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SOME MICROELEMENTS PROFILE IN CYCLIC NON-BREEDING COW SYNDROME (REPEAT BREEDER)

BY

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مستوى بعض العناعر النادره في الأبقار التي تعاني من ظاهرة التفويت الجنسي

مجمع العزب ، غاطف بطر ، جمال شوقى ، سيطات غبط المجيط

أجريت هذه الدراسه على قياس مستوى بعض العناصر النادره (النحاس ، الزنك ، السيلنيوم والمانجنيز) في سيرم الأبقار التي تعانى من التقويت الجنسى . لذلك أستخدم عدد ٣٧ من الأبقار الفريزيان والخليطة لهذا البحث ، منهم ١٦ بقره تعانى من التقويت الجنسى و١٦ بقره أخرى طبيعيه (مجموعه ضابطه) . ثم أخذت عينات دم من كل هذه الحيوانات أثناء فترة الشبق (وجود حويصلة جراف على المبايض) وبعد ذلك فصل السيرم من عينات الدم لقياس مستوى النحاس والزنك والسيلنيوم والمانجنيز .

وقد أشارت النتائج التي نقص ملحوظ في مستوى كل من النحاس والزنك والسيلنيوم والمانجنيز في الحيوانات التي تعانى من التفويت الجنسي عنه في المجموعة الضابطة.

من هذه النتائج نقترح بأن نقص مثل هذه العناصر النادره تقوم بدور مسبب وتكون مسئوله عن حالة التفويت الجنسي في مثل هذه الأبقار .

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SUMMEARY

The present study was conducted to elucidate serum profile of some microelements (copper, zinc, selenium and manganese) in cyclic non-breeding (CNB) dairy cows. Thirty two Friesian and Crossbred dairy cows were used (16 CNB cows and 16 normal cyclic herdmates served as control). Blood samples were collected from all animals during follicular phase, serum was harvested and assayed determination of copper, zinc, selenium and manganese. The results revealed significant (P < 0.05) lower serum levels of copper, zinc and manganese in CNB than control herdmates. Likewise serum selenium showed highly significant (P < 0.01) decrease in comparison to their contemporaries. It could be suggested that deficiency of copper, zinc, selenium and manganese might play a contributing vital factor incriminating in cyclic non-breeding dairy cows syndrome.

INTRODUCTION

The introduction of the metabolic profile test for dairy and beef cattle (PAYNE et al., 1970; DOWNIE 1975) has provided a tool which can be used in the diagnosis of nutritional infertility. Metabolic profiles have been used in an attempt to monitor the health (ZAMET et al., 1979), reproductive (ROWLANDS et al., 1980) and nutritional (LEE et al., 1978) status of dairy cows.

Ruminants frequently are subjected to severe dietary deficiencies of trace elements such as copper, cobalt, selenium, iodine, manganese and zinc. Concomitant infertility in cattle is believed to be associated with enzymatic dysfunction resulting from these deficiencies (HIDIROGLOU 1979) in virtue of this, trace elements may function as co-factors, as activators of enzymes, or stabilizers of secondary molecular strcture (VALEE and WACKER 1976).

The importance of trace elements for optimum reproductive function is widely discussed. Copper and zinc in particular have received significant attention (MILLS and DAVIES 1979). Both elements occur as prosthetic groups in a variety of metallo-enzyme. Moreover, hypocuprosis in cattle is associated with female reproductive disorders mostly early embryonic loss. Also, zinc, manganese and selenium deficiencies have been reported to cause reproductive failure in the female or disturbances in spermatogenesis, depresses conception rate and

fertilization rate in cattle (McCHOWELL and HALL 1970; SEGERSON et al., 1977 and HIDIROGLOU 1979).

