WORLD RABBIT SCIENCE, 2 (2), 67-73

EFFECTS OF DIETARY INCLUSION OF PACIFLOR® (BACILLUS CIP 5832) ON THE MILK COMPOSITION AND PERFORMANCES OF DOES AND ON CAECAL AND GROWTH PARAMETERS OF THEIR WEANLINGS

MAERTENS L. *, VAN RENTERGHEM R. **, DE GROOTE G. *

Government Agricultural Research Centre Ghent
* Research Station for Small Livestock Husbandry
Burg. Van Gansberghelaan, 92, MERELBEKE - Belgium

** Research Station for Dairy Products
Brusselsesteenweg 370, MELLE - Belgium

SUMMARY : Sixty does and their 461 weanlings were used to study the effect of dietary supplementation (1.0 x 106 CFU) of the probiotic Paciflor® (Bacillus CIP 5832) on their performances, does' milk composition and caecal fermentation pattern. The weanlings were partly housed under favourable and less favourable housing conditions. The addition of Paciflor to a reproduction diet resulted in a significantly (P<0.05) higher weaning weight (+ 8 %). Pre-weaning performances (weight, litter size, feed intake) and milk composition (e.g. γ-globulin level) showed small and not significant differences. Under favourable housing conditions daily weight gain during the fattening period was 42.3 g for the control rabbits versus 43.4 for the Paciflor treated rabbits. Under less favourable conditions these values were 41.7 and 41.8 g, respectively. Overall weight gain was not significantly affected by the treatment. However, covariated feed efficiency was 2.3 % more favourable when fed the Paciflor diet (P<0.05). At 70 d, 10 rabbits were euthanised in each group. Paciflor treated rabbits showed a significant heavier caecum weight. However pH, VFA and NH3 concentrations were not significantly different (P>0.05). Mortality tended to be higher in the Paciflor treated group.

RESUME : Effet de l'addition alimentaire de PACIFLOR® (BACILLUS CIP 5832) sur les performances et la composition laitière des lapines, et sur la croissance et les paramètres caecaux de leurs descendance.
Soixante lapines et leurs 461 jeunes ont été utilisés pour étudier l'effet de la supplémentation alimentaire (1.0 x 106 CFU) avec le probiotique Paciflor® (Bacillus CIP 5832) sur leurs performances, la composition laitière et la fermentation caecale. Les lapereaux sevrés ont été logés sous des conditions favorables et moins favorables. L'addition de Paciflor à un régime maternel a un effet favorable sur le poids au sevrage des lapereaux (+ 8 %, P<0,05). Les autres performances des femelles (poids, taille de la portée, l'ingestion) et la composition laitière (eg. taux en γ-globulines) ne diffèrent pas significativement entre les deux lots. La croissance journalière était 42,3 g sous des conditions favorables de logement et 41,7 g sous des conditions moins favorables pour les lapins du lot témoin. Ces valeurs étaient respectivement 43,4 et 41.8 g pour le lot Paciflor. Le gain de poids n'était pas significativement différent entre les deux lots bien que l'indice de consommation fût 2,3 % favorable (P<0,05) dans le lot Paciflor. Dix lapereaux ont été sacrifiés dans chaque lot, à l'âge de 70 jours. Le poids caecal des lapins du lot Paciflor était significativement plus élevé. L'acidité et les concentrations en AGV et NH3, par contre, n'étaient pas significativement (p>0,05) différent. La mortalité tend à être plus élevée dans le lot Paciflor.

INTRODUCTION

Last years, an increasing number of probiotics are available for animal production. Probiotics are usually defined as microbial cells given to animals to get a more stable and optimum bacterial flora in the gut. They are added to the diet with the intention of having these organisms to colonize the gut. Their mode of action is generally ascribed to their ability to stimulate the digestion process and/or to contribute to the microbial equilibrium of the gut (for a review see VANBELLE *et al.*, 1990). In contrast with antibiotics, their objective is not to destroy pathogenic bacteria, but to exercise a barrier effect against pathogens by preventing their development and colonisation in order to secure optimum utility of the feed. For this reasons, research on the use of these alternative natural products has been enhanced in recent years because of public concern about utilization of antibiotics in animal feeding.

