Effects of *in Ovo* Injection and Inclusion a Blend of Essential Oils and Organic Acids in High NSPs Diets of Broiler Breeders on Performance of Them and Their Offspring

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Two factorial completely randomized design trials 2×2 and 2×2×2 were conducted to evaluate the effect of a blend of essential oils and organic acids (Biacid™) in broiler breeder diets at two levels, two dietary non-starch polysaccharides (NSPs) levels and *in ovo* injection of Biacid™ on their progenies performance, respectively. 240 broiler breeders of Ross 308 strain were housed from the age of week 44th for 12 weeks in four groups. 120 produced eggs from each group were divided in two groups of 60 eggs for injecting by 0.5 ml of Biacid™ or distilled water. Injection was done during transferring from setter to hatcher in day 18th of incubation. Twenty-five cockerels from each of 8 treatments were housed into separate pens. Using Biacid™ and high NSPs in broiler breeders’ ration affected hatchability, embryo mortality, weight of day old chicks and progenies’ carcass yield significantly (p<0.05) whereas *in ovo* injection of Biacid™ did not show significant effects in this regards (p≥0.05). Offspring’s abdominal fat was neither affected by broiler breeders’ rations not *in ovo* injection of Biacid™ (p≥0.05). Biacid™ and high NSPs content in broiler breeders’ ration affected all primary and secondary humoral immune responses of progenies against sheep red blood cells (p<0.05). *In ovo* injection of Biacid™ increased the primary IgG, primary IgT and secondary IgG responses (p<0.05). The interaction of the effects of Biacid™ and high NSPs in broiler breeders’ ration and also *in ovo* injection of Biacid™ affected progenies’ weight gain, feed intake, feed conversion ratio and European production index significantly (p<0.05). It seems that using Biacid™ in broiler breeders’ diet can modify the undesirable effects of high NSPs content of breeders’ ration on performance of their offspring.

**Key words:** essential oils, *in ovo* injection, organic acids, non-starch polysaccharides

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**Introduction**

The idea of this study was based on nutrigenomics concepts which indicate that performance of offspring can be affected by breeder’s rations. Due to the rising cost of corn, poultry feed industry tend to use cheaper sources of cereals that naturally contain high NSPs. Carbohydrates are the main energy source of poultry rations. Apart from glucose, fructose, sucrose and starch, other carbohydrates cannot be digested by poultry digestive enzymes and mainly fermented by gut microflora (Yasar, 2003; Wu *et al*., 2004; Masey *et al*., 2005; Masey O’Neill *et al*., 2014). Researches have indicated that only water-soluble carbohydrates can be fermented by gut microflora (Jozefiak *et al*., 2004; Moharrery *et al*., 2005). It is known that NSPs are viscous water-soluble compounds which their anti-nutritional effects in poultry diets lead to decreasing the performance via decreasing the digestibility of nutrients (Garcia *et al*., 2003; Moharrery *et al*., 2005; Masey O’Neill *et al*., 2014). Undigested nutrients are the main reason of disrupting the balance of gut microflora. Since gut microflora affects gut morphology, digestion and immune system directly and indirectly, it can influence the poultry health status. Because of being relatively unstable, gut microflora can be affected simply by nutritional factors (Antongiovanni *et al*., 2007; Deriu *et al*., 2008; Isabel and Santos, 2009; Khodambashi *et al*., 2013; Masey O’Neill *et al*., 2014). Many additives are being used in modern poultry industry for stimulating growth, improving performance and...
health enhancing (Velasco et al., 2010; Toit, 2011; Milbradt et al., 2014). These additives not only improve the performance and feed efficiency, but also influence the health status. By considering the probability of side effects of some of additives on disrupting gut microflora and also residue of them in poultry meat, using of them should be careful (Isabel and Santos, 2009; Bravo et al., 2014; Milbradt et al., 2014).

