Proceedings 5th World Rabbit Congress, 25-30 July 1992, Corvallis – USA, 965-972.

THE INFLUENCE OF FEEDING AND PROTEIN LEVELS ON ENERGY AND PROTEIN UTILIZATION BY RABBIT DOES

G. Xiccato, R. Parigi-Bini, M. Cinetto and Antonella Dalle Zotte

Dipartimento di Scienze Zootecniche, Via Gradenigo, 6 - 35131 Padova, Italy

Abstract

In order to study the utilization of digestible energy (DE) and digestible protein (DP) and their partitioning between maternal body, milk and foetal tissues, 70 primiparous rabbit does were used. Fourteen does were slaughtered immediately after their first parturition (preliminary slaughter group) to estimate initial body composition. The 56 remaining does were divided into 4 groups (HL, HR, ML and MR) offered two pelleted diets given at two different feeding levels: H groups were fed a high protein diet (201 g/kg DM of CP); M groups were fed a medium protein diet (184 g/kg DM of CP); L groups were fed *ad libitum*; R groups were fed restricted (0.75 of *ad libitum*). The females were kept in individual metabolism cages and remated 4 to 5 days after parturition. Those that got pregnant were controlled until their 2nd parturition (12 of HL group, 9 of HR group, 10 of ML group and 9 of MR group). They were then slaughtered with their new-born litters.

Dietary protein level did not influence the milk yield and feed intake of the does and the growth of their litters. On the contrary, feeding levels significantly affected doe and litter performance. A large decrease in empty body weight was observed in all groups between the 1st and 2nd parturition. Body composition of the does changed substantially, with a considerable loss of fat and energy. Protein balance was positive in all groups. The average loss of energy corresponded to 0.38 and 0.34 of the initial body energy, in the H and M groups respectively ($P>0.10$), and to 0.32 and 0.40, in the L and R groups ($P<0.05$).

The DE requirement for maintenance was estimated to be 470 kJ/day/kg $W^{0.75}$ and the efficiencies of utilization of DE and body energy for milk production were 0.63 and 0.76, respectively. The efficiency of utilization of DE for foetal tissue synthesis was estimated to be 0.30.

The DP requirement for maintenance was 3.76 g/day/kg $W^{0.75}$ and the efficiency of utilization of DP and body protein for milk protein production were 0.80 and 0.60, respectively. The efficiency of utilization of DP for foetal protein synthesis was 0.42.

Introduction

Energy and protein metabolism in rabbit does have been recently investigated (Cheeke, 1987; Lebas, 1988 and 1989; Maertens and De Groote, 1988; Parigi-Bini, 1988). Partridge and co-workers performed a series of experiments on does in different physiological states and found high and very variable digestible energy (DE) requirements for maintenance and efficiencies of DE utilization for milk production (Partridge, 1986; Partridge and Allan, 1982 and 1983; Partridge, Fuller and Pullar, 1983; Partridge, Lobley and Fordyce, 1986).

Extended research has been carried out also in this laboratory on primiparous rabbit does in different physiological states (pregnancy, lactation, lactation and concurrent pregnancy). During pregnancy, a transfer of energy (as protein and fat) from the maternal body to the pregnant uterus was observed (Parigi-Bini, Xiccato and Cinetto, 1990). During lactation, a substantial deficit of energy in both non-pregnant and concurrently pregnant does was found (0.28 to 0.33 of the initial energy content). Concurrent pregnancy and lactation increased the requirement of energy for maintenance in comparison with non-pregnant lactating does. The efficiencies of utilization of DE and body energy for milk production were similar in both pregnant and non-pregnant lactating does. Nevertheless, protein demand was increased by overlapping pregnancy and lactation due to the high protein content of foetal tissues and the low efficiency of utilization of dietary protein for foetal growth (Parigi-Bini, Xiccato and Cinetto, 1991a and 1991b; Parigi-Bini, Xiccato, Cinetto and Dalle Zotte, 1992).

The present work was carried out to study the effect of different feeding and protein levels in order to guarantee the energy and protein requirements of reproducing does and consequently avoid a severe decrease of body reserves due to lactation.

