The effect of *Stachys inflata* on the performance of broiler chickens and Newcastle Disease Antibody Titer

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Abstract

This study shows how performance and NDHI titers of broiler chickens are affected by the plant *Stachys inflatay*. One hundred and eighty, one day old Ross male broiler chickens were randomly divided into 5 groups of 36 (consisting of 3 replicates) and placed in 12 separate pens. Groups 1, 2, 3, 4 and 5 received 0, 0.15, 0.3, 0.45 and 0.6% *S.inflata* in their feed respectively from 8 to 42 days of age. Performance indexes were recorded on a weekly basis. At 42 days of age, serum and mid gut samples were taken for NDHI test and histopathological studies. The results were analyzed by the One Way ANOVA, Tukey test. The body weight of the second group was significantly ($P \leq 0.05$) higher than the control from the third week to the end. All treatment groups had HI titers higher than the control but the differences were not significant ($P > 0.05$). In the third group (0.3% *S.inflata*), relative liver and heart weight were significantly lower than control. No microscopic lesions were observed in mid gut in all experimental groups. It seems that *S. inflata* reduces harmful intestinal flora which causes better use of feed.

Key words: Broiler, NDHI titer, Performance, *Stachys inflata*

Introduction

The use of natural products is growing in the world, especially in developing countries such as China, India and Arabic countries. Parallel to the use of medicinal herbs in the treatment of human diseases, plants or plant originated compound have been used extensively within the veterinary field especially as natural growth promoters or feed supplement in the broiler industry.

One of the most important medicinal herb families is the *Lamiaceae*, whose related drugs have been used at times for different purposes. According to Skaltsa et al. the sub cosmopolitan genus known as *Stachys*, which is compromised of more than 270 species and is justifiably considered one of the largest genera of the *Lamiaceae* (Skaltsa et al., 2001). For several centuries, some *Stachys* species have been used as herbs for their potential health benefits (Zargari, 1990) and this would include the 34 species which are

found in Iran (Mozaffarian, 1996). Along with many other chemicals, Phytochemical lab tests on *Stachys* species have shown presence of phenylethanoid glycosides (Nishimura *et al.*, 1991; Miyase *et al.*, 1996), terpenoids, steroids (Ross and Zinchenko, 1975; Yamamoto *et al.*, 1994), diterpenes (Piozzi *et al.*, 1980) and flavonoids (Zinchenko, 1970; EL-Ansari *et al.*, 1991) along with some essential oils of some species (Skaltsa *et al.*, 1999; Cakir *et al.*, 1997). Pharmacological studies have shown that extracts of some *Stachys* species have anti-inflammatory, anti-toxic (Maleki *et al.*, 2001; Zinchenko *et al.*, 1981), anti-nephritic (Hayashi *et al.*, 1994a, 1994b), anti-hepatitis (Savchenko and Khvorostinka, 1978) and anti-anoxia (Yamahara *et al.*, 1990) properties. Phenylethanoid glycosides, triterpenoids and flavonoids are considered the active components responsible for biological reactions in the *Stachys* genus (Miyase *et al.*, 1996; Yamamoto *et al.*, 1994; Zinchenko, 1970; EL-Ansari *et al.*, 1991).

Related to this genus, the *S. inflata* is one of the known plants in which extracts have been used in Iranian folk medicine as treatment for infective, rheumatic and other inflammatory disorders (Maleki *et al.*, 2001). Several pharmaceutical effects have been proven for *S. inflata* related compounds and its different fractions in vitro and in vivo. For this reason, a study was designed to investigate the beneficial effects of this herb on the immune system by checking Newcastle disease (ND) antibody titer and performance of broiler chickens.

