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# Haemato-biochemical evaluation of long bone fracture healing in dogs treated with stainless-steel and titanium dynamic compression plates

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**Abstract**
The study assessed haemato-biochemical responses during fracture healing in dogs treated with stainless-steel and titanium dynamic compression plates (DCPs). Twelve dogs with diaphyseal radius-ulna fractures were randomly divided into two groups (n = 6 each) and managed by open reduction and internal fixation using either stainless-steel or titanium DCPs. Haematological and biochemical parameters were evaluated up to 90 postoperative days. Both groups showed transient postoperative decreases in haemoglobin, packed cell volume, and total erythrocyte count, which normalized by day 7. Serum alkaline phosphatase and phosphorus increased during early osteogenesis and declined as remodelling progressed, while calcium showed a biphasic trend. Titanium implants showed earlier normalization of leukocyte count, ALP, and phosphorus levels, indicating reduced inflammation and faster metabolic recovery. Both materials were biocompatible and effective for fracture stabilization; however, titanium DCPs demonstrated superior healing dynamics and clinical outcomes, supporting their preference for canine long bone fixation.

**Keywords:** Haematobiochemical, Osseointegration, canine fracture, radius-ulna, dynamic compression plate, titanium, stainless steel, response

## 1. Introduction

Fracture healing is a dynamic and complex process involving coordinated cellular, molecular, and biochemical interactions that restore bone integrity. In veterinary orthopaedics, long bone fractures particularly those of the radius and ulna are among the most common musculoskeletal injuries in dogs, typically resulting from vehicular trauma or falls (Gadallah *et al*., 2009; Rudd and Whitehair, 1992) [10, 24]. Successful management depends on achieving anatomical alignment, rigid stabilization, and early restoration of limb function. Dynamic compression plates (DCPs) are widely used for diaphyseal fracture fixation, providing stable reduction and mechanical support (Reddy *et al*., 2020; Hayashi *et al*., 2019) [22, 12]. However, the choice of implant material significantly influences both mechanical and biological outcomes. Stainless-steel plates, though effective, may induce stress shielding and corrosion (Uhthoff *et al*., 1981) [29], whereas titanium alloys such as Ti-6Al-4V offer superior biocompatibility, corrosion resistance, and osseointegration due to an elastic modulus closer to that of cortical bone (Niinomi, 2002; Golish and Mihalko, 2011) [18, 11].

Fracture healing is also accompanied by systemic physiological changes that can be assessed through haematological and biochemical parameters. Haematological indices such as haemoglobin concentration, packed cell volume, erythrocyte and leukocyte counts, and differential leukocyte profiles reflect the body's inflammatory and immune responses.

Similarly, biochemical markers including serum calcium, phosphorus, and alkaline phosphatase (ALP) levels provide insights into bone metabolism and osteoblastic activity (Beale, 2004; Arens *et al*., 1996) [5, 4].

Despite their diagnostic importance, limited data are available on the comparative haematobiochemical responses to different implant materials during fracture healing in dogs.
Hence, the present study was designed to evaluate the haematological and biochemical alterations associated with the use of stainless-steel and titanium DCPs in the treatment of canine radius-ulna fractures. The primary objective was to elucidate the systemic and metabolic responses during the healing process and to compare the biocompatibility and clinical performance of these two implant materials in canine orthopaedic practice.

## 2. Materials and Methods

### 2.1 Study Design and Case Selection

The present study aimed to evaluate and compare the haemato-biochemical responses during fracture healing in dogs treated with dynamic compression plates (DCPs) made of stainless steel and titanium alloy. Clinical cases of long bone fractures presenting with lameness of the thoracic or pelvic limbs were selected from the clinical wards. Twelve dogs with diaphyseal radius-ulna fractures, irrespective of age and sex, were included after thorough clinical, orthopaedic, neurological, and radiographic evaluation. Cases with compound fractures or neurological deficits were excluded. The animals were randomly allocated into two groups (N=6 each):

- **Group I (Control):** Open reduction and internal fixation using stainless steel DCPs.
- **Group II:** Open reduction and internal fixation using titanium alloy DCPs.

