



Growth performance, apparent nutrient digestibility, caecal fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing probiotics and prebiotics

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ARTICLE INFO

Article history:

Received 22 July 2012
Received in revised form
5 June 2013
Accepted 6 June 2013

Keywords:

Weaner rabbits
Mannan oligosaccharides
Arabinoxylan oligosaccharides
Probiotics
Prebiotics

ABSTRACT

Ninety eight male, mixed breed weaner rabbits were used in a 70 day feeding trial to study the performance, apparent nutrient digestibility, caecal fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing *Pediococcus acidilactis* (1×10^{10} cfu/g; 0.5 g/kg), *Bacillus cereus* (1×10^9 cfu/g; 0.5 g/kg), mannan oligosaccharides (MOS; 1 g/kg), arabinoxylans oligosaccharides (AX; 1 g/kg), oxytetracycline (1 g/kg), or synbiotics (TGI; 1 g/kg). A basal diet containing no feed additive was formulated. There were 14 rabbits per treatment. Rabbits fed diets containing MOS had the highest ($P < 0.05$) final live weight and weight gain. Dietary inclusion of prebiotics (MOS, AX) resulted in higher ($P < 0.05$) weight gains and improved feed to gain ratios. Rabbits fed diets containing MOS showed the highest ($P < 0.05$) while those fed diets containing probiotics (*Pediococcus acidilactis*, *Bacillus cereus*) had the least ($P < 0.05$) caecal total volatile fatty acid (VFA) concentration. Rabbits fed diets containing prebiotics (MOS, AX) had longer ileal villi than groups fed with other treatments ($P < 0.05$). Inclusion of various additives showed reduced caecal coliform counts. The lowest ($P < 0.05$) lactobacillus count was obtained in the caecal content of rabbits fed diets containing MOS. Poor growth response was obtained with rabbits fed diets containing *Pediococcus acidilactis* or *Bacillus cereus* while inclusion of prebiotics in growing rabbits resulted in improved growth and gut morphology. Rabbits fed diet containing MOS showed the highest overall final live weight, weight gain, total VFA concentration and reduced caecal lactobacillus count.

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1. Introduction

Antibiotics have been widely used in animal production to improve the health, growth rate and well-being of animals. The development of resistant strains of bacteria to antibiotics and the incidence of certain diseases in man

traceable to overuse of antibiotics led to the ban on the use of antibiotics as growth promoter by the European Union. In fact the scientific steering committee of the European Parliament in its opinion reported that 'regarding the use of antimicrobials as growth promoting agents, the use of agents from classes which are or may be used in human or veterinary medicine (i.e. where there is a risk of selecting for cross resistance to drugs used to treat bacterial infections) should be phased out and ultimately abolished' (Regulation of the European Parliament and Council, 2003). This situation stimulated research to explore

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alternatives to antibiotic growth promotants. Prebiotics, probiotics and synbiotics have since been used as alternative growth promotants in poultry (Spring et al., 2000; Fernandez et al., 2002; Xu et al., 2003; La Ragione et al., 2004), pigs (Lee et al., 2009; Chu et al., 2011) and ruminant feeds (Philippeau et al., 2010).

Probiotics are mono- or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous microbiota (Fuller, 1992). Probiotics are direct-fed microbial feed supplements which modulate the gut microflora by successfully competing with pathogens through a competitive exclusion process (Mountzouris et al., 2007). Available probiotics include the colonising (*Lactobacillus* and *Enterococcus* spp) and free flowing, non-colonising (*Bacillus* spp, *Saccharomyces cerevisiae*) microorganisms. The mode of action of probiotics is that they produce specific and intermediate metabolites which stimulate the body immune systems (Sherman et al., 2009). Previous studies with rabbits indicated that dietary supplementation of probiotics improved growth rate and enhanced efficiency of feed conversion (Amber et al., 2004).

Prebiotics are nondigestible food ingredient(s) that beneficially affects host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). They are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota which confers benefits upon host well-being and health (Gibson et al., 2004). Most interest in the development of prebiotics has been focused on the use of nondigestible oligosaccharides and polysaccharides (Mussatto and Mancilha, 2007) which cannot be digested but are readily fermented by anaerobic, colonic bacteria which are regarded as beneficial (Zhang et al., 2003). Prebiotics have shown considerable promise in promoting health and performance of rabbits (Fonseca et al., 2004; Pinheiro et al., 2009). There are few studies on the use of probiotics and prebiotics in rabbit nutrition.

