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FIELD INVESTIGATIONS ON SOME TRACE ELEMENTS, VITAMINS AND BIOCHEMICAL VALUES IN BUFFALO CALVES WITH DIARRHEA IN ASSIUT GOVERNORATE

(With 5 Tables and 9 Figures)

By

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فحوصات حقلية على بعض العناصر النادرة والفيتامينات وبعض القيم البيوكيميائية المصاحبة للاسهال فى عجول الجاموس فى محافظة أسيوط

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أجريت هذه الدراسة على عدد ١١٨ عجل جاموس تراوحت أعمارهم ما بين ٦-٩ شهور فى محافظة أسيوط، كان من بينهم عدد ٨٠ عجل جاموسى تعانى من الاسهال الذى تختلف درجاتة من اللين الى المائى والذى كان فى بعض الحالات مختلطا بالدم مع الجفاف والهزال الشديد. بعض هذه العجول لا تستطيع القيام والبعض الاخر فى حالة رقاد جانبا. وقد قسمت العجول المستخدمة في هذا البحث بعد الفحص الإكلينيكى والبكتر يولوجى والطفيلى الي ثلاثة مجموعات:

المجموعة الأولى: وقد اشتملت على عدد ٣٨ عجل جاموسى اصحاء ظاهريا واكلينيكيا استخدمت كمجموعة ضابطة.

المجموعة الثانية: وقد اشتملت على عدد ٦٤ عجل جاموسى تعانى من اسهال ناتج عن الاصابة البكتيرية، حيث أظهر الفحص البكتريولوجى وجود *الا شريشيا كولاى- السالمونيلا- الكلبسيلا-البروتيس- السودومونس ارجنوزا والشجيلا* بنسب مختلفة.

المجموعة الثالثة: وقد اشتملت على عدد ١٦ عجل جاموسى تعانى من اسهال ناتج عن اسباب غذائية وأظهر الفحص البكتريولوجى نتائج سلبية.

أظهر الفحص الطفيلى لجميع العجول الجاموسى المستخدمة فى هذا البحث نتائج سلبية. بالتحليل الكيميائى الحيوى تبين الآتى:

فى المجموعة الثانية: عند مقارنتها بالمجموعة الضابطة تبين وجود نقص معنوى فى مستويات العناصر النادرة (الحديد- الحديد الكلى- النحاس)، فيتامين أ- البيتا كاروتين وفيتامين ج، البروتين الكلى والألبيومين، زيادة معنوية فى مستوى كلا من اليوريا والكرياتينين فى مصل دم العجول الجاموسى مع ارتفاع معنوى جدا فى حجم كرات الدم المصمتة والذى يعكس مدى اصابة هذه العجول بالجفاف والهزال والرقاد.

فى المجموعة الثالثة: عند مقارنتها بالمجموعة الضابطة كان النقص غير معنوى فى مستويات العناصر النادرة (الحديد- الحديد الكلى- النحاس)، بينما كان هناك نقص معنوى فى مستوى كلا من فيتامين أ- البيتا كاروتين وفيتامين ج مع وجود ارتفاع معنوى فى حجم كرات الدم المصمتة. وقد استنتج من هذه الفحوصات وجود تغيرات جوهرية فى قيم الحيوانات المصابة باسهال ناتج عن عدوى بكتيرية مما يستدعى وضع تلك التغيرات فى خطة العلاج حيث يراعى رفع الكفاءة الوظيفية للكلى واعادة بناء الانسجة المتهتكة للامعاء مع رفع الكفاءة المناعية للجسم بجانب العلاج التقليدى بالمحاليل.

SUMMARY

In this investigations 118 buffaloe calves ranging from 6 to 9 months old were carefully clinically and laboratory examined. Levels of some serum trace elements such as iron, total iron binding capacity copper and vitamin A, Beta- carotene and vitamin C were determined. In addition PCV and some biochemical values include serum total protein, albumin, creatinine and urea were also determined. Significant changes in these values in diarrhoeic buffalo calves due to bacterial infection were higher than those due to dietary causes which showed insignificant variations. It could be concluded that a substantial biochemical changes occured in diarrhoeic buffalo calves and may be due to bacterial causes and that must put in consider to increase renal function efficiency, repair the damaged intestinal epithelium and increase the body immune status during the infection alongside with the traditional electrolytes therapy.