Material and methods

In this study, 180 day old male Ross 308 broiler chickens were randomly divided into 5 groups of 36 with three replicates of 12. The feed rations were formulated according to the standard of NRC. The flowering aerial parts of *S. inflata* including stems and leaves have been collected during the flowering period (June 2009), identified in Central Herbarium of Iran (Research Institute of Forest and Rangelands, Tehran). *S. inflata* were collected from Khalkhal, in the province of Ardabil, Iran. Dried plant materials were grounded to fine powder. Groups 2, 3, 4 and 5 received a balanced ration with 0.15%, 0.3%, 0.45% and 0.6% of *S. inflata* respectively. Group 1 as the control, received a balanced diet without any

S. inflata added to their feed until the end of the 42 days experiment. Chicks received dietary treatments at free access from 1 to 42 days of age. All chicks were vaccinated against ND, Infectious bronchitis (IB) and Infectious bursal disease (IBD) according to the regional standard vaccination program. All other management procedures were the same for all groups. The following parameters were recorded on a weekly basis: feed intake, mean body weight, feed conversion ratio (FCR) and mortality rate. Processing criteria obtained was body weight, liver weight, heart weight, gizzard weight, breast weight, thigh weight and abdominal fat weight. Four chicks from each pen were randomly selected and blood samples were collected for NDHI tests at 31 and 42 days of age (10 days after ND vaccination). Mid-guts of sacrificed birds of all groups were examined for histopathological changes. Other special conditions such as clinical signs and diarrhea index (by visiting the birds 3 times a day to access the probable incidence of diarrhea) were investigated throughout the experiment.

Statistical analysis

All data were analyzed by One Way ANOVA and in case of a significant difference also by Tukey's test.

Results:

Effects of *S. inflata* on Body Weight: At the end of the 3rd, 4th, 5th and 6th weeks, the mean body weight of group 2 (0.15%) was significantly higher than the control group ($P \leq 0.05$). In the 6th week, also among group 2 with groups 4 and 5, a significant difference was observed. At the end of the second week, weight gain had been more in all treatment groups than in the control group, but significant differences between any of the groups were not observed. At the 3rd, 4th and 5th weeks the statistical significant difference between the groups receiving 0.15 percent and the control group at the level of $P \leq 0.05$ was observed. At the 6th week the significant difference was observed between 0.15 percent recipient group among 0.45 and 0.6 percent recipient groups at the level of $P \leq 0.05$.

Effect of *S. inflata* on feed intake (FI): No significant difference on feed intake between groups was observed ($P > 0.05$). The feed consumption during 1-42 days was approximately the same in all groups, and there was no significant difference at the level of $P \leq 0.05$ in different groups (Table 2).

Effect of *S. inflata* on feed conversion ratio (FCR): Feed conversion ratio at 3 weeks of age was significantly improved when 0.15% of *S. inflata* was added to the control diet. According to table 3, observation showed that FCR at 21 days old chicks in the 0.15 percent received group was significantly ($P \leq 0.05$) less than in the control group. In the rest of the growing period 21 days through to 42 days, the 0.15 percent recipient group had a lower FCR, but there were no significant differences ($P > 0.05$).

Effects of *S. inflata* on NDHI titers: Newcastle antibody titers were also measured in two stages, once at day 31 and again at day 42 days where the results are expressed in table 4. According to these results, all groups which used *S. inflata* in their premixes had greater antibody titers than the control group at these two stages. No significant differences were observed in any of these groups ($P > 0.05$).

Effect of *S. inflata* on the relative weight of breast, thigh muscles, liver, gizzard, heart, abdominal and gizzard fat: As it is observed in table 5, the percentage of breast's weight and thigh muscle's weight between the groups were not significant ($P > 0.05$). Treatment groups 2 and 3 with 0.15% and 0.3% *S. inflata* had the lowest liver weight respectively. Between groups 3 with 0.3% *S. inflata* and the control group, there is a relatively noticeable liver weight difference ($P \leq 0.05$). The gizzard's weight from the groups that were treated showed no differences ($P > 0.05$). The highest and lowest heart weight was related to the control and 0.3% treatment group respectively ($P \leq 0.05$). No significant difference was observed among the experimental groups in abdominal and gizzard fat ($P > 0.05$).

