Efficacy of a bioflavonoid as partial replacement of retinol and α-tocopherol supplementation in broiler chickens and feedlot steers

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Abstract

A natural bioflavonoid antioxidant was investigated as partial replacement for vitamin A and E supplementation in broiler and feedlot steer rations. The results show that the inclusion of 50% of industry standard inclusion (ISI) of vitamin A and E and the bioflavonoid in both broiler and the steer resulted in equal 35-day body weight (broilers) or improved average daily gain (steers) when compared with broilers and steers on diets supplemented with 100% of ISI for vitamin A and E and zero bioflavonoid. The results in steers can perhaps be attributed to the fact that flavonoids are deglycocelated to a larger extent by microbial action in the digestive tract of ruminants than in broilers. The deglycocelated flavonoids are more hydrophobic and thus better absorbable, resulting in a superior average daily gain. The efficacy of deglycocelated bioflavonoids as partial replacement for vitamin A and E should be further investigated in monogastric animals.

Keywords: Vitamin A, vitamin E, antioxidant, poultry, ruminants

Abbreviations: ISI, Industry Standard Inclusion; ROS, Reactive Oxygen Species; SOD, Super-oxide Dismutase; ADG, Average Daily Gain

1. Introduction

The use of a natural water extracted antioxidant (Biored®) as partial replacement of vitamin A and E have been investigated in growth studies with both broilers and feedlot steers. These vitamins act as antioxidants and it was hypothesized that this natural antioxidant could partially fulfil this role without negatively influencing animal production. Antioxidants are used commercially to prevent oxidative stress. Oxidative stress in living cells is caused by oxidants which include reactive oxygen and reactive nitrogen species and free radicals (Jensen, 2003). Biological molecules such as proteins, DNA and lipids are especially susceptible to free radical attack (Machlin and Bendich, 1987; Middleton *et al*., 2000).

Living organisms naturally have systems in place in order to protect itself against tissue damage caused by reactive oxygen species (ROS) and free radicals. Antioxidants remove or stabilise the harmful reactive molecules thereby protecting cells and ensuring their structural integrity (Chew, 1996). They can do this either through enzymatic or non-enzymatic pathways (Kitts, 1997; Middleton *et al*., 2000). Mitochondria, for example, are very susceptible to ROS and to protect themselves they have enzymatic (superoxide dismutase, catalase and glutathione peroxidases) as well as non-enzymatic (glutathione redox cycle, α-tocopherol) antioxidant protection (Avanzo *et al*., 2001). The enzymatic system in the body includes super-oxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase. Some enzymes like SOD and catalase target specifically ROS, while other enzymes reduce thiols (Middleton *et al*., 2000). Non-enzymatic antioxidants consist of vitamins and micronutrients, which are less specific in their radical scavenging ability (Kitts, 1997; Middleton *et al*., 2000). The hydrophilic non-enzymatic antioxidants include vitamin C (ascorbic acid) and urate while lipid soluble antioxidants include vitamin E

