and had lower levels of blood urea and to a lesser extent serum albumin than untreated controls (**Galbraith and Watson, 1978)**. It was clear from the present results that the increase in body gain was attributed to the increase in serum total proteins and globulin which indicated improvement in lambs health and immunity and a decrease in protein breakdown. Since anabolism is defined as any 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)**. From the other point of view, plasma concentrations of amino acids and serum urea were similar in both conditions **(Christiansen et al.,2005)**.

Total cholesterol and HDL: Total cholesterol levels showed only significant difference between groups of animals during second injection (table 2). In control lambs, cholesterol levels were lower than those of treated groups during all injections except at the fifth injection. As well as, a significant increase of total cholesterol was observed in all treatment groups **(Jockenhovel et al., 1999)**. Plasma cholesterol concentrations in Suffolk males implanted with trenbolone acetate and oestradiol-17β exhibit a biphasic response to implantation and the magnitude of the response was directly related to the dose level of the ear implant (**Scaife et al., 1982)**. On the other hand, **Berg et al., (2002**) stated that total cholesterol did not increase. The high density lipoproteins were declined significantly after first injection of boldenone in only the half dose group (Table 2) but its levels increased more than those of first injection in full dose group at the second injection and in control group at the third one. In agreement with the present results, plasma concentrations of total cholesterol and high-density lipoprotein cholesterol, were not affected by trenbolone acetate (TBA) in either rams or ewes **(Lough et al., 1993)**, for rams, but high-density lipoprotein cholesterol showed a significant decrease **(Jockenhovel et al., 1999)** .In addition, **Hartgens and Kuipers (2004)** recorded a depression of serum high-density lipoprotein -cholesterol levels and significant time effects were noted for HDL cholesterol. Storage of frozen plasma might affect concentrations of plasma lipid metabolites, especially HDL cholesterol **(Bachorik et al., 1980).** Total and LDL-cholesterol were similar, HDL-cholesterol was distinctly lower in athletes were still abuse AAS (**Urhausen et al., 2003**).

Creatinine, Aspartate aminotransferase(AST) and Alanine aminotransferase(ALT).

Creatinine were increased significantly in both treated groups of lambs at the third and fourth injections compared to other injections but declined again at the fifth and after last injections to levels still higher than those of first and second injections. Although plasma creatinine concentrations after androgen administration were significantly higher than those before androgen administration but changes were not observed in plasma urea values (**van Miert et al., 1988**). ALT levels (table 2) decreased significantly in all groups after first injection but a significant increase was observed in only full dose group at the third injection. AST levels were low at the second injection in both treated groups compared to control but were higher in both treated groups at the fifth one than control lambs (table 3). AST levels were higher in control lambs at the 2nd and 4th injections compared to both treated groups. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were higher (**Urhausen et al., 2003**) in athletes abusing AAS.

Testosterone (T): In the present experiment (Table 3), testosterone concentrations were insignificantly higher than 1ng/ml in both treated groups increase after first injection but control lambs reached this level at the time of the fifth injection indicating the delayed puberty in control lambs. In agreement with our findings, serum testosterone levels in treated groups with AAS were significantly higher than that in control group **(Urhausen et al., 2003**;**Takahashi et al., 2004&Gabr and Shaker, 2006**). In contrast, the administration of T alone did not induce any variation in plasma testosterone **(Simontacchi, et al.,**

2004). Also, **Shimomura et al., (2005)** showed that the treatment of rats with ethinylestradiol alone significantly decreased testosterone levels in serum and the testis. Men treated with testosterone enanthate intramuscularly every 21 days had normal testosterone **(Jockenhovel et al., 1999)**. **Demisch and Nickelsen (1983)** referred the high testosterone levels to the disturbance in the distribution of the androgen between the plasma proteins.

Triiodothrnine(T_3)and Tetraiodothyronine (T_4): Levels of T_3 were higher at the fifth injection (Table 3) in the treated groups of lambs compared to pretreatment levels but during other injection T_3 levels were lower than at first pretreatment injection. A significant increase in T_4 was observed in control lambs (Table 3) at the third injection compared to both treated groups but a non significant increase was also observed at the last and after last injections. There were no significant treatment effects on mean basal plasma concentrations of thyroxine (T_4) or triiodothyronine (T_3) among horses treated with boldenone undecylenate, given twice weekly for 12 days but resulted in a significant time effect on overall mean basal plasma T_4 and T_3 concentrations **(Morris and Garcia, 1985).** In rams and ewes subcutaneously implanted with TBA, **Kahl et al. (1992)** found a decrease in plasma thyroxine and hepatic 5'-deiodinase activity. The enzyme, 5'-deiodinase, converts thyroxine to 3,5,3'-triiodothyronine (T_3), which was the metabolically active thyroid hormone. **Donaldson et al. (1981)** also noted a decrease in plasma thyroxine of growing wethers implanted with 140 mg of TBA. This might suggest a decrease in lipid metabolism and (or)turnover of lipid in the lambs implanted with TBA. At physiological concentrations, T3 was involved with lipogenesis in the liver (**Blennemann et al., 1992**).