Essential oils are volatile oils extracted mainly from plant products by steam water distillation or enzyme activity followed by steam water distillation. Essential oils comprise a multitude of components, such as terpenoids, alcohols, aldehydes, acyclic esters and etc. These substances are in fact the active components that give each essential oil their unique properties. Many of the components of essential oils have multifunctional properties. Cinnamaldehyde, eugenol and menthol as example, are known to increase voluntary feed intake as well as stimulating nutrient digestion and exhibiting anti-bacterial properties (Lee et al., 2004; Kamataou et al., 2005; Oussalah et al., 2007; Santurio et al., 2007; Rusenova and Parvanov, 2009; Bravo et al., 2014). As many essential oils have anti-microbial properties, many researches have been conducted to use them as alternative for antibiotics (Schelz et al., 2006; Santurio et al., 2007; Karadas et al., 2013; Shapiro et al., 2013; Bravo et al., 2014). The correct combination of essential oils can exhibit greater responses than when individual components are used alone, so in other word they express synergy (Deriu et al., 2008; Horosova et al., 2012; Shapiro et al., 2013; Bravo et al., 2014).

Volatile fatty acids (VFAs) have been used as feed and drinking water additives in poultry for many years. Their beneficial effects have been mainly limited to crop and gizzard by reducing bacterial growth in the ingesta but not having any significant effect in the intestinal tract since they are metabolized and absorbed (Van Immerseel et al., 2005; Zhonghong and Yuming, 2006; Antongiovanni et al., 2007; Khodambashi et al., 2013). Today, technology exists to coat VFAs in order to obtain a slower and gradual release in the intestine and ceca of poultry for preserving and extending their bacteriostatic activity (Al-Zenki et al., 2009; Zirelbeke and Belguom, 2013). VFAs are widely used in food and feed industry as preservatives because of their strong bacteriostatic action. The positive effects of VFAs can be explained by several mechanisms, including pH lowering effect, bacteriostatic activities and metabolic properties of their anionic part (Dibner and Boutin, 2002; Hernandez et al., 2006; Milbradt et al., 2014). A reduction in the pH value of the stomach by ingesting acids in feed can inhibit bacterial growth. Although pH reducing effect of VFAs in the stomach may not be significant, a reduction in pH value in crop can be important (Paul et al., 2007; Isabel and Santos, 2009; Khodambashi et al., 2013). Unlike inorganic acids, VFAs are easily absorbed through the cell wall of bacteria and damage the structure of genome in the cells which lead to disrupting bacterial multiply, so cell death can accrue (Van Immerseel et al., 2006; Isabel and Santos, 2009; Milbradt et al., 2014). Several studies indicated that metabolic effect is an important aspect of VFAs for poultry. For example butyric acid has several physiological functions such as improved cell proliferation and stimulation of protein synthesis (Zhonghong and Yuming, 2006; Antongiovanni et al., 2007; Kim and Paik, 2007). Generally, poultry industries intend to use mixture of VFAs or their salts, since they may express synergy (Isabel and Santos, 2009; Milbradt et al., 2014).

Due to the positive impact on microflora and health promotion properties of essential oils and VFAs, it seems that using them in broiler breeder diets may have positive effects on hatchability. Moreover, some nutrigenomics researches imply on influences of breeder rations on progeny performance, hence it would be expected that manipulation of breeder rations lead to better performance and improved health status in their offspring (Johanna et al., 2006; Rebel et al., 2006; Koedijk et al., 2010; Van Emous et al., 2015). Moreover in ovo injection has been done in this research for studying the probability of substituting in ovo injection of Biacid™ for feeding it to broiler breeders.

Since essential oils and VFAs have ability to control the gut microflora and also enhance the immune system (Van Immerseel et al., 2005, 2006; Kim and Paik, 2007; Oussalah et al., 2007; Rusenova and Parvanov, 2009; Khodambashi et al., 2013) and the other hand the negative effect of high NSPs content diets on immune system (Jozefiak et al., 2004; Lyte, 2004; Friedman and Bar-Shair, 2005; Liu et al., 2013; Masey O’Neill et al., 2014), the influence of using essential oils and VFAs in high NSPs content diets should be studied.

