

Full Length Research Paper

Effects of active, inactive and compounded *Saccharomyces cerevisiae* on growth-related hormones and performance of Japanese quails (*Coturnix Japonica*)

Tohid Vahdatpour^{1*}, Hossein Nikpiran², Arman Moshaveri³, Alireza Ahmadzadeh¹, Seyyed Reza Riyazi¹ and Sina Vahdatpour²

¹Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran.

²Department of Clinical Science, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

³Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran.

Accepted 22 July, 2011

The objective of this research was to determine the effects of active, inactive and compounded *Saccharomyces cerevisiae* (SC) as natural feed additives on growth performance, visceral organs weight, insulin, thyroxin and growth hormone of Japanese quails. One day old Japanese quails allocated in 4 treatments by 4 replicates and 12 birds per pen. The experimental diets consisted of a basal diet without additive (Control), 1 g/kg active SC (ASC) as a probiotic, 1 g/kg inactive SC (ISC) as a prebiotic and mixed of 0.5 g/kg active + 0.5 g/kg inactive SC (ASC+ISC) as a synbiotic added to the basal diet. At 42 days, growth performance values were calculated, then eight birds were weighted and slaughtered for visceral organs weight recording, and blood sampling were taken for analysis of serum hormones using ELISA technique. Consumption of each ASC and ISC caused elevation in feed intake and body weight and better feed conversion ($p < 0.05$). The dressing percentage in quails fed three types of each additive was increased ($p < 0.05$). Total viscera weight in males fed each additives was declined ($p < 0.05$). In females, the weight of total viscera fed ASC + ISC was higher than ASC fed group ($p < 0.05$). Consumption of ISC in males and ASC in females caused to depressing in proventriculus weight ($p < 0.05$). The gizzard weight in males fed ASC and ISC were decreased and in females fed ASC and ASC + ISC were elevated ($p < 0.05$). Females fed ASC have lighter intestines weight than control group ($p < 0.05$). The heart weight in males fed ASC and ISC and in females fed ISC were reduced ($p < 0.05$). In males fed ASC + ISC liver weight was lighter than other groups and in ASC and ISC fed groups lighter than control group ($p < 0.05$). In females fed ASC + ISC liver weight was reduced ($p < 0.05$). Consumption of ISC in males and ASC + ISC in females caused insulin elevation ($p < 0.05$). Growth hormone level was elevated by inclusion of ASC + ISC in basal diet of both genders ($p < 0.05$). It was concluded that consumption of *S. cerevisiae* in different form of activity could be used in quail's diet to improve growth performance values by increasing the feed intake and stimulating the insulin and growth hormone release and they also have implications in human health by organic meat and egg production.

Key words: *Saccharomyces cerevisiae*, hormone, growth performance, quail.

INTRODUCTION

Japanese quail had gained attention in poultry industry, as they are resistant to pathogens and a good producer

of organic egg and meat for human healthy nutrition, and is being used as beneficial animal model in researches (Minvielle, 2004; Huss et al., 2008; Bishop, 2009). The global paradigm is shifting from an emphasis on productive efficiency to one of public concerns (Cakir et al., 2008), as long term use of dietary antibiotics resulted

*Corresponding author. E-mail: tohid_vahdatpour@yahoo.com.
Tel: +98.411.4777882. Fax: +98.411.4777840.

