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INFLUENCE OF LOW AND HIGH-PROTEIN DIETS ON BODY GROWTH AND GLUCOSE INTENSITY IN *Rattus norvagicus*

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ABSTRACT

The influence of the protein content of the diet on glucose concentration (denoted by fluorescence intensity) and body weight was studied in the white rat. Rats of 30d of age received ad lib. a control diet containing 15% crude protein (Pl5), or a diet containing 23% crude protein (P23) or 35% crude protein (P35) and a fourth group of rats (P8) received the 8% crude protein contained diet. After 4 week on these diets, plasma glucose intensity and body weight were different in treatment groups P35, P23, P8 and control PI5 rats, but were lower in P8 rats. It is concluded from these studies that these types of dietary manipulation have a significant effect on plasma glucose intensity. Plasma glucose intensity was measured after the 28 days crude protein percentage diet treating by Benedict reagent and fluorescence spectrometry (Perkin Elmer LS 55).

Keywords:- Influence; intensity; glucose concentration; protein diet.

INTRODUCTION

Protein malnutrition is known to alter glucose homeostasis in man and in several animal species [1,2.]. Recognition that diabetes sometimes presents particular clinical features in regions of endemic protein-energy malnutrition has even led to the hypothesis that under nutrition may play a causal role in the pathogenesis of one form of the disease[3,4,5]. Recent experimental studies, using rats fed on a low-protein diet, have characterized some of these abnormalities of glucose homeostasis, and have determined their evolution with time and partial reversal on refeeding a control diet[6,7,8.]. However, it is still unclear to what extent these changes must be ascribed to protein restriction or to energy restriction, since young rats receiving a low-protein diet spontaneously reduce their food intake and body weight. This was the question addressed in the present study, in which we also investigated the influence of low, moderate and a high-protein diet on plasma glucose intensity.

MATERIAL AND METHODS

(i)Diets:

Four types of isocaloric diet with crude protein were prepared by mixing appropriate proportions of different food composition. The final composition of these diets is given in Table 1. The dietary treatment groups were: control diet (15%, P15); the low-protein diet (8% crude protein, P8); and the high-protein diet (35% crude protein, P35) fed ad lib. The fourth diet, containing approximately 23% crude protein, (P23), was given ad lib (see below Table -1).

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Table 1.
Composition of the diets (g/kg)

Ingradient	*8/.*	*15/.*	*23/.*	*35/.*
Starch	*53.31*	*45.60*	*37.60*	*30.00*
Ground corn	*21.67*	*21.35*	*21.59*	*20.94*
Soya bean meal	*7.56*	*16.16*	*24.46*	*32.66*
Animal fat	*12.09*	*11.89*	*11.42*	*11.63*
Dicalcium phosphate	*3.32*	*2.96*	*2.82*	*2.61*
*Vitamin mix**	*0.45*	*0.44*	*0.44*	*0.44*
Methionine(99%)	*0.45*	*0.44*	*0.44*	*0.44*
Salt(NaCl)	*0.45*	*0.44*	*0.44*	*0.44*
Limestone	*0.45*	*0.57*	*0.64*	*0.68*
*Trace mineral mix**	*0.15*	*0.15*	*0.15*	*0.15*

**Vitamin mixer- vitamin A (palmitate) 3,968,280 IU; cholecalciferol 1, 102,300 IU;vitamin E(dl-tocopherol, 13,228 IU;vitamin B12, 7.9 mg; riboflavin, 2,646 mg; niacin, 17,637 mg; d-biotin, 44 mg.*
**Trace mineral mixer - Ca 15.00%, Zn10.00%, Mn12.00%, Fe7.50%, Cu 1.00%,I 0.25%.*

(ii)Animals:

The animals used were *Rattus norvagicus* male female randomly. They were collected at 30d and received diet normal feed. They were then divided into four experimental groups of five rats, matched for initial body-weight, and housed in a temperature- and light-controlled room (22", 12 h light-12 h dark cycle with lights on at 07.00 hours). Between the ages of 4 and 8 weeks the animals were housed in group metabolism cages permitting daily measurement of food intake. Food intake was measured every 7th day. Food was provided ad lib. to P15, P8, P23 and P35 rats. However, they were fed once daily, at 17.00 hours, and it should be noted that these hungry animals ate their whole ration in less than 3 h. This experimental design approved by approval committee of CSJM University, Kanpur.

(iii)Sampling procedures:

From the all rats blood samples were collected (in the fed state) at the age of 8 weeks. On several occasions, blood was taken with EDTA from fed animals (between 08.00 and 09.00 hours). The collected blood was stored in -20^{0}C for glucose analysis.

(iv) Analytical procedures:

Plasma glucose concentration was measured after the 28 days crude protein percentage diet treated by Benedict reagent and fluorescence spectrometry (Perkin Elmer LS 55).Benedict reagent was synthesized in our own laboratory. It composed of copper sulphate, sodium carbonate, and sodium citrate (pH 10.5). The citrate will form soluble complex ions with Cu++, preventing the precipitation of CuCO3 in alkaline solutions. Method of preparation of Benedict reagent is as follows:

Solution A: Dissolve 1.73 g trisodium citrate (dihydrate) and 1.0 g anhydrous

Sodium carbonate in 8 ml of warm distilled H2O.

