

**SOME HAEMOSTATIC PARAMETERS OF BUFFALO-CALVES KEPT ON NATURAL MILK WITH REFERENCE TO THE ROLE OF VITAMIN-K**

**K.A. Attia**

*Department of Physiology, Faculty of Veterinary Medicine, University of Cairo, Giza, Egypt*

**SUMMARY**

The present investigation was carried out to study some haemostatic parameters, including prothrombin (PT) and activated partial thromboplastin times (APTT) as well as assays of clotting factors activities (II, VII, IX and X), in buffalo-calves fed on either natural milk alone (group I) or natural milk supplemented with vitamin K<sub>3</sub> (group II). Blood samples from calves of both groups were collected at the end of 1, 2, 4, 8 and 12 weeks of age and also from buffalo-bulls, which were considered as a standard group. The results revealed the following:-

1. In calves of the first group, inadequate haemostatic activity was recorded during the first four weeks of age manifested by long PT and APTT together with marked reduction in the activities of the measured clotting factors as compared to the standard buffalo-bulls.
2. During the 8<sup>th</sup> and 12<sup>th</sup> weeks of age, calves of the first group showed a significant decrease in PT and APTT together with a significant increase in the activities of clotting factors (II, VII, IX and X) as compared to their values during the first four weeks of age. No significant differences were recorded between the values taken at the 8<sup>th</sup> and 12<sup>th</sup> weeks of age.
3. Calves of the second group exhibited significant decrease in PT and APTT as well as significant increase in the activities of the measured clotting factors during the 2<sup>nd</sup> and 4<sup>th</sup> weeks of age as compared to those of the first group at the same age. Moreover, no significant differences were recorded between the studied haemostatic parameters in calves of both groups during the 1<sup>st</sup>, 8<sup>th</sup> and 12<sup>th</sup> weeks of age.

It could be concluded that, the newly born buffalo-calves during the first few weeks of age were characterized by defective haemostatic activity. This could be improved by administration of vitamin-K<sub>3</sub>. This study might be of value in

avoiding bleeding disorders that may occur due to any possible surgical interference or accidental trauma.

**Keywords:** Buffalo-calf, hemostasis, natural milk, vitamin K

## INTRODUCTION

Blood coagulation deficit during the neonatal periods in man and animals was recorded by many authors. In man, Lusher *et al.* (1983) reported cases of hypothrombinaemia in infants. In sheep and cattle, Nockels *et al.* (1978) found deficiency in blood clotting factors during the first few days of life. Haemorrhagic diseases that were recorded in calves fed on colostrum were manifested by thrombopenia, hypofibrinogenaemia and reduced plasma clotting factors (Dimmock *et al.*, 1976).

Haemorrhages and poor coagulation of blood were recorded in calves fed on milk substitute or cow's milk containing high doses of anticoccidial drugs (Develee and Hemker, 1985). In Japan, Sasaki *et al.* (1982) recorded high mortality rate in young piglets due to haemorrhages of castration wounds and subcutaneous haemorrhage. The prothrombin and activated partial thromboplastin times in those piglets were recorded to be prolonged with decreased level of vitamin K dependent factors (II, VII, IX and X). The authors attributed the cause of vitamin K deficiency to a combined effect of consanguinity and use of anti-microbial drugs. Wendt and Eymael (1990) mentioned that oral administration of anti-microbial drugs could destroy the intestinal flora. They recommended that medicated feed should be supplemented with vitamin K.

Radostits *et al.* (1994) reported that vitamin K is essential for production of the functional clotting factors number II, VII, IX and X (vitamin K dependent factors) by the liver. In addition, they mentioned that the common feed sources of vitamin K for adult dairy cattle and buffaloes are green leafy forages and it is synthesized in the digestive tract (rumen) of adult ruminants. Literature showed that colostrum, milk and milk replacers are deficient in vitamin K (Naylor and Ralston, 1991).

The present study aimed to clarify the haemostatic activity of buffalo-calves fed on natural milk with reference to the role of vitamin K supplementation.

