4

BARD

FINAL REPORT
PROJECT NO. IS-1234-87

הספריה המרכזית
לכתיבה חזקלאות
בית-17

**Growth Stimulation and Improved Feed
Efficiency by Feed Restriction in Chickens and
Turkeys**

**S. Hurwitz, M. Pines, I. Plavnik, J.P. McMurtry,
R.W. Rosenbrough**

1992

000000229883

36908
Date: 22/1/92

BARD

P.O. Box 6

Bet Dagan, ISRAEL

BARD Project No. IS-1234-87C

Title

Growth Stimulation and Improved Feed Efficiency by Feed Restriction in Chickens and Turkeys

RECEIVED
FEB 10 1992
11 11

<i>Investigators' Names</i>	<i>Investigators Institution</i>
S. Hurwitz	ARO, The Volcani Center, Bet Dagan, Israel
M. Pines	ARO, The Volcani Center, Bet Dagan, Israel
I. Plavnik	ARO, The Volcani Center, Bet Dagan, Israel
J.P. McMurtry	Nonruminant Research Laboratory, USDA, Beltsville MD
R. Rosebrough	Nonruminant Research Laboratory, USDA, Beltsville MD

Project's starting date: August 16, 1988

Type of Report : Final

Principal Investigator

Shmuel Hurwitz

Institutions Authorizing Official

D. Rimon

636.072
BAR/HUR
2nd copy

: 636.508.5 : 636.592.085 : 636.089.2398:
591.192.7

3-0-

b. TABLE OF CONTENTS

c. ABSTRACT.....	3
d. OBJECTIVES.....	4
e. RESEARCH REPORTS.....	5
1. Performance of broiler chickens and turkey poults subjected to feed restriction or to feeding of low-protein or low-sodium diets at an early age.....	6
2. Response of broiler chickens and turkey poults to food restriction of varied severity during early life.....	14
3. Characterization of growth and development of male British United Turkeys.....	24
4. Modeling of the dynamics of accelerated growth following feed restriction in chicks.....	30
5. Prediction of the response of chicks to early-age feed restriction: Model validation.....	41
6. Tibia development in turkeys and chickens as affected by early-age feed restriction.....	65
7. Sexual development in male turkeys as affected by early age feed restriction.....	87
8. The effect of quantitative feed restriction in young poults on the performance of meat-type turkeys (field study).....	97
9. Effect of feed restriction on growth, feed efficiency, carcass composition and metabolic hormones in chickens and turkeys.....	100
10. A comparison between constant-protein, calorie-restricted and protein-restricted diet on growth, <i>in vitro</i> lipogenesis and plasma growth hormone, thyroxine, triiodothyronine and somatomedin-C (Sm-C) of young chickens.....	120
11. <i>In vitro</i> lipogenesis and glucose production in chickens exposed to protein/amino acid restriction.....	127
f. COOPERATION.....	134
g. EVALUATION OF RESEARCH ACHIEVEMENTS.....	135
h. LIST OF PUBLICATIONS.....	136

c. ABSTRACT

The results of the present project, inclusive of a large field experiment, substantiate the findings of the previous BARD project (I-735-84) which suggested a practical benefit due to improved feed efficiency and carcass quality in chickens and turkeys as a result of early-age feed restriction. The existence of compensatory growth of variable magnitude, following a large range of feed restriction conditions in terms of severity, duration, and some nutritional deficiencies such as protein or sodium, was validated. Furthermore, compensatory growth and improved feed efficiency were observed in several broiler strains characterized by a different growth potential. In effect, the large range of responses could be predicted fairly accurately by a single mathematical model. Thus, for practical implementation, economic consideration remains the main determinant of a suitable restriction regime, within the general context of nutrition and environment. The restriction-compensation model has been now incorporated into a general computerized optimization algorithm previously constructed as part of BARD project US-777-86.

In the search for a physiological mechanism, we found no specific involvement of the epiphyseal growth plate of the long bones, and no change in mineral gain in the compensatory growth response. In turkeys, muscle gain was specifically promoted by feed restriction. In chicks, this is also suggested by the increase in gain of carcass water. During feed restriction, lipogenesis decreases, whereas during refeeding, the normal temporal pattern of *in vivo* fat deposition changes so that for the first six weeks, fat deposition is considerably reduced.

