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Specific Biochemical Test for Detecting Fatty Liver in Farm Animals

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Specific Biochemical Test for Detecting Fatty Liver in Farm Animals.

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SPECIFIC BIOCHEMICAL TEST FOR DETECTING FATTY LIVER IN FARM ANIMALS.

ABSTRACT

Studying fatty liver in farm and lab. animals revealed various biochemical changes in the liver. Fatty liver was studied in 5 models and was induced as following: geese - force feeding; hens (laying and non-laying) - estradiol injection and adding KI in the diet; broilers - biotin deficient diet; rats - sucrose rich containing orotic acid diet; cows - metabolic disturbances.

Biochemical changes, although not similar in all models were seen in the blood and livers of the fatty liver induced animals. The following enzymes were studied in the liver and serum: malic enzyme (ME), NADP dependent isocitric dehydrogenase (ICDH), glucose-6-phosphate dehydrogenase (G6PDH), 6 phosphogluconate dehydrogenase (6PGDH), lactic dehydrogenase (LDH), malic dehydrogenase (MDH), glutamic-dehydrogenase (GLDH), alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), gamma glutamyl transferase, and aldolase (ALD). In addition, albumine, total protein, and total lipids were measured in the serum. In most cases lipogenic enzymes generating NADPH were elevated in the fatty livers while SDH decreased. ME, MDH, LDH and ICDH were found to be a good biochemical marker for fatty liver in cows. In the case of geese and to some extent hens, it was possible to predict the degree of liver fattening, and the possibility of using these biochemical parameters as genetic markers is tried.

There were differences seen between gray and white geese in the degree of changes in the biochemical parameters which corresponded to the degree of liver fattening.

In the fatty liver of the rat, greater biochemical changes were seen in the non-fatty part in comparison to the fatty part.

The main lipid fraction accumulated in the liver in the process of liver fattening is triglycerides while in the serum the lipid fractions elevated were triglycerides, cholesterol, and free fatty acids.

INTRODUCTION

Steatosis is a common and nonspecific response of the liver to different forms of acquired injury or inherited metabolic derangement. The clinical impact of fatty liver is variable. It can be acute and fulminating or insidious and benign. Inasmuch as its presence is best detected by histological examination, the differential diagnosis may be aided to some extent by noting the distribution of fat in the hepatic lobule and the location of the nucleus in the hepatocyte. (hoyumpa et al. 1976).

Knowledge of the biochemical lesion(s) involved was fragmentary or nonexistent and consequently no clinical biochemical test was available. There was a need to further investigate and identify a specific process which is impaired or modified and which will provide a firm basis for specifically identifying this disease.

The liver has many complex functions, including formation of bile, ketone bodies, plasma proteins and urea, storage of carbohydrates, control of carbohydrate metabolism detoxification of many drugs and toxins, inactivation of polypeptides and metabolism of fats.

Because of the wide diversity of function and tremendous regenerative and reserve capacity of the liver, detection of specific hepatic diseases is difficult before there is a serious impairment of hepatic function.

The onset of hepatic disease can be insidious and the signs vague. Some of the signs frequently seen with hepatic disease (namely anorexia, emesis, diarrhea, depression, jaundice, ascites, anemia and hemorrhagic tendencies) may also occur as a result of disease processes in other body organs.

The functional status of the liver can be evaluated by the application of laboratory tests. Since the clinical manifestations of diseases of these organs are often not characteristic, it was required to apply certain laboratory tests

to detect the presence of the disease. Tests utilized for measuring the functional capacity of the liver are ultimately dependent on the total of many integrated enzymatic activities that take place within hepatic cells.

Estimation of the presence or absence of hepatic malfunction is further complicated by the tremendous functional reserve of the liver and its powers of regeneration.

Laboratory tests in hepatic disease are helpful in many different ways:

(1) They are of value in detecting hepatic abnormalities (2) these tests may suggest the underlying lesions behind the hepatobiliary dysfunction (3) the tests may provide an index of the extent to hepatic damage and hence a way of giving prognosis on the condition, and (4) these tests serve as a means of following the course of hepatic diseases.

Many tests have been proposed to study hepatic disease, however, no single laboratory procedure fulfills all the objectives just outlined. Therefore, a carefully selected combination of liver function tests and further investigation for new tests are important.

Laboratory findings in fatty metamorphosis of the liver are: blood glucose level is reduced, lower glycogen storage, prolonged prothrombine time, increased total cholesterol, decreased bromosulph^{-phthalein} clearance rate, increased thymol turbidity and lower albumine level. Serum bilirubin and alkaline phosphatase are sometimes increased and urobilin in the urine may be increased (Goodale and Widmann, 1970).

The liver profile includes tests based on bile pigments metabolism, tests based on evaluation of enzyme activity and of synthesis in the liver. The use of clinical laboratory tests in the liver profile may permit to gain insight into the type of liver problem that exists. However, information obtained requires several

tests and in most cases it is not conclusive because of the fact that many of the clinical tests are general and are not liver specific.

A lack of specific tests for liver diseases is standing in the way of early diagnosis and consequently treatment of the disease.

The liver plays a major role in fat metabolism as well as in carbohydrate and protein metabolism. It removes much of the free fatty acids released by adipose tissue and converts them into triglycerides which then are secreted into the circulation as lipoproteins. The synthesis of the moiety of the lipoproteins occurs only in the liver and is a rate limiting step for the hepatic release of lipoproteins. Thus there is an active shuttle of fatty substances between liver and adipose tissue. Except for ruminants, the liver also actively participates in liponeogenesis from amino acids and glucose.

Fat normally constitutes about 5 per cent of the liver weight, but in many instances it can rise to 30 per cent or more. Excessive fat in the liver is sometimes called a fatty infiltration or fatty degeneration, but this accumulation is not a disease per se, but rather a symptom of deranged fat metabolism. One may think of the problem in the general statement that fatty liver is to fat metabolism, what hyperglycemia is to carbohydrate metabolism. It may be due to an overproduction or an underutilization, that is, an increased transport of fat to the liver.

Fatty liver diseases occur widely in both man and animals. Fatty liver may be caused by any one of the following: (1) high fat or high cholesterol diet; (2) increased liponeogenesis by the liver from excessive carbohydrate intake or excessive administration of certain vitamins such as biotin and thiamine; (3) increased mobilization of fat from adipose tissue due to stress, starvation, insulin deficiency, hypoglycemia, hyperactivity of sympathoadrenal system or increased output of glucagon, STH, ACTH, and adrenal steroids; (4) liver damage due to

hepatitis, cirrhosis, necrosis from Vitamin E deficiency, or liver poisoning such as carbontetrachloride, phosphorus and chloroform and (5) deficient transport of fat from the liver due to excessive glucagon or to specific nutritional deficiencies such as lack of choline, inositol, protein, essential fatty acids, pyridoxine and pantothenic acid.

The rate of transport of fat away from the liver depends upon the ability of the liver to synthesize phospholipids and also the protein moiety of the lipoproteins. Without this ability, fat cannot be released into the circulation.

Exposure to toxic chemicals or infections, or even deficiencies which cause fatty livers, eventually can lead to excessive production of fibrous tissue in the liver, shrunked and distorted liver orange-brown in color. It is commonly seen in prolonged protein deficiency and begins after the central lobular cells have been ruptured by excessive fat. The ruptured cells have atrophied and left scars formed from their remnants. The scars may also spread and even include areas around the large portal veins. A reduction in portal blood flow and portal hypertension can occur.

The aetiology and pathogenesis of the fatty liver syndrome in the fowl has recently been reviewed (Butler, 1976; Brugere-Picoux and Brugere, 1974). Most of the studies were done with young chicks and laying hens. Fatty liver in chickens is well defined as two diseases known as fatty liver and kidney syndrome (FLKS) and fatty liver haemorrhagic syndrome (FLHS). The description of FLKS, biochemical studies concerning lipid and carbohydrates metabolism and the involvement of diets and other factors in the disease have been reviewed by Whitehead et al (1974a, 1974b, 1975a, 1975b) and Whitehead and Blair (1976), Evans et al (1975) and Bannister et al (1975). FLHS was also well described by Couch (1968) and by Wolford and Folin (1972, 1973, 1974).

Less information was available on fatty liver in geese; contrary to the case in chickens, fatty liver is intentional in these birds, where they are crammed by force feeding a rich diet. Research on geese steatosis has been mostly directed to the economical and nutritional needs and there are only a few studies of the biochemistry of the fattened birds (Labie and Tournut, 1970; LeClercq et al, 1968; Ivorec-Szylit and Szylit, 1968).

Leclercq et al (1968) studied the composition of the accumulated lipids in the liver during cramming. They reported that cramming causes intense metabolic activity with a large increase in total amount of protein, RNA and phospholipids in liver. Ivorec-Szylit and Szylit (1969), analyzed the activity of LDH in the liver and plasma during steatosis caused by cramming. They showed an increase of LDH activity in liver followed by an increase of the enzyme level in the plasma before the appearance of any histological change. Labie and Tournut (1970) reported an increase in lipemia, glycemia and in proteinemia. However, while lipemia continued to increase during the whole period of cramming and the level of blood sugar was kept at a high plateau level, serum protein fell after the second week of cramming and serum transaminases increased.

Blum et al (1970) studied blood composition in the crammed goose. They found that the degree of steatosis had no effect on serum sodium and chlorine levels, but drastic changes occurred in the serum level of metabolites which are essentially produced and/or regulated by the liver. Serum albumin level was closely correlated to liver size and to the degree of icterus as evaluated by the serum bilirubin level.

The plasma of the crammed goose becomes highly lipemic and globulins are increased (Labie and Tournut, 1970; Nir and Perek, 1971, 1972). Plasma protein fractions as determined by electrophoresis were differently affected during the cramming period. Beta globulines increased, gamma globulines decreased and alpha

globulines showed a transient decrease, followed by an increase (Nir and Perek, 1971, 1972). The decrease in the albumin fraction was probably a result of hepatic failure (Cornelius, 1970).

Nir (1972) reported a negative correlation between the lipid fractions and total plasma protein, β -globulins and albumin and attributed it to the insufficient lipoprotein formation. They also reported on differences in glucose level between individual geese and attributed it to the impairment in the glycemic regulation of the liver.

Compared to poultry, information available on fatty liver in mammals was limited and could be found mainly on rats, men, and to a lesser degree ruminants and dogs.

Because of the interplay of the various pathologic mechanisms by which excess fat is accumulated in the liver, it was hard to make a precise statement on the mechanism which operates in any given case. It was, however, possible to group the pathologic mechanisms into: 1. those which involve an imbalance of nutritional or metabolic factors such as starvation, especially in ruminants, and diet low in protein or high in fat or carbohydrates, also from endogenous imbalances especially those involving hormones; 2. those with toxic bases - known to cause fatty degeneration of the liver such as bacterial toxins, phytotoxins, chemical poisons and others; 3. those which are a result of anoxia.

Pathological degree of fatty change in the liver is commonly observed in animals. It is frequently occurring in sucklings and quite severe fatty change is very common in late pregnancy in cows. The most severe degrees of lipidosis occur in metabolic diseases, diabetes mellitus of dogs and cats, ketosis of cows and pregnancy toxemia of ewes and in poisoning by phosphorus and plants of the genus lupinus.

Studying fatty liver in cattle, Bogin and Sommer (1978) showed that fatty liver contained higher levels of LDH, glutamyl transferase (GT) and ALP (more than 200 per cent) while the activity of transaminases were lower. No change was seen in aldolase.

Reid et al (1977) described biochemical and serological fat composition in fasting induced fatty liver in non-lactating cows. They showed that the content of total lipids in liver doubled with 8 fold increase in triglycerides.

Rats have been used extensively for studying various aspects of fatty liver. While in birds the common procedure for introducing the syndrome was biotin deficiency diet, in rats the common procedure used was by the administration of orotic acid or alloxan or the administration of the hormone prolactine (Ozegovic and Milkovic, 1975). Most studies dealt with lipid content of the liver, preventive or reversal treatments, and some biochemical studies concerning some liver lipogenic enzymes. The data available however do not clearly describe the syndrome, and the various related aspects, especially clinical biochemistry aspects of the disease.

