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Immune Response, Antioxidant Biomarkers and Histology of Caecal Tonsils of quail Supplemented with Sodium Butyrate

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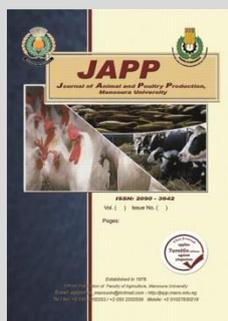
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ABSTRACT

This study aimed to evaluate the impacts of dietary supplementation of sodium butyrate (SB) on antioxidant biomarkers, immune response and caecal tonsils (CT) histomorphometry of quail. A total of 240 one-day-old quails were randomly allocated into four groups with three replicates each. The first group was fed a basal diet (BD) without SB (control, T1), the 2nd group fed 1 g SB/kg BD during the first period from 0-3 weeks, then BD until the sixth week as early short feeding SB (ESFSB, T2), the 3rd group fed 1 g SB/kg BD during the whole period from 0-6 weeks as long feeding SB (LFSB, T3), and the 4th group fed BD from 0-3 week then fed 1 g SB/kg BD during the second period from 3-6 weeks as late short feeding SB (LSFSB, T4). The SB supplementation in quail diet significantly increased ($P<0.05$) serum total antioxidant capacity and declined malondialdehyde level compared with the control group. The inclusion of SB had a higher immune response through the increase of SRBCs titer value ($P<0.05$) in ESFSB, LSFSB and LFSB groups than the control group. Histomorphometry parameters of CT were significantly improved in ESFSB and LFSB compared the control. The LFSB group fed a diet containing SB from 0-6 weeks had better antioxidant biomarkers, immune response and histomorphometry parameters of CT of quail. In conclusion, it is suggested feeding quail on diets containing SB through the whole growth period to display its positive influence on the antioxidant biomarkers and immunity of quail

Keywords: sodium butyrate; caecal tonsils; immunity; quail



INTRODUCTION

There are many approaches improve development of digestivetract and immune system. One of these approaches is the use of sodium butyrate (SB) in poultry diet. Once SB reaches the stomach of the bird, it rapidly releases the sodium ion (the first fraction) and owing to the low pH, the butyrate (the other fraction) is quickly converted to the undissociated form, termed the butyric acid (Elnesr *et al.*, 2020). The butyric acid or its sodium salt has received much attention and its supportive effects on the intestinal integrity and growth performance have been confirmed in poultry (Hu and Guo, 2007; Zhang *et al.*, 2011). The butyric acid is the major energy source to enterocytes and is necessary to the health of the intestinal mucosa (Isolauri *et al.*, 2004). Also, SB is commonly used to improve the general performance and health of the bird under commercial conditions (Ricke, 2003). Butyrate can be used by epithelial cells of the intestine as a direct energy source to stimulate their differentiation and proliferation and boost intestinal barrier function (Kinoshita *et al.*, 2002). SB increases the blood flow to the intestine that leads to better tissue growth and oxygenation (Reilly *et al.*, 1995). Butyric acid stimulates the functional development of the gastrointestinal tract (GIT) in terms of digestion and absorption of nutrients, and it induces peptide production in the distal GIT, as well as it encourages the development of the gut-associated lymphoid tissue and (Cox *et al.*, 2009).

Owing to the stated beneficial properties, the current study hypothesized that SB may be a possible candidate to enhance the immune status in quail. This product may improve the gut and modulate the systemic immune responses. The SB modulates the intestinal barrier function and immune-system (Bortoluzzi *et al.*, 2018). The GIT is not only an organ for digestion and absorption of nutrient, but also an organ for systemic immunity and at the same time performs a barrier function. The dietary SB may be conducive to the physiological functioning of the gut (Bortoluzzi *et al.*, 2017) and play an important role in maintaining the integrity of the intestinal mucosa (Zou *et al.*, 2019) as well as it can improve the balance of the intestinal microbiome (Yang *et al.*, 2018). The beneficial effects of SB were reported in previous studies, but there are still details to be clarified in poultry. Because of the best period of age in which the addition of SB is more effective on birds is not well understood, this work was being studied. The objective of the current study was to investigate the impacts of dietary supplementation of SB on caecal tonsils histomorphometry, antioxidant biomarkers and immune response of quail.

