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Performance, blood profile, carcass and meat traits and tissue morphology in growing rabbits fed mannanoligosaccharides and zinc-bacitracin continuously or intermittently

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The study aimed at evaluation the effect of mannanoligosaccharides (MOS) and zinc-bacitracin (ZnB) administered continuously (+) or intermittently (+/-) upon growing performance, carcass and meat traits, blood profile and tissue morphology of growing rabbits. One hundred and twenty five, 25 days-old rabbits were distributed among 5 groups fed the same basal diet: the control group did not receive any supplements; MOS+ group fed mannanoligosaccarides at 0.083 g/rabbit/day; MOS+/- group fed MOS at the same concentration as MOS+ group, but only two days/week; ZnB+ group received Zinc bacitracin at 0.083 g/rabbit/day; ZnB+/- group fed ZnB at the same level as ZnB+ group but only two days/week. The experiment lasted from day 25 to 81 of life. The mortality rate in the control group (36%) was higher (P<0.05) than in MOS+/- (12%) and ZnB+ groups (12%). MOS and ZnB administered intermittently increased (P<0.01) dressing percentage as compared to the control group. The liver percentage increased (P<0.01) due to use of MOS (+ or +/-) and ZnB (+). ZnB (+ or +/-) increased (P<0.05) the protein percentage of meat as compared to the control group, while the continuative administration of MOS was able to reduce (P<0.05) the fat percentage

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as related to the control group. Both supplements and both administration protocols increased (P<0.01) meat tenderness and water holding capacity in respect to the control group. Continuous ZnB administration had deleterious effects on liver, kidney and ileum morphology. MOS could replace ZnB from weaning to slaughter age of rabbits without negative effects on performance and blood profiles causing no alterations in tissue morphology as compared to the control group. In addition, the MOS can be administered intermittently, reducing the production costs.

KEY WORDS: blood profiles / growing and slaughtering performance / mannanoligosaccharide / rabbit / tissue morphology / zinc-bacitracin

Enteric troubles are the most frequent problem in rabbit husbandry, in particular during the post weaning period (up to day 56 of life) [Bovera *et al*. 2010ab]. To prevent post-weaning digestive disorders, prophylactic antimicrobial medication is normally used in growing rabbits [Attia *et al.* 2015]. However, the wide use of antibiotics (not only in animal production) resulted in the occurrence of antibiotic resistant bacteria [Falcao-e-Cunha *et al*. 2008]. As a consequence, the European Community placed (on January 2006) a general ban on antibiotics used as growth promoters (EC Reg. 1831/2003). So, great efforts were addressed in the world to the research alternatives to antibiotics able not only to maintain an adequate sanitary status of the digestive system, but also to promote rabbit growth. Prebiotics, and in particular mannanoligosaccharides (MOS) derived from the outer cell wall of the yeast *Saccharomyces cerevisiae*, are considered a promising alternative to antibiotics [Kocher 2006]. Several authors investigated the effect of MOS *vs*. antibiotics on *in vivo* rabbit performance and did not found significant differences [Fonseca *et al*. 2004, Pinheiro *et al*. 2004, Mourao *et al.* 2006]. Recently, Guedes *et al*. [2009] found that the addition of 2.0 g MOS/kg body weight to the diet increased VFA concentration in the caecum of growing rabbits, but Pinheiro *et al*. [2009] observed that 1.0 g MOS/kg live weight was not able to reduce the negative effect of low fibre diets on rabbit growth performance. However, all the studies showed that the addition of MOS to the diets resulted in a better intestinal integrity and had a protective effect against common pathogens. Mannanoligosaccharides are able to bind the mannose receptors on the type 1 *fimbriae* of some pathogen bacteria (as *Escherichia coli* and *Salmonella enteritidis*) in order to prevent their attach to intestinal mucosa [Spring *et al*. 2000]. Due to their interaction with microbes, MOS could have an effect on microbial components of intestinal microflora and/or on their activity. Bovera *et al*. [2010a] during an episode of enzootic entheropathy, observed that MOS had a stronger effect than antibiotics on lowering rabbit mortality rate.

Almost all the studies on the use of MOS as possible alternative to antibiotics, tested different level of inclusion in the diet but the supplement was administered day by day.

Present research aimed at comparing the effect of mannanoligosaccharide with that of zinc-bacitracin both administered continuously or intermittently for two days per week from 25-81 days of life on growth performance, blood profiles, carcass and meat traits and the morphology of some tissues in rabbits.

