The Effect of Amino Acid Chelates on Total Antioxidant Capacity, Oxidative Stress (Lipid Peroxidation), and SOD Activity in Serum of Feedlot Cattle

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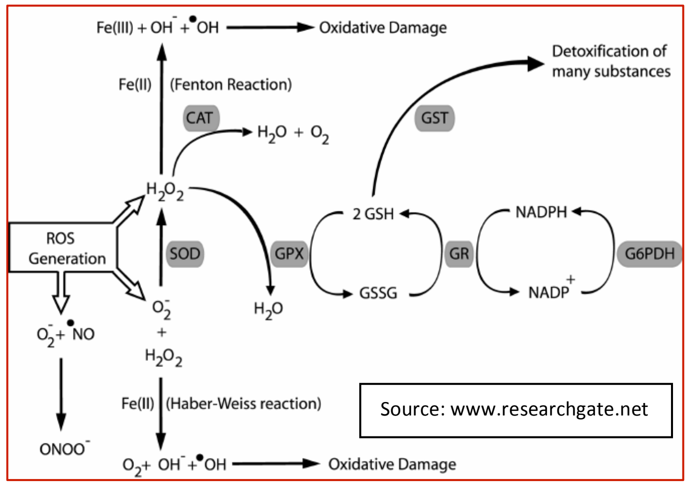
I. Introduction

There are a variety of reactive oxygen species (ROS) generated in cellular aerobic metabolism creating oxidative stress (OS). These ROS include superoxide, hydroxide, and lipid hydroperoxides. If left unchecked these ROS are damaging to cells and cellular systems leading to disease.

Figure 1



Antioxidant defense mechanisms (Fig. 1) rely on the essential trace elements Cu, Zn, Mn, and Se, and the macro elements Mg, K, and Ca for enzyme systems to function properly and for the formation of other antioxidants such as reduced glutathione. Animals undergoing oxidative stress (OS) will have a higher demand for antioxidant defense mechanisms.

The objective of this preliminary study is to demonstrate the beneficial physiological effect of **DuoPort GPX** when fed to feedlot cattle during the growing and finishing phase. The hypothesis is: amino acid chelates improve the metabolic state of the animal reducing the total antioxidant requirements thereby reducing oxidative stress. Simply stated, the metabolic engine runs cooler!

II. Product Description & Guaranteed Analysis (DuoPort GPX)

Zinc Amino Acid Chelate	2.4% Zinc
Copper Amino Acid Chelate	2.4% Cu
Manganese Amino Acid Chelate	1.0% Mn
Magnesium Amino Acid Chelate	2.0% Mg
Potassium Amino Acid Complex	2.0% K

Recommended Dosage:
7 gm/hd/day

Recommended Days to Feed:
Continuous

III. Cattle Groups

Reimplant Group	Total Head	Steers	Heifers	DOF	Days on DuoPort GPX
F1 Treated	35	20	15	67	67
F2 Non-Treated	29	15	14	Unknown	0

Kill Floor Group	Total Head	Steers	Heifers	DOF	Days on DuoPort GPX
F3 Treated	61	31	30	163	157
UKN Non-Treated	60	60	0	Unknown	0

(Note: F1, F2, UKN refer to the feedlots)

