Effects of Multi-enzyme Feed Additive "*Kemzyme*" or/and Sodium Bentonite "as a Feed Binder"on Sexual Activity and Some Fertility Parameters of Rabbit Bucks

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Abstract

The present study was conducted to clarify the effect of Kemzyme or Bentonite and their mix as feed additives on the main semen characteristics, testicular enzyme markers, plasma testosterone level and fertility indices of bucks. Twenty four mature NZW rabbit bucks of about 4-month age and average body weight 2.400 kg were equally divided into four groups (6 in each). The first group was kept untreated (C). The second group (K) was supplemented with 0.1% "*Kemzyme*", a multi-enzyme. The third group (B) was supplemented with 2% sodium bentonite while the fourth group (KB) was supplemented with Kemzyme plus bentonite. The dietary treatment lasted for 10 weeks to cover a complete spermatogenic cycle. Results obtained revealed better sexual activity and higher libido of (K) and (KB) bucks. Besides, significant improvement in their different semen parameters, blood testosterone level, seminal total lipids concentration and the activities of different seminal enzymes. Such improvement was complementary to the higher fertility indices that also noticed on bucks of the same groups. Practically, it could be considered that (K) supplementation is a good reproductive promotant tool in the field of rabbit production.

Keywords: Multi-enzyme, Bentonite, Reproductive performance, Sexual Behaviour, Rabbit

1. Introduction

Rabbits have been recognized to have a very important role to play in the supply of animal protein to humans, especially in tropical and subtropical areas. Moreover, rabbit occupies a vital midway between ruminants and monogastric animals and can effectively utilize cellulose rich feed with ration containing less than 20% grain. Simple biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place rabbit just below poultry (Hasanat *et.al*, 2006). Rabbits are herbivores and can be successfully raised on diets that are low in grains and high in roughage. The poor and Green grass *Ad-libitum* unbalanced quality of forage based diets, usually provided to rabbits in developing countries was described by Cheeke *et al.* (1985). Despite these obvious advantages,improved feed formulation and strategies for enhancing the production

and reproduction potentials of rabbit especially in tropical and subtropical regions of the world have not been fully exploited.

Several studies have been attempted for incorporating exogenous enzymes into rabbit diets to improve nutrients availability (Falcao-e-Cunha *et al.,* 2007). Some of them could not detect any significant effect of enzymes on rabbit performances Falcão-e-Cunha *et al.* (2004), Others succeeded to get lower mortality (García *et al.* 2005) besides improved feed conversion ratio (Eiben *et al.* 2004). In some trials when cellulase and enzyme pool (xylanase, b-glucanase, b-gluccosidase, pentosanase, myloglucosidase, acid and neutral protease) were added, the authors got significant improvements on NDF and ADF digestibilities, with getting reductions of digestible and metabolizable energies, and nitrogen balance, in comparison with the control diets (Bolis *et al.*1996). It appears that the conditions (pH and temperature) through which these enzymes act are critical for their activity (Colombatto *et al.* (2003) & Khan *et al.* (2001)). Mista (2007) suggested a decisive impact of bentonite on the pH reaction of rabbit cecum that was favorable on the bacterial processes that take place there and consequently may affect the enzymatic activities. Moreover Abdl-Rahman *et al*., (2010) suggests that, coupling bentonite to multi-enzyme feed additives for rabbits could lead to favorable modifications in cecal environment presumably, acidification of cecal contents and stabilization of ammonia nitrogen concentrations. They added that; decreased cecal pH value, increased total VFAs concentration, and increased propionate at the expense of acetate and butyrate, increased fermentation efficiency and VFAs utilization. Additionally, co-supplementation increased serum glucose concentration and decreased serum triglycerides and cholesterol concentration. These alterations should be considered as an advantage, as they improve the impact of the supplemented enzymes on cecal fermentation pattern and rabbit metabolism.

In turkey toms & cockerels Saleh *et al*., (2006 a & b) have observed a significant increase in serum total lipid concentration in association with a significant increase in both testicular total lipids and cholesterol concentrations in both *Kemzyme* supplemented turkey toms and cockerels at 42 and 30 weeks of age (after 6 successive weeks experimental period). The authors concluded that *Kemzyme* in turkey tom or cockerels breeder diets improved the performance and fertilizing capability of such species either through reducing the viscosity of digesta and improving nutrient utilization specially lipids or through stimulating the synthesis and the secretions of hormones involved in steroidogenesis or spermatogenesis.

Depending on the overmentioned findings, the present experiment was therefore, designed to compare the effect of supplementing Kemzyme, or Bentonite and their combination on some sexual behaviour and certain reproductive parameters of rabbits.

2. Materials and methods

2.1 Animals, and location

The study was conducted in the experimental rabbitry of Physiology Department, Faculty of Veterinary Medicine Cairo University. Twenty four mature male New Zealand White bucks of about 4-month age and average body weight 2.400 kg were used during June to August. The rabbits were housed individually in commercial cages (55×60×34cm), equipped with automatic drinkers and j-feeders. Daily lighting regime was 10-12 hour photoperiod /day through both natural and fluorescent lighting. A commercial pelleted diet of 16.7% crude protein, 13.7% crude fiber and 2590 kcal of digestible energy per kg (Atmida Feed Company, Egypt) was offered *ad libitum*. Clean, fresh water was available all times. The diet subjected to chemical analysis according to AOAC (1999).

