CHARACTERISATION OF DEER BLOOD BIOACTIVITY IN COMPARISON WITH THAT OF SHEEP AND PIG

Clara S.F. Bah[1], Alaa El-Din A. Bekhit[1], Alan Carne[2], Michelle A. McConnell[3]

[1] Department of Food Science, University of Otago, Dunedin, New Zealand

[2] Department of Biochemistry, University of Otago, Dunedin, New Zealand

[3] Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

Abstract – There is considerable interest in the potential of extracting bioactive compounds from animal blood. In the current study, cervine, ovine and porcine blood was obtained from local slaughterhouses and their biochemical parameters were characterised. Fractionated blood was assayed for antioxidant and antimicrobial activities. Red blood cell fractions exhibited higher DPPH radical scavenging activity ($p < 0.05$) compared to the plasma fractions of all three animal species. Deer plasma had slightly higher ORAC values compared to sheep and pig plasma while pig red blood cells had higher ORAC values compared to deer and sheep red blood cells. Only sheep white blood cells exhibited inhibitory activity towards *E. coli, S. aureus* and *P. aeruginosa*.

Key Words – animal blood, antioxidant, antimicrobial, bioactivity, biochemical parameters

I. INTRODUCTION

Animal blood is a valuable protein resource with large amounts produced annually as a by-product from the meat industry. Blood from slaughtered animals can represent up to 4% of the live weight of the animal [1]. While the functions and applications of bovine (cow), porcine (pig) and ovine (sheep) blood have been examined to various extents, the potential of cervine (deer) blood remains largely unexplored. Blood obtained from New Zealand red deer is currently exported and sold in capsule form with a focus on its ability to heal human ailments, a therapeutic treatment based on the medicinal use of natural products obtained either directly from animals or ultimately derived from them. With the New Zealand deer farming industry being the largest and most advanced in the world, deer blood represents an important resource which needs to be evaluated.

In the current study, deer blood was evaluated for its potential biological activity in *in-vitro* antioxidant and antimicrobial systems in comparison to the biological activities of blood from other (ovine and porcine) animal species.

II. MATERIALS AND METHODS

(i) Materials

Fresh animal blood (from deer, sheep, and pig) was obtained from local slaughterhouse facilities. The blood was collected with sodium citrate (3g/L) added to prevent coagulation. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, and fluorescein sodium salt were obtained from Sigma-Aldrich (USA). 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Cayman Chemicals (USA).

(ii) Blood separation

The collected blood was separated into red blood cell, plasma and white blood cell fractions. To separate the red blood cells (RBC) from the plasma, the blood was centrifuged at 3,000 rpm for 15 min at 4°C. Ficoll-Paque™ (an erythrocyte aggregating medium) was used to separate white blood cells (WBC) [2]. All blood fractions (plasma, red blood cells and white blood cells) were stored at -20°C until use.

(iii) Biochemical composition

Measurement of deer, sheep and pig blood biochemical parameters such as ferritin levels, serum iron, total protein, albumin and globulin (alpha, beta and gamma) levels, as well as prothrombin time, were outsourced to a commercial lab.

(iv) Assays for bioactivity

The antioxidant assays were carried out using the red blood cell (RBC), and plasma fractions while the antimicrobial assay was carried out using all fractions (RBC, plasma and WBC).

a. DPPH radical scavenging assay

The DPPH radical scavenging activity by the animal blood samples was carried out as described by [3] with a reaction time of 1 hour. The DPPH free radical scavenging activity was calculated using the following formula:

$$\%\, radical\ scavenging = \left(\frac{(C - Cb) - (S - Sb)}{C - Cb} \right) \times 100$$

Where C is the absorbance of water plus DPPH in methanol,
Cb is the absorbance of water plus methanol,
S is the absorbance of the sample added to DPPH in methanol,
Sb is the absorbance of the sample plus methanol.