Blood haemogram: The haemoglobin concentration in blood of both treated groups was decreased after the second injection (Table 4) while its concentration decreased in control lambs after the third one. The neutrophils count was higher in full dose lambs at the third injection and was high in half dose group in the second injection compared to other injections. Conversely, lymphocyte count was lower in full dose lambs at the third injection and was low in half dose group in the second injection compared to other injections. In contrast to the presented data of lambs, hemoglobin,leucocytes and platelets were significantly higher in athletes were still abusing AAS **(Urhausen et al., 2003**). Large doses of androgens have been employed in the treatment of refractory anemias and have resulted in some increase in reticulcytosis and haemoglobin levels **(Katzung, 1989)**.Anabolic steroids could also stimulate erythropoiesis the mechanism for this effect may occur by stimulating erythropoietic stimulating factor **(Donald, 1989**).

Conclusion

The use of anabolic androgenic steroids in animals could be recommended in breeding animals to enhance puberty and increase body gain in animals of low body gain and during nutritional stress but its use in fattening animals must be done under control and the recommended withdrawal must not be less than three months before slaughter to avoid its side effect on humans consumers. There is a necessity for further research to distinguish between naturally occurring and illegally used boldenone forms.

Table (1): Effect of different doses of BOL on body weight of weaned male lambs (Mean ±SE)

Treatment	Full dose**	Half dose*	control
Initial body weight/kg	27.25±1.18 [a]	25.50±2.25a	26.25±1.75
At second injection	26.75±1.38a	24.5±2.06a	25.75±1.49
At third injection	26.75±1.38a	25.0±2.04a	26.25±1.65
2 weeks post last injection	32.25±1.32b	30.00±2.27ab	28.25±1.97
4 weeks post last injection	35.0±1.78bc	32.50±2.10b	30.75±2.09
7 weeks post last injection (Final)	37.75±2.25c	35.50±2.10 b	33.25±1.44
Final gain/kg*	10.5±1.19[y]	10.5±0.50[y]	7.0±0.71[x]
Final body gain%(Final/InitialX100)	38.5 [y]	41.2[y]	26.7 [x]

Means with different superscripts a, b, c within column and x, y, z within row are significantly different at p<0.05,*P<0.001, ** P<0.0001

Table (2): Effect of different injections and doses of BOl on total proteins, albumin, globulin, urea total cholesterol, HDL, creatinine, ALT and AST(Mean ±SE)

