Received: 14 November 2017 | Revised: 8 April 2018 | Accepted: 25 April 2018

DOI: 10.1002/bit.26724

ARTICLE

WILEY BIOTECHNOLOGY BIOENGINEERING

Improving culture performance and antibody production in CHO cell culture processes by reducing the Warburg effect

Maria Buchsteiner[1] | Lake-Ee Quek[1,2] | Peter Gray[1] | Lars K. Nielsen[1,3]

[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, Queensland, Australia

[2]Charles Perkins Centre, The University of Sydney, Sydney, New South Wales, Australia

[3]Novo Nordisk Foundation Center for Biosustainability, The Technical University of Denmark, Kongens Lyngby, Denmark

Correspondence
Lars K. Nielsen, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Queensland 4072, Australia.
Email: lars.nielsen@uq.edu.au

Abstract

Lactate is one of the key waste metabolites of mammalian cell culture. High lactate levels are caused by high aerobic glycolysis, also known as the Warburg effect, and are usually associated with adverse culture performance. Therefore, reducing lactate accumulation has been an ongoing challenge in the cell culture development to improve growth, productivity, and process robustness. The pyruvate dehydrogenase complex (PDC) plays a crucial role for the fate of pyruvate, as it converts pyruvate to acetyl coenzyme A (acetyl-CoA). The PDC activity can be indirectly increased by inhibiting the PDC inhibitor, pyruvate dehydrogenase kinase, using dichloroacetate (DCA), resulting in less pyruvate being available for lactate formation. Here, Chinese hamster ovary cells were cultivated either with 5 mM DCA or without DCA in various batch and fed-batch bioreactor processes. In all cultures, DCA increased peak viable cell density (VCD), culture length and final antibody titer. The strongest effect was observed in a fed batch with media and glucose feeding in which peak VCD was increased by more than 50%, culture length was extended by more than 3 days, and the final antibody titer increased by more than twofold. In cultures with DCA, lactate production and glucose consumption during exponential growth were on average reduced by approximately 40% and 35%, respectively. Metabolic flux analysis showed reduced glycolytic fluxes, whereas fluxes in the tricarboxylic acid (TCA) cycle were not affected, suggesting that cultures with DCA use glucose more efficiently. In a proteomics analysis, only few proteins were identified as being differentially expressed, indicating that DCA acts on a posttranslational level. Antibody quality in terms of aggregation, charge variant, and glycosylation pattern was unaffected. Subsequent bioreactor experiments with sodium lactate and sodium chloride feeding indicated that lower osmolality, rather than lower lactate concentration itself, improved culture performance in DCA cultures. In conclusion, the addition of DCA to the cell culture improved culture performance and increased antibody titers without any disadvantages for cell-specific productivity or antibody quality.

KEYWORDS

Chinese hamster ovary cells, fed batch, lactate, monoclonal antibody, pyruvate dehydrogenase kinase, Warburg effect

1 | INTRODUCTION

Mammalian cells are the preferred production host for therapeutic proteins such as monoclonal antibodies due to their ability to perform posttranslational modifications, which are essential for biological efficacy and safety (Durocher & Butler, 2009; Wurm, 2004). Most cultured mammalian cells display aerobic glycolysis also known as the Warburg effect, that is, inefficient energy metabolism, characterized by increased glucose consumption and lactate production (Warburg, Wind, & Negelein, 1927). Rapid lactate accumulation adversely affects culture performance and productivity (Dietmair, Nielsen, & Timmins, 2011; Gambhir et al., 2003; Lao & Toth, 1997; Lim et al., 2010). In fermentation, pH is controlled, and growth inhibition is attributed to the concomitant osmolality increase (Cruz, Freitas, Alves, Moreira, & Carrondo, 2000). Increased osmolality can also affect antibody quality (Li, Wong, Vijayasankaran, Hudson, & Amanullah, 2012; Pacis, Yu, Autsen, Bayer, & Li, 2011).

Many strategies involving process optimization (Altamirano, Paredes, Illanes, Cairó, & Gòdia, 2004; Gagnon et al., 2011; Glacken, Fleischaker, & Sinskey, 1986; Kuwae, Ohda, Tamashima, Miki, & Kobayashi, 2005) or genetic engineering (Fogolín, Wagner, Etcheverrigaray, & Kratje, 2004; Irani, Wirth, Van Den Heuvel, & Wagner, 1999; Wlaschin & Hu, 2007; Zhou et al., 2011) have been explored to reduce lactate production and hereby improve cell growth, productivity, and process robustness. Interestingly, although a knockdown of lactate dehydrogenase (LDH) reduces glucose consumption and lactate production (Kim & Lee, 2007), our attempt to perform a double knock-out of the LDHA gene failed presumably because of lethality; one allele was readily interrupted by a zing finger nuclease, but no double knock-out could be produced (unpublished). It is possible that the TCA cycle is unable to absorb additional carbon. Consistent herewith, the glucose consumption declined in a knockdown study and no additional carbon was redirected into the TCA cycle as lactate to glucose yield was unaltered.

The pyruvate dehydrogenase complex (PDC) plays a crucial role in glucose metabolism linking glycolysis to the TCA cycle, as it converts pyruvate to acetyl-CoA. The PDC activity is regulated by reversible phosphorylation of three serine residues, phosphorylation being catalyzed by pyruvate dehydrogenase kinase (PDK), which inactivates PDC (Yeaman et al., 1978). RNA interference (RNAi)-mediated knockdown of PDK1, 2, and 3 caused a significant downregulation in lactate production, but complete knockout of the LDHA gene remained impossible (Yip et al., 2014).

RNAi-mediated knockdown of PDK1, 2, and 3 displays substantial clonal variation (Yip et al., 2014). In the current study, we used the PDK inhibitor dichloroacetate (DCA) (Whitehouse, Cooper, & Randle, 1974), which has been used for decades in the treatment of human diseases, such as acquired or congenital lactic acidosis, congestive heart failure, and ischemic heart disease, because of its ability to lower blood lactate levels (Stacpoole, Henderson, Yan, Cornett, & James, 1998). DCA has previously been explored to improve mammalian cell culture. In hybridoma cell cultures without pH control, DCA improved peak viable cell density (VCD) and final antibody titer by delaying glutamine depletion without any effect on glucose consumption or lactate production (Murray, Gull, & Dickson, 1996). In Chinese Hamster Ovary (CHO) cultures without pH control, DCA reduced lactate production leading to higher VCD (Follstad, 2002). However, no improvements in culture performance were observed in pH-controlled bioreactor fermentations.

In this study, we successfully used DCA to reduce lactate production and glucose consumption in commercially relevant, pH-controlled CHO fed-batch cultures, in which no essential macronutrients were depleted. We compared cell growth as well as nutrient consumption and metabolite production, productivity, and antibody quality. Proteomics, metabolomics, and fluxomics were used to explore the system-wide effect of DCA. Finally, sodium lactate and sodium chloride feed experiments were performed to establish if lower lactate accumulation itself or lower osmolality levels are responsible for improvements in culture performance.

2 | MATERIAL AND METHODS

2.1 | Cell line and medium

CHO-XL99 is a derivative of CHO-K1 (ATCC 61-CCL) and expresses a full IgG1 antibody (Ab2). It was adapted to CD CHO medium supplemented with 8 mM GlutaMAX, 400 µg/ml Geneticin, and 0.2% v/v Anticlumping agent (all Invitrogen). Cells are routinely maintained in a shake flask at 37°C, 7.5% CO_2, and 130 rpm. Sodium DCA (Sigma-Aldrich) was added to respective precultures 2 days before transfer to the bioreactor. Separate shake flask experiments were performed for the western blot, DCA response curve, and DCA stability in the cell culture analysis. Two milliliters per liter of Pluronic F-68 (Invitrogen) was added to the medium used for bioreactor cultures.

2.2 | Bioreactor cell culture

A total of 12 bioreactor cultures were performed (Table 1). All cultures were carried out in a 3-L sparged bioreactor (Applikon) with 1-L initial working volume at 37°C, pO_2 at 50% air saturation, stirrer speed at 200 rpm, and pH 7.0 controlled by CO_2 sparging and addition of 1 M sodium bicarbonate. Oxygen uptake rate (OUR) was determined using the dynamic method (Singh, 1996).

In *fed-batch* cultivations, a 1:1 mixture of CHO CD EfficientFeed A and CHO CD EfficientFeed B (FeedAB) (Gibco) was fed four times: before inoculation (15% of volume) and approximately 90 hr (15%), 109 hr (10%), and 118 hr (10%) after inoculation. In the *fed batch plus glucose* cultures, concentrated glucose (450 g/L, Sigma) was added to the culture as needed to keep glucose concentration above 11 mM after the last FeedAB feed and until the end of the culture.