Effect of *S. inflata* on pathological examination in mid gut: No microscopic lesions were discovered in the mid-gut of all the groups. Observations show that the lowest diameter of mid gut was related to 0.45% and 0.15% groups respectively but no significant difference was observed between the groups ($P > 0.05$). Also between the diameter of mucosal and sub-mucosal layer, the lowest measurement of mucosal and sub-mucosal layer of mid-gut belongs to the 0.45% group. (Table 6)

Discussion:

Body weight gains by using *S. inflata* could be in relation to higher anti-oxidants, anti-microbial properties, and anti-inflammatory properties. It seems that *S. inflata* reduces harmful intestinal flora which causes better use of feed. On the other hand, *S. inflata* can prevent the feed from harmful effects of mycotoxins. Furthermore, *S. inflata* with its high anti-inflammatory properties, can reduce layers of intestinal inflammation and lead to better feed absorption. Anti-oxidant activities of the plant may also prevent and protect the chickens ration from oxidation and save the value of vitamins and proteins of the diet.

There was no significant difference on feed intake among the treatment groups and the control group ($P > 0.05$). Lack of significant differences between the control group and all of the groups receiving *S. inflata* showed that improvement in overall weight in the recipient groups is not due to higher feed consumption, but due to the better use of food components and increase efficiency of the feed. It could be a postulated improvement in FCR by reducing inflammation and harmful intestinal bacteria and improve the feed absorption from the gastro-intestinal tract.

Although there were no significant differences in NDHI observed in any of the groups ($P > 0.05$), antibody titers in all groups which had used *S. inflata* as a premix in ration was greater than the control groups antibody titer. Since stress and sickness are two factors that cause vaccination failure, perhaps sedative, anti-inflammatory and anti-microbial properties of this plant could protect chickens against pathogens and stress.

Considering that the relative weight of carcasses, breasts and thigh muscles are mainly affected by genetic factors, inclusion of the feed with *S. inflata* cannot significantly alter these relative weights of thigh muscles and breasts simultaneously.

There was no significant difference between the abdominal fat and the fat around the gizzard of treatment groups and the control group ($P > 0.05$). This could be (evidence) postulated that adding *S. inflata* to the feed has no detrimental effect on the abdominal fat metabolism.

In 2007, the effect of *Satureja hortensis* (*S. h.*) on performance of broiler chickens and NDHI titers were investigated. The results showed birds that received 0.3% *S. h.* had a higher body weight and lower FCR, but the differences were not significant ($P \geq 0.05$). All groups that received *S. h.* in ration had higher HI titers than the control group. Adding *S. h.* to the diet had no effect on the relative weight of breasts, thigh muscles, abdominal fat and histological structures of the liver and kidney samples (Zamanimoghaddam et al., 2007).

Dulger and Gonuz (2004., 2005) reported the antimicrobial activity of some endemic *Stachys* species which includes *S. sivasica*, *S. anamurensis*, *S. cydnia*, *S. aleurites* and *S. pinardii*. In 2008, research was done on the antimicrobial activities of the methanol extracts from dried flowering aerial parts of *S. byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* (Labiatae). According to this research, “Flavonoids may be responsible for their antibacterial activity,” which is extracted from the aerial parts of the genus; *Stachys* (Saeedi et al., 2008).

Hydro-alcoholic extract of *S. inflata* has been demonstrated to exert potent anti-inflammatory effects in peripheral inflammation which is induced by carrageenan or formalin in rat paw (Maleki et al., 2001). In another study, the Hydro-alcoholic extracts of *S. inflata* reduced infarct size with a low dosage. This corresponds with the findings of Maleki and co-workers (2001) who revealed that the higher dose of the extract had no anti-inflammatory effects. This result suggests that hydro-alcoholic extracts from aerial parts of *S. inflata* may play a major role in reducing the infarct size (Garjani et al., 2001).

