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BIOCHEMICAL AND PHYSIOLOGICAL INFLUENCES OF BOLDENONE UNDECYLENATE AS A GROWTH PROMOTOR ON DIFFERENT AGES OF MALE WHITE NEW-ZEALAND RABBITS.

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ABSTRACT

The objectives of the current study were to investigate the effects of high dose administration of boldenone undecylenate as a growth promoter on some biochemical and hematological parameters in both immature and mature male rabbits. Forty male New Zealand rabbits (20 immature and 20 mature) were randomly assigned into four equal groups. Group 1: control, injected with sesame oil. Group 2: injected with 5 mg Equi-gan®/Kg. b. wt, Group 3: injected with 10 mg Equi-gan® /Kg. b. wt., Group 4: injected with 15 mg Equi-gan® /Kg. b. wt. All animals were intramuscularly injected. After one and two months of administration, animals were weighed to obtain body gain of each group then two blood samples were collected from each animal; the first sample was collected on EDTA and the other was collected on a sterile tube for serum separation. The obtained results indicated that, intramuscular injection of boldenone resulted in a significant increase in body weight gain in both mature and immature rabbits after one and two months of treatment. This was accompanied with significant increase in growth hormone in immature and testosterone in mature rabbits. Boldenone significantly improved RBCs, Hb and PCV, significantly decreased MCH and MCH while MCV showed non-significant changes. Significant increases in NO and MDA concentrations and SOD activity were observed after one and two month of treatment while GSH concentration significantly decreased in both immature and mature rabbits after two months of treatment. Boldenone administration significantly increased interleukin-2, interleukin-6 and creatinine but did not affect sodium or potassium concentrations. It could be concluded that, although intramuscular injection of boldenone undecyclenate to male New Zealand rabbits enhances body gain and increased some hematological parameters, it increased oxidative stresses biomarkers levels, pro-inflammatory cytokines and creatinine indicating side effects on the liver and kidneys.

Keywords: Boldenone undecylenate, hematology, antioxidants, interleukin-2, interleukin-6, creatinine, rabbits.

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1. INTRODUCTION

Boldenone undecylenate is one of the anabolic steroid hormones (synthetic androgenic steroid) that derived from testosterone (Cannizzo et al., 2007 and Tousson et al., 2012 and 2013). Boldenone has a very long half-life up to 1.5 years due to the long undecylenate ester attached to the parent steroid so; trace amounts of this drug can be easily detected for several months after discontinued use (Hoffmann, 2002 and Brookhouse, 2007). Moreover, boldenone undecylenate is applied as a growth promoter in meat producing farms in order to increase growth, productivity and feed conversion, to achieve more efficient meat production and to reduce breeding expense (De Brabander et al., 2004). Furthermore, it is used to improve athletic, body builders and racing horse's performance (Ho et al., 2004) as it is well known for increasing vascularity in preparation for body building contests (Mohammed et al., 2016).

There is a growing disquiet that, these synthetic hormones used in veterinary treatments are making their ways into surface water and even ground water via human and animal wastes (Soto et al., 2004). They exhibit relatively more stability in aqueous media and more resistance to microbial degradation leading to accumulation and persistence in the environment and endanger consumers with a permanent exposure beside the danger of consuming the meat of animals treated with them (Schiffer et al., 2001).

According to the International Agency for Research on Cancer (IARC), Boldenone undecylenate is classified in class 2A (growth promoter steroids) as a probable human carcinogen with a high carcinogenic index (IARC Monograph, 1987). In light of this obvious human health risks, the European Community banned the use of steroid hormones as growth-promoting agents in livestock breeding (European Community, 1996). Hence, the presence of boldenone or its metabolites in biological samples is proposed to be a marker for illegal hormone administration in various animal species especially in fattened cattle (De Brabander et al., 2004). However, the illegal use of boldenone undecylenate as a growth promoter to increase body mass and enhance physical conditioning is becoming widespread (Evans, 2004). In Egypt, boldenone has been used heavily in the field of animal production. It is represented under the trade name Equi-gan®.