2.2 Experimental design

The animals were equally divided into four groups (6 in each). The first group was the control group (C) the animals were kept untreated and were fed the basal diet without additives. The second group (K) was supplemented with 0.1% "*Kemzyme*", a multi-enzyme blend of Kemin Agrifoods Europe, composed of cellulases**,** amylases, proteases and lipases. The third group (B) the animals were supplemented with 2% sodium bentonite (Ghanem, 1995) which purchased from (Morgan for chemicals - Egypt) while the fourth group (KB) was supplemented with 0.1% Kemzyme plus 2% sodium bentonite. Doses of supplemented additives were mixed with the basal ration pellets. The dietary treatment lasted for 10 weeks to cover complete spermatogenic cycle.

2.3 Behavioural measurements

Sexual activity of the bucks was evaluated through behavioural testing "mating test". On the test day, a receptive female was introduced to the male'cage and the following behavioral parameters were recorded according to Theau-Clement *et al.*, (1995) and Agmo *et al*., (2004) during 10 min testing period for each buck: latency to

mount, time from introduction of the female until the first mount with pelvic thrusting; mating latency "reaction time", time from introduction of the female until the first ejaculation; interval between the first and second matings as a measure of libido. The previously mentioned parameters were measured in seconds using a stopwatch. Total number of mounts and ejaculations were also recorded. Each male in the different groups was tested three times, two days after each semen collection.

2.4 Semen collection and evaluation

At the beginning of the ninth week of the treatment, Semen collection was done by using a teaser female and artificial vagina that was locally fabricated as described by Herbert & Adejumo (1995). Semen was collected weekly for three consecutive times and in every collection two successive ejaculations (with a lag of 15 min.) were obtained from each buck between 8:00 to 10:00 h to ensure optimum quality of semen obtained (Castellini, 2008 & Ogbuewu *et al.,* 2009). The volume of each ejaculate was recorded (using a graduated collection tube) after removal of the gel mass. A weak eosin solution (Smith & Mayer, 1955) was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH & Co., Brands twiete 4, 2000 Hamburg 11, Germany). Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. Assessment of live, dead, and abnormal spermatozoa were performed using an eosin–nigrosin blue staining mixture (Rodríguez -De Lara *et al.,* 2008).The percentages of motile sperm and motility grade were estimated by visual examination under high-power magnification (40×) using an ordinary microscope with heated stage. Motility was scored as follows: 0 = no movement; 1 = twitching, no forward progressive movement (fpm); 2 = slow fpm; 3 = good fpm; and 4 = fast fpm.

The two motility parameters were combined to yield; Sperm motility index: SMI = percentage motile X motility grade (Yousef *et al.,* 1996). Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and total sperm outputs.

Seminal plasma was obtained by centrifugation of semen samples at 860 X g for 20 min, and was stored at −20 ◦C until analysis. The activities of seminal plasma lactate dehydrogenase /LDH (Allain,1973), Alkaline phosphatase / ALP (Roy,1970) and Gamma glutamyle transferase /GGT (Shaw,1982), were measured spectrophotometerically by using kinetic mode. Total lipids were also measured in the seminal plasma according to method of Schmitt (1964).

2.5 Bucks fertility indices:

For evaluation of their fertility parameters, bucks of the different groups were bred with 24 receptive nulliparous female rabbits and their parameters were recorded for each group according to IRRG (2005): kindling rate, total born and total born alive litter also stillborn kits were monitored

2.6 Blood Sampling

Blood samples were collected from the ear vein of all animals in the morning before accesses to feed and water. Heparin was used as anticoagulant. Plasma was obtained by centrifugation of samples at 860×g for 20 min and was stored at -20°C until used for determination of total testosterone (Abraham, 1981&Tietz, 1995).

2.7 Statistical analysis

Data of the experiment for all variables were subjected to ANOVA as a completely randomized design according to Snedecor & Cochran (1982). Means were compared by Least Significant Difference (LSD) test at 0.05 significant level (Steel &Torrie, 1980).

3. Results

Results tabulated in table (1) &figures (1-6) reveal that bucks of (K) group showed statistically ($P<0.05$) the highest plasma testosterone level and sexual activity among the different control and treated groups as indicated by reduced latency to mount (2.12±0.22s), to mate (4.77±0.64 s) and the shortest interval between 1st and 2nd mating (63.43-3.07s) with the highest number of mountings (3.33±0.28) and successful mating (3.5±0.22) throughout the testing period. Compared to C group, bucks of KB group showed insignificantly improved sexual performance however no statistical differences ($P<0.05$) were recorded between those of B and C groups. Testosterone level was significantly increased in bucks supplemented with either (K) alone or (KB). However, no significant alteration was recorded in testosterone level in (B) supplemented bucks.