Liver enzymes related to lipid metabolism - In avian and mammalian species, fatty acids are synthesized in the cytoplasm (adipose tissue, liver, mammary gland, heart, lung and brain) from acetyl-CoA. Acetyl CoA is generated in the mitochondria but cannot cross the inner mitochondrial membrane. Citric acid, the initial intermediate in the citric acid cycle carries acetyl groups across the membrane. In the cytoplasm, citric acid is cleaved by citrate lyase, the enzyme that catalyses the reaction of citric acid with ATP and CoA to produce acetyl CoA, oxalacetic acid, ATP and Pi. The complexed enzyme system also requires reducing compound NADPH, which is derived from pentose phosphate pathway and from the cytoplasmic oxidation of malate to pyruvate (Scott Allen, R. 1976).

In modern medicine, clinical enzymology plays an important role in the diagnosis, prognosis and evaluation of different diseases and metabolic disorders (Wacker and Coombs, 1969; Goodale and Widman, 1970; Freeland and Kremer, 1970; Hoffman, 1966) and the determination of enzyme activity has been an established procedure. The enzymes used are mainly organ-specific cellular enzymes which leak from the cells to the blood.

The correlation between the degree of damage and the extent of the increase in enzyme activity in the serum is well established (Goodale and Widman, 1970; Hoffman, 1966; and Wacker and Coombs, 1969). In acute tissue damage, the enzyme profile of the tissue damaged and of the serum resemble each other. This however is not necessarily so in chronic tissue diseases such as liver cirrhosis, since levels of enzymes and metabolites may be changed quantitatively and qualitatively within the cells during the process of liver damage.

Since the blood enzyme profile is so greatly affected by the enzyme makeup of the tissue, it is important to know the enzyme profile of the pathological tissue. Furthermore, the blood enzyme profile could change greatly by a change in the enzyme content of the tissue, even if there is no excessive damage to the cells.

Work done in our laboratory (Bogin, 1978) clearly showed changes in some of the liver and serum enzymes in fatty liver in cows and geese. The data strongly suggest that some of these changes could serve as good blood parameters which could indicate the existence of fatty liver.

This research project proposed to study this problem and establish methods for early detection of the disease, and a tool to evaluate the degree of the liver fattening.

Materials and Methods

The research was done in 4 stages:

1. Establish the biochemical changes in blood at the/ or close to the peak of fatty liver development.
2. Kinetic studies with parameters showing the most significant changes by following the levels of these parameters at various times during liver fattening.
3. Study the changes in the liver.
4. Testing the results with cases from the field.

Animals - The following animals were studied.

1. Rats - weighing 250-350 grams were used in both the control and experimental groups. Fatty liver was induced according to Novikoff (1974) in two steps.

1) sucrose rich diet (consisting of 69.8% sucrose, 20% vitamin free casein, 4% corn oil, 4% salt mixture and 2.2% vitamin mixture) given to the rats for 2-3 weeks and 2) the same diet containing 1% orotic acid, for 3-5 weeks. The controls received a commercial chow diet.

Blood samples were taken from the tail vein at 0 time before the experiment started and, at the change between diet 1 and 2 and sacrifice. Liver samples were taken at sacrifice, for enzymatic analysis and for proteins and lipid composition.

Fatty liver in laying hens - The experiment was conducted with two groups.

1. Mature hens at their laying peak. The birds were about 12 months old, weighing 1.50 ± 0.15 kg. Fatty liver was induced by intramuscularly injecting estradiol-propionate (8.0 mg/kg body weight) and adding KI (3.25 gr/5.0 kg) into the feed. The birds were fed with KI containing diet for 10 days prior to the injection of the hormone. Three days after the injection, the birds were slaughtered, and the blood and liver were taken for the biochemical analysis.

II. Immature young non-laying hens. The birds were 7 weeks old, weighing about 300 gr. Fatty liver was induced by injecting 1 ml estradiol-propionate (8.0 mg/kg body weight) and a second injection 46 hours later. 26 hours after that, the birds were slaughtered and the blood and liver were collected for the biochemical analysis.

The separation of serum, homogenization of the liver and preparation of the enzymes for analysis and measurements of enzymes, proteins and metabolites were done as described in the methods.

Fatty liver in broilers

Chickens Two hundred, one day old broiler chicks type "Anak" (Poultry Breeding Union, Israel) were used. The chicks were divided into two groups of 100 each. During the entire experiment the poults in both groups were kept under similar conditions, except for feed composition. The experimental group received a biotin-deficient feed. The birds were kept in brooder hovers for the first 4 weeks and then transferred to cages until the end of the experiment. At 28, 40, 57 and 75 days, 10-15 poults from each group were taken at random, weighed and sacrificed. The blood was collected, and the entire liver was removed and weighed. The liver was kept in the cold (0-4°C) for homogenization and enzyme extraction.

Feed. The experimental, biotin deficient group, received a diet according to Whitehead et al (1974) from day one for the entire experimental period, while the control group received a similar diet containing biotin.

Fatty liver in geese - Four different experiments were conducted;

1) on a commercial farm which specialized in geese cramming. Blood samples were taken from the wing vein at 0, 12, 18, 25, 33 of cramming and at slaughter. Liver samples were taken for enzymes assay and lipid composition.

2) genetic differences between families. Blood and liver samples were taken from gray and white geese, kept under similar nutritional and cramming regimes. The experiment was conducted in the "ACO" poultry experimental station. Gray geese which produced larger livers, were compared to white ones. Blood samples were taken at 1, 13, 27, 33-34 and slaughter and the various blood parameters were analyzed. The liver was weighed and evaluated by commercial referees.

3) Prediction of liver size. Blood samples were taken from the wing vein at 25 and 33 days of cramming and the liver sizes were predicted

based on data obtained from previous experiments.

4) Correlation of blood constituents and liver size at slaughter. Blood samples were taken in the slaughter house for biochemical analysis and correlations to liver size were done.

fatty liver in cows- blood and liver samples from cows, were taken in the slaughter house. Immediately after slaughter, the cows were checked by the veterinarian for fatty liver. Samples of normal and fatty livers were taken for biochemical analysis. Blood (or serum) were taken from the heart for the enzymes determinations and chemical composition.

The control group consisted of cows showing no fatty liver or any other pathological signs^{of the liver}. The liver samples were divided into 3 groups according to the degree of fattening 1) control with normal livers, 2) light to moderate fatty liver and 3) livers with high fatty infiltration. Enzyme levels in the livers were assayed and a correlation between the enzymes and degree of liver fattening was done.

Blood taken was allowed to clot and serum was obtained. The levels of the enzymes: lactic dehydrogenase (LDH), malic dehydrogenase (MDH), isocitric dehydrogenase NADP specific (ICDH), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), alkaline phosphatase (ALP), aspartate aminotransferase (AST), glutamic dehydrogenase (GLDH), sorbitol dehydrogenase (SDH) and glutamyl transferase (GGT). In addition the following were also measured: total lipids, total proteins, and albumin.

Liver tissue homogenization and enzyme preparation

Liver samples were put on ice immediately after sacrifice. The tissue was homogenized in 20 volumes of Tris-HCl buffer (0.02 M, pH 7.4), according to Bogin et al (1978). The homogenate was centrifuged at 100000 g for 60 minutes and the supernatant obtained was used for enzyme assays. The following enzymes were assayed: AST, ICDH, LDH, MDH, G6PDH, 6-phosphogluconate dehydrogenase (6PGDH), ME, GLDH, ALP, SDH, GGT and ALT.

Protein determination was done according to the Lowry method (1951).

Total lipid was determined with the phosphovanilin method according to Frings et al (1972).

Lipid extraction and chromatography. Liver tissue was extracted according to Folch et al (1957). Serum was extracted according to Chedid et al (1972), and the lipids were separated and identified by thin layer chromatography according to Hojnacki and Smith (1974).

Statistics. Data analysis was done according to SAS User's Guide (1979). Significance (p) was analyzed according to t test methods.

Effect of animals transportation on enzymes level. Blood was taken from geese on the farm prior to their transportation to the slaughter house. The geese were marked and shipped to the slaughter house, 20 km away. Blood samples were taken again from the same animal at slaughter.

Liver damage caused by common bile duct ligation (CBDL) and CCl₄ injection. In order to compare the blood picture due to acute liver disease, rats weighing 200-250 gr were used. In one case the rats were operated, the abdomen was opened and the common-bile duct was ligated. Success of operation was evaluated by the development of jaundice and elevation of bilirubin at 6 days after the operation. In another case, CCl₄ (0.2 ml/ rat) were injected e.p and blood was taken 48 hours afterward.

RESULTS

Fatty liver in hens - The results are shown in tables 1-2 and pictures 3,4. In the sera of laying hens with fatty liver, there were significant changes in the levels of the enzymes AST which was higher by 36% and ICDH which was lower by 34%. There were very large increases in the levels of total lipids (112%) and HDL-cholesterol (1300%) (table 1). As shown in picture 3 the fraction which contributed mainly to this increase was triglycerides, while the level of the cholesterol and cholesterol-esters was similar in both the experimental and control groups.

The liver enzyme levels in both the control and the fatty liver are shown in table 2. There were significant decreases in the levels of AST (31%) and ME (73%) while no significant changes were seen in the levels of the other enzymes (ALP, MDH, LDH and ICDH). Picture 3,4 shows the distribution of the main lipid constituents in the liver of both groups. There were big increases in the levels of triglycerides, and cholesterol with smaller changes in the levels of cholesterol esters, free fatty acids and phospholipids.

The changes were somewhat different in the process of liver fattening in the non-laying hens. As shown in tables 3,4, there were very large increases in the serum levels of the ME and total lipids. While the level of ME was non-detectable in the controls, it was 30.9 ± 3.6 u/l in the sera from fatty liver birds. Total lipids in the controls were 1.03 ± 0.20 gr/l, it reached levels of 34.54 ± 1.64 gr/l in the fatty liver group (table 3). The changes in the fatty liver were relatively small. While ME slightly increased (22%), ICDH, LDH and SDH decreased (24%, 26% and 25% respectively). No significant changes were seen in the levels of ALP, AST and MDH (table 4).

Of interest are the changes in the liver enzymes during the maturation of the birds and changing from non-laying to laying. Except for MDH and ICDH

which did not change, the levels of ME, ALP, AST and LDH decreased significantly, (60%, 79%, 52% and 75% respectively) (tables 2,4). The process of liver fattening in laying hens, further caused enzyme decreases in the liver cells. (table 2,4).

Sera from poultry with fatty liver contained very high levels of total lipids. (tables 1,3). Following centrifugation at 100000 g for 60 minutes and removal of the fat layer on the top, total lipids in the sera were reduced from a mean of 28.72 gr/l to 23.54 gr/l. The activities of the enzymes in the sera following low and high speed centrifugation is described in table 5 . As seen the activities of ME and ICDH significantly increased, while the activities of MDH, LDH, ALP and AST were not affected.

Chicks given a biotin-deficient diet showed retarded development and growth rate up to about 60 days of age, after which there was very rapid growth and the chicks surpassed the control group at the age of 75 days. At 28 days, the mean weight of the chicks in the biotin-deficient group was 197 gr. and the controls 516 gr. At 75 days, the mean weight of the biotin-deficient birds was 1922 grams, while that of the control reaching only 1785 grams (Table 7). At 28, 40 and 57 days the mean liver weight of the biotin deficient chicks was significantly smaller ($p < 0.01$) than the control group. However, when the liver weight was calculated as a percentage of body weight, the biotin deficient chicks had a relatively larger liver (2.9 ± 0.2 ; $p < 0.01$) at age 28 days and to a lesser degree at the age of 40 days (2.7 ± 0.1 vs. 3.5 ± 0.1 ; $p < 0.01$). These differences were even smaller at 57 days and negligible at 75 days (Table 7).

Table 8 shows the levels of enzymes, proteins, metabolites and inorganic phosphorus in the blood of biotin-deficient and control chickens at 28, 40, 57 and 75 days. The greatest differences between the two groups were at 28 days.

