Influence of Supplemental Enzymes, Yeast Culture and Effective Micro-organism Culture on Gut Micro-flora and Nutrient Digestion at Different Parts of the Rabbit Digestive Tract

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ABSTRACT : An experiment of 10 weeks duration was carried out to study the influence of supplemental effective microorganism (EM) culture, yeast culture and enzymes on nutrient digestibility and gut microflora in rabbit gastrointestinal (GI) tract. Twenty four eight to nine weeks old, New Zealand White rabbits were allotted to four dietary treatments; a basal (control) feed, basal feed supplemented with either EM (1%), yeast culture or enzymes (400 ppm). Nutrient flow in digesta and their digestibility at ileum, caecum, colon and in the total tract as well as gut microflora distribution were studied. Feed dry matter was diluted from 92% to about 14% up to the ileum and about 95% of this water was reabsorbed by the colonic rectal segment followed by caecum (25%). EM and yeast improved protein digestibility at a lower rate than enzymes. Ileal, caecal, colonic and total tract digestibility of crude protein with enzymes were higher by 10.8, 9.4, 11.3 and 10.7%, respectively, as compared to the control. Yeast and enzymes increased crude fiber digestibility at ileum, caecum, colon and in the total tract by 8.5, 9.6, 9.0 and 8.3%, respectively, while EM improved them at a lower rate. Irrespective of treatments, total tract digestibility of crude protein (0.698-0.773) and fiber (0.169-0.183) were greater ($p<0.05$) than the ileal digestibility. Even though a post-caecal protein digestibility was observed, fiber digestion seemed to be completed in the caecum especially with yeast and enzymes. High precaecal digestibility of crude fiber (97%) and protein (95%) were observed even without additives probably due to caecotrophy. EM and yeast culture promoted the growth of lactic acid bacteria especially in the caecum but they did not influence gut yeast and mould. Present findings reveal that even though rabbits digest nutrients efficiently through hind gut fermentation, they can be further enhanced by EM, yeast and enzymes. Of the three additives tested, enzymes found to be the best. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 6 : 830-835)***

Key Words : Rabbit, Digestion, Enzymes, Probiotics, Gut-microflora

INTRODUCTION

Domesticated rabbit (*Oryctolagus cuniculus*) has a great potential as a source of meat. It is an established fact that rabbits are good converters of fibrous plant material into lean meat and they can tolerate a substantial fiber level in their diet (Cheeke, 1987). However, being a monogastric animal, rabbits utilize fiber by virtue of hind gut fermentation which in fact is less efficient compared to rumen fermentation (Aderibigbe et al., 1992a,b,c; Adjiri, et al, 1992; Aderibigbe and Cheeke, 1993). Rabbits can absorb and metabolize volatile fatty acids produced due to hind-gut fermentation which represents about 40% of the maintenance energy requirement (Marty and Vernay, 1984). Therefore, there is a great potential for using feed additives to enhance the digestibility of fibrous feeds in rabbits. Various scientists have demonstrated that supplemental probiotics and enzymes can increase the feed digestibility, nutrient utilization and thereby the performance of chicken and pigs (Feighner and Dashkevicz, 1988; Fengler and Marquardt, 1988; Campbell, 1989; Petterson and Aman, 1989; Salih et al., 1991; Samarasinghe and Wenk, 1993). However the effect of such additives in rabbit diets is yet to be established.

Probiotics, such as yeasts (Maertens and De Groote, 1992) and *Bacillus* (McCartney, 1994) are finding a place in rabbit feeding. Some of them have been shown to benefit rabbit performance (Blas et al., 1991). Effective Microorganisms (EM; a live microbial culture of Japanese origin) may also be able to play an important role in rabbit feeding with their ability of degrading organic materials as reported by Anuar et al. (1993). According to the producers EM culture contains photosynthetic bacteria, lactic acid bacteria, yeast (*Saccharomyces cerevisiae* and *Candida utilis*), ray fungi (*Streptomyces albus* and *Streptomyces griseus*) and fungi (*Aspergillus oryzae* and *Mucor hiemalia*). On the other hand, the microbiology of the rabbit gut has not been extensively studied as that of some other species (Straw, 1988). Therefore, the present study was carried out to investigate the effect of exogenous enzymes (cellulases and proteases), yeast culture (Yea-Sacc^{1026}) and effective micro-organism (EM) culture on nutrient digestibility and gut micro-flora of rabbits fed diets based on rice bran and coconut poonac.

