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EFFECT OF FEED DEPRIVATION ON THE BLOOD LEVELS OF GLUCOSE, β-HYDROXYBUTYRATE, TRIGLYCERIDES, AND CHOLESTEROL AND ON BODY WEIGHT AND YOLK SAC WEIGHT IN ONE-DAY-OLD BROILER CHICKS

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RUNNING HEAD: Feed Deprivation and blood metabolites in day-old broiler chicks

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The effect of feed deprivation on mean serum levels of glucose, β- hydroxybutyrate, triglycerides and cholesterol and on body and yolk sac weights was determined using three hundred and twenty one-day-old broiler chicks. The chicks were either fed ad libitum or deprived of feed for 5 days. Observations were made on chick behaviour. Feed deprived and fed chicks had similar serum glucose levels, except for day 5, when serum glucose levels decreased sharply in the feed deprived chicks, suggesting a shift from fats to glucose as a source of energy. The mean levels of β-hydroxybutyrate in the serum of the deprived chicks were higher ($P<.05$) and triglycerides were lower ($P<.05$) than those in the fed group on days 1,2 and especially on day 3. A significant ($P<.05$) decrease was observed in serum cholesterol levels in the fed group throughout the experimental period. The fed group exhibited heavier ($P<.01$) body weights than the starved chicks. However, no significant difference was found in yolk sac weight between the two groups, which suggests that the rate of utilization of the yolk sac was not different. Feed deprivation in newly-hatched chicks results in stressful behavior and mortality rates were 2.5, 21.25, and 15 per cent on the 3 rd, 4th and 5th day of feed deprivation, respectively. None of the birds died in the control group. The study suggests that metabolism in newly-hatched feed-deprived broiler chicks might be different from mammals and adult chickens. Further investigations are needed.

Key words: Day-old chicks, blood glucose, β-hydroxybutyrate, cholesterol, triglycerides, body weight.

INTRODUCTION

An estimated 10% of imported day-old chicks die from starvation in transit in Jordan. Feed deprivation in day-old chicks increases their susceptibility to diseases and can result in poor productivity and decreased profitability. The World Bank (1990) suggested that if the production efficiency indicators (feed conversion efficiency, daily gain and mortality) are improved, productivity of the poultry sector in Jordan could be increased by 5-10 per cent.

A relationship between fasting during the first few days of life and subsequent survival rate and productivity of broiler chickens is expected to exist. Warris et al. (1992) suggested that transportation of newly hatched chicks from hatcheries to rearing units caused weight loss and increased mortality as a result of starvation and stress. It is important to understand the physiological changes that occur in the starved day-old chick and the possible implications on chick survival and productivity.

Day-old chicks that are deprived of feed for up to 48 hours tend to rely on fatoriented metabolism to satisfy their energy needs. The chicks will lose weight, metabolise fat and have a concomitant increase in plasma β-hidroxybutyrate (BHB) levels (Warris et.al., 1992). During periods of glucose deprivation, circulating glucose concentrations fall and fatty acid mobilization and oxidation increase (Radcliffe et al., 1983).

In comparison with mammals, birds generally resist starvation over very long periods (Hazelwood, 1972). Fasting chickens have been reported to maintain high levels of glucose (Evans and Scholz, 1971; Belo et al., 1976 and Brady et al. 1978). In the emperor penguin male, which endures a 4-month fast during the breeding cycle, lipids are the major fuel (LeMaho et al., 1976). Additionally, in starving geese, lipid metabolism accounted for 95% of the energy expenditure (Benedict and Lee, 1937). In mammals, such as humans and dogs, the fasting state is characterized by a decrease in plasma glucose levels accompanied by an increase in ketone levels (Cahill et. al., 1966; Owen et al., 1969; Brady et al., 1977) indicating that fat metabolism is the principal source of energy for the fasted animals.

This experiment was designed to investigate the effect of feed deprivation for up to 5 days on body weight, mortality rate, and on the blood levels of glucose, BHB, cholesterol and triglycerides (TG). Observations on behavioral changes of the starved birds are reported.

