Pakistan J. Zool., vol. 54(3), pp 1379-1383, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20160925170957

Effect of Dietary Natural Growth Promoters on the Liver Function Enzymes of Chicken Challenged with *Clostridium perfringens*

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Article Information
Received 25 September 2016
Revised 30 October 2016
Accepted 22 November 2016
Available online 22 February 2022 (early access)
Published 28 February 2022

Authors' Contributions
AMA and ASA presented the concept, planned the methodology and wrote the manuscript. NJS, SZ, AMA and AHA performed investigation, validation, and data curation. NJS and SZ did formal analysis.

Key words
Antibiotic, Broilers, Liver enzymes, Prebiotic, Probiotic, Symbiotic.

ABSTRACT

In this study four groups of chicken challenged with *Clostridium perfringens* were fed on Neoxyval, GalliPro, TechnoMos and GalliPro + TechnoMos. A group fed on normal diet was used as control and the other challenged with bacterium was maintained. Three hepatic enzymes *viz.,* lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased after feeding on growth promoters. However, total serum protein, gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) showed no significant differences between the groups. Thus, it can be concluded that probiotic, prebiotic and symbiotic in feed may cause some degree of liver damage as indicated by the release of AST and ALT in the serum.

INTRODUCTION

Antibiotics are substances which are produced by microorganisms and which exhibit either an inhibitory or lethal effect on other microorganisms (Apata, 2012). Usually, antimicrobial compounds are added to animal feeds for treatment / prevention of infectious diseases and for growth promoting effects (Redondo *et al*., 2014). The most accepted mechanism of action of antibiotic growth promoters (AGPs) is their modulation of the gut microbiota, which are responsible for maintaining the bird health (Tuohy *et al*., 2005; Abudabos *et al*., 2016). However, AGPs used for a long period of time in sub-therapeutic doses favor the selection of antimicrobial drug resistance microorganisms (Redondo *et al*., 2014). The occurrence of the antimicrobial resistance as well as the resistance genes transfer from animal to human strains caused major concerns (Salyers *et al*., 2004; Mathur and Singh, 2005). The use of antibiotics as growth promoters was banned by the European Union as a result of the concerns of antimicrobial resistance generation and transmission (Redondo *et al*., 2014; Alzawqari *et al*., 2016). However, the ban of antibiotics may increase the incidence of poultry infectious diseases (Inborr, 2001; Casewell *et al*., 2003; Grave *et al*., 2006).

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0030-9923/2022/0003-1379 $ 9.00/0

Salmonella spp., *Campylobacter jejuni* and *Clostridium perfringens* are well known threats for poultry and human health (Humphrey *et al*., 2007). *Clostridium perfringens,* which causes necrotic enteritis is now recognized to cause a spectrum (Wilson *et al*., 2005). Alternatives to AGPs include nutritional interventions such as probiotics, prebiotics, synbiotic, acidifiers, antioxidants and phytogene additives (Abudabos *et al*., 2020; Aljumaah *et al*., 2020). Formulation of diets that have specific effects on gut health is being used in poultry industry because of its importance in the welfare and productivity of the birds in the absence of antibiotics (Redondo *et al*., 2014). Alternatives to antibiotics promote gut health by several possible mechanisms including altering gut pH, maintaining protective gut mucins, selecting for beneficial intestinal organisms or against pathogens, enhancing fermentation acids, enhancing nutrient uptake, and increasing the humoral immune response (Inborr, 2001).

A probiotic is a viable microbial feed supplement that has positive impacts on the host by improving the microbial balance in the intestine (Fuller and Gibson, 1997). The main probiotics in use are those of lactic acid excretes, like *Lactobacilli* and *Bifidobacteria* (Ziemer and Gibson, 1998). GalliPro® is a *Bacillus Subtilis* based DMF (4 x 10^9 CFU/g). A prebiotic is a non-viable food constituent that travels to the colon and causes selective fermentation (Ziemer and Gibson, 1998). The synbiotics are mixtures of probiotics and prebiotics (Gibson and Roberfroid, 1995).

TechnoMos® is an extract of *Saccharomyces cerevisiae* and is rich in mannanoligosaccharides (MOS) and Beta-1,3-Glucans.

There are limited reports on the effect of probiotics, prebiotics and synbiotics on chicks' liver enzymes under a bacterial challenge. This study was conducted to evaluate the impact of these feed additives on serum protein and liver enzymes in broilers under a subclinical *C. perfringens* challenge (CPC).

Table I.- Dietary composition of broiler chick starter and finisher diets.