It was demonstrated that the crude protein content of the diet could alter concentrations in the uterine secretion of urea, magnesium, potassium, phosphorus and zinc (JORDAN et al., 1983). Moreover, embryonic mortality was associated with marked changes in the concentration of several ions in the uterine fluid (AYALON 1984). Concomitantly, the binding of progesterone to plasma and endometrial cytosol is markedly influenced by the concentration of zinc and that the protein concentration influences these zinc-induced effects. The action of zinc in the target tissue is a protein mediated process whereby the metal and protein parameters may influence the degree of uptake, thus progesterone there is a three-way, zinc-progesterone-protein interaction (HABIB et al., 1980) Evidently, fertility appears to be related to progesterone concentration in the cycle preceding the insemination, greater fertility being associated with higher blood progesterone concentrations (GERLOFF and MORROW 1986). substantiated by MARCUS and AYALON (1981) who got significant improvement in fertility of Friesians with supplemental progesterone given in a slowly absorbed form post-insemination.

Among the microelements selected for this study, zinc. copper, manganese and selenium were included as a deficiency of these minerals have been shown to adversely affect a variety of reproductive functions in animals.

MATERIAL AND METHODS

The present investigation included thirty Friesian-Holstein and crossbred dairy cows. Sixteen of them were cyclic non-breeding cows where they had been bred by normal tested fertile bull for more than 3 times in successive estrus phases but failed to conceive. Moreover, these cases were thoroughly examined and proved to be free from any detectable genital tract abnormalities. The selected control group (16) had no previous history of repeat breeding and they were clinically normal without any detectable genital abnormalities. All animals were milked thrice daily. animals were housed in open yards and were stall fed and had unrestricted access to hay and 8-10 Kg concentrate feed of the following formula: 11% crude protein, 50% bran, 15% cotton seed cake, 15% yellow maize, 9.5% rice polish, 3% soya bean cake, 3% molase, 3% lime salt and 1.5% sodium chloride. All animals had been regularly tested for freedom from brucellosis. camylobacteriosis, trichomoniasis, IBR-IPV and were routinely vaccinated against brucellosis and IBR-IPV.

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Blood samples were collected from all animals during follicular phase. The control cows were followed up until diagnosed and confirmed to be pregnant following 1-2 services and the non-pregnant ones were excluded from the control group. Serum was harvested and stored deep frozen until assayed for copper, zinc, selenium and manganese. Serum copper, zinc, selenium and manganese were assayed with atomic absorption spectophotometer (OSER 1965) by the center biochemistry laboratory, College of Agricultrue, Zagazig University. Statistical analysis was done according to SNEDECOR and COCHRAN (1967).

RESULTS

Concentration of serum copper, zinc, selenium and manganese values are presented in table (1). Serum copper concentrations in the CNB cows were lower (P < 0.05) in all cases $(0.56 \pm 0.0.4 \text{ ug/ml})$ than the control cows $(0.73 \pm 0.04 \text{ ug/ml})$ and statistical analysis did notice significant difference (table 1).

Significant variation (P < 0.05) was observed between serum values of zinc of the CNB cows (1.48 \pm 0.17 Ug/ml) and

the control herdmates (1.95 ± 0.15 ug/ml).

As for selenium serum values in repeat breeding cows $(0.096\pm0.008~\text{ug/ml})$ compared with the control group $(0.142\pm0.01~\text{ug/ml})$, statistically significant difference (P < 0.01) was noticed between the two groups.

Difference in serum levels of manganese between the CNB group (0.21 \pm 0.03 ug/ml) and the control ones (0.33 \pm 0.04

ug/ml) was found to be significant.

Table (1): Serum levels of copper, zinc, selenium and manganese in the cyclic non-breeding and control dairy cows.

