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Evaluation of Platelet Storage Lesion (PSL) Markers in SSP+ Platelet Additive Solution Added Canine Platelet Concentrates (CPC)

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ABSTRACT

Background: To evaluate the platelet storage lesion (PSL) markers and extended storage time for SSP+ added canine platelet concentrates (CPC) beyond five days at 22°C. Fresh canine CPC undergo certain metabolic and morphologic changes during storage. Estimation of PSL markers helps us better understand quality of the canine PC and to estimate the efficacy of SSP+ in increasing the life span of stored CPC.

Methods: CPC was prepared from 350 ml of whole blood, collected from 6 apparently healthy donor dogs by buffy coat method in a quadruple bag closed system. CPC was divided into four aliquots of 15 ml each with one control and three test groups with SSP+ additive solution in different concentrations (65%, 75%, 85%). The *In vitro* PSL markers including swirling, pH, glucose, lactate, bicarbonate, pO_2, pCO_2, platelet concentration, MPV and PDW were evaluated on day 1, 5, 9 and 13 of storage.

Result: The results were analysed by Two-way ANOVA wherein swirling, pH, glucose, lactate, bicarbonate, pO_2, pCO_2, platelet concentration, MPV showed significant difference ($p<0.01$) between the groups. Interaction between the additive concentrations and the days of storage revealed a pH on day 9 for 65% SSP+ to be similar to day 5 for plasma stored CPC while swirling, lactate and bicarbonate were also better maintained for 65% SSP+ PAS added CPC till day 9. Addition of 65% SSP+ PAS to CPC evidenced increased shelf life up to 9 days at 22°C under agitation without significant deterioration in product quality.

Key words: Canine, Platelet additive solutions (PAS), Platelet Concentrate, Platelet indices, Platelet storage lesions (PSL), Swirling.

INTRODUCTION

Thrombocytopenia (mild, moderate and severe), a quantitative disorder of reduction in number of circulating platelets is the most commonly occurring haematological disorders in small animal medicine (Hux and Martin, 2012).

Severe thrombocytopenia (Platelets < 25,000 cells/µl) due to haemoprotozoal and rickettsial diseases with clinical signs of haemostatic abnormalities are highly prevalent in dogs with an incidence of 32.2% which were presented to the Critical Care Unit, MVC, Chennai and the TANUVAS Animal Blood Bank, MVC, Chennai (Baranidharan, 2015).

Conventional fresh CPC stored at 22°C showed optimal post transfusion platelet recovery and platelet functions when compared to chilled platelets, cryopreserved platelets or lyophilised platelets that can be used in severely thrombocytopenic dogs to provide immediate and short-term haemostasis.

Globally, CPC transfusions are seldom practiced on account of unavailability of stored platelets due to its short period of storage (5 days) and lack of donors at emergency crisis which are shortcomings that are encountered in veterinary blood banks (Callan *et al*., 2009). Hence, in this study it was hypothesised that the addition of SSP+ PAS to the CPC stored at 22°C under continuous agitation would increase the storage life and quality of canine PC.

Henceforth, the objective of this study was to increase the shelf life of CPC by the use of SSP+ PAS.

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MATERIALS AND METHODS

The study was conducted at the TANUVAS Animal Blood Bank (TABB) facility at Madras Veterinary College and Teaching Hospital, TANUVAS, Chennai during the period

from April 2020 to February 2021. CPC was prepared from eligible donor dogs (*n = 6*) brought to the TANUVAS Animal Blood Bank of the Madras Veterinary College. Jugular phlebotomy and blood collection was performed using 350 ml Quadruple blood bag system[1] as per standard protocols. The tubing was then sealed was sealed using an automated tube sealer[2] and the blood bag was allowed to rest at ambient temp (22-24°C) for one hour until further processing. The blood bag was then centrifuged at 3400 rpm for 11 minutes at 22°C in a refrigerated centrifuge[3] and an automated component separator[4] was used to extract the Packed Red Blood Cells into the SAGM containing satellite bag and Plasma component into another satellite bag. Buffy Coat layer was suspended in the bag for at least 2 hours at room temperature which was further centrifuged at 900 rpm for 6 minutes at 17°C and the supernatant PC was extracted into the platelet storage satellite bag. CPC was kept at room temperature for 30 minutes before storing it in the Platelet Agitator[5] at 22°C.

The total amount of CPC was divided into four aliquots of 15 ml to which SSP+ was added at concentrations of 65%, 75% and 85% of the total final volume (Table 1).