(tocopherols), vitamin A (retinoids), carotenoids and ubiquinols (Middleton *et al*., 2000). Vitamin E is one of the most important natural antioxidants and being fat soluble it is involved in processes preventing lipid peroxidation. It is an essential nutrient that needs to be supplemented in the diet of broilers as they do not synthesise the vitamin in their bodies (Fellenberg and Speisky, 2005). Continuous intake of vitamin E is also required in order to maintain vitamin E concentrations in cellular membranes throughout the body in ruminants. There is little or no pre-intestinal absorption of dietary tocopherol and rumen microbial destruction of tocopherol has been reported (McMurray and Rice, 1982; Weiss, 1998). However, in more recent studies, using the stabilised form of vitamin E (dl-alpha-tocopheryl acetate), little if any degradation of vitamin E in the rumen have been reported (Weiss, 1998). Hymøller and Jensen (2010) also showed no degradation of dl-alpha-tocopheryl acetate in the rumen of high producing dairy cows. In most species, including ruminants, vitamin E absorption is proportional to the vitamin E status and requirement of the animal (Hidiroglou *et al*., 1992; Scherf *et al*., 1996; Traber and Sies, 1996). The clinical signs of a vitamin E deficiency typically include retarded growth, exudative diathesis, encephalomalacia and several other diseases as well as a high morbidity rate (Avonzo *et al*., 2001; Yuming *et al*., 2001). The role of vitamin A as antioxidant is more controversial. Kartha and Krishnamurthy (1977) reported that large doses increased the antioxygenic potential of the tissues of rats and suggested that it might be considered as an antioxidant in animal nutrition. It has also been suggested that the precursors to vitamin A have a more important antioxidant function than retinol and carotenoids. These precursors, such as beta-carotene, lycopene, and some oxycarotenoids, e.g. zeaxanthin and lutein, exert antioxidant functions in lipid phases by quenching singlet molecular oxygen ($^1O_2$) that targets functional groups such as unsaturated fatty acids, proteins, enzymes and DNA

or free radicals (Sies and Stahl, 1995; Di Mascio *et al*., 2016). Synergy between beta-carotene, vitamin E and vitamin C in the protection of lipids in membranes has been demonstrated (Niki *et al.,* 1995; Zhang *et al.*, 2000).

Bioflavonoids, polyphenolic compounds found in plants, have been shown in numerous studies to contain antioxidative properties. Flavonoids possess potential health-enhancing effects (Kamboh *et al*., 2015) and are generally regarded as natural replacers of synthetic growth promoters in poultry production (Kamboh *et al*., 2016). Heim *et al*. (2002) listed the antioxidative functions of flavonoids as follows: ROS scavenging, chain breaking antioxidants, metal chelators and reducing agents, quenchers of the formation of singlet oxygen and providing protection to vitamin C. They can also react with $OH^{\cdot}$ and can function as chain-breaking antioxidants (Heim *et al*., 2002). Flavonoids might be able to have an additive effect on the antioxidant capacity of the body (Nijveldt *et al*., 2001). In the studies reported here Biored®, a bioflavonoid composed of 7.8 g/100 g flavonoids and 39.8 g/100 g pro-anthocyanidins, was used as a feed additive.

2. Material and methods

Two trials were conducted to determine if vitamin A and E could be partially replaced with Biored® in the diet of broiler chickens and feedlot steers.

2.1 Broiler growth trail

In the first trial, broiler chickens were subjected to different levels of vitamin A and E, supplemented with different levels of Biored®. This was done for a grow-out cycle of 35 days. One thousand two hundred and eighty male Ross broiler chickens were randomly distributed among 64 pens, 20 birds per pen, at a stocking density of 13 birds/m^2. Eight different dietary treatments were used in the trial (Table 1) with each of the treatments replicated eight times. Pen replicates were arranged according to a randomised complete block design with each block containing one replication of each treatment. The same maize-soya based basal diet was mixed for all treatments after which the premixes with the different concentrations of vitamin A, vitamin E and Biored® were added to the basal feed and remixed to form the eight different dietary treatments. A three-phase feeding regimen was followed consisting of a starter crumble and grower and finisher pellets. Representative feed samples were analysed for dry matter and ash (AOAC, 2000, Official method of analysis 942.05), crude protein (AOAC, 2000, Official method of Analysis 988.05, using a Leco FP-428), ether extract (AOAC, 2000, Official method of Analysis 920.39), crude fibre (AOAC, 2000, Official method of Analysis 962.09), calcium (AOAC, 2000, Official method of Analysis 935.13), total phosphorus (AOAC, 2000, Official method of Analysis 965.17) and selenium (AOAC, 2000, Official method of Analysis 996.16) content (Table 2). Body weight (BW) of broiler chickens was measured per pen replicate on a weekly basis from day old till 35 days of age and averages were calculated. Feed intake was