A synbiotic is a mixture of probiotics and prebiotics that benefits the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract. The synbiotic concept has been suggested to give synergistic effects of both prebiotics and probiotics and thus offer a number of health-promoting effects, stimulate growth and improve the welfare of the host (Gibson and Roberfroid, 1995; Awad et al., 2006). Dietary inclusion of Biomin (a synbiotic produced by a combination of the probiotic strain *E. faecium* and prebiotic derived from chicory and sea algae) showed a greater growth-promoting effect in broilers than control group and group fed with probiotic *Lactobacillus* sp (Awad et al., 2009). Previous studies have also shown improved growth and health status of swine fed with synbiotics over groups fed with probiotics (Nemcová et al., 1999). Studies on the use of synbiotics in rabbit nutrition are rare. This study therefore seeks to investigate the growth performance, apparent nutrient digestibility, caecal fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing probiotics and prebiotics.

2. Materials and methods

2.1. Experimental animals and management

The study was carried out at the rabbit unit of the Teaching and Research Farm of the University of Agriculture, Abeokuta, Nigeria. Rabbits were kept in a well ventilated building in which the maximum temperature was 29.7 °C and the minimum temperature was 26.2 °C. A cycle of natural 12 h of day light and 12 h of dark was used throughout this trial. SimLac (a product containing 1×10^{10} cfu viable strain of *Pediococcus acidilactis per gram*) and SimPro (a product containing 1×10^9 cfu viable strain of *Bacillus cereus per gram*) supplied by a commercial company (Simbiyotec Biological Product Inc. Tuzaistanbul) were used in this study. Mannose oligosaccharides (MOS) and arabinoxylans oligosaccharides (AX) (Alltech Inc. Kentucky, USA) were used as prebiotics. Total Gut Integrity® (TGI) (a commercial product from Polchem Hygiene Laboratory, PVT. Ltd, Pune, India) was used as synbiotic. The TGI supplement used was based on natural raw materials in combination with a probiotic (*Bacillus cereus*) along with a bacterial cell wall preparation, a non-digestible oligosaccharides (fructo-oligosaccharides), immune-stimulating substances and phycophytic substances that stimulate cellular immunity. Ninety eight male, mixed breed weaner rabbits were used for the study.

2.2. Experimental diets

A basal diet containing no additive (control diet) was formulated while an additional six diets were formulated as shown in Table 1 to include SimPro (0.5 g/kg), SimLac (0.5 g/kg), AX (1 g/kg), MOS at (1 g/kg), TGI (1 g/kg) or feed grade oxytetracycline (1 g/kg). The inclusion level of SimPro and SimLac used were according to manufacturer's specifications. Inclusion level of prebiotics (AX, MOS) used in the current study was based on previous studies (Bovera et al., 2010) while therapeutic dosage of oxytetracycline was used as a negative control. The additives were added to the basal diet and thoroughly mixed to formulate the experimental diets. The rabbits were allotted to the seven dietary treatments with 14 rabbits each. Rabbits were housed individually in cages. The housing and the description of the rabbit hutch was as reported by Oso et al. (2010). Each cage unit contained a feeder and drinker providing free access to feed and water. Fresh experimental diets and clean water were offered. Except for the oxytetracycline group, no medication was administered to the rabbits throughout the 70 day study. Proximate composition (A.O.A.C, 1990) and fibre fraction (Van Soest et al., 1991) of the basal diet was determined.

2.3. Growth response and apparent nutrient digestibility

Feed intake and live weight per cage were recorded on a weekly basis. The average feed intake, weight gain and the feed to gain ratio were also computed.

At the end of the feeding trial, six rabbits per treatment were randomly selected, housed individually in metabolic cages and used to measure apparent nutrient digestibility.

Table 1
Gross composition of basal diet.

