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MICHELLE RENEE' SMIRICKY

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CHARACTERIZATION OF THE CHANGES IN ANTIOXIDANT ENZYME ACTIVITY PROFILES IN GROWING SWINE

By

Michelle Renee' Smiricky

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ABSTRACT

CHARACTERIZATION OF THE CHANGES IN ANTIOXIDANT ENZYME ACTIVITY PROFILES IN GROWING SWINE

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Two experiments were conducted to evaluate the effects of common production methods on the changes in the antioxidant enzymes, Cu/Zn superoxide dismutase (CZSOD), Se-dependent glutathione peroxidase (GPX), catalase (CAT), and ceruloplasmin (CP). In Exp. 1 (n=16, d 2 of age, 1.6 kg BW), antioxidant enzymes were evaluated prior to iron injection, 12h, 24h, 36h, and 48h after injection; prior to weaning, 3h, 12h, 24h, and 48h after weaning; prior to moving and comingling, 6h, 24h, 48h after moving. In Exp. 2 (n=12, d 21 of age, 10.5 kg BW), antioxidant enzymes were evaluated prior to weaning, 3h, 6h, 12h, 18h, 24h, 36h, 48h, 60h, and 72h after weaning.

Iron administration did not increase CZSOD activity, increased GPX activity, did not increase CAT activity and increased CP activity. Weaning (Exp. 1) increased CZSOD and CAT activity and decreased GPX and CP activity. Weaning (Exp. 2) decreased then increased CZSOD activity. GPX activity decreased, increased then decreased post weaning (Exp. 2). Weaning (Exp. 2) did not change CAT and CP activities. Moving and comingling increased CZSOD and CAT activities, decreased GPX activity, and did not change CP activity until an increase 48 h post move. In summary, the antioxidant enzyme profiles change during different sources of production stress and these changes must be further researched to better ascertain how these enzymes respond to production stress.

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INTRODUCTION

Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's ability to dispose of them (Miller and Brzezinska-Slebodzinska, 1993). A free radical is any atom or molecule with an unpaired electron that is capable of existing independently in the body (Halliwell, 1987; Halliwell et al., 1995). There has been interest in the oxygen-centered radicals, such as the superoxide and hydroxyl radicals because of the necessity and abundance of oxygen, its relationship in metabolism, and its toxic effects on cells.

Free radicals can be produced both inadvertently and deliberately. Inadvertently, free radicals can result from electron transfer to molecular oxygen from metabolic processes (Halliwell et al., 1995). These processes include the electron transport chain, beta-oxidation, glucose metabolism, and by the activity of oxidative enzymes, such as xanthine oxidase, cytochrome P450 oxidase, and others (Thomas, 1995). Free radicals also result from the autoxidation of hormones, such as catecholamines (Halliwell et al., 1995). Deliberately, free radicals are produced by the process of phagocytosis (Thomas, 1995). Phagocytic cells produce the superoxide radical in the process of killing invading organisms.

The body has a major defense mechanism to stop the proliferation of the damaging free radicals: antioxidant enzymes and nonenzymatic antioxidants. Enzymatic antioxidants function to convert toxic radicals to less toxic species. Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical to hydrogen peroxide and oxygen (Bannister and Bannister, 1987). Hydrogen peroxide has the

capability to react with free transition metals, such as iron and copper, then further free radical propagation is a result. Therefore, glutathione peroxidase (GPX) catalyzes the decomposition of hydrogen peroxide to water and oxygen (Bannister and Bannister, 1987). While GPX is considered the primary hydrogen peroxide scavenger, catalase (CAT) also decomposes hydrogen peroxide to water and oxygen (Yu et al., 1998). However, CAT functions at higher concentrations of hydrogen peroxide.

Ceruloplasmin (CP), a serum protein that functions as a ferroxidase, is a nonenzymatic antioxidant (Percival and Harris, 1990). Its ferroxidase activity allows it to oxidize iron (II) to iron (III) so iron can be attached to transferrin (Percival and Harris, 1990). If iron is not bound to a protein, it could initiate peroxidative damage to the lipid component of the cellular membranes. Ceruloplasmin also scavenges free radicals produced during the immune response by functioning as an acute phase reactant protein (Cousins, 1985).

The characterization of the changes in swine antioxidant enzyme activities throughout production practices, such as the iron injection, weaning, etc., has not yet been reported. Many studies have characterized the changes in antioxidant enzyme activities due to dietary restriction, dietary manipulation, hormonal modulation, age, exercise, or the intake of oxidants, such as drugs or pollutants (Bolzan et al., 1997; Bolzan et al., 1995; Brzenzinska-Slebodzinska et al., 1994; Chan and Decker, 1994; Chung et al., 1988; deRosa et al., 1980; Duthie et al., 1992; Frank and Sosenko, 1987; Halliwell, 1987; Ji et al., 1990; Ledwozyw and Kadziolka, 1989; Luhtala et al., 1994; Starcher and Hill, 1965; Venkatraman et al., 1998). In this study, we characterized the changes in CAT, CP, GPX, and SOD throughout production.

LITERATURE REVIEW

Free radicals and Oxidative stress

Free radicals, or reactive oxygen metabolites (ROM), are atoms or molecules that have one or more unpaired electrons in an outer orbital and are capable of maintaining independent existence (Halliwell et al., 1995). The imbalance in electron pairing causes the increased reactivity of ROM. There are many biologically relevant types of free radicals. First, the hydrogen centered radicals, for example the hydrogen atom, often initiate radical chain reactions (Halliwell, 1987). Second, the sulfur centered radicals, for example the thiyl radical, are produced during the oxidation of thiol compounds (Halliwell, 1987). Third, the inorganic oxygen centered radicals, such as the superoxide radical and hydroxyl radical (Halliwell, 1987). The superoxide radical is an oxygen molecule containing an extra electron. The hydroxyl radical is a hydroxyl group containing an extra electron. Fourth, the organic oxygen centered radical, such as the alkoxy and peroxy radicals, which are produced during peroxidation and by metal decomposition of lipid peroxides (Halliwell, 1987). Fifth, transition metals, such as copper and iron, are catalysts of free radical reactions by accepting and donating single electrons (Halliwell, 1987).

Free radicals result from both inadvertent and deliberate processes. Inadvertently, the generation of free radicals is a result of the incomplete reduction of molecular oxygen to water during oxidative metabolism (Halliwell et al., 1995; Thurnham, 1990). During the reduction, molecular oxygen can gain an electron as a result of an escaped electron from the mitochondrial electron transport chain, microsomal cytochrome P450, or

autoxidation reactions including catecholamines, ascorbic acid, or reduced flavins (Halliwell et al., 1995). In addition exogenous oxidants, such as drugs or pollutants, can generate radicals (Camhi et al., 1995). Deliberately, infection or inflammation causes respiratory bursts of phagocytic cells and these cells produce ROM while they are consuming the infectious cells (Halliwell, 1987; Halliwell and Gutteridge, 1986; Thurnham, 1990). Finally, it is the imbalance between production and removal of these ROM that can initiate oxidative chain reactions and lipid peroxidation (Miller and Brzezinska-Slebodzinska, 1993).

An excess production of free radicals is defined as oxidative stress (Halliwell, 1987). The superoxide radical is produced as a result of the normal biological processes previously described. If the generation of the superoxide radical exceeds the regulatory mechanisms to rid the cell of this radical, then a cascade of reactions can result in the generation of more free radicals and increase oxidative stress. The superoxide radical is a weak radical with low affinity towards lipids (Niki, 1988). Therefore, its direct contribution to lipid peroxidation of the superoxide radical is small. However, it does play a key role in the peroxidation pathway. First, the superoxide radical aids in the reduction of iron from the ferric to the ferrous form (Niki, 1988). Second, it can react with hydrogen and form hydrogen peroxide (Halliwell et al., 1995). Hydrogen peroxide itself is not a free radical. However, in the presence of free metal ions, the hydrogen peroxide will be decomposed into active oxygen radicals, such as the hydroxyl radical and initiate free radical oxidation (Niki, 1988). The hydroxyl radical is possibly the most powerful oxidant that can be formed in biological systems (Minotti and Aust, 1987).

Oxidative stress manifests itself when free radicals produce oxidative damage in aerobic organisms. All components of DNA can be attacked by the hydroxyl radical (Breimer, 1991). Many ROM can oxidize sulfhydryl groups and attack amino acid residues (Thomas, 1995). The hydroxyl radical can also initiate lipid peroxidation. Lipid peroxidation results in the weakening and eventual destruction of the cell membrane (Halliwell et al., 1995). Halliwell (1987) summarized the interrelationships of free radical induced damage beginning with DNA. Free radicals cause DNA strand breaks and base modifications. This can cause the depletion of ATP and NADPH and the inability to maintain low intracellular calcium. High intracellular calcium stimulates calcium-stimulated proteases and these proteases digest metalloproteins. The decrease in ATP and NADPH results in increased inability to maintain compartmentalized intracellular iron pools, which increases the availability of catalytically active iron. Therefore, there are more radical reactions, lipid peroxidation, cell wall damage, and calcium release. The result is cell destruction.

Antioxidants

Regulating oxidative stress is the role of the antioxidant system. The enzymes and proteins involved in this system stop the proliferation of free radicals and prevent release of free metal ions. An antioxidant is defined as any substance that prevents the transfer of free electrons to and from molecular oxygen or stops free radical production (Bray and Bettger, 1990). Antioxidants can be divided into two classes: enzymatic and nonenzymatic. Enzymatic antioxidants function to convert toxic free radicals to less reactive species. Some examples of enzymatic antioxidants include superoxide dismutase,

glutathione peroxidase, glutathione reductase, and catalase. The antioxidant enzymes, superoxide dismutase (EC 1.15.1.1), glutathione peroxidase (EC 1.11.1.9) and catalase (EC 1.11.1.6), work together to eliminate toxic products of one and two electron reductions of oxygen.

Nonenzymatic antioxidants or radical-trapping antioxidants can be dietary nutrients, proteins, or other substances, such as Vitamin A, Vitamin C, Vitamin E, albumin, ceruloplasmin, transferrin, glutathione, haptoglobin, beta carotene, unithiol, uric acid, N-acetyl cysteine, allopurinol, oxypurinol, and glucose. Radical-quenching antioxidants function to prevent free radical propagation (Thurnham, 1990). According to Thurnham (1990), "the main characteristics of a radical-quenching antioxidants are 1) that they will be more easily oxidized than the other substances in the area and 2) that once oxidized they are a relatively stable compound."

Superoxide Dismutase

The superoxide dismutase enzymes, copper, zinc dependent SOD (CZSOD), manganese dependent SOD (MNSOD), and extracellular SOD (ECSOD), work as antioxidants to eliminate the superoxide radical (Quinlan et al., 1994). The superoxide radical can be produced during cellular metabolic processes, such as ATP generation, the oxidation of catecholamines, detoxification of pollutants, or the immune response (Quinlan et al., 1994). Copper, zinc dependent superoxide dismutase is a blue, copper-containing protein that contains .34% copper and had a weight of 34 kD (Mann and Keilin, 1939). In 1969, McCord and Fridovich purified a protein from bovine blood with superoxide dismutase activity, or superoxide oxidoreductase behavior, which was found

to be identical to the compound described by Mann and Keilin. They reported that the protein had 2 copper atoms per molecule of protein that could be reversibly removed, yet were required for enzymatic activity. Carrico and Deutsch (1969) reported that the protein also contained 2 zinc atoms per molecule that were required to stabilize the protein and were not for enzymatic function. They also reported that the copper and zinc were tightly bound to the protein and there was little change in the tertiary structure when the metals were removed. Later, Keele et al. (1971) reported that the CZSOD protein contained 2, identical, 16 kD subunits that were held together by disulfide bonds. They further characterized the molecule by noting that the 2 subunits contained 151 amino acid residues and the 2 active sites were facing away from each other. Additionally, copper was located on the bottom of a deep cleft in the protein and zinc was buried within the protein. Beem et al. (1974) showed that the zinc component could be replaced by other transition metals, such as cadmium, mercury, and cobalt with only slight activity impairment.

The SOD gene contains 5 exons and 5 introns with an unusual dinucloetide sequence at the first intron (G-C instead of the common G-T) (Levaron et al., 1985). The 5' end of the gene contains both TATTA and CAT promoter sequences (Levaron et al., 1985). Transcription in the nucleus results in 2 mRNA species one, which is 200 base pairs longer than the other (Levaron et al., 1985). The shorter mRNA species is expressed approximately 4 times as much as the longer one, indicating that the shorter may be the mRNA further processed in the endoplasmic reticulum for future enzymatic activity (Levaron et al., 1985). The CZSOD is present in the cytoplasm, nuclear matrix and peroxisomes (Chang et al., 1988; Crapo et al., 1992; Keller et al., 1991).

Many scientists have characterized regulation of CZSOD. Chung et al. (1988) reported that in copper deficiency, the liver still synthesized the apoprotein. They reported that dietary copper was a requirement for enzymatic activity but not protein synthesis. They also discovered that supplementation with other divalent metals, such as zinc and cadmium, increased CZSOD protein and mRNA concentrations. The researchers theorized that divalent metals other than copper had a distinct role in the expression of the protein moiety of CZSOD. Prohaska and Sunde (1993) reported that animal species or sex did not influence CZSOD activity and mRNA concentration.

Nutrition has been reported to be a major factor in CZSOD activity. Prohaska (1997) and L'abbe and Fischer (1984) both reported that rats experiencing a copper deficiency had lower heart CZSOD enzyme activities. L'abbe and Fischer (1984) also reported that a diet high in zinc and marginal in copper would further depress the activity of CZSOD. Pimentel et al. (1992) reported a depression in CZSOD activity in chickens when a diet high in zinc and low in copper was fed, and a subsequent increase in enzyme activity when additional dietary copper was added. Additionally, Williams et al. (1975) reported the CZSOD activity of both the liver and red cells in swine decreased when the diet was deficient in copper.

Manganese dependent SOD is also a purple-red tetrameric protein with 2 interacting dimers (Keele et al., 1970; Salin et al., 1978). The protein is 89 kD with 4 subunits of approximately 22 kD each (Salin et al., 1978). Each subunit contains an active catalytic site that has 1 atom of manganese (Salin et al., 1978). The manganese is required for enzymatic activity, similar to the copper, zinc dependent SOD (Quinlan et al., 1994). Salin and coworkers (1978) reported that this was a cyanide-insensitive

enzyme that accounted for 10% of the total SOD activity in the rat liver. Manganese dependent SOD is produced in the mitochondria to detoxify superoxide from cellular processes, such as electron transport chain and fatty acid oxidation (Heffner and Repine, 1989).

Numerous of investigators have reported that a consequence of manganese deficiency is the depression of MNSOD activity (Davis et al., 1990; deRosa et al., 1980; Malecki et al., 1994). Malecki and Greger (1996) reported that when the diet was deficient in manganese, the concentration of conjugated dienes increased. This indicated increased lipid peroxidation. They also reported that as dietary manganese decreased, MNSOD activity decreased and CZSOD increased. Paytner (1980) reported that in heart and kidney of rats, MNSOD decreased when the diet was deficient of manganese.

Extracellular SOD is a 155 kD tetrameric metalloprotein (Tibell et al., 1987). It is glycosylated and secreted from the endothelial cells in humans (Tibell et al., 1987). Extracellular SOD contains 2 dimers of 222 amino acids suggesting that ECSOD and CZSOD have distant, but common ancestors (Marklund, 1982). The amino acids in the copper, zinc dimer are nearly identical to the corresponding ones in ECSOD (Marklund, 1982). Extracellular SOD is regulated by proteolytic processing (Sandstrom et al., 1992). It is upregulated in response to cytokine release because leukocytes in plasma are directly responsible for superoxide production (Marklund, 1992). Extracellular SOD functions to protect cells against superoxide radicals produced in the plasma, lymph, and synovial fluids (Tibell et al., 1987; Marklund, 1992).

Glutathione Peroxidase

Glutathione peroxidase (GPX) is a tetramer with 4 identical 23-kilodalton subunits with one selenium molecule located in each active site (Bannister and Bannister, 1987; Epp et al., 1983). The discovery in 1973 that GPX contains an integral amount of selenium was important because it demonstrated a biochemical role for the trace element as well as providing a biochemical marker for accessing selenium status (Rotruck et al., 1973).

For all known selenoproteins, like GPX, selenium is present as a selenocysteine and is incorporated during translation at specific positions encoded by in-frame UGA codons (Low et al., 1995). A selenocysteine insertion sequence element stem-loop (SECIS) has been shown to be necessary for the recognition of the UGA as a selenocysteine codon rather than a nonsense or stop codon during translation (Berry et al., 1991). This element is located in eukaryotes in the 3' untranslated region (Berry et al., 1993). The formation of the carbon skeleton of the selenocysteine was determined by Evenson and Sunde (1986) to be derived from a serine molecule. The synthesis of the carbon skeleton begins with the esterification of the serine to a unique tRNA, and the synthesis of a selenophosphate by selenophosphate synthetase (Burk and Hill, 1993). This leads to translational incorporation of the selenocysteine into the polypeptide chain and formation of the entire GPX protein, which is then released into the cytosol of the cell (Kromayer et al., 1996). Glutathione peroxidase is found in virtually all cells, but its specific activity varies greatly between species and tissues (Burk and Hill, 1993).

The glutathione peroxidase family has two distinct functions: 1) to catalyze the reduction of hydrogen peroxide to water; and 2) to destroy lipid hydroperoxides generated in the hydrophobic region of the cell membrane (Bannister and Bannister, 1987). Glutathione peroxidase is the initial detoxifier of hydrogen peroxide at physiological concentrations (Kaneko, 1980). However, there are 3 other glutathione peroxidases with some antioxidant characteristics: plasma GPX, phospholipid hydroperoxide glutathione peroxidase (PHGPX), and non-selenium dependent GPX.

Nutritional regulation of GPX activity differs from mRNA synthesis. Weiss et al. (1996) reported that the liver GPX mRNA concentration plateaued at .05μg/ kg diet, whereas the GPX activity plateaued at .1μg/ kg diet in growing female rats. Therefore, the requirement for maximal GPX mRNA synthesis is half that of the requirement for maximal GPX activity. Hafeman et al (1974) reported that erythrocyte GPX responded more gradually to selenium deficiency than liver GPX. As repletion with different amounts of dietary selenium occurred, GPX activity increased correspondily. Sex also regulates GPX mRNA concentration and GPX activity. Prohaska and Sunde (1993) reported that GPX activity and GPX mRNA concentrations were lower in male rats when compared to female rats. They also reported differences in GPX activity and mRNA concentration within species.

Plasma GPX was first purified in 1987 from human plasma and was found to be structurally, enzymatically, and antigenically distinct from cellular GPX (Avissar et al., 1989; Takahashi et al., 1987). Plasma GPX is an extracellular tetrameric protein with 23 kD subunits with 4 atoms of selenium per mole of protein (Takahashi et al., 1987).

Avissar et al. (1989) reported that plasma GPX had increased mobility on the gel and therefore it was concluded that the polypeptide backbones differed between plasma and cellular GPX.

Plasma GPX is a glycoprotein unlike cGPX, which is to be expected for plasma protein species (Takahashi et al., 1987). Plasma GPX has a distinct amino acid sequence from GPX, and is thought to be a product of a different gene (Avissar et al., 1990; Sunde, 1990). Both GPX and plasma GPX are synthesized by hepatocytes in culture and are reported to originate in the liver (Sunde, 1990). The function of plasma GPX is unknown. The regulation of plasma GPX also has not been described. However, Avissar et al. (1989) reported that during selenium repletion plasma GPX activity increased at a faster rate than GPX. Takahashi et al. (1987) reported that copper, zinc, and mercury inhibited the enzymatic activity of plasma GPX.

Phospholipid hydroperoxide GPX was reported in 1985 and was purified from porcine heart and liver (Ursini et al., 1985). They described PHGPX as a monomeric species of 22 kD with 1 atom of selenium per mole of protein and is a polypeptide containing 170 amino acids (Sunde et al., 1993). It is unknown at this time if there is any relationship between PHGPX and GPX, however they have different amino acid compositions and are speculated to result from different genes (Sunde, 1990). Ursini et al. (1987) reported the apparent function of PHGPX is to protect against lipid peroxidation. PHGPX is capable of reducing fatty acid hydroperoxides that were esterified to phospholipids (Ursini and Bindoli, 1987). PHGPX, therefore, does not reduce any substrates of GPX.

PHGPX mRNA concentrations were neither affected by a selenium deficiency, nor elevated in females as with GPX (Sunde et al., 1993). Lei et al. (1995) reported that the amount of dietary selenium required to plateau PHGPX activity is one-half of the amount required to plateau GPX activity. Additionally, these researchers reported that selenium deficiency did not decrease PHGPX mRNA, whereas GPX mRNA did decrease.

Non-selenium dependent GPX was discovered in 1976 in the liver (Lawrence and Burk, 1976). Prohaska and Ganter (1977) reported that its activity was due to the activity of the glutathione-S-transferases (GSH-S-transferases), one of the most abundant liver proteins. Non-selenium dependent GPX catalyzes glutathione conjugation and reduces hydroperoxides with a lower affinity than GPX (Prohaska and Ganter, 1977). It therefore can decrease lipid peroxidation and protect the liver from damage due to organic hydroperoxides (Gibson et al., 1980). Jenkinson et al (1983) reported that non-selenium dependent GPX was present in the rat lung even during a selenium deficiency. They speculated that it protected the oxygen-rich environment of the lung from oxidative damage. Lawrence et al. (1978) reported that selenium deficiency upregulated GSH-S-transferase activity that apparently induced non-selenium dependent GPX activity.

Catalase

Catalase, or hydrogen peroxide oxidoreductase, was one of the first enzymes to be isolated which contained iron (Sumner and Dounce, 1937). Catalase has a molecular weight of 230 kD and a heme protein oligomer containing four tetrahedrally arranged 60 kD subunits (Herbert and Pinsent, 1948). Each subunit consists of a single polypeptide

chain that is associated with a single prosthetic group, ferric protoporphyrin IX (Herbert and Pinsent, 1948; Yu et al., 1998).

The CAT gene has been mapped to chromosome 11 and is 34 kb in length and consists of 13 exons and 12 introns (Quan et al., 1986). Transcription of the CAT gene results in a single, 2.4 kb mRNA species (Quan et al., 1986). The biosynthesis of CAT in the peroxisome is governed by a single autosome and occurs in three distinct phases: 1) synthesis of 60 kilodalton apocatalase subunits; 2) intercalation of heme; and 3) formation of the tetramers (Herbert and Pinsent, 1948).

Catalase is found in the peroxisomes, mitochondria, endoplasmic reticulum, and cytosol (Yu et al., 1998). In the red blood cell (RBC), CAT appears to exist in microgranules in the cytoplasm during the maturation of the erythroblast (Bannister and Bannister, 1987). The microgranules disappear when the red cell reaches maturity and CAT is completely soluble within the cell cytoplasm (Bannister and Bannister, 1987).

Catalase was initially thought to be the primary hydrogen peroxide scavenger (Heffner and Repine, 1989). It is now known that GPX is the primary scavenger until levels of hydrogen peroxide increase (Heffner and Repine, 1989). Like GPX, CAT functions to detoxify hydrogen peroxide to water and molecular oxygen. The scavenging ability of CAT is limited because of its discrete localization in the peroxisomes (Ishikawa et al., 1998). Catalase activity has been reported to fluctuate due to physiological levels of hydrogen peroxide (Yu et al., 1998). Also, the activity of CAT has been shown to be variable between species, strains, tissues and even developmental stage within a species (Yu et al., 1998).

Bulitta and coworkers (1996) reported another isoform of CAT, cytosolic CAT, which was reported to account for 50 to 60% of the total CAT activity in the guinea pig liver. They reported a 500 to 1000 kD difference in size between the cytosolic and peroxisomal CAT enzymes. The two isoforms have different amino acid compositions and distinct isoelectric points. Thus, if pre-translational mechanisms are operating-expression of separate genes or allelic variants-they could be considered isoenzymes. However, if it were just post-translational modification, these two isoforms would represent variants of the same polypeptide.

Age related changes in SOD, GPX, and CAT

In humans it has been reported that the activity of erythrocyte CZSOD was negatively correlated with age (Narayanan, 1996). The study also showed a positive correlation of GPX activity and age. Niwa and coworkers (1993) showed no change in the CZSOD activity between young (18-65 yr) and older (65-92 yr) humans. This study saw higher activities of both CAT and GPX activity in the young versus the older participants.

There has been extensive research concerning the relationship between aging and antioxidants in rats. Perez and coworkers (1991) reported a decrease in both CZSOD and GPX activity in the lungs of old (24 mo) vs. young (3 mo) Wistar rats. However, the CAT activity increased in the older rats. In contrast, Ji and coworkers (1990) reported that CZSOD decreased, but GPX activity increased, in the 4 vs. 31-month-old Wistar-Furth rats. All three enzymes increased with age in the skeletal muscle.

Yam et al. (1978) examined the changes in antioxidant pulmonary activity of the fetal and neonatal Sprague-Dawley albino rat. First, they found that there is no difference in enzyme activity due to the sex of the rat. Second, the pulmonary CZSOD and pulmonary GPX activities increased from two days prepartum to birth, decreased for 2 d postpartum, and increased progressively until 62 d of age. Pulmonary CAT activity, however, increased significantly before birth, decreased until 2 d of age, and then increased to prepartum values, only to start decreasing again at 10 d. After 10 d of age, the CAT activity continued to decrease through 62 d.

Tanswell and Freeman (1984) examined the changes of these enzymes in the pulmonary tissues of fetal, neonatal and adult rats. They observed an increase of CZSOD throughout the lifecycle. However, both GPX and CAT activities increased from d 18 gestation to d 3. The adult rats' enzyme activities decreased significantly compared to the fetal and neonatal activities. Yoshioka and coworkers (1980) also studied the developmental changes in the pulmonary tissue of fetal versus neonatal Wistar rats. They reported that SOD activity in the tissue on d 10 was higher than the activity on d 1, but the activity of the tissue on d 1 was higher than the activity of the fetal tissue. The activity they found on d 10 was identical to the value they observed at the adult stage (24 mo).

Paytner and Caple (1984) reported the changes in antioxidant enzyme activity in newborn versus adult sheep. In the erythrocytes, they found no age-related change in CZSOD. However, in the heart, lung and liver an age-related increase of CZSOD activity was observed. The newborn lamb had one-half the heart, lung and liver CZSOD activity when compared to the 4 and 16 wk old sheep.

In swine, Das and coworkers (1987) reported the age-related changes in the heart. Heart CZSOD activity increased 20 % the first 7 d of life, but increased only a small fraction during the later phases of growth. Glutathione peroxidase activity in the heart increased the first wk as well, but then plateaued. Loudenslager and coworkers (1986) found that piglets are born with a low activity of GPX. The activity increased with the consumption of colostrum. Additionally, they found that iron supplementation, in the form of an injection, increased GPX activity. Also, Jorgensen and Wegger (1979) found that an increased GPX concentration was associated with increased resistance to disease. Yu and coworkers (1998) reported no change in the CAT activity in the first 24 h, but for the next 10 d they saw a significant increase in activity in the liver, heart, and kidney of pigs. The CAT activity gradually continued increase until 5 mo of age.

Ceruloplasmin

Superoxide dismutase, GPX, and CAT are the major components of the intracellular antioxidant defense system. However, there is an important protein that accounts for some of the antioxidant defense of plasma. Ceruloplasmin is a 132-kilodalton alpha-two protein (Chang et al., 1975; Pan and Failla, 1998; Wooten et al., 1996). Holmberg and Laurell (1948) first discovered CP by isolating a copper protein in pig and human serum. They distinguished it from the other serum proteins based upon its intense blue color. Ceruloplasmin consists of two major components: 1) 6 atoms of copper and 2) an alpha globulin glycoprotein, called apoceruloplasmin (Chang et al., 1976; Wooten et al., 1996). Originally, it was thought that CP was only synthesized by the liver parenchymal cells (Chang et al., 1976). However, Skinner and Griswold (1983)

discovered that CP is also produced in the sertoli cells. Ceruloplasmin is formed in two steps: 1) the synthesis of a 65 kilodalton preproceruloplasmin protein in the Golgi apparatus; and 2) the synthesis of an 84 kilodalton proceruloplasmin protein in the endoplasmic reticulum (Cousins, 1985). Prozorovski et al. (1982) reported that CP maturation requires the ligation of the two precursor proteins in the endoplasmic reticulum before release into the blood.

Stevens et al. (1984) first established that CP specifically binds to the cell membrane in chick heart tissue. Additionally, Barnes and Frieden (1984) reported evidence for the existence of a CP receptor on erythrocyte membranes. They characterized the receptor's binding as specific because when CP was added to an equilibrated system of labeled CP and erythrocytes, 40 % of the labeled CP was displaced. Also, they reported that the receptor was a cell surface protein because when the red cells were exposed to trypsin, they did not bind CP thereafter. Using a Scatchard analysis they determined a binding affinity of 5nM. This is a higher affinity than the 10-50nM reported by Stevens and coworkers (1984). However, they were using chicken aorta and heart tissue instead of red cells. Finally, Barnes and Frieden (1984) reported that the CP must bind the receptor to functionally protect the cell from metal lysis or lipid peroxidation. It has since been reported that CP binds liver endothelial cells (Kataoka and Tavassoli, 1984), granulocytes and lymphocytes (Kataoka and Tavassoli, 1985b), membranes of the heart, brain, liver and kidney (Orena et al., 1986), and human erythroleukemic K562 cells (Percival and Harris, 1989b). Tavassoli et al. (1986) reported that receptors might mediate copper uptake into the cell. They reported that labeled CP binds a receptor on the endothelial surface and then is internalized. Percival and Harris

(1990) described the mechanism of copper transfer from CP. First, the transformed cell culture line, K562, binds CP. Secondly, the binding initiates a copper transfer from the CP molecule to the cytosol. By following both the protein fraction and the copper simultaneously, the researchers confirmed that dissociation of CP occurs before uptake. So it seems clear that CP's function is to transport the copper to the cell surface, but not to mediate copper uptake into the cell. They then reported that the copper entering the K562 cell was later bound to CZSOD. Their research verified earlier reports by Marceau and Aspin (1973) and Dameron and Harris (1987), who reported that labeled copper from CP was found in the copper fraction of aortic CZSOD.

Ceruloplasmin is responsible for carrying 70-90 percent of the copper in circulation (Chang et al., 1975; Pan and Failla, 1998). Ceruloplasmin can inhibit iron-catalyzed lipid peroxidation by acting as a ferroxidase, and it may have a biological role in promoting iron saturation of transferrin by oxidizing iron (II) to iron (III), so iron can be attached to the transferrin molecule (Chan and Decker, 1994; Chang et al., 1975; Lee and Matrone, 1969). Ceruloplasmin inhibits iron's ability to convert molecular oxygen into the superoxide radical by converting iron to its storage form where it is bound to the transferrin molecule (Niki, 1988). Another important point is the fact that CP completes this conversion without the release of oxy-radical intermediates and thus inhibits iron/copper lipid peroxidation (Halliwell and Gutteridge, 1986). Additionally, physiological stress, such as infection or inflammation, increases the plasma concentrations of acute phase reactant proteins, such as CP (Cousins, 1985). Increased CP mRNA expression and CP activity during physiological stress appears to be mediated by the proinflammatory cytokines (Fleming et al., 1991).

