Acta vet. scand. 1990, *31*, 137-143.

Adenosine Deaminase and Porcine Meat Quality

I. Effect of Dipyridamole on Plasma Free Fatty Acids, Glucose, Lactate and c-AMP in Pigs Representing High and Low Red Cell Adenosine Deaminase Activity

By *J. F. Hyldgaard-Jensen*

Department of Anatomy and Physiology, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Hyldgaard-Jensen, J.F.: Adenosine deaminase and porcine meat quality. I. Effect of dipyridamole on plasma free fatty acids, glucose, lactate and c-AMP in pigs representing high and low red cell adenosine deaminase activity. Acta vet. scand. 1990, 31, 137-143. - The effect of dipyridamole - an adenosine uptake inhibitor - on the plasma concentration of free fatty acids (FFA), glucose, lactate and cyclic adenosine monophosphate (cAMP) has been examined in 2 groups of Landrace pigs representing low (Ada 0) and high (Ada A) red cell adenosine deaminase (Ada) activity. Pigs fitted with a jugular vein catheter were given dipyridamole (0.16 mg/kg/min) over a period of 30 min. The infusions were performed 22 h after the last meal at a time where pigs were found to show steady increase and decline in rates of lipolysis and glycogenolysis, respectively. The results showed that lipid mobilization as identified by the plasma FFA concentration was markedly depressed. During the infusion of dipyridamole similar degree of inhibition was seen in Ada 0 and Ada A pigs, however, in the period following the infusion, a significantly stronger suppression persisted in the Ada 0 pigs. Both the blood glucose and lactate level rose distinctly as a result of the dipyridamole treatment. This stimulation of the glycolysis rate was significantly more expressed in Ada 0 pigs compared to that of the Ada A pigs. When theophylline, an antagonist of adenosine, was given together with dipyridamole, the rise in the lactate level was considerably diminished. Dipyridamole also produced a distinct rise in the plasma cAMP levels.

lipolysis; glycolysis; pigs.

Introduction

An association of the polymorphic enzyme system adenosine deaminase (Ada) with porcine meat quality has previously been established (*Hyldgaard-Jensen et al.* 1982, *Vögeli et al.* 1982). Thus pigs lacking red cell Ada activity (Ada 0 type pigs) are found to be particularly prone to develop inferior meat quality notably of the type called DFD (dark, firm, dry) meat. Whereas the more common type of inferior meat quality - PSE (pale, soft, exudative) is characterized by a low ultimate pH, DFD is typically showing a high ultimate pH and being mainly confined to red muscles. Biochemically the dominating feature of DFD muscles is a severe ante mortem glycogen depletion as a result of a stressful preslaughter treatment thus explaining the high ultimate pH observed post mortem. Although PSE and DFD are problems encountered post mortem their development is clearly related to an abnormal physiology and stress susceptibility of the live animal (c.f. *Eikelenboom* 1985). Genetic liability for PSE and DFD is usually explained by the presence of a single locus (Hal) si-

tuated within a linkage group of blood type loci (c.f. *Webb et al.* 1985).

However, as a close linkage between the Ada gene and aforementioned genes can be excluded (*Hyldgaard-Jensen et al.* 1982), it is necessary to look for a different and perhaps more direct mechanism to explain the Ada effect on meat quality. Accordingly a theory involving a direct effect of red cell Ada activity through its naturally occurring substrate-adenosine- on porcine muscle metabolism was advanced. The theory is based on 3 major observations, firstly a lowered ability to mobilize free fatty acids (FFA) as energy source was recently linked to the occurrence of DFD meat in pigs and sheep (*Spencer et al.* 1983, *Lister & Spencer* 1983). Secondly adenosine is proven to be a potent inhibitor of lipolysis in vitro as well as in vivo (c.f. *Fredholm* 1981) and thirdly in pigs lacking red cell Ada activity the catabolic rate of adenosine was shown to be delayed (*Hyldgaard-Jensen* 1983).

As it has been proposed that reliance on muscle glycogen results when FFA are not so readily available from fat, attempts were done to show whether a potentiated effect of endogenously generated adenosine might have an effect on glucose and fat mobilisation as identified by plasma FFA, glucose, lactate and c-AMP concentration and whether this is differently expressed in fasted pigs representing high (Ada A) and low (Ada 0) red cell Ada activity.

