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EFFECT OF ADDING ACTIVE DRIED YEAST INTO JAPANESE QUAIL DIETS ON PERFORMANCE, SOME IMMUNITY AND MICROBIOLOGICAL ASPECTS

Wakwak,Magda M.[1]; Sh.F. El-Afifi[2]; Nagla K. Soliman[2] and M. Attia[3]

1- Dept. of Biol. App., Nuclear Res. Cen., Atomic Energy Autho., Egypt
2- Poultry Prod. Dep. Faculty Agric., Ain-Shams Univ., Cairo, Egypt
3- Agric. Microbiology Dep. National Research Center

ABSTRACT

The present study was carried out to investigate the effect of adding 0.5%, 1%, 1.5% and 2% active dried yeast (Saccharomyces Cerevisiae) into diets of Japanese quails on their performance. As well the effect on small intestine thickness, some immunity characteristics and ileal content of total bacteria, fungi and yeast were investigated. Five hundred day old Japanese quails were used and divided into five experimental treatments as 100 birds each.

The results indicated that, average body weight of Japanese quails fed yeast-supplemented diets were higher than the control. This improvement may be related to the increase in nutrients availability resulted from antagonistic effect of yeast against harmful enterobacteria.

There was a slight reduction in feed consumption values of yeast feed groups. Feed efficiency ratios improved by 6, 9, 12 and 3% due to adding yeast by levels of 0.5%, 1%, 1.5% and 2% into diets respectively.

Small intestine wall thickness was clearly reduced by feeding yeast.

There was increase in bursa and spleen size associated with elevation WBC's, Haemagglutination Inhibition (HI) titre against sheep RBC's and blood globulin.

Ileal content of total bacteria and fungi was significantly decreased due to supplementation yeast diets. The count of yeast cells in ileal content increased as dietary yeast levels increase.

It can be suggested that, yeast may improve the performance of Japanese quails due to its antagonistic effect against enteropathogenic bacteria. As well it may also stimulate immunity characteristics in Japanese quails.

Keywords: Yeast- Saccharomyces – Japanese quail – Performance – Immunity measurements – Ileal micro flora

INTRODUCTION

In Egypt awareness has been created recently towards addition of chemically synthesized growth promoters into poultry rations, due to their harmful residual in the products (meat & eggs). The government as well encourages production of chemicals–free food which is called organic production. Therefore there is a progressive invitation for using natural growth promoters instead of antibiotics and chemically synthesized additives for producing safe poultry products.

In this connection active yeast has been successfully used as a natural, biological growth promoter (Wray and Wray 2000). Supplementation of active yeast into rations increase the performance of broiler chicks (Onifade *et al* 1999). In Egypt Soliman (2003) observed an enhancement in

performance of laying hens fed diets enriched with active yeast. Likewise Ali *et al* (2000) showed that, addition of yeast culture can improve the growth performance of Japanese quail fed on low protein diets.

Mode of action of yeast as growth promoter in poultry diets has received recently some consideration. Bradley and Savage (1995) suggested that, yeast culture may exert their influence by improving nutrients availability to the animal. On the other hand Stanley *et al* (1993) attributed the beneficial effect of yeast to the effectiveness of yeast in counteracting aflatoxin or reducing aflatoxicosis in the small intestine. Another possibility was introduced by Eric Line *et al* (1998) who stated that, oral administration of yeast can reduce colonization of some pathogenic bacteria, due to its antagonistic effect against these bacterial strains.

However, in this respect information is still lacking in various aspects of mode of action of yeast in Japanese quail diets.

Therefore the present study aimed to investigate the effect of feeding graded levels of active, dried yeast on the growth performance of japans quail. As well, the effect on gut micro flora and some immunity organs were investigated.

MATERIALS AND METHODS

This study was carried out at Biological Application Department of Egyptian Atomic Energy Authority, Inshas.

It was conducted using five hundred day-old Japanese quails, which were divided into five experimental groups of 100 birds each. The experimental group was sited in two battery cages as 50 birds per cage, which was represented as replicate group. For five weeks experimental period the birds were fed on a basal diet (Table 1) supplemented with five graded levels of active dried yeast (saccharomyces Cerevisiae produced by starch, yeast and cleaner co., Alex.) as 0% (control), 0.5%, 1%, 1.5% and 2%. The basal diet satisfied nutrient requirements of growing Japanese quails according to NRC (1994).

The birds were kept under same environmental conditions of light and temperature, also feed and water were provided ad lib.