Materials and Methods

Two factorial completely randomized design trials 2×2 and 2×2×2 were conducted. Experimental factors were included using Biacid™ or lack of it in breeder rations, high NSPs in breeder rations and in ovo injection of Biacid™ or distilled water in produced eggs. Treatments are mentioned in Table 1. Blend of essential oils and VFAs have been provided by Provimi® Company. Biacid™ consist of citric acid, calcium butyrate, calcium lactate, calcium fumarate, cinnamaldehyde, thymol and carvacrol. The density of Biacid™ is 600–800 kg/m³ and pH is 4–4.5.

An in ovo injection of Biacid™ have been done to be sure that it is not lethal for fetuses afterwards vitality and performance of produced chicks were checked, prior to starting the trial. Weight gain and production level of hens were controlled for 2 weeks. Then 240 unified Ross 308 broiler breeders were selected at the end of week 43 of age for starting the first step of the trial. Hens were distributed in 20 pens consist of 4 treatments with 5 replicates after weighting starting the first step of the trial. Hens were distributed in 20 pens consist of 4 treatments with 5 replicates after weighting and were fed for 12 weeks. Twelve hens and 2 roosters were put in each pen. Group 1 were fed with diets without Biacid™ and NSPs, diet of second group was without Biacid™ and contained high NSPs, diet of third group contained Biacid™ and without NSPs and the fourth group was fed with diet contained Biacid™ and high NSPs. Table 2 is the breeder rations which were formulated based on CVB standards (2009). Wheat grain which was used in experiment contained 4% NSPs. Since the measured NSPs of corn were...
negligible (0.04%), rations without wheat were considered as without NSPs diets. In the second phase, eggs produced by each 4 groups at the age of 55 weeks were collected for four days. The second step of trial consisted of 8 treatments in such a way that 120 fertilized eggs of each treatment of first step were divided in two groups of 60 eggs for incubation. One group was injected by 0.5 ml of Biacid™ and the other group was injected by 0.5 ml of distilled water during trans-

Table 1. Experimental treatments

| Treatment No. | Biacid™ | NSPs content | In ovo injection |
|---------------|---------|--------------|------------------|
| 1 (control)   | Not applied | Without | Distilled water |
| 2             | Not applied | Without | Biacid™          |
| 3             | Not applied | High    | Biacid™          |
| 4             | Not applied | High    | Distilled water  |
| 5             | Applied    | Without | Biacid™          |
| 6             | Applied    | Without | Distilled water  |
| 7             | Applied    | High    | Biacid™          |
| 8             | Applied    | High    | Distilled water  |

Table 2. Broiler breeder rations formulations and nutrients

| Ingredients (%) | With high NSPs | Without NSPs | Rooster Diet |
|-----------------|----------------|--------------|--------------|
|                 | With Biacid™  | Without Biacid™ | With Biacid™  | Without Biacid™ | |
| Corn grain      | 15.818        | 15.858       | 66.65        | 66.68       | 56.9 |
| Soybean meal    | 15.73         | 15.75        | 21.74        | 21.75       | 14.5 |
| Wheat grain     | 57            | 57           | —            | —           | —   |
| Wheat bran      | —             | —            | —            | —           | 24.15 |
| Soybean oil     | 1             | 1            | 1            | 1           | 1   |
| Oyster shell    | 5.21          | 5.24         | 5.34         | 5.38        | —   |
| Limestone       | 2.61          | 2.62         | 2.67         | 2.69        | 1   |
| DCP             | —             | —            | —            | —           | 0.35 |
| NaHCO₃          | —             | —            | —            | —           | 0.1  |
| DL-Methionine   | 0.032         | 0.032        | —            | —           | —   |
| Concentrate 2.5%* | 2.5         | 2.5          | 2.5          | 2.5         | 2   |
| Biacid™         | 0.1           | —            | 0.1          | —           | —   |

