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Effect of a Commercial Yeast-Based Product (Maxigen®) on Intestinal Villi Morphology and Growth Performance of Broiler Chickens

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Abstract: Yeast products contain nucleotides that are essential for cellular functions and growth and may therefore enhance intestinal villi morphology and growth performance. Accordingly, two 42-day experiments were conducted to evaluate the efficacy of Maxigen® (a novel yeast-based product) in enhancing intestinal villi morphology, growth performance and flock uniformity. Experiment 1 utilized 480 (day-old) female chicks obtained from a commercial hatchery, weighed and randomly assigned to 2 dietary treatments. Treatment 1 (CX, control) consisted of chicks fed corn-soybean meal (SBM) diet that was not supplemented with Maxigen®. Treatment 2 (MG) consisted of chicks fed corn-SBM basal into which Maxigen® was added at 0.075% level. Each treatment consisted of 12 replicate pens, with each pen housing 20 chicks. On days 21 and 42 of experiment, growth performance (body weight and feed conversion) and flock uniformity were assessed. Experiment 2 was conducted in a similar manner, except that male chicks were used. Intestinal tissue samples were also collected on day 10 of experiment for histological evaluation of villi morphology. From the results, only Experiment 2 showed differences between CX and MG treatments for the parameters assessed. Specifically, at day 42, feed conversion ratio of birds in MG treatment (1.67) was superior ($P < 0.05$) to that of birds in CX treatment (1.71). In addition, flock uniformity of MG birds (66.4%) was better than the uniformity of CX flock (56.8%; $P = 0.0527$). It was concluded that Maxigen® supplemented at 0.075% level of the diet enhanced growth performance and flock uniformity of broiler chickens.

Key words: Maxigen®, yeast-based product, Intestinal villi morphology, broiler chicks, flock uniformity

INTRODUCTION

The intestine of the newly hatched chick is not fully functionally developed and therefore restricts optimal chick growth (Uni, 1999; Lee *et al.*, 2010). Consequently, Antibiotic Growth Promoters (AGP) are typically administered in feed as prophylactics to enhance intestinal development, chick growth and flock (body weight) uniformity (Engster *et al.*, 2002; Huyghebaert *et al.*, 2010). However, in recent years, the emergence of microbes that are resistant to antibiotics used to treat human and animal infections, along with increasing consumer demand for drug-free poultry products have initiated the search for non-antibiotic effective alternatives.

Yeast products are non-antibiotic functional products that are naturally obtained from yeast strains such as *Saccharomyces cerevisiae* and *Kloeckera apiculata* (Owens and McCracken, 2007). Although the composition of yeast products is variable, they essentially contain some nucleotides and beta-glucans which are growth-promoting and immunostimulatory (Leblanc *et al.*, 2006; Solis de los Santos *et al.*, 2007; Ferreira *et al.*, 2011). In poultry, the efficacy of yeast products as growth promoters has produced inconsistent results. In some studies, yeast was found to improve feed utilization and body weight in broiler chickens (Madrigal *et al.*, 1993). On the hand, Morales-

López *et al.* (2009) observed no effect when he evaluated the effect of various yeast cell wall components on growth performance parameters in broiler chicks.

The inconsistency in the efficacy of yeast products as growth promoters in broilers is probably due to the inevitable variation in the levels of nucleotides, beta-glucans and other nutrients in various yeast products. It is therefore imperative to evaluate each yeast product formulation for efficacy as a growth promoter in the animal species for which its use is intended.

Maxigen® is a novel yeast-based heat-stable feed additive that essentially contains dehydrated brewer's yeast and corn distillers dried grains with solubles (Canadian Biosystems, Calgary, Canada). The objective of this study was to evaluate the effect of dietary Maxigen® supplementation on intestinal villi morphology, broiler growth and flock uniformity.

MATERIALS AND METHODS

All the procedures used in this study were approved by the Auburn University Institutional Animal Care and Use Committee.