Materials and methods

Experiments were performed on 10 catheterized (jugular vein) Landrace pigs representing high (Ada A) and low (Ada 0) red cell Ada activity. The pigs originating from 2 litters were fed twice daily using a 16% protein pig ration. At 50-60 kg body weight pigs were subjected to the following treatments:

1. Diurnal variation of the blood levels of FFA, glucose and lactate: pigs were fed 08.30 and 15.00 h and thereafter fasted until 15.00 h the following day.
2. Dipyridamole to fasted pigs: following a fasting period of 22 h pigs received an infusion of 0.3 g dipyridamole (Sigma) dissolved in 60 ml sterile saline. Infusion was done over a period of 30 min. The experiment was repeated in pigs receiving a bolus injection of 0.6 g theophylline (Sigma) dissolved in 20 ml sterile saline before the dipyridamole infusion.

Blood samples were taken prior to treatment and at short intervals up to 70 min after the infusion. A 3% sodium citrate solution was used to flush the catheter as heparin strongly interferes with the FFA results. Blood was centrifuged within 1-2 h after its collection and plasma stored at -20°C until analysis were performed.

Analytical methods

FFA determination was done on EDTA plasma using the Wako - NEFA C test kit (Wako Chemicals GmbH). This method proved to be both simple and accurate. The high cost was minimized by reducing the size of the reaction volume.

Blood glucose and lactate were determined enzymatically using the Sigma kits No. 16-UV and No. 826-UV, respectively. Plasma c-AMP estimations were carried out using a commercially available kit (Trk 432, Amersham).

Results

Diurnal variation of plasma FFA, glucose and lactate

The variation in plasma FFA, glucose and lactate concentration during a period of 30 h in which pigs were fed twice (08.00 and 15.00 h) is shown in Fig. 1. Since the changes in plasma FFA, glucose and lactate level did not differ between the 2 groups of pigs (Ada

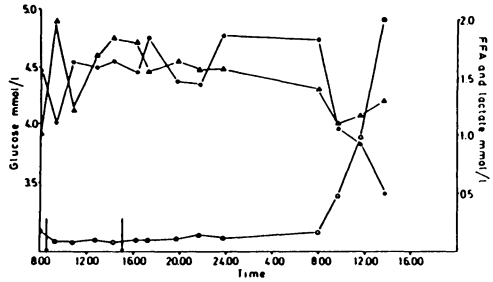

Figure 1. The mean plasma content of FFA (○-○), glucose (•-•) and lactate (△-△) in catheterized pigs fed twice (see arrows) within a period of 30 h.

A and Ada 0), the curves in Fig. 1 represent the mean of all pigs. As may be seen the plasma FFA level remained low and fairly stable during the day (0.1-0.2 mmol/l). During the night the FFA level showed a steady increase to a level between 0.2-0.4 mmol/l. When the fasting period was extended until the afternoon, the FFA level rose steeply to a niveau about 1.5-2.0 mmol/l. Coinciding with the marked increase in plasma FFA, the glucose level showed a marked and steady fall. The blood lactate level did not follow the same definite pattern seen for plasma FFA and glucose, but remained fairly stable throughout the sampling period. The relatively small and irregular fluctuations observed could not be related to the nutritional status of the pigs.

Effect of dipyridamole on plasma FFA, glucose, lactate and c-AMP

Dipyridamole administration produced a marked suppression of the FFA concentration (Fig. 2). The lowest levels were found at the end of the infusion period, thereafter the FFA content gradually increased to pretreatment values over the following 40-50 min. The rate of this increase was significantly slower in Ada 0 Pigs. As seen from Fig. 2 dipyridamole infusion produced a moderate hyperglycaemia of similar magnitude in Ada

