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Haematological Traits of Induced Hypovolemic Large White Yorkshire Piglets as Affected by Feeding them Milk of Pantja Goats and Black Badri Cows

M. Dineshkumar, D.V. Singh, S.K. Rastogi, Sanjay Kumar, S.K. Singh, Anil Kumar

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ABSTRACT

Background: Pig is recognized as an advantageous non-rodent animal model within a large number of biomedical research areas and of xenotransplantation of animal organs into humans. People prefer taking goat milk during dengue fever like conditions for recovery from low blood constituents, particularly the blood platelets, without any scientific proof.

Methods: Haemoglobin, erythrocyte sedimentation rate (ESR) and blood platelets count were studied in 2.5 months old 18 experimentally induced hypovolemic large white yorkshire (LWY) piglets at CVASc., GBPUAT, Pantnagar after feeding them milk of Pantja goats and black Badri cows over a period of 30 days during Oct.-Nov. 2019 and May-June 2020. Hypovolemia in piglets was achieved by withdrawing their 15% of total estimated blood volume (@ 7.5% in each time on day 1st and 3rd of experiment), followed by providing them with milk, which was double the amount of blood withdrawn, for 30 days. Control group (T1) piglets (6 no.) were maintained on basal diet, whereas group T2 and T3 piglets were given milk of Pantja goats and black Badri cows, respectively. Blood samples for testing were collected on 1st, 3rd, 7th, 15th and 30th day.

Result: Average normal blood picture of weaned LWY piglets for haemoglobin (g/dl), ESR (mm/hr) and platelets count (10^5 cells/ mm^3) was 11.36 ± 0.20, 10.00± 2.01, 3.1767 ± 0.2577, respectively. For haemoglobin and ESR, the values on testing days and the overall values for group T1, T2 and T3 piglets did not show any significant variation. However, the pooled values for haemoglobin were significantly different (P<0.01) on various testing days, being higher on day 1st and 30th than on 3rd, 7th and 15th day, implying that normal haemoglobin level was regained on 30th day of hypovolemia. The pooled values for ESR were significantly different (P<0.05) on various testing days, being higher on day 1st and 3rd only, which may be due to simultaneous increase in total erythrocyte counts. Overall mean platelets count (x10^{5}cells/ mm^3) for LWY piglets was 3.2570± 0.0890 and their values in group T1, T2 and T3 piglets were 3.0983±0.1675, 3.0820±0.1490 and 3.5907±0.1885, respectively, being significantly higher (P<0.05) for black Badri cow milk fed group piglets. This may imply usefulness of black Badri cow milk over Pantja goat milk in improving blood platelets count in human, considering pig a good animal model for human within a large number of biomedical researches.

Key words: Badri cow, ESR, Haemoglobin, Hypovolemia, Large white yorkshire piglets, Pantja goat, Platelets.

INTRODUCTION

Milk from goats and cows is invariably consumed by human beings throughout the globe. Goat milk has been said to be beneficial due to its unique nutritional and medicinal values (Holmes *et al*., 1946; Sharma and Kumar, 2004) and many times it has been prescribed as perfect alternative to cow and human milk, particularly for infants and aged people (Devendra and Burns, 1970, Chandan *et al*., 1992, Park and Haenlein, 2007). Goat milk is enriched with selenium and also improves digestive and metabolic utilization of various minerals (Gunjan *et al*., 2011). Cow milk is a rich, cheap and easy source of protein and calcium. It has important growth supporting minerals (K, Mg, P, Zn), which are absorbed in gut due to high lactose content. Further, favourable role of indigenous cow milk has been frequently mentioned in ancient and modern literature (Kalyan, 1945 and 1995). Old generation people have signified role of black Indian cow's milk for maintaining better health and this notion is still prevalent in Indian society (Kalyan, 1945a). In India, dengue fever has been a common health issue during mosquito breeding season where-in there becomes a complication of selenium deficiency and reduced blood platelet count. Most of the people prefer goat milk during dengue fever and they give it upper hand for recovery without having any scientific proof.

Other than laboratory rodents, pig is recognized as an advantageous non-rodent animal model within a large

College of Veterinary and Animal Science, G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, Uttarakhand, India.