IV. Tissue Sampling

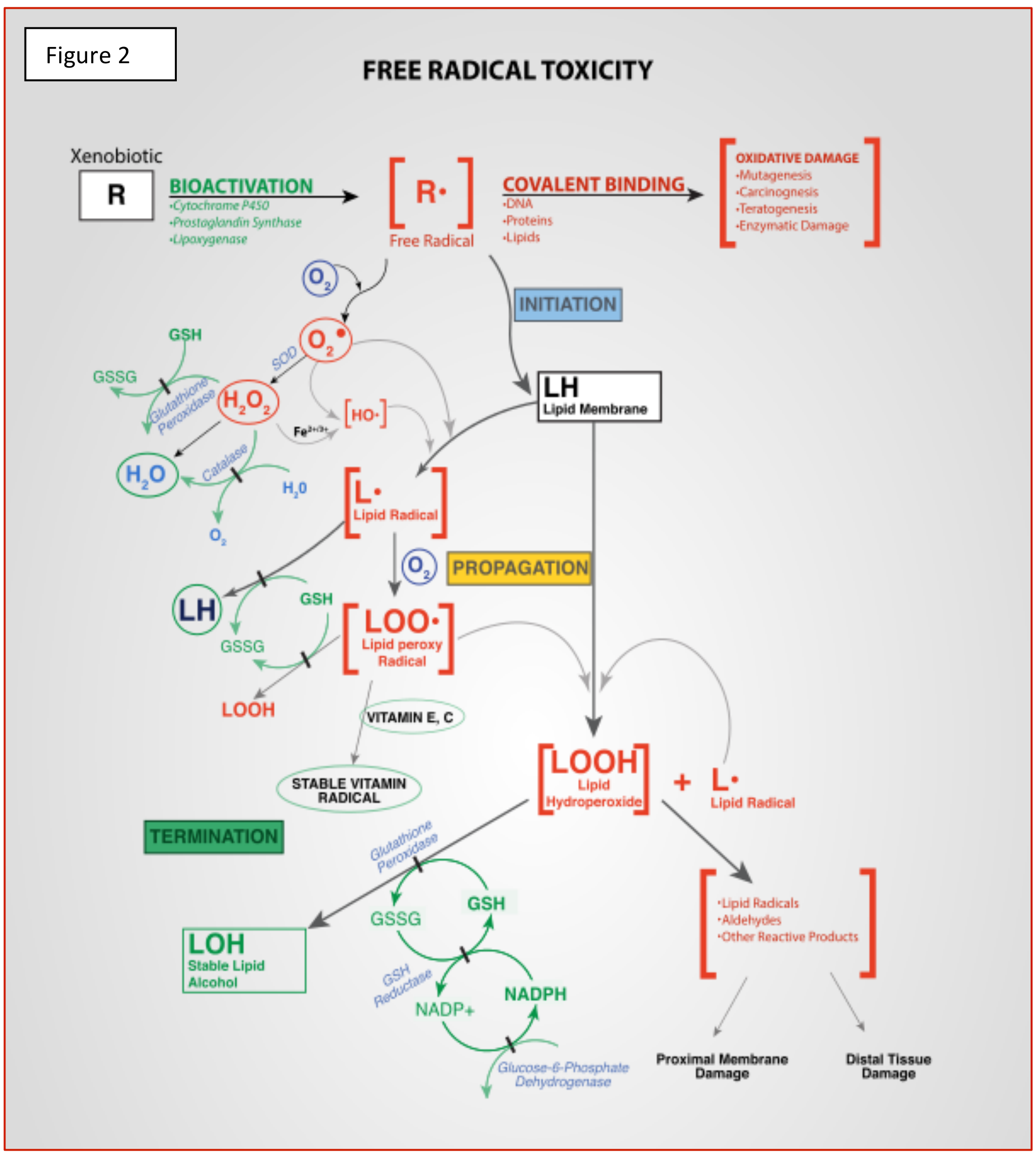
Whole blood samples were collected from all cattle at reimplant time or on the kill floor and centrifuged for 14 minutes at 3,000 RPM at 4^0C to collect serum for the assays.

V. Biological Assays

There are three different biological assays utilized to determine the physiological benefits of DuoPort GPX and its effect on total antioxidant capacity and a reduction in oxidative stress caused by lipid hydro-peroxides and other ROS.

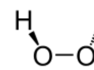
1.) Total Antioxidant Assay[1]: The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS® · + by metmyoglobin. The capacity of the antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox, a water-soluble tocopherol analogue, and is quantified as molar trolox equivalent antioxidant capacity (TEAC) and reported as mM TROLOX (Cayman Chemical Co.).