Solution B: Dissolve copper sulphate (pentahydrate) (1.73 g) separately in 20 ml of distilled H2O.

Immediately before using, prepare Benedict reagent by mixing 0.8 ml of Solution A with 0.2 ml of Solution B. To test glucose intensity, add 200ul of Plasma solution to 1 ml of Benedict reagent and heat in a boiling water bath for 5 minutes. Remove the tubes from the heat and allow them to cool. Glucose intensity was taken under fluorescence spectrometry by slit 5,5 and excitation 535nm (Perkin Elmer LS 55). A calibration graph of known glucose concentration was also made by using the same.

(v)Presentation of results:

Results are presented as means with their standard errors. Comparisons between groups of rats receiving different types of diet were carried out by analysis of variance, and where this showed a treatment effect groups were compared by the test of Tukey Test. Differences were considered statistically significant at $P < 0.05$, $P<0.5$. $P< 0.8865$ and $P<0.999$ using commercial software

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(Originpro 7.5, Microcal Software, Northampton, MA, USA).

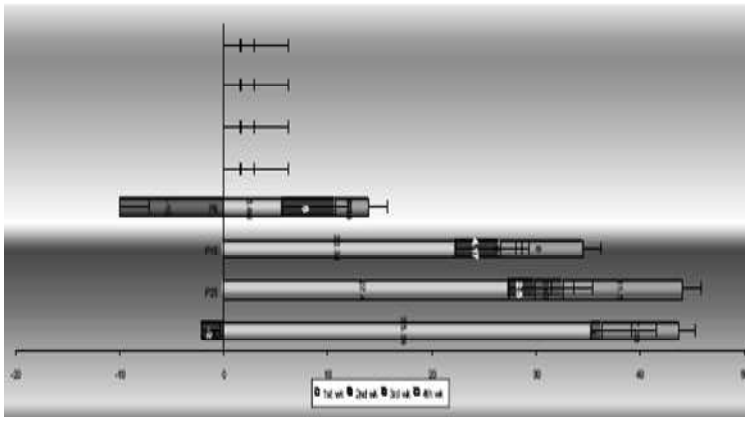
RESULTS AND DISCUSSION

1.Influence of dietary protein level on body-weight gain and on food intake:

At the age of 30d, before being given the different types of diet, the rats had an average body-weight of 69.75g. As shown in Fig. 1, Body-weight gain was significantly different ($p<0.8656$) from control P15 to other treatment groups, very low in P8 rats and higher in P23 rats.

Graph-1.

Body-weights of rats receiving different crude protein percentage diet after 4weeks treatment.



Values are means with their standard errors represented by vertical bars for groups of rats.

Per day average body-weight gain and food intake for 4 week on the diets are presented in Table 2. Feed conversion energy were significantly fluctuated ($P<0.8$) from control to other dietary group rats while P23 and P35 groups were not significantly varied ($P<0.8$) and P8 dietary groups feed conversion energy is very low from control and other group rats. Feed intake capacity were not significantly changed ($P<0.05$) among dietary treatment groups P8, P23 and P35 from control group P15 (Table-2) but P8 group rats showed high diversity from P23 and P35 group rats. After 4week on the respective diets (8-week-old rats), variation had been reported on the mean (with SE) body-weight (g) of P8, P15, P23, and P35 rats ($P < 0.05$) (Table-3).

Table-2.

Food intake, Body-weight (BW) gain, and energy conversion efficiency in rats receiving diets with different crude protein contents as per day. (30 to 58-day-old rats)

Dietary Treatment groups…..	P15		P8		P23		P35	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Feed intake(FI) (g/d)	7.07*	0.04041	6.13*	0.07506	7.75*	0.43301	7.29*	0.16743
BW gain (g/d)	1.23**	0.13279	0.38**	0.04619	1.61**	0.35218	1.49**	0.28290
Energy conversion efficiency (g BW/FI)	0.17**	0.04041	0.06**	0.01155	0.21**	0.00577	0.20**	0.00577

Mean values were significantly different from those for control P15 rats; * P<0.05, ** P< 865.

2. Influence of dietary protein level on glucose intensity:

In fed P8, P23 and P35 rats, plasma glucose levels were significantly different from control (P15) after 4 week diet treatment ($P < 0.999$(Table 3 and Fig-2). Plasma glucose intensity ratio of P8 and P35 group rats were not

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significantly associated (P<0.5) with control (P15) while P23 groups were not significantly varied (P<0.5) (Table 3 and Fig-2)**.** Fig. 2 illustrates the changes in plasma glucose intensity level measured during and after 28 days diet treatment in the four divergent rat groups. P8 glucose intensity ratio levels were higher than those in control P15 rats (Table-3 and Fig-3). These differences may be partly due to the particular pattern of body growth gaining of these rats. They intake 'less feed' than fed controls at the time of treatment (Fig.-3). The influence of the diet on glucose intensity reserves is shown in Fig 3. Relative to P15 controls, body weight was lower in P8 (P < 0.01) and P35 rats (P < 005) and higher in P23 rats (P < 0.01).