In other studies, there were anti-inflammatory effects of hydro-alcoholic and ethyl acetate extracts from *S. schtschegleevii* in both the flowering and sterile tops (Nazemiyeh et al., 2007; Maleki et al., 2008). The anti-inflammatory and anti-nociceptive properties of total methanolic extracts of the flowering aerial parts of two *Stachys* species (Rezazadeh et al., 2005). Anti-inflammatory and anti-hyperalgesic activities of *Stachys athorecalyx* extracts on CFA-induced Inflammation (Rezazadeh et al., 2009) were also observed.

The stabilizing effects of methanolic extract of *S. byzantina*, *S. inflata* and *S. laxa* on sunflower oil as antioxidant agents were observed by Morteza-Semnani et al., 2006 and the results indicated that *S. laxa* had a potential source of antioxidants. In 2009, *S. persica* and *S. fruticulosa* revealed anti-oxidant effects approximately two times greater than *S. laxa*

(Khanavi et al., 2009). There were also many other studies on the anti-oxidant effects of *S. inflata*, *S. byzantina*, *S. setifera* and *S. laxa* (Erdemoglu et al., 2006; Morteza-Semnani et al., 2006). However, because various methods have been used, the results are not fully comparable.

References

Cakir A, Duru ME, Harmandar M, Izumi S, Hirata T (1997): The volatile constituents of *Stachys balansae* L. from Turkey. Flavour Fragr J 12, 215-218.

Dulger B, Gonuz (2004): Antimicrobial activity of some endemic *Verbascum* , *Salvia* and *Stachys*. Pharm Biol 42, 301-304.

Dulger B, Ugurlu E, Aki C, Suerdem TB, Camdeviren A, Tazeler G (2005): Evaluation of antimicrobial activity of some endemic *Verbascum* , *Sideritis* and *Stachys* species from Turkey. Pharm Biol 43, 270-274.

EL-Ansari MA, Barron D, Abdalla MF, Saleh NAM, LE Quere JL (1991): Flavonoid constituents of *Stachys aegyptica*. Phytochemistry 30, 1169–1173.

Erdemoglu N, Turan NN, Cak_c I, Sener B, Ayd_n A (2006): Antioxidant activities of some Lamiaceae plant extracts. Phytother. Res. 20, 9-13.

Garjani A, _Maleki N, Nazemieh H, _Mansori A (2001): The effects of *stachys inflata* on myocardial infarct size in rats. Journal of Molecular and Cellular Cardiology 33, A38.

Hayashi K, Nagamatsu T, Ito M, Hattori T, Suzuki Y (1994): Acteoside, a component of *Stachys sieboldii* MIQ, may be a promising antinephritic agent (1): Effects of acteoside on crescentic-type anti-GBM nephritis in rats. Jpn J Pharmacol 65, 143–151.

Hayashi K, Nagamatsu T, Ito M, Hattori T, Suzuki Y (1994): Acteoside, a component of

Stachys sieboldii MIQ, may be a promising antinephritic agent (2): Effects of acteoside on leukocyte accumulation in the glomeruli of nephritic rats. *Jpn J Pharmacol* 66, 47–52.

Khanavi M, Hajimahmoodi M, Cheraghi-Niroomand M, Kargar Z, Ajani Y, Hadjiakhoondi A, Oveisi MR (2009): Comparison of the antioxidant activity and total phenolic contents in some *Stachys* species. *African Journal of Biotechnology* 8, 1143-1147.

Maleki N, Garjani A, Nazemiyeh H, Nilfouroushan N, Eftekharsadat AT, Allameh Z, Hasannia N (2001): Potent anti-inflammatory activities of hydroalcoholic extract from aerial parts of *Stachys inflata* on rats. *J Ethnopharmacol* 75, 213–218.

Maleki N, Nazemiyeh H, Maddah N, Mehmani F, Garjani A (2008): Screening of extracts and fractions from aerial parts of *Stachys schtschegleevii* sosn. for anti-inflammatory activities. *Pak J Pharm Sci* 21, 338-343.