Sodium lactate and sodium chloride (Sigma) feeding experiments were carried out, starting the bioreactor cultivation as with the

TABLE 1 Overview of experiments presented in this work

Study	Conditions[a]	Cultures	No. of runs
Batch	CD CHO medium	5 mM DCA	2
	No feeding	No DCA (control)	2
Fed batch	CD CHO medium	5 mM DCA	1
	FeedAB	No DCA (control)	1
Fed batch plus glucose	CD CHO medium	5 mM DCA	2
	FeedAB + glucose feed	No DCA (control)	2
Sodium lactate feed	CD CHO medium	No DCA	1
	FeedAB + sodium lactate		
Sodium chloride feed	CD CHO medium	No DCA	1
	FeedAB + sodium chloride		

Note. DCA, dichloroacetate.
[a]FeedAB = CHO CD EfficientFeed A and B (1:1).

fed-batch cultures. One day after inoculation, sodium lactate (40 mM/day) or sodium chloride (30 mM/day) was fed continuously until the end of the culture.

2.3 | Cell count, metabolite, and IgG measurements

VCD, viability, and cell diameter were measured using a CedeX cell counter (Innovatis AG). Ammonia, sodium, and glucose concentrations used to determine time points of glucose feeding were quantified using a Nova Bioprofile FLEX (Nova Biomedical). Extracellular glucose, lactate, GlutaMAX, and amino acid concentrations were measured by high-performance liquid chromatography (HPLC) as described previously (Dietmair, Timmins, Gray, Nielsen, & Krömer, 2010), whereas antibody titers were determined using surface plasmon resonance on a Biacore T-100 with a human antibody capture kit (GE Healthcare). Selected intracellular metabolites from glycolysis, TCA cycle, and pentose phosphate pathway were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Dietmair et al., 2010) in midexponential growth phase of fed-batch cultivations (Figure 2).

2.4 | Metabolic flux analysis

Metabolic flux analysis (MFA) was performed as previously described for HEK293 cells (Quek et al., 2014), here deriving the reduced flux model from a *Mus musculus* genome-scale metabolic reconstruction (Quek & Nielsen, 2008). The average dry weight was measured to be 350 pg/cell, and precursor compositions obtained from previous CHO cell studies (Martínez et al., 2013) were used for the biomass equation. An Ab2 antibody production reaction was added (Ab2 amino acid composition shown in Supporting Information Table 2). GlutaMAX (L-alanyl-L-glutamine dipeptide) was treated as free glutamine and alanine. Reactions for phosphoenolpyruvate carboxykinase and serine degradation by serine dehydratase were removed from the model as both enzymes' main role is in gluconeogenesis, which can be considered inactive when glycolysis is highly active (Quek, Dietmair, Krömer, & Nielsen, 2010). Estimated fluxes were also used to estimate the ATP production (Martínez et al., 2013).

2.5 | Proteomics

Samples were collected during the midexponential growth phase of fed batch and fed batch plus glucose cultures (Figure 2), extracted, and analyzed using LC-MS/MS as described previously (Orellana et al., 2015). ProteinPilot v4.5 (ABSciex) and the Paragon algorithm were used to analyze MS/MS data. The protein sequences for CHO-K1 were downloaded from the Chinese hamster genome database (http://www.chogenome.org). A false discovery rate of 5% was used and only proteins identified with at least 2 peptides at 95% confidence interval were included in the statistical analysis. For SWATH-MS, MS/MS data were analyzed with the SWATH-MS processing script in PeakView v1.2 (ABSciex) using discovery data as the spectral library for protein identification and fragment ion peak areas were used for quantification. Summed protein peak areas were used for statistical analysis. Raw data were log2 transformed, quantile normalized, and analyzed for statistically differentially expressed proteins using limma (Smyth, 2005) in R (R Core Team 2013). Expression differences with an adjusted p value <0.05 were considered statistically significant.

2.6 | Antibody product quality analysis

Samples were collected during the early stationary phase of fed batch plus glucose cultivations (Figure 2) and protein-A purified. A LabChip GXII (Caliper) was used to analyze charge variants (HT Protein Charge Variant Kit) and various glycoforms (ProfilerPro Glycan Profiling Kit). Except for a longer PNGase digestion step of 3 hr, the kits were used according to manufacturer's instructions. Antibody aggregation, monomer, and fragment levels were determined using standard size exclusion chromatography on an Agilent HPLC system.

3 | RESULTS

3.1 | DCA is stable in CHO cell culture, decreases PDC phosphorylation, and reduces lactate formation

Shake flask experiments with different DCA concentrations were performed to analyze the effects of DCA on cell growth, lactate production, and PDC phosphorylation. DCA can be degraded by glutathione S-transferase ζ1 (Lantum, Cornejo, Pierce, & Anders, 2003; Li et al., 2011), and we first established that DCA concentration in CHO culture decreased less than 5% of its initial concentration over a period of 12 days and no additional DCA supplementation is required (Figure 1a). Lactate production decreased with increasing DCA concentration (Figure 1b), whereas the growth rate was unaffected up to a concentration of 5 mM DCA (Figure 1c), and this concentration was chosen for subsequent bioreactor cultures. The western blot analysis confirmed a decrease in PDC E1α phosphorylation with increasing DCA concentration with a 17% reduction observed at 5 mM DCA (Figure 1d).

3.2 | Growth, glucose consumption, lactate production, and antibody titer profiles in different bioreactor culture processes

In batch fermentations, cell growth ceased once glucose was depleted in both control and 5 mM DCA cultures (Figure 2, top panel). DCA delayed depletion, maintained high viability for another day, and enhanced Ab2 titer (Table 2). Depletion can be avoided using fed batch, and we first used a rich feed to the maximum recommended total of 50% of culture volume (Figure 2, middle panel). Although feeding extended the culture, cell growth still ceased because of glucose depletion, and DCA again extended the culture and increased the titer (Table 2). Glucose depletion was avoided in the subsequent fed-batch cultures by feeding glucose as

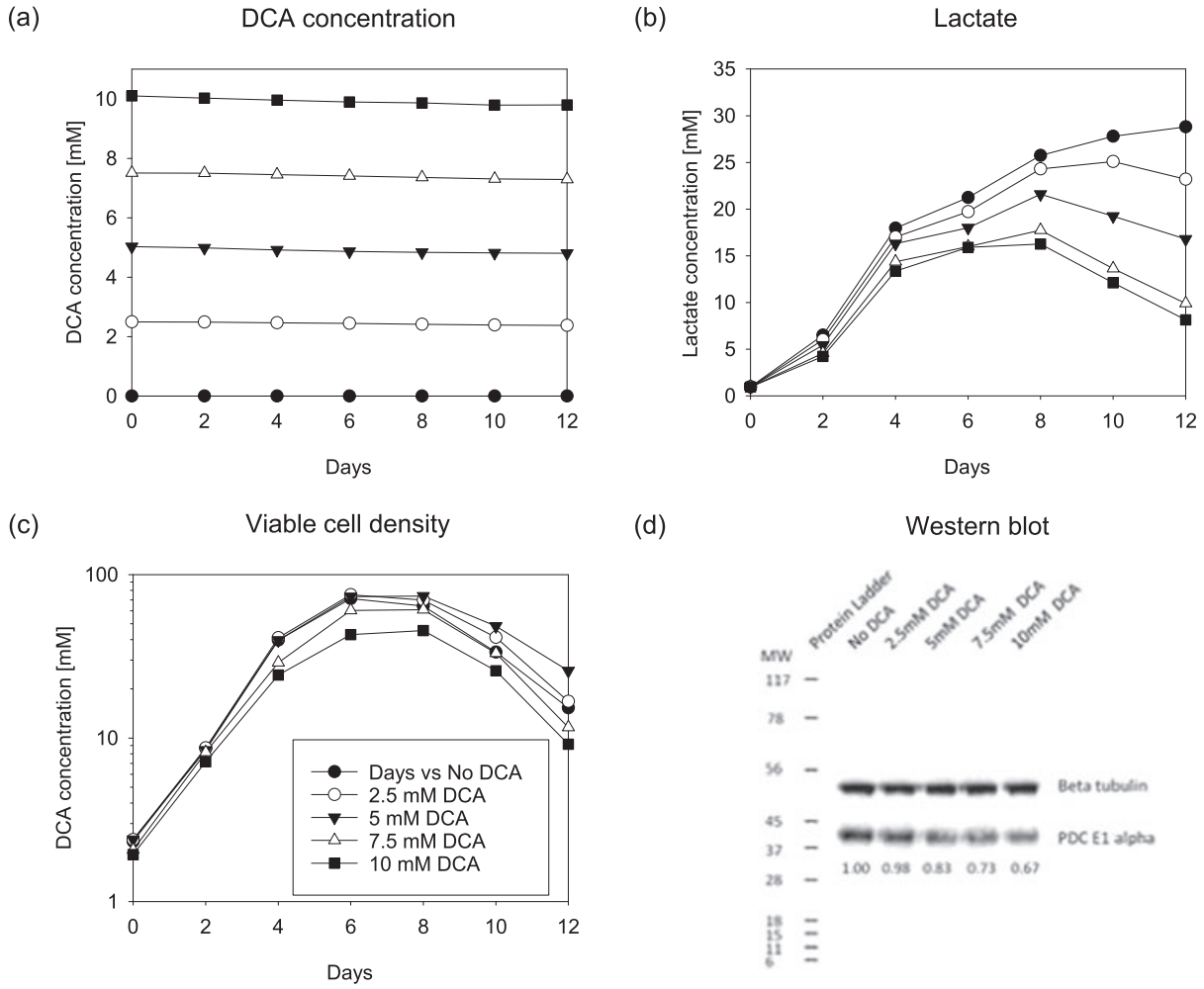

FIGURE 1 Growth curve, lactate and DCA concentration profile, and the western blot analysis. CHO cells were cultured in a shake flask with different DCA concentrations. DCA concentration decreased less than 5% in CHO cell culture over a 12-day culture period (a). Lactate production decreased with increasing DCA concentration during exponential growth (b). Growth rate was unaffected by DCA up to 5 mM and decreased at higher DCA concentrations (c) and with increasing DCA concentration PDC E1α phosphorylation decreased (d). Western blot and DCA measurements using HPLC were performed as described in Materials and Methods of Supporting Information. CHO, Chinese hamster ovary; DCA, dichloroacetate; HPLC, high-performance liquid chromatography; PDC, pyruvate dehydrogenase complex

