Alopecia in Rabbits Fed Semi-purified Diets with Marginal Protein Content

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Summary
A marginal (i.e. less than optimal) protein intake by rabbits has been suggested to cause hair loss. Our recent study with rabbits offered the opportunity to test the suggestion under controlled conditions. Young growing rabbits were fed one of four semi-purified diets. The diets were high (21.6 energy % protein) or low in casein (13.0 energy % protein) with either high or low level of corn oil (21.1 instead of 5.3 energy %). On various body parts of the rabbits, the degree of alopecia was scored. Upon slaughter, the amount of hair in the stomach was assessed. The low-fat diets with either high or low protein content induced similar growth rates, indicating that the low protein level was not limiting growth. It was found that a decrease in the protein level of the low-fat diet was associated with significant alopecia on legs and belly and less hair accumulation in the stomach. It is concluded that signs of alopecia that are observed in rabbits fed experimental diets could relate to low dietary protein concentration.

Introduction
Our feeding studies in rabbits have shown that dietary fats promote growth more efficiently than identical amounts of metabolizable energy in the form of carbohydrates (*Alhaidary et al., 2010a,b*). We then tested the hypothesis that dietary fat spares protein for growth more effectively than do carbohydrates which would imply that the growth-promoting effect of fat is greater on a marginal than normal-protein diet (*Alhaidary et al., 2010c*). The dietary protein requirement of growing rabbits has been set at 16% (*National Research Council, 1977*). However, with the use of properly formulated semi-purified diets containing casein as protein source, 11% protein in the diet will support growth (*Cheeke, 1987*). It has been suggested that a marginal protein intake by rabbits may be associated with hair loss (*Cheeke, 1987*), but no reference to published observations was made. Our recent study (*Alhaidary et al., 2010c*) offered the opportunity to test the suggestion under controlled conditions, and thus we decided to assess the degree of alopecia in the rabbits.

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Materials and Methods
Experimental details have been described elsewhere (*Alhaidary et al., 2010c*). Random-bred, male rabbits of the New Zealand strain were used. The rabbits were housed individually. Food and demineralised water were provided *ad libitum*.
On arrival in the animal house, the rabbits, which were aged about 6 weeks, were maintained on commercial rabbit pellets for two weeks. Subsequently, the rabbits were allocated to one of the four experimental diets given in Table 1. The diets contained two levels of casein as protein source and two levels of corn oil as fat source. To the low-protein, low-fat diet extra corn oil and/or extra protein was added at the expense of an isoenergetic amount of cornstarch and dextrose in a 1:1 ratio. The diets were in pelleted form. Each dietary group consisted of 12 animals. The experimental period lasted 56 days.

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Table 1. Composition of the experimental diets.

	Energy percentage of dietary fat/protein[1]			
	5.3/21.6	21.1/21.6	5.3/13.0	21.1/13.0
Ingredient	g			
Corn oil	20	80	20	80
Casein	177.8	177.8	106.7	106.7
Corn starch/dextrose, 1/1	487.25	352.25	557.43	422.43
Constant components[2]	314.95	314.95	315.87	315.87
Total	1000	925	1000	925

[1]Percentage of metabolisable energy from fat as calculated on the basis of the ingredient composition. The following energy values for metabolisable energy were used (kJ/g): protein, 16.5; fat, 35.8; fiber, 4.1; carbohydrates, 15.9.
[2]The constant components are given elsewhere (Alhaidary et al., 2010c).

Body weights and feed intake were measured.
One day before the end of the experiment, the degree of alopecia on various parts of the body was assessed in random order by author ACB while being blinded to treatment modality. Scores of 0-3 were assigned to each variable, with 0 given if no abnormal variation was detected. The procedure of scoring has been described (*Beynen et al., 1992*). At the end of the experiment, the rabbits were transported to a commercial slaughterhouse. They were stunned in random order by electrocution and killed manually by cutting the carotid arteries and jugular veins. The stomachs were collected to score the content of hair on a 0-3 scale as described (*Beynen et al., 1992*).

Body weight and feed intake data were subjected to two-way ANOVA to identify statistically significant effects of fat type, fat level and the interaction between type and level. The scores for alopecia and hair content of stomach were compared by the Mann Whitney U test. The level of statistical significance was pre-set at $P < 0.05$.

Results

Table 2 shows that final body weights were similar for the two low-fat diets with either high or low protein content. When corn oil was added to the high-protein diet, there was a significant increase in final body weight and an increase in group mean feed in-

Table 2. Body weights and feed intake in rabbits fed the experimental diets.