Haemato-biochemical parameters were assessed on the preoperative day and on the immediate postoperative, 7th, 15$^{th}$, 30$^{th}$, 60$^{th}$, and 90$^{th}$ postoperative days. Whole blood was collected aseptically in EDTA-coated vials for haematological analysis, including haemoglobin concentration, packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total erythrocyte count (TEC), total leukocyte count (TLC), and differential leukocyte count (DLC). Serum separated from clot activator tubes was analyzed for calcium, inorganic phosphorus, and alkaline phosphatase (ALP) concentrations. Data were statistically analyzed using SPSS v20, employing independent t-tests for between-group comparisons and repeated-measures ANOVA with Turkey's HSD for periodic variations.

## 3. Results and Discussion

### 3.1 Haematological Parameters

The mean haemoglobin (Hb), packed cell volume (PCV), and total erythrocyte count (TEC) values (Tables 1-3) showed a non-significant decline from the preoperative to immediate postoperative periods in both groups, followed by a gradual rise from the 7th postoperative day onwards. This transient decline can be attributed to intraoperative haemorrhage and haemodilution due to intravenous fluid administration. Restoration of these.

**Table 1:** The mean + SE values of haemoglobin in both groups during various periodic intervals.

| Group | Pre-operative day | Immediate-P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| I | 13.17±0.42 | 12.57±0.33 | 13.15±0.26 | 12.75±0.34 | 13.08±0.24 | 12.88±0.21 | 12.48±0.29 | 12.87±0.11 |
| II | 13.07±0.24 | 12.48±0.13 | 12.48±-0.34 | 12.63±0.22 | 12.32±0.31 | 12.92±0.24 | 12.58±0.44 | 12.63±0.11 |

**P.O.D: Postoperative day**
*Normal physiological range of haemoglobin in dogs is 12-19g/dL.

**Table 2:** The mean + SE values of packed cell volume in both groups during various periodic intervals

| Group | Pre-operative day | Immediate-P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| I | 37.62±1.21 | 35.92±0.94 | 35.57±0.74 | 36.43±0.95 | 37.37±0.69 | 36.80±0.62 | 35.67±0.82 | 36.77±0.33 |
| II | 37.32±0.71 | 35.65±0.36 | 35.67±-0.97 | 36.08±0.62 | 35.20±0.87 | 36.90±0.68 | 35.97±1.24 | 36.11±0.31 |

**P.O.D: Postoperative day**
*Normal physiological range of packed cell volume in dogs is 35%-55%.

**Table 3:** The mean + SE values of total erythrocyte count in both groups during various periodic intervals

| Group | Pre-operative day | Immediate-P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| I | 6.27±0.20 | 5.98±0.16 | 6.26±0.12 | 6.07±0.16 | 6.23±0.11 | 6.13±0.10 | 5.94±0.14 | 6.13±0.05 |
| II | 6.22±0.12 | 5.94±0.06 | 5.94±0.16 | 6.01±0.10 | 5.86±0.14 | 6.15±0.11 | 5.99±0.21 | 6.02±0.05 |

*Normal physiological range of total erythrocyte count in dogs is 5-7.9 millons/μL
Means bearing different superscripts within a row (A, B) differ significantly ($p$<0.05).

**Table 4:** The mean + SE values of Erythrocyte Sedimentation Rate (ESR) in both groups during various periodic intervals.

| Group | Pre-operative day | Immediate-P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| I | 6.67±0.49$^{AB}$ | 7.33±0.33$^{A}$ | 6.67±0.21$^{AB}$ | 5.33±0.33$^{BC}$ | 4.33±0.49$^{CD}$ | 4.00±0.52$^{CD}$ | 3.33±0.61$^{D}$ | 5.38±0.27 |
| II | 6.83±0.40$^{A}$ | 7.50±0.34$^{A}$ | 6.67±0.21$^{AB}$ | 5.17±0.47$^{BC}$ | 3.83±0.30$^{CD}$ | 3.61±0.21$^{CD}$ | 3.00±0.45$^{D}$ | 5.23±0.28 |

*Normal physiological range of erythrocyte sedimentation rate in dogs is 1-8 mm/hr.