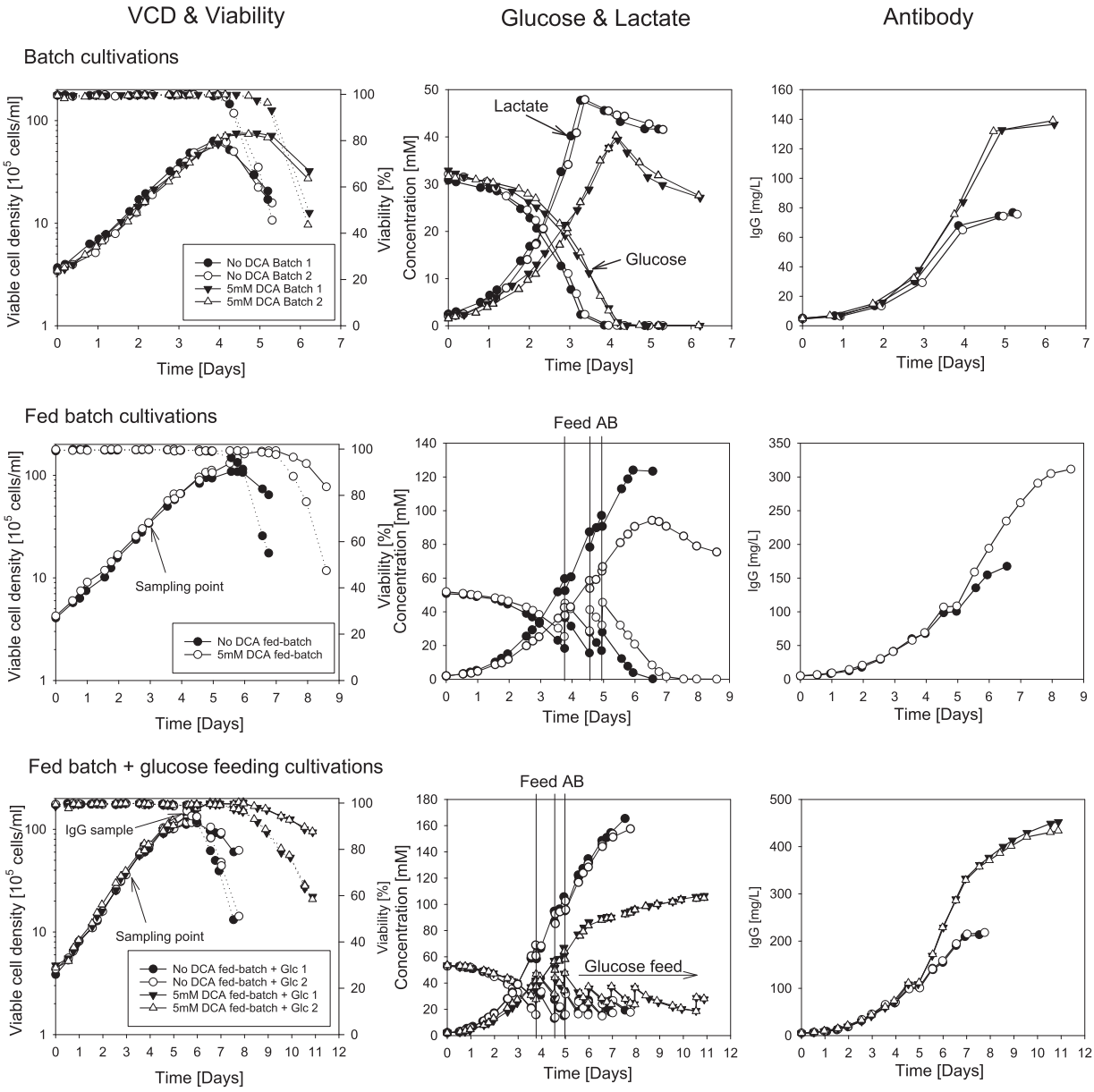

FIGURE 2 Impact of DCA on cell growth, glucose consumption, lactate production, and antibody titer. CHO-XL99 cells were cultivated in a bioreactor with 1-L initial working volume in batch, fed batch, and fed batch plus glucose processes either with 5 mM dichloroacetate (DCA) or no DCA as control cultures. In fed batch and fed batch plus glucose, the feeding medium (FeedAB) was added before inoculation followed by three more feedings during the cultivation. In fed batch plus glucose cultures, glucose feeding started on Day 6 and continued until the end of the culture as needed to keep glucose concentration above 11 mM. In all processes, DCA extended the culture time, led to a higher peak viable cell density (VCD), decreased glucose consumption and lactate production, and increased final antibody titer. In the left column, solid lines are VCD and dotted lines are viability

required to avoid depletion after the completion of the rich medium feed (Figure 2, bottom panel). Surprisingly, glucose feeding had a greater benefit in DCA cultures, extending these by 2.5 days compared with 1 day without DCA, and the Ab2 titer improvement was 105% (Table 2).

During exponential growth, DCA reduced glucose consumption and lactate production by approximately 35% ± 3% and 40% ± 3%, respectively, resulting in a decrease in molar lactate to glucose yield of 7% (Table 2). The growth rate during exponential growth for all 10 cultures was the same ($0.033 \pm 0.001\,hr^{-1}$, $p > 0.05$; a two-way ANOVA [analysis of variance]). Similarly, there was no difference in the cell-specific productivity (8.80 ± 0.12 pg·cell·day), $p > 0.05$, a two-way ANOVA). Thus, the improved titers seen with DCA are exclusively attributable to maintaining cultures viable for longer.

3.3 | DCA does not affect the antibody quality

The CHO-XL99 cell line expresses a full IgG1 antibody (Ab2). Changes in culture conditions and cell metabolism can influence the antibody quality. However, DCA had no major impact on the

TABLE 2 Culture length, peak viable cell density, lactate to glucose yield, and final antibody titer of cultures with DCA and control cultures in different processes

Culture condition	Culture time (days)	Peak VCD ([a]10^5 cells/ml)	$Y_{lactate/glucose}$	Ab2 titer (μg/ml)	Ab2 titer increase[a]
Batch, no DCA	~4	62.5	1.75	77.2	
Batch, 5 mM DCA	~5	74.7	1.62	137.8	78%
Fed batch, no DCA	~7	108.5	1.74	167.3	
Fed batch, 5 mM DCA	~8.5	172.9	1.61	311.5	86%
Fed batch + glucose, no DCA	~8	118.0	1.68	215.6	
Fed batch + glucose, 5 mM DCA	~11	181.5	1.56	442.9	105%

Note. DCA, dichloroacetate; VCD, viable cell density.
[a]Compared with the control culture.

aggregation level, acidic and basic variants, or glycoform pattern (Table 3). The percentage of monomers was slightly lower, whereas the percentage of dimers and multimers was higher. Similarly, a slight shift from basic to acidic variants was observed in DCA cultures. The glycan profile analysis was similar for both cultures and showed no change in % Man5, a variable previously found to be affected by osmolality (Pacis et al., 2011).

3.4 | Growth cessation is caused by high osmolality

During fed batch plus glucose cultures, cell growth slowed down and cultures entered the stationary phase despite neither glucose nor any of the essential amino acids being depleted. Although depletion of a micronutrient is a possibility, a more likely explanation, given the effect of DCA, is the accumulation of growth inhibiting by-products. Comparing growth curves and lactate concentrations of control and DCA cultures showed that in both growth started to slow down once lactate concentration reached approximately 90 mM (Figure 3a,b, respectively).

A sodium lactate feeding experiment was performed to confirm that high lactate concentration was responsible for growth inhibition (Figure 4a). Sodium lactate was fed at 40 mM/day, which is higher than the maximum volumetric lactate production rate observed in DCA cultures (~24 mM/day) but slightly lower than in control cultures (~42 mM/day).

