Effects of Protein Restriction on the Live Weight Gain and Some Blood Parameters in Mature and Immature Rats

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SUMMARY

The aim of this study was to compare the effects of different levels of protein restriction on live weight gain, feed intake and some blood parameters in immature and mature rats. Twenty – four 20 day-old (immature group) and twenty – four 65 day-old (mature group) male Wistar Albino rats were selected as the experimental subjects. All rats were weighed bi-weekly and amounts of feed consumed were recorded for each cages. Blood samples were collected from the neck and then, plasma total protein, glucose, and cholesterol levels were determined. As CP concentration of diets increased, both live weight gain and feed intake significantly increased in both mature and immature rats (P<0.05). Plasma glucose and cholesterol levels were similar among rats (P>0.05). Plasma total protein concentrations linearly increased as protein content of diets increased in both mature and immature rats (P<0.05). It can be concluded that immature rats can be affected from protein restriction more compared with mature rats based on growth performance of rats on protein-restricted diets.

Key words: *Protein restriction, feed intake, growth, glucose, rat*

Puberta Öncesi ve Puberta Sonrası Ratlarda Protein Kısıtlamasının Canlı Ağırlık ve Bazı Kan Parametreleri Üzerine Etkisi

ÖZET

Bu çalışma, puberta öncesi ve puberta sonrası ratların rasyonlarına farklı oranlarda protein kısıtlamasının canlı ağırlık kazancı, yem tüketimi ve bazı kan parametreleri üzerine etkisini karşılaştırmak amacı ile yapıldı. Denek olarak , puberta öncesi grup 20 günlük ve puberta sonrası grup 65 günlük olacak şekilde 24'er adet erkek Wistar Albino rat seçildi. İki aylık deneme süresince, iki haftada bir her gruptaki ratların canlı ağırlık artışı ve yem tüketim miktarı belirlenerek kaydedildi. Boyundan alınan kan örneklerinde plazma total protein, glikoz ve kolesterol seviyeleri belirlendi. Dietteki CP konsantrasyonu arttıkça puberta öncesi ve puberta sonrası ratlarda hem canlı ağırlık kazancı hem de yem tüketimi önemli derecede arttığı gözlendi (p<0.05). Plazma glikoz ve kolesterol seviyeleri gruplar arasında birbirine benzerdi (p>0.05). Plazma total protein konsantrasyonu, hem puberta öncesi hem de puberta sonrası ratlarda diyetin protein içeriğindeki artışa bağlı olarak artmıştır (p<0.05). Sonuç olarak, protein kısıtlaması yapılan diyetin büyüme performansı üzerine olan olumsuz etkisi puberta öncesi ratlarda, puberta sonrası ratlarla karşılaştırıldığında daha belirgin görüldü.

Anahtar kelimeler:Protein sınırlaması, yem tüketimi, büyüme glikoz,rat

INTRODUCTION

Nutrient deficiency results in impairment in organism throughout the life, especially at fast growing infancy period (4). Malnutrition may develop as a result of the deficiency of one or several nutrients (16) and can cause irreversible impairment at growing period characterized by cell accumulation (18). Tissue turnover and synthesis of nutrient required for growth might impaired during protein deficiency, therefore, weight loss or lack of weight gain can be observed due to protein deficiency (7).

In addition to delay of growth, protein deficiency has been reported to affect metabolism of animal by changing hormonal statues (3, 9).When protein intake is not sufficient enough protein synthesis in liver decreases, resulting in significant alteration in cellular protein (2).

In order to reveal the effects of malnutrition on health, especially in young, this study was aimed to compare the effects of different levels of protein restriction on live weight gain, feed intake and some blood parameters in immature and mature rats.