Material and methods

One hundred and twenty five, 25 day-old V-line mixed sex rabbits, weighing 445.6±98.4 g, were randomly distributed among 5 experimental groups of 25 animals each (12 males and 13 females). All rabbits were fed the same basal diet, formulated according to NRC [1977], which ingredients and calculated chemical composition are presented in Table 1. The groups were submitted to different experimental dietary treatments as follows: the control group did not receive any feed supplements; MOS continuously group (MOS+) received mannanoligosaccarides (ALLTECH INC., Nicholasville, Kentucky, USA) at 0.083 g/rabbit/day; MOS intermittently group (MOS+/-) received MOS at the same concentration, but only two days per week; ZnB continuously group (ZnB+) received Zinc bacitracin (10%; Pucheng Lifecome Biochemistry Co., Ltd. No.19, Nanpu Ecological Industrial Park, Pucheng, Fujian, P.R. China) at 0.083 g/rabbit/day; ZnB intermittently group (ZnB +/-) fed ZnB at the same level as ZnB+ group, but just two days per week. The experiment lasted 8 weeks, from 25 to 81 days of life.

Table 1. Ingredients and calculated chemical composition of the basal diet

Ingredients	kg/ton
Clover hay	395
Soybean meal	175
Wheat bran	150
Barley	130
Yellow corn	100
Molasses	30
Dicalcium phosphate	8
Limestone	5
Sodium chloride	3
Vitamin and mineral mixture*	3
DL-methionine	1
Chemical composition	**g/kg**
Dry matter	897.1
Organic matter	801.4
Crude protein	169.6
Crude fibre	137.3
Ether extract	23.8
Nitrogen free extract	573.6
Ash	95.7

*Provides per kg of diet: Vit. A 6000 IU; Vit. D 450 IU; Vit.E 40 mg; Vit. K 1mg; Vit. B1 1mg; Vit. B2 3 mg; Vit. B3 180 mg; Vit. B6 39 mg; Folic acid 2.5 mg; Vit. B12 5 µg; Pantothenic acid 10 mg; Biotin 10 µg; Choline Chloride 1200 mg; Zn 35 mg; Fe 38 mg; Cu 5 mg; I 0.2 mg; Se 0.05 mg and Mn 15 mg.

Rabbits were raised in an open system, housed individually in galvanized wire cage Italian battery with standard dimension. All cages were provided with a manual feeder and clean fresh water was available continuously through an automatic system of nipple

drinkers. The commercial pelleted diet was offered *ad libitum*. Rabbits were kept under the same hygienic and environmental conditions during the experimental period.

The rabbits were weighted on 25, 53 and 81 days of life. The weight gain was calculated by subtracting body weight at beginning of each period from the body weight at the end of the same period. Mortality was recorded daily throughout the experimental period. Feed intake was calculated as the difference between the weight of the feed offered and the weight of the remained at same day of weighing the animals. Feed conversion ratio was computed as the ratio between feed intake and weight gain per period.

At week 3, 5 and 7, six rabbits from each treatment were randomly chosen for blood analysis. Blood samples were collected from marginal ear vein under vacuum in clean tubes with heparin for determination of hemoglobin concentration, red blood cell counts, white blood cell counts packed cells volume, differential white blood cell count and the phagocyte activity (PA) and phagocyte index (PI). Plasma was obtained by centrifugation the blood at 4000 rpm for 20 min for analysis the blood biochemical parameters (total plasma protein, albumin, cholesterol, aspartate amino transferase, alanine amino transferase and total antioxidant capacity).

Hemoglobin concentration (g/dl) was determined of fresh blood samples using hemoglobinometers as the method described by Tietz [1982]. Red blood cells were counted on bright line hemocytometer using light microscope at 400X magnification. RBC'S were counted according to the method of Helper [1966] and Hawkeye and Dennett [1989]. White blood cells were counted according to Helper [1966], and Hawkeye and Dennett [1989] using a light microscope at 100X magnification. Blood was withdrawn by wintrobe hematocrite tubes and centrifuged at 4000 rpm for 20 minutes. PCV (%) was recorded directly according to Wintrobe [1965]. Blood film was prepared according to the method described by Lucky [1977]. Ten drops from any Gunwale stain stack solution a dry, unfixed smear were added to equal amount of distilled water, then mixed and left for 1 minute for staining. The dye was de courted without rinsing. Diluted Giemsa solution (10 drops of the day were added to 10 ml distilled water) was poured over the film as counter stain and left for 20 minutes, then rounded in current water and absolute values for each type of cells were counted and calculated relative to total WBC's. Phagocytic activity was determined according to Kawahara *et al*. [1991]. Fifty µg *Candida albicans* culture were added to 1 ml of citrated blood and shaken in water bath at 23-25°C for 3-5 hours. Smears of the blood were then stained with Giemsa solution. Phagocytic activity (PA) was estimated by determining the proportion of macrophages containing intracellular yeast cell in a random count of 300 macrophages and expressed as a percentage. The number of phagocytized organisms was counted in the phagocytic cells and called phagocytic index (PI).

Plasma total protein and albumin were determined according to Doumas *et al*. [1981] and Reinhold [1953], respectively, using commercial kits produced by DIAMOND diagnostics. Plasma globulin was determined by subtracting plasma albumin from total plasma protein according to Coles [1974].

Plasma total lipids were determined according to Chabrol and Charonnat [1973] using commercial kits produced by DIAMOND diagnostics. Plasma total cholesterol was determined according to Watson [1960] using specific diagnostic kits produced by DIAMOND diagnostics.