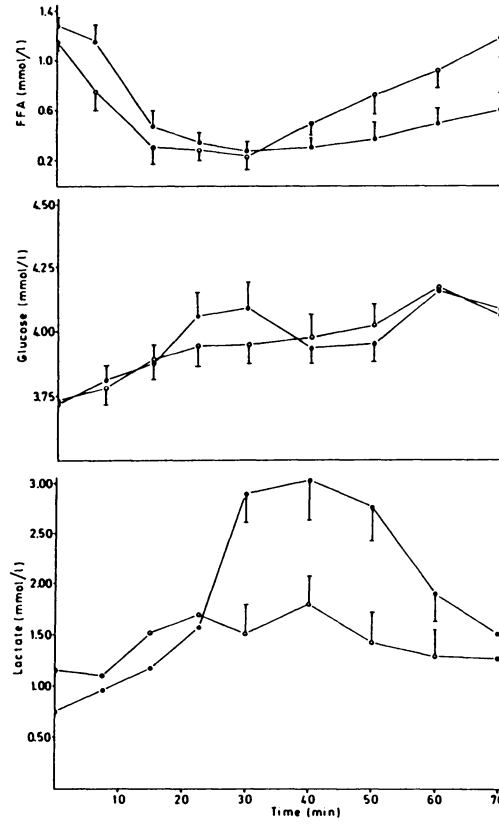

Figure 2. The mean plasma FFA, glucose and lactate levels in Ada A (○-○) and Ada 0 (•-•) pigs following an infusion of dipyridamole (0.16 mg/kg/min) over a period of 30 min.

A and Ada 0 type pigs and also affected the blood lactate level in that it produced a distinct rise in this level. The increase was significantly higher in Ada 0 pigs than in the Ada A pigs. When the dipyridamole infusion was preceeded by an infusion of theophylline, the rise in blood lactate was significantly less pronounced (Fig. 3).

The dipyridamole infusion produced a distinct elevation of the plasma c-AMP level (Fig. 4). Peak levels were obtained at the end of the infusion period and preceeded the peak levels of blood lactate.

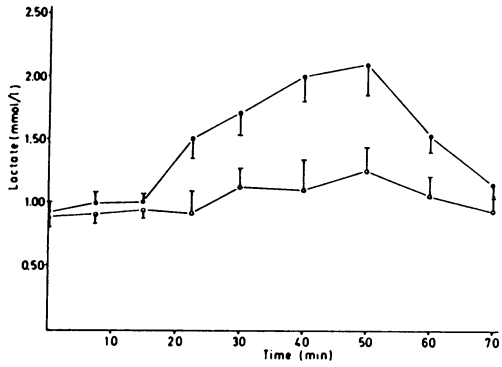

Figure 3. The mean plasma lactate levels (•-•) in Ada 0 pigs infused with dipyridamole (0.16 mg/kg-/min.) over a period of 30 min. Results are compared with an experiment, where a preinfusion of 0.6 g theophylline (○-○) was given 10 min. before the administration of dipyridamole.

Although the trend of curves obtained for Ada A and Ada 0 pigs were quite similar, significantly higher c-AMP levels were reached in Ada 0 pigs during the infusion of dipyridamole.

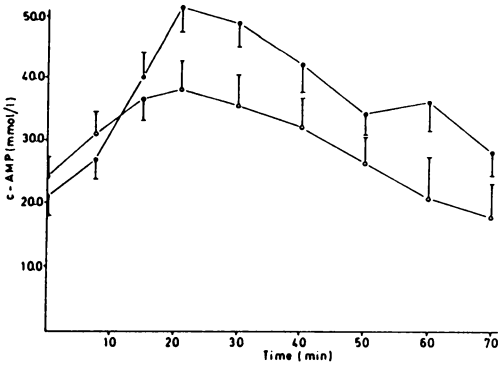

Figure 4. The mean plasma c-AMP content in Ada A (○-○) and Ada 0 (•-•) pigs following an infusion of dipyridamole (0.16 mg/kg.min.) over a period of 30 min.

Discussion

The results of this study show that dipyridamole, an adenosine uptake inhibitor, markedly supresses fasting-induced lipolysis in pig. While the degree of suppression was similar in Ada A and Ada 0 pigs during the infusion of dipyridamole, the return to preinfusion levels of FFA was found to be significantly delayed in the latter mentioned group of pigs. Dipyridamole in therapeutic plasma concentrations has been found to inhibit the uptake of adenosine into various types of cells such as blood cells (*Schrader et al.* 1972, *Newby* 1986) and furthermore to elevate the endogenous venous plasma level of adenosin in man and pig (*Sollevi* 1986, *Hyldgaard-Jensen* 1984). Although adenosine was not determined in this study, it is very likely that an elevated adenosine concentration remained for a longer time in Ada 0 pigs, thus explaining the prolonged antilipolytic effect seen in these pigs. The antilipolytic effect induced by dipyridamole is therefore likely to have been caused by a potentiating effect of adenosine. An antilipolytic effect for adenosine is well established both in vitro using animal fat cells (cf. *Fredholm* 1981) and in vivo using rats (*Hoffman et al.* 1986) and pigs (*Hyldgaard-Jensen* 1986). From these results and the previously found lower catabolic rate of exogenously administered adenosine in Ada 0 pigs (*Hyldgaard-Jensen* 1984) it could well be that this type of pig has a lower ability to mobilize fat and to a higher extent than Ada A pigs must rely on muscle glycogen as energy source during stressful situations such as transport and preslaughter treatment of pigs.

