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Haemato-biochemical alterations in cattle with plastic impaction

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Abstract
Six cows suffering from plastic impaction have been evaluated for various haemato-biochemical parameters pre and post surgically on the day of surgery and 3rd, 5th, 7th and 10th day after surgery. Pre surgical haematological evaluation revealed mild anaemia, low PCV, neutrophilic leucocytosis and lymphocytopenia. Biochemical evaluation of impacted cows established hyperglycemia, high BUN, mild hypoproteinaemia, hypocalcaemia, mild hypophosphataemia, normal AST, ALT levels. However, the values reached base level by the end of study.

Keywords: cattle, plastic impaction, haemato-biochemical alterations

Introduction
Menace of plastic impaction in different species of animals alarmingly increased with vast and indiscriminate use of polythene bags, polythene wrappings, *etc.* (single use plastic) all over the world (Wright and Kelly, 2017) [51]. Plastic is non-biodegradable and hence, it can stay in animal stomachs asymptomatically till either the animal show impaction signs or die. On entering the rumen, the fate of plastics was not studied properly, other than removing it by rumenotomy (Vanitha *et al*., 2010) [49]. They break into micro or nano-plastics and release many toxins like PCBs, dioxins, phthalates, *etc*. that enter the biological components of body like rumen liquor, milk, blood, meat and eggs (Priyanka and Dey, 2018) [28] resulting in many adverse endocrine disorders (Vandenberg *et al*., 2012) [48].
Cattle with plastic impaction may suffer from off feed, dehydration, recurrent bloat, mineral and vitamin deficiency and ultimately die, if untreated timely (Ramu, 2015) [33]. Because of the chronicity of nutritional deficiencies and plastic impaction, the cattle show alterations in various haemato-biochemical parameters like anaemia, marked neutrophilia, leucocytosis, hyperglycemia, elevated BUN levels, altered electrolyte concentrations, *etc*. (Boodur, 2008; Akinrinmade and Akinrinde, 2012; Dodia, *et al*., 2014 and Fani *et al*., 2019) [8, 3, 10, 11]. As a part of our study of estimating plastic derived residues in milk, we evaluated various haemato-biochemical parameters at different intervals pre and post surgically in plastic impacted cattle and is been presented in this communication.

Materials and Methods
The present study was carried out among six cows presented to large animal surgery facility, Dept. of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru, Karnataka, India. All cows were subjected to rumenotomy to retrieve plastics after diagnosing plastic impaction based on history, various clinical, physiological, ultrasonographic and haemato-biochemical examinations. Two millilitres of blood each was collected by venipuncture into vacutainers (both plain and EDTA mixed) and various haematological parameters like TEC, TLC, PCV, Hb and DLC' various biochemical parameters like glucose, BUN, creatinine, total protein, calcium, phosphorus, sodium, chloride, potassium, ALT and AST were estimated before surgery and on 3rd, 5th, 7th and 10th post operative days by fully automatic analyzers.

Results and Discussion
I. Haematological evaluation
1. Total Erythrocyte Count (TEC)
Mild anaemia was observed on the day of presentation in all animals owing to nutritional,

mineral and vitamin deficiencies (Spaeis, 1975 and Ramprabhu *et al*., 2006) [40, 32] and prolonged anorexia or off feed (Dodia *et al*., 2014) [10]. The levels further decreased by 3rd post operative day owing to surgical stress and cased oral feeding. The TEC levels gradually improved to near normalcy by day 10th after proper nutritional and medical management (Table 1). Concurrent findings were also reported by Abdelal and El-Maghawry (2014) [1], Dodia *et al*. (2014) [10], Ramu (2015) [33], Akraiem and Abd Al-Galil (2016) [4] and Fani *et al*. (2019) [11]. Contrary to this, unaltered RBC counts were published by Tiwari (2012) [42], Behera *et al*. (2013) [6] and Rouabah *et al*. (2017) [36] indicating that those animals were not chronic sufferers. Hailat *et al*. (1996) [14] opined that various potential and non-potential foreign bodies present in the rumen resulted in erosions, haemorrhages, ruminitis and consequent sloughing off of ruminal papillae were the reasons for anaemia in such animals.

