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EFFECT OF DIETARY SUPPLEMENTATIOM OF ZINC, COPPER AND SELENIM PRE- OR POST-PARTUM ON REPRODUCTIVE PREFORMANCE OF FRIESIAN COWS

Abu El-Hamd, M. A.; M. S. Sayah; M. A. Tag El-Dein; A.M.A. Salama and S.A. Ibrahim
Animal Production Res. Inst., Agric. Research Center, Dokki, Egypt.

ABSTRACT

The objective of this study was to evaluate the effect of adding zinc (Zn), copper (Cu) and selenium (Se) to the ration on ovarian activity and reproduction performance of Friesian cows. A total of 39 Friesian cows between the 1st and 3rd parity and average 532.7±23.5 kg LBW was divided into three similar groups (n=13 each). Multiparous cows (n=10 in each group) were divided according to their LBW, parity and milk production of the previous season, while primiparous cows (n=3 in each group) were allotted on their LBW. Cows of the 1st group (G1) were fed concentrate feed mixture (CFM), rice straw and corn silage (control). Cows of the 2nd group (G2) were received 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg CFM on day 30 prepartum to calving, while those of the 3rd group (G3) were received the same diet of (G2) from calving to 60 days postpartum. Throughout the experimental period, oestrus was detected and cows in heat were inseminated. Also, number and length of ovulatery cycles from calving up to conception were recorded. Post-partum 1st estrus and 1st service intervals, number of services per conception (NSC), days open (DO) and conception rate were assessed. Pregnancy was diagnosed by rectal palpation on day 60 post-insemination.

Results revealed that Pre- and postpartum supplementation of Zn, Cu and Se (G2 and G3) increased ($P<0.05$) body weight gain and body condition score as compared to G1. Interval from calving to 1st oestrus was significantly ($P<0.01$) shorter in G2 and G3 (23.5 and 27.45 days, respectively) than the G1 (39 days). Post-partum 1st service interval was significantly ($P<0.05$) shorter in G2 and G3 (42.5 and 44.15 days) than that in G1 (59.1 days). Supplementation of Zn, Cu and Se in G2 and G3 significantly ($P<0.05$) reduced NS/C to 1.7 and 1.8 services and DO to 77.6 and 79.8 days as compared to 3.6 services and DO of 128.0 days in the control group, respectively. The Zn, Cu and Se treatment increased conception rate (CR), being significantly ($P<0.05$) the highest in G3 (84.15%), modest in G2 (76.92%) and the lowest in G1 (53.85%). Supplementation of Zn, Cu and Se in G2 and G3 significantly ($P<0.05$) decreased average number of total ovulations and ovulatory cycles per cow compared with the control group. Average P4 concentration during the ovulatory cycles and P4 level prior to estrus incidence were significantly ($P<0.05$) increased in G2 and G3 as compared to G1. Ovulatory cycle length and interval to P4 peak during the ovulatory cycles were not affected by dietary supplementation.

Keywords: Friesian cows, reproductive performance, ovarian activity, Zn, Cu and Se.

INTRODUCTION

Elements such as Ca, P, Zn, Mg, and Cu are essential for the growth and reproduction and are involved in a large number of digestive, physiological, and biosynthetic processes within the body (Maas, 1987 and Spain *et al*., 1997).

The most obvious function is to be components of body organs and tissues and to provide structural support. In addition, they act as electrolytes, as constituents of body fluids, and as catalysts in both enzyme and hormone systems. Therefore, they fulfil several important functions for the maintenance of animal growth and reproduction as well as the health status (Boland, 2003).

Common copper deficiency symptoms in cows include delayed or suppressed oestrus, impaired ovarian function, decreased conception, increased incidence of retained placentas, infertility, and early embryonic death (Madhavan and Iyer, 1993 and Graham *et al*., 1994).