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as (U/L) in blood plasma were determined according to Reitman and Frank [1957] using commercial kits produced by DIAMOND diagnostics. Total antioxidant capacity was determined using commercial kits produced by DIAMOND diagnostics.

On day 81 of life, five male rabbits from each group were randomly selected, fasted for 6 hours, individually weighed and slaughtered according to Islamic procedure. After bleeding, rabbits were weighed to obtain blood percentage as the differences between body weight before and after slaughtered divided by body weight before slaughtered. Rabbits were skinned and then, they were weighed and the carcasses were eviscerated. The following carcass traits were determined: dressing percentage (weight of hot eviscerated carcass including liver, heart, abdominal fat and head divided by the live body weight); organs weight as a percentage of live body weight (head, liver, pancreas, kidney, spleen, heart, lungs and testes).

Samples of meat were taken individually from each slaughtered rabbit and chemical compositions and physical characteristics of meat were determined. Meat chemical composition was determined according to AOAC [1995]. Water-holding capacity (WHC) and meat tenderness were measured according to Volvoinskiaa and Kelman [1962]; pH was measured by pH meter as described by Aiken *et al*. [1962]; color intensity of meat was determined according to Husani *et al*. [1960].

Samples of liver, kidney, testes, spleen and ileum were collected from the slaughtered animals for microscopic examination. The collected specimens were fixed in 10% neutral buffered formalin solution for at least 24 hrs. After fixation specimens were washed in tap water and then passed through the routine paraffin embedding technique (dehydration in ascending grads of the ethyl alcohol, clearing in a series of xylene and then passed through a series of melted paraffin wax, embedded and put in paraffin blocks). Later on, the paraffin blocks were subjected for microtome to prepare paraffin sections of 3-5 microns thick which were stained with Mayer's hematoxylin and eosin [Culling 1983] and then examined with the light microscopy.

Statistical

Data were statistically evaluated using General Linear Model (GLM) procedure of the statistical analysis system of SAS Institute [SAS 2002] using one way ANOVA. Data of blood constituents were analyzed using factorial design and presented based on main effect of supplements only. Before analyses, arcsin transformation was done to normalize distribution of data. Mean differences were tested with Student Newman Kelus of SAS [2002]. Mortality rate was analysed using the chi-square test.

Results and discussion

Mortality rate in the control group (36%) was higher (P<0.05) than those recorded in MOS+/- (12%) and ZnB+ groups (12%). MOS+ group showed a mortality rate of 20% while ZnB+/- group of 28% statistically not different from the other groups. The number of rabbits per group is not high enough to allow firm conclusions from our data on the effect of supplements and way of their administration on mortality rate. However, both MOS and ZnB were both able to reduce the mortality rate in relation to the control group even if the antibiotic is needed to be administered continuously to reach this goal, while MOS has to be administered intermittently.

The *in vivo* performance of rabbits is given in Table 2. Body weight gain, feed intake and feed conversion ratio were affected by supplements only in the post-weaning period (25-53 days of age). Mannanoligosaccharides administered continuously or intermittently increased (P<0.05) BWG in respect of the control group, while ZnB had a positive effect on BWG of rabbits *vs*. the control group when administered continuously. MOS+ group showed a higher feed intake (P<0.01) than the ZnB+ group, while no differences were recorded among the other groups. FCR was improved (P<0.01) due to the continuative use of ZnB in respect to the control group, but no effect was observed due to the other supplements. However, considering the entire experimental period, rabbit performance was unaffected by dietary treatments and growth parameters at slaughter (day 81 of life) were not different among groups.

Table 2. Effect of mannanoligosaccharides (MOS) and zinc-bacitracin (ZnB) administered continuously or intermittently on performance of growing rabbits

Trait	Treatment					RMSE	P
	Control	MOS+	MOS+/-	ZnB+	ZnB+/-		
BW 25d (g)	443	450	440	439	446	92.4	0.9931
BW 81d, g	1600	1703	1662	1647	1694	229.9	0.6154
BWG 25-53d (g)	465^{b}	527^{a}	520^{a}	526^{a}	513^{ab}	3.88	0.0316
BWG 54-81d (g)	672	718	688	676	730	130.4	0.5477
BWG 25-81d (g)	1137	1240	1210	1203	1249	188.4	0.4781
FI 25-53d (g)	2200^{ab}	2266^{a}	2196^{ab}	1974^{b}	2214^{ab}	322.1	0.0001
FI 54-81d (g)	2791	2834	2834	2856	2818	273.9	0.9511
FI 25-81d (g)	4994	5054	5045	4836	5033	550.1	0.6754
FCR 25-53d	4.84^{a}	4.43^{ab}	4.27^{ab}	3.86^{b}	4.40^{ab}	3.77	0.0068
FCR 54-81d	4.25	4.05	4.20	4.34	3.98	0.66	0.3950
FCR 25-81d	4.46	4.13	4.19	4.07	4.08	0.259	0.0840

BW – body weight; BWG – body weight gain; FI – feed intake; FCR – feed conversion ratio; RMSE – root mean square error.
^{a,b}Means in each row with different superscripts are significantly different.