MATERIALS AND METHODS

An experiment was conducted using eight to nine weeks old, New Zealand White rabbits of both sexes (average

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Table 1. Ingredient composition (as fed basis) and analysed nutrient contents (on DM basis) of the basal feed

Component	Amount	Component	Amount
Rice bran, %	43	Molasses, %	4
Coconut poonac, %	15	Dicalciumphosphate, %	1.2
Grass meal, %	15	Salt, %	0.25
Maize meal, %	12	Vitamin-mineral premix[1], %	0.25
Soybean meal, %	5	Lysine-HCl, %	0.25
Fish meal, %	4	DL-methionine, %	0.05
Analysed nutrients:			
Dry matter, %	92.08	Ether extract, %	5.52
Ash, %	6.25	Nitrogen free extract, %	49.28
Crude protein, %	19.75	Gross energy, kcal/kg	4,900
Crude fiber, %	13.01		

Contained 150,000 IU, vit. A; 300,000 IU, vit. D_3; 0.3 g, vit. B_1; 0.6 g, vit. B_2; 0.5 g, vit. B_6; 1.5 g, vit. B_{12}; 2.5 g, vit. E; 1.5 g, vit. K; 0.3 g, calcium pantothenate; 0.15 g, folic acid; 3 g, nicotinic acid; 15 g, choline chloride; 0.1 g, cobolt; 5 g, selenium; 4 g, iron; 8 g, manganese; 1 g, copper; 5 g, zinc; 0.1 g, iodine 5 g, antioxidants and 50 g DL-methionine per kg.

body weight 1,986 g) over a period of ten weeks to study the effect of enzymes and probiotics on gut micro-flora and nutrient digestibility at different parts of the GI tract. They were housed in individual wire net cages having a floor space of 2,160 sq.cm (72 cm×30 cm), equipped with nipple drinkers and a nylon net fixed under each cage for faeces collection.

A basal feed (Table 1) was formulated using locally available ingredients to contain all the nutrients as required by growing rabbits (NRC, 1977). Guinea grass (*Panicum maximum*) was cut at pre-bloom stage, dried under shade for few days, ground to a meal and used to obtain the required fiber level in the basal ration. The total amount of the basal feed required was mixed as one batch using a horizontal feed mixer. Then the basal feed was divided in to four equal portions and one portion was taken as the control feed. The remaining three portions were supplemented either with effective micro-organisms (1%), yeast culture (Yea Sacc^{1026} at 200 ppm) or enzymes (a mixture of cellulases and proteases from Alltech Inc. USA, at 400 ppm) to get three test diets. All four rations were separately pelleted using a 3.5 mm die in the pelleting machine (CPM LP 4001-Aerphilly Mid-Glam-Eriez magnetics). In the case of EM treatment, EM culture solution was sprayed on pellets immediately before feeding every day. EM could not be added to the feed before pelleting because the viability of bacteria gets reduced when exposed to sunlight. As recommended by producers, EM solution was also added to drinking water every day at the rate of 1:2,000 (v/v). Rabbits in other treatments received fresh water without EM. Each dietary treatment was then assigned to six rabbits in a Complete Randomized Deign (CRD). Feed and water was given *ad libitum*.

Samples of faeces from individual rabbits were collected separately during three consecutive days for three collection periods starting from 31st, 38th and 45th days after commencement of the experiment in order to estimate total tract digestibility of nutrients (indicator method). Soon after collection, the samples were stored in a deep freezer until they were taken for analyses. Feed samples were also collected during each collection period and stored for subsequent analyses. No attempt was made to study the sex effect on parameters tested since it was known that the feed digestion in rabbit is not influenced by their sex (Fekete and Bokori cited by Fekete, 1988; Fekete and Lebas cited by Fekete, 1988).

