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ALBINO RABBITS CAN SUFFER FROM MEGACOLON-SYNDROME WHEN THEY ARE HOMOZYGOUS FOR THE "ENGLISH SPOT" GENE (EnEn)

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SUMMARY : Segregation patterns, reproduction rate, mortality, fattening performance and gut parameters of albino (cc) rabbits heterozygous (Enen) or homozygous (EnEn) for the English Spot gene have been investigated to test the hypothesis whether albinos can suffer from Megacolon-Syndrome, which is a characteristic predisposition of EnEn spotted animals. Whereas the weight of the gut (brutto) of cc Enen - animals reached 8.5 ± 0.7 % of live weight at slaughter and 17.2 ± 1.6 % of the carcass weight, cc-EnEn animals exhibited a relative gut weight of 16.5 ± 6.5 % and 37.0 ± 17.4 % respectively. In addition to these findings one cc-EnEn breeder buck suffered chronically from Megacolon-Syndrome. Thus there is evidence that the En - gene segregating in cc - genotypes may induce enteric problems in albinotic stocks.

RÉSUMÉ : Les lapins albinos peuvent souffrir du syndrome du mégacolon quand ils sont homozygotes pour le gène "papillon anglais" EnEn.
Les lapins "papillon anglais" EnEn ont une prédisposition à souffrir du syndrome du méga côlon (hyper développement du gros intestin). Les auteurs ont cherché à déterminer si cette prédisposition existe aussi chez des lapins albinos homozygotes EnEn. Pour cela, chez des lapins albinos (cc) hétérozygote pour le gène papillon anglais (Enen) ou homozygote (EnEn), ils ont étudié la ségrégation des gènes, le taux de reproduction, la mortalité, les performances d'engraissement et les caractéristiques du tube digestif. A 20 semaines chez les lapins cc - Enen, le poids brut du tube digestif représente 8,5 ± 0,7 % du poids vif et 17,2 ± 1,6 % du poids de la carcasse ; chez les lapins cc - EnEn, il représente au même stade 16,5 ± 6,5 % du poids vif et 37,0 ± 17,4 % du poids de la carcasse. En outre, un mâle reproducteur cc - EnEn souffrait du syndrome chronique du méga côlon. Compte tenu de ces éléments, il apparaît évident que le gène En ségrégeant chez les animaux de génotype cc peut induire des problèmes digestifs dans les troupeaux de lapins albinos.

INTRODUCTION

In several rabbit breeds a spotting pattern is caused by the incompletely dominant "English Spot" – gene (En) which was recently found by this research group to be associated with an inherited predisposition for a malady from Megacolon–Syndrome in a homozygous state (EnEn), thus rendering an explanation for the maldevelopment and the disease-proneness of this genotype which has been known since long (WIEBERNEIT *et al.*, 1991). A leading characteristic of the Megacolon-Syndrome is not a congenital but obviously an obstipation and stasis–induced significant gut augmentation of later onset, especially an increase of the absolute and relative weights of the large intestine (brutto and netto) with the exception of the appendix (Fig. 2). Consequently these animals do not thrive and they have lower body weights at slaughter, reduced carcass weights and an unsatisfactory carcass yield. Though the trigger point of the pathogenesis and the initial site of the deleterious gene's action still remains to be elucidated, a hypoganglionotic state in certain layers of the distal colon (GERLITZ *et al.*, 1993) and a tendency to hypothyreotic conditions (FLEMMING *et al.*, 1994) concomitant with increased adrenal size (MAHDI *et al.*, 1992) were found in EnEn – genotypes as possible pathogenetic factors. This megacolon–proneness of purebred rabbits cannot be totally inhibited by heterotic effects but was modifiable by breed, hybrid (from breed hybridization) state or sex to a certain degree. The clinical features have some similarities with other commercially important enteric diseases of rabbits, as for instance "Intestinal Paresis" of breeding does and "Rupture of the Bowels", less so with "Mucoid Enteropathy" of broiler rabbits (BERNHARDT, 1992 ; JACKSON, 1991 ; OKERMAN, 1988), the causes of which are still discussed controversially. Besides the relevance of this genetic syndrome for aspects of animal protection in fancy breeding (WEGNER, 1991) from a differentiating diagnostical point of view it therefore seemed to be of interest if there exists a possibility to import or to maintain the En – allele in

albino rabbit populations (cc) and to foster in this way an EnEn – linked disease predisposition in rabbitries.