Key Words: *Buffalo-Calves-Diarrhea-Trace elements-Assiut.*

INTRODUCTION

Buffalo calves are susceptible to many enteric affections, which contribute to higher economic losses through their high mortality rates as well as its cost of therapy (Bhullar and Tiawana, 1985). Moreover

enteric diseases of buffalo calves, whatever the cause may constitute the main factors of direct and indirect losses of body weight gain (Caldow et al., 1988). The statistical data recorded in the animal reports of the Egyptian General Organization for Veterinary Service showed high mortality rates among buffalo calves (47.2 %) and 75 % of the total mortalities were due to enteric infections (Abou-Zaid and Nasr, 1995).

Dietary overload is also being a common cause of diarrhea in both calves and adult buffaloes (Whitlock, 1981). Nutritional disorders, seasonal and environmental changes, faulty management and bad hygiene were claimed to facilitate the occurrence and spreading of infections in buffalo calves (Sadiek, 1987). Diarrhea in buffalo calves usually occur, when the delicate balance between environmental factors, the calf and the causative agent is lost (McGurik, 1992).

Calf's demeanour is influenced by the severity of dehydration and the clinical signs of diarrhoeic calves can help in predilection of metabolic acidosis in situation where blood gas analysis is not available (Naylor, 1989). The author added that acidosis increases in seriousness with the level of depression.

Biochemical abnormalities in diarrhoeic calves are so consistent in occurrence that they form the basis for a rational therapeutic plan, and the clinician can sometimes treat a diarrhoeic calf successfully without knowledge of the specific etiologic agent (s) (Kasari, 1990).

During the course of enteritis in buffalo calves remarkable disturbances appear in hematological and biochemical values through direct or indirect effects due to impaired defense mechanisms. These in turn reflect the disturbance in general health condition of the affected calves (Aly et al., 1996). The present investigation was designed to monitoring the serum biochemical changes in diarrhoeic buffalo calves with dietary and bacterial enteritis during various degree of dehydration.

MATERIALS AND METHODS

A total number of 118 buffalo calves aging from 6 to 9 months old were involved in this investigation. The calves were belonged to individual farms in different villages of Assiut Governorate. Signs of enteritis appeared on 80 buffalo calves, where some calves showed soft to watery feces which contaminate the skin at the anal region, depression, loss of weight and signs of dehydration which varied from loss of elasticity, roughness of the coat to sunking of the eye balls. Some

calves showed sternal while others showed lateral recumbency. Buffalo calves, which appeared apparently healthy (n = 38) were used as control. Both diseased and apparently healthy calves were clinically examined after Rosenberger et al. (1979).

Samples and adopted methods:

Fecal samples:

Two fecal samples were taken directly from the rectum of all animals in the investigation. One sample was taken in a clean dry plastic packs for parasitologic examination to detect gastrointestinal parasites (Coles, 1986) and the second using sterile swabs for further bacteriological analysis. These swabs were immediately inoculated on Carry and Blair's transport medium and were cultured on selective and differential culture media at 37 °C for 24 hours and the isolated colonies were then identified according to Carter (1984) and Baily and Scott (1990) as follows: Isolated colonies from MacConky's agar plate were examined to be either lactose fermenting or non-lactose fermenting. Lactose fermenting colonies appeared to be rose pink in color and non-lactose fermenting as pale yellow colonies. Isolated colonies were then examined by Gram staining. Colonies, which appeared as Gram negative bacilli were then described for further identification of Gram negative isolates. These were then subjected to biochemical reactions such as indol production, methyl red Voges Proskauer test (MR/VP), citrate utilization, hydrogen sulphide production, reaction of triple sugar iron agar (TSI), urase production and oxidase test.