Ingredient	Starter¥	Finisher¥
Yellow corn	62.45	69.90
Soybean meal	31.0	26.73
Palm oil	2.19	2.80
Di-calcium phosphate	2.50	2.0
Ground limestone	0.73	0.59
Choline chloride	0.05	0.04
DL-methionine	0.26	0.20
L- lysine	0.18	0.24
Salt	0.25	0.25
Threonine	0.07	0.25
V-M premix[1]	0.20	0.12
Total	100	100
Analysis		
ME (kcal/kg)	3000	3100
Crude protein (%)	21.5	21.0
Non phytate P (%)	0.50	0.40
Calcium (%)	1.0	0.9
Lysine (%)	1.20	1.1
Methionine (%)	0.55	0.47
Sulfur amino acids (%)	0.90	0.80
Threonine (%)	0.85	0.80

[1]Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

¥For starter period, the control diet was supplemented with 0.005% Neoxyval (treatment 3); 0.02% Gallipro (treatment 4); 0.075% TechnoMos (treatment 5) and 0.02% Gallipro + 0.06% TechnoMos (treatment 6). For finisher period, the diet was supplemented with 0.005% Neoxyval (treatment 3); 0.02% Gallipro (treatment 4); 0.05% TechnoMos (treatment 5) and 0.02% Gallipro + 0.05% TechnoMos (treatment 6).

MATERIALS AND METHODS

A total of 210, one-day-old Ross 308 broiler chicks were purchased from a local commercial hatchery. The chicks were allotted to 42 experimental cages (50 cm length, 60 cm width and 36 cm depth) in a four-deck cage system. Five chicks were placed in each cage and received the experimental diets in electrically heated battery brooders. The chicks were vaccinated against Marek's disease, Newcastle disease and Infectious Bronchitis. The experiment was conducted in the environmentally controlled battery room at the Animal Production Department, College of Food and Agriculture Sciences, King Saud University. The temperature during the first week was kept at 34 °C and then decreased to 24 °C up to the end of the experimental period. The experiment was conducted from day 1 to day-42. Feed and water were provided *ad libitum* and the chicks were maintained on a 24 h light schedule. Typical starter (d1-d14) and finisher (d15- d42) diets based on corn-soybean meal were formulated which met the recommendations in commercial practice in Saudi Arabia and were provided in mash form. The control starter and finisher diets contained 3000 and 3100 kcal/kg ME and 21.5% and 21.0% crude protein, respectively (Table I). Then the experimental materials were added over the top. Chicks received one of six dietary treatments for each growing period (starter and finisher) as follows: Control diet (positive control); Control diet + CPC (negative control); CPC + 0.05 g Neoxyval / kg; CPC + 0.2 g GalliPro / kg; CPC + 0.75 (starter) and 0.6 (finisher) g TechnoMos / kg; and CPC + 0.2 g GalliPro / kg + 0.6 g TechnoMos / kg for starter and 0.2 g GalliPro / kg + 0.5 g TechnoMos / kg for starter. On d 14, birds which had received T2, T3, T4, T5 and T6 were subjected to CPC by using an overdose of 10-fold dose of the anticoccidial vaccine, Paracox-8, followed by 1 ml of a cocktail containing *C. perfringens* inoculations (4 x 10^8 CFU) on days 15 and 16 according to the procedure described by Abudabos and Yehia (2013). Culture of *C. perfringens* was obtained commercially (MicroBiologics, Cloud, MN-U.S.A) for oral gavage of chicks. Random samples were obtained at the end of the trial.

At day 42, random blood samples were taken from 6 randomly chosen chicks for each treatment via brachial venipuncture into plain tubes for enzyme analysis. Serum was separated by centrifugation at 3000 g for 10 min and stored at -20°C until analysis. Serum gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using specific commercial kits (United Diagnostics). Protein content was determined using the modified method of

Markwell *et al*. (1978) with bovine serum albumin as a standard. The amount of protein was calculated from the standard curve.

Statistical analysis

Data were analyzed in accordance to SAS, 2010 (version 9.1. SAS Institute, Cary, NC), using a randomized complete block design with seven replicates. Significantly different means were compared using the PDIFF option and differences were considered significant using P <0.05. Means ± standard errors are presented.

RESULTS AND DISCUSSION

Serum level of liver enzymes is an important tool to determine the degree of liver damage. Disruption of cells causes the enzymes to leak into the blood where their levels help to determine the extent of cell injury. There were no significant changes in the serum total protein content between the groups studied. Cakir *et al*. (2008) have also reported no significant change in the serum protein levels of Japanese quails treated with prebiotics, probiotics and an antibiotic. The absence of any significant changes in serum protein level between the groups may indicate an absence of antibody or inflammatory response to CPC.