Experimental animals and treatments: Two experiments were conducted in this study. In experiment 1, four hundred and eighty female broiler chicks (day-old;

Table 1: Composition of experimental diets (% "as is")

Ingredient	Starter diets ¹		Grower diets ¹	
	CX	MG	CX	MG
Corn	54.51	54.44	64.98	64.91
Soybean meal	36.90	36.90	29.48	29.48
Poultry oil	3.70	3.70	1.54	1.54
DL-Methionine	0.19	0.19	0.07	0.07
Limestone	1.68	1.68	1.43	1.43
Dicalcium Phosphate	1.74	1.74	1.32	1.32
Vitamin Premix ²	0.50	0.50	0.50	0.50
Mineral Premix ³	0.38	0.38	0.38	0.38
Salt	0.40	0.40	0.30	0.30
Maxigen® (%)	----	0.075	----	0.075
Calculated nutrient composition				
Metabolizable energy (Kcal/kg)	3200	3200	3200	3200
Crude protein,%	23.0	23.0	20.0	20.0
Total Sulfur Amino Acids,%	0.90	0.90	0.72	0.72
Lysine,%	1.25	1.25	1.05	1.05
Calcium,%	1.10	1.10	0.90	0.90
Available phosphorus,%	0.13	0.13	0.12	0.12

¹Starter and grower diets used in this study included i) the CX diet comprising of corn-soybean meal (SBM) basal without Maxigen® added, (ii) the MG diet comprising of corn-SBM basal into which Maxigen® was added at 0.075% level.

²Vitamin Premix, supplied per kilogram of diet: Vitamin A (retinyl acetate), 7356 IU; vitamin D₃ (cholecalciferol), 2, 205 ICU; vitamin E (8 IU); vitamin B₁₂ (cyanocobalamin), 0.2 mg; riboflavin, 5.5 mg; niacin, 36 mg; D-pantothenic acid, 13 mg; choline, 501 mg; vitamin K (menadione sodium bisulfate), 2 mg; folic acid, 0.5 mg; vitamin B₆ (pyridoxine), 2.2; vitamin B₁ (thiamin), 1.0 mg; D-biotin, 0.5 mg; and ethoxyquin, 0.13 mg.

³Mineral Premix, supplied per kilogram of diet: manganese, 65 mg; zinc, 55 mg; iron, 55 mg; copper, 6 mg; iodine, 1 mg; and selenium, 0.3 mg.

Ross x Ross 708) were obtained from a commercial hatchery and utilized in a floor-pen trial that simulated industry settings. Chicks were weighed, wing-banded and randomly assigned to two dietary treatments. Treatment 1 (CX) consisted of chicks fed a control diet (Table 1) consisting of corn-soybean meal (SBM) basal that was not supplemented with Maxigen®. Treatment 2 (MG) consisted of chicks fed the corn-SBM basal into which Maxigen® was added at 0.075% level (750g/Tonne of complete feed). Compositions of experimental diets are presented in Table 1. Starter diets were fed to chicks from day-old to 3 weeks as a crumble after steam-pelleting, while grower diets were fed as whole pellets from 3 to 6 weeks of age. Experimental diets were formulated to meet the recommendations of the National Research Council (1994).

Each treatment consisted of 12 replicate pens, with each pen housing 20 chicks. In each pen, chicks were placed on fresh pine shaving litter in a house with cross ventilation and temperature control. Chicks were given ad libitum access to feed and water and continuous lighting commensurate with day (1, 400 to 2, 800 lx) and night (4 to 17 lx) intensities was provided. Duration of experiment was 42 days. Experiment 2 was performed using similar experimental design and procedures described for Experiment 1, except that male broiler chicks (day-old; Ross x Ross 708) were used instead of females.

Assessment of growth performance: On days 21 and 42 of experiment, body weight, Feed Conversion Ratio

(FCR) and flock uniformity were calculated for the evaluation of broiler growth performance. Flock uniformity is a measure of body weight variation within a flock (Abbas *et al.*, 2010) and it is calculated as "% within±10% of BW mean" using the following equation:

$$\text{uniformity} = 100 - [(\text{standard deviation}/\text{mean}) \times 100]$$

(Jackson *et al.*, 2004)

Mortality was also recorded on daily basis.

Tissue sampling for the assessment of intestinal villi morphology: Tissue sampling was done only in Experiment 2 on day 10. From each pen, one chick was randomly taken for the collection of intestinal tissue samples. Accordingly, 12 chicks were taken from each treatment and euthanized by cervical dislocation. The small intestine was then aseptically excised, placed on ice and tissue sections (approximately 2 cm long) were taken from the mid-portion of duodenum (from the gizzard to the point of entry of the pancreo-biliary ducts), jejunum (from the pancreo-biliary ducts to the yolk stalk) and ileum (from yolk stalk to the ileo-cecal junction). Each tissue section was fixed by careful immersion in 10% neutral buffered formalin (Fisher Scientific, Pittsburgh, PA) until time for tissue processing and examination of villi morphology.