Weekly body weight of birds was recorded individually in grams and average body weight gain was calculated for each replicate and treatment group. Feed intake were determined weekly for each replicate group and averaged per bird in gram. Efficiency of feed utilization was calculated as gram feed per gram gain.

Slaughter traits and samples collection

At the end of experimental period at 35 days of age, five birds of each replicate group (10 birds/treatment) were randomly slaughtered, allowed to bleed, defeathered, eviscerated and internal organs were separated. Spleen and bursa of fabricius weights were determined. Small intestine wall thickness was determined according the procedure described by Stutz *et al* (1983b) and calculated as small intestine weight (g.) /small intestine length (cm.) Ileal samples content were collected by pressing the outer wall of ileum to push its content into sterile glass bottle.

Table 1. Composition and calculated analysis of experimental diet

Ingredients	Percentage (%)
Yellow corn	54.8
Soybean meal	34.0
Corn gluten meal	7.0
Bone meal	2.4
Oil	1.0
Common salt	0.3
Lysine Hcl	0.2
Premix*	0.3
Total	**100**
Calculated analysis	
ME (kcal/kg)	2907
Protein %	23.96
Calcium %	0.85
Av. Phosphorus %	0.43
Meth. + Cyst %	0.82
Lysine %	1.33

***Vitamins and minerals Premix: each 1kg ofsupplied the following per kilogram of diet; vit. A; 12000 Iu, vit. D_3; 1100 Iu, vit E 12mg, vit B_{12} 0.02mg, vit B_1 1 mg, Choline chloride 0.16mg, Copper 3mg, Iron 30mg, Manganese40mg, Zinc 45mg, and Selenium 3mg.**

Chemical analysis and blood parameters

Blood samples were collected in heparinized tubes. White blood cells (WBC's) were counted according to the method of Natt and Herrick (1952). For chemical analysis of blood constituents, blood samples were centrifuged (400rpm/minute). Plasma total protein was determined according to Biuret method (Henery 1964), albumin according to Doumas *et al* (1971). Plasma globulin was calculated by subtracting albumins from total protein values, also, globulin to albumin ratios were calculated. Triiodothyronine (T3) and thyroxin (T4) were measured by radioimmunoassay (RIA) technique (Chopra 1972) and (Larsen, 1972) while the purification method of iodinated T3 and T4 was conducted according to Ibrahim *et al* (1982). Transaminase enzymes activities GPT, GOT were determined according to the method of Reitman and Frankel (1957). The immune response was determined by Haemagglutination Inhibition Test (Wegman and Smithies, 1966) against sheep red blood cells (SRBC's) and antibody titre (log_2) was estimated.

Microbiological examination of ileal content

The microbiological study aimed to determine count of total bacteria, total fungi and yeast in one gram of ileal content. The examination procedure was done by transferring one gram of ileal content into test tube containing 9ml of buffered peptone and serial dilutions were prepared.Total bacteria was examined with nutrient agar medium. Total fungi were counted by martin's medium while yeast was estimated by Wackerham's agar medium.

Statistical analysis

Statistical analysis was computerized by statistical program SAS (1988). Duncan's multiple range tests were used to separate means.

RESULTS AND DISCUSSION

Effect of active yeast on the performance of Japanese quails

1- Body weight

Average body weight of Japanese quail birds are shown in (Table2). It is clear that, yeast supplementation into diets had almost a positive effect on body weight of Japanese quails at different weeks of age. As well as the body weight values were significantly higher by 4% and 6% due to feeding diets with 1% and 1.5% yeast respectively. These results are in agreement with those obtained by Ali *et al* (2000) who stated that; supplementing quail diets with 1.5% yeast culture significantly increased body weight gain. Similarlry Onifade *et al*; (1999) and Abdel-Azeem, (2002) who observed an enhancement in body weight of broiler chicks fed diets inoculated with yeast. However Sarkar *et al* (1996) and Ernest; (1996) did not observe any improvement in the performance of broiler chicks fed yeast-supplemented diets. This could be due to the low levels of yeast in their experiments being less than 0.5% levels of yeast which was probably insufficient to improve growth compared with the current experiment which employed graded yeast levels beyond 0.5%. The growth promotive effect of active yeast in this experiment may be attributed to the increase in nutrients availability a long the small intestine of birds as reported by (Bradley and Savage 1995). This increase in nutrients availability resulted probably from the antagonistic effect of yeast against harmful microflora which produce metabolic substances, which irritate the gut wall and cause malabsorption associated with retardation in growth (Eric Line *et al* 1998, Wray and Wray 2000).