As precipitates were formed in the samples when the hydrolysates were added to DPPH-methanol solutions, the samples were centrifuged prior to measurement of absorbance at 517 nm [4].

b. ORAC assay

The antioxidant capacity of blood fractions was determined using the Oxygen Radical Antioxidant Capacity (ORAC) assay according to [5]. The plasma fractions were diluted 200-fold and red blood cell fractions diluted 800-fold in 75 mM PBS, pH 7.4, prior to measurement.

c. Antimicrobial activity

Mueller Hinton agar plates were inoculated with *Escherichia coli O157*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at a 0.5 McFarland standard concentration of each test isolate. A 10 µl aliquot of the blood samples was then applied to the inoculated plates in one spot. The plates were then incubated at 37°C for 24 hours. Sabouraud dextrose agar plates were used for *Candida albicans* and they were incubated for 48 hours.

The E Test (AB Biodisk, Sweden) method was used as a control to determine antimicrobial susceptibility. It consists of an impervious carrier (5- by 50-mm strip) with a predefined antimicrobic gradient which is placed on an inoculated agar plate. The MIC was read at the point where the zone of inhibition intersected the MIC scale on the strip [6].

(v) Statistical Analysis

Statistical analysis was performed with Microsoft Excel 2010 (using the Analysis ToolPak Add-in). The statistical differences between the means were determined by T test and the results are presented as means ± standard deviation.

III. RESULTS AND DISCUSSION

Deer blood had higher levels of ferritin and total plasma protein than sheep and pig blood (Table 1). Ferritin is a major iron-storing protein found in reticuloendothelial cells. Total plasma protein of New Zealand red deer blood was similar to the value reported by Padilla *et al.* [7] in Mexican captive red deer (66.0 ± 6.6 g/L) and for Norwegian free ranging (64.8 ± 6.1 g/L) red deer [8]. Sheep blood had the highest amount of serum iron. Both deer and pig blood had similar amounts of albumin. Deer albumin levels were similar to values (36.6 ± 4.3 g/L) reported for Norwegian free ranging red deer [8].

Table 1. Biochemical composition of animal blood

Analyte	Deer	Sheep	Pig
Ferritin (µg/L)	213.7 ± 43.7	133.5 ± 4.9	140.3 ± 11.6
Serum Iron (µmol/L)	16.3 ± 2.8	29.5 ± 4.9	23.3 ± 5.7
Total Plasma Protein (g/l)	66.3 ± 15.0	57.3 ± 11.0	46.3 ± 6.0
Albumin (g/L)	33.3 ± 8.3	25.3 ± 2.3	33.7 ± 4.9
Globulin (g/L)	33.0 ± 7.2	32.0 ± 13.2	12.7 ± 2.1
Alpha 1 (g/L)	3.2 ± 0.7	2.7 ± 1.3	0.7 ± 0.2
Alpha 2 (g/L)	6.3 ± 1.5	5.9 ± 2.4	3.8 ± 0.5
Beta 1 (g/L)	7.1 ± 1.8	5.6 ± 3.4	1.9 ± 0.2
Beta 2 (g/L)	6.2 ± 0.4	14.1 ± 4.0	3.8 ± 0.9
Gamma (g/L)	10.3 ± 3.4	3.7 ± 3.4	2.5 ± 0.5
Prothrombin Time (sec)	12.3 ± 0.6	11.0 ± 0.0	14.3 ± 0.6

Both deer and sheep blood had higher amounts of total globulins compared to pig blood, however the composition of the globulins were different. Deer blood had a higher level of gamma globulins while sheep blood was higher in beta 2 globulins. Prothrombin time (which is a measure of phase III of the clotting process) among the 3 animal species was similar.

The DPPH radical scavenging activity of the three animal blood plasmas and red blood cell fractions are shown in Figure 1. Sheep plasma had the highest DPPH radical scavenging activity (27.6 ± 2.0%) compared to deer (21.9 ± 2.2%) and pig plasma (8.9 ± 4.7%). Albumin, a major protein in plasma, is considered to be a major circulating antioxidant [9].

Red blood cell fractions of all three animal species had higher DPPH scavenging activity compared to the plasma fraction. Sheep RBC had a DPPH radical scavenging activity of 81.9 ± 1.4%, followed by pig RBC (76.4 ± 2.6%) and deer RBC (73.5 ± 4.7%). This higher antioxidant scavenging activity could be attributed to enzymatic antioxidant systems within the red blood cells such as superoxide dismutase, catalase and glutathione peroxidase [10].