Blood Samples:

Heparinized blood samples were collected for determination of packed cell volume. Those without anti-coagulant for obtaining a clear, non-hemolysed serum. Serum total protein, albumin, urea, creatinine, iron, total iron capacity and copper levels were estimated spectrophotometery using chemical kits supplied by Boehringer Mannheim (Germany) after the methods of Weichselbaum (1946); Doumas et.al., (1971); Chaney and Marbach (1963); Selling and Wust (1969); Trinder (1956), Ramsay (1958) and Zak (1958) respectively. Serum vitamin A and Beta carotene levels were estimated colorometery after the method of Carr and Price (1926), while serum vitamin C was estimated colorometery after the method of Lowery et.al., (1945).

RESULTS

Buffalo calves were divided after careful clinical examination, parasitologic and bacteriologic examinations of the fecal samples into three groups I, II and III as shown in table 1.

Bacteriologic examination of the fecal samples of diarrhoeic buffalo calves (80) revealed that 64 fecal samples were positive for pathogenic bacteria. The distribution of these indicated that enteropathogenic E. Coli and Salmonella constituted the high incidence, while Shegella and Pseudomonas aeurginosa recorded the lowerest incidence (Table 2).

There was a remarkable drop in the serum levels of iron, total iron binding capacity and copper in diarrhoeic buffalo calves than in apparently healthy group. The decrease in these levels were correlated with the type of enteritis. However the drop in serum copper was higher than in serum iron and total iron binding capacity (Table 3).

There was a significant decrease in serum levels of serum vitamin C, vitamin A and Beta-carotene in diarrhoeic buffalo calves than those in apparently healthy control group. The significance of decreased levels was correlated with the type of enteritis. However the decrease in serum level of vitamin C was higher than in serum vitamin A and Beta-carotene (Table 4).

The packed cell volume (PCV) reflected the severity of dehydration occurred in the diarrhoeic buffalo calves, where higher PCV values were observed in buffalo calves with bacterial enteritis (group II) than that in buffalo calves with dietetic enteritis (group III) and in apparently healthy buffalo calves (group I) (Table 5).

There was a significant ($P< 0.01$) increase in serum level of total protein in buffalo calves with bacterial enteritis, while diarrhoeic buffalo calves due to dietary imbalance showed insignificant decrease in total protein level. A significant ($P< 0.01$) decrease in albumin level with significant ($P< 0.01$) increase in globulin level were found in group II. There was an elevation in serum creatinine and urea in diarrhoeic buffalo calves. However, the elevation was highly significant in group II (Table 5).

DISCUSSION

The predominant severe systemic changes and high degree of dehydration with depression, incoordination or recumbancy in diarrhoeic buffalo calves made the possibility of bacterial effects higher than dietary causes for the observed diarrhea. This became clear after culturing the fecal samples of diarrhoeic calves, where 64 (80 %) fecal samples out of 80 were positive for pathogenic bacteria, while 16 (20 %) fecal samples were negative for pathogenic bacteria. Calf's demeanour was influenced by the severity of dehydration (Naylor, 1989). In addition the characters of the feces in diarrhoeic buffalo calves in which feces was watery and contained mucus, or blood occult or both and was having a putrefied odour could explain the high incidence of isolated *enteropathogenic E. coli* (45 %) and *Salmonella* (15 %). However the presence of other pathogenic bacteria was also suggested but their incidence were low as *Klebsiella* (8.75 %) and *Proteus* (5.0 %) and very low as *Shegella* (2.5 %) and *Pseudomonas aeurginosa* (3.75 %). Radostits (1992) reported that diarrhoeic calves with *enteropathogenic E. coli* often pass large quantities of liquid feces containing billions *enteropathogenic E. coli* per gram feces , and recovered calves may shed bacteria for several months. According to McLaren and Wray (1991) *Salmonella species* can persist in calf rearing units for up to 2 years. They may play direct or indirect role as a source of infection to other animals (Farid et. al., 1987). *Shegella* and *Pseudomonas aeurginosa* were found in diarrhoeic buffalo calves in the form of mixed infection either with *E. coli* or with *Salmonella* (Manaa et.al. (1993). *Klebsiella* and *Proteus* were isolated in low percentage from diarrhoeic buffalo calves (Aly et al., 1996).