	Energy percentage of dietary fat/protein				
	5.3/21.6	21.1/21.6	5.3/13.0	21.1/13.0	**Significance**[1]
Body weight, kg					
Day 0	1.52 ± 0.10	1.53 ± 0.16	1.53 ± 0.18	1.53 ± 0.16	NS
Day 56	2.48 ± 0.32	3.01 ± 0.38	2.44 ± 0.45	2.36 ± 0.30	F, P, FxP
Feed intake, g/day	81.6 ± 9.3	95.4 ± 11.7	85.3 ± 14.2	76.3 ± 9.1	P, FxP

Results are expressed as means ± SD for 12 rabbits per dietary group, except for the rabbits fed the 21.1/13.0 diet, which consisted of 11 animals.
[1]Significance was calculated by analysis of variance. F = effect of amount of fat; P = effect of protein; FxP = effect of interaction; NS = no significant effect.

Table 3. Degree of alopecia and hair in stomach in rabbits fed the experimental diets.

	Energy percentage of dietary fat/protein																		Significance[1]
	5.3/21.6				21.1/21.6				5.3/13.0				21.1/13.0						
Alopecia, absolute frequency on 0-3 scale																			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**			
Head	12	-	-	-	12	-	-	-	12	-	-	-	11	-	-	-	NS		
Cervix	12	-	-	-	12	-	-	-	8	1	2	1	9	1	-	1	NS		
Back	12	-	-	-	12	-	-	-	11	-	-	-	11	-	-	-	NS		
Legs	10	2	-	-	8	3	1	-	2	7	2	1	5	6	-	-	P_{LF}		
Belly	11	1	-	-	7	4	1	-	2	4	2	4	7	1	3	-	P_{LF}		
Hair in stomach, absolute frequency on a 0-3 scale																			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**			
	1	5	3	3	3	2	3	3	6	5	-	-	3	4	2	-	P_{LF}		

The number of animals per variable equals the sum of the frequency distributions. [1]Significance was calculated with the Mann Whitney U test. NS = not significant; P_{LF}= effect of protein at low fat level.

take. The addition of corn oil to the low-protein diet did not influence final body weight and it lowered the group mean level of feed intake.

There was no diet effect on the fur quality on head, cervix and back (Table 3). Feeding the low-fat, high-protein diet was associated with least signs of alopecia on legs and belly. However, the incidence of alopecia was raised by both increasing fat intake and lowering protein intake. A decrease in the protein level of the low-fat diet produced statistically significant hair loss on legs and belly (Table 3).

Rabbits fed the low-fat, low-protein diet had the lowest degree of hair in the stomach (Table 3). In the other dietary groups, most animals had hair in their stomach. A decrease in the protein level of the low-fat diet caused statistically significant lower amounts of hair in the stomach.

Discussion

This study confirms our earlier studies in that the addition of corn oil to a diet with about 21 energy % casein increases weight gain (*Alhaidary et al., 2010a,b*). However, the addition of corn oil to a diet with a protein level of 13.0 energy % protein did not enhance weight gain. Because the low-fat diets with either 21.6 or 13.0 energy % protein had induced similar growth rates, the low protein level of 13.0 energy % was not limiting growth. Our hypothesis that the growth-enhancing effect of the addition of corn oil would be greater on a low-protein diet has to be rejected.

Feeding of the low-fat, low-protein diet instead of the low-fat, high-protein diet was associated with significantly less hair on legs and belly and less hair accumulation in the stomach. These two effects are indicative for hair loss. However, it may not be due to a protein deficiency because the rabbits with signs of alopecia on legs and belly attained growth rates similar to their counterparts fed the low-fat, high-protein diet. Furthermore, the effect of marginal protein intake on hair loss did not reach statistical significance for the high-fat diets. It is difficult to see why only the combination of low fat and low protein intake caused alopecia and why it only involved the legs and belly.

In rabbits housed individually, fur chewing can be related to excessive grooming habits. Stimulation

of fur chewing in rabbits fed the low-protein, low-fat diet can be excluded because there was less hair accumulation in the stomach. In rabbits housed in groups, certain rabbits may pull off fur from cage mates. It was found earlier in group-housed rabbits that lack of supplemental roughage induced fur chewing as indicated by both alopecia and hair accumulation in the stomach (*Beynen et al., 1992*).
This study with rabbits shows that a low-fat diet with marginal protein content that was adequate for growth had caused hair loss on legs and belly. Thus, the presence of signs of alopecia in rabbits fed experimental diets could point at low protein intake. If signs of alopecia do occur in rabbits fed experimental diets, it may be checked whether the dietary protein is low and whether this is intentional or not.

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