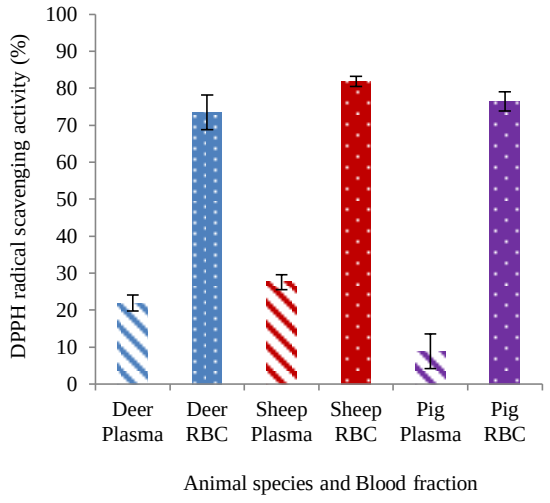

Figure 1. DPPH radical scavenging activity of animal blood fractions

In the ORAC assay, oxidation occurs due to exposure of the flurophore to the peroxyl radical AAPH, leading to the decay of the fluorescence emission over time. The ORAC value refers to the net protection area under the curve (AUC) of fluorescein in the presence of blood samples, minus a blank performed with phosphate buffer (Figures 2 and 3). The ORAC value of samples is expressed as micromole Trolox equivalents (TE) per liter for liquid samples or per gram for solid samples [5]. The ORAC antioxidant capacity of deer plasma which was diluted 200-fold, was the highest followed by sheep and then pig plasma (Figure 2).

ORAC values of 11,374 ± 734 µM have been reported for pig whole plasma (1:100 diluted), and 16,521 ± 1140 µM for sheep whole plasma (1:100 diluted), however this was using B-Phycoerythrin (B-PE) as the fluorescent probe [11].

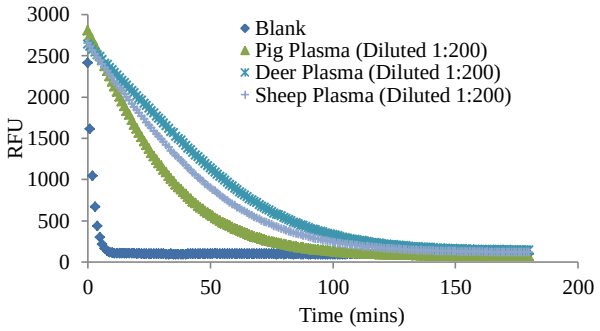

Figure 2. Time dependent fluorescence decay curves in the presence of blank and animal plasmas

Pig red blood cells (diluted 800-fold) had the highest ORAC antioxidant capacity followed by sheep and deer red blood cells (Figure 3).

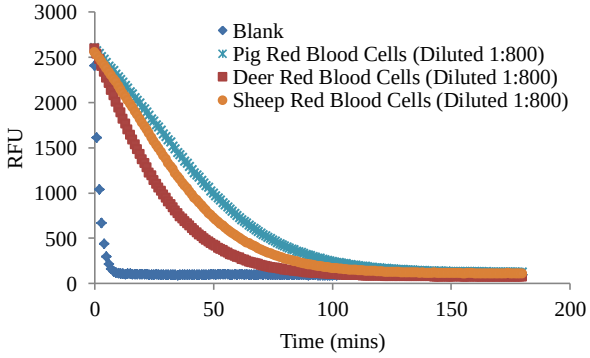

Figure 3. Time dependent fluorescence decay curves in the presence of blank and animal red blood cells

Antimicrobial activity was detected with sheep white blood cells which demonstrated slight inhibition towards the growth of *E. coli*, *S. aureus* and *P. aeruginosa* (Figure 3), but not with the other species or fractions or towards *C. albicans*. Results from other studies have indicated antibacterial activity from the red blood cell fraction, however this activity appeared after the red blood cells had been hydrolysed by proteases into smaller peptides [12].