Copper nutrition is vital to maintaining CP activity in the plasma. Prohaska (1997) reported that in rats fed inadequate dietary copper, the plasma CP activity decreased. When dietary copper was adequate, the CP activity increased. This research confirmed earlier data reported by Prohaska (1991) and Bettger et al. (1978) that stated that dietary copper restriction in rats resulted in a reduction of copper containing enzymes, such as CZSOD and CP. Milne (1998) reported a decrease in CP activity in rats when the diets were deficient in copper. Panemangalore and Bebe (1996) also reported a decrease in CP activity in rats fed a copper deficient diet. Therefore, copper nutriture is critical in maintaining optimum CP activity.

Ceruloplasmin activity can be modulated by trauma or stress because these events cause leukocytes to release interluken-1, which stimulates an increase in plasma glucagon and glucocortcoids (DiSilvestro and Cousins, 1984). Both glucagon and glucocortcoids increase CP activity (DiSilvestro and Cousins, 1984). When infection or inflammation occurs, the body releases acute-phase proteins to modulate the inflammatory process. In infection or inflammation, phagocytosis of invading organisms generates superoxide radicals. DiSilvestro (1988) reported that in response to inflammation, plasma CP increases. Additionally, the release of glucocortcoids during times of stress has also been reported to increase plasma CP activity (Alias, 1971; Curtis and Butler, 1980; Starcher and Hill, 1965). Therefore, CP works to scavenge the free radicals and superoxide anions produced during this process and is by definition, an antioxidant enzyme (Cousins, 1985).

Age-related changes in CP

Some studies have tried to determine the limiting factors of CP synthesis in the newborn pig. Chang and coworkers (1975) reported that the CP protein was not transferred from the sow to the piglets because no apoceruloplasmin protein was present in the plasma of the newborns. However, they reported that the liver copper concentrations in the neonatal pig were nearly 10 fold that of an adult pig. By 10 to 15 hr after birth, the piglets were synthesizing the CP protein because it was present in the plasma.

Chang and coworkers (1976) reported that at birth there was no apoceruloplasmin protein found in piglet plasma. They also reported that when piglets are removed from the sow at birth and fed either a copper deficient or copper supplemented diet, there is a steady increase in CP synthesis regardless of dietary treatment. However at two weeks of age if the diet was adequate in copper, CP synthesis and activity increased, but if the diet was deficient in copper, the liver copper stores became the limiting factor in CP synthesis and activity.

CHAPTER 1

CHARACTERIZATION OF THE CHANGES IN ANTIOXIDANT ENZYME ACTIVITY IN GROWING SWINE

Abstract

Two experiments were conducted to evaluate the effects of common production methods on the changes in the antioxidant enzymes, Cu/Zn superoxide dismutase (CZSOD), Se-dependent glutathione peroxidase (GPX), catalase (CAT), and ceruloplasmin (CP). In Exp. 1 (n=16, d 2 of age, 1.6 kg BW), antioxidant enzymes were evaluated prior to iron injection, 12h, 24h, 36h, and 48h after injection; prior to weaning, 3h, 12h, 24h, and 48h after weaning; prior to moving and comingling, 6h, 24h, 48h after moving. In Exp. 2 (n=12, d 21 of age, 10.5 kg BW), antioxidant enzymes were evaluated prior to weaning, 3h, 6h, 12h, 18h, 24h, 36h, 48h, 60h, and 72h after weaning.

Iron administration did not increase CZSOD activity, increased GPX activity, did not increase CAT activity and increased CP activity. Weaning (Exp. 1) increased CZSOD and CAT activity and decreased GPX and CP activity. Weaning (Exp. 2) decreased then increased CZSOD activity. GPX activity decreased, increased then decreased post weaning (Exp. 2). Weaning (Exp. 2) did not change CAT and CP activities. Moving and comingling increased CZSOD and CAT activities, decreased GPX activity, and did not change CP activity until an increase 48 h post move. In summary, the antioxidant enzyme profiles change during different sources of production stress and these changes must be further researched to better ascertain how these enzymes respond to production stress.

Keywords: Gilts, Production Methods, Antioxidant Enzymes

Introduction

Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's ability to dispose of them (Miller and Brzezinska-Slebodzinska, 1993). A free radical is any atom or molecule with an unpaired electron that is capable of existing independently in the body (Halliwell, 1987; Halliwell et al., 1995).

Free radicals can be produced both inadvertently and deliberately. Inadvertently, free radicals can result from electron transfer to molecular oxygen from metabolic processes (Halliwell et al., 1995). Deliberately, free radicals are produced by the process of phagocytosis (Thomas, 1995).

The body has a major defense mechanism to stop the proliferation of damaging free radicals: antioxidant enzymes and nonenzymatic antioxidants. Enzymatic antioxidants, including superoxide dismutase, glutathione peroxidase, and catalase, function to convert toxic radicals to less toxic species. Ceruloplasmin (CP), a serum protein that functions as a ferroxidase, is a nonenzymatic antioxidant (Percival and Harris, 1990). Ceruloplasmin also scavenges free radicals produced during the immune response by functioning as an acute phase reactant protein (Cousins, 1985).

The characterization of the changes in swine antioxidant enzyme activities during early production, such as the iron injection, weaning, etc., has not yet been reported. The objective of this study was to characterize the changes in CAT, CP, GPX, and CZSOD during production.

Materials and Methods

Experiments 1 and 2 were approved by the Michigan State All University Committee on Animal Care and Use prior to initiation of the study. Both experiments were completed at Michigan State University swine teaching and research facility.

Experiment 1

Sixteen Yorkshire gilts from four litters were assigned to the trial at 2 d of age and an average weight of 1.64 kg. On d 2 each gilt was administered 2 cc iron (Iron dextran complex, 100 mg elemental iron per mL; Fermenta Animal Health Co., St. Louis, Mo.) intramuscularly (i.m.). Gilts remained with dams until 11 d of age, at which time they were weaned into an off-site, mechanically temperature controlled room and allotted by weight to one of four pens with ad libitum access to feed and water. The nursery room temperature was set at 25.6°C for the first 3 wk and then decreased .56°C each wk for 5 wk to a temperature of 22.8°C. The first 2 wk after weaning, the gilts were provided supplemental heat from heat lamps hung .92 m above the floor of the pen. Nursery pens were equipped with stainless steel, single-sided, five-hole feeders (Circle B, Marcellus, MI) and single cup waterers (La Buvette, Paris, France). At weaning, pigs were administered .5 cc of penicillin G procaine (Agri-cillin, Agri Labs, St. Joseph, MO) i.m., and fed four times per day on a plastic tray on the floor during the first week to stimulate feed intake. Phase 1, 2, 3, and 4 diet (Table1) were fed from 11-25, 25-39, 39-46, and 46-60 d of age respectively. At 60 d of age, the gilts were moved to a single pen in a temperature controlled grow-finish room. The initial temperature was 21.1°C and

remained the same until project termination at 63 d of age. Grow-finish pens were equipped with stainless steel, Crystal Springs two hole wet/dry feeders (Growmaster, Inc., Omaha, NE). Phase 5 diet (Table 1) was fed from 60 d of age until project termination at 63 d of age. The chemical analysis of each diet is shown in Table 3. Pig weights and feed disappearance were determined weekly in the nursery period. These data (Table 4) were used to calculate average daily gain (ADG), average daily feed intake, and feed efficiency (Gain to Feed).

Experiment 2

Twelve (Yorkshire x Landrace) x Duroc crossbred gilts from four litters were assigned to the trial at an average of 20 d of age and an average weight of 10.3 kg. They were weaned into a mechanically temperature-controlled room and allotted by weight and co-mingled across litters to one of four pens with ad libitum access to feed and water. The nursery room temperature was set at 25.6°C. The nursery pens were equipped with the same feeders and waterers as in Exp. 1. At the time of weaning, pigs were administered .5 cc of penicillin (Agri-cillin, Agri Labs, Inc., St. Joseph, MO) i.m. Diet composition and chemical analysis are shown in Tables 2 and 3.

Blood collection

Blood was collected in Exp. 1 by venapuncture from the anterior vena cava into 5 mL heparnized (72 units sodium heparin/ tube) vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) using 21 gauge, 1 inch needles until the gilts were 14 d of age. After 14 d, blood was collected by venapuncture from the anterior vena cava into 10 mL heparnized (143 units sodium heparin/ tube) vacutainer tubes (Becton Dickinson,

Franklin Lakes, NJ) with 20 gauge, 1.5 inch needles. During Exp. 2, blood was collected by venapuncture from the anterior vena cava into 10 mL heparnized (143 units sodium heparin/ tube) vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) with 20 gauge, 1.5 inch needles. Blood was immediately centrifuged at 4°C, 2,000 x g for 10 min (Beckman GS-6KR, Palo Alto, CA). Plasma was pipetted with disposable, glass Pasteur pipettes (VWR Scientific Products, South Plainfield, NJ) into polypropylene tubes and stored at -80°C until analysis could be performed. In Exp. 1, blood was collected from the gilts prior to the iron injection and 12h, 24h, 36h, and 48h after the iron injection; prior to weaning, 3h, 12h, 24h, and 48h, after weaning; and prior to co-mingling in a single grow-finish pen, 6h, 24h, and 48h after the move. In Exp. 2, blood was collected from the gilts prior to weaning, 3h, 6h, 12h, 18h, 24h, 36h, 48h, 60h, and 72h after weaning.

Superoxide Dismutase Analysis (Appendix A)

Red blood cell Cu/Zn superoxide dismutase (CZSOD; EC 1.15.1.1) activity was determined (Hill et al., 1999) by a modification of the method of Marklund and Marklund (1974).

Glutathione Peroxidase Analysis (Appendix B)

Red blood cell glutathione peroxidase (GPX; EC 1.11.1.9) activity was determined by the coupled assay procedure first published by Paglia and Valentine (1967) and described by Lawrence et al. (1974).

Catalase Analysis (Appendix C)

Red blood cell catalase (CAT; EC 1.11.1.6) activity was determined by the method of Aebi (1984).

Ceruloplasmin Analysis (Appendix D)

Plasma ceruloplasmin (CP; EC 1.12.3.1) activity was determined by the method of Schosinsky et al. (1974) and Lehmann et al. (1974).

Statistical Analysis

Data for Exp. 1 were analyzed using the PROC MIXED procedure of SAS and least squares of means by time (SAS, 1996). Enzyme type (CZSOD, GPX, CAT, and CP) was the dependent variable and litter, pig, and pig within litter were the independent variables. For the blood analysis, individual pig was the experimental unit. Separate repeated measures on each pig blocked by litter over a specific time (production event: iron injection, weaning, or move to grow-finish) for each enzyme were analyzed using PROC MIXED. Tukey's test was used to control for experimentwise error rates on all pairwise comparisons (Neter et al., 1996). Differences were considered significant at $P < .05$. Data for Exp. 2 were analyzed using the PROC MIXED procedure of SAS and least squares of means by time (SAS, 1996). Enzyme type (CZSOD, GPX, CAT, and CP) was the dependent variable and litter, pig, and pig within litter were the independent variables. For the blood analysis, individual pig was the experimental unit. Separate repeated measures on each pig blocked by litter over a specific time after weaning for each enzyme were analyzed using PROC MIXED. Tukey's test was used to control for experimentwise error rates on all pairwise comparisons (Neter et al., 1996). Differences were considered significant at $P < .05$.

Results

Effects of iron injection on enzyme activities (Exp. 1)

Catalase activity at 48 h was 21% higher (P<.05; Table 5) than CAT activity at 24 h, but no other differences were observed. Twelve hours after the iron injection CP activity increased (P<.05; Table 5) 50%. The activity at 12 h did not differ statistically from the activity at 24 h. However, CP activity increased (P<.05) 30% from 12 to 36 h and increased (P<.05) 20% from 36 to 48 h. Glutathione peroxidase activity was not statistically different during the first 12 h post injection (Table 5). The GPX activity at 24 h was 33% higher (P<.05) than the activity at 12 h and remained elevated throughout the remainder of the study. Superoxide dismutase activity was not altered from the time of injection until 48 h (Table 5), when activity decreased (P<.05) 15 % from 0 h activity.

Effects of weaning on enzyme activities (Exp. 1)

Catalase activity remained unchanged (Table 6) at 3 and 12 h post weaning, but the activity at 24 h increased (P<.05) 18 % above the 0 h activity. Catalase activity at 48 h was significantly lower (P<.05) than any other time in the study. At 24 h post weaning, CP activity decreased (P<.05; Table 6) 15% compared to the activity prior to weaning. At 48 h CP activity was significantly lower (P<.05) than any other time period in the study. Glutathione peroxidase activity decreased (P<.05; Table 6) and remained at this lower activity throughout the remainder of the study. The activity of CZSOD enzyme was elevated at 12 h post weaning compared to the activity at other times of observation in this study.

Effect of weaning on enzyme activities (Exp. 2)

Catalase activity at 12 h (Table 7) was significantly different than at 48 h. At no other time of observation did CAT activity differ. Ceruloplasmin enzyme activity did not differ statistically between any bleeding periods during this study (Table 7). Glutathione peroxidase activity (Table 7) decreased at 12 h and increased at 36 h compared to all other times of measurement. Activity at 36 h and 48 h did not differ. The CZSOD activity (Table7) decreased at 12 h and increased at 60 h compared to the activity at other times of observation ($P<.05$).

Effects of moving and co-mingling on enzyme activities (Exp. 1)

Catalase activity increased 165% at 6 h compared to the activity at 0 h ($P<.05$; Table 8). At 24 h enzyme activity decreased ($P<.05$) 53% from the 6-h activity. The CAT activity (Table 8) was decreased ($P<.05$) at 24 h and 48h. Ceruloplasmin activity (Table 8) was increased ($P<.05$) at 48 h compared to other periods of observation. Glutathione peroxidase activity was statistically decreased ($P<.05$; Table 8)at 24 h compared to the activity at 0 h and 48 h. Superoxide dismutase activity increased ($P<.05$; Table 8) at 6 h compared to other times of observation.

Discussion

In the swine industry, a common practice is the administration of iron to prevent anemia in neonatal pigs (Miller, 1981). However, iron if not bound to a protein may initiate lipid peroxidation via the Fenton reaction (Minotti and Aust, 1987). This reaction cascade may produce toxic free radicals, such as superoxide, hydrogen peroxide, and hydroxyl radicals (Minotti and Aust, 1987). For the present study (Exp. 1), CP activity increased at 12 h, as did GPX at 24 h after the iron injection. The activity of CZSOD and CAT remained unchanged until 48 h. Superoxide dismutase converts the superoxide radical to hydrogen peroxide and oxygen (Quinlan, et al., 1994). Therefore, if the concentration of superoxide radicals had been increased by iron administration and CZSOD could be synthesized in 48 h, one might have expected an increase in activity.

Glutathione peroxidase and CAT convert hydrogen peroxide to water (Bannister and Bannister, 1987). Catalase activity did not change after the iron injection. It is possible the hydrogen peroxide production remained at low enough concentration that an increase in CAT activity was not needed since it remains at a relatively high activity during the lifecycle. Bannister and Bannister (1987) reported that GPX is the primary hydrogen peroxide scavenger and CAT has low affinity for hydrogen peroxide at low concentrations. Also, CAT is compartmentalized in the peroxisome, whereas GPX is located in the cytosol (Bannister and Bannister, 1987). Therefore, GPX may be more readily available to detoxify hydrogen peroxide produced by free iron than CAT. Catalase activity has been reported not to fluctuate early in life. Rickett and Kelly (1990)

reported that CAT activity remained constant until 10 d of age and then dropped 60% by 66 d of age or adulthood in the guinea pig.

Ceruloplasmin activity increased throughout this portion of the study (0-5 d of age). Ceruloplasmin functions as a ferroxidase to oxidize iron (II) to iron (III) so it can be attached to the transferrin molecule and inhibit further free iron catalyzed lipid peroxidation (Chang, et al., 1975). Ceruloplasmin also functions as an acute phase reactant protein (Cousins, 1985). Therefore, CP activity increases during times of stress. DiSilvestro (1988) reported that injecting rats with turpentine resulted in inflammation and increase in CP activity. Chang and coworkers (1975 and 1976) reported that the newborn pig had low concentrations of the CP apoprotein present in the plasma and Gomez-Garcia and Matrone (1967) reported that CP activity increased in the first 2 wk after birth reaching the adult activity level by 2 wk of age. Therefore, the gradual increase in CP activity observed in this study may be a result of age and not physiological stimulus.

Early weaning pigs into an offsite facility is utilized in today's swine industry to minimize disease transfer between older pigs, especially the sows, to the younger pigs. However, early weaning (>d 8) as well as traditional weaning (d 14-21), creates a variety of challenges, such as reduced feed due to the differences in the form of the diet, water intake and the comingling in a different environment.

Superoxide dismutase activity increased shortly after weaning (12 h) in Exp. 1. This may or may not indicate an increase in oxidative stress associated with weaning. If moving the pigs into a different environment resulted in oxidative stress, the release of the superoxide radical could have occurred. However, CZSOD activity prior to weaning

was lower than the activity prior to the iron injection. Rickett and Kelly (1990) reported that CZSOD activity decreased from 10 d of age until 66 d of age in the guinea pig. The CZSOD activity prior to weaning (Exp. 1) was 155 EU/mg protein and Carlson and coworkers (1999) reported 143 EU/mg protein mean CZSOD activity for early-weaned pigs (11.5 d of age).

Superoxide dismutase activity in Exp. 2 behaved distinctly different from Exp. 1 The basal and final activities were similar, however, CZSOD activity after weaning decreased (12 h) instead of increasing as in Exp. 1. Superoxide dismutase activity increased (60 h) after the initial decrease in Exp. 2, but the trend of the two experiments differed. The gilts used in Exp. 2 were of different genetic origin. There were many laboratory complications during this study (Appendix E). The protein concentrations for the CZSOD extract fluctuated greatly between and within pigs and bleeding periods around weaning. Superoxide dismutase activity is standardized with the protein concentration of the blood extract. If the extraction process is not complete, hemoglobin could remain in the extract and increase the total protein concentration. Also, if the phosphate buffered saline is not removed, dilution of the total sample will occur. The protein concentrations of the standards did not fit a linear standard curve. For example, the .1 standard read .155 on the standard curve. This error may have prevented accurate determination of sample protein concentrations.

Glutathione peroxidase activity responded differently to weaning than it did after the iron injection. The GPX activity in Exp. 1 decreased initially and similar activity for this enzyme remained through 48 h. Glutathione peroxidase activity in Exp. 2 decreased at 12 h instead of at 3 h as in Exp. 1. The activity increased at 18 and 36 h and then

decreased again at 60 h. Jorgenson and Wegger (1979) reported GPX activities between 7 and 288 EU/g hemoglobin for 14 d old pigs. The values for Exp.1 and 2 fall within this range. However, due to the large amount of variation between activities reported by Jorgenson and Wegger, little comparison can be made between experiments. The difference could also be due to laboratory error. The extreme changes in hemoglobin concentration during this experiment (Appendix F) are not physiologically possible in this short period of time, therefore error must have occurred. If the samples were not accurately pipetted, hemoglobin concentration and GPX activity would have been altered. Additionally, if any plasma or saline had remained with the cells after the washing of the RBC, the concentration of red cells would have been diluted. The higher activity overall of GPX in Exp. 2 could be the result of sample management. Zhang and coworkers (1986) reported that storage of plasma samples at -15°C for 56 d caused a 57% reduction in GPX activity. All samples were stored at -80°C, but major reductions in the activity of Exp. 1 samples because they were stored for 2 mo vs. 1 wk in Exp. 2.

Activity of CAT in Exp. 1 increased 24 h then declined significantly below the activity observed at any other time in this study. Venkatraman and coworkers (1998) reported that food restriction increased CAT activity in rats, but Yu and coworkers (1998) reported that CAT activity did not decrease with food restriction. Yu and coworkers (1998) studied newborn pigs that had not suckled, 1 d old pigs that had not suckled, 10 d, 21 d, and 5 mo old pigs fasted for 24 h prior to sampling. At weaning, the feed intake of pigs was low; the pigs in Exp. 1 consumed approximately 150 g per pig per day the first week after weaning. Therefore, it could be that the dietary restriction caused the increase

in CAT activity. However, this does not account for the drastic drop in CAT activity 24 h later.

Catalase activity did not change throughout Exp. 2. There was an increase in activity between the 12h and 48h activities, but the basal and final activities were not statically different from each other. Additionally, the CAT activity in Exp. 2 was twice that of Exp. 1. This may have been due to age or the higher feed intake of the older pigs (later weaning) in Exp. 2. Early-weaned pigs (10-14 d of age) should consume 250 g per day and traditionally weaned pigs (14-21 d of age) should consume 500 g per day (NRC, 1998).

Ceruloplasmin activity in Exp. 1 remained the same until a significant decrease at 48 h. However, the CP activity at weaning is 2 to 4 times higher than the final activity measured after the iron injection (4 d of age). Milne and Matrone (1970) reported that CP activity doubles between 2 and 4 days of age in pigs. Ceruloplasmin activity at weaning (21 d of age) in Exp. 2 was similar to that of weaning in Exp. 1. However, CP activity did not change from 0 to 72 h post weaning. Chang and coworkers (1976) reported that CP activity of the pig reached adult amounts by wk 2 of age. Gilts in Exp. 2 were 2 wk of age and this may explain the lack of change in CP activity throughout the duration of the experiment.

Pigs are moved from the nursery to a grow-finish room or facility and usually co-mingling with unfamiliar pigs occurs. Six hours after moving the gilts in Exp. 1 from four pens to a single pen CZSOD activity increased. This could be a result of adapting to a new environment similar to weaning in Exp. 1, and/or the large amount of fighting between the pigs. By 24 h, SOD activity had returned to the basal activity (0 h).

Glutathione peroxidase activity decreased (0 vs. 24 h) after moving the pigs, but returned to 0 h activity at 48 h. Activity of CAT increased at 6 h and then decreased to premove (0 h) activity by 48 h. The feeder and waterer style was different than it was in the nursery. The feeder was a wet/dry feeder with a water nipple design that may have initially resulted in a lower feed intake. As previously stated, the decreased in feed intake could be an explanation for the increased CAT activity.

Ceruloplasmin activity was unchanged until an increase at 48 h post move. Milne and Matrone (1970) reported an increase (.727 U/mg protein to 1.18 U/mg protein) in CP activity between 4 d old and 10 wk old pigs. Our study showed an increase (.03 U/mL to .16 U/mL) between 4 d of age and 9 wk of age. Finally, the increase at 48h could have been a result of the fighting.

In conclusion, these experiments may indicate that some changes in swine antioxidant enzyme profiles occur in relation to current production practices that could be sources of oxidative stress to pigs. However, laboratory errors in Exp.2 will not allow further conclusions to be drawn from that experiment.

Implications

The antioxidant enzyme activity may change as a result of common production practices. It may be important to know if production practices alter these enzymes. However, more research in this area is needed.

Table 1. Percentage compositions of Exp. 1 diets (as-fed basis)

Ingredient	Phase 1[ab] (%)	Phase 2[cd] (%)	Phase 3[e] (%)	Phase 4[f] (%)	Phase 5[g] (%)
Corn, dent yellow	33.21	40.245	57.744	65.205	62.225
Soybean meal, 44%	15.0	19.08	21.048	27.854	30.27
Blood plasma	7.0	----	----	----	----
Dried whey	25.0	20.0	10.0	----	----
Fish meal, 60%	3.0	3.0	5.0	----	----
Spray dried blood cell	----	3.5	----	----	----
Lactose	10.0	10.0	----	----	----
Soy protein concentrate	2.75	----	----	----	----
DL-methionine	.05	.1	----	----	----
L-lysine	----	----	.15	.15	----
Dicalcium phosphate, 21% P	.6	1.2	1.352	1.616	2.065
Ground limestone	.9	.7	.629	1.098	.839
Choice white grease	----	----	3.0	3.0	3.0
Soy oil	1.0	1.0	----	----	----
Trace mineral premix 96[h]	.22	.22	.22	.22	----
Trace mineral premix 98[i]	----	----	----	----	.5
Plain white salt, NaCl	----	----	----	----	.5
Vitamin premix[j]	.6	.6	.6	.6	.6
Carbadox[k]	.25	.25	.25	.25	----

[a]Formulated to contain 22% CP, 1.5% lysine, .9% Ca, and .7% P.
[b]Diet contained 42 mg Zn/kg of diet as zinc oxide.
[c]Formulated to contain 20% CP, 1.3% lysine, .9% Ca, and .7% P.
[d]Diet contained 10 mg Cu/kg of diet as copper sulfate.
[e]Formulated to contain 19% CP, 1.2% lysine, .9% Ca, and .7% P.
[f]Formulated to contain 19% CP, 1.1% lysine, .8% Ca, and .7% P.
[g]Formulated to contain 19% CP, 1.1% lysine, .8% Ca, and .7% P.
[h]Provided per kg complete diet: 4.5 mg Cu, 4.5 mg Zn, 4.5 mg Mn, 45 mg Fe, .0675 mg I, and .135 mg Se.
[i]Provided per kg complete diet: 10 mg Cu, 100 mg Zn, 10 mg Mn, 100 mg Fe, .15 mg I, and .3 mg Se.
[j]Provided per kg complete diet: 1136.4 IU vitamin A, 113.6 IU vitamin D_3, 13.6 IU vitamin E, 2.8 mg vitamin K_3, .908 mg menadione, .0068 mg vitamin B_{12}, .908 mg riboflavin, 3.64 mg d-pantothenic acid, .22 mg thiamin, .204 mg pyridoxine, 5.4 mg niacin.
[k]Antibacterial agent, 2.2% carbadox (Mecadox®-10, Pfizer Animal Health, Inc., New York City, NY)

Table 2. Percentage composition of Exp. 2 diet (as-fed basis)

Ingredient	Diet[a] (%)
Corn, dent yellow	45.89
Soybean meal, 44%	20.81
Dried whey	20
Spray dried blood cell	3.5
Lactose	5
DL-methionine	.2
L-lysine	.1
Dicalcium phosphate, 21% P	1.55
Ground limestone	.85
Soy oil	1
Trace mineral premix 98[b]	.5
Plain white salt, NaCl	.25
Vitamin premix[c]	.6

[a]Formulated to contain 19% CP, 1.3% lysine, .9% Ca, and .7% P.
[b]Provided per kg complete diet: 10 mg Cu, 100 mg Zn, 10 mg Mn, 100 mg Fe, .15 mg I, and .3 mg Se.
[c]Provided per kg complete diet: 1136.4 IU vitamin A, 113.6 IU vitamin D_3, 13.6 IU vitamin E, 2.8 mg vitamin K_3, .908 mg menadione, .0068 mg vitamin B_{12}, .908 mg riboflavin, 3.64 mg d-pantothenic acid, .22 mg thiamin, .204 mg pyridoxine, 5.4 mg niacin.

Table 3. Chemical analysis of Exp. 1 and Exp. 2 diets, ppm (as-fed basis)

	NRC Requirement			Analyzed Concentration		
Diet	Zn requirement, mg/kg diet	Cu requirement, mg/kg diet	Fe requirement, mg/kg diet	[Zn], mg/kg diet	[Cu], mg/kg diet	[Fe], mg/kg diet
Exp. 1						
1	100.0	6.0	100.0	3373.0	17.27	226.32
2	100.0	6.0	100.0	29.89	261.08	281.16
3	80.0	5.0	80.0	41.57	21.97	251.39
4	60.0	4.0	60.0	38.1	16.15	256.69
5	60.0	4.0	60.0	54.63	19.16	228.86
Exp. 2						
1	100.0	6.0	100.0	249.06	22.12	446.45

Table 4. Growth characteristics of gilts (Exp. 1)

Parameter[a]	Pen 1	Pen 2	Pen 3	Pen 4
Avg. Daily Gain, g/d				
d 11-18	-39.0	-55.2	9.7	-39.0
d 18-25	196.4	227.3	199.7	134.7
d 25-32	211.0	332.8	297.1	254.9
d 32-39	433.4	488.6	439.9	469.2
d 39-46	449.7	555.2	487.0	556.8
d 46-53	496.8	392.9	391.2	423.7
d 53-60	563.3	545.5	425.3	514.6
Overall ADG	330.2	355.3	321.4	330.7
Avg. Daily Intake, g/d				
d 11-18	712.1	482.9	630.7	502.9
d 18-25	816.0	891.1	690.1	699.0
d 25-32	2857.1	3714.3	2857.1	2857.1
d 32-39	4110.0	5652.9	4307.1	4190.0
d 39-46	2981.4	4238.6	2762.1	3887.1
d 46-53	6429.5	6605.5	6394.6	6555.3
d 53-60	10285.7	11714.2	10285.7	11714.2
Overall ADFI	4027.4	4757.1	3989.6	4343.7
Gain to Feed				
d 11-18	-.055	-.11	.015	-.078
d 18-25	.24	.26	.29	.19
d 25-32	.074	.09	.10	.09
d 32-39	.11	.09	.10	.11
d 39-46	.15	.13	.18	.14
d 46-53	.08	.06	.06	.07
d 53-60	.06	.05	.04	.04
Overall G:F	.08	.07	.08	.08

[a]Means are reported in d of age from 4 pigs per pen.

Table 5. Least squares means of enzyme activity after iron injection (Exp. 1)

	Time[a]					
Enzyme	0	12	24	36	48	SEM[b]
CAT[c]	.0070[gh]	.0072[gh]	.0063[g]	.0075[gh]	.0076[gh]	.00033
CP[d]	.030[g]	.045[h]	.052[h]	.066[i]	.079[j]	.003
GPX[e]	18.72[g]	15.60[g]	24.81[h]	25.26[h]	26.15[h]	1.05
CZSOD[f]	155.1[g]	150.0[g]	163.3[g]	152.0[g]	128.0[h]	5.2

[a]Hours after iron injection.
[b]Standard error of the least squares means.
[c]Activity expressed as a rate constant (k).
[d]Activity expressed as enzyme units per mL of serum.
[e]Activity expressed as enzyme units per g hemoglobin.
[f]Activity expressed as enzyme units per mg protein.
[g,h,i,j]Means within row with different superscripts differ (P<.05).

