DEVELOPMENTAL CHANGES ON PROTEIN TURNOVER IN GROWING RATS FED ON DIETS CONTAINING FIELD BEANS (*Vicia faba* L.) AS SOURCE OF PROTEIN

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SUMMARY

The effects of nutrition with *Vicia Faba* L. administered at two different levels (12 and 18% protein) on the developmental changes of protein turnover were investigated in the rat.

The myofibrillar gain and synthesis values were lower in the animals fed on legume protein as compared with casein-fed controls, while no differences were found in myofibrillar degradation during the three periods of time evaluated (0-14, 14-28 and 28-45 days).

The fractional myofibrillar gain, breakdown and synthesis calculated as the sum of both, decreased with age in all the dietary groups. The antinutritional effects of the inclusion of *Vicia faba* L. in diets were more evident in the first 28 days, and attributed to a decreased muscle protein synthesis.

INTRODUCTION

The protein deposition in any tissue is a delicate balance between the synthesis and degradation rates, which can be altered by changes in the diet, hormonal status and pathological conditions (1). Furthermore, the changes in protein synthesis and breakdown, which accompany growth, are not the same in all the tissues. In that context, special attention has been given to muscle as the largest protein mass in the body (2) and therefore the major determinant of the growth of whole body protein (3).

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The inclusion of raw field bean (*Vicia faba* L.) seeds in the diet of productive animals has a depressing effect on growth rate, as well as on the feed conversion efficiency rate (4). However, the mechanism of action of this phenomenon remains unknown (5, 6).

The present work has been focussed to determine the influence of intake of *Vicia faba* L., as the protein source at two different levels, on the quantitative aspects of muscle protein turnover during development.

MATERIALS AND METHODS

Animals and Diets

Male Wistar rats weighing approximately 90 g (4-5 weeks of age) were randomly assigned to four dietary groups of six animals each, housed in metabolic cages in a temperature regulated room at 22°C. Animals were fed *ad libitum* over 45 days on diets containing casein or whole *Vicia faba* L. seeds as the protein source at two different levels: 12 an 18% (Table 1). No 3-Methylhistidine (3-Mehis) was found in either sources of protein. Water was also provided *ad libitum.*

TABLE 1

COMPOSITION OF THE EXPERIMENTAL DIETS
(Expressed in %)

Diet crude protein (N x 6.25)	Casein 18.8	*V. faba* 18.6	Casein 12.8	*V. faba* 12.4
Casein*	20.80	—	14.2	—
V. faba	—	76.0	—	51.0
Saccharose	31.20	8.0	34.8	19.5
Starch	31.20	8.0	34.8	19.5
Olive oil	4.50	5.0	5.0	5.0
Cellulose	6.00	—	6.0	—
Mineral mix[1]	4.50	2.5	4.5	3.6
Vitamin mix[2]	1.60	1.6	1.6	1.6

* One per cent methionine was added to the casein diets.

1 *Harper mixture* containing the following percentages of salts: sodium chloride, 13.93; potassium iodide, 0.08; potassium phosphate dibasic, 38.91; magnesium sulfate, 5.73; calcium carbonate, 38.14; ferric sulfate, 2.70; manganese sulfate, 0.40; zinc sulfate, 0.06; cupric sulfate, 0.05; cobalt chloride, 0.058.

2 *Harper mixture* containing the following vitamins per g: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 10 IU; choline, 200 mg; p-aminobenzoic acid, 10 mg; inositol, 10 mg; niacin, 4 mg; thiamine HCl, 0.5 mg; folic acid, 0.2 mg; D-biotin, 0.04 mg; calcium pantothenate, 4 mg; pyridoxine, 0.5 mg; riboflavin, 0.8 mg; vitamin K, 0.5 mg; and sucrose csp, 1 g.