Group	No.	Copper (ug/ml)	Zinc (ug/ml)	Selenium (ug/ml)	Manganese (ug/ml)
Control	16	0.73±0.04	1.95±0.15	0.142±0.01	0.33±0.04
cyclic non- breeding	16	0.56±0.04*	1.48±0.17*	0.096±0.008*	0.21±0.03*

Mean±SEM * (P<0.05)

^{** (}P<0.01)

DISCUSSION

A differenitial diagnosis on infertility covers a wide range of pathological conditions and, after eliminating infection known to affect fertility, infestations, stress in its several manifestations, congenital abnormalities and poor quality semen, the problem of poor conception rate to first service remains unsolved and faulty nutrition appears to play a significant role in the malfunctioning of the reproductive system (DOWNIE and GELMAN 1976). Some reports on improved fertility following dietary supplementation in the (MUSTAKIMOV and SIROTENKO 1974), Israel (FRANCOS 1974) and New Zealand (SCALES 1976) confirm the growing interest in this problem. Undernutrition has been shown to suppress reproductive function in the bovine, although it is generally believed that the resulting infertility is due to altered levels of gonadotrophins and/or ovarian steroids although, experimental studies have produced conflicting results (EASDON and CHESWORTH 1980).

The present investigation disclosed a significant difference between the cyclic nonbreeding cows and the control one in serum levels of zine and copper. This conclusion has been emphasized by most studies that have shed considerable light on the importance and role of the trace elements on reproductive functions (HUNTER 1977 and HIDIROGLOU 1979) and fetal development (MILLS and DAVIES 1979). This vital role of trace elements in particular of zinc and copper is now widely recgnized and has received considerable attention as both metals occur as prosthetic groups in a variety metalloenzymes and in a number of species a maternal deficit of either element during pregnancy has been demonstrated to be highly teratogenic (HURLEY 1981). However, zinc has been recognized as a cofactor or constituent of certain enzymes for example carbonic anhydrase, activity of zinc-dependant enzyme in reproductive processes is unknown (BRYAR 1974). Although, some of these involvements may be mediated by influences on pituitary gonadotropin production (YARMOL CHUCK and MESCHYSHEN 1976). Moreover, hypocuprosis and zinc deficiency in cows, ewes and goats are associated with lower conception rate, either prevented implantation or induced early embryonic death (McCHOWELL and HALL 1970; ANKE et al ., 1978 and MORROW 1986). In virtue of these findings the fertility of cows, ewes and goats has been rapidly and dramatically improved when received both zinc and copper supplementation (MAHADEVAN and ZUBAIRY 1969; KOROVINA & MOROZOW 1976 and HUNTER 1977).

Evidently, the levels of manganese and selenium obtained in the present study were significantly lower in the cyclic

non-breeding cows than in the control ones (Table 1). Apparently, these results strongly suggests that the role of these elements is urgently prerequisite for normal reproductive functions. This latter deduction has been substantiated by a considerable amount of data showing the vital role of both elements in runminants for normal fertility since a deficiency of both has been reported to result in decreased conception, delayed ovulation, lowerd fertilization rate and embryonic loss (SEGERSON et al., 1977; HIDIROGLOU 1979 and Morrow 1986). The precise loci of specific manganese involvement in reproductive process remain unknown, though, some evidence suggest that manganese plays a role in the activity of certain endocrine organs (HIDIROGLOU 1979). It was established that maximal uptaKe of manganese occurred during the luteal phase when progesterone production was greatest, it also has been implicated explicity in the synthesis of steroids and hence, of gonadal hormones (BENEDICT et al., 1965). Thus it may be specifically involved in luteal tissue metabolism and/ or activity (HIDIROGLOU & SHEARER 1976 and HIDIROGLOU 1979). Therefore manganese deficiency can in result luteal dysfunction which might explain the connection between manganese imbalance and impaired fertility. Concurrently, supplementation of progesterone, given in a slowly absorbed form 6-18 days postinsemination signigicantly improved fertility in Israeli Friesians (MARCUS and AYALON 1981). Since fertility is related to progesterone concentrations in the cycle preceding the insemination and greater fertility being associated with higher blood progesterone concentrations (GERLOFF and MORROW 1986).