Early on Day 4, the lactate concentration reached 90 mM and cell growth slowed down, followed by stationary phase and entering death phase late on Day 4. Na^+ concentration was around 180 mM when the cell growth rate decreased. Cells entered stationary phase at an Na^+ concentration of approximately 220 mM. Feeding sodium lactate to the culture not only increased the lactate concentration but also increased osmolality. Therefore, it is not clear whether lactate itself or the increased osmolality caused growth inhibition.

A sodium chloride feeding experiment in which osmolality is increased at a higher rate while lactate concentration only increases at the rate produced by the cells was carried out to determine which factor is growth inhibitory. The growth rate declined on Day 4 at around 180 mM Na^+; however, cell growth continued until Day 5. Cells started to die once the Na^+ concentration reached approximately 220 mM (Figure 4b). The Na^+ concentration was similar to the concentration observed in sodium lactate feeding experiment at time point of growth rate reduction; however, lactate concentrations were significantly lower (final lactate concentration was 44 mM), indicating that high osmolality, rather than the lactate concentration per se, was growth inhibitory in fed batch plus glucose fermentations.

3.5 | DCA significantly decreases lactate production without affecting oxidative metabolism

To understand global effects of DCA, a comprehensive omics comparison was performed during the exponential phase of fed.

TABLE 3 Aggregation, charge variant, and glycan profiles of control and DCA cultures

	Aggregation (%)			Charge variant (%)		
	Monomers	Dimers	Multimers	Acidic	Main	Basic
Control	90.4 ± 0.3	8.6 ± 0.4	1.0 ± 0.1	12.5 ± 0.4	50.0 ± 0.7	37.5 ± 0.4
5 mM DCA	88.4 ± 0.4	10.2 ± 0.4	1.4 ± 0.1	13.7 ± 0.8	51.0 ± 0.6	35.3 ± 0.2
	Glycan (%)					
	Man5	G0	G0f	G1f/G1f′	G2	G2f
Control	1.04 ± 0.12	5.43 ± 0.49	60.14 ± 4.98	22.15 ± 2. 12	0.79 ± 0.05	1.40 ± 0.32
5 mM DCA	1.15 ± 0.10	5.31 ± 0.86	59.76 ± 1.74	22.38 ± 1.93	0.81 ± 0.07	1.41 ± 0.18

Note. Samples were collected in an early stationary phase of fed batch plus glucose cultivations (Figure 2; "IgG samples") and analyzed using size exclusion chromatography for aggregation, charge variants (HT Protein Charge Variant Kit; LabChip GX2), and glycoforms (ProfilerPro Glycan Profiling Kit; LabChip GX2). Table shows mean ± standard deviation (n = 3 for each condition). DCA, dichloroacetate.

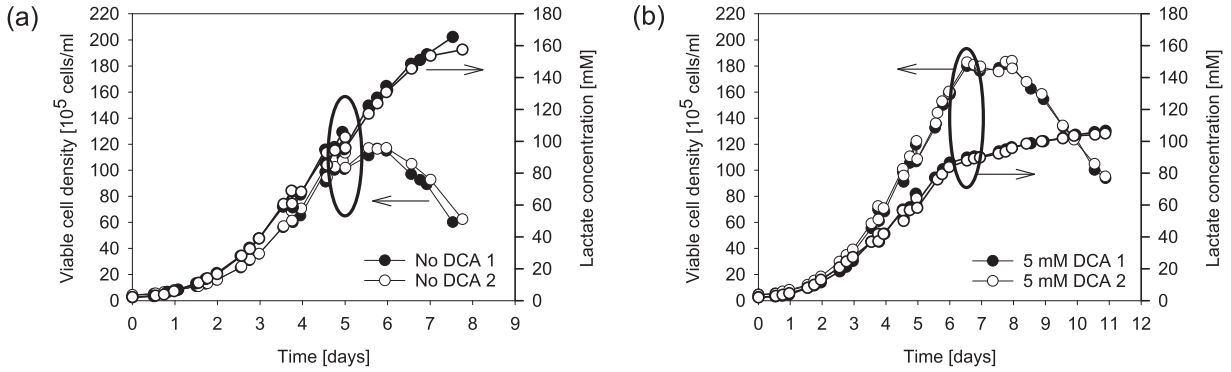

FIGURE 3 Viable cell density (VCD) and lactate concentration of fed batch plus glucose cultures. VCD and lactate concentration profile of fed batch plus glucose control cultures (a) and with 5 mM DCA (b). Circles indicate the start of growth rate reduction. DCA, dichloroacetate

batch cultures. Because this precedes postinoculation feeding, data from fedbatch cultures with and without glucose were used as biological replicates. Samples for proteomics and metabolomics were obtained on Day 3, whereas metabolic rates were determined between Days 1 and 3.5 (Figure 2).

Using SWATH for quantitative proteomics, less than 1% (20) of 2016 identified proteins were differentially expressed (Supporting Information Table S1) with the highest fold change being 2.31. None of the genes in central carbon metabolism appeared in the differentially expressed list, nor did gene enrichment analysis identify central metabolic processes such as glycolysis. Evidently, both direct and indirect effects of DCA are at a posttranslational level, and observed metabolic changes are the result of posttranslational regulation.

Glucose consumption and lactate production rates were significantly reduced in DCA cultures, whereas the OUR was unchanged, indicating similar TCA cycle activity (Table 4). Apart from a 30% decrease in glycine production in DCA cultures, amino acid metabolism was very similar between the two conditions. The rates in Table 4 were used to perform MFA (Figure 5). Samples for intracellular metabolite analysis were collected in triplicate (technical) on Day 3 from fed batch plus glucose cultures only. One of the DCA samples had varying results without a clear trend and was omitted from the analysis, leaving $n = 5$ for DCA cultures and $n = 6$ for control cultures. A total of 43 metabolites were measured whereof 14 were either not detected or below the lower limit of quantification in at least one of the samples (Figure 6).

As expected from reduced glucose consumption and lactate production (Table 3), glycolytic fluxes and flux through LDH were significantly lower in cultures with DCA (Figure 5). The concentrations of glycolytic intermediates downstream of phosphofructokinase (PFK) and aldolase were lower in DCA cultures compared with control cultures as was lactate. In contrast, glucose 6-phosphate, glucose 1-phosphate, and fructose 6-phosphate were similar. MFA cannot resolve fluxes in the oxidative pentose phosphate pathway as the need for NADPH can also be met by the cytosolic malic enzyme (Quek et al., 2010). The concentrations of ribose 5-phosphate and ribulose 5-phosphate were higher in DCA cultures, suggesting a more active pentose phosphate pathway.

Apart from glycolysis, the only significant flux change was reduced flux in DCA cultures through the serine transhydroxymethyltransferase enzyme that converts serine and tetrahydrofolate (THF) to glycine and 5,10-methylene-THF, which is consistent with the reduced production of glycine observed in Table 4.

In both cultures, the majority of glycolytic-derived pyruvate was converted to lactate though the percentage was lower for cultures with DCA (68% and 78% for DCA and control cultures, respectively). DCA reduces phosphorylation of PDC (Figure 1), and data does support a small shift in concentrations with lower pyruvate and higher acetyl CoA (Figure 6). However, fluxes for pyruvate transport into the

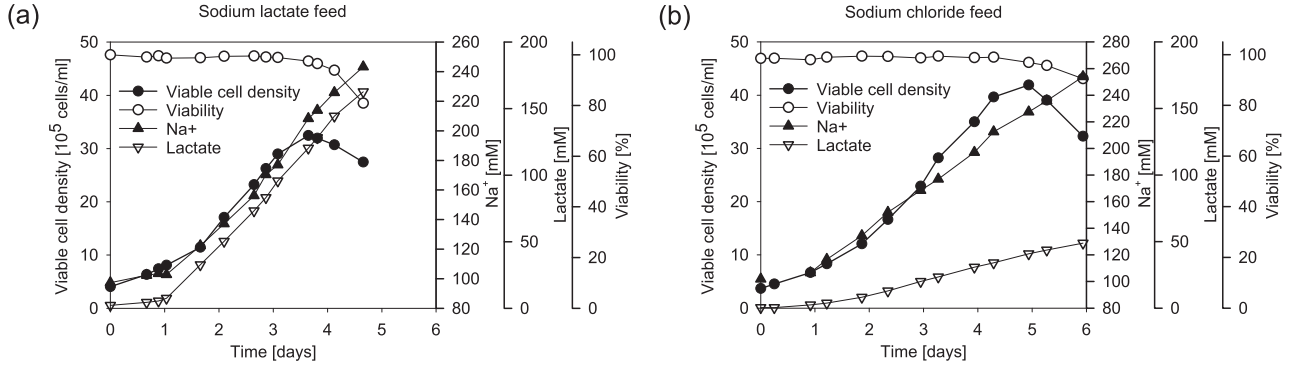

FIGURE 4 Impact of sodium lactate and sodium chloride feeding on cell growth. Culture had same starting conditions as fed batch without DCA. One day after inoculation sodium lactate feeding (a, 40 mM/day) or sodium chloride feeding (b, 30 mM/day) started. DCA, dichloroacetate