Assay Procedure

At the end of each experimental period, all the rats were sacrificed by decapitation, and the gastrocnemius muscle was carefully excised and frozen at −20°C prior to analysis. Nitrogen in muscle was measured by the Kjeldahl method (7) while the myofibrillar fraction and the urinary and myofibrillar 3-Mehis content were determined according to a previous report (8). The myofibrillar protein was measured on rats sacrificed after 14, 28 and 45 days of feeding, and the urine samples collected in the last two days of each experimental period were pooled.

Calculations

The rate of myofibrillar degradation (D) was calculated from the urinary output of 3-Mehis and its concentration in the myofibrillar pool, by using the equation:

$$D = 0.8 \quad \frac{\text{3-Mehis (urine)}}{\text{3-Mehis (myofibrillar pool)}}$$

The coefficient 0.8 was chosen to represent the contribution of muscle 3-Mehis, to total urinary 3-Mehis excretion (9). The myofibrillar gain rate (G), was calculated from the differences between the initial and final content of myofibrillar protein in the three experimental periods studied in this trial. Since the rate of protein gain is the difference between the rates of protein synthesis and degradation, the protein synthesis rate was estimated as the sum of breakdown rate (D) and gain rate (G), as follows:

$$S = G + D$$

The data were statistically evaluated by one way of analysis of variance (ANOVA–1) among all the dietary groups, and during the three experimental periods (10).

RESULTS

Changes in body and gastrocnemius weight, as well as nitrogen content of the myofibrillar fraction along the experimental periods are shown in Table 2.

The myofibrillar gain, daily gain and fractional rate of myofibrillar gain –calculated as a percentage of the myofibrillar nitrogen content on the first day of each experimental period– are presented in Table 3. The absolute values of myofibrillar gain decreased with age in all the diets, except in the animals fed on diets containing 18.8% casein, in which a decrease took place only during the third period. The myofibrillar protein breakdown rate diminished in the three stages studied.

Table 4 shows the urinary and myofibrillar 3-Mehis content data. The values of fractional rate of myofibrillar protein breakdown were calculated as indicated in Material and Methods. No differences were found in this parameter among the diets in all the experimental periods,

TABLE 2

BODY AND GASTROCNEMIUS WEIGHT (g) AND MYOFIBRILLAR NITROGEN CONTENT (mg N_2 g tissue) OF THE DIFFERENT DIETARY GROUPS IN THE THREE EXPERIMENTAL PERIODS (0-14, 14-28 and 28-45 days)

		Days	Casein 18.8o/o	*Vicia faba* 18.6o/o	Casein 12.8o/o	*Vicia faba* 12.4o/o	Anova-1 (diets)
Initial body weight	(g)		89.2 ± 2.1	89.4 ± 2.1	89.4 ± 1.0	89.9 ± 1.1	NS
Body weight	(g)		121.4 ± 2.1	118.0 ± 3.2	124.6 ± 1.6	120.6 ± 2.4	$P < 0.05$
Gastrocnemius weight	(g)	14	0.71 ± 0.04	0.69 ± 0.03	0.75 ± 0.04	0.71 ± 0.02	$P < 0.05$
Myofibrillar nitrogen	(mg/g)		20.7 ± 1.2	19.9 ± 0.9	20.7 ± 0.9	20.3 ± 0.4	NS
Body weight	(g)		158.2 ± 1.5	145.0 ± 3.0	154.4 ± 3.6	146.0 ± 2.2	$P < 0.01$
Gastrocnemius weight	(g)	28	0.92 ± 0.04	0.82 ± 0.02	0.90 ± 0.03	0.84 ± 0.03	$P < 0.05$
Myofibrillar nitrogen	(mg/g)		20.0 ± 1.3	20.2 ± 0.8	20.4 ± 1.1	21.2 ± 0.8	NS
Body weight	(g)		197.4 ± 3.4	162.4 ± 2.6	181.0 ± 4.1	166.0 ± 2.8	$P < 0.01$
Gastrocnemius weight	(g)	45	1.17 ± 0.05	1.01 ± 0.03	1.08 ± 0.04	0.98 ± 0.02	$P < 0.01$
Myofibrillar nitrogen	(mg/g)		20.8 ± 0.8	21.0 ± 0.7	20.2 ± 1.2	20.7 ± 0.9	NS
Anova-1 (time) Myofibrillar nitrogen			NS	NS	NS	NS	