MATERIALS AND METHODS

The matings ♂ cc enen x ♀ Cc EnEn and ♂ cc EnEn x ♀ Cc EnEn have been performed in order to compare cc rabbits with Cc rabbits that are homozygous or heterozygous for the English Spot gene (En). The albino gene (c) came from the NZW (New Zealand White) breed. The following matings and tests were carried out to obtain the genotypes of the breeder bucks (cc enen, cc EnEn) and does (Cc EnEn), mentioned above :

1. NZW - males were tested whether they were occult carriers of the En-gene (by mating them to self coloured does i.e. enen/CC-genotype of the hybrid F1-generation (F1-HYS, HYS : HYbrids of Spot breeds) from first crosses between the "Giant German Spot" (DRS) breed and the "English Spot" (ES) breed).

2. Proven NZW - bucks (cc enen) were mated to a sample of the heterozygous spotted fraction of hybrid does from the F1-generation. As a result within two non related strains (F2-HYA, HYA : HYbrids of NZW Albinos) we got 50 % of the progeny being double heterozygous (Cc Enen) animals.

3. Line crossings of these double heterozygous bucks and does were carried out (Cc Enen x Cc Enen), thus producing three quarters of progeny being pigmented and of the three possible "spotting" types enen (self coloured), Enen (standard spotted animal) and EnEn (depigmented "White Spot" rabbits). In addition one quarter of the progeny was albinotic but its spotting type was unsecured (F3-HYA). All the progeny was not inbred.

4. Segregation patterns in the progeny from testmatings with pigmented En/En - does and albino bucks allowed to recognize the spotting type of albino males and were indicating heterozygosity at the C-locus of the EnEn-does, too. Thus we identified four

Table 1 : Breeding performance of hybrid "White Spot" does (Cc-EnEn) and segregation of the offspring in dependence on the genotype of the buck (cc-enen or cc-EnEn)

	♀ Cc -EnEn x ♂ cc - enen	P	♀ Cc -EnEn x ♂cc - EnEn	Total
Spotting type of the litter	Enen		EnEn	
No. of litters	5		8	13
Born, total	37		68	105
No. of born/litter	7.40		8.50	8.08
Weaned rabbits, abs.	7.20		6.38	6.69
Mortality, absolutely (1)	1	0.0002	17	18
Mortality %	2.7		25.0	17.1
Portions of born rabbits cc : Cc	19 : 18		35 : 33	54 : 51
Ratio	1.06		1.06	1.06
Mortality of albino rabbits (cc), abs.	1		12	13
Relative (% of born albinos)	5.3		34.3	24.0
P =			0.0896	0.0593
Mortality of pigmented ones (Cc), abs.	0		5	5
Relative (% of born pigmented ones)	0		15.2	9.8
Portions of sexes in weaners ♀ : ♂, abs.	24 : 12	0.0633	23 : 29	47 : 41
P =	0.0455		0.4054	0.5224
Ratio	2.00		0.79	1.15

(1) up to slaughter with 21 weeks of age ; with exception of one albino EnEn animal which was narcotized for euthanasia due to paraplegia with unknown aetiology, death occurred during the nesting period.

Table 2 : Body weight from birth to slaughter and relative carcass weight in EnEn and Enen - rabbits.

