

Selenium Sources Affect Protein Concentration, Thioredoxin Reductase Activity and Selected Production Parameters in Reovirus Infected Broiler Chickens

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Abstract: Successful Avian Reovirus (ARV) infections ultimately result in decreased weight gains coupled with increased mortality. Selenium (Se) is a structural component of Thioredoxin Reductase (TRX), an enzyme capable of quenching intracellular free radicals and influencing redox balance. The aim of this study was to determine the various effects that Se-containing diets fed to ARV-infected broilers had on protein concentrations, TRX activity, body weights and mortality. Eggs were obtained from Cobb breeders that had been maintained on isocaloric Torula yeast diets containing either no supplemental Se, sodium selenite at 0.3 ppm, or organic Se (Sel-Plex®, Alltech, Inc., Nicholasville, KY) at 0.3 ppm. Chicks hatched from those eggs were placed on Torula yeast broiler diets containing 1) no supplemental Se, 2) 0.3 ppm sodium selenite, or 3) 0.3 ppm organic Se similar to their respective parents' diets. On day of hatch, 60 chicks per dietary Se treatment were placed into either Control or Virus-infected groups in heated-growing batteries in separate isolation rooms. Chicks in the Virus-infected groups were given each an oral gavage of 0.5 ml of ARV-CU98 ($10^{4.2}$ pfu/ml/chick), and Control chicks were given the medium only. At 14 and 21 days of age, the birds were weighed, bled, killed by CO₂ asphyxiation, and tissues collected for analyses. Data from this 2 X 3 factorially arranged, completely randomized experimental design were analyzed using the GLM procedure of SAS. ARV-infected birds had significantly lower average body weights (ABW) at 14 and 21 days ($P < 0.0001$), three times higher mortality rates, and decreased tissue protein concentration ($p < 0.001$) than controls. Se treatments did not affect ABW and mortality, but did significantly improve plasma protein concentration ($p < 0.05$) and TRX activity in both, healthy and virus-challenged bird. Our findings suggest that ARV infection depresses growth, increases mortality and reduces protein concentration in various tissues, whereas Se is beneficial against ARV infection in broilers through an improved antioxidant status.

Key words: Reovirus, selenium, broilers, thioredoxin reductase, mortality

Introduction

Reoviruses (RV) are members of the *Orthoreovirus* genus within the *Reoviridae* family; which also include Rotavirus, Coltivirus, Seadornavirus and Orbivirus (Tyler, 2001). A non-enveloped dual concentric icosahedral capsid layer enclosing a 10-piece double-stranded RNA segmented genome structurally characterizes RV features (Nibert *et al.*, 1996). Avian Reovirus (ARV) is specie-specific and mildly virulent to chickens (Robertson *et al.*, 1984), turkeys (Page *et al.*, 1982), geese (Palya *et al.*, 2003) and ducks (Kaschula, 1950; Gaudry *et al.*, 1972). ARV is typically associated with viral arthritis/tenosynovitis (Robertson and Wilcox, 1986), moderate and self-limiting enteric and respiratory infections, (Jackson *et al.*, 1961; Guy, 1998) and also suggested to be partially responsible in several organ-distressing syndromes (London *et al.*, 2002; Heggen-Peay *et al.*, 2002). ARV replication occurs mainly in heart, liver, hock joints, and intestines (Menendez *et al.*, 1975;

al Afaleq and Jones, 1990). Successful ARV infection ultimately result in decreased weight gains coupled with increased mortality leading to potentially substantial economic loses in established commercial poultry operations.

Selenium (Se) is a trace mineral discovered by Swedish chemist Berzelius in 1817 (Rayman, 2000) that exists as both, organic and inorganic forms in soil, plant and animal tissues (Johnson *et al.*, 1999). Schwarz and Foltz (1957) established its essentiality in rats, which ignited initial research into its elemental properties. Se is a structural component of several synthetic and naturally occurring compounds, some of which have antioxidant (AOX) properties (Gladyshev *et al.*, 1998; Kirsi *et al.*, 1983). In chickens, Se is involved with Glutathione Peroxidase (GPX) and Thioredoxin Reductase (TRX), both of which are ubiquitous enzymes whose function, among others, is to quench oxygen and nitrogen free radicals (FR) that can damage proteins, lipids and

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nucleic acids (Brigelius-Flohe, 1999; Arner and Holmgren, 2000; Rayman, 2000). Unchecked and sustained FR generation affects cellular and tissue functions that deleteriously result in organ dysfunctions and physiological disruptions (Floyd and Carney, 1992; de Zwart *et al.*, 2001; Halliwell and Gutteridge, 1985; Beckett *et al.*, 1992).

The AOX and antiviral effects of Se have been studied extensively in human and non-human primates, less so in other species and cell cultures (Patrick, 1999; Schrauzer, 2000; Schrauzer and Sacher, 1994; Beck, 1997; Spallholz *et al.*, 1990). Its effects have been tested against HIV (Dworkin, 1994), FIV (Mortola *et al.*, 1998), Coxsackie viruses (Beck *et al.*, 2003) and influenza viruses (Beck *et al.*, 2001). Repeated scientific literature searches reveal that little is known about how dietary Se, either organic or inorganic, affects ARV infection in broiler chickens.