1-Feed Consumption

Supplementing of active yeast into quail's diets have no significant effect on average feed consumption (Table3). However there was a slight decrease in feed consumption values due to yeast addition into diets. This result is partially similar with the results obtained of Bradley and Savage; (1995) and Brake (1991) who did not find any significant effect on feed consumption values of broiler chicks fed diets supplemented with yeast.

2- Feed efficiency ratio (g feed/g gain)

Feed efficiency ratios were obviously improved by adding active yeast into Japanese quail diets (Table3). The improvement in feed efficiency values as compared with the control diets were 6%, 9%, 12% and 3% by supplementing the diets with 0.5%, 1%, 1.5% and 2% active yeast respectively. The current results are in harmony with those obtained by Madrigal *et al* (1993) and Abdel-Azeem (2002) who reported that, addition of yeast into poultry diets improved the efficiency of feed utilization. The enhancement in feed efficiency ratios may be related to the increase in nutrients absorption associated with incorporating yeast into diets. Stanley *et al* (1993) stated that, the absorption rate of nitrogenous compounds increase with added yeast into diets. Bradley and Savage (1995) observed an improvement in energy utilization and percentage retention of calcium and phosphorus due to adding yeast into turkey diets.

Effect on small intestine thickness

Small intestine thickness (g. weight/cm. Length) values were reduced (Table4 & Fig 1) by feeding different graded levels of active yeast. As percentage to control the values of small intestine thickness were 88%, 76%, 73% and 78% for 0.5%, 1%, 1.5% and 2% yeast levels respectively. This result is in consistence with the result of Bradley *et al* (1994) who observed a reduction in ileal wall thickness of male poults due to adding yeast in their diets. Also they carried out a histological examination of ileal section and attributed the reduction in ileal wall to the decrease in Goblet cells per millimeter of villus high and crypt depth. The reduction in small intestine thickness is a good indication for the inhibition effect of active yeast against harmful microorganisms which produce toxins or metabolic products that cause irritation and increase thickness of intestinal wall, leading to reduction in nutrients absorbability (Scott *et al* 1982).

Effect on some immunity measurements, organs and blood constituents

Table (4) shows bursa of fabricius and spleen weights of the Japanese quails at five weeks of age. It will be noted that, both bursa and spleen weights increased due to supplementation of yeast into diets. In this concern the information in the literature appears to be lacking. However the previous studies with other antmicrobial agents indicated that, bursa and spleen weights are increased by feeding diets supplemented with antibiotics (Dafwang *et al* 1985), onion and garlic (El Afifi 1997) and black Cumin seeds (Ali *et al* 2000).

Simultaneously the count of white blood cells (WBC's) and heamoagglutination inhibiting (HI) antibody titre against sheep RBC'S were significantly higher by adding yeast into Japanese quails diets(Table 5&Fig.2)

These results are well expected because a functioning bursa and spleen are essential for normal immuno-competence (Yamamoto and Glick, 1982). Consequently the positive effect of yeast on bursa and spleen size would be inherent with increase in antibody titre and count of WBC's. Sabria (2000) stated that adding black cumin seeds into Japanese quail diets increase the total count of WBC's and antibody titre against sheep RBC's.

Active yeast has no obvious effect on blood total protein or albumin. These results are in accordance with Stanley *et al* (1993) who did not find any effect on the values of blood total protein or blood albumin due to including live yeast into broiler diets.

The values of blood globulin and globulin to albumin ratios were higher at 1%, 1.5% and 2% yeast levels than control. Eric Line *et al* (1998) suggested that, live yeast stimulates the immune system to increase the intestinal secretion of secretary (Ig) immuno globulin.

Generally, it can be suggested that, the increase in bursa and spleen size associated with elevation in WBC's, HI titre and globulin may indicate the ability of yeast in stimulating the immune system of Japanese quails. Santin *et al* (2002) reported that, live yeast can ameliorate the humeral immunity, that was impaired by aflatoxin. Administration of active yeast into Japanese quail diets significantly reduced the levels of triiodothyronine hormone (T3)

while the tetraiodothyronine hormone (T4) was not affected (Table5). The level of T3 hormone in chicks is related to the basal metabolic rate value, therefore the relatively low levels of T3 is inherent with the relatively high body weight (Wakwak 2002).