Body weight (g)	n(2)	Enen	n(2)	EnEn	p(1)
p.p	37	65.6 ± 8.9	68	54.9 ± 14.4	0.0(t)
At day 35	36	803 ± 116	52	762 ± 232	0.2562(t)
At day 98	29	3236 ± 313	49	2681 ± 334	0.0
At day 140	29	4303 ± 398	28	3433 ± 538	0.0
At day of slaughter	29	4363 ± 454	28	3485 ± 532	0.0
Carcass weight, abs.	29	2167 ± 243	28	1597 ± 376	0.0 (t)
Carcass weight, rel.	29	49.7 ± 1.6	28	45.3 ± 5.4	0.0002 (t)

Carcass weight : warm, without head, perirenal fat tissue and subcarpal/tarsal parts of the legs.
(1) (t) indicates differences of the variances of the groups by the variance ratio (f)test. In these cases P is given from a modified t-test used by the program. Furthermore (t) symbolizes that P from Barlett's test of homogeneity of variances is lower than 0.10, thus the assumption is supported that the groups in comparison belong to populations with different means.
(2) Decreasing numbers of tested animals from birth to slaughter are due to missing data either due to mortality or due to a hitherto incomplete fattening period. Moreover the guts of three pigmented animals of each strains were used for special investigations.

Cc EnEn does and two bucks in each case of cc EnEn and cc enen genotype producing the final inbred generation (F4-HYA) which was investigated.

Since the foundation of this experimental rabbit stock which was established in spring 1990 from purebred rabbits (DRS and ES) from fancy breeders all descendants were bred, reared, fattened and evaluated under standardized conditions. Hybrid rabbits were born in big nest boxes (80 x 40 x 40 cm) affixed to breeding boxes of 80 x 80 x 60 cm of width. They were completely strewed. Feeding was with pellets (18 % CP) and water *ad libitum*. and hay was given additionally. The litters were weaned at day 35 p.p. and were caged in boxes comparable to that of the breeder does but pellets fed had 16 % of crude protein only. The same ration was fed during the fattening period from day 56 to 98 and during an additional time of observation up to slaughter at 20 weeks of age. The broilers were caged individually in modified breeder cages like they are in use for industrial rabbit production but, instead of the nest box a dark "retreat" room was furnished as well as a piece of metal sheet as a floor. Straw and hay was given also. All rabbits were housed in one air-conditioned and lightened room (15 ± 3 °C and 15 h light regime).

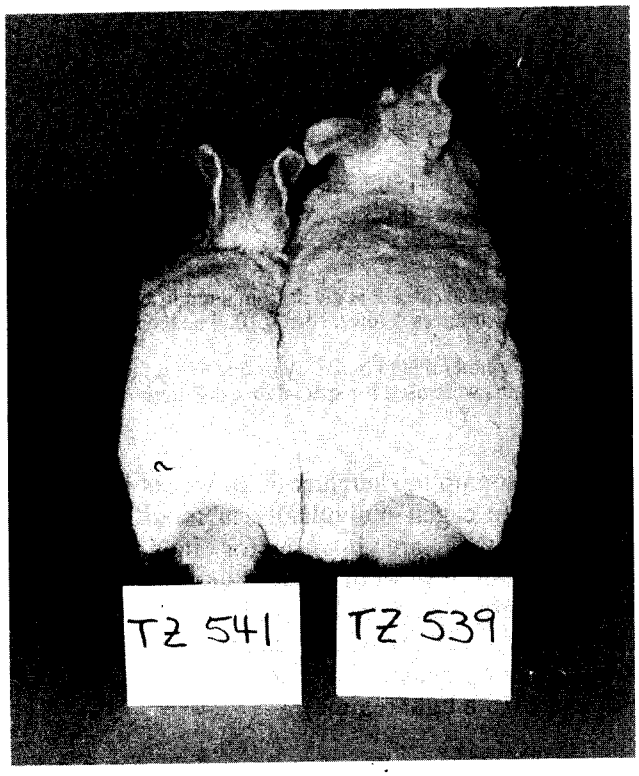
Investigation was performed as a routine for all animals : Parameters of reproduction, of fattening performance and carcass traits were recorded and a diagnostical, esp. a morpho-histometrical and gravimetrical analysis of organs was accomplished. Statistical evaluation of the results was done by chi square method resp. a 2 x 2 contingency table (segregation patterns) and by t-test for quantitative traits.