Miyase T, Yamamoto R, Ueno A (1996): Phenylethanoid glycosides from *Stachys officinalis*. *Phytochemistry* 43, 475–479.

Morteza-Semnani K, Saeedi M, Shahani S (2006) Antioxidant activity of the methanolic extracts of some species of *Phlomis* and *Stachys* on sunflower oil. *Afr. J. Biotechnol* 5: 2428-2432.

Mozaffarian V (1996): A dictionary of Iranian plant names. Farhang Moaser Press, Iran, pp.522-524.

Nazemiyeh H, Maleki N, Mehmani F, Kumarasamy Y, Shoeb M, Garjani A, Sarker SD (2007): Assessment of anti- inflammatory properties of ethyl acetate extract of *Stachys schtschegleevii* Sosn. *DARU* 15, 174-182.

Nishimura H, Sasaki H, Inagaki N, Chin M, Mitsushashi H (1991): Nine phenethyl alcohol glycosides from *Stachys sieboldii*. *Phytochemistry* 30, 965–969.

Piozzi F, Savona G, Hanson JR (1980): Kaurenoid diterpenes from *Stachys lanata*. *Phytochemistry* 19, 1237.

1 Rezazadeh S, Kebryaezadeh A, Pirali-Hamedani M, Shafiee, A, Gharuni Isfahani S
2 (2005): Anti-inflammatory and analgesic activity of methanolic extracts of aerial parts of
3 *Stachys Schtschegleevii* Sosn. and *Stachys Balansae* Boiss. and *Kotschy ex Boiss* in rats.
4 DARU

5 Rezazadeh SH, Zaringhalam J, Manaheji H, Kebryaezadeh A (2009): Anti-inflammatory
6 and anti-hyperalgesic activities of *Stachys athorecalyx* extracts on CFA-induced
7 Inflammation. *Journal of Medicinal Plants Research* 3, 368-376.

8 Ross SA, Zinchenko TV (1975): Study of triterpenoids and steroids of *Stachys palustris* L.
9 *Farm Zh (Kiev)* 30, 91–92.

10 Saeedi M, Morteza-Semnani K, Mahdavi MR, Rahimi F (2008): Antimicrobial studies on
11 extracts of four species of *Stachys*. *Indian journal of Pharmaceutical science* 70, 403-406.

12 Savchenko VM, Khvorostinka VM (1978): Effects of a preparation from *Stachys inflata* on
13 the course of experimental hepatitis in rats. *Farm Zh (Kiev)* 33, 50–53.

14 Skaltsa HD, Lazari DM, Chinou IB, Loukis AE (1999): Composition and antibacterial
15 activity of the essential oils of *Stachys candida* and *S. chrysanthra* from southern Greece
16 [letter]. *Planta Med* 65, 255–256.

17 Skaltsa HD, Mavrommati A, Constantinidis T (2001): A chemotaxonomic investigation of
18 volatile constituents in *Stachys* subsect: Swainsonianeae (Labiatae). *Phytochemistry* 57,
19 235-44.

- 1 Zanimoghaddam AK, Ghannadi AR, Gaffaian A, Shojadoost B (2007): The effect of
- 2 *Satureja hortensis*(*S.h*) on performance of broiler chickens and NDHI titers. XVI European
- 3 Symposium on Poultry Nutrition, August 2007, France.
- 4 Zargari A (1990): Medicinal Plants, Vol.4. Tehran University Publication, Tehran. pp. 123.
- 5 Zinchenko TV (1970): Flavonoid glycosides of *Stachys neglecta*. Farm Zh (Kiev) 25, 81–
- 6 82.
- 7 Zinchenko TV, Voitenko GN, Lipkan G.N (1981): Anti-inflammatory, antitoxic and
- 8 hypoazotemic effect of a *Stachys recta* preparation, stachyrene. Farmakol Toksikol 44,
- 9 191–194.
- 10 Yamahara J, Kitani T, Kobayashi H, Kawahara Y (1990): Studies on *Stachys sieboldii*
- 11 MIQ.II. Antianoxia action and the active constituents. Yakugaku Zasshi 110, 932–935.
- 12 Yamamoto R, Miyase T, Ueno A (1994): *Stachys* saponins I –VIII, new oleanane-type
- 13 triterpene saponins from *Stachys riederi* Chamisso. Chem Pharm Bull 42, 1291–1296.