Table 6. Least squares means of enzyme activity after weaning (Exp. 1)

	Time[a]					
Enzyme	0	3	12	24	48	SEM[b]
CAT[c]	.0077[g]	.0071[g]	.0076[g]	.0091[h]	.0029[i]	.0004
CP[d]	.126[g]	.113[gh]	.114[gh]	.107[h]	.095[i]	.007
GPX[e]	24.13[g]	19.22[h]	19.32[h]	19.86[h]	21.42[h]	.97
CZSOD[f]	140.2[g]	138.0[g]	158.5[h]	138.6[g]	146.5[g]	4.19

[a]Hours after weaning.
[b]Standard error of the least squares means.
[c]Activity expressed as a rate constant (k).
[d]Activity expressed as enzyme units per mL of serum.
[e]Activity expressed as enzyme units per g hemoglobin.
[f]Activity expressed as enzyme units per mg protein.
[g,h,i,j]Means within row with different superscripts differ (P<.05).

Table 7. Least squares means of enzyme activity after weaning (Exp. 2)

	Time[a]										
Enzyme	0	3	6	12	18	24	36	48	60	72	SEM[b]
CAT[c]	.0129^{gh}	.0134^{gh}	.0137^{gh}	.0119^{g}	.0126^{gh}	.0135^{gh}	.0124^{gh}	.0143^{h}	.0141^{gh}	.0142^{gh}	.0005
CP[d]	.124	.122	.120	.123	.132	.129	.129	.129	.128	.128	.006
GPX[e]	46.99^{g}	49.94^{g}	48.71^{g}	34.56^{h}	52.37^{g}	47.30^{g}	69.13^{i}	58.53^{gi}	52.82^{g}	56.27^{g}	3.47
CZSOD[f]	108.88^{g}	104.13^{g}	108.60^{g}	80.66^{h}	104.20^{g}	110.37^{g}	103.62^{g}	108.37^{g}	126.26^{i}	117.46^{g}	3.48

[a]Hours after weaning.
[b]Standard Error of the least squares means.
[c]Activity expressed as a rate constant (k).
[d]Activity expressed as enzyme units per mL of serum.
[e]Activity expressed as enzyme units per g hemoglobin.
[f]Activity expressed as enzyme units per mg protein.
[g,h,i]Means within row with different superscripts differ (P<.05).

Table 8. Least squares means of enzyme activity after move to grow-finish room (Exp. 1)

	Time[a]				
Enzyme	0	6	24	48	SEM[b]
CAT[c]	.0034[g]	.0090[h]	.0042[i]	.0041[i]	.0002
CP[d]	.160[g]	.152[g]	.166[g]	.183[h]	.005
GPX[e]	34.90[g]	30.26[gh]	24.53[h]	36.00[g]	1.94
CZSOD[f]	127.6[gh]	195.0[i]	147.0[h]	117.6[g]	5.71

[a]Hours after move to grow-finish room.
[b]Standard error of the least squares means.
[c]Activity expressed as a rate constant (k).
[d]Activity expressed as enzyme units per mL of serum.
[e]Activity expressed as enzyme units per g hemoglobin.
[f]Activity expressed as enzyme units per mg protein.
[g,h,i]Means within row with different superscripts differ (P<.05).

Figure 1. CZSOD activity post iron injection (Exp. 1)

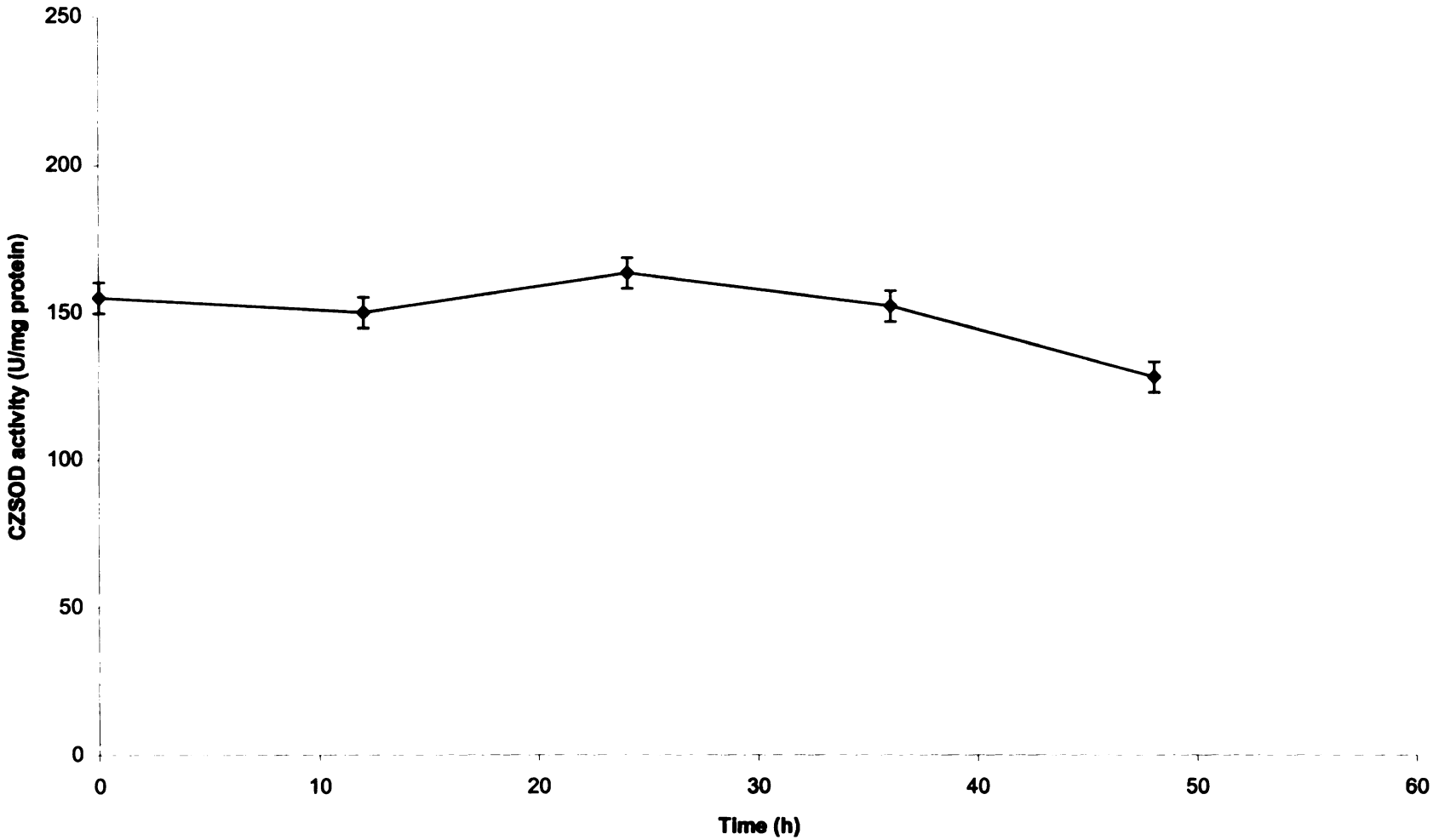


Figure 2. GPX activity post iron injection (Exp. 1)

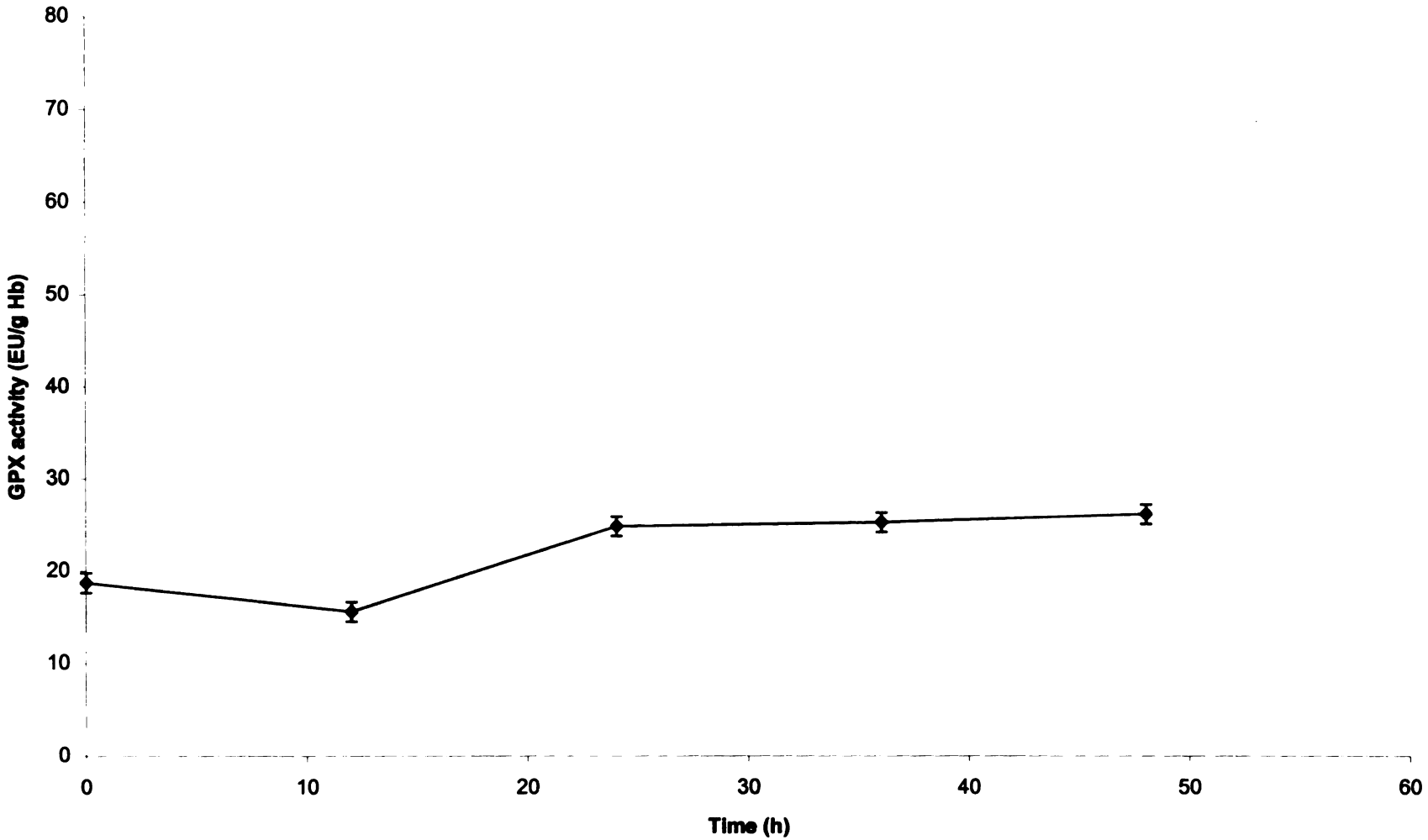


Figure 3. CAT activity post iron injection (Exp. 1)

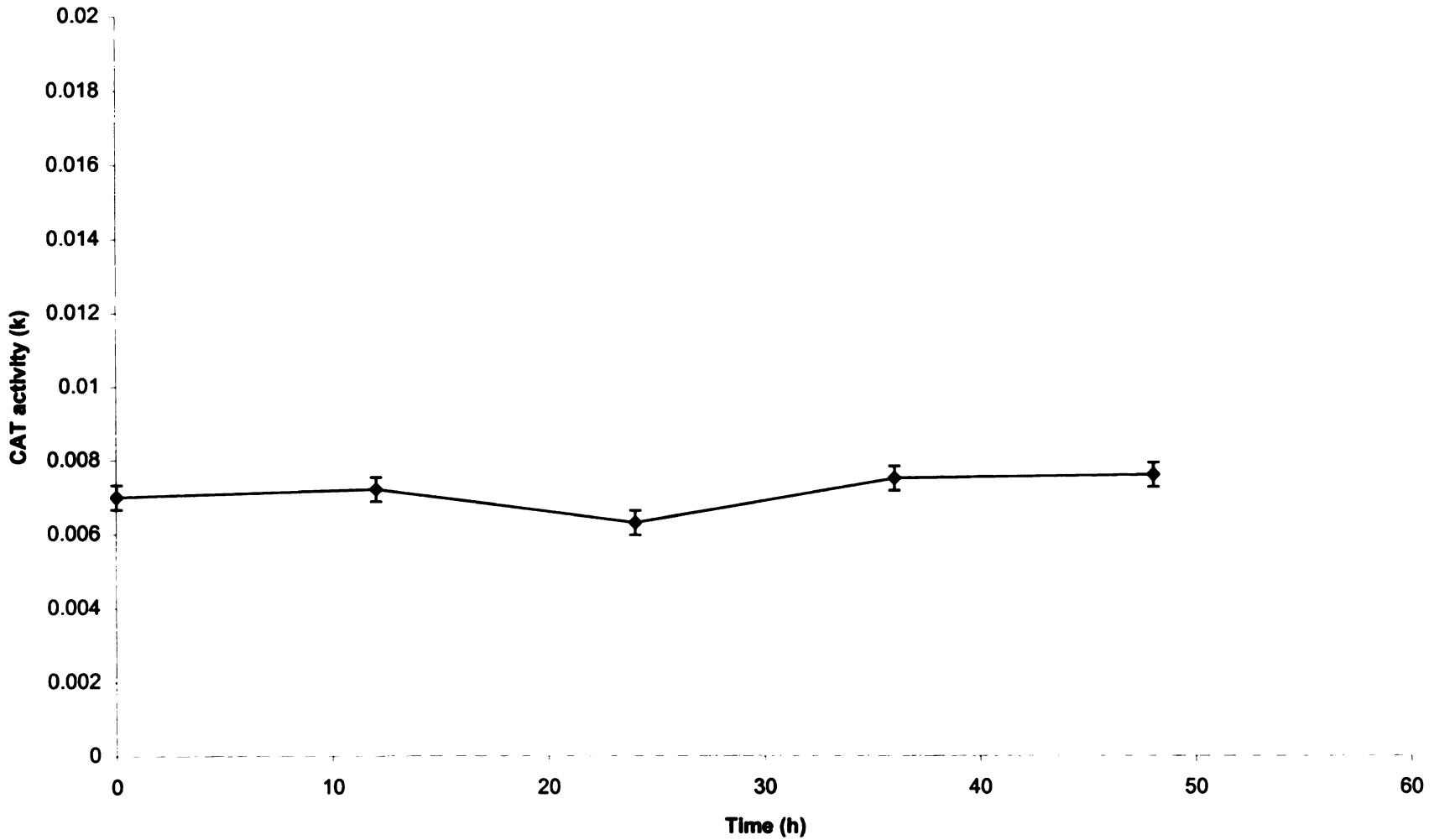


Figure 4. CP activity post iron injection (Exp. 1)

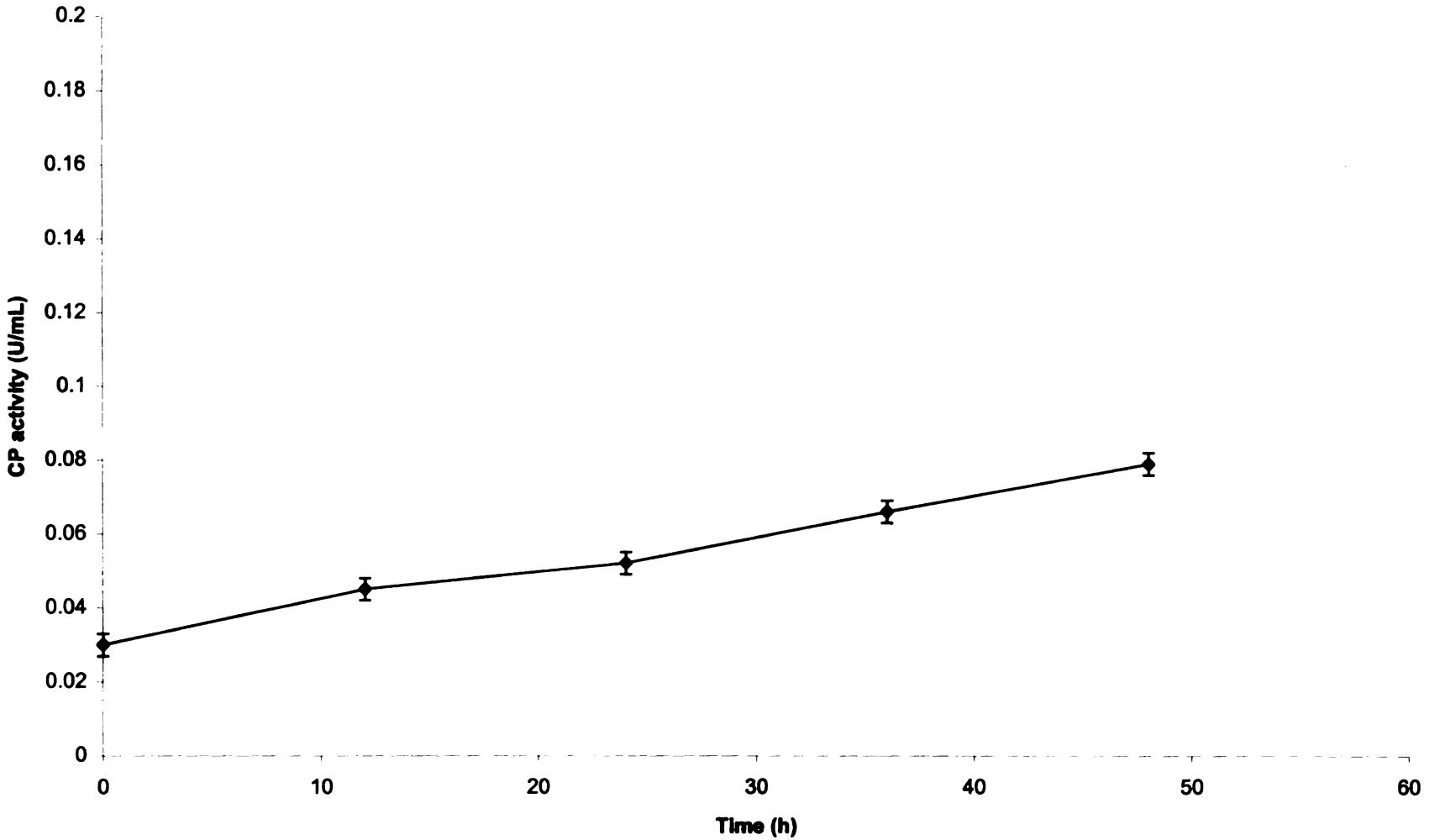


Figure 5. CZSOD activity post weaning (Exp. 1)

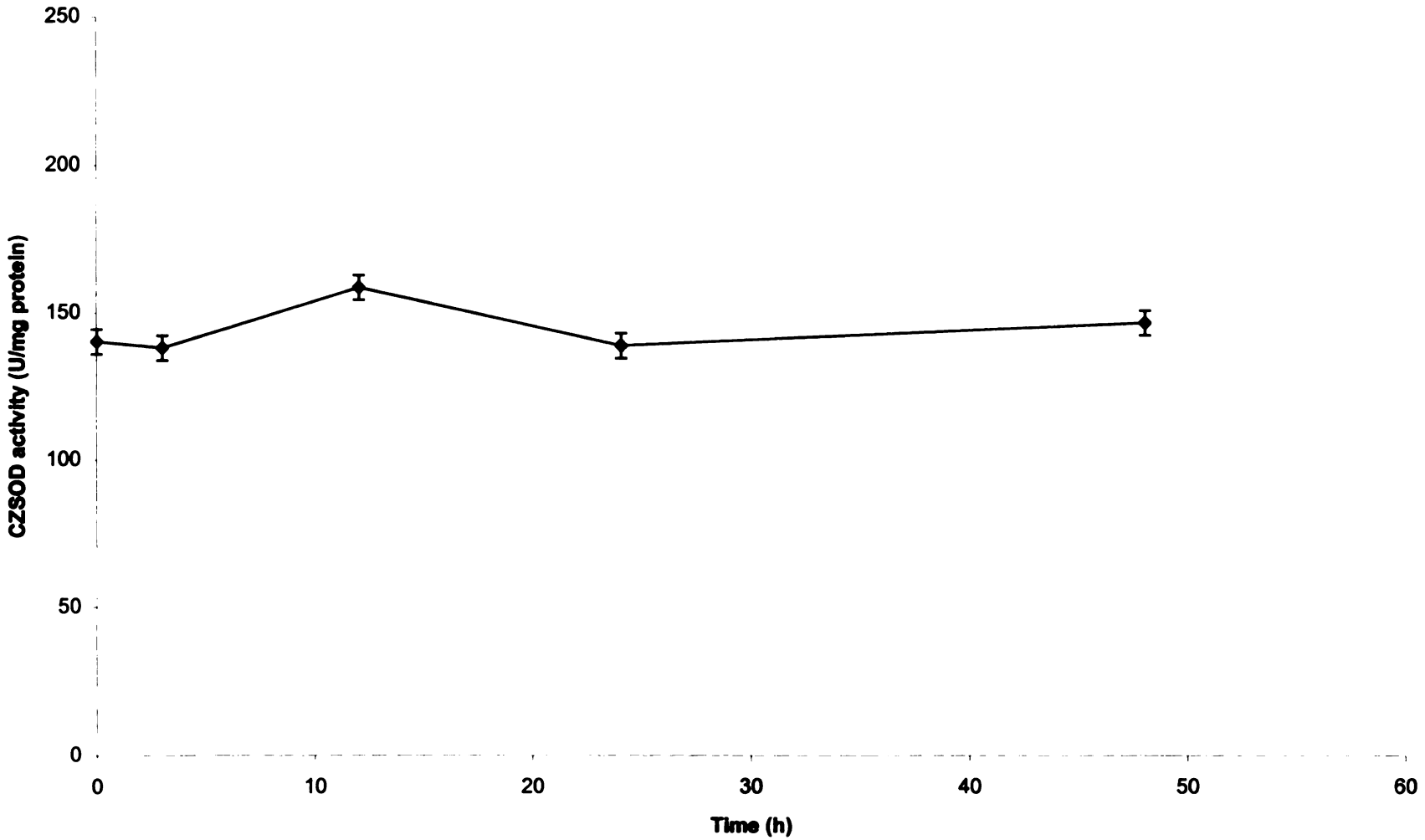


Figure 6. GPX activity post weaning (Exp. 1)

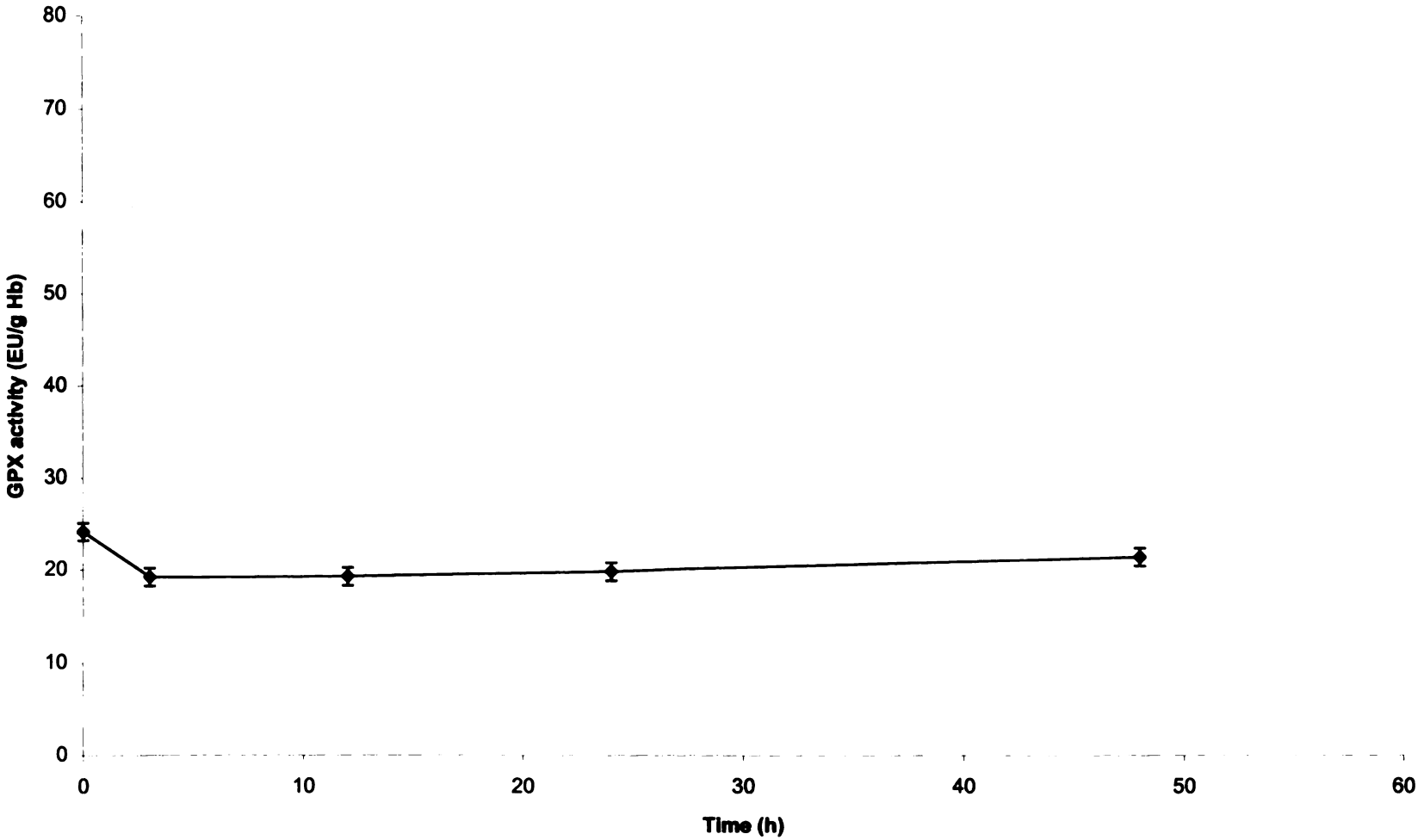


Figure 7. CAT activity post weaning (Exp. 1)

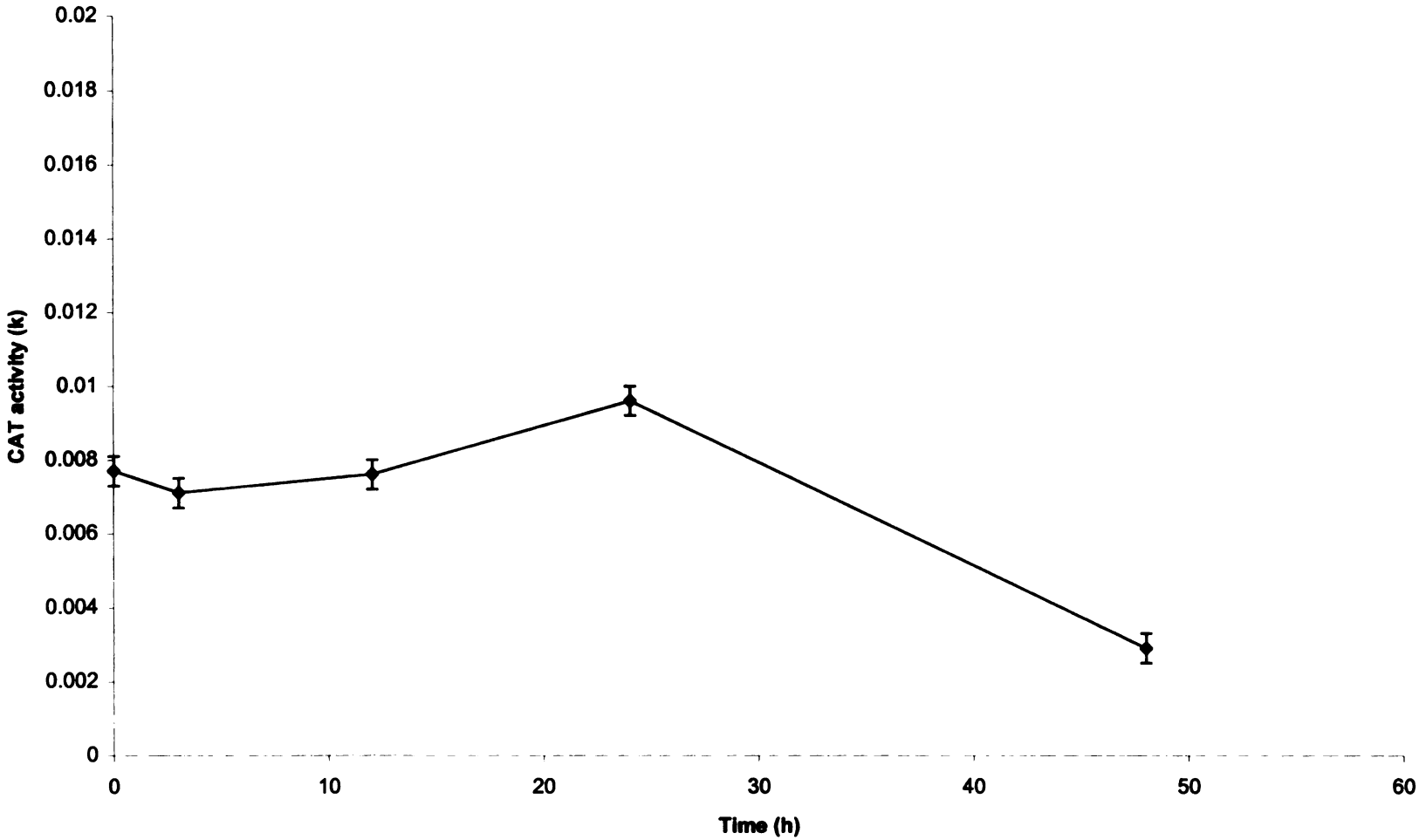


Figure 8. CP activity post weaning (Exp. 1)