2.) TBARS Assay[1]: Oxidative stress (OS) in the cellular environment results in the formation of highly reactive and unstable lipid hydroperoxides. Decomposition of the unstable peroxides derived from PUFAs results in the formation of malondialdehyde (MDA), which can be quantified colorimetrically following its controlled reaction with thiobarbituric acid. The results are expressed as uM MDA and commonly referred to as the TBARS assay. A schematic of lipid peroxidation and the antioxidant systems to control it is as follows: (Source: Wikipedia, the free encyclopedia; Lipid Peroxidation):



The important understanding of lipid peroxidation (Fig. 2) is that the initiation begins with the superoxide, hydroxide, and/or hydroperoxyl free radical attacking the cellular lipid membrane. Adequate glutathione (GSH) and glutathione peroxidase (GPx) are necessary for the termination of lipid peroxidation. If left unchecked lipid radicals, aldehydes, and other reactive products accumulate causing tissue damage. The TBARS assay demonstrates the level of OS from Lipid Peroxidation.

3.) Superoxide Dismutase[1]: Significant amounts of superoxide dismutase (SOD) in cellular and extracellular environments are crucial for the prevention of diseases linked to oxidative stress. Quantification of SOD activity (Cu/Zn-SOD, Mn-SOD, Fe-SOD) is essential in order to fully characterize the antioxidant capabilities of a biological system. SOD activity is assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine. The standard curve generated using this enzyme provides a means to accurately quantify the activity of all three types of SOD (Cu/Zn-, Mn-, and Fe^{-}SOD). Results are expressed as U/ml with a sensitivity of 0.005 U/ml.

As superoxide radicals are generated  the main function of SOD is to form  hydrogen peroxide. Catalase, glutathione peroxidase, and GSH are required to terminate the reaction to water and oxygen (Fig. 1). Left unchecked, hydrogen peroxide can cause cellular damage.

VI. Summary: Total Antioxidant Capacity, MDA (TBARS), and SOD Activity

Table 1

Reimplant Group				
Summary Averages (Mean) Reimplant				
	n	mM TROLOX	uM MDA	U/ml SOD
F1 Reimp – Treated – All Cattle	**35**	**1.500**	**2.331**	**21.1**
Steers	20	1.211	2.643	19.5
Heifers	15	1.885	1.916	23.2
F2 Reimp - Non-trt – All Cattle	**29**	**1.805**	**2.555**	**36.7**
Steers	15	1.625	2.656	38.6
Heifers	14	1.997	2.447	34.8
Kill Floor Group				
Summary Averages (Mean) Kill Floor				
	n	mM TROLOX	uM MDA	U/ml SOD
F3 Kill Floor-Treated – All Cattle	**61**	**1.229**	**7.206**	**13.5**
Steers	31	1.220	7.505	9.9
Heifers	30	1.238	6.897	17.3
UKN-Kill Floor-Non-trt – Strs	**60**	**1.434**	**9.468**	**11.0**

VII. All Groups Summary

The treated Reimplant Group – All Cattle have less TEAC and less MDA (lipid peroxidation) than the non-treated Reimplant Group – All Cattle (Fig. 3).

The treated Kill Floor Group – All Cattle have less TEAC and less MDA (lipid peroxidation) than the non-treated Kill Floor Group – All Cattle (Fig. 3).

The assumption is that the treated cattle have less need for total antioxidant capacity because there is less ROS and oxidative stress due to the beneficial effect of **DuoPort GPX**.

