Full Length

EFFECT OF NATURAL SYNBIOTIC CONSORTIUM ON BROILER MEAT QUALITY

K. Divya Manjari[1], M. Parthiban[2]*, T.M.A. Senthilkumar[3], R. Karunakaran[4] and V. Appa Rao[5]

Department of Animal Biotechnology,
Facultry of Basic Sciences, Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University,
Chennai - 600 007

ABSTRACT

Synbiotics are synergistic combinations of prebiotics and probiotics. This study was aimed to design synbiotic in vitro and validate them in broiler chickens upon in vivo delivery. Based on the in vitro assays and extracellular assays for estimation of the antioxidant potential, a scoring system was evaluated to screen the probiotic isolated with maximum antioxidant potential. A consortium of probiotic bacteria was formulated based on the scoring system that comprised E. hirae-2, E. faecium-2, E. fecalis-1, E. durans, and P. acidilactici. The probiotic inoculum was scaled up in a fifty-liter pilot scale fermenter supplemented with MRS broth under optimum growth conditions. The product was then spray-dried in a low-temperature spray drier. The lyophilized product was stored at room temperature for further use. Two hundred and forty day sold broiler chicks (Vencobb) were randomly allotted to one of five treatments (prebiotic alone, probiotic alone, symbiotic, and commercial probiotic and control treatments) on the basis of body weight in a randomized complete block design. Dietary supplementation of probiotics significantly enhanced growth performance by improving body weight gain, performance index, and protein efficiency ratio. Growth performance and nutrient retention of the Lactiflora-supplemented group was significantly better than that of the control and Provisacc-supplemented group but comparable to the combination group. It was concluded that combined supplementation of Lactobacillus acidophilus and Saccharomyces cerevisiae supplementation at the rate of 0.05% each is beneficial in improving body weight gain, growth performance, nutrient utilization, and immune response of broiler chicken.

Key words: Broiler, synbiotic consortium, meat quality assessment, probiotics, antioxidant

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[1]Ph.D Scholar
[2]Professor and Head, *corresponding author Email : drparthiban66@gmail.com
[3]Professor and Head, Zoonoses Research Laboratory, Centre for Animal Health Studies, TANUVAS
[4]Professor, Department of Animal Nutrition
[5]Director of Extension Education, TANUVAS

INTRODUCTION

The term 'probiotics' is derived from the Greek word "probios" meaning "for life." It is a beneficial microorganism or combination of such microorganisms, which would quickly establish in the gut to suppress colonization and growth of harmful bacteria. This improves the health and well-being of animals, birds, or human beings. Poultry is the cheapest source of animal protein, contributing significantly to supply the growing demand for animal food products around the world (Farrell, 2013). The consumption and trade in poultry products are increasing rapidly as the human population increases, making it the second largest source of meat after pork (Sharma Bajagai *et al*., 2016).

Probiotics, as a feed additive, have been acclaimed to be a safe growth promoter in animals (O'dea *et al*., 2006; Bansal *et al.*, 2016). Probiotics were reported to improve body weight gain and feed efficiency and reduce mortality in broiler chickens (Panda *et al*., 2008). Synbiotics are synergistic combinations of prebiotics and probiotics. Beneficial bacteria can deliver synbiotics i*n-vivo* to chickens to expedite gut colonization. Therefore an experiment was aimed to design synbiotics *in-vitro* and validate them in broiler chickens upon *in-vivo* delivery.

MATERIALS AND METHODS

Formulation of consortium

A scoring system was evolved to normalize the expression levels of efficiency of every probiotic isolate based on their antioxidant potential. A consortium was formed based on the scores obtained from the scoring system (Manjari *et al*., 2019).

Bulk production of probiotic consortium

The individual probiotic isolates (n=6) were produced in bulk in a pilot-scale fermenter (5 L) until the CFU reached 1012 CFU/ml of the multispecies probiotic culture. They were then spray dried at 40°C, using a low-temperature spray drying method. The spray-dried product was infused with milk powder until the CFU reached 1012 CFU/ gram dry weight.