In the recent years, domestic rabbits have been identified as an economy livestock for solving the meat shortage problem in high human population developing countries such as Egypt. Rabbit meat has a very good nutritive value, high in protein, low in fat, calories and sodium, and so could bridge the wide gap in dietary protein intake (Adeyinka, et al., 2007). Unfortunately, the rabbit producing farms begin to use boldenone undecylenate in high doses as a growth promoter. Therefore, the objectives of the present study were to investigate the effects of high dose administration of boldenone undecylenate as a growth promoter on some biochemical and hematological parameters in both immature and mature male rabbits.

2. MATERIALS AND METHODS

The experiment was conducted at the experimental house of the Faculty of Veterinary Medicine, Benha University, Egypt.

2.1. Growth promoter:

The growth promoter used in this experiment was Equi-gan®, each 1 ml contains 50 mg boldenone undecylenate in 1ml sesame oil (Equi-gan®; Lab Tornel, Co., Mexico)

2.2. Experimental animals:

Forty male white New-Zealand rabbits (20 immature of 6-8 weeks old and of average body weight 0.600 - 0.750 kg and 20 mature of 12-14 weeks old and of average body weight 2.250 - 2.500 kg) were used in the experimental investigation of this study. Rabbits were housed in individual metal cages. Fresh and clean drinking water was supplied *ad- libitum.* The experimental diet was commercial pelleted rabbit ration, containing 18% crude protein and 2700 cal/Kg ration metabolizable energy (ME).

2.3. Experimental design:

Both mature and immature rabbits were randomly assigned into four equal groups (five animals per group). Group 1: control, injected with 0.25 ml Sesame oil/Kg. b. wt.; Group 2: injected with boldenone undecylenate at a dose of 5 mg/Kg. b. wt.; Group 3: injected with boldenone undecylenate at a dose of 10 mg/Kg. b. wt.; Group 4: injected with boldenone undecylenate at a dose of 15 mg/Kg. b. wt. All

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animals were intramuscularly injected (Gabr et al., 2009). After one and two months, animals were weighed to obtain body gain of each group.

2.4. Blood sampling:

Blood samples were collected after an overnight fasting by vein puncture of the marginal ear vein. Blood samples were collected from all animals of each group after one and two months from the beginning of administration of boldenone undecylenate. Two blood samples were collected from each animal; the first sample was collected on EDTA for determination of nitric oxide (NO), reduced glutathione (GSH), malondiadehyde (MDA) concentrations, super oxide dismutase activity (SOD) and hematological examination (Hb, PCV, RBCs, MCV, MCHC, and MCH). The second blood sample was collected in a clean sterile tube for serum separation. Serum was separated by centrifugation at 3500 r.p.m for 20 minutes after blood clotting for determination of interleukin-2, interleukin-6, creatinine, sodium (Na^+), potassium (K^+), testosterone and growth hormone,

2.5. Biochemical and hematological analysis:

Nitric oxide (NO), super oxide dismutase activity (SOD), reduced glutathione (GSH) and malondi adehyde (MDA), sodium (Na^+), potassium (K^+), creatinine, testosterone, growth hormone, interleukin -2 and interleukin- 6 were determined using the methods described by Montgomery and Dymock (1961); Nishikimi et al., (1972); Beutler (1963); Ohkawa et al., (1979); Maruna (1958); Terri and Sesin (1958); Henry (1974); Tietz (1995); Henry (1996) ; Gearing and Thorpe (1988) and Chan and Perlstein (1987), respectively.

Whereas, RBCs counts were estimated by hemocytometer neubauer slide method according to Harvey (2001); hemoglobin was measured calorimetrically according to Haemat (1967) and MCV, MCHC, and MCH were calculated according to Dacie and lewis (2001).

