Reprod Nutr Dev (1995) 35, 201-212

Original article

Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks

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(Received 26 September 1994 ; accepted 24 January 1995)

Summary — Day-old male meat-type chicks were fed a commercial starter diet supplemented with 2 levels of enzyme preparations containing amylase and proteases up to 14 d of age. Enzyme supplementation had no significant effect on feed intake or growth rate, and was accompagnied by a significant decrease in gizzard content and small intestine weight. The intestine contents increased and this increase was accompanied by a significant decrease in its pH. Enzyme supplementation depressed the activity of chymotrypsin in the pancreas and the activity of amylase, trypsin and chymotrypsin in the intestinal contents. Some carry-over effects were observed on d 42, 4 weeks after the cessation of the enzyme supplements. These were mainly a significant depression in the activity of trypsin in the intestinal contents. In a balance study, diets supplemented with 0, 250 and 1 000 μg/kg enzyme preparations were supplied. Exogenous enzyme supplements had no significant effect on the digestibility of all the nutrients studied except for the highest level of enzyme supplementation, which improved slightly but consistently the digestibility of amino acids. Some age effects were observed, mainly a decrease in the digestibility of fat and starch, and in the ME of the diet from weeks 1 to 2 followed by an increase during week 3. Protein digestibility and retention of nitrogen decreased with age.

amylase / protease / meat-type chicks / digestibility / amino acids

Résumé — Effet de l'âge, de l'amylase et des protéases sur la digestion, l'activité enzymatique du pancréas et la digestibilité de l'aliment chez des poulets de chair. *Des poulets de chair ont été nourris d'un aliment supplémenté avec 2 niveaux d'un concentré enzymatique contenant de l'amylase et des protéases jusqu'à l'âge de 14 j. L'addition d'enzymes n'a eu aucun effet significatif sur la prise alimentaire ou le taux de croissance. L'addition d'enzymes a cependant causé une réduction significative*

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du contenu du gésier et du poids de l'intestin grêle, le poids du chyme intestinal a augmenté et son pH a été réduit. L'apport d'enzymes a réduit l'activité de l'amylase au niveau du pancréas et celle de l'amylase, de la trypsine et de la chymotrypsine au niveau du chyme intestinal. Un effet résiduel observé était la réduction de la trypsine dans le chyme intestinal à l'âge de 42 j, 4 sem après la cessation d'apport d'enzymes. Dans une étude subséquente, l'effet de l'addition de 0, 250 ou 1 000 μg/kg de préparation enzymatique sur la digestibilité de l'aliment à l'âge de 1, 2 et 3 sem a été étudié. L'addition d'amylase et de protéases n'a eu aucun effet sur la digestibilité des lipides totaux, de l'amidon, des protéines, de l'énergie métabolisable (ME), ou de l'énergie métabolisable apparente corrigée pour la rétention azotée (AMEn). La digestibilité des acides aminés a été légèrement améliorée par l'addition de 1 000 μg/kg de préparation enzymatique. Les effets de l'âge observés étaient une réduction de la digestibilité des lipides totaux, de l'amidon et de l'ME durant la deuxième semaine et une amélioration de la digestibilité des lipides totaux durant la troisième semaine. La digestibilité des protéines ainsi que la rétention azotée ont été réduites avec l'âge.