Figure 3. **Total Antioxidants & TBARS All Groups Summary**

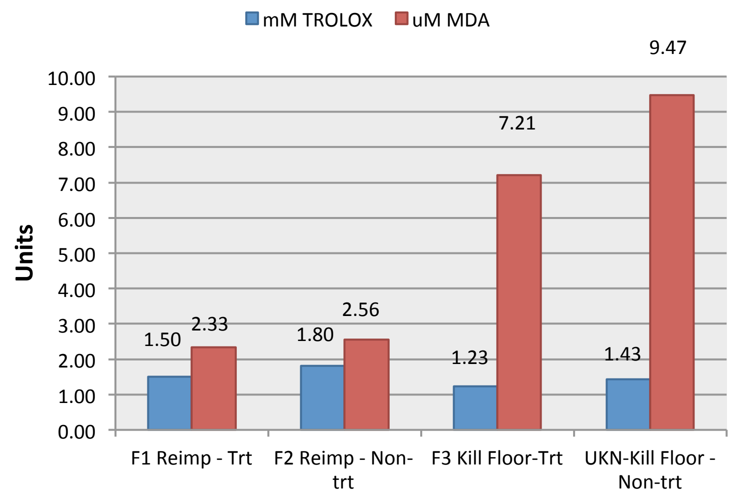
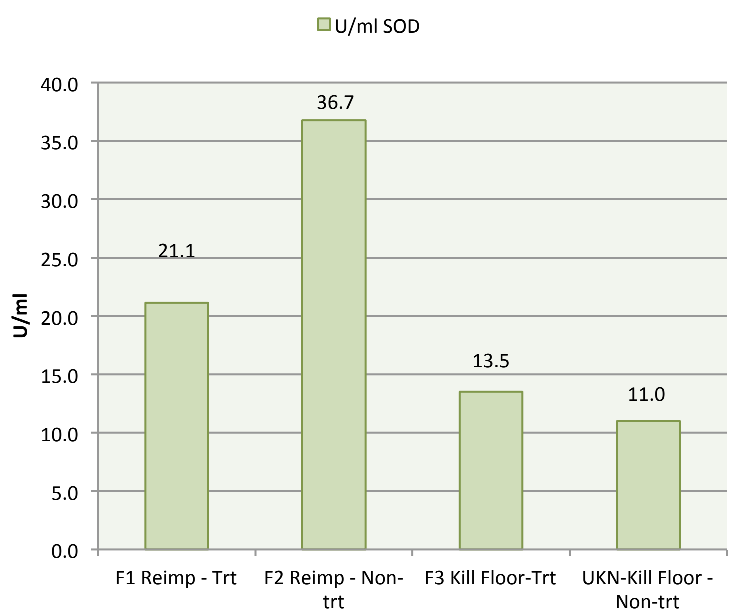


Fig. 4 The treated Reimplant Group – All Cattle have less SOD activity than the non-treated Reimplant Group – All Cattle because there are fewer ROS in the serum. The SOD activity is accelerated in the non-treated Reimplant Group – All Cattle because of the greater amount of ROS. If that activity remained elevated into the finishing phase, chronic OS would become an issue.

The treated Kill Floor Group – All Cattle has maintained a higher SOD activity than the non-treated Kill Floor Group – All Cattle. It is suspected that with chronic ROS generation and sustained lipid peroxidation, i.e. chronic oxidative stress (OS), the SOD activity will decrease due to protein damage or depletion.

Figure 4. **SOD Acivity - All Groups Summary**



"The activity of SOD has to be lower if the OS is chronic. This is because OS could damage [the] mitochondrial engine producing more OS and decreasing energy production so the cell could produce less activity and less OS defenses. On the other hand, if the OS is for a short period the defense mechanisms will increase its [SOD] activity to counteract it. So it [SOD activity] depends on the amount of OS and the time of the exposure to the OS." Rodolfo Pinto-Almazan, PhD, University of California – Los Angeles, Neurology and Chemical Biology. (this will be explained further in the discussion section).

VIII. Reimplant Group

The treated Reimplant Steers had less TEAC than the non-treated steers. Both treated and non-treated Steers have similar MDA (lipid peroxidation) (Fig. 5).

The treated Reimplant Heifers had similar TEAC as the non-treated Heifers. The treated heifers have less MDA (lipid peroxidation) than the non-treated heifers (Fig. 5).

The TEAC observation in heifers may, in part, be due to the fact that heifers demonstrate a higher OS requiring more total antioxidants.