Corresponding Author: D.V. Singh, Department of Livestock Production Management, College of Veterinary and Animal Science, G.B. Pant University of Agriculture and Technology, Pantnagar-263 145, Uttarakhand, India. Email: singhdvs56@gmail.com

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number of biomedical research areas (Roth and Tuggle, 2015). Considering the above facts present work was undertaken to study effect of feeding milk of Pantja goats and black Badri cows to experimentally induced hypovolemic large white yorkshire (LWY) weaned piglets to ascertain superiority of goat milk over cow milk or *vice-versa,* that may be recommended for improving haematological traits during the conditions as prevailing during dengue fever, accidental hypovolemia and the like.

MATERIALS AND METHODS

Before conducting the experiment, an approval (IAEC-CVASc-LPM-385) was obtained from duly constituted Institutional Animal Ethics Committee. The experiment was conducted at Pig unit of LPM Department of College of Veterinary and Animal Sciences, GBPUAT, Pantnagar during October -November 2019 and May-June 2020. The place is situated in Tarai plains at the foothills of Shivalik ranges of Himalayas at 29.5°N and 79.5°E at 243.84 m MSL, experiencing ambient temperature exceeding 42°C in summer and below 2°C in winter and relative humidity range of 15 to 95 per cent.

A total of 18 LWY weaned piglets, aged 2.5 months, littered by 3 sows were available for the study. They were individually sexed and weighed and divided into three groups to conduct the experiment in randomized block design for a period of 30 days. All these groups had 6 piglets in each (having 4 males and 2 females in each group), with an average body weight of 8.91± 3.33, 8.78 ± 2.09 and 9.00 ± 2.42 kg for T1, T2 and T3 groups, respectively. All the piglets were reared under intensive system in well ventilated pens on concrete flooring and provided with similar environmental and managemental conditions.

In order to attempt for induced hypovolemia, first total volume of blood in circulation in the piglet was estimated as per IACUC (2007), followed by taking out 15% of the piglet's total estimated blood volume [@ 7.5% on day 1st (75th day age) and again 7.5% on day 3rd (78th day age)], as suggested by IACUC (2007). All the 18 piglets of the three groups were subjected to induced hypovolemia by withdrawing required amount of blood at a fixed schedule (Table 2; Dineshkumar, 2020).

Feeding schedule of induced hypovolemic LWY piglets

The control group piglets (T1) were maintained on basal diet only during the experimental period. Whereas, the treatment groups piglets, T2 and T3, were provided with freshly collected milk of farm reared Pantja goats and black Badri cows (Table 1), respectively, in addition to the usual basal diet for a period of 30 days (*i.e.* from 75th to 135th day age of piglets). The milk was hand fed through pail and its quantity was double the volume of blood, withdrawn from the piglets to induce hypovolemia (Table 2). *Ad libitum* drinking water was made available to all piglets throughout the period of experiment.

Sampling of blood (5 ml) from each piglet was done on day 1st, 3rd, 7th, 15th and 30th of the experiment (*i.e.* 75th, 78th, 82nd, 90th and 105th day age of piglets) using a sterilized disposable syringe and blood was immediately transferred to EDTA rubber capped 10 ml tube. On first two occasions, the samples were taken from the removed blood while attempting for induced hypovolemia. However, the blood for the remaining three samples was collected by following the usual procedure. All the 90 samples, so collected, were kept at room temperature in thermos and analysed through Auto-haem Analyser (Celltac-α, MEK-6510K) on the same day of their collection. The values so obtained for haemoglobin (g/dl), erythrocyte sedimentation rate (mm/h) and blood platelets count (x10^5 cells/ mm^3) were subjected to statistical analysis as per Snedecor and Cochran (1994).

Table 1: Nutrients' composition of Badri cow milk and Pantja goat milk.

Major constituents	Unit	Pantja goat milk[1]	Badri cow milk[2]
Milk fat	%	4.96	5.67
Protein	%	3.82	3.24
Carbohydrate	%	5.09	4.59
Total solids	%	14.67	13.53
Solids not fat	%	10.06	8.94

1-Surya (2019); 2- Dar (2019).