14 **Table 1- Effect of *S.inflata* on Body Weight**

Mean Body Weight (gr) ± SD						
	1 st	2 nd	3 rd	4 th	5 th	6 th
0	113.19 ± 3.18	265.27 ± 20.56	538.19±101.05 ^a	988.14±141.04 ^a	1575.06±218.91 ^a	2089.54±277.69 ^a
1.5kg/ton	115.27 ± 2.40	288.88 ± 13.39	616.64 ± 81.47 ^b	1088.51±114.83 ^b	1730.30±164.44 ^b	2274.69±215.21 ^b
3 kg/ton	111.11 ± 2.41	283.33 ± 14.43	579.17±103.35 ^{ab}	1061.41±136.10 ^{ab}	1612.12±240.14 ^{ab}	2166.68±263.34 ^{ab}
4.5kg/ton	110.41 ± 5.51	281.94 ± 25.12	578.06±120.73 ^{ab}	1050.40±144.96 ^{ab}	1612.29±254.57 ^{ab}	2102.69±283.92 ^a
6 kg/ton	113.19 ± 4.34	293.05 ± 10.48	580.64 ± 15.91 ^{ab}	1044.28±104.49 ^{ab}	1590.69±192.42 ^{ab}	2081.11±253.97 ^a

* In each column, different letters indicates of significant differences ($P \leq 0.05$)

Table 2- Effect of *S.inflata* on Feed intake (FI)

16

Feed intake $\pm$ SD					
	2 nd	3 rd	4 th	5 th	6 th
0	443.05 $\pm$ 13.39	395.16 $\pm$ 19.24	682.09 $\pm$ 21.00	1015.85 $\pm$ 42.86	946.28 $\pm$ 99.36
1.5 kg/ton	444.44 $\pm$ 19.24	404.38 $\pm$ 9.58	694.25 $\pm$ 34.67	1025.53 $\pm$ 86.87	975.57 $\pm$ 14.63
3 kg/ton	441.66 $\pm$ 12.50	397.44 $\pm$ 4.57	695.66 $\pm$ 14.74	1011.54 $\pm$ 54.13	966.19 $\pm$ 30.04
4.5 kg/ton	429.16 $\pm$ 11.02	392.61 $\pm$ 8.74	692.74 $\pm$ 35.61	1020.83 $\pm$ 66.81	966.99 $\pm$ 66.31
6 kg/ton	429.16 $\pm$ 12.50	400.83 $\pm$ 2.87	683.66 $\pm$ 33.42	990.44 $\pm$ 31.16	910.10 $\pm$ 72.64

1 * In each column, different letters indicates of significant differences ($P \leq 0.05$)

Table 3- Effect of *S.inflata* on Feed Conversion Ratio (FCR)

2

Feed Conversion Ratio $\pm$ SD					
F.C.R	3 rd	4 th	5 th	6 th	Total FCR
0	1.63 $\pm$ 0.1 ^a	1.59 $\pm$ 0.03	1.68 $\pm$ 0.09	1.81 $\pm$ 0.01	1.68 $\pm$ 0.01
1.5 kg/ton	1.41 $\pm$ 0.02 ^b	1.52 $\pm$ 0.03	1.60 $\pm$ 0.03	1.73 $\pm$ 0.01	1.57 $\pm$ 0.14
3 kg/ton	1.53 $\pm$ 0.01 ^{ab}	1.53 $\pm$ 0.07	1.66 $\pm$ 0.02	1.82 $\pm$ 0.08	1.64 $\pm$ 0.14
4.5 kg/ton	1.57 $\pm$ 0.08 ^{ab}	1.54 $\pm$ 0.07	1.69 $\pm$ 0.07	1.98 $\pm$ 0.24	1.69 $\pm$ 0.20
6 kg/ton	1.49 $\pm$ 0.05 ^{ab}	1.61 $\pm$ 0.15	1.69 $\pm$ 0.03	1.98 $\pm$ 0.25	1.69 $\pm$ 0.21