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There were significantly ($p < 0.05$) lower levels, ranging between 24 and 39 percent, of the enzymes ALP, MDH and LDH in the serum of the biotin-deficient chicks. These differences disappeared later when the birds were older. ALP, which was 32% lower in the biotin-deficient chicks at the age of 28 days, was 19% higher at 40 days, 54% higher at 57 days and 70% higher at 75 days. Levels of serum ME, which were similar in both groups at 28, 40 and 57 days, were 41% lower in the biotin-deficient chickens at 75 days (Table 8). Activities of ALP in the serum of the control group decreased and total proteins and globulins increased as the birds grew older. Albumin levels, on the other hand, remained constant at all ages studied (Table 8). Levels of liver enzymes in the biotin-deficient and control birds at ages of 28, 40, 57 and 75 days are shown in Table 9. All dehydrogenases studied (LDH, MDH and ICDH), were generally somewhat higher in the biotin-deficient birds at all ages, with the largest differences with ICDH occurring in the older birds. A large and significant difference ($p < 0.01$) was seen in the level of ME. Livers in biotin-deficient birds contained higher levels of the enzyme, ranging from 23 to 156%. Levels of liver ALP in both groups at all ages were similar. While the levels of this enzyme in the liver increased with age (Table 9), an opposite pattern was seen in the blood (Table 8), decreasing as the birds grew older. The enzyme GOT in the liver of both groups was similar, decreasing with age.

As shown in table 10 there were significant changes in the serum levels of ICDH, G6PDH and LDH, from cows with fatty liver, which were higher than in the controls. On the other hand, serum total lipids was significantly lower in the fatty liver cows. Although not significant, higher serum levels were seen with MDH, ALP and ACP. Significant changes were also seen in the levels of liver enzymes (table 11). Levels of ME, ICDH, G6PDH, LDH, MDH and ALP were higher, while

the level of SDH was lower in the fatty liver in comparison to the controls.

There was also a decrease in the protein concentration in the fatty liver in comparison to the controls. Of interest is the change in the level of G6PDH, which was very greatly elevated in the moderately fattened liver (+1270%) and decreased in the greatly fattened liver to levels of 85% higher than the controls (table 11). Except for G6PDH, there was a direct correlation between the degree of liver fattening and the degree of enzyme changes in the liver. The opposite was seen with SDH, where a negative correlation was seen (table 11). Whenever the livers were divided according to the degree of fattening (which was a subjective evaluation) on a scale of 1-6 and a correlation (r) between the enzymatic changes and the degree of fattening were calculated, value of $r = 0.7085$ with significance of $p < 0.001$ were obtained for LDH and 0.5210 with $p < 0.001$ for ICDH.

As shown in picture 5, there were significant changes in lipid composition in both the sera and liver from fatty liver cows. In the sera there were no major changes in the distribution of the various lipid components and there was a decrease in the absolute levels of the various lipid fraction, leading to lower total lipids in cows with fatty liver (table 10). In the liver, however, there were significant changes in the pattern of distribution of the various lipid components. While in the light fatty infiltration there seemed to be some increases in the free fatty acid and triglycerides fractions, there were very large increases in the triglycerides and cholesterol esters in the medium-heavy fatty infiltration (picture 5 numbers 5-8). From the pattern of the triglycerides migration in chromatography it is possible that there are new types of lipids formed which caused the tailing seen. It is however clear that the major lipid constituent in the fatty liver is the triglycerides (picture 5).

Fatty liver in crammed geese - The results of the various blood constituents in crammed geese at various times of force feeding and at slaughter are shown in table 12;18 and in figures 1-21. As shown there were significant changes in the fatty liver geese starting at 11-18 days of cramming and reaching a peak at 25-32 days. Very large increases in the serum levels of enzymes were seen with ME (7.60 ± 4.8 vs. 212 ± 4.5 units/liter) at 25 days, and ICDH (12.2 ± 1.7 vs. 90 ± 13 units/liter) at slaughter, and SDH (1.80 ± 1.20 vs. 21.1 ± 8.2). Other changes, but to a lesser degree, were seen in the levels of LDH, MDH, ALP, GOT, ACP, albumin, glucose and total lipids. (table 12;18).

Comparing enzyme levels in serum and liver at various times of force feeding showed the gradual biochemical changes in the liver which took place during fattening (table 13). In the liver, there were also significant increases in the levels of ME, MDH and LDH and to a lesser extent AST. Of interest is the induction of the enzyme G6PDH which was undetectable up to 19 days of force feeding and then appeared in the liver. No activity of G6PDH was seen in the serum during the entire experimental period. Studying the correlation coefficient between liver and serum enzymes at slaughter day, showed relatively poor correlations which in most cases were insignificant (table 14). Even correlations between similar enzymes in the liver and serum did not show good correlations. Significant correlations of approximately 0.5000 were seen between serum ICDH and liver's MDH and AST and between serum's MDH and liver's LDH and vice versa serum's LDH and liver's MDH. (table 14).

Correlations between serum constituents and liver's weight at slaughter, are shown in table 15. There were good significant correlations between liver's weight and several biochemical parameters. Correlations of 0.8353 and 0.8033 were seen between liver's weight and serum MDH/ALB and $TL/(LDH + MDH)$ respectively. Negative

r of -0.7820 and -0.7383 were seen between liver weight to serum albumine and ALP respectively. Similarly good r were also seen with ALP/MDH and ALP/LDH. There was no correlation between the weight of the animal and the liver. Good correlations however between blood constituents and days of force feeding were seen (table 15).

Fatty liver in genetically different white and gray geese. There were significant differences between white and gray geese (table 16, Fig. 14-21) while the mean liver weight at slaughter of white geese was 565 gr., in gray geese the mean value was 747 gr. As seen, there were initial differences in the levels of ME, ALP, LDH, MDH and AST, which were lower in the white geese in comparison to the gray ones. The differences became more pronounced at a later stage of liver fattening with the biggest differences seen with ME at 27 days of liver fattening. While most enzyme's level was higher at the end of the cramming, the level of ALP decreased reaching a similar level at the end (table 16).

During liver fattening, there was a gradual increase in blood glucose, total proteins and total lipids and a gradual decrease in albumine level (table 16).

Effect of transportation of geese on blood constituents - Since blood sampling at slaughter day was done in the slaughter house, we have checked the effect of transportation of geese from the farm to the slaughter house on the levels of various blood constituents. Blood samples were taken before transporting the geese to the slaughter house, and at slaughter and the levels of the various constituents were determined in both samples. As seen in table 17 there were some (GOT, LDH) differences in the values obtained if taken on the farm and at slaughter day. This study was conducted at 33-35 days of fattening with at least 7 geese in each group.

Fatty liver in rats -

The levels of enzymes in the sera from normal and fatty liver rats are given in table 19. As shown, there were significant increases in the levels of ALT(+68.7%), MDH (+77.8%) and GGT(-53.4%). There were some smaller insignificant changes in the levels of AST (+18.7%), G6PDH (+13.9%), LDH (9.8%) and ALD (+22.3%) (table 19). There were no changes in the level of albumin, but the levels of total lipids were significantly higher (+26.6%). (table 20). Liver size and liver/body weight ratio were significantly higher, increasing by 18.9% and 19.3% respectively.

As shown in table 21, there were large significant changes in the enzyme levels from fatty liver in comparison to the controls. The largest differences were seen in the levels of G6PDH and ME which were +234% and 170% respectively. The levels of GGT(+113%), G6PDH (+63%), AST (+35.6 %), MDH (+38%) and LDH (+37%) were also significantly elevated. There was a small but significant difference in the level of ALT (-22.8%).

Comparing enzyme levels in the fatty and non-fatty parts of the fatty liver revealed that the largest changes seen were in the non-fatty parts. The most dramatic change was seen in the level of G6PDH. The level of this enzyme in the control group was 12.7 ± 2.1 units/gram protein, 52.7 ± 8.0 units/gram protein in the ^{non}fatty part and 27.0 ± 5.0 in the fatty part (table 22). Similar patterns were seen in the levels of ME, ICDH, G6PDH, MDH and LDH. SDH, which was lower in the fatty liver, showed a similar pattern but in the opposite direction (table 22).

Correlations between enzymes from liver and sera of rats with fatty liver are shown in table 23. The best correlation was seen between liver GGT and serum triglycerides ($r = -0.9341$, $p < 0.05$). This however was made on a small population ($n=4$) and therefore requires additional work. There was also a correlation of -0.8134 ($p < 0.05$), between liver AST and serum GGT. There were some correlations between liver GGT and serum MDH, and between liver ME and serum total lipids ($r=0.6332$, $p < 0.001$). Other correlations between 0.4820 and 0.7000 are also shown in table 23.

The levels of serum enzymes in geese at 18 and 25 days of cramming divided according to liver size are shown in table 18. As shown, the levels of the enzymes were higher in geese with a larger liver.

A summary of the results obtained with the various animals and the pattern of enzyme changes in serum and livers are given in tables 25-27.

DISCUSSION

The present study revealed some of the biochemical aspects taking place in the process of liver fattening. Although the induction of fatty liver was different in the models used in this study, there were some similarities in certain biochemical aspects. These include the accumulation of lipids in the liver, increased levels of lipogenic enzymes followed by larger destruction of hepatocytes and the release of intracellular enzymes into the blood.

The large amounts of lipids accumulated in the liver leading to its enlargement are not from exogenous sources only, but also and to a large extent from its synthesis by the liver. This can explain the large increases by the lipogenic enzymes (ME, ICDH, MDH) which generate NADPH necessary for fatty acids synthesis. In the geese, where the liver size can increase by over 1000%, this phenomena was most pronounced in comparison to the other models, where liver increases were smaller. There was a constant increase in the liver levels of ME in all animals studied. Similarly, there were also increases in MDH. This enzyme was suggested to take part in the generation of NADPH required for fatty acids synthesis by NADH/NADP transhydrogenation. Another enzyme which generates NADPH is NADP dependent ICDH. This enzyme however was not elevated in the liver in all the animals studied. This is a key enzyme in regulating the overall rate of fatty acids degradation and synthesis.

The elevated level of lipogenic enzymes on one hand and increased rate of enzymes leakage from the cells into the blood in fattening liver are the principles of the specific test for fatty liver. This was very clearly seen in geese where serum ME was elevated from an initial level of 6.0 ± 2.2 U/l to over 200 U/l. Similarly it was also seen in chicks where the enzyme's level went up to 30.9 ± 3.6 from non-detectable levels in the normal birds. In cows, although existing, this change was not seen to that great extent. In laying hens however, no significant changes in the serum level of this enzyme were seen. The reason for it

is not clear, but it could be due to the fact that initially the estrogens levels in laying hens were already high, and additional injection of the hormone did not have the enzymatic effects seen in the other models. This can explain the commonly seen fatty liver in the laying hens at the peak of production. Thus addition of estrogens to the already high blood levels may result in an opposite effect, thus leading to a situation in which protein synthesis is inhibited instead of stimulated. It was shown that under certain conditions such as supplementation of a diet deficient in choline, estrogens may inhibit liver lipid accumulation (White et al 1978).

Studying the enzyme profile in the livers of the various animals studied, revealed some similarities and variations in the various enzymes. Liver's AST in all the animals studied showed approximately the same levels. Similarly, also the levels of ICDH, MDH and ALP were within a close range not exceeding a factor of 5. On the other hand levels of ME, G6PDH, LDH, SDH and ALT showed greater differences between the different animals.

There was a relatively low level of the enzyme ALT in the cows' liver. This is in comparison to a closely related enzyme - AST - in the same liver. This low level seen in the cow's liver, can explain the poor usefulness of the enzyme in clinical biochemistry for the evaluation of liver disease in cattle. On the other hand, the relatively high level of this enzyme in rats' liver make it a useful enzyme for liver diseases in the rat.

Another example is ALP. This enzyme which is very low in the cows' liver, is of little use for liver disease evaluation. In man and dogs however, this enzyme is a good marker for liver diseases.