METHODS

Three hundred and twenty one-day-old broiler chicks were obtained from a commercial source (Khalil Alia Co., Co., Irbid, Jordan) and kept in a room at an ambient temperature of 28-30^0 C. The chicks were divided into two groups of 160 chicks each and were assigned at random to either an experimental or a control treatment. Chicks in the control group were fed ad libitum a standard chick starter mash, while those in the experimental group were fasted for 5 days. Water was available at all times to chicks in both groups, and the light was on for 24 hours a day.

At the beginning of the experiment (day 0), and after weighing, 1 ml blood samples were drawn from the jugular vein of each of 20 chicks in each group. Chicks were then killed by cervical dislocation and the weight of the yolk sac was obtained.

Similarly, on the next 4 consecutive days of fasting,blood samples, body weight and yolk sac weight were obtained each day from 20 chicks randomly selected from each group. On the 5th day, all chicks that remained in the fasted group (10 chicks) and 10 chicks from the fed group were individually weighed, blood samples were collected and yolk sac weight was obtained from each bird. Sera were separated by centrifugation for 5 minutes at 3,000 X g.

Serum levels of glucose were determined by an enzymatic colorimetric reaction, namely, the glucose oxidase para-amino antipyrine peroxidase (GOD-PAP) method using a commercial kit (Arab Company for Medical Diagnostics, Amman, Jordan). Serum levels of BHB were determined by a kinetic enzymatic method using a commercial kit (Randox Lab., Ltd., Ireland) TG levels were determined by an enzymatic, liquid colorimetric test, namely, glycerol-3-phosphate oxidase para-amino antipyrine peroxidase (GPO-PAP) using a commercial kit (Crescent Diagnostics, Amman, Jordan). Cholesterol levels were determined by a similar method employ ing cholesterol oxidase para-amino antipyrine peroxidase (CHOD-PAP) using a commercial kit (Crescent Diagnostics, Amman, Jordan).

Behaviour assessments were made following the description employed by Houpt (1958) for fasted chicks. The criteria used were: activity level, distribution, posture, chirping, consumption of water, voiding of watery urine-feces, pecking on objects.

Statistical Analysis: Statistical analyses were performed using SYSTAT (Wilkinson, 1986). The Tukey-Kramer test in the STATS module of SYSTAT was utilized to examine the differences in the mean levels of glucose, BHB, triglycerides, cholesterol, body weight and yolk sac weight between and within the experimental and control groups. Within group comparisons of day 0 values for each variable were also made.

RESULTS

Figure 1 presents changes in the mean serum levels of glucose in feed-deprived (treatment) and fed chicks. In the fed group, the levels of serum glucose increased on days 1,2,3 and then decreased on days 4 and 5. Within the groups, the mean glucose levels in the fasted group were significantly higher ($P<0.05$) on days 2 and 3 than on day zero. Mean serum glucose levels were slightly but significantly ($P<0.05$) less on days 1 and 2 in the treatment group compared with the fed group. On day 5, the difference ($P<0.05$) in serum glucose levels between the two groups was approximately 4 mmol/ml in favour of the fed group.

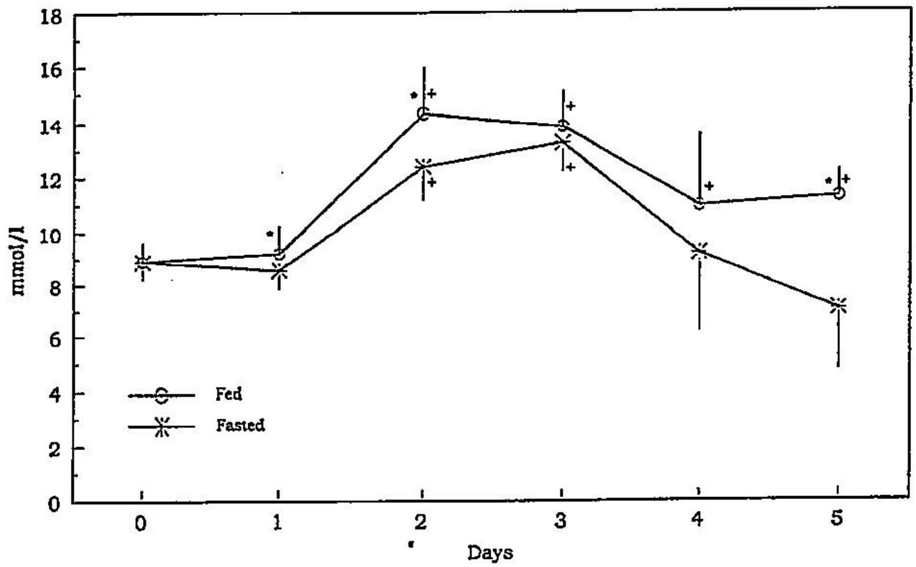

Figure 1. Changes in mean serum glucose level in fasted and fed newly-hatched broiler chicks.
*= Significant between groups, and
+ = Significant within groups, (P<0.5)