Swirling was assessed for the CPC by holding the bags against a source of bright light and gently agitating the bag. The intensity of the white turbulent cloudy appearance was graded as nil (No swirling), + (mild), ++ (moderate) and +++ (Extensive).

The PSL markers were evaluated using a portable arterial blood gas analyses machine[6] while the platelet indices like MPV and PDW were measured using a auto-haemoanalyser[7]. All samples were studied for the above parameters on days 1, 5, 9 and 13 during storage and were evaluated for bacterial contamination.

Statistical analysis

The level of swirling was assigned ranks and statistical analysis was done using Kruskal Wallis analysis (Table 2). The statistical analysis was performed using SPSS software. Two Way ANOVA was used determine the effect of the treatments (Control, 65% SSP+ PAS, 75% SSP+ PAS and 85% SSP+ PAS) (Table 3) and days (Table 4) on the PSL markers and the interactions between the treatments and days (Table 5) on the PSL markers using 95% confidence interval.

RESULTS AND DISCUSSION

There was statistically significant ($p<0.05$) difference between the swirling observed in the control and 65% SSP+ PAS added CPC group and that the swirling quality of 65% SSP+ PAS added CPC was well maintained till day 9 (Table 2) (Fig 1) The difference in mean pH between the control group and the test groups was significant and the plasma stored control CPC showed similar pH to that of 65% SSP+ PAS added CPC group (7.27±0.021) and (7.22±0.021) (Table 3). The mean pH of the control CPC on day 5 was 7.28±0.0384 which similar to that 9th day of 65% SSP+ PAS added CPC. (Table 5) (Fig 2).

The mean glucose in the control CPC (441±5.19 mg/dl) was significantly high ($p<0.01$) when compared to that of the SSP+ PAS added PC groups (Table 3). The depletion in glucose concentration was significant ($p<0.01$) in control CPC when compared to the PAS added test groups (Table 5) (Fig 3). Similarly, mean lactate concentrations evidenced a statistically significant difference ($p<0.01$) between the control group and the SSP+ PAS added CPC group (Table 3). The increase in the lactate concentrations and difference in the mean lactate concentrations through the days was high for the 65% SSP+ added PAS group (Table 4 and 5) (Fig 4) The difference in the mean pO_2 and pCO_2 concentration between the control group, 65%, 75% and 85% SSP+ PAS added CPC group was significant ($p<0.01$) (Table 3) (Fig5 and Fig 6). The difference between the mean bicarbonate concentration during storage in the control group was statistically significant ($p<0.01$) as was the difference in the mean bicarbonate concentration across the days of storage. (Table 3 and 4). The control CPC showed a rapid decline in the plasma bicarbonate concentrations when compared to the PAS added test groups (Table 5) (Fig 7).

The mean platelet concentration during storage in the control was significantly higher ($p < 0.01$) when compared to the mean platelet concentration of the 65%, 75% and 85% SSP+ PAS added CPC groups and we observed that the platelet concentration remained fairly stable during storage for all the SSP+ PAS added CPC (Table 5) (Fig 8).

The MPV increased over time during storage across all groups and mean MPV for the control group, 65%, 75% and 85% SSP+ PAS added CPC evidenced a significant difference ($p<0.01$) across the treatment group (Table 3) The mean MPV at 9 days during storage was highest for 75% SSP+ PAS added CPC group while the lowest was observed in the control CPC group (Table 5) (Fig 9). The PDW also increased over time during storage across all groups and mean PDW for the control group, 65%, 75% and 85% SSP+ PAS added CPC group were not statistically significant (Table 3) interaction study between the additive concentration and the days for storage for PDW was unable to yield significant results (Table 5) (Fig 10).

All CPC samples under our study were subjected to bacterial culture examination and were negative for any bacterial growth.

It was observed that swirling was well maintained at moderate level in plasma stored control CPC up to day 6 of

[1]Donato 350 ml CPDA/SAGM Top - and - bottom bag.
[2]TERUMO PENMOL™ XS1010 Tube Sealer
[3]Thermo Scientific HERAEUS CRYOFUGE 5500i™ centrifuge
[4]HiCARE™ – CX
[5]TERUMO PENMOL™ PI200 Platelet Incubator
[6]EPOC™

storage while it was maintained at moderate levels till day 9 in 65% SSP+ PAS added CPC group while the pH was within the range of 6.4-7.4 (Bertolini and Murphy, 1996). Swirling reduced drastically in plasma stored control CPC when compared to the 65% SSP+ PAS added group (Hlavac *et al*., 2017).