## MATERIALS AND METHODS

Eighteen male buffalo-calves raised at "Benha station for buffalo veal production, Kaluobia" were used in the present investigation. All calves were fed colostrum from their dams for three days after birth and then classified into two equal groups, group (1): Calves were fed on natural milk through open bucket feeding system. The fresh milk was fed diluted 2 : 1 with water and fed twice daily at a total daily intake of about 8 - 10% of their body weight

(Jonathan, 1991). Beginning from the 3<sup>rd</sup> month of age the amount of milk intake was reduced to half the amount and given once/day, group 2: Calves were subjected to the same regimen of feeding as in group (1); in addition each calf received vitamin K<sub>3</sub> during a.m. feeding, after being dissolved in the water and added to the milk, in a dose equal to 25 mg / calf / day, twice weekly (Nockels *et al.*, 1978). Vitamin K<sub>3</sub> was obtained as Menadione sodium bisulfite powder (25%) from "Muvco-Abu-Sultan-Ismaalia".

Starter calf ration based on grains, bran and cotton seed cake together with green fodder (*Trifolium alexandrinum*) and hay were offered to all calves for *ad libitum* consumption beginning at 45 days of age. Fresh water and mineral mixture were also available. All calves were weaned completely from milk feeding at the end of the 3<sup>rd</sup> month of age.

Blood samples for coagulation tests and clotting factor assays were collected from calves of both groups at the end of 1, 2, 4, 8 and 12 weeks of age by venipuncture of jugular vein into one-ninth volume of 3.8 % sodium citrate solution. In addition, blood samples from seven buffalo-bulls were collected to deliver standard determinant values for clotting factors. Plasma was obtained by centrifugation of blood at 3000 r.p.m. for 15 minutes and used for determination of PT and APTT as well as activities of clotting factors II, VII, IX and X according to the method of Dacie and Lewis (1984) using the fibrometer apparatus. Activities of clotting factors assayed were determined as a percentage value of control pooled plasma prepared from five normal buffalo-bulls (Jacobs *et al.*, 1989).

Statistical analysis of data was done according Snedecor and Cochrane (1980). Difference between means was compared by the protected least significant difference test (PSD) at the 5% level of probability.

## RESULTS

Analyses of data tabulated in Table (1) and represented by figures (1-6) show that, in calves fed on milk alone, PT and APTT were significantly higher with a significantly lower values in the clotting factors activities during the first four weeks of age as compared to the standard buffalo-bulls. However, by the end of the 8<sup>th</sup> and 12<sup>th</sup> weeks of age, PT and APTT were significantly decreased and the activities of the clotting factors were significantly increased as compared to their values during the first four weeks of age. No significant differences were found between these values and those of the standard buffalo-bulls.

During the first week of age, no significant differences were recorded in the haemostatic parameters of calves fed on milk alone or calves supplemented by vitamin K<sub>3</sub>. In contrast, addition of vitamin K<sub>3</sub> significantly decreased both

Table 1. Haemostatic parameters of buffalo-calves fed on natural milk or natural milk supplemented by vitamin K<sub>3</sub>

