Influence of overfeeding on growth, obesity and intestinal tract in young chicks of light and heavy breeds*

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1. Heavy-breed (HB) chicks differed from light-breed (LB) ones in their propensity to be overfed. Whereas in the LB chicks the amount by which they could be overfed reached 70 % more than the food consumed daily by the *ad lib.*-fed chicks, in the HB chicks the maximal excess was only 13 %.

2. Overfeeding caused a slight but statistically significant increase in the linear growth rate (shank length) of the LB chicks, with an opposite effect in the HB chicks.

3. Overfeeding increased the weight of the crop, proventriculus, small intestine, pancreas, liver and adipose tissue but had no such effect on the heart, cerebrum or cerebellum.

4. Overfeeding had no effect on the specific activities of the pancreatic digestive enzymes, liver xanthine dehydrogenase, or tryptophan oxygenase (*EC* 1.13.1.12). The increase in the total activities was due entirely to organ hypertrophy.

5. Obesity induced in young chicks had no residual effects on the adult LB chicks, but reduced the linear growth of the adult HB chicks.

6. An explanation for the difference between breeds in response to overfeeding at an early age is discussed.

The effects of nutrition during early life on the development of the adult have been widely studied in human beings and rats. According to Winick & Noble (1966) and Winick & Rosso (1975), cellular effects of malnutrition depend on the stage of growth of the animal and on the length of the malnutrition period. At an early age, malnutrition impedes irreversibly cell division (mainly in the brain), whereas at a later stage of growth, malnutrition causes only a reversible reduction in cell size. Adult rats that had been overnourished before weaning were similar in many respects to undernourished rats and usually inferior to control rats in behavioural responses (Frankova, 1970).

Overnutrition at an early age has attracted attention as a by-product of the general concern with adult obesity. The fear that the fat baby is on a path to adult obesity (and atherosclerosis and other cardiovascular disorders) is common, in spite of contradictory evidence (Garn, Clark & Guire, 1975).

Overfeeding rats by the tube technique during the preweaning period caused a marked obesity at weaning which was not maintained at adulthood when evaluated by carcass composition (Czajka-Narins & Hirsch, 1974).

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In light-breed (LB) chicks overfeeding at an early age (2 weeks) caused a marked increase in weight due partly to an increase in lean body substance and partly to fat deposition (Nir, Shapira, Nitsan & Dror, 1974). The increase in the mass of the digestive tract and in the activity of enzymes involved in digestion and protein metabolism was found to be parallel to the increase in food and protein consumption (Nitsan, Dror, Nir & Shapira, 1974). It was considered worthwhile to conduct a comparative study in which LB and heavy-breed (HB) chicks were overfed, the objective being to evaluate the limit of food assimilation in the two breeds. Force-feeding was started at 3 d of age in order to observe whether such a practice at this early age would cause obesity at a later age, and whether some other changes caused by overfeeding were maintained after its cessation.

EXPERIMENTAL

Animals and diets

Cross-bred New Hampshire × White Leghorn (LB) and White Rock (HB) male chicks were each wing-banded and numbered. All the chicks had free access to water and food throughout the experimental period. At the age of 3 d, twenty birds of each breed were force-fed and thirty chicks served as *ad lib.*-fed controls. Overfeeding involved force-feeding of food by intubation in addition to voluntary food intake. Force-feeding and management were carried out as described earlier (Nir *et al.* 1974). Force-feeding lasted for 18 d. At the end of the force-feeding period, five chicks from each treatment and breed were selected for autopsy, taking care that the mean body-weights and variances of the resulting sub-groups were close to the corresponding values for the complete groups. All remaining chicks were subsequently fed *ad lib.* up to 59 d (HB) or 66 d of age (LB) to determine whether overfeeding caused permanent changes in food intake, body-weight and shank length. Two other groups of LB chicks (seven chicks/group), one fed *ad lib.* and the other overfed from 14 to 29 d as described by Nir *et al.* (1974), were subsequently fed *ad lib.* up to adult-hood (254 d), when autopsies were carried out for determination of organ weights and other values.