amylase / protéase / poulets de chair / digestibilité / acides aminés

INTRODUCTION

Gastrointestinal tract (GIT) development during the post-hatching growth period has been widely investigated in birds (Dror *et al,* 1977; Lilja, 1983; Katanbaf *et al,* 1988; Nitsan *et al,* 1991a, b; Sell *et al,* 1991; Nir *et al,* 1993). All of these studies emphasize the major role of the GIT in inducing growth during the early post-hatching period. During this period, the GIT segments increase in size much more rapidly than the rest of the body. Limited synthesis of digestive enzymes in the pancreas during the first days after hatching and their increase to maximum values around d 10 when relative growth is maximal, indicate a possible relationship between these 2 traits (Nitsan *et al,* 1991a,b). The GIT has been implicated as a limiting factor in food intake, and subsequently growth, more so in meat-type chicks than in egg-type Leghorn chickens (Nir *et al,* 1978). It was difficult to overfeed post-hatching meat-type chicks using an intubation technique, in contrast to Leghorn chicks where overfeeding was accompanied by increased lean-body growth and fat deposition. The above was confirmed when stocks selected for high body weight were compared with those selected for low body weight (Barbato *et al,* 1984). In another study, Nir *et al* (1993) found that although the allometric growth of the small intestine and intestinal contents and liver was greater in meat-type than egg-type chickens, the allometric growth of the pancreas was higher in egg-type birds. The intestinal contents of meat-type chicks exhibited lower activities of amylase, lipase, trypsin and chymotrypsin. These results did not agree with those of Nitsan *et al* (1991b) who assayed Plymouth Rocks selected over 30 generations for high (HW) or low (LW) body weight (Dunnington and Siegel, 1985). In this case HW chicks had higher enzymatic activities in their intestinal contents than LW ones.

The potential of industrial enzyme products as animal feed additives has recently been reviewed by Campbell and Bedford (1992). Early studies showed a beneficial effect of using crude amylase and protease preparations (Jensen *et al,* 1957; Fry *et al,* 1958; Burnett, 1966) which were later attributed to the β-glucanase activity found in these preparations (Rickes *et al,* 1962).

Assuming that post-hatching meat-type chicks have limited synthesis and secretion of digestive enzymes, in the present work a preparation containing amylase and proteases of bacterial origin was supplemented to a starter diet and fed to meat-type chicks to the age of 14 d. Their effect on performance, development of the GIT including

the digestive enzyme activities, and nutrient retention was studied. In order to verify any carry-over effects, the enzymes were removed from the diet at the age of 14 d and the same variables were determined at the age of 42 d.

MATERIALS AND METHODS

Experiment 1. Performance and GIT

Diets and chickens

Day-old male meat-type chicks were placed in heated battery cages (3 per cage) under continuous artificial lighting. Each cage was equipped with a feeder and nipple drinker to provide free access to food and water. The chicks were fed a commercial starter diet (crumbles) containing 21% protein and 13.0 MJ/kg nitrogen-corrected apparent metabolizable energy (AME_n) composed essentially of sorghum and soybean meal (table I).

Enzyme preparations

Enzyme preparations were produced by *Bacillus subtilis* and *Penicillium emersonii*. Activities of the supplemented enzymes were determined as follows. Amylase was determined as in the digestive tract (Nitsan *et al*, 1974) and was expressed as glucose produced per min from starch per milligram of enzyme preparation. The values were 0.38 and 0.96 mg in EI and EII, respectively. The total activity of the proteases was measured by recording tyrosine produced from casein according to Colowick and Kaplan (1955). The values were 38 and 48 μeq/mg for enzyme preparations EI and EII, respectively.

Treatments

Five treatments were studied: 1) diet not supplemented with enzymes; 2) supplementation with 250 μg/kg enzyme preparation I (EI250); 3) supplementation with 1 000 μg/kg enzyme preparation I (EI1000); 4) supplementation with 250 μg/kg enzyme preparation II (EII250); and 5) supplementation with 1 000 μg/kg enzyme preparation II (EII1000). There were 9 replicates per treatment for week 1 (45 cages x 3 chicks per cage for

Table I. Composition of the experimental diet (%).

