final report

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Prepared by: Dr. John Friend

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Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Pre-slaughter manipulation – Project management

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1 Introduction

1.1 Key Strategic Issue

Conventional co-products of animal slaughter contribute a significant amount of revenue to the producer and processor and the potential exists to further increase the profitability of the industry by developing high value bioactive co-products. Conventional co-products tend however to be commodity products and are subject to commodity pricing.

The need exists to identify ways in which the red meat industry can increase its share of the profits within the valuable bioactive co-product industry. In order to secure a larger share of the added value the industry must be able to add some further value. In a competitive environment this requires an understanding of how increases in the value of the bioactive to the end user can be effected by actions taken on farm or during processing.

1.2 Background

The red meat bioactives industry in Australia is estimated to be worth $200mpa.

MLA is aiming to increase the profitability of the red meat industry by supporting the growth of the bioactives industry. Our strategy is to facilitate growth by a combination of identifying new opportunities, identifying new technologies and co-investing in R&D with potential value adders. Our priority is to maximise the share of the added value that can be realised by the red meat producers and processors.

The largest sector of the Australian red meat bioactives industry currently is blood products, valued at $100m pa, which includes serum, plasma, fatty acids and growth factors. The greatest potential for significant impact on the red meat industry in the short to medium term is therefore seen to lie in growth of this sector.

The highest value blood products are those used by the pharmaceutical industry for the manufacture of therapeutic and diagnostic chemicals. This is an expensive and capital intensive production process and it is known that there is natural variation in the efficacy of sera in these applications. The conventional solution by serum manufacturers to this variability is to combine many batches of blood in order to “even-out” the variation. The opportunity exists however to take advantage of this natural variation, and even to exploit it, in order to create products of greater value to the pharmaceutical and diagnostic manufacturers.

It is envisaged that genetic and physiological differences between the donor animals which underlie this variation could be exploited to the benefit of the whole value chain, with producers and processors able to make the biggest contribution and therefore claim a significant share of this benefit. It is recognised that only by developing products of real value to the pharmaceutical and diagnostic manufacturers can profitability of the red meat bioactives industry be developed. Before investing in the development of this capability, it is necessary to objectively assess both the potential for impact and the likely value of the impact on pharmaceutical and diagnostic compound manufacturers.

1.3 Scope of the current work:

The current project lies within stage 1 of a 3 stage process:

Stage 1: Demonstrate the potential for pre-slaughter interventions to add significant value to blood products used in the manufacture of therapeutic and diagnostic compounds. This is to be a desk based research project involving critical literature reviews and perhaps some interviews.

Stage 2: If the results are favourable, the second stage (a separate project) will be around identifying (empirically) breed, environment and other factors affecting yield and productivity. This work will involve laboratory studies with a range of cell lines, probably using a combination of fermentation and cell culture techniques. It is intended to seek involvement from the major antibody manufacturers in this stage. The outcome of this work would be experimental demonstration that it is possible to make a significant and valuable difference to serum functionality by pre-slaughter manipulation. It is expected that this will involve establishment of a cell culture capability for the red meat value adding industry in Australia at a neutral location such as a university.

Stage 3: The third stage would be the development of processes, supply chains, methodology, traceability etc to provide optimum serum to the antibody manufacturers. This will involve further laboratory based R&D using the expertise developed in earlier stages. It is not clear at this early stage whether donor herds or slaughter would be the best model for blood collection.

There were 3 parallel projects within stage 1.

Project 1: Nature and Nurture impact on bioactivity: A project aimed at reviewing the evidence for both “nature and nurture” (genetics and environment) being able to affect biochemical factors which are able to impact cell growth and protein production.

Project 2: Biochemical impacts on the production of recombinant proteins in mammalian systems. A project aimed at reviewing the evidence for the impact of biochemical components on the production characteristics of importance to Pharmaceutical and diagnostic manufacturers, such as yield, productivity, reliability.

Project 3: The economic potential for on-farm manipulation of bioactivity. A project designed to combine the results of projects 1 & 2 in order to estimate the potential value of on-farm manipulation to the pharmaceutical and diagnostic manufacturers.

It is project 3 above that is covered by the current report.

1.4 Issues to be Addressed

This project draws together the outputs from Projects 1 and 2 above in order to estimate the potential value of on-farm manipulations to the pharmaceutical and diagnostic manufacturers. It involved the following activities:

- Coordinate and manage projects 1 and 2 (see above) with University of Queensland and University of Adelaide.
- Identify the potential increase in profit or cost of production that could accrue to manufacturers of recombinant proteins via mammalian cells through selective use of the “best” sera products.
- Facilitate communication between researchers in projects 1&2 in order to ensure that the biochemicals (or biochemical fractions) used in each study are compatible with each other and with the need to calculate value and impact on pharmaceutical manufacturers.