Enzymes like many other cellular constituents are constantly being made and destroyed. The amount of enzyme may therefore be changed by altering the rate of synthesis or the rate of destruction. Changes in the rate of synthesis of an

enzyme appear to be more common than changes in the rate of destruction. Animals respond to altered biochemical environments by changes in enzymes content. This is especially true in animals which are built to live on widely different diets. ~~The amount of enzymes related to fatty acids synthesis in a rat eating high glucose diet.~~ These include ME (NADP-specific malate dehydrogenase) of the cytosol, NADP-specific G6PDH and 6PGDH (McGilvery 1975; Freedland and Szepesi 1971). There are 3 main sources of NADPH generation in the animal required for fatty acids synthesis. The pentose phosphate pathway, NADP-linked MDH, and NADP-linked ICDH. These findings were also seen in the present study using the rat model, but only partially in the other models. This could be the result of different causes for liver fattening. While in rats it was a high sucrose diet, in poults and hens it was hormonal and in cows probably dietary imbalances together with metabolic disease associated with high production.

In cows with fatty liver, the changes seen in the liver and also in the sera were correlated to the degree of liver fattening. There were larger changes seen in livers with heavier fatty infiltration in comparison to the light fatty infiltration. Similarly, the degree of enzyme levels and changes in the geese were also correlated to the degree of liver fattening and consequently size, seen in the geese. Level of ME at 25 days of cramming was significantly lower in geese yielding livers of 300-500 grams, in comparison to geese yielding livers weighing 600-1000 gr. Similar patterns were seen with the enzymes ICDH, MDH, LDH, and AST. An opposite trend was seen with serum albumin which was higher in geese with small liver in comparison to geese with large livers. This picture was not seen with the enzyme ALP and serum total lipids.

Serum enzymes in the different birds vary from one group to another. The largest difference was seen in the level of ALP which was as low as 50.7 ± 2.7 in the geese and as high as 1074 ± 100 in the broilers. Large differences were also seen in the

level of AST, while the differences in the levels of the dehydrogenases were not as big. One of the big factors influencing the serum levels of ALP is age. The level of this enzyme is greatly influenced by the activities of the bones and intestines, which are more active in young animals than in old ones. Insults of any kind, which affect nutrition and growth of the birds will be seen by a decrease of this enzyme in the serum. In birds, it is a useful clinical biochemistry parameter for bones, intestine and general metabolism, but of little value for liver evaluation.

A pronounced difference was seen between cows and chicken in the levels of serum total proteins and albumin. The levels of these constituents were significantly lower in birds, reaching values of about 50% from those of cows. The physiological reasons for this phenomenon are not clear.

The present study also revealed the enzymatic profile changes which take place, not only due to liver fattening but also due to age change and maturation. This is seen both in the liver and blood. This is most clearly seen in the young poults and to a lesser degree in the geese and rats. The most striking change is seen in the level of serum ALP, which decreased very rapidly with age. Serum ALP in poults originated mainly from bones and intestine and only little from the liver. During growth of the animals there is apparently greater release of enzymes into the blood, and whenever growth has stopped this release of enzymes into the blood will slow down. Indeed, any metabolic insult in birds resulting in growth retardation will be shown by the lower levels of serum ALP (Bogin unpublished observation). It is of interest that in the biotin-deficient chickens, whenever growth was retarded in the first two weeks, ALP was lower than in the controls. On the other hand, toward the end, when the growth rate of the biotin deficient birds exceeded that of the controls, the level of serum ALP was also higher. Qualitative analysis of the fat constituents revealed that in all models, the stored lipids in the fatty liver were triglycerides and to some extent also cholesterol esters. The origin of these lipids depending on the animal could be from either endogenous or exogenous fat. The endogenous fat

could be either mobilized fat from various body depots or newly synthesized fat. In the case of geese, it seems that the origin of the lipids in the liver is endogenously synthesized fat, since the crammed geese received a diet which is rich in carbohydrate and protein and poor in fat. This assumption can explain the dramatic increases in the lipogenic enzymes.

Looking carefully on the chromatograms may reveal some differences in the levels of both liver and serum phospholipids. The phospholipids as part of the lipoproteins, have an important role in the transport of lipids from the liver outside. In the fatty liver, where it looks like lower levels of phospholipids exist, may suggest that it could be a cause in poorer ability of lipids transport from the liver, thus leading to its accumulation there.

Additional problems may arise from the change in the total lipid to albumine in the serum. While this ratio normally in geese is 0.396, it has changed to 2.72 in serum of fatty liver geese. One of the roles of serum albumin is to serve as a carrier of insoluble lipids constituents in the blood. In normal geese albumin levels are high and lipids are relatively low. During liver fattening, serum albumin levels declined (Probably as a result of liver dysfunction) and lipids increased, leading to a possibility of poorer ability of lipid transportation, once again leading to enhanced lipid deposition.

There were two enzymatic changes seen in the serum of all animal models studied. SDH which was reduced and ME was elevated (except for rats). It seems that sorbitol which is metabolized by SDH in the liver is inhibited in fatty liver regardless of the causes leading to it.

There were significantly different enzyme levels in parts of rats' liver showing different degrees of fattening. In the course of these changes in fatty liver, greater changes were seen in the less fatty parts of the liver in comparison to the more fatty parts. In table 19, the given values of enzymes in fatty liver

are the average of the fatty and non-fatty parts, while in table 22, the values represent different liver parts. If one excludes the possibility that in normal liver, there are differences in metabolism and enzyme levels in various parts of the liver, then the differences seen in the parts with various degrees of fattening represent some qualitative and quantitative differences. Since the changes seen in the liver parts with low degree of fattening were greater than those seen in the parts with high degree of fattening, it seems that the main changes in the liver occur prior to the stage of liver hypertrophy and lipids accumulation. At the stage of high degree of fattening there could be: 1) a reverse in the pattern due to lower synthesis of enzymes resulting from liver degeneration 2) larger leakage of enzymes from the fattened hepatocytes 3) inhibition of enzyme activities by the high lipids content - namely the cellular levels of enzymes are the same but they are inhibited and thus giving lower activity values. Lipids were shown to cause enzyme inhibition, This was also shown in the present study. Enzyme activities in serum obtained following centrifugation at low speed showed lower activities in comparison to the activities seen in the same serum following high speed centrifugation. In evaluating this possibility one should take into consideration that enzyme activities in the liver were conducted in homogenates centrifuged at a high speed, thus lowering the lipid content and enzyme inhibition caused by lipids. The possibility however could be correct only in cases where the activities of the enzymes were lower, and not in cases where enzyme activity was higher. It seems that the present data suggest that the ongoing process is according to the first possibility.

An important outcome of the present study in addition to better understanding the metabolic processes which take place in liver fattening, is the use of the selected biochemical parameters as genetic markers for the selection of geese for better fatty liver production. A farmer does not know until the goose is slaughtered the real size of the liver. It has been shown that the degree of blood changes are

correlated to size of liver (in a blind trial, to predict liver size, there was a success of over 70%). At this stage, the process of liver fattening is still reversible and stopping cramming and changing it to a normal diet, will lead back to normal liver. A screening test of a large geese population can lead to a selection of families having the ability to produce better liver, thus improving production and profit of the farmer.

In the study where gray geese, which have a better liver production, were compared to white geese, which have a lower liver production, the biochemical parameters measured in their blood were shown to correlate to these characteristics. Gray geese showed a larger change in the parameters selected in comparison to the white ones, thus supporting the suggestion of using these parameters as genetic markers.

In the present study serum levels of fatty and non-fatty liver cows are somewhat higher from values obtained whenever blood is taken on the farm. Comparing the levels of several enzymes in the serum of non-fatty cows showed the values to be higher. This could be attributed to the transportation of the animals from the farm to the slaughter house. This was also seen with geese.

It seems that there is a correlation between estrogen levels and tendency for fatty liver. Estrogen administration results in an increased lipid metabolism. The activity of the intracellular lipoprotein lipase which hydrolyzes the triglycerides to fatty acids and glycerol, is controlled by cyclic-AMP, which is affected by various hormones including estrogens. This can lead to lipid mobilization from adipose tissue. A massive release of lipids from the adipose can lead to its accumulation in the liver, and formation of fatty liver. The information on how it is possible to control fat mobilization is of great importance. In the case of geese, it would be of great economic value if body fat is mobilized and accumulated in the liver thus giving a larger liver on the one hand and a better ^{low fat} meat

on the other hand, all this in addition to a shorter cramming period and savings in labor and feed. Presently experiments are being conducted to find ways to mobilize adipose fat and its deposition in the liver. Similarly, experiments are being conducted to find ways to avoid fatty liver in farm animals.

The present research project accomplished its objectives by a) further elucidating and understanding the biochemical aspects of liver fattening b) establish biochemical parameters which can indicate occurrence of fatty liver and degree of fattening c) led to additional studies to further understanding and establishing ways to prevent and promote liver fattening.

The biochemical parameters for specifically detecting fatty liver are somewhat different for the various animals, which are probably resulting from different causes and metabolism of these animals.

Table 1: Levels of enzymes and other blood constituents in sera from normal and fatty liver laying hen

B l o o d c o n s t i t u e n t (Enzymes U/l, Lipids gr/l)									
Group	statistics	AST	ALP	ME	ICDH	MDH	LDH	HDL chol	Total lipids
normal	x	91	151	6.7	11.4	453	918	15	23.5
	sem	6	23	0.7	1.5	39	67	5	2.8
fatty liver	x	124*	165	7.6	7.6*	506	1177*	212**	64.1**
	sem	5	46	1.8	0.8	62	77	40	5.9

n = 10 ; * $p < 0.05$; ** $p < 0.01$

Table 2: Enzyme levels in normal and fatty liver of laying hens. (units /gr protein).

		E N Z Y M E S					
	statistics	AST	ALP	ME	MDH	LDH	ICDH
Normal	$\bar{x}$	194	1.82	70.3	3090	814	134
	sem	21	0.18	14.1	265	80	10
Fatty liver	$\bar{x}$	134*	1.95	19.3**	2817	810	158
	sem	13	0.28	2.2	258	48	15

n=10

*p < 0.05

** p < 0.01

Table 3: levels of enzymes and blood constituents in sera of non-laying young chicks. (4/1).

		Blood						
		constituent						
	statistics	ME	ICDH	ALP	AST	LDH	MDH	Total lipids (gr/1)
Normal (n=8)	$\bar{x}$ sem	0.0 0.0	11.3 1.1	834 80	126 8	670 49	590 24	1.03 0.20
Fatty liver (n=12)	$\bar{x}$ sem	30.9* 3.6	8.7 0.7	1170 114	108 9	577 48	359 26	34.54** 1.64

* $p < 0.05$ ** $p < 0.01$

Table 4: Enzyme levels in normal and fatty liver of non-laying young chicks (units/gram protein).

Group	statistics	E N Z Y M E						
		ME	ICDH	ALP	AST	LDH	MDH	SDH
Normal (n=8)	$\bar{x}$ sem	174 17	149 7	8.64 0.39	407 32	3304 245	3200 374	106 9
Fatty liver (n= 12)	$\bar{x}$ sem	212* 10	113* 5	9.59 1.29	376 29	2439* 179	3474 315	80* 10

* $p < 0.05$

Table 5: Effect of serum lipids on serum enzyme activity in chicken poult

Group	statistics	E N Z Y M E (u/l)					
		ME	ICDH	MDH	LDH	ALP	AST
Low Speed Centrifugation	x sem	28 4.2	5.11 0.79	955 135	666 98	296 61	138 38
High Speed Centrifugation	x sem	37.4 3.7	12.89* 3.38	1130 169	644 52	317 28	111 8

n = 7

* p < 0.01

Table 6: Effect of ccl_4 on serum enzyme levels in chicks with normal and estradiol induced fatty liver.
(non-laying hens).