Ingredient		*Calculated analysis*	
Sorghum	57.26	Metabolizable energy (MJ/kg)	13.39
Sunflower soapstock oil	5.10	Protein	21.06
Soybean meal (44% protein)	24.60	Lysine	1.16
Meat meal (60% protein)	5.00	Methionine	0.54
Corn gluten meal (62% protein)	2.00	Sulfur amino acids	0.9
Dicalcium phosphate	1.00	Total fat	7.03
Limestone	0.64	Calcium	1.00
NaCl/limestone 3:7	0.36	Total phosphorus	0.66
Choline chloride (5%)	1.56	Non-phytate phosphorus	0.45
Premix vitamins-microelements [a]	0.57	Sodium	0.15
Premix lysine (5%)	1.66	Potassium	0.71
Methionine hydroxy analogue [b]	0.25	Chloride	0.18

[a] Provides per kilogram of diet: vitamin A, 10 500 IU; cholecalciferol, 2 500 IU; vitamin E, 30 IU; B12, 0.1 mg; riboflavin, 7 mg; niacin, 37 mg; D-pantothenic acid, 14 mg; choline, 600 mg; menadione, 2.5 mg; folic acid, 1 mg; thiamin, 1 mg; pyridoxine, 3 mg; D-biotin, 0.125 mg; ethoxyquin, 125 mg; Mn, 80 mg; Zn, 50 mg; Fe, 20 mg; Cu, 5 mg; I, 1.2 mg, Se, 0.2 mg. [b] Alimet, estimated to have 88% DL-methionine activity (Novus).

a total of 135 chicks). At the beginning of week 2 the chicks were transferred to individual cages, 18 chicks per treatment, and from the beginning of week 3 all chicks were fed the unsupplemented diet.

Autopsy and digestive enzyme activities in the pancreas and intestine contents

On d 7 and 14, 8 chicks per treatment were weighed and necropsied. The chicks were killed sequentially, one chick from each treatment group. Each necropsy lasted approximately 6 min. Digestive organs and liver were weighed full and empty and the contents were calculated by difference, pH was determined in the gizzard and intestinal contents using a glass electrode. The pancreas and small intestinal contents were immediately frozen in liquid nitrogen and stored at –20°C. Amylase, trypsin and chymotrypsin activities were measured in the pancreas and intestinal chyme essentially as described previously (Nitsan *et al*, 1974). On d 42 the above determinations were carried out on chicks supplied up to the age of 14 d with or without EII1000. Enzyme activities were expressed in units, one unit being defined as a change of 10^{-3} absorbency units for trypsin and chymotrypsin and 10^{-6} absorbency units for amylase, under the conditions specified for each assay system.

Experiment 2. Balance experiment

This experiment was carried parallel to *Experiment 1*, the chicks, feed and enzymes were similar. Balance was carried out using diets supplemented with 3 levels of EII: 0, 250 and 1 000 µg/kg. During week 1, balance was carried out in metabolic cages with 3 chicks per cage, during week 2, with 2 chicks per cage and during week 3, with 1 chick per cage. There were 4 replicates per treatment. Excreta were collected daily at 08.00 h from days 3 to 7 (week 1), 10 to 14 (week 2) and 17 to 21 (week 3), and freeze-dried. Feed intake was determined by weighing the feeders at the begining and end of each period which lasted exactly 96 h. Dry matter, crude protein (N x 6.25) and ash contents were analyzed using methods described by the Association of Official Agricultural Chemists (1984).

Total lipid was determined gravimetrically after acid hydrolysis (6 N HCl, 1 h, 100°C) and extraction with petroleum ether (60–80°C bp) after cooling. Starch content was determined by an enzymatic method using amyloglucosidase from *Rhizopus* mold (Sigma A7255) and glucose content by the glucose oxidase procedure (Sigma diagnostic kits and reagents, catalog number 510-DA). Using soluble starch, almost theoretical yields of glucose were obtained by this procedure. Uric acid in the excreta was determined according to Marquardt (1983). Amino-acid contents of feed and excreta were determined by ion-exchange chromatography using an autoanalyzer (Amino Acid Analyzer LC 5000, Biotronik), after 24 h acid hydrolysis with 6 M aqueous HCl at 115°C. Methionine and cystine were determined on samples oxidized with performic acid (Moore, 1963). Energy was determined with an adiabatic bomb calorimeter (Parr Instrument Co, Moline, IL, model Parr 1261).