- Liaise with sera manufacturers in Australia to gather anecdotal evidence of changes of significance to the project in order to include these in the factors studied in projects 1&2.
- Develop a high level spreadsheet model of mammalian recombinant protein manufacture which is capable of quantifying the benefit of yield and productivity improvements. The model should be based on manufacturer data.

1.5 Project Objectives

To estimate the potential value of pre-slaughter manipulation of blood product bioactivity to the manufacturers of pharmaceuticals and diagnostics who use these products as process ingredients.

2 Economic Analysis

Section 9 of the Uniquest's (UQ) final report (MLA Project A.BIT.0003) presents an analysis of the impact of variations in bioreactor yield on production costs for a typical monoclonal antibody process generating an annual revenue stream of US$1B p.a. The primary data[1], (from employees of Boehringer-Ingelheim and Amgen) is summarised in Tables 14, 15 and 16 of the UQ report (see Appendix 1 below). Table 16 contains in that report assumed scenarios extrapolated by UQ from an Amgen conference-presented case study[2].

The key facts and/or assumptions in the above three tables are summarised in Table 1.

Table 1 – Key facts / assumptions underlying calculation of benefits of a 50% increase in productivity of a notional target recombinant vaccine or monoclonal antibody

Table number in report A.BIT.0003	Key fact / assumption
14	$1B Sales Capacity 1 g/L at 70% yield; $100M capital cost Assume for 1.5 l and 70% yield, capital cost falls to $70M
15	$1B Sales Capacity Cost of Cell Culture at 1g/L is 1.1% of sales price i.e. very small indeed
16	50% increase in productivity from 1 to 1.5 g/L results in increase in GM from 82.4 to 88.0% and increase in Gross Margin ($) of 6.8% equals to $56M for $1B sales

[1] Werner RG. (2004) Economic aspects of commercial manufacture of biopharmaceuticals. Journal of Biotechnology 113: 171-182.

[2] Croughan M The silver anniversary of clinical protein production from recombinant CHO cell culture. Cell Culture Engineering XI Conference, Coolum, Queensland, Australia (2008) Abstract P68

The UQ analysis only considers the impact of bioreactor productivity improvements on Direct Costs. It is necessary however, particularly for such high Gross Margin (GM) bioscience products to consider process fixed costs (overheads) in determining the economic bottom line (EBIT = earnings before interest and tax).

To do this the data from UQ's Tables 14-16 has been used and extended using certain assumptions to forecast the bottom line impact of a 50% increase in bioreactor productivity from 1 to 1.5 g/L. Table 2 summarises this analysis in which the following assumptions have been made:

Assumptions:

1	$1B sales plant
2	Average BioScience EBIT 20%. This assumed figure is taken from published financial results such as those for Medimmune's humanized Mab Synagis (a $1B product) with a GM of at least 70%
3	Capital Funding Costs 8%
4	Depreciation 10%
5	Table 16 Sales Figures for 1 and 1.5g/L.
6	Table 14 Capex $100M for 1 g/L. and $70M for 1.5 g/L.
7	Other Fixed Costs remain Constant
8	Table 15 Doubling Cost of Cell Culture adds 6.1 % to COGS
9	Improvement A below assumes no increased raw material costs for the fermentation, while Improvement B assumes that the 50% yield improvement will require the use of a Premium grade of FCS thereby doubling the direct cost of the fermentation stage.

Table 2[3] Calculated impact on EBIT of productivity improvements arising from one medium component such as an improved FCS, at standard FCS cost (A) and at double direct fermentation cost (B)

		A	B	Improvement A		Improvement B	
Sales ($M)	1000	1000	1000	(%)	($M)	(%)	($M)
Yield (g/L)	1	1.5	1.5				
COGS ($M)	177	120	127.3				
Capex ($M)	100	70	70				
GM(%)	82.3%	88.0%	87.3%				
GM ($M)	823	880	872.7	**6.9**	**57**	**6.0**	**49.7**
Depreciation ($M)	10	7	7				
Financing ($M)	8	5.6	5.6				
Other Fixed ($M)	605	605	605				
EBIT	**200**	**262.4**	**255.1**	**31.2**	**62.4**	**27.6**	**55.1**

This analysis suggests that the overall impact of such a productivity improvement is far greater than the direct costs analysis suggest. In fact the above indicates that of the overall EBIT

[3] By assuming a Bioscience EBIT of 20%, EBIT for a $1B sales plant becomes $200M. From this, after deducting the assumed financing and depreciation costs, an assumed fixed cost is calculated.

improvement 22% would come from direct cost savings and 78% from those in the fixed cost area, specifically related to the savings in capital expenditure.

