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EFFECT OF DEWORMING ON SEMINAL CHARACTERISTICS OF KARAN FRIES BULLS

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ABSTRACT

Present investigation, conducted on Holstein Friesian crosses maintained at Artificial Breeding Complex, NDRI, Karnal, India, was aimed at to assess the impact of deworming on various semen characteristics of Karan Fries (KF) bulls. Before deworming, no significant (p = 0.122) difference in worm load was observed between control (272.66±6.69 egg g^{-1}) and treated (256.00±5.29 egg g^{-1}) groups. The semen characteristics after deworming were on higher side and significant improvement was noticed only in reaction time (69.00±9.18 to 46.21±5.11 seconds) and osmolality (279.56±2.69 to 292.68±3.04 mOsmol kg^{-1}). Semen volume, mass activity (MA), sperm motility, sperm concentration, non-eosinophilic count, pH, total sperm per ejaculate, total motile sperms per ejaculate, total non-eosinophilic count per ejaculate, total non-eosinophilic count mL^{-1} semen and semen dose harvested $week^{-1}$ though on higher side did not show any significant (p>0.05) improvement in KF bulls after deworming. Significant analysis of covariance for MA (p = 0.03) and individual motility indicate that the significant differences may be masked due to pre-treatment effect. Present study showed significant group differences between 3 and 5 weeks after Doramectin application, which almost corresponds to the duration of spermatogenesis in bovines.

Key words: Bulls, crossbreds, deworming, semen quality, spermatogenesis

INTRODUCTION

Deworming agents not only affect worms, but may also affect the host itself. Some of these effects are related to the same biochemical mechanism that operates against the parasite and some of them are peculiar to the host (Schroder *et al.*, 1986). Deworming is probably beneficial if it happens at the onset of any concentrate feeding period aimed at improving the body condition of breeding bulls (Volkmann, 2011). Volkmann (2011) revealed that commonly used pyrethroid insecticides have devastating effects on semen quality of bulls and rams. Aerial sprays and pour-on formulations have been implicated in the development of severe secondary sperm defects and very poor sperm motility in exposed breeding animals. It has recently been reported that many pyrethroids act as endocrine disruptors and show host-species specific effects (Perry *et al*., 2007; Sun *et al*., 2007; Zhang *et al*., 2008). There is need to assess the effects of deworming on spermatogenesis in bulls. Keeping this in view, the present investigation was carried out to assess the effect of deworming on various semen characteristics.

MATERIALS AND METHODS

The present study was conducted on Holstein Friesian crosses, maintained at Artificial Breeding Complex, NDRI, Karnal, India. Six Karan Fries (KF) bulls each were randomly allotted to control and treatment groups of KF breeds. Semen was collected twice a week upto 4 weeks {a total of 96 ejaculates (2 ejaculates x 12 animals x 4 weeks)} from both the groups. After semen collection for 4 weeks, deworming was done with doramectin inj. (Dectomax) (Pfizer Ltd., Guarulhos, Brazil) which contained 10 mg Doramectin mL^{-1}. The injection was given intramuscularly to all the bulls under treatment at prophylactic dose @ 200 mcg Doramectin kg^{-1} (1 mL 50 kg^{-1} body weight), whereas control animals received no deworming treatment. A total of 216 ejaculates (2 ejaculates x 12 animals x 9 weeks) were included, which were collected over 9 weeks after deworming. The reaction time (RT) (Kilgour *et al*., 1984) and semen parameters viz., semen volume (Roberts, 1986), pH (Steinbach and Foote, 1967), osmolality by WECOR vapour pressure osmometer (Liu *et al*., 1998), mass activity (MA) as per Matharoo *et al*. (1985), individual motility (IM) as visual observation (Lasley, 1951), sperm concentration by haemocytometer (Neubauer's chamber) method (Murugavel, 1997), percent eosinophilic count (PEC) by eosine-nigrosine staining (Makler *et al*., 1980) and semen dose harvested (DOSE) [calculated by dividing the total motile sperms (TMS) per ejaculate by 20×10^6] were recorded. Seven days before and after Doramectin administration, faecal samples were collected from all the bulls. Quantitative examination of faecal samples for parasitic egg output was performed using Stoll's method (Rim, 2005) and calculation of eggs g^{-1} faeces (epg) done by using following formula:

epg = Number of eggs counted x 100

The total sperms (TS), TMS and total eosinophilic sperms (TES) values were obtained by multiplying the volume with sperm concentration, per cent individual motility and per cent live spermatozoa, respectively. The data was statistically analyzed (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

