

FEED ADDITIVES AS THEY AFFECT THE FATTENING PERFORMANCE OF RABBITS

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ABSTRACT

This work compared the separate effect of probiotic, prebiotic, organic acids and herbal extract dietary supplementation on growth performance. Rabbits were fed a diet without additives in the Control group (C). The same diet was supplemented with 1000 mg/kg of probiotic bacteria of *B. subtilis* and *B. licheniformis* (B group); 0.3% prebiotic inulin (I group); 0.3% organic acids (O group); or 0.3% tannin (T group). Each diet was antibiotic free, but supplemented with anticoccidial robenidine. New Zealand White rabbits aged 35 d were weighed, weaned and assigned to one of the five dietary groups. A fattening trial was carried out with 150 individually housed rabbits (n=30/group) and 360 housed collectively (3 rabbits/cage, n=72/group) and reared up to 63 d of age. Feed intake was not affected by the diets. The 63-d live weight tended to be higher (P=0.081) both in the I and T groups (2043 and 2051 g), intermediate in the C and B groups (1990 and 1996 g) and lower in the O group (1964 g). The additives exerted a significant influence on 49-63 d growth rate (P=0.006) that was the best in the I and T groups, moderate in the O and C groups and poorer in the B group. The 35-63 d feed conversion was not affected (3.14, 2.99, 2.94, 3.02, 2.95 in the C, B, I, O, T groups, respectively; P>0.05). Health risk was the lowest in the C group and highest in the O group (33.3, 43.0, 37.9, 49.5, 39.8% in order of C, B, I, O, T groups; P=0.035). In conclusion, both herbal extract (tannin) and prebiotica (inulin) can be useful as natural additives in antibiotic-free rabbit diets because growth rate improved while health status was not adversely affected. The impact of probiotics (*B. subtilis* and *B. licheniformis*) is ambiguous. The supplemental blend of organic acids (formic, acetic, propionic) was not effective since it increased the health risk and reduced 63 d body weight.

Key words: *B. subtilis*, *B. Licheniformis*, Inulin, Organic acid, Tannin.

INTRODUCTION

The ban on using antibiotic growth promoters in the EU poses a serious challenge for rabbit meat producers. Because of the very complex and peculiar digestion of rabbit (caecotrophy, microbial fermentation), this species is susceptible to enteric diseases, particularly after weaning. Accordingly, there have been several studies with alternatives, i.e. natural feed additives to replace dietary antibiotics. These results have recently been summarized (Falcão-e-Cunha *et al.*, 2007). The seriousness of the problem is indicated by the 18-20% mortality rate (Kurze *et al.*, 2003; Krieg and Rodehutschord, 2003; Briens *et al.*, 2005; Maertens and Štruklec, 2006) and 40-55% health risk (Zimmermann and Bessei, 2001; Volek *et al.*, 2007) with antibiotic-free diets despite different natural substitutions under suboptimal conditions. Data for this issue on rabbits are scarce when compared to pigs or poultry (Falcão-e-Cunha *et al.*, 2007). The lack of consistency in the results obtained with additives such as probiotics, prebiotics, enzymes and organic acids can be partly explained by different experimental protocols and hygienic conditions (Falcão-e-Cunha *et al.*, 2007). Studies with complex preparations are useful but explanations of the results can be difficult.

Therefore, this study aimed to compare more precisely the single effects on growth and health status of rabbits during the fattening period of four commercially available natural feed additives (i.e. probiotics, prebiotics, organic acids, herbal extract) under the same conditions.

MATERIALS AND METHODS

Dietary groups and animals

Rabbits received a diet without additives in the Control group (Table 1). This feed was supplemented with 1000 mg/kg of *B. subtilis* and *B. licheniformis* probiotic bacteria (B group) or 0.3% prebiotic inulin (I group) or 0.3% organic acids (O group) or 0.3% tannin (T group). Each feed was antibiotic free but supplemented with anticoccidial robenidine.