Table (2) &figures (7-12) show the different semen characteristics of control and supplemented bucks. A significant improvement in the measured semen parameters was recorded in bucks supplemented with either (K) alone or (KB) as compared with those of (C) or (B) supplemented bucks. However, supplementation with (B)

alone did not show any significant alterations in the semen parameters of treated bucks as compared with those of control one

Table (3) & figures (13-16) show that in bucks supplemented with either (K) alone or (KB) activities of seminal enzymes and lipids concentration were significantly decreased as compared with those of (C) or (B) supplemented bucks. However (B) alone did not induce any significant alterations in the activities of seminal enzymes while, a significant increase in seminal total lipids concentration was recorded as compared with control bucks.

Table (4) Showed that; the improved semen traits of (K) and (KB) supplemented groups were accompanied with better reproductive performance than those of (B) or (C) groups; higher kindling rate (83% & 80%) and total litter size at birth (10.17±0.31 & 9.17±0.02) of females bred with both K and KB bucks than those bred with either (B) or (C) groups, however, no significant differences were recorded between the different groups for stillborn kits.

4. Discussion

4.1 Sexual behaviour and libido

Good sex drive "libido" of male rabbits and high quality semen are required year-round to achieve the maximum productivity either through artificial insemination (Rodríguez-De Lara *et al*., 2008) or natural mating.

As libido in male rabbits depends on the reaction time; observation of time elapsed between the exposure of a buck to a doe and the first mounting and and/or copulation (Chenweth, 1981& Rodríguez-De Lara *et al.,* 2008), the interval between the first two ejaculates (Moustafa, 1997) also it depends on number of ejaculations achieved before exhaustion and the interest in receptive female (Hafez, 1962), so it was clear that males of K followed by those of KB groups had better sexual performance and higher libido than either B or C groups; The improved sexual activity of both K and KB groups is parallel to the higher testosterone level of both groups which reflected in higher libido and improved sexual performance. Angel *et al.,* (2008) found that, in male rabbits, androgens are involved in chinning stimulation and other sexual behavior. This might confirm the results of Cross & Roselli (1999) who suggested that testosterone acts synergistically with estradiole to stimulate male sexual behaviour. Testosterone was found to acts in part through in situ conversion to estradiol by aromatase in the preoptic area stimulating mounting, ultimately improved the copulatory behaviour.

4.2 Semen characteristics, seminal enzymes, total lipids concentration, and Fertility indices

In the present study, kemzyme supplemented, either alone or combined with bentonite, in bucks was associated with a significant improvement in the semen parameters and significant decrease in the activities of seminal enzyme markers and total lipids concentration as compared to control or bentonite- supplemented bucks. Such improvement was complementary to the activated sexual behaviour and improved fertility indices that also noticed on the same bucks and further more was augmented by the recorded elevated level of male sex hormone, testosterone.

Similar results were obtained with Brun *et al.* (2002) who found that the mass motility significantly influenced the kindling rate. They added that taken separately, volume, percentage of motile sperm and concentration didn't influence the kindling rate but their product, the number of motile sperm per ejaculate did. They found also that litter size (total born) was significantly influenced by concentration and all variables depending on it, particularly the number of total and motile sperms. However, Castellini *et al.* (2006) didn't find a significant effect for number of spermatozoa inseminated on reproductive performance.

The effect of bentonite on fertility parameters of bucks was clearly insensible. The obtained results might be attributed based on the suggestion that kemzyme should have a stimulatory role on the digestion and absorption processes that consequently enhance nutrient availability reflecting positively on the synthetic pathways, including gonadal one. Other stimulatory effect for kemzyme on the level of thyroid-gonadal axis, insuline and IGF-1 synthesis was also suggested by (Jones & Clemmons, 1995).Such explanations couldn't extend toward the effect of bentonite as its action as a ration binder might be localized on GIT environment and couldn't affect the main metabolic pathways that reflect on the main biological activities of male animals, including sexual and fertility elements.

Campell & Bedford (1992) has reported that the benefits of adding enzymes to diets of non ruminants animals has become more common. Recently, several studies have been attempted for incorporating exogenous enzymes into rabbit diets to improve nutrients availability (Falcão-e-Cunha *et al.,* 2007). Some of them could not detect any significant effect of enzymes on rabbit performances (Falcão-e-Cunha *et al.* 2004). Also enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as

barley, oats, rye and wheat (Marquardt *et al.,* 1994). The beneficial effects of adding enzymes have been attributed to a reduction in viscosity of digesta in the intestine which results from arabinoxylans; the non starch polysaccharides (NSP) present in the endosperm cell walls and which represent 70% of the total non starch polysaccharides in wheat (Zijstra *et al.,* 1999).