Diarrhoeic buffaloe calves with no systemic changes, low degree of dehydration and which were negative for parasitologic and bacteriolgic examination could be suggested to be of dietary imbalance and bad management. Dietary overload is also being a common cause of diarrhea in both calves and adult buffaloes (Whitlock, 1981).

There was a temporary decrease in serum levels of trace elements in diarrhoeic buffalo calves (Table 3) and this was related to the cause of this diarrhea, where it was significant in bacterial enteritis and insignificant in dietary causes. This could be explained by impaired absorption of these trace elements through the damaged intestinal

epithelium resulting from enterotoxins produced by *enteropathogenic bacteria* proliferating in the anterior small intestine. Mean values of serum iron, total iron binding capacity and copper in healthy buffalo calves according to Ali (1991) were 282.1 ± 4.8 µg/dl,765.3± 5.9 µg/dl and173.6±7.4 µg/dl respectively. The author found a significant decrease in serum iron and total iron capacity from 282.1 to 167.7 µg/dl and 765.3 to 708.3 respectively and in serum copper from 173.6 to 136.1 µg/dl in diarrhoeic buffalo calves. The decrease in serum level of copper was higher than the decrease in serum iron and total iron binding capacity may be due to rapid assimilation and reutilization of iron than copper.

The decrease in serum levels of vitamin A, Beta-carotene and vitamin C was higher in bacterial diarrhea than in dietary ones and in serum vitamin C than in serum vitamin A and Beta-carotene. This could be due to the malabsorption of vitamin A and Beta-carotene from the intestinal epithelium damaged by enterotoxins produced by *enteropathogenic bacteria* proliferating in the small intestine and due to the high consumption of vitamin C in response to stress conditions in dietary diarrhea and to enterotoxins in *enteropathogenic bacterial infection* of the intestine. Our view can be supported by Axt et. al., (1968), who concluded that consumption of ascorbic acid increases in the adrenals during stress conditions and that lead to temporary drop in its plasma concentration. Moreover Bendich (1987) reported that vitamin C concentration is reduced in fever and it is useful in detoxification of toxins and chemicals. Belschner and Edwards (1984) reported that the conversion of Beta-carotene to vitamin A occurs in the intestinal epithelium. A low serum levels of vitamin A (40.99±0.74) and Beta-carotene (121.91±3.99) were found in diarrhoeic buffalo calves (Ali, 1991). In addition, Seifi et. al., (1996) stated that calves with enteric infection showed lower ascorbic acid concentration.

An increase in PCV values was observed in diarrhoeic buffalo calves and this increase was highly significant ($P<0.01$) in buffalo calves with bacterial enteritis (group II) than those with diarrhea due to dietary causes (group III). This reflect the severity of diarrhea caused by enterotoxins produced by *enterotoxigenic bacteria* proliferating in the intestine, which in addition lead to toxemia accompanied with fever that in turn aggravates the dehydration.

The increased value of PCV together with hyperproteinemia observed in diarrhoeic buffalo calves in group II indicated a high degree of dehydration occurred in this group than those with diarrhea due to

dietary causes. There was however significant ($P<0.01$) decrease in albumin level with significant ($P<0.01$) increase in globulins level in group II, which can be explained by severe malabsorption of albumin through the damaged intestinal epithelium. The increase in globulins level may be compensatory due to the hypoalbuminic status or due to response of the body immune system to infection with *enterotoxigenic bacteria*. A low albumin and raised œ-1and œ2-globulin concentration were previously suggested by Joan (1982) to occur in more severe acute infection.

The elevation of serum creatinine and urea levels was highly significant ($p<0.01$) in diarrhoeic buffalo calves in group II, while the elevation in group III was insignificant. This can suggest that enterotoxins due to *enterotoxigenic bacteria* can directly or indirectly affect the functions of the kidneys. Diarrhea causes substantial changes in renal function in calves. These changes occur even when the impact on extra cellular fluid volume is still quite modest (Michell, et. al., 1992).