The pH dropped drastically during the period of storage in the plasma stored control CPC group which could be attributed to the increased utilization of glucose for platelet metabolism and subsequent increase in the lactate concentration leading to rapid decrease in the pH (Milford and Reade, 2016). The pH was maintained at a constant level and the decrease in the pH was not statistically significant for the CPC stored in 65% SSP+ PAS group which had a mean of 7.28 at day 9 of storage, similar to that of day 5 of the control group signifying that the quality of CPC was well maintained up to 9th day of storage for the 65% SSP+ PAS added CPC group (Hoareau *et al*., 2014). Mean pH of 75% and 85% SSP+ PAS added CPC were above 7.4 which could be associated with loss of platelet viability (Tynngård, 2009).

Table 1: Volume of additive solution for 65 per cent, 75 per cent and 85 per cent SSP+ PAS added PC groups respectively.

Percentage PAS	Amount added to 15 ml PC (ml)	Final volume
65 per cent PAS	27.7 ml PAS	42.7 ml
75 per cent PAS	32.02 ml PAS	47.02 ml
85 per cent PAS	36.3 ml PAS	51.3 ml

Table 2: Effect of Mean±SE on platelet swirling (Kruskal Wallis test).

	Control	65% PAS	75% PAS	85% PAS	p value
Swirling	1.83±1.090^{a}	2.41±0.653^{b}	1.75±0.793^{ab}	1.7±0.750^{a}	< 0.05*

Values bearing the same superscript do not vary significantly.

*Significant, **Highly significant, NS- Non-significant.

Table 3: Effect of additive concentration on Mean±SE of platelet storage lesion (PSL) markers (n=6).

Platelet storage lesion markers	Treatments				
	Control	PAS 65%	PAS 75%	PAS 85%	p value
pH	7.22±0.021^{b}	7.27±0.021^{b}	7.47±0.021^{a}	7.47±0.021^{a}	< 0.01**
Glucose (mg/dl)	441±5.19^{a}	192±5.19^{b}	171±5.19^{c}	169±5.19^{c}	< 0.01**
Lactate (mmol/l)	6.5±0.286^{a}	1.49±0.286^{b}	2.16±0.286^{b}	2.08±0.286^{b}	< 0.01**
pO_2 (mmHg)	166±1.73^{b}	178±1.73^{a}	171±1.73^{ab}	174±1.73^{a}	< 0.01**
pCO_2 (mmHg)	23.6±1.66^{a}	11.7±1.66^{c}	18.1±1.66^{ab}	15.1±1.66^{bc}	< 0.01**
Bicarbonate (mmol/l)	9.95±0.431^{a}	6.87±0.431^{b}	4.77±0.431^{c}	4.58±0.431^{c}	< 0.01**
Platelet concentration (× 10^3/µl)	381±9.62^{a}	135±9.62^{b}	121±9.62^{b}	113±9.62^{b}	< 0.01**
MPV (fL)	5.50±0.059^{c}	5.65±0.059^{bc}	5.78±0.059^{ab}	5.91±0.059^{a}	< 0.01**
PDW	14.8±0.0981^{a}	14.9±0.0981^{a}	14.8±0.0981^{a}	14.8±0.0981^{a}	NS

Values bearing the same superscript do not vary significantly.

*Significant, **Highly significant, NS- Non-significant.

Table 4: Effect of days of storage on mean±SE of platelet storage lesion (PSL) Markers (n=6).

Platelet storage lesion markers	Days of storage				
	Day 1	Day 5	Day 9	Day 13	p value
pH	7.36±0.021^{a}	7.38±0.021^{a}	7.36±0.021^{a}	7.32±0.021^{a}	NS
Glucose (mg/dl)	260±5.19^{a}	249±5.19^{ab}	239±5.19^{bc}	225±5.19^{c}	< 0.01**
Lactate (mmol/l)	1.58±0.286^{a}	4.69±0.286^{b}	2.52±0.286^{bc}	3.44±0.286^{c}	< 0.01**
pO_2 (mmHg)	163±1.73^{c}	170±1.73^{b}	175±1.73^{ab}	181±1.73^{a}	< 0.01**
pCO_2 (mmHg)	21.0±1.66^{a}	17.6±1.66^{ab}	15.8±1.66^{ab}	14.1±1.66^{b}	< 0.01**
Bicarbonate (mmol/l)	8.35±0.431^{a}	6.92±0.431^{ab}	5.97±0.431^{bc}	4.93±0.431^{c}	< 0.01**
Platelet concentration (× 10^3/µl)	216±9.62^{a}	192±9.62^{ab}	181±9.62^{ab}	161±9.62^{b}	< 0.01**
MPV (fL)	5.55±0.059^{b}	5.60±0.059^{b}	5.72±0.059^{b}	5.98±0.059^{a}	< 0.01**
PDW	14.5±0.0981^{c}	14.6±0.0981^{bc}	14.9±0.0981^{b}	15.3±0.0981^{a}	< 0.01**

Values bearing the same superscript do not vary significantly.