RESULTS AND DISCUSSION

Several physical signs to indicate hypovolemia [postural vital signs such as increase in heart rate by >30/min, decrease in systolic blood pressure by >20 mmHg, dryness of mouth, nasal mucous membranes, axillae and tongue and neurological signs like alterations in mental status, weakness of upper or lower extremities] have been reported (Gergely *et al*., 2011). However, as a scope of present study following paragraphs discuss changes in haemoglobin, ESR and blood platelets counts of experimentally induced hypovolemic weaned LWY piglets as affected by feeding them with milk of Pantja goats and black Badri cows.

Haemoglobin

The overall value for haemoglobin in LWY piglets was 10.85±0.11 g/dl. The values for control, Pantja goat milk fed and black Badri cow milk fed group of piglets for haemoglobin were 10.66±0.27, 10.95±0.16 and 10.93±0.17 g/dl, respectively, which did not vary significantly. However, the values showed significant (P<0.01) variation on different days following hypovolemia, being higher on 1st and 30th day and the lowest and insignificantly different on 3rd, 7th and 15th day. Overall haemoglobin value on 30th day became similar to normal value, 11.36 ± 0.20 g/ dl and was in consonance with reported values (Reece *et al*., 2015). About 8-12% of initial haemoglobin level was reduced due to blood withdrawal. These low values may be a consequence to experimental hypovolemia till 15th day (Table 3, Fig 1).

The values for haemoglobin for control, Pantja goat milk fed and black Badri cow milk fed groups piglets did not show any significant variation on various days of hypovolemia,

Table 2: Feeding schedule of induced hypovolemic LWY piglets.

Group	Piglet no.	Body weight at 75th day age (kg)		Amount of blood withdrawn (ml) to create hypovolemia			Milk fed to piglets (ml/ day) during	
		Piglet's	Group's average	on 75th day age	on 78th day age	Total	75-77th day age	78-105th day age
T1	2F	12.10	8.91±3.33	55.5	55.5	111	-	-
(no milk fed)	4M	13.10		60.0	60.0	120	-	-
	10F	7.30		34.0	34.0	68	-	-
	13M	6.10		28.0	28.0	56	-	-
	15F	10.00		45.0	45.0	90	-	-
	18M	4.90		22.5	22.5	45	-	-
T2	3M	12.00	8.78±2.09	55.0	55.0	110	110	220
(Pantja goat milk fed)	6F	10.50		48.0	48.0	96	96	192
	11M	7.60		35.0	35.0	70	70	140
	12M	6.40		30.0	30.0	60	60	120
	14M	7.50		35.0	35.0	70	70	140
	16F	8.68		40.0	40.0	80	80	160
T3	1M	11.50	9.00±2.42	53.0	53.0	106	106	212
(Badri cow milk fed)	5F	12.30		56.5	56.5	113	113	226
	7F	5.95		27.5	27.5	55	55	110
	8M	8.20		37.5	37.5	75	75	150
	9M	7.85		36.0	36.0	72	72	144
	17M	8.20		37.5	37.5	75	75	150

Table 3: Haemoglobin (g/ dl) of LWY hypovolemic piglets under different treatments.

Day of induced hypovolemia	Treatment groups			Overall (n=18)
	T1, Control (n=6)	T2, Pantja goat milk fed (n=6)	T3, Black Badri cow milk fed (n=6)	
1st	11.60 ± 0.36	11.25 ± 0.44	11.22 ± 0.33	11.36^{A} ± 0.20
3rd	11.03 ± 0.16	10.63 ± 0.43	10.82 ± 0.25	10.83^{B} ± 0.16
7th	9.63 ± 0.97	10.53 ± 0.10	9.88 ± 0.35	10.02^{B} ± 0.32
15th	10.55 ± 0.30	10.75 ± 0.13	10.92 ± 0.47	10.74^{B} ± 0.17
30th	10.50 ± 0.87	11.58 ± 0.54	11.82 ± 0.07	11.30^{A} ± 0.33
Overall	10.66 ± 0.27	10.95 ± 0.16	10.93 ± 0.17	10.85 ± 0.11
	(30)	(30)	(30)	(90)

Values with different superscripts row wise differ significantly (A, B; P< 0.01).
Figures within parentheses indicate number of observations.