| Nutrients       | Hen Diets | Rooster Diet |
|-----------------|-----------|--------------|
| Metabolisable Energy (kcal/kg) | 2750 | 2650 |
| Crude Protein (%) | 15.01 | 15.11 |
| Calcium (%)      | 3.46     | 0.88         |
| Av. Phosphorous (%) | 0.35 | 0.35 |
| Sodium (%)       | 0.16     | 0.14         |
| Chloride (%)     | 0.20     | 0.18         |
| Lysine (%)       | 0.72     | 0.67         |
| Methionine (%)   | 0.34     | 0.29         |
| Met+Cys (%)      | 0.61     | 0.58         |
| Threonine (%)    | 0.53     | 0.52         |
| Tryptophan (%)   | 0.18     | 0.17         |
| Isoleucine (%)   | 0.60     | 0.57         |
| Arginine (%)     | 0.93     | 0.93         |
| Valine (%)       | 0.70     | 0.69         |

*Broiler breeder concentrate 2.5% consist of 1109 kcal/kg metabolisable energy, 14.52% calcium, 9.31% Av. phosphorous, 4.87% sodium, 0.18% chloride, 17.61% crude protein, 1.81% lysine, 3.07% methionine, 3.26% met+cys, 0.51% threonine and 0.17% tryptophan and supply 12500 IU vitamin A, 3000 IU vitamin D₃, 100 IU vitamin E, 3.3 mg vitamin K, 2mg thiamine, 9mg riboflavin, 12mg pantothenic acid, 40mg niacin, 5mg pyridoxine, 0.03mg cobalamin, 2mg folic acid, 0.2mg biotin, 50mg Fe, 10mg Cu, 125mg Zn, 100mg Mn, 1.8mg I, 0.4mg Se, 600mg choline chloride, 300 FTU phytase, 70 units β xylanase and 100 units β glucanase per kg of diet.
feeding from setter to hatchery in day 18th of incubation. Dilution rate of Biacid\textsuperscript{TM} for injection was 1% and injection was done in amniotic fluid by using 2 ml syringe with needle 22G (Salahi et al., 2011). The average weight of the eggs was 66.1 ± 4.2 g.

In the third phase, 25 cockerels from each of 8 groups were housed into separate pens and reared for 42 days. The average weight of the cockerels was 44.2 ± 5.8 g. Pens sizes were 1.7 × 1.8 m. All pens were fed with the same diets based on soya‒corn without NSPs during all rearing periods till the variations in chick’s performance was only due to the effects of their broiler breeders’ rations and \textit{in ovo} injection. Table 3 is the offspring rations which were formulated based on CVB standards (2009).

Characteristics of broiler breeders including hatchability, embryo mortality, weight of day old chicks as well as their progenies’ performance, including carcass yield, proportion of abdominal fat per live weight, weight gain, feed intake, feed conversion ratio, European production index, and humoral immune response using titer of antibody against sheep red blood cell (SRBC) were studied. Measurement of antibody titer against SRBC was done via micro-titer hemagglutination assay (Wegmann and Smithies, 1996). The European production index was calculated via below formula:

\[ \frac{\text{Live weight (kg)} \times \text{Survival rate } \times 100}{\text{FCR} \times \text{Rearing period (day)}} \]

The data obtained in the research was statistically analyzed by Proc GLM of SAS 9.1 (Statistical Analysis System) software package system. Statistical model was as below and error level of alpha was 0.05%.

\[ X_{ijkm} = \mu + P_i + R_j + M_k + (PR)_{ij} + (PM)_{ik} + (RM)_{jk} + (PRM)_{ijk} + \varepsilon_{ijkm} \]