Saleh *et al*. (2004) have recorded a significant increase in serum total lipid concentration in association with a significant increase in both testicular total lipids and cholesterol concentrations in kemzyme- supplemented turkey toms and cockerels at 42 and 30 weeks of age, after 6 successive weeks experimental period, respectively. They attributed their findings to the reduction in digesta viscosity that might lead to a subsequent increase in nutrient digestibility specially lipids. In the present study, seminal total lipids were significantly increased in bucks supplemented with bentonite. Such finding could suggest that bentonite might improve the availability of nutrients, including fatty acids (Abdl-Rahman *et al*., 2010); this reflecting on the level of blood lipids and consequently on its level in the seminal fluid. On the other hand, the persistant higher level of lipids in the semen may confirm the suggestion that bentonite did not possess a regulatory metabolic role on the level of testicular tissues. Meanwhile, it could be claimed that kemzyme may have such systemic stimulatory metabolic effect on testicular steroidogenesis that might reflect on the decreased level of seminal lipids, an essential element for steroidogenesis, and the increased testosterone level in the seminal plasma that also recorded in the present study.

Concerning lipids and fertility, a fundamental relation between dietary lipids, spermatozoa, fatty acid composition, sperm quality and fertility has been reported. In mammals, lipid composition of sperm membrane was found to play a major role in the physicochemical modification leading to fertilization (Langlais & Robert, 1985). In all species, the phospholipids are the major lipid components of spermatozoa; they contain large amounts of polyunsaturated fatty acids (PUFAs). A diet with specific fatty acid and antioxidant composition has been successfully used to improve the reproductive performance of boar (Penny *et al.,* 2000 & Maldjian *et al.,* 2002).

The significant decrease observed in testicular enzyme markers of bucks supplied with either kemzyme alone or combined with bentonite, including ALP, GGT and LDH activities may suggests that kemzyme might adjust the testicular environment minimizing the rate of testicular cell tear, denoting that it may have anabolic rather than catabolic effect, providing adequate levels of elements necessary for proper steroidogenesis and spermatogenesis. Saleh *et al.(*2004) reported that the acrosome system of sperm head was found to contain alkaline phosphatase (Kumar *et al.,* 2003) and lactate dehydrogenase which was found to be expressed in premeiotic, meiotic and postmeiotic cells including spermatids and spermatozoa of both mammalian and avian species (Arias *et al.,* 2000).

5. Conclusions

Kemzyme supplementation in bucks was associated with improved sexual activity indices, improved semen parameters and steroidogensis. Its application as fertility enhancer should not be neglected. Bentonite, although it was claimed to have a growth promoting effect, its influence on animal sexuality and fertility was unsatisfactory

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Table 1. Serum testosterone level and sexual activity traits in control and treated bucks

Group / Parameters	Control	Kemzyme	Bentonite	Kemzyme +Bentonite
Testosterone level (ng/l)	2.39 ± 0.18[a]	3.90 ± 0.19 [b]	2.14 ± 0.18[a]	3.78 ± 0.21[b]
Latency to mount (sec.)	6.5 ± 2.12 ±[b]	2.12 ±0.22[a]	8.00 ± 2.29[b]	3.62 ± 0.65[a]
Latency to mate (sec.)	12.88 ± 2.62 [b]	4.77 ± 0.64[a]	25.33 ±7.88 [b]	8.88 ± 1.03[b]
Interval between 1st & 2nd matings (sec.)	83.43 ± 4.91	63.43 ± 3.07	82.85 ± 7.78	75.42 ± 9.85
NO. of mountings	2.33 ± 0.4[b]	3.33 ± 0.28[a]	2.11 ± 0.26[b]	2.22 ± 0.32[b]
NO. of matings	2.2 ± 0.2[b]	3.5 ± 0.22[a]	1.5 ± 0.16[b]	1.9 ± 0.23[b]

Data presented as means ± SE, N =6

Means with different superscript in the same row are statistically different at (P<0.05)

Table 2. Semen characteristics in control and treated bucks

Group / Parameters	Control	Kemzyme	Bentonite	Kemzyme+entonite
Ejaculate volume (EV/ ml)	0.50±0.14[a]	0.73±0.24[b]	0.52±0.32[a]	0.76±0.20[b]
Sperm concentration (Sp. Conc. ×10^6 ml)	305.0±55.9[a]	342±77.5 [b]	296±66.8[a]	332.0±44.8 [b]
Total sperm output (T. Sp. O. ×10^6)	152.5±55.0[a]	249.66±74.0[b]	153.92±98.0[a]	252.32±70.0[b]
Sperm motility index (SMI)	2.16±0.33[a]	2.72±0.26[b]	2.22±0.92[a]	2.66±0.37[b]
Total motile sperm (TMS ×10^6)	141.4±46.3[a]	198.7±88.8[b]	138.60±69.3[a]	201.0±56.0[b]
Dead sperm (D. Sp. %)	26.53±4.24[a]	19.98±4.25[b]	25.42±7.98[a]	20.52±3.35 [b]
Abnormal sperm (Ab. Sp. %)	16.08±2.37[a]	12.55±2.80[b]	18.10±3.04a	12.07±2.82[b]

Data presented as means ± SE, N =6

Means with different superscript in the same row are statistically different at (P<0.05)