Table 3 reports the data recorded at rabbit slaughtering (day 81 of life). MOS and ZnB administered intermittently increased (P<0.01) dressing percentage in respect to the control group. However, ZnB+/- group showed higher (P<0.01) dressing percentage than MOS+ and ZnB+ groups. The liver percentage increased (P<0.01) due to the use of MOS (+ or +/-) and ZnB administered continuously. MOS administration + or +/-

reduced (P<0.01) the pancreas percentage in respect to the other groups. The testes percentage was minimal (P<0.01) in MOS+ group, followed by control, MOS+/- and ZnB+ groups together and then by ZnB+/- group. The group fed MOS continuously had the lowest (P<0.01) heart percentage than the other groups. Finally, MOS administered continuously and ZnB administered intermittently increased (P<0.01) lung percentage as related to ZnB+ group. Results of the present study indicate that MOS can replace ZnB in the diet of early-weaned growing rabbits (25-81 days of life) without negative effects on growing performance, carcass and meat traits. This partially agree with the finding of Bovera *et al*. [2012] and Attia *et al*. [2015] who replaced apramicin with MOS in the rabbit diet from 60 to 82 days of life. These authors recorded no effect on carcass and meat traits but revealed an improvement in rabbit growth.

Table 3. Effect of mannanoligosaccharides (MOS) and zinc-bacitracin (ZnB) administered continuously or intermittently on carcass characteristics of rabbits

Trait	Treatment					RMSE	P
	Control	MOS+	MOS+/-	ZnB+	ZnB+/-		
Blood (%)	3.45	3.31	3.18	3.24	2.89	0.31	0.0945
Dressing (%)	46.81^{c}	48.9^{bc}	50.02^{ab}	47.21^{bc}	51.09^{a}	2.21	0.0001
Head (%)	6.00	5.87	5.95	5.37	5.98	0.39	0.1038
Liver (%)	2.69^{b}	3.11^{a}	3.16^{a}	3.08^{a}	2.62^{b}	0.17	0.0001
Spleen (%)	0.048	0.058	0.041	0.049	0.063	0.011	0.0504
Pancreas (%)	0.222^{a}	0.181^{b}	0.161^{b}	0.220^{a}	0.257^{a}	0.022	0.0001
Kidney (%)	0.610	0.661	0.653	0.632	0.626	0.038	0.3004
Testes (%)	0.193^{b}	0.161^{c}	0.213^{b}	0.221^{b}	0.301^{a}	0.019	0.0001
Heart (%)	0.326^{a}	0.269^{b}	0.322^{a}	0.338^{a}	0.319^{a}	0.028	0.0089
Lungs (%)	0.634^{ab}	0.796^{a}	0.628^{ab}	0.499^{b}	0.691^{a}	0.10	0.0030

^{a,b}Means in each row with different superscripts are significantly different.
RMSE – root mean square error.

The positive effect of the intermittently administration of MOS and even more of ZnB on dressing percentage is not in late with other findings in literature [Piccolo *et al*. 2009, Bovera *et al*. 2012, Yalcinkaia *et al.* 2012]. Unfortunately, it was not possible to measure the percentage of intestinal tract and, considering that the main effects of prebiotic and antibiotic are on this system, we can hypothize than in early weaned rabbit there is a strong effect of these feed supplements on gut development. On this regard, Piccolo *et al*. [2009] showed a decrease in the percentage of empty gastrointestinal tract in respect of the antibiotics, even if this does not affect the dressing percentage of rabbits weaned at 35 days of life. Bovera *et al*. [2012] showed that the inclusion of antibiotic growth promoter in diets fed to rabbits results in a reduction of the enteric mass. This partly agrees with our results on intestinal morphology that showed a mild stunting due to the continuative administration of ZnB (Fig. 11) which could lead to the reduction of nutrients absorption, even if this does not modify *in vivo* performance along the entire experimental period.

Bovera *et al*. [2012] found a progressive decrease in liver percentage as compared to the unsupplemented group due to the increase of the inclusion level of MOS in the diet. At the lowest concentration used by the above mentioned authors (around 0.142 g/rabbit/day) no differences were reported in respect to the control group. The use of even lower levels of MOS, as in our trial, increased the liver percentage. However, from the post-mortem examination of the liver no signs of enlargement (as rounded edges) were observed. In addition, the blood concentration of liver enzymes AST and ALT falls for all the groups in the physiological range [Archetti *et al*. 2008]. The AST concentration was reduced due to use of the antibiotic, however, we have to consider that AST (mitochondrial enzyme) is considered a less specific of liver function than other enzymes since it can also be found in many peripheral tissues (as muscles) and hence had a very high variability [Moniello *et al*. 2005, Bovera *et al*. 2007].