Age (weeks)	Groups	Haemostatic parameters						
		PT / sec.	APTT / sec.	F. II (activity %)	F. VII (activity %)	F. IX (activity %)	F. X (activity %)	F. X (activity %)
	Standard	16.07 ± 0.13	37.33 ± 0.22	98.79 ± 0.38	97.36 ± 0.44	96.86 ± 0.71	99.29 ± 0.39	
1	G1	22.57 ± 0.61	46.50 ± 1.08	56.00 ± 0.53	57.57 ± 0.99	52.28 ± 0.71	49.71 ± 0.80	
	G.2	23.57 ± 1.06	48.00 ± 0.89	56.14 ± 2.64	58.28 ± 1.68	53.28 ± 2.12	50.57 ± 1.13	
2	G.1	23.07 ± 0.71	47.00 ± 1.06	57.00 ± 1.64	55.85 ± 1.68	51.00 ± 1.54	51.57 ± 1.06	
	G.2	16.28 ± 0.68	38.71 ± 0.86	85.57 ± 2.21	85.28 ± 1.34	87.00 ± 1.57	80.14 ± 1.59	
4	G.1	22.00 ± 0.97	45.85 ± 0.67	55.71 ± 1.35	56.71 ± 1.47	50.14 ± 1.38	50.85 ± 1.63	
	G.2	15.85 ± 0.45	38.28 ± 1.45	84.42 ± 0.89	85.14 ± 1.01	86.32 ± 0.88	85.57 ± 1.19	
8	G.1	15.88 ± 0.28	36.42 ± 0.75	97.92 ± 1.23	96.00 ± 1.19	97.85 ± 1.68	99.14 ± 1.62	
	G.2	15.71 ± 0.44	36.42 ± 0.84	97.14 ± 1.81	96.85 ± 1.68	97.28 ± 1.72	98.85 ± 1.68	
12	G.1	15.40 ± 0.37	37.71 ± 1.53	99.71 ± 1.45	97.28 ± 1.01	98.57 ± 1.91	100.00 ± 1.48	
	G.2	15.37 ± 0.60	37.14 ± 1.35	99.00 ± 1.95	97.80 ± 1.56	97.82 ± 2.74	99.14 ± 2.05	
	F-value	6.28	3.83	31.13	27.29	31.51	31.09	
	PSD	1.60	2.14	2.42	2.38	2.69	2.41	

\* Standard values represent means of 7 buffalo-bulls which were considered as 100% activities.

Data indicate Mean ± S.E., (n) : number of calves.

G1: calves fed on natural milk, G.2: (n=9) calves fed on natural milk + vit. K<sub>3</sub>.

Clotting factor activities are expressed as a percentage of control pooled plasma from 5 buffalo-bulls.

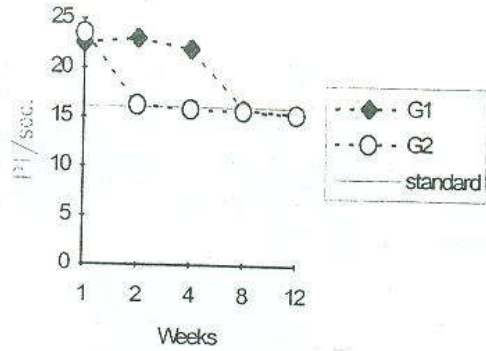


Fig. 1. PT of calves fed on natural milk (G1) or natural milk + vit. K<sub>3</sub> (G2).

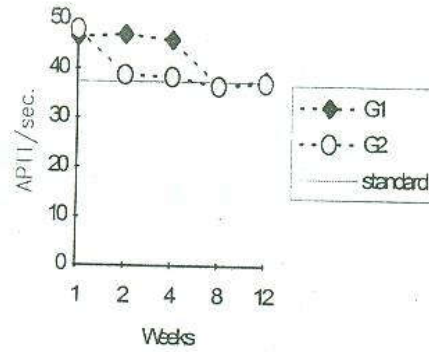


Fig. 2. APTT of calves fed on natural milk (G1) or natural milk + vit. K<sub>3</sub> (G2).

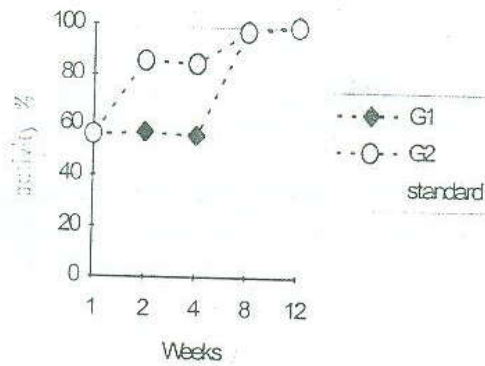


Fig. 3. Factor (II) activity of calves fed on natural milk (G1) or natural milk + vit. K<sub>3</sub> (G2).

