

Full Length Research Paper

Postpartum serum biochemical profile of Sudanese cystic ovarian crossbred dairy cattle

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Received 27 March, 2017; Accepted 12 May, 2017

Cystic ovarian disease (COD) is an ovarian dysfunction in cows resulting in a serious economic loss in the dairy industry. This study was conducted to examine the hemoglobin (Hb) concentration, serum total protein (TP), phosphorus (P), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) levels of Sudanese crossbred (Friesian x Kenana) cows with COD in semi-closed condition. Forty-five dairy cows were divided into two groups. Group A (n= 30) were the cows with COD, and group B (n= 15) were healthy normal cycling cows (NC) that served as healthy control. Diagnosis of COD was based on history of frequent prolonged signs of estrus and per rectal palpation. Per rectal palpation for the uterus and ovaries was done weekly. A cow having a large follicle in the ovary that remained at the same position for three successive palpations or more was considered having COD. Results of the blood analysis showed that the serum levels of P, Cu, Zn and Mn of cows with COD were significantly lower ($P < 0.05$) than those of NC cows (5.2 ± 1.3 vs. 6.7 ± 2.5 mg/dl, 0.41 ± 0.3 vs. 0.72 ± 0.3 ppm, 0.5 ± 0.3 vs. 0.7 ± 0.3 ppm and 0.4 ± 0.2 vs. 0.6 ± 0.2 ppm, respectively). No differences ($p > 0.05$) in Hb concentration (7.5 ± 1.2 vs. 7.4 ± 1.1 g/dl), serum TP (6.8 ± 1.2 vs. 6.5 ± 0.7 g/dl) and Fe (3.7 ± 1.3 vs. 3.7 ± 1.9 ppm) were observed between the two groups. This study reported reduced serum minerals (P, Cu, Zn and Mn) levels in Sudanese crossbred dairy cows with COD as compared to NC cows. Future studies are still needed to highlight the contribution of these minerals in inducing COD.

Key words: Cystic ovarian disease, deficiency of minerals, dairy cow.

INTRODUCTION

Over the past few decades, milk yield per cow has relatively increased due to a continuous genetic selection, improvement of nutrition and herd management (Oltenucu

and Broom, 2010). Simultaneously, dairy cow fertility has significantly declined (Butler, 2003). Reproductive performance is an essential factor for assessing the dairy

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Table 1. Proximal composition of supplementary feed provided to dairy cows.

DM	CP	EE	NFE	Ash	Moisture
92.40	20.80	6.40	52.20	4.90	7.60

Table 2. Proximal composition of forages of Abu 70 (*Sorghum vulgare*) and Alfalfa/Burseem grown at river Nile state as DM% (AbuDamir et al., 1983).

Feed	DM (%)	CP (%)	CF (%)	EE (%)	NFE (%)	Ash (%)
Abu70	93.50	10.73	40.14	1.37	37.15	10.66
Burseem	93.48	22.93	30.40	1.63	32.70	12.35

Table 3. Mineral composition of forages of Abu 70 and Barseem grown at river Nile state as DM% (AbuDamir et al., 1983).

Feed	Ca	Mg	P	Na	K	Cu	Zn	Mn
Abu70	0.44	0.30	0.24	0.02	2.64	7.84	25.4	53.1
Burseem	2.1	0.28	0.22	0.034	3.18	10.38	22.0	55.6