TABLE 4 Metabolic rates of CHO-XL99 cultures treated with 5 mM DCA and control cultures

Metabolite	No DCA Rate ± SE		5 mM DCA Rate ± SE	
Glucose*	−575	10	−372	9
Lactate*	1,005	15	600	14
$Y_{lactate/glucose}$*	1.749	0.004	1.616	0.001
NH_4^+*	27.0	0.3	30.0	0.7
OUR	−291.4	3.5	−289.2	3.5
Consumed amino acids				
Asp*	−4.83	0.04	−3.83	0.09
Glu	−4.04	0.04	−3.82	0.17
Asn	−43.02	0.38	−42.30	0.54
Ser*	−47.95	0.23	−44.30	0.76
Arg*	−10.03	0.21	−8.44	0.13
Essential amino acids				
His	−4.45	0.13	−4.16	0.03
Thr	−10.70	0.25	−10.10	0.21
Tyr	−4.56	0.11	−4.76	0.21
Val	−13.89	0.23	−13.18	0.38
Met	−4.90	0.15	−4.79	0.07
Trp*	−3.67	0.09	−3.26	0.09
Phe*	−6.77	0.10	−5.52	0.12
Ile*	−11.43	0.12	−10.55	0.26
Leu	−17.79	0.23	−16.62	0.45
Lys*	−13.94	0.11	−13.05	0.25
Pro	−11.16	0.28	−11.88	0.31
Produced amino acids				
Gly*	23.73	0.30	16.61	0.24
Ala	97.4	1.4	95.0	1.9
Gln*	22.77	0.27	19.05	0.53
GlutaMax*	−56.8	1.1	−50.8	1.4
Growth rate	0.0328	0.0004	0.0322	0.0007
Productivity	8.86	0.04	8.77	0.10

Note. Mean (n = 3; fed batch and fed batch plus glucose) metabolic uptake and production rates of cultures with 5 mM DCA and control cultures during exponential growth. Rates are in µM·gDW·hr, the growth rate is in hr^{-1}, the antibody (Ab2) production rate is in pg·cell·day. Negative rates represent consumption, whereas positive rates represent the production of the corresponding metabolite. The asterisks indicate rates that were statistically significantly different ($p < 0.05$; t test) in cultures with DCA compared with control cultures. DCA, dichloroacetate; OUR, oxygen uptake rate; SE, standard error; Y, yield

mitochondrion and the PDC reaction were unchanged (Figure 5), indicating that DCA cultures did not direct more pyruvate into the TCA cycle but rather reduced the flux to pyruvate and the fraction of pyruvate converted to lactate. Consistent herewith, the concentrations of TCA cycle intermediates (citrate, aconitate, fumarate, and malate) were similar between the two conditions (Figure 6).

ATP production was calculated using the estimated optimum fluxes. ATP production via the TCA cycle was similar in the cultures. A total of 1,284 µmol·gDW·hr and 1,256 µmol·gDW·hr for control and DCA cultures, respectively, is expected, given the unchanged OUR (Table 3). However, the amount of ATP generated in glycolysis is approximately 35% lower in DCA cultures, 1,155 µM·gDW·hr and 741 µM·gDW·hr for control and DCA cultures, respectively. Accordingly, cultures with DCA produced 18% less ATP overall, 2,440 µmol·gDW·hr for control cultures and 1,997 µmol·gDW·hr for DCA cultures despite having similar growth rates and cell-specific productivity.

ATP concentration was lower in DCA cultures; however, the adenylate energy charge (Atkinson & Walton, 1967) was similar for control and DCA cultures with 0.91 ± 0.03 and 0.92 ± 0.01, respectively, indicating no lack of energy in either condition (Figure 6). Other nucleotides and nucleotide sugars were also elevated under control conditions. $NADP^+$ concentrations were similar, whereas NAD^+ concentration was slightly reduced in DCA cultures (Figure 6). The latter may be attributed to reduced LDH activity in DCA cultures.

4 | DISCUSSION

4.1 | DCA improves culture performance without affecting productivity and product quality

Cultured mammalian cells, such as CHO cells, consume glucose and glutamine beyond their stoichiometric needs and produce large amounts of waste products, such as lactate and ammonia (Altamirano, Paredes, Cairo, & Godia, 2000; DeBerardinis et al., 2007; Warburg, 1956). Lactate negatively impacts cell growth as well as antibody production and quality (Dorai et al., 2009; Hassell, Gleave, & Butler, 1991; Li et al., 2012; Pacis et al., 2011). In this study, we investigated the effects of a DCA-mediated increase of PDC activity on culture performance, cell metabolism, and antibody production and quality in a CHO cell line.

DCA is an inexpensive (a few cents per liter of medium at 5 mM) inhibitor of PDK and thus indirectly an activator of PDC. DCA reduced PDC phosphorylation in CHO-XL99 cells, and cells were unable to degrade DCA, ensuring exposure of the cells to a constant DCA concentration throughout the cultivation (Figure 1). PDC phosphorylation and lactate production were reduced in a dose-dependent manner, whereas cell growth was only affected at concentrations above 5 mM DCA, which was used in subsequent studies.

In all bioreactor fermentations, aerobic glycolysis was reduced and the lactate to glucose yield was approximately 7.4% lower in DCA cultures (Figure 2). DCA increased peak VCD, culture duration, and final antibody titer. The largest effect was observed in fed batch plus glucose cultures in which culture duration was extended by 3 days and final antibody titer doubled.

Culture conditions can impact productivity and antibody quality (Li et al., 2012; Lao & Toth, 1997; Lim et al., 2010; Pacis et al., 2011). The cell-specific productivity during exponential growth was the same in control and DCA cultures with 8.80 ± 0.12 pg·cell·day. The differences seen between the two conditions regarding antibody