2.6. Statistical analysis:

The obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 16.0 software, 2009), for obtaining mean and standard error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

3. RESULTS AND DISCUSSION

Data represented in table 1 and 3 showed that, intramuscular injection of boldenone resulted in a significant increase in body weight gain (in a dose dependent manner) in both mature and immature rabbits after one and two months of treatment. These results are consistent with previous reports obtained by Thabet et al. (2010) who recorded that, the growth performance improved in boldenone treated groups relative to the control group and Tousson et al. (2012) who stated that the use of boldenone resulted in obvious improvement in the growth rate and also the results of Mohammed et al. (2016) who reported that, boldenone injection in male rabbits resulted in an increase in total final BW gain. However, Tawfeek et al. (1994) reported that the growth performance of rabbits was not affected by testosterone injection.

The obtained increase in body weight gain may be attributed to that, anabolic steroids increase muscle size by the promotion of positive nitrogen balance by stimulating protein production and decreasing destruction (Guan et al., 2010 and El-Moghazy *et al*., 2012). Also, the increased body weight gain may be ascribed to the increment in serum total proteins and globulin, which indicated improvement in wellness and immunity (Tousson *et al*, 2013).

The mechanism of action of boldenone was explained by Fahey (1998) who reported that; boldenone is a steroid hormone works by stimulation of receptor molecules in muscle cells, which activate specific genes to produce proteins. They also affect the activation rate of enzyme system involved in protein mechanism. Thus enhancing protein synthesis and inhibiting protein degradation.

However, the effect of boldenone on weight gain 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

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reduction of glucocorticoid receptor levels and sensitivity to endogenous glucocorticoids (Melloni et al. 1997 and Thienpont et al. 1998). Moreover, sex hormones may increase the cellular protein biosynthesis indirectly by stimulation of growth hormone and insulin like growth factor secretion, or equally as a result of skeletal muscle hypertrophy (Weissberger and Ho, 1993; Bondanelli et al., 2003; Veldhuis et al., 2005 and Johnson *et al*, 2013). That may explain the results obtained in the present study which revealed that, significant increases in serum growth hormone levels were observed in immature rabbits treated with boldenone undecylenate as compared with the control group (tables1 and 3).

The significant increase in testosterone observed in (G3 and G4) of mature rabbits treated by boldenone may be in accordance with the findings of Urhausen et al., (2003), Takahashi et al. (2004) and Gabr and Shaker (2006) who founded that, serum testosterone levels in treated groups with androgenic steroids were significantly higher than that in control group. 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 the hematological studies presented in tables1and 3 revealed significant increases in RBCs, Hb and PCV in boldenone administered groups as compared to the control groups after one and two months of treatment. As a consequence of the elevated PCV % and relatively stable Hb concentrations and RBCs counts, the MCH and MCHC values significantly decreased during the drug administration while MCV showed non-significant changes as compared to the control groups.

The obtained findings agree with the results of Battista et al., (2003) and Liewellyn (2006) who founded that, testosterone dosage and its entrance to the body caused an increase in hematocrit %. Moreover, Gagnon, et al. (1994) founded that, the raised hematocrit and hemoglobin persist for extended periods after the cessation of androgenic steroids use. Similarly, Ahmed (2014) recorded significant increase RBCs, Hb and PCV in boldenone administered groups as compared to the control one. These results may be attributed to that; anabolic steroids could stimulate erythropoiesis (Gabr et al., 2009) through the direct positive effect of androgenic steroids on erythropoietin production in renal tissues (Liewellyn, 2006). This reaction is mainly driven by the androgen receptor stimulation in renal tissue, leading to the stimulation of erythropoietin production directly. Androgens may also affect the stem cells directly, perhaps by enhancing the stem cell's responsiveness to erythropoietin (Snyder, 2008).