Morphological evaluation of intestinal villi epithelium: Formalin-fixed tissue samples were dehydrated, cleared and embedded in paraffin. Serial sections (5 µm) of the

tissue were then obtained, placed on glass slides and stained with haematoxylin and eosin. As previously described by Fasina *et al.* (2006), sections were examined by light microscope and morphometric parameters were taken. Parameters recorded include villus height (from tip of villus to the crypt opening), villus width at half height, villus area (calculated from villus height and width at half height), crypt depth (from the base of the crypt to the level of crypt opening) and villus-crypt ratio (V:C ratio; calculated by dividing villus height with crypt depth). For each bird, two slides, each with a mounted tissue section containing 10 vertically oriented and adjacent villus-crypt units were used for analysis. All measurements were performed with an Olympus light microscope (model BH-2) with Image J software (National Institutes of Health, Bethesda, MD). From the examination of serial sections, mean values for each morphometric parameter was calculated for each chick.

Statistical analysis: Data collected were subjected to one-way ANOVA Using the General Linear Models (GLM)

procedure of SAS (SAS Institute, 2004). Significant differences among means were determined using the Duncan option of the GLM procedure as a post hoc test and statements of statistical significance were based upon $P < 0.05$.

RESULTS AND DISCUSSION

Results for broiler growth performance are presented in Table 2 and 3. In Experiment 1, there was no difference between CX and MG ($P > 0.05$) for all parameters evaluated (Table 2). However, in Experiment 2, at 6 weeks of age, birds in the MG treatment had superior ($P < 0.05$) FCR (1.671) compared to birds in the CX treatment (FCR = 1.706; Table 3). A similar growth-promoting effect of yeast product was observed by Gao *et al.* (2008) when they supplemented a yeast culture product into the diets of broiler chicks at 0, 0.25, 0.50 and 0.75% from day-old to 42 days. They found that supplementing the yeast culture at 0.25% level of the diet improved body weight gain and FCR ($P < 0.05$) throughout the experiment. More recently, Ghosh *et al.*

Table 2: Effect of Maxigen® supplementation on broiler growth performance (Experiment 1)

Treatments ¹	Body weight (kg/chick) ²	FCR (g:g) ³	Uniformity (%)
Day 21 (0 to 3 weeks)			
CX	0.724	1.440	64.47
MG	0.723	1.465	66.38
SEM	0.009	0.024	3.122
P-value	0.9476	0.4741	0.6698
Day 42 (0 to 6 weeks)			
CX	2.33	1.741	82.87
MG	2.30	1.740	82.30
SEM	0.024	0.022	2.311
P-value	0.4874	0.9618	0.8639

^{a,b}Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

¹Treatment 1 (CX) consisted of female chicks fed corn-soybean meal (SBM) diet that was not supplemented with Maxigen® yeast product. Treatment 2 (MG) consisted of female chicks fed corn-SBM basal into which Maxigen® yeast product was added at 0.075% level.

²Values are based only on weight of live birds.

³FCR: feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

Table 3: Effect of Maxigen® supplementation on broiler growth performance (Experiment 2)

Treatments ¹	Body weight (kg/chick) ²	FCR (g:g) ³	Uniformity (%)
Day 21 (0 to 3 weeks)			
CX	0.752	1.418	56.82
MG	0.750	1.437	51.57
SEM	0.009	0.012	3.838
P-value	0.8503	0.2522	0.3440
Day 42 (0 to 6 weeks)			
CX	2.57	1.706 ^a	56.82
MG	2.59	1.671 ^b	66.41
SEM	0.028	0.011	3.309
P-value	0.6682	0.0401	0.0527

^{a,b}Mean values bearing different superscript letters within a column are significantly different ($P < 0.05$).

¹Treatment 1 (CX) consisted of male chicks fed corn-soybean meal (SBM) diet that was not supplemented with Maxigen® yeast product. Treatment 2 (MG) consisted of male chicks fed corn-SBM basal into which Maxigen® yeast product was added at 0.075% level.

²Values are based only on weight of live birds.

³FCR: Feed conversion ratio calculated as feed-to-gain ratio and adjusted for mortality by including the gains of dead birds in the calculations.