Table 3. Seminal enzymes activity and total lipids of control and treated bucks

Group / Parameters	Control	Kemzyme	Bentonite	Kemzyme + Bentonite
Lactate dehydrogenase (LDH -IU/l)	2020 ± 110 [a]	1920 ± 111[b]	2068 ± 123[a]	1903 ± 122 [b]
Gamma glutamyl transferase (GGT-IU/l).	48 ± 2.33 [a]	28.11 ± 3.33 [b]	42.42 ± 4.01 [a]	32.05 ± 3.04 [b]
Alkaline phosphatase (ALP- IU/l)	54.11 ± 4.00 [a]	27.23 ± 5.01 [b]	49.55 ± 5.33 [a].	37.45 ± 4.90 [b]
Total lipids (mg/dl)	218.2 ± 21.79 [a]	189.55 ± 22.46 [b]	234 ± 18.9[a]	177 ± 42.11 [b]

Data presented as means ± SE, N =6

Means with different superscript in the same row are statistically different at (P<0.05)

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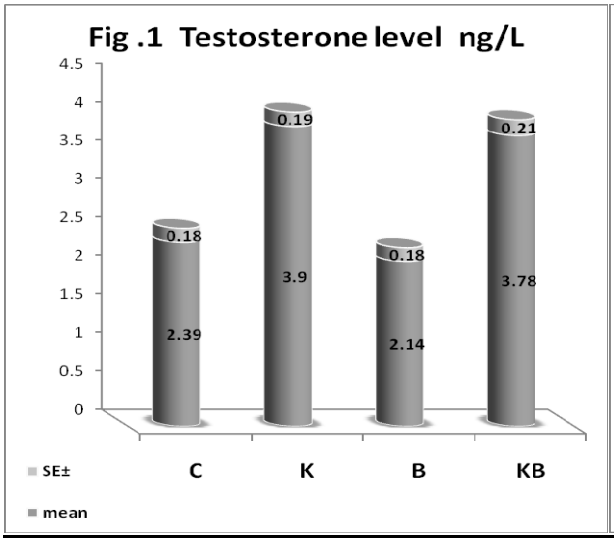
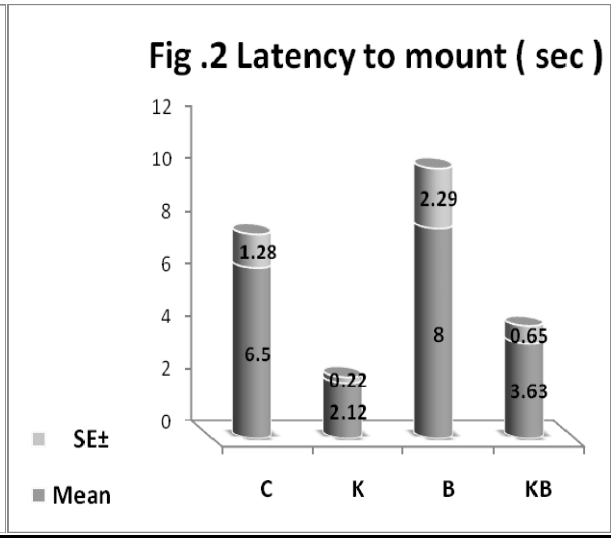
Table 4. Fertility indices of control and treated bucks

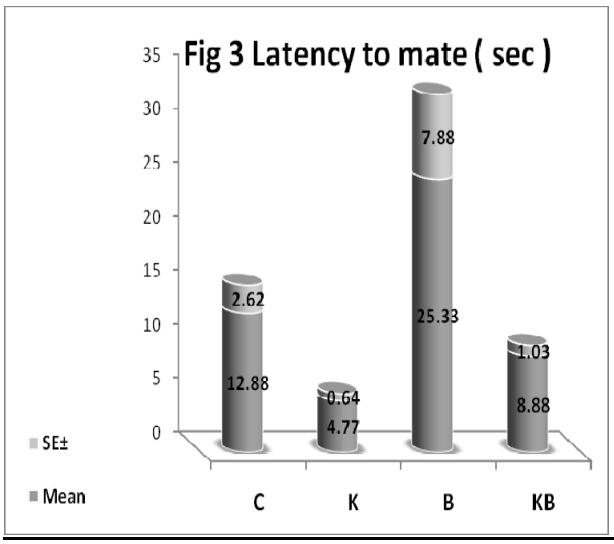
Parameters \ Group	Control	Kemzyme	Bentonite	Kemzyme + Bentonite
Kindling rate %	66[a]	83[b]	60[a]	80[b]
Total litter size	7.33± 0.33[a]	10.17±0.31[b]	9.17±0.02[b]	7.17±0.18[a]
Still born kits %	2.4	1.6	2.1	2.3