cow profitability. It is known that the end product of the reproductive process is a result of a close and well-orchestrated interaction between hypothalamus, pituitary, ovary and the uterus (Carruthers et al., 1980). The complexity of fertility suggests that any factor that interferes with the function of one or more organ would be influential to the general reproductive health (Christensen et al., 2012). One of the most common ovarian dysfunctions during early postpartum period (PPP) is ovulation failure, and consequent formation of ovarian cyst (Opsomer et al., 1998). The cystic ovarian disease (COD) is an important cause of subfertility in dairy cows as it extends the calving interval (Vanholder et al., 2006). This extension, in addition to the treatment cost and the increasing involuntary culling rate, would result in considerable loss for the dairy farmers (Bartlett et al., 1986; Vanholder et al., 2006). It has become clear that COD is the consequence of malfunction of the hypothalamic-pituitary-gonadal axis (Peter, 2004). The cows with high milk yield were more susceptible to develop infertility (Lucy, 2001). High milk production associated with negative energy balance (NEB) during the early PPP, was reported as a predisposing factor for the COD (Vanholder et al., 2006). The role of the NEB in cyst formation remains inconclusive (Butler, 2003). However, it was suggested that cows with a longer period of NEB, poor liver functions and low circulating insulin-like growth factor-I (IGF-I) concentrations in the early PPP were likely to develop inactive or cystic ovaries and persistent corpus luteum (Zulu et al., 2002a; Zulu et al., 2002b). The genetic factor may also be involved (Hooijer et al., 2001). Certain animal lines such as the Holstein Friesian were genetically predisposed to develop COD

(Vanholder et al., 2006). Moreover, it has been reported that single or combined mineral (Cu, Co, Se, Mn, I, Zn and Fe) deficiency can induce reproductive failure (Hidiroglou, 1979; Ahmed et al., 2017).

In the Sudan, to fill the gap of shortage in milk production, some local cow breeders had imported Holstein-Friesian cattle since 1976 (Rahman and Alemam, 2008). Due to the widespread distribution of crossbred dairy cows, more research is required to investigate the incidence and prevalence of all infertility problems. This study was conducted to estimate some minerals serum levels in Sudanese crossbred dairy cows with COD.

MATERIALS AND METHODS

This study was carried out in the River Nile State, Sudan, during the year 2014. Forty-five crossbred (Friesian x Kenana) dairy cows were included. They were under semi-closed system as they were allowed to graze from 7 to 10 am. Their ages ranges between 5 and 11 years, and their body condition scores (Wildman et al., 1982) were from 3.0 to 3.50. The cows were milked twice a day. They were fed roughages, composed of Abu 70 (*Sorghum vulgare*) and Alfalfa (AbuDamir et al., 1983) (Tables 2 and 3), in addition to a supplementary feed that was prepared to meet their production requirements (Table 1). The cows were divided into two groups. Cows in group A (n=30) were diagnosed having cystic ovarian disease (COD), whereas group B cows (n=15) were healthy and normally cycling (NC) that served as control. The COD was diagnosed based on history of frequent prolonged signs of estrus, and further by per rectal palpation (Hafez and Hafez, 2000; Noakes et al., 2001). Per rectal palpation for the uterus and ovaries was done every week starting from the third postpartum week as a routine practice for each cow. A cow having a large sac-like fluid filled structure in the ovary that remained at the same position for

Table 4. Hemoglobin (Hb) concentration (g/dl), serum total protein (TP) (g/dl) and serum levels of P, Cu, Zn, Fe and Mn in cows with COD (group A) and NC cows (group B).

Parameter	Group A	Group B
Hb (g/dl)	7.4 ± 1.1 ^a	7.5 ± 1.2 ^a
TP (g/dl)	6.5 ± 0.7 ^a	6.8 ± 1.2 ^a
P (mg/dl)	5.2 ± 1.3 ^a	6.7 ± 2.5 ^b
Cu (ppm)	0.4 ± 0.3 ^a	0.7 ± 0.3 ^b
Zn (ppm)	0.5 ± 0.3 ^a	0.7 ± 0.3 ^b
Fe (ppm)	3.7 ± 1.3 ^a	3.7 ± 1.9 ^a
Mn (ppm)	0.4 ± 0.2 ^a	0.6 ± 0.2 ^b

Results are shown as mean ± standard deviation (SD). Values with different superscripts in the same row differ significantly ($P < 0.05$).

three or more successive palpations was considered having COD.