It can be concluded that a substantial biochemical changes occur in diarrhoeic buffalo calves, when the cause of this diarrhea is *enterotoxigenic bacteria*, which means that therapeutic plan must put in consider the decreased renal functions, damaged intestinal epithelium and supporting the body immune status during infection alongside with the traditional electrolytes therapy.

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Table 1: Distribution of animal's groups after clinical examination, parasitologic and bacteriologic examinations of fecal samples.

Groups	Number of animals	Parasitologic examination of the feces	Bacteriologic examination of the feces	Clinical signs
I	38	Negative	Negative	Apparently healthy, no signs of gastro-intestinal disturbances. Physiologic mucus membranes, body temprature, pulse and respiratory rates.
II	64	Negative	Positive	Severe depression, animal were unable to stand or move, some showed sternal or lateral recumpancy. Soft to watery feces, which contaminate the skin at the anal region. The feces contained sometimes mucus, or blood occult or both and which sometimes putrified odour. Severe loss of skin elasticity, rough hairs, dry muzzle. increase in body temprature, pulse and respiratory rates.
III	16	Negative	Negative	Depression, pasty feces, which conta-minated skin at the anal region. There was no mucus or blood occult in the feces. Low degree of skin elasticity, rough hairs. No systemic changes.

Table 2: Bactetrial examination of fecal samples of diarrhoeic buffalo calves.

The organism	Number of isolates	% of isolates	% of incidence
E. coli	36	56.25	45
Salmonella sp. Sp.	12	18.75	15
Klebsiella sp. SP.	7	10.93	8.75
Proteus sp. SP.	4	6.25	5.0
Pseudomonas aeurginosa SP.	3	4.69	3.75
Shigella sp. Sp.	2	3.13	2.5
Total	64	100	80
No growth	16		

Table 3: Serum trace elements levels in diarrhoeic and clinically healthy buffalo calves.

Groups	Number of animals	Iron µg/dl	T.I.B.C. µg/dl	Copper µg/dl
I	38	207.515 ± 2.410 (175.322 - 243.620)	729.170 ± 3.823 (670.270 - 803.460)	165.880 ± 2.520 (150.80 - 188.220)
II	64	170.290 ± 1.660 ** (145.822 - 193.340)	644.038 ± 1.362 ** (610.220 - 692.228)	140.660 ± 4.170 ** (128.700 - 155.600)
III	16	196.730 ± 4.220 (163.350 - 225.970)	721.480 ± 2.820 (643.620 - 788.310)	159.728 ± 2.230 (147.700 - 176.670)

**: P < 0.01
I: Clinically healthy buffalo calves.
II: Buffalo calves with bacterial enteritis.
III: Buffalo calves with dietetic enteritis.

Table 4: Serum Vitamin A, Beta-carotene and vitamin C levels in diarrhoeic and clinically healthy buffalo calves.

Groups	Number of animals	Vitamin C mg/dl	Vitamin A µg/dl	Beta-carotene µg/dl
I	38	1.102 ± 0.045 (0.823 - 1.464)	78.487 ± 3.123 (65.77 - 102.633)	176.510 ± 3.398 (124.690 - 212.670)
II	64	0.745 ± 0.039 ** (0.618 - 1.012)	43.154 ± 2.25 ** (36.420 - 70. 935)	99.931 ± 4.675 ** (111.660 - 173.193)
III	16	0.941 ± 0.027 * (0.811 - 0.984)	60.915 ± 4.738 * (53.420 - 86.680)	154.865 ± 6.549 * (93.210 - 186.283)

*: P < 0.05 **: P < 0.01
I: Clinically healthy buffalo calves.
II: Buffalo calves with bacterial enteritis.
III: Buffalo calves with dietetic enteritis.

Table 5: Packed cell volum and serum biochemical values# in diarrheic buffalo calves and healthy control ones.