Table 1: Ingredients, chemical composition and calculated nutritive value of diets

	Control (C) (no additive)	B (probiotics)	I (prebiotics)	O (organic acids)	T (herbal extract)
Probiotic bacteria ¹	-	0.1	-	-	-
Prebiotic inulin ²	-	-	0.3	-	-
Organic acids ³	-	-	-	0.3	-
Tannin ⁴	-	-	-	-	0.3
Ingredients (%):					
Alfalfa meal	53.7	53.6	53.4	53.4	53.4
Oats	10.0	10.0	10.0	10.0	10.0
Barley	8.0	8.0	8.0	8.0	8.0
Extr. sunflower meal	7.5	7.5	7.5	7.5	7.5
Wheat grain	5.0	5.0	5.0	5.0	5.0
Dried apple pulp	5.0	5.0	5.0	5.0	5.0
Full fat soybean meal	4.5	4.5	4.5	4.5	4.5
Wheat bran	1.5	1.5	1.5	1.5	1.5
KNP-843 premix ⁵	4.0	4.0	4.0	4.0	4.0
Chemical composition (%)					
Dry matter	89.67	89.67	89.67	89.40	89.68
Digestible energy (MJ/kg)	9.74	9.74	9.72	9.72	9.72
Crude protein	16.78	16.78	16.75	16.75	16.75
Ether extract	3.83	3.83	3.82	3.82	3.82
Crude fibre	16.78	16.77	16.72	16.72	16.72
NDF	30.61	30.57	30.49	30.49	30.49
ADF	21.92	21.89	21.83	21.83	21.83
ADL (lignin)	4.68	4.68	4.66	4.66	4.66
Lysine	0.75	0.75	0.75	0.75	0.75
Methionine	0.34	0.34	0.34	0.34	0.34
Met+Cis	0.58	0.58	0.58	0.58	0.58

¹BioPlus 2B[®] (Chr. Hansen A/S, Hørsholm, Denmark): 1000 mg per kg diet that corresponds to 3.2×10^6 colony forming units (CFU) per g feedstuff, i.e. 1.6×10^6 CFU/g of *Bacillus subtilis* and 1.6×10^6 of *Bacillus licheniformis*. ²Raftifeed IPX[®] (ORAFIT, Tienen, Belgium): min. 75% inulin (semi-refined inulin powder from chicory). ³Rabbitstat (Agil Ltd., Aldermaston, Reading, England): a blended mix of carboxylic acids found naturally in the caecum of the healthy adult rabbit (Na salt stabilized blend of formic, acetic, propionic and butyric acids). ⁴Farmatan 75[®] (TANIN Sevnica, Slovenia): natural extract from sweet chestnut wood (*Castanea sativa* Mill.), a mixture of ester (hydrolysable) and glycosidic (condensed) tannins, where the hydrolysable ones are predominant. ⁵Supplemental medication: 50 mg/kg robenidine. Each diet contained 10000 NE/kg Vitamin A, 1000 NE/kg Vitamin D₃ and 60 mg/kg Vitamin E

New Zealand White rabbits aged 35 days were weighed, ear-tagged and weaned and assigned to one of the five dietary groups taking into account the body weight and litter. They were transferred from Alsóold rabbit farm of Lab-Nyúl Ltd. to the Gödöllő experimental unit (65 km distance). A fattening trial was carried out with 150 rabbits caged individually (30 rabbits/group) and 360 collectively (3 rabbits/cage, 72 rabbits/group) in all-wire cages (61x30x28 cm, i.e. 1830 and 610 cm² individual area) in a climatized building (20-23°C, 16L:8D) up to 63 d of age between August and October 2007. Feed and drinking water were provided *ad libitum*.

Data collection and management

Feed intake and feed conversion rate were precisely determined on an individual basis. Since group rearing is usual and advised in commercial farms because rabbits are highly social, the housing effect on production was also studied. Feed consumption was only estimated for group housing (total intake of group/alive animals) Morbidity corresponded to frequency of enteric disease or severe loss of weight. Health risk was the sum of morbidity and mortality. Growth data of ill and dead rabbits were excluded from the analysis.

