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EFFECT OF HOUSING HYGIENE CONDITIONS AND FEED INTAKE ON SPECIFIC AND INFLAMMATORY IMMUNE RESPONSE, BACTERIAL CECAL FERMENTATION AND SURVIVAL OF RABBITS

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ABSTRACT

Limiting the post-weaning intake of the young rabbit is known to improve its resistance to digestive troubles, while a degradation of housing hygiene is supposed to have a negative impact on health. A 2 X 2 factorial design from 28d of age (weaning) to 64 d of age was set up to study these two factors: *ad libitum* intake or restricted at 70% of *ad libitum*, and high *vs* low hygiene of housing (n=105 per group). Body weight was recorded weekly and mortality and morbidity were checked daily. At 36 and 45 d, 15 animals per group were subcutaneously immunized with ovalbumin (OVA) to assess their specific immune response. Blood were sampled at 36, 45, 57 and 64 d (slaughter) to determine total and anti OVA IgG and haptoglobin levels. At 64 d, rabbits were sacrificed to collect cecal contents (18 per group). The cecal pH, NH3, volatile fatty acid (VFA) concentration were measured to characterize microbial fermentative activity. A 30 % reduction in feed intake led to a 17% growth decrease ($p<0.001$), and improved the feed conversion ratio by 15 % ($p<0.001$). The degradation of hygiene condition decreased feed intake in rabbits fed *ad libitum* (-3.5 % $p<0.02$). Poor hygiene condition did not affect weight gain, but feed conversion between 42 and 64 d was improved ($p<0.05$). Restricted feeding led to a lower on mortality between 28 and 40 days of age (5 vs 13 deads/210 resp for restricted and ad libitum groups, p=0,047) while degraded hygiene conditions decreased overall morbidity (7.8% vs 16.6% $p<0.01$). Cecal VFA balance was not affected by treatments, while total VFA and pH were respectively increased (+19% $p<0.001$) and decreased (-0.1 pH unit $p<0.05$) in restricted fed rabbits. Nor specific anti OVA IgG neither haptoglobin were affected by treatments. Total IgG concentrations were the highest in LH-R animals after 8 days of restriction but decrease after 19 days of restriction in high hygiene condition (-2.15 % $p<0.05$). Our results suggest that a degradation of environmental hygiene had no negative impact on growth but led to an unexpected morbidity reduction. We demonstrated that neither systemic specific nor systemic inflammatory responses were impacted by the feed restriction. Moreover feed intake limitation might penalize IgG production.

Key words: rabbit, housing hygiene conditions, feed intake level, specific and inflammatory immune response, cecal microbial activity

INTRODUCTION

A high incidence of digestive diseases is observed in young mammals, particularly around the weaning period (feed transition, stresses, etc…). The etiology is often related to distortions and instability in microbiota and incapacity of innate or adaptive immune system to fight efficiently against opportunistic pathogen bacteria. Thereby, administration of antimicrobial is a common practice to control digestive diseases. However such practice led to the emergence and dissemination

of antibiotic-resistant bacteria and represents a public health threat for human as well as for animal. In this context, feed restriction is one of the most powerful alternative to reduce incidence of digestive diseases in growing rabbits and particularly epizootic rabbit enteropathy syndrome (for review Gidenne et al., 2012). In growing pigs, Le Floch et al. (2014) challenged restricted growing pig with a degradation of environmental hygiene in order to induce a moderate inflammatory status (Le Floc'h et al., 2006). They demonstrated that feed restriction reduced the occurrence of digestive disorders (Le Floc'h et al., 2014) but did not reduce the systemic inflammatory response caused by poor hygiene conditions. To get more insight in the mechanism of action of feed restriction in rabbit, this study was undertaken to evaluate the interaction between feed intake level and hygiene condition on growth performance, health, specific and inflammatory immune response and digestive microbiota functioning.

