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Effect of Feed Supplementation with Probiotics and Antimicrobial Agents on Meat Quality of Broiler Chickens

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ABSTRACT

Background: This study was conducted to determine the effect of supplementing probiotics and antimicrobial agents on the meat quality of broiler chickens.

Methods: A complete randomized design was used and 90 male chickens were randomly assigned to five treatments which were replicated 3 times with each replicate having 6 chickens. The treatments had the same nutrients (20% CP and 12MJ/kg) but different supplementation levels of the probiotics and antimicrobial agents.

Result: Antimicrobial and effective microorganism supplementations did not have any effect ($p>0.05$) on feed intake, growth rate and live weights. A significantly lower ($p<0.05$) feed conversion ratio was observed with supplementation of the antimicrobial. Blood glucose levels were optimized at an effective microorganism supplementation level of 29.00 ml of EM/l of drinking water. Supplementation with 100 ml of EMs per litre of drinking water reduced significantly ($p<0.05$) the pH of ileum. Effective microorganism supplementation level of 85.00 ml per litre of drinking water optimized the crop pH value. Antimicrobial and effective microorganism supplementations did not have any influence ($p>0.05$) on live weight, carcass weight, breast weight, drumstick weight ad thigh weight. Similarly, antimicrobial and effective microorganism supplementations did not have influence on meat tenderness, juiciness and flavour. There were no antibiotic and effective microbe residues in the meat. It is, therefore, concluded that effective microorganism supplementation did not have much effect on production parameters, carcass characteristics and meat quality of Ross 308 broiler chickens.

Key words: Effective microorganisms, Meat quality, Performance, Poultry, Supplementation.

INTRODUCTION

The International Poultry Council, IPC, took a rare decision in 2017 to publish a united position statement on the responsible use of on-farm antibiotics against a background of mounting pressure on the industry. The position statement is as follows: the IPC will follow a science-based course to encourage industry to reduce the use of antimicrobials while also ensuring that, when antibiotics are used, it is in compliance with guidelines set by international organisations (IPC, 2019). IPC (2019) stated that the statement set out safeguards for the efficacy of antimicrobial use, while also recognising issues of antimicrobial resistance, animal welfare, food safety and consumer concerns. As a result, this led to application of new feed supplements and biotechnological products in science as well as practice in the poultry industry. Research has since highlighted the role of effective microorganisms (Ems) sometimes referred to as probiotics as sound alternatives to antibiotic growth promoters (Mountzouris *et al*., 2007). This, however, requires maintenance of appropriate nutritional and sensory properties in meat and meat products because different supplements can cause the deterioration of meat quality in terms of sensory properties which are important to the consumer (Melen *et al*., 2014).

MATERIALS AND METHODS

The experiment was conducted at the University of Limpopo, Animal Unit during the period April 2020 to October 2020. The experiment included 90 male Ross 308 broiler chicks supplied by a reputable hatchery in Limpopo province, South Africa. The chicks were vaccinated at the hatchery, against New Castle and Infectious bronchitis with Vitabron at day old. The chicks were divided into 5 groups (n=18) with each group further randomly divided into 3 groups of 6 chicks per pen. The chicks were fed grower mash that had been formulated by a local milling company. The chicks were allowed to feed and drink water *ad libitum*. Light was provided for 24 hours per day throughout the experiment. The mash was in three different forms, one containing antibiotics, oxytetracycline and a coccidiostat; the second one had neither antibiotics nor EMs and the third form had different

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concentration of EMs. The EMs were a mixture of lactic acid bacteria with 8.3×10^6 CFU/ml (T2) (*Lactobacillus planetarium* species), yeast with 1.8×10^5 CFU/ml (T3) (*Candida valida* species which is good in producing amino acids), actinomycetes with 3×10^3 CFU/ml (T4) (*Streptomyces albus* species) and fermenting fungi with 1.1×10^5 CFU/ml (T5) (*Aspergillus oryzae* species) while the basal diet with no inclusion of probiotics was labelled as T1. They were added daily to the drinking water in the chick fountains in concentrations indicated as shown in Table 1. Feed intakes, live weights, growth rates, feed conversion ratios and digestibility were measured weekly until day 42. For digestibility, faeces voided by the chickens were collected daily at the same time while avoiding any contamination from feathers, scales, debris and feeds. Apparent digestibility of the nutrients was calculated according to the procedures of McDonald *et al*. (2010). The chickens were subsequently slaughtered by decapitation and de-feathered by placing them in hot water. The carcasses were cut open at the abdominal site and de-gutted. The pH levels of the crop, gizzard, proventiculus, ileum and large intestines were measured. Particular attention was paid to the pH of the caecum and the large intestines before freezing the carcasses for 4 days at -40°C.

