

Full Length Research Paper

Blood biochemical profile of Sudanese crossbred repeat breeder cows

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Repeat breeder (RB) is a cow that fails to conceive from three or more successive insemination, but with normal estrus and absence of detectable clinical abnormalities. It is one of the major problems in dairy production. In the present study, the plasma biochemistry of Sudanese crossbred RB cows was examined. Plasma glucose, total cholesterol (TC), triacylglycerol (TG), total protein (TP) and urea nitrogen were estimated with the spectrophotometer while the plasma levels of Cu, Zn, Fe, and Mn were estimated using the atomic absorption spectroscopy. The results indicate the plasma levels of glucose, Zn, Mn and Fe of RB were significantly lower ($P < 0.05$) than that of the normal cyclic (NC) cows. Moreover, the plasma level of urea nitrogen of RB was significantly higher ($P < 0.05$) than that of the NC cows. According to the obtained results, it can be concluded that correcting the plasma glucose, Zn, Mn and Fe as well as lowering the urea nitrogen may be a part of an effective strategy for treatment of repeat breeding syndrome in Sudanese crossbred cows.

Key words: Repeat breeder cows, plasma biochemistry, Sudanese crossbred cows, fertility of cows.

INTRODUCTION

Repeat breeder (RB) cow is the one that cycles normally and with no clinical abnormality, but fail to conceive from three or more successive insemination (Gustafsson and Emanuelson, 2002). The economic loss associated with RB is high, and is due to the cost of insemination, decreased productivity and the losses due to the involuntary culling (Bonneville-Hebert et al., 2011). The good nutritional condition is vital to the animal health and reproduction. Changes in plasma biochemical or

hematological parameters may be the cause of the reproductive insufficiency (Noakes et al., 2001). For instance, the blood glucose level was shown to be lower in RB than normal cycling (NC) cows and buffaloes (Guzel and Tanriverdi, 2014; Sabasthin et al., 2012). In addition, it has been reported that there are lower plasma cholesterol levels in RB than NC buffaloes and cows (Sabasthin et al., 2012; Amle et al., 2014). Nevertheless, Guzel and Tanriverdi (2014) have reported that there is

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no significant difference in blood cholesterol levels between the RB and NC cows. The plasma total protein TP was also estimated in RB cows and buffaloes in previous studies. However, the results were not consistent. It has been reported that there is no significant difference in the plasma TP levels between the RB and NC cows (Guzel and Tanriverdi, 2014; Kurykin et al., 2011). However, some studies have reported that the plasma TP of RB is significantly lower than that of the NC buffaloes and cows (Amle et al., 2014; Sabasthin et al., 2012). Moreover, the plasma urea was shown to be higher in RB than NC cows in previous studies (Kurykin et al., 2011; Sabasthin et al., 2012). Deficiencies of Mn, Cu, Fe and Zn have been associated with immune suppression, anemia, and poor fertility in dairy animals (Ingraham et al., 1987; Akhtar et al., 2014).

In the present study, certain plasma biochemical parameters of the RB have been examined and compared them with that of the NC crossbred cows in Khartoum State, Sudan. The crossbred cattle of Sudan are unique in being hybrids between Kinnana or Butana (local breeds) and the Holstein or Jersey. To the best of our knowledge, this study is the first one that has examined the Sudanese RB cow from a biochemical point of view. Therefore, it is probably helpful in treatment of the repeat breeding syndrome by correcting the plasma levels of glucose, Zn, Mn, and Fe, in addition to lowering the urea nitrogen.