Materials and methods

Seventy hybrid rabbit does in their first pregnancy were used. They were the female-line parent of a commercial hybrid (Provisal S.p.A., Molinella (BO), Italy) based on the N.Z.W. breed. Fifteen days before parturition (live weight 4101±374 g), the does were allocated to individual metabolism cages. Cages and breeding house characteristics have been described previously (Parigi-Bini *et al.*, 1991a and 1992).

Within a few hours after parturition, 14 does and their litters were slaughtered. The does were emptied of gut content and analyzed to estimate the initial empty body composition of the 56 remaining does (comparative slaughter technique) (Parigi-Bini *et al.*, 1991a and 1992).

The litters of the remaining does were standardized to eight pups and separated from their mothers whom they were allowed to suckle once a day. Milk yield was measured daily for the entire lactation of 30 days by weighing the doe immediately before and after suckling. The litters were also weighed daily before feeding.

Since the first kindling, the does were divided into four groups of 14 animals each, which were assigned to two protein levels and two feeding levels, according to a 2x2 factorial design:

- group HL, fed a high protein diet (H), containing 186 g/kg of crude protein, given *ad libitum* (L);
- group HR, fed H diet, given at restricted level (0.75 of *ad libitum*) (R);
- group ML, fed a medium protein diet (M), containing 165 g/kg of crude protein, given *ad libitum*;
- group MR, fed M diet, given at restricted level.

Feed intake was recorded daily and the quantity of feed to be offered to the R does was determined on the basis of the average voluntary feed intake measured the previous day for the L groups.

Between the 4th and the 5th day after parturition, all does were remated (artificial insemination). Twelve days after parturition, the does were tested for pregnancy by palpation. Forty does had their second parturition (12 of HL group, 9 of HR group, 10 of ML group and 9 of MR group). The remaining does were excluded from the experiment (9 for unsuccessful pregnancy, 3 for abortion and 4 for sickness).

The digestible energy content of the diets and the digestibility of other components were determined by digestibility and nitrogen balance trials. Total collection of faeces and urine was carried out on 40 does (10 for each group) for a period of 9 days from the 12th day of lactation (Parigi-Bini *et al.*, 1991a and 1992).

At the 20th day of lactation, the sucking pups were allowed to eat the maternal diet from their own trough separate from that of the mother. The pups were completely weaned at 30 days of life. Immediately after the 2nd parturition, all does and the new-born litters were slaughtered.

The empty body of the does and their litters were analyzed separately. Chemical analyses of animals, diets, faeces and urine were performed as described previously (Parigi-Bini *et al.*, 1991a and 1992).

The composition and energy value of milk were considered equal to those determined in previous research carried out on does of the same commercial hybrid and age (Parigi-Bini *et al.*, 1991a): water 699 g/kg, protein (N x 6.38) 115 g/kg, fat 133 g/kg, ash 23 g/kg, lactose (by difference) 30 g/kg, gross energy 8.27 MJ/kg.

The difference between the final and the initial empty body composition was used to calculate the energy balance of the does. Initial empty body composition was estimated by means of linear regression equations on live body weight determined from the 14 does slaughtered at the 1st parturition.

Statistical analyses were performed by analysis of variance using the least-square method (Harvey, 1987).

To estimate the efficiencies of utilization of DE intake and of body energy for milk production and pregnancy, the following multiple regression model was adapted (all variables are expressed in kJ/day/kg $W^{0.75}$):

$$Emilk_{ijk} = b_0 + P_i + F_j + b_1DEI_{ijk} + b_2REd_{ijk} + b_3REf_{ijk} + \varepsilon_{ijk}$$

where $Emilk_{ijk}$ = milk energy production of k^{th} doe of i^{th} protein group and j^{th} feeding group; b_0 = constant term; P_i = effect of i^{th} protein diet (i: 1 = H diet; 2 = M diet); F_j = effect of j^{th} feeding level (j: 1 = *ad libitum*; 2 = restricted); b_1, b_2, b_3 = efficiency of utilization of DEI, REd and REf; DEI_{ijk} = digestible energy intake; REd_{ijk} = retained energy in doe empty body; REf_{ijk} = retained energy in foetal body (2nd parturition); ε_{ijk} = residual error (0, σ_e^2).