Circulating levels of growth hormone and IGF-I were reduced by feed restriction. In another study, however, growth hormone level was increased during restriction. A severe energy restriction resulted in a reduction in triiodothyronine and an increase in the ratio of thyroxine to triiodothyronine. During refeeding, the normal decline in growth hormone was attenuated while IGF-I concentration remained similar to that the non-restricted birds. The level of testosterone was higher in restricted-refed than in *ad libitum*-fed turkeys and may thus play an important role in promoting muscle growth.

c. OBJECTIVES

- a. To elucidate the mechanism of growth stimulation by early restriction.
- b. To determine breed differences in the response to feed restriction.
- c. to evaluate the responses in terms of growth, feed efficiency and carcass composition
- d. To examine the feed restriction technique under semi-practical conditions.

e. RESERACH REPORTS

***In vitro* Lipogenesis and Glucose Production in Chickens
Exposed to Protein/ Amino Acid Restriction**

R. Rosebrough

*Nonruminant Animal Nutrition Laboratory, United States Department of Agriculture-
Agriculture Research Service, Beltsville Agricultural Research Center, Beltsville MD
20705, U.S.A.*

FINAL REPORT

There are findings characterizing physiological responses to meal feeding and intermittent feeding in rodents. Both these feeding regimens increase *de novo* lipogenesis, carcass and liver fat, and improve dietary energy utilization. Early reports showed that periodic feeding regimens increase *in vitro* lipid metabolism in chickens but do not improve energetic efficiency. We found that intermittent-feeding cycles or daily changes in the dietary protein level also change lipid metabolism. The alteration in metabolism is similar to the intermittent-feeding response noted in older chickens.

An overall comparison between the effects of diet composition and feeding regimens reveals that feeding low-protein diets as a part of an alternating-high-low-protein feeding cycle and refeeding fasted chickens result in similar responses. In both cases, *de novo* lipid synthesis increases. In contrast, feeding high-protein diets and fasting chickens decrease *de novo* lipogenesis. It is of interest if both these types of nutritional regimens can be used on a chronic basis to adapt intermediary metabolism in the broiler to further increase lipogenesis during the respective refeeding and low-protein phases of the two feeding programmes.

The purpose of the following section was to examine *in vitro* lipogenesis and glucose production in chickens subjected to chronic or acute bouts of either an intermittent- or alternating-protein feeding regimen. The overall hypothesis was that an alternating-protein diet series would result in a metabolic response similar to the refeeding response noted during the intermittent-feeding regimen. Circulating metabolic hormone levels were also measured during chronic or acute dietary treatment regimens. We were especially interested in the adaptation chickens subjected to repeated bouts of high-low-protein feeding and fasting-refeeding compared to chickens subjected to single bouts of these two regimens.

Broiler chickens growing from 7 to 28 d of age were fed: (1) a 210 g protein/kg control diet for the entire experimental period, (2) an intermittent regimen (210 g protein/kg diet for either 1 or 2 d followed by a 1-d fast) or 3) a daily change in the dietary protein level from 120 to 300 g/kg diet. Treatment variables examined were *in vitro* lipogenesis, glucose production, circulating concentrations of insulin, triiodothyronine (T₃), and thyroxine (T₄) to determine the effects of chronic or acute dietary

treatments. Feeding the 300 g protein/kg diet or withholding feed for 1 d decreased ($P<0.05$) *in vitro* lipogenesis compared to controls. Feeding the 120 g protein/kg diet or refeeding a 210 g protein/kg diet for 1 or 2 d increased ($P<0.05$) *in vitro* lipogenesis compared to controls. Glucose production was affected in the same manner. Fasting decreased ($P<0.05$) plasma insulin and T_3 and increased T_4 . Both refeeding or a low-protein diet increased T_3 . Refeeding increased and a low-protein diet decreased insulin. Chronic use (7-28 d of age) of either an alternating-protein or intermittent-feeding regimen caused greater responses compared to acute bouts (single cycle) of either of the regimens.