This notion is supported by the additionel findings in this study that dipyridamole evidently has a stimulatory effect on glycolysis as identified by the rise in the blood lactate concentration. The stimulation was distinctly more expressed in Ada 0 pigs than in Ada A pigs, which suggest that the dipyridamole effect also in this case was mediated through an increased action of adenosine. Support for this notion may be taken from the fact

that theophylline- an adenosine antagonist- significantly counteracted the glycolytic effect of dipyridamole. It is well known that a decrease in ATP or an increase in AMP are potent stimulators of glycolysis. No account for changes in these nucleotides was made in the present study, however a distinct rise in plasma c-AMP was observed as a result of the dipyridamole infusion. This increase in plasma c-AMP reflecting an intracellular increased synthesis or decreased catabolism might well explain the enchanced rate of glycolysis. If the increased c-AMP is responsible for the increased glycolysis rate, the question arises, what mechanism(s) causes the c-AMP level to rise. Apart from being an uptake inhibitor of adenosine, dipyridamole is also a phosphodiesterase inhibitor (*Gresele et al.* 1986) and this activity, although weak, can explain at least a part of the increased c-AMP level. However, the fact that theophylline an adenosine receptor antagonist was found to abolish the increase in lactate and that this increase was distinctly more marked in Ada 0 pigs both suggest that the principal mediator of the activity of dipyridamole is adenosine.

Since dipyridamole is a potent inhibitor of cellular uptake of adenosine the continuously produced adenosine accumulates and reaches concentrations high enough to affect adenylate cyclase hence the concentration of c-AMP via its specific receptors (*Wolff et al.* 1981). It is now well established that adenosine acts on various physiologic processes through two types of receptors A_1 and A_2 located on the extracellular surface of adenosine's target cells (*Daly* 1985). A_1 receptors that are particularly found in fat, brain cells and heart has a high affinity for adenosine and is inhibitory to adenylate cyclase. A_2 receptors are more ubiquitous since they are found in brain, cardiac muscle, gastrointestinal smooth muscle, presynaptic nerve terminals and liver. A_2 receptors have a lower affinity for adenosine and is stimulatory to adenylate cyclase. Methylxanthines like theophylline and caffeine are competitive inhibitors of adenosine at its A_1 and A_2 receptors.

Based on these informations it seems plausible that adenosine in the present study was responsible for the increased level of c-AMP via receptors that stimulate adenylate cyclase i.e. A_2 receptors. This notion is supported by some recent in vitro observations demonstrating that adenosine accelerates glycogenolysis in brain (*Laborit & Bonifacj* 1984, *Magistretti et al.* 1986) and liver cells (*Hoffer & Lowenstein* 1986, *Buc et al.* 1986) via A_2 receptors. As yet the kind of adenosine receptor located on skeletal muscle cells is unknown, however the presence of A_2 receptors in other muscle types like heart and smooth muscle makes it plausible that this receptor type also is present in skeletal muscles. In this case an increase in extracellular adenosine known to occur during skeletal muscle contraction particularly under restricted blood flow or hypoxic conditions (cf. *Klabunde* 1986) might promote adenylate cyclase hence produce an increase of the c-AMP concentration. The latter event will accelerate glycogen breakdown and increase the production of lactate. The mechanism by which dipyridamole alters carbohydrate metabolism is unknown. Recent studies on isolated soleus muscle of the rat show that dipyridamole may inhibit the transport of glucose (*Lozeman et al.* 1987). Whether this effect explains the presently found hyperglycaemia is not possible to say, however, such inhibition of glucose transport might in addition to the found stimulated glycolysis rate seriously limit energy generation in the cells.