A similar regression model was adapted to estimate the efficiencies of digestible protein (DP) intake and body protein for milk and foetal protein synthesis (all variables are expressed in g/day/kg $W^{0.75}$):

$$Pmilk_{ijk} = b_0 + P_i + F_j + b_1DPI_{ijk} + b_2RPd_{ijk} + b_3RPf_{ijk} + \varepsilon_{ijk}$$

where: $Pmilk_{ijk}$ = milk protein production of k^{th} doe of i^{th} protein group and j^{th} feeding group; b_0 = constant term; P_i = effect of i^{th} protein diet (i: 1 = H diet; 2 = M diet); F_j = effect of j^{th} feeding level (j: 1 = *ad libitum*; 2 = restricted); b_1, b_2, b_3 = efficiency of utilization of DPI, RPd and RPf; DPI_{ijk} = digestible protein intake; RPd_{ijk} = retained protein in doe empty body; RPf_{ijk} = retained protein in foetal body (2nd parturition); ε_{ijk} = residual error (0, σ_e^2).

DE and DP requirements for maintenance (DE_m and DP_m) were calculated as $-b_0/b_1$.

Results and discussion

Feed composition and nutritive value

The composition and the nutritive value of the diets are given in table 1. The diets were prepared with the same ingredients, with slight differences in the proportions of a few feedstuffs, mostly protein concentrates. The compound feeds had a very similar chemical composition, apart from crude protein content. DE content was 11.41 and 11.20 MJ/kg dry matter (DM), for H and M diets respectively. On the contrary, DP content differed substantially (143 *vs* 125 g/kg DM). Therefore, the H diet had a DP/DE ratio higher (12.5 g/MJ) than that proposed for lactating does (11.5-12) (Lebas, 1989), whereas the M diet showed a value slightly lower (11.2) than that recommended. Nevertheless, the latter is a very common value in commercial feed for reproducing rabbits.

Performance and food intake

During lactation, protein levels did not influence the performance and feed intake of does and their litters (table 2). The restriction of feeding resulted in a lower average milk yield (171.4 *vs* 133.3 g/day, for groups L and R respectively, $P<0.001$) and in a loss of live weight. The lower milk intake caused a decrease in the litter weight gain and in the weaning live weight ($P<0.01$). The litters of R does ingested a quantity of pelleted feed slightly lower than the litters of the L does, whereas, in a previous research, the litters of does given restricted feed consumed more pelleted feed and compensated for the lower amount of milk available (Parigi-Bini *et al.*, 1992).

Body composition of does and litters

As in previous research, a significant increase in the gut fill was observed between the 1st and the 2nd parturition (table 3). Large variations in the chemical composition and in the energy content of the empty body of the lactating does were also confirmed (Parigi-Bini *et al.*, 1991a, 1991b and 1992).

The composition and energy value of the empty body were not influenced by the level of dietary protein. On the contrary, feeding level affected body composition at the 2nd kindling. In particular, water and fat content significantly differed between L and R groups ($P<0.05$).

Protein level significantly affected reproductive performance of does at their 2nd parturition. In fact, all abortions and the highest number of unsuccessful pregnancies (6 of 9) were observed with the M diet. Moreover the new-born litter weight was reduced in does receiving the medium protein diet ($P<0.05$). The number and weight of pups born alive per litter were also reduced, whereas the average weight of pups was not influenced. The chemical composition of litters was slightly affected by protein level, with higher protein content in H litters.

Feed restriction reduced the reproductive performance of does at the 2nd parturition. The weight of new-born litters was significantly decreased in the R does ($P<0.01$), as well as the average weight of pups born alive per litter ($P<0.10$). Only the protein content of pups was influenced by the maternal level of feeding, with higher content in L litters ($P<0.001$). Similar results were observed previously (Parigi-Bini *et al.*, 1992).

Table 4 shows the composition of empty body gain between the 1st and the 2nd parturition. The does showed a large decrease in empty body weight and a lower increase of gut content. The empty body weight loss was due to a very large decrease in fat, only partially compensated by some retention of water.

No differences were observed due to protein levels, with similar losses of body fat (0.70 and 0.63 of total initial body fat content, in H and M groups, respectively) and body energy (0.38 and 0.34 of the initial body energy). Protein balance was slightly positive with both diets, indicating that the feeds were adequate in terms of protein quantity and quality. In previous research on lactating and pregnant does given a commercial feed (CP: 189 g/kg DM), a slightly negative balance was observed (Parigi-Bini *et al.*, 1991b and 1992), probably due to an inadequate quality of dietary protein.