MATERIALS AND METHODS

Animals

This study has been approved by the research board of the Faculty of Veterinary Medicine, University of Khartoum. It was conducted at Khartoum State, in private dairy farms during the period from the first of April until the end of May 2015. Ninety dairy uniparous and multiparous lactating crossbred cows (Friesian or Jersey × Kenana or Butana). The age range from 5 to 8 years old and body condition score was from 3 to 3.5, according to the five-scale point system (Ahmed and Elsheikh, 2014). Cows were divided into two groups, each contains about 45 cows. The first group (group 1) is the RB cows. The RB cows were recruited according to the case history from the owners, farm records, visual diagnosis and by rectal examination as the RB cows had regular estrous, multiparous, young (3 to 12 years) without anatomical defects in the genital tract and free from vaginal discharges (Warriach et al., 2008). The second group (group 2) serves as a reference group including healthy NC cows.

Collection of blood samples

Ten milliliters of blood samples were collected from the jugular veins of RB and NC cows in anticoagulant-coated tubes. Four milliliters in sodium fluoride-coated tubes for estimation of glucose and 6 ml in EDTA-coated tubes for estimation of all others biochemical parameters. The tubes were transferred in an ice container to the laboratory. Cells were removed from plasma by centrifugation at 2000 rpm for 10 min. Plasma was stored at -20°C until further analysis. Blood glucose, total protein TP, triacylglycerol

TG, total cholesterol TC, and urea nitrogen were determined with the Spectrophotometer using commercial kits (Spinreact, Spain). The blood glucose was estimated using glucose oxidase method (GOD-POD, liquid kit). The plasma total protein was estimated using Biuret method (Biuret, Colorimetric kit). Plasma triacylglycerol was estimated using lipoprotein lipase method (GPO-POD, liquid kit). Plasma total cholesterol was estimated using cholesterol esterase method (CHOD-POD, Liquid kit). Blood urea was estimated using the urease method (Urease –GLDH, Kinetic UV kit). The plasma levels of Cu, Zn, Fe, and Mn were measured using the Phoenix -986 AAS Atomic Absorption Spectrophotometer.

Statistical analysis

The data were analyzed using the independent sample T test to compare means between the two groups. Result was expressed as mean ± standard deviation (SD); P-value < 0.05 was considered as significant. The analysis process was done using SPSS version 22.

RESULTS AND DISCUSSION

The results obtained during this study indicate that the plasma glucose levels of RB cows were lower than that of the NC cows. The difference is significant with p-value = 0.001 (Table 1). The plasma levels of TC and TG of RB cows were not significantly different from that of the NC cows (p-values = 0.075 and 0.063, respectively, Table1). TP plasma levels of RB were significantly higher (p-value= 0.02) than that of the NC cows, but still within the normal range (Table 1). Results indicate that the plasma levels of urea nitrogen of RB cows were significantly higher (p-value = 0.04) than that of the NC cows (Table 1) while the plasma levels of Cu was not significantly different (p-value= 0.3) in RB cows compared to that of the NC cows (Table 2). Plasma levels of Zn, Fe, and Mn were significantly higher (p-values = 0.04, 0.03 and 0.000, respectively) in NC cows than that of the RB cows (Table 2).

The reproduction and nutrition interplay was confirmed from early studies (Noakes et al., 2001; Saraswat and Purohit, 2016). It has been reported that single or combined mineral (Cu, Co, Se, Mn, I, Zn, and Fe) deficiency can induce reproductive failure (Hidiroglou, 1979). Furthermore, the quality of the oocytes depends on the metabolic and endocrine status of the cow (Kurykin et al., 2011). In the present study, the plasma biochemistry of RB have been investigated and compared with that of their respective healthy NC cows. It was found that the plasma glucose levels of RB cows were significantly lower ($P < 0.05$) than that of the NC cows (Table 1). This result is consistent with previous studies (Guzel and Tanriverdi, 2014; Kurykin et al., 2011; Sabasthin et al., 2012). Hypoglycemia in RB cows may be attributed to one of the three causes. First, is the increased peripheral glucose uptake; second, is the failure of gluconeogenesis or glycogenolysis; third, is endogenous hyperinsulinemia (Mukherjee et al., 2011). No significant difference was found between the plasma

Table 1. Mean \pm SD of the plasma of glucose, total cholesterol (TC), triacylglycerol (TG), total protein (TP) and urea nitrogen (mg/dl) in the repeat breeder (RB) and normal cyclic (NC) cows.