The changes in the mean serum levels of BHB in feed-deprived and in the fed groups are shown in figure 2. The levels of BHB in serum of the fasted group were significantly higher (P<0.05) than those in the fed group on days 1 and 2 especially on day 3. Within the treatment group there were no significant (P>.05) differences in the BHB values compared with the reading on day 0. In the fed group BHB levels were significantly less (P<0.05) on days 1,2,3,4 and 5 than on day zero.

Figure 3 shows changes in the mean values of serum triglyceride levels in tretment and fed groups. In the feed-deprived group, serum triglyceride levels were increased on day 1 of fasting and then declined on days 2 and 3. A slight increase in serum triglycerides was observed on days 4 and 5 in the fasted group. In the fed group, the levels of serum triglyceride showed an increase on day 1 which was sustained at the same level till the 3rd day, after which they sharply decreased on day 4. In the starved group the levels of triglyceride were significantly less (P<0.05) than those in the fed group on days 1,2 and 3. No significant difference was observed in serum TG levels between the two groups on days 4 and 5. When comparing the values during days of fasting with the zero reading for each group, significant differences (P<0.05) were found on days 1, 3 and 4 in the treatment group and on days 1,2 and 3 in the fed group.

While a slight decrease in the serum cholesterol level was observed in the feeddeprived chicks, a significant decrease was observed in the fed group throughout the experimental period. The differences in serum cholesterol levels

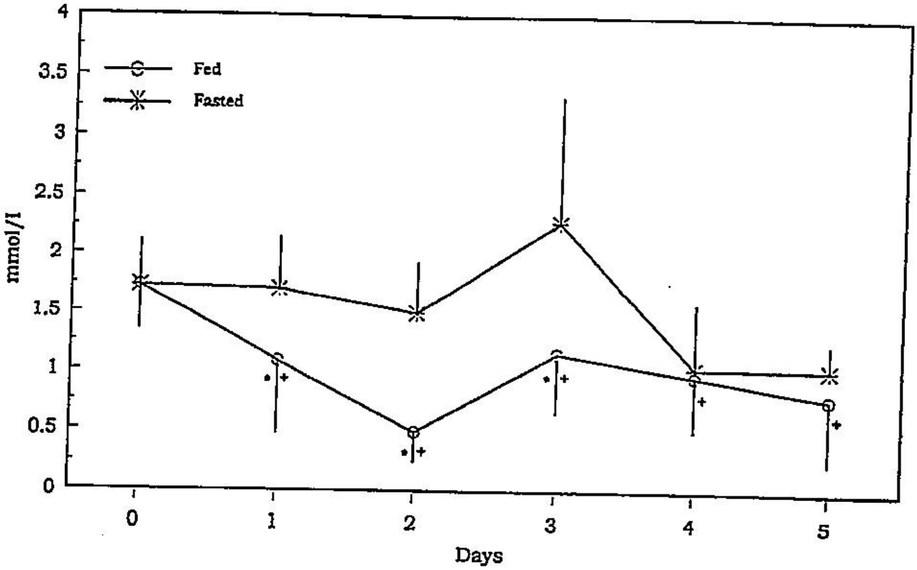

Figure 2. Changes in mean serum β-hydroxybutyrate level in fasted and fed chicks * = Significant between groups, and
+ = Significant within groups, (P <.05)