Group		statistics	Enzyme (U/l)					
			ME	ICDH	MDH	LDH	ALP	AST
fatty liver	control	x sem	28 4.2	5.11 0.79	955 135	666 98	296 61	138 38
	ccl_4	x sem	45.0* 9.0	7.5 2.8	1245 232	902 186	361 67	157 3
normal liver	control	x sem	0.0 0.0	11.27 1.38	1111 156	794 154	374 57	133 16
	ccl_4	x sem	0.0 0.0	14.5 3.8	2206* 244	1353* 201	317 21	214* 22

n = 6 * $p < 0.01$

TABLE 7. Body and liver weights in control and biotin-deficient birds at various ages ($\bar{X} \pm \text{SEM}$)

Age of Birds (days)	Weight of Birds (grams)		Weight of Liver (grams)		Liver weight/Body weight (%)	
	Control	Experiment	Control	Experiment	Control	Experiment
28	516 $\pm$ 27	197 $\pm$ 21 *	15.0 $\pm$ 0.75	8.6 $\pm$ 0.7 *	2.9 $\pm$ 0.2	4.4 $\pm$ 0.2 *
40	858 $\pm$ 31	534 $\pm$ 21 *	22.7 $\pm$ 1.1	18.4 $\pm$ 1.0 *	2.7 $\pm$ 0.1	3.5 $\pm$ 0.1 *
57	1546 $\pm$ 92	806 $\pm$ 93 *	44.6 $\pm$ 5.4	25.8 $\pm$ 2.0 *	2.9 $\pm$ 0.1	3.5 $\pm$ 0.2 *
75	1785 $\pm$ 68	1922 $\pm$ 105	47.2 $\pm$ 1.7	46.8 $\pm$ 2.0	2.6 $\pm$ 0.1	2.5 $\pm$ 0.1

* $p < 0.01$

TABLE 8: Levels of enzymes, proteins, metabolites and inorganic phosphorus in blood of control and biotin deficient chicken at various ages ($\bar{X} \pm \text{SEM}$)

Blood constituent	AGE OF BIRDS (DAYS)									
	28		40		57		75			
	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment		
Total proteins (gr/l)	33.6 ⁺ 1.1	38.8 ⁺ 2.0	38.6 ⁺ 1.2	35.5 ⁺ 0.90	43.8 ⁺ 0.80	37.0 ⁺ 1.0	51.0 ⁺ 2.3	51.6 ⁺ 0.80		
Albumin (gr/l)	15.6 ⁺ 1.3	18.9 ⁺ 3.3	16.0 ⁺ 0.30	17.7 ⁺ 0.90	17.0 ⁺ 0.40	13.3 ⁺ 0.40	15.6 ⁺ 0.5	17.0 ⁺ 0.50		
Globulin (gr/l)	18.0 ⁺ 1.6	18.9 ⁺ 2.1	22.6 ⁺ 1.4	17.8 ⁺ 1.2	26.8 ⁺ 1.1	23.7 ⁺ 1.3	35.0 ⁺ 2.2	34.6 ⁺ 1.0		
Total lipids (gr/l)	3.66 ⁺ 0.20	3.84 ⁺ 0.24	3.94 ⁺ 0.19	4.38 ⁺ 0.25	3.71 ⁺ 0.20	4.48 ⁺ 0.29	3.82 ⁺ 0.13	4.40 ⁺ 0.17		
Glucose (mmol/l)	----	----	----	----	8.32 ⁺ 0.94	8.99 ⁺ 0.39	9.60 ⁺ 0.50	10.0 ⁺ 0.56		
GOT (U/l)	120 ⁺ 13	119 ⁺ 15	82 ⁺ 4	80 ⁺ 9	76 ⁺ 4	74 ⁺ 4	74 ⁺ 3	77 ⁺ 3		
ALP (U/l)	1397 ⁺ 229	957 ⁺ 220*	1074 ⁺ 100	1287 ⁺ 148	463 ⁺ 88	712 ⁺ 105*	253 ⁺ 60	429 ⁺ 24*		
ICDH (U/l)	31 ⁺ 1.5	27 ⁺ 2.4	28.7 ⁺ 2.1	26.1 ⁺ 4.3	14.8 ⁺ 1.4	12.6 ⁺ 1.2	15.3 ⁺ 1.3	14.3 ⁺ 0.8		
ME (U/l)	5.7 ⁺ 0.7	5.9 ⁺ 1.2	5.4 ⁺ 0.4	4.8 ⁺ 0.6	5.1 ⁺ 0.4	5.0 ⁺ 0.6	8.2 ⁺ 1.1	4.8 ⁺ 0.7*		
MDH (U/l)	1615 ⁺ 242	1226 ⁺ 106	1318 ⁺ 100	900 ⁺ 135	1014 ⁺ 104	1001 ⁺ 113	2082 ⁺ 191	1786 ⁺ 277		
LDH (U/l)	1435 ⁺ 123	880 ⁺ 104*	1421 ⁺ 142	1558 ⁺ 171	1178 ⁺ 172	1016 ⁺ 139	1375 ⁺ 133	1347 ⁺ 202		
Phos. inorg. mmol/l	1.87 ⁺ 0.07	1.89 ⁺ 0.17	----	----	1.67 ⁺ 0.17	2.00 ⁺ 0.13	1.74 ⁺ 0.05	1.80 ⁺ 0.13		

*p < 0.01

TABLE 9: Levels of enzymes in livers of control and biotin-deficient chicks at various ages ($\bar{x} \pm \text{SEM}$)

ENZYME U/gram	AGE OF BIRDS (DAYS)									
	28		40		57		75			
	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
LDH	1834 $\pm$ 151	1999 $\pm$ 167	1044 $\pm$ 157	1558 $\pm$ 109	2667 $\pm$ 316	2709 $\pm$ 226	2901 $\pm$ 312	3048 $\pm$ 253		
MDH	2422 $\pm$ 275	3303 $\pm$ 192 *	-----	-----	2936 $\pm$ 425	3177 $\pm$ 239	4633 $\pm$ 332	5020 $\pm$ 120		
ICDH	255 $\pm$ 25	305 $\pm$ 20	344 $\pm$ 25	334 $\pm$ 24	173 $\pm$ 42	266 $\pm$ 9 *	225 $\pm$ 19	323 $\pm$ 33 *		
ME	80 $\pm$ 8	117 $\pm$ 12 *	65 $\pm$ 11	134 $\pm$ 10 *	44 $\pm$ 8	113 $\pm$ 10 *	56 $\pm$ 4	86 $\pm$ 5 *		
GOT	275 $\pm$ 35	235 $\pm$ 19	232 $\pm$ 31	238 $\pm$ 16	193 $\pm$ 15	180 $\pm$ 7	143 $\pm$ 9	145 $\pm$ 11		
ALP	2.43 $\pm$ 0.21	2.73 $\pm$ 0.24	3.1 $\pm$ 0.4	3.8 $\pm$ 0.3	5.7 $\pm$ 0.4	5.5 $\pm$ 0.3	9.7 $\pm$ 0.8	8.0 $\pm$ 1.9		

* p < 0.01

Biochemical Parameters (enzymes - u/l ; others - gr/l)															
Group	statistics	ME	ICDH	G6PDH	LDH	MDH	GLDH	AST	ALT	ALP	ACP	SDH	Total prote-in	Album-in	Total Lipids
Control	$\bar{x}$	8.7	28.5	2.58	1690	1735	8.5	176	8.0	26.9	6.7	6.7	70.6	38.8	6.86
	sem	1.5	2.8	0.39	285	151	3.1	42	1.5	4.5	1.4	3.8	0.5	0.9	0.94
	N	9	9	6	8	9	14	9	8	8	7	7	7	9	8
light to moderate fatty infiltration	$\bar{x}$	13.9	30.9	2.05	2272	2882	3.07	309	12.6	22.0	8.4	3.8	69.3	35.1	3.99*
	sem	2.1	2.7	0.39	370	590	0.47	56	4.6	2.6	1.1	1.2	2.2	1.7	0.42
	N	16	16	8	13	14	10	15	8	12	9	11	13	15	13
Heavy fatty infiltration	$\bar{x}$	12.8	49.8*	4.03**	3106*	3769	8.39	229	7.8	45.2	12.1	3.1	70.8	37.0	4.18*
	sem	3.0	5.9	0.51	460	647	2.61	27	1.7	9.3	2.1	0.9	1.6	1.0	0.67
	N	16	17	5	17	17	10	18	14	16	15	13	15	17	16

**p < 0.01

*p < 0.05

Table 10 Level of enzymes in normal and fatty liver from cows.

E N Z Y M E S (u / gr protein)												
Group	statistics	ME	ICDH	G6PDH	LDH	MDH	SDH	GLDH	AST	ALT	ACP	ALP
Control	$\bar{x}$	0.94	92	1.85	517	1016	79.1	12.4	229	1.71	11.33	3.60
	sem	0.09	9	0.52	42	56	13.3	2.3	14	0.33	0.61	0.57
	N	15	18	8	18	18	16	14	18	14	16	17
Light to moderate fatty liver	$\bar{x}$	1.67	112	25.3 *	814	1171	69.6	18.9	231	2.21	12.21	4.56
	sem	0.38	11	5.9	123	132	24.0	5.5	20	0.28	0.79	0.52
	N	11	13	7	13	13	11	12	13	12	12	13
Heavy fatty liver	$\bar{x}$	2.16 **	133 *	3.43 **	1292 *	1358 **	27.5 *	17.3	217	2.93	10.78	6.36 *
	sem	0.47	7	0.38	100	117	5.11	6.7	9	1.00	0.59	0.72
	N	12	16	12	16	16	14	8	16	14	14	14

*p < 0.01

**p < 0.05

Table 12: Levels (mean[±]sem) of geese body and liver weights and blood composition at various times of (a) force feeding (mean[±]sem).

Parameter	Group	Time in days of force feeding					
		0	11	18	25	32	36 - 41
Weight of geese (kg)	CONT	4.00 [±] 0.12	4.28 [±] 0.16	4.64 [±] 0.19	4.17 [±] 0.2	4.72 [±] 0.17	4.92 [±] 0.14
	EXP	3.97 [±] 0.08	5.13 [±] 0.09	5.94 [±] 0.09	6.72 [±] 0.09	7.70 [±] 0.17	8.0 [±] 0.26
Malic Enzyme (U/l)	CONT	2.50 [±] 1.57	6.00 [±] 0.93	-	7.60 [±] 4.8	6.40 [±] 3.9	6.0 [±] 2.2
	EXP	3.35 [±] 0.59	3.94 [±] 0.87	13.4 [±] 2.0	212 [±] 24.5	189 [±] 22.4	202 [±] 62
Isocitric Dehydrogenase (U/l)	CONT	18.6 [±] 3.5	10.4 [±] 2.2	10.4 [±] 1.3	13.0 [±] 0.9	11.8 [±] 1.2	12.2 [±] 1.7
	EXP	13.9 [±] 1.4	13.1 [±] 0.7	17.4 [±] 1.3	58.7 [±] 5.0	62.5 [±] 3.7	90 [±] 13
Lactic Dehydrogenase (U/l)	CONT	476 [±] 67	528 [±] 73	557 [±] 76	451 [±] 65	418 [±] 45	321 [±] 71
	EXP	475 [±] 27	306 [±] 22	454 [±] 27	654 [±] 61	745 [±] 99	1730 [±] 518
Malic Dehydrogenase (U/l)	CONT	344 [±] 41	277 [±] 13	332 [±] 41	370 [±] 36	309 [±] 22	232 [±] 34
	EXP	357 [±] 16	251 [±] 11	410 [±] 21	1130 [±] 119	1103 [±] 85	1550 [±] 400