Ingredients	Quantity
Maize	195
Maize starch	135
Wheat offal	140
Rice bran	130
Rice husk	100
Soyabean meal	250
Bone meal	25
Oyster shell	15
Lysine-HCL	2
DL-Methionine	2
Salt	3
Vitamin/mineral premix ^a	3
Composition (in g/kg)	
Crude protein	174.5
Crude ash	90.4
NDF	370.79
ADF	189.61
ADL	55.91
Ca ^b	12.20
p ^b	6.30
Gross energy (MJ/Kg)	16.95
Digestible energy (MJ/Kg) ^c	10.84
Lysine ^b	8.20
Methionine ^b	4.20
Threonine ^b	6.04

^a Each kg of diet contained 120 IU, Vit A, 400 g Vit E, 30 mg Vit K2, 30 mg Vit B1, 80 mg Vit B2, 60 mg Vit B6, 3 mg Vit B12, 400 mg Niacin, 120 mg Pantothenic acid, 10 mg Folic acid, 1 mg Biotin, 1 mg Antioxidant, 3000 mg Choline chloride, 800 mg Mn, 400 mg Fe, 600 mg Zn, 3 mg Co, 10 mg I, and 2 mg Se.

^b Calculated composition based on standard table

^c Calculated using deBlas et al. (1992) ($DE = GE \times (0.867 - 0.0012ADF)$)

Selected rabbits were given feed at a level that matched their previous mean daily intake. Sample collection was done for four days. Excreta (free of feed particles and other contaminants) were collected daily from each cage and dried overnight (at 60 °C for 12 h). Feed samples and ground excreta samples were used for chemical analysis.

2.4. Ileal morphology

At the end of the 70 day feeding trial, six rabbits were selected at random from each treatment, slaughtered for histological studies and collection of caecal contents. Ileum samples were separated from each of the selected rabbits. Sample portion of the ileum segment was taken from each rabbit for histological measurements. A sample about five cm long was taken from the middle part of the ileum of each rabbit for histological studies. The sample was opened longitudinally, rinsed with cold saline and fixed in a buffered formaline solution for about 4 h and later stored in ethanol/water, 70/30 (v/v) until further analysis. Histological analysis was done according to the procedure of Goodlad et al. (1991). The preparations were mounted between slide and strip. Intestinal villi with their crypts were individually separated under a dissecting microscope.

The length and width of the villi were measured according to the procedure described by Hampson (1986) using an optical microscope, camera and an image analysis.

2.5. Caecal fermentation

Caecal content collected from each sampled rabbit was divided into two equal subsamples. One subsample was stored at –20 °C for analysis of VFA while the other was immediately used for caecal microbial analysis. For the measurement of VFA, the caecal contents were centrifuged (at 25,000g) at 0 °C for 10 min, a solution of 5% orthophosphoric acid (v/v) plus 1% mercury chloride (w/v) was added to the samples. Volatile fatty acids were measured by gas chromatography, with a gas column: free fatty acids and phenols 10% H₃PO₄, 1% acid-washed chromosorb W, 100–120 mesh. The carrier gas was N₂ with a flow rate of 40 ml/min: H₂ and air flows to the detector were 60 ml/min. Injector and detector temperatures were 250 °C while the oven temperature was 150 °C.

2.6. Caecal microflora

A 1 mg sample of caecal contents from each rabbit was placed under a stream of CO₂ in bottles and transported immediately to the laboratory for microflora count. Microbial counts were measured according to the methods of Xia et al. (2004). For this purpose, approximately one gram of fresh sample was dispersed in 9 ml phosphate buffered saline solution supplemented with 0.5 g/l Cysteine-hydrochloride, was thoroughly mixed and further diluted to a factor of 10⁻⁸. From this material, each 0.1 ml was spread on sterile Petri dishes containing selective media and incubated for analysis of total microbial colony count using the Plate-count agar incubated at 30 °C, for 72 h, Coliform (ES agar, 37 °C, 24 h, Merck, Darmstadt), lactobacilli (MRS-agar 37 °C, 72 h, Merck, Darmstadt), clostridia (Wilkins-Chalgren Agar+Novobiocin (8 mg/l)+Colistin Sulphate (8 mg/l), 37 °C, 72 h). Colony forming units (CFU) were counted. Colony counts were calculated per one gram of sample and then transformed as log 10 of viable bacteria per gram of fresh matter.