Although the level of dietary protein and of limiting amino acids influence both lean and adipose tissue development in broiler chickens, interpretation of effects can be confounded by dietary energy intake. Interpretation of the effects of both dietary carbohydrate and protein on lipogenesis in chickens is further complicated, even in isocaloric diets, by difficulties in dietary formulations. For example, an increase in dietary protein is normally accompanied by a decrease in the dietary carbohydrate level. The importance of dietary carbohydrate was illustrated by Clark *et al.* (1980) who found little relationship between the level of the dietary fat and *de novo* lipogenesis when

chicks were provided the same amount of dietary carbohydrate in all dietary treatments. In contrast, the role of dietary protein in the regulation of lipid metabolism in the chick appears to be more complex than that of dietary fat. For example, it can be shown that an improvement in dietary protein quality (addition of the limiting amino acid lysine to a low protein diet) depressed *de novo* lipogenesis in chicks. Confounding the role of amino acid supply as a regulator of lipogenesis in all birds was the observation that turkey poults overeat diets containing a wide calorie:protein ratio ($>17:1$), even when these diets are supplemented with limiting amino acids to meet requirements (NRC, 1984). The net effect is still an increase in lipogenesis even in the presence of an ideal dietary amino acid profile.

Thus, in defining the mechanisms surrounding the role of dietary protein in avian lipid metabolism, one must look farther than just meeting the amino acid requirement for a particular stage of growth. The liver is main site of *de novo* lipid synthesis in birds as well as a major point of degradation and transamination of dietary amino acids. We have found active net synthesis of glucose (gluconeogenesis) in chicks fed high-protein diets ($>23\%$) and have speculated that carbon skeleton rearrangement

during gluconeogenesis directed substrates from lipid synthetic pathways and required energy which could be used for lipogenesis. We are unaware any of work describing gluconeogenesis, lipogenesis and endocrine function under defined energy and protein intakes because some form of limited feeding regime is necessary to separate the effects of dietary protein and energy intake. The experiments described in the next section were designed to test the effects of absolute protein and energy intakes on avian carbohydrate and lipid metabolism.

Ross male broiler chicks growing from 14 to 28 days of age were fed 14 and 20% protein diets (4 Kcal day⁻¹/BWt^{0.66}) or 20 and 28% protein diets (2.8 Kcal day⁻¹/BWt^{0.66}) in a 2 x 2 factorial arrangement to determine the effects of protein and energy intakes on *in vitro* lipogenesis (IVL) and net glucose production (NGP). Plasma concentrations of insulin, glucagon, thyroid hormones (T₃ and T₄) and somatomedin-C (Sm-C) were estimated by radioimmunoassay. There was a significant (P<0.05) decrease in IVL in the chicks given the higher daily protein intake. The higher protein intake increased (P<0.05) NGP while the lower energy intake decreased (P<0.05) NGP. Insulin, both thyroid hormones and Sm-C were affected by dietary energy and protein intakes.

Diets containing large energy to protein ratios (>13 MJ, 72 MJ/kg crude protein) promote high rates of *in vitro* lipogenesis as well as *de novo* carcass lipid synthesis by the liver. Diets with small energy to protein ratios (<13 MJ, 56 MJ/kg crude protein) promote lean broiler carcasses. We have recently examined energy to protein ratios and have found different possible interpretations of results. For example, feeding a very small energy:protein diet (13 MJ, 43 MJ/kg crude protein) will result in a very lean carcass (crude protein as a % of dry matter) when compared to results attained by feeding a larger energy:protein diet (13 MJ, 65 MJ/kg crude protein). When the data were analyzed on the basis of grams of nitrogen deposited over the course of the experiment, it became obvious to us that the diet containing a larger energy: protein ratio favoured the deposition of more carcass protein as well as lipid. Thus, diets containing very small energy:protein ratios may promote lean carcasses by naturally restricting feed intake. Bartov (1979) has also proposed that the excretion of excess excess amino acid nitrogen would require energy.

Both the quantity (crude protein concentration) and quality (amino acid composition relative to the required balance) of the dietary protein affect the body composition of chicks. The effective protein level can be calculated according to the lysine content

relative to the crude protein level. It was assumed that a completely effective protein contained 60 g of lysine per kg of protein. Although the crude protein level (g/kg diet), as well as the effective dietary protein level (% of the requirement of the first limiting amino acid present in the diet), may regulate lipogenesis, it is unknown if the effect is due to the presence of a limiting amino acid at the ribosomal level or to a shift in cellular metabolism caused by a need to process excess amino acid carbon. In addition the process of converting gluconeogenic amino acid carbon into glucose carbon, reducing equivalents (NADH-NADPH) normally required for the addition of acetyl residues during *de novo* lipogenesis are utilised and may be unavailable for lipogenesis.