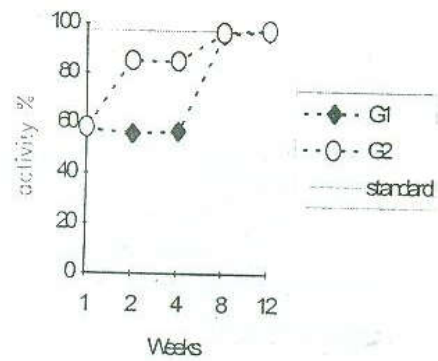


Fig. 4. Factor (VII) activity of calves fed on natural milk (G1) or natural milk + vit. K<sub>3</sub> (G2).

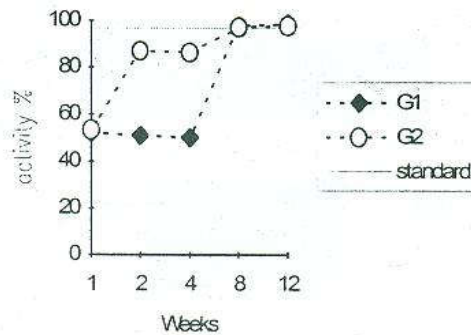


Fig. 5. Factor (IX) activity of calves fed on natural milk (G1) or natural milk + vit. K<sub>3</sub> (G2).

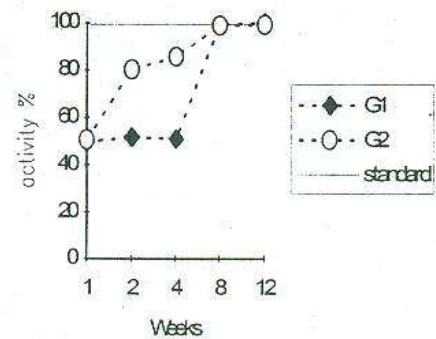


Fig. 6. Factor (X) activity of calves fed on natural milk (G1) or natural milk + vit. K<sub>3</sub> (G2).

PT and APTT and significantly increased the activities of measured clotting factors at the end of the 2<sup>nd</sup> and 4<sup>th</sup> weeks of age. The values were almost the same as those of the standard values.

In addition, no significant differences were recorded between the haemostatic parameters of calves of the 1<sup>st</sup> and 2<sup>nd</sup> groups at the end of the 8<sup>th</sup> or 12<sup>th</sup> weeks of age which were also nearly the same as those of the standard values.

## DISCUSSION

The present work was carried out to study some haemostatic parameters in buffalo-calves fed either on natural milk alone or natural milk supplemented with vitamin K<sub>3</sub> during the first 12 weeks of age. A pronounced decrease in PT and APTT together with a marked increase in the activities of clotting factors number II, VII, IX and X were recorded during the first four weeks of age due to vitamin K<sub>3</sub> supplementation. The improvement in the haemostatic parameters following vitamin K<sub>3</sub> administration indicated that the defect in the haemostatic activity in newly born buffalo-calves might be due to vitamin K deficiency. This deficiency in turn might affect the synthesis of vitamin K dependent clotting factors (Katayama *et al.*, 1979).

The reduced activities of clotting factors might be the cause of prolonged PT and APTT. Troy (1988) reported that prolongation of PT and APTT could be due to a deficiency in a single factor in the common coagulation pathway (I, II, V and X) or to multiple deficiencies of factors in the intrinsic and extrinsic pathways.

Colostrum, natural milk and milk replacer were reported to be deficient in vitamin K (Nestor and Conrad, 1990 and Naylor and Ralston, 1991). Vitamin K is found to be synthesized by rumen microorganism of adult ruminants (Wiss and Gloor, 1966) and this synthesis was minimal in pre-ruminants that were fed exclusively on natural milk. Katayama *et al.* (1979) found that vitamin K is involved in the synthesis of blood clotting factors (vitamin K dependent factors: II, VII, IX and X) by the liver, so its deficiency would reflect their levels in plasma. Moreover, Shapiro (1977) added that the disorder of the vitamin K dependent factors, generally, is due to parenchymal hepatic diseases, abnormalities in absorption of vitamin K, defect in vitamin K biosynthesis and unavailability of vitamin K (vitamin K antagonist).