Table-3

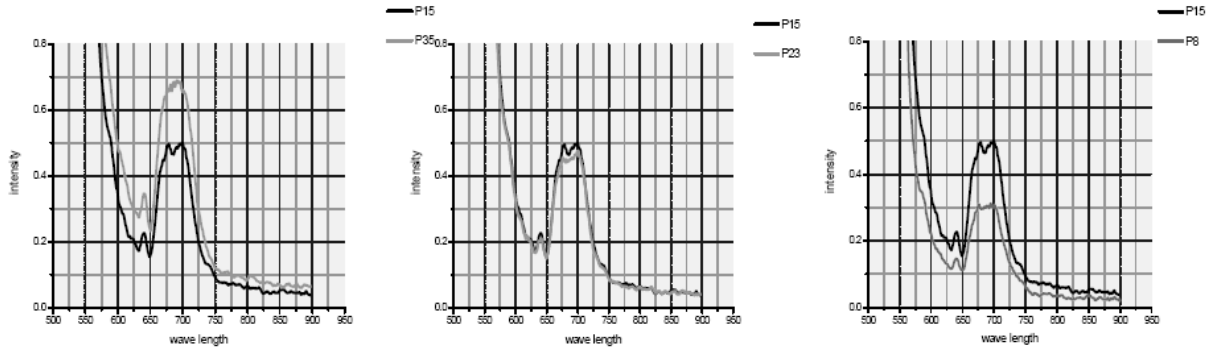
Plasma glucose intensity, body weight gain and glucose intensity ratio in rats receiving diets with different crude protein percentage after 28 days treatment.

Dietary Treatment groups…..	P15		P 8		P 23		P35	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body weight gain (BWG)	34.51*	0.386	10.67*	0.294	45.07*	0.040	41.67*	0.386
Glucose intensity (GI)	5.582**	1.592	3.982**	1.152	5.431**	1.553	6.657**	1.861
Glucose intensity ratio(GI/BWG)	0.16***	4.12	0.37***	3.91	0.12***	38.44	0.16*[a]	4.81

Mean values were significantly different from those for control P15 rats; * P<0.05, ** P< .999, ***P<0.5. *[a] P<0.5

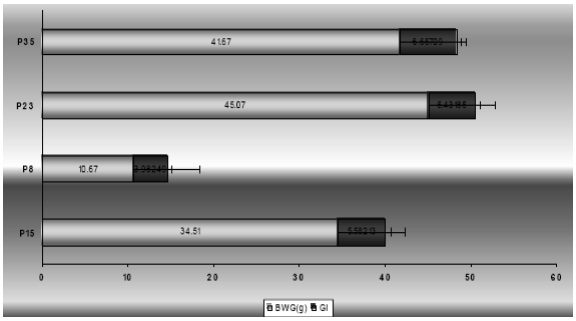
Graph-2.

Glucose intensity compares with controls P15 to P35, P23 and P8 respectively



Graph-3.

Glucose intensity ratio (GI/BWG- glucose intensity /Body weight gain (g)) After 28 days different crude protein diet treatment.



Means error bar in the graph represents +_standard error.

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CONCLUSION

The protein content of the diet markedly influenced spontaneous food consumption, bodyweight gain and gross efficiency of protein and energy utilization. Rats given a low-protein diet voluntarily reduced their food intake, as reported by others [9,10,11]. The severely reduced rate of growth can thus be explained by a combined shortage of protein and energy. That both deficiencies play a role is demonstrated by the intermediate body-weight gain of fed P35 rats, which received the same amount of diet as control P15 rats. Energy consumption and body-weight gain of rats given a high-protein diet (P35) were similar to those of controls. **A** decrease in food intake with high-protein diets has sometimes been reported in other studies, but it was either transient (a few days) or significant only when the protein content exceeded 500-600 g/kg diet [9,10,12]. The efficiencies of energy and protein utilization for growth were very different in the four groups of rats, in agreement with previous reports [9.10,11,13]. Fed protein-deprived P8 rats had slightly lower plasma glucose levels and markedly lower body weight than controls. The plasma glucose intensity ratio is higher among lowest dietary protein groups (P8).Where P23 and P35 showing more or less similar glucose intensity ratio with control (P15) and P23 exhibit highest body weight gain among all dietary groups. So from our experiment we can conclude that both the very low and very high protein diets (8% and 35%) are not appropriate for the development of animal body and to maintain glucose level in plasma. But a protein percentage like 23% showing moderate plasma glucose intensity level with highest body weight gain within 28 days among all protein groups.

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