TABLE 3

TOTAL NITROGEN (mg N_2), DAILY (mg N_2/day) AND FRACTIONAL RATE OF MYOFIBRILLAR GAIN (o/o) OF RATS ON DIFFERENT DIETS IN THE THREE EXPERIMENTAL PERIODS (0-14, 14-28 and 28-45 days)

	Days	Casein 18.8o/o	*Vicia faba* 18.6o/o	Casein 12.8o/o	*Vicia faba* 12.4o/o	Anova-1 (diets)
Nitrogen myofibrillar gain (o/o)		269 ± 16	242 ± 22	289 ± 10	257 ± 11	P < 0.01
Daily myofibrillar gain (mg N_2/day)	0-14	19.20 ± 1.16	17.30 ± 1.61	20.63 ± 0.60	18.40 ± 0.80	P < 0.05
Fractional gain rate (o/o)		2.60 ± 0.13	2.30 ± 0.22	2.80 ± 0.09	2.41 ± 0.10	P < 0.01
Nitrogen myofibrillar gain (mg N_2)		303 ± 21	177 ± 12	250 ± 17	232 ± 32	P < 0.01
Daily myofibrillar gain (mg N_2/day)	14-28	21.66 ± 0.42	12.66 ± 0.89	17.82 ± 1.22	16.71 ± 2.16	P < 0.01
Fractional gain rate (o/o)		2.16 ± 0.08	1.19 ± 0.10	1.70 ± 0.09	1.65 ± 0.25	P < 0.01
Nitrogen myofibrillar gain (mg N_2)		303 ± 14	132 ± 25	177 ± 10	176 ± 18	P < 0.01
Daily myofibrillar gain (mg N_2/day)	28-45	17.84 ± 0.82	7.63 ± 1.40	10.42 ± 0.57	10.35 ± 1.05	P < 0.01
Fractional gain rate (o/o)		1.31 ± 0.06	0.60 ± 0.11	0.78 ± 0.07	0.85 ± 0.09	P < 0.01
Anova-1 (time)						
Daily myofibrillar gain		P < 0.01	P < 0.02	P < 0.01	P < 0.01	
Fractional gain rate		P < 0.01	P < 0.01	P < 0.01	P < 0.01	

TABLE 4

URINARY (pM/day) AND MYOFIBRILLAR (pM/mg N_2) 3-Mehis, DAILY MYOFIBRILLAR DEGRADATION (mg N_2/day) AND THE CALCULATED FRACTIONAL RATE OF DEGRADATION (o/o) OF RATS FED ON DIFFERENT DIETS IN THE THREE EXPERIMENTAL PERIODS (0-14, 14-28 and 28-45 days)