* In each column, different letters indicates of significant differences ($P \leq 0.05$) 3

Table 4- Effect of *S.inflata* on NDHI titer

4

HI	At 31 days	At 42 days
0	3.45 $\pm$ 1.51	4.17 $\pm$ 1.27
1.5 kg/ton	4.10 $\pm$ 1.79	4.73 $\pm$ 1.10
3 kg/ton	4.11 $\pm$ 1.54	4.82 $\pm$ 1.33
4.5 kg/ton	3.67 $\pm$ 1.12	4.50 $\pm$ 1.45
6 kg/ton	4.54 $\pm$ 1.92	4.50 $\pm$ 0.80

* In each column, different letters indicates of significant differences ($P \leq 0.05$) 1

Table 5 – Effect of *S.inflata* on the relative weight of breast and thigh muscles, liver, gizzard, heart, abdominal and gizzard fat 3

the relative weight $\pm$ SD							
	Breast	Thigh	Liver	Gizzard	Heart	Abdominal fat	Gizzard fat
0	21.00 $\pm$ 2.13	21.00 $\pm$ 1.17	2.39 $\pm$ 0.23 ^a	1.60 $\pm$ 0.23	0.46 $\pm$ 0.05 ^a	0.88 $\pm$ 0.29	0.48 $\pm$ 0.13
1.5 kg/ton	21.30 $\pm$ 0.73	21.68 $\pm$ 1.15	1.90 $\pm$ 0.23 ^{ab}	1.58 $\pm$ 0.13	0.43 $\pm$ 0.05 ^{ab}	0.89 $\pm$ 0.38	0.53 $\pm$ 0.34
3 kg/ton	21.51 $\pm$ 0.60	21.36 $\pm$ 0.46	1.81 $\pm$ 0.56 ^b	1.31 $\pm$ 0.17	0.33 $\pm$ 0.11 ^b	0.92 $\pm$ 0.55	0.29 $\pm$ 0.06
4.5 kg/ton	20.67 $\pm$ 0.74	20.99 $\pm$ 0.73	2.22 $\pm$ 0.26 ^{ab}	1.52 $\pm$ 0.15	0.42 $\pm$ 0.05 ^{ab}	0.94 $\pm$ 0.30	0.60 $\pm$ 0.31
6 kg/ton	21.60 $\pm$ 0.68	20.76 $\pm$ 0.80	2.14 $\pm$ 0.23 ^{ab}	1.51 $\pm$ 0.17	0.43 $\pm$ 0.06 ^{ab}	1.06 $\pm$ 0.16	0.46 $\pm$ 0.31

* In each column, different letters indicates of significant differences ($P \leq 0.05$) 4

Table 6- Effect of *Stachys inflata* on pathological examination in mid gut 5

	diameter of mid gut	diameter of mucosal and sub mucosal layer
0	169.67 $\pm$ 23.50	129.36 $\pm$ 23.82 ^{bc}
1.5 kg/ton	159.58 $\pm$ 39.05	125.38 $\pm$ 30.25 ^b
3 kg/ton	187.13 $\pm$ 34.50	153.23 $\pm$ 25.15 ^{ac}
4.5 kg/ton	158.83 $\pm$ 31.64	115.45 $\pm$ 14.09 ^b
6 kg/ton	180.20 $\pm$ 28.46	160.65 $\pm$ 16.49 ^{ac}

* In each column, different letters indicates of significant differences ($P \leq 0.05$) 6