The reduction effect of yeast on T3 (active hormonal form in poultry) hormone is still obscure and the information in the literature is still lacking.

The values of blood transaminases enzymes (GOT, GPT) were not affected by adding yeast into Japanese quail diets. These results are in agreement with those obtained by Abdel-Azeem (2002) who did not find any effect on GOT and GPT enzymes due to adding yeast into broiler diets. Because GOT and GPT enzymes are related to the nutrients bioavailability in liver (Stanley et *al* 1993), therefore it can be suggested that, the addition of yeast into Japanese quail diets had no harmful effect on liver function. In contrast, the levels of GOT and GPT enzymes were affected by feeding other antimicrobial growth promoters such as zinc baciteracin (El Gendi *et al* 2000) and neomycin (Abde-Azeem 2002).

Effect on total bacteria, fungi and yeast

There was a significant sharp reduction in total count of bacteria and fungi per gram of ileal content (Table 6 & Fig.3). This result confirms the result of Eric Line *et al* (1998); Wray and Wray (2000) and Spring *et al* (2000) who reported that; yeast has inhibitory effect on intestinal, microflora. Eric Line *et al* (1998); explain the antagonistic action of yeast against intestinal microflora, as the enteropathogenic bacteria appear to adhere to the yeast surface and are removed from the birds when the yeast is voided.

Yeast counts were duplicated as dietary yeast levels increase (Table 6 & Fig. 3). This result is expected and indicated that, live yeast cells can withstand the acidic action of stomach juice and pass into small intestine.

Eric Line *et al* (1998); stated that, yeast has been shown to survive passage through low Ph environment of the proventriculus and gizzard of chickens to reach intestines Therefore it can be suggested that the addition of yeast into diets is a suitable mean for use as growth promoter.

Table (2): Effect of feeding different yeast levels on body weight of Japanese quail at various ages.

	Age in week				
Yeast levels	**1**	**2**	**3**	**4**	**5**
0.0%	31.3^{a}	51.9^{c}	84.0^{b}	131.9^{b}	171.4^{b} **(100%)**
0.5%	29.2^{ab}	55.2^{b}	90.5^{a}	131.1^{b}	170.5^{b} **(99%)**
1.0%	28.5^{b}	56.6^{b}	91.4^{a}	135.8^{b}	178.6^{ab} **(104%)**
1.5%	30.8^{ab}	60.3^{a}	94.2^{a}	146.9^{a}	181.7^{a} **(106%)**
2.0%	29.8^{ab}	59.9^{a}	84.2^{b}	136.5^{b}	174.0^{ab} **(101%)**

a,b means with different superscripts are significantly different ($p \leq 0.05$)
. ( ) % body weight as percentage of control al five weeks of age.

Table (3): Effect of feeding different yeast levels on feed consumption and feed efficiency ratio at two intervals

Yeast levels	Feed consumption		Feed efficiency (g.feed/g.gain)	
	Age in weeks			
	(0-3)	(0-5)	(0-3)	(0-5)
0.0%	207	520	2.73[a]	3.19 (100%)
0.5%	199	489	2.42[ab]	3.01 (94%)
1.0%	204	497	2.45[ab]	2.91 (91%)
1.5%	198	490	2.31[b]	2.82 (88%)
2.0%	206	514	2.70[a]	3.10 (97%)

a,b means with different superscripts are significantly different ($p \leq 0.05$).
( ) body weight as percentage of control al five weeks of age.

Table (4): Effect of feeding different yeast levels on small intestine thickness and some immunity organs.

Yeast levels	Small intestine			Immunity organs	
	Leng. (Cm)	Wt. (g)	Thick. (g/cm)	Bursa Wt. (g)	Spleen Wt.(g)
0.0%	71.2	3.20	0.045 (100%)	0.145	0.070
0.5%	71.3	2.85	0.040 (88%)	0.158	0.062
1.0%	76.8	2.61	0.034 (76%)	0.167	0.078
1.5%	74.3	2.45	0.033 (73%)	0.164	0.081
2.0%	72.8	2.62	0.036 (80%)	0.171	0.077

( ) % as percentage of control.