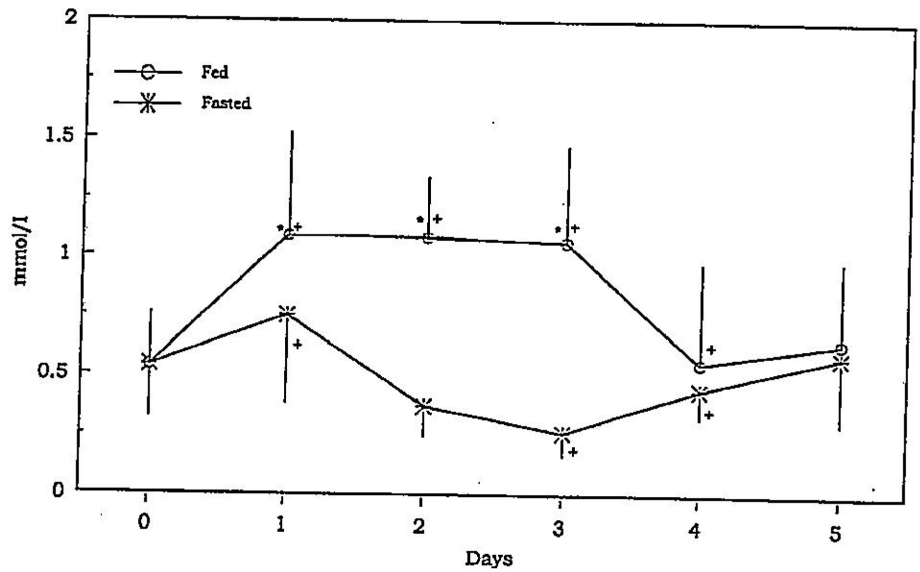

Figure 3. Changes in mean serum triglyceride level in fasted and fed groups of chicks.
* = Significant between groups, and
+ = Significant within groups, (P<0.5)

between groups were significant on each day (P<0.01). A greater decline (from 13 to 4 mmol/l) in mean serum cholesterol level was observed in the fed group compared with that of the treatment group (Figure 4). Within group comparison revealed that there were significant differences (P<0.05) in mean serum cholesterol levels on days 1,2,3,4 and 5 in the fed group.

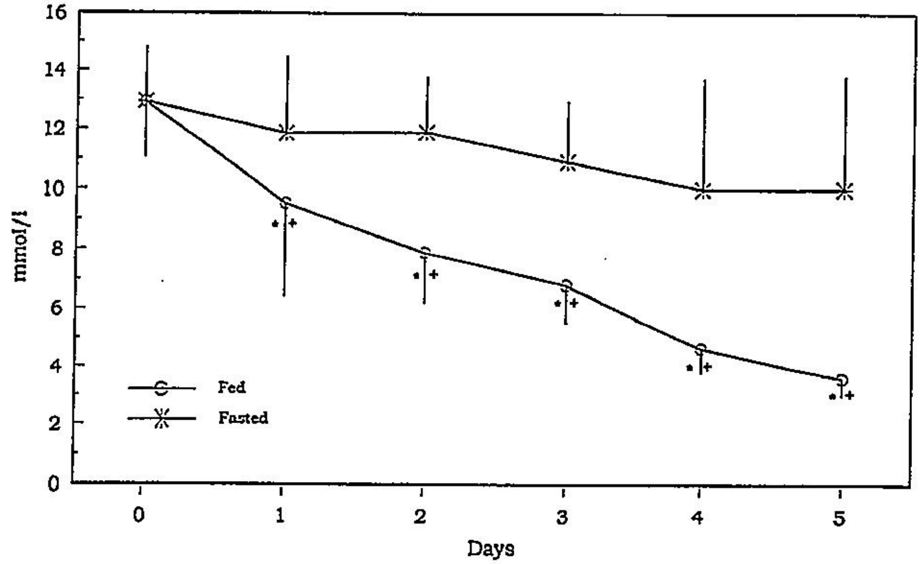

Figure 4. Changes in mean serum cholesterol level in fasted and fed chicks
* = Significant between groups, and
+ = Significant within groups, (P<.05)

Body Weight: Changes in the mean body weight in the feed-deprived and fed groups are shown in figure 5. The treatment group lost weight and the fed group gained weight until the end of the experimental period. There were significant (P<.01) differences between mean body weight of treatment and fed groups on days 1,2,3,4 and 5. In the treatment group, mean body weights on day 3,4 and 5 differed slightly (P<0.05) from those obtaind on day 0, while in the fed group body weights progressively increased.