There was no significant ($p>0.05$) difference in worm load between control and treatment groups of cattle bulls before deworming (272.66±6.69 and 256.00±5.29 egg g^{-1}, respectively at $p = 0.122$) [Table 1]. In majority cases, maximum improvement in semen quality was observed 9 weeks after deworming treatment. Analysis of weekly difference between control and treatment groups revealed absence of pre-treatment effect in KF bulls (Table 2). The effect of deworming started from 4^{th} week by change in pH only which was significant ($p<0.05$). Significant improvement ($p<0.05$) in reaction time (RT) and sperm concentration was observed from 6^{th} week.

Table 1: Mean egg output in faecal samples collected before and 7 day after Doramectin in control and treated Karan Fries bulls

Faecal collection	Control	Treatment	p-value
Before Doramectin	272.66± 6.69	256.00±5.29	0.122
After Doramectin	222.33±22.82	0.00±0.00	0.001

For the assessment of the effect of deworming treatment, pre-treatment data was compared with post-treatment data (Table 3). The semen characteristics after deworming in KF bulls (Figs. 1-4) were on higher side, however, significant improvement was observed only in RT ($p = 0.04$) and osmolality ($p = 0.0023$). The semen volume was on higher side; however, it showed no significant ($p>0.05$) improvement in KF bulls after deworming. The MA, IM, sperm concentration, PEC, pH, TS, TMS, TES and DOSE though on higher side after deworming showed no significant improvement. Production of spermatozoa is a continuous process in sexually mature bulls. Concentration of sperm reflects the status of testicular functions. In present study the overall mean sperm concentration and mean sperm concentration was almost similar in this cattle breed and the findings are in line with Rao *et al*. (1996), Murugavel *et al.* (1997) and Mondal (1998).

Table 2: Weekly p-values of t-test between control and deworming treatment groups in Karan Fries bulls

Parameters*	Weekly p-values												
	Pre-treatment					Post-treatment							
	1	2	3	4	5	6	7	8	9	10	11	12	13
RT (sec.)	0.051	0.447	0.163	0.914	0.587	0.426	0.880	0.081	0.738	0.027	0.115	0.544	0.143
Semen volume (mL)	0.019	0.012	0.846	0.122	0.433	0.652	0.484	0.627	0.966	0.774	0.157	0.356	0.468
MA (0-4 scales)	0.199	0.328	0.228	1.000	1.000	0.393	0.293	0.207	0.461	0.368	1.000	0.870	0.504
IM (%)	0.385	0.213	0.199	0.956	0.721	0.206	0.664	0.687	0.788	0.745	0.832	0.931	0.734
Sperm conc. (10^6 mL^{-1})	0.608	0.950	0.537	0.254	0.354	0.659	0.893	0.795	0.522	0.017	0.837	0.406	0.292
NEC (%)	0.358	0.212	0.273	0.908	0.669	0.247	0.727	0.269	0.714	0.911	0.904	0.815	0.922
pH	0.047	0.308	0.552	0.775	0.860	0.300	0.152	0.289	0.393	0.770	0.069	0.911	0.187
Osmolality (mOsmol kg^{-1})	0.954	0.329	0.068	0.701	0.219	0.385	0.355	0.036	0.745	0.088	0.739	0.049	0.231
TS (x 10^6)	0.466	0.069	0.951	0.015	0.969	0.076	0.571	0.916	0.404	0.116	0.282	0.360	0.770
TMS (10^6)	0.691	0.063	0.510	0.122	0.752	0.060	0.972	0.901	0.472	0.263	0.564	0.558	0.887
TES (10^6)	0.622	0.052	0.606	0.088	0.758	0.059	0.893	0.807	0.610	0.188	0.454	0.502	0.985
TES (10^6 mL^{-1})	0.867	0.334	0.695	0.392	0.555	0.266	0.590	0.945	0.896	0.098	0.909	0.530	0.597
DOSE (No.)	0.691	0.063	0.510	0.122	0.752	0.060	0.972	0.901	0.472	0.263	0.564	0.558	0.887

