SELENIUM AND VITAMIN E SUPPLEMENTATION OF HIGH N-ALKANE GROWN YEAST IN CHICK DIETS

TUNDE OGUNTONA AND R. NEALE

Department of Applied Biochemistry and Food Science, University of Nottingham Sutton,
Bonington LE 12 5RD, U.K.

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ABSTRACT

A total of four experiments have been conducted to evaluate performance of chicks given high (300/kg diet) n-alkane grown yeast diet supplemented with vitamin E and selenium. Four levels (0,10,20,30 mg) of vitamin E were examined in experiment 1 while five levels (0,12.5,15.0,17.5,20mg) were examined in experiment 2. Performance was also measured at four selenium supplementation levels (0, 50,100, 150 μ g Se/kg diet) initially and finally at five other (0,100, 125, 150, 175 μ g/kg diet) more detailed levels. Supplementation of a 300g/kg yeast diet with 15mg Vitamin E produced significantly ($P < 0.05$) higher body weight than unsupplemented high yeast diet. Higher levels produced no further growth response. At this level of vitamin E supplementation every level of selenium examined gave significant ($P < 0.05$) increase in weight but optimum growth response was obtained at 100 μ g Se/kg yeast diet. There were no significant treatment effects on feed intake at all levels of vitamin E and selenium examined. Nitrogen retention also showed no response at the highest levels (100 - 175 μ g) of selenium supplementation. The results show that supplementation with appropriate levels of vitamin E and selenium significantly reduced the depression in growth rate caused by high levels of inclusion of n-alkane grown yeast in chick diet.

Key Words: Selenium, Vitamin E, N-alkane, Single - cell protein

INTRODUCTION

Yeast grown on n-alkane is currently receiving renewed interest especially in Eastern Europe where more than 500,000 tonnes are now produced annually

(Rimmington, 1985). An initial nutritional problem with this material has been that high dietary levels lead to reduced growth in monogastric animals like rats and pigs (Oguntona, 1976) as well as chickens (Oguntona, Neale and Lewis, 1977). Studies from our station have sought to rectify this growth retardation by supplementation with some nutrients (Oguntona, Neale and Lewis, 1983; Oguntona, Neale and Lewis, 1986), and physical manipulation of the diet (Oguntona, Neale and Lewis, 1985), but the effect of selenium and vitamin E supplementation has not been investigated. It has long been known that when torula yeast (*Candida utilis*) is used in any appreciable quantity, above 200 g/kg, in the diet to replace fishmeal, supplementation with selenium and vitamin E becomes necessary (Schwarz and Foltz, 1985) to achieve maximum growth and prevent deficiency diseases as selenium content of yeast is very low. Yeast grown on purified substrates like sulphate liquor and wood hydrolysate contains virtually no selenium and diets containing such yeast at dietary levels of 300 - 400 g/kg are routinely used to either deplete animals of selenium or precipitate deficiency symptoms (Stockstad *et al.* 1957, Kelleher *et al.* 1959). Scott *et al.* (1955) have also described a 300 g/kg torula yeast diet that produced, in addition to exudative diathesis, considerable growth reduction and death in chicks even when the liver was microscopically normal. Yeast grown on n-alkane substrate is also generally low in selenium (Schwarz and Fredga, 1969) and growth reduction is commonly observed when high (300 g/kg) dietary levels of the yeast are given to chicks (Oguntona *et al.* 1985, Daghir & Sell, 1982). It is not known what roles the adequacy of selenium and vitamin E play in this phenomenon.

¹ Present Address: Department of Animal Nutrition, University of Agriculture, P.M.B. 2240, Abeokuta

The objective of the present studies was to examine the effects of supplementing high n-alkane grown yeast diet with selenium and vitamin E on the growth and nitrogen retention of chicks.

throughout the experiment was a Soya/Fish meal practical type diet and the composition is given in Table 1. The basal high yeast diet was a 300/kg formulation in which the fishmeal and soyabean meal of the control have been replaced by n-alkane grown yeast. Table 1

