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Comparative Evaluation of Platelet Rich Plasma and Zinc Fluorophosphate Along with LCP for Healing of Long Bone Fractures in Dogs

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ABSTRACT

Background: Objective of present study was to evaluate the efficacy of synthetic (zinc fluorophosphate) and biological (platelet rich plasma) bone substitutesalong with locking compression plate fixation for repair of long bone fractures in dogs.

Methods: In this study, 36 clinical cases of fractures of different long bones were divided in three groups having 12 animals each.In group I animals, fracture was stabilized with Locking Compression Plate (LCP), while in groupsIIand III, platelet rich plasma (PRP) and zinc fluorophosphates, respectively were additionally used at the fracture site along with LCP.

Result: Different clinical, radiographic and haemato-biochemical parameters were evaluated up to 3 months post-operatively, the progress of the fracture healing was assessed using a radiographic scoring system for bone formation and union. Highest values of bone formation, union and weight bearing score were observed in group III at all intervals as compared to other groups. Functional outcome, lameness grading and radiographic observations revealed that LCP with zinc fluorophosphate had superior outcomes, early bone formation, bone union, better weight bearing and improved gait, as compared to LCP alone or LCP with PRP. Results suggested that zinc fluorophosphate, along with LCP, is better than PRP and only LCP for osteosynthesis of long bones in dogs. In present study, Group III yielded best result followed by Group II and Group I.

Key words: Locking compression plate, Long bone fracture, Dog platelet rich plasma, Zinc fluorophosphate.

INTRODUCTION

The Locking Compression Plate (LCP) offers distinct advantages during fracture healing by preserving the vascularity and geometric benefits of the Limited Contact Dynamic Compression Plate (LC-DCP) system with the elimination of the need to apply compressive forces to the bone. The LCP features a combi-hole that can serve as either a locking or compression hole (Siddaraju *et al.*, 2021). Bone grafting with locking compression bone plate and barrier membranes as guided bone regeneration aids in enhanced fracture healing with minimal complications (Preethi *et al.,* 2025).

Bone grafting is one of the most frequently employed surgical techniques to enhance bone regeneration in orthopedic surgery. Bone grafts are intended to be fully degraded and replaced by host like bone through osteogenesis and remodeling (Su *et al*., 2019). However, autograft has significant disadvantages, such as donor site morbidity and potential complications like haematoma formation, nerve injury, blood loss and unaesthetic defects. To circumvent the drawbacks of biologically sourced bone grafts, synthetic grafts can be used to treat bone defects. One of the most crucial properties of synthetic bone graft substitutes is their porous structure and complete biodegradability. Ideally, the biodegradation products should support bone regeneration at a rate that matches new bone formation. Bioactive glass based bone graft substitutes can possess all these properties (O'Connor *et al*., 2020).

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The graft materials applied at fracture site had hasten the healing process. The rate of fracture union is increased when it implanted at fracture site (Dwivedi *et al.,* 2024). There is significant scope in veterinary surgical practice for the therapeutic use of zinc fluorophosphate graft for long bone fracture repair (Deshpande, 2023). Platelet rich plasma (PRP) is a simple, easy to apply, cost effective and minimally invasive procedure that delivers concentrated autologous growth factors (GFs) and cytokines into injured tissues in natural proportions. This blood derived product is applied directly to damaged tissue, either surgically or via injections and has been extensively studied across various medical disciplines (Cavallo *et al*., 2016). PRP is abundant in growth factors that are essential for bone healing, including platelet-derived growth factor (PDGF), Transforming Growth Factor-beta (TGF-β) and insulin-like growth factor-1 (IGF-1). These factors are helps to promote the bone healing process. These growth factors and cytokines play key roles in regulating inflammation, angiogenesis and osteoblastic activity, making them vital to the various phases of bone repair (Zhang *et al*., 2021). Looking to above mention facts and promising role of bone substitute in fracture healing present study was planned with the objective to evaluate efficiency of bone substitute for healing of long bone in dogs.

MATERIALS AND METHODS

Groups

In this study, total 36 dogs were divided in three groups (Table 1). In Group I, fractures were immobilized using a LCP. While in Group II and III, long bone fractures were immobilized using LCP along with additional use of PRP and zinc fluorophosphate granules as a bone substitute, respectively at the fracture site.