Zinc is an essential trace element found to be an integral component of over 300 metabolic enzymes ((Dibley, 2001). The element plays a critical role in the repair and maintenance of the uterine lining following calving, speeding the return to normal reproductive function and oestrus. Inadequate Zn levels have been associated with decreased fertility, abnormal oestrus, and abortion (Lotthammer, 1983; Maas, 1987 and Kılıç, *et al*., 2007). Furthermore, Zn is involved in the formation of prostaglandins because Zn enzymes control the arachidonic acid cascade (Wauben *et al*., 1999).

Mineral deficiencies and imbalances are often cited as causes of poor reproduction. It is clear that adequate amounts of minerals must be provided, but little is known about the effects of marginal deficiencies and imbalances (Maas, 1987). Because of their role in the endocrine system and in tissue integrity, minerals may have a beneficial role to play in the resumption of follicular growth and fertility in dairy cows. Reproductive failure may be induced by deficiencies of single or combined elements and by their imbalances.

Trace elements such as Manganese (Mn), Cu, Iron (Fe), Iodine (I), Se and Zn are essential in animal nutrition and are needed in very small amounts for essential metabolic reactions in the body. Their deficiencies are often associated with alterations in many metabolic processes and cause various kinds of diseases. Deficiency of these trace elements causes severe economic loss due to increased susceptibility to oxidative stress, growth retardation in young animals, anemia (Bureau *et al*., 2008), decrease in feed efficiency and fertility (Grenier *et al*., 2003).

The objective of this study was to evaluate the effect of adding Zn, Cu and Se to the ration of dairy cows pre- or post-partum on ovarian activity and reproductive performance in Friesian cows.

MATERIALS AND METHODS

The experimental work of the present study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt during the period from July to November 2010.

Animals and the experimental group:

A total of 39 healthy Friesian cows with an average of 532.7±23.5 kg body weight (BW), between 28 and 56 months of age and 1-3 parities was used in the present study. All cows were chosen at late pre-partum period (8 months gestation). At the beginning of the experimental period, the experimental cows were divided into three similar groups, 13 in each. The multiparous cows (n=10 in each group) were divided according to their body weight (BW), parity and milk production of the previous season, while primiparous cows (n=3 in each group) were allotted based only on their BW.

All cows were fed according to their BW and milk production. The 1st group (G1) was served as a control. Cows of the 2nd group (G2) were fed diet supplemented with 60 mg Zn, 20 mg Cu and 0.3 mg Se /kg diet from 30 day prepartum to calving, while those of the 3rd group (G3) were treated with the same dose of (G2) but after parturition up to 60 days of postpartum. All cows were housed separately in semi-open yards.

Feeding system:

Cows in all groups were fed diets containing concentrate feed mixture (CFM), rice straw and corn silage according to the recommendation of NRC (2001) for dairy cows based on their live body weight and milk yield. The CFM was composed of 37.5% yellow corn, 20% soybean meal, 15% corn gluten, 22.5% wheat bran, 3% molasses, 0.5% and 1.5% common salt. Chemical analysis of representative monthly samples of foodstuffs was analyzed for CP, CF, EE, NFE and ash on DM basis according to A.O.A.C. (1995). Chemical composition of CFM, rice straw and corn silage as well as calculated chemical composition of the basal diet used in feeding cows in all groups is shown in Table (1).

Table (1): Chemical analysis of different feedstuffs (on dry matter basis) used in feeding cows in all groups.

Item	Chemical composition (%)		
	CFM	**Rice straw**	**Corn silage**
Dry matter, DM	90.42	88.74	36.14
Organic matter, OM	89.54	82.83	92.4
Crude protein, CP	15.34	1.61	9.35
Crude fiber, CF	11.46	37.36	17.15
Other extract, EE	5.02	1.51	3.04
Nitrogen free extract	57.72	42.35	56.8
Ash	10.46	17.18	7.6

Experimental procedures:

The BCS was measured by the method reported by Ferguson *et al*. (1994).

