PENGARUH PEMBERIAN PAKAN BERPROTEIN TINGGI SELAMA REFEEDING TERHADAP AKUMULASI LEMAK PADA BROILER

U. Santoso[1]

INTISARI

Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian pakan berprotein tinggi selama pemberian pakan bebas setelah periode pembatasan pakan terhadap akumulasi lemak pada broiler. Broiler betina umur 7 hari didistribusikan ke 5 kelompok perlakuan dengan 3 replikasi dan setiap replikasi terdiri dari 15 ekor. Satu kelompok diberi pakan bebas sebagai kontrol dan 4 kelompok lainnya dibatasi pakannya menggunakan metode "a skip-a-day feeding" selama 6 hari. Dari umur 14-21 hari, broiler diberi pakan bebas dengan kadar protein 21, 21, 25, 30 dan 35%. Hasil penelitian menunjukkan bahwa pembatasan pakan tidak berpengaruh terhadap kadar *cholesterol ester*, *triglyceride*, *free cholesterol* dan *phospholipid* di hati, dan secara nyata menurunkan kadar triglyceride, free cholesterol dan lemak karkas ($P<0,05$). Pakan berkadar protein 35% menaikkan kadar free cholesterol hati ($P<0,05$) dan *phospholipid* ($P<0,01$). Kadar protein pakan 25-35% mempunyai kadar *triglyceride* dan *free cholesterol* karkas yang lebih rendah dibandingkan kadar protein dengan 21%. Kadar protein pakan 35% menurunkan kadar lemak karkas ($P<0,05$) dengan kadar protein karkas yang meningkat. Dapat disimpulkan bahwa pemberian pakan berprotein tinggi mampu menghambat peningkatan akumulasi lemak yang disebabkan oleh pembatasan pakan.

(Kata kunci: Pakan berprotein tinggi, Refeeding, Akumulasi lemak, Broiler).

Buletin Peternakan 26 (1) : 7 - 12, 2002

[1] Jurusan Peternakan, Fakultas Pertanian, Universitas Bengkulu, Bengkulu.

EFFECT OF HIGH-PROTEIN REALIMENTATION DIET ON LIPID ACCUMULATION IN BROILER CHICKS

ABSTRACT

This study was conducted to evaluate effect of high-protein realimentation diet on lipid accumulation of broiler chicks. Seven-day-old female broiler chicks were distributed to five treatments with three replicates of 15 chicks each. One treatment was fed *ad libitum* as a control, while the other four treatments were subjected to feed restriction from 7-13 day of age. From 14 to 21 day of age, the chicks of the five treatment groups were fed diets containing 21, 21, 25, 30 or 35% protein. Feed restriction had no effect on liver cholesterol ester, triglyceride, free cholesterol and phospholipids ($P<0.05$), but it significantly reduced carcass triglyceride, free cholesterol and fat ($P<0.05$) and significantly increased carcass moisture ($P<0.05$). However, fat accumulation in liver and carcass were stimulated upon refeeding. The 25% protein realimentation diet increased liver free cholesterol ($P<0.05$) and phospholipids ($P<0.01$). The 25-35% protein realimentation diet increased liver cholesterol ester ($P<0,01$). The 25%-30% protein realimentation diet reduced carcass triglyceride and free cholesterol as compared with 21% protein. The 35% protein realimentation diet reduced carcass fat, whereas carcass protein was increased. In conclusion, feed restriction for 6 day reduced carcass fat content, and increased carcass fat content during refeeding. The 35%-protein realimentation diet could prevent an increase in fat content during refeeding period.