There are conflicting opinions about the effect of selenium deficiency on reproductive performance in runminant, in those studies of impairment of fertility associated with selenium deficiency, the locus of the impairment generally has been embryonic or fetal loss (HIDIROGLOU 1979). IT has been demonstrated that low selenium (< 0.05ppm) appears to account, at least in part, for infertility of dairy herds in some areas in Uganda (LONG and MARSHALL 1973). Also effects of selenium and Vit E alone or in combination on fertilization of ova have been shown that beef cows on an adequate plane of nutrition given a supplement of selenium and Vit, E had 100% fertilization rate (SEGERSON et al., 1977). In other studies, however, prebreeding selenium treatment of beef cows in areas deficient in selenium did not improve subnormal pregnancy rate (SOUTHCOTT et al., 1972 and SCALES 1976).

The aforementioned obtained results open new horizons and have brought forth some additive contributing etiological factors and insights on this complicated, multifaceted problem.

The present results must therefore be regarded as preliminary, and suggestive rather than conclusive, therefore future studies on the direct and indirect etiological or predisposing causes for a cyclic non-breeding syndrome are indicated.

REFERENCES

- Anke M., Grum M., Gropple B., Ludke H. and Partschefeld (1978):
 Der Fiasalz standorstspezifischer mineral staffgemische
 fur wiederkauer in den Mangan Zink und Kupfermangel
 gobieten der DDR. Tierermahrung, Futterung, 9:18.
- Ayalon N. (1984): The repeat breeder problem. Proc. 10 th Int. Cong. on Anim. Reprod. & A.I., Urbana, Vol. III: 41-50 (A review).
- Benedict C.R., Kett J. and Poter J.W. (1965): Properties of funnesyl pyrophosphate synthetase of pig. Liver. Arch. Biochem. Biophys., 110:611.
- Bryar D.P. (1974): Zinc in male sex accessory organs: Distribution and hormonal response in male accessory organs. D. Brandes, ed. Academic Press. New York.
- Downie J.G. (1975): A study of some factors affecting blood components of beef cows. Ph.D. Thesis University of Aberdeen.
- Downie J.G. and Gelman A. (1976): The relationship between changes in body weight, plasma glucose and fertility in beef cows. Vet Rec, 99: 210-212.
- Easdon M.P. and Chesworth J.M. (1980): Effect of undernutrition on the levels of some hormones in the blood of beef heifers. 9 th. Int. Cong. on Anim. Reprod., Fertil. & A.I. (Madrid) Spain. Vol. V.
- Francos G. (1974): Dt. Tierarztl W. Schr. 81: 135. Cited by Downie J.G. and Gelman A. (1976). Vet. Rec. 99: 210-212.
- Gerloff B.J. and Morrow D.A. (1986): Effect of nutrition on reproduction in dairy cattle. In Current Therapy in Theriogenology, 2:310.
- Habib F.K. Muddy S.Q and Stitch S.R. (1980): Acta Endocrinol. 94: 99. Cited by Ayalon (1984) Proc. 10th Int. Cong. on Anim. Reprod. & A.I., Urbana, Vol. 111:41-50(A review).
- Hidiroglou M. (1979): Trace elements deficiencies and fertility in ruminants. J. Dairy Sci. 62: 1195.
- Hidiroglou M. and Shearer D.A. (1976): Concentration of manganese in tissues of cycling and anoestrus ewes. Can J. Camp. Med. 40: 306.
- Hunter A.P. (1977): Some nutritional factors affecting the fertility of dairy cattle. New Zealand Vet. J. 25:305.
- Hurley L.S. (1981): Teratogenic aspects of manganese, zinc and copper in nutrition. Physiol. Rev. 61: 249.