Table (5): Effect of yeast levels on some blood constituents and parameters

Items	Yeast levels				
	0.0	0.5%	1%	1.5%	2%
WBC's (10^3/mm)	14.33[b]	16.33[b]	18.33[ab]	21.67[a]	18.67[ab]
HI titre log_2	7.4[bc]	8.2[bc]	9.6[ab]	11.2[a]	6.80[c]
Total Protein (g/dl)	3.70[a]	3.31[a]	3.67[a]	3.82[a]	3.30[a]
Albumin (g/dl)	2.23	2.17	2.00	1.71	2.10
Globulins (g/dl)	1.47	1.14	1.67	2.11	1.20
Glob./Albu. ratio	0.66	0.53	0.84	1.2	0.57
GPT (u/ml)	25.0	26.2	27.0	27.0	27.2
GOT (u/ml)	79.4	76.4	75.6	73.4	70.4
T3 (ng/ml)	422[a]	358[b]	360[b]	276[c]	354[b]
T4 (ng/ml)	9.27	10.12	10.90	9.68	11.8

a,b,c means with different superscripts are significantly different ($p \leq 0.05$).
HI =Haemogglutination inhibition titre.

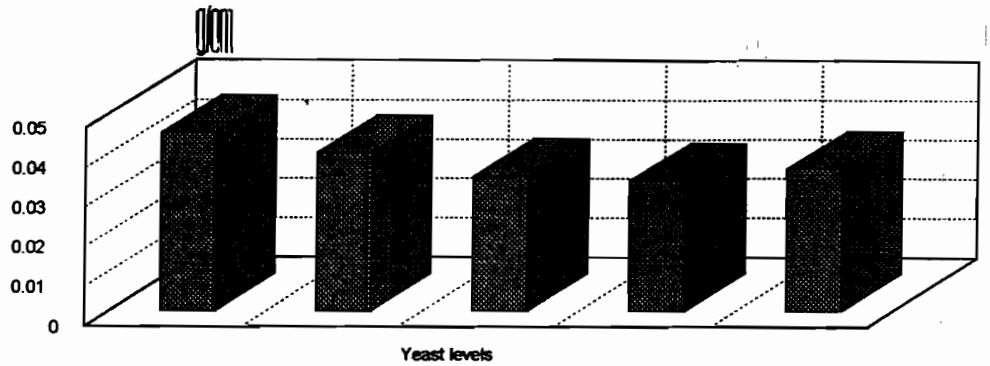

Fig 1. Effect of yeast levels on small intestine thickness (g/cm)