MATERIALS AND METHODS

Experimental design, birds, management and diets

The present study was conducted at Poultry Research Station belonging to the Poultry Production Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. A total of 240 one-day-old quail chicks

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were randomly allocated into four treatment groups with three replicates each. Birds were kept for 6 weeks on a floor system in separate pens. The study was included four experimental groups (3 replicates per group of 20 chicks in each). The first group fed basal diet without SB (control, T1), the 2nd group fed 1 g SB/kg basal diet during the first period from 0-3 weeks, then basal diet until 6 weeks as early short feeding SB (ESFSB, T2), the 3rd group fed 1 g SB/kg basal diet during whole period from 0-6 weeks as long feeding SB (LFSB, T3), and the 4th group fed basal diet from 0-3 week then fed 1 g SB/kg basal diet during the

second period from 3-6 weeks as late short feeding SB (LSFSB, T4), as shown in Figure 1. Sodium Butyrate was produced by Norel-Misr, Egypt. Experimental diets were formulated to cover the recommended requirements of Japanese quail birds during the growing period according to NRC (1994). Ingredients and chemical analysis of the basal diet were shown in Table 1. A lighting schedule was 24 hours daily through the experimental period, which lasted for 6 weeks. Then, birds were allowed to access *ad libitum* to feed and water.

Table 1. Ingredients and chemical analysis of the basal diet.

Ingredients		Chemical analysis	
Items	Amount (%)	Items	
Yellow corn	55.10	*Crude protein %	23.9
Soybean meal	33.45	*Ether extract %	4.5
Broiler concentrate meal	10	*Crude fiber %	3.9
Sodium chloride	0.15	Metabolizable energy (Kcal/kg)	2850
Limestone	0.9	Available phosphorus %	0.35
Lysine	0.10	Calcium %	0.80
Premix ¹	0.30	Total sulphur amino acids%	0.92
Total	100 %	Lysine%	1.30

¹Analysed composition

¹ Each 3 kg of premix supplies one ton of the diet with: Vit. D3, 2000000 IU; Vit. A, 12000000 IU; Vit. K3, 4g; Vit. E, 40g; Folic acid, 1.5g; Niacin, 30gm; Vit. B1, 3g; Vit. B2, 6g; Vit.B6, 4g; Biotin, 80mg; Vit.B12, 30mg; Pantothenic acid, 12g; Cu, 10g; Zn, 70g; Fe, 40g; Mn, 70g; Co, 250mg; I, 1.5g; Choline, 350g; Se, 200mg and complete to 3.0 Kg by calcium carbonate.

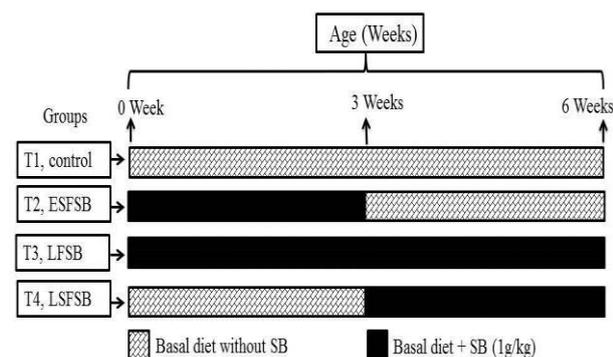


Figure 1. The design of the experimental treatments. Groups: T1 = Control (without SB); T2= ESFSB = early short feeding SB (0-3 weeks); T3 = LFSB = long feeding SB (0-6 weeks); T4 = LSFSB = late short feeding SB (3-6 weeks).

Immune response: Serum antibody level produced in response to sheep red blood cells (SRBCs) was measured to evaluate the immune response in SB-treated quails. At the 2 and 5 weeks of age, three birds from each treatment group were injected in the wing vein, using 0.1 ml of solution (10 % suspension SRBCs in phosphate buffer saline). At 3 and 6 weeks of age, the same birds slaughtered by severing the jugular vein, and the blood sample of each bird was collected in tubes and centrifuged at 3,000 rpm for 10 minutes to separate serum, and stored in -20°C until the time of antibody titer of SRBCs determination. The antibody response to SRBCs was measured in serum using the micro haemagglutination technique as described by Yamamoto and Glick (1982) and Dix and Taylor (1996). The reciprocal of highest dilution giving visible agglutination was the end point of titer and the values were expressed as log 2.