Reduction in the pancreas percentage due to use of MOS are in line with the findings of Sherief *et al*. [2011] and Yang *et al*. [2007] in broiler. It is not easy to explain the other effects of MOS and antibiotic on heart and lung percentage as not enough data are reported in literature and very often are given together as a sum. The percentage of testes decreased due to continuative administration of MOS and the result agree with histological findings that found low level of spermatogenic cells (Fig. 6); this may lead to delay the sexual maturity of young rabbits and could affect fertility after maturity. Even if these considerations are not important when rabbits have to be addressed to slaughtering, this point has to be taken into account and object of further studies in the farms which breed males for reproduction. However, if MOS are administered intermittently, no alterations are recorded in testes tissue (Fig. 7). The relative weight of the testes increased when ZnB was administrated intermittently confirming the morphological changes. These results indicate that the intermittent administration of ZnB stimulated spermatogenesis (Fig. 12).

Table 4 shows chemical and physical characteristics of rabbit meat. ZnB + or +/- increased ($P<0.05$) the protein percentage of meat as related to the control group, while

Table 4. Effect of mannanoligosaccharides (MOS) and zinc-bacitracin (ZnB) administered continuously or intermittently on meat characteristics of rabbits

Trait	Treatment					RMSE	P
	Control	MOS+	MOS+/-	ZnB+	ZnB+/-		
Dry matter (%)	27.31	27.35	27.35	27.50	27.49	0.141	0.1419
Protein (%)	21.64^{b}	21.94^{ab}	21.91^{ab}	22.01^{a}	22.05^{a}	0.19	0.0232
Lipids (%)	4.13^{a}	3.79^{b}	3.94^{ab}	4.03^{ab}	3.98^{ab}	0.16	0.0408
Ash (%)	1.39	1.41	1.38	1.38	1.38	0.062	0.8681
pH	6.02	6.06	6.04	5.97	6.01	0.17	0.9210
Color intensity	0.306	0.306	0.296	0.292	0.313	0.018	0.3737
Tenderness ($cm^{2.g}$)	2.59^{b}	2.79^{a}	2.73^{a}	2.76^{a}	2.72^{a}	0.056	0.0020
WHC ($cm^{2/g}$)	5.33^{b}	5.87^{a}	5.78^{a}	5.92^{a}	5.77^{a}	0.24	0.0090

WHC – water holding capacity; RMSE – root mean square error.
^{a,b} means in each row with different superscripts are significantly different.

the continuative administration of MOS was able to reduce (P<0.05) the percentage of fat when compared to the control group. Reduction in muscular fat due to the use of MOS continuously in respect to the control group agrees with the findings of Ghosh *et al*. [2008] and Bonos *et al*. [2010] in Japanese quail meats. The result can probably be ascribed to the effect of MOS in the dilution of bile salt and reducing lipid digestibility [Maisonnier *et al*. 2003, Ghosh *et al*. 2008]. However, only the continuative administration along the trial was able to induce a valuable effect on fat content of meat.

Both supplements and both administration protocols increased (P<0.01) meat tenderness and WHC as related to the control group showing an improvement in the meat sensory quality. These agree with Zhang *et al*. [2005] and Sherief *et al*. [2011] who reported that MOS are able to improve the meat tenderness of broilers. In addition, Zhang *et al*. [2005] also found that the supplementation of probiotics to broiler diets improved the meat quality both at pre-freezing and post-freezing storage, due to an increase of the water holding capacity of meat. Interestingly, ZnB had a similar effect to MOS on tenderness and WHC of meat.

Table 5 illustrates the effects of dietary treatments on blood profiles. MOS administered continuously increased (P<0.01) red blood cell count when compared to ZnB administered continuously or intermittently. MOS+/- group showed a lower

Table 5. Effect of mannanoligosaccharides (MOS) and zinc-bacitracin (ZnB) administered continuously or intermittently on blood profiles of rabbits