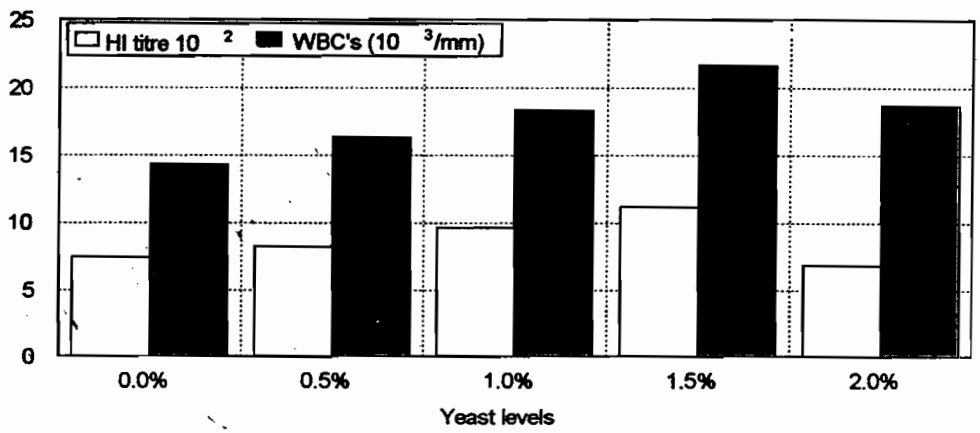

Fig 2. Effect of yeast levels on WBC's and HI titre

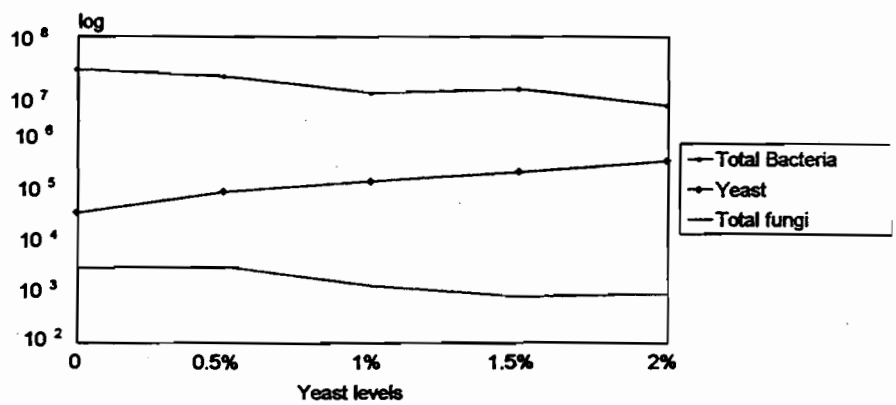

Fig 3. Effect of yeast levels on Total bacteria, fungi and yeast

Table (6): Effect of yeast levels on ileal content of total bacteria, fungi and yeast

	Yeast levels				
Items	**0.0**	**0.5%**	**1%**	**1.5%**	**2%**
Total Bacteria. $\times10^6$/g.	22.6^{a}	16.0^{ab}	7.6^{bc}	9.0^{bc}	4.2^{c}
Total fungi $\times10^3$/g.	2.9^{a}	3.1^{a}	1.3^{b}	0.8^{b}	0.9^{b}
Yeast $\times10^4$/g.	3.5^{c}	9.0^{bc}	14.5^{b}	22.0^{ab}	35.8^{a}

a,b,c means with different superscripts are significantly different ($p\leq0.05$).

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تأثير أضافة الخميرة النشطه لعلائق السمان اليابانى على الأداء الإنتاجى وبعض الصفات المناعيه والميكروبيولوجيه

ماجده محمد وكوك[1] – شعبان فتوح العفيفى[2] – نجلاء كمال سليمان[2] –مجدى عطيه[3]
هيئة الطاقه الذريه – مركز البحوث النوويه – قسم التطبيقات البيولوجيه[1]
قسم إنتاج الدواجن[2] – كليه الزراعه –. جامعه عين شمس – القاهره – مصر
قسم الميكروبيولوجيا الزراعيه[3] – المركز القومى للبحوث.

أجريت هذه الدراسه لمعرفة تأثير أضافة الخميره النشطه بمستويات متدرجه إلى علائق السمان اليابانى النامى على الأداء الإنتاجى وسمك الأمعاء وبعض الصفات المناعيه والميكروبيولوجيه.

تم إضافة الخميره للعلائق بمستويات ٠٪ (كونترول) و٠,٥ ٪ و١٪ و١,٥٪ و٢٪ وإستخدم فى الدراسه عدد ٥٠٠ طائر عمر يوم تم تقسيمهم إلى ٥ مجاميع وتم تغذيتهم على العلائق المختبره لمدة ٥ أسابيع.

وأشارت النتائج إلى مايلى:-

١- تحسن النمو خلال أسابيع التجربه المختلفه نتيجه لإضافة الخميره إلى علائق السمان وربما يرجع هذا التحسن إلى زيادة معدل الإستفاده من العناصر الغذائيه المختلفه فى الأمعاء نتيجه للتأثير المضاد للخميره على الميكروبات الضاره بالامعاء.

٢- تحسن معدل الإستفادة من الغذاء بمعدلات ٦٪ و٩٪ و١٢٪ و٣٪ نتيجة لإضافة الخميره بمستويات ٠,٥٪ و١٪ و١,٥٪ و٢٪ على التوالى. وكان هناك إنخفاض طفيف فى الإستهلاك الغذائى للمجاميع المغذاه على علائق الخميره.

٣- كذلك أوضحت الدراسه أن إضافة الخميرة لعلائق السمان يؤدى إلى:-
(أ)- إنخفاض فى سمك الأمعاء الدقيقه بصوره ملحوظه.
(ب)- زيادة وزن غدة البرزا والطحال المصاحب بالإرتفاع فى عدد كرات الدم البيضاء والأجسام المضاده ونسبة الجلوبيولين.

٤- أوضحت نتائج الفحص الميكروبيولوجى لمحتويات الأمعاء أن هناك إنخفاض شديد فى العدد الكلى للبكتريا والفطريات بالتوازى مع زيادة مستوى الخميره بالعلف والتى صاحبها زيادة عددخلايا الخميره فى الأمعاء.

هذا ويمكن الإشاره إلى أن الخميره أدت لتحسين الأداء الإنتاجى للسمان اليابانى نتيجة لتأثيرها المضاد على العترات الميكروبيه الضاره فى الأمعاء وكذالك تأثيرها الإيجابى فى تحسين المناعه.