\( X_{ijkm} \): observation m in level i of factor P, level j of factor R and level k of factor M
\( \mu \): average
\( P_i \): level i of factor P (i = 0, 1)
\( R_j \): level j of factor R (j = 0, 1)
\( M_k \): level k of factor M (k = 0, 1)
\( (PR)_{ij} \): interaction of level i of factor P and level j of factor R
\( (PM)_{ik} \): interaction of level i of factor P and level k of factor M
\( (RM)_{jk} \): interaction of level j of factor R and level k of factor M
\( (PRM)_{ijk} \): interaction of level i of factor P, level j of factor R and level k of factor M
\( \varepsilon_{ijkm} \): error

Table 3. Broilers rations formulations and nutrients

| Ingredients (%) | Starter Phase | Grower Phase | Finisher Phase |
|-----------------|---------------|--------------|---------------|
| Corn grain      | 54.75         | 57.5         | 66.3          |
| Soybean meal    | 37.3          | 35.3         | 28.5          |
| Soybean oil     | 3.35          | 3.55         | 1.6           |
| Limestone       | 1.6           | 1.15         | 1.1           |
| Broiler Concentrate 2.5%* | 3 | 2.5 | 2.5 |

Nutrients

| Ingredients (%) | Starter Phase | Grower Phase | Finisher Phase |
|-----------------|---------------|--------------|---------------|
| Metabolisable Energy (kcal/kg) | 2900 | 2950 | 2950 |
| Crude Protein (%) | 21.28 | 20.51 | 18.5 |
| Calcium (%) | 0.97 | 0.76 | 0.65 |
| Av. Phosphorous (%) | 0.57 | 0.45 | 0.40 |
| Sodium (%) | 0.17 | 0.15 | 0.15 |
| Chloride (%) | 0.18 | 0.16 | 0.16 |
| Lysine (%) | 1.28 | 1.21 | 1.10 |
| Methionine (%) | 0.57 | 0.52 | 0.49 |
| Met+Cys (%) | 0.91 | 0.86 | 0.81 |
| Threonine (%) | 0.82 | 0.79 | 0.72 |
| Tryptophan (%) | 0.24 | 0.24 | 0.21 |
| Isoleucine (%) | 0.87 | 0.79 | 0.74 |
| Arginine (%) | 1.34 | 1.21 | 1.14 |
| Valine (%) | 1.08 | 0.98 | 0.92 |

*Broiler concentrate 2.5% consist of 1750 kcal/kg metabolisable energy, 8.04% calcium, 10.47% Av. phosphorous, 5.16% sodium, 4.53% chloride, 26.28% crude protein, 5.31% lysine, 8.58% methionine, 8.81% met+cys, 1.16% threonine and 0.19% tryptophan and supply 10000 IU vitamin A, 4167 IU vitamin D\textsubscript{3}, 40 IU vitamin E, 2.3 mg vitamin K, 1 mg thiamine, 5 mg riboflavin, 8 mg pantothenic acid, 30 mg niacin, 2 mg pyridoxine, 0.02 mg cobalamin, 0.5 mg folic acid, 0.1 mg biotin, 50 mg Fe, 10 mg Cu, 50 mg Zn, 65 mg Mn, 1.3 mg I, 0.23 mg Se, 400 mg choline chloride, 500 FTU phytase, 70 units β xylanase and 100 units β glucanase per kg of diet.
Results

The results of this study (Table 4) indicated that including Biacid™ in broiler breeders’ ration increased hatchability whereas high levels of NSPs in broiler breeders’ ration decreased hatchability significantly \((p<0.05)\). In ovo injection of Biacid™ do not show significant effects in this regard \((p\geq 0.05)\). Using Biacid™ and high levels of NSPs in broiler breeders’ ration affected embryo mortality significantly \((p<0.05)\) in such a way that including Biacid™ in broiler breeders’ ration led to decrease and high levels of NSPs in their diets increased embryo mortality (Table 4). In ovo injection of Biacid™ did not show significant effects in this regard \((p\geq 0.05)\).