Because these additives have a cost–increasing effect on the feed, only products which improve significantly animal performances are interesting for practical application. Although encouraging results are reported with numerous livestock species, the zootechnical efficiency is still disputed. Therefore zootechnical trials are necessary to evaluate the efficiency of probiotics as growth promoter or as buffer against pathogenic microorganisms. For this purpose rabbits are extremely suitable. Because of the digestive gut complexity and fragility, commercial rabbit production is characterised, among others, by its high losses due to enteric diseases (PEETERS, 1988).

The aim of this experiment was to study the effect of the dietary inclusion of the probiotic Paciflor, composed of spores of the Bacillus CIP 5832 strain, on the performances of does and their weanlings as well. Weanlings were housed under different housing conditions in order to evaluate the efficacy of Paciflor in different housing conditions.

MATERIALS AND METHODS

Animals

Seventy–two pregnant does (14 days post insemination), belonging to the female line of the Institute (MAERTENS, 1992a), were randomly allotted to two groups with equal numbers of primiparous and multiparous does. They were previously inseminated with semen from the male line of the Institute. At parturition, litters were standardized to 8 youngs by cross fostering (only intra experimental group). Two days after kindling, 30 does per group with 8 healthy youngs were retained for the experiment. The does were re–inseminated 11 days post partum. Weaning was performed when youngs were 28 days old. All weaned rabbits were used for the fattening trial. At the end of the fattening trial, 10 rabbits of both treatments were euthanised for caecal determinations. They were randomly selected out (one rabbit per cage) of the 10 first replicates, housed under favourable conditions.

Experimental diets

Experimental diets were prepared at the Institute, in accordance with the recommendations of the INRA (LEBAS, 1989) and MAERTENS (1992b). One batch of both meals was prepared and afterwards separated in two equal parts. Bacillus spores (Paciflor-Prodeta Co., Vannes, France) were added to

Table 1. Ingredient and chemical composition of diets

Ingredients(%)	Reproduction diets		Fatteners diets	
	Control	Paciflor	Control	Paciflor
Alfalfa meal	24.0		31.5	
Wheat meal	23.5		13.0	
Wheat middlings	16.4		29.0	
Sunflower meal (29%)	11.5		4.75	
Soybean full–fat	9.0		7.0	
Soybean meal	5.0		4.0	
Flax chaff	4.0		4.0	
Molasses (cane)	4.0		4.0	
Salt	0.1		0.05	
Min.Vit. pre–mix	2.5		2.5	
Coccidiostat	–		0.1	
Paciflor	–	**0.01**	–	**0.01**
Composition (%)				
Dry matter	88.4	88.6	88.6	88.5
Crude protein	18.1	18.4	17.0	17.2
Crude fibre	14.5	14.5	16.0	16.5
Crude fat	4.4	4.3	4.4	4.1
ME (MJ/kg)*	10.13		9.55	
Lysine*	0.84		0.80	
Methionine + cystine*	0.60		0.55	
Ca*	0.88		0.98	
P*	0.58		0.66	

* calculated values, based on the Dutch feedstuffs table (CVB, 1991)

the diets before the pelleting process at a dose of 100 g of Paciflor C.10. per ton of feed. This is equivalent to 1.0 x 106 colony forming units (CFU) per g. In order to avoid contamination, control diets were pelleted (∅ 3.2 mm) before the experimental diets. Temperature was always lower than 70°C during pelletization. The analysed/calculated chemical and raw material composition of both diets is shown in Table 1. Does received experimental diets ad libitum from 14 days post insemination until their youngs were three weeks old. Afterwards the growing diets were fed ad libitum. All youngs were weaned at the same day (28 - 29 days of age). All weanlings maintained the same treatment as before weaning, and litters were separately housed.

Housing

Does were housed on wire flat–deck cages (60 x 43 cm), equipped with a nipple drinker, an outside placed feeder and nestbox. Temperature was maintained at 18°±2°C. using a central water heating system. Does were maintained on a 16h light–8h dark schedule but weanlings were provided only 9 h light per 24 h. At weaning, 3 youngs were randomly selected out of each litter and transferred to a separate room (fattening trial 1). In total 30 replicates of 3 rabbits per pen were housed in cages as described above. The experimental room was artificially heated and ventilated in order to create optimum environmental conditions (± 18°C). Formerly this room was cleaned, disinfected and during 3 months non–occupied. Housing density of rabbits was low (3 rabbits/m2 floor area). All other weaned youngs (No.= 281) were transferred to a fattening unit (fattening trial 2) in order to judge the effect on mortality. They were caged per litter on a three–level battery (70 x 60 cm). This unit was occupied with other fattening and used continuously during several months. Housing density was much higher (± 14 rabbits/m2 floor area).

