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# Clinico-biochemical alterations in zero-grazed riverine buffaloes on dry roughage based ration

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**ABSTRACT**

The clinical signs of inappetance, poor body condition, pityriasis and decubital skin lesions in 22 stall-fed buffaloes of 18 to 20 months of age warranted the present clinical investigation. The animals were clinically examined and history was collected on feeding and management conditions. The animals had been held on wheat straw based diet supplemented with 0.5 to 0.8 kg concentrate (devoid of vitamin premix) without access to green forage for the last seven months. Six animals had skin sloughing, rough coat, abrasion and two buffaloes were on sternal recumbency and were unable to get up. Blood samples were collected by jugular venepuncture from eight randomly selected from the 22 affected buffaloes, with varying degrees of clinical signs, and from five control buffaloes of a similar age group, who were provided with *ad lib* green fodder to serve as control. Serum samples were analyzed for blood glucose, total protein, albumin, urea, uric acid, creatinine, calcium, and phosphorous. Aspartate animo-transferase (AST) and alanine amino-transferase (ALT) activities were significantly ($P<0.01$) lower in serum from affected animals, indicating reduced hepatic function. Alkaline phosphatase activities (AP), along with serum cholesterol level were also significantly ($P<0.05$) lower in zero-green fed animals compared to the controls. Blood copper and zinc concentrations were statistically comparable in both the groups, but the mean values for cobalt and iron were significantly lower in affected animals. Mean plasma level of vitamin A, ß-carotene and α-tocopherol was significantly ($P<0.01$) lower in affected animals than in the controls. This is the first report documenting the combined deficiency of vitamin A and its precursor ß- carotene along with α-tocopherol, and the affected animals had reduced hepatic function. The present investigation strongly suggests provision of green fodder or supplementation of vitamins in the diet of buffaloes to avoid poor health and clinical signs associated with deficiency.

**Key words**: vitamin A, ß-carotene, α-tocopherol, micro-minerals, buffaloes

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**Introduction**

The shortage of good quality feed in the tropics and subtropics has been implicated as the major cause of poor productivity in ruminants. Crop residues, such as straw, constitute the bulk of the feed supply in tropical farming systems, and its importance may rise due to increased population pressure, bringing more land acreage under crop production and, thus, limiting the size of grazing areas and supply of green fodder to livestock (RAGHUVANSI et al., 2007). In traditional animal husbandry practices, 80-95% Indian farmers utilize local feedstuffs as regular supplementation (DEVENDRA, 1992).

Green pasture contains adequate vitamin E and precursor of vitamin A as provitamin A carotenoids (especially, ß- carotene). Cereal grains, except yellow maize, are deficient in vitamin A precursors (DIVERS et al., 1986). The stall fed animals without access to grazing and fed diets, not adequately supplemented with these vitamins (or precursor), may suffer from their deficiency (RADOSTITS et al., 2000). Vitamin A and α-tocoferol are essential micronutrients for several life processes including proper vision, growth, reproduction, cellular differentiation, development and immunity (SARMA et al., 2009). Deficiency of vitamin A results in clinical signs of blindness, decreased appetite, poor growth, change in gait, ataxia, scaling, rough hair coat, diarrhea, dermatitis, xerophthalmia, pneumonia in growing calves, convulsion, anasarca in adult and feedlot steers (WHITEHAIR, 1984; YAVUZ et al., 1989). Pregnant dams with lowered serum retinol and ß-carotene have been reported to give birth to blind calves (MASON et al., 2003). Ocular changes in hypovitaminosis A include retinal degeneration, papilledema, corneal thickening, corneal opacity, anopthalmos and exophthalmus (BAHR et al., 2003; DONKERSGOED and CLARK, 1988). Decreased utilization of dietary nitrogen and the accompanying loss of body fat has been suggested for the slow weight gain in vitamin A deficit animals (HAYES, 1971), and the deficiency or clinical picture may be associated with liver or kidney function (KANEKO, 1997)