Table 3 : Weight of the gut in filled resp. emptied condition and in relation to the body - resp. carcass weight in EnEn - and Enen - genotypes

Weight (g)	n(2)	Enen	n(2)	EnEn	p
gut brutto, absolute	29	372 ± 46	28	574 ± 217	0.0(t)
1. rel. weight	29	8.6 ± 0.8	28	17.3 ± 8.3	0.0(t)
2. rel. weight	29	17.2 ± 1.8	28	41.2 ± 26.2	0.0(t)
gut net w., absolute	26	118 ± 19	25	136 ± 25	0.0055
1. rel. weight	26	2.7 ± 0.3	25	4.0 ± 1.1	0.0(t)
2. rel. weight	26	5.4 ± 0.7	25	9.1 ± 3.6	0.0(t)

gut brutto : weight of the bowel (± ingesta) after separation from the mesenterium ; gut net weight : weight of the complete wall of the bowel without ingesta
1. rel. weight : relative weight refers to the body weight at slaughter ; 2. rel. weight : relative weight refers to the carcass weight.

Figure 1 : Both albino rabbits are "Chaplins" (EnEn) from the same litter (brothers) ; only the left one showed already a marked retardation of development during the fattening period.



RESULTS AND DISCUSSION

The results revealing the manifestation of a defective development in EnEn genotypes on a NZW background are given in Table 2 and 3. It becomes quite clear, that these animals do not thrive after birth as well as animals from the Enen strain. In addition to this poor p.p. development there is good reason to believe that this retardation of growth of inbred EnEn HYA rabbits (F4) already starts *ante partum* because their body weights at birth are significantly lower compared with Enen rabbits of the same genetical background. This effect has not been found for EnEn genotypes of the former generations. On the other hand the relative carcass weight of inbred F4-EnEn HYA rabbits was not as unfavourable as that of outbred F3-EnEn HYA rabbits (Table 2 : 45.3 % and 42.8 %) though the F3 and rabbits of earlier generations were descendants from vital mothers (GÖRSE 1994) but the F4 came from EnEn does as described above. EnEn animals of the other origins exhibited a relative carcass weight of 49.8 (F2-HYS), 48.9 (F1-HYS), 47.7 (DRS) and 52.0 % (ES) but vital genotypes of these groups had a relative carcass weight between 49.2 and 51.9 %. These unequal differences of the fattening performance between the genotypes in dependence on the breed or hybrid state are indicating that some further genetical effects (inbreeding ; maternal genotype ; segregation of genotypes within the litters : F3 = enen and Enen and EnEn, F4 = EnEn or Enen only, etc.) are to be considered and may intensify the deleterious actions of the defective gene(s) or may widen the range of defect manifestations. This view is supported by different mortality rates between the strains Enen and EnEn (Table 1), being much higher (24.0 %) in EnEn HYA rabbits than in heterozygous spotted genotypes (2.7 % only), thus demonstrating the influence of the genetic background, though the mean number of born/litter should be taken into account in future investigations. Nevertheless the poor development becomes evident by virtue of the leading symptoms of the Megacolon-Syndrome : these are increased absolute and relative weights of the bowel in a filled (brutto) and emptied (netto) condition. Most values for these traits of the F4-EnEn HYA strain are nearly twice as high as the values of the heterozygous spotted strain (Table 3). Such drastic differences between the genotypes occurred since the NZW genome was introduced (F3 and F4). This is indicated by the relative gut weight (brutto) of the homozygous spotted progeny of the ES and DRS breed and the F1-HYS (ES x DRS), F2-HYS (ES x DRS)^2, F3 and F4 generations : 9.0, 12.3, 10.1, 10.6, 16.9 and at least 18.3 % within the final generation (Table 3). The corresponding values for the vital genotypes of these purebred and crossbred rabbits were 7.7, 10.1, 8.4, 8.4 and 8.5 for F2-HYA (in this part of the F2 generation no EnEn could have been born), 9.3 and a final 8.6 %. Beside these pathologic gut developments in broiler "Chaplins" further accentuations of the symptomatology of this megacolon-disease occurred demonstrated by one diseased animal with nearly completely liquefied ingesta within the large intestine (Fig. 3). These variable manifestations of Megacolon-Disease indeed induce a much broader standard deviation of quantitative traits in EnEn - animals, which is obvious from the tables.