2.7. Statistical analysis

The data generated from this study was laid out in a completely randomized design and analysed using the General Linear Models procedure of SAS (SAS Institute, 1991). Significant means were separated using the Duncans' multiple range test (Duncan, 1955). Statements of statistical significance are based upon a probability of $P < 0.05$.

3. Results

3.1. Growth response and apparent nutrient digestibility

The growth responses and apparent digestibilities of rabbits fed diets containing probiotics, prebiotics and synbiotic is shown in Table 2. The final live weights, weight

Table 2

Growth response and apparent nutrient digestibility (\pm SEM) of rabbits fed diets containing prebiotics (MOS or AX) or probiotics (*Bacillus cereus* or *Pediococcus acidilactis*).

Treatments	Control	<i>Bacillus cereus</i>	<i>Pediococcus acidilactis</i>	MOS	AX	Synbiotic	Oxytetracycline	Pooled SEM	P- value
Initial live weight (g/rabbit)	566.7	570.8	566.7	575.8	566.7	546.7	520.8	9.15	0.74
Final live weight (g/rabbit)	1466.7 ^d	1200.0 ^f	1375.0 ^e	1916.7 ^a	1533.3 ^c	1608.3 ^b	1650.0 ^b	75.14	0.02
Total feed Intake (g/rabbit)	4916.7	6050.0	5358.3	5654.2	5275.0	5333.3	5283.3	96.21	0.08
Average weight gain (g/rabbit)	900.0 ^e	629.2 ^g	808.3 ^f	1340.9 ^a	966.7 ^d	1061.6 ^c	1129.2 ^b	63.10	0.02
Feed conversion ratio	5.46 ^b	9.61 ^d	6.63 ^c	4.22 ^a	5.46 ^b	5.02 ^{ab}	4.68 ^{ab}	0.40	0.03
Coefficient of apparent nutrient digestibility									
Dry matter	0.44	0.44	0.44	0.46	0.45	0.45	0.44	0.05	0.23
Organic matter	0.43	0.43	0.42	0.45	0.44	0.44	0.43	0.05	0.37
Ether extract	0.53	0.53	0.54	0.55	0.54	0.54	0.55	0.06	0.47
Crude protein	0.48	0.47	0.47	0.44	0.49	0.50	0.47	0.07	0.44
Neutral detergent fibre	0.25	0.24	0.23	0.25	0.26	0.24	0.25	0.06	0.60
Acid detergent fibre	0.19	0.18	0.18	0.25	0.19	0.18	0.18	0.05	0.59

MOS=Mannan oligosaccharides, AX=Arabinoxylan oligosaccharides

^{a-g} Means in the same row with unlike superscripts differ ($P < 0.05$)

Table 3

Caecal fermentation (mmol/100 ml) and ileal morphology (μ m) of rabbits fed diets containing prebiotics (MOS or AX) or probiotics (*Bacillus cereus* or *Pediococcus acidilactis*).

Treatments	Control	<i>Bacillus cereus</i>	<i>Pediococcus Acidilactis</i>	MOS	AX	Synbiotic	Oxytetracycline	Pooled SEM	P- Value
Parameters									
Total volatile fatty acid	7.19 ^b	5.91 ^c	5.91 ^c	7.61 ^a	7.34 ^b	7.07 ^b	6.91 ^b	0.99	0.03
Acetic acid	5.21	4.07	4.07	5.67	5.37	5.17	5.00	0.17	0.19
Propionic acid	0.64	0.51	0.50	0.62	0.60	0.57	0.55	0.05	0.09
Butyric acid	1.34	1.33	1.34	1.32	1.37	1.33	1.36	0.02	0.10
Ileal morphology									
Villi length	404 ^b	402 ^b	400 ^b	414 ^a	411 ^a	404 ^b	400 ^b	11.90	0.02
Crypt depth	135	131	130	140	137	133	127	7.22	0.95
Villi/crypt ratio	2.99	3.07	3.08	2.96	3.00	3.04	3.15	0.12	0.35