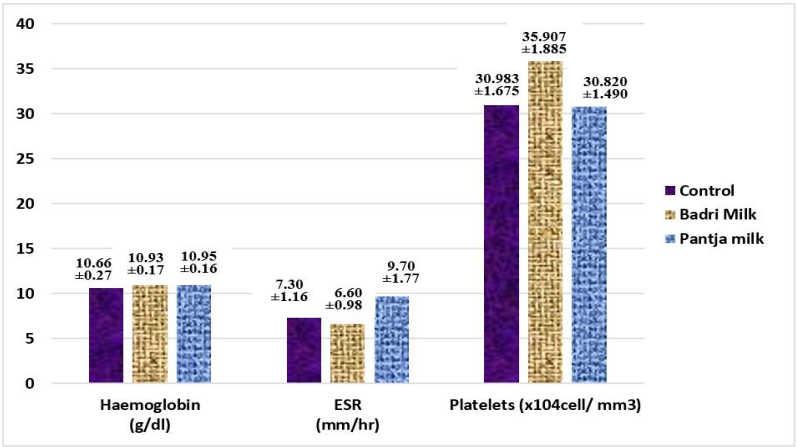

Fig 1: Haematological traits of LWY hypovolemic piglets under different treatments.

implying that all the group piglets behaved similarly in declining and then regaining the haemoglobin level over the study period. The overall values were numerically, but not statistically, highest in the order of "Pantja milk fed > black Badri cow milk fed > Control group" (Table 3).

Haemoglobin is a complex iron containing and oxygen carrying pigment conjugated with protein in red blood cells. Its low amount leads to anaemia, causing depletion of oxygen in tissues and overall inactivity and other related problems. Normal haemoglobin range in pig is reported as 10.0-16.0 g/dl (Reece *et al*., 2015).

Erythrocyte sedimentation rate (ESR)

Overall values for erythrocyte sedimentation rate was 7.87± 0.74 mm/hr. Control, Pantja goat milk fed and black Badri cow milk fed groups had ESR values of 7.30±1.16, 9.70±1.77 and 6.60±0.98 mm/hr, respectively and they didn't show any significant variation among the groups. However, on different days following hypovolemia these values had a significant variation (P<0.05), being higher on day 1st and 3rd and lower on day 7th, 15th and 30th (Table 4, Fig 1). These low values may be due to simultaneous increase in total erythrocytic count which in turn increases viscosity of blood (Dineshkumar, 2020). Reduction in ESR value indirectly indicates improvement and thus was a good sign for healthy animal.

Control, Pantja goat milk fed and black Badri cow milk fed groups piglets' ESR values on different days of hypovolemia did not show any significant variation, implying that all the treatment groups behaved similarly during the study period (Table 4).

ESR in an anticoagulated whole blood is the rate at which red blood cells descend in a vertically placed standard tube over a period of time. Generally, it is measured in millimetre per hour (mm/ h) as the distance in uppermost layer of erythrocyte descends in certain length. It is mainly based on degree of agglutination of erythrocyte and level of plasma proteins (fibrinogen, albumin, globulin, *etc*.). In swine, ESR normally ranges from 1-14 mm/hr (Reece *et al*., 2015).

Platelets count (x10^{5}cell/ mm^3)

Platelets are the tiny blood cells that help to stop bleeding by forming clots. The process of spreading across the surface of damaged blood vessels to stop bleeding is adhesion by mash work with fibrin. Platelets count in domestic animals range from 200,000-400,000 cells/mm^3. Reduction in platelets count is termed as thrombocytopenia, which may lead to haemorrhage in internal organs (Reece *et al*., 2015).

The overall mean platelets count (x10^5 cells/ mm^3) for LWY piglets was 3.257± 0.089. The values of platelets count in control, Pantja goat milk fed and black Badri cow milk fed groups piglets were 3.0983±0.1675, 3.0820±0.1490 and 3.5907±0.1885, respectively, being significantly higher (P<0.05) for black Badri cow milk fed group piglets. Whereas, the values for platelets count were statistically indifferent for control and Pantja goat milk fed group piglets (Table 5, Fig 1). This implies that use of indigenous cow milk (black Badri cow milk), but not the goat (Pantja) milk, may be helpful in improving platelets count in human beings too, as pig is a good model for human medical studies. However, further studies are required to validate this finding. Jung *et al.* (2008) observed the platelets (x 10^{5}cell/mm^3) as 13.74, 6.55, 4.53, 4.77 on 4, 8, 12 and 20 weeks of age in miniature growing pigs.