Histological Technique:

Six quails per group were slaughtered at 3 and 6 weeks of age. Samples from *caecal tonsils* were collected

and immersed directly in Bouin's fixative, dehydrated in ascending grades of ethyl alcohol and processed using paraffin technique. They were cut using rotatory microtome at 4-6 µm and mounted on clean, dry glass slides. The obtained sections were stained with Harris Haematoxylin and Eosin.

Histomorphometry of caecal tonsils

Most thick and thin parts of caecal tonsils, lymphatic follicles diameter and number and muscular layer width were measured. These measurements were obtained by the aid of Image J analysis software program, Microsoft Company using LEICA DFC290 HD system digital camera connected to the light microscope using 10X objective lens.

Antioxidant biomarkers

Total antioxidant capacity (TAC) was determined according to Koracevic *et al.* (2001). Malondialdehyde (MDA) was determined according to Ohkawa *et al.* (1979). Kits for estimation of these parameters were purchased from Biodiagnostic Company.

Statistical analysis:

All data were subjected to one-way ANOVA using IBM SPSS Statistics for Windows (IBM SPSS 22; IBM Corp., Armonk, New York, USA) and the means were compared for significance by post hoc Duncan's multiple range tests.

RESULTS AND DISCUSSION

Results

Serum antioxidant biomarkers

Results of serum antioxidant biomarkers as affected by dietary SB supplementation are shown in Table 2. At 3 weeks of age, serum total antioxidant capacity was significantly higher (P = 0.018) in the SB-treated groups (ESFSB and LFSB) than the untreated groups. At 6 weeks of age, the group supplemented with SB through the whole period had the highest (P<0.001) serum total antioxidant

capacity followed by the ESFSB and LSFSB groups compared with the control group. At 3 weeks of age, the SB-treated group recorded significantly high MDA level (P

$= 0.001$) compared with the untreated groups. At 6 weeks of age, MDA levels in ESFSB, LFSB and LSFSB groups were significantly lower ($P < 0.05$) than the control.

Table 2. Effects of sodium butyrate (SB) on serum antioxidant biomarkers of Japanese quail at 21 and 42 days of age.

Item	Treatments				SEM	P-value
	Control(T1)	ESFSB (T2)	LFSB(T3)	LSFSB (T4)		
At 21 Days						
TAC (mM/L)	0.522 ^b	0.626 ^a	0.643 ^a	0.523 ^b	0.015	0.018
MDA (nmol/ml)	14.97 ^a	12.89 ^b	12.70 ^b	14.04 ^a	0.156	0.001
At 42 Days						
TAC (mM/L)	0.615 ^c	0.726 ^b	0.774 ^a	0.689 ^b	0.007	<0.001
MDA (nmol/ml)	15.47 ^a	14.13 ^b	13.45 ^b	14.29 ^b	0.153	0.004

^{a-c}Means having different superscripts within each effect in the same row are significantly different at accompanied probability.

T1 = Control (without SB); T2= ESFSB = early short feeding SB (0-3 weeks); T3 = LFSB = long feeding SB (0-6 weeks);

T4 = LSFSB = late short feeding SB (3-6 weeks)

TAC = Total antioxidant capacity; MDA = Malondialdehyde; SEM = Standard Error Means.

Immune response

Significant changes in immune response among groups were presented in Table 3. At 3 weeks of age, SRBCs titer values in ESFSB and LFSB groups were

higher ($P = 0.006$) than the control and LSFSB groups.. At 6 weeks of age, SRBCs titer in LFSB group was higher than group ESFSB and LSFSB levels, and all were higher ($P = 0.002$) than the control group.

Table 3. Effects of sodium butyrate (SB) on immune response of Japanese quail at 21 and 42 days of age.

Item	Treatments				SEM	P-value
	Control(T1)	ESFSB (T2)	LFSB(T3)	LSFSB (T4)		
At 21 Days						
SRBCs titer	3.50 ^b	5.75 ^a	6.25 ^a	3.75 ^b	0.263	0.006
At 42 Days						
SRBCs titer	4.00 ^c	6.00 ^{ab}	7.25 ^a	5.75 ^b	0.222	0.002

^{a-c}Means having different superscripts within each effect in the same row are significantly different at accompanied probability.