measured weekly on the same day that the chicks were weighed. During twice daily inspections in the broiler house, dead birds were collected and the weight of dead birds recorded. BW and feed intake data were used to calculate feed conversion (FCR), corrected for mortalities, on a weekly basis (results not reported) and for the total grow-out period of 35 days. On day 35, five birds of each replicate group were euthanised and their livers excised. The left lobe of the five livers were removed and placed together in marked plastic bags to form a pooled sample, placed in a black container (to protect samples from sunlight) and immediately frozen. Two days after slaughter the samples were moved to a -80°C freezer where it was stored till they were ready to be sent for analyses. The 64 pooled liver samples were shipped to an independent laboratory (V&M Analytical Toxicology Laboratory Services (Pty) Ltd., George, South Africa) for analysis of retinol and α-tocopherol.

2.2 Steers growth trail

In the second growth trail, 30 Holstein steers were reared under feedlot conditions for a period of 117 days. The steers (one or two per pen of 3m x 6m) were randomly allocated by pen to four different treatments (Table 3). A pen was the replicate unit for statistical analysis of live performance data. The steers used in this trial were pen-paired since birth, and mortalities during two preceding trial periods (pre-weaning growth and post-weaning growth), and one mortality during the growth period of this trial, resulted in the unequal number of steers per pen (Table 3). The steers were fed *ad libitum* of a commercial complete finisher diet (Table 4) in pellet form. Feed intake (per pen) was recorded on a weekly basis as the difference between feed offered and orts, and the cumulative feed intake was used to calculate average daily feed intake over the 117-day growth period. Feed intake was corrected for the mortality that

occurred in one pen (in the treatment combination of the 50% vitamin A and E level and not receiving the bioflavonoid). Average daily gain was determined over the growth period of 117 days. Only steers weighing 375 kg or more at the end of the growth period were slaughtered (Table 3), and the individual steer was the replicate unit for statistical analysis of slaughter trait data and tissue vitamin data. The caudal lobe of the liver was collected after slaughter for subsequent vitamin analysis. For the same purpose, a sample of sub-cutaneous fat was collected after slaughter with a core sampler, at a point between the 10^{th} and 11^{th} ribs of the left half of the carcass, and 10 cm laterally from the carcass midline. Liver and fat concentrations of retinol and α-tocopherol were determined by HPLC (Plavolab, Bloemfontein, South Africa).

2.3 Statistical analysis

Data were analysed statistically as a randomized block design with the GLM model

(Statistical Analysis Systems, 2018) for the average effects over time. Means and standard error of the mean were calculated and significance of difference ($P < 0.05$) between means was determined by Fischer's test (Samuels, 1989).

The linear model used is described by the following equation:

$$Yij = \mu + Ti + Lj + TLij + eij$$

Where Y = variable studied during the period

μ = overall mean of the population

T = effect of the ith treatment

L = effect of the jth level

TL = effect of the ijth interaction between treatment and level

e = error associated with each Y

3. Results

3.1 Broiler growth trail

Production performance data for the different treatments at the end of the 35-day grow-out period is presented in Table 5. Vitamin A and E supplementation level had a significant effect ($P < 0.05$) on BW and cumulative feed intake. Broilers that received no vitamin A and E supplementation (Treatment 1 and 4) had significantly lower BW at the end of the period than the other treatments. Body weight of broilers that received 50% of the vitamin supplementation without Biored® was significantly lower compared to the 100% vitamin supplementation level. However, no differences in BW of broilers receiving either 100% or 50% vitamin supplementation in combination with Biored® were observed ($P > 0.05$). Total cumulative feed intake for the broilers that received no vitamin A and E supplementation, and also 50% vitamin supplementation without Biored®, was significantly lower ($P < 0.05$) than the feed intake of all other treatments. No treatment differed in FCR from that of the positive control (Treatment 2) at the end of the grow-out period. From Table 6 it is clear that the highest levels of retinol and α-tocopherol were observed in the livers of broilers that received the 100% vitamin supplementation (Treatments 2 and 5; $P < 0.05$). No significant differences ($P > 0.05$) were observed between Treatments 1 and 4 (0% vitamin supplementation) and between Treatments 3, 6, 7 and 8 (50% vitamin supplementation).