TABLE 1: COMPOSITION OF CONTROL AND 300g/kg N - ALKANE GROWN YEAST DIETS

Ingredients (g/kg)	Control	Yeast Diet
Wheat	514.0	470.0
Barley	50.0	50.0
Fishmeal	125.0	-
Soya bean meal	205.0	-
Yeast	-	300.0
Fat	35.0	55.0
Premix*	48.8	48.8
DL - Methionine	1.0	3.0
Limestone	-	13.16
Glucose	21.2	60.04
Calculated Composition		
Metabolisable energy (MJ/Kg ⁻¹)	12.77	12.80
Crude Protein (%)	24.85	24.70
Selenium (ug/kg)**	499.0	250.0
Vitamin E (ug/kg) from premix	4.0	4.0

* Contribution of premix to final diets: Ca, 0.85%; P, 0.30%; Na, 0.12%; Cl, 0.18%; Mn 70 mg/kg; Zn, 50 mg/kg; Fe, 20 mg/kg; I, 1 mg/kg; Co, 0.1 mg/kg; Mo, 2 mg/kg; Vitamin a, 6000 IU/kg; Vitamin D3, 1500 IU/kg; Vitamin E, 4 mg/kg; choline chloride, 500 mg/kg; menadione sodium bisulphate, 5 mg/kg; calcium pantothenate, 10 mg/kg; riboflavin, 4 mg/kg; folic acid, 2 mg/kg; vitamin B12, 0.1 mg/kg; nicotinic acid, 20 mg/kg; Pancoxin (coccidiostat, 0.0125%, butylated hydroxytoluence, 0.0125%.

* Values are based on Selenium content of feed stuffs given by Scott (1975)

MATERIALS AND METHODS

The yeast sample used in this study was produced on n-alkane substrates by a commercial company and supplied as a proprietary product. The composition of the yeast has been described previously (Oguntona, 1976) and the selenium content was 10mg/kg. The control diet used

also gives the composition of this basal yeast diet.

Throughout the studies, male Cobb broiler chicks were used. They were received at day-old and fed a commercial starter diet for 7 days. Excessively heavy or light chicks were rejected at this age and the remainder divided into groups of 12 chicks, and each was then allocated to a treatment. The birds were

TABLE 2: PATTERN OF VITAMIN E AND SELENIUM SUPPLEMENTATION OF DIETS HIGH IN N-ALKANE GROWN YEAST

Experiment 1:	(a)	Control Soya/fishmeal based diet
	(b)	300g/kg Yeast diet
	(c)	300g/kg Yeast diet + 10mg vit. E/kg diet
	(d)	300g/kg Yeast diet + 20mg vit. E/kg diet
	(e)	300g/kg Yeast diet + 30mg vit. E/kg diet
Experiment 2:	(a)	Control Soya/fishmeal based diet
	(b)	300g/kg Yeast diet
	(c)	300g/kg Yeast diet + 12.5mg vit. E/kg diet
	(d)	300g/kg Yeast diet + 15.0mg vit. E/kg diet
	(e)	300g/kg Yeast diet + 17.5mg vit. E/kg diet
Experiment 3:	(f)	300g/kg Yeast diet + 20.0mg vit. E/kg diet
	(a)	Control Soya/fishmeal based diet
	(b)	300g/kg Yeast diet
	(c)	300g/kg Yeast diet + 50 ug Se/kg diet
	(d)	300g/kg Yeast diet + 100ug Se/kg diet
Experiment 4:	(e)	300g/kg Yeast diet + 150ug Se/kg diet
	(a)	Control Soya/fishmeal based diet
	(b)	300g/kg Yeast diet
	(c)	300g/kg Yeast diet + 100ug Se/kg diet
	(d)	300g/kg Yeast diet + 125ug Se/kg diet
	(e)	300g/kg Yeast diet + 150ug Se/kg diet
	(f)	300g/kg Yeast diet + 175ug Se/kg diet

TABLE 3: FEED UTILISATION BY CHICKS FED N-ALKANE GROWN YEAST DIET SUPPLEMENTED WITH THREE LEVELS OF VITAMIN E

(Values are means of 4 cages of three chicks per cage)

Diet	Body Weight gain (g/d)	Food intake (g/d)	FCR	Nitrogen retention
Control	23.9 ^a	36.3 ^a	0.658 ^a	0.54 ^a
300g/kg Yeast	20.1 ^c	35.3 ^a	0.569 ^c	0.47 ^b
" + 10mg vit. E/kg	20.2 ^c	35.1 ^a	0.574 ^c	0.46 ^b
" + 20mg vit. E/kg	21.1 ^b	35.7 ^a	0.590 ^b	0.47 ^b
" + 30mg vit. E/kg	20.9 ^b	35.1 ^a	0.597 ^b	0.47 ^b
SEM	±0.2044	±0.3633	±0.0045	±0.006