The diet used up to 66 d of age was a commercial mash containing (/kg) 210 g protein, 3 g fat and 11·7 MJ metabolizable energy (ME). From 66 d, a mash containing less protein (170 g/kg) but the same amount of fat and ME was fed. The amino acid requirements and the ME of the diet were calculated according to the (US) National Research Council (1971). Autopsies and plasma preparation for analysis were done as described earlier (Nir *et al.* 1974).

Chemical analyses

Liver and abdominal adipose tissue total lipids were determined on aqueous homogenates (1:10, w/v) by the sulpho-phosphovanillin procedure (Zöllner & Kirsch, 1962). Blood plasma lipids were extracted using chloroform–methanol (2:1, w/v), triglycerides were determined according to Haux & Natelson (1971), phospholipids (phosphorus × 25) according to Ames & Dubin (1960) and cholesterol according to Searcy & Bergquist (1960), DNA and RNA concentrations by the method of Hatcher & Goldstein (1969) and total nitrogen by the micro-Kjeldahl procedure.

Determination of enzyme activities

Liver tryptophan oxygenase (*EC* 1.13.1.12; TO) activity was determined essentially according to Knox, Yip & Reshef (1970); liver xanthine dehydrogenase (XDH) activity according to Strittmatter (1965) as detailed by Nitsan *et al.* (1974); plasma XDH activity was determined essentially as for the liver enzyme: 10 μl plasma was admixed in 1·2 ml reaction mixture.