Table 12- b

Cholin- Esterase (U/l)	CONT	-	659 ± 64	457 ± 34	466 ± 29	460 ± 26	454 ± 21
	EXP	-	567 ± 24	458 ± 17	505 ± 17	545 ± 26	517 ± 36
Alkaline Phosphatase (U/l)	CONT	50.7 [±] 2.7	-	55.8 [±] 6.2	46.6 [±] 6.0	60.0 [±] 7.3	34 ± 8
	EXP	55.0 [±] 2.2	93.9 [±] 1.6	55.5 [±] 2.5	36.4 [±] 2.4	20.4 [±] 1.5	16 ± 2.3
Glutamic- Oxalacetic Transaminase (U/l)	CONT	17.8 [±] 2.0	-	12.8 [±] 2.9	8.6 ± 1.4	13.6 [±] 1.5	8.0 ± 2
	EXP	17.0 [±] 1.8	16.0 [±] 1.4	19.7 [±] 2.1	45.2 [±] 3.5	46.8 [±] 4.7	55.0 [±] 3.0
Acid Phosphatase (U/l)	CONT	12.3 [±] 1.4	-	9.5 ± 1.2	18.1 [±] 1.8	18.3 [±] 1.8	13.5 [±] 1.0
	EXP	13.0 [±] 1.2	-	9.5 ± 0.5	26.0 [±] 0.9	25.7 [±] 1.8	31.3 [±] 4.7
Sorbitol Dehydrogenase (U/l)	CONT	1.20 [±] 0.73	-	-	0.80 [±] 0.20	-	1.80 [±] 1.20
	EXP	0.95 [±] 0.42	-	-	24.6 [±] 4.32	-	21.1 [±] 8.2
Albumine (gr/dl)	CONT	1.54 [±] 0.05	1.52 [±] 0.02	1.52 [±] 0.11	1.62 [±] 0.06	1.88 [±] 0.05	1.59 [±] 0.07
	EXP	1.56 [±] 0.03	1.71 [±] 0.06	1.82 [±] 0.04	1.62 [±] 0.07	1.42 [±] 0.06	1.04 [±] 0.08
Glucose (mg/dl)	CONT	135 ± 14	116 ± 3	129 ± 3	127 ± 4.1	124 ± 3	128 ± 3
	EXP	120 ± 2	160 ± 4	154 ± 4	164 ± 3.4	138 ± 3	161 ± 17

Table 12- c

Total lipids (mg/dl)	CONT	624 ± 42	599 ± 25	816 ± 59	654 ± 57	603 ± 79	630 ± 70
	EXP	578 ± 15	769 ± 37	1042 ± 38	1291 ± 97	1284 ± 80	2830 ± 1127
Total proteins (gr/dl)	CONT	5.13 [±] 0.11	5.42 [±] 0.09	5.63 [±] 0.21	5.76 [±] 0.08	5.96 [±] 0.26	5.87 [±] 0.26
	EXP	5.07 [±] 0.05	5.38 [±] 0.08	5.76 [±] 0.10	5.58 [±] 0.08	5.40 [±] 0.15	5.06 [±] 0.35
Inorganic -P (mg/dl)	CONT	5.39 [±] 0.43	5.55 [±] 0.14	6.16 [±] 0.20	5.71 [±] 0.23	4.74 [±] 0.34	6.40 [±] 0.30
	EXP	5.65 [±] 0.34	6.05 [±] 0.12	4.65 [±] 0.16	6.11 [±] 0.10	6.20 [±] 0.25	6.76 [±] 0.11
Hematocrit	CONT	38.6 [±] 1.4	38.2 [±] 0.7	37.5 [±] 4.0	40.3 [±] 1.8	39.7 [±] 0.33	42.7 [±] 3.7
	EXP	38.1 [±] 0.8	35.8 [±] 0.6	37.7 [±] 0.5	36.5 [±] 0.5	36.8 [±] 0.47	30.6 [±] 4.1
Weight of liver (gr)	CONT	-	-	-	-	-	-
	EXP	105 ± 10	-	143 ± 21	313 ± 31	687 ± 109	822 ± 76
Globuline gr/dl	CONT	3.59 [±] 0.06	3.90 [±] 0.03	4.11 [±] 0.11	4.14 [±] 0.06	4.08 [±] 0.06	4.28 [±] 0.08
	EXP	3.51 [±] 0.04	3.67 [±] 0.07	3.94 [±] 0.05	3.96 [±] 0.07	3.98 [±] 0.07	4.02 [±] 0.09

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Table 13: Levels (means $\pm$ sem) of liver enzymes and blood constituents from geese at various times after the start of force

force feeding (days)	weight of geese (kg)	weight of liver (gr)	L I V E R							S E R U M							
			ME U/1	ICDH U/1	LDH U/1	MDH U/1	G6PDH U/1	ALP U/1	AST U/1	ME U/1	ICDH U/1	LDH U/1	MDH U/1	G6PDH U/1	ALP U/1	AST U/1	ALB gr/dl
0	4.10 0.20	96 12	35 1		918 89	1173 68	- -	- -	152 9	2.5 1.6	18 2.1	289 53	276 34	0	50.7 2.7	17.8 2.0	1.54 0.05
19	5.83 0.28	143 21	134 10	69 4	1425 147	2934 222	- -	207 12	215 12	24.8 7.8	19.5 1.7	137 25	366 53	0	51.8 3.0	19.5 4.9	1.85 0.04
26	6.05 0.17	312 32	242 9	86 7	1417 186	2412 168	119 11	129 2	273 41	72.5 24.2	33.3 4.7	185 57	572 148	0	44.6 9.9	41.3 12.5	1.86 0.17
30	7.01 0.18	575 60	196 18	83 3	2645 190	5101 368	130 7	105 7	496 69	161 61	70.7 18.8	604 174	1030 311	0	29.5 1.8	79.0 31.0	1.77 0.08
33	7.58 0.24	688 109	191 19	77 4	- -	3374 378	127 17	110 6	358 36	- -	37.3 3.7	547 77	958 65	0	20.3 3.4	49.5 5.1	1.30 0.11
41	8.32 0.28	900 32	214 8	79 10	5741 344	3271 342	78 3	208 30	391 58	143 26	43.0 7.3	643 106	1191 152	0	24.0 3.1	50.2 3.7	1.15 0.09

Table 14: Correlation coefficients and significance between serum and liver from geese with fatty liver.

Liver Enzymes	Wt Geese	S E R U M						
		ME	ICDH	Lipids	MDH	AST	ALP	LDH
LDH	0.6973**	0.4846*	0.2149	0.3677	0.5401*	0.1518	-0.3005	0.4284
MDH	0.2079	0.3516	0.5639*	0.4026	0.2770	0.4278	-0.2183	0.4893*
AST	0.4015	0.4948	0.5142*	-0.0266	0.4511	0.4257	-0.3533	0.4107
ICDH	-0.0145	0.2011	0.2357	-0.2317	0.1902	0.1452	-0.0965	0.2956
ME	0.2239	0.2590	0.2924	-0.1976	0.2093	0.2615	-0.1481	0.1998
ALP	0.0025	-0.1792	-0.2254	0.0702	-0.1601	-0.3893	0.2047	-0.3481
G6PDH	-0.5648*	-0.0627	0.0966	-0.5708	-0.2508	0.1035	0.2273	-0.1161
mg prot/ gr tissue	-0.7623**	-0.4516*	-0.4686*	-0.1494	-0.6541**	-0.4145	0.7791**	-0.6740**

*p < 0.05

**p < 0.01

Table 15: Correlations* between days of force feeding, weight of geese, weight and quality of liver to various blood parameters at slaughter

Parameter	Correlation to			
	days	wt geese	wt liver	liver grade
albumine	-	-	-0.7820	0.7638
GOT	0.600	-	-	-
LDH	-	-	0.6954	-
ALP	-0.8771	-0.7359	-0.7383	0.6678
MDH	0.7750	0.7075	0.6413	-
ICDH	0.7086	0.6715	-	-
ME	0.6626	0.6946	-	-
ME/GOT	0.5136	0.5669	-	-
ME/LDH	-	0.5913	-	-
ME/ALB	0.7510	0.7267	0.6483	-
ME/MDH	0.5537	0.6000	-	-
(serum lipids	0.4313	0.4608	0.2706 NS	-0.3442 NS)
ALP/LDH	-0.6974	0.6453	-0.7103	0.6169
ALP/MDH	-0.8337	-0.7229	-0.7676	0.6666
ALP/glucose	-0.8515	0.6629	-	-
ALP/ALB	-0.7690	0.6591	-	-
TL/ALB	0.6481	-	0.6527	-0.7250
TL/LDH	-	-	-	0.6158
TL/(LDH+MDH)	0.8102	0.7619	0.8033	0.7591
MDH/ALB	0.8224	0.7027	0.8353	0.7173
LDH/ALB	0.6526	-	0.7954	-0.6490

* Only r above 0.55 showing significance higher than 0.001 (except for serum lipids N.S. = not significant)

Table 16 a: Enzyme levels in the sera of white and gray geese during various times of force feeding.

E N Z Y M E (U/L)								
Days	Group		AST	ALP	MDH	LDH	ICDH	ME
1	white	$\bar{x}$	19.9	78.0	304	543	16.3	1.1
		sem	2.2	3.8	20	33	0.8	1.1
	gray	$\bar{x}$	24.3	93.6	379	664	16.6	3.1
		sem	2.8	6.5	24	49	1.6	2.1
		n	20	20	20	19	20	9
13	white	$\bar{x}$	20.2	84.8	419	393	42.6	6.6
		sem	2.2	3.9	21	30	2.2	1.3
	gray	$\bar{x}$	20.5	109.2	469	440	44.1	8.4
		sem	2.2	4.6	24	32	1.7	1.4
		n	20	20	20	20	17	20
27	white	$\bar{x}$	44.3	39.5	780	598	29.5	140.9
		sem	4.3	3.5	75	44	3.8	18.9
	gray	$\bar{x}$	56.2	32.7	953	675	44.8	226.5
		sem	5.9	3.3	45	50	4.0	20.7
		n	19	20	20	20	18	20
32	white	$\bar{x}$	39.1	25.2	714	640	47.9	158.6
		sem	5.2	2.2	91	90	5.6	26.8
	gray	$\bar{x}$	47.6	24.1	900	749	66.9	218
		sem	5.5	2.3	114	77	10.2	34
		n	15	15	15	15	10	15

Table 16b: Levels of blood constituents in white and gray geese during various times of force feeding.

Days	Group	Blood Constituent					
		IP (mmol/l)	Glucose (mmol/l)	PCV %	Total proteins (gr/l)	Albumine (gr/l)	Total lipids (gr/l)
1	White	$\bar{x}$	2.60	7.60	35.4	49.7	17.4
		sem	0.04	0.33	0.5	0.9	0.7
	Gray	$\bar{x}$	2.54	7.16	36.4	51.3	18.6
		sem	0.04	0.11	0.4	1.1	0.6
13	White	$\bar{x}$	2.23	10.04	34.7	50.8	19.2
		sem	0.06	0.28	0.6	1.2	0.5
	Gray	$\bar{x}$	2.32	10.21	35.1	51.8	18.8
		sem	0.06	0.29	0.7	1.0	0.8
27	White	$\bar{x}$	1.81	8.10	36.4	58.7	13.7
		sem	0.08	0.44	0.7	1.7	0.4
	Gray	$\bar{x}$	1.91	8.21	36.8	54.6	13.2
		sem	0.07	0.39	0.7	1.6	0.4
32	White	$\bar{x}$	2.01	12.54	37.7	61.0	12.6
		sem	0.14	0.39	0.6	2.4	1.2
	Gray	$\bar{x}$	2.20	12.04	39.0	60.0	12.7
		sem	0.15	0.89	0.8	2.5	1.5

Table 17: Levels of blood constituents in fattened geese taken on the farm before shipment and at slaughter after shipment

Group		Blood Constituent						
		ME	ICDH	ALP	LDH	GOT	MDH	ALB Total Lipids
slaughterhouse	$\bar{x}$	(u/l)	(u/l)	(u/l)	(u/l)	(u/l)	(u/l)	(gr/l)
		131	50.4	28.7	1645	47.3	2319	7.9
	sem	16	4.1	2.2	113	5.0	186	0.5
farm	$\bar{x}$	124	51.4	29.2	1347	31.7	1954	7.8
		13	3.2	1.9	105	2.6	142	0.4
	sem	13	3.2	1.9	105	2.6	142	0.4
	p	n.s.	n.s.	n.s.	0.05	0.01	n.s.	n.s.

1000		
0+2.0	3	
25	6	
+2.9	3	
+7.6	6	
112	3	
131	6	
111	3	
69	6	
+8.7	3	
+6.9	6	
+12.5	3	
+7.0	6	
+0.17	3	
+0.10	6	
+111	3	
+515	4	

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Table 18: Enzyme levels in serum of force fed geese Divided According To Liver Weight and Time.