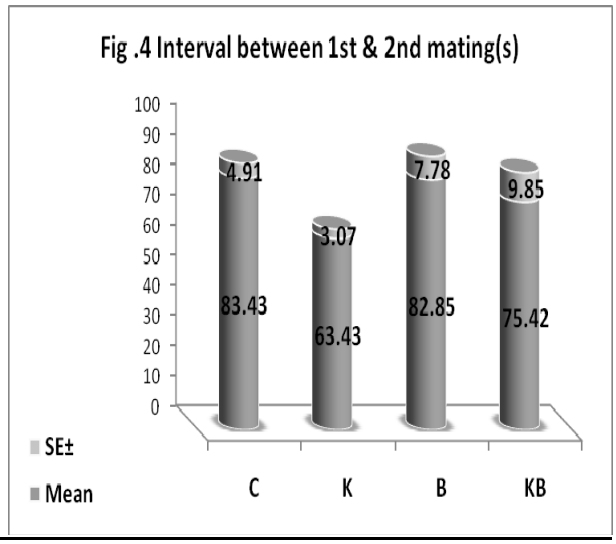
Data presented as means ± SE or %, N = (5- 6)

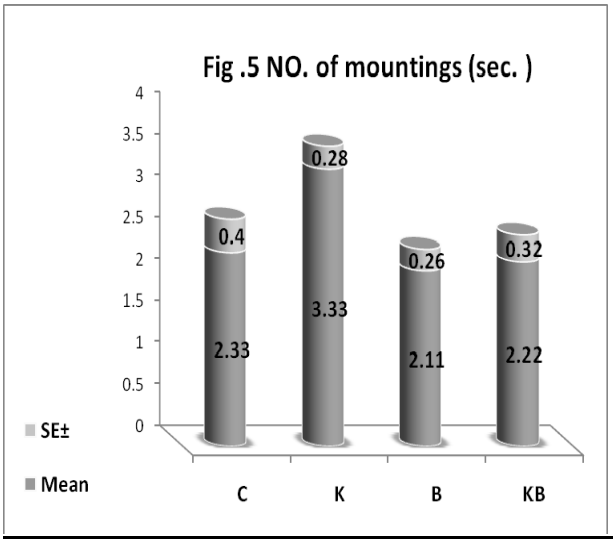
Means with different superscript in the same row are statistically different at (P<0.05)