	Days	Casein 18.8o/o	*Vicia faba* 18.6o/o	Casein 12.8o/o	*Vicia faba* 12.4o/o	Anova-1 (diets)
Urinary 3-Mehis (μM/day)		1.10± 0.05	1.01± 0.03	1.05± 0.06	1.00± 0.05	P <0.05
Myofibrillar 3-Mehis (pM/mg N_2)	0-14	36.6 ± 2.0	35.0 ± 1.3	36.9 ± 1.4	36.0 ± 1.0	NS
Daily degradation (mg N_2/day)		24.07± 1.26	23.01± 0.91	22.78± 0.89	22.18± 0.38	NS
Fractional rate* (o/o)		3.19± 0.20	3.08± 0.13	3.10± 0.16	2.92± 0.18	NS
Urinary 3-Mehis (μM/day)		1.31± 0.07	1.16± 0.05	1.20± 0.04	1.16± 0.16	P <0.05
Myofibrillar 3-Mehis (pM/mg N_2)	14-28	35.5 ± 2.3	34.6 ± 2.1	36.0 ± 2.0	34.4 ± 1.3	NS
Daily degradation (mg N_2/day)		29.03± 1.70	26.86± 0.86	26.72± 1.40	27.01± 0.74	NS
Fractional rate* (o/o)		2.80± 0.20	2.66± 0.14	2.56± 0.16	2.53± 0.16	NS
Urinary 3-Mehis (μM/day)		1.49± 0.08	1.37± 0.07	1.42± 0.08	1.33± 0.16	P <0.05
Myofibrillar 3-Mehis (pM/mg N_2)	28-45	36.1 ± 1.3	35.1 ± 1.1	36.7 ± 1.7	35.7 ± 1.2	NS
Daily degradation (mg N_2/day)		33.09± 1.13	32.96± 1.08	31.61± 1.50	30.21± 1.19	NS
Fractional rate* (o/o)		2.44± 0.09	2.56± 0.11	2.42± 0.14	2.45± 0.32	NS
Anova-1 (time)						
Daily degradation		P<0.01	P<0.01	P<0.01	P<0.01	
Fractional rate		P<0.01	P<0.01	P<0.01	P<0.01	

* Assuming that 80o/o of the total 3-Mehis excretion is derived from myofibrillar protein.

TABLE 5

MYOFIBRILLAR PROTEIN SYNTHESIS (mg N_2/day) AND FRACTIONAL SYNTHETIC RATE (%) OF RATS FED ON DIFFERENT DIETS IN THE THREE EXPERIMENTAL PERIODS (0-14, 14-28 and 28-45 days)

		Days	Casein 18.8%	*Vicia faba* 18.0%	Casein 12.8%	*Vicia faba* 12.4%	Anova-1 (diets)
Myofibrillar gain	(mg N_2/day)		19.20± 1.16	17.30± 1.61	20.63± 0.69	18.40± 0.80	$P < 0.05$
Myofibrillar degradation	(mg N_2/day)	0-14	24.07± 1.26	23.01± 0.90	22.78± 0.89	22.18± 0.38	NS
Myofibrillar synthesis	(mg N_2/day)		43.27± 1.83	40.31± 1.59	43.41± 1.14	40.59± 1.02	$P < 0.05$
Fractional rate*	(%)		5.78± 0.24	5.37± 0.21	5.89± 0.18	5.32± 0.13	$P < 0.01$
Myofibrillar gain	(mg N_2/day)		21.66± 0.42	12.60± 0.89	17.82± 1.22	16.71± 2.16	$P < 0.01$
Myofibrillar degradation	(mg N_2/day)	14-28	29.03± 1.70	27.01± 0.74	26.72± 1.40	26.86± 0.86	NS
Myofibrillar synthesis	(mg N_2/day)		50.69± 2.01	39.67± 0.85	44.54± 0.74	43.57± 3.50	$P < 0.01$
Fractional rate*	(%)		4.69± 0.20	3.92± 0.08	4.26± 0.07	4.24± 0.33	$P < 0.01$
Myofibrillar gain	(mg N_2/day)		17.84± 0.82	7.63± 1.40	10.42± 0.57	10.35± 1.05	$P < 0.01$
Myofibrillar degradation	(mg N_2/day)	28-45	33.09± 1.13	32.96± 0.86	31.61± 1.59	30.21± 1.20	NS
Myofibrillar synthesis	(mg N_2/day)		51.93± 1.12	40.59± 1.15	42.03± 1.42	40.56± 1.21	$P < 0.01$
Fractional rate*	(%)		3.75± 0.18	3.23± 0.09	3.23± 0.11	3.34± 0.10	$P < 0.01$
Anova-1 (time) Fractional rate			$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	

* Calculated as the sum of myofibrillar gain and myofibrillar degradation.

although within the same diet, the values decreased with age.

The absolute and fractional rate of myofibrillar synthesis, estimated as the sum of myofibrillar gain and breakdown, is reported in Table 5. As the data show, protein synthesis was influenced more than the catabolic rate, by the different quality and level of protein intake. Figure 1 summarizes graphically the results obtained.