Blood sampling:

Blood samples were collected with anticoagulant (heparin) from the jugular vein of each animal in all groups at 3-4 day-interval from 10 days after calving up to conception through 120 days post-partum. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate blood plasma and stored at -20^{o}C for determination of progesterone (P4) concentration in blood plasma.

Detection of oestrus and insemination:

Beginning of day 10 post-partum, an infertile bull was introduced to cows of each group for 20 minutes three times daily at 6, 12 and 15 h to recognize the onset of the 1st oestrus. Oestrus was identified when cows showed complete receptivity to the teaser and stood quietly to be mounted. Cows recognized to be on heat were artificially inseminated.

Number and length of estrous cycles from calving up to conception were recorded. Postpartum 1st ovulation (PPOI), 1st oestrus (PPEI) and 1st service (PPSI) intervals, number of services per conception (NSC), days open (DO) and conception rate (CR%) were calculated. Conception rate was calculated as the proportion of conceived cows relative to inseminated cows multipliable by 100.

Pregnancy was diagnosed by rectal palpation which taken place on day 60 post-insemination.

Progesterone assay:

Direct radioimmunoassay technique (RIA) was performed for determination of plasma progesterone concentration using antibody-coated tubes kit (Diagnosis systems, laboratories Texas, USA) according to the procedure outlined by the manufacture. According to the manufacture's information, the radioimmunoassay of progesterone is a competition assay. Sample and standards are incubated with ^{125}I-labeled progesterone, as tracer, in antibody-coated tubes. After incubation the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are calculated by interpolation from the curve. The standard curve of progesterone concentration ranged from 0 to 2.4 ng/ml. The intra and inter assay coefficient of variation were 5.4 and 9.1%, respectively.

Statistical analysis:

The obtained data were statistically analyzed using SAS (1990). The significant differences among treatment groups were tested using Duncan's Multiple Range Test (Duncan, 1955). The statistical model was

$$Y_{ij} = U + A_i + e_{ij}.$$

Where:

Y_{ij} = Observed traits

U = Overall mean

A_i = Experimental group 1-3 (1= G1, 2= G2 and 3=G3)

$\mathbf{e_{ij}}$ **=** Random error

Conception rate values were statically analyzed using Chi square test.

RESULTS AND DISCUSSION

Change of body weight and body condition score:

Data in Table 2 showed that pre- and post-partum supplementation of Zn, Cu and Se (G2) increased (P<0.05) body weight gain and body condition score as compared to G1. However, the differences between G2 and G3 were not significant.

Appropriate trace mineral supplementation is essential for maintaining optimum level of growth and performance of the animal (Šrejberová *et al*., 2008). So, the present results indicated some deficiency in mineral content of cows in G1 In study of Nocek *et al*. (2006) using diet supplemented with a mixture of complexed minerals and supplemented in excess of NRC (2001) requirements, and do not permit the improvement in performance to be identified to one specific mineral.

Table (2): Effect of Zn, Cu and Se treatment on body weight and body condition score (BCS) of Friesian cows.

Item	Treatment group			SEM
	G1	G2	G3	
Initial body weight, kg	521.8	523.1	522.4	22
Final body weight, kg	530.0	539.6	538.4	23
Change weight, kg	8.2[b]	16.5[a]	16.0[a]	2.1
Initial BCS	3.61	3.62	3.62	0.18
Final BCS	3.63	3.68	3.69	0.14
Change BCS	0.02[b]	0.06[a]	0.07[a]	0.006

[a and b:] **Means having different superscripts within the same row are significantly different at P<0.05.**

Reproductive traits:

Data in Table (3) show that interval from calving to 1st oestrus was significantly (P<0.05) shorter in G2 and G3 (23.5 and 27.45 days, respectively) than the control group (39 days). Bonomi *et al*. (1986) indicated that mean first estrus occurred of Parma dairy herd was 16.8 days sooner when Cu, Zn, and Mn amino acid chelates were supplemented compared with cows receiving against Cu, Zn, and Mn inorganic metal salts. Kropp (1990) similar trend was reported by Bosseboeuf (2006) reported that, the number of days from calving to first estrus may be less in cows receiving Cu, Zn, and Mn amino acid chelates supplementation.