Calculations

Digestibility of fat or starch (%) = [(ingested – excreted) / ingested] • 100

Digestibility of protein (%) = {[ingested N – (excreted N – uric acid N x 1.2)] / ingested N} • 100

The coefficient 1.2 was chosen according to Larbier and Leclercq (1992).

Metabolizability ME (%) = [(gross energy intake – gross energy in excreta) / gross energy intake] • 100

AME_n was calculated according to Hill and Anderson (1958).

Statistical analysis

Data were analyzed by the General Linear Model procedure of the Statistical Analysis System (SAS Institute, 1985). Duncan's multiple range test was used to compare means between treatments.

RESULTS

No differences were obtained between the effects of EI and EII on performance, pan-

creatic enzyme activites and digestibility of nutrients, and no interactions were observed between the source of enzyme and its level. We thus present pooled data for effects of enzyme supplementation levels.

At the age of 42 d, 4 weeks after enzymes were removed from the feed, the only carry-over effects observed was a lower trypsin activity in the intestinal contents of the group supplied with the enzyme preparation (control 7.5 units/g *vs* 3.2 in the treated chicks, $P < 0.02$). Therefore the data obtained at day 42 are not presented.

Performance

Although enzyme supplementation had no significant effect on growth rate or feed intake during the period of the supplementation (d 1–14, table II), it caused a depression in feed utilization from 1 to 7 d. This depression was significant at the supplement level of 250 µg but not at the 1 000 µg level. No effect was observed later than 7 d.

Relative weight of the duodenum and small intestine, the contents of the gizzard, duodenum and small intestine, and the pH of gizzard and intestine contents

Enzyme supplementation was accompanied by a significant decrease in duodeum and small intestine (jejunum and ileum) weights at 7 and 14 d of age (table III). Removal of the enzyme from the diet at the age of 15 d canceled the weight differences of the small intestine between treatment groups, determined at 42 d of age. Enzyme supplementation had no effect on the weight of all the other organs examined (liver, pancreas, crop, proventriculus, gizzard, not presented in table III).

Table II. Body weight, feed intake and feed utilization at 7, 14 and 42 d of age.

	Enzyme level (µg/kg) [a]			*Pooled SEM*	*Significance*
	0	*250*	*1 000*		
Body weight (g)					
7 d of age	148	151	150	3.30	NS
14 d of age	384	384	388	5.36	NS
42 d of age	1 939	1 982	1 982	24.0	NS
Cumulative feed intake (g)					
7 d of age	108	126	116	6.1	NS
14 d of age	415	420	423	6.1	NS
42 d of age	3 307	3 325	3 347	80	NS
Cumulative gain/feed					
7 d of age	0.85 [b]	0.80 [c]	0.82 [bc]	0.013	0.04
14 d of age	0.78	0.78	0.77	0.008	NS
42 d of age	0.61	0.60	0.61	0.013	NS

[a] $n = 16$ per each age and enzyme level. [b–c] Means within rows with no common superscript differ significantly ($P < 0.05$).

Table III. Relative weight (g/100 g body weight) of the duodenum and small intestine, the contents of the gizzard, duodenum and small intestine (g/100 g body weight), and the pH of gizzard and intestinal contents.

Organ	*Enzyme level (μg/kg)* [a]			*Pooled SEM*	*Significance*
	0	*250*	*1 000*		
7 d of age					
Duodenum	1.89 [b]	1.45 [c]	1.56 [c]	0.09	0.01
Small intestine	4.94 [b]	4.05 [c]	3.66 [c]	0.19	0.01
GIT content and pH					
Gizzard	3.56	3.36	3.35	0.04	NS
Duodeum	0.46	0.44	0.55	0.05	NS
Small intestine	3.70 [c]	3.86 [c]	4.68 [b]	0.21	0.01
pH in gizzard	2.89	2.80	2.90	0.07	NS
pH in intestine	6.46	6.33	6.19	0.12	NS
14 d of age					
Duodenum	1.19 [b]	0.97 [c]	0.93 [c]	0.04	0.002
Small intestine	3.39 [b]	3.16 [bc]	2.85 [c]	0.09	0.004
GIT content and pH					
Gizzard	2.11 [b]	1.93 [b]	1.69 [c]	0.04	0.001
Duodenum	0.38 [c]	0.46 [bc]	0.49 [b]	0.03	0.1
Small intestine	2.08 [c]	2.53 [bc]	2.63 [b]	0.15	0.05
pH in gizzard	2.96	3.08	3.13	0.06	NS
pH in intestine	6.88 [b]	6.64 [c]	6.52 [c]	0.07	0.01