Storage viability studies

The spray-dried product was checked for viability at monthly intervals for 4 months. It was dissolved in sterile PBS and serially diluted. Then, it was spread on MRS agar and then incubated at 37°C overnight in triplicates. The CFU were counted on the next day to assess the viability of the product.

Experimental design for broilers

Day-old birds were procured from hatcheries, maintained in ventilated cages with ad libitum water, and vaccinated against NDV. The chicks were divided into 5 different treatment groups as per table 1. The poultry feed was rationed according to broiler feed guidelines.

Growth performance

Feed intake and body weight were measured on days 7, 14, 21, 28, and 35 after 4 hours of fasting to calculate body weight

gain (BWG) for each period and the overall experimental period.

Assessment of shelf life

Tyrosine value (TV) was determined by the modified method of Strange *et al*. (1977). With reference to the standard graph, the tyrosine value was calculated as mg of tyrosine per 100 g of sample. The standard graph was prepared with the known concentration of tyrosine. The shelf life of meat was determined by assessing the tyrosine value of meat before and after storage for 10 days.

Water holding capacity and pH of broiler meat

The breast and thigh meat samples were subjected to a pH test on the day of slaughter, from 6 birds randomly selected from each treatment. The pH of the meat sample was measured using a digital pH meter (Digisun Electronic System, Model: 2001), following the procedure of Troutt *et al*. (1992). The pH was recorded by immersing the meat pH meter in the homogenized meat sample.

The water-holding capacity of the meat was calculated on the day of slaughter following the protocol outlined by Grau and Hamm (1957). The meat samples were collected from 6 birds, with two birds per replicate selected randomly from each treatment. About 300 mg of the given meat sample was placed on a Whatman No. 41 filter paper, which in turn was placed between two glass slides. On the top of the upper glass slide, a 100 g weight was placed for 3 minutes so as to exert a downward force. This arrangement was kept on a hard top plate. The released water from the meat sample was absorbed in the filter paper, which left an impression. With a sharp pencil, the boundary of the impression was carefully demarcated. The area was measured by using a compensation polar planimeter (Model KP – 90 N, PLACOM). From the impression, the area of the outer circle indicating the water expressed, and the area of the inner circle indicating the area of meat before pressing was measured.

Water Holding Capacity (%) = (Area of inner circle /Area of outer circle) x 100

Shear force value and colour of broiler meat

The shear force value of the meat sample was recorded by using Warner Bratzler Shear Press as per Bratzler (1954). At the time of slaughter, 1 cm thickness of breast muscle was collected from 48 broiler carcasses with,two birds selected randomly from each replicate, contributing six birds per treatment. The breast muscle samples were stored at –20 °C till the measurement. On the day of measurement, the sample was placed on the blade of the Warner – Bratzler press in such a way that the fiber was perpendicular to the blade. The meat sample was cut slowly by the machine blade, and the needle was deflected due to the force of shearing the sample. The shear force value was measured in kg/cm^2. The colour of meat was determined by Munsell's scale of colouration.

Proximate composition of broiler meat

The proximate composition, such as moisture, protein, fat, and ash of broiler meat, was analysed by following the standard procedure of AOAC (2000). Fat estimation was done in SOCS plus (Model SCS 4, Pelican Equipment Pvt. Ltd., Chennai) and protein estimation in KEL plus (Model Classic DX, Pelican Equipment Pvt. Ltd., Chennai).