3.2 Steers growth trail

The body weight of steers at the start and end of the growth period of 117 days, as well as their average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) over that period, are shown in Table 7. Neither of the main

effects nor the interaction between them had a significant effect on the live performance traits. Certain treatment combinations nevertheless differed significantly ($P < 0.05$) or trended towards differences ($P < 0.10$), as indicated in Table 7. With respect to ADG, steers supplemented with vitamins at the 50% level and Biored® at 200 mg/kg tended to have a higher ADG ($P < 0.10$) than steers supplemented with vitamins at the 100% level and no bioflavonoid. ADFI of steers supplemented with vitamins at the 50% level in combination with Biored® at 200 mg/kg, was significantly higher than that of steers supplemented with vitamins at the 100% level and no bioflavonoid, and tended to be higher ($P < 0.10$) than that of steers in the remaining two treatment groups (receiving respectively vitamins at the 50% level and no Biored® or vitamins at 100% level with Biored® at 200 mg/kg). Treatment combinations did not differ in respect of the FCR of steers ($P > 0.05$). In Table 8, the effect on the average liver and fat concentrations of retinol and α-tocopherol for steers on the different diets are displayed. Retinol and α-tocopherol concentrations in the subcutaneous fat were significantly lower ($P < 0.05$) for steers on the 50% vitamin diet with no Biored® supplementation than for the other diets. There was no significant difference ($P > 0.05$) in retinol and α-tocopherol between steers on 100% vitamin diet with no Biored® and steers on the 50% vitamin and 200 g/ton Biored® diet. Steers on the 100% vitamin plus 200 g/ton Biored® had significantly higher ($P < 0.05$) retinol and α-tocopherol concentration in their fat than any of the other treatments. The same pattern was observed for retinol and α-tocopherol concentrations in the liver, although the group that received 100% vitamin plus 200 g/ton Biored® did not have significantly higher ($P > 0.05$) retinol concentrations than the group that received 50% vitamin and 200 g/ton Biored®.

4. Discussion

The results indicate that the efficacy of Biored® as partial replacement for vitamin A and E is different for steers and for broilers. There is a biological and economical advantage in replacing 50% of the vitamin A and E supplementation with 150 g and 200 g Biored® per ton feed, respectively, for steers and broilers. Steers receiving Biored® and supplemented with vitamin A and E at the 50% level tended to have a higher ADG ($P<0.10$) when compared to the steers on the diet containing no Biored® and 100% inclusion of industry standard supplementation of vitamin A and E. Growth performance in broilers was not impeded when replacing 50% of the vitamin A and E with Biored®. In broilers, liver levels of retinol and α-tocopherol were lower in the 150 and 200 g Biored® with 50% vitamin A and E groups when compared with 100% inclusion of industry standard supplementation of vitamin A and E. It is clear that in broilers, differences in retinol and α-tocopherol concentrations were determined by the inclusion levels of vitamin A and E in the ration and not by the inclusion level of Biored®.

Flavonoid glycosides are commonly hydrolysed to their aglycones to produce effects *in vivo* and the deglycocelation by small intestinal epithelial cell β-glucosidases is a critical step in the absorption and metabolism of flavonoid glycocides (Walle *et al*., 2005). The rate of absorption and the availability at the site of action is of utmost importance for a flavonoid to be effective within biological systems (Thilakarathna and Vasantha Rupasinghe, 2013). The absorption of flavonoids mainly depends on their permeability but glycosylated flavonoids are too water-soluble to diffuse across the cellular membrane. The absorption of flavonoid glycosides therefore requires hydrolysis of their sugar group as the flavonoid aglycones are more hydrophobic and can easily be absorbed by the epithelial cells through passive diffusion (Kottra and Daniel, 2007; Chen *et al*., 2011). Bokkenheuser *et al*. (1987) and