Parameter	Cows	Number	Mean \pm SD	P-value
Glucose	RB	45	58.4 \pm 17.8 ^a	0.001
	NC	45	75.2 \pm 26.9 ^b	
TC	RB	41	87.94 \pm 30.2 ^a	0.075
	NC	45	74.5 \pm 37.9 ^a	
TG	RB	41	68.9 \pm 13.8 ^a	0.063
	NC	45	62.4 \pm 17.6 ^a	
TP	RB	48	4.5 \pm 0.3 ^a	0.02
	NC	45	4.3 \pm 0.6 ^b	
Urea	RB	48	33 \pm 15 ^a	0.04
	NC	45	24 \pm 14 ^b	

Values with different superscripts within a parameter differ significantly ($P < 0.05$).

Table 2. Mean \pm SD of plasma copper, zinc, manganese and iron (μ g/dl) in the repeat breeder (RB) and normal cyclic (NC) cows.

Parameter	Cows	Number	Mean \pm SD	P-value
Copper	RB	43	99.7 \pm 23.8 ^a	0.3
	NC	43	91.8 \pm 38.3 ^a	
Zinc	RB	45	18.8 \pm 17.7 ^a	0.04
	NC	45	26.4 \pm 17.4 ^b	
Iron	RB	42	124.6 \pm 27.6 ^a	0.03
	NC	36	136.6 \pm 18.1 ^b	
Manganese	RB	42	0.1 \pm 0.04 ^a	0.000
	NC	45	0.22 \pm 0.18 ^b	

Values with different superscripts within a parameter differ significantly ($P < 0.05$).

TC levels of RB ($P < 0.05$) and the NC cows (Table 1). This finding is consistent with a previous study (Guzel and Tanriverdi, 2014). However, it is inconsistent with other reports (Amle et al., 2014; Sabasthin et al., 2012). Cholesterol is a steroid and is a precursor of the steroid hormones that include sex hormones (Berg et al., 2002). In addition, our study demonstrates that RB cows have no significant different ($P < 0.05$) plasma TG compared to that of the NC cows (Table 1). Lipids are rich energy source and required for oocyte maturation (Dunning et al., 2014). Nevertheless, our results indicate that plasma TG and TC have no effect on repeat breeding syndrome, at least in our case.

The plasma TP levels of RB cows in our study was significantly higher than that of the NC cows but still

within the normal range (Table 1). The plasma TP may be caused by high protein content in the diet. We also found that the plasma urea nitrogen of the RB cows was significantly higher ($P < 0.05$) than that of the NC cows (Table 1). This result comes in a line with previous studies (Godden et al., 2001; Kurykin et al., 2011). The high plasma level of urea nitrogen may be the cause of production of abnormal oocytes in RB cows (Kurykin et al., 2011). The elevated plasma urea nitrogen can change the uterine fluid composition, lowering the uterine pH, and reducing the conception rates (Butler et al., 1996; Jordan et al., 1983). This elevated plasma urea nitrogen level may be caused by the high protein content in the diet.

Copper supplementation was used to correct the

subnormal fertility of cows (Ingraham et al., 1987). One important role of copper is being a cofactor for enzymes like the amine oxidase, copper-dependent superoxide dismutase, cytochrome oxidase, and tyrosinase. Furthermore, copper plays a critical role in female fertility (Ingraham et al., 1987). It has been reported that copper makes complexes with gonadotropin-releasing hormone (GnRH) (Michaluk and Kochman, 2007). These complexes are more efficient in the releasing of the luteinizing hormone and follicle-stimulating hormone than the GnRH alone (Michaluk and Kochman, 2007). However, no significant difference was found in the blood copper levels between the RB and NC cows (Table 2). This result is in agreement with that of Ceylan et al. (2008). However, it is not in agreement with a previous report (Ahmed et al., 2010). This may indicate that other factors may induce repeat breeding syndrome rather than copper deficiency alone.