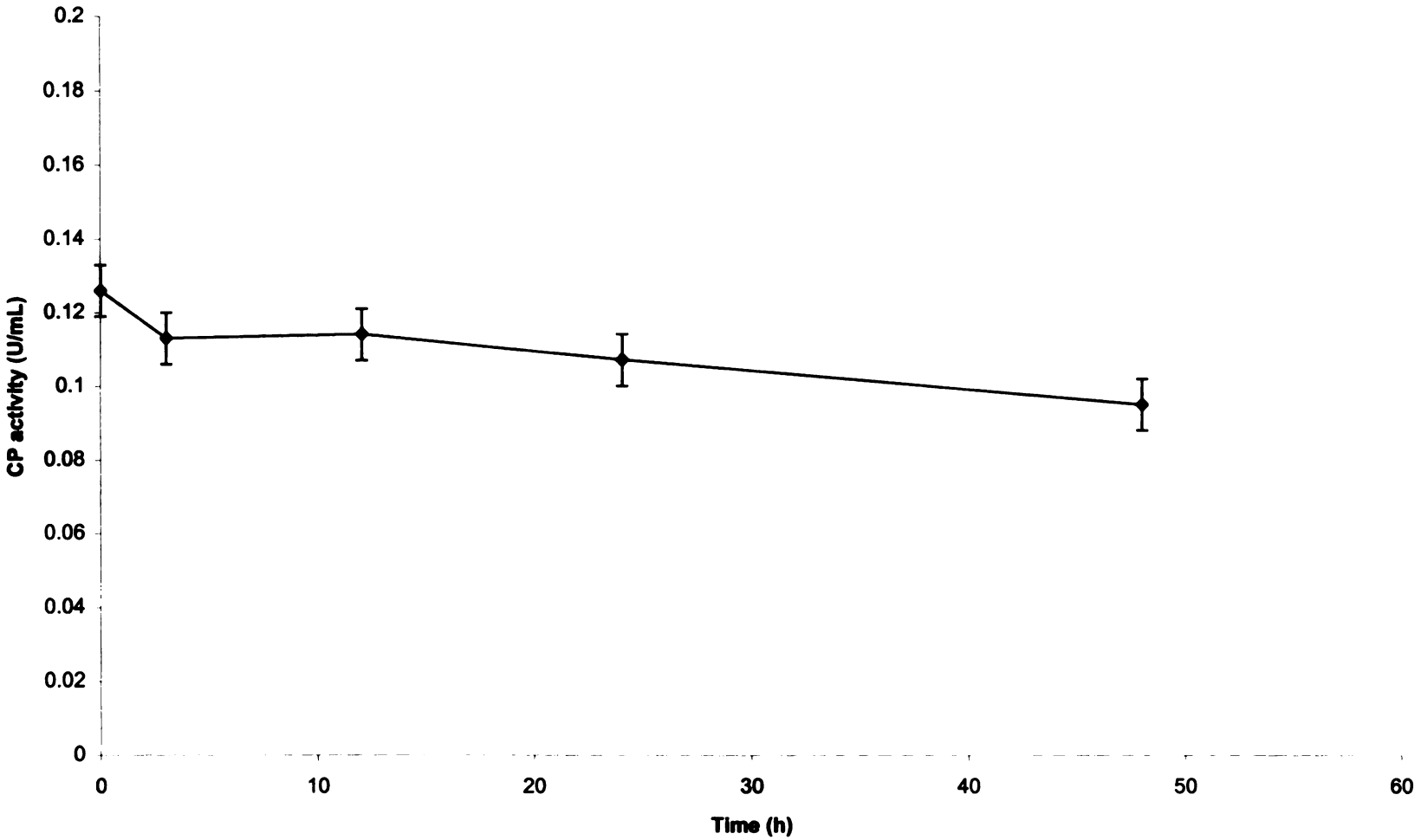
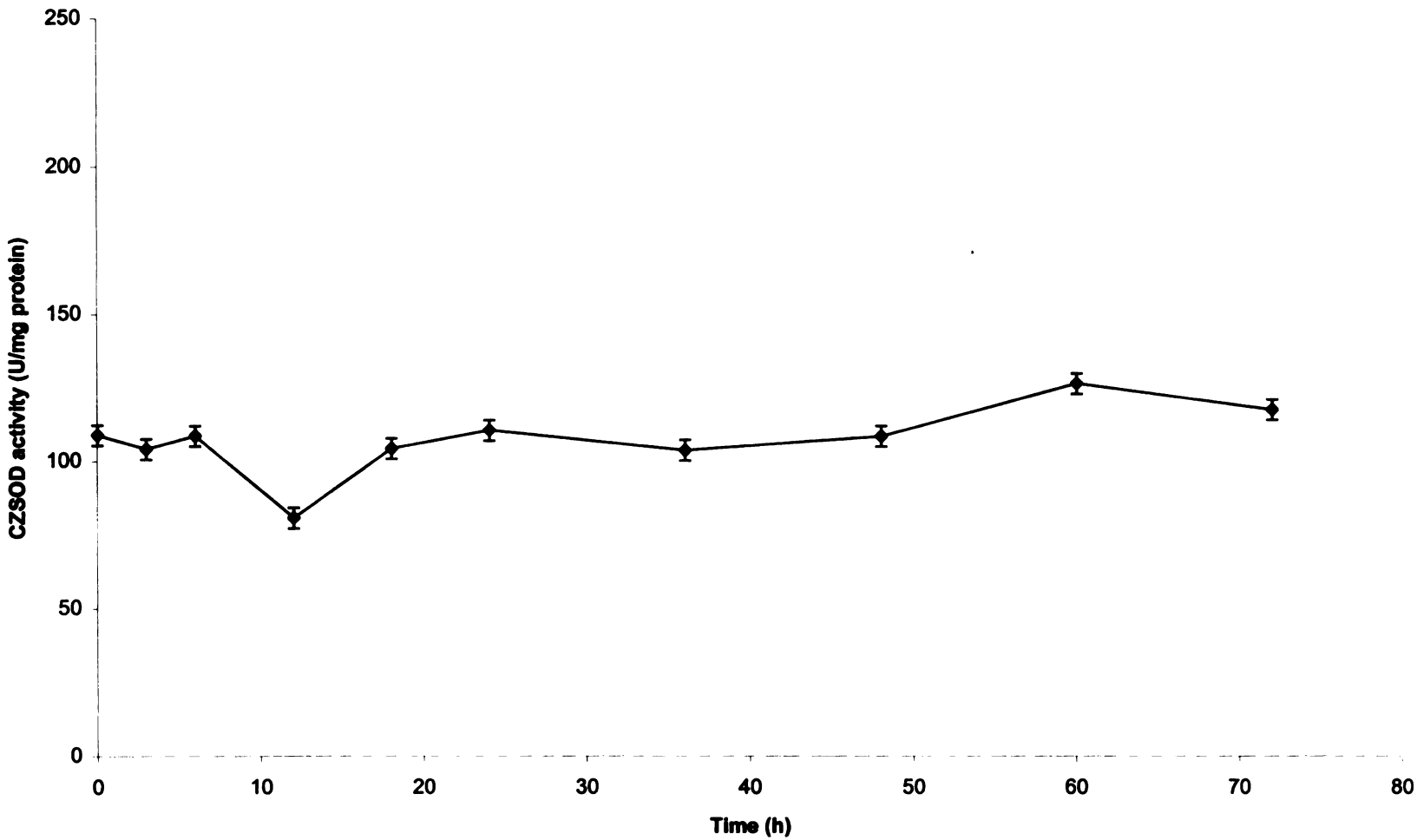


Figure 9. CZSOD activity post weaning (Exp. 2)



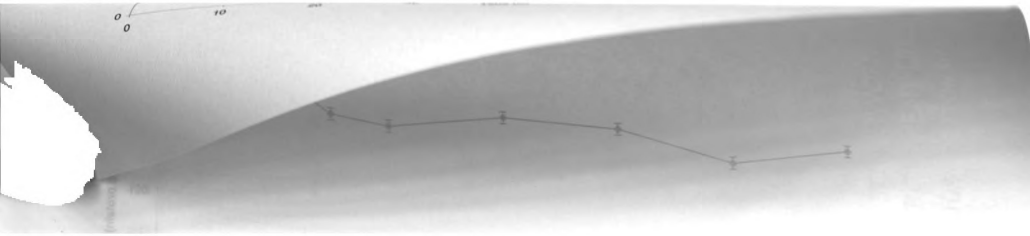

0
0
10

Figure 10. GPX activity post weaning (Exp. 2)

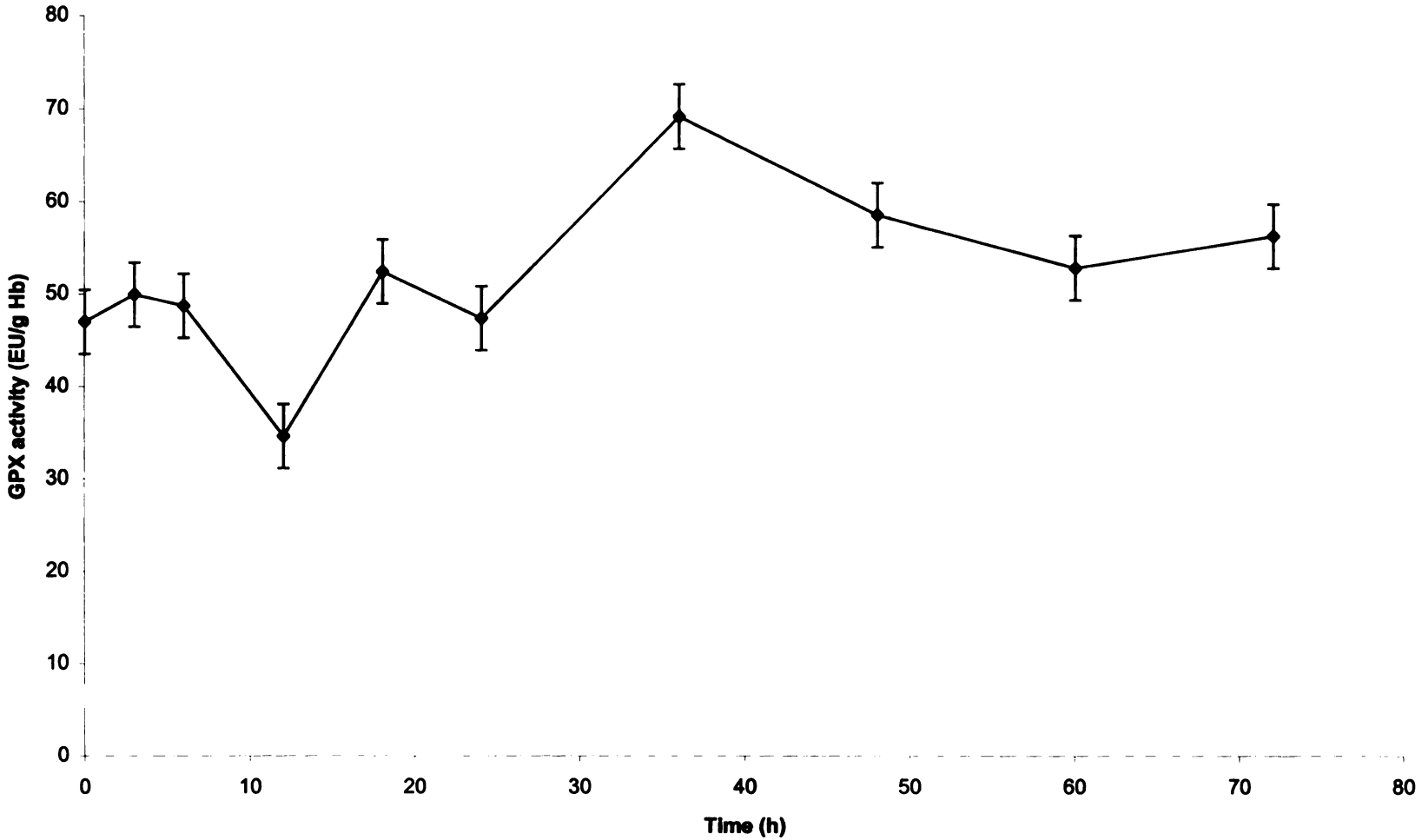


Figure 11. CAT activity post weaning (Exp. 2)

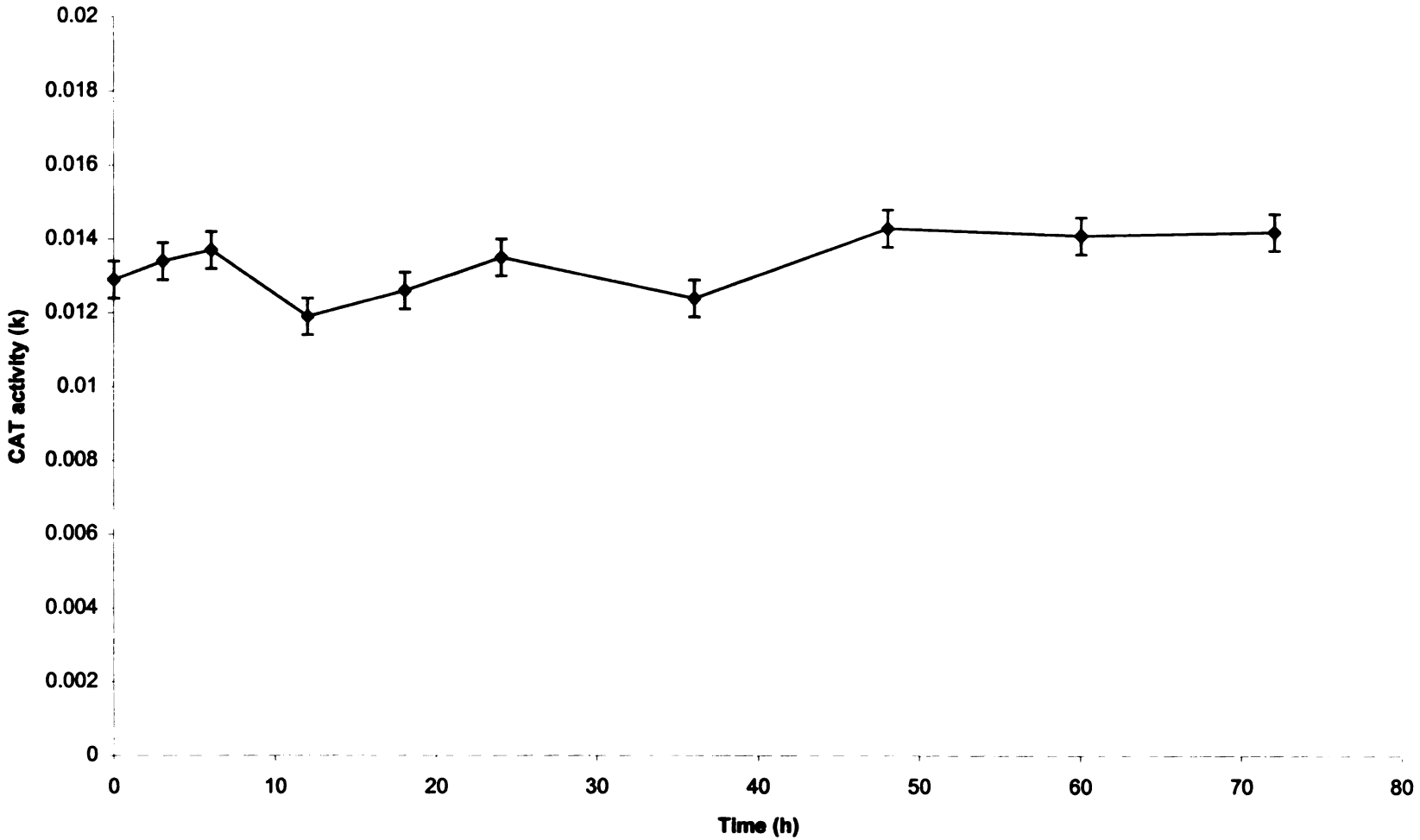


Figure 12. CP activity post weaning (Exp. 2)

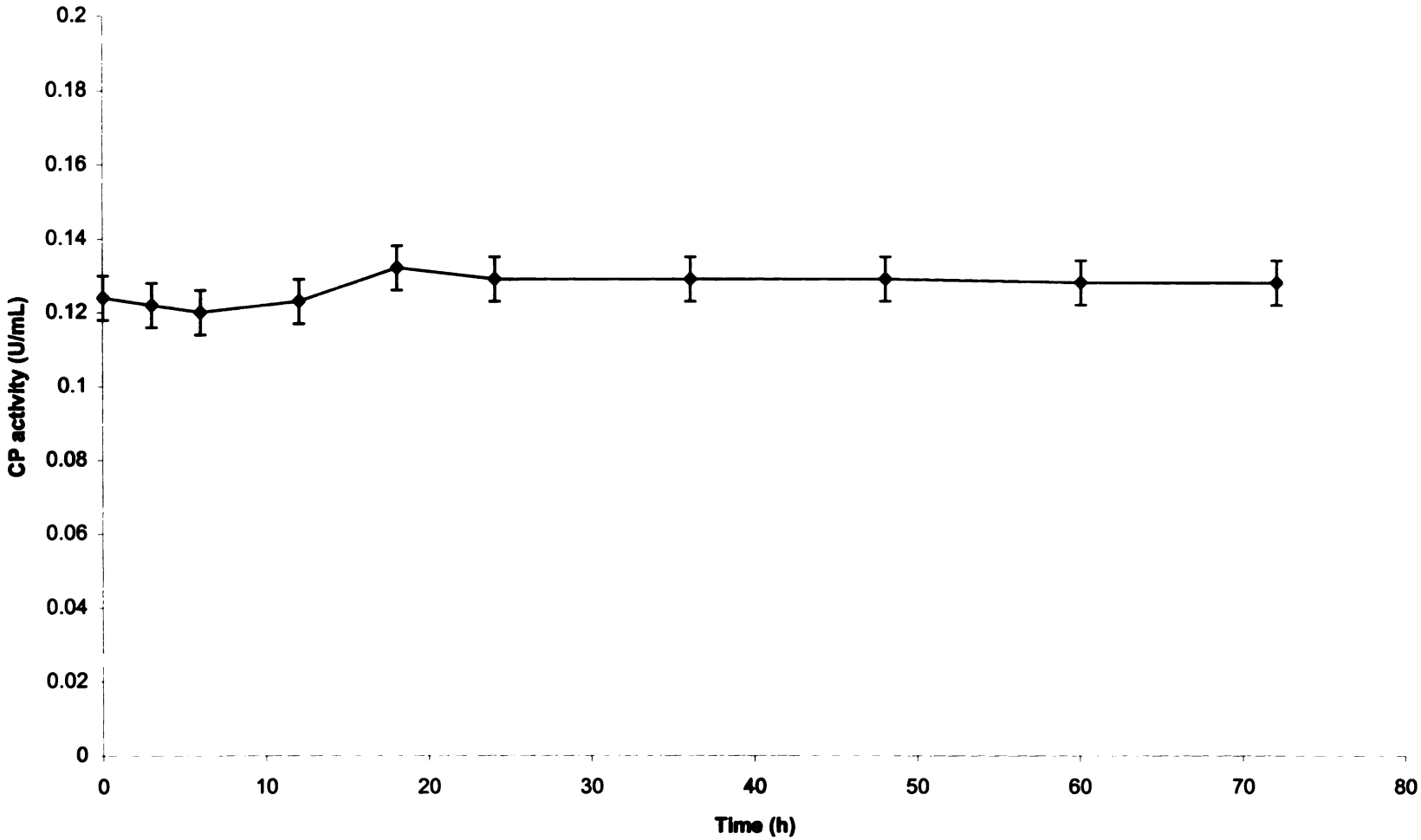


Figure 13. CZSOD activity post moving and comingling (Exp. 1)

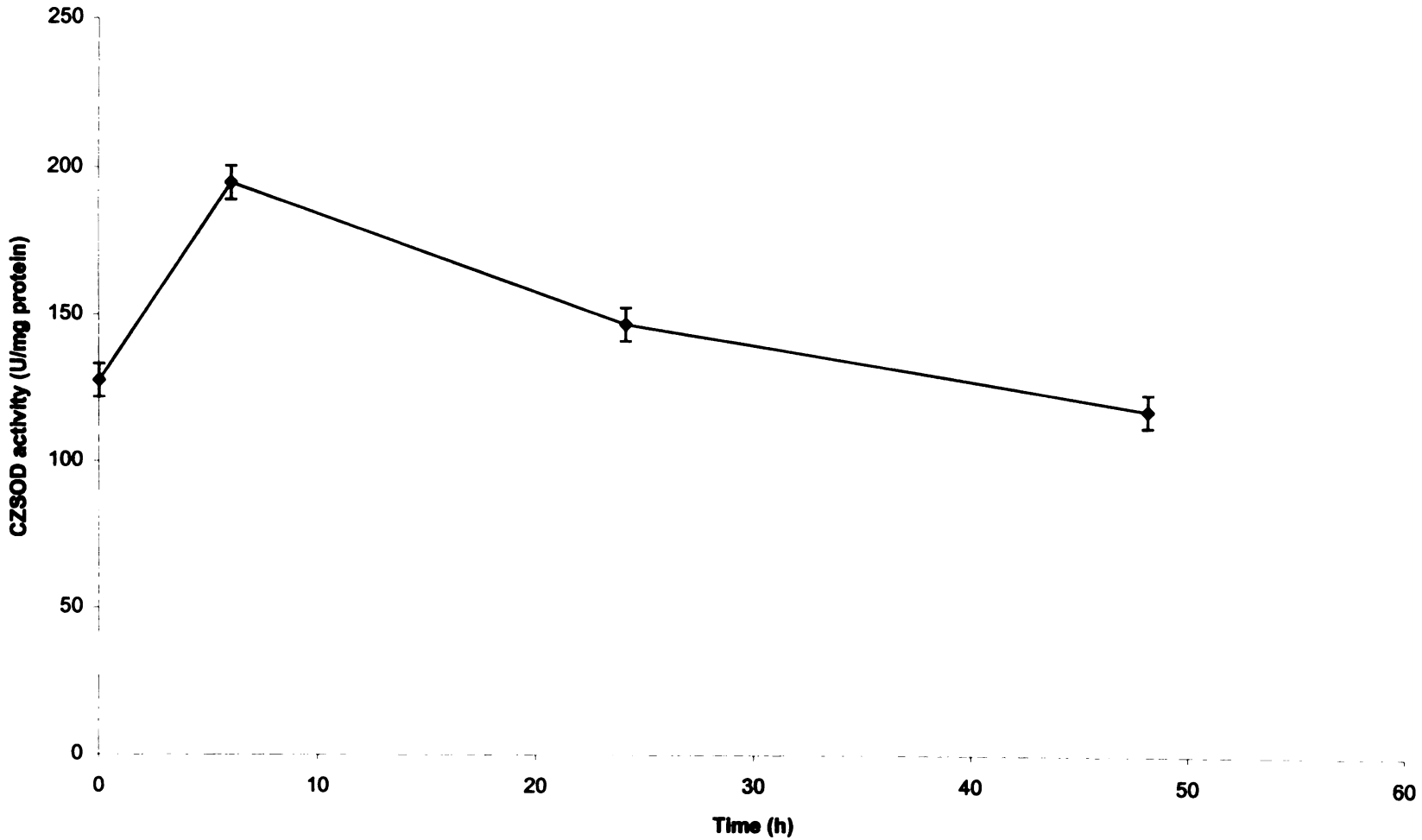


Figure 7. CAT activity post weaning (Exp. 1)

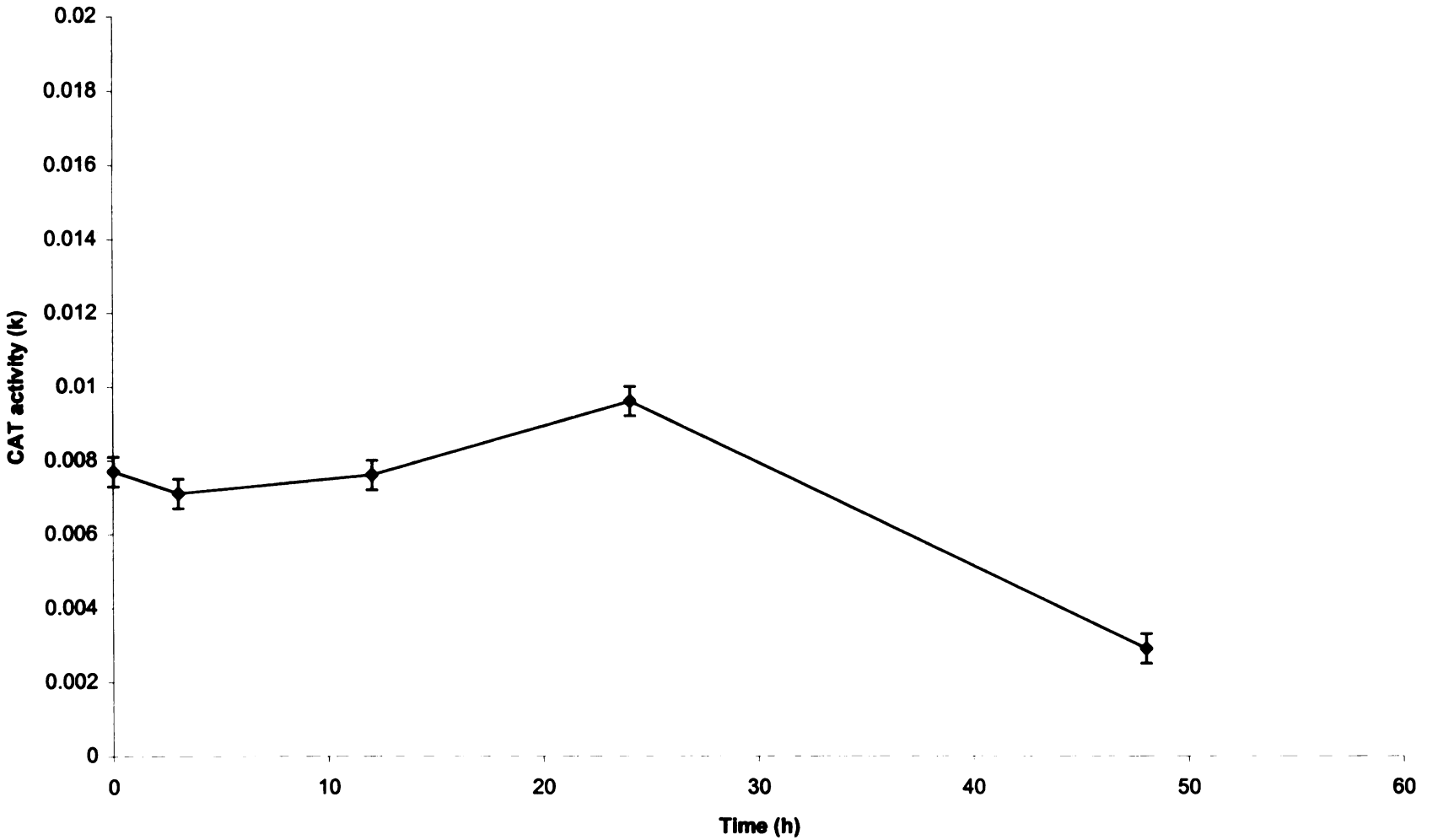


Figure 8. CP activity post weaning (Exp. 1)

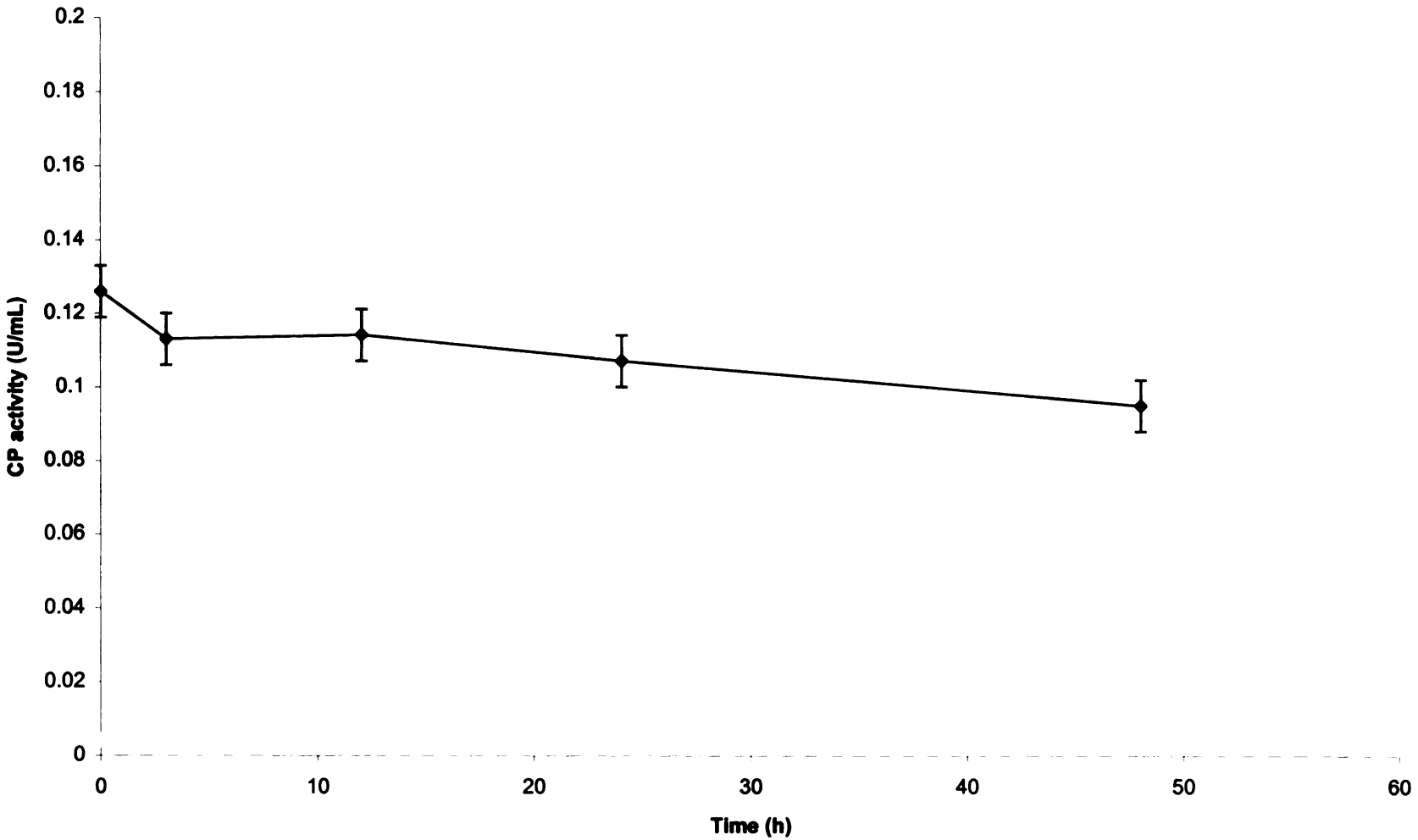


Figure 9. CZSOD activity post weaning (Exp. 2)