in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001), accumulation of drug residues in the body of the birds (Burgut, 1999), imbalance of normal microflora (Andremont, 2000), and finally, harmful effects on human health (Abaza et al., 2008; Attia et al., 2011). Therefore, in the poultry industry, the application of antibiotics as growth promoters and prevention of the poultry diseases by competing with the pathogens are gradually being lost. Challenges for finding safe alternatives have been started and continued. Three types of novel feed additives/ functional foods including probiotics, prebiotics and synbiotic are applied (Roberfroid, 2000; Chen et al., 2009). A probiotic was defined as a live microbial feed additive that beneficially affects the host animal by improving its intestinal microfloral balance (Fuller, 1989). A prebiotic was defined as non digestible food ingredient that beneficially affects the host, selectively stimulating the growth or activity or both of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). This approach which seems to be the best efficacy of use in these products is synbiotic, which is a combination of both probiotics and prebiotics and which synergistically affects the host by improving the survival and implantation of live microbial dietary additives and their necessary nutrients and suitable environment in the gastrointestinal tract (Awad et al., 2009; Attia et al., 2011). These feed additives promote gut health by several possible mechanisms including altering gut pH, maintaining protective gut mucus, selecting beneficial intestinal organisms acting against pathogens, enhancing fermentation acids, enhancing nutrient uptake and increasing the humoral immune response (Cakir et al., 2008). Finally, these metabolic changes in bird's physiology results in enhanced growth and performance of the host. Live yeast is a probiotic, but yeast cell-wall is prebiotic (Santos and Ferket, 2006). The active *Saccharomyces cerevisiae* (ACS) yeast has biologically valuable proteins, vitamin B-complex, important trace minerals and several unique "plus" factors (Paryad and Mahmoudi, 2008). The inactive *S. cerevisiae* (ISC) yeast has lesser biological compounds but has high level of mannan-oligosaccharides and fructo-oligosaccharide in the cell wall (Santin et al., 2006). Compounded ASC and ISC (ASC + ISC) may be as a synbiotic. Already, some studies were conducted to evaluate active yeast on poultry performances, but not comparing the effects of active and non-active yeast especially on quails performance and hormones. Therefore, the present study was conducted to investigate the effects of ASC (probiotic), ISC (prebiotic) and a mixture of ASC and ISC (ASC + ISC) (synbiotic) from commercial production on Japanese quail's growth performance and three growth-related hormones in birds.

MATERIALS AND METHODS

Experimental design and housing

One hundred and ninety-two (192) newly-hatched and healthy

Japanese quail chicken (mean body weight 7.78 ± 0.39 g) were provided from the Damavand Quail Co. flock and kept in a specially designed farm in the city of Tabriz, Iran. The chicken were randomly placed in 4 treatment groups with 4 replicates and 12 chicks (48 birds/group) per wire cages ($0.3 \times 0.5 \times 0.35$ m) and were established with a distance from one another at one room with controlled standard temperature, humidity, ventilation for quails housing and 24 h of light (2.5 watt/m^2) kept for 42 days (Figure 1).

Dietary treatments and additives

The basal diet (conventional mash) was formulated to meet and exceed the nutrient requirements of grower Japanese quails following NRC, 1994 (Table 1). Each feed additives in standard high level of manufactory recommendation was added to the basal diet and completely mixed with water were available *ad libitum* in length of the experiment (1 to 42 days of age). The four treatment groups were as follow: 1) basal diet without additive (control group) 2) basal diet + 1000 g/ton Fariman® IRAN (the active yeast of *S. cerevisiae* 1026 has been known as a probiotic, 2×10^9 cfu/g) 3) Basal diet + 1000 g/ton Thepax®, DOX-AL ITALIA SPA (the inactive yeast of *S. cerevisiae* Var. ellipsoideus were inactivated in order to avoid further fermentation and stabilized in order to be protected from the birds digestive actions by an osmotic treatment as a prebiotic) 4) basal diet + 500 g/ton Fariman® + 500 g/ton Thepax® (as a synbiotic).

Measurements and blood sampling

Feed intake (FI) of each experimental unit (each cage) was recorded. At the end of experimental period (42 days), the total body weight (BW) of birds in each cage was measured and then feed conversion (FC) was calculated. At 42 days of fasting state (feed was removed 12 h before sampling), one male and one female bird from each cage (8 birds/treatment) were randomly selected, weighed, slaughtered and blood samples by cervical cutting and without anti coagulant for serum separation were collected (one sample/bird). The total viscera, proventriculus, gizzard, intestine, liver, and heart were excised, weighed and calculated as a percentage of the live body weight and then recorded. The sera were separated by centrifugation at 3000 rpm for 8 min after 2 h incubation at room temperature and were analyzed by ELISA technique (AWAKNESS technology Inc., USA) including ELISA microplate reader (stat fax 2100) and automatic ELISA plate washer (state fax 2006) for growth related hormones including, insulin (Biosource Inc, EASIA Kit, Belgium), growth hormone (Monobind Inc, ELISA Kit, USA) and thyroxin (T_4 ELISA Kit, Pishtaz Teb, IRAN).

Statistical analysis

Analysis of variance (ANOVA) for completely randomized design (CRD) using the general linear model (GLM) procedure, Bartlett's test for homogeneity of data variance and standard deviation were calculated. Duncan's new multiple range test was used to compare the means treatments at $P < 0.05$ level (SAS Institute, 1994).