The plasma concentration of zinc was significantly higher ($P < 0.05$) in NC than in RB cows. This result is in agreement with other studies (Akhtar et al., 2014; Marai et al., 1992). In fact, zinc supplementation was used to improve the conception rate for up to 80% in RB buffalos (Marai et al., 1992). Zinc is a cofactor for more than 300 metalloenzymes (McCall et al., 2000). These metalloenzymes are spanning all the enzyme classes. Zinc metalloenzymes are involved in almost every biologic process (Beyersmann and Haase, 2001; Maret, 2013; Park et al., 2004). For instance, the DNA transcription and protein bio-synthesis (Ebisch et al., 2007). Since DNA transcription is a major part for the development germ cells, zinc is vital for reproduction (Ebisch et al., 2007). It has been reported that zinc is essential for the maintenance and repairing of uterine lining following the calving, and it accelerates the return to normal reproductive efficiency and estrus (Yasothai, 2014). This is probably because zinc (and also copper) diffuses through the uterine epithelial cells into the lumen of the reproductive system. This diffusion creates osmosis that causes the transport of water out of the epithelial cells into the lumen of the uterus (Alavi-Shoushtari et al., 2012). Moreover, zinc finger proteins are implicated in the gene expression of the receptors of the steroid hormones. In addition, zinc has antioxidant as well as anti-apoptotic properties (Ebisch et al., 2007).

Significant difference ($P > 0.05$) was found in the plasma Mn levels between the RB and the NC cows (Table 2). This result is consistent with other studies (Das et al., 2009; Kalita and Sarmah, 2006). Manganese is involved in all metabolic processes (Davis et al., 1990; Hansen et al., 2006; Watts, 1990). The earliest studies demonstrated that a manganese deficiency causes defective ovulation and subfertility of female and male (Tuormaa, 1996). It is suggested that Mn act as a cofactor for mevalonate kinase and farnesyl pyrophosphate synthase; enzymes involved in the production of squalene, a precursor of cholesterol

(Davis et al., 1990; Hansen et al., 2006; Hidioglou, 1979; Tuormaa, 1996; Watts, 1990). However, in a more recent study, it is reported that treating heifers with different concentrations of Mn did not affect the serum cholesterol (Hansen et al., 2006). This study indicates that even low serum levels of Mn are sufficient for cholesterol synthesis (Hansen et al., 2006). This result is quite consistent with our data of plasma cholesterol that showed no significant difference between the NC and the RB cows (Table 1). Varying concentrations of Mn did not affect the heifer pregnancy rate, conception rate, age at conception, and services to conception (Hansen et al., 2006). The mode of action by which Mn influences reproductive performance is unclear (Hansen et al., 2006).

Our result demonstrates that the iron plasma level of RB was significantly lower ($P < 0.05$) than that of the NC cows. This result is consistent with recent works (Ahmed et al., 2010; Akhtar et al., 2014). The role of iron is the formation of hemoglobin and myoglobin required for the oxygen transport and storage. Moreover, iron is also required for the cytochromes and iron-sulfur protein which are part of the respiratory chain (Murray et al., 2003). Iron is also involved in ferritin formation. Low levels of plasma iron will result in anemia and change in the molarity of the oviduct which will be a cause for failure of conception and embryonic death (Kumar et al., 2011; Modi et al., 2013).

Conclusion

In the current study, the plasma biochemistry of crossbred Sudanese RB cows has been examined. The results demonstrate that the plasma glucose, Zn, Mn and Fe were significantly lower ($P < 0.05$) in RB than in NC cows. The plasma urea nitrogen of RB was significantly higher ($P < 0.05$) than that of the NC cows. It was hypothesized that correcting these biochemical parameters may be an effective strategy for the treatment of the repeat breeding syndrome, however, this hypothesis still needs to be tested in future studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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