*Significant, **Highly significant, NS- Non-significant.

The low glucose concentration in the PAS added CPC groups can be explained by the dilution of the plasma glucose by the addition of a PAS which contains very low amount of glucose and contains acetate instead as a fuel for platelet metabolism which leads to decreased consumption of glucose (Hlavac *et al*., 2017). The low mean lactate concentrations in the PAS added CPC groups can be attributed to the presence of potassium and magnesium in the additive solution which provides a buffering effect, influences the lactate production and preserves pH (Shanwell *et al*., 2003; Kiminkinen *et al*., 2016).

Unlike previous studies (Haines *et al*., 2020) the levels of pO_2 concentration were high when compared to the PAS added CPC groups which could be attributed to sampling error due to time duration between the collection of sample and time of analysis. Mean pO_2 increase during the period of storage indicate an increased anaerobic metabolism over aerobic even in the presence of oxygen leading to decreased

Table 5: Mean±SE of platelet storage lesion (PSL) markers with interaction between additive concentration and days of storage (n=6).

		Platelet storage lesions markers				
		pH	Glucose (mg/dl)	Lactate (mmol/l)	pO_2 (mmHg)	pCO_2 (mmHg)
Control	Day 1	7.33±0.0384^{abcdef}	490 9.04^{a}	3.652±0.535^{cd}	157±3.6^{e}	31.98±3.42^{a}
	Day 5	7.28±0.0384^{cdef}	457 9.04^{ab}	5.62±0.535^{bc}	162±3.6^{de}	23.8±3.42^{ab}
	Day 9	7.21±0.0384^{fg}	428 9.04^{bc}	7.19±0.535^{ab}	168±3.6^{bcde}	20.70±3.42^{ab}
	Day 13	7.05±0.0384^{g}	390 9.04^{c}	9.555±0.535^{a}	176±3.6^{abcd}	17.92±3.42^{ab}
PAS 65%	Day 1	7.25±0.0384^{def}	197 9.04^{d}	0.913±0.535^{ef}	166±3.6^{bcde}	15.80±3.42^{ab}
	Day 5	7.29±0.0384^{bcdef}	194 9.04^{d}	1.295±0.535^{def}	175±3.6^{abcd}	12.53±3.42^{b}
	Day 9	7.28±0.0384^{cdef}	191 9.04^{d}	1.518±0.535^{def}	181±3.6^{abc}	10.17±3.42^{b}
	Day 13	7.24±0.0384^{efg}	186 9.04^{d}	2.227±0.535^{def}	189±3.6^{a}	8.47±3.42^{b}
PAS 75%	Day 1	7.42±0.0384^{abcde}	176 9.04^{d}	1.000±0.535^{def}	163±3.6^{de}	19.22±3.42^{ab}
	Day 5	7.47±0.0384^{abc}	173 9.04^{d}	1.64±0.535^{def}	171±3.6^{abcd}	18.38±3.42^{ab}
	Day 9	7.47±0.0384^{abc}	169 9.04^{d}	2.592±0.535^{def}	174±3.6^{abcde}	17.83±3.42^{ab}
	Day 13	7.51±0.0384^{a}	164 9.04^{d}	3.417±0.535^{cdef}	178±3.6^{abcd}	16.88±3.42^{ab}
PAS 85%	Day 1	7.44±0.0384^{abcd}	179 9.04^{d}	0.765±0.535^{f}	165±3.6^{cde}	17.02±3.42^{ab}
	Day 5	7.50±0.0384^{abc}	172 9.04^{d}	1.512±0.535^{def}	171±3.6^{bcde}	15.70±3.42^{ab}
	Day 9	7.47±0.0384^{ab}	166 9.04^{d}	2.471±0.535^{def}	177±3.6^{abcd}	14.55±3.42^{b}
	Day 13	7.48±0.0384^{a}	161 9.04^{d}	3.562±0.535^{cde}	183±3.6^{ab}	13.03±3.42^{b}
	p value	< 0.01**	< 0.01**	< 0.01**	< 0.01**	< 0.01**