The aim of this study was to determine the effects that organic and inorganic Se-containing diets fed to ARV-infected broilers had on protein concentrations, TRX specific activity, body weights and mortality. Our findings suggest that ARV infection depresses growth, increases mortality and reduces protein concentration in various tissues, whereas Se is beneficial against ARV infection in broilers through an improved antioxidant status.

Materials and Methods

Eggs, birds, diets, virus and conditions: Eggs were obtained from Cobb¹ breeders that had been maintained on isocaloric Torula yeast diets containing either no supplemental Se, sodium selenite at 0.3 ppm, or organic Se (Sel-Plex®, Alltech, Inc., Nicholasville, KY) at 0.3 ppm at the Coldstream Research Facility within the University of Kentucky's campus and transported to North Carolina State University (NCSU). Eggs were placed in Jamesway² incubators at 99 F for 21 days until hatch. Chicks hatched from those eggs were placed on three Torula yeast broiler diets containing 1) no supplemental Se, 2) 0.3 ppm sodium selenite, or 3) 0.3 ppm organic Se (as Sel-Plex®³) similar to their respective parents' diets which were manufactured and provided by Alltech, Inc., a Nicholasville, KY-based multinational biotechnology firm. Avian Reovirus was procured at Cornell University (ARV-CU98) by M. A. Qureshi from K. A. Shat, and kindly provided by M. D. Koci's lab for this study. Two isolation rooms located at Roy S. Dearstyne Avian Research Center at NCSU were preheated at 95 F for 24 hours prior to chick placement, and after a week, room temperature was reduced to 90 F. Throughout the experiment room temperature ranged between 85-97 F. This study was conducted under animal care and use guidelines established by North Carolina State University's Institutional Animal Care and Use Committee (IACUC) that governs all animal use in experimental procedures.

Experimental design, procedures and parameters: The experimental features of this study result in a 2 x 3 factorially arranged, completely randomized design: virus-challenged and unchallenged groups and three dietary Se treatments. 360 Day-old male and female broiler chicks were randomly assigned into 6 experimental treatments with 4 replicates each, totaling 24 pens (15 birds per pen), distributed among two separate isolation rooms (virus room = V and control room = C) with two wire-mesh-floored Petersime batteries in each room. Only the middle six pens of the batteries were used to house the birds to reduce statistical noise. Twenty hours after placement, 180 chicks were orally inoculated with 0.5 ml gavages of ARV-CU98 suspended in a 1:5 dilution with PBS at a concentration of $1 \times 10^{4.2}$ c.f.u./ml and the remaining chicks were mock inoculated with 0.5 ml of sterile water. Food and water were provided *ad libitum*. Pens were cleaned every other day, while mortality was recorded daily per treatment. At 14 and 21 days, five birds per treatment were weighed, bled by heart puncture, and euthanized by CO₂ asphyxiation. For chemical assays, approximately one gram of tissue samples from liver and middle-ileum were excised, placed in 0.85% ice-cold saline and frozen until assayed. Bird carcasses were disposed as stipulated by Environmental Health and Safety (EHS) and IACUC procedures.

Protein concentration determination: Tissue samples were thawed, thoroughly rinsed with PBS, minced, homogenized⁴ in a polytron blender⁵ with 2 ml of 50mM Tris-1mM EDTA buffer in each 17 x 100 polypropylene test tubes and spun for 30 min at 5000 x g in a Beckman⁶ Model J-6B centrifuge. Blood samples were collected in EDTA coated tubes and similarly centrifuged for plasma collection. Tissue supernatants were decanted into 1.5 ml polypropylene micro-vials and spun for 15 min at 12,500 rpm in Micromax RF⁷ micro-centrifuge. Plasma and pellet-free supernatant were used for protein concentration determination as described by the microassay procedure for microtiter plates in Bio-Rad Protein Assay Kit⁸. Samples were plated in triplicate, diluted in a 1:30 ratio and absorbance was measured at 595 nm (A₅₉₅) in DYNEX⁹ plate reader, Model MRK-TC, powered by DYNEX Revelation 4.06 software.

To calculate protein concentration in µg/ml a regression equation was established according to the calculated parameters as described below:

$$\mu\text{g/ml} = [(A_{595}) * (\text{slope}) + \text{intersection}] \times 30$$

The protein assay kit required six protein standards that were concocted using bovine serum albumin (BSA) and water. Coefficients of variation among replicates of the same sample were consistently maintained at < 5 percent.