Implant used in study

In this study, surgical procedure was performed to apply LCP on the tensile surface of the bone, *viz*. on the lateral surface of the femur, cranio-lateral aspect of the radius and cranio-medial aspect of the tibia. For fixation of the fractures, LCP with diameters of 2.7, 3.5 and 4.0 mm were employed, along with locking head screws ranging from 10 to 28 mm in length. These locking plate and screw combination was found to be effective in achieving rigid fracture fixation in this study.

- Locking compression plates 2.7 mm, 3.5 mm and 4.0 mm.
- Locking head screws 2.7 mm, 3.5 mm and 4.0 mm.
- Drill bit 2.5 mm, 3.0 mm and 3.5 mm.

Location of study

The present study was carried out during year of 2022-2023 at the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat

Radiographic examination

Pre-operative radiographs were taken before surgery and then at different intervals like on the 15th, 30th, 60th and 90th post-operative days healing of the fracture was evaluated using a radiographic scoring system based on Lane and Sandhu (1987).

Weight bearing

The weight bearing of the affected limb was recorded and scored during standing, walking and running on 7th, 14th, 30th, 60th and 90th days post-operatively, following the scoring system described by Aithal (1996).

Bone substitute

PRP was prepared by double centrifugation method as described by Shin *et al*. (2017). Strip of zinc fluorophosphate in form of granules were procured from Pandian Advanced Medical Centre Pvt. Ltd., Madurai, Tamil Nadu.

Preoperative preparation, anaesthesia and postoperative care

In this study, fractures were diagnosed based on pre-operative radiograph and clinical signs such as non-weight bearing, reluctance to move the limb, lifting the limb above ground level, or just touching the toe to the ground. A dangling limb indicated a broken bone (Fig 1). Immediate post-operative care in orthopedic surgery involved limb bandaging and a week-long course of antibiotics to promote favorable outcomes. Initially, movement was restricted and dogs were allowed to use their limbs approximately 10-15 days after the surgery. For surgical anaesthesia, atropine sulphate was administered as anticholinergic drug, followed by the use of intravenous ketamine and diazepam to induce anesthesia. Maintenance was carried out under isoflurane inhalant anaesthesia. This protocol adequately anesthetized the animals in all four groups, allowing sufficient muscle relaxation for surgical procedure. Post-operatively antibiotic was given for 7 days, while analgesic was administered for 5 days. Owners were also advised for regular wound care and limited movement of dogs.

Haemato-biochemical estimations

Blood samples were collected preoperatively and on the 15th, 30th and 60th post-operative days. These blood samples were obtained to estimate various parameters like Complete Blood Count (CBC)using fully automated hematology analyzer[-8]. While separated serum was used to estimate calcium (Ca) (mg/dL), phosphorus (P) (mg/dL) and alkaline phosphatase (ALP) (IU/L) using fully automated analyzer[-9].

Complications

Intra and post-operative complications up to 3 months related to wound, implant and fracture healing, if any were observed, recorded and treated accordingly in all the groups.

Statistical analysis

The data obtained from the study between and within the two groups of equal observation were statistically analyzed by using two-way analysis of variance (ANOVA) followed by TUKEY to check the significance difference between the mean.

RESULTS AND DISCUSSION

In this study, the main cause of fracture was road traffic accidents/automobile accidents (57%), followed by fall from height (31%), human inflicted trauma (10%) like hit by a stick and unknown causes (2%). Road traffic accidents as major cause of fracture followed by fall from height was also reported earlier by Singh *et al*. (2015); Jain *et al*. (2018) and Deshpande (2023). Highest incidence of fracture was recorded in femur (50%), followed by tibia (26%) and radius-ulna (24%), Similar findings have also been noted by Aithal *et al*. (1999) who have reported highest number of fractures in femur (38.56%), followed by tibia/fibula (17.16%), radius/ ulna (16.92%) and humerus (7.71%). Singh *et al*. (1999); Seaman and Simpson (2004), Fazili *et al.* (2005) and Jain *et al*. (2018) also reported more number of fractures in the femur bone followed by tibia-fibula, radius-ulna and humerus in dogs. Palpation of the fractured fragments revealed crepitus, as well as pain and swelling at the fracture site. Similar clinical findings were also earlier recorded by Shashikant *et al*. (2024) in tibial fractures.

Table 1: Data related to signalment of selected cases in all groups of present study.