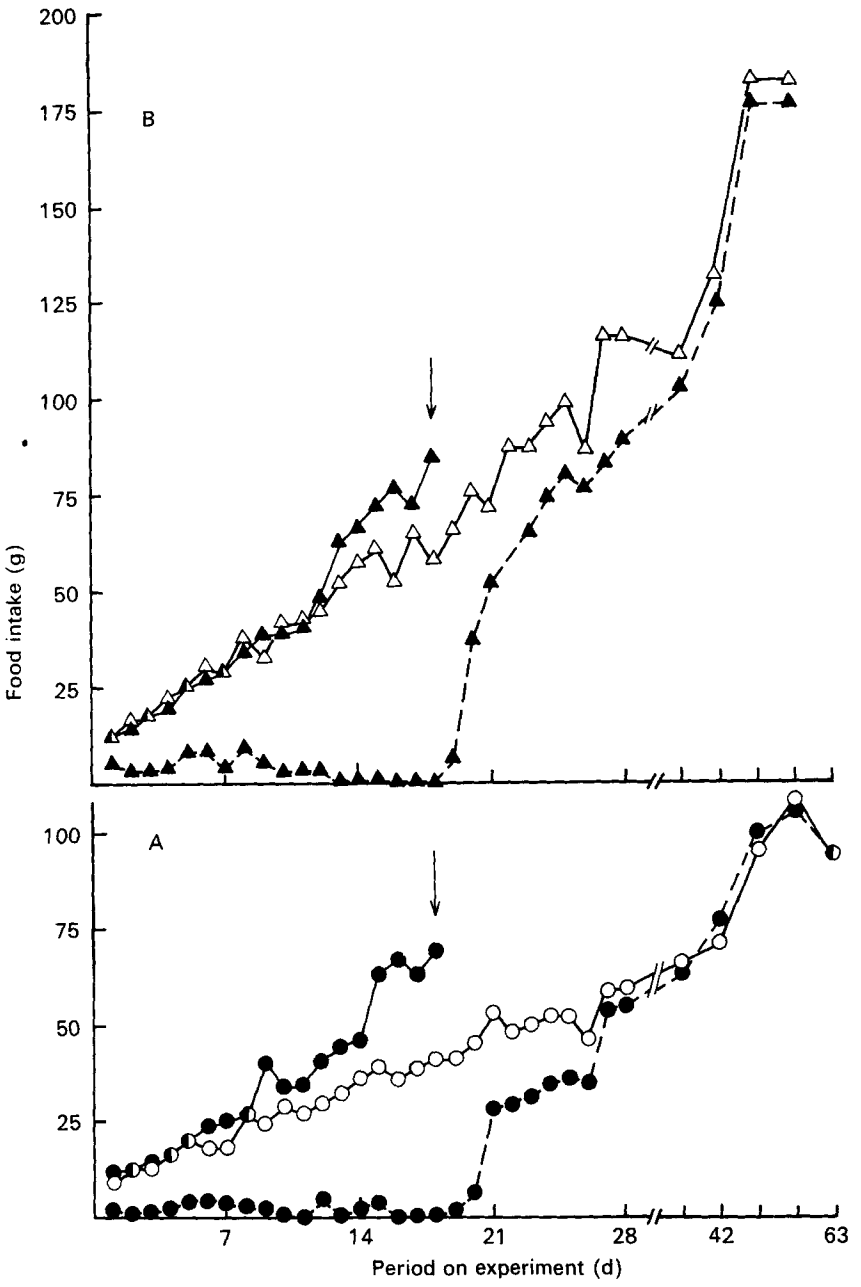

Fig. 1. Food intake of two breeds of chicks subjected to different feeding regimens at an early age and at adulthood. (A) Light breed: overfed (force-fed+voluntary intake) (●—●); voluntary intake of the force-fed chicks (●---●); *ad lib.*-fed controls (○—○). (B) Heavy breed: overfed (▲—▲); voluntary intake of the force-fed chicks (▲---▲); *ad lib.*-fed controls (△—△). The values shown are means for twenty overfed and thirty control chicks per breed up to 18 d and for fifteen overfed and twenty-five controls from 18 d. ↓, end of force-feeding. For details of feeding regimens, see p. 28.

Table 1. *Body-weight† gain and organ weight† (g), and shank length‡ (mm) of light- (LB) and heavy-breed (HB) chicks after* ad lib.*-feeding or overfeeding§ for 18 d*

(Empty weight for the gastrointestinal tract)

	LB			HB		
	Ad lib.-fed	Overfed	SE of mean	*Ad lib.*-fed	Overfed	SE of mean
Food intake (g)	467	668	—	691	799	—
Initial body-wt	46	48	1	53	50	1
Final body-wt	263	339	5 **	435	455	10
Body-wt gain	217	291	5 **	382	405	11
Shank length	52·3	54·3	0·6 *	59·6	56·6	0·6 **
Organ wt:						
Crop	1·06	4·47	0·30 **	1·41	4·87	0·14 **
Proventriculus	2·00	3·15	0·20 **	3·33	4·86	0·62
Gizzard	6·5	7·1	0·3	11·7	13·1	0·5
Duodenum	1·73	3·52	0·57	2·47	3·33	0·55
Small intestine	7·1	11·1	0·8 **	11·5	14·0	0·5 **
Caecum	1·22	1·35	0·13	2·02	1·86	0·17
Pancreas	0·96	1·46	0·06	1·42	1·67	0·09
Liver	7·1	21·7	0·9 **	12·1	22·2	2·1 **
Abdominal adipose tissue	0·86	7·80	0·93 **	2·83	4·95	0·58 *
Heart	2·03	2·10	0·06	2·93	3·23	0·22
Cerebrum	1·41	1·35	0·03	1·56	1·49	0·08
Cerebellum	0·292	0·271	0·010	0·286	0·293	0·012

Statistical significance of the differences between treatments within each breed: * $P < 0.05$, ** $P < 0.01$.
† Mean weight of the chicks at autopsy (five chicks/breed and treatment).
‡ Mean values for thirty chicks in the *ad lib.*-fed groups and twenty chicks in the overfed ones.
§ Force-feeding of food by intubation in addition to voluntary intake.