MATERIALS and METHODS

Animals

Twenty – four 20 day-old (immature group) and twenty – four 65 day-old (mature group) male Wistar Albino rats were selected as the experimental subjects. They were acclimatized for 7 d with laboratory conditions at 22-25 ºC with a 12 h light/dark cycle. Each groups of rats were randomly divided into three groups of eight animals each. Eight rats within each group and each treatment were also assigned to two cages of four rats. Animals were fed with diet containing 3 %, 10 % and 20 % protein (control). Compositions of these experimental diets were shown in Table 1. Diets were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), and ether extract (EE) (8). Percentages of organic matter (OM) and nitrogen free extract (NFE) were calculated. Rats were fed diets ad libitum intake and had free accsess to clean tap water troughout the experiment. Three of mature rats and four of immature rats fed diet containing 3 % protein died close to end of experiment. Rats were sacrificed by decapitation under ether anaeshesia two months after initiation of experiment. The rats received

humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Table 1. Composition of experimental diets

Ingredients	20% Group	10% Group	3% Group
	% of total diet		
Casein	25.0	12.5	3.0
Corn oil	7.5	7.5	7.5
Vitamin-mineral mix	7.0	7.0	7.0
Starch	57.5	70.0	79.5
Peanut hull	3.0	3.0	3.0
	Chemical composition of diet		
DM	95.25	94.98	93.91
Ash, % DM	12.59	12.27	12.52
OM, % DM	87.41	87.73	87.48
CP, % DM	18.33	9.18	2.96
EE, % DM	7.98	7.14	8.56
CF, % DM	0.36	0.26	0.34
NFE, % DM	60.74	71.15	75.62

Feed intake and Live weight gain

All rats were weighed bi-weekly. To determine feed intake, amounts of feed consumed were recorded for each cages.

Blood sampling

Blood samples were collected from the neck. Plasma was obtained after centrifugation (1500 g, 20 min, 4 ºC) and stored at ‑ 20 ºC for cholesterol, glucose and total protein analyses.

Plasma total protein, glucose, and cholesterol levels were determined by spectrophotometry (Shimadzu UV 1201V, Japan) using commericial kits (Randox®).

Statistical analysis

The results were analyzed by two-way ANOVA. The mean treatment differences were determined by Bonferroni's t-test with a level of statistical significance of 5 % for each maturities, separately (15).

RESULTS

Feed intake and live weight gain values are presented in Table 2 and 3, respectively. As CP concentration of diets increased, both feed intake and live weight gain significantly increased in both mature and immature rats ($P<0.05$).

Plasma glucose, cholesterol, and total protein levels are shown in Table 4. Plasma glucose and cholesterol levels were lower in rats fed diet containing 3 % protein compared with rats fed diet containing 10 % protein in both mature and immature groups, but these differences were not statistically significant ($P>0.05$). Plasma total protein concentrations linearly increased as protein content of diets increased in both mature and immature rats ($P<0.05$).

Table 2. Feed intake, g/rat.

Inition of Experiment	Immature				Mature			
	3(n=8)	10(n=8)	20(n=8)	SEM	3(n=8)	10(n=8)	20(n=8)	SEM
0-30 d	251.6^{c}	317.3^{b}	397.1^{a}	5.64	284.75^{b}	543.5^{a}	249.6^{a}	5.26
31-60 d	270.0^{c}	356.7^{b}	487.5^{a}	5.92	291.5^{c}	659.8^{b}	742.3^{a}	10.66

^{a,b,c,}: mean values in same rows with no common superscripts differ significantly ($p<0.05$).

Table 3. Live weight gain, g.

Inition of Experiment	Immature				Mature			
	3(n=8)	10(n=8)	20(n=8)	SEM	3(n=8)	10(n=8)	20(n=8)	SEM
7 d	52.10^{ab}	71.76^{a}	68.64^{a}	8.71	129.62^{a}	158.44^{a}	162.62^{a}	16.50
15 d	31.75^{b}	68.99^{a}	79.73^{a}	8.81	118.54^{b}	150.48^{ab}	170.75^{a}	17.04
30 d	34.66^{c}	68.68^{b}	100.66^{a}	10.01	100.28^{b}	166.05^{a}	183.79^{a}	19.76
45 d	33.16^{c}	70.01^{b}	101.12^{a}	5.42	90.96^{b}	169.33^{a}	187.2^{a}	22.34
60 d	33.91^{c}	72.74^{b}	126.06^{a}	5.42	80.73^{b}	164.5^{a}	200.19^{a}	21.49

^{a,b,c,}: mean values in same rows with no common superscripts differ significantly ($p<0.05$).