Recordings

Does were weighed at parturition and at 3 and 4 weeks post kindling. Youngs were weighed before standardization and at 3 and 4 weeks of age. Milk composition was determined on 5 does/treatment. These does were not selected, but the first 5 replicates were taken for collecting the milk samples. Milk samples (15–20 ml) of these does were collected weekly by handmilking after an injection with 0.1 ml oxytocin. In order to facilitate milking, nestboxes were closed in the evening prior to the sampling. At weaning all youngs received an ear tag. Post weaning recordings were done after one, two, four and six weeks (fattening trial 1). Feed consumption was recorded per pen but weight gain individually. In fattening trial 2, daily weight gain over the six–week fattening period was determined. Mortality was recorded daily. Dead rabbits were autopsied at the Provincial Animal Disease Control Laboratory, Drongen (Gent).

Analytical procedures

Analysis of diets and milk samples were made following AOAC (1991) for dry matter, ash, crude protein (N x 6,37 for milk) and ether extract. Immunoglobulin levels were further determined in milk samples as an indicator of the immunity status. γ–Globulins were determined by liquid chromatography, using a wide pore reversed phase column (RESMINI *et al.,* 1989). Samples of the experimental diets were assayed on their concentration of Bacillus CIP 5832 by the Hoechst laboratory and the Government Analysis Lab of Antwerp.

Caecal volatile fatty acid (VFA) concentrations were determined after diluting caecal samples 1:1 in distiled water. Three drops of toluene were added as protection against freezing before storing at –20°C. After thawing, aliquots of 5 ml were acidified with 1 ml of a mixture of 75 ml metaphosphoric acid (25%) and 25 ml of formic acid and centrifuged twice at 12,000 r.p.m. for 15 minutes. The concentration of VFA in the resulting supernatant was determined by gas chromatography (Perkin Elmer type 8500 gas chromatograph) using a chrompack WCOT fused silica column type (25 m x 0.22 mm) with a FFAP liquid phase. NH3 concentration was measured according to the microdiffusion method with Conway dishes (Voight and Steger, 1967).

Statistical analysis

Data were subjected to analysis of variance using the Statgraphics package 5.0 (1991). Post weaning performances were submitted to covariance analysis, with weaning weight as inter–class covariate. Mortality was judged using the X2–test.

RESULTS AND DISCUSSION

The number of viable spores after pelleting demonstrated a good agreement with the expected number of CFU. In all cases, the analytical values were within the error margin (20%) considered as acceptable. These results confirm those of MICHARD and LEVESQUE (1989) and DE BLAS *et al.* (1991).

Pre–weaning results

Weight of the does at different reproduction stages was not significantly affected by the treatment (Table 2). Does fed the control diet were somewhat heavier at parturition but the Paciflor treated does showed a higher body weight at weaning. However the increase in body weight between parturition and weaning was not significantly (P>0.05) different between both treatments.

Table 2 : Effect of Paciflor on pre–weaning performances

Diet	Paciflor	Control	SEM	Significance
No. of does	30	30	–	–
Weight of the does (g)				
at parturition	4187	4210	44.0	NS
day 21	4439	4474	48.9	NS
at weaning	4418	4394	55.1	NS
Weight of the youngs (g/young)				
at parturition	59.4	62.2	2.5	NS
day 21	364.6	340.5	6.8	+
at weaning	617.9	573.1	11.4	*
Weight of the litter (g)				
at parturition	542	503	20.9	NS
day 21	2783	2656	54.7	NS
at weaning	4716	4432	89.6	*
Litter size (alive)				
at parturition	9.13	8.07	0.4	NS
after standardization	8.00	8.00	–	–
day 21	7.63	7.80	0.1	NS
at weaning	7.63	7.73	0.1	NS
Feed intake (g/cage)				
parturition – 3 weeks	7337	7093	93.0	NS
3 weeks – weaning	3704	3489	62.1	+

+ : P<0.10; * : P<0.05; NS: non significant

Birth weight of youngs from control does was 5% higher than for those fed the Paciflor diet. This could be explained by the difference in litter size of 1 young in favour of the Paciflor. However, these differences can not be ascribed to the treatment, because does received the experimental diets only 14 days after conception.

