

The effect of β -carotene and nucleotide base supplementation on haematological, biochemical and certain immunological parameters in weaned pigs*

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ABSTRACT

The effect of synthetic β -carotene and of synthetic nucleotide bases was investigated in this experiment. Beginning one week prior to weaning, the diet fed to one experimental group of piglets was supplemented with 10 % Rovimix β -carotene at 875 mg/kg diet (10 times the recommendation of the NRC,1988). Synthetic uracil and adenine (98%, Sigma-Aldrich) were mixed into the diet for the other experimental group at quantities of 500 mg/kg diet of each substance. During the 3-week period of the experiment changes in the daily weight gain of the animals, their feed consumption and certain blood parameters were examined. Changes which could be observed with the progress of time in the haematological parameters (RBC, WBC, Hb, HTC, MCV, MCH, MCHC), in the ESR and in certain biochemical parameters (TP, ALB, CHOL, TRIG, UREA, ALT and AST) can be regarded as physiological. The LST values given for Phytohaemagglutinin and Concanavalin-A increased in the experimental groups by 50 and 130%, respectively (nucleotide), and by 60 and 30%, respectively, (carotene) respectively, while the blastic transformation of the lymphocytes did not vary in the control group. The supplements applied exerted no positive effect on *in vivo* cellular immune response. No significant degree of difference was observed between the plasma retinol concentrations determined at the end of the experimental period, (control: 376 ± 183 , nucleotide: 412 ± 268 and carotene: 349 ± 251 $\mu\text{g/L}$) while there was a decrease in plasma E-vitamin concentration due to carotene supplementation (control: 5.58 ± 2.36 , nucleotide: 5.83 ± 3.01 and carotene: 2.12 ± 1.60 mg/L).

KEY WORDS: nucleotide bases, beta-carotene, immune response, piglets

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INTRODUCTION

It is a generally known fact that there is a relation between stress conditions, the immune responsiveness of an animal and certain frequently occurring disorders (Kelley, 1980; Siegel, 1985; Blecha, 1988). In such cases stimulation of the immune response may manifest itself in the form of an increase in resistance to disease in animals.

A number of chemical substances (levamisol, imuthiol, avidine, isoprinosine, etc.) are known to exert a stimulating effect on the functioning of the immune system (Kende et al., 1984; Torrence, 1985; Blecha, 1988). Due to reservations about the application of such non-endogenous chemical substances attention has now turned to the determination of the immunomodulatory effect of, and possibilities for the application of, substances occurring in the organism even under natural conditions (vitamins, minerals, amino acids, etc.).

The present experiment investigated the effects of dietary supplementation with synthetic β -carotene and synthetic nucleotide bases on immune system function in the piglet.

MATERIAL AND METHODS

Animals

Unweaned 20-to-22-day-old piglets were divided into three groups such that 6 healthy piglets of approximately identical weight, taken from two litters, were selected for each group (12 per group). Feeding with the experimental diets was initiated 7 days prior to weaning; subsequently, after removal from the litters the animals were housed together in batteries, in which they were fed the experimental diets for a further two weeks. The state of health of the animals (the frequency and severity of diarrhoea and the number of days for which the animals were affected by this) was monitored, and the quantity of feed consumed per group and individual body weight gain were recorded.

Feeding

The basic diet of one of the experimental groups was supplemented with 10 % Rovimix Beta-carotene (Hoffmann-La Roche, 100 mg/g β -carotene) at 875 mg/kg diet (10 times the recommendation of the NRC, 1988). Synthetic uracil and adenine (98 %, Sigma-Aldrich) were mixed into the diet for the other experimental group at quantities of 500 mg/kg diet of each substance.

Collection of samples

Blood samples were taken from the *vena cava cranialis* on the 1st, 7th and 21st day of the experimental period into two tubes containing dipotassium ethylenediamine tetraacetate (K-EDTA) and sodium-heparin, respectively.

Haematological analyses

Cobas Minos STE equipment (Roche, Switzerland) was used to determine the concentration of red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb), haematocrit value (HTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Erythrocyte sedimentation rate (ESR) was also determined, in accordance with the method described by Westergreen (Horwath, 1979).

Biochemical parameters

Determination of total protein (TP), albumin (ALB), globulin (GLOB), blood urea nitrogen (BUN), cholesterol (CHOL), triglyceride (TG) and uric acid (UA) concentration in blood plasma was performed by means of a Centrifichem 600 analyser (Roche), while gamma glutamyl transferase (GGT), aspartic acid aminotransferase (AST) and alanine aminotransferase (ALT) activity determination involved the use of a Kodak Ectachem 750 x rc analyser with Johnson and Johnson test discs.

Immunological parameters

In order to investigate *in vitro* cellular immune responsiveness an examination of blastic transformation, induced by means of 200 gamma per ml Phytohaemagglutinin (PHA) or Concanavalin-A (Con A), was performed, by application of the lymphocyte stimulation test (LST). The rate of the blastogenic response was determined by fluorometric DNA assay (Nagahata et al., 1987).

The *in vivo* cellular immune response was examined by means of an intradermal probe. 0.1 ml of the PHA solution (500 mg Phytohaemagglutinin M dissolved in 1 ml PBS) was injected into the skin of the neck on the last day of the experiment. Skin fold thickness was measured at 24 h post-injection.

Statistical analysis

The various statistical analyses made were performed by application of the SPSS programme package (1996); in this, differences between the groups and

between blood samplings were examined by means of paired t-test and variance analysis.

RESULTS AND DISCUSSION

Subsequent to weaning (on days 7 to 21 of the experimental period) the more intensive body weight gain observed in the groups fed the experimental diets was assumed to be due to their higher feed consumption (Table 1).