(Key words: Feed restriction, High-protein realimentation diets, Fat accumulation, Broilers)

Introduction

Feed restriction was known to reduce lipid accumulation in broiler chicks (Santoso *et al.*, 1993, 1995a), and, therefore, it could reduce the risk of lipid abnormalities in chickens. However, a lower lipid accumulation during feed restriction period could not be maintained when broiler chicks were refed after feed restriction ended. Higher lipid accumulation during refeeding was partly caused by a drastic increase in fatty acid synthesis mainly during the fist week after feed restriction ended (Rosebrough *et al.*, 1986). Therefore, an attempt should be conducted to maintain lower lipid accumulation during the first week after feed restriction. Santoso (1995) found that lowering lipid accumulation during the first week after feed restriction would produce lower lipid accumulation at market age.

Toyomizu *et al.* (1992) found that high protein diet could reduce lipid accumulation in broiler chicks. Therefore, the present study was conducted to evaluate effect of high-protein realimentation diet on lipid accumulation during the first week after feed restriction ended.

Materials and Methods

One-day-old female broiler chicks were obtained from a commercial hatchery (225, strain Chunky), weighed, and randomly distributed among 15 pens with 15 birds per pen. From 1 to 14 day of age, supplemental heat was provided with a hanging heat lamp. The broilers were maintained on the floor in a windowless house, under continuous fluorescent lighting with feed and water available for ad libitum consumption except during the period of feed restriction.

At 7 day of age, three pens of 15 chicks each were randomly assigned to one of five treatments. One treatment (as the control) involved ad libitum access to a 21% crude protein (CP) diet, and four treatments were subjected to feed restriction (broilers were

deprived of feed 1 day and consumed a 21% CP feed ad libitum on the alternate day) from 7 to 13 day of age. From 14 to 21 day of age, chicks were fed realimentation diet containing 21, 21, 25, 30, or 35% protein realimentation diet for the ad libitum group and four restricted groups, respectively. Composition of experimental diets were published elsewhere (Santoso *et al.*, 1995b).

Six chicks at 7 day of age and 30 chicks each at 14 and 21 day of age were killed by decapitation, and the liver and abdominal fat pad were removed and weighed. All chicks were withheld from feed 12 hour prior to killing. The body without ingesta was removed, feathers, and liver were also obtained. Each body, including abdominal fat, was frozen and stored in a sealed plastic bag at –30 C prior to subsequent analysis. The frozen bodies were again cut into small pieces with a knife and ground through a 5-mm screen. The ground bodies were passed through the mincer five times to obtain uniform mixing. Fat, moisture, and protein contents of carcasses were determined by the method of AOAC (1980).

Various lipid fractions in the liver and carcass were separated by thin layer chromatography on silica gel chromarod using hexane-diethyl ether-formic acid (85:15:0.15) as developing solvent, and quantified by IATROSCAN TH-10 TLC/FID Analyzer (Santoso *et al.*, 1995b).

Data for all response variables were subjected to ANOVA (Shinjo, 1990) as one-way arrangement of treatments. Variables among the treatments were compared using Duncan's Multiple Range Test if there was a significant overall treatment effect.

Results and Discussion

Table 1 shows effect of high-protein realimentation diet on lipid accumulation in the liver. Feed restriction for 6 day had no effect on cholesterol ester, triglyceride, free cholesterol and phospholipid in the liver. High-protein realimentation diet significantly increased hepatic cholesterol ester. Total cholesterol in the liver was significantly increased in 30-35% protein groups.

Table 2 shows effect of high protein realimentation diet on lipid accumulation in the carcass. Feed restriction significantly reduced carcass triglyceride, free cholesterol ($P<0.01$), but had no effect on cholesterol ester and phospholipid. High protein realimentation diet (25-35% CP) was significantly effective to lower carcass triglyceride and free cholesterol ($P<0.01$). High protein realimentation diets significantly reduced carcass phospholipid.