MOS=Mannan oligosaccharides, AX=Arabinoxylan oligosaccharides

^{a-c} Means in the same row with unlike superscripts differ ($P < 0.05$)

gains and feed conversion ratios were affected ($P < 0.05$) by dietary inclusion of the additives. Rabbits fed the diet containing MOS had the highest final live weight and weight gain ($P < 0.05$) while the rabbits fed the diet containing *Bacillus cereus* (SimPro) had the lowest ($P < 0.05$). Dietary inclusion of prebiotics (MOS and AX) resulted in higher ($P < 0.05$) final live weight and weight gain than rabbits fed diets containing probiotics (*Pediococcus acidilactis*, *Bacillus cereus*). Rabbits fed diet containing prebiotics (MOS, AX) had lower ($P < 0.05$) feed conversion ratio (FCR) than those fed diet containing probiotics (*Pediococcus acidilactis* or *Bacillus cereus*). Rabbits fed the diets containing MOS, synbiotic and oxytetracycline had the same FCR. The highest ($P < 0.05$) FCR was obtained with rabbits fed the diet containing *Bacillus cereus*. The apparent nutrient digestibility values were not affected by dietary inclusion of prebiotics, probiotics and synbiotic.

3.2. Caecal fermentation and ileal morphology

Table 3 shows the caecal fermentation and ileal morphology of rabbits fed diets containing probiotics, prebiotics and synbiotics. Rabbits fed diets containing probiotics

(*Pediococcus acidilactis*, *Bacillus cereus*) had the lowest ($P < 0.05$) total volatile fatty acid (VFA) concentration. Rabbits fed diets containing MOS had the highest ($P < 0.05$) total VFA concentration. The concentration of the acetic acid, propionic acid and butyric acid produced were not affected by dietary inclusion of prebiotics, probiotics and synbiotic.

The crypt depth and villi/crypt depth ratio of the ileum samples as shown in Table 3 were not affected by dietary inclusion of various additives. Rabbits fed with diets containing prebiotics (MOS, AX) had longer ($P < 0.05$) villi than the other treatments.

3.3. Caecal microflora

Table 4 shows the caecal microflora of rabbits fed with probiotics, prebiotics and synbiotics. Caecal content of rabbits fed diets containing oxytetracycline showed higher ($P < 0.05$) TBC (Total bacterial count) than groups fed the control diet and the diets containing prebiotics (MOS, AX) and probiotics (*Pediococcus acidilactis*, *Bacillus cereus*). Rabbits fed the control diet however had the lowest ($P < 0.05$) TBC. Dietary inclusion of various additives showed reduced ($P < 0.05$) caecal coliform counts. Rabbits

Table 4

Caecal microflora (in Log10 Organisms/g wet weight) of rabbits fed diets containing prebiotics (MOS or AX) or probiotics (*Bacillus cereus* or *Pediococcus acidilactis*).

Treatments	Control	<i>Bacillus cereus</i>	<i>Pediococcus acidilactis</i>	MOS	AX	Synbiotic	Oxytetracycline	Pooled SEM	P-Value
Total bacterial count	5.20 ^c	5.74 ^b	5.60 ^b	5.63 ^b	5.82 ^b	6.63 ^{ab}	7.25 ^a	0.42	0.04
<i>Coliform Spp.</i>	6.25 ^a	5.00 ^b	5.01 ^b	5.11 ^b	5.42 ^b	5.40 ^b	5.03 ^b	0.27	0.03
<i>Lactobacillus Spp.</i>	5.34 ^b	5.66 ^a	5.54 ^a	3.70 ^c	5.18 ^b	5.03 ^b	5.82 ^a	0.39	0.04
<i>Clostridium Spp.</i>	4.02	4.10	4.25	4.30	4.20	4.28	4.13	0.09	0.75

MOS=Mannan oligosaccharides, AX=Arabinosyran oligosaccharides

^{a-c} Means in the same row with unlike superscripts differ ($P < 0.05$)

fed with the diets containing oxytetracycline and groups fed diets containing probiotics (*Pediococcus acidilactis*, *Bacillus cereus*) had the highest ($P < 0.05$) caecal lactobacillus but the lowest coliform counts. The caecal content of rabbits fed diets containing MOS had the lowest ($P < 0.05$) lactobacillus count. Clostridium count was not affected by dietary inclusion of various additives.