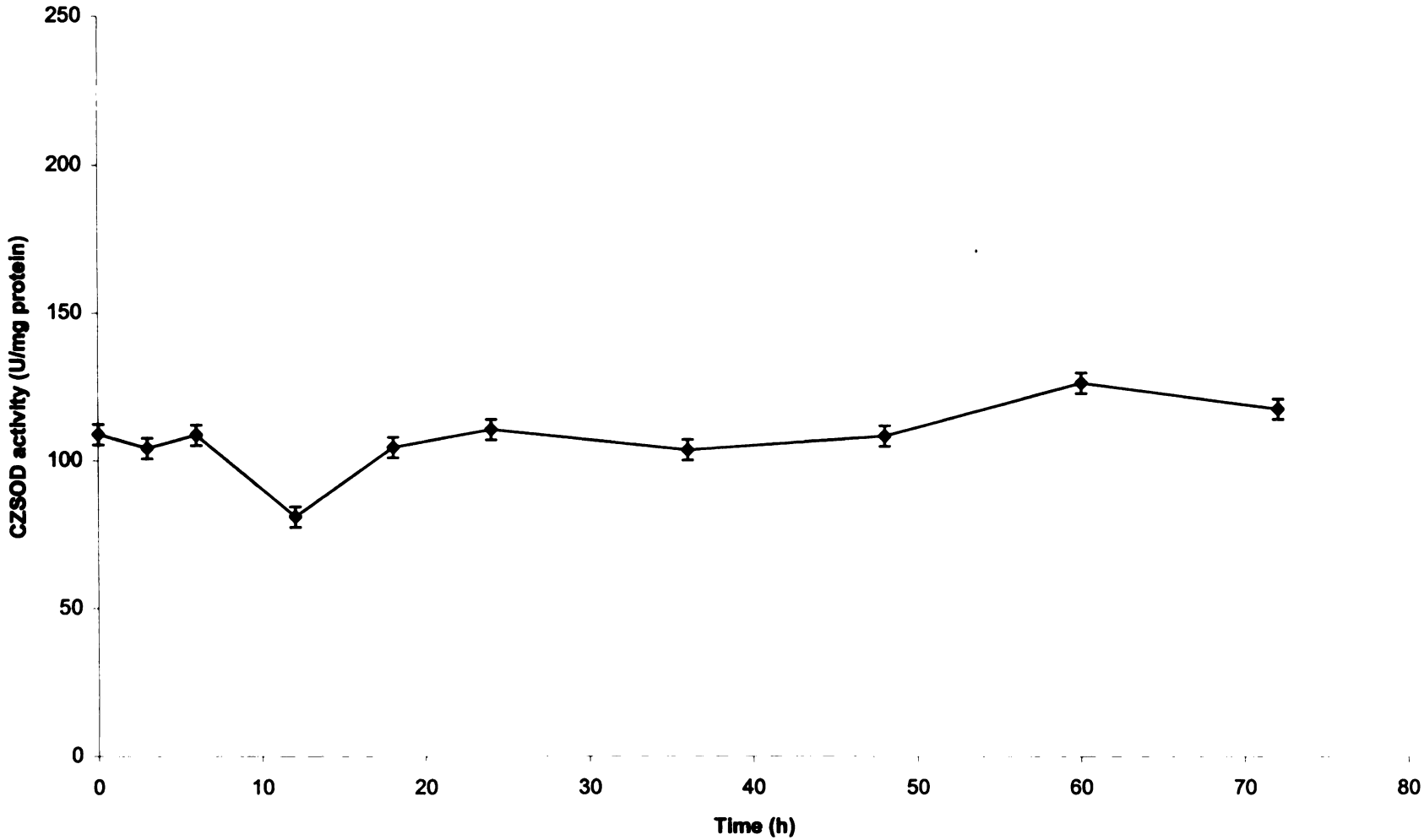


Figure 10. GPX activity post weaning (Exp. 2)

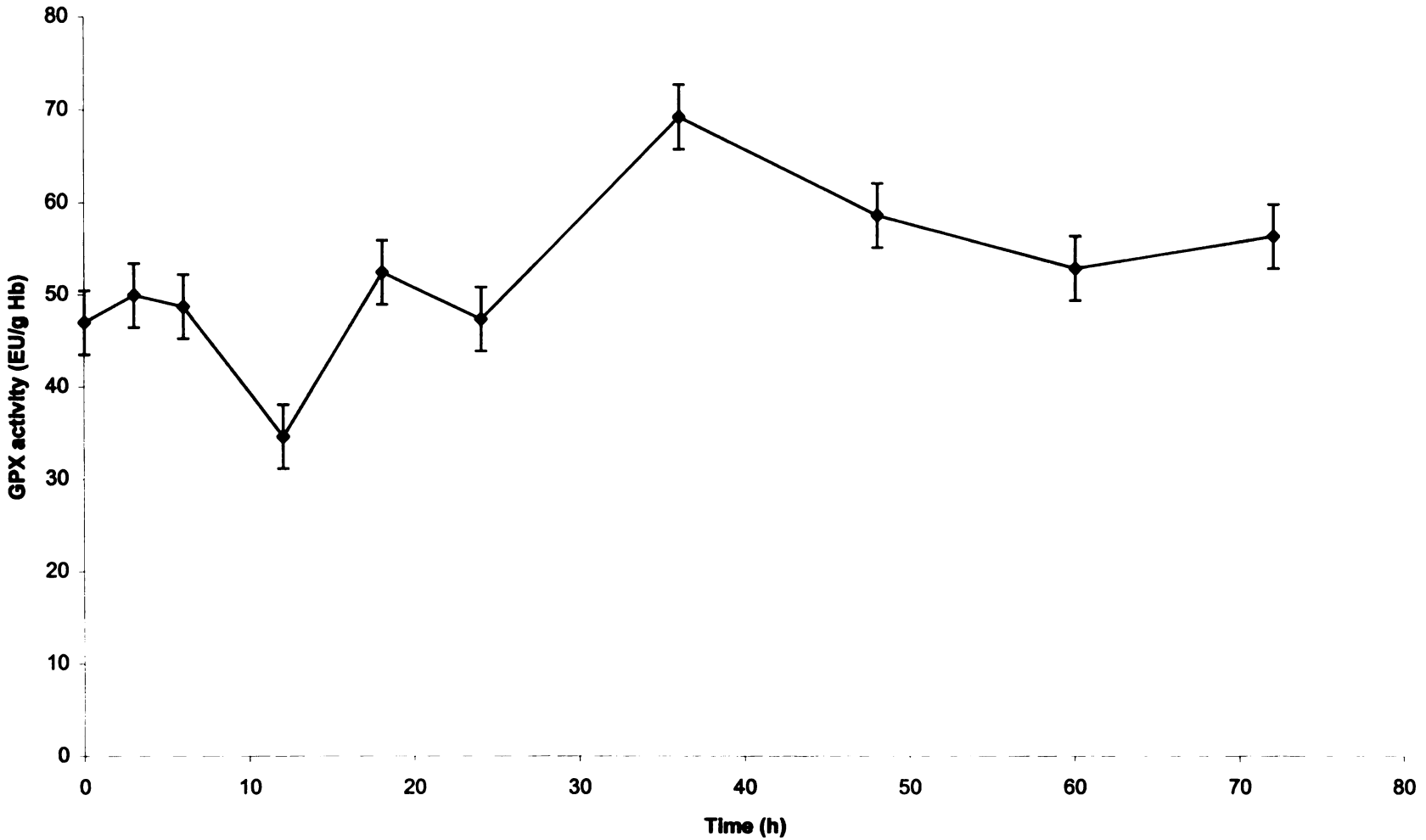


Figure 11. CAT activity post weaning (Exp. 2)

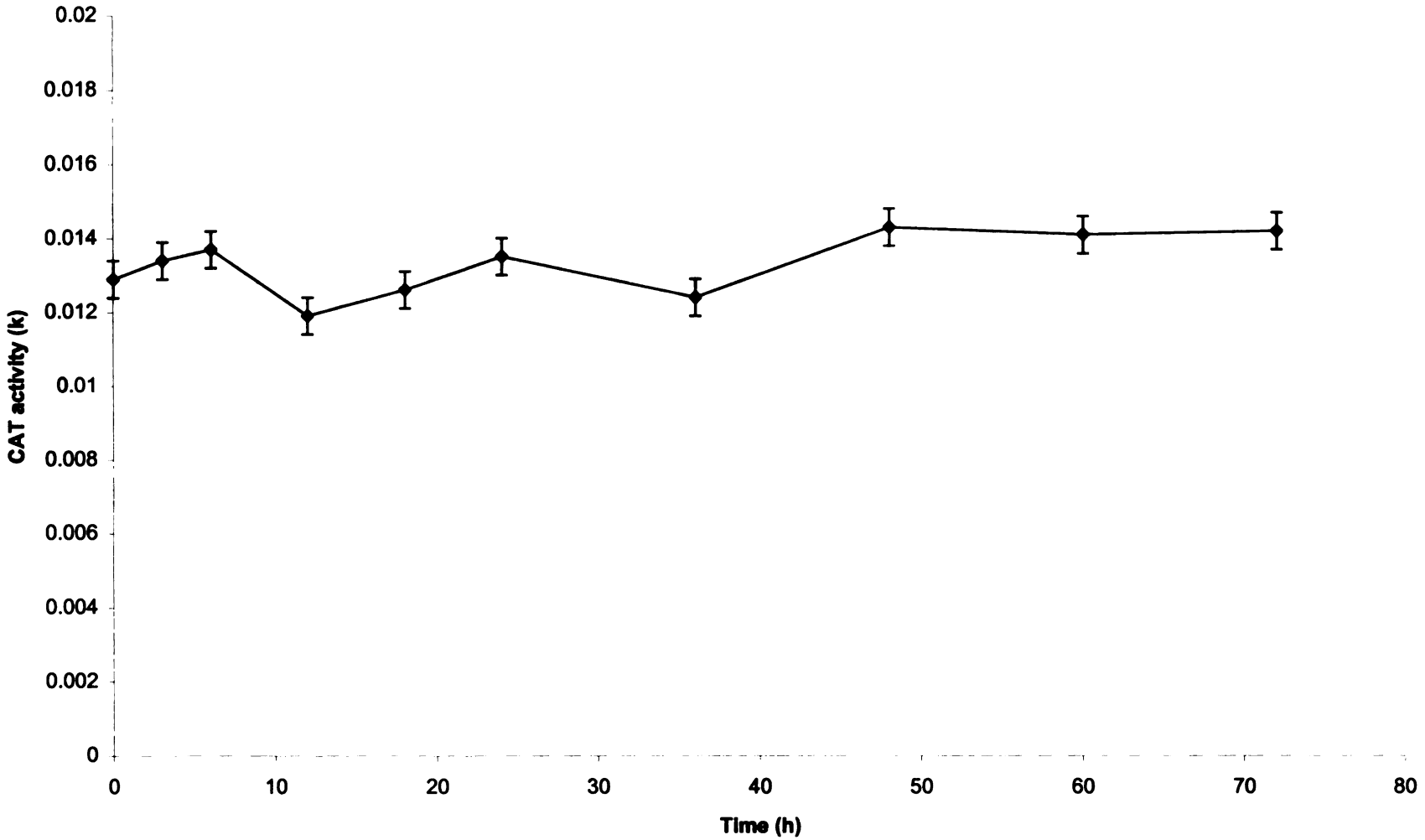


Figure 12. CP activity post weaning (Exp. 2)

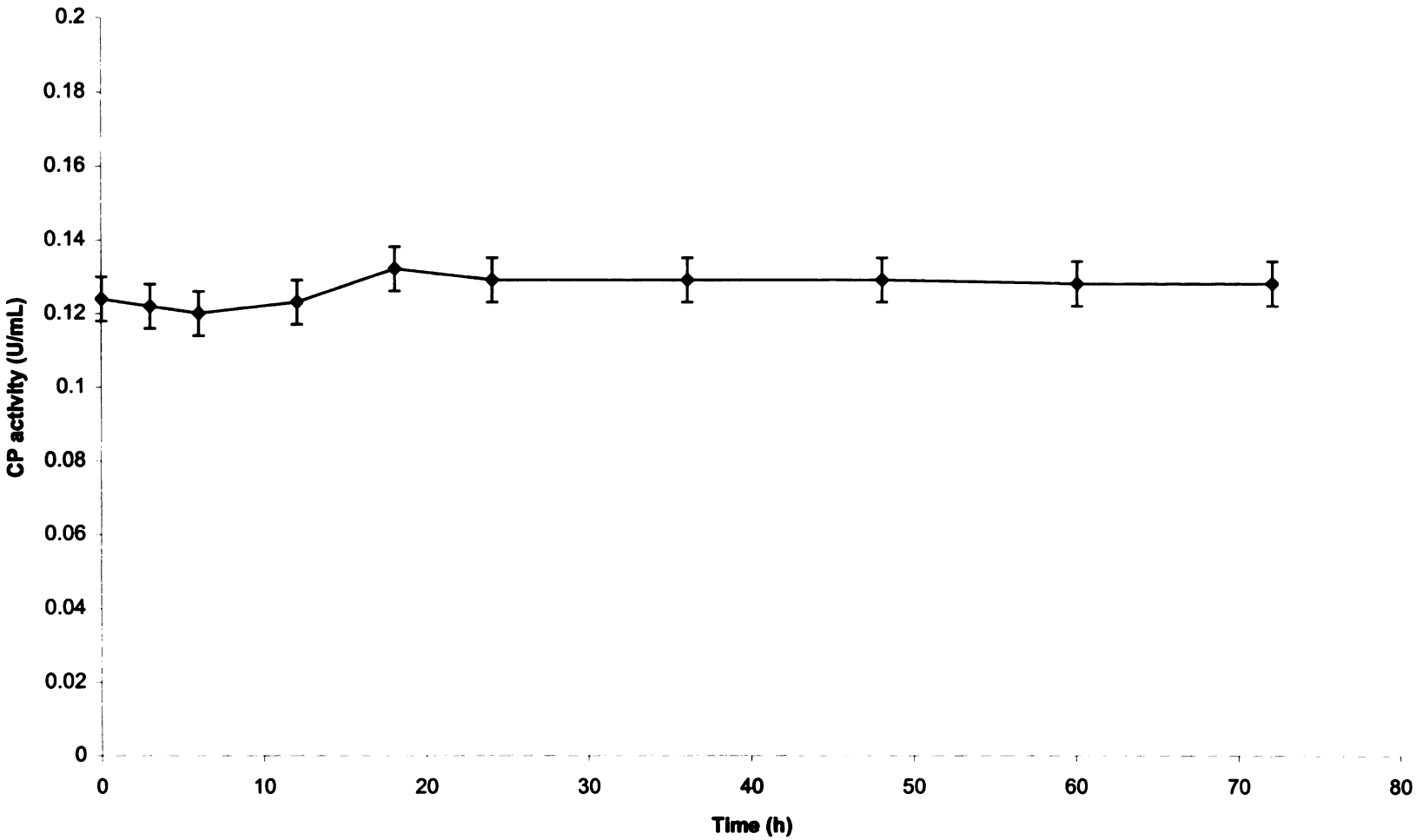


Figure 13. CZSOD activity post moving and comingling (Exp. 1)

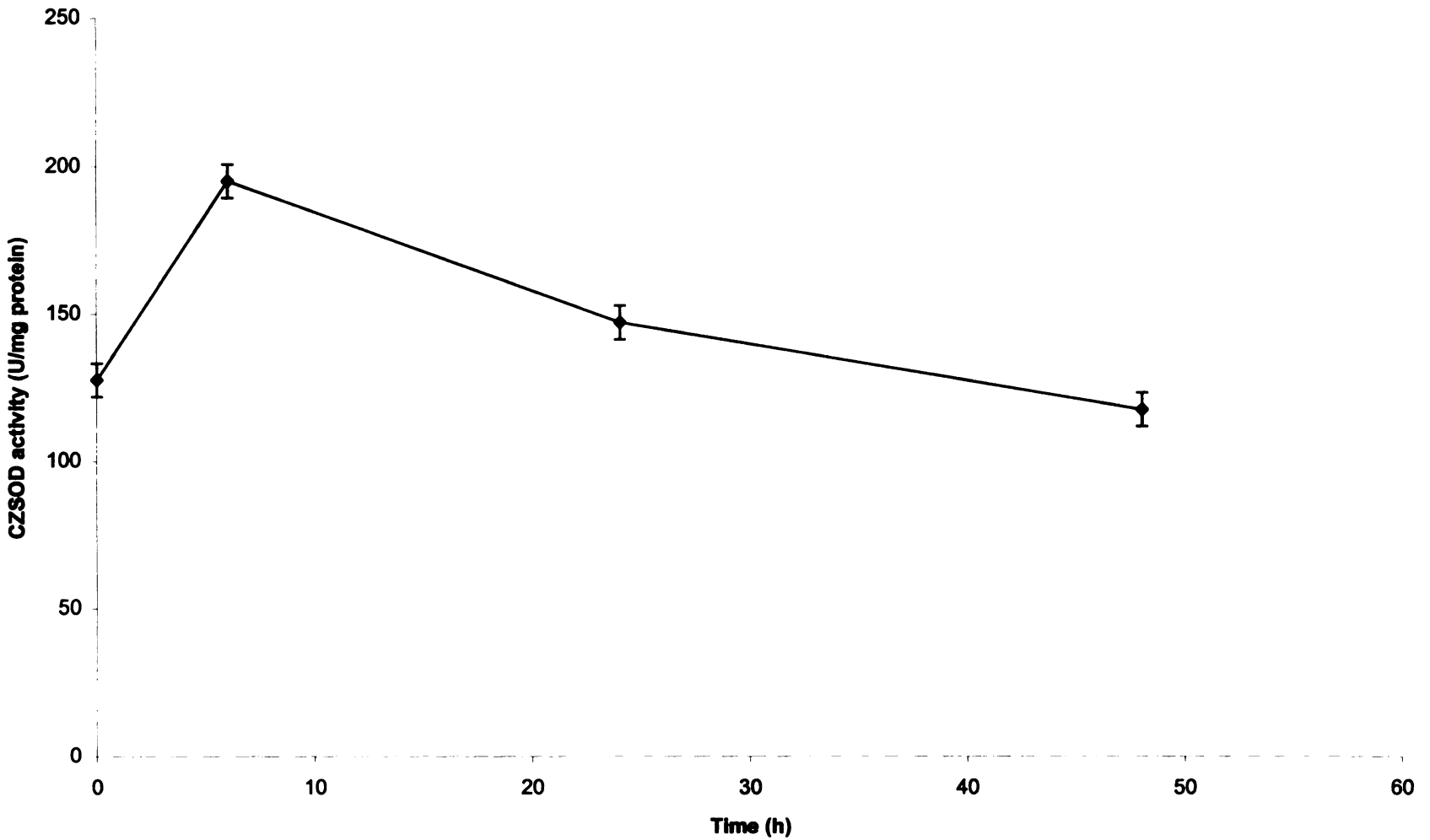


Figure 14. GPX activity post moving and comingling (Exp. 1)

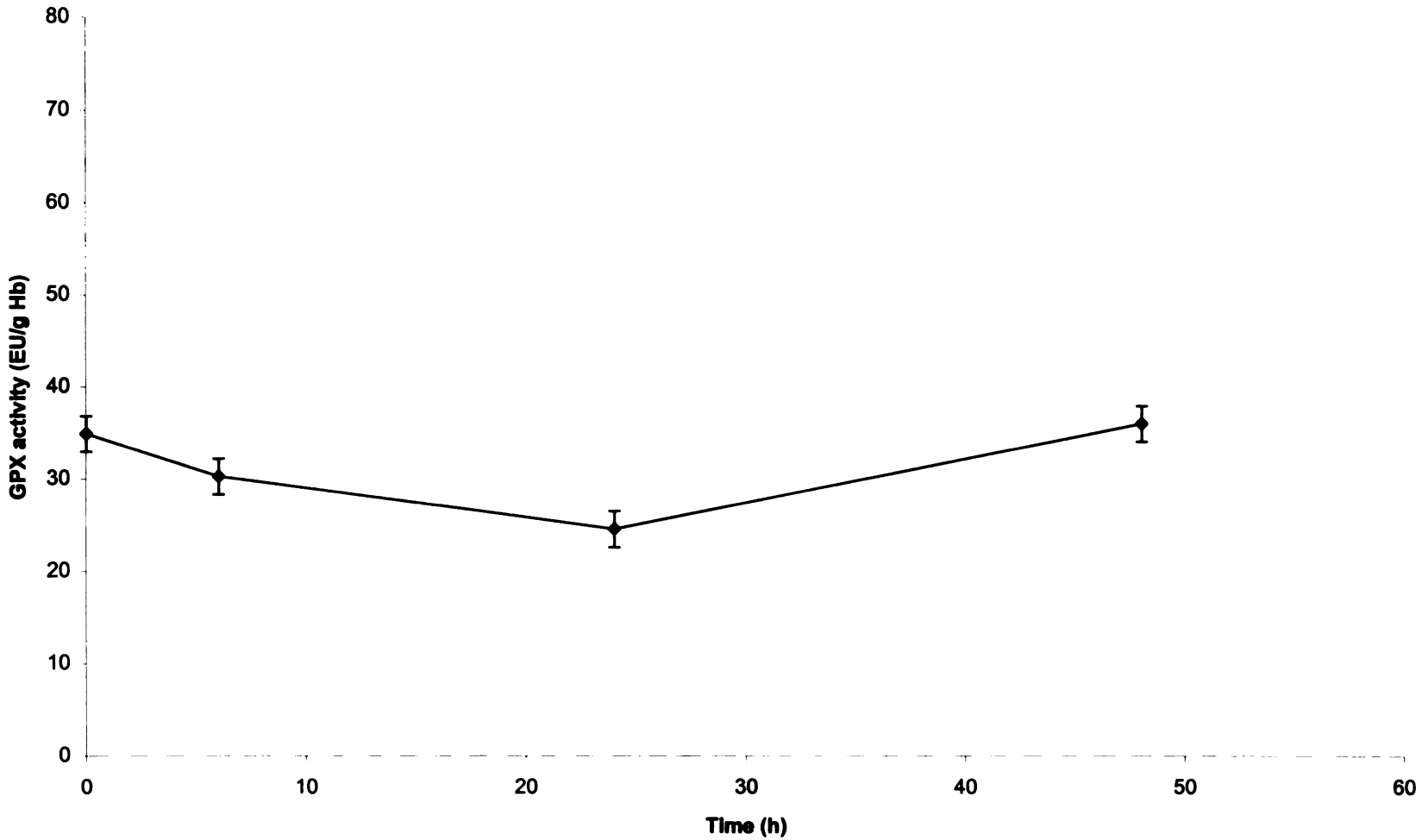


Figure 15. CAT activity post moving and comingling (Exp. 1)

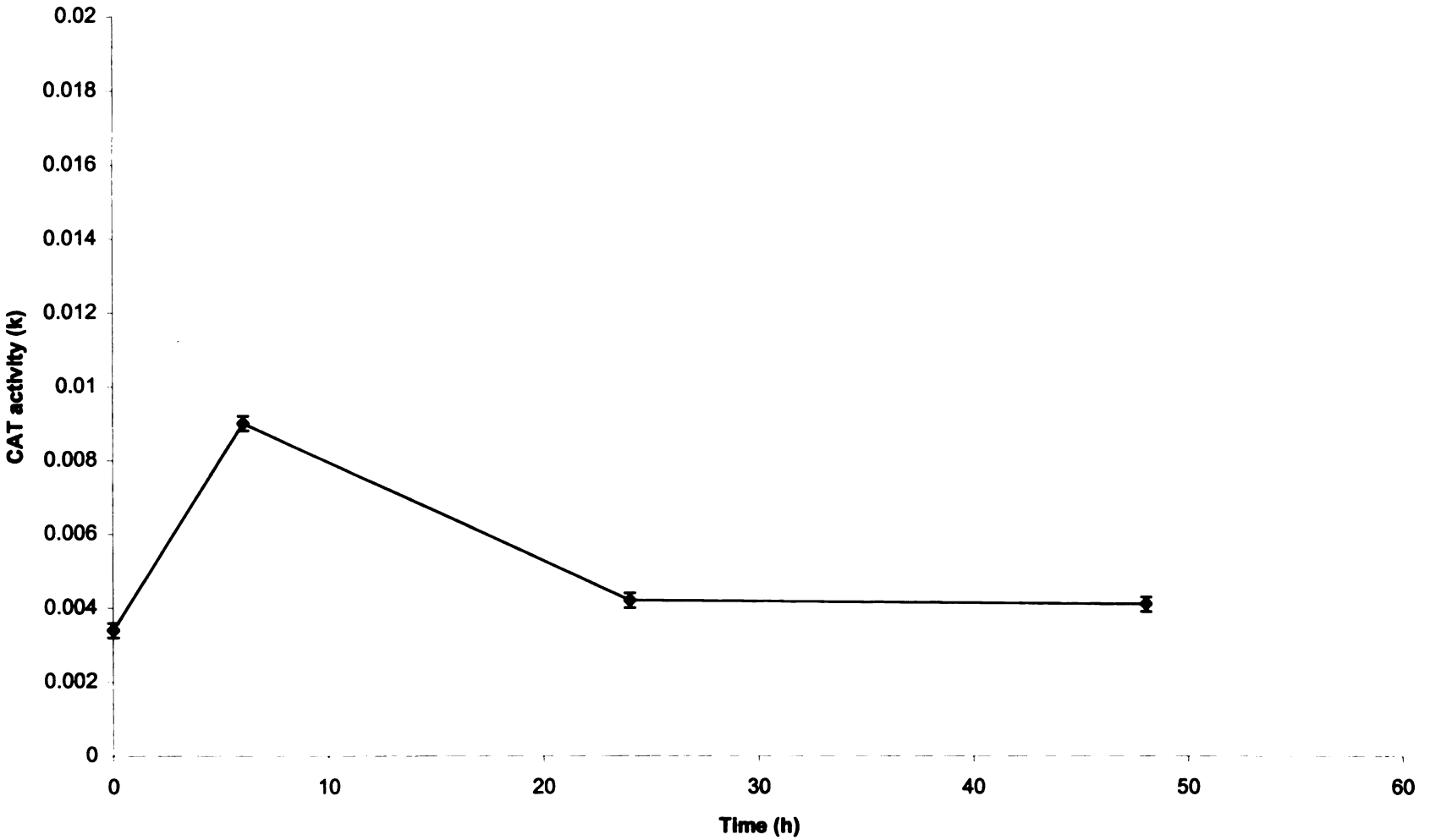
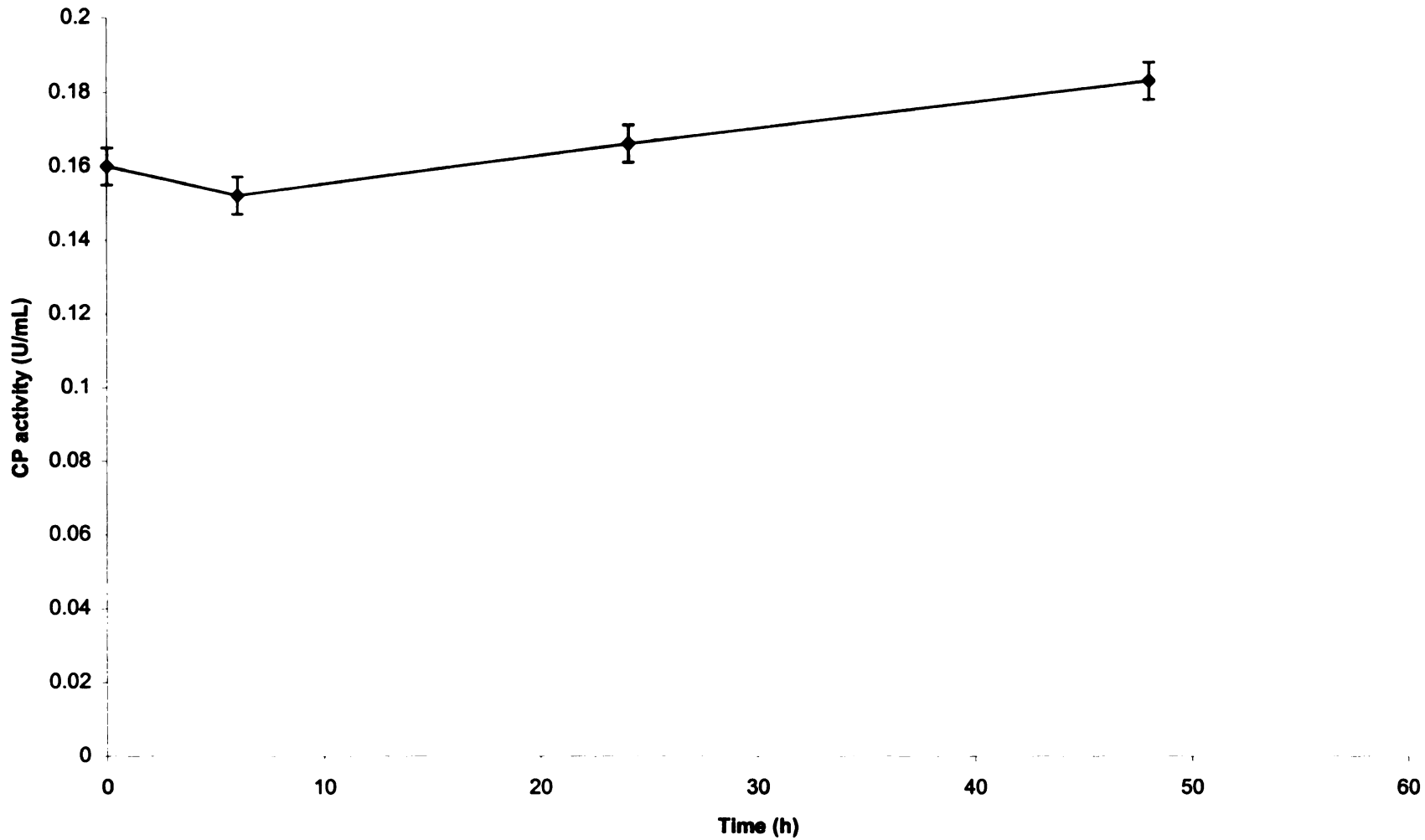


Figure 16. CP activity post moving and comingling (Exp. 1)



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APPENDICES

APPENDIX A

Copper, Zinc dependent superoxide dismutase method

Red blood cells for CZSOD activity analysis were obtained from centrifugation. Red blood cells were washed three times in two volumes of ice-cold 0.9% phosphate buffered saline. Between each wash, cells were centrifuged at 4°C, 2000 x g, for 10 min (Beckman GS-6KR, Palo Alto, CA) and saline was removed by aspiration. After the third and final wash, cells were hemolyzed in ice-cold deionized distilled water in a 1 to 2 ratio in Exp. 1 and 1 to 1 ratio in Exp. 2 and frozen at -80°C until analysis.

Red blood cell hemolysates were extracted with 0.6 volumes of ice-cold ethanol, 95% (Aaper Alcohol and Chemical Co., Louisville, KY): chloroform, 99.8% (JT Baker, Phillipsburg, NJ) (25:15) to inactivate the manganese dependent SOD (SOD; EC 1.15.1.1). After ethanol: chloroform addition, samples were mixed at speed 3 on a Fisher Vortex Genie 2 (Fisher Scientific, Bohemia, NY) and centrifuged at 4°C, 5000 x g, for 15 min (Beckman GS-6KR, Palo Alto, CA). Aliquots of clear supernatant from red blood cells were diluted in a range of 1:20 to 1:40 with 50 mM Tris-HCl, 1.0 mM diethylenetriamine pentaacetic acid (DTPA) buffer. The dilution factors increased as the gilts aged to ensure 50% inhibition of the autooxidation of pyrogallol. The reaction mixture in three semimicro cuvettes of the DU 7000 spectrophotometer (Beckman, Palo Alto, CA) contained varying amounts of Tris-HCl-DTPA buffer, 50 μL of 10 mM sodium azide, and varying amounts of supernatant to equal a total volume of 950 μL. Semimicro cuvettes were preincubated at 25°C for 5 min, and finally 50 μL of 4 mM pyrogallol was added to initiate the dismutation of the peroxyl radical. Protein concentration of the supernatant was determined by the method of Lowry et al. (1951) and results were expressed as units of SOD enzyme activity per mg protein.

