Efficacy of a Feed Probiotic Bacteria (*Enterococcus faecium* NCIMB 10415), Spore (*Bacillus subtilis ATCC PTA-6737*) and Yeast (*Saccharomyces cerevisiae*) in Japanese quails

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Abstract

Efficacy of a bacteria (*Enterococcus faecium* NCIMB 10415), spore (*Bacillus subtilis ATCC PTA-6737*) and yeast (*Saccharomyces cerevisiae*) probiotic was tested in quails from day 2 to day 35. A control diet (CON) was supplemented at the recommended dosages to obtain three diets containing one bacterium (D-bacteria), one spore (D-spore) and one yeast (D-yeast). The results indicated that the birds fed on D-yeast significantly (P<0.05) consumed more feed at the age of 16, 30 and 37 days than the birds fed on CON, D-bacteria and D-spore, whose food intake (FI) were almost similar throughout the fattening period. Body weights (BW) and weight gains (WG) of birds fed on probiotic diets were higher than the birds fed on CON diet, especially BW and GW of the birds fed on D-yeast were significantly (P<0.05) greater than birds on CON at the age of 9, 16 and 30 days, and than birds on D-spore at the age of 30 days. Feed conversion ratio (FCR) was (P<0.05) improved in the birds of all probiotic diets, compared to the control bird group at the age of 9 and 16 days. As the birds get older the differences in FCR between control group and probiotic groups were not significant; all FCR were almost similar. Carcass yield was significantly (P<0.05) high in the birds of D-bacteria. A significant increase in the weight and length of digestive tract was seen with the birds of D-spore group. The results indicated that the use of selected probiotics enhanced bird performance, and the effect of D-yeast probiotic was better.

Keywords

Japanese quail, fattening performance, probiotics

INTRODUCTION

Probiotics are known as live microbial feed supplements, digestive bio-regulators or direct-fed microbial (Fuller, 1995), and classified as gut stabilizing agents in a functional group of zoo-technical additives in European Registrar of Feed Additive used in animal nutrition (Anonymous, 2014). Irrespective of being an alternative to feed antibiotics, currently banned from animal feeds, the probiotic preparations are commercially used in animal nutrition to stimulate immune response of animals by increasing activity of host antibodies, to compete for important nutrients with pathogens and to block intestinal receptor for exclusion of pathogens (Stavric and Kornegay, 1995). Three groups of probiotics, most commonly used in animal nutrition are bacteria, spores and yeasts, e.g., *Bacillus*, *Bifidobacterium*, *Enterococcus*, *E. coli*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* species, and *Saccharomyces cerevisiae* (Patterson & Burkholder, 2003; Kabir *et al.,* 2004; Mountzouris *et al.,* 2007). Feed probiotics have long been tested in poultry species for their efficacies, which are measured from animal performance indicators such as feed intake-FI, weight gain-WG and feed conversion ratio-FCR. However, the results were, in general, found to be inconsistent: Kalavathy *et al.* (2003), Kabir *et al.*

(2004), Schocken-Iturrino *et al.* (2004), Huang *et al.* (2004), de Los Santos *et al.* (2005), Mountzouris *et al.* (2007) and Rigobelo *et al.* (2011) reported significantly improved broiler performance by the use of feed probiotics. In contrary, Pelicano *et al.* (2004) reported significantly improved FCR in broilers, only for the periods of 0-21 days old, but no improvements were reported for the period of 1 to 42 days old. Similarly, the works of Lima *et al.* (2003) and Willis & Reid (2008) reported no changes in bird's performance throughout entire fattening period. Unaffected performance was also reported by the use of single or combined mixtures of probiotic and prebiotic (symbiotics) in Japanese quails (Sahin *et al.,* 2008). A probiotic of *Lactobacillus sp*. given either through drinking water or feed to meat-type quails had no effects on quail's performance from 1 to 35 days old (Otutumi *et al.,* 2010). Recent emphasis on probiotic is, however, mostly given to the strains of probiotic bacteria (Wang & Gu, 2010).