Collection of blood samples

Ten milliliters of blood were collected from the jugular vein of each cow. Two milliliters in heparinized tube was used for estimation of Hb concentration, and 8 ml in sterile tube for estimation of TP and the minerals (P, Cu, Zn, Fe and Mn). The blood in the 8 ml-tube was allowed to clot by leaving it undisturbed at room temperature for about 30 min. The clot was then removed by centrifugation at 2000 g for 10 min and sera were stored at -20°C until analysis.

Measurement of Hb, total protein and minerals serum levels

The hemoglobin concentration was estimated within two hours from blood collection using the standard Sahli's method. The serum total protein concentration was estimated with a commercial kit (Biuret Colorimetric kit, Spinreact, Spain). The serum levels of P, Cu, Zn, Fe and Mn were measured using the Phoenix -986 atomic absorption spectrophotometer.

Statistical analysis

The statistical analysis was performed using the SPSS version 20. The Independent Sample T test was used to compare the means between the two groups. Results were expressed as mean ± standard deviation (SD). Significant difference was considered at $p < 0.05$.

RESULTS

The means ± standard deviation (SD) of Hb concentration, serum total protein and serum minerals levels of group A and group B are shown in Table 4. No differences ($p > 0.05$) were observed in Hb concentration (7.4 ± 1.1 vs. 7.5 ± 1.2 g/dl), serum TP (6.5 ± 0.7 vs. 6.8 ± 1.2 g/dl) and the level of serum Fe (3.7 ± 1.3 vs. 3.7 ± 1.9 ppm) between the two groups. However, the serum levels of P, Cu, Zn, and Mn were lower ($p < 0.05$) in COD

(group A) than those of NC cows (group B) (5.2 ± 1.3 vs. 6.7 ± 2.5 mg/dl, 0.41 ± 0.26 vs. 0.72 ± 0.29 ppm, 0.5 ± 0.3 vs. 0.7 ± 0.3 ppm and 0.4 ± 0.2 vs. 0.6 ± 0.2 ppm, respectively).

DISCUSSION

Cystic ovarian disease (COD) is one of the most important infertility problems in dairy cows. It occurs most frequently during the PPP one to two months after calving at a time when ovarian function usually restarts (Vanholder et al., 2006). It is characterized by the presence of one or more large anovulatory follicular cysts in the ovary, unilateral or bilateral, as well as abnormal pattern of estrus (Peter, 2004). The existence of such ovulatory follicular cysts would extend the calving-to-conception and calving intervals resulting in economic losses for dairy industry.

In an earlier study (Nadaraja and Hansel, 1976), COD was induced by suppressing bovine luteinizing hormone (LH) using either estradiol or antibodies against LH. Furthermore, exogenous cortisol was used to suppress the LH surge, ovulation and the behavior of estrus (Stoebel and Moberg, 1982). It has been proposed that there is a metabolic signal required for an efficient LH surge, and poor nutrition (stress) and NEB would interrupt this signal (Mwaanga and Janowski, 2000; Johnson, 2004). In the status of NEB, some hormonal and metabolic changes might increase the COD formation at the hypothalamus-pituitary as well as ovary-follicle levels (Diskin et al., 2003). During NEB, there are decreased blood levels of glucose, IGF-I, insulin and leptin (Beam and Butler, 1999; Block et al., 2001), and increased concentrations of metabolites such as non-esterified fatty acids and β -hydroxybutyrate (Vanholder et al., 2006). The IGF-I and insulin stimulate follicular development by enhancing the steroidogenesis, and differentiation of granulosa cell (Davoren et al., 1986; Zulu et al., 2002a). Leptin is a hormone produced by adipose cells, and is required to induce the first postpartum LH surge (Elias and Purohit, 2013). Nutrition and suckling were the two critical factors that delayed the onset of estrous cycles in postpartum cows (Lamb, 2012). It was reported that the postpartum period was found to be extended in Sudanese crossbred dairy cows due to many reasons and COD was one of them (Elzubeir and Elsheikh, 2004).