In conclusion the data presented show that dipyridamole inhibits lipolysis and stimulate glycolysis and glycogenolysis when given in-

to the circulation of live pigs. The role of dipyridamole as a metabolic modulator appear to be mediated by a potentiating effect of endogenously adenosine. Of particular interest was to find that the stimulation of glycolysis was significantly greater in Ada 0 pigs than in Ada A pigs. Thus lack of red cell Ada activity and delayed catabolism of adenosine both characteristic features of the Ada 0 pig may be linked to a higher reliance on muscle glycogen as energy source. If in addition to that dipyridamole and hence adenosine also interferes with the glucose transport and utilization further studies in this line could be an important step towards a better understanding of the association found between Ada, adenosine and meat quality in pigs.

Acknowledgements

This study was supported by the Danish Agricultural and Veterinary Research Council and the Leo Research Foundation.

References

Buc HA, Demaugre F, Cépanec C, Moncion A, Leroux J-P: The effect of adenosine and chloroadenosine on sex differences in the energy metabolism of rat hepatocytes. Biochim. biophys. Acta 1986, *887,* 222-228.

Daly JW: In: D.M.F. Cooper & K. B. Schman (eds.). Advances in cyclic mucleotide and protein phosphorylation research. Raven Press 1985, vol 19, pp. 29-46.

Eikelenboom G: Ways to improve mest quality. In: J. B. Ludvigsen (ed.) Stress Susceptibility and Meat Quality in Pigs. EAAP Publication No. 33. Jelling Press, Denmark 1985, pp. 68-79.

Fredholm BB: Adenosine and lipolysis. Int. J. Obesity 1981, *5,* 643-649.

Gresele P, Arnont J, Deckmyn H, Vermylen J: Mechanism of the antiplatelet action of dipyridamole in whole blood: Modulation of adenosine concentration and activity. Tromb. Hemostas, 1986, *55,* 12-18.

Hoffer LJ, Lowenstein JM: Effects of adenosine and adenosine analogues on glycogen metabolism in isolated rat hepatocytes. Biochem. Pharmac. 1986, *35,* 4529-4536.

Hoffman BB, Dall'Aglio E, Hollenbeck C, Chang H, Reaven GM: Suppression of free fatty acids and triglycerides in normal hypertriglyceridemic rats by the adenosine receptor agonist phenylisopropyladenosine. J. Pharmacol exp. Ther. 1986, *239,* 715-718.

Hyldgaard-Jensen J: Adenosin deaminase og malign hyperthermi hos grise (Adenosine deaminase and malignant hyperthermia in pigs). Årsberetn. Inst. Sterilitetsforsk. (Copenhagen) 1983, *26,* 85-97.

Hyldgaard-Jensen J: Effekt af dipyridamol på blodets adenosinindhold hos malign hyperthermi grise repræsenterende forskellige adenosin deaminase (Ada) typer (Effect of dipyridamole on the blood adenosin content in malignant hyperthermia (MH) pigs representing different adenosine deaminase (Ada) types). Årsberetn. Inst. Sterilitetsforsk. (Copenhagen) 1984, *27,* 83-96.

Hyldgaard-Jensen J: Adenosine deaminase and meat quality in pigs. Role of an antilipolytic action of adenosine. Isr. J. vet. Med. 1986, *42,* 278-286.

Hyldgaard-Jensen J: Adenosine deaminase and meat quality in pigs. Animal Genetics 1987, *18,* (Suppl. 1), 107-109 (Abstr.).

Hyldgaard-Jensen J, Fogd Jørgensen P, Bräuner Nielsen P: Adenosin deaminasens mulige betydning for svins kødkvalitet (Possible role of adenosine deaminase (Ada) for porcine meat quality). Årsberetn. Inst. Sterilitetsforsk. 1982, *25,* 42-52.

Klabunde RE: Conditions for dipyridamole potentiation of skeletal muscle active hyperemia. Amer. J. Physiol. 1986, *250,* H 62-67.

Laborit H, Bonifacj JF: Action of adenosine on energy metabolism and on glucose-6-phosphate dehydrogenase in rat brains. Res. Com. Chem. Path. & Pharmac. 1984, *44,* 123-130.