Significant differences in live and empty body gain and in fat and energy losses ($P<0.05$) were ascribed to feeding level, even though the greater utilization of body tissues and energy in the restricted does was mitigated by the reduction in milk production. The loss of body fat was 0.59 of total initial fat content in L does. As regards energy, such does showed a total loss of 11.78 MJ, equivalent to 0.32 of initial body energy. A loss of body energy (essentially as fat) of 0.32 was observed also in previous research on lactating and concurrently pregnant does. In lactating non-pregnant does the loss of energy was slightly lower (0.28) (Parigi-Bini *et al.*, 1991a and 1992).

Feeding restriction accentuated the deficit of energy and R does showed a very pronounced loss of fat (0.63 of the initial content) and energy (0.40 of the initial body energy).

Energy requirement for maintenance and efficiency of utilization of DE

The following multiple regression equation was calculated from the energy balance:

$$\text{Emilk} = -296 + 0.63\ (\pm 0.06)\ \text{DEI} - 0.76\ (\pm 0.20)\ \text{REd} - 2.07\ (\pm 0.82)\ \text{REf} \qquad (1)$$

residual s.d. = ± 43.1 $r = 0.881$ n = 38

(no significant effect was observed due to the levels of protein and feeding; all values are expressed in kJ/day/kg $W^{0.75}$; 2 does in positive energy balance were excluded).

This equation indicates an efficiency of utilization of DE intake for milk energy production equal to 0.63, while body energy reserves are used with an efficiency of 0.76 for the production of milk energy. The energy retained in the foetuses appears to be in competition with energy required for milk having a metabolic cost 2.07 times higher than that for milk. The efficiency of utilization of DE for foetal growth was calculated from the equation 1:

$$\text{REf/DEI} = (\text{Emilk/DEI})/(\text{Emilk/REf}) = 0.63/2.07 = 0.30.$$

In conditions of energy equilibrium, the DE requirement for maintenance (DE_m) is:

$$DE_m = 296/0.63 = 470 \text{ kJ/day/kg } W^{0.75}.$$

The estimate of utilization of DE found for milk production (0.63) corresponds to that observed in previous research in this laboratory both on lactating non-pregnant and lactating and concurrently pregnant does (Parigi-Bini *et al.*, 1991a and 1992) and confirms the estimates of Partridge (1986) and Lebas (1988).

The efficiency of utilization of maternal body energy for milk production determined in the present work (0.76) is equal to that found in previous research on lactating and concurrently pregnant does and slightly lower than that observed on lactating non-pregnant does (0.81) (Parigi-Bini *et al.*, 1991a and 1992).

The efficiency of utilization of dietary energy for foetal growth, *i.e.* 0.30, is slightly higher than that found previously on lactating and concurrently pregnant does (0.27) and confirms the value of 0.31 found on nulliparous pregnant does (Parigi-Bini *et al.* 1991b). No other published data on reproducing rabbits are available. Regarding pigs, De Wilde (1980) and Walach-Janiak, Raj and Fandrejewski (1986) reported similar or lower efficiencies (0.20 to 0.30), while Whittemore and Morgan (1990) supplied much higher values (0.40 to 0.70). The high cost of foetal growth may be explained by the very high protein content in the foetal body and its very fast turnover (Young, 1979).

The estimate of DE_m in this work (*i.e.* 470 kJ/day/kg $W^{0.75}$) agrees with that found in previous research in this laboratory for does in the same physiological state (*i.e.* 468 kJ/day/kg $W^{0.75}$). These values are higher than those observed on non-lactating does (398 kJ/day/kg $W^{0.75}$ for non-pregnant does and 431 kJ/day/kg $W^{0.75}$ for pregnant does) or non-pregnant lactating does (432 kJ/day/kg $W^{0.75}$) (Parigi-Bini *et al.*, 1990 and 1991b). Partridge observed DE_m values ranging from 442 to 495 kJ/day/kg $W^{0.75}$ for lactating does (pregnant or non-pregnant) (Partridge *et al.*, 1983 and 1986). Lebas (1989) indicated 400 and 460 kJ/day/kg $W^{0.75}$ as the DE_m requirements of non-lactating and lactating does, respectively.