T1 = Control (without SB); T2= ESFSB = early short feeding SB (0-3 weeks); T3 = LFSB = long feeding SB (0-6 weeks);

T4 = LSFSB = late short feeding SB (3-6 weeks)

SRBCs = sheep red blood cells; SEM = Standard Error Means.

Histomorphometry of caecal tonsils

Histomorphometry parameters of the caecal tonsils are offered in Table 4 and Figure 2. At 3 weeks of age, values of most thick and thin parts of caecal tonsils and lymphatic follicle diameter and number were significantly increased ($P < 0.05$) in the SB-treated groups compared with the untreated groups. In contrast, the values of muscular

layer width were significantly lower ($P < 0.0001$) in the SB-treated group than the other groups. At 6 weeks of age, the LFSB group fed a diet containing SB from 0-6 weeks had better histomorphometry parameters, followed by the ESFSB group fed SB from 0-3 weeks compared to other groups (the control and LSFSB).

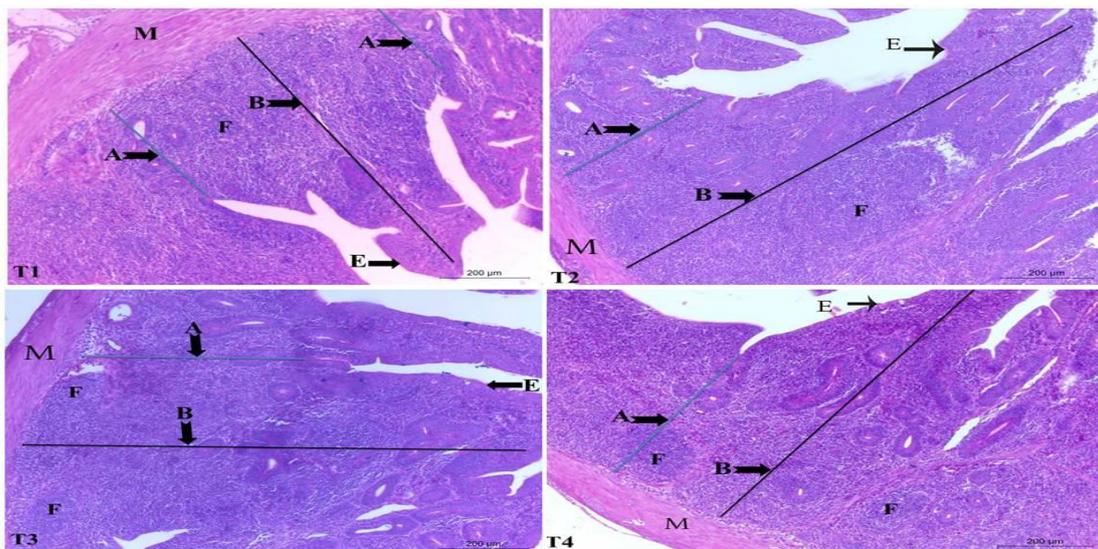


Figure 2. Photomicrograph of the caecal tonsil. The photomicrograph of caecal mucosa shows normal caecal tonsils. Note, thicker caecal mucosa in T2 and T3 groups in comparison to T1 and T4 groups. While the muscular layer was more thick in the T1 and T4 groups than T2 and T3 groups. H&E stain X100. Treatments: T1 = Control (without SB); T2= ESFSB = early short feeding SB (0-3 weeks); T3 = LFSB = long feeding SB (0-6 weeks); T4 = LSFSB = late short feeding SB (3-6 weeks). Abbreviations: A = the thinnest part of caecal submucosa; B = the thickest part of caecal submucosa; E = Epithelial lining of caecal tonsils; M = Muscular layer; F = lymphatic follicles

Table 4. Effects of sodium butyrate (SB) on histomorphometry of caecal tonsils of Japanese quail at 21 and 42 days of age.