2. Total Leucocyte Count (TLC)

The recorded total leucocyte counts (10^3/ cmm) before surgery and on 3rd, 5th, 7th and 10th day post surgery were 11.02 ± 1.03, 13.11 ± 2.10, 10.66 ± 1.08, 9.54 ± 1.83 and 9.01 ± 2.54 respectively. (Table 1). Marked leucocytosis was evident in all cases on the day of presentation, which became more severe by 3rd day post surgery ascribed to the infection, inflammation and stress caused by impaction and surgery (Hailat *et al*., 1996) [14]. These results were in congruence with that of Rouabah *et al*. (2017) [36], Rajput *et al*. (2018) [31] and Fani *et al*. (2019) [11] who recorded severe leucocytosis. By the end of study period, the values became almost normal except in two cows which suffered from mild peritonitis and abscess near sutured site.

3. Haemoglobin (Hb)

The Hb values were significantly lower on the day of presentation which gradually improved by day 10 (Table 1). Initial low Hb values (anaemia) in our study could be attributed to the poor nutrition, prolonged off feeding and gastrointestinal disease (Radostits *et al*., 2006) [30]. Authors like Bisla *et al*. (2002) [7], Turkar *et al*. (2010) [46], Vanitha *et al*. (2010) [49], Otsyina *et al*. (2017) [27] also recorded low Hb values in animals with plastic impaction. However, Boodur (2008) [8], Athar *et al*. (2010) [5], Hussain and Uppal (2012) [15] and Tiwari (2012) [42] reported unaltered Hb values in animals with plastic impaction. Significantly lower Hb values on day of presentation were gradually improved by day 10th. Similar trend was also recorded by Shankar (2015) [38] and Fani *et al*. (2019) [11].

4. Packed cell volume (PCV)

Packed cell volume values (%) recorded before surgery and on 3rd, 5th, 7th and 10th day post surgery were 26.23 ± 2.15, 28.25 ± 2.91, 28.60 ± 2.26, 31.68±1.15 and 35.47 ± 2.01 respectively. Low values were observed throughout the study period that altered non-significantly (Table 1) which might be ascribed to anaemia, off feeding or anorexia, hampered digestion process, reduced food conversion ratio (Abu-Seida and Al-Abbadi, 2014; Turkar *et al*., 2010, Tiwari, 2012 and Dodia *et al*., 2014) [2, 10, 46]. Contrary to this Ramu (2015) [33] observed normal PCV values and Akraiem and Abd Al-Galil (2016) [4] reported elevated PCV values in cattle with rumen impaction attributed to catecholamine release due to stress (Athar *et al*., 2010) [5]. After commencing proper oral feeding and watering post surgery, all the animals recorded improved PCV values which were in accordance with Boodur (2008) [8], Shankar (2015) [38] and Fani *et al*. (2019) [11].

5. Differential leucocyte count (DLC)

5.1. Neutrophils

Significant neutrophilia was observed throughout the study period in all the animals under study. The value on day 0 was significantly higher than that of day 10 (Table 1). Apart from stress, presence of the indigestible foreign bodies like plastics in rumen might have irritated the ruminal epithelium making it susceptible for secondary bacterial infections resulted in severe neutrophilia (Hailat *et al*., 1996) [14]. In congruence with our results, many authors published high neutrophil counts (Behera *et al*., 2013; Hussain *et al*., 2013; Nayak *et al*., 2014; Tripathi *et al*., 2016; Otsyina *et al*., 2018) [44, 6, 16, 26]. However, Boodur *et al*. (2008) [8] and Athar *et al*. (2010) [5] found neutrophil counts less than 35 % even before surgery. As days progress the neutrophils further increased by day 3rd due to initial surgical stress and infection, but post operative antibiotic and anti inflammatory therapy made the values come down to near normal by the end of study period.