Yolk Sac Weight: The mean yolk sac weight in both the fed and the treatment groups decreased steadily until the 5th day of fasting (Figure 6). No significant difference was found between the two groups in the mean yolk sac weight, which suggests that the rates of utilization of the yolk sac were not different in the fed and the fasted group. However, in both groups, the mean values of yolk sac weight were significantly lower (P<0.05) on all days of the experiment than the initial value obtained on day 0. The yolk sac weight decreased steadily with increased days of the experiment in both experimental groups.

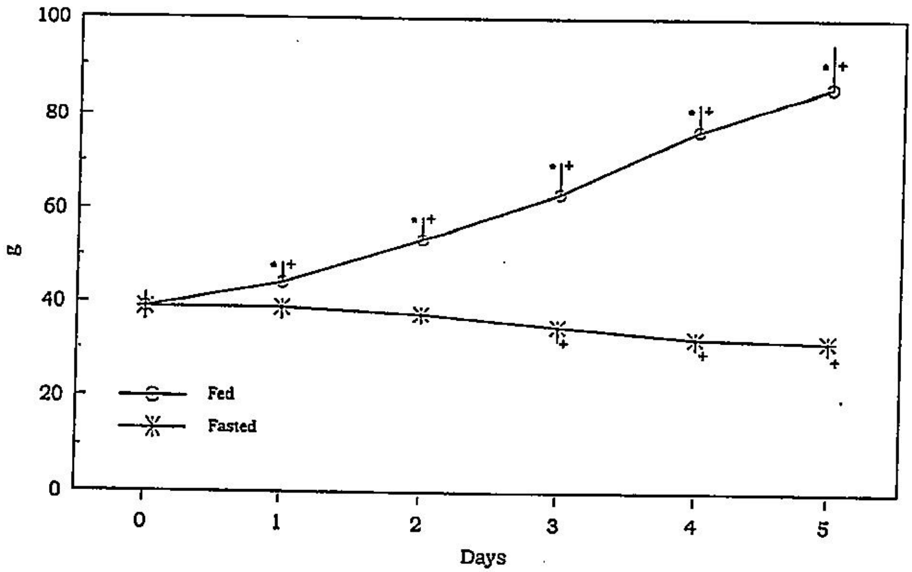

Figure 5. Changes in mean body weight in fasted and fed newly-hatched broiler chicks.
* = Significant between groups, and
+ = Significant within groups, (P <.05)

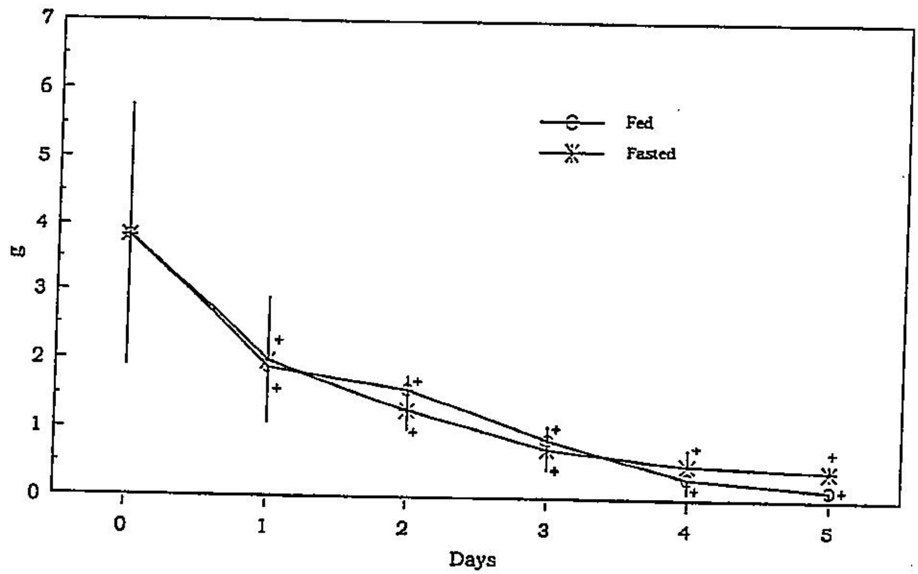

Figure 6. Changes in mean yolk sac weight in newly-hatched broiler chicks.
* = Significant between groups, and
+ = Significant within groups, (P<.05)

In the treatment group, significant positive correlations were observed between body weight and both blood triglyceride levels and yolk sac weight. However, in the fed group significant negative correlations were found between body weight and both triglyceride levels and yolk sac weight.