Meat samples were defrosted for 7 hours at room temperature prior to cooking. Only thighs and drumsticks were prepared and the skin was left on the meat samples. The samples were cooked following the method previously described by Pavelková *et al*. (2013). Briefly, chicken meat samples covered in aluminium foil to prevent water loss were prepared and cooked in a microwave at 180°C for 60 minutes. The samples were turned over every 10 minutes and subsequently cut into small pieces used for sensory evaluation by twenty-one randomly selected female students. Each panel member was given the chance to anonymously evaluate the different characteristics of the meat cuts from the 5 treatments. The method followed was that previously described by Pribela (2001). The following characteristics of the meat pieces: meat tenderness, juiciness and flavour were evaluated. Each member was requested to drink lemon juice after tasting the pieces of meat from each treatment before proceeding to the next treatment. This was done to wash out the taste of the previous treatment piece of meat. Acceptability of tenderness (fibres perceived during mastication), juiciness (water perceived during mastication) and flavour (smell and taste associated with cooked meat) were estimated using a modified hedonic scale ranging from 5 to 1 as shown in Table 2, where 1 was the worst and 5 the best of each characteristic. Sensory properties of fresh chicken breast meat were monitored over a 16 day period.

Data on feed intake, digestibility, growth rate, feed conversion ratio and meat quality were analysed using General Linear Model procedures of the statistical analysis system. Least Significant Difference test was applied for mean separation where there were significant differences ($p<0.05$) between treatment means. Regression analysis was used to determine the dose-related optimal responses

Table 1: Composition of basal feed mixtures.

	Treatments				
	T1	T2	T3	T4	T5
% Feed ingredient.					
Yellow maize	39.83	39.83	39.83	39.83	39.83
Soybean full fat	17.73	17.73	17.73	17.73	17.73
Wheat	15.00	15.00	15.00	15.00	15.00
Sunflower	12.39	12.39	12.39	12.39	12.39
Fishmeal	5.66	5.66	5.66	5.66	5.66
Vitamin+minerals premix	3.00	3.00	3.00	3.00	3.00
	2.50	2.50	2.50	2.50	2.50
Sunflower oil	1.50	1.50	1.50	1.50	1.50
Sodium bicarbonate	1.50	1.50	1.50	1.50	1.50
	0.30	0.30	0.30	0.30	0.30
Limestone	0.20	0.20	0.20	0.20	0.20
Sodium chloride	0.15	0.15	0.15	0.15	0.15
Monocalcium phosphate					
	0.15	0.15	0.15	0.15	0.15
DL methionine	0.10	0.10	0.10	0.10	0.10
	0	0	30	50	100
L threonine	0	0.01	0	0	0
Lysine	100	100	100	100	100
Effective microbes					
Terramycin					
Total					

for significantly different variables, feed intake, digestibility, feed conversion ratio, growth rate and meat quality to EMS supplementation levels.

RESULTS AND DISCUSSION

The results presented in table3 suggest that EM30 appeared to have negative effect on glucose levels which then rose to a comparative level with control chickens as EM concentrations were increased in the feed. The present study showed that EMs and antimicrobial supplementation had no effect ($p>0.05$) on feed intake, growth rate, live weight and food intake of male Ross 308 broiler chickens aged 22 to 42 days. Chickens supplemented with lower EMs appeared to be hypoglycaemic, however, there were no significant differences in FCR among the EM supplemented groups. Usually, hypoglycaemia prevention is associated with efficient FCR and good nutrient absorption to maintain stead levels of glucose. Hypoglycaemia is prevented during fasting by the formation of glucose synthesis via gluconeogenesis. Lactate is produced from glucose by the intestines and it has been reported that up to 37% of glucose taken up from chicken intestinal lumen may be converted to lactate before transferred to circulation. Lactase dehydrogenase is responsible for converting pyruvate to lactate. Therefore, the low levels of glucose in the chickens supplemented with EMs might have been caused by low blood levels of lactase dehydrogenase. *Lactobacilli*, in particular, in the feed were also responsible for the production of lactate through the fermentation of glucose in the intestines. All this created a healthy environment in the chickens which, however, made no differences in live, carcass, breast, drumstick and thigh weights ($p<0.05$) as shown in Table 4.

The gut is the main part of the body responsible for digestion and absorption of feed. Therefore, gut conditions have been subject of many researches. The digestive system

Table 2: Sensory evaluation scores used.

Score	Sensory attribute		
	Tenderness	Juiciness	Flavour
1.	Too tough	Too dry	Very bad flavour
2.	Tough	Dry	Poor flavour
3.	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good
4.	Tender	Juicy	Good flavour
5.	Too tender	Very juicy	Very good flavour

Table 3: Effect on feed intake (g of dry matter/bird/day), growth rate (g/bird/day), feed conversion ratio (FCR) (g, dry matter feed/g live weight gain) live weight (g/at day 42) and blood glucose level (mmol/l).