Table 4. Plasma glucose, cholesterol, and total protein levels.

	Immature				Mature			
	3(n=8)	10(n=8)	20(n=8)	SEM	3(n=8)	10(n=8)	20(n=8)	SEM
Glucose	96.20	121.63	105.26	14.50	73.12	93.79	75.21	15.31
Cholestrol (mg/dl	75.85	83.57	88.94	17.34	52.85	60.47	59.85	7.57
Total Protein (gr/dl)	2.68^{b}	5.20^{a}	5.81^{a}	1.67	5.36^{b}	7.18^{b}	12.2^{a}	1.95

^{a,b,c,}: mean values in same rows with no common superscripts differ significantly ($p<0.05$).

DISCUSSION

Protein metabolism is a dynamic process taking place throughout our life. Even after mature body-size is reached, protein is necessary for integrity of immune response, hormones, enzymes and cell.

Feed intake of rats fed diets containing different levels of CP were significantly different (Table 2; $p<0.05$). There was a vicious cycle between protein restriction and feed intake: the more severe protein restriction, the less feed taken, which are in agreement with the results of KONNO et al. (11) and KERN et al. (10).

The effect of protein restriction on body weight of rats is presented in Table 2. It seemed that effect of protein restriction were more severe on immature rats compared with mature rats. Feeding 10% CP did not significantly differed body weight of mature rats, but significantly altered body weight of immature rats at the end of the experiment. While body weight of mature rats fed 20% CP diet were 2.48 times greater compared those of mature rats fed 3% CP diet, body weight of immature rats fed 20% CP diet were 3.72 times greater compared those of immature rats fed 3% CP diet, revealing the effects of age differences on body weight change. Similarly, KONNO et al. (11) reported that while body weight of immature rats receiving protein free-diet decreased to a level less than 25% of controls, body weight of mature rats receiving protein free-diet decreased to a level between 75 and 85 of controls. Significant body weight gains have also been reported by KERN et al. (10) and SANTANA et al. (14), which support the results of our study. Nutrient required for growth and tissue-turnover can not be accomplished when protein synthesis is impaired. Thus, animals fed protein deficient diet gain less compared animals fed control diet (14).

Plasma glucose levels were lower in rats fed 3% CP diet compared with other groups, but were not significant. Even though carbohydrate content of diets were not restricted, because animal fed protein restricted diets had significantly less feed intake compared with control diet, thus, daily glucose intake were significantly different. Therefore, differences in plasma glucose levels were expected. Plasma glucose levels were approximately 21-22% greater in rats fed diets containing 10% CP compared with rats fed diets containing 3 % CP in both mature and immature group. However, rats fed 20% CP diet had numerically less plasma glucose levels compared with rats fed 10% CP diet in both maturities, which can not be explained. Similar to our results, WALFORD et al. (19) reported 21 % reduction in plasma glucose levels during energy restriction.

Plasma cholesterol levels of rats fed diets containing 3% diet tended to be lower compared with rats fed diets containing 10 or 20% CP, but these differences were not statistically significant. Three are contrasting reports in the literature regarding plasma cholesterol levels during protein restriction. While many researchers have reported significantly lower plasma cholesterol level in rats fed protein deficient diet compared with rats fed control diets (13, 5, 19), others reported not significant differences among rats fed diets containing different levels of protein (6, 12).

It has been reported that plasma protein levels were significantly lower in rats fed protein-free diets compared with rats fed control diets (17, 1). KERN et al. (10) also reported that protein restriction caused reduction in serum protein levels, which supports the results of our study.

It can be concluded that immature rats can be affected from protein restriction more compared with mature rats based on growth performance of rats on protein-restricted diets.