Deficiency of α- tocopherol, a cellular antioxidant, is common in young and growing farm animals fed on diets low in this vitamin. The deficiency of vitamin E and A has been linked to oxidative stress, mineral status and disease conditions in animals (BAGLEY et al., 2003; JANOSI et al., 2003). Vitamin E is essential for a properly functioning immune system. Dairy cattle that receive suboptimal amounts of vitamin E have increased incidence rates of mastitis and reproductive disorders (WEISS et al., 1994). Green pasture contains adequate vitamin E, but its concentration is highly variable in hay, silage and stored fodder that contain a much lower/minimal concentration due to its destruction, and it is negligible in straws (THAFVELIN and OKSANEN, 1996; RADOSTITS et al., 2000). Deficiency of vitamin E along with selenium has recently been identified as a major health concern of sheep producers in certain regions of the United States (VAN METRE and CALLAN, 2001). There are reports of deficiencies of individual vitamins in feedlot steers and calves (BOOTH et al., 1987; MASON et al., 2003), but there seem have been no reports on concurrent deficiency of vitamin A and α-tocopherol. In the present investigation, it was hypothesized that the lack of supplies of green fodder for a prolonged period in a stall fed buffaloes might have resulted in concurrent deficiencies of these vitamins and thus, plasma levels of vitamin A, β-carotene and α-tocoferol, the liver and kidney functions and the blood mineral status were assessed in moribund animals as an indicator of nutritional health.

## Materials and methods

*Animals.* Stall fed buffaloes (*Bubalus bubalis*) (n = 22) maintained in an intensive system of rearing, with the clinical signs of inappetance, poor growth, superficial skin wounds, scaling, dipigmentation, bran like deposits on the skin, corneal opacity of varied intensity, and recumbency, were used for the present investigation. These affected animals were taken from an actual feeding situation in which they had been routinely fed green-less rations for the last seven months. All the animals were clinically examined and the history collected about feeding and management practices. The animals were 18 to 20 months old. The routine diet was *ad libitum* wheat straw with little (0.5 to 0.8 kg) supplementation of concentrate mixture (wheat bran 55%, maize 18%, oil cake 25%, mineral mixture 1.5% and common salt 0.5% with no added vitamins). Five healthy buffaloes of almost identical age, fed on diet with *ad libitum* green fodder held in another shed were used as controls.

*Blood collection and processing.* Blood samples were collected by jugular venepuncture from 8 out of the 22 affected animals with varying degrees of clinical signs and the selection of animals for further laboratory analysis was random. Blood sampling was also done from 5 apparently healthy control buffaloes with different nutrition and provided with green fodder *ad libitum*. About 7 mL of blood was collected into heparinized vials and 3 mL, into tubes without anti-coagulants. Serum was harvested after clotting of blood. From the 7 mL anti-coagulated blood samples, 3 mL was centrifuged for collection of plasma and the rest (4 mL) was used for acid digestion for analysis of minerals. Both plasma and serum samples were stored frozen (-20 $^{o}$C) for further analysis within a week.

*Biochemical estimation.* Vitamins were estimated in plasma samples by HPLC (Model Shimadzu, Japan) using C18 reverse phase column, 250 × 4.0 mm$^2$, and 5 μm particle size. The Solvent system consisted of acetonitrile, tetrahydrofuran and HPLC water in the ratio of 47:42:11 at a flow rate of 1.7 mL/min. Vitamin A, α-tocopherol and ß-carotene were separated at 325, 290 and 450 nm wavelengths at 2.10, 3.25 and 4.50 min, respectively (CHAWLA and KAUR, 2001).

Serum biochemical parameters, such as glucose, cholesterol, total protein, albumin, globulin, urea, uric acid, creatinine, aspartate amino-transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (AP) along with calcium (Ca) and phosphorus (P) were taken using commercial kits supplied by SPAN Diagnostic kits (Surat, India).