RESULTS AND DISCUSSION

Formulation of consortium

Based on the in vitro assays and extracellular assays for estimation of the antioxidant potential, a scoring system was evaluated to screen the probiotic isolated with maximum antioxidant potential. A consortium of probiotic bacteria was formulated based on the scoring system that comprised *E.hirae-2, E.faecium-2, E.fecalis-1, E.durans*, and *P.acidilactici.*

Bulk production of probiotic consortium

The probiotic inoculum was scaled up in a fifty-liter pilot-scale fermenter supplemented with MRS broth under optimum growth conditions. The product was then spray-dried in a low-temperature spray drier. The lyophilized product was stored at room temperature for further use.

Storage viability studies

The freeze-dried probiotic samples were revived in sterile PBS, and the viability studies were done by serial dilution. The plate counts revealed that the viable count limit, as suggested by Sharma Bajagai *et al*. (2016), is 108, and it was maintained for 4 months. The probiotic consortium was found to be effectively viable at room temperature, which is an added advantage of low-temperature spray drying (Fig 1).

Growth performance

There was a significant gain of 200 ($P \leq 0.05$) grams in the symbiotic group compared to other groups in the fourth week of 45-day trial. The significance of synbioticsis shown in the weight gain that is depicted in Figure 2.

Conventional methods

Water holding capacity and pH of broiler meat

The pH of the meat was measured immediately post-slaughter since the degradation causes variations in the pH as time increases. The control group showed lower ultimate pH values (5.96 vs. 6.04; $P \leq 0.001$). However, there was no significant difference in the pH of the control and treated groups. Breast meat from the control group also exhibited significantly lower ability to retain liquid during refrigerated storage (drip loss, 2.96 vs. 2.02%; $P \leq 0.001$) (Table 2).

Shear force value and colour of broiler meat

The shear force value represents the tenderness of the meat. The tenderer the meat, the lesser the shear force value. The values of meat tendernessin correspondence with the shear force value of 1.85 for the

control meat sample and 1.74 for the treated meat sample ($p \leq 0.05$)(Table 2).

Proximate composition of broiler meat

The proximate composition of the breast meat was determined following the procedures of AOAC (2000) in triplicate samples of each treatment group.Moisture was determined by drying 1 g of meat in an oven at 100–105°C until a constant weight was obtained, which was found to be 73.07 % and 72.68 %in the control and symbiotic-treated group, respectively ($P \leq 0.05$). Crude protein was determinedby the Kjeldahl method and reported as 22.55% and 22.9 % in control and treated groups, respectively. The crude protein was obtained as6.25 × N%. The fat content of the meat was determined by the Soxhlet extraction method using petroleum ether and was found to be 1.54 % and 1.68 % in the control and treated groups, respectively. The ash content of the meat was determined by igniting the samplein a muffle furnace at 550°C for 3 hours and the ash percentage was found to be 1.35 and 1.22 in control and treated groups, respectively.

Tyrosine value of meat

The tyrosine value of meat immediately after slaughter and upon storage for 10 days at 4˚C was estimated and found to be 1.2 and 1.16 in fresh control and treated meat samples and 1.29 and 1.26 in 10 days stored meat. Twenty gram of product was blended with 50ml of pre-cooled 20% TCA (Trichloro-acetic acid) solution for 2 min. The blended contents were transferred to a beaker after rinsing with 50ml of cold water and mixed. The mixture was filtered through Whatman filter paper No. 42. The filtrate was named TCA extract, and 2.5ml of TCA extract was diluted with an equal amount of distilled water. 10ml of 0.5 N freshly prepared sodium hydroxide and 3ml of diluted phenol Folin-Ciocalteu's reagent (1:2 with distilled water) were added. After 30 min, OD was measured at 730 nm in a spectrophotometer. Tyrosine value was calculated by referring to the standard curve prepared following the procedure of Pearson (1968) and expressed as mg of tyrosine per gm of the test sample. (Table 2).

Probiotic consortium was formulated based on a ranking system assigned to all the probiotic isolates, as per Manjari *et al*. (2019). The isolates with maximum scores were selected based on the scoring in between the species and were produced in bulk for in vivo trials. According to the scores obtained by the in vitro assays and the antioxidant assays, a consortium was formulated with the best of the isolates viz., *E.hirae-1, E.faecium-1, E.fecalis-3, P.acidilactici*.