5.2. Lymphocytes

Lymphocytopenia was recorded in all six cows in study before surgery which was unaltered by day 3rd (Table 1) but, thereafter the values increased significantly owing to antibiotic and anti-inflammatory treatment. Release of the stress induced corticosteroids might be the probable cause for low lymphocyte values (Feldman *et al*., 2000) [12]. Many authors put forth lymphocytopenia in plastic impaction cases (Ismail *et al*., 2007; Turkar *et al*., 2010; Vanitha *et al*., 2010; Dodia *et al*., 2014; Ramu, 2015; Akraiem and Abd Al-Galil, 2016; Rouabah *et al*., 2017 and Fani *et al*., 2019) [49, 33, 14, 4, 11, 36, 46]. However, Boodur *et al*. (2008) [8] and Athar *et al*. (2010) [5] found normal lymphocyte counts even before surgery. Post operative antibiotic and anti inflammatory therapy made the values reach to near normal by the end of study period.

5.3. Eosinophils

The recorded eosinophil counts before surgery and on day 3rd were significantly higher than those of end of the study, although all were within physiological range (Table 1). Ruminitis and or concurrent parasitism might be reason for higher values (Thorat, 1999) [41]. Eotaxin is a specific chemokine derived from leucocytes or epithelial cells that recruits eosinophils would have been the reason for negligible eosinophilia initially (Vegad, 2008) [50] as was also reported by Tiwari (2012) [42]. However majority of authors reported normal eosinophil counts in cattle with plastic impaction *viz*., Athar *et al*. (2010) [5], Vanitha *et al*. (2010) [49], Boodur *et al*. (2008) [8] and Fani *et al*. (2019) [11].

5.4. Monocyte counts

The monocyte counts fluctuated non-significantly throughout the study period in all the animals except in case five, which recorded severe monocytosis initially and the values altered in higher levels, even by the end of the study period. In all other animals the values were almost in normal range and non-significantly varied (Table 1). Pre surgical monocytosis could be attributed to chronicity of the inflammation created by the presence of plastics in rumen. Usually monocytes appear lately in inflammation and persist at higher values till it subsides (Vegad, 2008) [50]. Comparatively lower values were also reported by authors like Boodur (2008) [8], Shankar

(2015) [38] and Fani *et al*. (2019) [11].

5.5. Basophil counts

No basophils (%) were recorded throughout the study period (Table 1). No basophils were recorded throughout the study period. Similar finding were also reported by Boodur (2008) [8] Tiwari (2012) [42] and Shankar (2015) [38].

II. Biochemical evaluation:

1. Glucose

Significant hyperglycaemia was observed in all the animals initially which gradually declined to normalcy by the end of study period. There was significant difference among the values on day 0, 5, 7 and 10 (Table 2). To compensate the energy requirements due to anorexia or off feed, nutritional imbalances and related stress, body might have responded by releasing adrenocorticosteroids that has glycogenolytic effect releasing more glucose into circulation and hence the elevated levels were recorded initially (Valdez *et al*., 1977) [47]. Mills-Thompson *et al*. (2017) [24] opined that the pressure exerted by the plastics in rumen created stress and reduced VFA production and possible stimulation of hypothalamo-adrenal axis led to high cortisol levels. Similar elevated glucose levels were also published by Hussain and Uppal (2012) [12], Tripathi *et al*. (2016) [44], Rouabah *et al*. (2017) [36], Otsyina *et al*. (2018) [26]. Contrary to this, Boodur (2008) [8], Vanitha *et al*. (2010) [49], Akinrinmade and Akinrinde (2012) [3], Dodia *et al*. (2014) [10], Fani *et al*. (2019) [11] observed low glucose levels attributed to failure of glucose production by meagre availability of free fatty acids and cholesterol.