Amylase (*EC* 3.2.1.1 and 3.2.1.2), trypsin (*EC* 3.4.4.4) and chymotrypsin (*EC* 3.4.4.5) in the pancreas were determined as described by Nitsan *et al.* (1974). *N*-benzoyl-DL-arginine-*p*-nitroanilide hydrogen chloride and *N*-glutaryl-L-phenylalanine-*p*-nitroanilide (Sigma Chemical Corp., St Louis, Missouri, USA) were used as substrates for trypsin and chymotrypsin, respectively. Lipase (*EC* 3.1.1.3) was determined according to Seligman & Nachlas (1963), using 2-naphthyl-laurate (Sigma) as substrate; the samples were incubated for 10 min at 37°.

Activity units were calculated as follows: for liver: TO, μmol kynurenin produced/min; XDH, μmol NAD reduced/min; amylase, extinction at 550 nm ($\times 10^{-2}$)/3 min at 37°. Lipase units were defined as μg ($\times 10^{-2}$) naphthol released/10 min at 37°; trypsin and chymotrypsin, extinction at 410 nm/30 min at 30°.

Statistical analysis was done by the method of Snedecor & Cochran (1967).

RESULTS

Food consumption, body-weight gain and organ weight

In the LB chicks the amount of food that could be overfed increased substantially only after 9 d and gradually reached up to 70% more than the food consumed by the *ad lib.*-fed control chicks (Fig. 1). The total food consumed (force-fed and voluntary intake) by the overfed LB chicks during the experimental period (18 d) was 43% more than the controls (Table 1).

In the HB chicks, the intake of the overfed group exceeded the *ad lib.* intake only after 13 d and thereafter increased gradually to 10–13% more than that of the *ad lib.*-fed chicks.

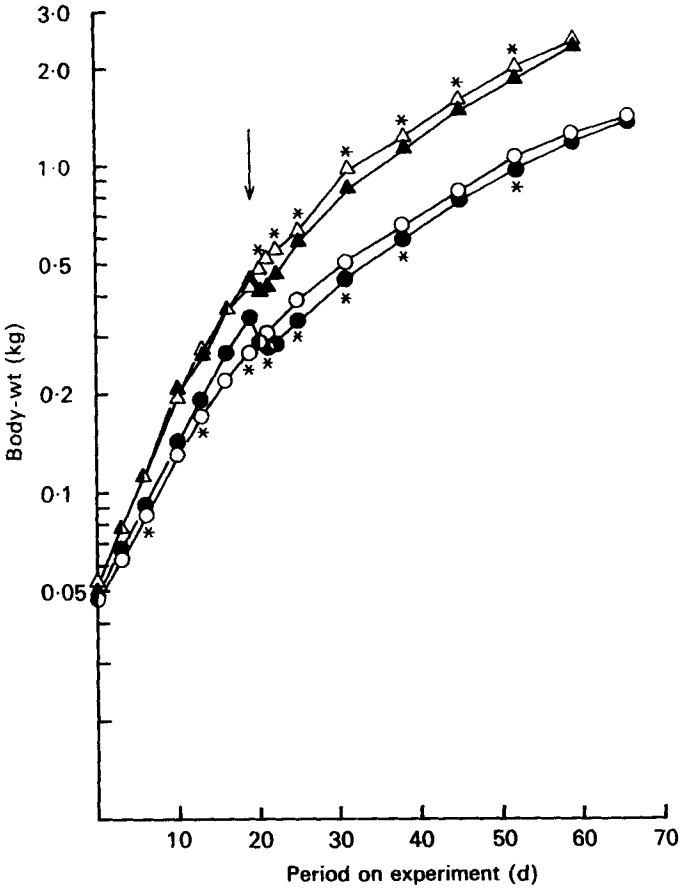

Fig. 2. Body-weights of two breeds of chicks subjected to different feeding regimens at an early age and at adulthood. Light breed: (●), overfed (force-fed+voluntary intake); (⊙), *ad lib.*-fed controls. Heavy breed: (▲) overfed, (△) *ad lib.*-fed controls. ↓, end of force-feeding. * Statistical significance of differences between overfed and corresponding control chicks: $P < 0{\cdot}01$ (except for the light breed on day 37, when $P < 0{\cdot}05$).