Sreeja and Prajapati (2013) reported that probiotics, particularly when included in dietary supplements, are commonly transported and stored at ambient temperatures and humidity. This may lead to loss of viability as compared to refrigerated/ frozen storage and handling. In order to provide the target dose until the end of shelf life and to compensate for potential losses during storage and handling, an overage is commonly included in the product. In this study, the storage viability studies revealed

that upon storage at room temperature, the probiotic consortium maintained a cell count of 108 CFU/gram of product for 4 months. These results clearly indicate that room temperature storage is ideal for storage of the spray-dried product.

A study was conducted by Kumar *et al*. (2013) to evaluate the effects of different probiotics, i.e.,Lactiflora (*Lactobacillus acidophilus*), Provisacc (*Saccharomyces cerevisiae*), on growth performance, nutrient retention, and immune responses of broiler chickens. Two hundred forty-day-old broiler chicks (Vencobb) were randomly allotted to one of four treatments on the basis of body weight in a randomized complete block design. Dietary supplementation of probiotics significantly ($P<0.05$) enhanced growth performance by improving body weight gain, performance index, and protein efficiency ratio. Growth performance and nutrient retention of the lactiflora-supplemented group were significantly ($P<0.05$) better than that of the control and Provisacc-supplemented group but comparable to the combination group. It was concluded that combined supplementation of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* supplementation at the rate of 0.05% each is beneficial in improving growth performance, nutrient utilization, and immune response of broiler chicken. Similarly, in this study, the growth performance was found to be alleviated by 200 grams ($p\leq0.05$) in the total body weight in the symbiotic-treated groups. This may be pertaining to the better feed conversion and performance that the synbiotic consortium has enhanced in the supplemented feed.

The pH of muscle/meat is a measurement of the acidity. In chickens, normal ultimate pH (pH) values are around 5.8 (Duclos *et al*., 2007). Thus, it can be concluded that the pH values observed in this study are within the pH range accepted for commercial poultry meats. In the case of probiotics, in the study described by Zheng *et al*. (2016), the influence of E. faecium on meat pH values (at 45 min and 24 hours post-mortem) was observed; both values were higher in treated groups ($P <0.05$) compared to control.

Colour or visual appearance is certainly one of the most important sensory attributes that influence consumer's acceptance of meat and meat products (Adeyemi and Sazili. 2014). The lightness of breast muscle could be used in the technological evaluation of meat with the standardized threshold value L* (lightness) to detect pre-slaughter or processing effects, with good reliability with different genetic strains (Saláková *et al.*, 2009). Higher values indicate lighter color, indicating that fillets have low pH (Garcia *et al*., 2010). The optimal lightness range of chicken and turkey fillets is around 49-50 (Barbut, 1997). Thus, it can be assumed that the recorded L* values measured post-mortem are within the acceptable range for commercial meats. In this study, the lightness values were found to be 53.87 ($p\leq 0.05$) in the control group and 54.02 ($p\leq 0.05$) in the treated group.

According to Sañudo (1998), meat quality is influenced by the alterations that occur in the pH during the rigor mortis. Meat

color alterations, which occur in swine, such as PSE (pale, soft, and exsudative) and DFD meat (dark, firm, and dry), are rare in birds. Considering these reference values, probiotics did not significantly affect meat tenderness in the present study.

Contrary to earlier reports on the increase in protein and ash contents in probiotic-fed birds (SN, 1992; Khaksefidi and Rahimi, 2005), the present results suggest that synbiotic feed supplemented feed mix did notsignificantly influence the protein, moisture, and ash content of breast meat in broiler chickens. The implication of this result is that mineral retention and protein efficiency ratio were unaffected by mix treatments. The disposition of the present findings supports the report of (Joy and Samuel, 1997) that carcass protein was not influenced by supplementation of *Lactobacillus sporogenes* in broiler diets.