Regarding antioxidants, the obtained results demonstrated in tables 2 and 4 revealed that, significant increases in NO, MDA and GSH concentrations and SOD activity were observed in boldenone treated immature and mature rabbits after a month of treatment as compared to the control group. However after two months of treatment, NO and MDA concentrations and SOD activity significantly increased and GSH concentration significantly decreased in immature and mature treated rabbits in comparison with control groups. These changes in total antioxidant capacity and the oxidative stress parameters increased with increasing the dose of boldenone. Our results are in agreement with the results of Pey et al. (2003) who reported that, the anabolic androgenic steroids induced changes in oxidative stress and Ahmed (2014) who found that, boldenone administration can induce an oxidative stress in the liver and kidney as indicated by elevation of serum MAD level. Furthermore, administration of the anabolic steroid boldenone induced changes in oxidative stress bio-marker levels and antioxidant defense systems in the liver and kidney (El Moghazy et al., 2012).

The results demonstrated in tables 2 and 4 revealed that, boldenone administration resulted in significant increase in interleukin-2 and interleukin-6 in both mature and immature rabbits after one and two months of treatment as compared with the control groups. Our results are in agreement with (Hughes et al., 1995 and Sullivan et al., 1998) who recorded many adverse effects associated with anabolic androgenic steroids such as the disturbance of the endocrine and immune functions.

The results of the present study showed in tables 2 and 4 revealed that, significantly higher concentrations of creatinine were observed in immature and mature rabbits treated by boldenone after one and two months of treatment as compared with the control groups. These results are in agreement with Anderson et al., (1997) who reported that, androgenic steroids are responsible for increase in muscle bulk and consequently rise in creatinine level and Taher *et al*. (2008) reported significantly higher serum creatinine concentrations in androgenic steroid user athletes. Also, similar results are obtained by Ahmed (2014) who reported that boldenone undecylenate injection caused elevation in serum creatinine level in New Zealand rabbits.

The obtained results demonstrated in tables 2 and 4 revealed that, there were no significant differences of serum sodium and potassium concentrations observed between groups in both mature and immature rabbits due to boldenone administration at any experimental period. These results came in accordance with the data recorded by Meiggiola et al. (1996) who stated that, administration of testosterone in combination with cyproterone acetate in men produced no change in sodium, potassium and phosphate concentrations. Also, Anderson et al. (1996) reported that, there were no significant changes or apparent trends in serum electrolytes levels in

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heterogeneous group of men supplemented with testosterone. On the other hand, these findings disagree with the results obtained by Hussein et al., (1999) who reported that testosterone causes moderate sodium, potassium, water, sulfate and phosphate retention.

4. CONCLUSION

From the obtained results it could be concluded that, although intramuscular injection of boldenone undecyclenate to male New Zealand rabbits enhances body gain and increased some hematological parameters, it increased oxidative stresses biomarkers levels, pro-inflammatory cytokines and creatinine indicating side effects on the liver and kidneys.

Table 1: Effect of boldenone undecylenate on some hormonal and hematological parameters in both immature and mature male rabbits after a month of administration (mean ± SE):