Values by the 7$^{th}$ postoperative day indicates activation of erythropoiesis as part of the physiological recovery process. Comparable trends were reported by Tembhurne *et al.* (2010) [27] and Chaurasia *et al.* (2019) [8], whereas Reddy *et al.* (2021) [23] observed delayed normalization by the 60th postoperative day. The stability of these parameters in the present study suggests minimal systemic blood loss and satisfactory postoperative recovery in both implant groups.
The erythrocyte sedimentation rate (ESR), (Table 4) was significantly ($p$<0.05) elevated during the preoperative,

immediate postoperative, and 7th postoperative days in both groups, followed by a gradual decline to baseline by the 90th postoperative day. The initial elevation represents the acute inflammatory phase triggered by trauma and surgical intervention, consistent with the observations of Phaneendra *et al*. (2018) [20]. The normalization of ESR by day 90 indicates resolution of inflammation and establishment of bone union. Ajadi *et al*. (2018) [2] also noted a positive correlation between ESR and the neutrophil-to-lymphocyte ratio during osteoarthritic inflammation, corroborating the present findings.

Total leukocyte count (TLC), (Table 5) values increased significantly ($p<0.05$) from the preoperative to 7$^{th}$ postoperative day and subsequently declined to normal levels by day 15. This biphasic trend aligns with the findings of Aiello and Mays (1998) [1], Mahendra *et al*. (2007) [16], and Chaurasia *et al*. (2019) [8]. The early leukocytosis can be attributed to the inflammatory response and stress-induced corticosteroid release following surgical trauma (Maiti *et al*., 1999; Toth *et al*., 2014) [17, 28]. The subsequent normalization reflects resolution of inflammation and progression of reparative bone formation.

Differential leukocyte counts (Table 6) revealed neutrophilia with relative lymphopenia during the preoperative and immediate postoperative periods, indicative of an acute inflammatory response (Schalm *et al*., 1975; Sastry, 1989) [26, 25]. Neutrophil counts declined significantly ($p<0.05$) up to the 30th postoperative day, while lymphocyte counts rose correspondingly from the 30$^{th}$.

**Table 5:** The mean + SE values of total leucocyte count in both groups during various periodic intervals

| Group | Pre-operative day | Immediate-P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean ± SE |
|---|---|---|---|---|---|---|---|---|
| I | 14.58±0.37$^{A}$ | 14.37±0.37$^{A}$ | 14.25±0.35$^{A}$ | 11.52±0.53$^{B}$ | 11.60±0.45$^{B}$ | 11.60±0.37$^{B}$ | 10.67±0.44$^{B}$ | 12.65±0.28 |
| II | 14.63±0.53$^{A}$ | 14.32±0.53$^{AB}$ | 14.17±0.52$^{AB}$ | 11.37±0.51$^{C}$ | 12.30±0.57$^{BC}$ | 10.85±0.43$^{C}$ | 11.18±0.40$^{C}$ | 12.68±0.29 |

*Normal physiological range of total leucocyte count in dogs is 5-14.1 thousands/µL.
Means bearing different superscripts within a row (A, B) differ significantly ($p<0.05$)

**Table 6:** The mean + SE values of white blood cells in both groups during various periodic intervals

| White blood cells | Group | Pre-operative day | Immediate-P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean ± SE |
|---|---|---|---|---|---|---|---|---|---|
| Neutrophils (%) | G-1 | 80.00±1.29$^{A}$ | 80.17±1.22$^{A}$ | 68.17±1.07$^{B}$ | 70.67±1.12$^{B}$ | 64.67±2.48$^{BC}$ | 59.00±1.03$^{C}$ | 64.50±2.64$^{BC}$ | 69.59±1.29 |
| | G-2 | 79.50±1.23$^{A}$ | 79.67±1.33$^{A}$ | 65.83±1.27$^{B}$ | 68.00±1.21$^{B}$ | 66.00±1.93$^{B}$ | 62.00±1.55$^{B}$ | 62.50±0.96$^{B}$ | 69.07±1.18 |
| Lymphocytes (%) | G-1 | 17.00±1.29$^{C}$ | 16.83±1.22$^{C}$ | 28.83±1.07$^{B}$ | 26.33±1.12$^{B}$ | 32.33±2.49$^{AB}$ | 38.00±1.03$^{A}$ | 32.50±2.64$^{AB}$ | 27.40±1..29 |
| | G-2 | 17.50±1.23$^{B}$ | 17.33±1.33$^{B}$ | 31.17±1.28$^{A}$ | 29.00±1.21$^{A}$ | 31.00±1.93$^{A}$ | 35.00±1.55$^{A}$ | 34.50±0.96$^{A}$ | 27.92±1.18 |
| Eosinophils (%) | G-1 | 1.50±0.22 | 1.33±0.21 | 1.33±0.21 | 1.35±0.33 | 1.17±0.17 | 1.35±0.21 | 1.33±0.21 | 1.33±0.08 |
| | G-2 | 1.33±0.21 | 1.33±0.21 | 1.33±0.21 | 1.33±0.21 | 1.50±0.22 | 1.50±0.22 | 1.50±0.22 | 1.40±0.07 |
| Monocytes (%) | G-1 | 1.50±0.22 | 1.67±0.21 | 1.67±0.21 | 1.67±0.33 | 1.83±0.17 | 1.67±0.21 | 1.67±0.21 | 1.67±0.08 |
| | G-2 | 1.67±0.21 | 1.67±0.21 | 1.67±0.20 | 1.67±0.21 | 1.50±0.22 | 1.50±0.22 | 1.50±0.22 | 1.59±0.08 |