APPENDIX B

Glutathione peroxidase method

Red blood cells were obtained in the same manner as for SOD and CAT analysis. The red cells were diluted and lysed 1:125 with ice-cold deionized distilled water. The reaction mixture in semimicro cuvettes of DU-7000 spectrophotometer (Beckman, Palo Alto, CA) consisted of 40 μL of deionized distilled water, 100 μL of diluted sample, 100 μL of 0.1 mM β-nicotinamide adenine dinucleotide phosphate, NADPH (Sigma Chemical, St. Louis, MO), 100 μL of 10 EU/ mL glutathione reductase (Sigma Chemical, St. Louis, MO), 100 μL of 10 mM sodium azide, and 50 μL of 40 mM reduced glutathione (Sigma Chemical, St. Louis, MO) in 500 μL 0.2 M phosphate buffer, pH 7.0 containing 6 mM ethylenediaminetetraacetic acid (EDTA) (Sigma Chemical, St.Louis, MO) for a total volume of 990 μL. Cuvettes were preincubated at 25°C for 5 min, and finally 10 μL of 12 mM hydrogen peroxide were added to the reaction mixture to initiate the oxidation of NADPH. To determine the nonenzymatic oxidation of NADPH, a blank was prepared by adding 100 μL of deionized distilled water instead of the diluted sample to the reaction mixture. Hemoglobin content of the red cells was determined using Sigma Diagnostics hemoglobin kit (Sigma Chemical, Catalog number: 525-A, St. Louis, MO). The reaction mixture contained 5 mL of Drabkin's Reagent (Sigma Chemical, Catalog number 525-2, St. Louis, MO), and 10 μL red blood cells. The hemoglobin concentration of the reaction mixture was determined on the DU700 spectrophotometer (Beckman, Palo Alto, CA) by a comparison to a standard curve. Working standards were prepared to determine the standard curve by pipetting and mixing varying amounts of Drabkin's solution (Sigma Chemical, Catalog number 525-2, St. Louis, MO) and Hemoglobin standard solution (Sigma Chemical, Catalog number 525-18, St. Louis, MO). Results were expressed as units of GPX enzyme activity per g hemoglobin.

APPENDIX C

Catalase method

Red blood cell hemolysates were obtained in the same manner as for the SOD analysis. Hemolysates were diluted 1:700 with ice-cold 50 mM phosphate buffer, pH 7.0 immediately before the assay was performed. A blank was prepared in one cuvette to contain 2 mL diluted sample and 1 mL 50 mM phosphate buffer, pH 7.0. The reaction mixture in two cuvettes of the DU 7000 spectrophotometer (Beckman, Palo Alto, CA) contained 2 mL diluted sample. The 3 cuvettes were preincubated in the 1, 2 and 3 positions of the DU700 spectrophotometer (Beckman, Palo Alto, CA) at 20°C for 5 min. The spectrophotometer was zeroed on the blank cuvette. The blank cuvette was then removed from position 1 and the first sample placed in position 1 and the duplicate in position 2. Finally, 1 mL of 30 mM hydrogen peroxide was added to initiate the reaction. Due to abnormal kinetics of CAT, it is not possible to define enzyme units. Therefore, results were expressed as a rate constant of the first-order reaction (k).

APPENDIX D

Ceruloplasmin method

The ceruloplasmin reaction mixture contained 0.75 mL of acetate buffer solution, pH 5.0 and 0.05 mL plasma in two polypropylene tubes (5 min tube and 15 min tube). The reaction mixture was preincubated for 5 min in a 30°C waterbath for temperature equilibration before 0.2 mL of 7.88 mM σ-diansidine dihydrochloride (preincubated at 30°C) reagent (Sigma Chemical, St. Louis, MO) was pipetted at timed intervals into each tube to initiate the reaction. At exactly 5 min the first tube was removed from the waterbath and 2 mL of 9 M sulfuric acid (JT Baker, Phillipsburg, NJ) was added to stop the reaction. At exactly 15 min the second tube was removed from the waterbath and 2 mL of 9 M sulfuric acid (JT Baker, Phillipsburg, NJ) was added to stop the reaction. The absorbance of the purple-red solution was measured at 540 nm on the DU-7000 spectrophotometer (Beckman, Palo Alto, CA) using deionized distilled water as a blank. Ceruloplasmin oxidase activity was determined by absorbance of the second tube minus absorbance of the first tube multiplied by $6.25*10^{-1}$ U/mL and results were expressed as units of CP enzyme activity per mL of plasma.