Table 1. Effect of high-protein realimentation diets on hepatic lipid accumulation (mg/g) in broiler chicks

Variable	Control	21% CP	25% CP	30%CP	35% CP
Restriction Period					
Cholesterol ester	0.10 ± 0.009[1]	0.15 ± 0.036			
Triglyceride	4.18 ± 0.02	4.72 ± 0.04			
Free cholesterol	1.54 ± 0.01	1.49 ± 0.02			
Phospholipid	21.70 ± 0.29	18.50 ± 0.14			
High-protein Realimentation Diet					
Cholesterol ester	0.16 ± 0.02^{a}	0.11 ± 0.01^{a}	0.59 ± 0.03^{b}	1.04 ± 0.03^{c}	1.31 ±0.04^{c}
Triglyceride	6.38 ± 0.05^{b}	6.60 ± 0.13^{b}	5.22 ± 0.35^{ab}	4.50 ± 0.02^{a}	4.22 ± 0.02^{a}
Free cholesterol	1.50 ± 0.09^{a}	1.41 ± 0.03^{a}	1.43 ± 0.02^{a}	1.41 ± 0.03^{a}	2.13 ± 0.02^{b}
Phospholipid	16.50 ± 0.79^{b}	20.70 ± 0.97^{ab}	16.40 ± 0.87^{a}	20.2 ± 0.49^{ab}	25.9 ± 0.73^{b}

[1] Values reported represent for 6 chicks ± SE.
Means within a row not followed by the same superscripts are significantly different.

Table 2. Effect of high-protein realimentation diets on carcass lipid accumulation (mg/g) in broiler chicks

Variable	Control	21% CP	25% CP	30%CP	35% CP
Restriction Period					
Cholesterol ester	0.30 ± 0.03[1]	0.27 ± 0.04			
Triglyceride	2860 ± 1.38[b]	14.12± 0.31[a]			
Free cholesterol	0.98 ± 0.19[b]	0.39 ± 0.02[a]			
Phospholipid	3.37 ± 0.26	3.25± 0.33			
High-protein Realimentation Diet					
Cholesterol ester	0.32± 0.06	0.30 ± 0.06	0.24 ± 0.02	0.36 ± 0.03	0.22 ± 0.04
Triglyceride	35.2 ± 0.88[c]	55.4 ± 0.7[d]	38.8 ± 0.83[c]	24.2 ± 0.26[b]	15.9 ± 0.8[a]
Free cholesterol	1.89± 0.08[b]	2.65 ± 0.04[c]	1.78± 0.05[b]	1.69 ± 0.08[b]	1.28 ± 0.07[a]
Phospholipid	8.44 ± 0.11[b]	5.21 ± 0.15[a]	4.80± 0.08[a]	3.79 ± 0.21[a]	4.16 ± 0.05[a]

[1] Values reported represent for 6 chicks ± SE.
Means within a row not followed by the same superscripts are significantly different.

Table 3. Effect of high-protein realimentation diets on body composition (%) in broiler chicks

Variable	Control	21% CP	25% CP	30%CP	35% CP
Restriction Period					
Moisture	72.9 ± 0.7[1]	75.3 ± 0.2			
Fat	7.7 ± 0.6[b]	5.2 ± 0.5[a]			
Protein	13.5 ± 0.2	14.5 ± 0.2			
Ash	5.9 ± 0.3	5.0 ± 0.3			
High-protein Realimentation Diet					
Moisture	69.2 ± 0.9	68.9 ± 0.4	68.8 ± 0.9	72.7 ± 0.8	72.1± 0.5
Fat	11.0 ± 0.7[b]	12.4 ± 0.5[b]	12.2 ± 0.4[b]	10.8 ± 0.5[b]	8.2 ± 0.4[a]
Protein	12.3 ± 0.6[a]	13.1± 0.6[a]	13.5 ± 0.6[a]	14.5 ± 0.8[ab]	15.5 ± 0.4[b]
Ash	6.5 ± 0.21[b]	5.6 ± 0.25[b]	5.5 ± 0.18[b]	2.0 ± 0.21[a]	3.8 ± 0.15[a]

[1] Values reported represent for 6 chicks ± SE.
Means within a row not followed by the same superscripts are significantly different.