Traits	Treatments	Injections					
		At first	At second	At third	At forth	At fifth	Post last
Total protein*** (g/dL)	Full **	6.74±0.26^{*abcy}	7.62±0.52^{**abcy}	7.78±0.44^{cdy}	7.60±0.21^{**bcdy}	7.29±0.41^{*abcy}	6.60±0.26^{ab}
	Half *	6.08 ± 0.46^{axy}	7.823±0.32^{by}	7.16±0.52^{abcxy}	7.05±0.37^{abcy}	7.71±0.42^{by}	7.02±0.18^{ab}
	control	5.29±0.16^{x}	5.94±0.10^{abx}	6.29±0.23^{bx}	5.71±0.32^{ax}	5.89±0.27^{x}	6.18±0.19^{b}
Albumin (g/dL)	Full	2.47±0.14	2.38±0.17^{*x}	2.61±0.47	2.41±0.37	2.41±0.37^{*xy}	2.57±0.11
	Half	2.42 ±0.11	2.27±0.07^{x}	2.45±0.42	2.42±0.22	2.86±0.29^{y}	2.72±0.13
	Control*	2.14±0.13^{b}	2.54±0.13^{by}	2.50±0.27^{b}	2.37±0.19^{b}	1.50±0.19^{x}	2.48±0.14^{b}
Globulin*** (g/dL)	Full **	4.27±0.31^{aby}	5.22±0.48^{**aby}	5.17±0 .37^{bc}	5.26± 0.14^{**bcy}	4.88± 0.71^{abc}	4.04±0.34^{ab}
	Half	3.66±0.47^{axy}	5.55±0. 30^{by}	4.70±0.74^{ab}	4.63±0.38^{aby}	4.85±0.69^{ab}	4.30±0.25^{ab}
	control	3.14±0.13^{x}	3.28±0.23^{x}	3.79±0.30	3.34±0.29^{x}	4.39±0.27	3.07±0.23
Albumin/ Globulin	Full	0.59± 0.07	0.48±0.09^{**x}	0.52±0.11^{x}	0.45 ±0.10^{*x}	0.56 ±0.15	0.86±0.31
	Half	0.70 ±0.11	0.42 ±0.06^{x}	0.59±0.17^{xy}	0.53±0.19^{xy}	068±0. 21	0.49±0.13
	control	0.67±0.12	0.76±0.27^{y}	0.68±0.11^{y}	0.73±0.17^{y}	035±0.10	0.65±0.22
Urea** (mg/dL)	Full ***	37.59 ± 1.63^{b}	32.71±1.24^{b*}	20.66±2.25^{*ax}	23.62±1.32^{**ay}	22.05±0.77^{*ax}	25.99±1.24^{*ax}
	Half ***	34.94±1.62^{c}	37.79±1.31^{c}	22.31±3.77^{abx}	17.68±1.46^{ax}	23.56±1.33^{bxy}	24. 78±0.82^{bx}
	Control*	36.06±1.6^{b}	35.23±2.48^{b}	31.50±2.07^{y}	30.99±2.11^{z}	26.96±1.3^{y}	30.94±1.2^{y}
Total ** cholesterol (mg/dL)	Full **	39.96±5.32^{ab}	55.68±3.62**^{by}	55.13±6.72^{b}	40.06±3.74^{ab}	29.67±2.89^{a}	45.14±4.15^{ab}
	Half	38.08±6.13^{ab}	40.96±3.24^{abx}	44.11±6.34^{ab}	32.09±2.95^{a}	30.67±2.69^{a}	44.61±2.62^{ab}
	control	35.22±4.92	37.02±2.96 ^{x}	40.99±2.81	29.74±2.79	29.86±3.42	41.56±1.3
HDL (mg/dL)	Full **	19.96±3.26^{ab}	23.39±2.43^{bxy}	15.47±1.09^{a}	17.85±2.39^{ab}	16.06±0.99^{a}	14.76±0.47^{a}
	Half **	23.18±1.89^{c}	21.01±1.10^{bcx}	19.98±2.78^{bc}	13.76±1.28^{a}	15.00±0.37^{ab}	15.91±1.07^{ab}
	control	22.46±2.55	22.29±1.66^{y}	23.25±3.77	18.34±1.82	16.19±2.22	14.96±0.4
Creatinine (mg/dL)	Full **	0.67±0.12^{a}	0.70±0.09^{a}	1.14±0.15^{b}	1.22±0.07^{b}	0.73±0.11^{**ay}	0.94±0.06^{*abx}
	Half ***	0.51±0.14^{a}	0.82±0. 80^{ab}	1.16±0.15^{b}	1.13±0.11^{b}	0.85±0.02^{abx}	0.92±0.10^{aby}
	control	0.60±0.09	0.75±0.08	1. 38±0.08	0. 79±0.21	0.88±0. 08^{y}	0.99±0.06^{x}
ALT(GPT) Units/ml	Full ***	51.50±4.79^{b}	37.13±6.39^{a}	59.50±2.36^{***by}	23.75±2.25^{a}	24.75±2.84^{a}	25.19±1.67^{a}
	Half *	47.25±3.33^{b}	33.75±3.29^{ab}	31.00±4.89^{ax}	20.75±0.75^{a}	32.25±1.03^{ab}	29.94±2.91^{a}
	control	41.00±4.55	35.25±3. 21	26.00±3.00^{x}	21.50±0.87	28.75±4.40	27.75±2.82
AST(GOT) Units/ml	Full *	76.75±3.66^{a}	76.13±3.64^{**acx}	80.25±3.82^{a}	62.50±3.75^{**ax}	138±24.47^{**by}	88.31±1.012^{a}
	Half **	62.75±6.75^{a}	64.13±2.38^{ax}	86.75±9.04^{ab}	59.25±3.25^{ax}	131±11.45^{cy}	109.3±14.3^{bc}
	control	66.75±6.60	92.38±8.29^{y}	91.50±17.3	97.3±1.01^{y}	55.0±5.93^{x}	84.94±6.63

Mean with different superscripts a, b, c within row and x, y, z within column are significantly different at P <0.05, *,**,** in the treatments indicate significance within groups and in other cells indicate significance between treatments. * P < 0.05,** P<0.001, *** P<0.0001

Table (3): Effect of different doses and injections of BOL on testosterone(T), T_3 and T_4 hrmones