Tyrosine values of fresh meat at 0^{th} day were recorded to be 1.2 ± 0.03(control group) and 1.16 ± 0.02(treated group), respectively (P≤ 0.05). Tyrosine values of stored meat 10^{th} day post storage in 4ºC were recorded to be 1.29 ± 0.11 (control group) and 1.26 ± 0.21 (treated group). Changes in tyrosine value were time-dependent and reported a significant (P <0.05) increase during refrigeration storage for all treatments, which was attributed to hydrolytic changes in meat by inherent tissue enzymes and bacterial proteolysis (Strange *et al*., 1977). This was coincidental with the observed values of this study.

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Table 1. Experimental design

Treatment	Feed	No of chicks
TG1	Prebiotic supplemented feed (chicory-2%)	30
TG2	Probiotic supplemented feed(1011 CFU per kg feed)	30
TG3	Synbiotic-supplemented feed (prebiotic-chicory-2%+ multispecies probiotic -1011 CFU per kg feed)	30
TG4	Commercial probiotic supplemented feed(as per manufacturers instruction-ProBee)	30
TG5	Control group feed without any prebiotic or probiotic	30

Table 2. Conventional parameters for meat quality analysis

Parameter		Control group	Synbiotic-treated group	P value
pH		5.96 ±0.45	6.04 ±0.22	≤ 0.001
Colour	Lightness (L)	53.87 ±2.5	54.02 ±3.2	≤ 0.05
	Redness (a)	0.03 ± 0.01	0.045 ±0.01	
	Yellowness(b)	15.36 ± 1.25	16.36 ± 1.65	
Drip loss (%)		2.96 ± 0.28	2.02 ± 0.22	≤ 0.001
Shear force (kg/g)		1.85 ± 0.02	1.74 ± 0.35	≤ 0.05
Chemical composition	Moisture (%)	73.07 ± 3.11	72.68 ± 3.46	≤ 0.05
	Protein (%)	22.55 ± 0.98	23.94 ± 0.75	≤ 0.001
	Lipid(%)	1.94 ± 0.22	1.68 ± 0.16	≤ 0.001
	Ash(%)	1.35 ± 0.02	1.22 ± 0.32	≤ 0.001
Tyrosine value	Fresh meat	1.2 ± 0.03	1.16 ± 0.02	≤ 0.05
	Frozen meat (10 days)	1.29 ± 0.11	1.26 ± 0.21	≤ 0.05