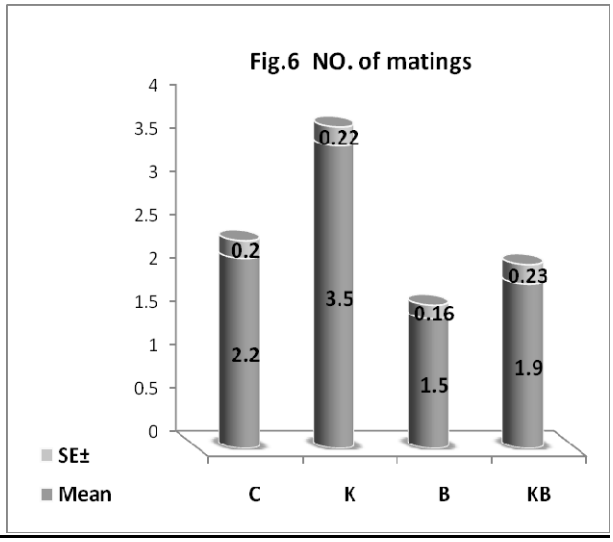


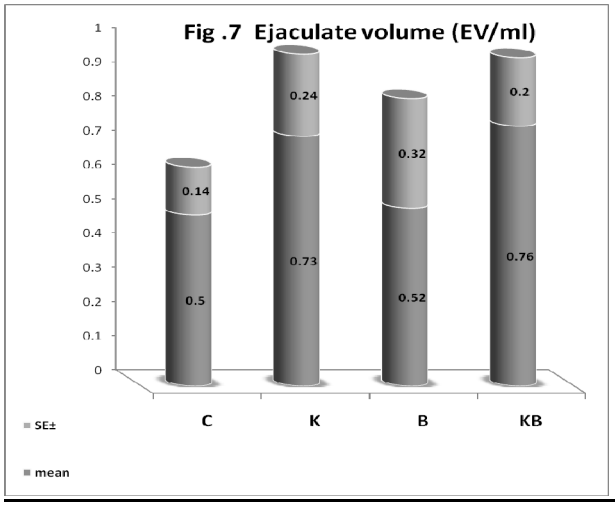


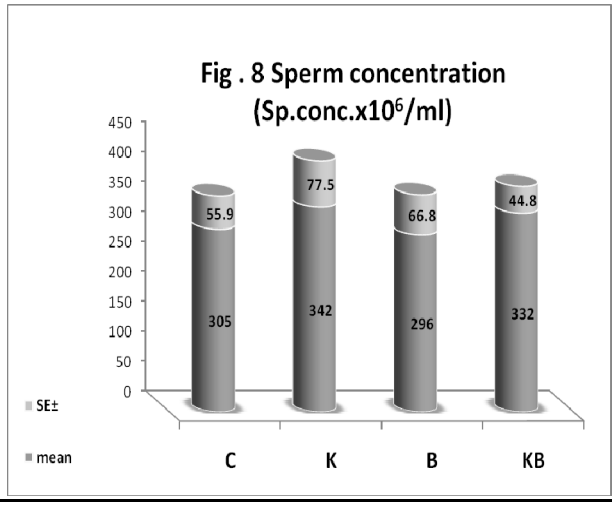


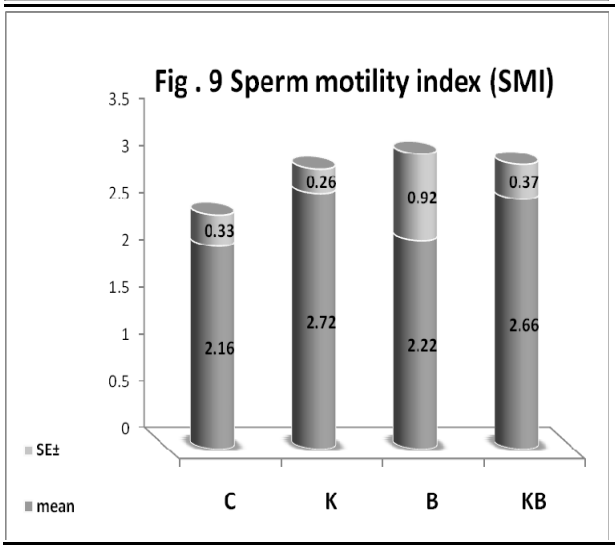


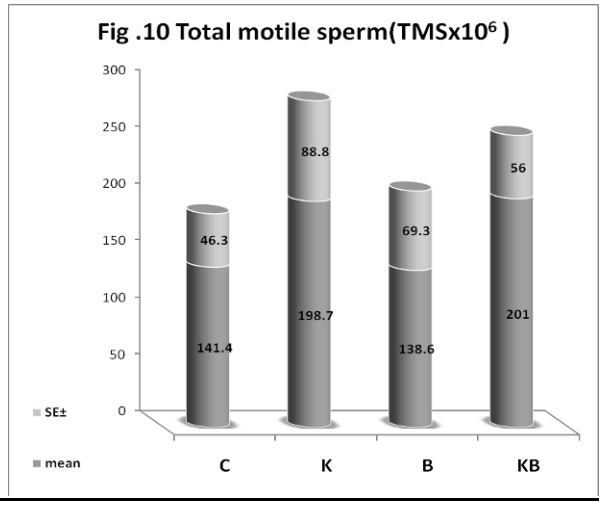












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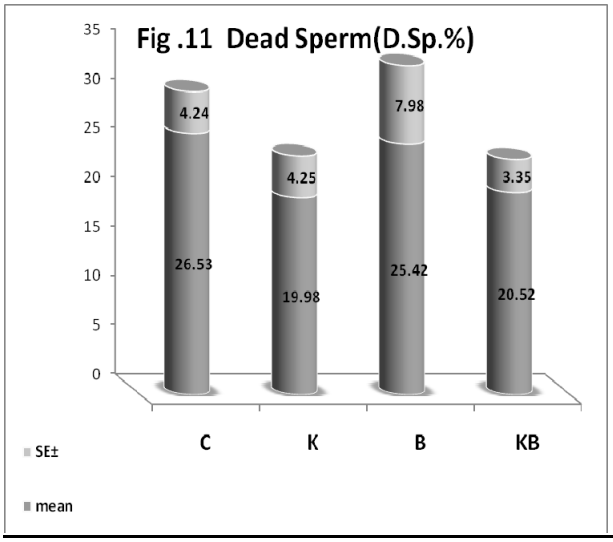
Fig .11 Dead Sperm(D.Sp.%)
35
30
25
20
15
10
5
0
4.24
26.53
4.25
19.98
7.98
25.42
3.35
20.52
C
K
B
KB
SE±
mean

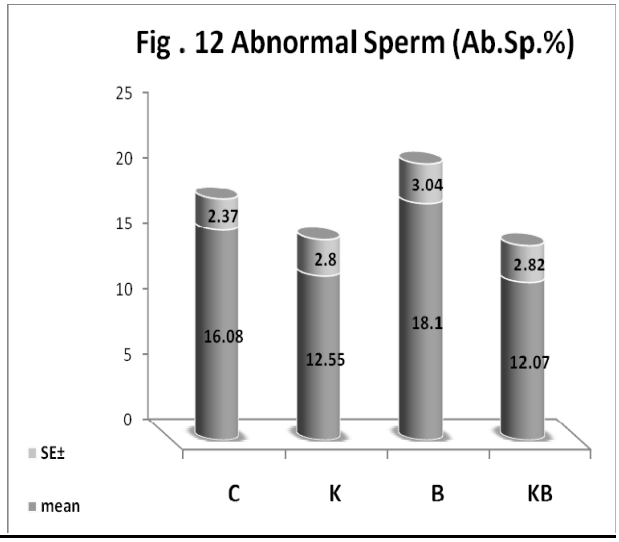
Fig . 12 Abnormal Sperm (Ab.Sp.%)
25
20
15
10
5
0
2.37
16.08
2.8
12.55
3.04
18.1
2.82
12.07
C
K
B
KB
SE±
mean