It is clear that administration of vitamin K<sub>3</sub> did not affect the haemostatic parameters during the first week of age. These results confirmed the recorded results of Nockels *et al.* (1978) who found that vitamin K administration did not improve clotting in bull calves less than one week old. They attributed this finding to an insufficient amount of vitamin given. Other explanation for such finding could be based on the defective hepatic microsomal enzyme system that metabolize the administered vitamin-K<sub>3</sub> at this age.

Comparing the haemostatic parameters of milk-fed calves during the first four weeks and 8<sup>th</sup> or 12<sup>th</sup> weeks of age, a significant decrease in PT and APTT together with a marked increase in clotting factors activities were recorded at 8<sup>th</sup> or 12<sup>th</sup> weeks old. The improvement in the haemostatic activity may be attributed to the availability of vitamin K via the microbial synthesis in the rumen which might be well developed at this period of age. Phillipson (1970) reported that the reticulum and rumen together reach an adult ratio in relation to the other parts of the stomach when calves are about six - eight weeks old. Another source for vitamin K supply at the larger age is the green leafy forage that was available for calves beside milk feeding during the 2<sup>nd</sup> month of age.

In conclusion, the present study revealed that buffalo-calves are characterized by defective haemostatic activity during the first four weeks of age. Haemostatic activity could be improved by administration of vitamin K<sub>3</sub>. Consequently, to avoid bleeding disorders, that may occur at this age due to any possible surgical interference or accidental trauma, vitamin K<sub>3</sub> is recommended to be added to the milk during the first four weeks of age.

## REFERENCES

- Dacie, J. and S. Lewis, 1984. *Practical hematology*, 6<sup>th</sup> ed., pp. 60 - 72, Churchill Livingstone, Edinburgh, London and New York.
- Devilee, P. and H. Hemker, 1985. Synthesis of vitamin K dependent clotting factors in newborn calves. *Thrombosis and Haemostasis*, 54 (1): 259-264.
- Dimmock, C., I. Clark and M. Hill, 1976. The experimental production of haemolytic diseases of the newborn calves. *Research in Veterinary Science*, 20 (3): 244-248.
- Jacobs, G., M. Lappin, A. Marks and E. Greene, 1989. Effect of clindamycin on factor-VII activity in healthy cats. *Am. J. Vet. Res.*, 50 (3): 393-395.
- Jonathan, M.N., 1991. Evaluating dietary management of hand-reared calves. In: *Large Animal Clinical Nutrition*, 1<sup>st</sup> ed., pp. 248-260, Mosby Year Book, St. Louis, London, Sydney and Toronto.
- Katayama, K., K. Fujikawa and K. Titani, 1979. Vitamin K-dependent blood coagulation factors- structure, function and biosynthesis. *Protein Nucleic Acid Enzyme*, 24: 924-941.
- Lusher, J., P. Blatt and J. Penner, 1983. Autoplex versus proplex: A controlled, double-blind study of effectiveness in acute hemarthroses in hemophiliacs with inhibitors to factor VIII. *Blood*, 62: 1135-1142.
- Naylor, J. and S., Ralston, 1991. Colostrum and feeding management of the dairy calf during the first two days of life, In *Large Animal Clinical Nutrition*, 1<sup>st</sup> ed. pp: 240-247, Mosby Year Book, St. Louis, London, Sydney and Toronto.
- Nestor, J. and H. Conrad, 1990. Metabolism of vitamin-K and influence on prothrombin time in milk-fed preruminant calves. *J. Dairy Sci.*, 73: 3291-3296.
- Nockels, C., J. Yokel, D. Jackson and V. Swanson, 1978. Factors affecting blood clotting in immature sheep and cattle. *Br. Vet. J.*, 134: 286-288.
- Phillipson, A.T. 1970. Ruminant digestion, In *Dukes' Physiology of Domestic Animals*, Swenson, M.J. (ed.), New York, pp. 428-429.
- Radostits, M., K. Leslie and J. Fetrow, 1994. Herd health. In: *Food Animal Production Medicine*, 2<sup>nd</sup> ed., pp. 377-382, by W.B. Saunders Company, London, Toronto, Sydney, Tokyo.
- Sasaki, Y., H. Kitagawa and K. Ishihara, 1982. Hemorrhagic disease in pigs associated with vitamin K deficiency. *Jpn. J. Vet. Sci.*, 44 (6): 933-940.
- Shapiro, S., 1977. Disorders of the vitamin-K-dependent coagulation factors. In: *Hematology*, 2<sup>nd</sup> ed., pp: 1441-1447, Williams, W.J., Beutler, E., Erslea, A.J. and Rundles, R.W. (ed.), McGraw-Hill Book Co., New York.
- Snedecor, G.M. and W.G., Cochran, 1980. *Statistical Method*. 7<sup>th</sup> ed. pp. 215-237., Oxford and J.B.H. Publishing Comp.
- Troy, G., 1988. An overview of hemostasis. *Vet. Clin. North. Am. Small Anim. Pract.*, 18: 5-20.