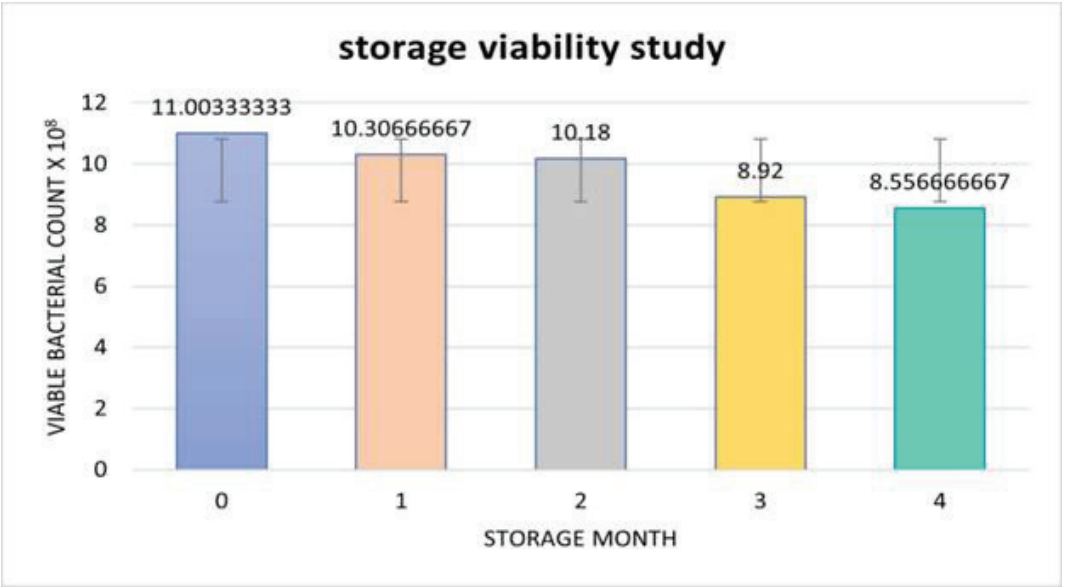

Fig 1. The storage viability of the lyophilized product estimated by microbial method

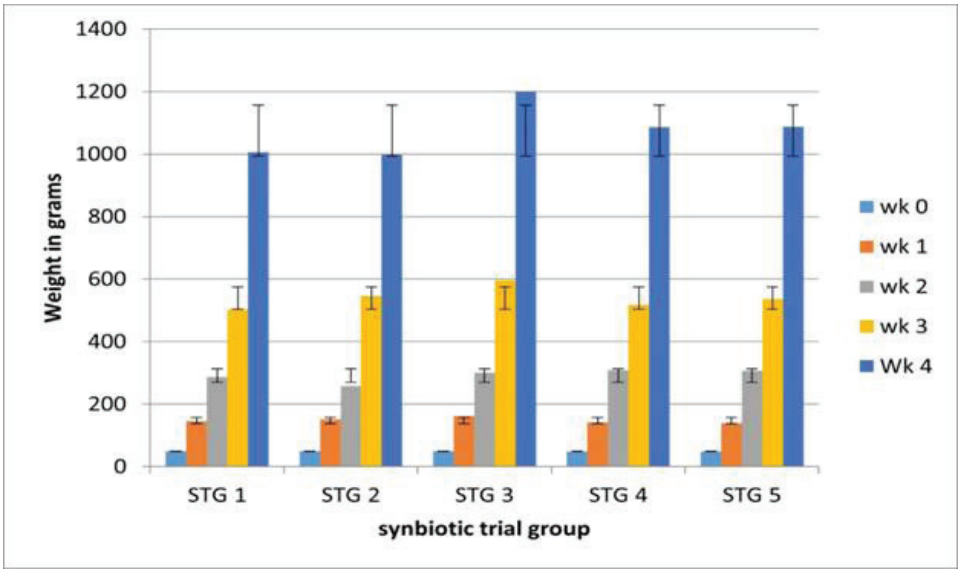

Fig 2. The weight gain chart of the experimental chicken.

REFERENCES

Adeyemi, K.D. and Sazili, A.Q., (2014). Efficacy of carcass electrical stimulation in meat quality enhancement: a review. *Asian-Australasian journal of animal sciences,* **27**(3):447.

AOAC. (2000). Official methods of analysis of AOAC International (17th ed.) Gaithersburg (MD): AOAC.

Bansal, S., Mangal, M., Sharma, S.K. and Gupta, R.K., (2016). Non-dairy based probiotics: a healthy treat for intestine. *Critical reviews in food science and nutrition*, **56**(11):1856-1867.

Barbut, S., (1997). Problem of pale soft exudative meat in broiler chickens. *British Poultry Science*, **38**(4):355-358.

Bratzler, L.J., (1954). Using the Warner-Bratzler shear. In Proc. Recip. *Meat Conf,* **7:** 154-160.

Duclos, M.J., Berri, C. and Le Bihan-Duval, E., (2007). Muscle growth and meat quality. *Journal of Applied Poultry Research*, **16**(1):107-112.

Farrell, D. (2013). The role of poultry in human nutrition. Poultry Development Review. Rome: *Food and Agriculture Organization* :2-9.