Enzyme	Liver weight		300 gr. $\bar{x} \pm \text{sem}$	N	400	500	600	700	800	900						
	Day															
MEZ	18		13.8 $\pm$ 5.2	4	14.5 $\pm$ 6.6	4	11.1 $\pm$ 2.2	7	12.7 $\pm$ 3.0	7	21.0 $\pm$ 5.2	6	27.8 $\pm$ 6.2	7	14.6 $\pm$ 3.9	5
	25		48.0 $\pm$ 17.5	4	105.0 $\pm$ 14.0	4	81.0 $\pm$ 15	9	119 $\pm$ 22	11	167 $\pm$ 23	17	215 $\pm$ 20	19	224 $\pm$ 24	13
ICDH	18		17.0 $\pm$ 4.7	4	17.0 $\pm$ 3.3	4	18.1 $\pm$ 3.4	7	18.6 $\pm$ 1.9	7	24.2 $\pm$ 1.4	6	21.0 $\pm$ 2.4	7	18.4 $\pm$ 2.7	5
	25		23.7 $\pm$ 1.7	4	46.3 $\pm$ 3.8	3	38.6 $\pm$ 9.4	9	46.2 $\pm$ 5.6	11	48.9 $\pm$ 5.0	16	54.7 $\pm$ 4.7	18	63.4 $\pm$ 5.9	13
MDH	18		414 $\pm$ 45	4	596 $\pm$ 136	4	614 $\pm$ 90	7	450 $\pm$ 37	7	561 $\pm$ 103	6	515 $\pm$ 40	7	349 $\pm$ 50	5
	25		373 $\pm$ 42	4	1016 $\pm$ 256	4	800 $\pm$ 167	9	1047 $\pm$ 212	11	1362 $\pm$ 166	17	1543 $\pm$ 157	17	1738 $\pm$ 294	12
LDH	18		464 $\pm$ 82	3	416 $\pm$ 92	3	338 $\pm$ 43	3	396 $\pm$ 50	5	585 $\pm$ 66	3	580 $\pm$ 125	4	449 $\pm$ 73	5
	25		310 $\pm$ 56	4	545 $\pm$ 132	4	455 $\pm$ 55	9	570 $\pm$ 104	11	625 $\pm$ 42	16	615 $\pm$ 81	19	678 $\pm$ 84	13
AST	18		13.8 $\pm$ 0.9	4	20.8 $\pm$ 2.5	4	17.9 $\pm$ 3.0	7	16.0 $\pm$ 2.6	7	17.2 $\pm$ 9.5	6	15.4 $\pm$ 3.6	7	18.4 $\pm$ 1.5	5
	25		25.3 $\pm$ 7.5	3	35.5 $\pm$ 7.4	4	35.5 $\pm$ 6.3	8	32.5 $\pm$ 4.5	11	43.6 $\pm$ 5.5	16	55.6 $\pm$ 5.2	19	54.5 $\pm$ 8.6	13
ALP	18		37.2 $\pm$ 6.9	4	62.9 $\pm$ 13.1	4	74.1 $\pm$ 5.5	7	58.1 $\pm$ 6.3	7	69.1 $\pm$ 7.2	6	75.1 $\pm$ 8.1	7	58.9 $\pm$ 7.7	5
	25		32.8 $\pm$ 11.2	4	28.3 $\pm$ 6.2	4	42.7 $\pm$ 4.7	9	36.6 $\pm$ 4.8	11	44 $\pm$ 4.8	17	37.1 $\pm$ 3.5	18	41.9 $\pm$ 5.0	13
ALB	18		1.60 $\pm$ 0.30	4	1.74 $\pm$ 0.11	4	1.60 $\pm$ 0.10	7	1.77 $\pm$ 0.09	7	1.78 $\pm$ 0.14	6	1.54 $\pm$ 0.12	6	1.72 $\pm$ 0.13	5
	25		1.87 $\pm$ 0.39	4	1.67 $\pm$ 0.15	4	1.53 $\pm$ 0.10	9	1.74 $\pm$ 0.15	11	1.34 $\pm$ 0.04	13	1.30 $\pm$ 0.05	19	1.47 $\pm$ 0.10	13
FATM	18		879 $\pm$ 67	4	966 $\pm$ 108	4	1137 $\pm$ 142	7	974 $\pm$ 63	7	1690 $\pm$ 317	6	1236 $\pm$ 151	7	1104 $\pm$ 88	5
	25		1617 $\pm$ 73	2	1340 $\pm$ 157	4	1246 $\pm$ 102	8	1334 $\pm$ 173	9	1248 $\pm$ 117	13	1236 $\pm$ 97	12	1355 $\pm$ 186	8

Table 19: Levels of enzymes in sera from normal and fatty liver rats.

Group	E N Z Y M E S (U / l)											
		AST	ALT	ALP	ME	G6PDH	6PGDH	ICDH	MDH	LDH	ALD	GT
control	$\bar{x}$	50.9	8.11	82.7	12.46	2.77	12.55	5.43	315	824	48.8	17.4
	SD	11.6	2.35	33.9	4.98	1.83	2.67	4.23	150	242	4.3	1.3
	sem	2.8	0.63	8.2	1.20	0.61	0.95	1.13	36	59	2.1	0.6
	N	17	14	17	17	9	8	14	17	17	4	4
fatty liver	$\bar{x}$	60.4	13.68 *	84.7	12.37	2.76	14.21	4.76	560 *	905	59.7	8.1 *
	SD	15.4	10.06	44.1	4.32	2.90	4.42	2.86	373	308	5.2	3.0
	sem	3.3	2.15	9.4	0.97	0.84	1.33	0.74	79	66	2.0	1.5
	N	22	22	22	20	12	11	15	22	22	7	4
change from control (%)		+18.7	+68.7 *	NC	NC	NC	+13.9	-12.3	+77.8 *	+9.8	+22.3	-53.4 *

* $p < 0.01$

Table 20 : Liver weight and serum albumin and total lipids levels from normal and fatty liver rats.

Group	Parameter				
	Statistics	Liver weight (gr)	Liver/body x100	Albumin (gr/l)	Total lipids (gr/l)
Control	$\bar{x}$	15.99	4.30	34.40	2.82
	SD	1.51	0.30	1.30	0.36
	sem	0.37	0.08	0.40	0.09
	N	17	17	12	15
Fatty liver	$\bar{x}$	19.02 *	5.13 *	34.40	3.57 *
	SD	3.52	0.80	1.20	0.96
	sem	0.75	0.17	0.30	0.21
	N	22	22	17	22
Change from control (%)		+18.9	+19.3	N.C.	+26.6

* $p < 0.01$

Table 21: Enzyme levels in livers from normal and fatty liver rats

Group	statistics	E N Z Y M E S (U / gr protein)												
		AST	ALT	ALP	ACP	ME	G6PDH	6PGDH	ICDH	MDH	LDH	ALD	SDH	G-GT
Control	$\bar{x}$	298	162	0.80	46.2	10.3	17.2	26.5	151	2468	1480	35.5	94.9	1.36
	SD	61	30	0.17	4.9	5.8	11.3	8.5	22	539	361	6.7	22.3	0.52
	sem	17	8	0.05	1.7	1.4	2.3	2.8	5	128	87	3.4	5.4	0.23
	N	13	13	12	8	17	16	9	17	17	17	4	17	5
Fatty liver	$\bar{x}$	404*	125*	0.72	42.0	27.8**	57.6**	43.1**	165	3405**	2033**	69.1**	72.5	2.89*
	SD	99	45	0.30	5.5	11.7	28.4	15.8	88	913	444	37.5	16.4	1.29
	sem	26	12	0.07	1.7	2.3	6.2	4.0	17	179	89	11.3	3.2	0.58
	N	14	14	21	10	25	21	16	26	26	25	11	26	5
Change from control (%)		+35.6	-22.8	-10	-9.1	+170	+235	+63	+9.3	+38	+37	+95	-24	+113

* $p < 0.05$ ** $p < 0.01$

Table 22 Enzyme levels in normal and parts of fatty livers from rats.

Group	E N Z Y M E S (u/gr protein).											
	AST	ALT	ALD	ALP	ME	ICDH	G6PDH	6PGDH	MDH	LDH	SDH	
Control	$\bar{x}$	246	79	35.5	0.60	5.8	135	12.7	35.0	2937	1459	91
	sem	34	28	3.9	0.07	0.6	12	2.1	3.0	208	125	13
	N	5	5	4	4	9	9	9	5	9	9	6
Fatty liver -non fatty part	$\bar{x}$	320	87	48.7	0.55	22.6	152	52.7	59.7	4326	1946	59.4
	sem	43	52	11.1	0.04	2.6	9	8.0	5.2	165	103	6.8
	N	4	4	4	4	7	8	8	5	8	7	8
Fatty liver -fatty part	$\bar{x}$	289	63	53.3	0.51	16.2	146	27.0	44.0	3682	1523	71.8
	sem	10	9	5.5	0.07	1.7	19	5.0	2.5	297	179	15.3
	N	4	4	4	4	8	8	8	5	8	8	8

* significantly different from control. $P < 0.05$

** " " " " $P < 0.01$

Table 23: Correlations and degree of significance between liver and serum enzymes from rats with fatty liver

Liver serum	ME	ICDH	G6PD	6PGD	AST	ALT	ALP	LDH	GGT
MDH					-0.5952 0.001 39				0.7223 0.018 10
LDH					0.6116 0.001 62	-0.5000 0.001 43			0.6436 0.104 10
ALP	0.5439 0.001 49		0.4820 0.001 41	0.5032 0.004 51			0.5441 0.001 39		
Total lipids	0.6332 0.001 63		0.6420 0.001 48					0.5242 0.001 63	
TGL	0.5952 0.001 33		0.6677 0.001 20					0.5578 0.001 33	-0.9341 0.05 4
GGT	-0.6195 0.1896 6				-0.8134 0.05 6				

T A B L E 24 Levels of various blood constituents in rats after liver intoxication with CCl_4 and during liver disease introduced by ligation of the common bile duct (CBDL)

		Blood constituents								
		AST U/1	ALP U/1	ACP U/1	ME U/1	ICDH U/1	G6PD U/1	MDH U/1	LDH U/1	lipids g/l
Control	X	37	35	17.7	6.7	5.4	2.77	315	824	257
	SD	3	6.0	1.4	4.6	4.2	1.83	150	242	030
	SEM	1.4	2.5	0.6	1.5	1.1	0.61	36	59	0.15
CCl ₄	X	121	62	55	6.8	15.8	-	354	1082	230
	SD	33	23	7	7.0	8.5	-	59	332	033
	SEM	15	10	3	3.1	3.8	-	26	148	0.14
CBDL (3 days)	X	563	84	23		74	-	971	1061	-
	SD	710	33	5		100	-	1000	1200	-
	SEM	300	15	2,2		44	-	450	540	-
fatty liver	X	60	85	-	12.4	4.8	2.76	560	905	3.57
	SD	15	44	-	4.3	2.9	2.90	373	308	0.96
	SEM	3	9	-	1.0	0.7	0.84	79	66	0.21

TABLE 25a: Enzymes (units / liter) and metabolites (grams / liter) in the blood of animals
with normal and fatty liver