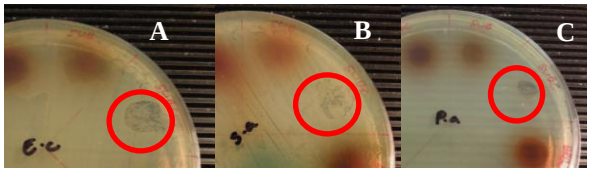

Figure 4. Antimicrobial activity of sheep white blood cells towards (A) *E. coli*, (B) *S. aureus*, (C) *P. aeruginosa*

The E test MIC values were read directly from the E test strip scales (Figure 4). For *E. coli*, the MIC using ampicillin was 4 µg/ml, while for *S. aureus* using ampicillin it was 0.64 µg/ml. MIC for *P. aeruginosa* using gentamicin was 1.0 µg/ml.

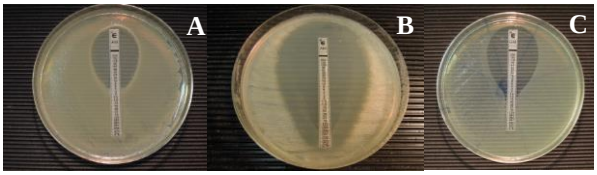

Figure 4. E test results of (A) *E. coli*, (B) *S. aureus*, (C) *P. aeruginosa*

IV. CONCLUSION

The biochemical parameters of deer blood in comparison to sheep and pig blood obtained from local slaughterhouses was determined. The blood from all three animal species demonstrated varying degrees of antioxidant activity, while sheep white blood cells demonstrated antibacterial activity. The present study benchmarked the activities in the different fractions of slaughterhouse animal blood and paves the way for future studies such as determining what compounds in the blood are responsible for contributing towards the bioactivity.

ACKNOWLEDGEMENTS

The authors thank Alliance Group Ltd. (Invercargill) for their kind support in supplying sheep blood and deer blood, and Bloem Highcliff Piggery (Dunedin) for providing pig blood.

REFERENCES

1. Wismer-Pedersen, J. (1988). Use of haemoglobin in foods–A review. Meat Science 24: 31-45.
2. Bøyum, A. (1976). Isolation of Lymphocytes, Granulocytes and Macrophages. Scandinavian Journal of Immunology 5: 9-15.
3. Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28: 25-30.
4. Janaszewska, A. & Bartosz, G. (2002). Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. Scandinavian Journal of Clinical & Laboratory Investigation 62: 231-236.
5. Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A. & Prior, R.L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. Journal of Agricultural and Food Chemistry 50: 4437-4444.
6. Baker, C.N., Stocker, S.A., Culver, D.H. & Thornsberry, C. (1991). Comparison of the E Test to Agar Dilution, Broth Microdilution, and Agar Diffusion Susceptibility Testing Techniques by Using a Special Challenge Set of Bacteria. Journal of Clinical Microbiology 29: 533-538.
7. Padilla, S., Bouda, J., Quiroz-Rocha, G., Davalos, J. & Sanchez, A. (2000). Biochemical and haematological values in venous blood of captive red deer (Cervus elaphus) at high altitude. Acta Veterinaria Brno 69: 327-331.
8. Rosef, O., Nystøyl, H.L., Solenes, T. & Arnemo, J.M. (2010). Haematological and serum biochemical reference values in free-ranging red deer (Cervus elaphus atlanticus). Rangifer 24: 79-85.
9. Ofori, J.A. & Y.H.P. Hsieh. (2012). The Use of Blood and Derived Products as Food Additives. In Y. El-Samragy, Food Additive (pp. 229 - 256). Croatia: InTech.
10. Maral, J., Puget, K. & Michelson, A.M. (1977). Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochemical and Biophysical Research Communications 77: 1525-1535.
11. Ninfali, P. & Aluigi, G. (1998). Variability of oxygen radical absorbance capacity (ORAC) in different animal species. Free Radical Research 29: 399-408.
12. Adje, E.Y., Balti, R., Kouach, M., Dhulster, P., Guillochon, D. & Nedjar-Arroume, N. (2011). Obtaining antimicrobial peptides by controlled peptic hydrolysis of bovine hemoglobin. International Journal of Biological Macromolecules 49: 143-153.