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Table 1: Effect of selenium sources and avian reovirus (ARV-CU98) inoculation on live body weights and mortality rates in 14 and 21 day old broilers*

Code	Source	Se ppm	Virus	Body weights (g)		Mortality %
				14d	21d	
C1	Torula	0.02	-	526	992.2	3/60 (5%)
C2	Organic	0.3	-	500.2	975.6	5/60 (8.33%)
C3	Inorganic	0.3	-	516.8	977	3/60 (5%)
<Control Average>				514.33A	981.6A	AVG=6.11%
V1	Torula	0.02	+	381.8	737.4	5/60 (8.33%)
V2	Organic	0.3	+	330.4	718.2	13/60 (21.67%)
V3	Inorganic	0.3	+	373.6	716.2	15/60 (25%)
<Virus Challenge Average>				361.93B	723.93B	AVG=18.33%

* n = 5; different letters within columns represent significance $p < 0.0001$; no dietary effects were found.

Thioredoxin reductase activity assay: Remaining pellet-free tissue supernatants were also used for TRX activity using the DTNB assay as previously described (Luthman and Holmgren, 1982). Working buffer (100mM Na phosphate, 10mM Na_4EDTA , 0.2 mM NADPH, 0.2 mg BSA/ml, 1% ethanol, 5mM DTNB) and 0.5 mM FAD (4.2 mg of FAD in 500mM Tris, pH 7.4) were added to 96 well plates before adding 10 μl of samples per well. Absorbency was read at 412 nm for 3 min. Samples were run in triplicate and statistical outliers automatically removed. Results were calculated based on the yield of 2 moles of 2-nitro-5-thiobenzoate per mole of NADPH consumed.

Statistical analysis: All data generated from this 2 x 3 factorially arranged, completely randomized experimental design were analyzed using the PROC GLM and ANOVA procedure of the Statistical Analysis Systems Institute software (SAS Institute, 1995). Significant differences were labeled and separated by the Student-Newman-Kuehls test at $p < 0.05$, and in some instances at $p < 0.001$ and $p < 0.0001$.

Results

ARV reduces 14-d & 21-d body weights and increases mortality: From Table 1, data suggests that broilers challenge with ARV had a significant detrimental effect on body weights (BW) at 14 and 21 days ($p < 0.0001$). At both sample dates, BW reduction is 152 grams and 257 grams, respectively, less than controls. Selenium supplementation, regardless of source and level, had no significant effect on BW. Mortality values were not subjected to statistical analysis; however, it is evident that birds afflicted with ARV experienced -on average- higher death rates (6.11%) than unchallenged birds (18.33%). Avian reovirus infection alone increased mortality in broilers by 3 folds before bird reached maturity, and most of it during the first 10 days after infection (data not shown). Death percentages from ARV-infected birds are far from the industry averages of less than 5%; validating the potential economic losses that are related with high mortality rates coupled with substantial weight gain depressions. Conclusively,

successful ARV infection reduces weight gains and increases mortality.

ARV reduces protein concentration; Organic Se may selectively reverse this effect: From Table 2, data suggests that ARV infection significantly reduces 14d plasma and ileum average protein concentration when compared to unchallenged birds ($p < 0.001$); however this effect is lost by day 21. Selenium supplementation, regardless of form, significantly ($p < 0.05$) increases 14d protein concentrations in liver compared to controls with no Se supplemented. Additionally, Organic Se significantly reduced ($p < 0.05$) 14d protein concentration in ileum of ARV infected birds. Liver is known to be a preferred site of viral replication. In this tissue average protein concentrations at 14 and 21d tends to increase in the presence of ARV, and much to our dismay, both organic and inorganic Se aid in maintaining protein levels in hepatocytes. Selenium, it seems, may have selective tissue-specific roles within an organism.

ARV reduces overall TRX activity, whereas Se increases TRX activity except in liver: From Table 3, there is a non-significant tendency for ARV-infected birds to reduce average TRX activity in ileum and liver at both sample dates, this may be due -most likely- by the fact that 5 birds were used per treatment; additionally, tissues were pooled for ease of enzymatic analysis, thus, variation within treatment is limited and conclusions cannot be fully accounted for in this study. Another possible reason is that colorimetric assays are highly variable as most enzyme assays are- and provide excessive background variations that make statistical accuracy cumbersome. Nonetheless, despite the lack of statistical power, the results are valid on their own accord, and truly reflect the effect of treatments; hence the forthcoming results are made with this caveat. In the middle ileum, regardless of age, Se supplementation increases TRX activity anywhere between 25 to 150 percent. In the liver, organic Se is slightly better than inorganic Se in improving TRX activity, but for the most part, overall selenium supplementation is only able to maintain enzyme activity, and in some cases it is

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Table 2: Effect of selenium sources and avian reovirus (ARV-CU98) inoculation on plasma, ileum and liver protein concentration in 14 and 21 day old broilers*

Code	Source	Se ppm	Virus	Protein Concentrations [ug/ml]					
				Plasma		Ileum		Liver	
				14d	21d	14d	21d	14d	21d
C1	Torula	0.02	-	29.16	34.17	16.4	28.77	14.19 ^a	26.52
C2	Organic	0.3	-	33.1	32.89	15.75	29.31	25.06 ^b	33.25
C3	Inorganic	0.3	-	31.65	32.85	11.54	28.32	27.19 ^b	34.87
<Control Average>				31.3A	33.3 ns	14.6A	28.8ns	22.1ns	31.5ns
V1	Torula	0.02	+	27.21	29.91	13.52 ^b	27.502	26.04	32.17
V2	Organic	0.3	+	29.92	35.54	9.07 ^a	30.26	19.74	35.76
V3	Inorganic	0.3	+	27.6	31.58	11.6 ^{ab}	30.82	26.51	33.52
<Virus challenged Average>				28.2B	32.3 ns	11.4B	29.5ns	24.1ns	33.8ns

* n = 5; Capital letters represent differences within columns at p<0.001; small letters at p<0.05. ns, not significant.

reduced below C1 and V1 levels. Similarly to the protein concentrations data set, selenium may have tissue-specific roles or is affected by *in situ* ARV replication.