RESULTS AND DISCUSSION

Growth performance

The effects of three different types of feed additives that were added to control basal diet on growth performance



Figure 1. Experimental room of Japanese quail, Tabriz-Iran.

values are presented in Table 2. Consumption of each ASC and ISC in basal diet of quails caused an elevation in FI ($p < 0.05$). Whereas, compounded additives (ASC + ISC) had no effect on feed intake. With increase of FI in each group fed by ASC and ISC, the BW showed elevation in comparison to control and ASC + ISC groups ($p < 0.05$). The FC in ASC and ISC fed groups was better than control and ASC + ISC groups ($p < 0.05$). The dressing percentage in quails fed ASC (74.9%), ISC (76.2%) and ASC + ISC (73.7%) was higher ($p < 0.05$) than control group (70.1%). By good management, in all period, groups faced no disease and mortality.

Yildiz et al. (2004) resulted that addition of live yeast SC to basal diet of Japanese quail caused numerically elevation of FI, significantly better FC and higher BW in comparison to control group ($p < 0.05$). Zhang et al.

(2005) reported that diets supplemented with 0.3% SC yeast cell components improved broiler BW, FI and FC, vice versa. Yalcinkaya et al. (2008) reported that BW, FI and FC of broilers were not significantly influenced ($p < 0.05$) by the addition of mannan oligosaccharide (MOS) which is derived from the cell wall of SC. Chen et al. (2009) and Guclu (2011) found that final BW of the quails fed 0.5 kg/ton MOS was significantly higher than control and microbial probiotic additive groups ($p < 0.01$). In agreement with present study, Goush et al. (2008) reported that consumption of MOS and active SC by Japanese quail motivated elevation of dressing percentage. The obtained results of present study confirmed the equal positives effects of both ASC as probiotic and ISC as prebiotic and depressed effects of mixed ASC + ISC as synbiotic on performance values.

Table 1. Ingredient and calculated analysis of basal diet.

Ingredient	Ration (%)
Yellow corn	53.00
Soybean meal, 44%CP	37.00
Fish Meal, 60%CP	5.50
Vegetable Oil	1.00
Oyster Shell	1.00
Mono calcium phosphate	1.50
DL-Methionine	0.15
Sodium chloride	0.15
Mineral-vitamin premix*	0.50
Vitamin A	0.10
Vitamin E	0.10
Calculated analysis	
ME (Kcal/Kg)	2900.00
CP (%)	24.00
Calcium (%)	1.20
Available phosphorus (%)	0.55
Methionine (%)	0.57
Lysine (%)	1.47

*Supplemented for kg of the diets: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3 mg; B12, 0.03 mg; Biotin, 0.1 mg; Choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu, 12 mg; I, 1 mg; Se, 0.2 mg, and adequate antioxidant.

This good performance in birds fed by ASC or ISC may be related to improved intestinal lumen health and increased digestion and absorption of nutrients by different enzymes and other unknown ingredients of SC structure. Certainly, stimulation effects of palatability and texture of ration and reduction of pathological microorganisms influenced by SC may be other hypothesis. In present study, the beneficial effects of probiotic and prebiotic on Japanese quail performance parameters including BW, FI, FC and dressing are in agreement with previous studies (Goush et al., 2008; Chen et al., 2009; Guclu, 2011). However, the mixtures of two additives that can be introduced by synbiotic do not display a synergistic effect on performance values.

Visceral organs weight

The effects of feed additives intake on visceral organs weight in male and female Japanese quail are presented in Table 3. In female quails, due to the presence of greater reproductive tract, the weight of total viscera was higher than males ($p < 0.01$). Total viscera weight in male quails fed each ASC, ISC and ASC + ISC were lighter than control group ($p < 0.05$). In females, the weight of total viscera fed ASC + ISC was higher than ASC fed