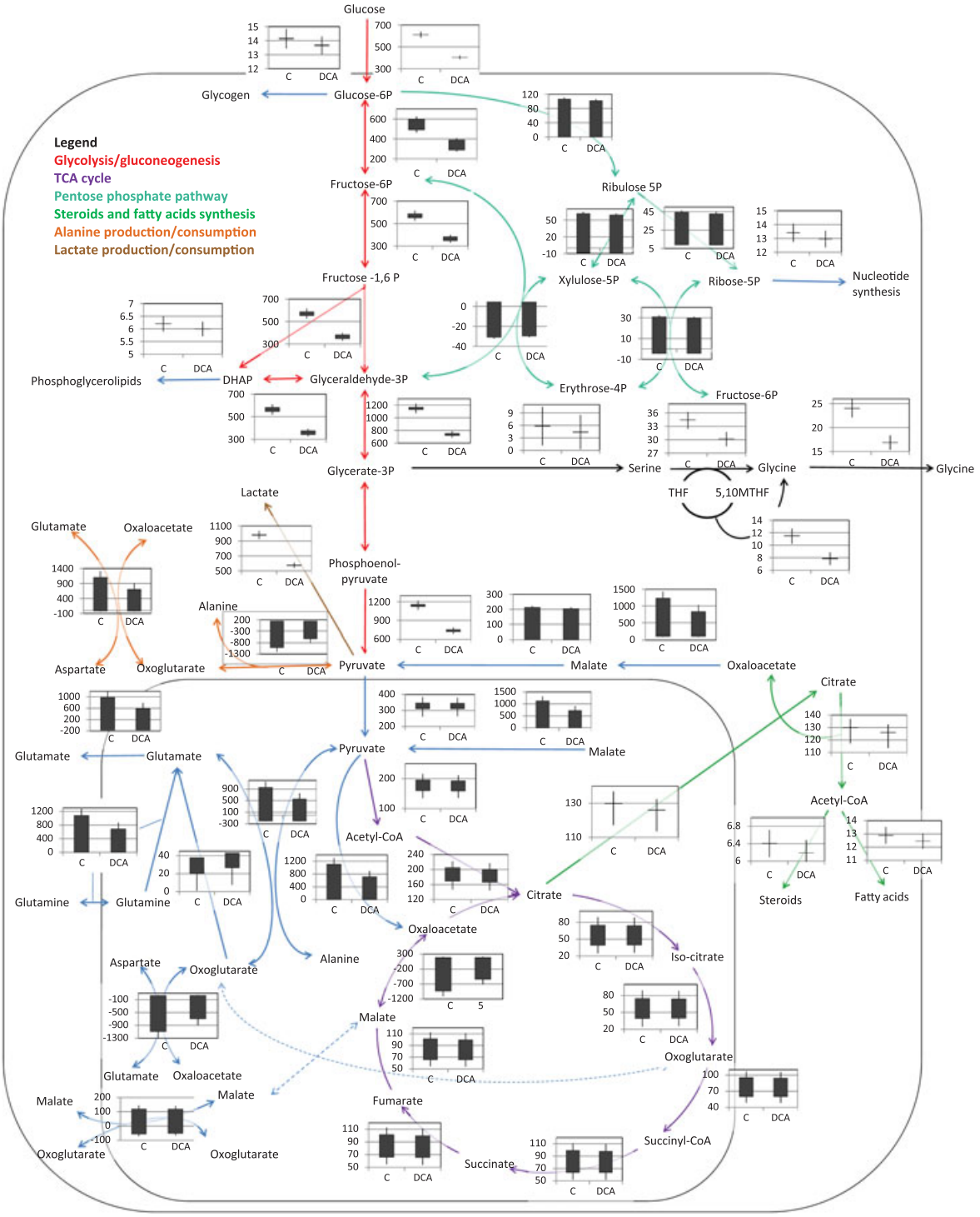

FIGURE 5 Metabolic flux analysis of CHO-XL99 cultures with 5 mM DCA and control cultures during exponential growth. Intracellular fluxes were estimated using metabolic flux analysis, as described in material and methods. Rates are shown as solid bars with 95% confidence intervals shown as vertical lines. Although glycolytic fluxes, fluxes through lactate dehydrogenase, and one carbon metabolism reaction were significantly lower in cultures with 5 mM DCA (DCA) compared with control cultures (C), fluxes through the TCA cycle, pentose phosphate pathway, and fatty acid and steroid production pathways were very similar. Rates are in μM·gDW·hr. 5,10MTHF = 5,10 methylenetetrahydrofolate; DCA, dichloroacetate; THF, tetrahydrofolate [Color figure can be viewed at wileyonlinelibrary.com]

quality were minor and within expected run-to-run variability (Table 3). We observed a small decrease in monomers, a slight reduction in basic charge variants and no change in glycan pattern (Table 3). In particular, no increase was observed in the Man5 fraction, a fraction that is readily cleared in the body (Goetze et al., 2011) and has previously been reported to increase under high-osmolality conditions (Pacis et al., 2011). Reduced aerobic glycolysis induced by DCA does not affect cell-specific productivity

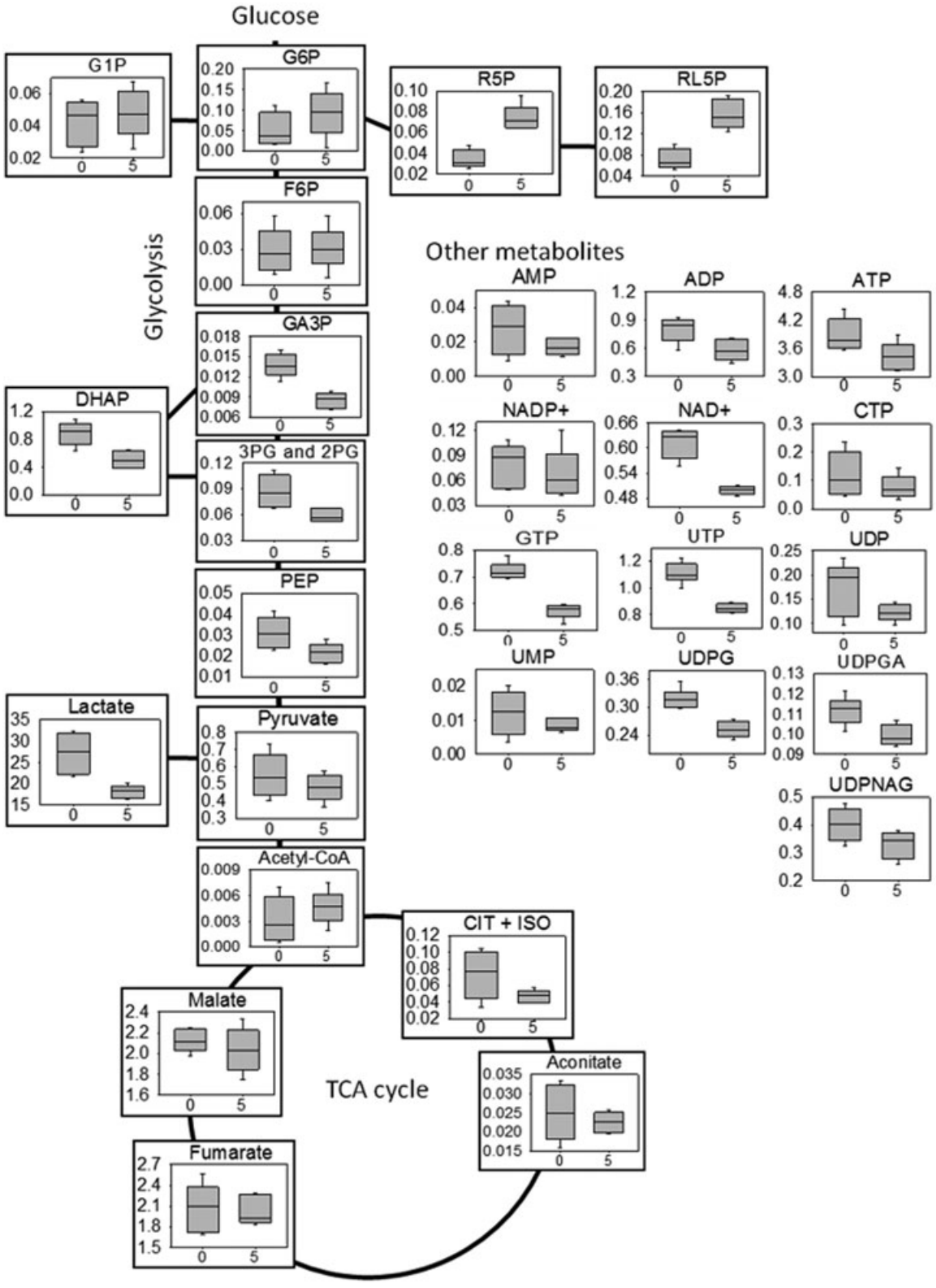

FIGURE 6 Comparison of intracellular metabolite concentrations. Concentrations are in femtomole per cell. $N = 5$ for DCA cultures, and $n = 6$ for control cultures. 5 = 5 mM DCA 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; C, control; CIT, citrate; DCA, dichloroacetate; DHAP, dihydroxyacetone phosphate; F6P, fructose 6-phosphate; G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; GA3P, glyceraldehyde 3-phosphate; PEP, phosphoenolpyruvate; PYR, pyruvate; R5P, ribose 5-phosphate; PL5P, ribulose 5-phosphate; UDPG, UDP-glucose; UDPGA, UDP-glucuronic acid; UDPNAG, UDP N-acetylglucosamine

or antibody quality in CHO-XL99 cells. Moreover, DCA has been used safely in the long-term treatment of congenital lactic acidosis (Abdelmalak et al., 2013); thus, minute carryovers from downstream purification would not be expected to have any adverse effect.

Excess lactate accumulation can be prevented by controlled feeding of glucose, but this requires significant cell-line-specific optimization of feed media and feedback control strategy. The patented high-end pH-controlled delivery of glucose (HiPDOG) feeding strategy illustrates that it is possible to control glucose feeding at scale (Gagnon et al., 2011), but more commonly excess lactate accumulation is avoided by switching CHO fed-batch cultures from growth to production phase by modulating pH and/or temperature (Oguchi, Saito, Tsukahara, & Tsumura, 2006) or by adding chemicals that induce arrest (Jiang & Sharfstein, 2008). DCA lowers lactate accumulation during the exponential phase and may be compatible with growth arrest strategies, thus enabling entry into the production phase at a higher cell density.

4.2 | Lactate inhibition results from increased osmolarity

Using fed batch, we demonstrated that the benefit of DCA is not merely to prevent depletion of glucose and amino acids. Indeed, the greatest improvement was observed in the fed batch plus glucose culture. In both DCA and control cultures, the reduction of growth rate was observed around 90 mM lactate concentration (Figure 3), which is higher than previously reported growth inhibitory lactate concentrations (Lao & Toth, 1997; Ozturk, Riley, & Palsson, 1992). Deliberately feeding sodium lactate, we confirmed that 90 mM lactate inhibits cell growth (Figure 4a). Sodium lactate was fed a rate (40 mM/day) just below the maximum rate of lactate production observed in the culture, to prevent stress responses related to the rate of change (Newland, Kamal, Greenfield, & Nielsen, 1994).

