Refinement of analytical technologies for detection of biomolecules of importance to the dairy sector

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Indu Sharma

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Abstract

There is a continuous push on the dairy industry to enhance livestock productivity to meet with the demands of ever increasing human population. This demand can be achieved by developing rapid and early diagnostic aids to help curtail various problems encountered in the livestock production. The first study of the present thesis was focussed on standardizing initial steps towards development of Surface Plasmon Resonance for progesterone and oestradiol 17-β, both of which are critically implicated in animal reproduction. For progesterone, the binding response of two different length linkers, and the antigen-antibody binding response of two different source monoclonal antibodies (P1922 from Sigma vs SE-7720-1430 from Serotec) were evaluated. It was concluded that the long length linker had better binding response than the short length linker. The antibody obtained from Serotec (SE-7720-1430) had greater sensitivity but its binding response was inconsistent. On the other hand, the sensitivity of the monoclonal antibody from Sigma (P1922) was lower, although its binding response was consistent. For oestradiol 17-β, antibody procured from Bio-trend (BT70-1020-06) was tested and its binding response was consistently low on all the test days. This study thus suggests that careful testing and selection of antibodies to achieve desired antigen-antibody binding response is a critical step towards development of SPR for progesterone and oestradiol 17-β. The second study was undertaken to refine the currently existing fluorescent techniques to measure phytoporphyrin in the peripheral circulation of cows. Phytoporphyrin is implicated in facial eczema (FE), which is a photosensitization disease of high economical importance. This occurs due to disturbances in the chlorophyll metabolism as a result of liver damage and bile duct occlusions caused by fungal

toxicity. The present study described new modified fluorescent methods to measure phytoporphyrin in the serum of cows. Further, the absorption and emission spectra of phytoporphyrin were compared with that of other chlorophyll metabolites and thus a currently existing anomaly in the chemical structure of phytoporphyrin was rectified.

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Publications

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List of Abbreviations

4TPH-P4	6-[3-[(pregn-4-ene-3, 20-dione-4-yl) thiopropanoyl] – amino] hexanoic acid
4TP-P4	3-(pregn-4-ene-3, 20-dione-4-yl) thiopropanoic acid
ALP	alkaline phosphate
AST	aspartate transaminase
CL	corpus Luteum
^{o}C	degree celsius
DMSO	dimethyl sulfo-oxide
E2	oestradiol 17-β
E2,C3	3 (propanoic acid)-17 β hydroxyl 1, 3, 5 (10) estratriene
EDC	N-ethyl-N-(3-dimethylaminopropyl)-carbodimide
ELISA	enzyme-linked immunosorbent assay
FC	flow cell
FE	facial Eczema
FSH	follicular stimulating hormone
GDH	glutamate dehydrogenase
GGT	γ-glutamyltransferase
GnRH	gonadotrophin releasing hormone
ID	Identification
L	litre
LH	luteinizing hormone
LOD	limit of detection
min	minute (s)
NHS	N-hydroxysuccinimide
NMR	nuclear magnetic resonance
OEG	oligoethylene glycol
OVA	ovalbumin
%	percent
P4	progesterone
RIA	radio-immuno assay
RU	response unit
sec	second (s)
SPR	surface plasmon resonance
vs	versus