MATERIALS AND METHODS

Animals and measurements

The experiment was carried out according to the European Union recommendations on the protection of animals used for scientific purposes (2010) at the INRA Experimental Unit (INRA PECTOUL, Toulouse, France). Young rabbits (New Zealand White x Californian), weaned at 28 days of age, were caged collectively (5 rabbits per cage) and allotted into 4 groups (n=105 rabbits per group), according to a 2x2 factorial design : two groups were housed in a room with low hygiene, and fed ad libitum (LH-F) or restricted to 70% of the ad libitum intake (LH-R), and two other groups of animals were housed in a room with a high level of hygiene, and fed freely (HH-F) or restricted to 70% of ad libitum intake (HH-R). The room with a high hygiene level was cleaned thoroughly before the animal's entry. In the room with poor hygiene conditions, previous animal manure was not removed and no cleaning or disinfection of cages was done (except a fur cleaning). The lighting schedule alternated 14 h of night with 10 h of light from 01:00 to 11:00. Restricted animals were fed daily in a single distribution between 09:00 and 9:30 in order to synchronize the ingestion profile. The amounts of feed distributed to the restricted groups were calculated on the basis of a theoretical ad libitum ingestion curve, and readjusted for each diet according to the real ingestion of the ad libitum fed rabbits. Rabbits were fed the same pelleted experimental diet (crude protein 17.4%, ADF=18.4% as fed ; dehydrated alfalfa 33.0%, barley 14.5%, wheat 15.0%, soybean meal 12.0%, beet pulp: 12.0%, sunflower meal: 11.0%). Rabbits were weighed individually once a week, feed consumption was measured per cage twice a week, and morbidity and mortality were checked once a day. At 36 days of age, fifteen rabbits per group were subcutaneously immunized with injection of 600µg ovalbumin (Sigma-Aldrich) in incomplete Freund's adjuvant (Sigma) and challenged 8 days later with 300 µg ovalbumin in incomplete Freund's adjuvant. Animals were handled according to the care of animals in experimentation, in agreement with European legislation (European Union, 2003).

Blood samples were taken on the fifteen immunized rabbits at 36, 45, 57, and 64 days of age. At 64 days of age, 18 rabbits per group were sacrificed. Thereafter, the full cecum was quickly removed and weighed, and cecal contents were sampled immediately. Lymphoid organs (spleen and vermiform appendix) were weighed. The pH was measured in the cecum and dry matter of cecal content was determined by heating a 10 g sample at 103 °C for 24 h. VFA was measured by gas chromatography and NH3 concentration was determined by a colorimetric method. The total and specific antibody anti-OVA concentration of the immunoglobulin type G (IgG) was measured by ELISA as previously described (Knudsen et al., 2015) Plasma concentration of haptoglobin, a major acute-phase protein, was measured by a colorimetric method (Phase Haptoglobin assay T801, Tridelta Development Limited, Maynooth, Ireland) on blood sampled from 45 d to 64 d.

Statistical Analysis

All statistical analyses were done using R software. Morbidity and mortality were analyzed using a chi-square test. Non-repeated numerical data (growth performance and fermentative parameters) were subjected to an analysis of variance (ANOVA) using a two-factor model —hygiene of the housing and

intake level — and their interaction. Repeated numerical data (specific and total IgG and haptoglobin) were analyzed by a linear mixed model, including age, and two-factor model —hygiene of the housing and intake level — and their interaction as fixed effects, and the rabbits as a random effect. To perform mean comparison the Tukey's HSD test was used.

RESULTS AND DISCUSSION

As expected, a reduction of 30% of the intake (87 g/day vs 123 g/day) led to a 17% reduction in daily weight gain (DWG 28-63d: 49.1 g/day vs 41.5 g/day) which resulted in a 15% improvement of the feed conversion ratio. No significant differences were observed on overall mortality (10.9 %). However, a positive effect of intake limitation on mortality was observed for the period 28-40d: 5 vs 13 dead rabbits /210 rabbits, respectively for [HH-F+LH-F] and [HH-R+LH-R] groups (p=0,047). Poor hygiene conditions resulted in a half reduction of morbidity for the whole period (7.8% vs 16.6% p<0.01).