Behavior of chicks and mortality: In contrast to the chicks, which were active, chirped normally, and were evenly distributed in the brooder after 24 hours, feed-deprived chicks appeared distressed, remained less active, and tended to huddle together near the heat source and started to peck each other and any objects in the pen. While ad libitum fed chicks voided normal feces, feed-deprived chicks voided clear watery feces-urine. They drank excessive quantities of water and often pecked at any object including other chicks. Feed-deprived birds chirped in a distressful fashion. As Houpt (1958) observed, and after another 2 to 3 days, the distress symptoms intensified and the birds started to look depressed. Thus, the wings drooped and the feathers became ruffled Many chicks assumed a hunched posture. The mortality rate in the fasted group was 2.5, 21.25, and 15 per cent on the 3rd, 4th and 5th day of feed deprivation, respectively. None of the birds died in the control group. The livers of the starved birds had a yellowish discoloration not observed in the birds in the control group.

DISCUSSION

Except for minor difference in serum glucose levels on the third day of the experiment, overall the results of this study showed that feed-deprived and fed newlyhatched chicks had similar mean serum glucose levels. These results are in agreement with those of Houpt, (1958); Halzelwood and Lorenz (1959) and Nir et al. (1973). Houpt (1958) observed that in newly-hatched chicks there was a gradual increase to near normal levels in the blood sugar by the 3rd day of fasting. However, the significant differences that were observed between the two groups on the 5th day might be due the fact that the chicks started to use glucose as an energy source instead of fat. Belo et al. (1976) found no significant changes in plasma glucose levels following starvation in older chickens fasted for 72 hours. The increase in glucose levels as feed-deprivation continued could largely be due to the shift in metabolism and the mobilization of body fat to be utilized as replacement for glucose as an energy source.

The higher levels of BHB and the lower serum levels of TG in the treatment group compared with those of the fed group, further indicates that fat was mobilized for energy. Our results are notin agreement with Belo et al. (1976) who found a 3-5 fold increase in BHB in 5 to 6-month-old chicks, fasted for up to 72 hours, and Brady et al. (1978) who found a 10-fold increase in BHB in 34-to 40-day-old chicks. Increases in mean serum BHB levels have also been observed in mammals, such as sheep (Bouchat et al., 1981), dogs (Brady et al., 1977) and humans (Ruderman et al., 1976). On the other hand, the increase in serum levels of TG, the decrease in BHB and glucose on the 4th and 5th day of feed deprivation indicates a shift from fat to glucose utilization as an energy source. Protein catabolism provides amino acids for glucose production (Young, 1970; Issekutz, 1977), so that body protein is important for maintaining carbohydrate status in fasted newly-hatched turkey poults (Donaldson et al., 1994).

It is well known that ketones may serve as an alternate energy substrate to glucose (Owen et al., 1967; Cahill et al., 1969), thus sparing body protein which would otherwise be used for glucose synthesis. In this study, the initial high level of BHB observed in both groups could have been due to the fact that the chick embryo used the yolk sac as the main source of energy, however further work is need to substantiate this (Warris et al., 1992). Although in both groups, changes in yolk sac weight were similar, the reliance of feed-deprived chicks on fat as a source of energy may explain the significant increase in BHB levels in their serum compared with thase of the fed group. On the other hand, the decline in PHB till the end of the fast in the deprived group may be attributed to the decrease in lipid stores. In the fed group, PHB decreived group may be attributed to the decrease in lipid stores. In the fed group, PHB decreased significantly from an intial level of 1.732 mmol/L to a lower level of 0.787 mmol/L on the 5th day. This decline can be attributed to the increased level of glucose supported by feeding, which means that fed chicks might have made a shift to wards glucose as a source of energy. This is supported by the findings of Warris et al. (1992) who indicated that in fed newly-hatched chicks the fast reduction in plasma BHB and the increase in plasma glucose concentrations indicates a shift to carbohydrate-oriented energy metabolism when newly-hatched chicks were fed. Further, Demigne and Remesy and Remesy (1977) observed that an injection of glucose into fasted sheep decreased ketogenesis. However, Brady et al. (1978) found that BHB values in fasted chickens increased dramatically, reflecting either increased production or decreased utilization.