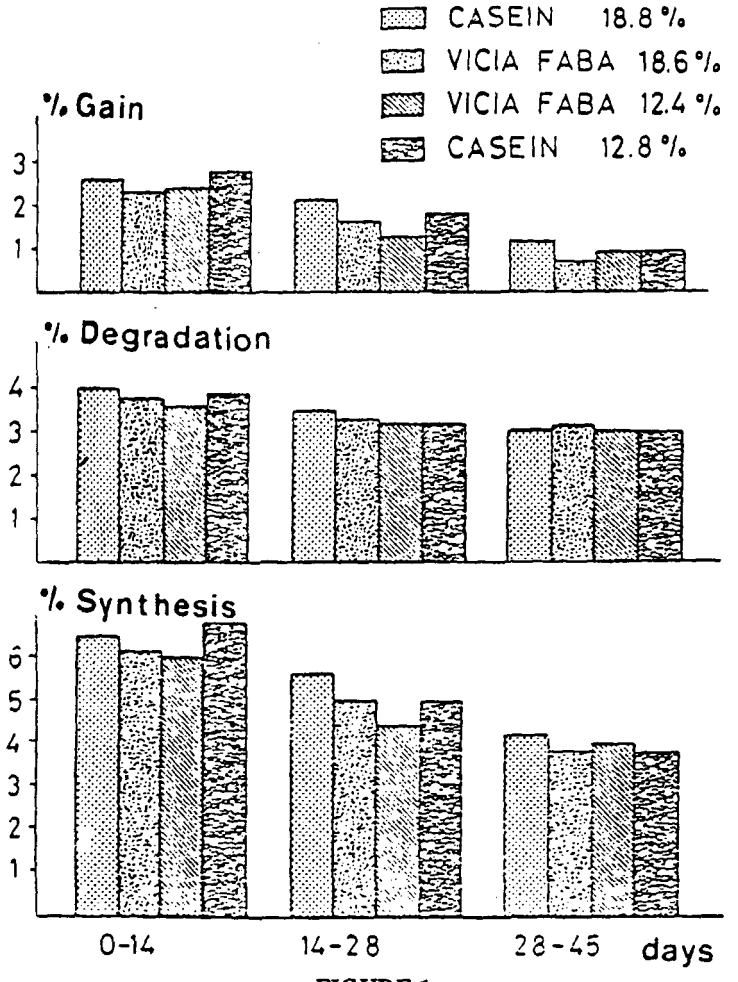

FIGURE 1

Summary of the myofibrillar protein gain values, degradation and synthesis, provided that the whole excretion of 3-Mehis was derived from muscle myofibrils

DISCUSSION

The raw field bean intake reduces growth performance in the rats, a phenomenon which has been atributted to a deficient content of sulfur

amino acids, or to the occurrence of some antinutritional factors, i.e., lectins, tannins, trypsin inhibitors (11). There is no published evidence on the intimate effect of this legume on muscle protein turnover, although Marquardt *et al.* (12) suggested that a growth inhibitor could be present in these seeds.

Changes in the structure, composition and metabolism of tissues occur continuously during growth. Therefore, it is not surprising that different diets can affect in different ways the developmental pattern of protein metabolism (13). Moreover, skeletal muscle, which is the largest simple tissue in the body of mammals, plays a significant role in protein metabolism and its net protein gain can be achieved by various combinations of changes in the protein synthesis and breakdown rates (14).

The stunting of growth in animals fed on diets containing raw field bean, affects the liver and muscle mass weight as well as the gastrocnemius muscle (15). Observation of the data on myofibrillar nitrogen composition –the main source of muscle protein– illustrates the fact that legume protein intake does not alter the protein deposition *per se;* however, the impaired performance of these animals clearly indicates that the protein turnover must be modified, as reported in a previous paper (15).