Weight of day old chicks has been affected significantly by existence of Biacid™ and high levels of NSPs in broiler breeders’ ration \((p<0.05)\). Negative effects of high levels of NSPs in broiler breeders’ ration on hatched chicks can be decreased by using Biacid™ in broiler breeders’ ration (Table 4) whereas in ovo injection of Biacid™ did not show significant effects in this regard \((p\geq 0.05)\). Results mentioned in Table 4 indicated that using Biacid™ in broiler breeders’ ration led to significant increase in progenies’ carcass yield whereas high levels of NSPs in broiler breeders’ ration decreased it significantly \((p<0.05)\). In ovo injection of Biacid™ did not have significant effects on progenies’ carcass yield \((p\geq 0.05)\). Offspring’s abdominal fat was neither affected by Biacid™ and high amounts of NSPs in broiler breeders’ ration nor in ovo injection of Biacid™ significantly \((p\geq 0.05)\).

Biacid™ and high NSPs content in broiler breeders’ ration affected all primary and secondary humoral immune responses of progenies against SRBC significantly \((p<0.05)\). Using Biacid™ in broiler breeders’ ration led to significant increase in progenies’ immune responses, whereas high levels of NSPs in broiler breeders’ ration decreased it significantly (Table 5). In ovo injection of Biacid™ showed different effects in this regard, in such a way that it increased primary response of IgG and IgY \((p<0.05)\) but had not significant effect on IgM responses \((p\geq 0.05)\). In ovo injection of Biacid™ only increased secondary responses of IgG significantly \((p<0.05)\).

Interaction of the effects of Biacid™ and high level of NSPs in broiler breeders’ ration and also in ovo injection of Biacid™ affected progenies’ weight gain, feed intake, feed conversion ratio (FCR) and European production index significantly. Based on the obtained results mentioned in Table 6, treatment 6 had the highest and treatment 4 had the lowest weight at the end of the first week of age \((p<0.05)\). Although high level of NSPs in broiler breeders’ ration besides using Biacid™ led to decrease the weight gain in treatments 7 and 8, in ovo injection of Biacid™ improved final weight gain in these treatments \((p<0.05)\).

Treatment 6 had the highest and treatments 3 and 4 had the lowest feed intake at the end of the period \((p<0.05)\). Using Biacid™ in the diets of broiler breeders with the lack of NSPs

|                | Hatchability (%) | Embryo mortality (%) | Weight of DOCs1 (%) | Carcass yield (%) | Abdominal fat (%) |
|----------------|------------------|----------------------|---------------------|------------------|------------------|
| **Biacid™ in b.b2 ration** |                  |                      |                     |                  |                  |
| Not applied    | 70.4b            | 15.2a                | 41.0b               | 66.1b            | 2.45             |
| Applied        | 82.9a            | 7.6b                 | 47.4a               | 67.9a            | 2.41             |
| SEM            | 1.09             | 1.21                 | 0.08                | 0.13             | 0.02             |
| **NSPs in b.b ration** |                |                      |                     |                  |                  |
| Without        | 80.2a            | 9.3b                 | 45.9a               | 67.6a            | 2.41             |
| High           | 73.1b            | 13.5b                | 42.5b               | 66.4b            | 2.45             |
| SEM            | 1.09             | 1.21                 | 0.08                | 0.13             | 0.02             |
| **In ovo injection** |                |                      |                     |                  |                  |
| Distilled water| 76.5             | 11.7                 | 44.3                | 67.1             | 2.44             |
| Biacid™        | 76.7             | 11.1                 | 44.1                | 67.0             | 2.42             |
| SEM            | 1.09             | 1.21                 | 0.08                | 0.13             | 0.01             |
| **P values**   |                  |                      |                     |                  |                  |
| Biacid™        | \(<0.0001\)      | \(<0.0001\)          | \(<0.0001\)         | \(<0.0001\)      | 0.007            |
| NSPs           | \(<0.0001\)      | 0.02                 | \(<0.0001\)         | \(<0.0001\)      | 0.008            |
| In ovo injection| 0.89             | 0.71                 | 0.31                | 0.58             | 0.28             |
| Biacid™×NSPs   | 0.76             | 0.79                 | 0.01                | 0.39             | 0.50             |
| Biacid™×In ovo injection | 0.74      | 0.71                 | 0.007               | 0.18             | 0.04             |
| NSPs×In ovo injection | 0.02     | 0.07                 | 0.01                | 0.004            | 0.78             |
| Biacid™×NSPs×In ovo injection | 0.07 | 0.07                 | 0.07                | 0.97             | 0.73             |