The total food consumed by the over-fed HB chicks during the force-feeding period was 11% more than the controls. When the amount overfed exceeded the amount consumed by the *ad lib.*-fed groups, voluntary food intake practically ceased. Overfeeding caused an increase of 30% in the body-weight gain of the LB chicks while no increase was noticed in the HB chicks (Fig. 2 and Table 1). The digestive organs and liver were increased in weight by overfeeding to a greater extent in the LB chicks than in the HB chicks, as shown in Table 1. The weights of the gizzard, caecum, pancreas, heart, cerebrum and cerebellum were not changed by overfeeding in the two breeds. The main difference between breeds was the 9-fold increase in the abdominal adipose tissue of the LB chicks compared with the 1·75-fold increase in HB chicks.

Liver, abdominal adipose tissue and blood plasma lipids concentration

The increase in the total fat deposition in liver and abdominal adipose tissue was 10- and 14-fold compared with 3- and 2-fold in the LB and HB chicks respectively (Table 2). Although the lipid concentration in the liver was similar in the *ad lib.*-fed chicks of the

Table 2. *Lipid concentrations in liver, abdominal adipose tissue and blood plasma of light- (LB) and heavy-breed (HB) chicks after* ad lib.*-feeding or overfeeding† for 18 d*

(Mean values for five chicks/group)

	LB			HB		
	Ad lib.-fed	Overfed	SE of mean	*Ad lib.*-fed	Overfed	SE of mean
Liver lipid:						
g/kg	48	161	17 **	49	91	16
g/liver	0·34	3·55	0·47 **	0·61	2·10	0·45 *
Abdominal adipose tissue lipid:						
g/kg	357	541	14 **	538	643	14 *
g/tissue	0·31	4·28	0·56 **	1·53	3·21	0·40 *
Blood plasma:						
Trigylcerides (g/l)	1·11	6·87	1·90 *	1·35	2·71	0·39 *
Phospholipids (g/l)	1·77	4·69	0·44 **	2·25	3·95	0·42 *
Cholesterol (g/l)	1·19	1·94	0·15 **	1·38	1·60	0·07

Statistical significance of differences between treatments within each breed: * $P < 0.05$, ** $P < 0.01$.
† Force feeding of food by intubation in addition to voluntary intake.

different breeds, the abdominal adipose tissue lipid concentration was much higher in the *ad lib.*-fed HB chicks and similar to the value obtained in the over-fed LB chicks. (A factorial statistical analysis performed between breeds and treatment revealed that the differences were statistically significant ($P < 0.01$).) Plasma lipids were increased by overfeeding, more in the LB chicks (triglycerides 6·2-fold, phospholipids 2·6-fold, cholesterol 1·6-fold) than in the HB (triglycerides 2-fold, phospholipids 1·8-fold, cholesterol 1·2-fold). The lipid concentration in the plasma of the *ad lib.*-fed HB chicks was higher than in the *ad lib.*-fed LB chicks.

Enzyme activities in the pancreas, liver and blood plasma

The total activity of pancreatic lipase was increased to a statistically significant extent in both breeds by force-feeding, whereas amylase and chymotrypsin were significantly increased in the LB chicks only. The increase in the total trypsin activity observed in both breeds was not statistically significant (Table 3).

The total activity of XDH in the liver was increased significantly in both breeds whereas TO activity was increased significantly in the LB chicks only. The specific activities of the enzymes assayed in the pancreas and liver were not affected by overfeeding.

Plasma XDH activity changes caused by overfeeding were not statistically significant in either breed.