*Trace elements analysis.* Four mL blood was wet digested separately, with nitric and perchloric acid mixture ($HNO_3$: $HClO_3$ = 4:1) (KOLMER et al., 1951). Two to three blank samples, where the bio sample was substituted by de-ionized triple distilled water, were run simultaneously with each batch of the digestion. The concentrations of trace elements such as copper (Cu), cobalt (Co), zinc (Zn) and iron (Fe) in the digested samples were estimated using an atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad, India) at wave lengths of 324.7, 240.7, 213.9, 248.3, with 6, 7, 7, 5 mA current, respectively. The Cu, Co, Zn, and Fe had the detection limit of 0.01, 0.02, 0.003, and 0.012 µg/mL, respectively. The standards procured (Sigma Aldrich Chemicals Corporation, New Delhi and Sisco Research Laboratory, Mumbai, India) for each element were used to calibrate the equipment and to check the analytical quality with serial dilutions of test-specific standard solution, and to measure the absorbance of the test samples in reference to that of the two fixed concentrations of the standard. The values were expressed as µg/mL of blood.

*Statistical analysis.* Data were analyzed to calculate the mean ± standard error, and students' 't' test and percent increase or decrease were used to compare the mean values of different parameters in affected and control animals (SNEDECOR and COCHRAN, 1994).

**Results**

The buffaloes (n = 22) maintained on wheat-straw based diets for the preceding seven months, with supplementation of 0.5 to 0.8 kg of concentrate per animal had clinical signs of inappetance, poor body conformation, wrinkles on the skin, pityriasis, dullness, unthriftiness, recumbency, scaling, rough hair coat and decubital wounds of varying degrees (Figures 1 to 3). Physical examination of the animals revealed a rectal temperature below the critical level. The pulse and respiration were normal in all the 22 affected animals. Six affected animals had skin sloughing, rough coat and abrasions or trauma in several body areas. Menace and pupillary light reflexes were absent in 3 animals. Two buffaloes were in sternal recumbency and one (12.5%) lateral recumbency. Closer examination of the animals revealed corneal opacity, poor eye lid reflex, with pallor conjunctiva in two affected animals (25%), suggestive of vitamin A deficiency.

The affected animals, with no access to green forage (green less diet), had significantly ($P<0.01$) reduced vitamin A, ß-carotene and $\alpha$-tocopherol levels (table 1). Each of the randomly selected 8 animals had either of these vitamins below the lower limit of the values in the control group. The mean plasma concentration of vitamin A, ß-carotene and α-tocopherol in the affected animals was 72, 9 and 35% of the respective level in the control group, suggesting that there was a very pronounced decline in ß-carotene level, followed by α-tocopherol and vitamin A.

Table 1. Plasma vitamin level in zero-grazed buffaloes maintained on green less diet

| Parameters | Range | | Mean ± SE | |
|---|---|---|---|---|
| | Control | Deficient | Control | Deficient |
| Vitamin A (µg/mL) | 0.51 - 0.65 | 0.24 - 0.48 | 0.57 ± 0.03 | 0.41 ± 0.03** |
| ß carotene (µg/mL) | 0.10 - 0.14 | 0.01 - 0.02 | 0.118 ± 0.007 | 0.017 ± 0.001** |
| α-tocopherol (µg/mL) | 0.23 - 0.34 | 0.03 - 0.15 | 0.292 ± 0.023 | 0.102 ± 0.015** |

** Significantly ($P<0.01$) different from respective control value, n = 5 for control group and n = 8 for deficient group

Table 2. Serum biochemical parameters of liver and kidney function in rats (Mean ± SE).