Groups	Number of animals	PCV %	T.P. gm/dl	Albumin gm/dl	Globulin gm/dl	Creatinine mg/dl	Urea mg/dl
I	38	36.4 ± 0.80 (35.5 - 37.8)	7.26 ± 0.25 (6.92 - 7.99)	4.36 ± 0.39 (4.11 - 4.88)	2.9 ± 011 (2.89 - 3.25)	1.28 ± 0.17 (1.12 - 1.59)	36.2 ± 3.5 (32.6 - 48.9)
II	64	50.3 ±2.55** (49.6-51.5.3)	8.84 ± 0.31** (4.88 - 5.82)	2.52 ± 0.21** (2.01 - 2.56)	6.32 ± 0.05** (2 95 - 3.88)	3.53 ± 0.33** (2.78 - 3.94)	133.6 ± 9.8** (129.5-178.8)
III	16	42.5 ± 1.75* (40.6 - 43.5)	6.24 ± 0.35 (6.12 - 6.96)	3.61 ± 0.23 (2.83 - 3.89)	2.63 ± 0.12 (2 44 - 3.94)	1.69 ± 0.11 (1.68 - 2.29)	51.9 ± 6.4 (44.9 - 78.7)

#: Value= mean ± standard error.
*: $P < 0.05$ **: $P < 0.01$
I: Clinically healthy buffalo calves.
II: Buffalo calves with bacterial enteritis.
III: Buffalo calves with dietetic enteritis.

Figure 1: Effect of diarrhoea on some trace elements.

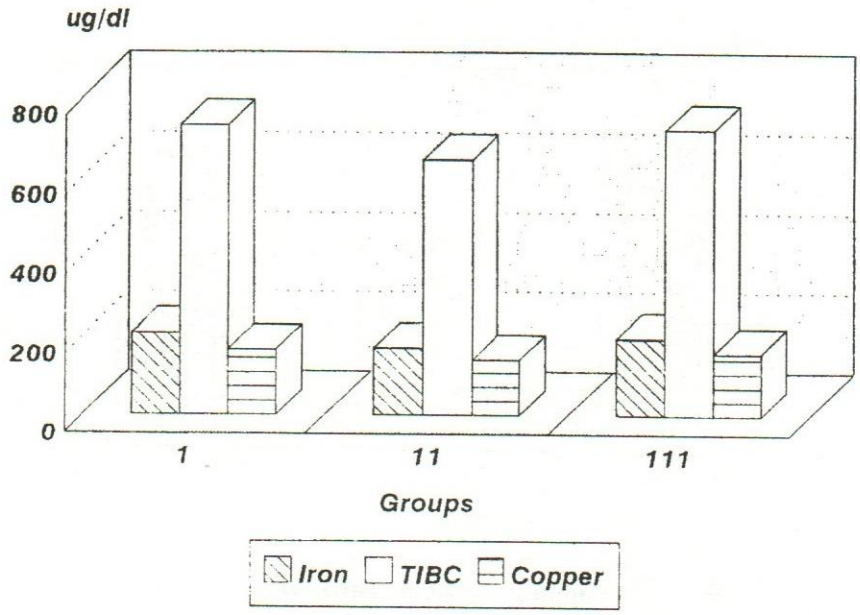

Figure 2: Effect of diarrhoea on vitamin C.