This means that post-partum 1st oestrus interval was 23.5 and 27.45 days in G2 and G3, respectivily, being significantly (P<0.01) shorter by 15.5 and 11.55 days than that of the control group (39.0 days). In accordance with the present results, in particular for cows in G2 treated with Zn, Cu and Se on pre-partum period. Several authors reported that Zn, Cu and Se treatment induced follicular growth and estrous in anovulatory dairy cows (Nayyar and Jindal, 2010).

Ceylan, *et al*. (2008) found that the deficiencies of copper, iron, zinc and selenium either singly or in combination could be responsible for anoestrus condition in buffaloes and by improving the nutritional status the fertility can be improved in females.

Post-partum 1st service interval was significantly (P<0.05) shorter in G2 and G3 (42.5 and 44.15 days, respectively) than that in G1 (59.1 days). Inspite these findings, Zn, Cu and Se treatment had beneficial effect on number of services per conception (NS/C) and days open (DO). Zn, Cu and Se treatment of cows in G2 and G3 significantly (P<0.05) reduced NS/C to 1.7 and 1.8 services and DO to 77.6 and 79.8 days as compared to 2.6 services and DO of 128.0 days in the control group. Such trends are in agreement with the results of DiCostanzo *et al*. (1986) who did report improved first-service conception rate in cows and heifers fed corn silage diets either with Mn or with Mn, Cu, and Zn as compared with unsupplemented (controls).

More benefits were obtained from Zn, Cu and Se treatment in term of conception rate (CR), being significantly (P<0.05) the highest in G3 (84.15%), the modest in G2 (76.92%) and the lowest in G1 (53.85%).

Table (3): Reproductive traits of cows in different experimental groups.

Item	Experimental group			MSE
	G1	G2	G3	
Post-partum 1st oestrus interval (day)	39.0^{a}	23.5^{c}	27.45^{b}	1.70
Post-partum 1st service interval (day)	59.1^{a}	42.5^{b}	44.15^{b}	2.70
Number of services per conception	3.60^{a}	1.70^{b}	1.8^{b}	0.35
Days open (day)	128.0^{a}	77.6^{b}	79.8^{b}	7.20
Conception rate (%)	53.85^{b}	76.92^{a}	84.15^{a}	

a and b: Means within the same row with different superscripts are significantly different at (P<0.05).

The impact of trace mineral supplementation on reproductive performance in cattle has been extensively researched. Supplementation of cattle with Cu, Zn, and Mn at NRC (1996) concentrations has been shown to enhance reproductive performance (Manspeaker *et al*., 1987 and Muehlenbein *et al*., 2001). Furthermore, a large amount of research comparing organic versus inorganic forms of trace minerals has also been conducted. Stanton *et al*. (2000) observed increased conception rates to AI in cows supplemented with organic minerals compared to those supplemented with inorganic minerals, yet no difference in pregnancy rate over the entire 60 d breeding season was observed.

Zn is an essential trace element found to be an integral component of over 300 metabolic enzymes (Dibley, 2001). The element plays a critical role in the repair and maintenance of the uterine lining following calving, speeding the return to normal reproductive function and oestrus. Inadequate Zn levels have been associated with decreased fertility, abnormal oestrus, and abortion (Lotthammer, 1983, Maas, 1987 and Kılıç, *et al*., 2007). Furthermore, Zn is involved in the formation of prostaglandins because Zn enzymes control the arachidonic acid cascade (Wauben, *et al*., 1999).