The result of this study shows that the serum TP of cows with COD is not different from that of the NC cows (Table 4). This result is consistent with a recent study by Yotov et al. (2014). Moreover, the Hb concentration is also not significantly different between the two groups (Table 4), which agrees with an earlier study (Larson et al., 1980). The serum Fe of cystic and control cows were nearly the same (Table 4). This result was expected as the Hb concentrations of the cystic cows were normal (Table 4), and neither anemia nor hemorrhage was

observed in both groups.

The serum phosphorus of the COD cows was significantly lower than that of the NC cows (Table 4). Similar results were reported by some recent studies (Bindari et al., 2014; Phiri et al., 2007; Yotov et al., 2014). Phosphorus is essential in every metabolic pathway, energy utilization and transfer as well as being part of nucleic acids structure (Murray et al., 2003).

This study also revealed that serum Cu of the cows with COD was lower than that of the NC cows (Table 4). This came in line with a previous research (Yasothai, 2014). Cu deficiency is associated with subfertility and delayed estrus or anestrus (Kumar et al., 2011; Yasothai, 2014). It is a co-factor for important enzymes like the amine oxidase, copper-dependent superoxide dismutase, cytochrome oxidase and tyrosinase (Murray et al., 2003). It was reported that Cu and gonadotropin releasing hormone (GnRH) complexes were more efficient in stimulating the secretion of the LH and FSH than the GnRH alone (Michaluk and Kochman, 2007).

The results also show that cows with COD had significantly lower serum Zn level than the NC cows (Table 4). Earlier studies reported that Zn deficiency was associated with reduced fertility, and that Zn supplementation was successfully used to increase the conception rate (Marai et al., 1992; Moellers and Riese 1988). Zinc was also found to be essential for recovery of the endometrium after calving and the accelerated return to estrus and normal reproductive performance (Yasothai, 2014). These different effects may be due to its metabolic effect on estrogen, progesterone and prostaglandins (Favier, 1992). Moreover, the nuclear steroid receptors are all Zn finger proteins (Favier, 1992). In addition, Zn has anti-apoptotic and antioxidant properties (Ebisch et al., 2007).

The results also showed that Mn serum level of cows with COD was significantly lower than that of their respective NC cows. This result was quite consistent with the results of previous studies (Corah, 1996; Yasothai, 2014). Deficiency of Mn was associated with occurrence of COD and poor follicular development with delayed ovulation (Corah, 1996). Mn can influence the reproductive efficiency in several ways. First, Mn is involved in all metabolic processes (Davis et al., 1990; Hansen et al., 2006; Tuormaa, 1996). Second, it acts as a co-factor for the enzymes that catalyze the biosynthesis of cholesterol (Tuormaa, 1996). Cholesterol is a precursor for all steroid hormones including the sex hormones (Murray et al., 2003). Mn was also reported to induce the hypothalamic secretion of the luteinizing hormone releasing hormone (Lee et al., 2007).

Conclusion

The current study examined the Hb concentrations and serum total protein (TP), phosphorus (P), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) levels of 30

dairy cows diagnosed having COD and compared with 15 normal cyclic (NC) cows. Results show that there were no differences in the Hb concentrations and the serum levels of TP and Fe between the two groups ($p > 0.05$). However, the serum levels of P, Cu, Zn and Mn of cows with COD were significantly lower than those of the NC cows ($p < 0.05$). This study reported decreased serum minerals (P, Cu, Zn and Mn) levels in Sudanese crossbred dairy cows with COD as compared to NC cows. Future studies with a larger sample size are recommended to highlight the contribution of these minerals in inducing COD in these cows.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the owners of the animals who allowed them to diagnose and take blood from their cows. Khalid Abdoun, Faculty of Food and Agriculture Science, King Saud University is acknowledged for fruitful discussion.

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