Case no.	Age (month)	Gender (M/F)	Breed	Etiology	Affected bone	Timesince injury
			Group I			
1	12	Male	Spitz	Accidental injury	L. femur	3 days
2	8	Female	Spitz	Automobile accident	L. tibia	5 days
3	48	Male	ND	Accidental injury	L. femur	7 days
4	14	Male	Great dane	Accident with cart wheel	L. tibia	6 days
5	48	Male	Golden retriever	Fall from height	R. radius	3 days
6	8	Male	GSD	Fall from height	L. femur	1 days
7	8	Male	Spitz	Fall from height	R. femur	2 days
8	6	Male	ND	Motor bike accident	R. femur	3 days
9	6	Female	ND	Fall from height	R. tibia	2 days
10	10	Male	ND	Accidental injury	L. radius	5 days
11	24	Female	ND	Automobile accident	L. radius	2 days
12	24	Female	GSD	Fall from height	L. tibia	6 days
			Group II			
1	48	Female	ND	Automobile accident	L. radius	3 days
2	42	Male	ND	Accidental injury	L. femur	4 days
3	12	Male	GSD	Jump from terrace	R. tibia	3 days
4	8	Male	ND	Fall from height	L. femur	6 days
5	7	Male	ND	Jump from height	L. femur	5 days
6	7	Female	GSD	Automobile accident	L. femur	3 days
7	24	Female	ND	Automobile accident	L. Tibia	2 days
8	12	Male	ND	Hit by car	L. femur	1 day
9	20	Female	ND	Automobile accident	L. femur	2 days
10	36	Male	Lab	Hit by stick	R. tibia	3 days
11	24	Male	Spitz	Automobile accident	L. tibia	3 days
12	24	Female	Spitz	Hit by stick	R. radius	3 days
			Group III			
1	8	Female	ND	Automobile accident	R. tibia	4 days
2	72	Male	GSD	Accidental injury	L. tibia	5 days
3	36	Male	ND	Automobile accident	R.radius	4 days
4	18	Male	ND	Automobile accident	L. tibia	2 days
5	12	Male	Spitz	Automobile accident	L. radius	5 days
6	84	Female	ND	Fall from height	R.radius	6 days
7	8	Male	Spitz	Fall from height	L. femur	2 days
8	36	Male	Spitz	Automobile accident	L. femur	3 days
9	7	Male	ND	Accidental injury	L. femur	3 days
10	12	Male	ND	Automobile accident	R. femur	3 days
11	24	Male	ND	Hit by stick	R. radius	5 days
12	24	Female	Spitz	unknown	L. radius	4 days

Bone formation score and union score

Highest values of bone formation and union score were observed in group III at all intervals as compared to group I and II. The radiographic scoring was higher in group 3, probably attributed to osteoinduction and osteoconduction properties of zinc fluorophosphate (Table 2) whereas, in group I, no biomaterial was used at the fracture site and in group II, PRP was administered at fracture site which had the property of osteoinduction. Similarly, Singh *et al*. (2020) obtained superior results when PRP was combined with scaffolds of β-tricalcium phosphate in dogs. On 15th post-operative day, radiographic examination of fracture site showed partial to full fracture line. It depicted non-significant difference between the all groups. The value of group III was higher as compare to I and II which revealed better osteogenic activity. Similar to 30th day time interval, a significant higher score was noticed in group III indicating almost absence of fracture line. These values were significantly different between the groups and conclusive of no fracture line during radiographic examination of affected dogs (Table 3). As per Sahu *et al*. (2017), fracture was considered healed when a visible bridging callus was present on at least one cortex or when the fracture line disappeared on both views. Additionally, it was observed that younger animals healed relatively earlier than older ones. By 60th post-operatively day, functional recovery was rated as good to excellent in most of the animals. On 90th day, radiograph showed complete radiographic union with remodeling of bone (Fig 2 to 17). During this study, post-operative radiographic examination showed displaced or bent plate in two cases of group II and two cases of group III (Fig 11 and 18). Further external immobilization were instituted for rapid healing without surgical intervention in such cases.

Post-operative care and wound healing

Immediate post-operative care in orthopedic surgery involved limb bandaging and a week-long course of antibiotics to promote favorable outcomes. Initially, movement was restricted and dogs were allowed to use their limbs approximately 10-15 days after the surgery. Surgical wounds healed by first intention in all dogs except in four dogs. In which surgical wound was healed by second intention after infection or self-mutilation (Sup. Fig 1 and 2). In group I, Case No.7 had wound dehiscence after 7 days of surgery. Whereas, in Case No. 5 mild discharge was observed due to self-mutilation over the surgical site. In group III, Case No. 11 had self-mutilated wound which was healed by second intention and in Case No. 1, tendency of self-mutilation led to exposure of bone plate from distal side after 3 month of surgery.