Mean values followed by similar letters down a column are not significantly different ($P > 0.05$)

TABLE 4: FEED UTILISATION BY CHICKS FED N-ALKANE GROWN YEAST DIET SUPPLEMENTED WITH FOUR LEVELS OF VITAMIN E

DIET	Body weight gain (g/d)	Food Intake (g/d)	FCR
1. Control	23.0 ^a	34.9 ^a	0.658 ^a
2. 300g/kg Yeast + 12.5mg vit. E/kg	19.3 ^c	34.5 ^a	0.561 ^d
3. 300g/kg Yeast + 12.5mg vit. E/kg	20.3 ^{bc}	35.7 ^a	0.568 ^d
4. 300g/kg Yeast + 15.0mg vit. E/kg	20.9 ^b	34.3 ^a	0.608 ^b
5. 300g/kg Yeast + 17.5mg vit. E/kg	20.8 ^b	35.1 ^a	0.593 ^c
6. 300g/kg Yeast + 20.9mg vit. E/kg	20.9 ^b	34.9 ^a	0.600 ^{bc}
SEM	±0.3717	±0.5746	±0.0028

Mean values followed by similar letter down a column are not significantly different ($P > 0.05$)TABLE 5: MEAN BODY WEIGHT GAIN, FOOD INTAKE, FOOD CONVERSION RATIO (FCR) AND NITROGEN RETENTION OF CHICKS FED A CONTROL DIET AND 300g/kg YEAST DIETS SUPPLEMENTED WITH 50, 100, AND 150 μ g Se/kg

Diet	Supplem entary (ug/kg)	Se Contrib- ution from premix (ug/kg)	Dietary Se i.e. supplem- entary Se + premix Se (ug/kg)	Body weight gain (g/d)	Food intake (g/d)	FCR	Nitrogen retention
1. Control	0	100	100	23.9 ^a	36.3 ^a	0.658 ^a	0.539 ^a
2. 300g/kg Yeast	0	100	100	20.1 ^c	35.3 ^a	0.571 ^b	0.465 ^b
3. 300g/kg Yeast	50	100	150	20.6 ^c	35.2 ^a	0.585 ^b	0.473 ^b
4. 300g/kg Yeast	100	100	200	20.5 ^c	35.1 ^a	0.585 ^b	0.480 ^b
5. 300g/kg Yeast	150	100	250	21.1 ^b	35.6 ^a	0.594 ^b	0.485 ^b
SEM				±0.2011	±0.8490	±0.0089	±0.005

The only supplementary vitamin E contained in each of these diets was the 4mg/kg from the premix *100 μ g Se/kg (0.1mg) supplied as 220ug (or 0.22mg) Na₂Se O₃/kg diet dissolved in water and added to diet before pelleting.

housed in stainless steel cages in a temperature controlled room with 22h/day (i.e. 03.00 to 01.00) lighting. The experimental period lasted from 7 to 21 days of age of the chicks. And each was carried out in two stages, and the investigation was carried out in two stages and each stage consisted of two experiments. Optimal vitamin E supplementary level of the 300g/kg yeast diet was examined in the first stage (Experiments 1 and 2). In the second stage (Experiments 3 and 4) the optimal level of selenium was studied. Details are shown in Table 2. In both experiments the diets were pelleted with the dl- α -tocopherol acetate dissolved in the oil before mixing with the other ingredients.

Feed and water were provided *ad libitum* and the experimental period lasted from 7 to 21 days. Body weight gain and feed intake of the chicks were measured over the whole period and nitrogen retention of the diets was determined during days 18 - 21 in the first experiment. In experiments 3 and 4, various levels of selenium (as sodium selenite, Na_2SeO_3) were added to the 300g/kg yeast diet in an attempt to determine the optimal supplementary selenium content in terms of chick growth and in the presence of a minimum of 19 mg vitamin E per kg. (Table 2) In experiment 4, there were six treatments. Nitrogen retention was measured in both experiments 3 and 4 and the selection and management of the birds were as described for the first two experiments. The method employed for nitrogen retention has been previously described (Oguntona *et al.*, 1985) and statistical significance of data was evaluated by the analysis of variance (Snedecor and Cochran, 1967).