Growth inhibition may be a direct effect of lactate itself or a result of the increased osmolality due to the lactic acid as well as the addition of base to neutralize the produced lactic acid (Cruz et al., 2000; Irani et al., 1999; Langheinrich & Nienow, 1999). Feeding sodium lactate has the same effect on lactate and osmolality as cells producing lactic acid, which is neutralized by sodium hydroxide addition. Feeding NaCl, we can separate the two effects, and the Na^+ concentration is a surrogate measure for the culture osmolality. Deliberately feeding NaCl at 30 mM/day, growth inhibition commenced at approximately 180 mM Na^+ concentration with complete arrest around 220 mM Na^+ before onset of cell death (Figure 4b). These numbers are similar to those observed in the sodium lactate feeding experiment (Figure 4a), while the final lactate concentration was only 43.6 mM, less than half of that observed in sodium lactate feeding experiments. It appears that high osmolality rather than lactate per se is the cause of growth inhibition and arrest. As DCA reduces lactate production and associated base addition, it delays culture reaching growth inhibiting osmolality levels, and increased antibody titer is a direct result of the extended cultures.

4.3 | Warburg effect is posttranslationally mediated

DCA reduces the production of lactate under aerobic conditions and may therefore provide insights into the Warburg effect. SWATH proteomics revealed only few differentially expressed proteins (20 of 2016 identified proteins) and none in central carbon metabolism. This indicates that at 5 mM, where DCA does not affect growth, both DCA and follow-on effects are at a posttranslational level.

PFK is considered the major regulatory glycolytic enzyme (Schöneberg, Kloos, Brüser, Kirchberger, & Sträter, 2013), and our data are consistent with control at the PFK node. Intracellular concentrations of glycolytic intermediates before PFK were similar or slightly higher, whereas concentrations of glycolytic intermediates downstream of PFK were significantly lower in DCA treated cultures (Figure 6). Reduced PFK activity causes an accumulation of fructose 6-phosphate and glucose 6-phosphate, which inhibits hexokinase and reduces glucose assimilation.

Inhibition of PFK is normally attributed to high ATP and citrate levels, reducing PFK activity when cells contain sufficient storage of ATP and oxidizable substrates (Costa leite, Da silva, Guimarães coelho, Zancan, & Sola-Penna, 2007; Marinho-Carvalho, Costa-Mattos, Spitz, Zancan, & Sola-Penna, 2009; Zancan, Almeida, Faber-Barata, Dellias, & Sola-Penna, 2007; Zancan, Marinho-carvalho, Faber-barata, Dellias, & Sola-Penna, 2008). However, the ATP and citrate levels were lower under DCA conditions and there was no change in the adenylate energy charge (Figure 6). Arguably, control is very tight and metabolomics is not sensitive enough to pick up the subtle concentration changes required for control. However, even at the most basic level, the regulatory logic does not work; there is no evidence of increased PDC flux leading to more pyruvate through the TCA cycle (Figure 5) nor is there an increase in oxidative metabolism as measured by OUR (Table 4). The molecular link between de-repressed PDC and reduced glycolytic flux remains to be resolved.

The data does indicate that energy metabolism is inefficient under normal conditions. Without increasing oxidative metabolism, DCA caused a 35% reduction in glucose uptake, a 40% reduction in lactate production, a 30% reduction in glycine production, and an 18% reduction in ATP production, all without affecting adenylate energy charge, growth rate, or productivity. The energy requirements may be lower in DCA culture due to the lower lactate concentration, which may lower the maintenance energy requirements for maintaining ion gradients. It is possible that the impact of DCA on cell growth at concentrations greater than 5 mM (Figure 1) represents the limit of reduction in ATP production possible, given that aerobic metabolism apparently does not increase.

5 | CONCLUSIONS

Lactate metabolism is one of the most important characteristics of industrial mammalian cell culture and because of its adverse effects on cell culture performance and productivity, reducing lactate production is a major focus in cell line and process development.

Decreasing PDK activity using DCA is an easy and simple approach to increase PDC activity and thereby reduces aerobic glycolysis and decreases lactate production in an industrial relevant setting. Reduction in aerobic glycolysis had positive effects on cell culture performance and final antibody titer, whereas cell-specific productivity and antibody quality were unaffected. The increase in final antibody titer in DCA cultures was the result of higher cell density and longer cultivation periods. DCA extended the culture duration by reducing the lactate production rate; thereby, the growth inhibiting osmolality level was reached later in DCA cultures. In addition, the data demonstrate that the DCA concentration used here decreased glycolytic fluxes, whereas the TCA cycle activity was unaffected and DCA mainly acted on a posttranslational level.

ACKNOWLEDGMENTS

The authors acknowledge the Queensland node of Metabolomics Australia at The University of Queensland, an NCRIS initiative under Bioplatforms Australia Pty Ltd.

ORCID

Lars K. Nielsen http://orcid.org/0000-0001-8191-3511

REFERENCES

Abdelmalak, M., Lew, A., Ramezani, R., Shroads, A. L., Coats, B. S., Langaee, T., … Stacpoole, P. W. (2013). Long-term safety of dichloroacetate in congenital lactic acidosis. *Molecular Genetics and Metabolism*, *109*(2), 139–143.

Altamirano, C., Paredes, C., Cairo, J. J., & Godia, F. (2000). Improvement of CHO cell culture medium formulation: Simultaneous substitution of glucose and glutamine. *Biotechnology Progress*, *16*(1), 69–75.

Altamirano, C., Paredes, C., Illanes, A., Cairó, J. J., & Gòdia, F. (2004). Strategies for fed-batch cultivation of t-PA producing CHO cells: Substitution of glucose and glutamine and rational design of culture medium. *Journal of Biotechnology*, *110*(2), 171–179.

Atkinson, D. E., & Walton, G. M. (1967). Adenosine triphosphate conservation in metabolic regulation. Rat liver citrate cleavage enzyme. *Journal of Biological Chemistry*, *242*(13), 3239–3241.

Costa Leite, T., Da Silva, D., Guimarães Coelho, R., Zancan, P., & Sola-Penna, M. (2007). Lactate favours the dissociation of skeletal muscle 6-phosphofructo-1-kinase tetramers down-regulating the enzyme and muscle glycolysis. *Biochemical Journal*, *408*(1), 123–130.

Cruz, H. J., Freitas, C. M., Alves, P. M., Moreira, J. L., & Carrondo, M. J. T. (2000). Effects of ammonia and lactate on growth, metabolism, and productivity of BHK cells. *Enzyme and Microbial Technology*, *27*(1-2), 43–52.

DeBerardinis, R. J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S., & Thompson, C. B. (2007). Beyond aerobic glycolysis: Transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(49), 19345–19350.

Dietmair, S., Nielsen, L. K., & Timmins, N. E. (2011). Engineering a mammalian super producer. *Journal of Chemical Technology and Biotechnology*, *86*(7), 905–914.

Dietmair, S., Timmins, N. E., Gray, P. P., Nielsen, L. K., & Krömer, J. O. (2010). Towards quantitative metabolomics of mammalian cells: Development of a metabolite extraction protocol. *Analytical Biochemistry*, *404*(2), 155–164.

Dorai, H., Kyung, Y. S., Ellis, D., Kinney, C., Lin, C., Jan, D., … Betenbaugh, M. J. (2009). Expression of anti-apoptosis genes alters lactate metabolism of Chinese Hamster ovary cells in culture. *Biotechnology and Bioengineering*, *103*(3), 592–608.

Durocher, Y., & Butler, M. (2009). Expression systems for therapeutic glycoprotein production. *Current Opinion in Biotechnology*, *20*(6), 700–707.

Fogolín, M. B., Wagner, R., Etcheverrigaray, M., & Kratje, R. (2004). Impact of temperature reduction and expression of yeast pyruvate carboxylase on hGM-CSF-producing CHO cells. *Journal of Biotechnology*, *109*(1-2), 179–191.

Follstad B. D. (2002). Improvement of cell culture performance by inhibitors of pyruvate dehydrogenase kinase. WO2002004598A2.

Gagnon, M., Hiller, G., Luan, Y. T., Kittredge, A., Defelice, J., & Drapeau, D. (2011). High-end pH-controlled delivery of glucose effectively suppresses lactate accumulation in CHO fed-batch cultures. *Biotechnology and Bioengineering*, *108*(6), 1328–1337.

Gambhir, A., Korke, R., Lee, J., Fu, P. C., Europa, A., & Hu, W. S. (2003). Analysis of cellular metabolism of hybridoma cells at distinct physiological states. *Journal of Bioscience and Bioengineering*, *95*(4), 317–327.

Glacken, M. W., Fleischaker, R. J., & Sinskey, A. J. (1986). Reduction of waste product excretion via nutrient control: Possible strategies for maximizing product and cell yields on serum in cultures of mammalian cells. *Biotechnology and Bioengineering*, *28*(9), 1376–1389.

Goetze, A. M., Liu, Y. D., Zhang, Z., Shah, B., Lee, E., Bondarenko, P. V., & Flynn, G. C. (2011). High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans. *Glycobiology*, *21*(7), 949–959.

Hassell, T., Gleave, S., & Butler, M. (1991). Growth inhibition in animal cell culture. The effect of lactate and ammonia. *Applied Biochemistry and Biotechnology*, *30*(1), 29–41.

Irani, N., Wirth, M., Van Den Heuvel, J., & Wagner, R. (1999). Improvement of the primary metabolism of cell cultures by introducing a new cytoplasmic pyruvate carboxylase reaction. *Biotechnology and Bioengineering*, *66*(4), 238–246.