*RT = Reaction time; MA = Mass activity; IM = Individual motility; NEC = Non-eosinophilic count; TS = Total sperms; TMS = Total motile sperms; TES = Total non-eosinophilic count and DOSE = Semen dose harvested

Table 3: Treatment effect on semen characteristics in treated and control KF bulls (n = 3 each) considering pre-treatment effect as a covariate

Parameters	Pre-treatment (mean ± SE) (n = 16)	Post-treatment (mean ± SE) (n = 38)	p-value	p-value of covariate (pre-treatment effect)	% change
RT (Sec)	69.00±9.18	46.21±5.11	0.0401	0.7553	-33.03
Semen volume (mL)	3.06±0.43	3.63±0.28	0.2869	0.1365	18.63
MA (0-4 Scales)	1.78±0.21	1.94±0.11	0.4578	0.0373	8.99
IM (%)	40.31±4.45	45.52±2.92	0.3363	0.0503	12.92
Sperm conc. (million mL^{-1})	554.37±41.61	560.78±38.60	0.9106	0.2914	1.16
NEC (%)	49.62±4.32	54.65±2.92	0.343	0.0707	10.14
pH	6.24±0.04	6.29±0.02	0.3191	0.3477	0.80
Osmolality (m Osmol kg^{-1})	279.56±2.69	292.68±3.04	0.0023	0.2530	4.69
TS (x 10^6)	1664.06±214.87	2004.21±212.19	0.2664	0.6987	20.44
TMS (x 10^6)	798.84±170.68	959.04±157.56	0.4944	0.4581	20.05
TES (x 10^6)	949.71±187.09	1129.13±163.89	0.4751	0.5095	18.89
TES (x 10^6 mL^{-1})	281.61±36.98	299.94±26.77	0.6908	0.9109	6.51
DOSE (No.)	39.94±8.53	47.95±7.87	0.4944	0.4581	20.06

(RT = Reaction time; MA = Mass activity; IM = Individual motility; NEC = Non-eosinophilic count, TS = Total sperms; TMS = Total motile sperms; TES = Total non-eosinophilic count; and DOSE = Semen dose harvested, n = Number of semen samples analyzed)

A single intramuscular application of Doramectin in therapeutic dose did not negatively influence the quality of cattle semen. Most parameters examined were significantly higher in treated than in non-treated cattle. Probably high worm infestation was not a possible cause of reduced semen quality in our study as only a small fecal egg count was seen in all bulls before treatment.

Both semen volume and sperm concentration were higher after deworming in KF bulls, which indicated that increase in volume was associated with greater number of spermatozoa rather than an

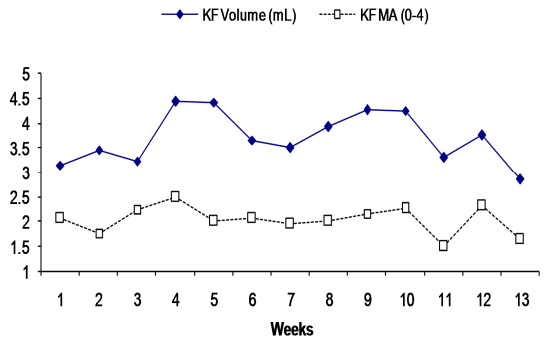

Fig. 1: Effect of deworming on semen volume and mass activity in KF bull semen

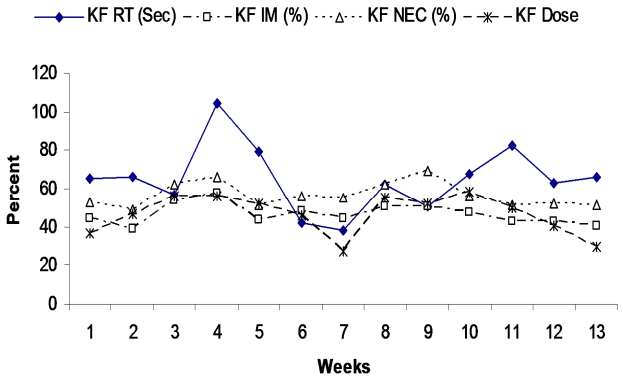

Fig. 2: Effect of deworming on reaction time (KFRT), individual sperm motility (KFM), non-eosinophilic count (KF NEC) and semen dose harvested (KF Dose) in KF bull semen