| Parameters | Range | | Mean ± SE | |
|---|---|---|---|---|
| | Control | Deficient | Control | Deficient |
| Glucose (mM/L) | 2.24 - 4.02 | 2.90 - 4.26 | 3.09 ± 0.34 | 3.74 ± 0.14 |
| Cholesterol (mg/mL) | 0.155 - 0.204 | 0.126 - 0.153 | 0.173 ± 0.089 | 0.137 ± 0.032** |
| Total protein (mg/mL) | 56.1 - 85.2 | 57.7 - 80.3 | 67.7 ± 5.5 | 68.4 ± 2.4 |
| Albumin (mg/mL) | 27.9 - 37.9 | 23.0 - 32.2 | 31.2 ± 1.8 | 28.0 ± 1.2 |
| Globulin (mg/mL) | 28.2 - 47.3 | 34.7 - 48.4 | 36.5 ± 3.9 | 40.4 ± 1.7 |
| Albumin:globulin ratio | 0.78 - 0.97 | 0.57 - 0.80 | 0.86 ± 0.039 | 0.69 ± 0.046* |
| Urea (mg/mL) | 0.23 - 0.56 | 0.24 - 0.49 | 0.39 ± 0.065 | 0.41 ± 0.034 |
| Uric acid (µg/mL) | 10.6 - 16.6 | 10.6 - 13.6 | 13.2 ± 1.0 | 12.3 ± 0.4 |
| Creatinine (µg/mL) | 6.0 - 12.0 | 4.9 - 13.8 | 9.3 ± 1.0 | 8.1 ± 0.9 |
| AST (U/mL) | 22.0 - 36.0 | 15.0 - 24.0 | 28.2 ± 2.3 | 18.0 ± 1.1** |
| ALT (U/mL) | 32.0 - 40.0 | 19.0 - 31.0 | 35.8 ± 1.3 | 23.4 ± 1.4** |
| ALP (IU/L) | 54.8-125.2 | 22.4 - 51.7 | 81.9 ± 12.2 | 38.6 ± 3.9** |

** Significantly ($P<0.01$) different from respective control value. n = 5 for control group and n = 8 for deficient group

Table 3. Macro and micro mineral levels in buffaloes maintained on dry roughage based diet

| Parameters | Range | | Mean ± SE | |
|---|---|---|---|---|
| | Control | Deficient | Control | Deficient |
| Blood Copper (µg/mL) | 0.59 - 0.71 | 0.43 - 1.01 | 0.64 ± 0.02 | 0.59 ± 0.07 |
| Blood Cobalt (µg/mL) | 0.10 - 0.20 | 0.04 - 0.14 | 0.16 ± 0.02 | 0.09 ± 0.01** |
| Blood Iron (µg/mL) | 0.80-1.00 | 0.47 - 0.94 | 0.89 ± 0.042 | 0.61 ± 0.058* |
| Blood Zinc (µg/mL) | 0.10 - 0.72 | 0.07 - 1.43 | 0.35 ± 0.13 | 0.58 ± 0.19 |
| Serum Calcium (µg/mL) | 65.4 - 93.1 | 76.0 - 90.2 | 76.3 ± 5.0 | 83.9 ± 2.2 |
| Serum Phosphorus (µg/mL) | 44.5 - 77.8 | 43.0 - 82.0 | 63.1 ± 3.7 | 62.7 ± 4.6 |
| Calcium: Phosphorous ratio | 1.16-1.33 | 1.24-1.48 | 1.21 ± 0.06 | 1.34 ± 0.08 |

** Significantly (P<0.01) different from respective control value, n = 5 for control group and n = 8 for deficient group

Fig. 1. Wrinkles on the skin, pityriasis, dullness, unthriftiness, recumbency in reverine buffalo maintained on a green less diet

Table 2 shows the serum biochemical profile suggestive of liver and kidney functions. The serum glucose, total protein, albumin, and globulin levels between green fed and green-less groups were statistically non-significant (P>0.05). However, there were relatively higher globulin and lower albumin levels in affected animals, thereby widening the albumin:globulin ratio (P<0.05) compared to respective controls. The total cholesterol level was significantly (P<0.01) lower in the affected animals. The activities of liver specific enzymes, ALT and AST, were significantly (P<0.01) lower in affected buffaloes. The serum urea, uric acid and creatinine, suggestive of kidney function, were comparable in both the groups, but AP activity was significantly lower (47%) in the affected animals.

Fig. 2. Corneal opacity and pallor conjunctiva

Fig. 3. Rough hair coat and decubital skin lesions in affected buffalo

Table 3 presents the levels of macro and micro minerals in the blood of both green fed and green less animals. The levels of Cu and Zn were similar, but those of Fe and Co were significantly higher in the normal group compared to the affected one (Table 3). The Ca and P levels and their ratio in normal and affected animals were non-significantly different.