After the final faeces collection, all the animals were sacrificed to take gut contents. They were stunted by dislocating the neck and decapitated. After bleeding for few minutes, the head, skin and feet were removed and a cut was made carefully with a sterilized knife ventrally. All the instruments used in the sample collection for microbiological study were sterilized before using them. Gut contents were collected aseptically from caecum and colon, put it in to sterile bottles and kept in a refrigerator until they were taken for culturing. Then samples of gut contents for digestibility determinations were collected from ileum, caecum and colon into polythene bags, sealed and stored in a deep freezer until taken for chemical analysis.

Dry matter (DM), ash, crude protein (CP), crude fiber (CF) and ether extract (EE) of feeds were analysed (AOAC, 1985). Samples of faeces and gut contents were tested for DM, CP and CF. Acid insoluble ash (AIA) contents of feed, faeces and gut contents were determined (AOAC, 1985) and the digestibility values were then calculated according to an indicator method using AIA as the indicator (Prabucki et al., 1975; Scott and Boldaji, 1997).

In the microbiological study, plates were prepared with potato dextrose agar and milk agar using Oxoid Kit-2000 to culture lactic acid bacteria (LAB) and yeast and mould. Serial dilutions were prepared using Ringer's solution. As the average range of microbial count of rabbits fed with different additives were not well known, three dilutions from each sample were inoculated. After inoculating the media with either caecal or large intestinal samples, the

Table 2. Nutrient composition of gut contents in ileum, caecum and colon and in faeces of rabbits

Dietary treatment	Dry matter				Crude protein (on DM)				Crude fiber (on DM)			
	Ileum	Caecum	Colon	Faeces	Ileum	Caecum	Colon	Faeces	Ileum	Caecum	Colon	Faeces
Control	14.52	36.29	39.59	96.67	16.30	17.30	16.95	17.10	27.09	27.10	28.69	31.50
EM culture	13.92	35.59	39.12	96.01	14.75	16.10	16.12	16.00	27.00	27.00	28.60	31.35
Yeast culture	13.69	34.43	38.55	95.91	14.25	16.00	14.00	13.78	26.95	26.85	28.56	31.20
Enzyemes	13.12	34.12	38.13	95.84	13.55	14.60	13.20	13.15	26.88	26.85	28.55	31.20

Table 3. Crude protein and crude fiber digestibility values at ileum, caecum, colon and in the total tract of rabbits

Dietary treatment	Crude protein digestibility				Crude fiber digestibility			
	Ileum	Caecum	Colon	Total tract	Ileum	Caecum	Colon	Total tract
Control	0.668 $^{a, y}$	0.649 $^{a, x}$	0.675 $^{a, y}$	0.698 $^{a, z}$	0.164 $^{a, x}$	0.166 $^{a, y}$	0.167 $^{a, y}$	0.169 $^{a, z}$
EM culture	0.702 $^{b, y}$	0.678 $^{b, x}$	0.712 $^{b, y}$	0.723 $^{b, z}$	0.172 $^{b, x}$	0.175 $^{b, y}$	0.175 $^{b, y}$	0.179 $^{b, z}$
Yeast culture	0.713 $^{b, y}$	0.676 $^{b, x}$	0.736 $^{c, y}$	0.762 $^{c, z}$	0.177 $^{c, x}$	0.181 $^{c, y}$	0.182 $^{c, y}$	0.183 $^{c, y}$
Enzymes	0.740 $^{c, y}$	0.710 $^{c, x}$	0.751 $^{c, y}$	0.773 $^{c, z}$	0.178 $^{c, x}$	0.182 $^{c, y}$	0.182 $^{c, y}$	0.183 $^{c, y}$

$^{a, b, c}$ Means with different superscripts within the same column are significantly different ($p<0.05$).

$^{x, y, z}$ Means with different superscripts within the same row are significantly different ($p<0.05$).

plates with milk agar were kept in an incubator at 36°C for two to three days and the plates with potato dextrose agar were kept in room temperature (25-27°C) for three to four days. At the end of incubation period, the number of colony forming units (cfu) of lactic acid bacteria and yeast and mould were counted.