group ($p < 0.05$). Consumption of ISC in males and ASC in females caused a depression in proventriculus weight as compared to control group ($p < 0.05$). The means of gizzard weight in males fed ASC and ISC were decreased and in females fed ASC and ASC + ISC were elevated as compared to control group ($p < 0.05$). Females fed ASC have lighter intestines weight than control group ($p < 0.05$). The means of heart weights in males fed ASC and ISC and in females fed ISC were lighter than control group ($p < 0.05$). Additives intake caused decreased weight of liver in male and females. In males fed ASC + ISC, liver weight (1.82%) was lighter ($p < 0.05$) than other groups, while the liver weight of males fed ASC (2.18%) and ISC (2.48%) was lighter ($p < 0.05$) than the control group (2.93%). In females fed ASC + ISC, liver weight was lighter than control group ($p < 0.05$) and other two groups have intermediate state. In general, consumption of ASC and ISC additives in basal diet of Japanese quails resulted in decreasing most of the relative organs weight. Whereas, in quails fed ASC + ISC, only in liver weights of both gender and weight of total viscera in males this reduction was existence. Food contamination, nutrient imbalance, environmental factors (stress) and mycotoxins are known to irritate the proventriculus, gizzard and intestines of the gastrointestinal tract, thus causing an increase in the relative weights of these organs (Abousadi et al., 2007; Vahdatpour et al., 2009). Victoria and Richard (1988) reported that liver weights were significantly heavier in birds that died from sudden death syndrome (SDS) and although this might be a reflection of the various diets of the field cases, it could be an indication of metabolic disease predisposing to SDS. EL-Banna et al. (2010) showed that the weight of internal organ weights except small intestine was significantly reduced by consumption of synbiotic ($p < 0.01$) and prebiotic ($p < 0.05$). In agreement with this study, Bozkurt et al. (2009) resulted that the liver and small intestine weights of male broilers with consumption of MOS prebiotic (Nutri-Mos®) decreased ($p < 0.05$), but females were not influenced compare to control group. These results suggested that effects of both ASC and ISC additives on the relative weight of internal organs were related to sex. Probably, due to existence of sexual hormones, males were sensitive to additives. Anti-microbial action for prebiotics (Ferket, 2004) and probiotics (Wenk, 2000) is an important reason for decreasing relative weight and pathological activity on internal organs in birds.

Hormones

The effects of feed additives intake on three growth-related hormones in Japanese quail are presented in Table 4. Consumption of ISC in males caused elevation of insulin as compared to control group ($p < 0.05$). Whereas, in females, insulin elevated by ASC + ISC intake as compared to control group ($p < 0.05$). Certainly,

Table 2. Means of growth performance values and mortality of quails fed additives at 42 days of age.

Diet (treatment)	Feed intake (g/bird)	Body weight (g)	Feed conversion (g/g)	Dressing (%)	Mortality (%)
Basal diet (control group)	697 ± 7 ^b	222 ± 6 ^b	3.14 ± 0.10 ^b	70.1 ± 2 ^b	0.0
Basal diet + ASC	706 ± 4 ^a	232 ± 7 ^a	3.04 ± 0.11 ^a	74.9 ± 2 ^a	0.0
Basal diet + ISC	703 ± 3 ^a	232 ± 4 ^a	3.03 ± 0.08 ^a	76.2 ± 3 ^a	0.0
Basal diet + ASC + ISC	700 ± 3 ^{ab}	222 ± 4 ^b	3.15 ± 0.07 ^b	73.7 ± 1 ^a	0.0

ASC: Active *Saccharomyces cerevisiae*; ISC: Inactive *Saccharomyces cerevisiae*. Means ± standard deviation (n = 8) within each column with no common superscript differ significantly (p < 0.05).

Table 3. Means visceral organs weight of quails (Percentage of live body weight) fed additives at 42 days of age.

Visceral organ (%)	Gender (male or female)	Basal diet (control group)	Basal diet + ASC	Basal diet + ISC	Basal diet + ASC+ISC
Total viscera	M	9.92 ± 2.07 ^a	8.97 ± 1.36 ^b	8.36 ± 0.22 ^b	8.80 ± 0.43 ^b
	F	10.95 ± 0.99 ^{ab}	9.36 ± 0.03 ^b	10.49 ± 1.13 ^{ab}	11.78 ± 1.03 ^a
Proventriculus	M	0.41 ± 0.12 ^a	0.36 ± 0.05 ^{ab}	0.32 ± 0.07 ^b	0.35 ± 0.09 ^{ab}
	F	0.36 ± 0.02 ^a	0.32 ± 0.02 ^b	0.36 ± 0.01 ^a	0.35 ± 0.01 ^a
Gizzard	M	1.91 ± 0.27 ^a	1.75 ± 0.17 ^b	1.81 ± 0.18 ^b	2.06 ± 0.13 ^a
	F	1.76 ± 0.19 ^b	1.97 ± 0.17 ^a	1.86 ± 0.20 ^{ab}	1.91 ± 0.20 ^a
Intestines	M	2.82 ± 0.75	2.36 ± 0.36	2.23 ± 0.35	2.84 ± 0.33
	F	3.30 ± 0.70 ^a	2.54 ± 0.73 ^b	3.03 ± 0.68 ^{ab}	3.12 ± 0.69 ^{ab}
Heart	M	0.98 ± 0.15 ^a	0.86 ± 0.15 ^b	0.88 ± 0.10 ^b	0.98 ± 0.09 ^a
	F	0.75 ± 0.04 ^a	0.75 ± 0.07 ^a	0.63 ± 0.09 ^b	0.71 ± 0.04 ^a
Liver	M	2.93 ± 0.42 ^a	2.18 ± 0.29 ^b	2.48 ± 0.53 ^b	1.82 ± 0.04 ^c
	F	2.73 ± 0.48 ^a	2.66 ± 0.45 ^{ab}	2.61 ± 0.75 ^{ab}	2.06 ± 0.22 ^b