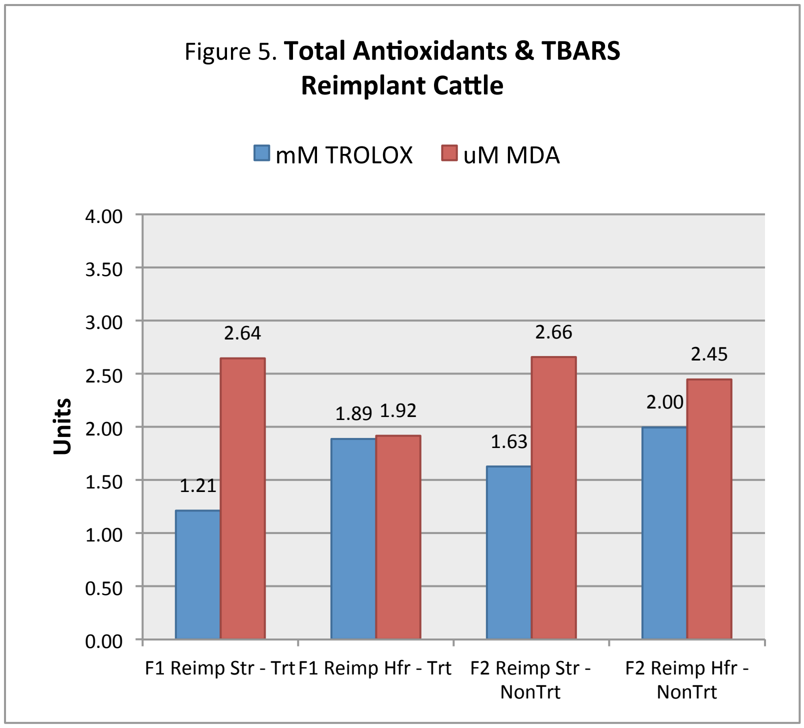
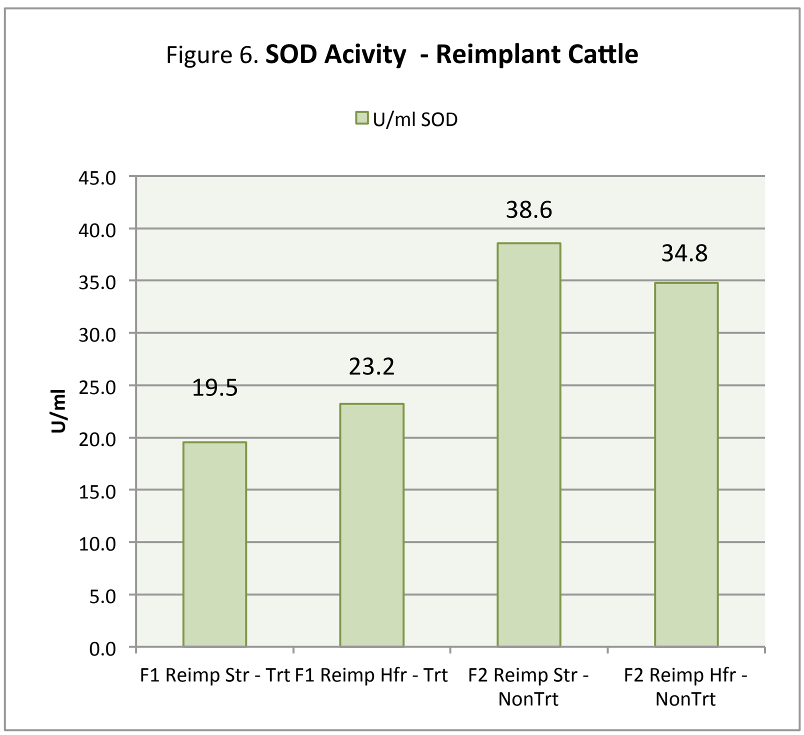


Fig. 6 The treated Steers and treated Heifers have less SOD activity than the non-treated Reimplant Steers and Heifers. The implication is because there is less OS and are fewer ROS in the serum.

If the accelerated SOD activity in non-treated steers and heifers remained elevated into the finishing phase, chronic OS would likely become an issue. Recall that the primary purpose of SOD is to convert oxygen free radicals to hydrogen peroxide.

The assumption of the observations in Figure 5 and Figure 6 is that the treated cattle have less need for total antioxidants because there is less ROS and less OS due to the beneficial effect of **DuoPort GPX**.