RESULTS

The results of experiment 1 (Table 3) show that increasing supplementary vitamin E beyond 20mg/kg feed did not produce further increase in body weight gain as no significant difference existed between the values obtained for chicks fed 20mg/kg and 30mg of the vitamin per kg feed. The higher level (30mg vitamin E) of supplementation did not

produce additional increase in body weight gain. The same pattern could be observed in the feed conversion ratio although nitrogen retention was similar for all the groups fed N-alkane grown yeast. These results suggest that the optimum level of vitamin E supplementation lies between 10mg and 20mg vitamin E per kg yeast diet. Body weight gain, food intake and food conversion ratio data in experiment 2, are shown in Table 4. At this level (15.0mg vit. E) weight gain of the chicks was still significantly ($P < 0.05$) lower than those of the chicks fed the soya-fish meal control diet. Higher levels of vitamin E supplementation above 15.0mg/kg to the basal yeast did not elicit further improvements in body weight gain and consequently at no level of vitamin examined was weight gain equal to that of yeast free control. Mean food intake by the chicks was identical for all dietary treatments irrespective of vitamin E supplementation level, consequently the food conversion ratio followed the same trend as the body weight gain. Nitrogen retention determination were not carried out in this experiment but results from the first experiment (Table 3) suggest that the levels of vitamin E supplementation of the yeast diet examined did not affect overall nitrogen retention by the chicks. Growth rate and efficiency of food conversion data from this experiment however indicate that in a practical - type chick diet containing 300g/kg yeast, a minimum of 15mg supplementary vitamin E was established. Safety margin was recognised and a dietary level of 19mg of supplementary vitamin E is being recommended.

SELENIUM SUPPLEMENTATION OF HIGH YEAST DIETS

The results of Experiment 3 are given in Table 5. This indicated a positive growth response when a 300g/kg yeast diet was supplemented with 150 μ g selenium per kg diet. The nitrogen retention data from this preliminary experiment also suggested that nitrogen was increasingly retained by the chicks with increasing level of selenium in the yeast diet.

SE AND VIT. E SUPPLEMENTATION IN CHICK DIETS

The mean body weight gain, food intake and nitrogen retention of the chicks in experiment 4 are given in Table 6. Chicks fed the Se Supplemented yeast diets gained weight

($P < 0.05$) higher nitrogen retention than any of the Se supplemented yeast diet (Table 6). Increasing the level of supplementary selenium from 100 ug to 175 μ g/kg in the 300g/kg yeast

TABLE 6: BODY WEIGHT GAIN, FOOD INTAKE, FOOD CONVERSION RATIO (FCR) AND NITROGEN RETENTION OF CHICKS FED CONTROL DIET AND 300g/kg YEAST DIETS CONTAINING 19mg VITAMIN E PER PLUS GRADED LEVELS OF SELENIUM

(Values are means of twelve chicks per treatment)

	Diet	Body weight gain (g/d)	Food intake (g/d)	FCR	Nitrogen retention
1.	Control	22.6 ^a	35.0 ^a	0.645 ^a	0.540 ^a
2.	300g/kg Yeast	20.0 ^c	34.7 ^a	0.576 ^c	0.469 ^d
3.	300g/kg Yeast + 100ug Se/kg	21.5 ^b	35.0 ^a	0.615 ^{ab}	0.481 ^c
4.	300g/kg Yeast + 125 ug Se/kg	21.5 ^b	34.5 ^a	0.623 ^{ab}	0.478 ^c
5.	300g/kg Yeast + 150ug Se/kg	21.3 ^b	35.5 ^a	0.600 ^{bc}	0.486 ^{bc}
6.	300g/kg Yeast + 175 ug Se/kg	21.6 ^b	35.3 ^a	0.613 ^b	0.490 ^b
	SEM	± 0.2878	± 0.3834	± 0.0102	± 0.003