Item	Treatments				SEM	P-value
	Control (T1)	ESFSB (T2)	LFSB(T3)	LSFSB (T4)		
At 21 Days						
Most thick part of CT (μm)	788.66 ^b	922.21 ^a	964.44 ^a	811.24 ^b	14.244	<0.001
Most thin part of CT (μm)	418.14 ^b	632.64 ^a	638.46 ^a	432.12 ^b	5.112	<0.0001
Follicle diameter (μm)	159.32 ^b	276.12 ^a	284.39 ^a	142.03 ^b	4.620	<0.0001
Follicle number per microscopic field	3.800 ^b	5.600 ^a	5.400 ^a	4.000 ^b	0.0523	0.0009
Muscular layer width (μm)	167.42 ^a	142.16 ^b	138.88 ^b	171.66 ^a	2.048	<0.0001
At 42 Days						
Most thick part of CT (μm)	1069.8 ^d	1500.7 ^b	1701.5 ^a	1249.4 ^c	15.27	<0.0006
Most thin part of CT (μm)	460.83 ^c	614.03 ^b	759.32 ^a	501.36 ^c	7.832	<0.0001
Follicle diameter (μm)	125.93 ^d	231.62 ^b	314.27 ^a	155.03 ^c	5.240	<0.0001
Follicle number per microscopic field	3.800 ^c	6.000 ^b	7.600 ^a	4.000 ^c	0.0374	0.0009
Muscular layer width (μm)	175.81 ^a	163.24 ^b	152.46 ^c	188.31 ^a	2.619	<0.0001

^{a-c}Means having different superscripts within each effect in the same row are significantly different at accompanied probability.

T1 = Control (without SB); T2= ESFSB = early short feeding SB (0-3 weeks); T3 = LFSB = long feeding SB (0-6 weeks);

T4 = LSFSB = late short feeding SB (3-6 weeks)

CT= caecal tonsils; SEM = Standard Error Means.

Discussion

In the quail life, the growth period is an important stage in realizing the long-term great health. The current study provides an additional knowledge to the inclusion of SB in growing quail diets. Many dietary supplements such as organic acids or their salts have been studied as potential replacements to maintain functions of the immune system. Butyrate carries multiple benefits for the gut health and integrity by stimulating the intestinal blood flow, absorption of water and electrolyte and mucin secretion (Canani *et al.*, 2011). SB has potential anti-inflammatory and immune-enhancing properties (Sunkara *et al.*, 2011), affecting the expression of inflammatory cytokines (Xu *et al.*, 2016). With regard to the results of immune response, it could say that the inclusion of SB in the quail diet through any period of growth (ESFSB, LFSB or LSFSB) offered an increase in SRBCs titer value compared to the control (without SB). These findings are similar to results of Sikandar *et al.* (2017) who noted higher geometric mean HI titers in SB-treated broiler chicks on day 35 compared to the untreated group, and thus it can be concluded that SB has a potential stimulatory effect on the immune system of chickens. Therefore, butyrate displays activity against certain the gut pathogenic bacteria supports the poultry immune system (Elnesr *et al.*, 2020). The dietary inclusion of SB showed remarkable benefits on immunestimulatory properties of broiler chicks, which has been highlighted by inducing host defense peptides (Sunkara *et al.*, 2011). In the birds, SB is readily transformed into butyric acid within the gut where it enhances the intestinal health (Ahsan *et al.*, 2016). It is known as an acidifier and used a worthy tool in maintaining the gut health (Elnesr *et al.*, 2020). As well, supplementation of SB in broiler diet has been related to improved immunity of these birds (Zhou *et al.*, 2014). Park *et al.* (2015) illustrated that SB supports the regulation and growth of the cells that maintain the immune system. SB showed augmenting immunity through the increase in phagocytosis and phagocytic index (El-Sheikh *et al.*, 2018). SB could stimulate host defense in chicken and regulates the macrophage activities in the intestine (Sunkara *et al.*, 2011).

Butyrate is of special importance because of its several affirmative impacts on the health of the gut and

extraintestinal tissues. The study on the effect of butyric acid or its sodium salt on antioxidant capacity of is limited, especially in poultry. The results of the present study declared that dietary SB supplementation in any period from the growth phase of quail improved serum antioxidant biomarker. In agreement with study of Zhang *et al.* (2011) who clarified that dietary SB addition declined the level of MDA and boosted the activities of catalase and superoxide dismutase in serum. In broiler chicks, dietary SB enhanced antioxidant properties and retarded damage of the mucosa by scavenging free radicals, where decreased MDA concentrations and demonstrated greater TAC (Wu *et al.*, 2018). The antioxidant property of butyric acid or its sodium salt remains unknown, therefore, it needs more interest and further studies.