Fig 1: Preoperative radiographs of various fractured bone of different groups.

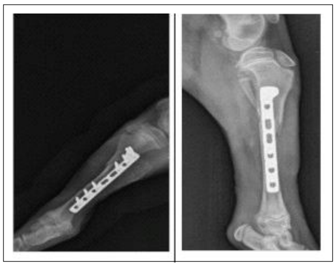


Fig 2: ML and CC view of 30th day radiograph of group I.

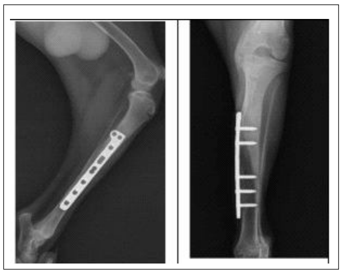


Fig 4: ML and CC view of 90th day radiograph of group I.

Fig 3: ML and CC view of 60th day radiograph of group I.

Fig 5: 15th day radiograph of group II.

Fig 6: 60th day radiograph of group II.

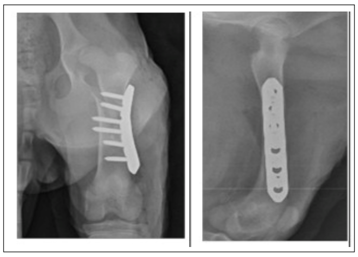


Fig 7: ML and CC view of 15th day radiograph of group II.

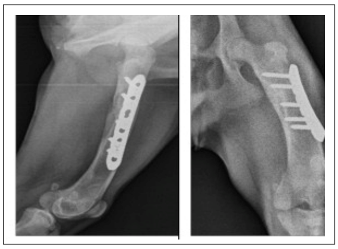


Fig 8: ML and CC view of 30th day radiograph of group II.

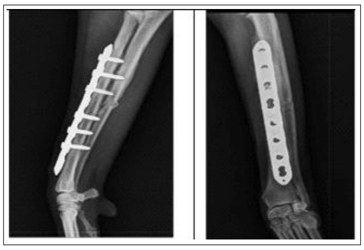


Fig 9: ML and CC view of 60th day radiograph of group II.

Fig 10: ML and CC view of 90th day radiograph of group II.

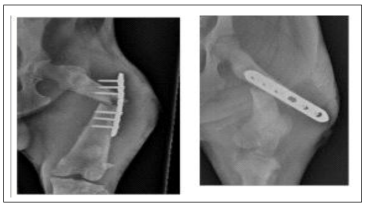


Fig 11: Post-operative plate displaced group II.

Fig 12: 15th day radiograph of group III.

Fig 13: 90th day radiograph of group III.

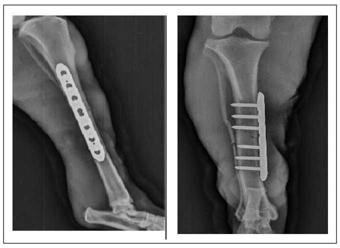


Fig 14: ML and CC view of 15th day radiograph of group III.

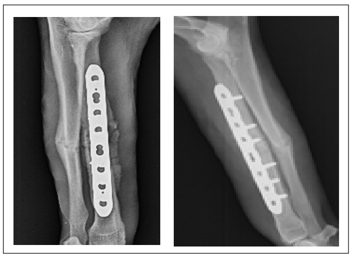


Fig 15: ML and CC view of 30th day radiograph of group III.

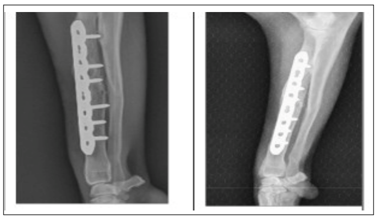


Fig 16: ML and CC view of 60th day radiograph of group III.

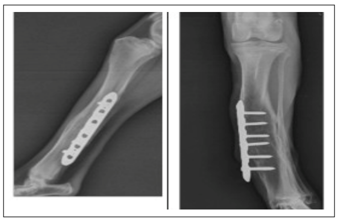


Fig 17: ML and CC view of 90th day radiograph of group III.