(2012) reported that in a 42-day experiment, broilers given a corn-soybean meal basal diet that was supplemented with yeast cell wall (at 0.1% level) had superior ($P<0.05$) FCR (1.97) compared to broilers that consumed the un-supplemented corn-soybean meal basal (FCR = 2.06). There were no differences ($P>0.05$) in mortality among treatments in both experiments and total mortality was 2.71% and 1.04% for Experiments 1 and 2, respectively.

Improvement in flock body weight uniformity is one of the most important economic factors in broiler production. This is because birds from a more uniform flock cause less disruptions for the machinery during slaughter and downstream carcass processing. For instance, growth-promoting feed additives such as prophylactic antibiotics have been established to enhance body weight uniformity (Engster *et al.*, 2002). In like-manner, other growth-promoting feed additives such as yeast products are expected to furnish similar benefits to broilers. It is therefore not surprising that in Experiment 2 of this study, birds that consumed the Maxigen®-supplemented diet (MG treatment) had superior ($P<0.05$) body weight uniformity (66.4%) compared to birds in the CX treatment (Uniformity = 56.82%; Table 3). The inherent growth-promoting ability of yeast products has been attributed to its constituent nucleotides, beta-glucans and mannan oligosaccharides (Cox *et al.*, 2010; Fasina and Thanissery, 2011; Shanmugasundaram *et al.*, 2013). Throughout this study, body weight uniformity (flock uniformity) ranged from 64.47 to 82.87 in Experiment 1 and from 51.57 to 66.41% in Experiment 2. These values were lower than 88 to 92% reported by Jackson *et al.* (2004) for Cobb male broilers over a 42-day experiment. The differences in uniformity values may be inherent in broiler strain differences because this study utilized Ross 708 chicks, while Jackson *et al.* (2004) utilized Cobb chicks in his study.

Assessment of intestinal villus morphology is important because the small intestine is the primary site for nutrient assimilation and is therefore sensitive to

changes in the diet (Pacha, 2000). Measuring villus height, villus width, crypt depth and calculating villus area and villus: Crypt ratios are established ways of investigating intestinal morphology and functional capacity (Fasina *et al.*, 2010). Furthermore, continuous proliferation, migration, differentiation and maturation of intestinal crypt stem cells is regulated by a variety of factors which include luminal nutrients (Uni *et al.*, 2001), such as the yeast product (Maxigen®) added into the starter and grower diets for chicks in MG treatment.

Because morphometrical parameters such as villus height, villus width and crypt depth are used in the calculation of villus area and villus: Crypt ratio, results for villi morphology (Table 4) will be interpreted and explained using the latter composite parameters. Accordingly, morphometric differences were observed in the jejunum and ileal villi, while no differences were observed in the duodenum. Villus area in the jejunum and ileum was similar ($P>0.05$) for both CX and MG treatments. However, birds in the MG treatment had deeper crypts ($P<0.05$) compared to birds in the CX treatment in both jejunum and ileum. Furthermore, jejunal villus: Crypt ratio was lower ($P<0.05$) for the MG treatment compared to the CX treatment. A similar trend was observed in the ileum but the difference between MG and CX was not significant ($P = 0.0960$). Differential responses of intestinal segments (duodenum, jejunum and ileum) to dietary supplementation of yeast products have also been reported for similar studies. For instance, Solis de los Santos *et al.* (2007) observed that supplementation of Alphamune (yeast extract) at 0.045% or 0.09% level of broiler chick diets from day-old to 21 days of age had no effect on duodenum epithelial morphology and had mixed (or inconclusive effects) on jejunal morphology, while significantly influencing only ileal morphology. Specifically, they observed that dietary supplementation of Alphamune at up to 0.09% level of the diet enhanced villus height, villus surface area, lamina propria thickness and crypt depth in the ileum at 7 and 21 days ($P<0.05$) of experiment.