2. Blood urea nitrogen (BUN)

Significant difference was observed in BUN values (mg/ dL) estimated before surgery and on 3rd, 5th, 7th and 10th day post surgery (27.72 ± 2.53, 21.21 ± 0.48, 18.77 ± 0.69, 14.53 ± 1.32 and 12.08 ± 0.71 respectively (Table 2). Elevated BUN levels were observed in cows with plastic impaction on the day of presentation. Decreased ruminal microbial activity, consequent hampered fermentation and reduced renal perfusion might have led to raised BUN levels (Dodia *et al*., 2014) [10]. Various authors *viz*., Rani *et al*. (1995) [34], Singh (2011) [31], Chanie and Tesfaye (2012) [9] and Kumar *et al*. (2018) [22] also recorded increase in BUN levels and attributed it to the renal insufficiency caused by starvation and anorexia. Excess ammonia produced in forestomachs would have been absorbed into blood and then metabolized into urea by liver also might be a cause for high BUN levels (Fani *et al*., 2019) [11]. Once the plastics were removed by rumenotomy, the values came down and reached normalcy by the end of study period (Fani *et al*., 2019) [11].

3. Serum creatinine

Mean serum creatinine levels estimated before and after surgery varied non-significantly in the study period and were within normal physiological range (Table 2). Similar findings were also reported by Vanitha *et al*. (2010) [49], Dodia *et al*. (2014) [10], Rajput *et al*. (2018) [31]. However, Dain *et al*. (1995), Shankar (2015) [38], Tripathi *et al*. (2016) [44] and Kumar *et al*. (2018) [22] observed high creatinine content in cattle suffering from plastic impaction which was ascribed to starvation, dehydration, muscular damage, failure of reabsorption of creatinine by kidneys and consequent excretion into urine (Kaneko *et al*., 2008) [20-21].

4. Serum total protein

Initially, there was significant hypoproteinemia in all the animals on day 0th which was more marked by day 3rd and all the animals recovered and reached normal levels by day 10 (Table 2). Prolonged anorexia, malnutrition, reduced appetite, inflammatory conditions and stress might have resulted in hypoproteinemia which persisted throughout our study period as it would take longer time to cope up the original body conditions. Similar marked low levels of serum total protein were also reported by Igbokwe *et al*. (2003) [17], Akinrinmade and Akinrinde (2012) [3], Shankar (2015) [38] and Otsyina *et al*. (2018) [26]. However, Turkar and Uppal (2007) [45], Athar *et al*. (2010) [5], Dodia *et al*. (2014), Tripathi *et al*. (2016) [44] and Rajput *et al*. (2018) [31] found hyperproteinemia in cows retrieved with plastics by rumenotomy.

5. Serum calcium

Hypocalcaemia was clearly evident in all the cases on the day of presentation, which recouped and reached normal levels by day 10th in all the animals (Table 2). Failure of calcium absorption through gut due to atonic or hypotonic rumen, malnutrition, extended anorexia duration and severe alkalosis were the factors contributed to the low calcium levels in present study (Kaneko *et al*., 2008; Shankar, 2015) [38, 20-21]. The results were in congruence with that of Bisla *et al*. (2002) [7], Boodur (2008) [8], Toor and Saini (2008) [43], Reddy and Kumari (2010) [35], Tripathi *et al*. (2016) [44]. No data was available indicating hypercalcemia in cattle with plastic impaction, which indicates the pathognomonicity of hypocalcemic factor in diagnosis.

6. Serum phosphorus

Mild hypophosphataemia was clearly observed before surgery which became normal by day 10th. The phosphorus levels were in normal physiological range and fluctuated non-significantly in the study period (Table 2). Pica due to malnutrition, poor energy levels, was stated as one of the most important factor causing depraved appetite in cattle, which made them to feed on many indigestible foreign bodies (McDonald *et al*., 1998) [23]. In agreement with our results, Toor and Saini, 2008) [43], Vanitha *et al*. (2010) [49], Hussain *et al*. (2013) [16] also informed hypophosphatemia in cattle with rumen impaction. However, Dodia *et al*. (2014) [10] reported hyperphosphatemia in cattle with rumen impaction and ascribed it to compensatory hypocalcemia. Boodur (2008) [8], Tripathi *et al*. (2016) [44] and Kumar *et al*. (2018) [22] found no change in phosphorus levels.