Food intake, growth and body organs at the end of overfeeding

The overfed chicks ceased to eat voluntarily during the force-feeding period but gradually started to eat again after the cessation of force-feeding and returned to the food intake of the *ad lib.*-fed chicks, the LB after 11 d and the HB after 13 d (Fig. 1). The reduced food consumption was coincident with a sharp loss in body-weight (Fig. 2), which brought the experimental groups to weights lower than those of the respective control groups. At the end of the experiment (66 and 59 d of age for the LB and HB chicks, respectively), the body-weight of the overfed groups reached the weight of their *ad lib.*-fed controls (Fig. 2). This was also confirmed by comparing two groups of seven cockerels, one group fed *ad lib.* throughout and one group force-fed between 14 and 29 d of age and then fed *ad lib.* up to 254 d. In the LB groups the shank of the overfed chicks, which was longer at the end of the overfeeding period (Table 1), was the same length (104 mm) as that of the control chicks at 66 d of age. In the HB chicks, the reduction in the shank length caused by over-

Table 3. *Enzyme activities in the pancreas, liver, and blood plasma of light- (LB) and heavy-breed (HB) chicks after* ad lib.*-feeding or overfeeding† for* 18 *d*

(Mean values for five chicks/group)

	LB			HB		
	Ad lib.-fed	Overfed	SE of mean	*Ad lib.*-fed	Overfed	SE of mean
Pancreas (units‡/pancreas)						
Lipase (*EC* 3.1.1.3)	40	80	4 **	70	94	7 *
Amylase (*EC* 3.2.1.1)	63	95	8 *	115	126	9
Trypsin (*EC* 3.4.4.4)	36	51	6	29	45	5
Chymotrypsin (*EC* 3.4.4.5)	25	61	9 *	61	89	11
Liver (units‡/liver)						
Xanthine dehydrogenase (XDH)	10·6	26·6	2·0 **	16·5	31·2	3·9 *
Tryptophan oxygenase (*EC* 1.13.1.12; TO)	5·0	17·0	2·6 *	9·2	24·2	9·8
Plasma (units‡/ml)						
Xanthine dehydrogenase (XDH)	4·26	5·04	1·37	4·73	2·21	1·62

Statistical significance of difference between treatments within each breed: * $P < 0.05$, ** $P < 0.01$.

† Force-feeding of food by intubation in addition to voluntary intake.

‡ Lipase, μg ($\times 10^{-2}$) naphthol released/10 min at 37°; amylase extinction at 550 nm ($\times 10^{-2}$)/3 min at 37°; trypsin and chymotrypsin, extinction at 410 nm/30 min at 37°; xanthine dehydrogenase, μmol NAD reduced/min at 30°; tryptophan oxygenase; μmol kynurenin produced/min at 30°.

feeding was not overcome; it remained shorter to a statistically significant extent at 59 d of age (117 mm *v.* 124 mm; $P < 0.01$).

Autopsies were carried out 42 or 211 d after the cessation of overfeeding in LB chicks only. No differences were found between the overfed groups and the controls in the weights of gastrointestinal organs, liver, abdominal adipose tissue, heart, kidneys and testis.

The following measurements, which were made also in the 254-d-old cocks, were similar for the experimental and control groups (mean ± SE of both groups): body-weight (g) 2850 ± 30; shank length (mm) 113 ± 2; comb weight (g) 71 ± 6; brain (g) 3·65 ± 0·06; cerebrum (g) 1·94 ± 0·64; cerebellum (g) 0·482 ± 0·007; cerebellum RNA (mg/g) 2·73 ± 0·10; cerebellum DNA (mg/g) 3·20 ± 0·13; cerebellum N (mg/g) 25·9 ± 0·6; liver XDH (units/g) 1·34 ± 0·15.

DISCUSSION

The interpretation of the different responses to overfeeding obtained in baby LB and HB chicks is complicated by the multiple factors that could be involved. However, three consistent differences between these breeds could contribute to the susceptibility of the LB chicks and to the resistance of the HB chicks to overfeeding at an early age.

First the breeds differ in the physical capacity and the enzymatic response of the intestinal tract. The weights (g/kg body-weight) of the duodenum and jejunum were found to be approximately 10% greater in the LB chicks than in the HB chicks fed *ad lib.* between hatching and 21 d of age (Dror, Nir & Nitsan, 1977). It was reported that the anterior part of the intestinal tract is the main absorptive site in chicks (Hurwitz, Shamir & Bar, 1972; Hurwitz, Bar, Katz, Sklan & Budowski, 1973). In the present work, the relative weight of the duodenum was also greater in the LB chicks than in the HB chicks (6·6 *v.* 5·7 g/kg body-weight respectively). Moreover, during the overfeeding period, the relative weight of the duodenum increased by 60% in the LB chicks and only by 30% in the HB chicks. The response of the pancreatic digestive enzymes to force-feeding was more pro-

nounced in the LB chicks than in the HB chicks (Table 3). This difference could be associated with the relatively higher amount of food consumed by the LB chicks and it could be also a limiting factor in the HB chicks, affecting their ability to digest excessive amounts of food.