**Discussion**

The clinical signs of rough hair coat, scaling, blindness and corneal opacity, sternal and lateral recumbency in a herd of stall-fed growing buffaloes warranted further investigation. The observed clinical signs were suggestive of vitamin A deficiency (RADOSTITS et al., 2000). The study documented lowered plasma vitamin A, ß-carotene, α-tocopherol level in buffaloes fed on a green less diet for a period of seven months, along with liver and kidney specific biochemical changes in the serum, and blood macro and micro-minerals levels.

Primary hypovitaminosis occurs in animals when the reserves are depleted due to rations deficient in vitamin A or carotene, or prolonged subsistence on drought stricken pastures, poorly cured and stored forages, and the clinical symptoms develop within 5-18 months (BOOTH et al., 1987). There are reports of hypovitaminosis A in calves fed on a green less diet, since the requirement is higher in young animals compared to adults (MASON et al., 2003). The plasma vitamin A concentration ranges from 0.25-0.60 µg/mL, <0.2 µg/mL results in deficiency disorders (CHEW et al., 1984; RADOSTITS et al., 2000). Slow weight gain and night blindness have been reported at a concentration of 0.08-0.1µg/mL, and optic damage and convulsion at 0.05 µg/mL (HODATE et al., 2004). Animals, deficient of vitamin A, have been reported to have decreased utilization of dietary nitrogen and accompanying loss of body fat (THURNHAM and NORTHROP-CLEWES, 1999). This might be attributed to poor body confirmation and emaciation in the animals which remained deficient of vitamin A due to the lack of green forage for a prolonged period. HODATE et al. (2004) reported a decline in feed intake in steers with plasma retinol level below 0.2 µg/mL. Similarly, there was a significant reduction in serum ß-carotene levels, primarily due to the long absence of green forage in the diet. It is stated that in contrast to plasma vitamin A, plasma carotenoids reflect the dietary intake of plant foods (THURNHAM and NORTHROP-CLEWES, 1999). Vitamin A cannot be synthesized *de novo* by animals and thus has to be taken up from animal food sources or as provitamin A carotenoids from green forage, and buffaloes are considered to be good converters of ß-carotene to vitamin A compared to other ruminant species (BONDI and SKLAN, 1984). However, the lack of access to green forage and the absence of dietary supplementation might have exhausted the liver fat soluble vitamin store, resulting in their significantly lower levels compared to green fed animals.

In the present investigation, reduced vitamin A and carotene was associated with low levels of vitamin E in the serum. In many recent studies it has been demonstrated that higher serum levels of vitamin A concomitant to high dietary vitamin A had a negative effect on the serum concentration of α-tocopherol and vice versa in young calves (WESTENDORF et al., 1990; SORENSON et al., 1997; AMETAJ et al., 2000). The plausible explanation for this interaction has been suggested to be the limited availability of fat-soluble vitamin carriers for absorption (AMETAJ et al., 2000). But contrary to these reports, SWANSON et al. (2000) failed to observe any negative effects on serum α-tocopherol concentrations resulting from feeding high vitamin A concentrations (44,000 IU/kg). However, in the present study, such a negative association between plasma concentrations of vitamin A and vitamin E was not observed and thus the deficiency of both vitamins can be attributed to primary dietary deficiencies in the absence of dietary supplementation in animals maintained on a green less diet. Fresh forage contains ample amounts of the vitamin A precursor ß-carotene as well as vitamin E. Irrespective of the dietary amount, however, the availability of vitamins A, and E, as well as beta-carotene, can be adversely influenced by poor fat digestion (HERDT and STOWE, 1991), which generally occurs in low fat diets, as in the case of buffaloes fed only with limited concentrate and ad libitum wheat straw that is low in fat as well as vitamin A precursors.

Serum cholesterol concentration is reported to be positively associated with serum *α*-tocopherol concentration (HERDT and SMITH, 1996). Cholesterol levels are highly correlated with circulating lipoprotein concentration and high-density lipoproteins are the main carrier of *α*-tocopherol in circulation. This could explain the observed lower serum values of cholesterol in the affected animals.