Parameter	Age	G1	G2	G3	G4
Body gain (kg)	Immature	0.737 ± 0.012^{d}	0.827 ± 0.013^{c}	1.170 ± 0.034^{b}	1.602 ± 0.02^{a}
	Mature	0.666 ± 0.009^{d}	0.785 ± 0.031^{c}	1.090 ± 0.047^{b}	1.447 ± 0.045^{a}
Growth hormone (µl U /ml)	Immature	3.26 ± 0.07^{b}	6.83 ± 0.59^{a}	6.87 ± 0.74^{a}	6.84 ± 1.11^{a}
Testosterone (ng/ml)	Mature	1.85 ± 0.05^{b}	2.48 ± 0.26^{b}	3.54 ± 0.35^{a}	3.77 ± 0.41^{a}
Hb (g/dl)	Immature	19.00 ± 0.11^{c}	23.27 ± 0.18^{b}	21.55 ± 0.17^{b}	22.05 ± 0.21^{a}
	Mature	25.42 ± 0.19^{c}	28.00 ± 0.23^{b}	27.50 ± 0.13^{a}	26.35 ± 0.21^{a}
PCV (%)	Immature	30.65 ± 0.05^{d}	40.08 ± 0.03^{c}	35.12 ± 0.04^{b}	37.59 ± 0.02^{a}
	Mature	36.82 ± 0.08^{d}	42.53 ± 0.21^{c}	41.86 ± 0.02^{b}	40.86 ± 0.06^{a}
RBCs (10^{12}/L)	Immature	4.54 ± 0.04^{d}	5.36 ± 0.08^{c}	5.12 ± 0.03^{b}	5.05 ± 0.02^{a}
	Mature	5.14 ± 0.02^{d}	6.90 ± 0.02^{c}	6.75 ± 0.07^{b}	5.76 ± 0.18^{a}
MCV(fl)	Immature	73.00 ± 0.40^{b}	74.75 ± 0.47^{ab}	74.25 ± 0.47^{ab}	$\pm73.25\pm0.47^{a}$
	Mature	71.50 ± 0.28^{b}	73.25 ± 0.25^{ab}	72.25 ± 0.47^{ab}	72.00 ± 0.57^{a}
MCHC (%)	Immature	63.75 ± 0.06^{a}	62.09 ± 0.05^{b}	60.68 ± 0.04^{c}	55.60 ± 0.20^{d}
	Mature	63.24 ± 0.15^{a}	62.38 ± 0.14^{b}	61.42 ± 0.03^{c}	59.77 ± 0.05^{d}
MCH(pg)	Immature	42.77 ± 0.03^{a}	40.14 ± 0.05^{b}	39.65 ± 0.04^{c}	39.03 ± 0.02^{d}
	Mature	42.60 ± 0.06^{a}	40.79 ± 0.03^{b}	$39.620.04^{c}$	38.82 ± 0.04^{d}

Data are represented as $\bar{X}\pm$ SE $\bar{X}$: Mean values SE: Standard Error

Mean values with different superscript letters in the same column are significantly different at $P<0.05$.

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Table 2: Effect of boldenone undecylenate on some biochemical parameters in both immature and mature male rabbits after a month of administration (mean ± SE):

Parameter	Age	G1	G2	G3	G4
NO (µmol/l)	Immature	24.25±1.37^{c}	36.25±2.21^{b}	46.75±4.59^{b}	70.75±6.51^{a}
	Mature	24.00±1.29^{d}	37.00±2.19^{c}	44.75±2.83^{b}	62.75±2.49^{a}
SOD activity (u/g Protein)	Immature	5.16±0.75^{c}	8.86±0.53^{b}	10.04±0.53^{ab}	11.56±0.65^{a}
	Mature	4.96±0.73^{c}	8.33±0.46^{b}	10.33±0.12^{a}	11.19±0.45^{a}
GSH (mmol/g protein)	Immature	3.89±0.32^{c}	5.15±0.71^{b}	7.69±0.40^{b}	11.57±0.52^{a}
	Mature	3.95±0.34^{c}	6.73±0.68^{b}	7.80±0.31^{b}	9.81±0.53^{a}
MDA (nmol/ml)	Immature	5.24±0.54^{d}	9.59±0.85^{c}	19.06±1.24^{b}	27.27±1.62^{a}
	Mature	5.03±0.28^{d}	8.82±0.71^{c}	15.29±1.38^{b}	19.29±0.73^{a}
Interleukin-2 (pg/ml)	Immature	0.36±0.02^{c}	1.32±0.29^{b}	2.37±0.63^{b}	4.25±1.78^{a}
	Mature	0.15±0.01^{c}	0.16±0.02^{c}	1.07±0.03^{b}	2.00±0.10^{a}
Interleukin-6 (pg/ml)	Immature	5.65±0.93^{d}	14.05±2.85^{c}	17.18±5.14^{b}	18.77±4.39^{a}
	Mature	4.90±0.40^{d}	6.08±0.20^{c}	7.70±1.59^{b}	13.80±1.59^{a}
Creatinine (mg/dl)	Immature	0.78±0.02^{c}	0.87±0.02^{b}	0.95±0.11^{b}	1.08±0.05^{a}
	Mature	0.74±0.05^{c}	0.90±0.01^{b}	0.91±0.03^{b}	1.13±0.04^{a}
Sodium (Na^{+}) (mmol /l)	Immature	1.24±8.10^{a}	1.35±8.97^{a}	1.36±5.60^{a}	1.28±5.43^{a}
	Mature	1.22±3.09^{a}	1.27±9.10^{a}	1.34±8.31^{a}	1.26±6.35^{a}
Potassium (K^{+}) (mmol /l)	Immature	3.86±0.24^{a}	4.62±0.26^{a}	4.82±0.64^{a}	4.44±0.71^{a}
	Mature	3.77±0.28^{a}	4.04±0.31^{a}	4.81±0.08^{a}	4.64±0.08^{a}