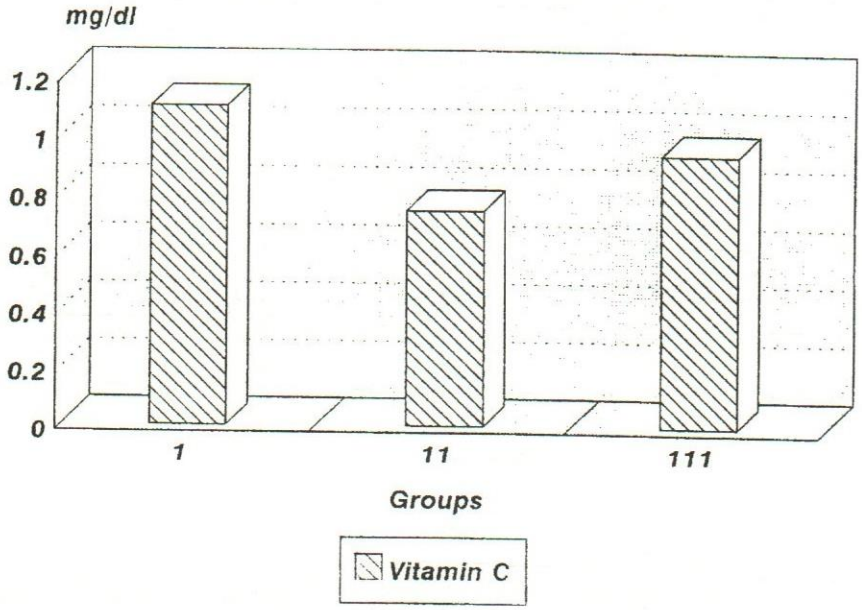

Figure 3: Effect of diarrhea on vitamin A & Beta-carotene.

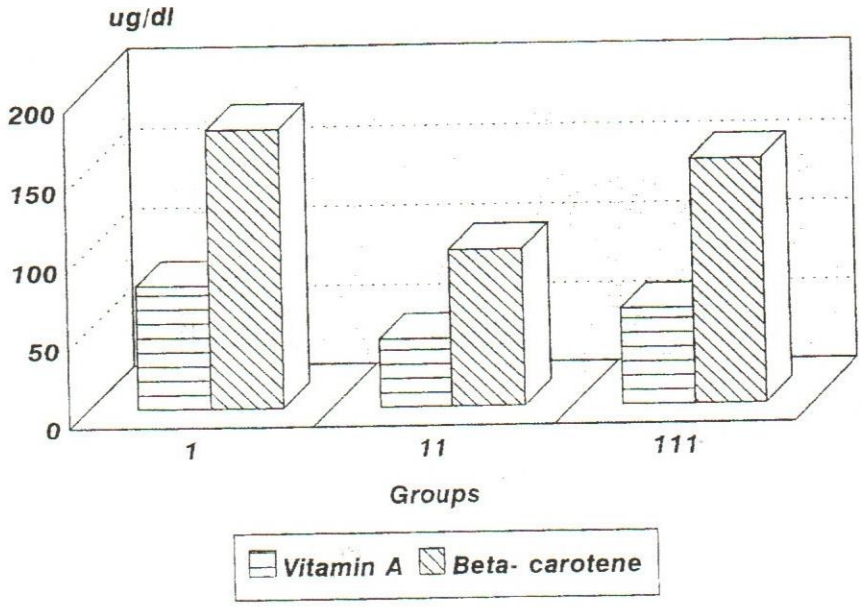

Figure 4: Effect of diarrhoea on serum creatinine level

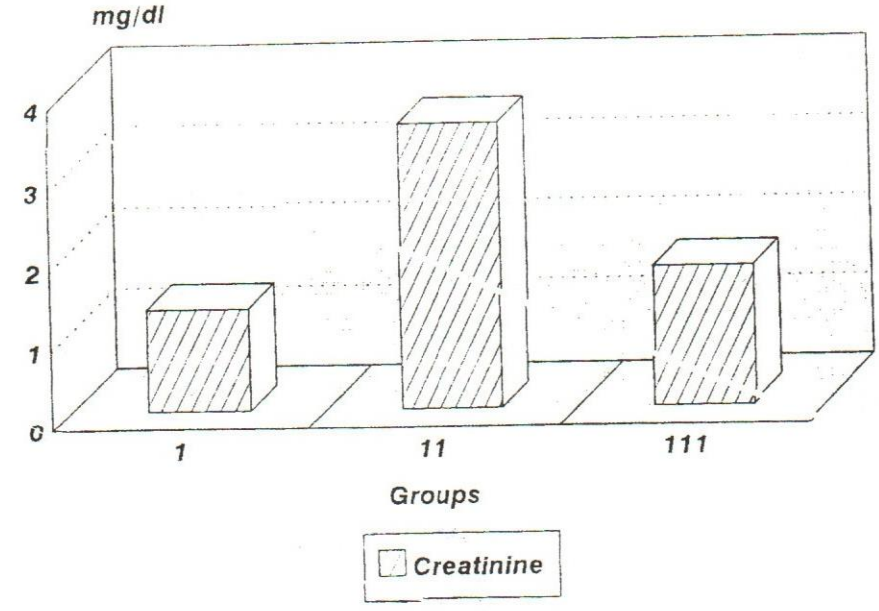

Figure 5: Effect of diarrhoea on serum urea level.