7. Serum sodium

Unaltered serum sodium levels within physiological range were observed throughout the study period (Table 2). Saxena (2003) [377], Boodur (2008) [8], Ramu (2015) [33] and Kumar *et al*. (2018) [22] also published no changes in serum sodium levels in animals with plastic impaction. But, Igbokwe *et al*. (2003) [17], Turkar and Uppal (2007) [45] and Tiwari (2012) [42] recorded hyponatremia which was supposed to be due to anorexia, reduced feed and water intake, loss of sodium through excretion and malnutrition (Radostits *et al*., 2006) [30]. Kaneko *et al*. (2008) [20-21] suggested the impaired sodium ionic pump as the reason for hyponatremia.

8. Serum chloride

Serum chloride levels also were found to be unaffected by the presence of plastics in rumen (Table 2). Usually,

hypochloraemia was observed in case of any gastrointestinal tract obstruction that prevents omasal and abomasal emptying and consequent pooling of chloride ions in rumen (Radostits *et al*., 2008) [30]. In the same way, Igbokwe *et al*. (2003) [17] and Kumar *et al*. (2018) [22] reported hypochloraemia. As there was no stasis of ingesta or dung in present study, the chloride levels were near normal as was also reported by Athar *et al*. (2010) [5] and Boodur (2012) [8]. However, Tiwari *et al*. (2012) [42] found hypochloraemia in cattle suffering from rumen impaction.

9. Serum potassium

Mild hypokalemia was observed in cows with plastic impaction, which was further lowered on day 3rd and recovered by 10th day post-surgery (Table 2). Saxena (2003) [37], Turkar and Uppal (2007) [45], Boodur (2008) [8], Athar *et al*. (2010) [5], Tiwari (2012) [42] and Kumar *et al*. (2018) [22] also reported similar findings. Dehydration and prolonged anorexia might be reasons for the hypokalaemia (Foster, 2017) [13]. Diarrhoea, upper gastro-intestinal tract obstruction also was the reason for low potassium levels (Radostits *et al*., 2006) [30]. However, diarrhoea was not the constant finding in our study.

10. Alanine Aminotransferase (ALT)

The estimated mean ± SE levels of ALT (U/ L) in serum before surgery and on 3rd, 5th, 7th and 10th day post surgery were 37.31 ± 6.73, 31.35 ± 5.38, 31.56 ± 1.73, 27.47 ± 5.23 and 31.93 ± 5.88 respectively. The values fluctuated non-significantly in all the animals in various time periods (Table 2). Similar trends in ALT levels were also reported by Boodur (2008) [8] Rouabah *et al*., (2017) [36] and Kumar *et al*. (2018) [22]. Elevated levels could be attributed to starvation, constipation and toxins absorption. Mucosal necrosis caused by continuous pressure or irritation by plastics in rumen would have released toxins that affected liver parenchyma (Kumar *et al*., 2018) [22].

11. Aspartate Aminotransferase (AST)

Low AST levels were observed in normal physiological range on all days of study (Table 2). Boodur (2008) [8] and Rajput *et al*. (2018) [31] also observed low levels of AST. Contrary to this, Rouabah *et al*. (2017) [36] and Kumar *et al*. (2018) [22] recorded elevated AST values in their study and ascribed them to the liver damage caused by the toxins released from rumen mucosal necrosis.