Lister D, Spencer GSG: Energy substrate provision in vivo and the changes in muscle pH post mortem. Meat Science 1983, *8,* 41-51.

Lozeman FJ, Challiss RAJ, Leighton B, Newsholme EA: Effects of dipyridamole on adenosine concentration, insulin sensitivity and glucose utilization in soleus muscle of the rat. Pflügers Arch. 1987, *410,* 192-197.

Magistretti PJ, Hof PR, Martin J-L: Adenosine stimulates glycogenolysis in mouse cerebral cortex: A possible coupling mechanism between neuronal activity and energy metabolism. J. Neuroscience 1986, *6,* 2558-2562.

Newby AC: How does dipyridamole elevate extracellular adenosine concentration? Biochem. J. 1986, *237,* 845-851.

Schrader JR, Berne RM, Rubio R: Uptake and metabolism of adenosine by human erythrocyte ghosts. Amer. J. Physiol. 1972, *223,* 159-166.

Sollevi A: Cardiovascular effects of adenosine in man, possible clinical implications. Progress in Neurobiology 1986, *27,* 319-349.

Spencer GSG, Wilkins L, Lister D: Caffeine stimulated lipolysis protects pigs against catecholamine induced dark cutting. Meat Science 1983, *8,* 53-64.

Webb AJ, Southwood OI, Simpson SP, Carden AE: Genetics of porcine stress syndrome. In: J. B. Ludvigsen (ed.) Stress Susceptibility and Meat Quality in Pigs. EAAP Publication No. 33, Jelling Press, Denmark 1985 pp. 9-30.

Wolff J, Londos C, Cooper DMF: Adenosine receptors and the regulation of adenylate cyclase. Adv. Cyclic Nucleotide Res. 1981, *14,* 199-214.

Vögeli P, Gervig C, Schneebeli H, Wäfler P: Genfrequenzen wichtiger polymorpher Systeme des Veredelten Landschweines bei Indexelektion in positiver und negativer Richtung (Gene frequencies of important polymorphic systems of two divergens lines of Landrace pigs using index selection procedures). Schw. Landw. Monatsh. 1982, *516,* 234-240.

Sammenfatning

Adenosin deaminase og kødkvalitet hos svin: Effekt af adenosin på plasma frie fede syrer, glucose, laktat og c-AMP hos grise repræsenterende høj og lav erytrocyt adenosin deaminase aktivitet.

Som led i at forklare den fundne sammenhæng mellem det polymorfe enzymsystem adenosin deaminase (Ada) og kødkvaliteten hos svin er en direkte effekt af erytrocyt Ada aktiviteten og adenosin på skeletmuskelstofskiftet søgt underbygget. Landrace grise repræsenterende høj (Ada A) og lav (Ada 0) erytrocyt Ada aktivitet er via et kateter indlagt i V. jugularis tilført dipyridamol (0,16 mg/kg/min) over en periode på 30 min. Infusionerne blev foretaget på et tidspunkt, hvor dyrene som følge af faste udviste en stigende og faldende hastighed af henholdsvis lipolysen og glykogenolysen. Resultaterne af dipyridamoltilførslen viste, at fedtmobiliseringen udtrykt ved plasmaets FFA indhold blev markant hæmmet. Hæmningen varede signifikant længere hos Ada 0 grisene. Samtidig med faldet i plasma FFA sås en distinkt stigning i såvel glucose- og laktatindholdet. Stigningen i blodets laktatindhold og dermed i glykolysehastigheden var signifikant mere udtalt hos Ada 0 grisene. Ved at tilføre theophyllin - en antagonist til adenosin - sammen med dipyridamol sås en markant mindre stigning i laktatindholdet. Dipyridamol fremkaldte også en markant stigning i plasmaets cAMP indhold.

De opnåede resultater støtter antagelsen, at dipyridamol via en forstærket effekt af endogen dannet adenosin hæmmer lipolysen og stimulerer glycolysen hos grise. Den stærkere metaboliske effekt af adenosin påvist hos Ada 0 grise er måske en del af forklaringen på, hvorfor denne type gris er særlig tilbøjelig til at udvikle dårlig kødkvalitet.

(Received January 6, 1989; accepted July 7, 1989).

Reprints may be requested from: J. F. Hyldgaard-Jensen, Department of Anatomy and Physiology, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1870 Frederiksberg C, Denmark.