APPENDIX E

CZSOD activity and Protein concentrations for gilts in Exp. 2

Figure 17. SOD activity and Protein concentrations for gilt 43-1

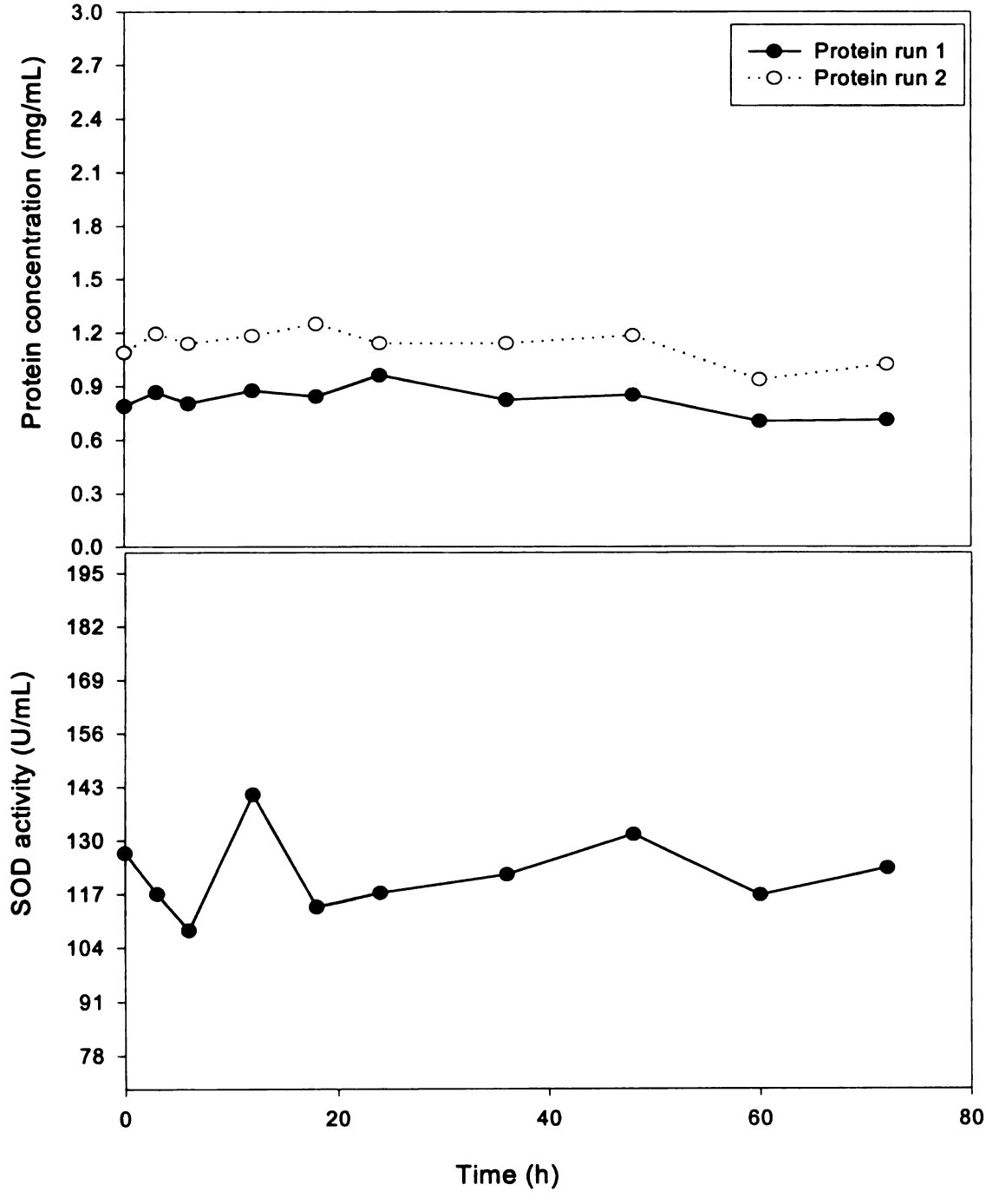


Figure 18. SOD activity and Protein concentrations for gilt 44-1

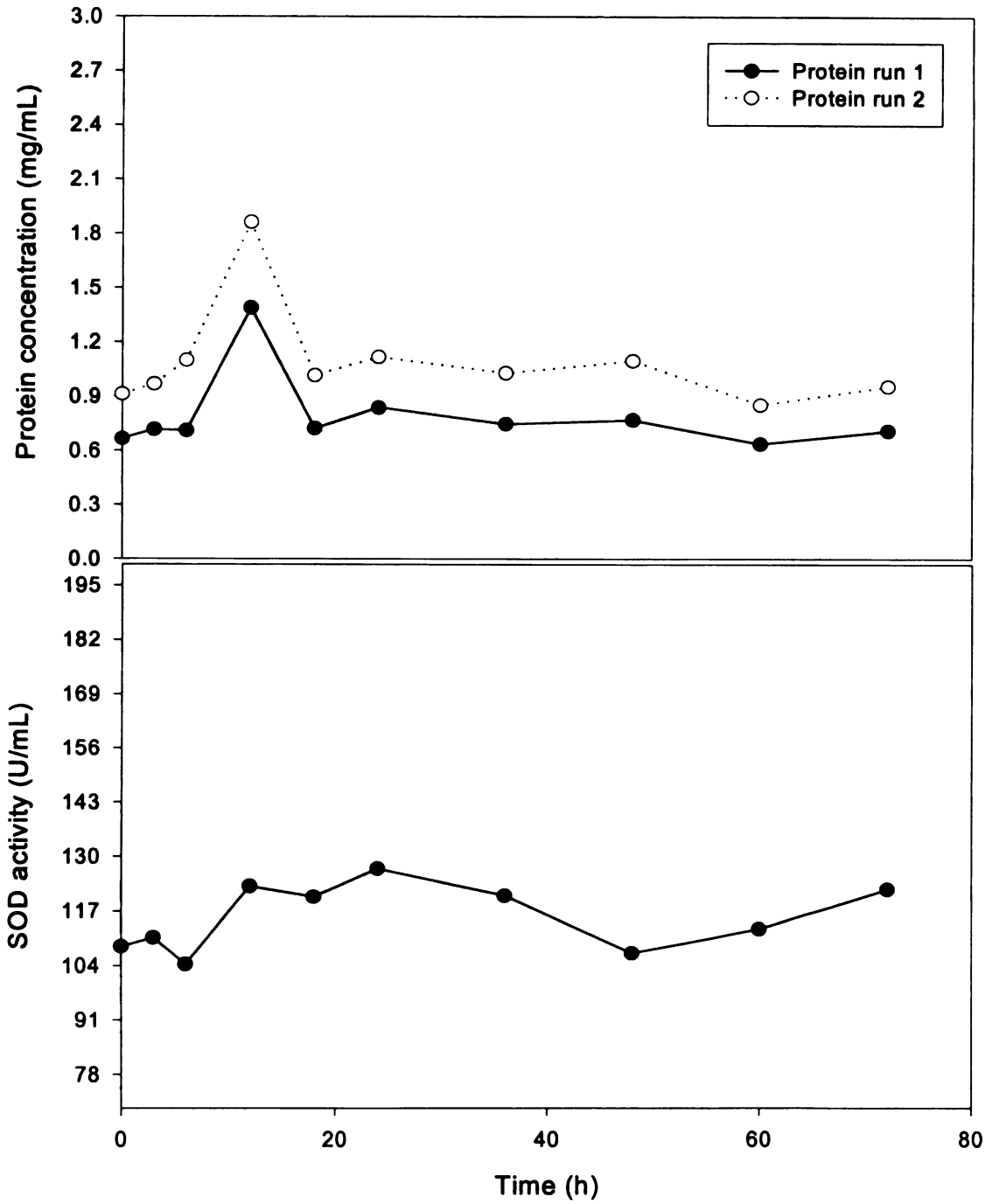


Figure 19. SOD activity and Protein concentrations for gilt 44-2

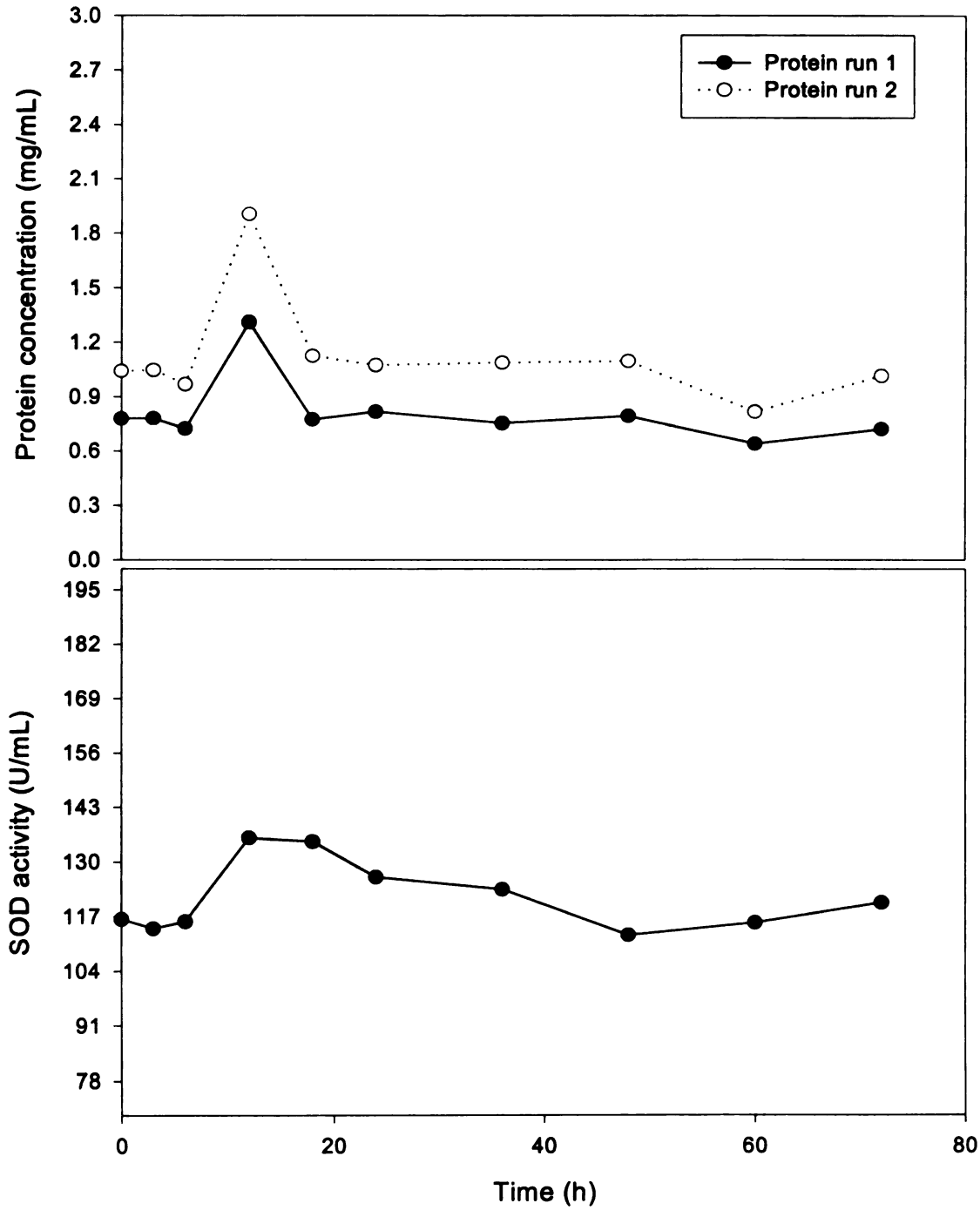


Figure 20. SOD activity and Protein concentrations for gilt 44-3

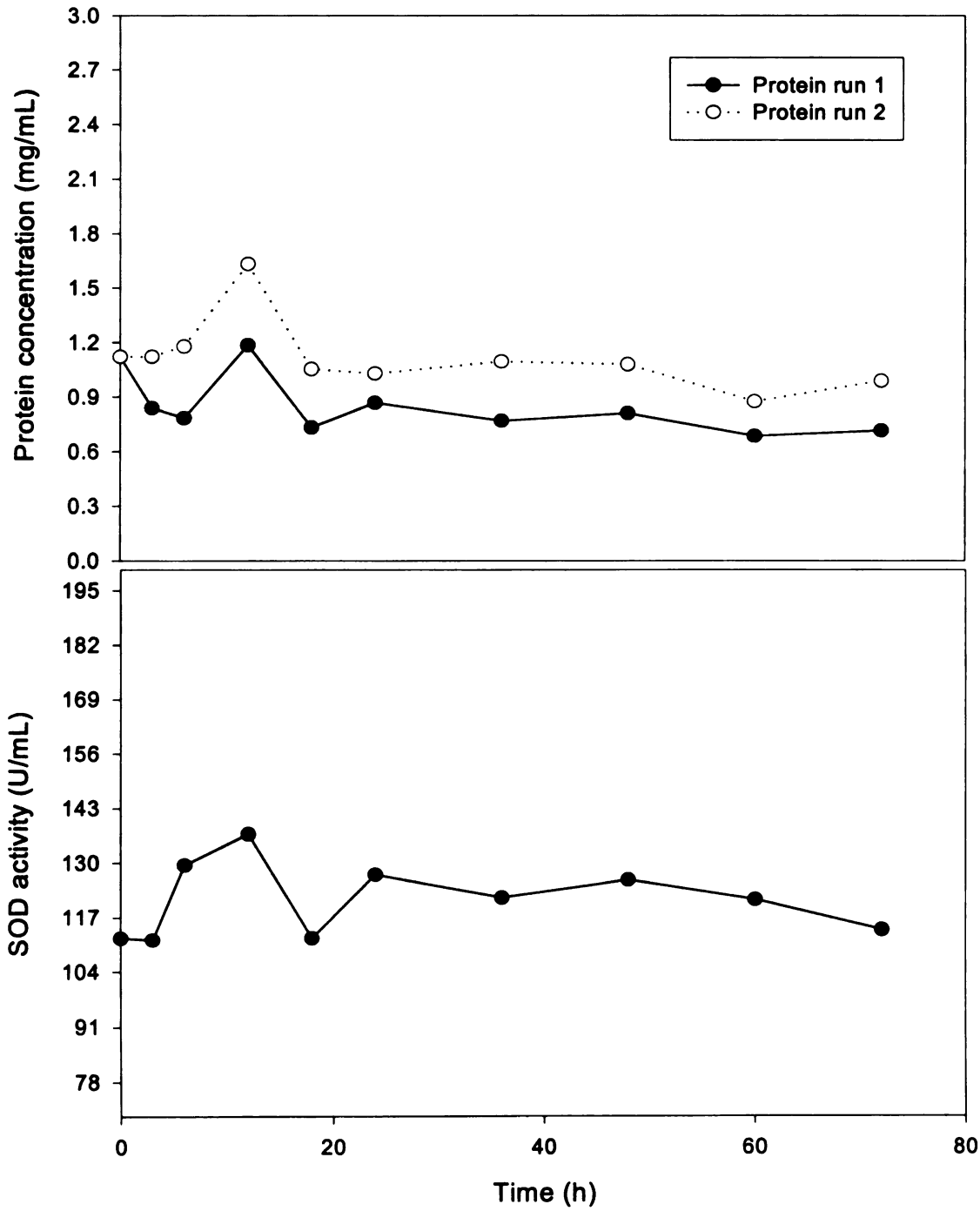


Figure 21. SOD activity and Protein concentrations for gilt 45-1

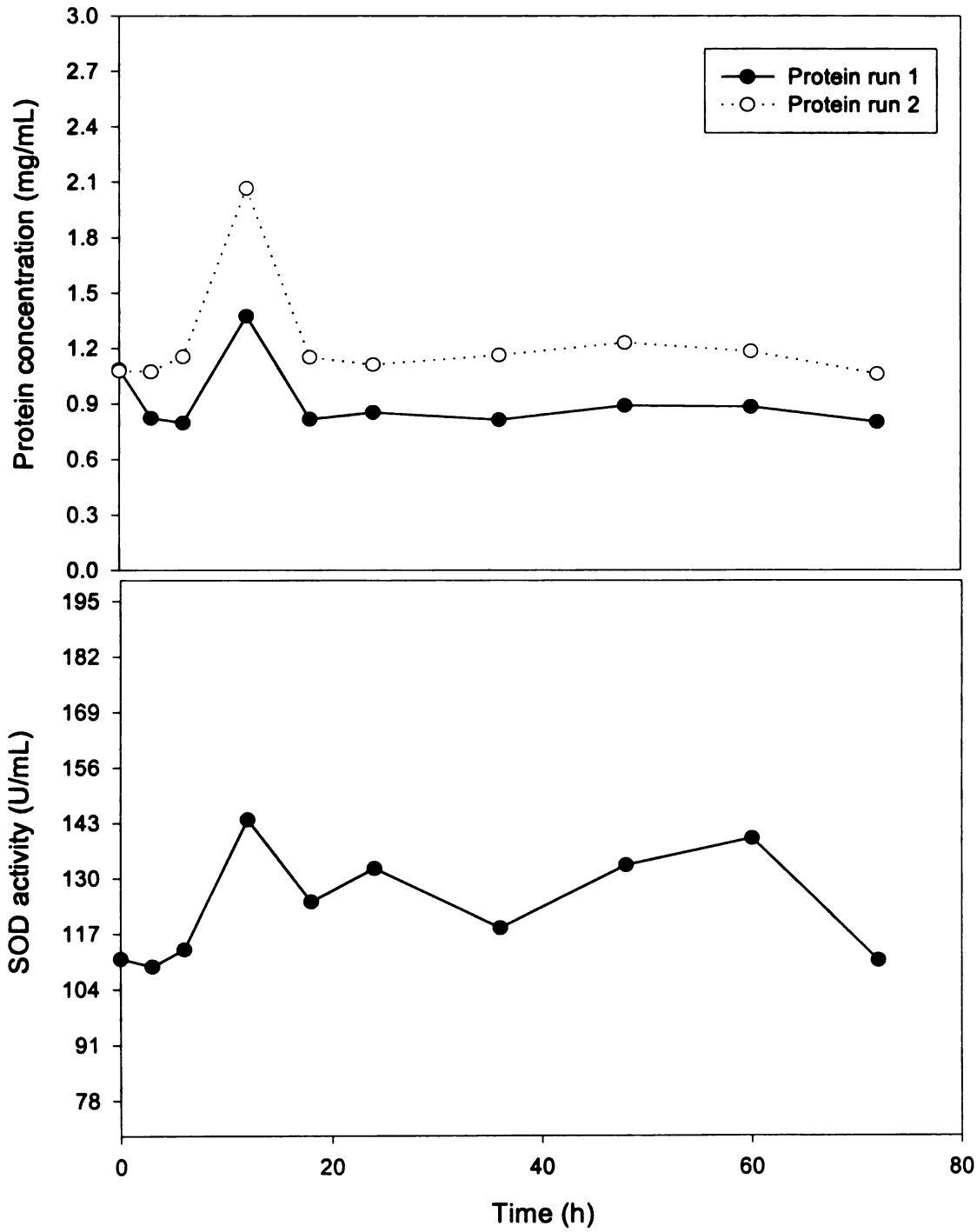


Figure 22. SOD activity and Protein concentrations for gilt 45-2

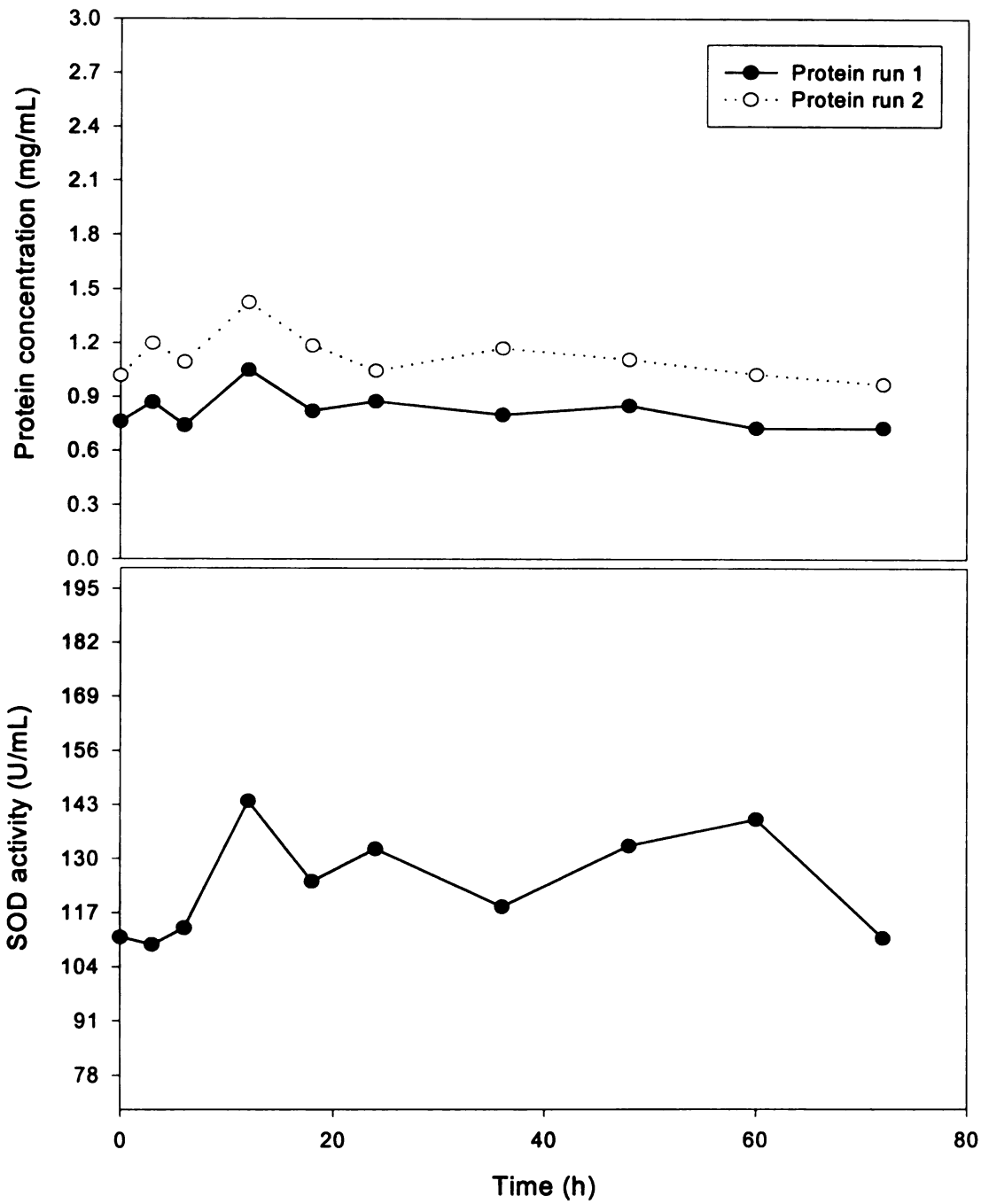


Figure 23. SOD activity and Protein concentrations for gilt 46-1

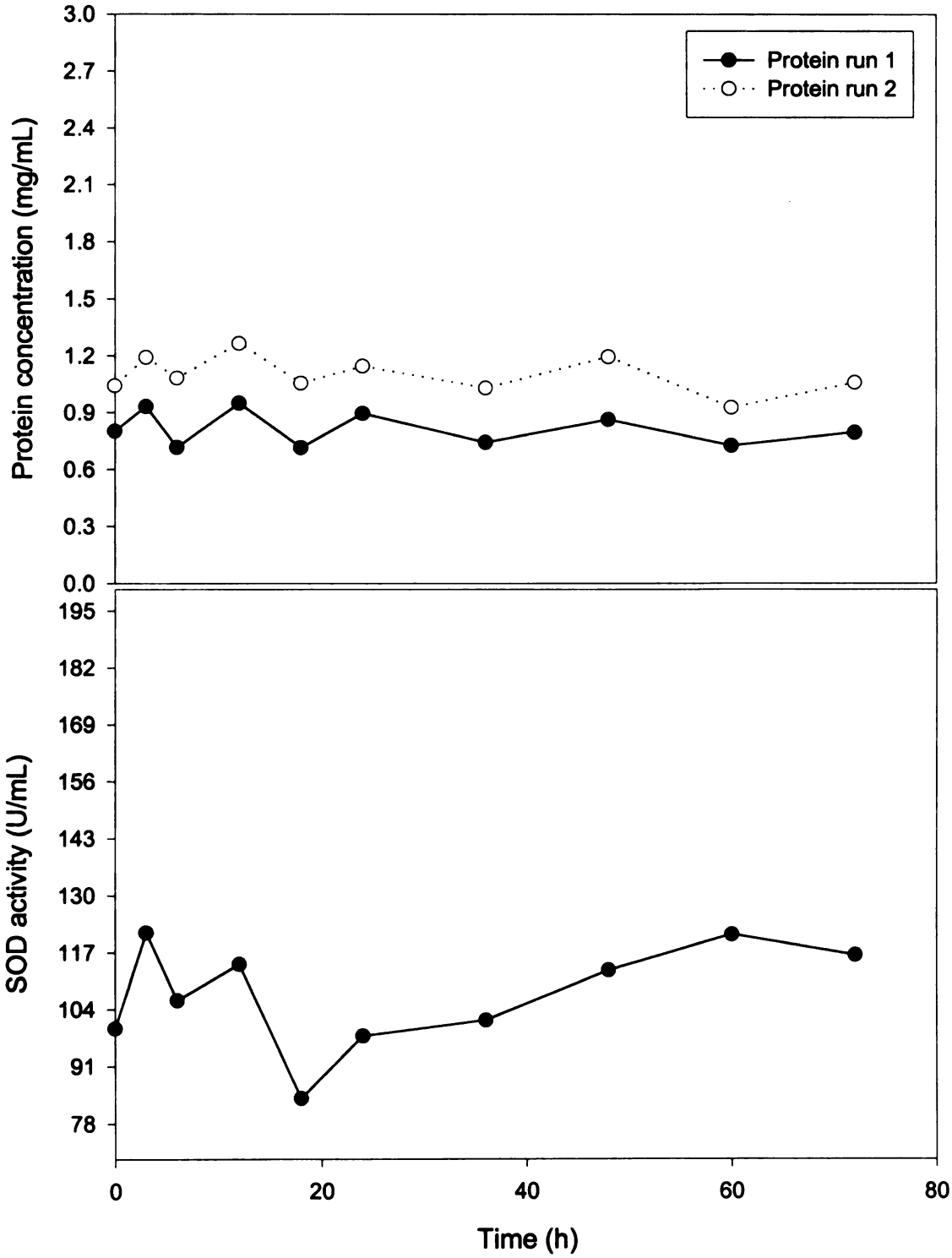


Figure 24. SOD activity and Protein concentrations for gilt 46-2

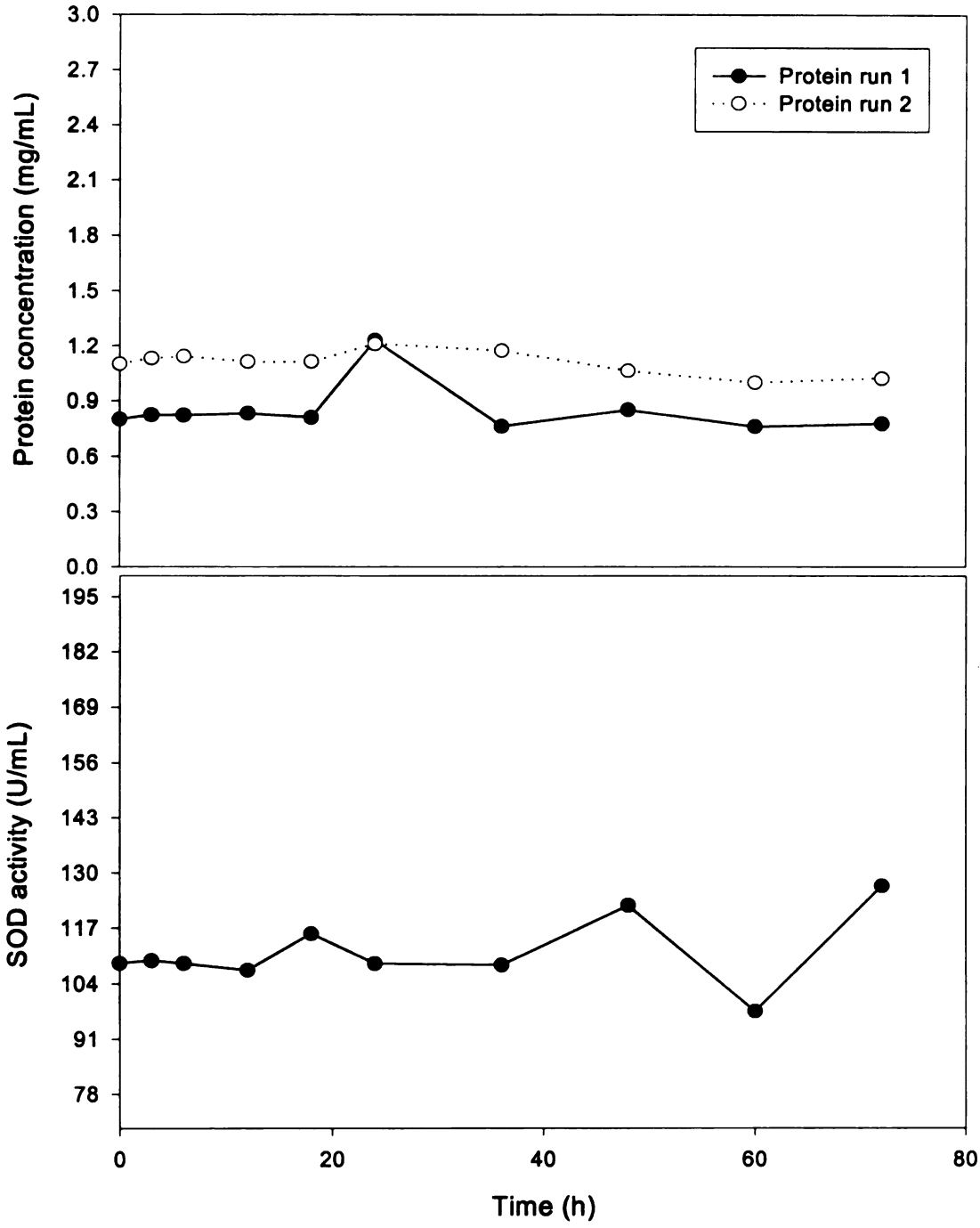


Figure 25. SOD activity and Protein concentrations for gilt 46-3

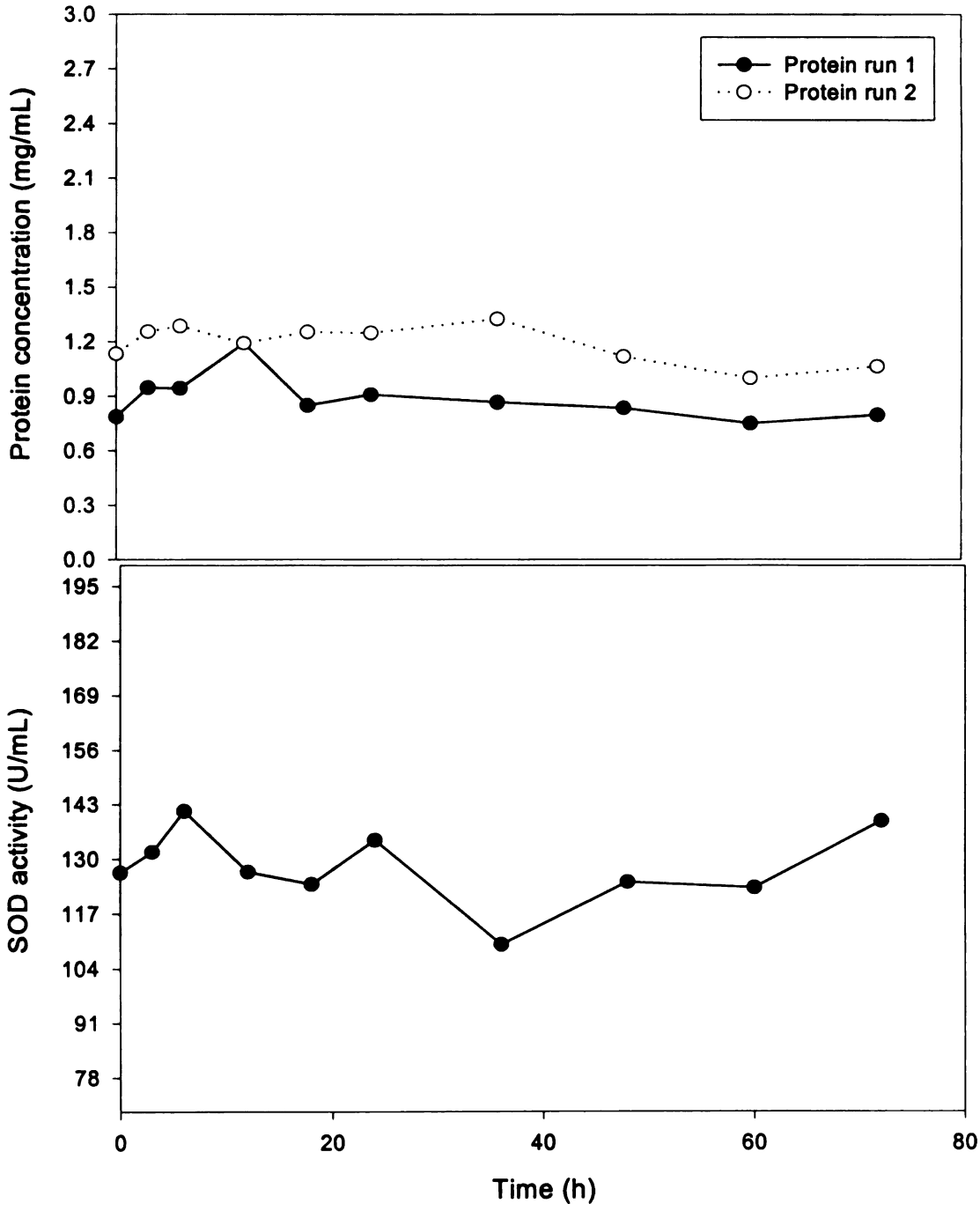


Figure 26. SOD activity and Protein concentrations for gilt 47-3

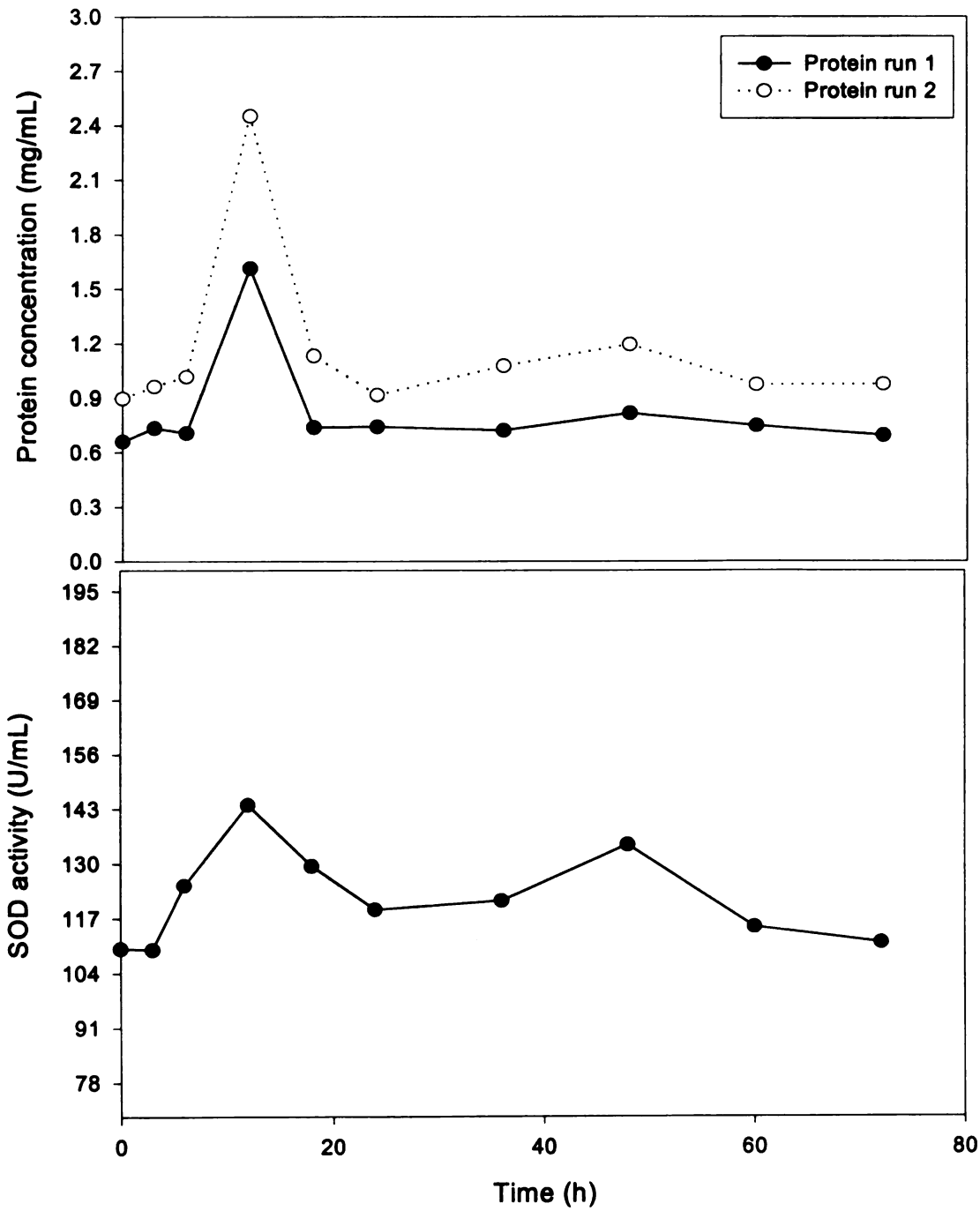


Figure 27. SOD activity and Protein concentations for gilt 47-4

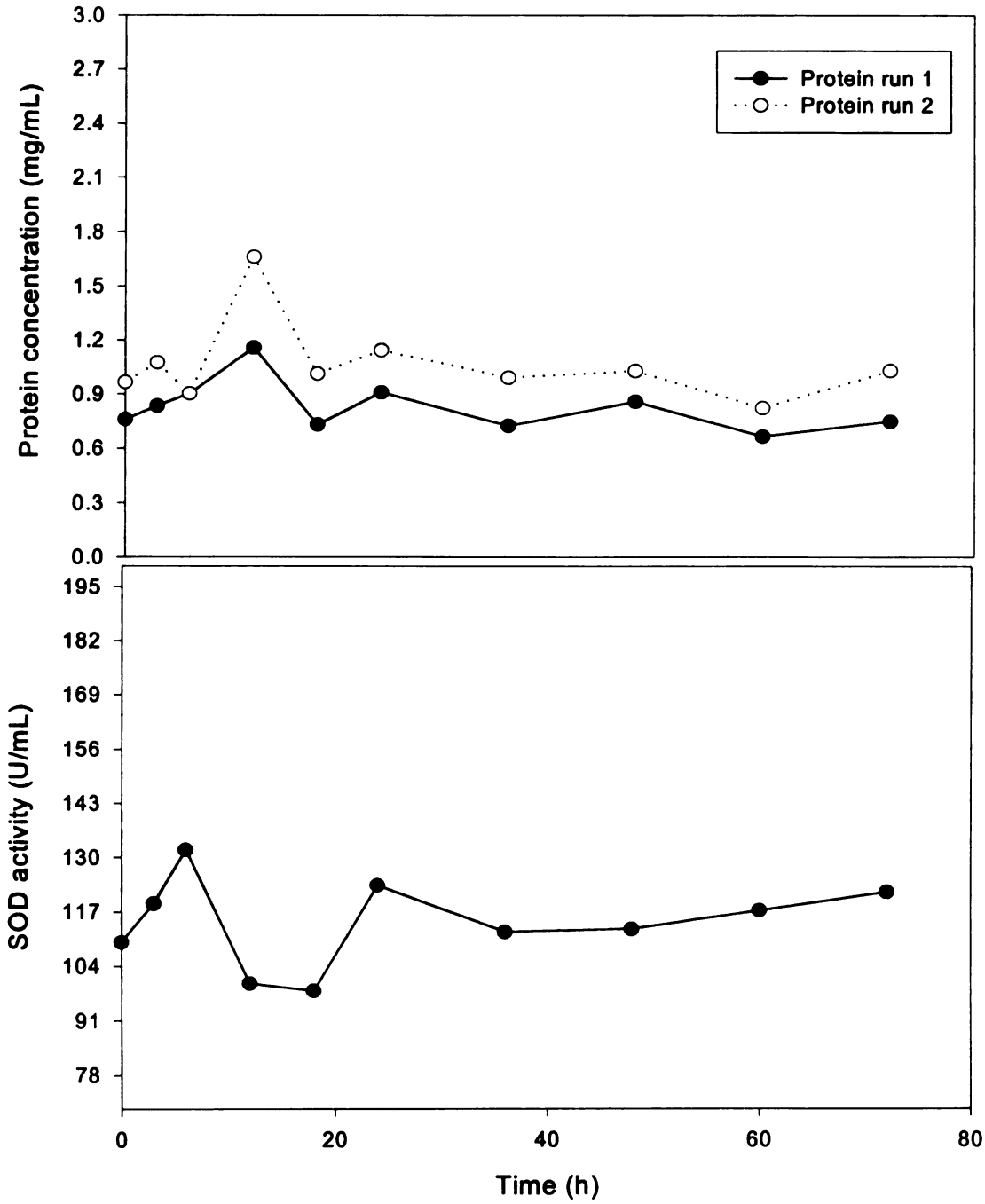
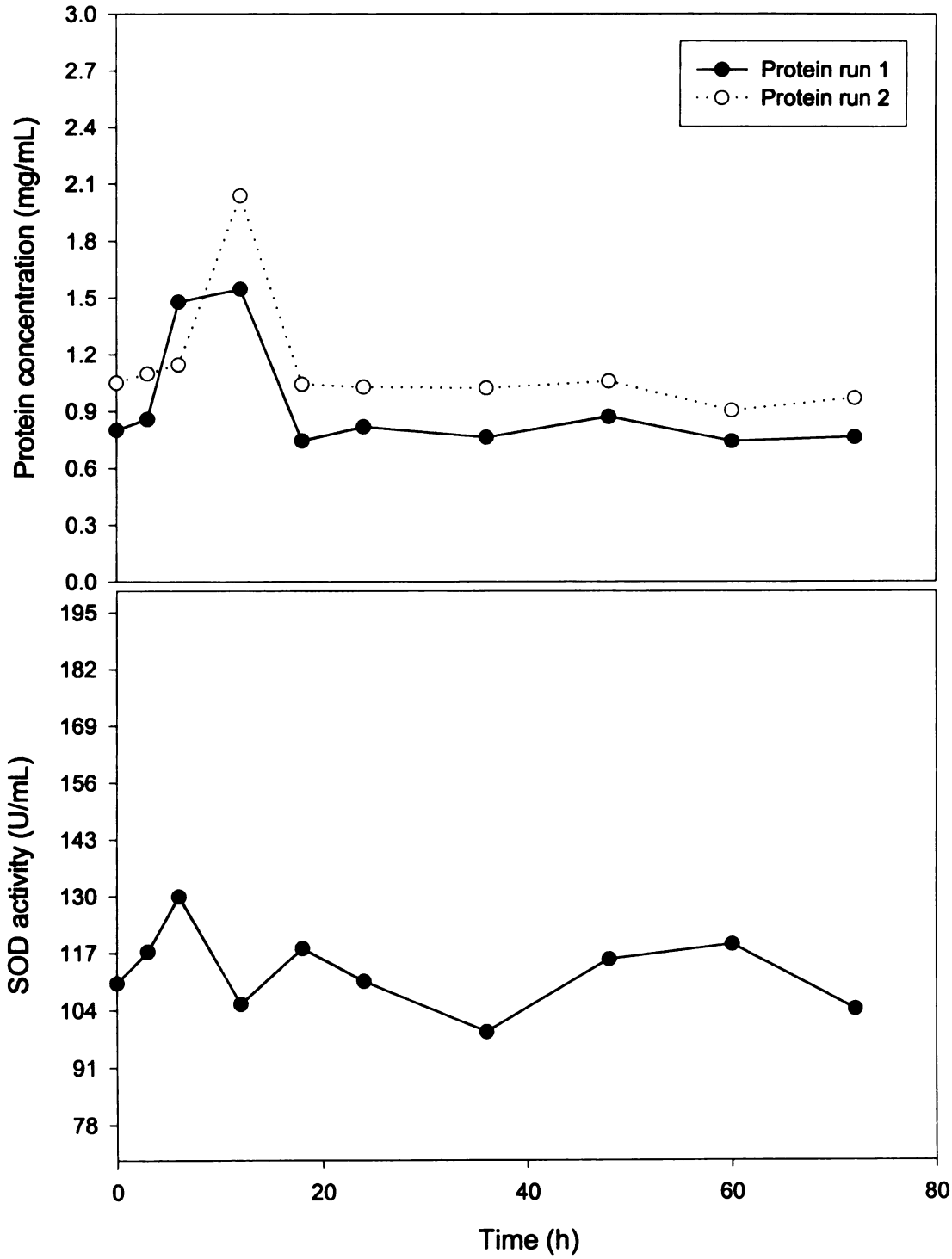