Protein requirement for maintenance and efficiency of utilization of DP

The maintenance requirement and the efficiency of utilization of DP for milk protein synthesis and foetal growth were estimated by multiple regression as follows:

$$Pmilk = -3.01 + 0.80\ (\pm 0.12)\ DPI - 0.60\ (\pm 0.33)\ RPd - 1.90\ (\pm 0.58)\ RPf \qquad (2)$$

residual s.d. = ± 0.85 $\quad r = 0.805 \quad$ n = 38

(no significant effect was observed due to the level of protein and feeding; all values are expressed in g/day/kg $W^{0.75}$).

The efficiency of utilization of DP for milk protein was 0.80, whereas the efficiency for transforming body protein in milk protein was 0.60. The DP required for foetal growth appears to have a cost higher (1.66 times) than that for protein milk synthesis. As a result, the efficiency of utilization of DPI for pregnancy was estimated to be 0.42 (0.80/1.90).

The protein requirement for maintenance (PD_m) can be estimated, assuming a protein equilibrium:

$$PD_m = 3.01/0.80 = 3.76 \text{ g/day/kg } W^{0.75}.$$

The efficiencies of utilization both of DP and of maternal body protein for milk protein synthesis agree with the results of previous works on lactating non-pregnant does (0.77 and 0.59, respectively) and on lactating and concurrently pregnant does (0.76 and 0.61, respectively) (Parigi-Bini *et al.*, 1991a and 1992). The efficiency of DP utilization for foetal protein synthesis, *i.e.* 0.42, is similar to that found previously (0.46) (Parigi-Bini *et al.*, 1992). These efficiencies are much lower than those found in growing rabbits by De Blas, Fraga and Rodriguez (1985) and by Parigi-Bini and Xiccato (1986), probably due to the very high turnover of foetal protein, as previously mentioned.

Also the DP requirement for maintenance estimated here confirms the values observed in previous research (3.73 to 3.80 g/day/kg $W^{0.75}$) (Parigi-Bini *et al.*, 1991a and 1992).

Conclusions

This research confirms that primiparous lactating does have a voluntary feed intake inadequate to maintain energy equilibrium. Consequently, they show a considerable reduction in empty body weight and a high loss of body fat and body energy. Feeding restriction accentuated the loss of empty body weight, body fat and energy, even though a significant decrease in milk production occurred.

Diets with 165 and 186 g/kg of good quality crude protein permitted a similar performance to be obtain (milk yield and litter growth). However, the lowest protein diet reduced reproductive performance in terms of fertility, size and weight of litters at 2nd parturition. A higher level of dietary protein was not able to reduce the energy deficit, but improved reproductive performance.

Protein requirement was increased by overlapping pregnancy and lactation because of the high protein content of foetal tissues and the low efficiency of utilization of dietary protein for foetal growth.

This research was carried out with a grant from the Consiglio Nazionale delle Ricerche (C.N.R.), Italy (C.T. 90.002884.CT06).

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Table 1: Chemical composition (g/kg DM) and nutritive value of feeds(1)

	High protein (H)	Medium protein (M)
Dry matter	912	911
Crude protein (Nx6.25)	204	181
Ether extract	35	34
Crude fibre	161	164
Ash	90	87
NDF	338	347
ADF	188	189
ADL	42	43
Gross energy (MJ/kg DM)	18.40	18.27
Digestible energy (MJ/kg DM)(2)	11.41	11.20
Digestible protein (g/kg DM)(2)	143	125
DP/DE ratio (g/MJ)	12.5	11.2

1) Ingredients (H vs M diet) (g/kg): dehydrated lucerne meal (360 vs 360), wheat middlings (230 vs 230), barley meal (140 vs 190), dried beet pulp (50 vs 80), soybean meal (110 vs 50), sunflower meal (60 vs 40), cane molasses (20 vs 20), dicalcium phosphate (12 vs 13), limestone (6 vs 4), salt (3.3 vs 3.3), vitamin-trace element supplement (6.7 vs 6.7), dl-methionine (1 vs 1.3), l-lysine-HCl (0 vs 0.7), Cycostat (1 vs 1).

2) Determined on the basis of average digestibility of two feeding levels: energy (0.620 for H diet and 0.613 for M diet); crude protein (0.702 and 0.690).