The platelets count (x10^5 cell/ mm^3) during different days of testing were 3.1767±0.2577, 2.7606±0.2022, 3.9717± 0.2045, 3.3172±0.1592 and 3.0589±0.2057 on day 1st, 3rd, 7th, 15th and 30th, respectively. These values were significantly higher (P<0.01) on day 1st, 7th, 15th and 30th day, but not on 3rd day of experiment. Initially, on 1st day platelets count was in normal range and after blood withdrawal there was 11-13% reduction in cells from the initial level. Its low value on day 3rd of testing was a sequel to induced hypovolemia. Level of platelets counts became higher on 7th day and later normal in all the treatment groups piglets. However, platelets count was significantly higher (P<0.05) in black Badri cow milk fed piglets than the rest groups, indicating positive role of black Badri cow's milk feeding on improving platelets in hypovolemic piglets (Table 5). Since pig is a good model for human medical researches, present findings could be beneficial for human beings also for improving blood platelets counts in Dengue fever and like situations where thrombocytopenia becomes the major health issue.

Table 4: ESR (mm/ hr) of LWY hypovolemic piglets under different treatments.

Day of induced hypovolemia	Treatment groups			Overall (n=18)
	T1, Control (n=6)	T2, Pantja goat milk fed (n=6)	T3, Black Badri cow milk fed (n=6)	
1st	7.00 ± 1.50	14.17 ± 6.10	8.83 ± 0.77	10.00^{a} ± 2.01
3rd	10.50 ± 2.66	11.83 ± 1.78	11.67 ± 3.09	11.33^{a} ± 1.32
7th	3.83 ± 1.40	2.83 ± 1.34	4.67 ± 2.63	3.78^{b} ± 0.98
15th	6.17 ± 2.20	8.33 ± 3.31	4.67 ± 1.57	6.39^{c} ± 1.31
30th	9.00 ± 4.77	11.33 ± 5.81	3.17 ± 0.91	7.83^{c} ± 2.39
Overall	7.30 ± 1.16	9.70 ± 1.77	6.60 ± 0.98	7.87 ± 0.74
	(30)	(30)	(30)	(90)

Values with different superscripts row wise differ significantly (a, b; P<0.05). Figures within parentheses indicate number of observations.

Table 5: Platelets counts (x10^5cell/ mm^3) of LWY piglets under different treatments.

Day of induced hypovolemia	Treatment groups			Overall (n=18)
	T1, Control (n=6)	T2, Pantja goat milk fed (n=6)	T3, Black Badri cow milk fed (n=6)	
1st	2.7667 ± 0.5333	3.2917 ± 0.6398	3.4717 ± 0.0969	3.1767^{A} ± 0.2577
3rd	2.8967 ± 0.3318	2.0983 ± 0.3749	3.2867 ± 0.2141	2.7606^{B} ± 0.2022
7th	3.5833 ± 0.4776	3.8983 ± 0.3364	4.4333 ± 0.2314	3.9717^{C} ± 0.2045
15th	3.1550 ± 0.1699	3.1767 ± 0.1758	3.6200 ± 0.4474	3.3172^{A} ± 0.1592
30th	3.0900 ± 0.4456	2.9450 ± 0.3039	3.1417 ± 0.4397	3.0589^{A} ± 0.2057
Overall	3.0983^{a} ± 0.1675	3.0820^{a} ± 0.1490	3.5907^{b} ± 0.1885	3.2570 ± 0.0890
	(30)	(30)	(30)	(90)

Values with different superscripts row wise (A, B, C; P<0.01) and column (treatment) wise (a, b; P<0.05) differ significantly.
Figures within parentheses indicate number of observations.

Other than laboratory rodents, pig is recognized as an advantageous non-rodent animal model within a large number of biomedical research areas (Roth and Tuggle, 2015) and it is a leading animal species within studies of xenotransplantation of animal organs into humans (Schook *et al.,* 2005). So far there seems no similar study that has been carried out with regard to using goat milk, indigenous cow milk or indigenous black cow milk to increase count of blood platelets or other blood components *in vivo*. At present such nature of studies are rare and the proofs for review or validation were scanty in literature, so further studies are recommended on these lines with larger observations to recommend use of black Badri (indigenous) cows to improve blood picture. However, in India people are seen buying and taking goat milk vaguely once they are affected with Dengue fever.

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