- Jordan E.R., Chapman T.E., Holton D.W. and Swanson L.V. (1983):
 J. Dairy Sci. 66: 1854. Cited by Ayalon (1984) Proc. Lnt.
 Cong. on Anim. Reprod. & A.I., Urbana, Vol. III: 41-50 (A review).
- Korovina E.K. and Morozow A.Z. (1976): Influence of zinc supplement on the fertility of sheep under zinc deficiency conditions. Sov. Agr. Sci. 1:49.
- Lee A.J., Twardoch A.R. and Bubar R.H. (1978): Blood metabolic profiles: Their use and relation to nutritional status of dairy cows. J. Dairy Sci. 61: 1652.
- Long M.E. and Marshall B. (1973): The selenium status of pastures in Uganda. Trop. Agr. (St. Augustine) 50: 121.
- Mahadevan V. and Zubairy A.W. (1969): The influence of copper sulphate supplement feeding on cows for early reproduction and reducing intercalving period. Indian Vet. J. 46: 892.
- Marcus S. and Ayalon N. (1981): Refush Vet. 38:55. Cited by Ayalon (1984) Proc. 10th. Cong. on Anim. Reprod. & A.l., Urbana, Vol. III: 41-50(A review).
- McChowell J. and Hall G.A. (1970): Infertility associated with experimental copper deficiency in sheep, guinea pigs and rats. Page 106 in Trace Element Metabolism In Animals. C.F. Mills ed. E.S. Livingstone, Edinburg.
- Mills C.F. and Davies N.T. (1979): Perinatal changes in the absorption of trace element In: Development of mammalian absorptive process. CIBA Foundation Symposium N, 70, Excerpta Medica, Amsterdam, 247-266.
- Morrow D.A. (1986): Diagnosis, treatment and prevention of reproductive diseases in small and large animals. In Current Therapy In Theriogenology, 2: 317.
- Mustakimov R.G. and Sirotenko Z.P. (1974): Vet. Sel. Khoz. Nauki. Mosk. 1: 41. Cited by Downie J.G. and Gelman A. (1976). Vet. Rec. 99: 210-212.
- Oser B.L. (1965): In Hawks Physiological Chemistry 14 th edition, Tata McGrow Hill Publishing LTD, Bombay-New Delhi, PP: 1128.
- Payne J.M., Sally M., Manston R. and Faulks M. (1970): The use of a metabolic profile test in dairy herds. Vet. Rec. 87:
- Rowlands G.J., Manston R. and Stark A.J. (1980): Changes in albumin, globulin, glucose and cholesterol concentration in the blood of dairy cow in late pregnancy and early lactation and relationships with subsequent fertility. J. Agric. Sci. 94: 517.
- Scales G.H. (1976): Selenium and beef cow fertility. New Zealand J. Exp. Agr. 4: 297.

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- Segerson E.C. JR.; Murray F.A.; Moxon A.L.; Redman D.R. and Conrad H.R. (1977): Selenium, vitamin E: role in fertilization of bovine ova. J. Dairy Sci. 60:1001.
- Snedecor G.W. and Cochran W.G. (1967): Statistical Methods. 6th Ed. Iowa Stat. Univer. Press, Iowa U.S.A.
- Southcott W.H.; Wheller J.L.; Hill M.K. and Hedges D.A. (1972):

 Effect of subdivision stocking rate, anthelmintic and selenium on the productivity of Hereford heifers. Proc. Australian Soc. Anim. Prod. 9:408.
- Valee B.L. and Wacker W.E.C. (1976): In The Proteins. H. Neurath, ed. Vol. 5. Academic Press. New York.
- Yarmol, Chuk H.M. and Meschyshen I.F. (1976): The effect of corticotrophin, somatotrophin and thyrotrophin on the distribution of mineral substances in the tissues of hypophysectomized rats. UK. Biokhimzh, No. 4:517.
- Zamet C.N.; Coleabrander V.F. and Erb R.E. (1979): Variable associated with peripartum traits in dairy cows. 111. Effect of diets and disorders on certain blood traits. Theriogenology 11:2

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ومن فيروسيد. الاولون الدين ودين فن الأناب و التنسيد . الدين و في أميط الويقون وقع الجويء الدين ب السيرا بين الانتخاب الطباعي و المعاطفة ويا عند القرائل فوروس الوينو المستوع له الطباع الطباعة المعاطفة المعاطون المستوية عن مكس السيالات الذي تنو في الواقية الوين في المعينة الوينو والتنسيدة ، في التشويدة . التنسيدة .

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