Variable	Treatments					SEM
	Grower mash (T1)	Mash with antibiotic (T2)	Mash with EM_{30} (T3)	Mash with EM_{50} (T4)	Mash with EM_{100} (T5)	
Feed intake	111	130	114	120	116	15.64
Growth rate	61.9	58.9	58.1	58.1	55.4	6.97
FCR	1.8^{b}	2.2^{ab}	2.0^{ab}	2.1^{ab}	2.1^{ab}	0.18
Live weight	1703	1681	1685	1677	1626	161.48
Glucose	11.4^{ab}	12.3^{a}	8.9^{b}	10.8^{ab}	12.0^{a}	1.45

$^{a, b, c}$: Means in the same row not sharing a common superscript are significantly different ($p<0.05$).

SEM: Standard error of the mean.

Table 4: Effect of supplementing diets with antimicrobials and effective microorganisms on live and carcass weights (g) of male Ross 308 broiler chickens.

Variable	Treatments					SEM
	Grower mash (T1)	Mash with antibiotic (T2)	Mash with EM_{30} (T3)	Mash with EM_{50} (T4)	Mash with EM_{100} (T5)	
Live weight	1621.9	1630.2	1462.9	1518.1	1401.3	244.25
Carcass weight	1055.8	1092.9	966.1	993.0	965.6	181.88
Breast weight	312.6	324.4	276.6	277.2	290.1	56.77
Drumstick weight	149.3	151.5	142.1	149.3	131.0	24.84
Thigh weight	174.8	166.5	145.4	157.3	157.3	31.28

SEM: Standard error of the mean.

of the avian species like other animals has a dynamic property which regulates itself depending on the physiological requirements and present circumstance. This dynamic situation of the gastrointestinal tract (GIT) is dependent on many factors including pH as well (Rhamani *et al*., 2005). Gut pH is dependent on the health of the chicken, kind of nutrients and more importantly microflora content of the GIT. The pH levels in the specific areas of the GIT are the main factors which establish a specific microbiota and also affect the digestibility and absorption of nutrients. It was previously shown that decreasing the pH in Ross 308 chicken guts significantly affected their performance by acting on microbial populations of their GITs (Rhamani *et al*., 2005). From the results shown in Table 5, it can be seen that 50 ml of EMs supplied in drinking water increased the pH in the crop while for the ileum, higher pH values were recorded for the chickens under the control diet. These pH differences did not, however, have any significant effect on the weights shown in Table 4. The pH levels in the broiler digestive systems were generally acidic and ideal for lactic acid bacteria such as *Lactobacillius sp*.

As shown in Table 5, a negative relationship was observed between effective microorganism supplementation levels and pH levels of the ileum. The results of the sensory evaluation (Table 6) indicate that supplementing the diet with antimicrobials and the probiotics did not affect ($p>0.05$) the tenderness, juiciness and flavour of the male Ross 308 broiler chicken thighs and drumsticks. Though, flavour appeared to have been more acceptable in EM supplemented chickens compared to the antibiotic supplemented and control groups. This agreed with what Brzóska *et al*. (2010) found with similar a breed of broilers. There were no significant differences in the meat quality of the treatment regimens after storage of the carcasses. This could mean that there were no significant differences in the post-mortem aging which normally leads to biochemical and physical changes in the chicken muscles. These changes are as a result of endogenous proteolytic systems in the muscles which in turn improve the quality of meat attributes such a tenderness, juiciness and flavour (Huff-Lonergan and Lonergan, 2005). The results of the present study agree with those reported by Pelicia *et al*. (2004) and Zhang *et al*. (2013). They noted that there was no synergistic effect of prebiotics, oligosaccharides and probiotics on chicken meat quality.

With regards to meat tenderness, it has been reported that, generally, after slaughter, chicken meat takes less time compared to beef, lamb and pork due to rapid development of rigor mortis. The major factors affecting meat tenderness are the maturity of the connective tissues and contractile state of the myofibrillar proteins. The maturity of the connective tissue is a function of chemical cross bonding of collagen in the muscles which increases with age (Mir *et al*., 2017). Probiotics for growth promotion have, however, been postulated to have adverse impact on tenderness development during post-mortem storage (Kim *et al*., 2016) which appear not to have been the case in the present study. The study did not separate the meat pieces from the breasts and the thighs for the tenderness evaluation test in order to have a better fidelity in simulation of what actually happens when chicken meat is ordinarily eaten. However, higher scores for tenderness have been obtained with thigh meat

Table 5: Effect of supplementing diets with antimicrobials and effective microorganisms on pH levels of the different sections of the gut after optimization of effective microorganisms in drinking water of 85.0 ml per litre.