It seems that after early development the gut and the digestive enzymes adjust readily to overfeeding. Excessive amounts of food were shown to be digested normally in geese (Nir, Nitsan & Vax, 1973) and humans (Strong, Shirling & Passmore, 1967); moreover, increases in the mass of the gastrointestinal tract and the amount of the digestive enzymes in the pancreas and intestinal contents of chicks were parallel to the excess of food supplied (Tables 1 and 3; also Nir *et al.* 1974). The same is true for liver XDH and TO, which are highly dependent on the nutritional regimen (Mukhtar, Sahib & Murti, 1973) and increased parallel to the excess of the protein consumed.

Secondly, the fat concentration in the adipose tissue in the *ad lib.*-fed HB chicks was much higher than in the *ad lib.*-fed LB chicks (Table 2). In the LB chicks, overfeeding brought the adipose tissue fat concentration to that of the *ad lib.*-fed HB chicks. A limiting factor to overfeeding could be the ability of the adipose tissue to incorporate the surplus fatty acids and glucose resulting from the excessive food absorbed, as proposed by Lepkovsky (1973). When fat accumulates in excess of a certain limit, the adipocyte is probably resistant to further incorporation; the reduced incorporation could contribute to a reduced absorption of food from the intestine and eventually to a slower evacuation of the 'crop content'. The residual 'crop content' between meals may be the last link in a causative chain which determines the disposition of the chick to be overfed.

It has been suggested that the adipose tissue may be involved not only in food intake regulation (Lepkovsky, 1973) but also in the disposition of the animal to digest and assimilate excessive amounts of food, i.e. to be overfed.

Thirdly the breeds differ in the response of NADP-malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40; MD) to force feeding. It was found that in LB chicks the hepatic and abdominal adipose tissue lipogenic enzyme activities (acetyl-CoA carboxylase (*EC* 6.4.1.2), fatty acid synthetase, ATP citrate lyase (*EC* 4.1.3.8) and MD) increased during overfeeding; in overfed HB chicks MD failed to respond (Shapira, Nir & Budowski, 1977). MD is a lipogenic enzyme involved in the generation of NADPH; it could be, therefore, a limiting factor to the capacity of the HB chicks to divert excess substrate, resulting from overfeeding, to fat synthesis.

The LB chicks could be overfed substantially earlier than the HB chicks, presumably because the LB chick was not selected for maximal growth and food intake, and therefore, its *ad lib.* food intake did not approach its assimilation potential. In this breed, overfeeding had a positive effect on the linear growth (shank length). The attempt to surpass the genetic potential of the HB chicks was probably a strong stress which caused a reduction in the linear growth (Table 1). In the LB chicks, after cessation of overfeeding, the linear growth increment returned to that of the *ad lib.*-fed chicks. This can be explained by the transient reduction in food consumption, which was equivalent to the excess consumed during the overfeeding period (Fig. 1). Force-feeding chicks on a protein and mineral concentrate after an overfeeding period would be of help in deciding whether the reduced protein intake is mandatory in the restoration of 'normal' size or whether other regulatory mechanisms are concerned.