Jiang, Z., & Sharfstein, S. T. (2008). Sodium butyrate stimulates monoclonal antibody over-expression in CHO cells by improving gene accessibility. *Biotechnology and Bioengineering*, *100*(1), 189–194.

Kim, S. H., & Lee, G. M. (2007). Down-regulation of lactate dehydrogenase-A by siRNAs for reduced lactic acid formation of Chinese hamster ovary cells producing thrombopoietin. *Applied Microbiology and Biotechnology*, *74*(1), 152–159.

Kuwae, S., Ohda, T., Tamashima, H., Miki, H., & Kobayashi, K. (2005). Development of a fed-batch culture process for enhanced production of recombinant human antithrombin by Chinese hamster ovary cells. *Journal of Bioscience and Bioengineering*, *100*(5), 502–510.

Langheinrich, C., & Nienow, A. W. (1999). Control of pH in large-scale, free suspension animal cell bioreactors: Alkali addition and pH excursions. *Biotechnology and Bioengineering*, *66*(3), 171–179.

Lantum, H. B. M., Cornejo, J., Pierce, R. H., & Anders, M. W. (2003). Perturbation of maleylacetoacetic acid metabolism in rats with dichloroacetic acid-induced glutathione transferase zeta deficiency. *Toxicological Sciences*, *74*(1), 192–202.

Lao, M. S., & Toth, D. (1997). Effects of ammonium and lactate on growth and metabolism of a recombinant Chinese hamster ovary cell culture. *Biotechnology Progress*, *13*(5), 688–691.

Li, J., Wong, C. L., Vijayasankaran, N., Hudson, T., & Amanullah, A. (2012). Feeding lactate for CHO cell culture processes: Impact on culture metabolism and performance. *Biotechnology and Bioengineering*, *109*(5), 1173–1186.

Li, W. J., James, M. O., McKenzie, S. C., Calcutt, N. A., Liu, C., & Stacpoole, P. W. (2011). Mitochondrion as a novel site of dichloroacetate

biotransformation by glutathione transferase zeta 1. *Journal of Pharmacology and Experimental Therapeutics*, *336*(1), 87–94.

Lim, Y., Wong, N. S. C., Lee, Y. Y., Ku, S. C. Y., Wong, D. C. F., & Yap, M. G. S. (2010). Engineering mammalian cells in bioprocessing—Current achievements and future perspectives. *Biotechnology and Applied Biochemistry*, *55*, 175–189.

Marinho-Carvalho, M. M., Costa-Mattos, P. V., Spitz, G. A., Zancan, P., & Sola-Penna, M. (2009). Calmodulin upregulates skeletal muscle 6-phosphofructo-1-kinase reversing the inhibitory effects of allosteric modulators. *Biochimica Et Biophysica Acta*, *1794*(8), 1175–1180.

Martínez, V. S., Dietmair, S., Quek, L. E., Hodson, M. P., Gray, P., & Nielsen, L. K. (2013). Flux balance analysis of CHO cells before and after a metabolic switch from lactate production to consumption. *Biotechnology and Bioengineering*, *110*(2), 660–666.

Murray, K., Gull, K., & Dickson, A. J. (1996). Dichloroacetate increases cell and antibody yields in batch cultures of a hybridoma cell line. *Biotechnology and Bioengineering*, *49*(4), 377–382.

Newland, M., Kamal, M. N., Greenfield, P. F., & Nielsen, L. K. (1994). Communication to the editor ammonia inhibition of hybridomas propagated in batch, fed-batch, and continuous culture. *Biotechnology and Bioengineering*, *43*(5), 434–438.

Oguchi, S., Saito, H., Tsukahara, M., & Tsumura, H. (2006). pH Condition in temperature shift cultivation enhances cell longevity and specific hMab productivity in CHO culture. *Cytotechnology*, *52*(3), 199–207.

Orellana, C. A., Marcellin, E., Schulz, B. L., Nouwens, A. S., Gray, P. P., & Nielsen, L. K. (2015). High-antibody-producing Chinese hamster ovary cells up-regulate intracellular protein transport and glutathione synthesis. *Journal of Proteome Research*, *14*(2), 609–618.

Ozturk, S. S., Riley, M. R., & Palsson, B. O. (1992). Effects of ammonia and lactate on hybridoma growth, metabolism, and antibody production. *Biotechnology and Bioengineering*, *39*(4), 418–431.

Pacis, E., Yu, M., Autsen, J., Bayer, R., & Li, F. (2011). Effects of cell culture conditions on antibody N-linked glycosylation-what affects high mannose 5 glycoform. *Biotechnology and Bioengineering*, *108*(10), 2348–2358.

Quek, L. E., Dietmair, S., Hanscho, M., Martínez, V. S., Borth, N., & Nielsen, L. K. (2014). Reducing Recon 2 for steady-state flux analysis of HEK cell culture. *Journal of Biotechnology*, *184*, 172–178.

Quek, L. E., Dietmair, S., Krömer, J. O., & Nielsen, L. K. (2010). Metabolic flux analysis in mammalian cell culture. *Metabolic Engineering*, *12*(2), 161–171.

Quek, L. E., & Nielsen, L. K. (2008). On the reconstruction of the *Mus musculus* genome-scale metabolic network model. *Genome Informatics*, *21*, 89–100.

Schöneberg, T., Kloos, M., Brüser, A., Kirchberger, J., & Sträter, N. (2013). Structure and allosteric regulation of eukaryotic 6-phosphofructokinases. *Biological Chemistry*, *394*(8), 977–993.

Singh, V. (1996). On-line measurement of oxygen uptake in cell culture using the dynamic method. *Biotechnology and Bioengineering*, *52*(3), 443–448.

Smyth, G. K. (Ed.), *Limma: Linear models for microarray data* 2005. New York, NY: Springer.

Stacpoole, P. W., Henderson, G. N., Yan, Z., Cornett, R., & James, M. O. (1998). Pharmacokinetics, metabolism, and toxicology of dichloroacetate. *Drug Metabolism Reviews*, *30*(3), 499–539.

Warburg, O. (1956). On the origin of cancer cells. *Science*, *123*(3191), 309–314.

Warburg, O., Wind, F., & Negelein, E. (1927). The metabolism of tumors in the body. *Journal of General Physiology*, *8*(6), 519–530.

Whitehouse, S., Cooper, R. H., & Randle, P. J. (1974). Mechanism of activation of pyruvate dehydrogenase by dichloroacetate and other halogenated carboxylic acids. *Biochemical Journal*, *141*(3), 761–774.

Wlaschin, K. F., & Hu, W. S. (2007). Engineering cell metabolism for high-density cell culture via manipulation of sugar transport. *Journal of Biotechnology*, *131*(2), 168–176.

Wurm, F. M. (2004). Production of recombinant protein therapeutics in cultivated mammalian cells. *Nature Biotechnology*, *22*(11), 1393–1398.

Yeaman, S. J., Hutcheson, E. T., Roche, T. E., Pettit, F. H., Brown, J. R., Reed, L. J., … Dixon, G. H. (1978). Sites of phosphorylation on pyruvate-dehydrogenase from bovine kidney and heart. *Biochemistry*, *17*(12), 2364–2370.

Yip, S. S. M., Zhou, M., Joly, J., Snedecor, B., Shen, A., & Crawford, Y. (2014). Complete knockout of the lactate dehydrogenase A gene is lethal in pyruvate dehydrogenase kinase 1, 2, 3 down-regulated CHO cells. *Molecular Biotechnology*, *56*(9), 833–838.

Zancan, P., Almeida, F. V. R., Faber-Barata, J., Dellias, J. M., & Sola-Penna, M. (2007). Fructose-2,6-bisphosphate counteracts guanidinium chloride-, thermal-, and ATP-induced dissociation of skeletal muscle key glycolytic enzyme 6-phosphofructo-1-kinase: A structural mechanism for PFK allosteric regulation. *Archives of Biochemistry and Biophysics*, *467*(2), 275–282.

Zancan, P., Marinho-Carvalho, M. M., Faber-Barata, J., Dellias, J. M. M., & Sola-Penna, M. (2008). ATP and fructose-2,6-bisphosphate regulate skeletal muscle 6-phosphofructo-1-kinase by altering its quaternary structure. *IUBMB Life*, *60*(8), 526–533.

Zhou, M., Crawford, Y., Ng, D., Tung, J., Pynn, A. F. J., Meier, A., … Shen, A. (2011). Decreasing lactate level and increasing antibody production in Chinese hamster ovary cells (CHO) by reducing the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases. *Journal of Biotechnology*, *153*(1–2), 27–34.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Buchsteiner M, Quek L-E, Gray P, Nielsen LK. Improving culture performance and antibody production in CHO cell culture processes by reducing the Warburg effect. *Biotechnology and Bioengineering*. 2018;115:2315–2327. https://doi.org/10.1002/bit.26724