REFERENCES

1. Alkan M (2002): Proteinden yetersiz diyetle beslenen ratlarda serum lipid, sialik asit ve vitamin E düzeylerinin araştırılması. PhD Thesis, Y.Y.U. Sağ. Bil. Enst., Van.

2. A.O.A.C. (1980): Official Methods of Analysis (13th Ed.). Association of Official Analytical Chemists, Washington DC

3. **Baysal A (1999):** Beslenme. Hatipoğlu Yayınevi, 8. Baskı, Ankara, 1999.

4. Bhattacaryya A K (1986): Protein – energy malnutrition (Kwashiorkor-Marasmus Syndrome); terminology, classification and evolution. World Rev. Nutr. Diet., 47, 80-133.

5. Bouziane M, Prost J, Belleville J (1994): Changes in fatty acid compositions of total serum and lipoprotein particles, in growing rats given protein-deficient diets with either hydrogenated coconut or salmon oils as fat sources. Br. J. Nutr., 71, 375-387.

6. Bydlowski S P, Stivaletti V L G, Douglas C R (1986): Biochemical observations on rat aorta: interaction of dietary protein and cholesterol. Br. J. Nutr., 55, 295-304.

7. **Dülger H (1998):** Van ve çevresinde yaşayan çocuklarda ve gençlerde açlık insulin seviyesi ile serum lipid ve lipoproteinleri arasında ilişkilerin araştırılması. PhD Thesis, Y.Y.U. Sağ. Bil. Enst., Van.

8. Huang C H, Fwu M L (1993): Degree of protein deficiency affects the extent of the depression of the antioxidative enzyme activities and the enhancement of tissue lipid per oxidation in rats. J. Nutr., 123, 803-810.

9. Karaca F, Dönmez H H, Karsli M A (2003): Effects of protein deficiency on testosterone levels, semen quality and testicular histology in the developing male rat. Scand. J. Lab. Anim. Sci., 1(30), 7-13.

10. **Kern M, Beuttenmuller D, Diehl S, Mccormick C, Ambrose M (2002):** The effects of protein repletion at varied levels on the growth and nutritional status of protein restricted rats. Nutr. Research, 22, 957-963.

11. **Konno A, Utsuyama M, Kurashima C, Kasai M, Kimura S, Hirokawa K (1993):** Effects of a

protein –free diet or food restriction on the immune system of Wistar and Buffalo rats at different ages. Mechanism of Ageing and Development, 72, 183-197.

12. Meghelli-Bouchenak M, Boguillon M, Belleville J (1987): Time course of changes in rat serum apolipoproteins during the consumption of different low protein diets followed by a balanced diet. J. Nutr., 117, 641-649.

13. Ogunkeye O O, Ighogboja I S (1992): Increase in total serum triglyceride and phospholipids in kwashiorkor. Ann. Trop. Pediatr., 12, 463-466.

14. Santana D M G, Molinan S L, Miranda-Neto M H (2001): Effects of protein and vitamin B deficiency on blood parameters and myenteric neurons of colon of rats. Arg. Neuropsiquiatr., 59, 3-A, 493-498.

15. **SPSS. (1999):** SPSS 10.0 for windows. SPSS Inc., Chicago, IL.

16. **Rafael J F, Mora M D (1999):** Malnutrition; Organic and functional consequences. World J. Surg., 23, 530-535.

17. Rana S, Sodhi C P, Mehta S, Vaiphei K, Katyal R, Thakur S, Mehta S K (1996): Protein- energy malnutrition and oxidative injury in growing rats. Human and Exp. Toxic., 15, 810-814.

18. Udani P M (1992): Protein energy malnutrition, brain and various facets of child development. Indian J. Pediatr., 59, 165-186, 1992.

19. Walfrod R L, Mock D, Verdery R, Mccallum T (2002): Calorie restriction in biosphere 2: alterations in physiologic, hematological, hormonal and biochemical parameters in humans restricted for a 2. year period. J. Gerontol. A Biol. Sci. Med. Sci., 57(6), B211-224.