Hur *et al*. (2000) consider the colonic microflora as a key source of hydrolase for the hydrolysis of flavonoid glycocides. Erener *et al.* (2011) argued that highly digestible diets influence the total coliform bacteria counts in the caecal intestine and this may limit the growth promoting effect of bioflavonoids. These findings indicate that the difference in the effect of Biored® in broilers and feedlot steers should be further investigated and that it can be attributed to differences in deglycosylation caused by differences in the microflora in the ruminant and monogastric digestive tract.

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Declaration of interest

Biored® is a product of Nandrea Health Products. SC Slippers declares *pro bono* provision of technical advice to Nandrea Health Products with respect to research trial planning and registration of products containing Biored® in terms of South African Farm Feed Regulations (Act 36 of 1947). PNW Groenewald is a shareholder in Nandrea Health Products and were not involved in performing the analyses or the taking of measurements related to the reported trials. GV Kriel is the Manager: Research of Nandrea Health Products and were not involved in performing the analyses or the taking of measurements related to the reported trials. A. Marshall, R. Coertze and C. Jansen van Rensburg: none.

Ethics statement

The protocol for the project was approved by the University of Pretoria's Animal Ethics Committee (Project number: EC095-13) before commencement of the trial.

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Table 1

Eight dietary treatments containing different levels of vitamin A and E (IU/kg) as well as different inclusion levels of a bioflavonoid antioxidant, Biored® (mg/kg feed), fed to broiler chickens. The highest inclusion levels of vitamin A and E were deemed the industry standard inclusion levels in South Africa for the different periods

		Starter		Grower		Finisher	
		Vitamin		Vitamin		Vitamin	
Treatment	Biored®	A	E	A	E	A	E
0% vitamin A and E	0	0	0	0	0	0	0
100% vitamin A and E	0	11.15	117.1	9.65	97.6	9.6	97.4
50% vitamin A and E	0	5.58	58.55	4.83	48.8	4.8	48.7
0% vitamin A and E	150	0	0	0	0	0	0
100% vitamin A and E	150	11.15	117.1	9.65	97.6	9.6	97.4
50% vitamin A and E	100	5.58	58.55	4.83	48.8	4.8	48.7
50% vitamin A and E	150	5.58	58.55	4.83	48.8	4.8	48.7
50% vitamin A and E	200	5.58	58.55	4.83	48.8	4.8	48.7

Table 2

Raw material inclusion and calculated and analysed nutrient composition of the basal diets for the broiler trial

Raw material (%)	Starter	Grower	Finisher
Yellow maize	59.09	62.53	66.74
Soya oilcake meal	26.50	18.98	14.39
Sunflower oilcake meal	3.00	3.00	3.00
Full fat soya	4.66	9.25	10.11
Gluten 60	3.00	3.00	3.00
Limestone ($CaCO_3$)	1.42	1.34	1.18
Sodium bicarbonate ($NaHCO_3$)	0.073	0.038	0
Salt (Fine)	0.43	0.43	0.43
Mono-calcium phosphate	0.949	0.560	0.313
Lysine HCL	0.314	0.299	0.286
DL Methionine	0.231	0.212	0.197
DL Threonine	0.063	0.051	0.038
Broiler Premix (excl. Vit A, E and Se)	0.3	0.25	0.25
Calculated nutrient values (g/kg)			
Dry matter	891.5	894.3	890.4
AME (MJ/kg)	11.5	11.95	12.25
Crude protein	222.2	207.4	192.7
Crude fibre	37.3	38.6	38.3
Crude fat	35.6	44.3	46.6
Ash	47.9	44.4	40.4
Calcium	9.4	8.3	7.2
Total phosphorus	7.9	6.9	6.3
Analysed nutrient values (g/kg)			
Dry matter	895.5	888.1	885.1
Crude protein	214.0	203.0	189.0
Crude fibre	37.9	40.3	37.1
Crude fat	37.1	49.0	47.8
Ash	56.3	45.1	41.4
Calcium	8.4	7.0	6.0
Phosphorus	5.8	4.9	4.3
Selenium (mg/kg)	0.210	0.305	0.238