ASC: Active *Saccharomyces cerevisiae*; ISC: Inactive *Saccharomyces cerevisiae*. Means ± standard deviation (n = 8) within each rows with no common superscript differ significantly (p < 0.05).

Table 4. Means serum hormones of quails fed additives at 42 days of age.

Diet (treatment)	Insulin (µU/ml)		Thyroxin (µg/dl)		Growth hormone (µU/ml)	
	Male	Female	Male	Female	Male	Female
Basal diet (control group)	8.15 ± 0.78 ^b	6.58 ± 0.42 ^b	2.78 ± 0.39	2.35 ± 0.61	0.40 ± 0.11 ^b	0.45 ± 0.17 ^b
Basal diet + ASC	7.50 ± 0.98 ^b	7.75 ± 1.05 ^{ab}	2.25 ± 0.51	2.13 ± 0.80	0.43 ± 0.12 ^b	0.41 ± 0.09 ^b
Basal diet + ISC	12.58 ± 1.90 ^a	7.33 ± 1.15 ^{ab}	2.03 ± 0.48	2.15 ± 0.41	0.38 ± 0.16 ^b	0.45 ± 0.12 ^b
Basal diet + ASC+ISC	8.28 ± 1.11 ^b	8.50 ± 1.91 ^a	2.23 ± 0.36	1.97 ± 0.33	0.61 ± 0.20 ^a	0.80 ± 0.14 ^a

ASC: Active *Saccharomyces cerevisiae*; ISC: Inactive *Saccharomyces cerevisiae*. Means ± standard deviation (n = 8) within each column with no common superscript differ significantly (p < 0.05).

consumption of each ASC and ISC amount to elevated insulin in females that were not significant (p > 0.05). The level of blood thyroxin (T₄) do not influenced by additives feeding, but numerically was depressed in both genders. Growth hormone level was significantly elevated by inclusion of ASC + ISC in basal diet of both genders as

compared to control group (p < 0.05).

One can emphasize on the role of insulin in growth in respect with its role in entrance of five necessary amino acid to cells (phenylalanine, leucine, isoleucine, valine and tyrosine), and so, elevation of insulin in blood is positive for bird growth. Numerically, reduction of thyroxin

could have been due to the positive effects of additives in alleviation of the negative effects of environmental condition (petty high temperature at summer) (Sabriea et al., 2006). Li et al. (2007) showed that chito-oligosaccharide supplementation as prebiotic in broiler chicken diet resulted in significant ($P = 0.02$) elevation of growth hormone level as compared with the control group and chlortetracycline fed group at 42 days. In the study of Li et al. (2007), improvement values of BW, FI and FC were in harmony with growth hormone level, higher growth hormone and better growth performance were in prebiotic fed group. Zhengkang et al. (2006) showed that by using isoflavonic phytoestrogens as new prebiotic, the growth hormone significantly increase ($p < 0.05$) in sow colostrum, but insulin was constant.

Conclusion

Based on the results, the consumption of diets containing *S. cerevisiae*, especially inactive *S. cerevisiae* (Thepax©) improved quail's growth performance and mixed active and inactive (as synbiotic) elevated growth related hormones to production of healthier and organic meat and egg for human nutrition. Thereby, stimulating of growth related hormones release may be one new theory for promotion of growth performance in poultry emphasis on quails. Certainly, most researches are needed for confirmation of this theory.

ACKNOWLEDGEMENTS

The authors would like to thank Damavand Quail Co. (www.dquail.ir) Tehran-Iran for equipment of the Experimental Room of Japanese Quail in Tabriz –Iran and The A. T. Darusazan Iran Co. (www.darusazaniranco.com) Tehran-Iran for providing Thepax© used to present research project.

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