[a] $n = 16$ per each age and enzyme level. [b-c] Means within rows with no common superscripts differ significantly ($P < 0.05$).

Enzyme supplementation had no effect on the amount of crop and proventriculus contents (not presented). Supplementation with 1 000 μg/kg enzyme preparation caused a significant decrease in gizzard content and an increase in duodenum and intestinal content (table III), as compared to non-supplemented chicks on d 14, while the 250 μg/kg supplemented group was intermediate. Only the increase in intestinal contents was significant on d 7. On d 14, a significant decrease in the pH of the intestinal contents was found. Removal of the enzyme preparation abolished these differences by d 42.

Activity of digestive enzymes in pancreas and intestine contents

Enzyme supplementation at 250 μg/kg had no effect on enzyme activities in the pancreas on d 7 and 14. However, diets supplemented with 1 000 μg/kg significantly depressed the activity of chymotrypsin in the pancreas at both 7 and 14 d (table IV). In the intestinal contents, the 250 μg/kg supplemented diet reduced all the enzyme activities on d 14, whereas 1 000 μg/kg reduced the enzyme activities at both 7 and 14 d (except for trypsin on d 14). After removal of

Table IV. Activities of amylase, trypsin and chymotrypsin in the pancreas and intestine contents (units/g).

	Enzyme level (μg/kg) [a]			*Pooled SEM*	*Significance*
	0	*250*	*1 000*		
7 d of age					
Pancreas					
Amylase	19.6	21.2	19.1	2.1	NS
Trypsin	75.2	79.0	73.1	3.4	NS
Chymotrypsin	20.3 [b]	20.5 [b]	16.4 [c]	1.0	0.01
Intestine contents					
Amylase	0.624 [bc]	0.697 [b]	0.506 [c]	0.045	0.05
Trypsin	12.2 [bc]	13.6 [b]	11.4 [c]	0.7	0.08
Chymotrypsin	1.09 [b]	1.22 [b]	0.77 [c]	0.09	0.01
14 d of age					
Pancreas					
Amylase	25.5	27.5	24.5	1.8	NS
Trypsin	83.8	89.6	85.9	4.6	NS
Chymotrypsin	36.2 [b]	36.1 [b]	30.8 [c]	1.8	0.06
Intestinal contents					
Amylase	1.024 [b]	0.762 [c]	0.750 [c]	0.076	0.03
Trypsin	8.5 [b]	8.0 [c]	8.5 [b]	0.14	0.02
Chymotrypsin	2.48 [b]	1.87 [c]	1.79 [c]	0.17	0.01

[a] $n = 16$ per each age and enzyme level. [b-c] Means within rows with no common superscripts differ significantly ($P < 0.05$).

the enzyme preparation on d 15, some carry-over effects were observed on d 42, mainly a significant depression in the activity of trypsin in the intestine contents and a reduction in amylase activity in the pancreas ($P < 0.02$ and $P < 0.08$ respectively).

Digestibility of fat, starch, protein and amino acids, metabolizability (ME), AME_n and retention of nitrogen in the diet

Exogenous enzyme supplements did not significantly affect the digestibility of fat, starch, protein, the metabolizability or AME_n of the diet (table V). However, some age effects were obtained, mainly a decrease in the digestibility of fat on week 2 followed by an increase on week 3. The digestibility of starch decreased slightly but highly significantly with age ($P < 0.001$). The metabolizability of the diet was reduced from weeks 1 to 2 ($P < 0.07$), followed by an increase during week 3 (not significant). When corrected for uric acid, protein digestibility decreased with age ($P < 0.001$). When calculated according to the mean digestibility of amino acids, the age reduction was slight and non-significant.