IX. Kill Floor Group

The treated Kill Floor cattle had less TEAC than the non-treated cattle. The treated Kill Floor cattle had less MDA (lipid peroxidation) (Fig. 7).

Figure 7 also illustrates that treated Kill Floor steers compared to treated Kill Floor heifers had similar TEAC and MDA levels which were significantly less than the non-treated Kill Floor.

At harvest the **DuoPort GPX** treated cattle had less total antioxidant requirement and less oxidative stress than non-treated cattle. The metabolic engine is running cooler.

Figure 7. **Total Antioxidants & TBARS - Kill Floor Cattle**

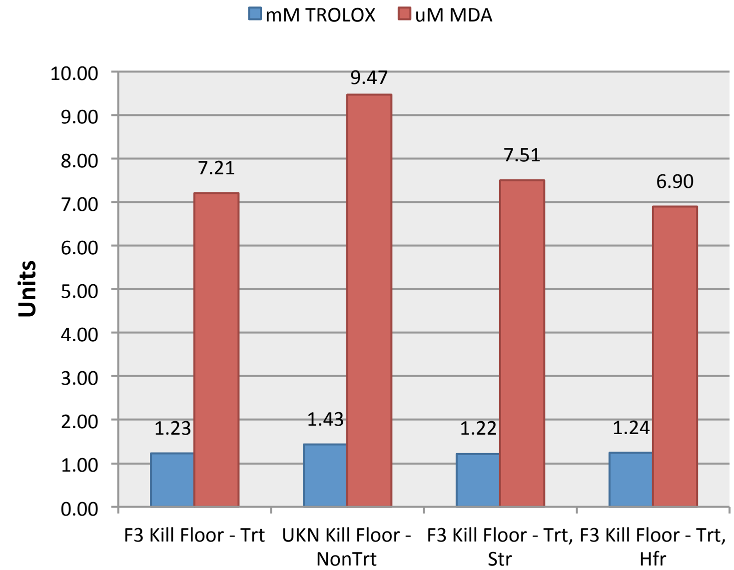


Figure 8: The signifcant negative difference in SOD activity in un-treated Kill Floor vs treated Kill Floor cattle is suspected to be SOD shutdown due to chronic oxidative stress in the un-treated cattle as illustrated by the increased uM MDA shown in Figure 7.

In comparing SOD activity in treated Kill Floor steers and treated Kill Floor heifers, the heifers have almost 2X the SOD activity. First, it is apparent that the treated heifers did not experience SOD shutdown; they are able to mount a defense against oxidative stress late in the finishing phase. Second, this 2X SOD activity may also demonstrate the additional need in heifers to counteract oxidative stress.

Figure 8**. SOD Acivity - Kill Floor Cattle**

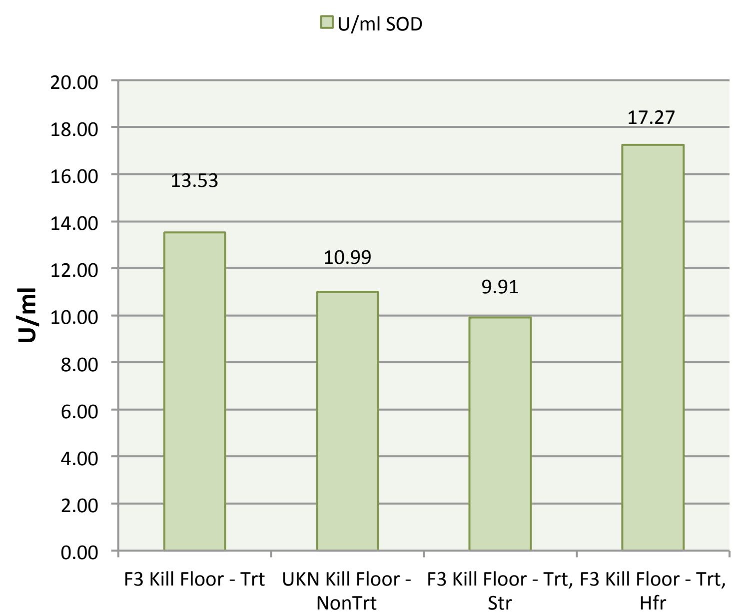


Figure 6 also shows a similar comparison in the reimplant treated steer vs the reimplant treated heifer.