*Normal physiological range of neutrophil count in dogs is 58-85%, lymphocyte (8-21%), eosinophil (0-9%) and monocyte (0-9%)
G-1: Group-I, G-2: Group-II

Means bearing different superscripts within a row (A, B) differ significantly ($p<0.05$). To 90$^{th}$ postoperative days. This reciprocal pattern signifies the transition from the acute inflammatory to the chronic reparative phase (Patil *et al*., 2017; Chaurasia *et al*., 2019) [19, 8]. Eosinophil and monocyte counts fluctuated within physiological limits, confirming the absence of hypersensitivity or implant rejection. The normal eosinophil trends in both groups further support the biocompatibility of titanium and stainless-steel implants.

### 3.2 Biochemical Parameters

Although clinical and radiographic evaluations are routinely used for fracture assessment, biochemical markers such as serum alkaline phosphatase (ALP), calcium, and phosphorus provide valuable insights into the metabolic activities associated with bone regeneration (Breur *et al*., 2004; Allen *et al*., 1998) [7, 3].

Serum ALP levels (Table 7) were significantly elevated ($p<0.05$) during the early postoperative period (up to day 7) in both groups, followed by a gradual decline to near-normal levels by day 90. This pattern corresponds to the period of active osteoblastic proliferation and matrix mineralization (Komnenou *et al*., 2005; Bhavani *et al*., 2022) [14, 6]. Elevated ALP levels in the early healing phase reflect the osteogenic response at the fracture site, while the subsequent decline indicates callus maturation and cortical bridging (Rani and Ganesh, 2003; Chaurasia *et al*., 2019) [21, 8]. Interestingly, the titanium DCP group (Group II) recorded comparatively lower ALP activity during the 30$^{th}$ and 60$^{th}$ postoperative days than the stainless-steel DCP group (Group I), suggesting faster osteoblastic stabilization and superior osseointegration with titanium implants.

Serum phosphorus levels (Table 8) increased up to the 7$^{th}$ postoperative day, followed by a gradual, non-significant decrease toward baseline values by the 90$^{th}$ day. This early rise corresponds to active mineralization during callus formation, while the later decline indicates transition to bone remodelling (Chaurasia *et al*., 2019; Bhavani *et al*., 2022; Chhatrola *et al*., 2022) [8, 6, 9]. The lower phosphorus levels observed in Group II during the late healing phase suggest faster attainment of metabolic equilibrium, reflecting efficient osteoblastic activity associated with titanium implants.

Serum calcium levels (Table 9) demonstrated a biphasic trend, showing a mild decline from the preoperative to 7th postoperative day, followed by a steady rise up to the 90th day. This decrease may be due to calcium deposition in the developing callus and temporary hypocalcaemia associated with postoperative bone metabolism (Hegde *et al*., 2007; Patil *et al*., 2017) [13, 19]. The subsequent rise represents bone remodelling and mineral consolidation. The calcium values in both groups remained within the physiological range throughout, indicating balanced calcium metabolism and absence of metabolic complications.

While both implant materials exhibited similar haematological and biochemical trends, the titanium DCP group consistently showed earlier normalization of leukocyte, ALP, and phosphorus values, indicating a reduced inflammatory response and faster metabolic stabilization. These findings reinforce titanium's superior biocompatibility, corrosion resistance, and enhanced osseointegration compared to stainless steel. No significant deviations in physiological parameters were observed, confirming that both materials are safe and effective for internal fixation in canine long bone fractures.