This finding that the major benefit of use of premium FCS could be in freeing up existing plant (improved production flexibility) or reducing capital expenditure requirements for new or expanded plant, was suggested in discussions with an animal vaccine manufacturer.(Appendix 2).

3 Conclusions:

- Overall then it would appear that if reproducible productivity improvements of the order of 50% can be demonstrated in e.g. a pilot study, it should be possible to attract commercial collaborators (both monoclonal and vaccine producers) in a larger scale project leading to production of premium grades of foetal calf serum.

- The financial modeling has shown that productivity gains of this order should generate sufficient additional process profit to interest the manufacturers of vaccines and/or recombinant proteins.

- There is a need therefore to confirm that sufficient evidence exists supporting the hypothesis that a 50% productivity improvement is probable. This should then justify further R&D investment in an appropriately designed and cost-effective pilot study to prove the hypothesis.

- There was consensus that there was still insufficient evidence to persuade value-adders to make substantial financial commitment to a complex and costly R&D program at the farm level in which the impact of a number of identified parameters could be studied in their impact on FCS quality.

- It was felt that a better value proposition could be generated if MLA (alone or collaboratively) could investigate and attempt to quantify the natural variability of FCS quality when used in mammalian cell culture.

- Quantification of such natural variation range should assist the design of the overall on farm R&D program by:
    - showing what increase in average quality might be possible if median quality FCS could be increased to that of the best naturally occurring;
    - allowing focus on the factors (breed, foetus size, maternal diet with or without supplementation etc) which were seen to be important in the superior FCS samples.

- It was therefore suggested that such a study of FCS variability would be a smaller and more digestible preliminary experimental stage of the overall on-farm project.

4 The Way Forward

FCS Natural Variability Investigation (Simple Outline)

Project Objectives

1). Determine natural range of FCS variability when used in mammalian cell culture for vaccines and/or recombinant proteins.

2). Develop rapid and improved testing methodology to facilitate 1).

Project Activities

1). Review available testing methodologies and select the most appropriate in terms of cost-effectiveness.

2). Collect a range of single foetus FCS samples. These should be collected and processed under controlled conditions. There should also be sufficient documented information re the pre-slaughter environment and source. The samples should attempt to cover a comprehensive range of all possible variables impacting on FCS quality.

3). Establish a FCS quality data set through a comprehensive testing program.

Project Outcomes

1). Better knowledge of the actual FCS quality variations.

2). Improved rapid test method for assessing FCS quality. Such a test should have considerable commercial value in itself. Its development may therefore attract external financial support from value adders.

3). Information re pre-slaughter conditions (breed, foetus size, maternal diet with or without supplementation etc), for good and bad FCS samples, which should assist in experimental design of the larger on-farm R&D project.

All three outcomes have the potential to significantly enhance the value proposition of the on-farm R&D project, thereby increasing the probability of financial investment from collaborators.

Conclusions and Recommendations of the Two Academic Groups.

At a meeting of researchers from the University of Adelaide (UA) and University of Queensland presentations based on their respective final reports were made. The two sets of recommendations were:

A. University of Adelaide

1. The effects of intersubspecies crosses, gestation, foetal sex and weight and maternal weight and condition on foetal serum levels of candidate bioactives (Tables 1, 2; prioritising IGFs and related factors) be investigated (multiplex assays are available for most of these candidate bioactives).
2. The feasibility of defining the effects of genetic, environmental and physiological factors on foetal serum bioactives be investigated by survey of
    a. MLA supported projects and other relevant projects in the bovine to identify those which offer archived samples for analysis.
    b. On farm and harvesting practices, which would enable collection of samples classified according to some factors.

3. Concentrations of candidate bioactives be measured in batches of FBS and correlated with bioactivity in various culture systems.
4. The effects of IVF on foetal serum concentrations of candidate bioactives and bioactivity in different culture systems be determined and correlated.
5. The effects of selected micronutrient supplementation of the mother and its timing (targeting epigenetic state) on foetal serum concentrations of candidate bioactives and bioactivity in different culture systems be determined and correlated.

B. University of Queensland

1. Further research should focus on IGF I and II (with associated binding proteins) and b- FGF as lead biomarker candidates for assessing the quality of FBS.

2. Second line potential biomarker candidates include EGF, PDGF, FGFs and TGFs.

3. Contact industrial manufacturers, such as Merck, Johnson & Johnson and Genzyme, currently using US-sourced FBS to discuss changing to Australian-sourced FBS.

4. Present a case to international biopharmaceutical companies for potentially increasing earnings by replacing FBS with a premium grade FBS in bioprocesses, based on a process economic analysis.