Figure 28. SOD activity and Protein concentrations for gilt 47-5



APPENDIX F

GPX activity and Hemoglobin concentrations for gilts Exp. 2

Figure 29. GPX activity and Hemoglobin concentrations for gilt 43-1

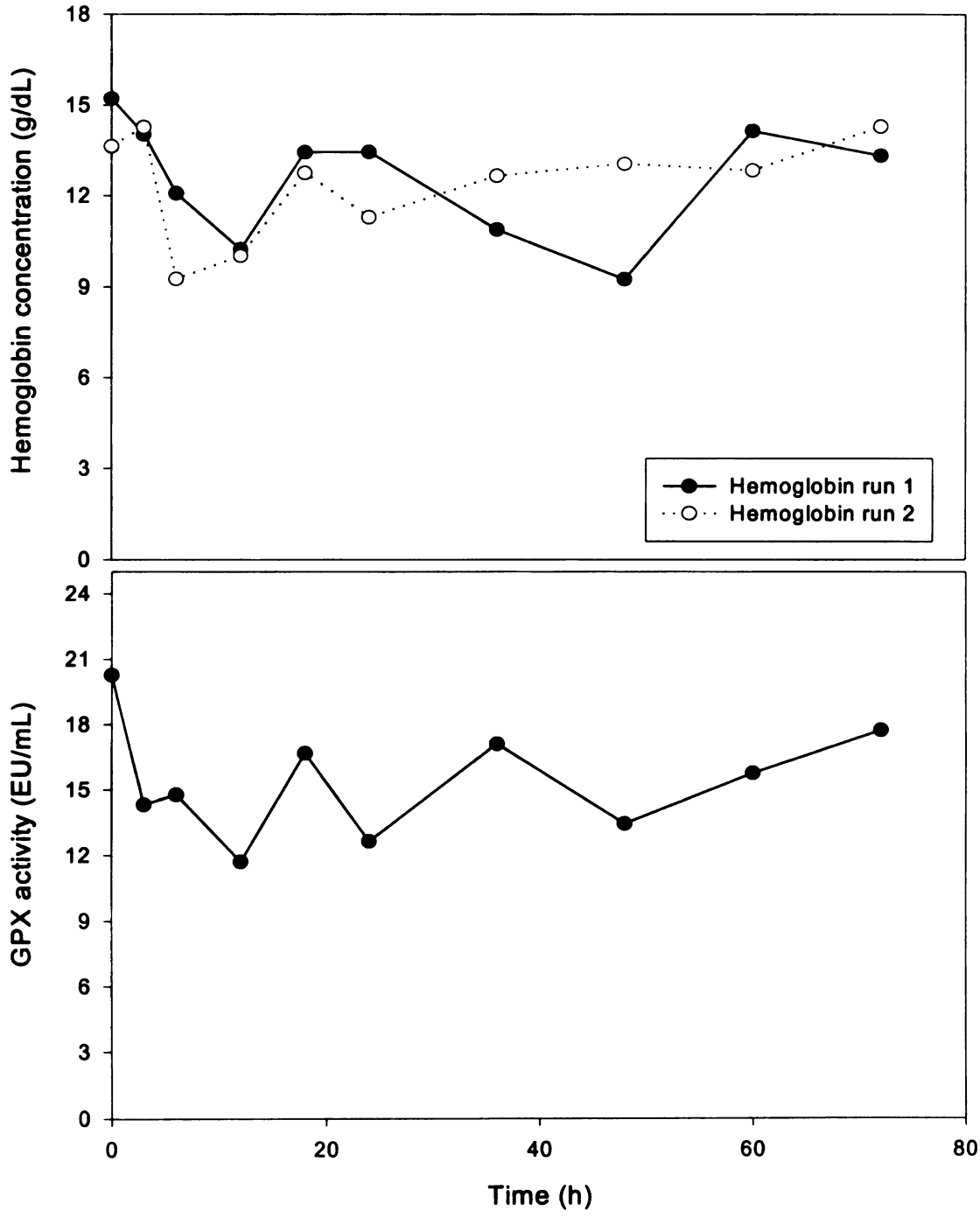


Figure 30. GPX activity and Hemoglobin concentrations for gilt 44-1

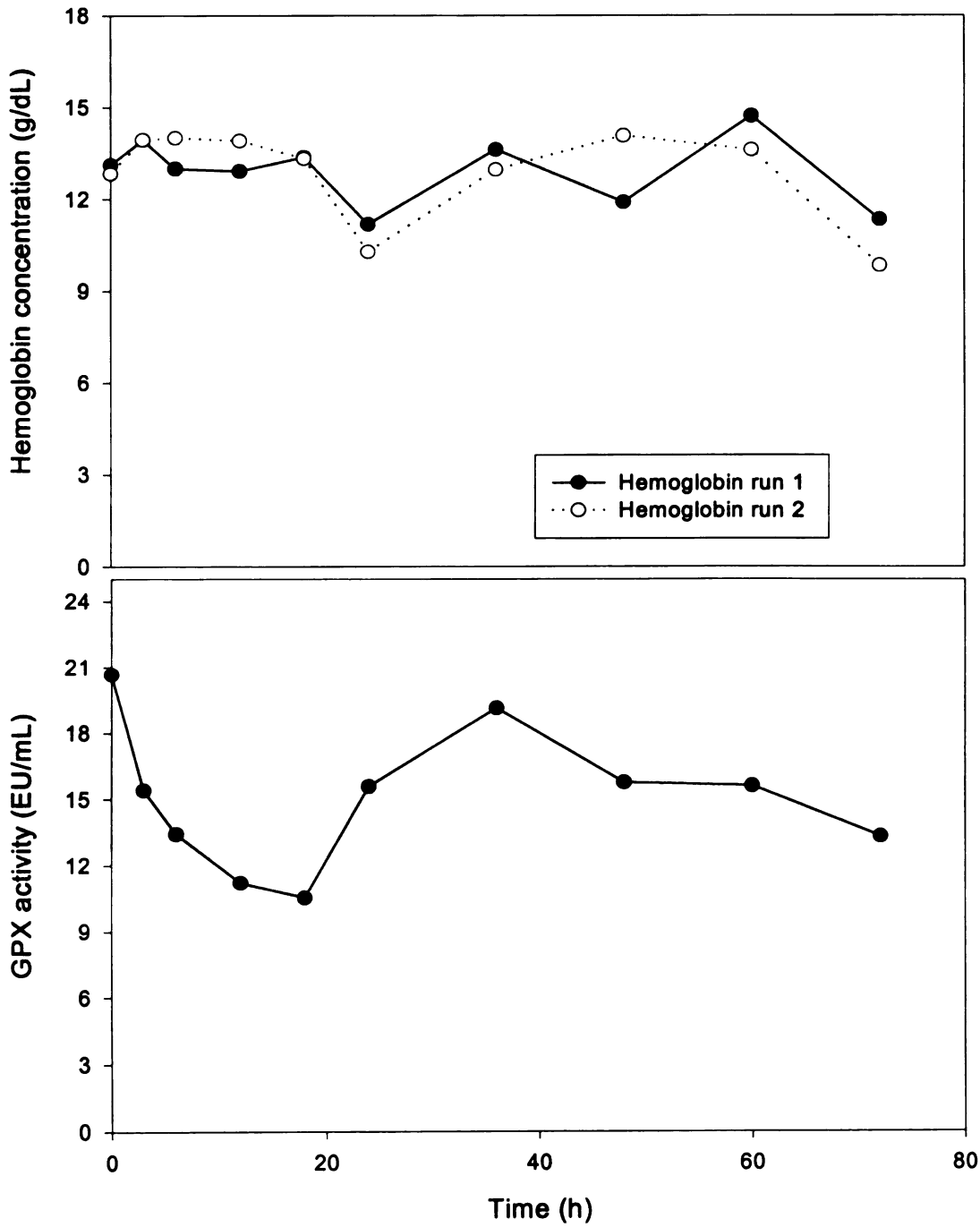


Figure 31. GPX activity and Hemoglobin concentrations for gilt 44-2

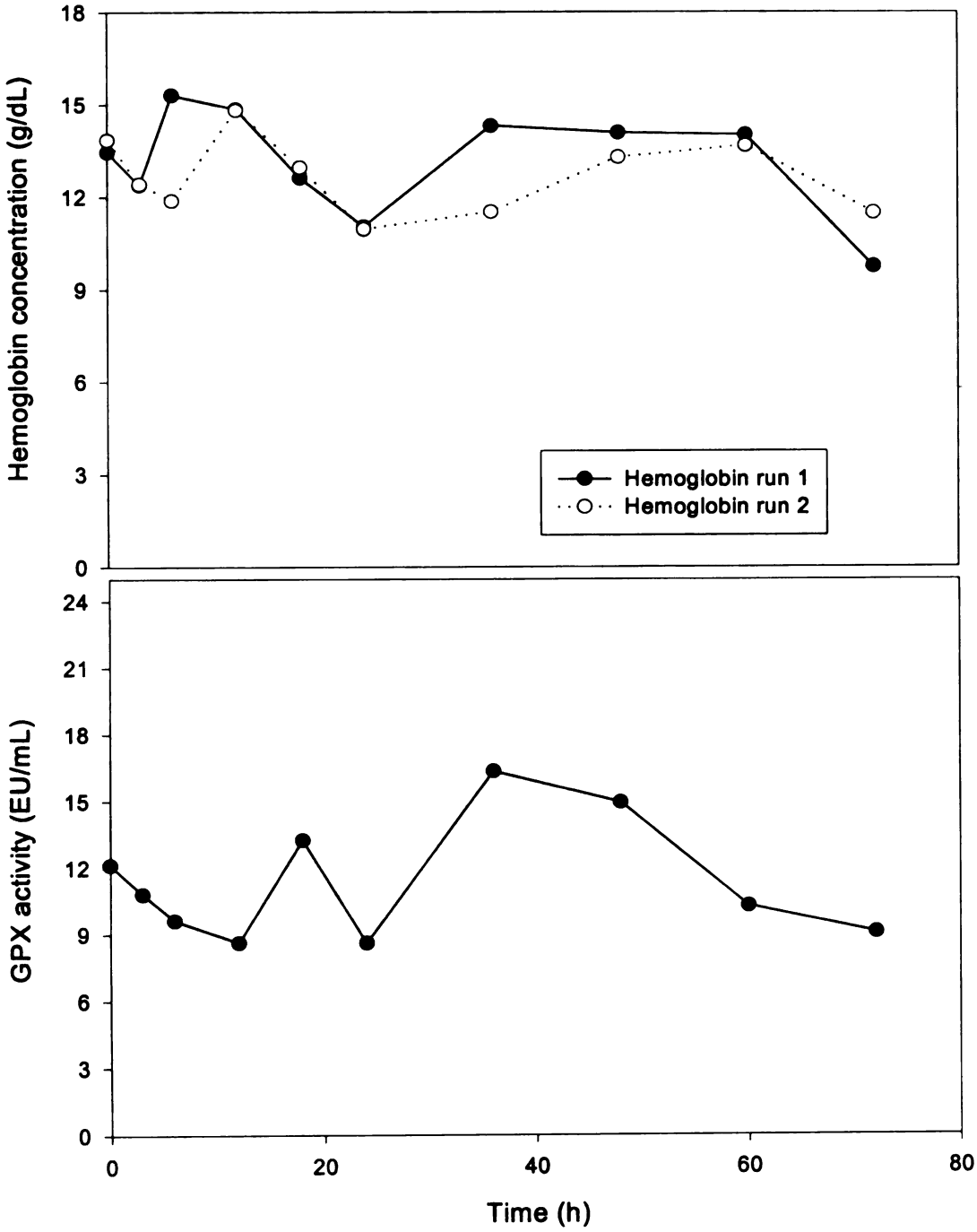


Figure 32. GPX activity and Hemoglobin concentrations for gilt 44-3

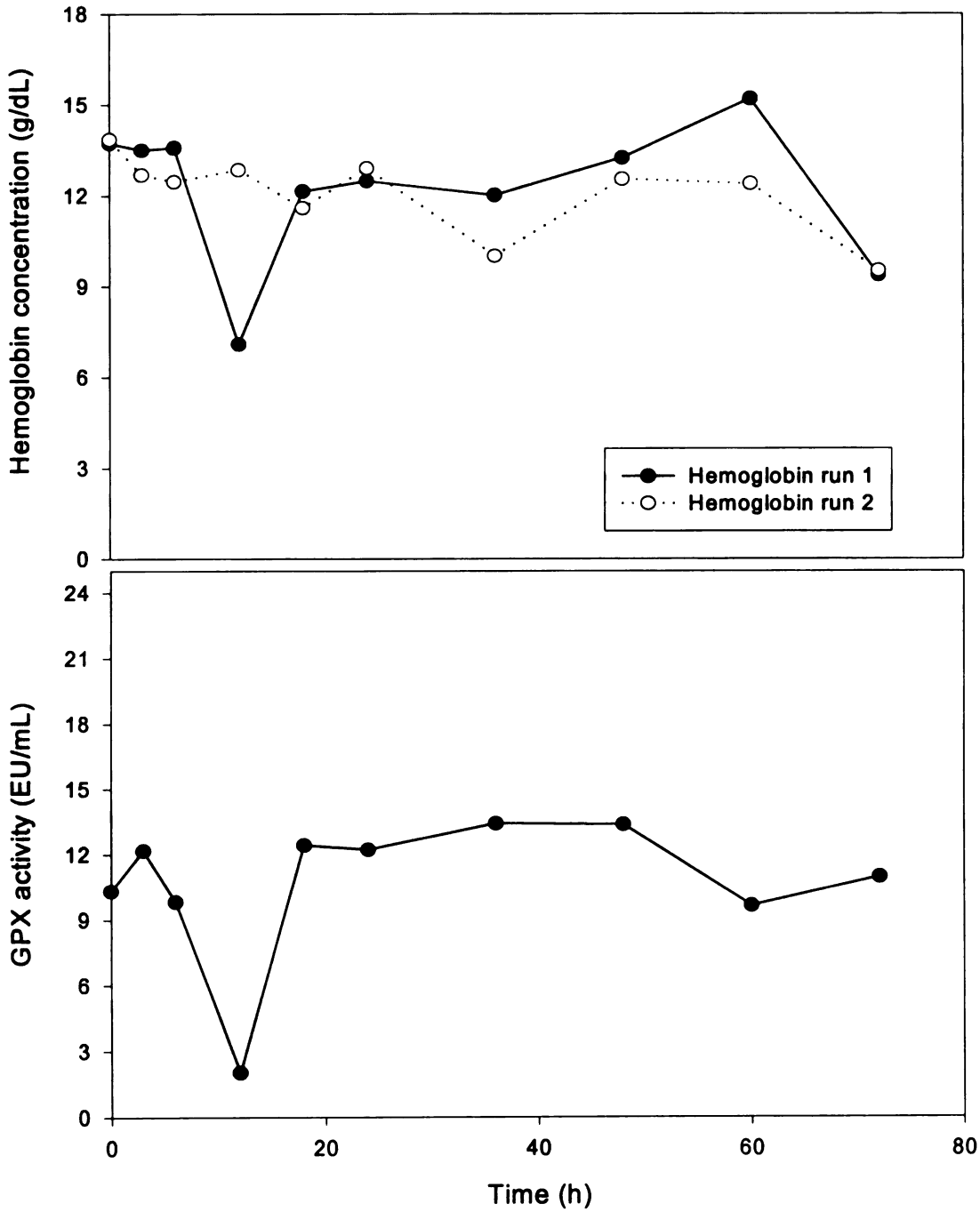


Figure 33. GPX activity and Hemoglobin concentrations for gilt 45-1

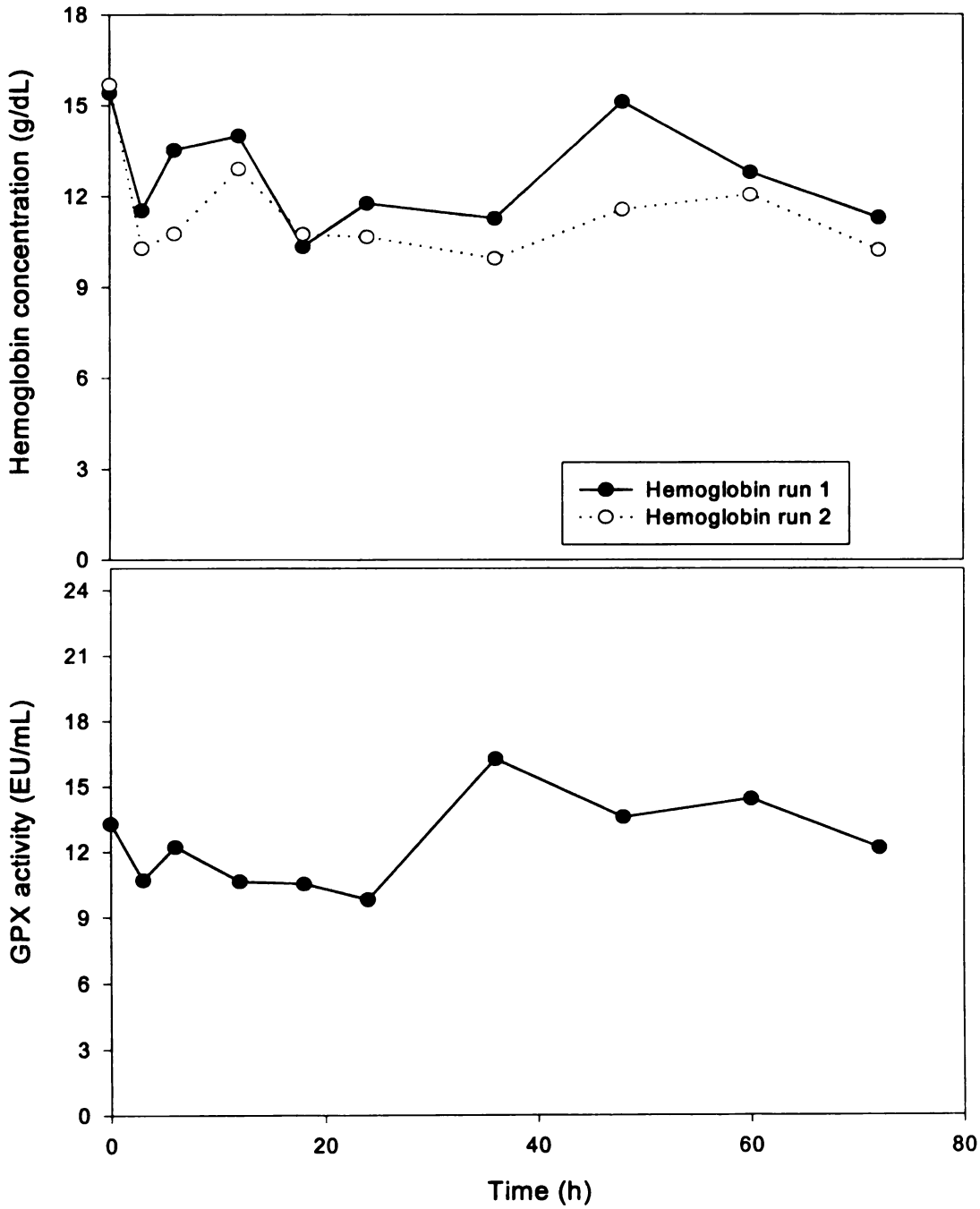


Figure 34. GPX activity and Hemoglobin concentrations for gilt 45-2

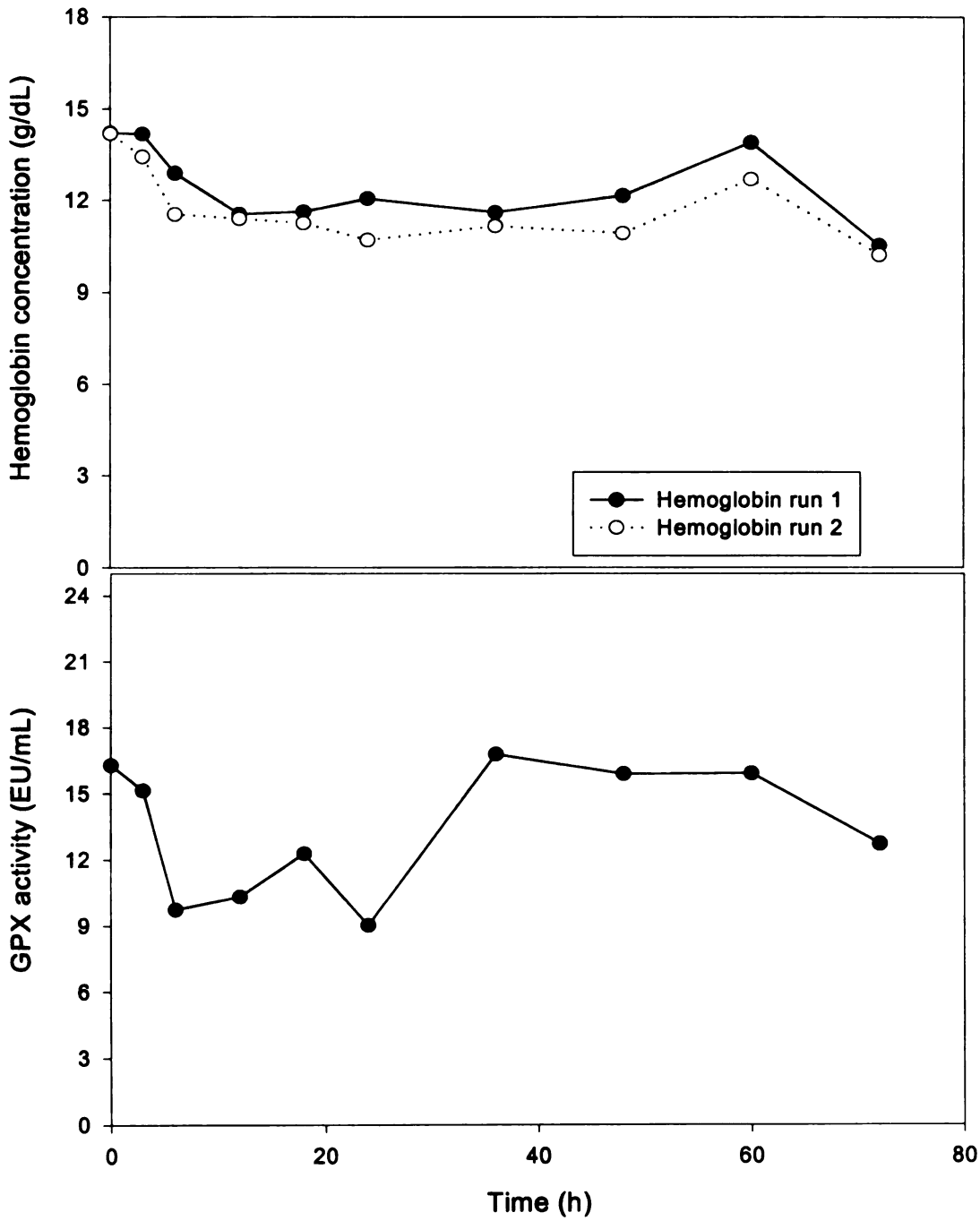


Figure 35. GPX activity and Hemoglobin concentrations for gilt 46-1

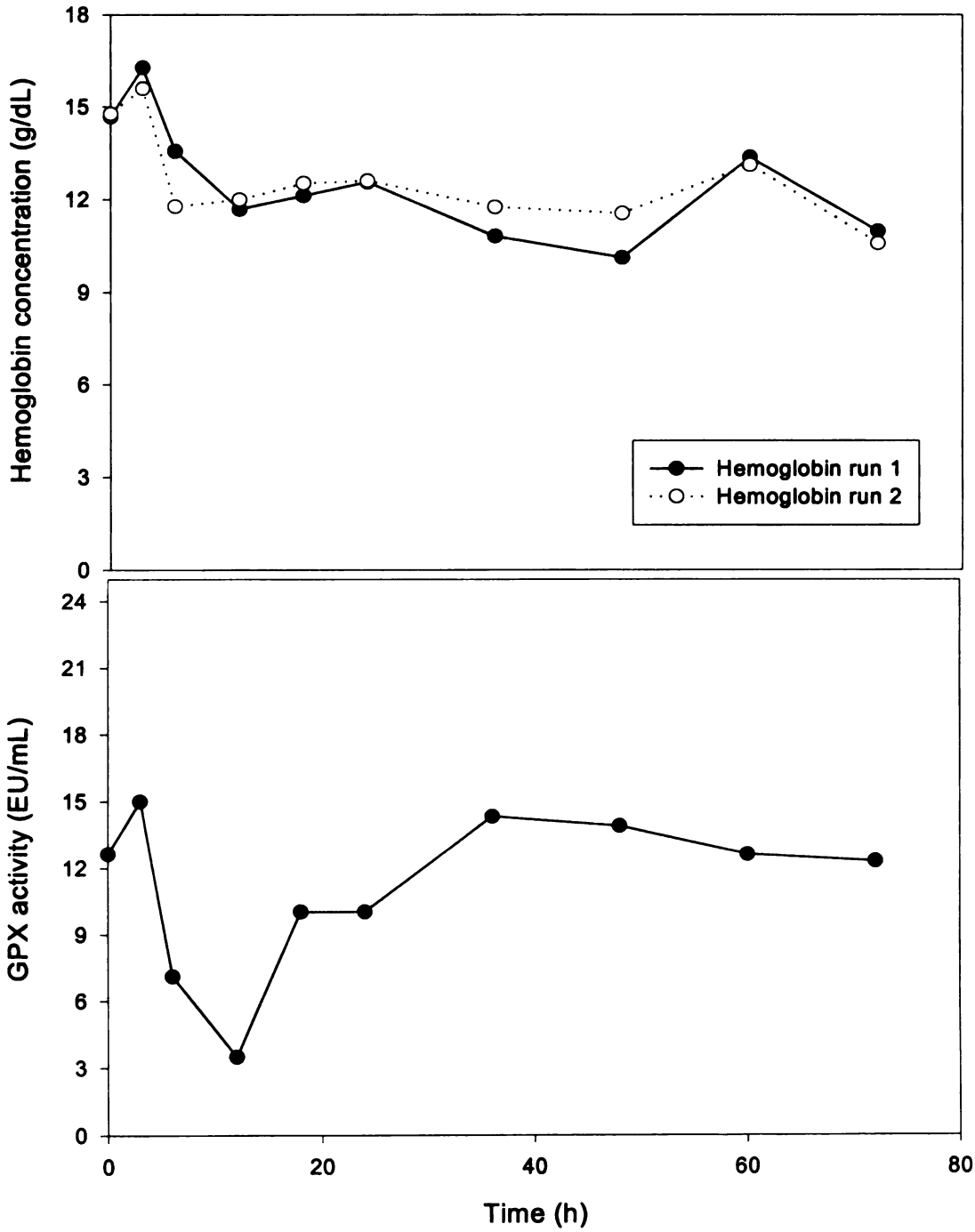


Figure 36. GPX activity and Hemoglobin concentrations for gilt 46-2

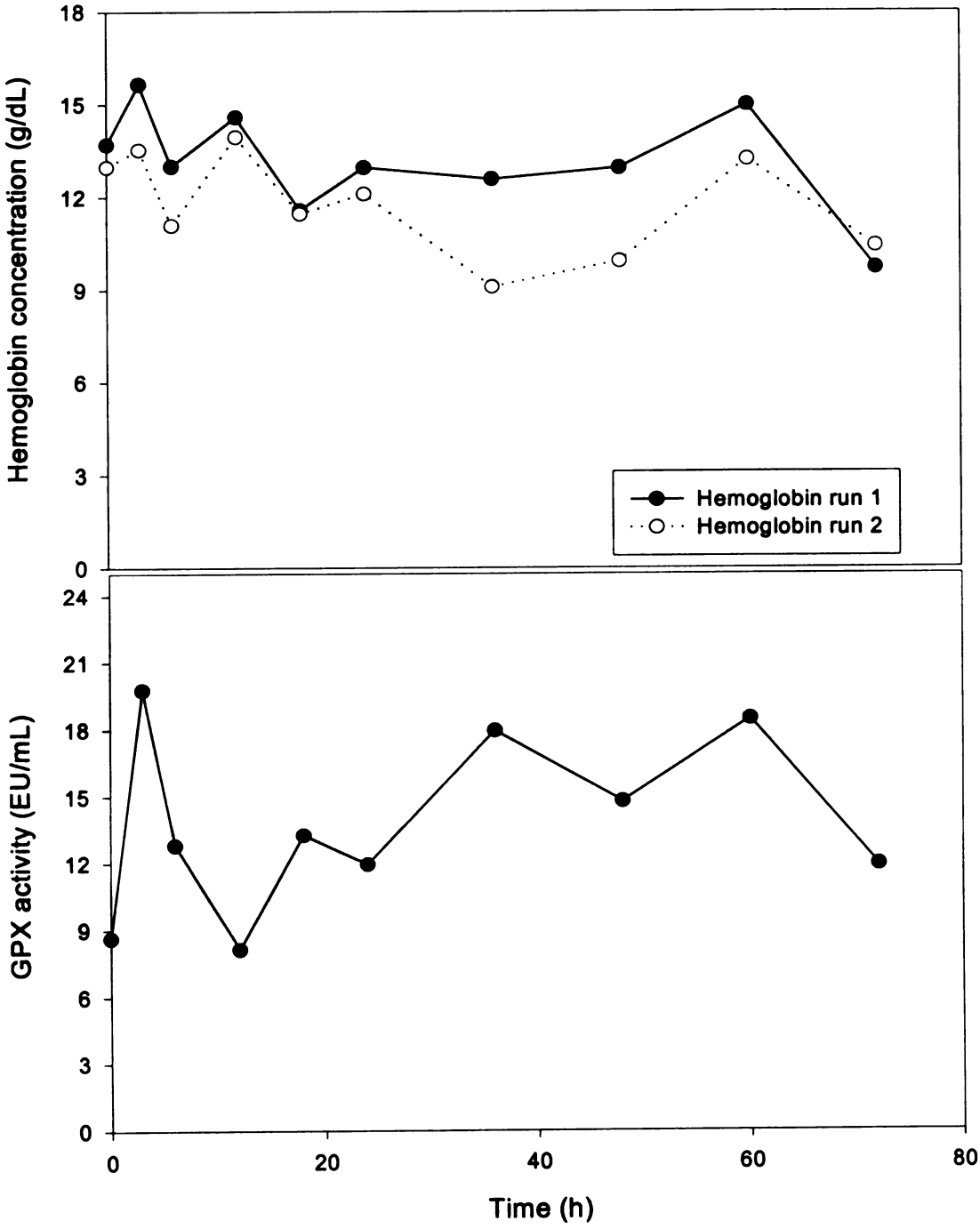


Figure 37. GPX activity and Hemoglobin concentrations for gilt 46-3

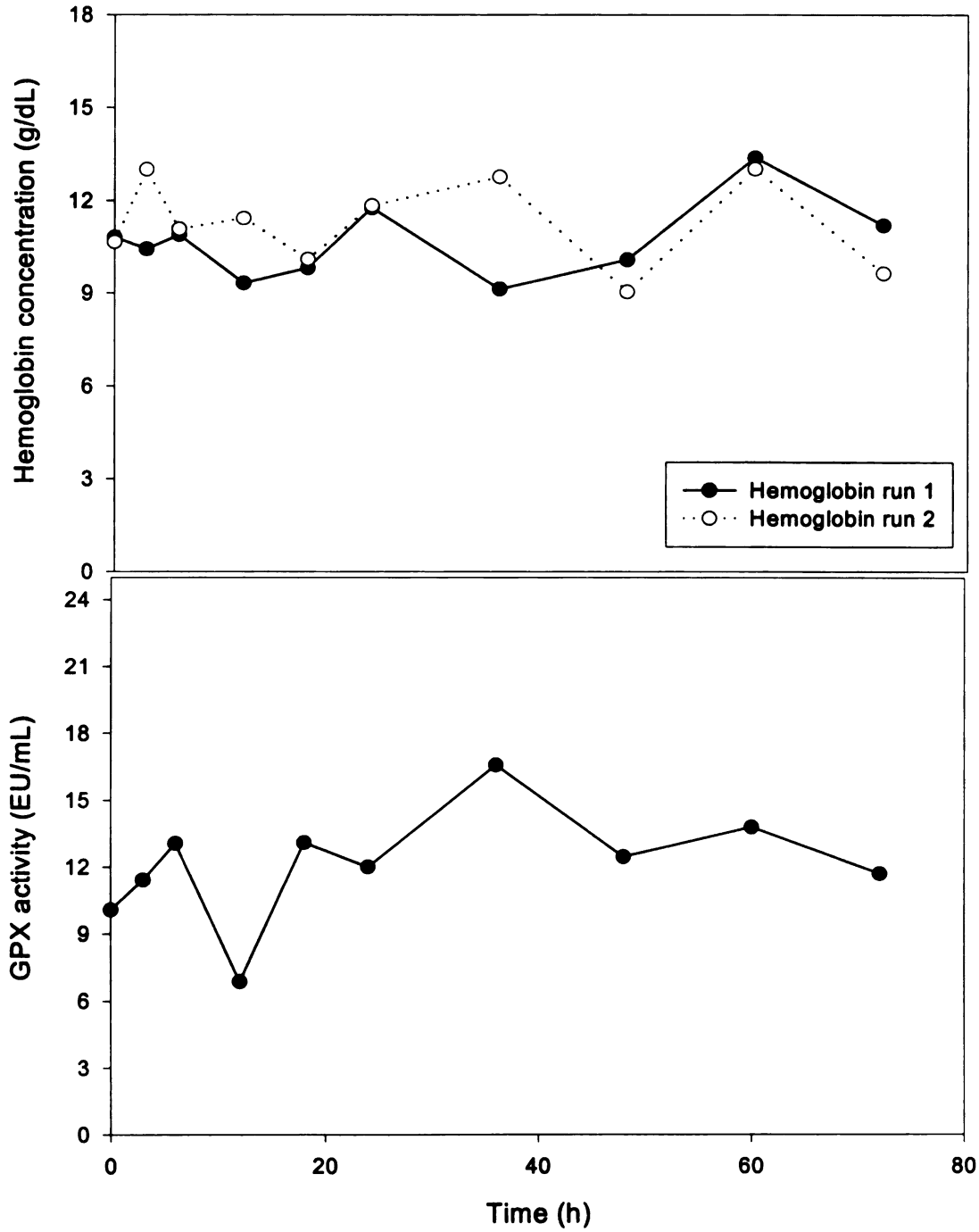


Figure 38. GPX activity and Hemoglobin concentrations for gilt 47-3

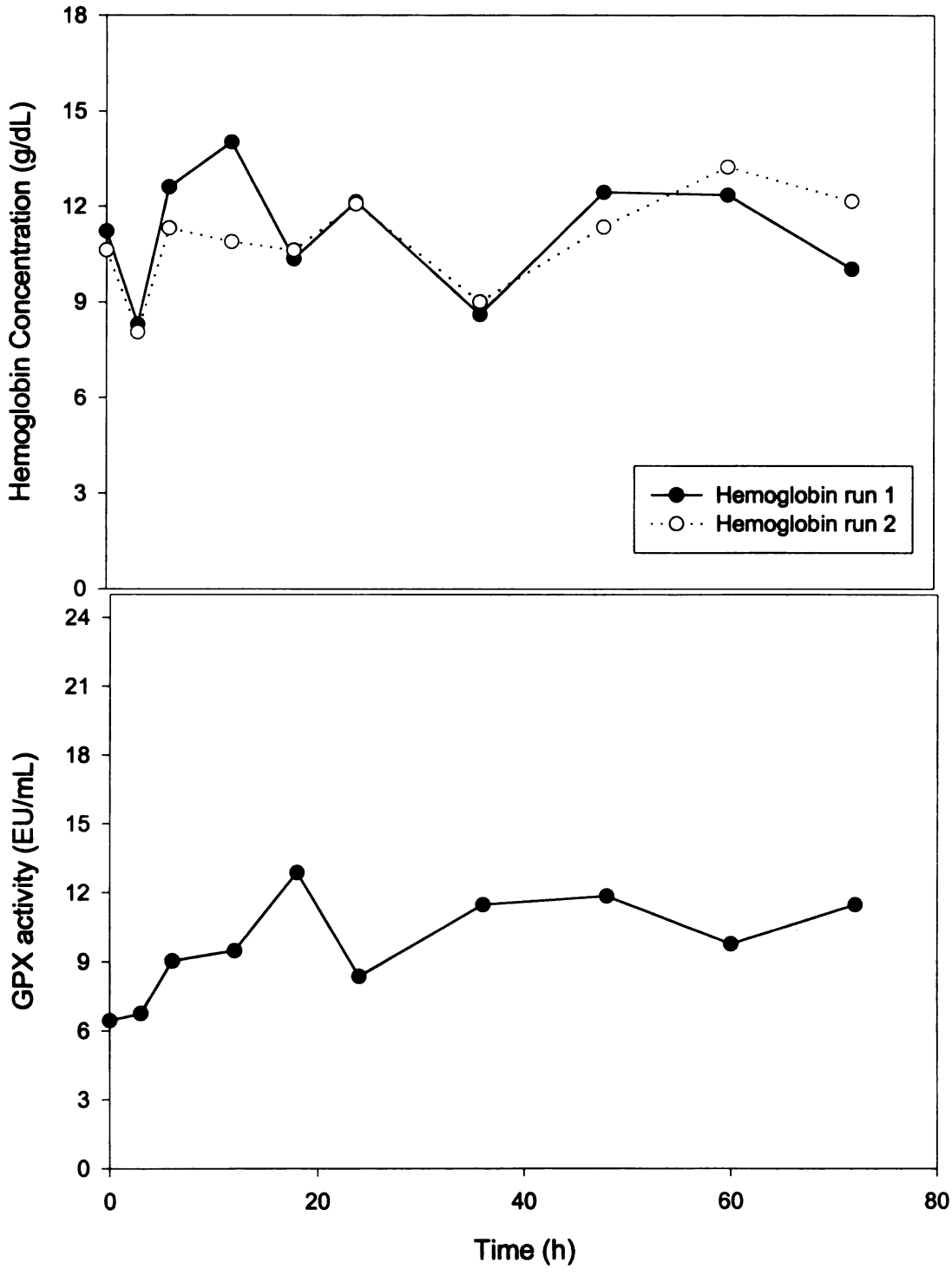


Figure 39. GPX activity and Hemoglobin concentrations for gilt 47-4

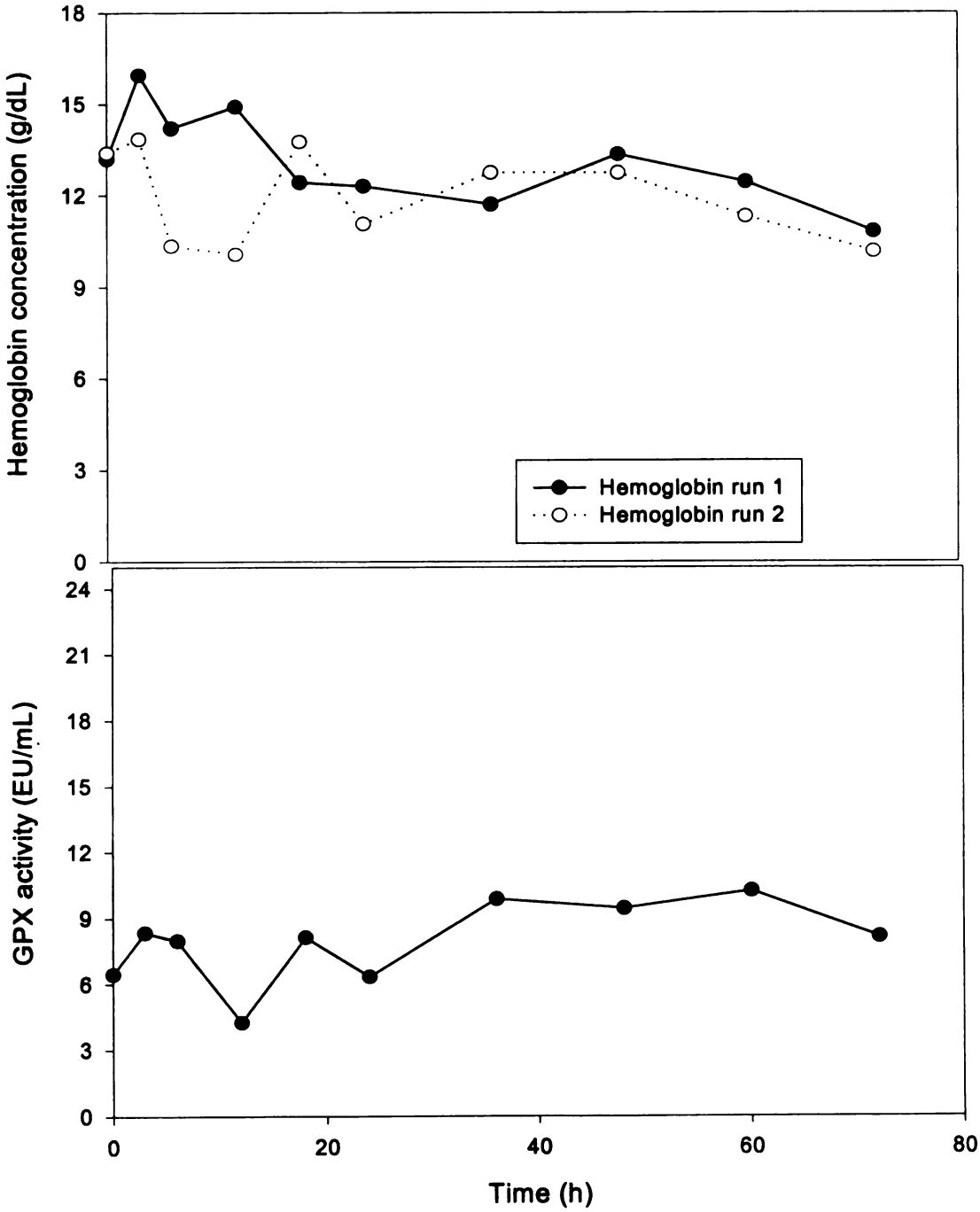


Figure 40. GPX activity and Hemoglobin concentrations for gilt 47-5