Table 4: Effect of Maxigen® supplementation on intestinal villi morphology of broiler chicks at 10 days of age (Experiment 2)

Parameters	Duodenum			
	CX ¹	MG ¹	SEM	P-value
Villi area (mm ²)	0.47	0.46	0.021	0.8983
Crypt depth (mm)	0.30	0.39	0.020	0.0511
Villus:Crypt ratio	8.31	8.11	0.759	0.8624
Jejunum				
Villi area (mm ²)	0.16	0.17	0.011	0.4791
Crypt depth (mm)	0.25 ^b	0.30 ^a	0.013	0.0220
Villus:Crypt ratio	4.42 ^a	3.68 ^b	0.244	0.0440
Ileum				
Villi area (mm ²)	0.12	0.11	0.007	0.2073
Crypt depth (mm)	0.20 ^b	0.27 ^a	0.014	0.0061
Villus:Crypt ratio	3.99	3.15	0.322	0.0960

^{a,b}Mean values bearing different superscript letters across a row are significantly different ($P<0.05$). No. of observations per mean $n = 12$. ¹CX represent chicks fed Maxigen®-free corn-soybean meal basal diet throughout the experiment, while MG represent chicks fed corn-soybean meal basal diet supplemented with Maxigen® at 0.075% level.

The crypt in the intestinal epithelium is the proliferating compartment that gives rise to cells undergoing differentiation and maturation on the villi (Potten and Loeffler, 1990; Fasina *et al.*, 2010). It has been proposed that a deeper crypt is indicative of a faster tissue turnover and perhaps, a higher demand for new tissue (Yanson *et al.*, 1987). Furthermore, it has been established that a high villus: Crypt ratio is associated with a well-differentiated intestinal mucosa with high digestive and absorptive capabilities (Jeurissen *et al.*, 2002). However, compared to CX treatment, the presence of deeper crypts in all intestinal segments for the MG treatment does not correlate with or support the observed superior FCR in Experiment 2. It is possible that the intestinal epithelium of birds in MG treatment compensated for the deeper crypts through an unknown mechanism that upregulated the function of another microstructure in the intestine.

The response of intestinal microstructure to dietary or luminal factors can be affected by bird strain, dietary level of yeast product inclusion, differences in composition of the yeast product and age of bird when taking tissue samples for histology. It is therefore imperative to always determine the optimum dietary inclusion level of each yeast product for its intended use, prior to inclusion in poultry or animal diets.

In summary, we evaluated the efficacy of Maxigen® (a novel yeast product) in enhancing intestinal villi morphology and broiler growth performance in a 42-day experiment. Although differences were not observed in Experiment 1 (female chicks), results from Experiment 2 (male chicks) showed that dietary supplementation of Maxigen® at 0.075% level influenced intestinal jejunal and ileal villi morphology and improved broiler FCR and body weight uniformity. Inclusion of higher levels (>0.075%) of Maxigen® in the diet may induce more pronounced beneficial differences between the CX and MG treatments. It was concluded that dietary inclusion of Maxigen® at 0.075% level showed potential to enhance broiler growth performance and body weight uniformity.