The dietary SB can improve the mucosal function and intestinal morphology of broiler chickens (Jiang *et al.*, 2015). The structure of the caecal tonsils mucosal reflects gut health and immune status. In the current study, supplementation of SB showed beneficial effects on caecal tonsil structure. The improvement in the histomorphometry parameters of the caecal tonsil indicates an improvement in the immunity of birds, because the increase of the thickness of the caecal submucosa indicating increase the immunocompetant cells which increase the immunity. In addition, the increase in the number and diameter of lymphatic follicles indicating the increase of the immune cell accumulation and the increase of immunity. Generally, there was a converse relationship between the thickening of the caecal submucosa and the thickening of the muscle layer. Awaad *et al.* (2019) indicated that SB had marked immunostimulatory influence of the cecal tonsils that exhibited lymphoid activation and mitosis of lymphoid tissues that corresponded to those described by Vanhoutvin *et al.* (2009) who revealed that butyric acid or its sodium salt mediated the immune response. Probably, the immunomodulatory properties of SB vary depending on the GIT segment wherein the molecule is present because of metabolic differences among cell types along the avian GIT (Moquet *et al.*, 2016). From present findings, the improvement of antioxidant biomarkers and immune response of quail fed SB supplementation may lead to the improvement of general health of the birds.

CONCLUSION

The SB supplementation in quail diet significantly improved the antioxidant biomarkers and histomorphometry parameters of the caecal tonsils, as well as increased SRBCs titer value compared with the control. The LFSB group fed a diet containing SB from 0-6 weeks had better results in these parameters. Finally, it is suggested feeding quail on diets containing SB through the whole growth period.

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الاستجابة المناعية والمؤشرات الحيوية لمضادات الأكسدة وهستولوجي اللوز الأوروية للسمان المدعم بالصوديوم بيوتريت

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استهدفت الدراسة تقييم فاعليه اضافته الصوديوم بيوتريت لعلائق السمان على المؤشرات الحيوية لمضادات الاكسده والاستجابة المناعية ومقاييس الهستومورفوميترى للوزنين الاورينين للسمان. تم استخدام 240 ككتوت سمان عمر يوم ووزعت الى اربعة مجموعات كلا منها ثلاث مكررات. المجموعه الاولى تغذت على عليقه قاعديه بدون اضافته صوديوم بيوتريت (ككنترول)، المجموعه الثانيه تغذت على عليقه تحتوى 1 جرام صوديوم بيوتريت لمدته ثلاث اسابيع (0-3 اسابيع) ثم عليقه قاعديه حتى الاسبوع السادس، والمجموعه الثالثه تغذت على عليقه تحتوى 1 جرام صوديوم بيوتريت اثناء الفتره الكليه من 0-6 اسابيع ، والمجموعه الرابعه تغذت على عليقه قاعديه اول ثلاث اسابيع ثم عليقه تحتوى 1 جرام صوديوم بيوتريت خلال الفتره من 3-6 اسابيع . أوضحت النتائج أن اضافته الصوديوم بيوتريت فى علائق السمادات الى زياده معنويه فى مضادات الاكسده الكليه بالسيرم وقللت من مستوي المالونداي الدهيد مقارنة بالمجموعه الكنترول. ادخال الصوديوم بيوتريت فى العلائق زادت من مستوي الاستجابة المناعية من خلال زياده قيمة الاجسام المضادة ضد كرات دم الغنم فى الثلاث مجموعات المغذاه على الصوديوم بيوتريت خلال الفترات المختلفه (0-3 و 3-6 و 6-3 اسابيع) مقارنة بالكنترول. تحسنت معنويا مقاييس المورفوميترى الخاصه باللوز الأوروية فى المجموعات المغذاه علي صوديوم بيوتريت خلال الفتره من 0-3 او 0-6 اسابيع. المجموعه المغذاه على عليقه تحتوى صوديوم بيوتريت فى الفتره من 0-6 اسابيع كان لها افضل المؤشرات الحيوية لمضادات الاكسده والاستجابة المناعية والمقاييس الهستومورفوميترية للوز الأوروية. وقد خلصت الدراسة الى أنه يمكن التوصية بتغذيه السمان على علائق تحتوى على صوديوم بيوتريت طوال فتره النمو (0-6 اسابيع) ليظهر تأثيرها الايجابى على المؤشرات الحيوية لمضادات الاكسده والاستجابة المناعية فى طيور السمان.

الكلمات الداله: صوديوم بيوتريت، اللوز الأوروية، المناعه، السمان