Therefore, one could speculate that specificity of probiotic is important for the expected benefits: the strain originating from the host species to be given supposedly had more ability to adhere to epithelial cells when colonization is reached (Fuller, 1986) in one hand or the natural micro biota of chicken and turkeys were shown to provide protection for chicks in other hand (Schneitz & Nuotio, 1992). The expected impact from a probiotic product is highly dependent upon a wide range of microorganism strains and their methods of product production with different metabolic activities, with their assertive action of modes and with their varied capacity of colonizing the gut, possibly responsible for variation in their impact on the desired results (Jin *et al.,* 1998). One must focus on the colonizing (*Lactobacillus* sp., *Enterococcus* sp.) and non-colonizing species (*Bacillus* sp. -spores and *Saccharomyces cerevisiae*) (Perić *et al.,* 2009). Even the same species from different strains and different strains from various species may lead to different impacts. The active component of probiotic products is live strain of bacteria, spore or yeast; its quantity per g of product is expressed in a colony-forming unit (c.f.u). It has been recommended that the animal feeds should not solely be dosed for a desired quantity of c.f.u per kg of feed, simply calculated from the c.f.u quantity of the product. The recommended dosage is usually declared from the authorized holders on the product label. It is therefore significantly important to buy the authorized probiotics under EU regulation (Anonymous, 2003), proven to be safe for animals and humans, stable (properly coated) and efficacious for the intended purpose of use in animal nutrition.

In this study three probiotics (bacteria, spore or yeast) were selected and added to the diets of quails according to the recommendation by the authorized holder under European Union Register of Feed Additives, pursuant to Regulation (EC) No 1831/2003 (Anonymous, 2003 and 2014). The objective of this study was, therefore, to test the efficacy of safe and stable probiotics in Japanese quails.

MATERIALS AND METHODS

One control diet (CON) was formulated (*Tab. 1*) with corn, soybean meal and fishmeal to meet nutrient requirements of quail chicks from hatching to 35 d-old, according to the standards of NCR (1994). The control diet was isocaloric and isonitrogenous, but contained no probiotic supplements. Probiotic preparations used in this experiment were well-characterized, safe, stabile and authorized for selling at European market (Anonymous, 2014). The control diet was supplemented with commercially available three probiotic preparations to produce three test diets; D-bacteria, D-spore and D-yeast, respectively. The diet of "D-bacteria" was supplemented to contain 3×10^9 c.f.u per kg of feed at a dosage of 0.28 g/kg with a probiotic bacteria preparation of *Enterococcus faecium* NCIMB 10415 containing a minimum of 1×10^{10} c.f.u/g in microencapsulated form. The diet of "D-spore" was supplemented to contain 1×10^7 c.f.u per kg of feed at a dosage of 0.50 g/kg with live probiotic spores of *Bacillus subtilis* ATCC PTA-6737 containing a minimum of 2×10^7 c.f.u/g in beige free-flowing powder form. The diet of "D-yeast" was supplemented to contain 9×10^9 c.f.u per kg of feed at a dosage of 0.45 g/kg with aviable probiotic yeast of *Saccharomyces cerevisiae* containing a minimum of 2×10^{10} c.f.u/g in dried granulated form. All probiotic products were homogenously pre-mixed with smaller proportion of corn and fishmeal together in a kitchen mixer before homogenously mixing with the remaining parts of diet ingredients.

Each diet was offered to 48 chicks in four replicated cages, each with 12 chicks, giving a

Table 1. Composition of quail diets (g.kg^{-1}, as fed), formulated according to NRC (1994)

Formulation	CON
Corn	600
Soya bean meal (48% CP)	303
Fish meal (68% CP)	70
Dicalciumphosphate	20
Sodium chloride	3.5
Vitamin and mineral premixture*	3.5
Total mixture, g/1000	1.000,00
Nutrient composition, calculated	
Dry matter (DM), g/kg	898,00
Crude Protein (CP), g/kg	239.60
Metabolisable energy (ME), MJ/kg	12.25
Calcium, g/kg	8.39
Available Phosphorous, g/kg	5.76
Methionine + cystine, g/kg	8.18
Lysine, g/kg,	1.40
Threonine, g/kg	9.37

Note: *Each kg of the premix contained: 5 000 000 IU Vitamin A; 750 000 IU Vitamin D_3; 25 000 mg Vitamin E; 2 000 mg Vitamin K_3; 2 500 mg Vitamin B_1; 5 000 mg Vitamin B2; 2 500 mg Vitamin B_6; 30 000 mg Niasin; 10 000 mg calcium D-pantothenate; 1 000 mg Folic acid; 100 mg Biotine; 37 500 mg Manganese; 50 000 mg Iron; 40 000 mg Zinc; 7 500 mg Cupper; 250 mg Iodine; 100 mg Cobalt; 100 mg Selenium.