These significant observations start to shed some light on how oxidative stress can initiate certain disease processes (see discussion section).

X. Statistics

The significance threshold (p value) is set at .05 for this trial data. Alpha values are .05, .01, and .001. The t-test has reliable normal distributions.

Table 2

	Figure	n		Means		Statistics	
Group	**Ref.**	**Trt**	**Non-Trt**	**DuoPort GPX**	**Non-Trt**	**ANOVA**	**t-test**
Reimplant – All Cattle	3,4						
mM TEAC		35	29	1.4997	1.8046	**p< .05**	**p< .01**
uM MDA, TBARS		35	29	2.3312	2.5550	p> .05	p> .05
U/ml SOD Activity		35	29	21.1197	36.747	**p< .001**	**p< .001**
Reimplant – Steers	5,6						
mM TEAC		20	15	1.2105	1.6251	**p< .05**	**p< .05**
uM MDA, TBARS		20	15	2.6427	2.6556	p> .05	p> .05
U/ml SOD Activity		20	15	19.5421	38.7272	**p< .05**	**p< .05**
Reimplant –Heifers	5,6						
mM TEAC		15	14	1.8852	1.9997	p> .05	p> .05
uM MDA, TBARS		15	14	1.9157	2.4471	p> .05[1]	**P< .05**[1]
U/ml SOD Activity		15	14	23.2230	34.7902	**p< .05**	**p< .05**
Kill Floor – All Cattle	3,7,8						
mM TEAC		61	60	1.2288	1.4339	**p< .05**	**p< .01**
uM MDA, TBARS		61	60	7.2059	9.4676	**p< .01**	**p< .001**
U/ml SOD Activity		61	60	13.5257	10.9945[3]	**p< .05**	**p< .05**
Kill Floor – Steers	7,8						
mM TEAC		31	60	1.2204	1.4339	p> .05[2]	**P< .05**[2]
uM MDA, TBARS		31	60	7.5053	9.4676	**p< .01**	**p< .001**
U/ml SOD Activity		31	60	9.9071	10.9945	p> .05[3]	p> .05[3]

[1] The uM MDA for treated vs non-treated reimplant heifers had an ANOVA p< .08 which is above our significance threshold, but the t-test p< .05 indicates there is a significant difference.

[2] The mM TROLOX (TEAC) for treated vs non-treated kill floor steers had an ANOVA p< .07 which is above our significance threshold, but the t-test p< .05 indicates there is a significant difference.

[3] See discussion section "SOD Activity shutdown in chronic oxidative stress (OS)".

Table 3

Comparison of Kill Floor treated steers vs Kill Floor treated heifers							
	Fig. ref	n		Means		Statistics	
KF Trt Steers v Trt Heifers	7,8	**Steers**	**Heifers**	**Steers**	**Heifers**	**ANOVA**	**t-test**
mM TEAC		31	30	1.2204	1.2375	p> .05	p> .05
uM MDA, TBARS		31	30	7.5053	6.8965	p> .05	p> .05
U/ml SOD Activity		31	30	9.9071	17.2650	**P< .001**[1]	**P< .001**[1]

[1] See discussion section "Kill Floor treated steers vs treated heifers have significantly different SOD activity".

XI. Discussion

Measuring serum physiological parameters in this initial feed trial with DuoPort GPX treated cattle vs non-treated cattle has demonstrated that in treated cattle there is a reduced need for circulating total antioxidants and an improvement in handling oxidative stress (OS) and reactive oxygen species (ROS). The hypothesis is validated:

> *"amino acid chelates improve the metabolic state of the animal reducing the total antioxidant requirements thereby reducing oxidative stress. Simply stated, the metabolic engine runs cooler!"*

As demonstrated in reimplant cattle and in harvest cattle, quantified OS and ROS levels were significant on both treated and untreated cattle.