Table 1: Details of various Haematological parameters recorded at different intervals in six bovines with plastic impaction

Parameter	Day 0	Day 3	Day 5	Day 7	Day 10
TEC (10^6/ cmm)	5.53±0.64^a	5.31± 0.60^a	5.55± 0.53^a	5.68± 0.54^a	6.02± 0.52^a
TLC (/cmm)	11.02±1.03^a	13.11±2.10^a	10.66±1.08^{ab}	9.54±1.83^{ab}	9.01±2.54^b
Hb (%)	7.30±0.99^a	7.78±1.34^a	8.57± 0.84^a	9.53±0.59^a	9.85±0.79^a
PCV (%)	26.13±2.15^a	28.25±2.91^a	28.60±2.26^a	31.68±1.15^{ab}	35.47±2.01^b
DLC N (%)	51.40±5.76^{ab}	55.35±4.11^b	48.67±5.34^a	44.72±4.12^a	45.40±5.45^a
L (%)	38.65±3.81^a	37.59±3.12 ^{a}	44.01±4.08 ^{b}	47.22±5.04 ^{b}	48.03±5.63 ^{b}
E (%)	5.15±1.92^a	5.12±2.06^a	2.70±0.44^a	2.63±0.60^a	1.95±0.30^a
M (%)	6.13±3.21^a	4.33±1.76^a	4.93±2.32^a	6.38±1.98^a	4.71±1.36^a
B (%)	-	-	-	-	-

^{a,b,c} Values bearing different superscripts differ significantly ($p<0.05$).

Table 2: Details of various Biochemical parameters recorded at different intervals in six bovines diagnosed with plastic impaction

	Day 0	Day 3	Day 5	Day 7	Day 10
Glucose (mg/dL)	115.63± 8.87 ^{a}	100.32± 9.02 ^{a}	88.40± 19.29 ^{b}	79.48± 12.36 ^{b}	73.46± 11.12 ^{b}
BUN (mg/dL)	27.72± 2.53^b	21.21± 0.48^{ab}	18.77± 0.69^{ab}	14.53± 1.32^a	12.08± 0.71^a
Serum Creatinine (mg/dL)	1.08± 0.08^b	0.83±0.05^{ab}	0.90±0.09^{ab}	0.98±0.13^{ab}	0.80±0.05^a
Total protein (g/dL)	5.80±0.16^a	5.57±0.14^a	6.68±0.16^b	6.61±0.36^{ab}	6.98±0.41^{ab}
Calcium (mg/dL)	9.03±0.40^a	9.30±0.41^a	9.35±0.36^a	9.38±1.39^a	10.33±0.28^{ab}
Phosphorus m(g/dL)	5.15±0.52^a	6.30±0.25^{ab}	6.11±0.45^{ab}	6.33±0.74^{ab}	6.62±0.17^b
Sodium (mEq/ L)	137.83±1.22 ^{a}	136.00±1.34 ^{a}	137.00±1.91 ^{a}	137.17±1.35 ^{a}	134.67±2.13 ^{a}
Chloride (mEq/L)	103.33±2.52 ^{a}	106.5±0.81 ^{a}	103.67±2.88 ^{a}	99.50±5.20 ^{a}	100.11±0.74 ^{a}
Potassium (mEq/L)	4.10±0.29 ^{a}	3.93±0.18 ^{a}	4.15±0.29 ^{a}	4.43±0.41 ^{a}	4.53±0.19 ^{a}
ALT (IU/L)	37.31±6.73 ^{a}	31.35±5.38 ^{a}	31.56±1.73 ^{a}	27.47±5.23 ^{a}	31.93±5.88 ^{a}
AST (IU/L)	70.50±21.56 ^{a}	65.53±21.07 ^{a}	55.56±13.84 ^{a}	59.85±12.20 ^{a}	67.93±9.48 ^{a}

^{a,b,c} Values bearing different superscripts differ significantly ($p<0.05$).

Conclusion

The presence of plastics not severely affected the haemato-biochemical parameters of the animals. This might be the reason that the animals did not exhibit the signs of obstruction. Further studies have to be conducted regarding the fate of plastics in rumen of the impacted animals.

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