Ovarian activity:

Results in Table (4) show that Zn, Cu and Se treatment in G2 and G3 significantly (P<0.05) decreased average number of total ovulations, ovulatory cycles per cow compared with the control group. However, average P4 concentration during the ovulatory cycles and P4 level prior to estrus incidence were significantly (P<0.05) increased in G2 and G3 as compared to G1. On the other hand, the differences in, ovulatory cycle length and P4 peak and interval to P4 peak during the ovulatory cycles among experimental groups were not significant.

The marked elevation in P4 concentration during the interval from treatment to estrus incidence in G2 and G3 than in G1 is in agreement with several authors. The role of Cu and Zn in augmenting production and reproduction is well documented and are known to have a significant correlation with reproductive hormones (progesterone and estradiol), as they are specific activators of enzyme systems that assist in maintaining the activity of hypophyseal hormones in blood (McDowell, 1992 and Underwood and Suttle, 1999). Estrogen hormone has been reported to increase copper level (Sato and Henkin, 1973) and the lower level of copper in anoestrus buffaloes in the present study may be due to lower estrogen level in anoestrus animals (Rajkumar *et al*., 2006). Dutta *et al*. (2001) also reported low zinc level in anoestrus heifers. Fall in zinc level was associated with fall in steroid hormone concentrations which indicated that there was some co-relation between plasma zinc levels and progesterone-estrogen levels for proper reproductive processes (Akhtar *et al*., 2009). Zn may enhance conceptus development through its effects on PGF2α synthesis (Ceylan *et al*., 2008). It was reported that GnRH increased LH and FSH release from rat pituitaries when Cu was present in the portal blood (Kochman, *et al*., 1992) possibly by influencing GnRH receptor binding (Kochman, *et al*., 1992).

Table (4): Postpartum ovarian activity and progesterone (P4) concentration of cows in different experimental groups.

Item	Experimental group			MSE
	G1	G2	G3	
Total number of ovulations/cow	4.75^a	3.25^b	3.50^b	0.32
Number of ovulatory cycles/cow	3.75^a	2.50^b	2.75^b	0.22
Ovulatory cycle length (day)	22.05	20.95	21.64	3.42
Average of P4 prior to estrus activity	0.324^b	0.465^a	0.459^a	0.03
Average P4 concentration (ng/ml)[1]	1.758^b	2.465^a	2.724^a	0.201
P4 peak (ng/ml)[1]	5.185^b	6.865^a	6.231^{ab}	0.49
Interval to P4 peak (day)[2]	11.43	10.65	11.25	1.85

[1] **During the ovulatory cycles.**
[2] **From the beginning the ovulatory cycle**

The ovarian activity of ruminants is influenced by mineral deficiency (Corah and Ives, 1991; Ansotegui, *et al*. 1999 and Boland, 2003). Mineral deficiencies and imbalances are often cited as causes of poor reproduction. It is clear that adequate amounts of minerals must be provided, but little is known about the effects of marginal deficiencies and imbalances (Maas, 1987 and Graham, 1991). Essential trace elements are required for normal growth and development of animals. The nutritional requirements for these elements are small; however, these nutrients can greatly affect reproduction (Hostetler *et al*. 2003). Trace elements are required for the synthesis of many proteins and activation of a vast array of enzyme systems (Hidiroglou, 1979). Metalloenzymes, of which essential trace elements are constituents, are important in bone formation (Leach, 1967), lipid metabolism (Cunnane, *et al*. 1993 and Hostetler *et al*. 2003), glucose utilizations (Jovanovic-Peterson and Peterson, 1996), iron transport (Raub *et al*., 1985), DNA synthesis and transport (Townsend *et al*., 1994), and free radical metabolism (DeHaan, *et al*. 1994). Through one or more of these mechanisms, the trace elements may directly affect embryonic and fetal development. Se and vitamin E affect reproduction through antioxidant role as well as involvement in prostaglandin synthesis and its preferentially accumulates in ovaries, pituitary and adrenals for their proper function (Spears, 2000).

Conclusion

The current study concluded that adding Zn, Cu and Se supplementation to the ration of Friesian cows during pre-partum improved ovarian activity and reproduction performance and increased conception rate.