total number of 192 chicks. Two days-old chicks were weighed and randomly allocated to the cage groups with a similar body weights (mean ± standard deviation of 11.8±0.4 g/chick). All chicks were reared in groups in electrically heated battery brooders with wire-mesh floor. All the groups were subjected to similar management practices (brooding, lighting, feeding and watering) throughout the experiment. Lighting regime was 24 h and brooding temperature gradually decreased from 34°C during the initial 7 days to 26°C by 21 days of age. Water and experimental diets were supplied *ad libitum* from day 2 to day 35. No vaccination was performed.

FI of birds in each group were recorded daily, but body weights (BW) were measured weekly intervals. Mortality and health inspection were monitored daily before and after feeding. FCR is calculated by dividing the amount of consumed feed by the WG at a specified interval of time. Dressing percentage is calculated dividing the carcass weight by live weight and multiplying by 100. The length and weight of total digestive organs were measured at the end of experiments for all birds. All data were analyzed to study the effect of dietary treatments using one-way analysis of variance, according to Snedecor and Cochran (1980). The treatment means for studied parameters found significant (P<0.05) in one-way analysis of variance were separated for significance comparisons using Duncan's multiple range test (Duncan, 1955) at the 1% and 0.5% of probability.

RESULTS AND DISCUSSION

There was an increasing tendency in FIs of the birds fed with the birds fed on D-yeast (*Tab. 2*). These birds significantly (P<0.05) consumed more feed at the age of 16, 30 and 37 days than the birds fed on CON, D-bacteria and D-spore, whose FI were almost similar throughout the fattening period.

The birds fed on the diets supplemented with bacteria (D-bacteria) and yeast (D-yeast) probiotics had numerically higher growth rate in terms of BW and WG than the birds fed on the diet with no probiotic and with spore probiotic (*Tab. 2*). Especially BW and GW of the birds fed on D-yeast were significantly (P<0.05) greater than that of birds on CON at the age of 9, 16 and 30 days, and than that of birds on D-spore at the age of 30 days.

FCR was significantly (P<0.05) improved in the birds of all probiotic diets (bacteria, spore and yeast), compared to the control bird group at the

Table 2. FI, BW, WG and FCR of quails fed on experimental diets from 0 to 35 d-old

	CON	D-bacteria	D-spore	D-yeast	*S.E.M	P
At 9 d-old						
**n	48	48	48	48	--	**NS**
Initial BW at 2 day-old, g	12.0	12.0	11.7	11.8	0.4	**0.91**
FI, g	39.4a	41.7a	39.7a	44.5a	1.5	0.134
BW, g	30.8a	34.4ab	32.7ab	35.1b	1.2	0.059
WG, g	18.2a	22.8ab	21.4ab	23.1b	1.4	0.052
FCR	2.16a	1.83b	1.85b	1.92c	0.02	0.060
Number of dead bird	3	3	3	4		**NS**
At 16 d-old						
FI, g	110.5ab	110.0ab	106.7a	113.63b	2.7	0.063
BW, g	67.7a	70.0ab	68.8ab	72.2b	1.7	0.061
WG, g	55.1a	57.9ab	57.5ab	60.3b	1.6	0.055
FCR	2.00a	1.89b	1.85b	1.89b	0.04	0.052
Number of dead bird	4	3	3	3		**NS**
At 23 d-old						
FI, g	194.5a	195.8a	192.9a	197.6a	2.6	0.651
BW, g	94.3a	97.2a	98.3a	97.6a	2.1	0.438
WG, g	81.6a	85.5a	87.0a	85.7a	2.9	0.605
FCR	2.38a	2.29a	2.21a	2.30a	0.08	0.620
Number of dead bird	1	2	2	1		**NS**
At 30 d-old						
FI, g	292.3a	292.7a	291.2a	300.6b	3.1	0.001
BW, g	119.7a	122.9ab	118.0a	126.8b	2.8	0.047
WG, g	107.0a	111.3ab	106.7a	114.8b	3.1	0.055
FCR	2.73a	2.63a	2.73a	2.62a	0.07	0. 400
Number of dead bird	-	-	-	-		**NS**
At 37 d-old						
FI, g	399.7a	398.3a	394.6a	412.3b	4.1	0.052
Final BW, g	139.1a	142.8a	141.6a	143.7a	3.3	0.390
WG, g	126.5a	131.1a	130.3a	131.6a	3.9	0.786
FCR	3.16a	3.04a	3.03a	3.13a	0.09	0.735
Number of dead bird	-	-	-	-		**NS**