The purpose of the experiments that follow was to examine lipid and carbohydrate metabolism in chickens fed additional amounts of the the limiting amino acid lysine as either lysine.HCl or soya-bean meal. The null hypothesis tested was that lysine supplementation of a low-protein diet would influence metabolism similarly to a diet containing a greater amount of crude protein. In the former case, the effective protein level (g lysine per kg of crude protein) would increase and in the latter case, the effective level would remain at 60 g lysine per kg of crude protein. A maize-soya-bean meal basal diet was formulated to be marginally adequate for all essential amino acids with the exception of lysine. Crystalline lysine.HCl was then added to give a total of 12g/kg of diet.

Chickens were fed diets containing either 12.8 MJ, 150 g crude protein/kg or 12.8 MJ, 200 g crude protein/kg to determine differences in metabolism. The diet containing 12.8 MJ, 150 g crude protein/kg contained either 8 or 12 g lysine/kg. Treatment variables examined were *in vitro* lipogenesis, glucose production and hepatic enzyme activities to compare metabolism in chicks fed a low-protein, lysine-supplemented diet and a diet formulated to contain the required amount of lysine from intact protein. Growth was similar in chicks fed diets containing either 12.8 MJ, 154 g crude protein-12 g lysine/kg or 12.8 MJ, 200 g crude protein/kg. Net glucose production was greater ($P < 0.05$) in liver explants from chickens fed diets containing either 12.8 MJ, 154 g crude protein-12 g lysine/kg or 12.8 MJ, 200 g crude protein/kg than in explants from chickens fed 12.8 MJ, 150 g crude protein-8 g lysine/kg. Pyruvate use for glucose production was greater ($P < 0.05$) in chickens fed a diet containing 12.8 MJ, 150 g crude protein-8 g kg lysine/kg. The data in this study suggest that crystalline and "natural" lysine additions to chick diets may influence metabolism differently.

We have also used a severe nutrient restriction (6 d at maintenance energy intake beginning at 6 d of age) to study both short and long-term repletion patterns in broilers (Rosebrough *et al.*, 1986). In some cases, there is a disproportional reduction in abdominal fat in the 7 wk old broiler which can be attributed to a severe energy restriction during the second week of life. We have noted a 4 fold increase in the apparent rate of lipid synthesis during the short-term repletion period (5 d following relief from restriction). Thus, physically restricting a balanced diet containing a required level of protein does not appear to cause long-term growth retardation. This finding contrasts with those of Lauterio and Scanes (1987) describing an elegant pattern in the development of hormone metabolic profiles that can be severely altered by offering diets containing less than 100 g crude protein/kg. These authors also indicated that when protein restrictions were begun early in life, body weight gain did not approach that of controls several weeks after repletion. All studies, including our own, should be cautiously interpreted because repleted chickens never consume the same amount of feed as controls during a corresponding time period.

The purpose of the experiments in the following section was to examine the adaptation of chickens during repletion from low- and adequate-protein diets. A secondary purpose was to alter lipogenesis by dietary treatments and to then determine if a β -adreneregic agonist decreased *in vitro* lipogenesis equally. An equal decrease on a percentage basis. Allred and Roebrigg (1973) established that acetyl coenzyme A carboxylase (AcCbx) activity could be increased or decreased *in vitro* depending upon dibutyl cyclic adenosine monophosphate (cAMP) levels. Peptide hormones such as catecholamines or their synthetic analogs such as isoproterenol interact with cell membrane receptors to change intracellular concentrations of cAMP which change metabolic processes. It is true that describing the absolute mechanism involving cAMP and lipogenesis in chick liver appears to be more involved than just presuming the existence of a cAMP-dependent protein kinase-catalyzed phosphorylation of a rate-limiting enzyme (AcCbx) as is common in describing regulation in rat liver. For example, the level of cellular citrate is particularly important in the regulation of the interconversion of the protomeric to the polymeric form of AcCbx with the latter form being the so called active form. Therefore, regulation of glycolytic flux and subsequent formation of citrate influences *de novo* lipogenesis.