Whereas the enzyme supplements had no significant effect on the retention of nitrogen (table V), it was markedly reduced with

Table V. Digestibility of fat, starch, and protein and metabolizability (% of ingested), AME_n (MJ/kg) and nitrogen (% of ingested) retention in meat-type chicks fed diets supplemented with exogenous amylase and proteases from 1 to 3 weeks of age.

Ingredient	*Enzyme (μg/kg)*			*Age (weeks)*			*SEM*[a]	*Significance*	
	0	*250*	*1 000*	*1*	*2*	*3*		*Level*	*Age*
Fat	77.6	78.2	81.3	80.0 [c]	70.3 [d]	86.8 [b]	1.19	NS	0.001
Starch	94.9	94.1	95.8	96.7 [b]	94.4 [c]	93.7 [c]	0.70	NS	0.001
Protein [e]	84.8	84.2	82.5	91.5 [b]	84.0 [c]	75.9 [d]	1.51	NS	0.001
Amino acids [f]	86.3	86.5	87.5	87.2	86.7	86.4	0.99	NS	NS
Metabolizability	77.8	78.0	77.8	79.4	76.7	77.3	1.01	NS	0.07
AME	13.0	13.0	13.3	13.2	13.1	13.1	0.013	NS	NS
Nitrogen retention	60.8	63.4	63.3	71.2 [b]	62.3 [c]	53.9 [d]	1.23	NS	0.01

No interaction was found between enzyme level and age. [a] Pooled SEM, 12 replicates per enzyme level and per age. [b–d] Means within rows with no common superscript differ significantly ($P < 0.05$) [e] Nitrogen digestibility corrected for uric acid excretion (see *Materials and methods*). [f] Mean of amino-acid digestibility.

age. Nitrogen retention was much lower during week 3 than during week 1; during week 2 the values were intermediate.

Digestibility of amino acids

The higher level of enzyme supplements improved the digestibility of amino acids slightly but consistently (fig 1). When analyzed by a paired *t* test, the difference between the control group and the group supplemented with 1 000 μg/kg was significant ($P < 0.05$). The slightly higher digestibility of amino acids observed at the age of 1 week as compared to 2 and 3 weeks agrees with the results obtained for protein digestibility (table V).

DISCUSSION

Age effects

Development of the GIT in the present experiment was consistent with that described in earlier studies, *ie* a decrease in the relative weight of digestive organs from a peak observed at 7–10 d and a gradual increase in the activity of pancreatic enzymes (Nitsan *et al*, 1991a; Nir *et al*, 1993). The reduction of fat digestibility and metabolizability of the diet during week 2 and its subsequent increase is in accord with the reports of Zelenka (1968, 1973) and Murakami *et al* (1988, 1992). The lower digestibility of fat during week 2 has been attributed to a faster rate of passage of the diet as the chicks grow from 1 to 3 weeks of age (Golian and Polin, 1984). Although metabolizability was related to fat digestibility, ME, corrected for nitrogen, reduces the differences within ages (Hill and Anderson, 1958). The nitrogen-correction factor in the AME_n determination veiled the observed age differences in ME.

The apparent digestibility of starch was higher during week 1 than during weeks 2 and 3, whereas the activity of amylase in the pancreas or in the intestinal chyme increased with age. The higher digestibility of starch during week 1 also contrasted with the activity of maltase in the jejunum, which was lower during week 1 than during weeks