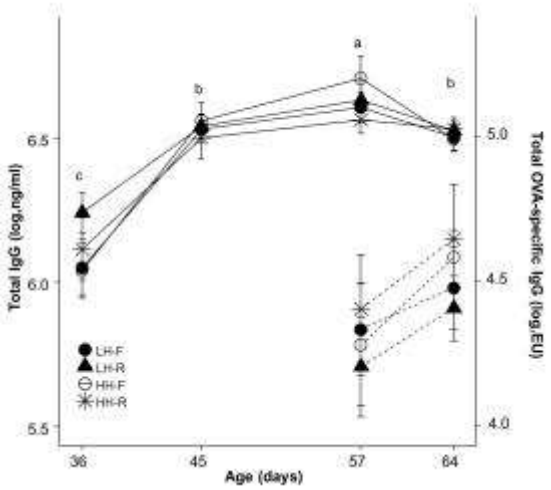

Figure 1 Effect of hygiene of housing and of intake level on the plasma anti-OVA IgG and the total plasma IgG concentrations from 36 to 64 days of age in rabbits

As previously shown by Knudsen et al. (2015), total plasma IgG concentrations evolved with age (p<0.001) to reach their maximum level at 57 d and decreased thereafter (p< 0.05) (Figure 1). At 36 d total IgG were the highest in LH-R (p<0.05). At 57 d, the rabbits in high hygiene condition with a limited feed intake had a decrease in the total IgG concentration (-2.15% p<0.05). The depletion in total IgG in restricted rabbit may result from the reduction of available energy for immune system, which are costly process or from a reduction in the metabolites available for synthesis, as suggested previously (Knudsen et al., 2015). As expected, plasma specific anti-OVA IgG concentrations were not detected prior immunization (36 d). Before the second OVA immunization (45 d), anti-OVA IgG could only be measured in 5 out of 60 rabbits and corresponded to the primary specific response. Thereafter anti-OVA IgG concentrations increased between 57 and 64 days of age. Nor hygiene condition neither intake level did affect anti-OVA IgG concentrations and haptoglobin (0.38 ± 0.18 mg/ml). This last observations is in agreement with a recent study that did not evidence any effects of the feeding level on the inflammatory response induced by LPS injection (Knudsen et al., 2016). The hygiene of the housing did not impact the relative weight of the cecum, the appendix or any of the cecal biotope parameters. The relative weight of cecum was greater in restricted fed rabbits than ad libitum groups (-1 point p<0.001 Table 1). The relative weight of the vermiform appendix was reduced in restricted rabbits (-0.3 points p<0.001). Cecal pH was lower for the animals under a restricted diet (5.60 vs 5.51 p<0.05). The total VFA concentration was 15% higher for restricted animals (101.7 vs 121.4 mMol/L, P<0.001), but molar proportion of acetate, butyrate and propionate was not different from free fed animals. Higher VFA concentration and the related lower pH observed

in restricted rabbits would mainly result from the difference in ingestion behavior compare to ad libitum one (Martignon et al., 2009; Gidenne et al., 2012).

Table 2: Cecal physico–chemical and fermentation parameters, according to housing hygiene and intake level, in the young rabbits

	Groups				SEM	P levels		
	HH-F	HH-R	LH-F	LH-R		Hygiene	IL	Hyg x IL
Caecum weight/live weight, %	6.6	7.8	7.2	8.0	0.13	0.09	<0.001	0.44
Appendix weight/live weight, %	0.31	0.30	0.33	0.28	0.01	0.88	<0.001	0.07
Spleen weight/live weight, %	0.072	0.074	0.082	0.071	0.002	0.28	0.19	0.06
Dry matter level, %	21.7	21.3	21.5	21.2	0.2	0.54	0.30	0.87
pH	5.64	5.49	5.55	5.54	0.02	0.58	0.014	0.06
Total VFA (mmol/L)	105.8	118.7	97.6	124.3	2.4	0.77	<0.001	0.10
Acetate (%)	80.5	80.6	79.0	79.9	0.3	0.11	0.46	0.60
Butyrate (%)	14.7	14.3	15.7	15.4	0.1	0.14	0.60	0.94
Propionate (%)	4.4	4.7	4.9	4.4	0.3	0.64	0.64	0.10

CONCLUSIONS

Our results confirmed the favorable effect of feed intake limitation on health and feed efficiency. A degradation of environmental hygiene had no negative impact on growth but led to an unexpected morbidity reduction. We hypothesized that the level of degradation was not sufficient enough to impair health status but instead seems to trigger mechanisms to preserve health. We demonstrated that neither systemic specific nor systemic inflammatory responses were impacted by the feed restriction. Moreover feed intake limitation might penalize IgG production.

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