Note: *S.E.M. refers to standard error of the means. **n, refers to the number of total birds in the groups at day 0.
a,b,c Mean values bearing different superscripts in the same row differ significantly (P<0.05).
NS=Non-significant (P>0.05).

age of 9 and 16 days (*Tab. 2*). There was a more significant improvement in FCR with D-bacteria and D-spore at the age of 9 days. As the birds get older the differences in FCR between the control group and probiotic groups were not significant; all FCR were almost similar (*Tab. 2*).

No significant changes in the weight of carcasses and carcass yield were observed with the birds in this experiment (*Tab. 3*), except that the carcass yield was significantly (P<0.05) higher in the birds of D-bacteria, compared to all the birds in remaining groups. No significant changes were observed in the total weights and lengths of digestive tract between the dietary treatments in this experiment. But, there is an increasing tendency in these parameters in the birds fed on the diets containing probiotics. Exceptionally there was a significant increase in the weight and length of digestive tract with the birds of D-spore group.

In this experiment, no significant changes were observed in FI of the birds fed on the diets of CON, D-bacteria and D-spore, whose BW and WG were not greatly differed from each other. In comparison to these groups of birds, BW, WG and FI of birds fed on the D-yeast were significantly

Table 3. Carcass characteristics and development of digestive tract of the quails fed on experimental diets at 35 d-old

	CON	D-bacteria	D-spore	D-yeast	*S.E.M	P
Live weight at 35-d old, g/b	139.1a	142.8a	141.6a	143.7a	3.3	0.390
Carcass weight, g/b	98.6a	102.1a	99.0a	100.6a	2.4	0.725
Dressing percentage, %	70.8a	71.5b	69.9a	70.0a	0.02	0.052
Whole digestive tract weight, g/b	17.6a	18.1a	24.3b	19.3a	1.5	0.037
Whole weight relative to BW, g per 100 g/b	12.6a	12.7a	17.0b	13.4a	0.7	0,050
Length of whole digestive tract, cm/b	48.0a	54.1a	66.8b	52.2a	3.8	0.024

Note: *S.E.M. refers to standard error of the means.
[a,b,c] Mean values bearing different superscripts in the same row differ significantly (P<0.05).

increased. In general the change in FI was well reflected on the changes in BW and WG under the influence of dietary treatments. These results of unaffected FI and WG indicators, except the case of the D-yeast, were strongly agreed with previously reported results (Miles *et al.,* 1981; Cakir *et al*., 2008; Akinleye *et al*., 2008; Sahin *et al.,* 2008; Sarica *et al.,* 2009; Sahin *et al.,* 2011; Sharifi *et al.,* 2012). The same performance indicators (except in the case of FCR, discussed later) of animals were not greatly changed by the dietary supplementation of feed probiotics during the fattening period: Sarica *et al*. (2009) tested the efficacy of 13 novel feed additives in Japanese quails. They found no significant change in FI, WG and FCR with probiotic and prebiotic preparations. Miles *et al.* (1981) reported no significant differences existed in growth, feed efficiency or mortality when quails were fed with the feed added with a probiotic culture of *L. acidophifus* and other *Lactobacilli*, but mortality was high with the probiotic added feed. In the current study, no significant differences in the number of dead birds were seen between the dietary treatments. Sharifi *et al.* (2012) demonstrated no improvements in quail's performance with the diet of low or high protein content; both supplemented with a combination of the probiotic strain *Enterococcus faecium*, a prebiotic (derived from chicory), and immune modulating substances (derived from sea algae). These results were similar to that of Cakir *et al.* (2008) and Akinleye *et al.* (2008). FI and FCR did not differ between the diets supplemented with and without probiotics (Sharifi *et al.,* 2012; Sahin *et al.,* 2008) in quails. Sahin *et al.* (2011) reported that FI, BW, WG and FCR of quails during the entire fattening period did not significantly affected by the use of a yeast probiotic, whereas the birds of yeast probiotic numerically had higher WG and FI, compared to the birds on non yeast diet. The later results were also observed with the yeast in this study.