Statistical analyses

The effects of five dietary treatments (Control, B, I, O and T) and two housing systems (one or three rabbits/cage) on growth performance were assessed by two-way ANOVA with interaction (not detailed here). Contrasts were evaluated by Student's *t*-test. Morbidity, mortality and health risk rates were subjected to Chi-square tests with Yates correction. All analyses were performed with Statgraphics 6.0 (1992) statistical software.

RESULTS AND DISCUSSION

Feed intake was not affected by the diets (Table 2). No significant differences were found for 49 d body weight ($P=0.185$) nor for 35-49 d weight gain ($P=0.173$). The 63 d body weight tended to be higher ($P=0.081$) both in the I and T groups, intermediate in the C and B groups and lower in the O group. The additives exerted a significant influence on 49-63 d growth rate ($P=0.006$). It was highest in the I and T groups, moderate in the O and C groups and poorest in the B group. The better 49-63 d feed conversion in the O and I groups than in the C group was not significant ($P=0.108$). Health risk was lowest in the C group and highest in the O group ($P=0.035$). In addition to omission of in-feed antibiotics, the reason for high health risk could be that the rabbits were exposed to higher stress (i.e. weaning and transfer from a more hygienic farm to a unit having poorer conditions). Rabbits housed in threes gained 6% less between 35 and 63 d of age and had 3.4% lower 63 d body weight than those caged singly. The reason could be the less time spent for feeding and more for moving with group vs. individual housing (Podberscek *et al.*, 1991). Health risk was not affected by housing ($P=0.642$).

Table 2: Effect of natural feed additives on growth of weaned rabbits

	day	Group					Prob.	Housing (rabbits/cage)			SEM
		Control n=96	B n=100	I n=95	O n=93	T n=98		One n=131	Three n=351	Prob.	
Body weight (g)	35	1010	1008	1016	1001	1002	0.781	1015	1000	0.106	4
	49	1518	1551	1508	1515	1544	0.185	1575 ^a	1480 ^b	0.001	8
	63	1990	1996	2043	1964	2051	0.081	2043 ^a	1974 ^b	0.008	13
Weight gain (g/d)	35-49	37.1	39.4	36.3	36.9	39.0	0.173	41.2 ^a	34.3 ^b	0.001	0.5
	49-63	35.1 ^{ab}	33.1 ^a	39.0 ^c	35.6 ^{ab}	37.3 ^{bc}	0.006	34.7 ^a	37.3 ^b	0.033	0.6
	35-63	36.4	36.3	37.8	36.6	38.1	0.327	38.2 ^a	35.9 ^b	0.004	0.4
Feed intake (g/d)	35-49	98	98	97	89	98	0.427	96	113	-	2
	49-63	121	132	132	131	136	0.450	130	133	-	3
	35-63	110	115	115	112	119	0.699	114	123	-	2
Feed conversion	35-49	2.37	2.38	2.39	2.53	2.39	0.797	2.41	-	-	0.04
	49-63	4.57	4.21	3.73	3.60	3.78	0.108	3.98	-	-	0.13
	35-63	3.14	2.99	2.94	3.02	2.95	0.252	3.01	-	-	0.03
Morbidity (%)	35-63	8.33	7.00	5.26	11.8	4.08	0.085	6.87	7.41	0.996	-
Mortality (%)	35-63	25.0	36.0	32.6	37.6	35.7	0.086	35.9	32.5	0.551	-
Health risk (%)	35-63	33.3 ^b	43.0 ^{ab}	37.9 ^{ab}	49.5 ^a	39.8 ^{ab}	0.035	42.7	39.9	0.642	-

Values in the same row within treatments with unlike superscripts differ

In our previous study (Kustos *et al.*, 2004) using the same probiotics but with a lower dose (0.04%) and with individual housing and thermal stress, growth was similarly unaffected. The health risk was reduced by 20% which was not observed here. Lui *et al.* (2006) found no effect on growth in response to added 0.15% *B. subtilis* (10^9 CFU/g) or symbiotics (0.15% *B. subtilis* plus 0.15% prebiotic MOS).