Discussion

The evidence of deleterious ARV infections to various avian species exemplifies the importance of finding ways to palliate its effects through nutritionally natural alternatives. Most negative consequences of RV in poultry include tenosynovitis leading to lameness, stunting and depressed growth (Islam *et al.*, 1988; van der Heide and Kalbac, 1975); intestinal villi damage leading to diarrhea and reduced nutrient absorption (Mebus *et al.*, 1976); uneven flocks, poor pigmentation, abnormal feathering, skeletal abnormalities and increased mortality (Kouwenhoven *et al.*, 1978a; Kouwenhoven *et al.*, 1978b; McFerran *et al.*, 1976; McNulty *et al.*, 1976).

Mineral and vitamin supplements in complete animal feeds may serve as potential health enhancers, with some antiviral properties ascribed to vitamins A, C and E, selenium, zinc, copper and manganese (Allard *et al.*, 1998; Pardue *et al.*, 1985; Peterhans, 1997). Se supplementation recommended by US Food and Drug Administration (FDA, 2003) guidelines is 0.3 ppm per ton. Considering the legally allowed inclusion rate, this study suggests that ARV infection depresses growth, increases mortality and reduces protein concentration on various tissues, whereas Se at 0.3 ppm is beneficial against ARV infection in broilers, with organic Se being a slightly better alternative than inorganic Se in feedstuffs.

The specific mechanisms that describe how Se improves health and performance of animals afflicted with ARV have not been completely elucidated, but some proposals have been put forward: A) Se is an essential cofactor for GPX and TRX, both AOX enzymes that decompose hydrogen and organic peroxides –natural intracellular free radicals, B) Se improves redox balance within the cell, thus improving host metabolic capacities and C) Se enhances iNOS expression that in turn

increases intracellular nitric oxide that has potential inhibitory effects on viral proteases (Saura *et al.*, 1999). It is not likely that Se is affecting ARV structure or its ability to attach to hosts cell membranes because RV's share characteristic of having segmented RNAs, which are continually evolving due to the lack of proofreading enzymes (Steinhauer *et al.*, 1992). This evolutionary survival advantage to rapidly mutate when faced with challenging environmental pressures allows them to adapt easily to new conditions (Beck *et al.*, 2003). Moreover, a series of clever experiments performed by Connolly and Dermody (2002) demonstrate that disassembly of RV virions to form infectious subviral particles (ISVPs), but not transcription or subsequent steps in viral replication, are required for reovirus to induce cell death, therefore, based on their findings, it is plausible to suggest that Se displays an intracellular mode of action.

In other animal species, dengue and respiratory syncytial virus induce the release of IL-8, which is a chemoattracting cytokine for neutrophils, thus promoting their degranulation (Jaovisidha *et al.*, 1999; Juffrie *et al.*, 2000). RV invites neutrophils invasion in rat lungs and intestines (Morin *et al.*, 1996). Cell culture studies indicate that RV infection releases IL-8 and other inflammatory cytokines (Hamamdzic *et al.*, 1999), suggesting RV is actively being sought by the immune system and that its detection ignites signaling cascades that attract hydrolase-releasing neutrophils and macrophages that can cause damage to epithelial tissues in an attempt to control viral infections.

In veterinary pathology, ARV is considered a viral stressor acting through nucleic factor activation (Clarke *et al.*, 2001; DeBiasi *et al.*, 2003). Stressors, inflammation and immune system energy expenditures against recurrent viral infections coupled with a diminished host protein synthesis invariably results in depressed growth rates as evidenced by slower body weight gains in virus-afflicted birds (Table 1).

Villus enterocytes are mature, non-proliferating cells covering the villi that are differentiated into digestive and

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Table 3: Effect of selenium sources and avian reovirus (ARV-CU98) inoculation on mid-ileum and liver Thioredoxin Reductase activity in 14 and 21 day old broilers*

Code	Source	Se ppm	Virus	TRX Activity [nmol/min/mg ptn]			
				Ileum		Liver	
				14d	21d	14d	21d
C1	Torula	0.02	-	19.85	40.5	20.54	54.86
C2	Organic	0.3	-	47.98	70.33	22.35	59.46
C3	Inorganic	0.3	-	50.15	57.04	17.83	43.7
<Control Average>				39.33	55.96	20.24	52.67
V1	Torula	0.02	+	19.03	41.36	19.94	26.11
V2	Organic	0.3	+	23.93	55.38	21.36	13.82
V3	Inorganic	0.3	+	42.27	67.81	13.04	16.62
<Virus Challenge Average>				28.41	54.85	18.11	18.85