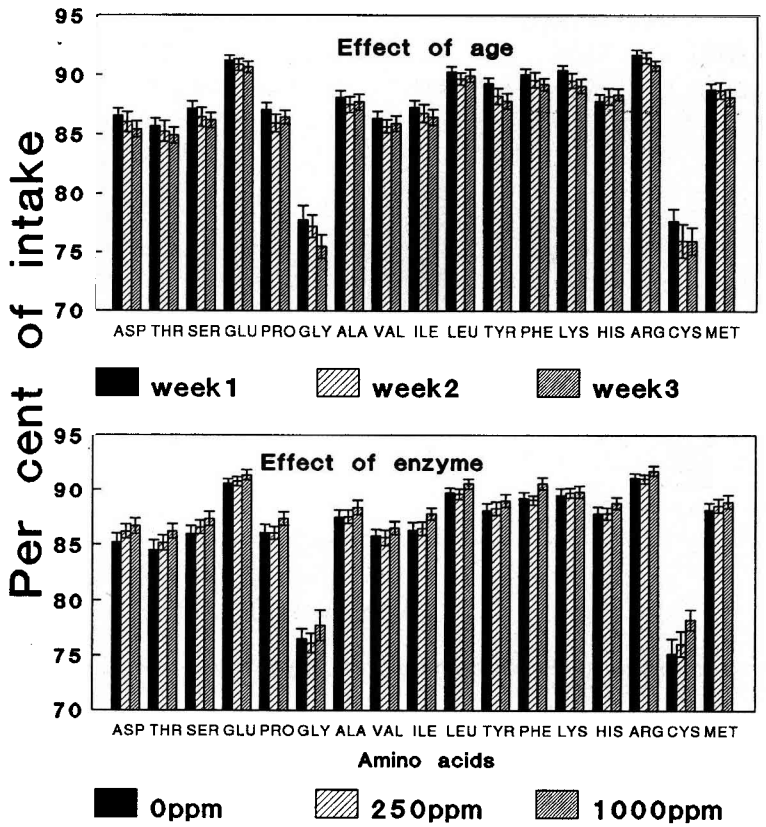

Fig 1. Digestibility of amino acids as affected by age or by enzyme supplementation. Age effects analyzed by paired *t*-test: week 1 > (week 2 = week 3), ($P < 0.01$). Enzyme supplementation effects: (no enzymes = EII250) < EII1000 µg/kg ($P < 0.01$). Vertical bars are the SEM.

2 and 3 (authors' unpublished results). Starch digestibility seems to be a dubious measurement because the disappearance of starch from the excreta may be partly the outcome of bacterial fermentation.

The reduction in digestibility of protein with age is in accordance with Zuprizal *et al* (1992) who reported a 4% decrease in protein digestibility between 3- and 6-week-old meat-type males when whole or dehulled rapeseed meal, or soybean meal were assayed. Fonolla *et al* (1981) also reported a decrease in protein digestibility as meat-type chicks grew older. Carre *et al* (1991) found that the digestibility of pea proteins was significantly higher in young than adult birds. Zelenka and Liska (1986) also reported that age has a considerable effect on the digestibility of individual amino acids, whereby the latter decrease with age. The reduction in protein digestibility with age observed in the present study and in that of Zuprizal *et al* (1992) was much higher than that observed for amino acids. The reason for this discrepancy remains to be determined.

In the present study, negative correlations between feed intake and digestibility of nutrients (protein excepted) during the