Within the two strains EnEn (Table 4, 5) and Enen (Table 6, 7) there are no significant differences of the analyzed traits between albino rabbits and pigmented spot rabbits. But whereas the mean values of all traits investigated were nearly identical in albino and pigmented animals of the heterozygous spotted strain, the severity of pathological effects seemed to be less pronounced in albino rabbits of the EnEn strain because they (cc EnEn) might have a better fattening performance but their relative carcass weight is nearly as bad as that of pigmented Cc EnEn animals. This stunted development of cc EnEn rabbits becomes evident also as the relative weights of the gut are in agreement with that of pigmented ones (Cc EnEn). In addition albino rabbits have a significantly heavier absolute weight of the gut in an emptied condition. However further components of variance (like sex etc.) must be considered again : less males were born within the Enen strain and only one albino rabbit died

Figure 2 : The bowels and some organs of the animals from Fig. 1. Heavy impaction esp. of the proximal colon and of the first spiral folds of the caecum, a smaller appendix, reduced amounts of separable fat tissue and enlarged adrenals are features of malady from Megacolon, whereas the thyroid glands, spleen, liver and kidneys showed no gross alterations.

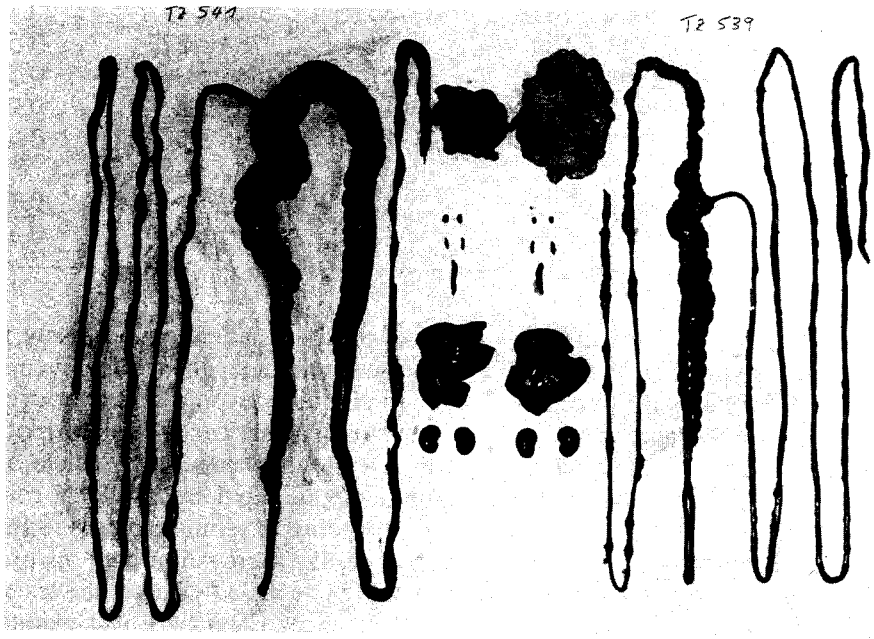


Figure 3 : Features of acute sickness due to megacolon-syndrome in a moribund homozygous spotted animal (F_4 from CC doe, Cc EnEn, 15 weeks old, forced slaughter) - an unusual picture of rapid progress of this malady.

Table 4 : Body weight from birth to slaughter and relative carcass weight in pigmented and albino rabbits of the EnEn - strain

Body weight (g)	n	Cc	n	cc	p
p.p.	33	55.4 ± 10.0	35	54.4 ± 17.7	0.7675(t)
at day 35	28	743 ± 189	24	784 ± 277	0.5234(t)
" day 98	28	2708 ± 316	21	2646 ± 361	0.5257
" day 140	16	3283 ± 520	12	3632 ± 514	0.0898
" day of slaughter	16	3327 ± 512	12	3696 ± 502	0.0683
Carcass weight, abs.	16	1511 ± 395	12	1712 ± 330	0.1655
Carcass weight, rel.	16	44.7 ± 6.3	12	46.0 ± 3.9	0.5213

perinatally. On the other hand the over-representation of weaned females was not marked within the EnEn strain. These effects might be due to other genetical factors acting in albino rabbits because there is a clear tendency to a higher mortality of albino rabbits within the "Chaplin" strain during the nesting period though its weights at birth are not different from Cc enen youngs. Thus there seem to be interactions between c and En. This view could be supported by the fact that clinical manifestations of this disease consistently occurred in females whereas males with serious problems used to be albino EnEn rabbits. Figures 1 and 2 demonstrate a comparison between a severely megacolon affected breeder buck and its still uneffected brother, both of cc EnEn genotype.