Traits	groups	At first	At second	At third	At forth	At fifth	Post last
Testo-sterone ng/ml	Full	0.59±0.43	1.79±0.62	1.79±0.42	1.34±0.23	2.38±0.64	6.07±2.76
	Half	0.49±0.37	1.06±0.19	1.27±0.32	1.35±0.41	4.47±2.42	2.74±0.49
	control	0.55±0.25	0.60±0.09	0.73±0.21	0.45±0.13	2.40±1.56	1.61±0.49
T_3 ng/dl	Full***	74.31±4.26^{bc}	58.86±6.57^{ab}	45.79±5.79^{a}	49.73±4.35^{a}	81.36±6.70^{c}	74.97±2.87^{bcy}
	Half***	79.45±4.29^{c}	60.17±4.26^{ab}	52.28±5.18^{a}	61.90±4.63^{ab}	90.62±5.95^{c}	76.62±4.12^{bcxy}
	control	77.23±3.56^{c}	60.94±2.29^{ab}	46.75±4.39^{a}	56.39±2.27^{ab}	85.31±7.66^{d}	60.83±3.21^{bx}
T_4 µg/dl	Full***	6.10±0.28^{c}	3.66±0.24^{b}	1.90±0.31^{**ax}	3.09±0.30^{**b}	3.17±0.35^{b}	2.72±0.23^{ab}
	Half***	5.17±0.44^{d}	4.05±0.32^{c}	1.65±0.15^{ax}	2.37±0.30^{ab}	2.95±0.37^{b}	2.64±0.20^{ab}
	control	5.45±0.28^{c}	3.36±0.12^{ab}	3.61±0.46^{aby}	3.02±0.24^{ab}	3.59±0.21^{ab}	2.92±0.32^{a}

Mean with different superscripts a,b,c,d within row , x,y,z within column are significantly different at P < 0.05, *P<0.05**P<0.001 , *** P<0.0001

Table (4) Effect of different doses and injections of BOL on White blood cell count, neutrophil , lymphocyte count and ahemoglobin g %

Traits	groups	At first	At second	At third	At forth	At fifth	Post last
Haemo-Globin g%	Full **	13.6±0.53^{b}	13.7±0.4^{b}	12.8±0.5^{b}	11.7±0.3^{ab}	9.9±0.7^{a}	11.6±0.7^{ab}
	Half **	13.4±0.29^{c}	13.3±0.4^{bc}	12.6±0.8^{abc}	11.5±0.5^{ab}	11.1±0.3^{a}	11.9±0.4^{abc}
	Control***	13.5±0.28	13.5±0.3	13.2±0.6	9.9±0.2	9.1±0.2	11.8±0.4
White blood cells/10^3	Full	12.8±1.49	12.2±2.1	11.4±1.2	10.7±.5	12.18±0.57	12.6±0.6
	Half	13.07±1.83	13.9±1.0	13.2±1.8	13.9±2.5	12.75±0.55	11.6±0.7
	control	12.95±1.09	13.1±1.2	12.7±1.5	10.7±1.0	11.32±0.63	11.1±0.9
Neutrophils %	Full*	32.00±2.55^{ab}	28.1±3.9^{a}	43.3±3.1^{b}	32.5±3.4^{ab}	34.7±5.2ab	39.0±1.9^{ab}
	Half **	25.25±1.55^{a}	42.1±3.6^{b}	28.3±1.9^{a}	31.5±3.1^{ab}	38.0±1.0^{ab}	27.2±4.4^{a}
	Control**	28.62±1.89	35.1±3.2	36.5±3.4	22.0±2.0	36.0±3.0	41.3±3.4
Lympho-Cytes %	Full **	64.25±2.25^{b}	64.3±1.6^{b}	52.5±4.5^{a}	64.8±3.2^{b}	62.7±5.8^{b}	55.8±2.7^{ab}
	Half **	67.25±1.65^{ab}	53.3±3.8^{a}	66.5±2.9^{ab}	62.5±3.3^{ab}	59.0±1.0^{ab}	68.8±4.7^{b}
	Control**	65.75±1.41	58.8±2.4	60.3±3.3	74.5±4.5	61.2±3.3	55.1±3.5
	Control**	28.62±1.89	35.1±3.2	36.5±3.4	22.0±2.0	36.0±3.0	41.3±3.4
Lympho-Cytes %	Full **	64.25±2.25^{b}	64.3±1.6^{b}	52.5±4.5^{a}	64.8±3.2^{b}	62.7±5.8^{b}	55.8±2.7^{ab}
	Half **	67.25±1.65^{ab}	53.3±3.8^{a}	66.5±2.9^{ab}	62.5±3.3^{ab}	59.0±1.0^{ab}	68.8±4.7^{b}
	Control**	65.75±1.41	58.8±2.4	60.3±3.3	74.5±4.5	61.2±3.3	55.1±3.5

Mean with different superscripts a,b,c,d within row , x,y,z within column are significantly different at P < 0.05, *P<0.05**P<0.001 , *** P<0.0001

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