		Platelet storage lesions			
		Bicarbonate (mmol/l)	Platelet concentration ($\times 10^3$/µl)	MPV (fL)	PDW
Control	Day 1	13.97±0.812^{a}	439±19.6^{a}	5.30±0.124 ^{abcd}	14.6±0.202^{ab}
	Day 5	10.88±0.812^{ab}	390±19.6^{ab}	5.42±0.124^{cd}	14.7±0.202^{ab}
	Day 9	8.58±0.812^{bc}	371±19.6^{ab}	5.50±0.124^{bcd}	14.8±0.202^{ab}
	Day 13	6.38±0.812^{cde}	325±19.6^{b}	5.78±0.124^{abcd}	15.1±0.202^{ab}
PAS 65%	Day 1	8.38±0.812^{bcd}	154±19.6^{c}	5.55±0.124^{bcd}	14.6±0.202^{ab}
	Day 5	6.87±0.812^{bcde}	138±19.6^{c}	5.55±0.124^{bcd}	14.8±0.202^{ab}
	Day 9	6.42±0.812^{cde}	130±19.6^{c}	5.67±0.124^{abcd}	14.9±0.202^{ab}
	Day 13	5,82±0.812^{cde}	117±19.6^{c}	5.85±0.124^{abcd}	15.2±0.202^{ab}
PAS 75%	Day 1	5.65±0.812^{cde}	140±19.6^{c}	5.58±0.124^{abcd}	14.4±0.202^{b}
	Day 5	5.03±0.812^{cde}	124±19.6^{c}	5.67±0.124^{abcd}	14.6±0.202^{ab}
	Day 9	4.52±0.812^{de}	114±19.6^{c}	5.80±0.124^{abcd}	14.9±0.202^{ab}
	Day 13	3.87±0.812^{e}	105±19.6^{c}	6.08±0.124^{ab}	15.4±0.202^{ab}
PAS 85%	Day 1	5.38±0.812^{cde}	132±19.6^{c}	5.75±0.124^{abcd}	14.4 0.202^{b}
	Day 5	4.92±0.812^{cde}	116±19.6^{c}	5.75±0.124^{abcd}	14.5±0.202^{ab}
	Day 9	4.37±0.812^{de}	108±19.6^{c}	5.93±0.124^{abc}	14.9±0.202^{ab}
	Day 13	3.66±0.812^{e}	95±19.6^{c}	6.20±0.124^{a}	15.5±0.202^{a}
	p value	< 0.001	< 0.001	< 0.001	< 0.01

Values bearing the same superscript do not vary significantly.

*Significant, **Highly significant, NS- Non-significant.

pH and increased lactate production (Lasta *et al*., 2020). A decline in pCO_2 over time and subsequent increase in the pO_2 were indicative of a shift to anaerobic metabolism which can be associated with decrease in the pH that can be detrimental to the storage quality and viability of platelets in the control CPC (Tynngård, 2009; Stiegler *et al*., 2009).

The reduction in the bicarbonate concentration was steep in the plasma control CPC when compared to the PAS

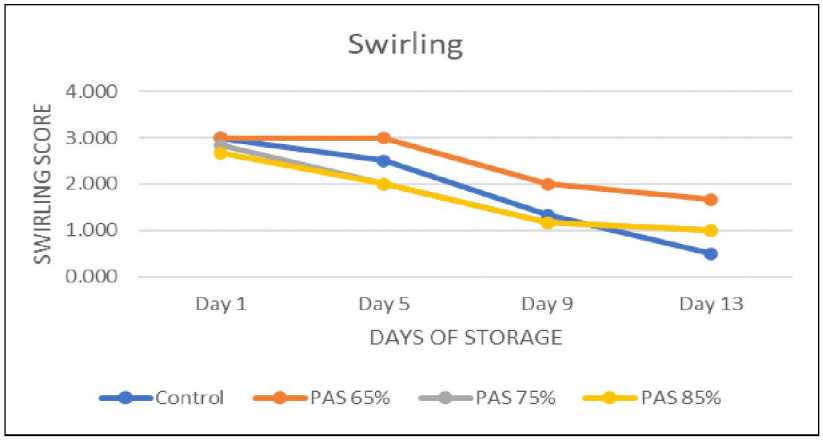

Fig 1: Day wise trend of mean swirling for control, 65%, 75% and 85% SSP+ PAS added PC.