Table 3

The different treatments used during the steer trail. The inclusion of 12000 IU/kg feed of vitamin A and 20 IU/kg feed of vitamin E (as fed-basis) are deemed to be the industry standard inclusion level for feedlot steers in South Africa

Treatment	Biored® (mg/kg feed)	Vitamin A (IU/kg feed)	Vitamin E (IU/kg feed)	Replicate pens $n_1 + n_2$	Steers slaughtered (n)
100% vitamin A and E	0	12000	20	2 + 3	5
100% vitamin A and E	200	12000	20	2 + 3	7
50% vitamin A and E	0	6000	10	3 + 2	5
50% vitamin A and E	200	6000	10	3 + 2	6

Table 4

Calculated nutrient composition of the commercial complete finisher pellet diet used during the steer trial (as fed-basis)

Nutrient (g/kg)	
Crude Protein	110.0
Crude Fat	2.5
Crude Fibre	11.0
Moisture	120.0
Calcium	12.0
Phosphorus	2.5
Urea	10.0

Table 5

Body weight, cumulative feed intake and feed conversion ratio (FCR; kg feed / kg weight gain) of broiler chickens that received feed containing various concentrations of vitamin A and E (% of industry standard) and a bioflavonoid antioxidant, Biored (mg/kg)

	Treatment									
Vitamin A and E[1]	0		50				100			Significance
Biored inclusion	0	150	0	100	150	200	0	150	RMSE	*P*-value
Body weight[2]										
Day 0 (g)	39.13^{ab}	39.75^{b}	39.00^{ab}	39.38^{ab}	39.19^{ab}	39.31^{ab}	38.63^{a}	39.38^{ab}	0.001	0.538
Day 35 (kg)	2.20^{c}	2.23^{c}	2.31^{b}	2.32^{ab}	2.33^{ab}	2.37^{ab}	2.39^{a}	2.34^{ab}	0.077	<0.0001
Feed intake[2]										
0 to 35 days (kg)	3.28^{b}	3.30^{bc}	3.37^{bcd}	3.44^{ad}	3.41^{ac}	3.47^{ad}	3.51^{a}	3.44^{ad}	0.115	0.003
FCR[2]										
0 to 35 days	1.52^{a}	1.51^{ab}	1.48^{c}	1.49^{ac}	1.48^{bc}	1.51^{ac}	1.49^{ac}	1.49^{ac}	0.031	0.138

RSME = Root mean square error.

[a-d]Values within a row not sharing a common superscript differ significantly (P<0.05).

[1]The highest inclusion level of vitamin A and E (100%) were deemed the industry standard supplementation levels in South Africa.

[2]Means represent replicate (pen) average values.

Table 6

Liver concentrations of retinol and α-tocopherol (µg/mL) in broilers that received feed containing various concentrations of vitamin A and E (% of industry standard) and a bioflavonoid antioxidant, Biored (mg/kg)

	Treatment									
Vitamin A and E[1]	0		50				100			Significance
Biored inclusion	0	150	0	100	150	200	0	150	RMSE	*P*-value
Retinol[2]	3.20[c]	3.70[c]	62.97[b]	61.88[b]	48.82[b]	77.48[b]	169.45[a]	150.66[a]	29.04	0.0004
α-Tocopherol[2]	9.43[c]	12.32[c]	44.03[b]	36.11[b]	40.18[b]	30.87[b]	68.16[a]	66.54[a]	18.45	0.189

RMSE = Root mean square error.