Weaning weight, in the standardized litters, was significantly higher (P<0.05) when fed the Paciflor diet. Youngs' weaning weight was 617.9 and 573.1 g, respectively. This significant positive effect (+ 8%) of the Paciflor treatment could not be ascribed to differences in litter size because pre–weaning mortality was very low (5% or less) in both groups.

Table 3 : Average milk composition according to treatment and lactation week.

Lactation	DM (%)		Ash (%)		Protein (%)		Fat (%)		γ globulin (g/l)	
Week	Paciflor	Control	Paciflor	Control	Paciflor	Control	Paciflor	Control	Paciflor	Control
1	–	–	–	–	11.5 * 0.5	11.8 0.4	12.9 1.6	12.9 1.9	10.1 1.0	9.9 1.4
2	–	–	–	–	11.3 0.3	11.9 0.8	11.9 1.2	12.4 1.4	9.9 1.5	11.0 1.4
3	28.3 1.6	30.3 0.6	2.1 0.1	2.1 0.1	11.1 0.6	11.4 0.5	11.2 1.0	13.6 1.2	10.1 1.4	10.6 0.8
4	33.4 2.5	33.9 1.8	2.5 0.1	2.7 0.3	13.0 0.3	13.2 1.6	15.2 2.0	17.1 2.6	12.2 2.2	13.5 1.9

* mean (n = 5) ± SD ; Differences between treatments are not significant.

The effect on weaning weight confirmed previous published data (NGUYEN *et al.*, 1988). They also found an increase of 8% when fed a Paciflor supplemented diet.

Feed consumption tended (P<0.10) to be higher in cages fed the Paciflor diet. An explanation could be searched in the increased weaning weight observed for this treatment. Especially the last week before weaning, when youngs show a significant intake of solid feed, a higher pellet intake became evident in the Paciflor group. However, our data are not sufficient to precise if the increased weaning weight (and feed intake) is due to a probably higher does' milk production or the result of a direct treatment effect.

Milk composition is given in Table 3. Dry matter and ash content were not determined on milk samples of week 1 and 2 because of an insufficient quantity sampled. Differences between treatments were not significant (P>0.05) for none of the parameters studied. Immunoglobulin levels were around 10 g/l, in the first lactation weeks, and seemed not to be influenced by the treatment. With increasing lactation stage, milk samples were more concentrated. This was evident at the end of the 4th week and in agreement with previous published data (LEBAS, 1971).

Post-weaning performances

Because weaning weight was significantly (P<0.05) different (Table 4), a covariance analysis was performed with weaning weight as covariate. The significance of the covariate was very high (P<0.001) for the weight at 70 days (r = 0.78), feed intake (r = 0.60) and feed conversion (r = 0.56) However, this was not the case with daily weight gain (r = 0.33 ; P>0.05).

Paciflor treated weanlings tended (P<0.10) to show a higher daily weight gain (DWG) under optimum housing conditions, 43.4 and 42.3 g respectively. Finishing weight was significantly higher (P<0.05) when fed the Paciflor diet. However, after covariance analysis final weight (70 days) was only slightly and not more significantly higher. On the other hand, feed conversion differed 2.3% in favour of the Paciflor treatment (P<0.05) after covariance analysis. Mortality was limited to 2 rabbits per group.

Also **under less favourable housing conditions**, DWG and final weight showed small and not significant (P>0.05) differences in favour of the Paciflor treatment (Table 5). Differences between treatments were less pronounced than under optimum housing conditions. Mortality was higher in the Paciflor treated litters. This non-significant difference was mainly due to mortality in only a small number of litters. Because litters were not divided at weaning over the two treatments, litter influence could be responsible for this difference. Mortality occurred in only 8 litters of the control group and in 11 litters of the Paciflor group. Causes for overall mortality were mainly due to respiratory problems (5 and 9 rabbits in the control and Paciflor group, respectively). Digestive disorders were responsible for the death of 5 (controls) and 10 (Paciflor) rabbits, respectively. However, compared to field data, a mortality rate of 6% is quite normal. This means that the infection pressure, even under less favourable housing conditions was not very high.

Table 4 : Summary of weight gain, feed intake and feed efficiency of weanlings under optimum housing conditions (fattening trial 1).