Data were subjected to Analysis of Variance (ANOVA) using statistical analysis system (SAS, 1990). Feed intake and total tract digestibility of nutrients were analysed by considering treatment and time as 2 factors. Ileal, caecal and large intestinal digestibility values and the cfu counts of lactic acid bacteria and yeast and mould were analysed in a single factor CRD. Means were compared using Duncan's Multiple Range Test.

RESULTS

Basal feed contained slightly higher crude protein (19.8%) and lower crude fiber (13%) (Table 1) than expected values (16 and 14%, respectively). As shown in Table 2, feed was highly diluted (13.8% DM) when it reached the ileum. Dry matter content of the digesta gradually increased up to the colon (38.9% DM) and thereafter rapidly to a level of about 95% before voided as faeces but there was no observed treatment effect. Crude protein content of rabbit gut contents and faeces which varied from 13.15 to 17.3% with slight differences between treatments were lower than that of feed. Caecal contents had more crude protein (16.0%) than ileal contents (14.7%). From ileum to colon (and in faeces), a slight reduction in crude protein of digesta was observed with yeast and enzymes while a slight increase was observed with the control and EM diets. Compared to feed, the gut contents and faeces contained higher crude fiber levels. Average crude fiber level of ileal contents was 27.0%. In crude fiber, there was no change from ileum to caecum but it gradually increased from caecum to colon. The highest crude fiber content (31.3%) was found in faeces. However, there were no significant differences in crude fiber contents between treatments.

Ileal, caecal, colon and total tract digestibility of crude protein (0.668, 0.649, 0.675 and 0.698, respectively, for the control) as well as crude fiber (0.164, 0.166, 0.167 and 0.169, respectively, for the control) were increased ($p<0.05$) with all additives. With regard to crude protein digestibility, there was no difference observed up to caecum between EM and yeast cultures which improved the protein digestibility by about 4 to 6%. However, yeast increased ($p<0.05$) the colon and total tract digestibility of crude protein at a rate similar to enzymes while the effect of EM was lower. The enzyme supplementation resulted in the highest improvement in crude protein digestibility throughout the digestive tract. As shown in Table 3, ileal, caecal, colon and total tract digestibility of crude protein with enzymes were higher by 10.8, 9.4, 11.3 and 10.7%, respectively, as compared to the control.

EM improved the fiber digestion at a lower rate compared to yeast and enzymes. Ileal, caecal, colon and total tract digestibility of crude fiber were increased ($p<0.05$) by 8.5, 9.6, 9.0 and 8.3%, respectively, when the diet was supplemented with either yeast or enzymes (Table 3).

The total tract digestibility values of crude protein and crude fiber were significantly ($p<0.05$) higher than those at ileum in all the dietary treatments showing the importance of hind gut fermentation in rabbits. Crude protein digestibility observed at caecum was significantly ($p<0.05$) lower as compared to the values observed at other sites irrespective of the dietary treatment while crude fiber digestibility was lowest at ileum.

Parallel to the improvements in nutrient digestibility, all the additives have enhanced the growth of lactic acid bacteria (LAB) in rabbit caecum and colon (Table 4). The counts of LAB in the caecum and in colon were lowest with

Table 4. Mean colony forming units (cfu) of lactic acid bacteria (LAB) in caecal and large intestinal contents of rabbits

Dietary treatment	Cfu of LAB/g of gut contents		Cfu of yeast and mould/g of gut contents	
	Caecum	Colon	Caecum	Colon
Control	$3.85\times10^{6\,a}$	$1.3\times10^{6\,a}$	1.16×10^{7}	2.57×10^{6}
EM culture	$1.65\times10^{8\,c}$	$1.14\times10^{7\,b}$	1.69×10^{7}	3.00×10^{6}
Yeast culture	$1.85\times10^{8\,c}$	$1.97\times10^{7\,b}$	6.17×10^{7}	3.14×10^{6}
Enzymes	$2.7\times10^{7\,b}$	$9.5\times10^{6\,a}$	1.42×10^{7}	1.05×10^{6}

[a, b, c] Means with different superscripts within the same column are significantly different ($p<0.05$).