Table 3 shows effect of high protein realimentation diets on carcass composition. Feed restriction significantly reduced carcass fat. 35% protein realimentation diet significantly reduced carcass fat ($P<0.05$) and increased carcass protein ($P<0.05$).

Yeh and Leveille (1970) found that feed restriction resulted in lower hepatic fatty acid synthesis in chicks. A lower hepatic fatty acid synthesis would lower hepatic triglyceride synthesis (Scorve *et al.*, 1993) and would result in lower hepatic triglyceride. However, the present study shows no change in hepatic triglyceride. It is possible that feed restriction resulted in lower VLDL-triglyceride release into the bloodstream, and therefore resulted in lower carcass triglyceride content with no change in hepatic triglyceride.

The present results agree with the observation of Boekholt *et al.* (1994) who found that feed restriction resulted in lower body fat and slightly higher body protein. They found that at lower energy intake body fat was mobilized whereas protein was deposited.

Ramirez *et al.* (1984) found that feed restriction decreased 3-hydroxy-3-methylglutaryl-CoA reductase activity, the rate-limiting enzyme for cholesterol synthesis, indicating a decrease in cholesterol synthesis in the liver. A

lower hepatic cholesterol synthesis may partly explain lower body cholesterol observed in the present study.

Refeeding resulted in a drastic increase in fatty acid synthesis (Rosebrough *et al.*, 1986). This may explain a higher triglyceride content of carcass in the present study. During this period, high-protein diet may able to decrease in an increase in fatty acid synthesis stimulated by refeeding, and therefore high-protein realimentation diet during refeeding could maintain normal body triglyceride and even could reduce below the control group. It is well established that fatty acid synthesis was depressed by high-protein diet (Mizuno *et al.*, 1979; Yeh and Leveille, 1972). Lower fatty acid synthesis may explain lower triglyceride content of carcass in restricted chicks fed high-realimentation diet. The Reduction of fatty acid synthesis by high-protein diet would also reduce the substrate for phospholipid synthesis. Therefore, it is logical if phospholipid content of carcass became lower when restricted chicks were refed with high-protein diet.

It is unknown why body cholesterol was higher during refeeding as compared with the control. It is possible that refeeding also resulted in a drastic increase in cholesterol synthesis or more rapid removal of cholesterol or both. High protein realimentation diets lower an increase in body cholesterol stimulated by refeeding. Yeh and Leveille (1973) found that the hypocholesterolemic effect of dietary protein is mediated in part through a more rapid removal of cholesterol from the blood and excretion in the feces as cholesterol and bile acids.

Hayashi *et al.* (1990) found that both the rates of muscle protein synthesis and breakdown were extremely stimulated by feed restriction followed by refeeding, but since the rate of synthesis was higher than that of the breakdown, higher body protein content resulted. High protein realimentation diet increases body protein content further. Reeds (1989) stated that increases in protein intake above those required for nitrogen equilibrium clearly accelerate body protein synthesis in young animals.

The present study shows that high-protein realimentation diet reduced body ash indicating that high-protein impaired mineralization (Hulan, 1980). Diets high in protein can interfere with folic acid metabolism and in so doing impair mineralization (Wong *et al.*, 1977). It also has been reported that a high-protein intake causes an increase in urinary calcium and phosphorus (Funaba *et al.*, 1989) and thus impaired mineralization (Funaba *et al.*, 1989; Weiss *et al.*, 1981).

Conclusion

In conclusion, early feed restriction program for 6 day from 7 day of age till 13 day of age lowered carcass fat accumulation in broiler chicks. High-protein realimentation diet could prevent an increase in fat accumulation stimulated by feed restriction during refeeding period. High-protein realimentation diet may reduce the risk of lipid abnormalities, but it increases the risk of leg abnormalities in broiler chicks.

Acknowledgment

We thank Dr. Keiichi Tanaka and Dr. Shigeru Ohtani for their supervisions during our experiment and writing dissertation. The technical assistance of all students of Animal Nutrition Section of Agriculture Faculty, Gifu University is gratefully acknowledged.

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