**Table 7:** Mean + SE values of alkaline phosphatase in different groups during various intervals

| Group | Pre-operative day | Immediate P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean± SE |
|---|---|---|---|---|---|---|---|---|
| I | 261.33±35.44$^{AB}$ | 259.50±26.61$^{AB}$ | 343.83±10.25$^{A}$ | 248.03±14.28$^{BC}$ | 191.17±15.18$^{BCD}$ | 163.67±15.32$^{CD}$ | 121.67±5.63$^{D}$ | 227.03±12.74 |
| II | 246.50±27.09$^{AB}$ | 253.17±24.43$^{AB}$ | 340.83±32.96$^{A}$ | 223.50±16.95$^{BC}$ | 177.83±25.60$^{BC}$ | 154.0±33.17$^{BC}$ | 120.00±13.5$^{C}$ | 216.55±13.93 |

*Normal physiological range of alkaline phosphatase in dogs is 1-114 units/L.
Means bearing different superscripts within a row (A, B) differ significantly ($p$<0.05).

**Table 8:** The mean + SE values of serum phosphorus in different groups during various periodic intervals

| Group | Preoperative day | Immediate P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean± SE |
|---|---|---|---|---|---|---|---|---|
| I | 4.14±0.56$^{AB}$ | 4.11±0.42$^{AB}$ | 5.45±0.16$^{A}$ | 3.93±0.23$^{BC}$ | 3.03±0.24$^{BC}$ | 3.48±0.33$^{BC}$ | 2.49±0.08$^{C}$ | 3.80±0.18 |
| II | 3.96±0.43$^{AB}$ | 4.01±0.39$^{AB}$ | 5.39±0.52$^{A}$ | 3.54±0.27$^{B}$ | 2.82±0.41$^{B}$ | 3.03±0.47$^{B}$ | 2.60±0.29$^{B}$ | 3.61±0.19 |

*Normal physiological range of serum phosphorus in dogs is 2.9-5.3 mg/ dL.
Means bearing different superscripts within a row (A, B) differ significantly ($p$<0.5)

**Table 9:** The mean + SE values of Serum calcium in different groups during various periodic intervals

| Group | Preoperative day | Immediate P.O.D | 7$^{th}$ P.O.D | 15$^{th}$ P.O.D | 30$^{th}$ P.O.D | 60$^{th}$ P.O.D | 90$^{th}$ P.O.D | Total Mean± SE |
|---|---|---|---|---|---|---|---|---|
| I | 10.47±0.56$^{BC}$ | 10.49±0.42$^{BC}$ | 9.16±0.16$^{C}$ | 10.67±0.23$^{AB}$ | 11.57±0.24$^{AB}$ | 11.12±0.32$^{AB}$ | 12.10±0.08$^{A}$ | 10.79±0.18 |
| II | 10.70±0.43$^{AB}$ | 10.59±0.39$^{AB}$ | 9.20±0.52$^{B}$ | 11.06±0.27$^{A}$ | 11.78±0.41$^{A}$ | 11.57±0.47$^{A}$ | 12.00±0.29$^{A}$ | 10.99±0.19 |

*Normal physiological range of serum phosphorus in dogs is 9.1-11.7 mg/ dL.

Means bearing different superscripts within a row (A, B) differ significantly ($p$<0.05). Intergroup differences were statistically non-significant, titanium DCPs exhibited faster biochemical normalization, earlier limb function, and fewer postoperative complications. Owing to their superior biocompatibility, corrosion resistance, and mechanical compatibility with bone, titanium DCPs are recommended over stainless-steel plates for internal fixation of canine long bone fractures, despite their higher cost.

## 4. Conclusion

Both stainless-steel and titanium dynamic compression plates (DCPs) effectively stabilized long bone fractures in dogs, supporting normal haematological and biochemical recovery throughout healing. Haematological parameters remained within physiological limits, indicating minimal systemic stress, while serum alkaline phosphatase and phosphorus increased during early osteogenesis and declined as healing progressed. Serum calcium showed an initial decrease followed by normalization, reflecting callus mineralization. Although.

**Conflict of Interest:** Not available

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