The activity of liver specific enzymes ALT and AST and kidney specific enzyme alkaline phosphates were significantly lower in affected animals compared to controls. Generally, these results may indicate degenerative changes and hypo-function of liver and kidneys (KANEKO et al., 1997; KAPLAN, 1987), which could be attributed to lowered vitamin levels, poor digestibility and debility in animals. Zinc deficiency has been implicated in the pathogenesis of liver diseases (STAMOULIS et al., 2007), which was not observed in the present investigation. The liver is important for the regulation of Zn homeostasis, while Zn is necessary for proper liver function. Similarly, there was a non-significant difference in blood Cu between the green fed and green-less groups. However, significantly low levels of blood Fe and Co in the green less group could not be explicitly defined and may be attributed to differential absorption from the gastrointestinal tract with differing body metabolism in clinically affected and emaciated animals. Further, long-term cobalt deprivation may primarily be due to a combination of reduced feed intake from a wheat straw based diet, resulting in tissue vitamin B12 deficiency, that could have an adverse effect on energy metabolism (STANGL, 1999). This may have a confounding effect on Fe absorption and metabolism leading to decreased blood Fe levels in the affected animals. STANGL et al. (1999) related Co deficiency induced diminished liver vitamin B12 level to reduced folate levels, that may have a depressing effect on heme-depending blood parameters.

It could be concluded from the present study that the lack of green fodder and dietary supplementation of vitamins led to reduced plasma levels of vitamin A, β-carotene and vitamin E, which was associated with the hypo-function of the liver and kidney and clinical signs of corneal opacity, emaciation, poor body confirmation and decubital lesions.

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**SAŽETAK**

U 22 stajski hranjena bivola, čija se dob kretala između 18 do 20 mjeseci, zabilježeni su klinički znakovi gubitka apetita, opadanja kondicije, te suhog ljuštenja i dekubitalnih oštećenja kože. Životinje su klinički pregledane i prikupljeni su podatci vezani za hranidbu i uvjete držanja. Osnovu obroka činila je pšenična slama kojoj je dodavano od 0,5 do 0,8 kg koncentrata (bez vitaminskoga premiksa). Bivolima je uskraćena mogućnost pristupa svježoj zelenoj krmi tijekom posljednjih sedam mjeseci. Šest životinja imalo je grubu kožu s pojavama ljuštenja i guljenja, a u dva bivola utvrđeno je ležanje na prsima s nemogućnošću ustajanja. Uzorci krvi iz jugularne vene prikupljeni su od 8 slučajno odabranih bivola zahvaćenih različitim stupnjem kliničkih promjena, te od 5 bivola koji su bili u približno istoj dobi i poslužili su kao kontrolna skupina hranjena svježom zelenom krmom po volji. U uzorcima seruma analizirani su glukoza, ukupni protein, albumin, mokraćevina, mokraćna kiselina, kreatinin, kalcij i fosfor. Aktivnosti aspartat amino-transferaze (AST) i alanin amino-transferaze (ALT) bile su značajno (P<0,01) niže u serumu životinja sa zahvaćenim promjenama što upućuje na smanjenu funkciju jetara. Aktivnost alkalne fosfataze (AP), zajedno s razinom serumskog kolesterola, bila je također značajno (P<0,05) snižena u životinja hranjenih suhom krmom u usporedbi sa životinjama kontrolne skupine. Koncentracije bakra i cinka u krvi bivola obje skupine bile su slične, a srednje vrijednosti za kobalt i željezo bile su značajno snižene u životinja zahvaćenih promjenama. Srednja razina vitamina A, β-karotena i α-tokoferola bila je značajno (P<0,01) niža u životinja zahvaćenih promjenama u odnosu na životinje u kontrolnoj skupini. Ovo je prvo izvješće koje potvrđuje da udruženi nedostatak vitamina A i njegova prekusora β-karotena, zajedno s nedostatkom α-tokoferola, u životinja zahvaćenih promjenama dovode do smanjene funkcije jetara. Kako bi se izbjeglo slabljenje zdravlja i pojave kliničkih znakova povezanih s navedenim deficijencijama, predlaže se u krmni obrok bivola dodavati svježu zelenu krmu ili vitamine.

**Ključne riječi:** vitamin A, β-karoten, α-tokoferol, mikrominerali, bivol