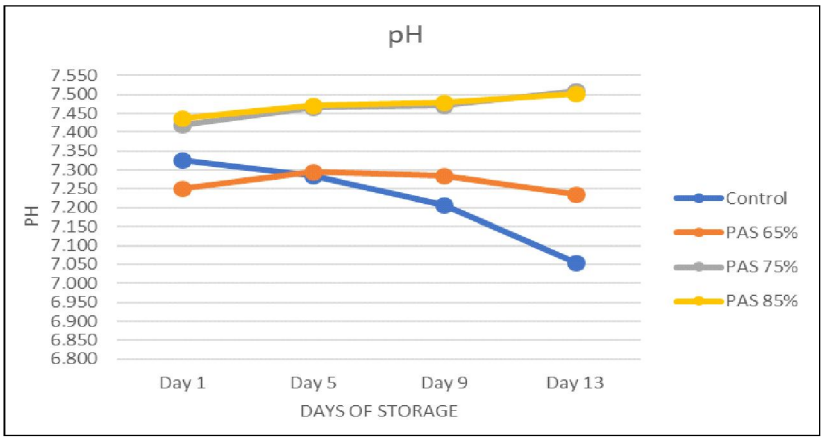

Fig 2: Day wise trend of mean pH for control, 65%, 75% and 85% SSP+ PAS added PC.

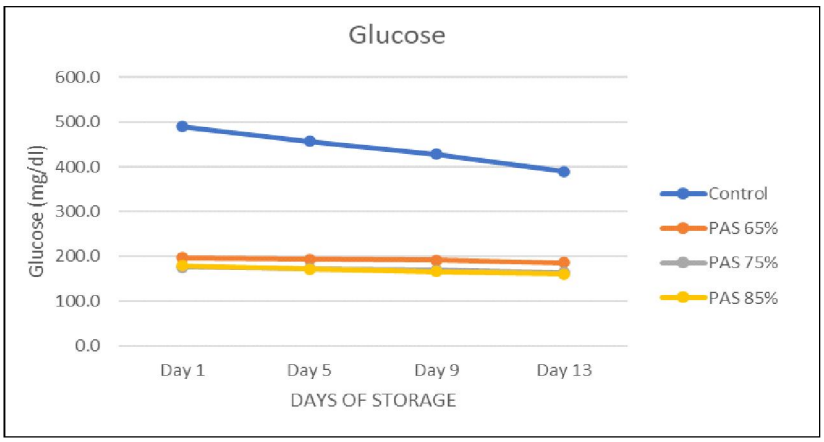

Fig 3: Day wise trend of mean glucose for control, 65%, 75% and 85% SSP+ PAS added PC.

added CPC groups because of increased need for buffering the increased lactate production due to anaerobic glycolysis in the plasma stored control CPC (Hlavac *et al*., 2017).

The high platelet concentration in the plasma stored CPC group when compared to the PAS added CPC groups could be attributed to the increased dilution volume in the PAS added CPC groups (Haines *et al*., 2020). The decrease in the platelet concentration in the plasma stored control CPC showed a rapid decrease as compared to SSP+ PAS added CPC units because of the increase in platelet fragility and decreased viability during in plasma after day 5 of storage (Jain *et al*., 2015).

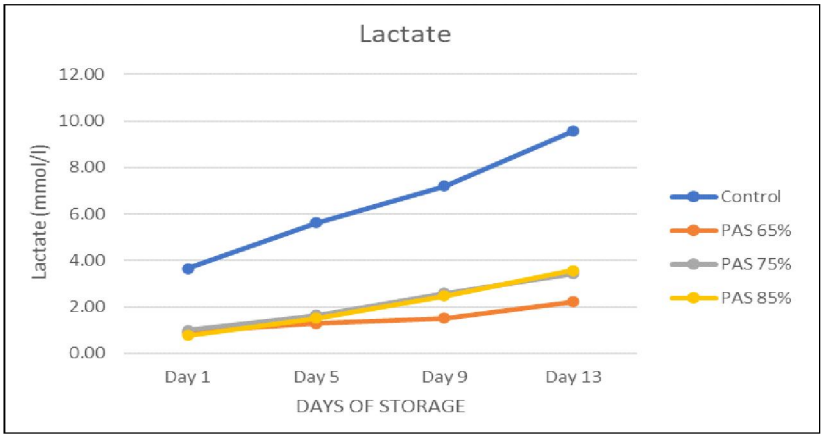

Fig 4: Day wise trend of mean lactate for control, 65%, 75% and 85% SSP+ PAS added PC.