All these results revealed the fact that overall bird's performances were not significantly affected by the dietary supplementation of probiotic feed additives in poultry species. However, there were significant influence of probiotic feed additives in certain periods, especially in early growing phases, similar to the present results with improved FCR with all probiotic treatments, and with increased FI and improved FCR and BW with the D-yeast treatment both in young and old ages.

In this study, the improved FCR with all probiotic products at early ages (day 9 and day 16), and that of increased FI, FCR and WG with D-yeast (except at the age of 23 and 37 days) were strongly supported by previously reported results: Pelicano *et al.* (2004) clearly showed that supplementation of diets with bacteria, spore or yeast probiotics significantly improved FCR of broiler aged at 14 d-old, whereas no improvements were observed during the entire fattening period (0-42 d-old). Tortuero (1973) reported an increase in growth rate in chicks given a *Lactobacillus acidophilus* culture in drinking water for 11 days from hatching. Similar results were also reported in the work of Jin *et al.* (1998) with an exception of the improved growth rate in the birds of yeast probiotic by the age of 28 day. Broilers fed on the diets containing a probiotic of *Enterococcus faecium NCIMB 10415* with or without dried whey product exhibited significantly greater WG and FCR from day 1 to day 21 (Samli *et al.,* 2007). A positive effect at 21 d-old on growth and FCR in broilers was obtained in the broilers fed on the diet with yeast products and/or hops (Sacakli *et al.,* 2011). This clearly indicated the fact that all types of probiotics including bacteria, spores and yeasts somehow work well enough to induce markedly favorable changes in animal performance from hatching to 21 days old in poultry species (broiler and quail). Unexpectedly, the enhanced performance was not repeated at the age of 30 days, with the yeast probiotic. It was more recently shown that the diet supplemented with 2 g/kg probiotic products of a 1.0×10^{10} viable spores/g of *Bacillus subtilis* endospores and 1.0×10^{9} viable spores/g of *Clostridium butyricum* increased BWG and FI throughout the experimental periods in broilers, but the effect was low with the diet of high content of crude protein (Chen *et al.,* 2013).

One could speculate the variations in animal performance by probiotic bacteria, spores and yeast were dependent on large variability of their persistence in the gut and in their stability in feed as well as lack of verification of their viability and definition of origin (Simon *et al.,* 2001; Siriken *et al.,* 2003; Huyghebaert *et al.,* 2011). This reasoning seems to be true because commercially available probiotic products are obtained from a wide range of microorganism strains; their metabolic activities, action modes and capacity of colonizing the gut are markedly different (Jin *et al.,* 1998). It would be easy and logical to compare and evaluate each of commercial probiotics within its purpose of usage in animal nutrition. In this experiment, all the probiotic products used lead to significantly improved performance in early ages. Of the probiotics the yeast probiotic continued its beneficial action on performance at old ages, while the bacteria and spore probiotic did not work. Thus they act differently in animal models with or without affecting the animal performance indicators. Therefore, it would not be a realistic approach to expect significant improvements in animal performance from the use of probiotics because they may exert some beneficial effects on the digestive tract (Santoso *et al.,* 1995; Stavric and Kornegay, 1995; Samli *et al.,* 2007).

Sahin *et al.* (2011) did not observe a change in the carcass yield of quails fed with the diet supplemented with yeast probiotic; similar with probiotic and prebiotic preparations used in broiler (Eren *et al.,* 1999; Sacakli *et al.,* 2011) and in quails (Akinleye *et al.,* 2008; Sahin *et al.,* 2008; Sharifi *et al.,* 2012). This is somehow found similar to the carcass yield data in the present study, except with the case of birds fed on D-bacteria probiotic. In this experiment the weight and length of digestive tract did not significantly differ amongst the dietary treatments, except that the birds on D-spore had longer and heavier digestive tract than the birds on other groups. This showed that this type of specific probiotics works better on the development of digestive tract, similar to the work of Samli *et al.* (2007).

CONCLUSION

It can be therefore concluded that each of the probiotics tested in this study improved animal performance from 1 to 21 d-old age; the probiotic yeast product significantly enhanced the animal performance during overall fattening period.

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