Experiments were conducted with Cobb male broiler breeder chickens to determine the metabolic effects of two levels of dietary crude protein fed during a repletion

regimen on subsequent responses to a β -adrenergic agonist (isoproterenol) *in vitro*. Day-old chickens were fed diets containing either 12 or 20% crude protein until 14 d of age and then switched to the opposite diets until 28 d. In Exp 1, chickens were killed at 28 d and in Exp 2, at intervals following the switch at 14 d. *In vitro* lipogenesis (IVL) and lipolysis (IVLY) were determined as the incorporation of 10 mM [2- 14 C]acetate $\pm$ 10^{-4} M isoproterenol into hepatic lipids and glycerol release from adipose tissue over the course of a 2-hr incubation at 37 C, respectively. Certain hepatic enzyme activities (isocitrate dehydrogenase; ICD, malic enzyme; ME, and fatty acid synthetase; FAS) were determined during the 14 to 28-day repletion period. Both IVL and IVLY were greater at 28 d when chickens were fed a 12% than a 20% protein diet. Neither 0 to 14-d dietary treatment influenced 28-d metabolic parameters. In contrast, the 12% protein diet depressed 14-d weight (160 g vs 320 g; $P < .05$) and gain during the 14 to 28-d period regardless of protein repletion levels (508 g vs 626 g; $P < 0.05$). Both processes were at their highest point 4 d following the dietary switch. Isoproterenol decreased IVL 70% in all dietary treatment groups. Stimulated lipolysis was not affected by nutritional status and was 2x the unstimulated rate. Enzyme activities followed trends noted in explants. It can be concluded from this study that most metabolic responses to severe dietary challenges occur within 1 to 3 d and that a steady state is reached within 4 d.

f. COOPERATION

The research program conducted in the two laboratories was coordinated. For example different strains of broilers were tested in both locations in order to obtain a larger range of conditions applicable in either country. Work with turkeys in the U.S. was started as a joint venture of the U.S.A. team and one of the Israeli coinvestigators (I. Plavnik) during a sojourn in Beltsville. Contact was kept for consultations between the teams.

g. EVALUATION OF RESEARCH ACHIEVEMENTS

Following the initial establishment of the feed restriction methodology and a basic optimization algorithm in the previous BARD projects, the main practical achievement of the present study is the establishment of the response to feed restriction under a large variety of conditions in terms of mode, severity and duration of feed restriction and in various strains. Formulation of the compensatory growth into a mathematical model is used to simulate the responses of body weight and feed efficiency so that the appropriate regimen can be selected on the basis of economic profitability. This economic optimization is facilitated by an improved computer program.

The simulated response to feed restriction was appropriately validated under laboratory conditions. Early-age feed restriction was field-tested in turkeys and found to be economic beneficial: Feed efficiency was improved by 2.9% and the productive index by 8.6%. A major American broiler producer (4 million broilers per week) adopted the method, and according to their nutritionist an improvement of 1 to 2 percentage points in feed efficiency is constantly obtained with a benefit of several million dollars annually. Details have not been disclosed due to commercial reasons.

At least a part of the mechanism of compensatory growth may be associated with growth hormone and/or testosterone. The latter explains the better response to feed restriction observed in males.

h. PUBLICATION LIST*

- Plavnik, I., and Hurwitz, S. Response of broiler chickens and turkey poults to feed restriction of varied severity during early life. *Brit. Poult. Sci.* 32: 347-356, 1991.
- Plavnik, I. and Hurwitz, S. Performance of broiler chickens and turkey poults subjected to feed restriction or to feeding of low-protein or low-sodium diets at an early age. *Poult. Sci.* 69:945-952, 1990.
- Hurwitz, S., Talpaz, H., Bartov, I., and Plavnik, I. Characterization of growth and development of male British United turkeys. *Poult. Sci.* 70: 2419-2424.
- Rosebrough, R., McMurtry, J., Proudman, J., and Steel, N. Comparison between constant-protein, calorie-restricted and protein-restricted, calorie-restricted diets on growth, *in vitro* lipogenesis and plasma growth hormone, thyroxine, triiodothyronine and somatomedin-C (SmC) of young chicks. *Comp. Biochem. Physiol.* 93A: 337-343, 1989.
- Rosebrough, R.W. Glucose production and glycogen cycle enzyme activities in avian liver explants: production optimization. *Comp. Biochem. Physiol.* 96B: 163-170, 1990.
- Talpaz, H., Sharpe, P.J.H., Wu, H.-I., Plavnik, I., and Hurwitz, S. Modeling of the dynamics of the accelerated growth following feed restriction in chicks. *Agric. Sys.*, 36: 125-135, 1991.

* Does not include abstracts, papers submitted for publications or in preparation.