TABLE 1
The influence of β -Carotene and nucleotide supplementation on body weight gain (means \pm SD) and feed consumption

Period investigated	Groups	Feed consumption kg/ group	Body weight gain, kg ($\bar{x} \pm$ SD)
0-7 days	Control	5.1	1.65 ^a \pm 0.56
	Carotene	4.3	1.16 ^a \pm 0.16
	Nucleotide	3.9	1.60 ^a \pm 0.36
7-21 days	Control	47.2	2.37 ^a \pm 0.62
	Carotene	56.9	3.74 ^b \pm 0.85
	Nucleotide	57.3	3.31 ^{ab} \pm 1.58

note: superscripts denote significant ($P < 0.05$) differences between groups

Of the haematological parameters, RBC and WBC count were observed to increase with time. MCH and MCV values decreased in all three groups. ESR increased in the control animals (from 1.9 to 3.4 mM/h), while showing a decrease by the 3rd blood sample ($P < 0.05$) in both experimental groups (carotene from 2.5 to 1.5, nucleotide from 2.3 to 1.2 mM/h). These changes, observed with the progress of time, can be regarded as physiological (Schalm, 1986). Neither carotene nor nucleotide supplementation exerted any significant effect on the parameters studied.

With respect to the biochemical parameters examined, a decrease, within the physiological limit values, was observed in TP, ALB, CHOL and TRIG concentration with the passage of time, while BUN concentration was seen to increase, as was AST and ALT activity. There was no substantial change in the quantity of uric acid present. In the group fed the diet supplemented with carotene, lower CHOL concentration ($P < 0.001$) and GGT activity ($P < 0.001$) was measured in comparison with that of the control group.

The LST values given for PHA and Con A increased in the experimental groups by 50 and 130 %, respectively (nucleotide), and by 60 and 30 %, respectively, (carotene), while the blastogenic transformation of the lymphocytes did not vary in the control group. In both groups the response for PHA was of a greater degree than the reaction elicited by Con A (Table 2).

TABLE 2

Changes in the LST values (%) after stimulation of the lymphocytes with PHA and ConA and the results of the intradermal PHA test (means + S.D.)

Groups	Sampling	LST (PHA)% %	LST (ConA)% %	Skin fold thickness mm
Control (n=12)	1 st	2.78 ± 1.18	1.19 ± 0.50	
	7 th	2.87 ± 0.38	1.22 ± 0.24	
	21 st	2.95 ± 0.62	1.13 ± 0.19	4.77 ^a ± 1.58
Carotene (n=12)	1 st	2.59 ^a ± 0.58	0.97 ± 0.22	
	7 th	3.34 ^{ab} ± 1.04	1.18 ± 0.25	
	21 st	4.20 ^b ± 1.17	1.27 ± 0.61	2.59 ^a ± 1.35
Nucleotide (n=12)	1 st	2.43 ^a ± 0.74	0.87 ^a ± 0.24	
	7 th	3.12 ^{ab} ± 1.27	1.85 ^{ab} ± 0.65	
	21 st	3.59 ^b ± 1.34	1.98 ^b ± 0.91	4.21 ^{xy} ± 2.21

note: different superscripts (a,b,c) denote significant ($P < 0.05$) differences between blood samples and (x,y) between groups

The two T-cell mitogens reflect the general cellular immune responsiveness of the organism. The results of this experiment correspond to data given in a number of reports in the literature, according to which both nucleotide and β -carotene supplementation result in an increase in T-cell immune responsiveness (Bendich and Shapiro, 1986; Barankiewicz and Cohen, 1987; Pérignon et al., 1987; Hoskinson et al., 1989; Daniel et al., 1991 a,b; Michal et al., 1994). β -carotene is deposited in the lymphocytes, and acts as an immunostimulator partly by virtue of its effect on membrane protection and partly via promoting blastogenesis (Chew et al., 1993). Exogenous nucleotides and purine/pyrimidine bases also bring about an increase in cellular immune response, since, by forming a pool, they have the capacity to fulfil the increased requirements, with respect to metabolism and nucleic acid synthesis, of proliferation and blastogenesis (Carver, 1994).

No positive effect on *in vivo* cellular immune response was found with the supplements applied in this study, presumably due to the low concentration in which they were administered. The reason that the cutaneous reaction observed in the group given β -carotene was of a lower degree than that produced in the control group and in the group given nucleotides may be that a negative effect was exerted on the level of vitamin E by β -carotene or by vitamin A formed from it. That is, at the time at which the intradermal test examination was performed, the concentration of vitamin E, which exerts a similar stimulatory effect on the functioning of the immune system, was in this group lower than half of that of the mean for the other two groups (Table 3). β -carotene was not detectable in the plasma even of the animals in the group consuming the carotene-supplemented diet. No significant differences were observed between the plasma retinol concentrations in the

TABLE 3

Changes in serum vitamin-E concentrations (mg/L) (means \pm S.D.) following β -carotene or nucleotide supplementation

Sampling days	Control n=12	Carotene n=12	Nucleotide n=12
7 th	2.32 ^{ax} \pm 1.15	2.76 ^x \pm 1.09	3.66 ^{xy} \pm 2.05
21 st	5.58 ^{bx} \pm 2.36	2.12 ^y \pm 1.60	5.83 ^{bz} \pm 3.01

note: superscripts (a,b) denote significant ($P < 0.05$) differences between blood samples and (x,y,z) between groups

different groups determined at the end of the experimental period (control 376 ± 183 , nucleotide 412 ± 268 and carotene 349 ± 251 mg/L). Thus, the level of vitamin A in the plasma was not altered by the excess carotene dosed into the diet.

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