No significant differences in activities or specific activities of GGH and ALP were due to treatment (p>0.05) as shown in Tables II and III. However, birds which had received the control (T1) or the TechnoMos (T5) diets had numerically higher GGH specific enzyme activity (p = 0.056) compared to other treatments. The lack of differences in activity of both enzymes among experimental groups demonstrates no significant effects of various feed regimens on the bone and body development in broilers (Silva *et al*., 2007).

Lactate dehydrogenase (LDH) activity and specific activity of the enzyme were influenced by treatment (p <0.05, 0.001, respectively) as shown in Tables II and III. Serum from birds which had received TechnoMos (T5) had the highest LDH activity, but it was not significantly different from birds which had received GalliPro (T4), negative control (T2) or control (T1). On the other hand, serum from birds which had received Neoxyval (T3) or symbiotic (T6) had the lowest LDH activity (p<0.05). Significant increase in LDH in TechnoMos group (T5) when compared to Neoxyval group (T3) may be due to stimulation of liver metabolism by prebiotic to CP challenged birds. However a decrease in LDH in symbiotic group (T6) when compared to TechnoMos (T5) may indicate the inhibitory effect of symbiotic on liver metabolism which was stimulated by prebiotic.

AST activity and specific activity of the enzyme were influenced by treatment (p <0.001, 0.001, respectively). Serum from birds which had received Gallipro (T4), TechnoMos (T5) or symbiotic (T6) had the highest AST activity and specific activity. No significant correlation was observed between protein levels and AST activity.

The liver cell damage leads to increase serum activities of both enzymes AST and ALT but in general, ALT elevation is more specific for liver damage than AST (Mohamed and Wakwak, 2014).

Alanine aminotransferase activity was influenced by treatment (p<0.05). Serum from birds which had received TechnoMos (T5) or symbiotic (T6) had the highest alanine aminotransferase activity, but it was not significantly different from birds which had received GalliPro (T4). On the other hand, serum from birds which had received the control

Table II.- Effect of various diet regimes on serum enzymes activity in broiler chickens.

Experimental Groups	GGT	LDH	ALP	AST	ALT
	Units/L	Units/Lx10^3	Units/L	Units/L	Units/L
T1 (Control)	7.28	1.21^{ab}	912.42	129.34^{b}	5.76^{c}
T2 (Negative control)	5.16	1.37^{ab}	667.58	150.18^{b}	4.66^{c}
T3 (Neoxyval)	2.62	0.75^{b}	648.55	178.48^{b}	7.12^{bc}
T4 (GalliPro)	4.95	1.39^{ab}	744.34	337.63^{a}	8.82^{abc}
T5 (TechnoMos)	6.56	1.71^{a}	769.54	337.63^{a}	11.28^{a}
T6 (GalliPro+ TechnoMos)	5.3	0.810^{b}	639.47	311.52^{a}	13.26^{a}
SEM	1.44	0.23	112.1	20.74	1.64
Level of significance	NS	*	NS	***	*

Abbreviations used: GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. ^{abc}Means in the column with different superscripts differ significantly, *P <0.05; ***P <0.001; NS, not significant.

Table III.- Effect of various diet regimes on serum enzymes specific activity in broiler chickens.

Experimental Groups	GGT	LDH	ALP	AST	ALT
	Units/mg protein x10^{-4}	Units/mg protein x10^{-2}	Units/mg protein	Units/mg protein	Units/mg protein
T1 (Control)	4.27	7.06^{bc}	60.98	7.34^{b}	0.53
T2 (Negative control)	2.19	5.30^{cd}	76.89	7.99^{b}	0.51
T3 (Neoxyval)	1.59	5.08^{cd}	46.68	10.91^{b}	0.47
T4 (GalliPro)	2.89	8.13^{b}	71.12	19.75^{a}	0.49
T5 (TechnoMos)	4.51	10.88^{a}	49.27	21.70^{a}	0.76
T6 (GalliPro+ TechnoMos)	3.08	4.66^{d}	37.77	17.98^{a}	0.78
SEM	0.69	0.82	19.77	1.47	0.13
Level of significance	NS	***	NS	***	NS

For abbreviations and statistical details see Table II.

(T1) or negative control (T2) diets had the lowest ALT activity ($P < 0.05$) but it was not different from those who received Neoxyval (T3). The increase in the serum levels of AST and ALT in groups T4, T5 and T6 may be due to disruption of hepatic cell as a result of necrosis or a consequence of altered membrane permeability (Ozer *et al*., 2008).

CONCLUSION

Feeding CP infected chicks with probiotic, prebiotic and synbiotic feed significantly increased the levels of aspartate aminotransferase and alanine aminotransferase in the serum which may indicate a certain degree of liver damage.

ACKNOWLEDGEMENTS

This project was funded by Researchers Supporting Project number (RSP-2022R439), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest statement

We declare that we have no conflict of interest.

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