Data are represented as $\bar{X}$ ± SE $\bar{X}$: Mean values SE: Standard Error

Mean values with different superscript letters in the same column are significantly different at P <0.05.

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Table 3: Effect of boldenone undecylenate on some hormonal and hematological parameters in both immature and mature male rabbits after two months of administration (mean ± SE):

Parameter	Age	G1	G2	G3	G4
Body gain (kg)	Immature	817±0.012^{d}	887±0.015^{c}	1305±0.064^{b}	1682±0.076^{a}
	Mature	765±0.006^{d}	880±0.014^{c}	1222±0.046^{b}	1597±0.048^{a}
Growth hormone (µl U /ml)	Immature	3.59±0.08^{b}	7.51±0.64^{a}	7.55±0.81^{a}	7.28±1.36^{a}
Testosterone (ng/ml)	Mature	2.03±0.08^{b}	2.73±0.28^{b}	3.89±0.38^{a}	4.10±0.48^{a}
Hb (g/dl)	Immature	19.40±0.09^{c}	23.82±0.18^{b}	22.17±0.15^{b}	22.55±0.25^{a}
	Mature	25.73±0.11^{c}	28.40±0.09^{b}	28.02±0.07^{a}	26.87±0.28^{a}
PCV (%)	Immature	31.61±0.29^{d}	41.53±0.24^{a}	36.87±0.04^{c}	38.53±0.25^{b}
	Mature	37.73±0.87^{d}	43.04±0.07^{a}	42.15±0.04^{b}	41.27±0.19^{c}
RBCs (10^{12}/L)	Immature	4.72±0.03^{d}	5.63±0.06^{a}	5.36±0. 05^{b}	5.1±0.02^{c}
	Mature	5.23±0.01^{d}	7.03±0.04^{a}	6.89±0.02^{b}	6.05±0.03^{c}
MCV(fl)	Immature	74.00±0.41^{b}	75.50±0.28^{ab}	75.00±0.57^{ab}	74.00±0.58^{ab}
	Mature	72.50±0.28^{b}	74.25±0.25^{ab}	73.25±0.48^{ab}	73.00±0.58^{ab}
MCHC (%)	Immature	64.46±0.05^{a}	63.25±0.04^{b}	61.35±0.2^{c}	56.43±0.19^{d}
	Mature	63.73±0.22^{a}	63.30±0.13^{b}	62.52±0.14^{c}	60.70±0.04^{d}
MCH(pg)	Immature	43.02±0.04^{a}	41.75±0.04^{b}	40.24±0.04^{c}	39.00±0.07^{d}
	Mature	42.86±0.04^{a}	41.03±0.04^{b}	39.93±0.05^{c}	39.01±0.04^{d}

Data are represented as $\bar{X} \pm SE$ $\bar{X}$: Mean values SE: Standard Error

Mean values with different superscript letters in the same column are significantly different at $P < 0.05$.