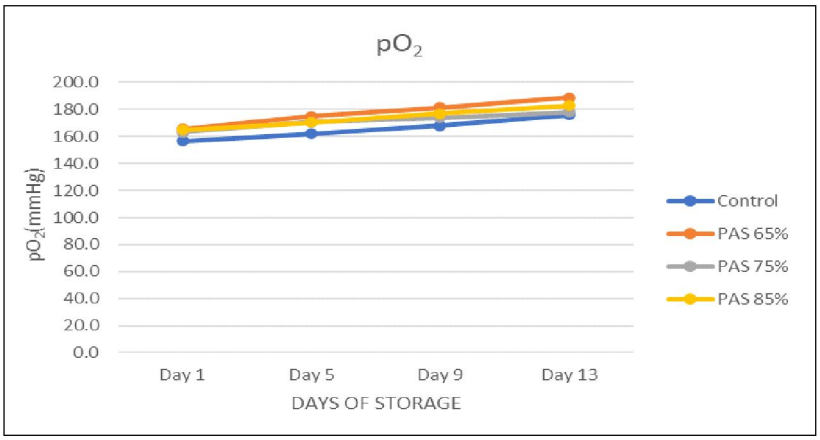

Fig 5: Day wise trend of mean pO_2 for Control, 65%, 75% and 85% SSP+ PAS added PC.

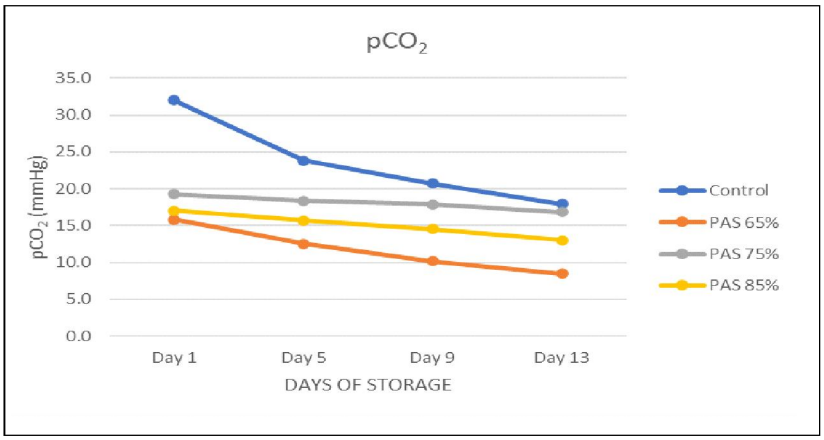

Fig 6: Day wise trend of mean pCO_2 for Control, 65%, 75% and 85% SSP+ PAS added PC.

The increase in the MPV was conversely associated with pH of the concentrates (Singh *et al*. 2003). The mean MPV for the CPCs under the study was less than that reported by many authors (Bommer *et al*., 2008; Lasta *et al*., 2020) the reason for which require further investigations. Increase in PDW during storage could be attributed to an increase in the platelet size and increased platelet activation during storage as it is a sensitive indicator of platelet shape change (Matos *et al*., 2008, Schwartz *et al*., 2014; Souza *et al*., 2016).

All CPC samples were negative for any bacterial growth indicating the importance of proper aseptic collection.

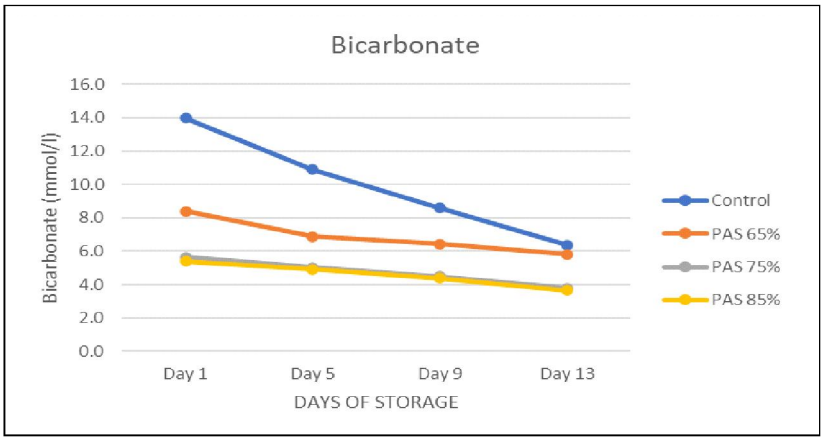

Fig 7: Day wise trend of mean bicarbonate for control, 65%, 75% and 85% SSP+ PAS added PC.