* n = 5; no statistical differences found. Tissues were pooled for analysis convenience, rendering dubious results.

absorptive roles. Rotaviruses, kindred to reoviruses, replicate in mature enterocytes suggesting that differentiated intestinal cells express factors required for efficient infection and replication. Additionally, modes of actions have been proposed, and most, if not all, agree that viral entry and uncoating signal intracellular calcium stores to be released, which further signal cell membrane protein pores to allow pericellular flow of water and electrolytes outside of the cell, hence ensuing diarrhea and nutrient malabsorption (Ramig, 2004). In our study, this is a plausible explanation for depressed growth rates and reduced body weights; as young-growing birds may be under severe mineral and water losses coupled with intestinal inflammation leading to a decreased nutrient absorbing capacity that ultimately results in smaller, weaker, unhealthy birds.

The mechanism of viral replication, regardless of tissue, is facilitated by an immediate hijack of the host transcriptional machinery in favor of viral protein manufacture (Piron *et al.*, 1998; Varani and Allain, 2002). RV infection can also be accounted for by evidence of enhanced viral protein synthesis and consequential shutoff of host protein synthesis within 12 to 48 hours after infection (Coffey *et al.*, 1998). Viruses use a surprising diversity of approaches to hijack G-protein-coupled receptors and harness their activated intracellular signaling pathways. These approaches function to ensure viral replicative success and often contribute to their pathogenesis (Sodhi *et al.*, 2004). Viral non structural proteins (VNSPs) have stronger affinity to transcription factors than host proteins, causing increased transnational activity of viral mRNA, while translation of host mRNAs is rendered less efficient (Michel *et al.*, 2000; Vende *et al.*, 2000). This is clearly evidenced in our study (Table 2), where plasma protein concentration is depressed in viral challenged birds, as it also is in middle ileum and liver to a lesser degree. Organic selenium was able to maintain and increase plasma protein concentration in ARV-infected birds and also increasing liver protein in unchallenged birds. This effect may be explained by two mechanisms: 1) ARV selectively replicates in liver cells and a higher AOX

profile resulting from Se supplementation, specifically GPX and TRX, is counteracting the deleterious effect of replication on this target tissues and 2) selenium increases Se-dependent enzyme activity in erythrocytes and hepatocytes as to minimize the extent of DNA damage by free radicals generation and viral replication. The latter mechanism has been demonstrated and reviewed by other researchers under different experimental settings and various AOX compounds (Anderson *et al.*, 1994; Bagchi *et al.*, 1993; Duthie *et al.*, 1996; Emonet-Piccardi *et al.*, 1998; Ueda *et al.*, 1996). Human immune deficiency virus (HIV) spread throughout the world has prompted an avalanche of studies into the beneficial supplementation of AOX to AIDS sufferers. Again, it has been repeatedly proven that selenium supplementation enhances Se-dependent enzymes in poor human and animal Se status (Brown *et al.*, 2000; Muller *et al.*, 2000); which explains a considerable yet not significant increase in ileum and liver TRX activity (Table 3). Thioredoxins are critical for redox regulation of protein function and signaling via thiol redox control. Transcription factor NF-KB requires thioredoxin reduction for DNA binding. The cytosolic mammalian thioredoxin, lack of which is embryonically lethal, has numerous functions including defense against oxidative stress, control of growth and apoptosis, donator of reducing equivalent to regenerate ascorbate into ascorbic acid, and has also co-cytokine and chemokine activities (Arnér and Holmgren, 2000). Besides nuclear factor (NF) kappa B, activator protein 1 (AP-1), an important transcription factor, was also identified to be regulated by intracellular redox states. Binding sites of the redox-regulated transcription factors NF-kappa B and AP-1 are located in the promoter region of a large variety of genes that are directly involved in the pathogenesis of debilitating diseases, such as AIDS, cancer, arteriosclerosis and diabetic complications. Many basic events of cell regulation such as protein phosphorylation and binding of transcription factors to consensus sites on DNA are driven by physiological oxidant-antioxidant homeostasis, especially by the thiol-disulfide balance. Endogenous glutathione and

thioredoxin systems, and the exogenous lipoate-dihydrolipoate couple may therefore be considered to be effective regulators of redox-sensitive gene expression (Sen and Packer, 1996). In summary, TRX activity functions, among other things, as a redox regulator with an array of effects in various cells. Our study of TRX as an AOX parameter-enzyme affected by ARV infection may render interesting observations as to its role in viral infections.

Knowledgeable consumer groups and regulatory agencies put pressure on animal factories to reduce the amount of synthetic, potentially deleterious, untested, lipid-soluble compounds that may remain biologically active in humans; as we, humans, hold the highest place in the food pyramid. Europe's ban on antibiotics and other strict additive inclusion regulations attest to the rapidly changing scene of feedstuffs to be fed to chickens and other avian species. Feed mill operators and feed additive firms may find themselves in a relentless search for naturally safe, organic, environmentally friendly compounds to remain competitive in a ruthless global market. In the case of minerals, organic Se may prove to be a highly preferable candidate not only for its documented superiority to other forms of selenium, namely sodium selenite, but also to be supplemented for its subtle antiviral activities and as an ameliorator of selenium-deficiency related illnesses.