The myofibrillar protein breakdown was assessed by the urinary excretion of 3-Mehis. This amino acid is not reutilized in protein synthesis and is quantitatively excreted in urine (16). So far, it seems to be the best method for evaluating the myofibrillar breakdown (17). The amount of myofibrillar protein degradation was calculated assuming that 80o/o of the urinary 3-Mehis was derived from myofibrils. A recent report indicates that the contributions of skin and gastrointestinal tract to the total 3-Mehis output, amounted to about 20o/o in normal adult rats (9). Although this is a point of controversy (18), according to Fairweather-Tait, Gee and Johnson (19), it appears that no changes were either observed in regard to gastrointestinal protein turnover after legume intake.

It is interesting to note that the differences in the values of myofibrillar gain are larger between the animals fed a higher protein level, either from casein or *Vicia faba* L., while such differences disappear with time in the other dietary groups. This fact agrees well with published evidence (4), indicative that the depressive effect on growth rate, is enhanced by increased levels of field beans in the diet.

When comparisons are made between both groups, *Vicia faba* L. as the protein source, the greater differences appear in the first 28 days of the experiment. These could be explained by the fact that young rats are more sensitive to a sulfur-deficient diet or have less resistance against the antinutritional substances of this legume. It is suggested that the small differences reported in the initial period can be explained by a similar antinutritional effect in the earliest experimental stage. Nevertheless, the high content of antinutritional factors in the diet prepared with 18.8o/o *Vicia faba* L., could also have an additional depressive effect, despite its higher protein content.

In all the dietary groups, the myofibrillar gain as well as the myofibrillar breakdown decreased with age, when the results are expressed as percentage of the total myofibrillar protein, although the absolute values continue to increase due to an enhanced body weight. This fact has been repeatedly notified by different authors using various methods (16, 20).

The differences on myofibrillar gain among the dietary groups are observed early (0-14 days), and continue throughout the following experimental periods (14-28 and 28-45 days).

In relation to the fractional rate of protein degradation, no statistical differences were found among the four dietary groups during the three experimental periods considered. Although the absolute values were lower in the rats fed on *Vicia faba* L., as compared to the casein controls, they seem to be a consequence of the lower growth rate of those animals, and not to a decreased myofibrillar breakdown.

The values of myofibrillar protein synthesis herein presented are slightly inferior to others previously reported by Millward, and Garlick (21) and by Waterlow *et al.* (1). These authors evaluated the protein synthesis in whole muscle protein, while our data strictly refer to the myofibrillar fraction, which constitutes the main source of muscle protein. Therefore, they can be considered to be in reasonable agreement.

The fact that the protein synthesis is affected more by the inadequacy of dietary protein than by the protein breakdown, and also that tissues in the early stages of life are sensitive to changes of dietary conditions, is widely supported by the literature (1, 3). Thus, in that context, the impairment of growth of the animals fed on *Vicia faba* L., as the source of protein over 45 days, was due to a decreased myofibrillar protein synthesis, particularly in the first 28 days of the experiment, rather than to changes in myofibrillar protein breakdown.

RESUMEN

CAMBIOS EN EL DESARROLLO DEL CICLO DE RENOVACION DE PROTEINAS EN RATAS EN CRECIMIENTO ALIMENTADAS CON DIETAS ELABORADAS A BASE DE HABAS COMO FUENTE PROTEINICA (*Vicia faba* L.)

Se investigó el efecto de la ingestión de dietas con diferente contenido de *Vicia faba* L., (12 y 18o/o proteína) en el metabolismo proteínico de la rata en tres períodos de tiempo (0-14, 14-28 y 28-45 días).

La ganancia y síntesis proteínica miofibrilar fueron inferiores en los animales alimentados con la leguminosa durante las tres etapas consideradas, mientras que no se observaron cambios en la degradación de la misma.

El efecto antinutricional de la *Vicia faba* L., fue particularmente evidente en los dos primeros períodos de vida (0-28 días). Según se constató, la tasa de síntesis de proteína miofibrilar se redujo con la edad en todos los grupos.

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