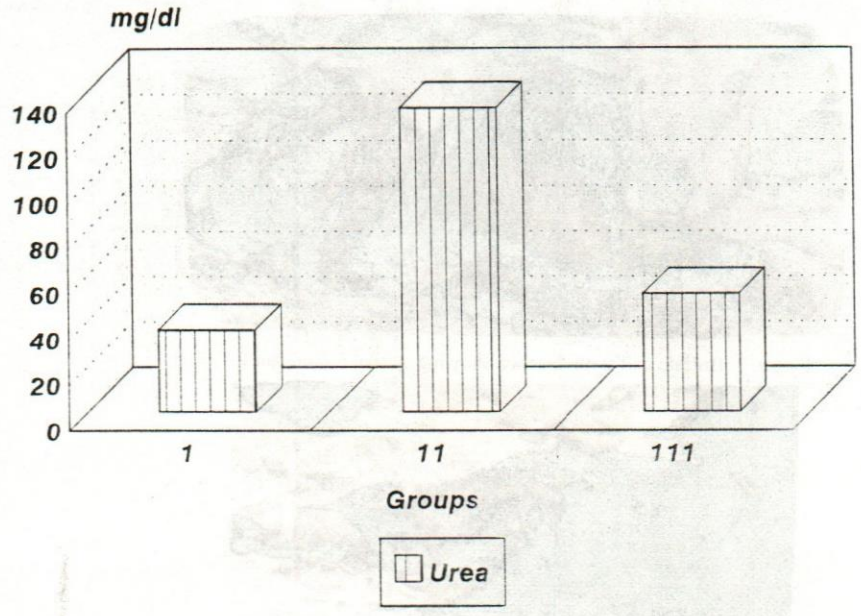

Figure 6:Effect of diarrhoea on serum total protein level.

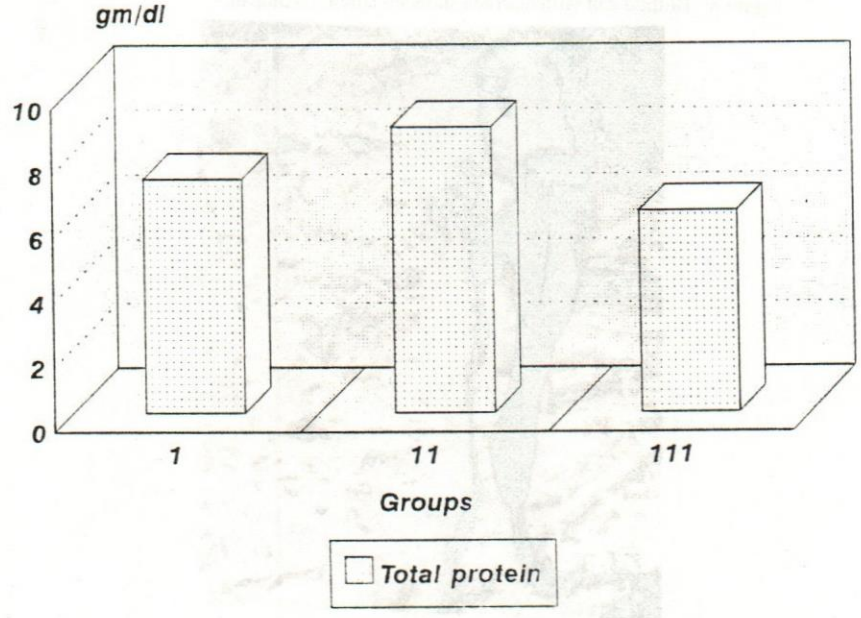

Figure 7: Buffalo calf with diarrhea showing sternal recumpancy.

Figure 8 : Buffalo calf with diarrhea showing lateral recumpancy.

Figure 9 : buffalo calf with diarrhea showin dehydration and contamination of the skin with fecal matter at the anal region.