Variable	Treatments					SEM
	Grower mash (T1)	Mash with antibiotic (T2)	Mash with EM_{30} (T3)	Mash with EM_{50} (T4)	Mash with EM_{100} (T5)	
Crop	5.45^{ab}	5.18^{b}	5.46^{ab}	6.01^{a}	5.26^{ab}	0.41
Gizzard	3.95	3.42	3.31	3.22	2.70	0.98
Proventriculus	3.90	4.25	4.30	4.14	3.69	0.46
Ileum	6.22^{a}	5.92^{ab}	5.85^{bc}	5.96^{ab}	5.59^{c}	0.46
L. intestines	5.89	5.66	5.62	5.71	5.47	0.40

$^{a, b, c}$: Means in the same row not sharing a common superscript are significantly different ($p<0.05$).

SEM: Standard error of the mean.

Table 6: Effect of supplementing diets with antimicrobials and effective microorganisms on tenderness, juiciness and flavour of male Ross 308 broiler chickens aged 42 days.

Variable	Treatments					SEM
	Grower mash (T1)	Mash with antibiotic (T2)	Mash with EM_{30} (T3)	Mash with EM_{50} (T4)	Mash with EM_{100} (T5)	
Tenderness	3.0	3.7	3.3	3.7	3.7	0,5
Juiciness	3.3	3.7	3.0	3.0	3.3	20.4
Flavour	2.3	3.0	3.7	3.7	3.7	80.75

SEM: Standard error of the mean.

than breast meat because thigh muscles contain more internal fat and blood capillaries (Melen *et al*., 2014). Earlier observation by Sonayia *et al*. (1990) had been that there was no age related differences in the tenderness of breast and thigh meat (5, 8 weeks of age) of broiler with more juiciness in the breast meat of older birds. Juiciness is said to be an important factor in the eating quality of meat. To get a tasty piece of meat requires some meat juice. The main factor determining the juiciness of meat is the end temperature. In the present study it was the same for all the pieces evaluated which appeared to be equally juicy implying that EMs nor terramycin in the feed had no effect on the intramuscular fat production. Intramuscular fat is said to dilute the connective tissue of the elements in the muscle in which it is deposited thereby enhancing tenderness and juiciness (Mir *et al*., 2017). It is reasonable to assume therefore that addition of EMs or terramycin did not have any effect on the chicken gut microbiota communication with the different organs and tissues.

The results obtained appear to suggest that the heating process had no effect on meat quality for all the treatment groups. This agrees with results obtained by Al-Khalaifa *et al*. (2019) from their organoleptic study with Cobb 500 broilers which were supplemented with *Lactobacillus* and *Bacillus coagulans*. Heating is regarded as the most destructive process in terms of meat quality as it results in changes such as flavour and taste enhancement (Mir *et al*., 2017). Flavour is a quality attribute that consumers use to determine the acceptability of poultry meat. Flavour development occurs during cooking due to sugar and amino acid interactions, lipid and thermal oxidation and thiamine degradation. Certain lipids and fats are unique in poultry and combine with odour to account for the characteristic poultry flavour (Northcutt, 2009) which did not appear to be affected by the presence of EMs or antibiotics in the feed. It is important, however, to note that the aroma of chicken meat is a result of a special assortment with specific relative quantities of a mixture of different metabolites (esters, aldehydes, alcohols, ketones and acids). Different probiotics can therefore affect the composition of flavour. In a study by Wang *et al*. (2017) they found that feed supplemented with *Pediococcus pentoseceus* resulted in chickens having more flavour characteristic compounds. *Pediococcus* is a genus of gram-positive lactic acid bacteria, *Lactobacillaceae* family. One would have expected to get similar results with *Lactobacillus planetarium* used in the present study. It is reasonable to conclude that there really was no need to use an antibiotic with the genetic strain of chicken and the combination of the EMs used in the study.

Hereditary estimates of various parameters such as meat quality traits among others suggest that genetic selection is the best tool for improvement of broiler meat. The results from the present study should therefore be interpreted with caution because genetics of the chicken and the microorganisms used play an important role in meat quality (Mir *et al*., 2017). Microorganisms currently being used in probiotic preparations are many and varied (Popova, 2017). It has been reported to be difficult to directly assess different studies using probiotics because the efficacy of probiotic preparations depends on many factors, the type of strains, administration level and concentration of probiotics, application method and basal diets provided (Wang *et al*., 2017).

CONCLUSION

Supplementing broiler poultry feed with the various inclusion levels of probiotics and antimicrobial agents used in the study did not have any adverse effects on meat quality and growth performance. It was concluded that probiotics and other antimicrobial agents could be safely used as replacements for conventional antibiotics for growth promotion in Ross 308 broiler chickens.

Conflict of interest: None.

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