Treated reimplant cattle demonstrated:

- 17% less antioxidant capacity requirement
- 8% less lipid peroxidation per the TBARS assay
- 42% less SOD activity requirement, i.e. less oxidative stress.

Treated harvest cattle demonstrated:

- 14% less antioxidant capacity requirement
- 24% less lipid peroxidation per the TBARS assay
- 23% increase in SOD activity over non-treated harvest cattle indicative of a shutdown in SOD activity in harvest cattle due to chronic oxidative stress.

Why this happens requires further investigation but some implied reasons are:

- The highly biologically available chelated trace minerals are readily incorporated into antioxidant enzyme systems in the liver creating better control of the normal production of ROS in metabolic processes;
- The magnesium (Mg) amino acid chelate is readily available for glutathione (GSH) production in the liver. GSH is essential in the control of lipid peroxidation both intracellular and extracellular as well as the breakdown of hydrogen peroxide by glutathione peroxidase to harmless water and oxygen;
- Subclinical potassium deficiency (hypokalemia) accelerates Mg loss through the kidneys. The potassium amino acid complex has a beneficial effect in this homeostasis.

SOD Activity Shutdown in Oxidative Stress

As mentioned in Section VII and shown in Figure 4 and Figure 8, in the field of oxidative stress and free radical physiology, researchers worldwide agree that there can be a negative feedback with chronic long term OS exposure and a shutdown of SOD activity:

> *"In my experience with in vivo systems, if the Oxidative Stress (OS) is not very strong, or very long-during, the SOD activity increases. If OS is persisting, or its level very high, the proteins damage became profound and a decreased SOD activity may occur (either via direct oxidative damage of the SOD molecules, or via OS-altered SOD gene expression, or both.)"* Maria Traykova**,** Medical University of Sofia, Sofia, Bulgaria.

> *"The activity of SOD has to be lower if the OS is chronic. This is because OS could damage [the] mitochondrial engine producing more OS and decreasing energy production so the cell could produce less activity and less OS defenses. On the other hand, if the OS is for a short period the defense mechanisms will increase its [SOD] activity to counteract it. So it [SOD activity] depends on the amount of OS and the time of the exposure to the OS."* Rodolfo Pinto-Almazan, PhD, University of California – Los Angeles, Neurology and Chemical Biology.

There are excellent reviews about ROS production, protection mechanisms, and oxidative stress in Physiological Reviews, Bhattacharyya et al [2] and in Veterinary Immunology and Immunopathology, Sordillo et al [3].

Kill Floor treated steers vs treated heifers have significantly different SOD activity
This is an interesting significant finding as shown in Figure 8. But also, the same significant finding exists between steers and heifers in the reimplant group. Part of the explanation may be that:

- heifers have a higher copper requirement (personal communication from BC Consulting, Brownstown, IN);
- heifer growth implants may have a different metabolic affect than in steers in regards to ROS production;
- monensin and beta-agonists (finishing phase) may have a different metabolic affect than in steers in regards to ROS production.

More research is needed to understand these differences.

Oxidative Stress and Disease
Just a couple comments:

Per steers and heifers having significantly different SOD activity, and non-treated heifers had significantly higher oxidative stress (TBARS), it is interesting that heifers are more prone to atypical interstitial pneumonia (AIP) [4] and higher incidence of dark cutters [5], both of which have an ROS causation and both of which can be, and are, issues in feedlots.

ROS impact meat tenderness in vivo and post mortem [6]. This can be a huge issue in marketing of specialty meats.

Mycoplasma pneumonia is influenced by H_2O_2 production free radical mediated cell stress [7]. As shown in Figure 6, non-treated reimplant cattle have higher SOD activity. If acute, the only end result of increased SOD activity is increased H_2O_2 production!

XII. Conclusion

Amino acid chelates-**DuoPort GPX**-improves the metabolic state of the animal reducing the total antioxidant requirements thereby reducing oxidative stress. Simply stated, the metabolic engine runs cooler.

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