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References

- al Afaleq, A.I. and R.C. Jones, 1990. Localization of avian reovirus in the hock joints of chicks after entry through broken skin. *Res. Vet. Sci.*, 48: 381-382.
- Allard, J., E. Aghdassi, J. Chau, C. Tam, C. Kovacs, I. Salit and S. Walmsley, 1998. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS*, 12: 1653-1659.
- Anderson, D., T. Yu, B. Phillips and P. Schmezer, 1994. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutat. Res.*, 307: 261-271.
- Arnér, E. and A. Holmgren, 2000. Physiological functions of thioredoxin and thioredoxin reductase. *FEBS J.*, 267: 6102-6109.
- Bagchi, D., E. Hassoun, M. Bagchi and S. Stohs, 1993. Protective effects of antioxidants against endrin-induced hepatic lipid peroxidation, DNA damage, and excretion of urinary lipid metabolites. *Free Radic. Biol. Med.*, 15: 217-222.
- Beck, M., 1997. Increased Virulence of Coxsackievirus B3 in Mice Due to Vitamin E or Selenium Deficiency. *J. Nutr.*, 127: 966.
- Beck, M., H. Nelson, Q. Shi, P. Van Dael, E. Schiffrin, S. Blum, D. Barclay and O. Levander, 2001. Selenium deficiency increases the pathology of an influenza virus infection. *FASEB J.*, 15: 1481-1483.
- Beck, M., O. Levander and J. Handy, 2003. Selenium Deficiency and Viral Infection. *J. Nutr.*, 133: 1463S-1467S.
- Beckett, G., A. Russell, F. Nicol, P. Sahu, C. Wolf and J. Arthur, 1992. Effect of selenium deficiency on hepatic type I 5-iodothyronine deiodinase activity and hepatic thyroid hormone levels in the rat. *Biochem. J.*, 282: 483-486.
- Brigelius-Flohe, R., 1999. Tissue-specific functions of individual glutathione peroxidases. *Free Radic. Biol. Med.*, 27: 951-65.
- Brown, K., K. Pickard, F. Nicol, G. Beckett, G. Duthie and J. Arthur, 2000. Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets and erythrocytes. *Clin. Sci.*, 98: 593-599.
- Clarke, P., S. Meintzer, C. Widmann, G. Johnson and K. Tyler, 2001. Reovirus Infection Activates JNK and the JNK-Dependent Transcription Factor c-Jun. *J. Virol.*, 75: 11275-11283.
- Coffey, M., J. Strong, P. Forsyth and P. Lee, 1998. Reovirus therapy of tumors with activated Ras pathway. *Sci.*, 282: 1332-1334.
- Connolly, J. and T. Dermody, 2002. Virion disassembly is required for apoptosis induced by reovirus. *J. Virol.*, 76: 1632-1641.
- DeBiasi, R., P. Clarke, S. Meintzer, R. Jotte, B. Kleinschmidt-Demasters, G. Johnson and K. Tyler, 2003. Reovirus-Induced Alteration in Expression of Apoptosis and DNA Repair Genes with Potential Roles in Viral Pathogenesis. *J. Virol.*, 77: 8934-8947.
- de Zwart, L., J. Meerman, J. Commandeur and N. Vermeulen, 2001. Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radic. Biol. Med.*, 1999: 202-26.
- Duthie, S., A. Ma, M. Ross and A. Collins, 1996. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res.*, 56: 1291.
- Dworkin, B., 1994. Selenium deficiency in HIV infection and the acquired immunodeficiency syndrome (AIDS). *Chem. Biol. Interact.*, 91: 181-186.
- Emonet-Piccardi, N., M. Richard, J. Ravanat, N. Signorini, J. Cadet and J. Beani, 1998. Protective effects of antioxidants against UVA-induced DNA damage in human skin fibroblasts in culture. *Free Radic. Res.*, 29: 307-313.