Trait	Treatment					RMSE	P
	Control	MOS+	MOS+/-	ZnB+	ZnB+/-		
RBC's (10^6)	1.89^{ab}	2.02^{a}	1.89^{ab}	1.81^{b}	1.79^{b}	0.17	0.0018
WBC's (10^3)	21.94	21.44	21.31	21.72	21.53	1.32	0.6346
PCV (%)	29.17	28.33	28.47	29.11	28.06	2.55	0.6191
Hgb (g/dl)	9.67	9.42	9.36	9.53	9.33	10.0	0.3998
Lymphocyte(10^3)	47.78	47.44	46.81	47.58	48.19	1.92	0.2911
Monocyte(10^3)	1.72	1.69	1.47	1.50	1.36	0.47	0.1112
Basophils (10^3)	6.14^{a}	6.08^{a}	5.31^{b}	6.14^{a}	6.08^{a}	0.84	0.0145
Eosynophils(10^3)	7.61	7.97	7.75	8.25	7.89	0.82	0.1888
Neutrophils (10^3)	36.75^{b}	36.81^{b}	38.67^{a}	36.53^{b}	36.47^{b}	2.28	0.0272
Phagocytic activity	21.64	21.56	20.67	20.94	20.94	1.43	0.1864
Phagocytic index	1.89	1.85	1.96	1.83	1.85	0.16	0.0757
Total protein (g/dl)	4.97	4.91	4.99	4.96	5.00	0.28	0.8581
Albumin (g/dl)	2.97	2.97	2.82	2.92	3.04	0.34	0.3761
Globulin (g/dl)	2.01	1.94	2.17	2.04	1.96	0.33	0.2369
Total lipid (g/dl)	102.8	103.8	102.1	104.2	105.2	3.84	0.1252
Cholesterol (g/dl)	200.8	200.5	201.9	192.3	186.0	18.82	0.0551
Glucose (g/dl)	81.7	80.9	81.7	80.5	80.4	2.48	0.3123
AST(U/L)	59.3^{a}	59.0^{a}	58.2^{ab}	57.0^{b}	56.9^{b}	2.39	0.0059
ALT(U/L)	64.5	64.3	65.2	63.8	63.4	2.12	0.1326
TAC (Mm/l)	143.7^{b}	152.8^{a}	154.4^{a}	145.8^{b}	144.1^{b}	2.12	0.0123

RMSE– root mean square error.
^{a,b} means in each row with different superscripts are significantly different.

(P<0.05) number of basophils and a higher (P<0.05) number of neutrophils than the other groups. The concentration of AST was lower (P<0.01) due to the use of ZnB (+ or +/-) than the control and MOS+ groups. MOS + or +/- increased (P<0.05) the total antioxidant capacity as related to other groups. Some blood parameter are affected by dietary treatments, however, all fall in the physiological range indicated for rabbits by Archetti *et al*. [2008]. The increase of the total antioxidant capacity due to the use of MOS administered continuously or intermittently indicates (in respect to the control and the ZnB groups) a reduction of rabbit oxidative stress and an improved general health status of the animals. Very interesting result could be reached when MOS is administered 2 days by week. The increase of TAC can also be justified when the high tenderness of meat for MOS groups as recent studies [Picard 2012] showed that proteins involved in oxidative stress, such as super oxide dismutase (SOD1) or peroxiredoxin 6 (PRDX6) have a negative relationship with meat tenderness.

The total white blood cells count was not different among groups. However, it is necessary to study the different leukocyte count to identify some different immune system activities [Bovera *et al*. 2012]. In the present study MOS administered intermittently gave the highest percentage of basophiles and the lowest of neutrophils. Neutrophils are responsible for phagocytosis of pathogenic microorganisms during the first few hours after their entry the tissues while basophiles count increase upon sensitization to an antigen (or allergen). In general, neutrophils are considered an indicator of infection and could indicate a worsening of sanitary status of rabbits fed MOS intermittently compared to the unsupplemented group.

Table 6. Results of microscopic examination for morphological characteristics of liver, kidney, testes, ileum and spleen

Item	Liver	Kidneys	Testis	Ileum	Spleen
MOS continuously	N	N	D+ low spermatogenesis	N	N
MOS intermittently	N	N	B++ high spermatogenesis	B++ increase intestinal villi height	N
ZnB continuously	D+ sinusoidal congestion	D++ mild glomeruli atrophy and tubular lesions	N	D+ mild stunting villi	N
ZnB intermittently	N	N	B+ high spermatogenesis	N	N

The results of organs morphology are shown in Figures 1-12 and summarized in Table 6. The control group exhibited normal morphology of liver and portal vein (Fig. 1), kidney (Fig. 2), testes (Fig. 3), ileum (Fig. 4) and spleen. The administration of MOS continuously or intermittently resulted in normal morphology of liver, kidney, ileum and spleen (Figures not shown), but MOS administrated continuously resulted in low spermatogenic cells (Fig. 6). MOS+/- stimulated spermatogenesis (Fig. 7). MOS administration also increased *villi* height in the ileum (Fig. 8).

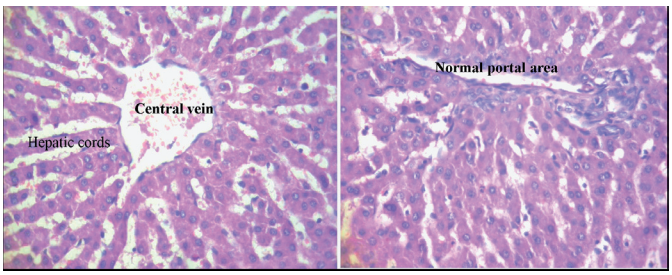

Fig 1. Hepatic histology of the control group showing normal heptocyte and portal vein H&E (X400).