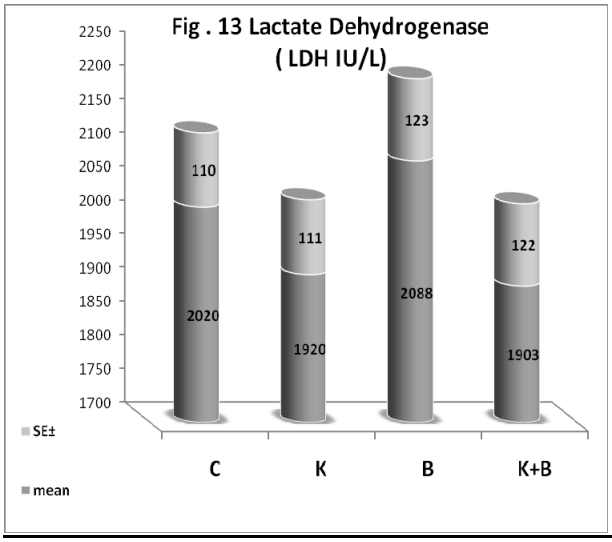
Fig . 13 Lactate Dehydrogenase
(LDH IU/L)
2250
2200
2150
2100
2050
2000
1950
1900
1850
1800
1750
1700
110
2020
111
1920
123
2088
122
1903
C
K
B
K+B
SE±
mean

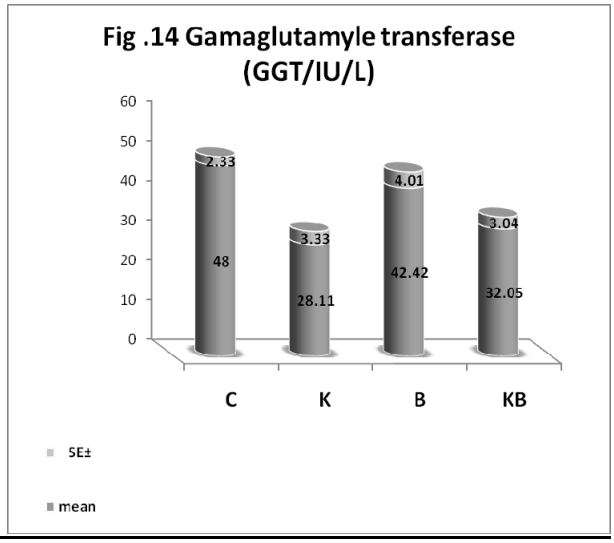
Fig .14 Gamaglutamyle transferase
(GGT/IU/L)
60
50
40
30
20
10
0
2.33
48
3.33
28.11
4.01
42.42
3.04
32.05
C
K
B
KB
SE±
mean

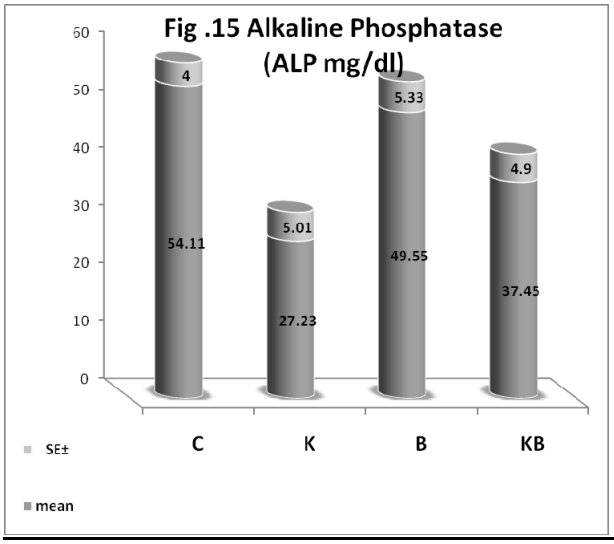
Fig .15 Alkaline Phosphatase
(ALP mg/dl)
60
50
40
30
20
10
0
4
54.11
5.01
27.23
5.33
49.55
4.9
37.45
C
K
B
KB
SE±
mean

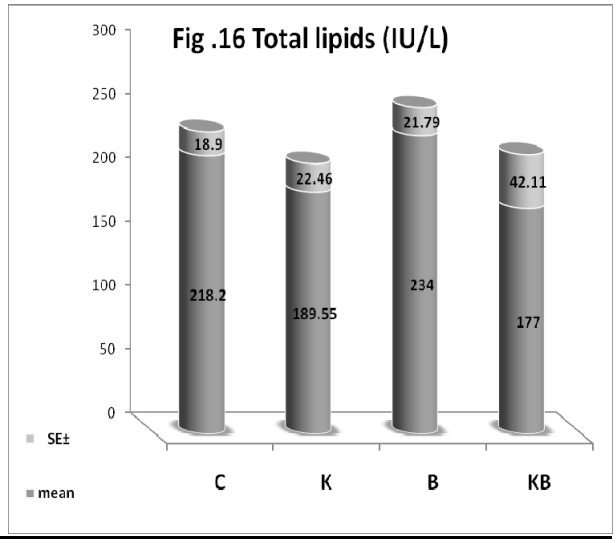
Fig .16 Total lipids (IU/L)
300
250
200
150
100
50
0
18.9
218.2
22.46
189.55
21.79
234
42.11
177
C
K
B
KB
SE±
mean