Table 5. Water reabsorption from different segments between ileum and anus of the GI tract of rabbit (as a % of total between ileum and anus)

Treatment	Moisture lost as a % of total loss between ileum and anus		
	Ileum-caecum	Caecum-colon	Colon-anus
Control	26.52	4.04	69.43
EM	26.43	4.26	69.31
Yeast	25.20	4.95	69.75
Enzymes	25.42	4.84	69.86

the control group (3.85×10^{6} and 1.3×10^{6} cfu/g, respectively) and highest with yeast culture (1.85×10^{8} and 1.97×10^{7} cfu/g, respectively). As compared to EM and yeast, enzymes had a lower effect on LAB growth. However the dietary treatments did not significantly influence the yeast and mould growth in rabbit caecum or colon (Table 4). The average cfu of yeast and mould in the caecum and colon were 2.61×10^{7} and 2.44×10^{6}, respectively, per g of gut contents. In general, higher microbial counts were seen in the caecum than in the colon.

DISCUSSION

Feed dry matter which was around 92% (Table 1) was reduced to approximately 14% when it reached the ileum (Table 2) due to the dilution effect of drinking water and digestive juices. The moisture lost within the GI tract of an animal is an estimation for water reabsorption into the body. Rabbits have reabsorbed about 95% of ileal digesta water when the digesta passed from ileum to anus as faeces (hard pellets). As shown in Table 5, the main site of water reabsorption is between colon and anus, the colonic-rectal segment. Previous studies also report that water reabsorption occurs mainly at colon and rectum (Burn, 1986; Reynolds and Bellward, 1989). A considerable amount of water (25.9%) has been reabsorbed from the caecum. Dietary treatments did not have any influence on water reabsorpion or the consistency of faeces in rabbits.

When the digesta enters the caecum of the rabbit, more fibrous particles are separated and moved to the lower parts of the large intestine which ultimately constitute the hard faeces (Gidenne et al., 1998). Some soluble particles coming with digesta can also be absorbed from the colon (Marty and Vernay, 1984). These must be the reasons for relatively high crude fiber contents found in digesta after the caecum. Though there was no treatment effect observed on the crude fiber content of digesta, the digestibility values were significantly influenced ($p<0.05$) by dietary treatments.

Results reveal that changes to digesta crude protein and crude fiber occur even up to the colonic-rectal segment of the rabbit. Therefore the total tract digestibility estimates were always higher than the digestibility values at any other segment of the GI tract. Possible reason might be the continuous degradation and absorption of digesta nutrients. Increased crude protein content in caecum (Table 2) has resulted in lower estimations for caecal protein digestibility values (Table 3). This was attributed to intensive microbial activity in the caecum (Gidenne et al., 1998). EM and yeast treatments which resulted the highest increase in LAB activity (Table 4) showed a greater uplifting of crude protein content from ileum to caecum thus justifying the above explanation. Similarly, as compared to the control and EM treatment, colon and total tract digestibility of crude protein were higher with yeast cultures and with enzymes corresponding to lower crude protein levels in colonic contents and faeces. However this kind of a relationship between gut protein concentration from ileum to colon and protein digestibility could not be seen with the control and EM treatment. The reingestion of soft faeces and the subsequent digestion of its crude protein in which about 31 to 68 % is from bacterial origin (Garcia et al. cited by Fraga, 1998) makes the interpretation of results of protein digestion by rabbits more complicated as stated by Merino (cited by Fraga, 1998).

Fermentation of dietary fiber has been considered to be a post-ileal activity of the endogenous microflora. However, there is an increasing evidence (Gidenne et al., 1998) that some components of structural carbohydrates disappear or are degraded prior to entering the caecum of rabbits. It is worthwhile to note that the precaecal fiber digestion in the present study has contributed about 96 to 97% of the total fiber digestion (0.164 to 0.178) of rabbits. Precaecal fiber digestion has also been observed in other non-ruminant species such as pigs and poultry also (Gidenne et al., 1998).