- Wendt, M. and A. Eymael, 1990. Adverse effects of medicated feed on pigs. *Deutsche-Tierarztliche-Wochenschrift*. 97 (1): 34-39.
- Wiss, O. and H. Gloor, 1966. Absorption, distribution, storage and metabolites of vitamin K and related quinones. *Vitam. Horm.*, 24: 575-581.

بعض مؤشرات تجلط دم عجول الجاموس المغذاه على اللبن الطبيعي مع الإشارة إلى دور فيتامين ك

كمال على أحمد عطيه

قسم الفسيولوجيا - كلية الطب البيطرى - جامعة القاهرة

أجرى هذا البحث لدراسة بعض مؤشرات تجلط دم عجول الجاموس المغذاه على اللبن الطبيعي فقط (المجموعة الأولى) أو مضافا إليه فيتامين ك<sub>3</sub> (المجموعة الثانية). هذا وقد قورنت نتائج المجموعتين بنتائج مؤشرات تجلط الدم فى عجول الجاموس البالغة كمجموعة قياسية. وقد أظهرت النتائج ما يلى:-

١- فى عجول المجموعة الأولى لوحظ زيادة فى زمنى البروثرومبين والثرموبلاستين الجزئى بصورة معنوية مع نقص واضح فى نشاط عوامل تجلط الدم الثانى والسابع والتاسع والعاشر وذلك خلال الأسابيع الأربعة الأولى من العمر بالمقارنة بقيم المجموعة القياسية.

٢- فى عجول نفس المجموعة وعند نهاية الأسبوع الثامن والثانى عشر من العمر لوحظ نقص معنوى فى زمنى البروثرومبين والثرموبلاستين الجزئى وكذا زيادة معنوية فى نشاط عوامل التجلط المقاسة بالمقارنة بنتائج الأسابيع الأربعة الأولى من العمر بينما لوحظ عدم وجود أى اختلافات معنوية بين قيم هذه المؤشرات لكلا العمرين والتي تتشابه مع قيم المجموعة البالغة القياسية.

٣- فى عجول المجموعة الثانية لوحظ نقص معنوى فى زمنى البروثرومبين والثرموبلاستين الجزئى وكذا زيادة معنوية فى نشاط عوامل التجلط المقاسة عند نهاية الأسبوع الثانى والرابع من العمر إذا ما قورنت بنتائج المجموعة الأولى عند نفس الأعمار. هذا ولم يلاحظ أية فروق معنوية فى مؤشرات تجلط الدم المقاسة بين المجموعتين عند نهاية الأسبوع الأول من العمر. وقد تشابهت نتائج مؤشرات تجلط الدم المقاسة لكلا المجموعتين عند نهاية الأسبوع الثامن والثانى عشر مع نتائج المجموعة البالغة القياسية.

مما سبق يتضح وجود خلل فى عملية تجلط دم عجول الجاموس المغذاه على اللبن الطبيعي فقط خلال الأسابيع الأربعة الأولى من العمر. هذا وقد أدى إضافة فيتامين ك<sub>3</sub> إلى اللبن إلى تحسن ملحوظ فى كفاءة عملية تجلط الدم مما ينصح بإضافته إلى اللبن خلال تلك الفترة من العمر لتفادى حدوث أى مشاكل نزفية نتيجة التدخلات الجراحية المتوقعة أو نتيجة الكدمات الطارئة.