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Table 4: Effect of boldenone undecylenate on some biochemical parameters in both immature and mature male rabbits after two months of administration (mean ± SE):

Parameter	Age	G_1	G_2	G_3	G_4
NO (µmol/l)	Immature	26.25 ± 1.39^{c}	39.50 ± 2.39^{b}	51.25 ± 5.51^{b}	76.75 ± 5.34^{a}
	Mature	25.75 ± 1.44^{c}	39.50 ± 3.59^{b}	46.25 ± 5.26^{b}	68.00 ± 2.61^{a}
SOD activity (u/g Protein)	Immature	5.67 ± 0.83^{c}	9.75 ± 0.58^{b}	11.04 ± 0.59^{ab}	12.72 ± 0.72^{a}
	Mature	5.45 ± 0.81^{c}	9.10 ± 0.50^{b}	11.33 ± 0.14^{a}	12.30 ± 0.49^{a}
GSH (mmol/g protein)	Immature	12.73 ± 0.57^{a}	8.56 ± 0.37^{b}	5.15 ± 0.78^{b}	4.28 ± 0.35^{c}
	Mature	10.78 ± 0.58^{a}	8.58 ± 0.43^{b}	7.40 ± 0.74^{b}	4.34 ± 0.37^{c}
MDA (nmol/ml)	Immature	5.77 ± 0.60^{d}	10.56 ± 0.93^{c}	20.97 ± 1.37^{b}	39.75 ± 1.41^{a}
	Mature	5.53 ± 0.31^{d}	9.70 ± 0.78^{c}	16.82 ± 1.52^{b}	21.12 ± 0.81^{a}
Interleukin-2 (pg/ml)	Immature	0.40 ± 0.22^{c}	1.68 ± 0.55^{b}	2.58 ± 0.67^{b}	4.84 ± 1.93^{a}
	Mature	0.17 ± 0.02^{c}	0.18 ± 0.02^{c}	1.18 ± 0.03^{b}	2.19 ± 0.11^{a}
Interleukin-6 (pg/ml)	Immature	6.21 ± 1.03^{d}	15.45 ± 3.13^{c}	18.90 ± 5.59^{b}	20.66 ± 4.83^{a}
	Mature	5.39 ± 0.44^{d}	6.69 ± 0.22^{c}	8.60 ± 1.69^{b}	15.27 ± 1.70^{a}
Creatinine (mg/dl)	Immature	0.82 ± 0.02^{c}	0.96 ± 0.03^{b}	1.05 ± 0.01^{b}	1.18 ± 0.06^{a}
	Mature	0.81 ± 0.05^{c}	0.98 ± 0.12^{b}	1.01 ± 0.03^{b}	1.24 ± 0.04^{a}
Sodium (Na^{+}) (mmol /l)	Immature	1.36 ± 8.95^{a}	1.45 ± 8.55^{a}	1.50 ± 6.68^{a}	1.42 ± 5.13^{a}
	Mature	1.29 ± 1.93^{a}	1.40 ± 9.91^{a}	1.47 ± 9.16^{a}	1.38 ± 6.98^{a}
Potassium (K^{+}) (mmol /l)	Immature	4.25 ± 0.40^{a}	5.06 ± 0.45^{a}	5.29 ± 0.24^{a}	4.88 ± 0.27^{a}
	Mature	4.15 ± 0.10^{a}	4.45 ± 0.11^{a}	5.29 ± 0.44^{a}	5.09 ± 0.46^{a}

Data are represented as $\bar{X} \pm SE$ $\bar{X}$: Mean values SE: Standard Error

Mean values with different superscript letters in the same column are significantly different at $P < 0.05$.

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