Post-operative inflammation, pain and swelling continued for up to 10 days after surgery. By the 15th day post-surgery, the animals began to show moderate weight-bearing, as evidenced by them touching their paw to the ground. Full weight bearing was observed from the 30th day onward (Table 4). Typically, early post-operative weight bearing occurs after bone plating (Sahu *et al*. 2017). Schwandt and Montavan (2005) reported full weight bearing in comminuted fractures of the radius-ulna and tibia-fibula treated with LCP by the 14th day. Weight bearing score during standing walking and running values were gradually increased in all groups at various intervals. Values of weight bearing increased on 14th day onward time interval with clinical examination depicted touching of toe to touching of paw in the operated limbs of dogs in group I. In group II and III, data showing significant improvement at 14th day onwards and complete weight bearing was observed with mean score of 2.0±0.17 in group III at the earliest (60th day) among all three groups. Moreover, scores of all groups displayed a steady and consistent increasing weight bearing pattern, with paw contact or touching on the

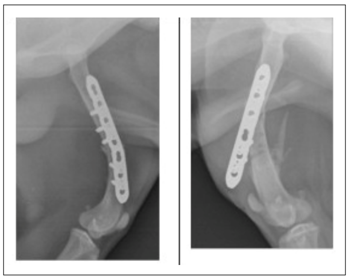
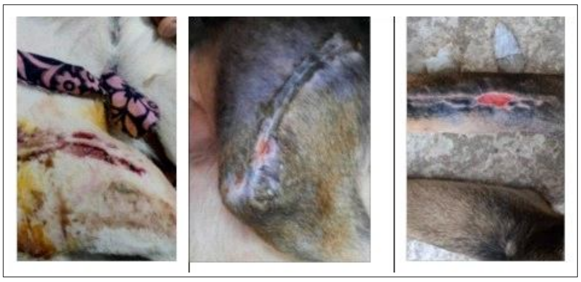


Fig 18: Bending and displaced plate.

Supplementary Fig 1: Skin wound healing by first intention.



Supplementary Fig 2: Skin wound complications (infection or self-mutilation).

Occasional touching of toe on 7th post-operative day in group II.

Weight bearing in operated limb on 7th post-operative day in group III.

Carrying of affected limb on 15th post-operative day in group I.

Carrying of affected limb on 15th post-operative day in group III.

Weight bearing (touching of paw) of affected limb on 15th post-operative day in group II.

Weight bearing (touching of paw) of affected limb on 15th post-operative day in group III.

Complete weight bearing (touching of paw) of operated limb on 30th post-operative day in group III.

Complete weight bearing (touching of paw) of operated limb on 30th post-operative day in group II.

Supplementary Fig 3: Weight bearing on different post-operative days in all groups.

ground post-operatively by day 60 (Sup. Fig 3). Scores of group III was higher at all intervals as compare to group I and II. Similar findings were also noted by Sahu *et al.* (2017).They noted early weight bearing while standing in all animals. They observed full weight bearing while walking and running, touching their paw with every step, by the 30^{th}post-operative day and beyond. Kumar *et al.* (2007) reported the early recovery of weight bearing in dogs with femur fractures stabilized using LCP. Kumar *et al*. (2024) also reported weight bearing scores, radiographic scores and functional usage of limb was better in dogs with long bone fractures treated with β-TCP and PRP. In this study, the clinical outcomes regarding weight bearing were excellent in group III as compare to other group. As the weight bearing scores increased gradually at various post-operative intervals and the lameness subsided entirely once the fractures had fully healed.

The haematological values varied non-significantly in normal physiological ranges. The biochemical values of serum calcium, phosphorus and alkaline phosphatase varied non-significantly in normal physiological ranges. Serum calcium values revealed a significant decrease on day 15 followed by a significant increase till day 60 in all groups. Serum alkaline phosphatase showed a significant increase on 15^{th} post-operative day followed by a significant decrease till 60^{th} post-operative day and the values returned to baseline level in all three groups. Values of alkaline phosphatase (ALP) varied non-significantly ($p>0.05$) among the groups at different time intervals during the study period. While values were significantly differs within groups on 15^{th} and 30^{th} day as compare to day 0 and day 60 in all groups. Similar type of findings previously also reported by Yadav *et al*. (2021) in long bone fracture cases. Fracture healing normally occurs through heightened activity of

Table 2: Mean±SE value of bone formation score in all groups at different intervals.