[a-c]Values within a row not sharing a common superscript differ significantly ($P<0.05$).

[1]The highest inclusion level of vitamin A and E (100%) were deemed the industry standard supplementation levels in South Africa.

[2]Means represent replicate average values, with the left lobe of five livers per replicate.

Table 7 *Body weight (kg), average daily gain (ADG; kg), average daily feed intake (ADFI; kg, as fed), and feed conversion ratio (FCR; kg feed / kg weight gain) of Holstein steers that received feed containing various concentrations of vitamin A and E (% of industry standard) and a bioflavonoid antioxidant, Biored® (mg/kg)*

	Treatment					Significance (*P*-values)		
Vitamin A and E[1]	50		100			Treatment effect		Interaction
Biored® inclusion	0	200	0	200	Model RMSE	Vitamin A and E	Biored®	Vitamin A and E x Biored®
Body weight[2]								
Day 0	201.6	209.4	198.6	198.9	19.7	0.46	0.65	0.67
Day 117	377.0	404.1	366.6	372.0	34.5	0.19	0.31	0.49
ADG[2]								
0 to 117 days	1.526^{AB}	1.693^{B}	1.461^{A}	1.505^{AB}	0.198	0.17	0.25	0.50
ADFI[2]								
0 to 117 days	9.355^{bA}	10.899^{bB}	9.494^{aA}	9.476^{bA}	1.148	0.23	0.16	0.15
FCR[2]								
0 to 117 days	5.722	5.159	5.993	6.139	1.035	0.20	0.66	0.46

RMSE = Root mean square error.

$^{a-b}$Contrasts in the same row without a common lower case letter in the superscript differ significantly (P<0.05)

$^{A-B}$Contrasts in the same row without a common upper case letter in the superscript tend to differ (P<0.10)

[1]The highest inclusion level of vitamin A and E (100%) were deemed the industry standard supplementation levels in South Africa.

[2]Means represent replicate (pen) average values – i.e. based on one or two steers per pen.

Table 8

Retinol and α-tocopherol concentration (µg/g wet tissue) of liver- and subcutaneous fat tissue samples, and slaughter weight (kg), carcass weight (kg) and dressing percentage (%) of Holstein steers that received feed containing various concentrations of vitamin A and E (% of industry standard) and a bioflavonoid antioxidant, Biored® (mg/kg)

	Treatment					Significance (*P*-values)		
Vitamin A and E[1]	50		100			Treatment effect		Interaction
Biored® inclusion	0	200	0	200	Model RMSE	Vitamin A and E	Biored®	Vitamin A and E x Biored®
Retinol[2]								
Liver tissue	7.035[a]	9.166[ab]	8.199[ab]	10.975[b]	1.831	0.06	0.00	0.68
Subcutaneous fat tissue	1.815[a]	2.435[b]	2.400[b]	2.737[c]	0.217	0.00	0.00	0.08
α-tocopherol[2]								
Liver tissue	12.682[a]	16.748[ab]	16.667[ab]	20.930[b]	3.681	0.02	0.01	0.95
Subcutaneous fat tissue	4.685[a]	6.494[ab]	6.325[a]	6.595[b]	0.812	0.04	0.01	0.04
Slaughter weight[2]								
117 days	390.4	405.9	388.0	396.7	24.7	0.55	0.27	0.75
Carcass weight[2]	205.9	202.0	210.5	197.6	15.1	0.95	0.19	0.48
Dressing percentage[2]	52.8	50.0	54.4	49.9	4.6	0.73	0.07	0.66

RMSE = Root mean square error.

[a-c]Values within a row not sharing a common superscript differ significantly ($P<0.05$)

[1]The highest inclusion level of vitamin A and E (100%) were deemed the industry standard supplementation levels in South Africa.

[2]Means represent values of replicate (individual steers with 117d body weight >375 kg) – varied from five to seven steers per treatment combination.