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- FDA, Department of Health and Human Services, 2003. Federal Register for Rules and Regulations. Food additives permitted in feed and drinking water of animals; Selenium-Final Rule. FDA Press, Washington, D.C., Vol., 68, No. 170.
- Floyd, R. and J. Carney, 1992. Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. *Ann. Neurol.*, 32: 22S-27S.
- Gaudry, D., J. Tektoff and J.M. Charles, 1972. A propos d'un nouveau virus isolé chez le canard de Barbarie. *Bulletin de la Société des Sciences Vétérinaires et de Médecine Comparée de Lyon.*, 74: 137-143.
- Gladyshev, V., V. Factor, F. Housseau and D. Hatfield, 1998. Contrasting Patterns of Regulation of the Antioxidant Selenoproteins, Thioredoxin Reductase, and Glutathione Peroxidase in Cancer Cells. *Biochem. Biophys. Res. Comm.*, 251: 488-493.
- Guy, J.S., 1998. Virus infections of the gastrointestinal tract of poultry. *Poult. Sci.*, 77: 1166-1175.
- Halliwell, B. and J. Gutteridge, 1985. Free radicals in biology and medicine. Clarendon Press, Oxford University Press, New York, NY.
- Hamamdžić, D., S. Altman-Hamamdžić, S. Bellum, T. Phillips-Dorsett, S. London and L. London, 1999. Prolonged Induction of IL-8 Gene Expression in a Human Fibroblast Cell Line Infected with Reovirus Serotype 1 Strain Lang. *Clin. Immunol.*, 91: 25-33.
- Heggen-Peay, C.L., M.A. Cheema, R.A. Ali, K.A. Schat, and M.A. Qureshi, 2002. Interactions of poult enteritis and mortality syndrome-associated reovirus with various cell types in vitro. *Poult. Sci.*, 81: 1661-1667.
- Islam, M., R. Jones and D. Kelly, 1988. Pathogenesis of experimental reovirus tenosynovitis in chickens: influence of the route of infection. *J. Comp. Pathol.*, 98: 325-336.
- Jackson, G.G., R.L. Muldoon and R.S. Cooper, 1961. Reovirus type I as an etiologic agent of the common cold. *J. Clin. Invest.*, 40: 1051.
- Jaovisidha, P., M. Peeples, A. Brees, L. Carpenter and J. Moy, 1999. Respiratory Syncytial Virus Stimulates Neutrophil Degranulation and Chemokine Release. *J. Immunol.*, 163: 2816-2820.
- Johnson, T., M. Herbel, T. Bullen and P. Zawislanski, 1999. Selenium isotope ratios as indicators of selenium sources and oxyanion reduction. *Geochim. Cosmochim. Acta.*, 63: 2775-2783.
- Juffrie, M., G. van Der Meer, C. Hack, K. Haasnoot, V. Sutaryo and L. Thijs, 2000. Inflammatory mediators in dengue virus infection in children: interleukin-8 and its relationship to neutrophil degranulation. *Infect. Immun.*, 68: 702-707.
- Kaschula, V.R., 1950. A new virus disease of the Muscovy duck (*Cairina moschata*) present in Natal. *J. South Afr. Vet. Med. Assoc.*, 21: 18-26.
- Kirsi, J., J. North, P. McKernan, B. Murray, P. Canonico, J. Huggins, P. Srivastava and R. Robins, 1983. Broad-spectrum antiviral activity of 2-beta-D-ribofuranosylselenazole-4-carboxamide, a new antiviral agent. *Antimicrob. Agents Chemo.*, 24: 353-361.
- Kouwenhoven, B., M. Vertommen and J. Van Eck, 1978a. Runting and leg weakness in broilers: involvement of infectious factors. *Vet. Sci. Commun.*, 2: 253-259.
- Kouwenhoven, B., F. Davelaar and J. Van Walsum, 1978b. Infectious proventriculitis causing runting in broilers. *Avian Pathol.*, 7: 183-187.
- London, L., E.I. Majeski, M.K. Paintila, R.A. Harley and S.D. London, 2002. Respiratory reovirus I/L induction of diffuse alveolar damage: a model of acute respiratory distress syndrome. *Exp. Mol. Pathol.*, 72: 24-36.
- Luthman, M., and A. Holmgren, 1982. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochem.*, 21: 6628-6633.
- McFerran, J., T. Connor and R. McCracken, 1976. Isolation of adenoviruses and reoviruses from avian species other than domestic fowl. *Avian Dis.*, 20: 519-524.
- McNulty, M., J. McFerran, D. Bryson, E. Logan and W. Curran, 1976. Studies on rotavirus infection and diarrhoea in young calves. *Vet. Rec.*, 99: 229-230.
- Mebus, C., R. Wyatt, R. Sharpee, M. Sereno, A. Kalica, A. Kapikian and M. Twiehaus, 1976. Diarrhea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. *Infect. Immun.*, 14: 471-474.
- Menendez, N., B. Calnek and B. Cowen, 1975. Localization of avian reovirus (FDO isolant) in tissues of mature chickens. *Avian Dis.*, 19: 112-7.
- Michel, Y., D. Poncet, M. Piron, K. Kean and A. Borman, 2000. Cap-Poly (A) Synergy in Mammalian Cell-free Extracts. *J. Biol. Chem.*, 275: 32268-32276.
- Morin, M., A. Warner and B. Fields, 1996. Reovirus infection in rat lungs as a model to study the pathogenesis of viral pneumonia. *J. Virol.*, 70: 541-548.
- Mortola, E., M. Okuda, K. Ohno, T. Watari, H. Tsujimoto, and A. Hasegawa, 1998. Inhibition of apoptosis and virus replication in feline immunodeficiency virus-infected cells by N-acetylcysteine and ascorbic acid. *J. Vet. Med. Sci.*, 60: 1187-93.
- Muller, F., A. Svardal, I. Nordoy, R. Berge, P. Aukrust and S. Froland, 2000. Virological and immunological effects of antioxidant treatment in patients with HIV infection. *Europ. J. Clin. Investig.*, 30: 905-914.
- Nibert, M.L., L.A. Schiff and B.N. Fields, 1996. Reovirus and their replication. Pages 1557-1623 in *Fundamental Virology*. B. N. Fields, D. M. Knipe and P. M. Howley, eds. 3rd ed. Lippincott-Raven Press, Philadelphia, Pa.