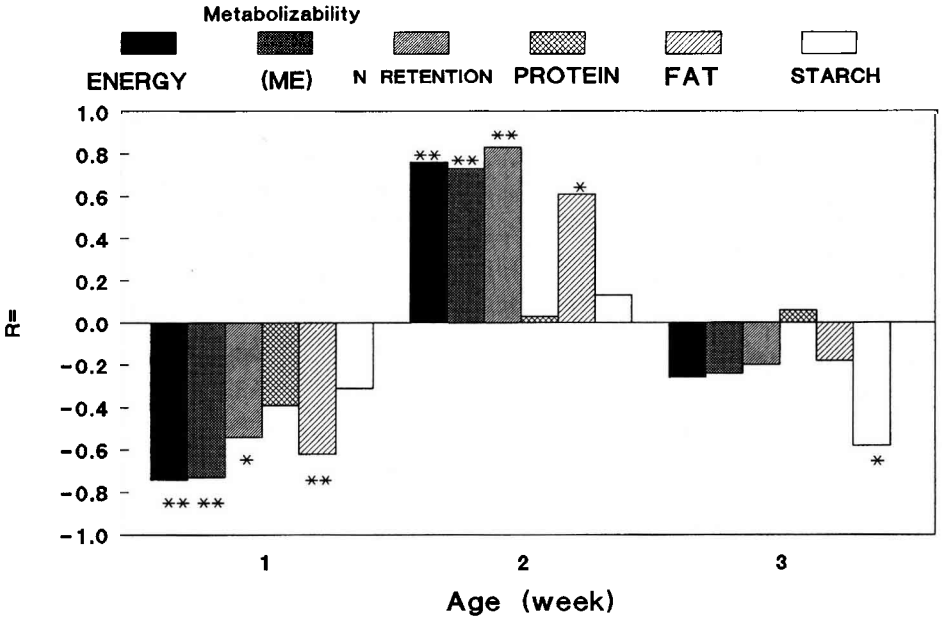

Fig 2. Correlation coefficients (R) between food intake, digestibility of some nutrients or nitrogen retention: *, $P < 0.05$; ** $P < 0.01$.

first week of life (fig 2) emphasize the meat-type chick's drive to consume larger amounts of food than their GIT can handle. During this period the GIT is at a stage of rapid development (Nitsan *et al,* 1991a,b; Nir *et al,* 1993). During the second week the correlations became positive, probably because the relative weight of the GIT segments and the activity of the digestive enzymes peaked faster in gluttonous chicks. During the third week the GIT reached a steady state, and did not limit the digestibility and absorption of nutrients. Thus there were no correlations between food intake and digestibility of nutrients (except for starch).

Supplemented enzyme effects

Meat-type chicks, selected over many generations for rapid early growth, may reach genetic limitations in digestive potential during the first few days after hatching, due to insufficient secretion of digestive enzymes (Nitsan *et al,* 1991a,b; Nir *et al,* 1993) which cannot match their drive to consume excessive amounts of feed. However the present study and earlier ones (Jensen *et al,* 1957; Fry *et al,* 1958; Willingham *et al,* 1959; Burnett, 1966), in which exogenous enzymes were added to the diets, do not support this view. Supplementation with amylases and proteases did not improve performance or digestibility.

Differences in *in vitro* activities of the supplemented enzymes did not correspond to the differences observed *in vivo*. Although the *in vitro* activities of amylase and proteases in the EII preparation were 2.5 and 1.25 times higher, respectively, than those in the EI preparation, the *in vivo* effects of the 2 preparations (tables III and IV) were quite similar. Supplementation of the enzyme

preparations did not affect digestibility, feed intake, growth rate or feed utilization in accordance with a subsequent study in which the same enzymes were added to the diet of stunting-syndrome-infected meat-type chickens (Shapiro *et al,* unpublished results). Lack of effect on performance cannot be attributed to inactivation of the enzymes in the digestive tract, because some significant effects of enzyme supplementation were observed in the GIT. Feed passage through the stomach was probably accelerated, as measured by a reduction in the contents of the gizzard and by an increase in the contents of the small intestine, whereas the weight of the small intestine was decreased. This could also result from a modification in the feeding pattern, an aspect which was not studied in the present work. The pH of the intestine contents was reduced by enzyme supplementation and the production and/or secretion of pancreatic amylase and proteases (trypsin and chymotrypsin) was also reduced. We propose that the secretion of pancreatic enzymes is affected by the concentration of enzymes in the small intestine and/or of substrates or products of hydrolysis (Twomby-Snook and Meyer, 1964a,b). In the present work, the reduction of pancreatic enzymes was most probably due to the presence of exogenous enzymes in the intestine. Supplementation of diets with enzymes should be performed with care, especially when the activity of the supplemented enzymes resembles that of the endogenous pancreatic ones.

ACKNOWLEDGMENT

Funding was provided by the Binational Agricultural Research and Development Project, US 1685 89.

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