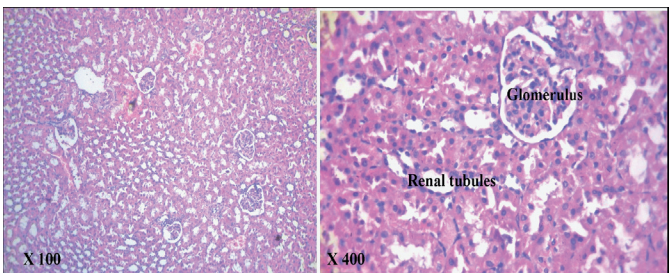

Fig 2. Renal histology of the control group showing normal renal tubules and glomeruli. H&E (X400).

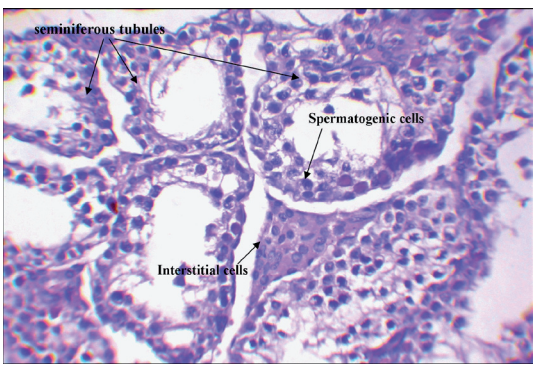

Fig 3. Histology of the testis of the control group showing normal testicular (seminferous tubules), spermatogenic cells and interstitial cells H&E (x400).

Continuous ZnB administration led to deleterious effects on liver (Fig. 9), kidney (Fig. 10) and ileum (Fig. 11). These effects showed multifocal dilatation in hepatic tissues, mild glomerular atrophy and severe dilatation of collecting tubules with atrophied lining epithelium in kidney and moderate stunting of intestinal *villi* while did not negatively affect testis morphology. The intermittent administration of ZnB did not negatively affect hepatic, renal and ileum tissues and induced a positive effect on testes tissue (Fig. 12). These results demonstrate that intermittent administration

of MOS and ZnB stimulate spermatogenesis, however, MOS had stronger effect than the ZnB (Fig 7 *vs*. Fig 12).

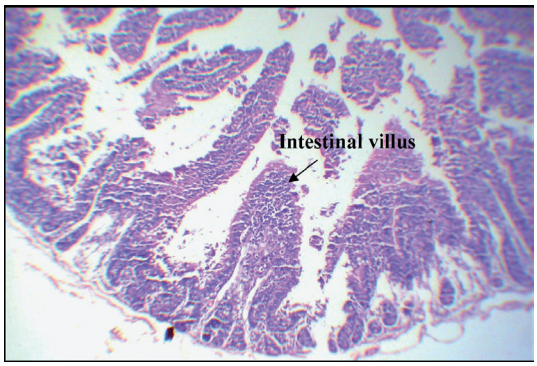

Fig 4. Histology of the ileum of the control group showing normal vilius H&E (x40).

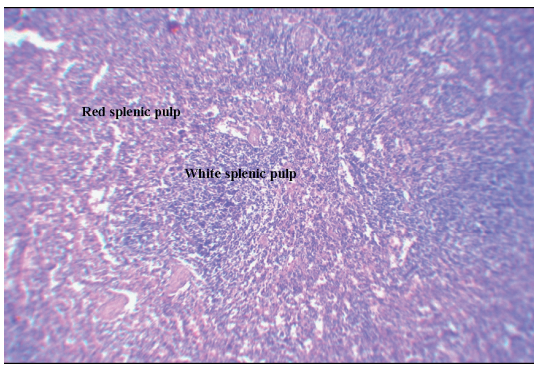

Fig 5. Histology of the spleen of the control group showing normal spleenic tissue H&E (x100)

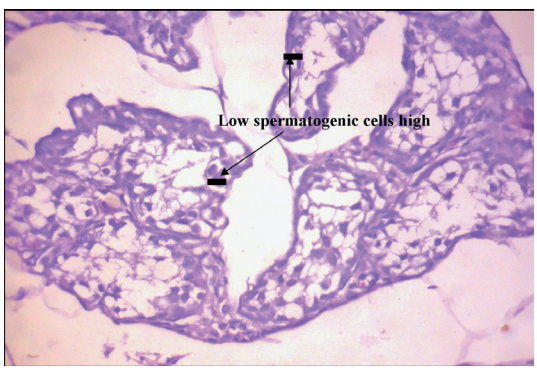

Fig 6. Histology of testis of rabbits given MOS contsinsouly for 15 days after weaning showing low spermatogenic cells which may lead to low spermatogenisis H&E (x400).

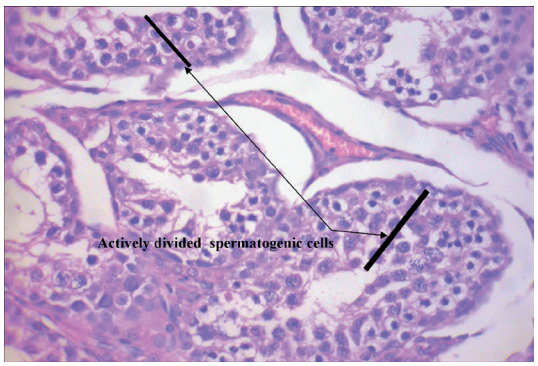

Fig 7. Histology of testis of rabbits given MOS intermittently for two days per week after weaning showing highly increase in the spermatogenic cells which may lead to high spermatogenisis H&E (x400).