5. Undertake a comprehensive proteomic analysis of FBS using state-of-the-art techniques such as multidimensional protein identification technologies (MuDPIT), which incorporates liquid chromatography interfaced with mass spectrometry. The analysis will identify factors not previously known and may potentially lead to the characterisation of new factors present in serum that are important in cell growth.

6. Carry out experimentation on selected batches of FBS “spiked” with various levels of factors (both existing and new factors identified in 4), as principal bioactives supporting cell growth and survival, using a factorial design to test precisely which factors are important in cell growth and productivity.

Outcomes of joint meeting of researchers.

Professors Owens (JO) and Mahler (SM) felt that, based on information currently available (both published and in-house), a reasonable hypothesis could be made that a 50% productivity improvement in recombinant protein production from mammalian cell culture was probable, through use of FCS of improved quality.

Action 1 : SM/JO to submit a technical case justifying the carrying out of a small pilot study

In terms of pilot study design it was felt that the key components could be
a) a partial chemical characterization of “good” and “bad” samples of FCS; and
b) impact on yield/productivity of sample spiking with growth factors.

UA recommendations 3. and 5. and UQ recommendations 1. and 6. above shared commonality in their impact on pilot plant design.

Action 2 : Subject to a reasonable case having been made (Action 1) SM/JO to submit a pilot study plan and resource budget

Appendix 1. (from UQ final report)

Table 14 Impact of innovation on economics and investments. Assumptions: 10,000 L scale; 250 kg per year, 50K US$ investment per bioreactor (from Werner, 2004).

Production Indices	No Innovation	With Innovation
Titer	100 mg L^{-1}	1000 mgL^{-1}
Yield	40%	70%
Capacity required	62,000,001 per year	3,400,001 per year
Number of bioreactors	31	2
Capital	US$ 1600 million	US$ 100 million
COGS per g	US$ 1500	US$ 260
COGS per year	US$ 375 million	US$ 65 million

Table 15 Breakdown for production of a biologic by mammalian (CHO) cell culture. Process: fed batch; amount of product in supernatant: 1g/L; selling price $4000 per gram.

PROCESS	COGS $	% of total COGS	% selling price ($4000/g)
1. Bioreactor; Cell Culture	**43**	**6.1**	**1.1**
2. Primary recovery	**48**	**6.8**	**1.2**
3. Purification	**107**	**15.2**	**2.7**
4. Viral exclusion/inactivation	**85**	**12.0**	**2.1**
5. Fill finish(vials)	**238**	**33.7**	**6.0**
6. Release assays	**185**	**26.2**	**4.6**
TOTAL	**706**	**100**	**17.7**

Table 16 Process economic metrics for production of a biologic in CHO cells. Figures in bold represent measured metrics.

Productivity g/L	COGS/g	% COGS with reference to 1g/L	Gross Margin	Gross Earnings ($m) for Revenue ($b):			% Increase in Earnings
				1	0.5	0.1	
0.5	**931**	**32**	**76.7**	**767.3**	**383.6**	**76.7**	**-6.8**
1.00	**706**	**0**	**82.4**	**824.3**	**412.1**	**82.4**	**0**
1.05	683	-3.2	82.9	829.3	414.6	82.9	0.7
1.10	661	-6.4	83.5	834.8	417.4	83.5	1.4
1.20	616	-12.8	84.6	846.0	423.0	84.6	2.7
1.50	480	-32	88.0	880.0	440.0	88.0	6.8

Appendix 2.

File Note: Meeting with vaccine manufacturer, June 2008.

- Australian FBS attracts a premium price
- claimed a number of big players had attempted to characterise FBS in the period 1978-1984; work by Daniel Wang reported at a cell culture engineering conference in San Diego was cited. Not much conclusive impact came out of this work
- Cell culture systems are quite fickle and a natural variability in yield/productivity of 20-25% is normal.
- In testing FCS quality the variability of test results (thought to be a function of the test methods used) is so great that no relationship with yield or productivity is seen. One simple test used is the Plating Efficiency Test; fairly crude but indicative of toxin presence
- Gross Margins vary greatly across the product range (20-80%) and clearly yield improvements would have a greater financial impact on low GM products; however it was felt that a demonstrable yield improvement of 20% would have the vaccine producers very interested, more for the increase in plant capacity that would result rather than the actual cost reduction. The major problem is to demonstrate that a 20% yield improvement is real as a 50%-200% yield range around the “Norm” is common experience.
- There was a suggestion that there may be an opportunity for a differentiated product in that there was a growing demand for Halal raw materials in vaccine production, as there had been cases in the industry of rejection of batches of vaccine being rejected in Malaysia or Indonesia due to Halal issues.