The difference in serum TG levels between the fed and feed-deprived chicks may be related to several factors. Although fat oxidation provides most of the energy needs after hatching (Moran, 1988), in the newly-hatched fed chicks there is an initial increase in TG in the first 3 days, which is transferred from the yolk sac to extrahepatic tissues during embryonic life, due to lack of an efficient mechanism for utilization of TG (Noble and Moore 1967, a&b; Noble and Shand, 1985). However, in the fasted chicks, TG remains the main source of energy (Best, 1966). On the fourth day, the fasted chicks probably shifted to glucose, provided by protein catabolism as mentioned earlier, as a source of energy. Therefore, no difference in mean serum TG levels were observed on day 4 and day 5 between the two treatment groups. Our results for TG are not consistent with those reported by Nir et al. (1973) who found no changes in plasma triglycerides of cockerels and geese during starvation.

The differences between the two groups in mean serum cholesterol levels can be explained by the fact that cholesterol is normally used in the liver to synthesize the bile acid, leading to decreased serum levels in the fed chicks. In feed-deprived chicks, the function of the hepatocytes might have been disturbed, leading to accumulation of fat in the liver. This could lead to accumulation of cholesterol in the liver and its consequent reabsorption in the blood (Al-Rawashdeh, 1988). Changes of cholesterol levels in newly-hatched chicks confirmed our previous findings in fasted sheep (Al-Rawashdeh, 1988).

The decrease in body weight which was proportional to the duration of the fast was in agreement with the findings of Houpt (1958). In our study we recorded an 18.64% body weight loss by the end of the fasting period. Nir et al. (1973) reported a body weight loss of 16.5% and 25.5% in geese and cockerels respectively after 8 days of fast. Also, Belo et al. (1976) found a 9.56% body weight loss in 4- to 5-month-old chicks fasted for 72 hours. Warris et al. (1992) demonstrated large body weight losses in feed deprived newly-hatched chicks that were in transit and attributed the weight loss mainly to the reliance of the chicks on fat metabolism.

In contrast to the findings of Bierer and Eleazer (1965) who observed that the weight of yolk sac in fed baby chicks was lower than feed and water deprived chicks the similarity in yolk sac weight on any day of this experiment, in the fed and fasted group suggests, that the utilization of the yolk sac in fasted and fed baby chicks was also similar. In both fed and fasted chicks the yolk had almost disappeared by the end of experiment. This is in agreement with the findings of Lesson et al. (1978) who indicated that in broiler chicks maternal yolk is absorbed and utilized by the first week after hatching. However, the manner in which absorbed fats are utilized might have been different in the fasted and fed groups.

This study confirmed earlier findings (Houpt, 1958) that feed deprivation in chicks results in stressful behavior and increased mortality as fasting progressed. The lipidoriented metabolism, especially with regards to BHB in chicks, might be different from older chickens. Further, the mechanism of metabolism in newly hatched chicks might be different from that in adult chickens and mammals, a premise that needs further investigation.

A c k n o w l e d g e m e n t:

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EFEKAT GLADOVANJA NA NIVOE GLUKOZE, BETA OKSI BUTERNE KISELINE, TRIGLICERIDA, HOLESTEROLA U KRVI, TELESNU MASU I TEŽINU ŽUMANČANE KESE KOD JEDAN DAN STARIH PILIĆA BROJLERA

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SADRŽAJ

U radu je ispitivan uticaj gladovanja na srednje vrednosti koncentracije glukoze, beta oksi buterne kiseline, triglicerida, i holesterola u krvi kao i na telesnu masu i masu žumančane kese kod jednodnevnih brojlerskih pilića. Utvrđeno je da kod piladi izloženoj u toku prvih 5 dana starosti gladovanju, dolazi do značajnog smanjenja koncentracije glukoze, triglicerida i holesterola u krvi dok se koncenracija beta oksi buterne kiseline značajno povećala. Ispitujući promenu težine žumančane kese kod ogledne i kontrolne grupe piladi u toku vremena trajanja ogleda, utvrđeno je da nije bilo značajne razlike.