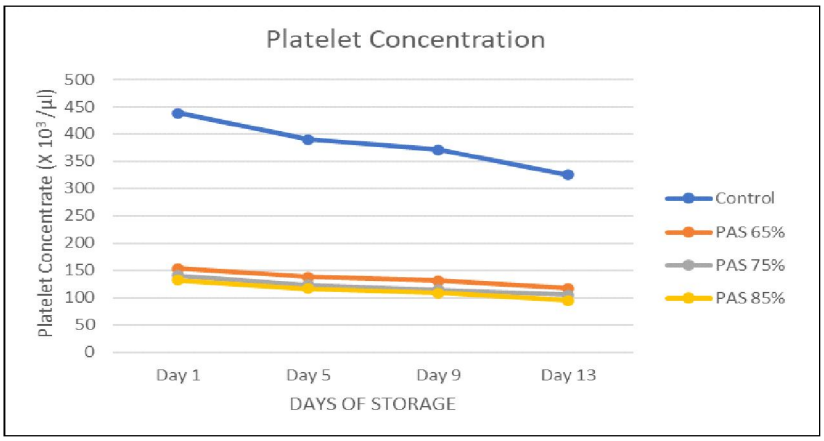

Fig 8: Day wise trend of mean platelet concentration for control, 65%, 75% and 85% SSP+ PAS added PC.

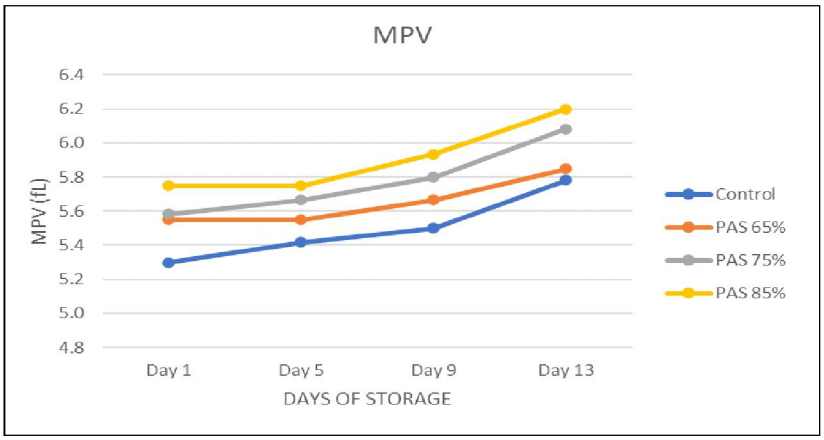

Fig 9: Day wise trend of mean MPV for control, 65%, 75% and 85% SSP+ PAS added PC.

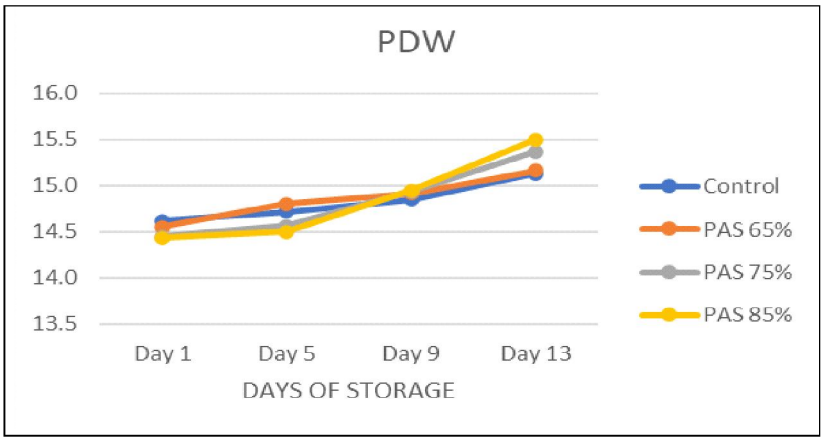

Fig 10: Day wise trend of mean PDW for control, 65%, 75% and 85% SSP+ PAS added PC.

CONCLUSION

The study of PSL markers in SSP+ added CPC concludes that 65% SSP+ PAS added CPC with 35% of plasma spill over was the ideal concentration to help maintain near ideal conditions for CPC storage with minimal deterioration in the quality and platelet viability. Further, ascertained by the retention of moderate swirling properties at day 9, maintenance of a constant pH within ideal limits when compared to day 5 CPC stored in plasma, flatter curve of increase in lactate and decreased bicarbonate consumption during storage. The use of 65% PAS in CPCs was found to be advantageous by limiting the levels of lactate production, decline in pH and limiting bicarbonate consumption, thereby reducing the production of storage lesions. The increased duration of storage of CPCs will help increase the availability of the CPCs for treatment of severe thrombocytopenia and various bleeding emergencies in a veterinary emergency and critical care set up.

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Conflict of interest

The authors declare that there was no conflict of interest.

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