	C O W	R A T	G E E S E	C H I C K	H E N	B R O I L L E R *	
ME	C	10.7 ⁺ -1.5	12.46 ⁺ -1.20	2.5 ⁺ -1.6	0	6.7 ⁺ -0.7	5.4 ⁺ -0.4
	E	12.8 ⁺ -3.0	12.37 ⁺ -0.97	182 ⁺ -12	30.9 ⁺ -3.6	7.6 ⁺ -1.8	4.8 ⁺ -0.6
ICDH	C	32.4 ⁺ -2.8	5.43 ⁺ -1.13	18.0 ⁺ -2.1	11.3 ⁺ -1.1	11.4 ⁺ -1.5	28.7 ⁺ -2.1
	E	49.8 ⁺ -5.9	4.76 ⁺ -0.74	57 ⁺ -3.6	8.7 ⁺ -0.7	7.6 ⁺ -0.8	26.1 ⁺ -4.3
G6PDH	C	2.58 ⁺ -0.39	2.77 ⁺ -0.61	0	-	-	-
	E	4.03 ⁺ -0.51	2.76 ⁺ -0.84	0	-	-	-
LDH	C	1690 ⁺ -285	824 ⁺ -59	289 ⁺ -53	670 ⁺ -49	918 ⁺ -67	1421 ⁺ -142
	E	3106 ⁺ -460	905 ⁺ -66	643 ⁺ -106	577 ⁺ -48	1177 ⁺ -77	1558 ⁺ -171
MDH	C	2888 ⁺ -425	315 ⁺ -36	276 ⁺ -34	590 ⁺ -24	453 ⁺ -39	1318 ⁺ -100
	E	3769 ⁺ -647	560 ⁺ -79	1191 ⁺ -135	359 ⁺ -26	506 ⁺ -62	900 ⁺ -135
GLDH	C	8.50 ⁺ -131	-	-	-	-	-
	E	8.39 ⁺ -2.6	-	-	-	-	-
SDH	C	6.7 ⁺ -3.8	-	-	-	-	-
	E	3.1 ⁺ -0.9	-	-	-	-	-
6PGDH	C	-	12.55 ⁺ -0.95	-	-	-	-
	E	-	14.21 ⁺ -1.33	-	-	-	-
GGT	C	-	17.4 ⁺ -0.6	-	-	-	-
	E	-	8.1 ⁺ -1.5	-	-	-	-

T A B L E 25b: Enzymes (units / liter) and metabolites (grams / liter) in the blood of animals with normal and fatty liver

	C O W	R A T	G E E S E	C H I C K	H E N	B R O I L E R *
ALD	C - E -	48.8 ⁺ 2.1 59.7 ⁺ 2.0	- -	- -	- -	- -
AST ^h	C 257 ⁺ -42 E 229 ⁺ -27	50.9 ⁺ -2.8 60.4 ⁺ -3.3	17.8 ⁺ -2.0 43 ⁺ -3.0	126 ⁺ -8 108 ⁺ -9	91 ⁺ -6 124 ⁺ -5	82 ⁺ -4 80 ⁺ -9
ALT	C 10.8 ⁺ -1.8 E 7.8 ⁺ 1.7	8.11 ⁺ 0.63 13.68 ⁺ 2.15	- -	- -	- -	- -
ALP	C 31.3 ⁺ -3.7 E 45.2 ⁺ -9.3	82.7 ⁺ -8.2 84.7 ⁺ -9.4	50.7 ⁺ -2.7 24.0 ⁺ -3.1	834 ⁺ -80 1170 ⁺ -114	151 ⁺ -23 165 ⁺ -46	1074 ⁺ -100 1287 ⁺ -148
AcP	C 7.8 ⁺ -1.1 E 12.1 ⁺ -2.1	- -	11.3 ⁺ -1.0 32.9 ⁺ -2.6	- -	- -	- -
TOT PROT	C 67.6 ⁺ -2.5 E 70.8 ⁺ -1.6	- -	58.7 ⁺ -2.6 50.6 ⁺ -3.5	- -	- -	38.6 ⁺ -1.2 35.5 ⁺ -0.90
ALB	C 36.8 ⁺ -0.9 E 37.0 ⁺ -1.0	34.4 ⁺ -0.40 34.40 ⁺ -0.30	15.4 ⁺ -0.50 11.55 ⁺ -0.99	- -	- -	16.0 ⁺ -0.30 17.7 ⁺ -0.90
TOT FAT	C 7.35 ⁺ -0.73 E 4.18 ⁺ -0.67	2.82 ⁺ -0.09 3.57 ⁺ -0.21	6.24 ⁺ -42 14.10 ⁺ -131	1.03 ⁺ -0.20 34.54 ⁺ -1.64	23.5 ⁺ -2.8 64.1 ⁺ -5.9	3.94 ⁺ -0.19 4.38 ⁺ -0.25

* 40 days old

T A B L E 26a: Enzyme levels (units / gram protein) in livers of animals.

	C O W	R A T	G E E S E	C H I C K (49 days old)	H E N	B R O I L E R (40 days old)
ME	C 0.94±0.09 E 2.16±0.47	10.3±5.8 27.8±11.7	35±1 235±19	174±17 212±10	70.3±14.1 19.3±2.2	80.2±7.5 117±12.3
ICDH	C 92±9 E 133±7	151±22 165±88	157±18 84±8	149±7 113±5	134±10 158±15	255±25 305±20
36PDH	C 1.85±0.52 E 3.43±0.38	17.2±11.3 57.6±28.4	86±19 59±5	- -	- -	- -
LDH	C 517±42 E 1292±100	1480±87 2033±89	918±89 5741±344	3304±245 2439±179	814±80 810±48	1834±151 1999±167
ADH	C 1016±56 E 1358±117	2468±128 3405±179	1173±68 3271±342	3200±374 3474±315	3090±265 2817±258	2422±275 3303±192
SDH	C 79.1±13.3 E 27.5±5.11	94.9±5.4 72.5±3.2	7.4±0.6 2.8±0.3	106±9 80±10	- -	- -
GLDH	C 12.4±2.3 E 17.3±6.7	- -	- -	- -	- -	- -
AST	C 229±14 E 217±9	298±17 404±26	152±19 391±58	407±32 376±29	194±21 134±13	232±31 238±16

T A B L E 26b: Enzyme levels (units / gram protein) in livers of animals

	C O W	R A T	G E E S E	C H I C K (35 days old)	H E N	B R O I L E R (40 days old)
ALT	C 1.71±0.33 E 2.93±1.00	162±8 125±12	28±6.4 40±5.4	- -	- -	- -
ACP	C 11.33±0.61 E 10.78±0.59	46.2±1.7 42.0±1.7	26.4±1.6 35.0±4.1	- -	- -	- -
ALP	C 3.60±0.57 E 6.36±0.72	0.80±0.05 0.72±0.07	5.4±0.8 5.1±0.8	8.64±0.39 9.59±1.29	1.82±0.18 1.95±0.28	2.43±0.21 2.73±0.24
ALD	C - E -	25.5±3.4 69.1±11.3	- -	- -	- -	- -
6PGDH	C - E -	26.5±2.8 43.1±4.0	- -	- -	- -	- -
GGT	C - E -	1.36±0.23 2.89±0.58	- -	- -	- -	- -

T A B L E 27: Change of liver enzymes in fatty liver

ANIMAL	E N Z Y M E											
	ME	ICDH	G6PDH	6PGDH	LDH	MDH	GLDH	ALP	AST	SDH	GT	ALT
Geese	↑	↓	↓		↑	↑			↑	↓		↑
Broiler	↑	↑				↑						
Hen	↓	↑							↓			
Non-laying hen	↑	↓			↓	↑				↓		
Rat	↑		↑	↑	↑	↑	ALD ↑		↑	↓	↑	↓
Cow	↑	↑	↑		↑	↑	↑	↑		↓		

FIG. 1 MALIC ENZYME LEVEL IN THE SERA OF GEESE AT VARIUOS TIMES OF FORCE FEEDING.

FIG. 2 ISOCITRIC DEHYDROGENASE LEVEL IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING.

FIG. 3 ALKALINE PHOSPHATASE IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING.

FIG. 4. ASPARTATE AMINOTRANSFERASE IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING.

FIG. 5 LACTIC DEHYDROGENASE IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING.

FIG. 6 ALBUMIN IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING.

FIG. 7 TOTAL LIPIDS IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING.

FIG. 8. RATIO OF MALIC DEHYDROGENASE TO ALBUMIN IN SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING

FIG. 9. RATIO OF MALIC ENZYME TO ASPARTATE AMINOTRANSFERASE IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING

FIG. 10 RATIO OF MALIC ENZYME TO ALBUMIN IN THE SERA OF GEESE AT VARIOUS TIMES OF FORCE FEEDING

FIG. 11. CORRELATION BETWEEN SERUM ME AT DAY 25 AND GOOSE LIVER SIZE AT SLAUGHTER.

FIG. 12. CORRELATION BETWEEN SERUM MALIC DEHYDROGENASE AT DAY 25 AND LIVER SIZE AT SLAUGHTER.

FIG. 13

Malic dehydrogenase in the sera of Geese
at Various time of force feeding

Figures 14-21: Levels of enzymes, albumin and lipids in the sera of
 fatty liver geese at various times of cramming.

⑧ gray (G) and white (W)

Fig 16

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Fig 17

Fig. 18.

Fig 19

Fig 20

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Fig 21

Pictures 1-5: Thin-layer chromatography of lipid extracts from liver and sera of geese, chicken and cows, with various degrees of liver fattening.

Pic. 1 GEESE - LIVER

NUMBERS RESPONDING TO LIVER WEIGHT:

1 = 30 DAYS - 700 GR. ;	2 = 30 DAYS - 500 GR.
3 = 19 DAYS - 110 GR. ;	4 = 26 DAYS - 400 GR.
5 = CONTROL - 105 GR. ;	6 = 19 DAYS - 200 GR.
7 = 26 DAYS - 300 GR. ;	8 = CONTROL - 100 GR.

Pic. 2. GEESE - SERUM

NUMBERS RESPONDING TO DAYS OF FORCE FEEDING:

- 1 = 1 ; 2 = 4 ; 3 = 8 ; 4 = 11 ; 5 = 24 ; 6 = 29

Pic 3

NON-LAYING HENS - SERUM

1 - 3 FATTY LIVER ; 4 - 6 CONTROL.

Pic 4

NON-LAYING HENS - LIVER

1 - 3 FATTY LIVER ; 4 - 6 CONTROL.

Pic. 5. COWS - LIVER

1 - 4 NORMAL ; 5-- 8 FATTY LIVER

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BENEFIT TO AGRICULTURE

The present study revealed some of the basics leading to liver fattening. The problem of fatty liver in farm animals can be divided into two: a) animals in which fatty liver is not desired and result from toxicological causes and metabolic disorders (cattle, poultry etc.) b) animals in which fatty liver is wanted (geese for "Pate" production).

The information in this study can lead to: a) detection of the syndrome by means of clinical biochemistry techniques b) establish means and ways to prevent its formation c) promote it and d) select some of the biochemical parameters as genetic markers for selecting animals with a tendency to form fatty liver (either for prevention or promotion).

Fatty liver in farm animals causes tremendous annual economic losses because of poor production and higher frequency of various other metabolic and production diseases. Early detection of the syndrome, before the appearance of the clinical signs, could allow the treatment and prevention of larger losses. Up to now no specific biochemical test was available for the detection of fatty liver. One of the accomplishments of the present study was in establishing such parameters (which varied for the different animals). These methods have been introduced to our laboratory practice with very good success (cattle, poultry and geese).

In case of geese on the other hand, with the aid of the described biochemical parameters can help in predicting the approximate live size at slaughter at about 2 weeks prior to the end of cramming. Predicting liver size can help in deciding the optimal time of slaughter, thus saving labor and feed and increasing profitability. Another research project which resulted from this study is the use of the biochemical parameters for selection of large liver producing geese. Doing so will lead to the selection of geese families with genetic characteristics of large liver production in a significantly shorter time.

Last, but not least, a great deal of information on liver fattening was gained which will allow further future studies on this topic for the promotion of agricultural animal farming.

DESCRIPTION OF COOPERATION

Cooperation between R. A. Freedland from University of California, Davis, School of Veterinary Medicine, U. S. A. and E. Bogin, from Kimron Veterinary Institute, Israel was restricted to exchange of information during the stages of planning the project and throughout the duration of the project. R. A. Freedland visited Israel, where the experiments were conducted, during which time the results obtained were evaluated and future experiments were prepared. At a later stage, E. Bogin visited U. C. D. campus with R. A. Freedland, and the results were analyzed and evaluated. During this time some enzymological techniques were learned by E. Bogin for later use in the research project.

R. A. Freedland, who has a great experience in the field of liver enzymes, especially in relation to liver fat metabolism, inspired and guided this research project all along the way.

LIST OF PUBLICATIONS

At present, after the data analysis and evaluation, several manuscripts are being prepared on the topics of biochemical changes associated with liver fattening and induction of enzymes, in the various animal models studied. Some of the data was presented in national scientific meetings. Reprints of the works will be sent at a later date.