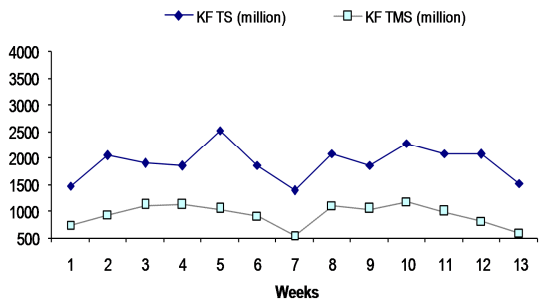

Fig. 3: Effect of deworming on total spermatozoa (KFTS) and total motile spermatozoa (KF TMS) in KF bull semen

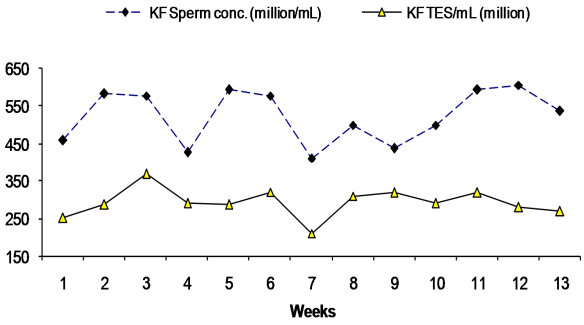

Fig. 4: Effect of deworming on sperm concentration and total non-eosinophilic count mL^{-1} in KF bull semen

increase in seminal fluid only, thus suggesting that deworming may have stimulatory effect on spermatogenesis to a great extent compared to its effect on accessory genital structures.

The higher TS, TMS and TES values after deworming, observed in present study, might be due to the fact that semen volume and sperm concentration were higher, although non-significant before deworming. So, numerical multiplication of two higher values might have produced difference between before-deworming and after-deworming groups in CB bulls. Results indicated beneficial effect of deworming on sperm concentration. However, pre-treatment effect was significant ($p = 0.05$) in MA ($p = 0.03$) and IM. These pre-treatment effects indicated that there may be significant difference for MA and IM (%) but that have been masked due to pre-treatment effect. Contrarily, Schroder *et al.* (1986) have reported increase in semen volume, post-thawed motility and sperm concentration and decrease in sperm abnormality after deworming treatment. There was no effect on testis and epididymis confirmed elsewhere by necropsy (Schroder *et al.*, 1986). The present results are in line with Schroder *et al.* (1986) who reported that repeated treatment with Ivermectin does not affect the semen volume, density, colour, motility, pH, percentage live sperm and sperm morphology. Schroder *et al.* (1986) further concluded that Eqvalan has favourable effect on stallion fertility as most sperm parameters examined were significantly improved in treated animals as compared to control. Following treatment in boars, there were no overall treatment differences in total number of sperm per ejaculate and no adverse treatment effects were exhibited on spermatogenesis or libido in boars as observed by Holste (1984).

Deworming is considered to be of benefit for improving the body condition of breeding bulls (Volkmann, 2011), however very commonly used insecticides can have devastating effects on semen

quality of bulls and rams (Kim *et al*., 2005; Wang *et al*., 2007; Zhang *et al*., 2008). The main activity of Doramectin and other members of this group reportedly is its agonistic function to γ-aminobutyric acid (GABA) (Turner and Schaeffer, 1989). In vertebrates, high concentrations of GABA and GABA-receptors have been found in hypothalamus, pituitary and some peripheral organs such as ovary (Elias, 1985). In bovine species, a positive effect of Ivermectin was observed by advancing the age of puberty (Larson *et al.,* 1995; Mejia *et al.,* 1999). Treated heifers were light in weight at sexual maturity and displayed increased follicular development compared to control animals (Whittier *et al.,* 1999). These findings together with our results support the hypothesis that Doramectin has a positive influence on male and female fertility. In our study the positive effect was most pronounced in RT (Table 2) showing significant group differences 5 weeks after Doramectin application ($p = 0.027$) which almost corresponds to the duration of spermatogenesis in bovines (Amann, 1981).

Conclusion: The present study demonstrates that a single intramuscular application of Doramectin does not negatively influence the quality of bovine semen. Rather it may be speculated that Doramectin has favourable effect on semen characteristics as most parameters examined were significantly improved in treated versus control bulls.

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