	Paciflor	Control	SEM	Significance [2]
No of weanlings (1)	90	90	–	
Mortality (number)	2	2	–	NS
Initial weight (g), 28 days	628 [2]	578	10.9	*
Weight at 70 days (g)				
determined	2453	2354	22.8	*
covariated [3]	2418	2387	17.4	NS
Daily weight gain (g)				
determined	43.4	42.3	0.42	NS
Feed intake (g/day) [4]				
determined	130.1	127.3	1.38	NS
covariated	128.3	129.2	1.12	NS
Feed conversion [4]				
determined	3.00	3.01	0.021	NS
covariated	2.97	3.04	0.017	*

[1] 30 litters of 3 rabbits ; [2] * : P<0.05 ; NS : non significant ; [3] : after covariance analysis with initial weight of covariate ; [4] : 30 data per group.

Table 5 : Fattening performances under less favourable housing conditions (trial 2).

	Paciflor	Control	SEM
No of weanlings	139	142	–
Mortality (number)	17	8	
Initial weight (g), 28 d	615	572	12.1
Weight at 70 days (g)			
determined	2369	2329	23.2
covariated	2350	2348	15.4
Daily weight gain (g)			
determined	41.8	41.7	8.8

Differences are not significant (P>0.05).

Caecal fermentation parameters are given in Table 6. No effect of the dietary inclusion of Paciflor on pH, VFA or NH3 concentration was found. Differences between treatments were small and not significant. Although live weight of rabbits differed only slightly, caecal weight was significantly higher when fed the Paciflor diet. A possible explanation could be searched in an increased intensity of fermentation. However, total VFA concentration was only slightly higher in the Paciflor treated rabbits.

Our results with fatteners are less favourable for Paciflor than those obtained in several other experiments (NGUYEN *et al.*, 1988; DUPERRAY, 1991; DE BLAS *et al.*, 1991; MOTTA FERRERA, 1992). Under our conditions DWG was not significantly increased by the probiotic while this was evident in the forementionned experiments. A possible explanation could be searched in the performance level of the rabbits. Even under less favourable housing conditions DWG of controls was higher than 41 g while in previous published studies DWG was always between 32 and 37 g. Greater responses to the use of Paciflor were found when rabbit growth was limited because of heat stress or low weight at weaning (DE BLAS *et al.*, 1991). This tendency has also been observed for other probiotics (HOLLISTER *et al.*, 1990; MAERTENS and DE GROOTE, 1992).

Although the effect on feed conversion was rather small (2.3%), it was significant. Previous work showed a more pronounced (DUPERRAY, 1991) effect on feed efficiency, mainly in the first weeks after weaning (DE BLAS *et al.*, 1991). However, it is not always clear if the effect on feed efficiency is the result of the difference in mortality rather than in their growing performances.

In contrast with previous published work (DUPERRAY, 1991, MOTTA FERRERA, 1992), when the efficiency of Paciflor was tested, we did not observe a tendency to reduced mortality. Although more than 400 weanlings were used in total, this number is probably too low to exclude the litter effects on mortality. Precaution was taken to avoid direct (contact) or indirect (faeces, weighing equipment) contamination, but it can not be excluded that controls could be infected by the Bacillus strain.

In conclusion, the addition of Paciflor to a reproduction diet resulted in a significant (P<0.05) higher weaning weight. Other parameters (e.g. γ-globulins) showed small and not significant differences. Post weaning growth was high, but not significantly different even under less favourable conditions. Feed efficiency was 2.3% (P<0.05) more favourable when fed the Paciflor diet. However, mortality tended to be higher in the Paciflor treated

Table 6 : Effect of dietary inclusion of Paciflor on caecal pH, NH_3 and Volatile Fatty Acids concentrations at 70 days.

	Paciflor	Control	SEM	Significance
Number	10	10	–	–
Live weight (g)	2458	2419	40.0	NS
Caecal weight (g)	159.0	136.9	4.68	0.03
pH	6.07	6.04	0.04	NS
Acetic acid (mmol/l)	48.4	53.3	1.78	NS
Propionic acid (mmol/l)	3.9	4.3	0.18	NS
Butyric acid (mmol/l)	12.0	13.8	0.78	NS
Isobutyric acid (mmol/l)	0.2	0.3	0.05	NS
Valeric acid (mmol/l)	0.7	0.7	0.05	NS
Total fatty acids (mmol/l)	65.2	72.4	2.61	NS
NH_3 (mmol/l)	13.3	11.5	0.80	NS

rabbits. In contrast with other experiments, a favourable effect on mortality was not observed.