CONCLUSIONS

Albinism is not able to amend or to inhibit the manifestations of the EnEn-induced megacolon predisposition thus rendering animals of the cc-EnEn genotype liable to this disease. Consequences of the cc–homozygosity (albinism) therefore do repress pigmentation and spotting but not so the deleterious effects of the En-allele (or a closely linked defective gene) when homozygous. Such events, if happening in albino rabbitries, would mimic or even be the cause of "Intestinal Paresis" or of "Rupture of the Bowel". But we have no knowledge of the allele frequency of En in albino rabbit populations which could be calculated after breeding of albino animals to rabbits of CC enen genotype. By segregation patterns of the progeny thus it would be revealed whether the tested bucks and does were of enen, Enen or EnEn genotype if no further spotting genes are present complicating the situation.

Clinical findings in broiler rabbits afflicted with "Mucoid Enteropathy" are different from symptoms due to this Megacolon-Syndrome and a previously assumed homology is not so likely, though breed or strain, sex and the environment can vary the symptoms of the disease (Fig. 3). In addition these results give hints that the severity or even the incidence of this disease essentially depends on the genetical background and therefore even very good environmental conditions may not prevent outbreaks.

We suppose that our findings may be of relevance for partially albinotic (e.g. acromelanistic) genotypes, too.

Table 5 : Weights of the gut in filled resp. emptied condition and in relation to the body-resp. carcass weight in pigmented and albino rabbits of the EnEn - strain

Weight (g, %)	n	Cc	n	cc	p
gut, brutto					
absolute	16	561 ± 232	12	590 ± 203	0.7329
1. rel. weight	16	18.0 ± 9.6	12	16.5 ± 6.5	0.6416
2. rel. weight	16	44.3 ± 31.4	12	37.0 ± 17.4	0.4896(t)
gut, net weight					
absolute	13	125 ± 19	12	149 ± 25	0.0122
1. rel. weight	13	3.9 ± 1.3	12	4.1 ± 0.9	0.6840
2. rel. weight	13	9.1 ± 4.4	12	9.1 ± 2.7	0.9933

Table 6 : Body weight from birth to slaughter and relative carcass weight in pigmented and albino rabbits of the heterozygous spotted strain (Enen)

Body weight (g, %)	Cc		cc		p
p.p.	18	65.2 ± 6.3	19	66.0 ± 11.0	0.7778(t)
day 35	18	797 ± 130	18	810 ± 103	0.7455
day 98	14	3236 ± 352	15	3235 ± 285	0.9975
day 140	14	4313 ± 420	15	4295 ± 391	0.9048
day of slaughter	14	4369 ± 445	15	4359 ± 478	0.9545
Carcass weight, abs.	14	2166 ± 216	15	2169 ± 273	0.9746
Carcass weight, rel.	14	49.6 ± 1.7	15	49.7 ± 1.5	0.9001

Table 7 : Weight of the gut in filled and emptied condition and in relation to the body - resp. carcass weight in pigmented and albino rabbits of the heterozygous spotted strain (Enen)

Weight (g, %)	Cc		cc		p
Gut, brutto					
absolute	14	375 ± 56	15	370 ± 34.8	0.7422(t)
1. rel. weight	14	8.6 ± 1.0	15	8.5 ± 0.7	0.8162
2. rel. weight	14	17.3 ± 2.1	15	17.2 ± 1.6	0.8083
Gut, netto					
absolute	11	116 ± 25	15	119 ± 13.5	0.7051(t)
1. rel. weight	11	2.6 ± 0.4	15	2.7 ± 0.2	0.4356(t)
2. rel. weight	11	5.3 ± 0.8	15	5.5 ± 0.5	0.4179(t)

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