REFERENCES

- Abbas, S.A., A.A. Gasm Elseid and M.K.A. Ahmed, 2010. Effect of body weight uniformity on the productivity of broiler breeder hens. *Int. J. Poult. Sci.*, 9: 225-230.
- Cox, C.M., L.H. Sumners, S. Kim, A.P. McElroy, M.R. Bedford and R.A. Dalloul, 2010. Immune responses to dietary beta-glucan in broiler chicks during an *Eimeria* challenge. *Poult. Sci.*, 89: 2597-2607.
- Engster, H.M., D. Marvil and B. Stewart-Brown, 2002. The effect of withdrawing growth promoting antibiotics from broiler chickens: A long-term commercial industry study. *J. Appl. Poult. Res.*, 11: 431-443.
- Fasina, Y.O. and R.R. Thanissery, 2011. Comparative efficacy of a yeast product and bacitracin methylene disalicylate in enhancing early growth and intestinal maturation in broiler chicks from breeder hens of different ages. *Poult. Sci.*, 90: 1067-1073.
- Fasina, Y.O., H.L. Classen, J.D. Garlich, B.L. Black, P.R. Ferket, Z. Uni and A.A. Olkowski, 2006. Response of turkey poults to soybean lectin levels typically encountered in commercial diets. 2. Effect on intestinal development and lymphoid organs. *Poult. Sci.*, 85: 870-877.
- Fasina, Y.O., J. Hoerr, S.R. McKee and D.E. Conner, 2010. Influence of *Salmonella enterica* serovar Typhimurium Infection on Intestinal Goblet Cells and Villous Morphology in Broiler Chicks. *Avian Dis.*, 54: 841-847.
- Ferreira, S.R., A.E. Murakami, T.G.V. Silveira, J.M. Goncalves dos Santos and J.I.M. Fernandes, 2011. Performance and macrophage activity of broilers fed with a sorghum meal with different yeast wall levels. *Braz. Arch. Biol. Technol.*, 54: 363-370.
- Gao, J., H.J. Zhang, S.H. Yu, S.G. Wu, I. Yoon, J. Quigley, Y.P. Gao and G.H. Qi, 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. *Poult. Sci.*, 87: 1377-1384.
- Ghosh, T.K., S. Halder, M.R. Bedford, N. Muthusami and I. Samanta, 2012. Assessment of yeast cell wall as replacements for antibiotic growth promoters in broiler diets: Effects on performance, intestinal histo-morphology and humoral immune responses. *Anim. Physiol. Anim. Nutr.*, 96: 275-284.
- Huyghebaert, G., R. Ducatelle and F. Van Immerseel, 2010. An update on alternatives to antimicrobial growth promoters for broilers. *Vet. J.*, 182-188.
- Jackson, M.E., I.K. Geronian, A. Knox, J. McNab and E. McCartney, 2004. A dose-response study with the feed enzyme beta-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. *Poult. Sci.*, 83: 1992-1996.
- Jeurissen, S.H.M., F. Lewis, J.D. van der Klis, Z. Mroz, J.M.J. Rebel and A.A.H.M. ter Huurne, 2002. Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity and functionality. *Curr. Issues Intest. Microbiol.*, 3: 1-14.
- Leblanc, B.W., J.E. Albina and J.S. Reichner, 2006. The effect of PGG- β -glucan on neutrophil chemotaxis in vitro. *J. Leukocyte Biol.*, 79: 667-675.
- Lee, K., H.S. Lillehoj and G.R. Siragusa, 2010. Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. *J. Poult. Sci.*, 47: 106-114.
- Madrigal, S.A., S.E. Watkins, J.T. Skinner, M.H. Adams, A.L. Waldroup and P.W. Waldroup, 1993. Effect of an active yeast culture on performance of broilers. *Poult. Sci.*, 72: 87-87.

- Morales-López, R., E. Auclair, F. García, E. Esteve-García and J. Brufau, 2009. Use of yeast cell walls; beta-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. *Poult. Sci.*, 88: 601-607.
- National Research Council, 1994. *Nutrient Requirements of Poultry*. 9th Rev. Edn., Natl. Acad. Press, Washington, DC.
- Owens, B. and K.J. McCracken, 2007. A comparison of the effects of different yeast products and antibiotic on broiler performance. *Br. Poult. Sci.*, 48: 49-54.
- Pacha, J., 2000. Development of intestinal transport function in mammals. *Physiol. Rev.*, 80: 1633-1667.
- Potten, C.S. and M. Loeffler, 1990. Stem cells: Attributes, cycles, spirals, pitfalls and uncertainties. Lessons from the crypt. *Development*, 110: 1001-1020.
- SAS Institute, 2004. *SAS/STAT User's Guide*. Version 9.1 for Windows. SAS Inst. Inc., Cary, NC.
- Shanmugasundaram, R., M. Sifri and R.K. Selvaraj, 2013. Effect of yeast cell product (CitriStim) supplementation on broiler performance and intestinal immune cell parameters during an experimental coccidial infection¹. *Poult. Sci.*, 92: 358-363.
- Solis de los Santos, F., A.M. Donoghue, M.B. Farnell, G.R. Huff, W.E. Huff and D.J. Donoghue, 2007. Gastrointestinal maturation is accelerated in turkey poult supplemented with a mannan-oligosaccharide yeast extract (Alphamune). *Poult. Sci.*, 86: 921-930.
- Uni, Z., 1999. Functional development of the small intestine in domestic birds: cellular and molecular aspects. *Poult. Avian Biol. Rev.*, 10: 167-179.
- Uni, Z., O. Gal-Garber, A. Geyra, D. Sklan and S. Yahav, 2001. Changes in growth and function of chick small intestine epithelium due to early thermal conditioning. *Poult. Sci.*, 80: 438-445.
- Yanson, C.V., B.A. Summers and K.A. Schat, 1987. Pathogenesis of notavirus infection in various age groups of chickens and turkeys: Pathology. *Am. J. Vet. Res.*, 6: 927-938.