Mean value followed by similar letter down do not differ significantly ($P > 0.05$)

at a rate higher than those given the unsupplemented yeast diet. This increase in growth rate was significantly ($P < 0.05$) higher at Se supplementation of 100 μ g/kg diet as compared to unsupplemented yeast diet. Above Se supplementation of 100 ug no extra stimulation in growth rate was observed, an indication that the Se requirement of the chicks on the 300g/kg yeast diet is satisfied at this Se level. Body weight gain by the chicks fed the soya/fed meal control diet was still superior compared with any level of Se-supplemented yeast diet. There was no significant treatment effect on feed intake thus resulting in significantly ($P < 0.05$) lower food conversion ratio on unsupplemented yeast diets, compared to those supplemented with selenium. At no level of selenium supplementation of yeast diet was food conversion equal that of control. Nitrogen retention by chicks fed the Se supplemented yeast diets was significantly higher ($P < 0.05$) than the unsupplemented yeast diet. The control chicks, however, still had significantly

diet did not significantly increase the percent nitrogen retention although there appears to be a trend towards an increase.

DISCUSSION

A 300g/kg yeast diet with only 4mg/kg vitamin E and 100 μ g/kg Se supplied in the premix, as used in this study contains enough of these two compounds to prevent deficiencies of either since no deficiency symptoms were observed in any of the experimental chicks. Weight gain responses to additional vitamin E as well as selenium, however, show that higher levels of these compounds are required for better performance. This suggests that low levels of vitamin E and selenium were partly responsible for the reduction in performance of chicks fed high yeast diets since a combination of supplementary 15mg vitamin E/kg and 100 ug/kg Se increased the rate of growth of chicks fed a 300g/kg yeast diet almost to the same extent as those fed a soya /fish control diet at the end of 2 experimental

weeks. The effect of vitamin E and selenium appears to be synergistic with the levels tested as weight gain with both supplements together was in excess of that by either alone. This is illustrated by the fact that with selenium supplementation of a yeast diet (Diet 2 Table 5) that already contained 4mg Vit. E/kg and 100 μ g/kg Se from the premix, significant body weight response was obtained only at 150 μ g Se/kg diet whereas with inclusion of 15mg vit. E/kg of the same yeast diet only extra 100 μ g Se/kg (Diet 3, Table 4) elicited the same response.

There are very few published works to compare these results with as most research had, in the past, been carried out with purified diets to determine either the requirements or toxic levels of selenium. The synergistic effect, however, has been demonstrated by several workers since the finding (Nesheim and Scott, 1958) that selenium had an effect on growth greater than that of vitamin E alone and equal to that obtained by giving both vitamin E and selenium. The level of Se (200 μ g/kg total dietary addition) found to be optimum for maximum growth in this study is relatively higher than values generally reported in the literature for requirement which usually range between 50 to 150 μ g/kg diet (Seier and Bragg, 1973; ARC, 1975). This might be due to the lower level of vitamin E (19mg/kg) used in this experiment compared to the total dietary level present in practical type diets in which more conventional sources of protein are used. In any case, the higher dietary selenium level (200 - 225 μ g/kg added) found to be optimal in this experiment may reflect differences in absolute minimum requirement to prevent deficiency symptoms and the level that supports maximum weight gain obtainable within the limitation of the diet. The processing treatment that the diets had undergone might also explain the need for a relatively higher selenium level. Pelleting is known to destroy vitamin E because of the severe conditions of high temperatures that the process involves especially in the absence of adequate antioxidant (Seier and Bragg, 1973). As the diets in the present experiments were pelleted,

although dl α -tocopherol acetate is known to be stable to most feed processing conditions, it is possible that a fraction of the vitamin is lost during pelleting and thus compensated for by selenium.

The level of selenium used in this work, at least, seemed to be more significant on nitrogen retention than the level of vitamin E examined. Addition of 30mg vit. E to the 300/kg yeast diet increased nitrogen retention to the same slight extent as 50 μ g/kg selenium alone but 150 μ g Se/kg alone or 100 μ g Se and 15mg vit. E/kg together significantly increased nitrogen retention more than either compound separately. The reasons for this are unknown and there are no published works with which to compare these values. However, if selenium is involved in the active centre of non-haem-Fe protein as selenide and vitamin E functions as a membrane localised antioxidant affording it (and not unsaturated fatty acyl residues) protection (Diplock and Lucy, 1973), it may be speculated that the levels of selenium and vitamin E found to support highest body weight gain in this experiment allow the accumulation of these proteins to an extent that is measurable as nitrogen retained. Why vitamin E additions did not increase nitrogen retention cannot be explained except that perhaps the levels of vitamin E were not high enough.

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