In the present study, the precaecal fiber (0.164) and protein (0.668) digestibility values observed with the control rabbits were 97 and 95%, respectively, of the total tract digestibility values. Such a high apparent nutrient digestion observed at precaecal segment of the rabbit GI tract can be attributed to reingestion of soft faeces (caecotrophy). According to Yu et al. (cited by Gidenne et al., 1998), the extent of precaecal fiber digestion in rabbits varies from 0.07 to 0.19 which is in agreement with present results. The precaecal fiber digestibility values obtained in the present study were more close to the upper limit since the rabbits used were somewhat matured. Precaecal fiber degradation as well as the caecal fermentation have been improved ($p<0.05$) by EM, yeast and enzyme supplementations. In parallel, crude protein digestibility was also improved by all three supplements. Of the three additives tested, enzymes gave the highest improvements in digestibility values. However, Aderibigbe and Cheeke (1993) and Aderibigbe et al. (1992a) did not find any improvement in *in vitro* caecal digestibility of various rabbit feeds as a result of probiotic supplementation. They however found that in vitro digestibilities were lower than the in vivo values and the source of fiber has a great influence on their digestibility. This it self explain the positive responses observed to probiotics in the present study.

EM and yeast must have promoted beneficial gut microbes thus resulting improved fiber and protein digestibility. As shown in Table 4, EM and yeast have remarkably increased ($p<0.05$) the cfu of LAB in caecum as well as in the colon. LAB is one of the major species of beneficial micro-organisms in the gut of monogastric animals and humans (Blaut, 2002). Glade (1991) has reported improved nutrient digestibility in mares fed diets supplemented with yeast cultures. The improvement in gut microbial population is attributed to the enhancing synergistic action of yeast and fungi on beneficial lactic acid bacteria both in caecum and colon. This may be due to provision of soluble nutrients especially B vitamins as suggested by Callaway and Martin (1997). Though reports on rabbits are scantly, positive effects of supplemental enzymes on nutrient digestibility in pigs and chicken have been well documented (Farrell et al., 1993; Samarasinghe and Wenk, 1993; Kopinski and Willis, 1996; Pack, 1996; Rostagno et al., 2000; Kocher et al., 2002; Owusu Asiedu et al., 2002). Therefore similar effects of supplemental enzymes in rabbits cannot be totally excluded.

It is a well-known fact that yeast could render the synergistic action on lactobacilli when allowed to grow together. On the other hand, enzymes also must have served as promoter for the microbial growth. Their growth promoting action on LAB would have been partly mediated through the ample availability of simple sugars generated through cellulolytic enzyme activity. This fact would have been determined by the analysis of gut contents of ileum, caecum, colon and faeces for such simple sugars and other relevant metabolites using caecotrophy prevented rabbits. In addition, the growth promoting action on LAB of supplemental enzymes did not exert the same activity in the colon (Table 4) indicating the importance of caecum in microbial fermentation.

According to Cole et al. (cited by Straw, 1988), the lactobacilli count in rabbit gut is 10^6 per gram of gut content which is in good agreement with the observations of the present study except for those fed with test diets.

Even though the LAB of gut contents showed significant ($p<0.05$) increase with the supplements, yeast and mould counts did not. However, the number of cfu of yeast and mould in the caecum was ten times higher than that of colon. This pattern was similar in all the treatments including the control. Therefore, strong indicaitons of colonisation of the probable probiotics in EM as well as in yeast culture were not found since the population counts decreased from caecum to colon in a similar way. If colonisation of any gut micro-organism had occurred, the yeast and mould counts must have reduced from caecum to colon significantly in EM or yeast culture treatments.