Addition of 4% chicory inulin (Frutafit®IQ) improved weight gain and lowered mortality (Volek *et al.*, 2005) but not in an enteropathy-infected environment (Volek *et al.*, 2007). Higher total caecal VFA concentration and lower pH were found in response to inulin in those trials. Maertens *et al.* (2004) reported reduced caecal pH with 2% inulin (Raftifeed®IPS) and Castellini *et al.* (2007) with adding fresh chicory. Molar proportion of acetic, propionic and butyric acids were modified by inulin in those studies. Rabbits efficiently utilized the diet containing dried chicory roots (Volek *et al.*, 2006). Use of fresh chicory seemed to promote growth and health (Cossu *et al.*, 2006). These reports agree with the positive impact of inulin observed here.

The stomach pH of young rabbits is higher than adults so administration of acidifiers may have beneficial effects. We did not find any literature for Rabbitstat studied in rabbit. Addition of 0.5% caprylic acid or 1% fatty acids (Akomed R) reduced *E. coli* counts in stomach and caecum contents but mortality was unaffected (Skřivan *et al.*, 2006). In a comparison of adding 0.4% FormaXol (formic and citric acids and essential oils); or 0.5% Formyl (formic and citric acids); or 0.4% Butyrol (butyric and citric acids), FormaXol seemed more effective in preventing the growth of pathogenic bacteria (Cardinali *et al.*, 2006; Cardinali *et al.*, 2007b). Good caecal fermentation was found with a combination of FormaXol and AciXol (Cardinali *et al.*, 2007a). Mortality rates were lower with oregano or sodium-butyrate supplementations but higher with their combination (Kurze *et al.*, 2003).

The dose-response of added 0.5% and 5% tannin (Farmatan 55%) was studied by Štruklec *et al.* (2001). Compared to control without tannin (95 g/d, 1580 g) 31-52 d feed intake and 52 d body weight tended to improve with the 0.5% inclusion (100 g/d, 1622 g). The response was greater with the higher dose of 5% (115 g/d, 1756 g) but feed conversion was unaffected (2.58, 2.53, 2.52 for 0, 0.5 and 5.0% respectively). Mortality occurred only in the control group (8%). Using the same product with 0.5% addition Maertens and Štruklec (2006) reported lower mortality (8-18%) and higher 57 d body weight (2005 g) than for controls without additive (17-29%, 1893 g). The use of 0.4% Farmatan or 5-10% of other tannin-rich substances, i.e. willowbark or oakbark was studied by Zimmermann and Bessei (2001). Final weight was 80 g higher with Farmatan than in the control but the advantage in mortality was not significant (4 vs. 9%). Addition of 2.5% willowbark did not affect body weight but reduced mortality compared to the control (12 vs. 30% and 36 vs. 43% in two farms).

CONCLUSIONS

Both herbal extract (tannin) and prebiotics (inulin) can be useful as natural additives in antibiotic-free rabbit diets because feed conversion and consequently growth rate improved while health status was not adversely affected. The impact of probiotics (*B. subtilis* and *B. licheniformis*) was ambiguous. Contrary to our hypothesis, a supplemental blend of organic acids (formic, acetic, propionic) was not effective because it enhanced health risk and reduced the 63 d body weight. It would be interesting to study the interactions of the pre- and probiotics and/or herbal extract used in this work.

ACKNOWLEDGEMENTS

This work was funded by GAK-OMFB-01336/ALAP1-00121/2004. The authors thank Végi B. and Váradi É. for technical assistance and Dr. James I. McNitt for checking the manuscript. Appreciation is expressed to TENDRE Feed Industry Ltd. for producing the experimental diets.

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