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- Page, R.K., O.J. Fletcher and P. Villegas, 1982. Infectious tenosynovitis in young turkeys. *Avian Dis.*, 26: 924-927.
- Palya, V., Glávits, R. b., Dobos-kovács, M. I., Ivanics, Á. v., Nagy, E. r., Báinyai, K. n., Reuter, G. b., Szács, G. r., Dájn, Á. d. m. and Benk, M. r. 2003. Reovirus identified as cause of disease in young geese. *Avian Path.*, 32: 129-138.
- Pardue, S., J. Thaxton and J. Brake, 1985. Role of ascorbic acid in chicks exposed to high environmental temperature. *J. Appl. Phys.*, 58: 1511-1516.
- Patrick, L., 1999. Nutrients and HIV: Part One-Beta Carotene and Selenium. *Altern. Med. Rev.*, 4: 403-413.
- Peterhans, E., 1997. Oxidants and antioxidants in viral diseases: Disease mechanism and metabolic regulation. *J. Nutr.*, 127: 962S-965S.
- Piron, M., P. Vende, J. Cohen and D. Poncet, 1998. Rotavirus RNA-binding protein NSP3 interacts with eIF4G1 and evicts PABP from eIF4F. *EMBO J.*, 17: 5811-5821.
- Ramig, R.F., 2004. Pathogenesis of intestinal and systemic rotavirus infection. *J. Virol.* 78:10213-10220.
- Rayman, M. 2000. The importance of selenium to human health. *Lancet.*, 356: 233-241.
- Robertson, M.D. and G.E. Wilcox, 1986. Avian reovirus. *Veterinary Bulletin*, 56: 155-174.
- Robertson, M.D., G.E. Wilcox and F.S.B. Kibenge, 1984. Prevalence of reoviruses in commercial chickens. *Aust. Vet. J.*, 61: 319-322.
- SAS Institute, 1995. SAS user's guide: statistics. Version 6.11. SAS, Cary, NC.
- Saura, M., C. Zaragoza, A. McMillan, R. Quick, C. Hohenadl, J. Lowenstein and C. Lowenstein, 1999. An antiviral mechanism of nitric oxide: inhibition of a viral protease. *Immunity*, 10: 21-28.
- Schrauzer, G.N., 2000. Anticarcinogenic effects of selenium. *Cell Mol. Life Sci.*, 57: 1864-1873.
- Schrauzer, G. and J. Sacher, 1994. Selenium in the maintenance and therapy of HIV-infected patients. *Chem. Biol. Interact.*, 91: 199-205.
- Schwarz, K. and C.M. Foltz, 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.*, 79: 3292-3293.
- Sen, C. and L. Packer, 1996. Antioxidant and redox regulation of gene transcription. *FASEB J.*, 10: 709-720.
- Sodhi, A., S. Montaner and J. Gutkind, 2004. Viral hijacking of G-protein-coupled-receptor signaling networks. *Nat. Rev. Mol. Cell Biol.*, 5: 998-1012.
- Spallholz, J., L. Boylan and H. Larsen, 1990. Advances in understanding selenium's role in the immune system. *Ann. NY Acad. Sci.*, 587: 123-139.
- Steinhauer, D., E. Domingo and J. Holland, 1992. Lack of evidence for proofreading mechanisms associated with an RNA virus polymerase. *Gene.*, 122: 281-288.
- Tyler, K.L., 2001. Mammalian reoviruses. Pages 1729-1745 in *Fields Virology*. D. M. Knipe, ed. 4th ed. Lippincott, Williams and Wilkins, Philadelphia, Pa.
- Ueda, J., N. Saito, Y. Shimazu and T. Ozawa, 1996. A comparison of scavenging abilities of antioxidants against hydroxyl radicals. *Arch. Biochem. Biophys.*, 333: 377-384.
- van der Heide, L. and M. Kalbac, 1975. Infectious tenosynovitis (viral arthritis): characterization of a Connecticut viral isolant as a reovirus and evidence of viral egg transmission by reovirus-infected broiler breeders. *Avian Dis.*, 19: 683-688.
- Varani, G. and F. Allain, 2002. How rotavirus hijacks human protein synthesis machinery. *Nature Struc. Biol.*, 9: 158.
- Vende, P., M. Piron, N. Castagné and D. Poncet, 2000. Efficient Translation of Rotavirus mRNA Requires Simultaneous Interaction of NSP3 with the Eukaryotic Translation Initiation Factor eIF4G and the mRNA 3'End. *J. Virol.*, 74: 7064-7071.

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