CONCLUSIONS

Rabbits (caecotrophy not prevented) show a high precaecal fiber and protein digestion which can be further enhanced by EM, yeast and enzymes. While protein digestion continues up to colon, fiber digestion seems to be completed in the caecum. EM and yeast culture promote the growth of LAB especially in the caecum but they do not influence gut yeast and mould indicating the absence of colonization. Present findings elucidate the fact that even though rabbits digest nutrients efficiently through hind gut fermentation and subsequent caecotrophy, nutrient digestibility can be further enhanced by appropriate feed additives. Of the additives tested, enzyme treatment found to be the best to improve feed digestibility in rabbits.

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REFERENCES

Aderibigbe, A. O. and P. R. Cheeke. 1993. Comparison of in vitro digestion of feed ingredients by rabbit cecal and bovine rumen fluids. An. Feed Sci. and Technol. 41:4, 329-339.

Aderibigbe, A. O., P. R. Cheeke and N. M. Patton. 1992a. *In vitro* rumen and rabbit cecum digestibilities. I. Effects of probiotics

and incubation time. J. Appl. Rabbit Res. 15:B, 947-957.

Aderibigbe, A. O., P. R. Cheeke and N. M. Patton. 1992b. *In vitro* rumen and rabbit cecum digestibilities. II. Effects of extended incubation time. J. Appl. Rabbit Res. 15:B, 1241-1248.

Aderibigbe, A. O., P. R. Cheeke and N. M. Patton. 1992c. *In vitro* rumen and rabbit cecum digestibilities. IV. Selected rabbit diets. J. Appl. Rabbit Res. 15:B, 1255-1262.

Adjiri, D., M. Bouillier Oudot, F. Lebas and M. Candau. 1992. Simulation *in vitro* des fermentations caecales du lapin en fermenteur a flux semi-continu. 2. Effet de la nature de l´inoculum. Reprod. Nutr. Development, 32(4):361-364.

Anuar, A. H., H. Sharifuddin, A. H. Sahabuddin and A. R. Zahara. 1993. Effectiveness of Effective Micro organisms (EM) on maize grown on sandy tintailings. Proc. 2nd Conference on EM, Saraburi, Thailand, pp. 1-6.

AOAC. 1985. Official methods of analysis. 14th Ed. Association of Official Analytical Chemist, Arlington, Washington, DC.

Blas, C. de, J. Garcia and S. Alday. 1991. Effect of dietary inclusion of a probiotic (Paciflor) on performance of growing rabbits. J. Appl. Rabbit Res. 14:148-150.

Burn, D. M. 1986. The digestive strategy and efficiency of the West Indian manatee, Trichechus manatus. Comparative Biochem. Physiol. 85(1):139-142.

Blaut, M. 2002. Relationship of prebiotics and food to intestinal microflora. European J. Nutr. 41(1)1-16.

Callaway, E. S. and S. A. Martin. 1997. Effect of Saccharomyces cerevisiae culture on rumial bacteria that utilise lactate and digest cellulose. J. Dairy Sci. 80(9)2035-2044.

Campbell, P. 1989. Nutritional value of Barley fed for broiler chicken supplemented with enzymes. Br. Poult. Sci. 41:35-37.

Cheeke, P. R. 1987. Rabbit Feeding and Nutrition. In: (Ed. T. J. Cunba). A Series of Monograph and Treatises. Interstate Printers and Publisher Inc, Danville, Illinois. pp. 15-36.

Farrell, D. J., E. Martin and D. J. Farrell. 1993. Recent advances in Animal Nutrition in Australia-1993. University of New England, Australia. pp. 266-276.

Feighner, S. D. and M. P. Dashkevicz. 1988. Effect of dietary carbohydrates on bacterial cholyltaurine hydrolase in poultry intestinal homogenates. Applied and Environmental Microbiology. 54:334-342.

Fekete, S. 1988. A review of rabbit nutrition and disease research in Hungary. J. Appl. Rabbit Res. 11(3):121-135.

Fengler, A. I. and R. R. Marquardt. 1988. Water-soluble pentosans from rye. In: Effects on rate of dialysis and retention of nutrients by the chick. Cereal Chemistry 65:298-302.

Fraga, M. J. 1998. Protein digestion. In: (Ed. C. de Blas and J. Wisemans). The nutrition of the rabbit. University Press, Cambridge, UK. pp. 39-54.