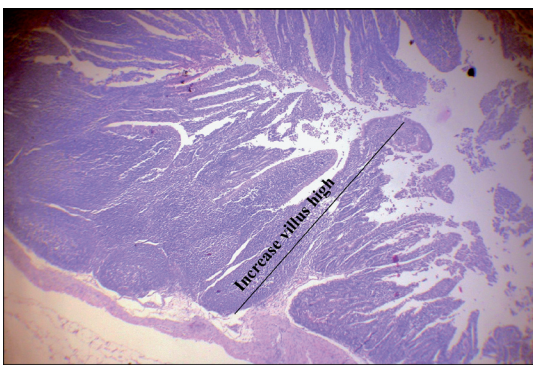

Fig 8. Histology of testis of rabbits given MOS intermittently for two days per week after weaning showing showing increased villi height which may lead to improve nutrient absorption H&E (x400)

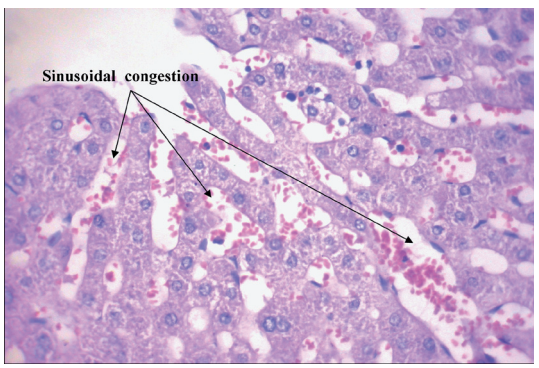

Fig 9. Histology of liver of rabbits given ZnB continsouly for 15 days after weaning showing multifocal sinusoidal dilatat which may affect negatively hepatic efficiency H&E (x400).

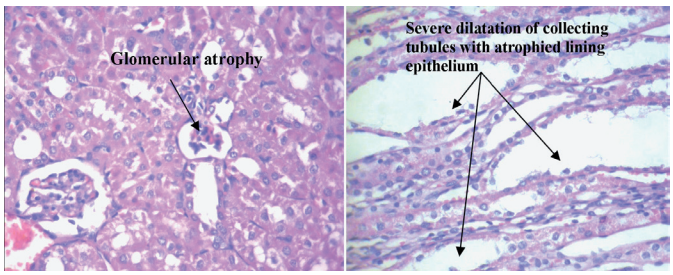

Fig 10. Histology of kidney of rabbits given ZnB continsouly for 15 days after weaning showing mild glomeruli atrophy and tubular lesions which may lead to deleterious effects on kidney H&E (x400).

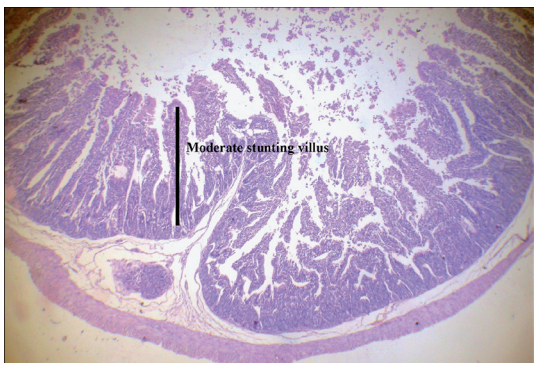

Fig 11. Histology of ileum of rabbits given ZnB continsouly for 15 days after weaning showing moderate stunting villi which negatively aect absorptive cacpcity H&E (x40).

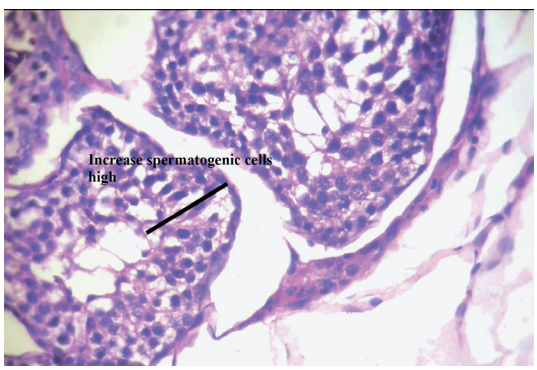

Fig 12. Histology of tests of rabbits given ZnB intermittently for 15 days after weaning showing highly increase in the spermatogenic cells which may lead to high spermatogenisis H&E (x40).

It can be concluded that mannanoligasaccharides can replace zinc-bacitracin from weaning to slaughter age of rabbits without negative effects on performance and blood profile and causing no alterations in tissue morphology (as ZnB) as compared to the control group. In addition, the MOS can be administered intermittently, reducing the production costs.

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