Table 2: Performance and feed intake of does and their litters

	Experimental group				Probability (1)		
	HL	HR	ML	MR	H-M	L-R	PxF
No. of does	12	9	10	9			
Live weight (g) at							
1st parturition	3621	3588	3748	3621			
30th day (end of lactation)	4017	3946	4118	3831		P<0.10	
2nd parturition	3557	3411	3743	3444		*	
Interval between parturition (days)	35.1	35.1	35.0	35.1			
Feed intake (g/day)							
0 to 30 days	321.0	242.2	316.0	243.9		***	
1st to 2nd parturition	306.4	233.7	298.6	235.2		***	
Milk production (g/day)							
0 to 30 days	174.9	132.3	167.8	134.4		***	
No. of litters (first parturition)	12	9	10	9			
Litter size							
Initial	7.91	8.00	7.78	7.78			
30th day (weaning)	7.64	6.63	7.33	6.67		*	
Live weight (g)							
Initial	434	454	455	440			
30th day	4997	3961	4717	4017		**	
Live weight gain (g/day)							
0 to 30 days	147.2	113.1	137.5	115.4		**	
Feed intake (g/day)							
20 to 30 days	152.6	129.4	144.4	144.7			

* = P<0.05; ** = P<0.01; *** = P<0.001

1) Contrasts: H-M = H protein vs M protein diet; L-R = ad libitum vs restricted feeding; PxF = Protein x Feeding level interaction.

Table 3: Comparative slaughter data of does and their litters

	1st parturition	2nd parturition (1)				Probability (2)			
	Preliminary slaughter group	HL	HR	ML	MR	I-II	H-M	L-R	PxF
No. of does or litters	13	12	9	10	9				
Live body weight (g)	3584	3556	3411	3743	3444			*	
Gut content (g)	241	303	282	331	311	*			
Empty body weight (g)	3343	3253	3129	3412	3133			*	
Empty body composition									
Water (g/kg)	602	692	696	672	702	***		*	
Protein (g/kg)	201	219	222	212	220	***			
Fat (g/kg)	164	57	49	83	44	***		*	
Ash (g/kg)	33	31	33	33	34				
Energy (MJ/kg)	10.78	7.17	7.00	8.02	6.85	***		P<0.10	
No. of pups born per litter	10.4	8.5	7.5	6.2	6.6	**			
New-born litter weight (g)	537	511	436	367	334	*	*	**	
No. of pups born alive per litter	8.6	8.2	6.9	5.2	5.9		P<0.10		
Wt. of pups born alive per litter(g)	441	496	413	320	305		*		
Avg. wt. of new-born pups (g)	53.6	61.2	59.3	58.9	52.5				
Avg. wt. of pups born alive (g)	56.0	62.5	60.9	62.0	52.9			P<0.10	
Composition of litters (3)									
Water (g/kg)	797	801	809	807	812	*		P<0.10	
Protein (g/kg)	127	131	123	126	121		*	***	
Fat (g/kg)	53	45	45	44	43	**			
Ash (g/kg)	22	23	23	23	23	P<0.10			
Energy (MJ/kg)	4.91	4.73	4.53	4.57	4.43	*			

* = P<0.05; ** = P<0.01; *** = P<0.001

1) The initial empty body weight and composition of the does slaughtered at the 2nd parturition were estimated by linear equations, determined from the empty body weight and composition of the does slaughtered at the 1st parturition (see text).
2) Contrasts: I-II = 1st vs 2nd parturition; H-M = H protein vs M protein diet; L-R = ad libitum vs restricted feeding; PxF = Protein x Feeding level interaction.
3) Pups born dead included.

Table 4: Effect of protein and feeding levels on empty body gain composition of does between the 1st and the 2nd parturition

	Experimental group				Probability (1)		
	HL	HR	ML	MR	H-M	L-R	PxF
No. of does	12	9	10	9			
Live weight gain (g)	-64	-177	-5	-177		*	
Gut content gain (g)	97	76	121	105			
Empty weight gain (g)	-161	-253	-126	-282		*	
Composition of empty body gain:							
Water (g)	200	147	171	147			
Protein (g)	24	10	10	-2			
Fat (g)	-375	-404	-306	-421		*	
Ash (g)	-10	-6	-1	-6			
Energy (MJ)	-13.06	-14.15	-10.49	-14.89		*	

* = P<0.05; ** = P<0.01; *** = P<0.001

1) Contrasts: H-M = H protein vs M protein diet; L-R = ad libitum vs restricted feeding; PxF = protein x feeding level interaction.