Acknowledgements : The authors are grateful to HOECHST AG for the support provided for this study. Appreciation is also expressed to Mr. Andre VERMEULEN for his skilled technical assistance and to R. LEMMENS for carrying the rabbits.

Received : April 20, 1994
Accepted : June 15, 1994.

BIBLIOGRAPHY

AOAC,1990. Association of Official Analytical Chemists, 1990. Official Methods of Analysis (15th edn.). *Washington, DC, vol. 1, 1230 pp.*

C.V.B., 1991. Veevoedertabel. Gegevens over voederwaarde, verteerbaarheid en samenstelling. *C.V.B., Lelystad, The Netherlands, 422 pp.*

DE BLAS C., GARCIA J., ALDAY S., 1991. Effects of dietary inclusion of a probiotic (Paciflor) on performance of growing rabbits. *J. Appl. Rabbit Res., **14**, 148-150.*

DUPPERAY J., 1991. Effects of Paciflor on performances, digestive microflora and sanitary conditions of rabbits. *In : Proc. 3th Hungarian Scientific Day on Rabbit Productions, Kaposvar, 191 - 206.*

HOLLISTER A.G., CHEEKE P.R., ROBINSON K.L., PATTON N.M., 1990. Effects of dietary probiotics and acidifiers on performance of weanling rabbits. *J. Appl. Rabbit Res., **13**, 6-9.*

LEBAS F., 1971. Composition chimique du lait de lapine, évolution au cours de la traite et en fonction du stade de lactation. *Ann. Zootech., **20(2)**, 185-191.*

LEBAS F., 1989. Besoins nutritionnels des lapins: revue bibliographique et perspectives. *Cuni-Sciences, 5(2), 1-28.*

MAERTENS L., 1992a. Selection scheme, performance level and comparative test of two lines of meat rabbits. Proc. 5th WRSA Congress, Oregon (Corvallis). In J. Appl. Rabbit Res., 15 : 206-212.

MAERTENS L., DE GROOTE G., 1992. Effect of a dietary supplementation of live yeast on the zootechnical performances of does and weanling rabbits. *J. Appl. Rabbit Res., **15**, 889-913.*

MAERTENS L., 1992b. Rabbit nutrition and feeding: a review of some recent developments. *J. Appl. Rabbit Res., **15**, 889-913.*

MICHARD J., LEVESQUE A., 1989. Stabilité du Paciflor dans des présentations commerciales et les aliments. Méthode de dénombrement. *Bulletin d'information, Station Expérimentale d'Aviculture de Ploufragan. Vol. **27**, 126-128.*

MOTTA FERRERA W., CESAR DE ARAUJO P., MOREIRA RIOS A., LEAO PINTO A., MIRANDA DE MANEZES L.D., 1992. Utilisation du biorégulateur PACIFLOR dans l'aliment des lapins en engraissement. XXVIème Réunion annuelle de la Sociedade Brasileira de Zootecnia (19-24 Juillet). *Département de Zootechnie Ecole Superieure d'Agriculture de Lavras, ESAL-37200 LAVRAS.*

NGUYEN T.H., DUPERRAY J., ECKENFELDER B., LECAMP B., LEFRANCOIS S., NEBOUT J.M., RIDREMONT B., SALLE F., SERGHERAERT R., 1988. Quelques probiotiques facteurs de croissance. *Revue de l'Alimentation Animale. **Mars 1988**, 31-37.*

PEETERS, J.E., 1988. Recent advances in intestinal pathology of rabbits and further perspectives. In : *Proc. 4th Congress of the World Rabbit Science Association, Vol. **3**, 293-315.*

RESMINI P., PELLEGRINO L., ANDREINI R., PRATI F., 1989. Determinazione delle sieroproteine solubile del latte per HPLC (cromatogrfia ad alta prestazione) in fase inversa. *Sci. Tecn. Latt., **40**, 7-24.*

STATGRAPHICS ® , 1991. Reference Manual, version 5. *Statistical graphics corporation, Rockville, MD, USA.*

VANBELLE M., TELLER E., FOCANT M., 1990. Probiotics in animal nutrition: a review. Arch. *Anim. Nutr., Berlin, **40(7)** : 543-567.*

VOIGHT J., STEGER H., 1967. Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörper in Biologischen Material mit hilfes eines modifizierten Mikrodiffusionsgefäßer. *Arch. Tierernährung, **17**, 289-293.*