The residual effects caused by overfeeding were more dependent on the breed than on the extent of obesity at an early age. The overfed LB chicks, in which obesity exceeded that obtained in the *ad lib.*-fed HB chicks, easily withstood overfeeding, and the organ weights and shank length returned to those of the controls after its cessation. The results obtained by overfeeding the LB chicks are in accordance with our previous work with

chicks (Nir *et al.* 1974), and with postnatal rats (Czajka-Narins & Hirsch, 1974). In both studies the marked obesity was accompanied by an increased body protein and ash mass and had no residual effects on the body-weight or carcass composition at adulthood. Increase in lean body mass by overfeeding at an early age has also been reported for humans. Residual effects of early obesity on the fat-free weight of humans at adulthood is mostly affected by the socio-economic level (Garn *et al.* 1975) and heredity (Shukla, Forsyth, Anderson & Marvah, 1972). Overfeeding did not alter the central nervous system of chicks, as judged by the weight of the cerebrum and cerebellum in young and adult chicks and by DNA and RNA concentrations at adulthood. It could be that the physiological age of the chicks challenged by overfeeding was higher than that of the rats, in which permanent cellular effects were obtained in the brain by different nutritional status at the early postnatal period (Winick & Noble, 1966, 1967).

REFERENCES

Ames, B. N. & Dubin, D. T. (1960). *J. biol. Chem.* **235**, 769.
Czajka-Narins, D. M. & Hirsch, J. (1974). *Biol. Neonate* **25**, 176.
Dror, Y., Nir, I. & Nitsan, Z. (1977). *Br. Poult. Sci.* **18**, 493.
Frankova, S. (1970). *Nutr. Metab.* **12**, 228.
Garn, S. M., Clark, D. C. & Guire, K. E. (1975). In *Childhood Obesity*, p. 23 [M. Winick, editor]. London: Wiley-Interscience Publication.
Hatcher, D. W. & Goldstein, G. (1969). *Analyt. Biochem.* **31**, 42.
Haux, P. & Natelson, S. (1971). *Microchem. J.* **16**, 68.
Hurwitz, S., Bar, A., Katz, M., Sklan, D. & Budowski, P. (1973). *J. Nutr.* **103**, 543.
Hurwitz, S., Shamir, N. & Bar, A. (1972). *Am. J. clin. Nutr.* **25**, 311.
Knox, W. E., Yip, A. & Reshef, L. (1970). *Meth. Enzym.* **17A**, 415.
Lepkovsky, S. (1973). *Am. J. clin. Nutr.* **26**, 271.
Mukhtar, H., Sahib, M. K. & Murti, C. R. K. (1973). *Biochem. J.* **135**, 225.
National Research Council (1971). *Nutrient Requirements of Poultry*, 6th ed. Washington, DC: US National Academy of Sciences.
Nir, I., Nitsan, Z. & Vax, A. (1973). *Annls Biol. anim. Biochim. Biophys.* **13**, 465.
Nir, I., Shapira, N., Nitsan, Z. & Dror, Y. (1974). *Br. J. Nutr.* **32**, 229.
Nitsan, Z., Dror, Y., Nir, I. & Shapira, N. (1974). *Br. J. Nutr.* **32**, 241.
Searcy, R. L. & Bergquist, L. M. (1960). *Clinica. chim. Acta* **5**, 192.
Seligman, A. M. & Nachlas, M. M. (1963). In *Methods of Enzymatic Analysis*, p. 776 [H. U. Begneyer, editor]. New York: Academic Press.
Shapira, N., Nir, I. & Budowsky, P. (1977). *Br. J. Nutr.* **39**, 151.
Shukla, A., Forsyth, H. A., Anderson, M. & Marvah, S. (1972). *Br. med. J.* iv, 507.
Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*, 6th ed. Ames, Iowa: Iowa State University Press.
Strittmatter, C. F. (1965). *J. biol. Chem.* **240**, 2557.
Strong, J. A., Shirling, D. & Passmore, R. (1967). *Br. J. Nutr.* **21**, 909.
Winick, M. & Noble, A. (1966). *J. Nutr.* **89**, 300.
Winick, M. & Noble, A. (1967). *J. Nutr.* **91**, 179.
Winick, M. & Rosso, P. (1975). In *Protein-Calorie Malnutrition*, p. 93 [R. E. Olson, editor]. New York: Academic Press.
Zöllner, N. & Kirsch, K. (1962). *Z. ges. exp. Med.* **135**, 545.

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