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تأثير التغذية علي علائق مضاف إليها النحاس والزنك والسيلينيوم قبل أو بعد الولادة على الكفاءة التناسلية لأبقار الفريزيان

محمد عوض أبو الحمد، محمود سيد صياح، محمد عبد الحليم تاج الدين، أحمد محمد أحمد سلامة و سمير علي ابراهيم
معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية- الدقي – الجيزة- مصر

تهدف هذه الدراسة إلى معرفة تأثير إضافة الزنك والنحاس والسيلينيوم خلال فترة ما قبل أو بعد الولادة على الكفاءة التناسلية في أبقار الفريزيان. استخدم في هذه الدراسة 39 بقرة فريزيان متوسط أوزانها 532.7 كجم وفى الموسم من 1-3 . وقسمت إلى ثلاث مجموعات متماثلة (ن=13). قسمت الأبقار إلى ثلاث مجموعات متماثلة في الوزن وموسم الحليب وإنتاج اللبن للموسم السابق. كانت الأبقار في المجموعة الأولى بدون معامله (كنترول) وأبقار المجموعة الثانية أضيف إلى علائقها 60ملليجرام زنك و 20ملليجرام نحاس و0.3 ملليجرام سيلينيوم / كجم عليقة وذلك قبل الولادة بثلاثين يوما حتى يوم الولادة ، في حين أن المجوعة الثالثة أخذت الجرعة نفسها ولكن المعاملة استمرت له بعد الولادة لمدة ستون يوما. خلال الفترة التجريبية كان يتم كشف الشياع والتلقيح وحساب عدد وطول دورات الشياع من الولادة حتى التلقيحة المخصبة. وكذلك تم حساب الفترة الفاصلة لأول شياع وأول تلقيح وعدد التلقيحات اللازمة للإخصاب ومعدل الحمل عن طريق الحس المستقيمي بعد 60 يوم من التلقيح.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

- إضافة كل من الزنك والنحاس والسيلينيوم(المجموعة الثانية والثالثة) إلى علائق الأبقار حسنت من التغير في وزن الأبقار في المجموعتين الثانية والثالثة بالمقارنة بمجموعة الكنترول(المجموعة الأولى).
- أدت إضافة كل من الزنك والنحاس والسيلينيوم (المجموعة الثانية والثالثة) إلى علائق الأبقار إلي تقصير الفترة من الولادة حتى أول شياع وأول تلقيح بالمقارنة بالكنترول.
- كما نقصت معنويا عدد التلقيحات اللازمة للإخصاب في المجموعات المعاملة(المجموعة الثانية والثالثة) 1.7 و1.8 مقابل
- 3.6 تلقيحة في الكنترول، كما أدت المعاملة إلي تقصير فترة الأيام المفتوحة 77.6 و79.8 مقابل 128 يوم في الكنترول(المجموعة الأولى).
- زاد معدل الحمل في المجموعات المعاملة (المجموعة الثانية والثالثة) 84.15 و 79.92 % مقابل 53.85% في الكنترول(المجموعة الأولى).
- كما أدت المعاملة إلي تقليل عدد دورات التبويض والشياع في الأبقار المعاملة(المجموعة الثانية والثالثة) مقارنا بالكنترول(المجموعة الأولى).
- زاد تركب هرمون البروجسترون تثناء دورات التبويض وخلال الفترات السابقة للشياع بدرجة معنوية في المجموعتين التانية والثالثة مقارنة بالمجموعة الأولي(المجموعة الأولى). في حين أن طول دورة التبويض وتركيز هرمون البروجسترون خلال دورة التبويض لم يختلف معنويا في كل المجاميع.

قام بتحكيم البحث

أ.د / عبد الخالق السيد عبد الخالق	**كلية الزراعة – جامعة المنصورة**
أ.د / علاء السيد بلاسي زيدان	**مركز البحوث الزراعية**