Gidenne, T., R. Carabano, J. Garcia and C. de Blas. 1998. Fiber digestion. In: (Ed. C. de Blas and J. Wiseman). The Nutrition of the Rabbit. University Press, Cambridge, UK. pp. 69-88.

Glade, M. J. 1991. Dietary yeast culture supplementation of mares during late gestation and early lactation. Effects on dietary nutrient digestibilities and faecal nitrogen partitioning. Equine Vet. Sci. 11:10-16.

Kocher, A., M. Choct, M. D. Porter and J. Broz. 2002. Effects of feed enzymes on nutritive value of soybean meal fed to broilers. Br. Poult. Sci. 43(1):54-63.

Kopinski, J. S. and S. Willis. 1996. Recent advances in utilization of sorghum for growing pigs. Proc. 3rd Australian sorghum conference. Tamworth, 20-22 February 1996. pp. 235-250.

Maertens, L. and G. de Groote. 1992. Effect of a dietary supplementation of live yeast on the zootechnical performance of does and weanling rabbits. J. Appl. Rabbit Res. 15:1079-1086.

Marty, J. and M. Vernay. 1984. Absorption and metabolism of the volatile fatty acids in the hind gut of the rabbit. Br. J. Nutr. 52(2)265-277.

Mc Cartney, E. 1994. Bacillus toyoi. Aplicacion en conejos de carne yen conejas reproductoras, Boletin de Cunicultura. 17:1-6.

NRC. 1977. Nutrient Requirements of Rabbits (2nd Ed.). National Academy Press. Washington, DC.

Owusu Asiedu, A., S. K. Baidoo and C. M. Nyachoti. 2002. Effect of heat processing on nutrient digestibility in pea and supplementing amylase and xylanase to raw, extruded or micronized pea-based diets on performance of early weaned pigs. Can. J. Anim. Sci. 82(3):367-374.

Pack, M. 1996. Nutritional impact of feed enzymes in broiler diets. Zootechnica Int. 19(12):82-85.

Petterson, D. and P. Aman. 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Br. J. Nutr. 62:139-149.

Prabucki, A. L., A. Rennerova, H. Voghtman, C. Wenk and A. Schurch. 1975. Die Verwendung von 4N HCl-unloeslicherAsche als Indikator zur Bestimung der Verdaulichkeit. Physiologie der Verdaung Misc. papers 11, Landbauwhogeschool, Wageningen, NL.

Raynolds, S. E. and K. Bellward. 1989. Water balance in Manduca sextra caterpillars: water recycling from the rectum. J. Experimental Biol. 141:33-45.

Rostagno, H. S., A. A. Tejedor, L. F. T. Albino and J. H. V. Silva. 2000. Enzyme supplementation of corn/soybean meal diets improves ileal digestibility of nutrients in broiler chicks. In: (Ed. T. P. Lyons and K. A. Jacques). Biotechnology in the Feed Industry. Proc. Alltech's 16th Annual Symp. Nottingham Univ. press, Nottingham. pp. 175-182.

Salih, M. E., H. L. Classen and G. L. Campbell. 1991. Response of chickens fed hull-less barley to dietary beta-glucanases at different ages. Anim. Feed Sci. Technol. 33:139-149.

Samarasinghe, K. and C. Wenk. 1993. Role of supplemental enzymes at low and conventional levels of protein in broiler diets based on cassava and maize. In: (Ed. C. Wenk and M. Boessinger). Proc. 1st Symp. Enzymes in Animal Nutrition. Switzerland. pp. 78-81.

SAS. 1990. SAS Institute Inc. Cary, North Carolina, USA.

Scott, T. A. and F. Boldaji. 1997. Comparision of inert markers [Chromic oxide or insoluble ash (CeliteTM)] for determining apparent metabolizable energy of wheat- or barley-based broiler diets with or without enzymes. Poult. Sci. 76:594-598.

Straw, T. E. 1988. Bacteria of the rabbit gut and their role in the health of the rabbit. J. Appl. Rabbit Res. 11(3):142-150.