Day	Group I	Group II	Group III	P value
15	$0.42±0.21^{D}$	$0.5±0.17^{C}$	$0.75±0.18^{C}$	0.247^{NS}
30	$1.5±0.21^{Cb}$	$1.5±0.17^{Bb}$	$2.25±0.18^{Ba}$	0.0263
60	$3.00±0.21^{B}$	$3.33±0.17^{A}$	$3.58±0.18^{A}$	0.2519^{NS}
90	$3.92±0.21^{A}$	$3.75±0.17^{A}$	$3.92±0.18^{A}$	0.4171^{NS}
P value	<.0001	<.0001	<.0001	

Table 3: Mean±SE value of union score in all groups at different intervals.

Day	Group I	Group II	Group III	P value
15	$0.67±0.29^{B}$	$0.67±0.28^{C}$	$0.83±0.24^{C}$	0.8952^{NS}
30	$1.33±0.29^{Bb}$	$2±0.28^{Bab}$	$2.83±0.24^{Ba}$	0.0068
60	$3±0.29^{A}$	$3.67±0.28^{A}$	$3.83±0.24^{A}$	0.0943^{NS}
90	$3.83±0.29^{A}$	$3.67±0.28^{A}$	$3.83±0.24^{A}$	0.7712^{NS}
P value	<.0001	<.0001	<.0001	

Table 4: Mean±SE value of weight bearing score in all groups.

Days	Weight bearing	Group I	Group II	Group III	P value
7	Standing	$0.58±0.2^{C}$	$0.83±0.17^{C}$	$1.00±0.17^{C}$	0.4382^{NS}
	Walking	$0.92±0.29^{C}$	$1.08±0.25^{C}$	$1.25±0.2^{C}$	0.7264^{NS}
	Running	$0.5±0.31^{D}$	$0.42±0.23^{C}$	$0.75±0.16^{C}$	0.4402^{NS}
14	Standing	$1.50±0.2^{B}$	$1.50±0.17^{BC}$	$1.67±0.17^{BC}$	0.7505^{NS}
	Walking	$1.83±0.29^{BC}$	$2.08±0.25^{BC}$	$2.5±0.2^{B}$	0.2556^{NS}
	Running	$1.42±0.31^{CD}$	$1.25±0.23^{C}$	$1.83±0.16^{B}$	0.3894^{NS}
30	Standing	$1.58±0.2^{B}$	$1.75±0.17^{B}$	$1.83±0.17^{B}$	0.4012^{NS}
	Walking	$2.67±0.29^{AB}$	$3.08±0.25^{AB}$	$3.25±0.2^{AB}$	0.3145^{NS}
	Running	$2.17±0.31^{BC}$	$2.42±0.23^{B}$	$2.5±0.16^{B}$	0.6886^{NS}
60	Standing	$1.75±0.2^{Bb}$	$1.92±0.17^{Aa}$	$2.00±0.17^{Aa}$	0.5290^{NS}
	Walking	$3.42±0.29^{A}$	$3.83±0.25^{A}$	$3.58±0.2^{A}$	0.2888^{NS}
	Running	$2.92±0.31^{ABb}$	$3.75±0.23^{Aa}$	$3.92±0.16^{Aa}$	0.0077
90	Standing	$1.83±0.2^{A}$	$2.00±0.17^{A}$	$2.00±0.17^{A}$	0.7626^{NS}
	Walking	$3.67±0.29^{A}$	$3.75±0.25^{A}$	$3.83±0.2^{A}$	0.8048^{NS}
	Running	$3.75±0.31^{A}$	$3.83±0.23^{A}$	$3.83±0.16^{A}$	0.9241^{NS}
P value		<.0001	<.0001	<.0001	

Different superscripts differ significantly between (Capital) and within (Small) groups ($p<0.05$), NS: Non significant.

osteoblasts. These cells play a key role in forming new bone tissue and mineralizing the bone matrix. As part of this process, they release significant amounts of alkaline phosphatase (ALP), which contributes to bone repair. ALP activity holds promise as a potential biomarker for assessing the progress and pace of bone healing Joshi *et al.* (2022).

CONCLUSION

LCP with zinc fluorophosphate was found better as the animals showed early bone formation and union, enhanced weight bearing and better gait improvement when compared to LCP and LCP with PRP during the study. The zinc fluorophosphate can be used as a potential synthetic bone substitute for accelerating bone healing in cases of long bone fractures in dogs.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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