Garcia, R.G., De Freitas, L.W., Schwingel, A.W., Farias, R.M., Caldara, F.R., Gabriel, A.M.A., Graciano, J.D., Komiyama, C.M. and Almeida Paz, I.C.L., (2010). Incidence and physical properties of PSE chicken meat in a commercial processing plant. *Brazilian Journal of Poultry Science*, 12:233-237.

Grau, R. and Hamm, R. (1957). About the water-binding capacity of the mammalian muscle. II. Commun. Z. Lebensm. Unters. Brisk, 105:446.

Joy, A.D. and Samuel, J.J. (1997). Effect of probiotic supplementation on the performance of broilers, **28**: 10-14.

Khaksefidi, A. and Rahimi, S., (2005). Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. *Asian-Australasian Journal of Animal Sciences*, **18**(8):1153-1156.

Kumar, L., Singh, P.K. and Kumar, M., (2013). Effect of dietary supplementation of combination of probiotics on the growth performance and immune response of broiler chickens. *Animal Nutrition and Feed Technology*, **13**(1):15-25.

Manjari, K.D., Divya, G., Saravanan, T.S. and Parthiban, M., (2019). In vitro selection of potential immunomodulatory multispecies lactic acid bacteria in broilers. *Indian Journal of Animal Research*, **53**(5):655-660.

O'dea, E.E., Fasenko, G.M., Allison, G.E., Korver, D.R., Tannock, G.W. and Guan, L.L., (2006). Investigating the effects of commercial probiotics on broiler chick quality and production efficiency. *Poultry science*, **85**(10):1855-1863.

Panda, A.K., Rama Rao, S.S., Raju, M.V. and Sharma, S.S., (2008). Effect of probiotic (*Lactobacillus sporogenes*) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. *Journal of the Science of Food and Agriculture*, **88**(1):43-47.

Pearson, D., (1968). Application of chemical methods for the assessment of beef quality. II. Methods related to protein breakdown. *Journal of the Science of Food and Agriculture*, **19**(7):366-369.

Saláková, A., Straková, E., Válková, V., Buchtová, H. and Steinhauserová, I., (2009). Quality indicators of chicken broiler raw and cooked meat depending on their sex. *Acta Veterinaria Brno*, **78**(3):497-504.

Sañudo, C., Nute, G.R., Campo, M.M., Maria, G., Baker, A., Sierra, I., Enser, M.E. and Wood, J.D., (1998). Assessment of commercial lamb meat quality by British and Spanish taste panels. *Meat Science*, **48**(1-2): 91-100.

Sharma Bajagai, Y., Klieve, A., Dart, P. and Bryden, W., (2016). Probiotics in animal nutrition: Production, impact and regulation.

SN, N., (1992). Effect of direct-fed microbials on nutrient retention production parameters of laying pullets. *Poultry Science*, **71**(1):111.

Sreeja, V. and Prajapati, J.B., (2013). Probiotic formulations: application and status as pharmaceutical A review. *Probiotics and antimicrobial proteins*, **5**:81-91.

Strange, E.D., Benedict, R.C., Smith, J.L. and Swift, C.E., (1977). Evaluation of rapid tests for monitoring alterations in meat quality during storage. *Journal of Food Protection*, **40**(12):843-847.

Troutt, E.S., Hunt, M.C., Johnson, D.E., Claus, J.R., Kastner, C.L. and Kropf, D.H., (1992). Characteristics of low-fat ground beef containing texture-modifying ingredients. *Journal of Food science*, **57**(1):19-24.

Zheng, A., Luo, J., Meng, K., Li, J., Bryden, W.L., Chang, W., Zhang, S., Wang, L.X.N., Liu, G. and Yao, B., (2016). Probiotic (Enterococcus faecium) induced responses of the hepatic proteome improves metabolic efficiency of broiler chickens (Gallus gallus). *BMC genomics*, **17**:1-12.