4. Discussion

4.1. Growth response and apparent nutrient digestibility

The highest final live weight and weight gain obtained with rabbits fed diet containing MOS agreed with previous findings that inclusion of MOS resulted in a positive effect on growth performance of rabbits (Fonseca et al., 2004; Mour-ao et al., 2006). In a related study, improved weight gain and feed to gain ratio were obtained with rabbits fed diet containing fructose-oligosaccharides (Aguilar et al., 1996). The similar feed to gain ratio obtained with rabbits fed diet containing MOS and those fed diets containing oxytetracycline in this study confirmed the ability of MOS to comfortably replace antibiotics as growth promoters in rabbit nutrition. Improved growth performance obtained with rabbits fed diets containing prebiotics could be due to improved gut health obtained while the poor performance noticed with inclusion of probiotics could be a result of instability in the gut integrity of the rabbits. Guts of rabbits are known to be colonised with balanced gut microbiota (Gouet and Fonty, 1979). However, introduction of supplemental live bacteria through the use of probiotics could lead to instability in the gut integrity. Rabbit fed control diets also showed improved growth compared to group fed diets containing *Pediococcus acidilactis* (SimLac) or *Bacillus cereus* (SimPro). The apparent nutrient digestibility of rabbits was not affected by various additives used in this study.

The findings of this study are contrary to the report of Amber et al. (2004) who showed improved daily weight gain and performance index with rabbits fed diet containing dried *Lactobacillus acidophilus* (probiotics) than groups fed control diet. The contradictions could be due to several factors like dosage used as reported by Sazawal et al. (2006) (in children and adult humans), timing of application as shown by Rosenfeldt et al. (2003) (in children) and duration of administration including age of the animals as reported by Siggers et al. (2008) (in piglets).

It could also be a result of the small sample size used in this study.

4.2. Caecal fermentation and ileal morphology

The low concentration of total VFA generated from the caecum content of rabbits fed diets containing probiotics indicates low available energy (Bellier and Gidenne, 1996). Acetic acid concentration produced from caecal content of rabbits fed diets containing probiotics also had numerically lower values. Dietary inclusion of MOS in the current study resulted in the production of highest total VFA concentration. This agreed with previous findings which showed that inclusion of prebiotics resulted in increased caecal VFA of rabbits (Peters et al., 1992; Morisse et al., 1993). High caecal VFA concentration in rabbits has been reported to result in a protective effect against experimental enteropathogenic *E. coli* infection (Peters et al., 1995).

Dietary components fed to rabbits have been shown to affect the intestinal morphology (Chiou et al., 1994). However, literatures on the gut morphology of rabbits fed prebiotics, probiotics or synbiotic is rare. The increased villi length obtained in the ileal samples of rabbits fed diets containing prebiotics (MOS, AX) suggested an increased surface area capable of greater absorption of available nutrients (Caspary, 1992). Reduced villi length and deeper crypts were reported to be implicative of poor performance and reduced nutrient absorption in poultry (Awad et al., 2009). The increased villus length obtained with dietary inclusion of prebiotics could be due to increased epithelial cell turnover and absorption of nutrients.

4.3. Caecal microflora

The effect of inclusion of probiotics or prebiotics on gut microflora was reported in previous findings (Fortun-Lamothe and Boullier, 2007). Rabbits fed diets containing various additives in the present study showed lower coliform count in their caecal content when compared with those fed the control diet. Investigation on the mode of action of mannan oligosaccharide pointed out that MOS is able to bind to mannose-specific lectin of gram-negative pathogens that express Type 1-fimbriae such as *Salmonella* and *E. coli*, resulting in their excretion from the intestine (Baurhoo et al., 2007). Rabbits fed diets containing MOS however showed the least lactobacillus counts.

In vivo trials with pigs also showed synergistic effect of synbiotic in the reduction of food-borne pathogenic bacterial populations (Estrada et al., 2001). The reduced caecal lactobacillus count obtained from rabbits fed synbiotics when compared to those fed probiotics could be implicative of positive health status (Casey et al., 2007).

In conclusion, inclusion of prebiotics (MOS and AX) in rations for growing rabbits showed improved growth and better gut morphology than rabbits fed diets containing probiotics. Rabbits fed diets containing MOS showed the highest overall final live weight, weight gain, total VFA concentration and reduced caecal lactobacillus count. In order to attain improved growth performance by rabbit producers, dietary inclusion of MOS is therefore recommended.

Conflict of interest

The author declare that there is no conflict of interest.

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