a and b: Different alphabetic words referred as existence of significant difference between treatments \((p<0.05)\)
1. DOCs: Day old chicks
2. b.b: Broiler breeder
content, led to significant increase in feed intake of progenies (Treatments 5 and 6). On the other hand, lacking Biacid™ in diets of broiler breeders containing high NSPs, led to significant decrease in feed intake of progenies (p<0.05) and it seems that in ovo injection of Biacid™ did not have significant effect in these treatments (treatments 3 and 4).

Table 5. Main effects of experimental factors on humoral immune responses of progenies

|                  | Primary titer | Secondary titer |
|------------------|---------------|-----------------|
|                  | IgT           | IgG            | IgM       |
|                  | IgT           | IgG            | IgM       |
| Biacid™ in b.b² ration |
| Not applied      | 4.6b          | 2.7b           | 1.8b      |
| Applied          | 5.1a          | 3.0a           | 2.0a      |
| SEM              | 0.01          | 0.01           | 0.02      |
| Biacid™ × In ovo injection          | 0.01          | 0.01           | 0.02      |

|                  | IgT           | IgG            | IgM       |
|                  | IgT           | IgG            | IgM       |
| SEM              | 0.01          | 0.01           | 0.02      |

|                  | IgT           | IgG            | IgM       |
|                  | IgT           | IgG            | IgM       |
| SEM              | 0.01          | 0.01           | 0.02      |

|                  | P values      | Biacid™        | NSPs       | In ovo injection |
|                  |              | <0.0001        | <0.0001    | <0.0001         |
|                  |              | <0.0001        | <0.0001    | <0.0001         |
|                  | NSPs         | <0.0001        | <0.0001    | <0.0001         |
|                  | In ovo injection | <0.0001    | 0.01       | <0.0001         |
|                  | Biacid™ × NSPs | 0.76          | 0.15       | 0.29            |
|                  | In ovo injection | 0.01          | 0.31       | 0.23            |
|                  | NSPs × In ovo injection | 0.04       | 0.004      | 0.79            |
|                  | Biacid™ × NSPs × In ovo injection | 0.47       | 0.26       | 0.24            |

|                  | P values      | Biacid™        | NSPs       | In ovo injection |
|                  |              | <0.0001        | <0.0001    | <0.0001         |
|                  |              | <0.0001        | <0.0001    | <0.0001         |
|                  | NSPs         | <0.0001        | <0.0001    | <0.0001         |
|                  | In ovo injection | <0.0001    | 0.35       | 0.0003          |
|                  | Biacid™ × NSPs | 0.0008        | 0.01       | 0.09            |
|                  | In ovo injection | 0.0008        | 0.01       | 0.09            |
|                  | NSPs × In ovo injection | 0.0008       | 0.0042     | 0.35            |
|                  | Biacid™ × NSPs × In ovo injection | 0.0008      | 0.0042     | 0.35            |

|                  | a, b, c, d, e, f and g: Different alphabetic words referred as existence of significant difference between treatments (p<0.05)

Due to significant interaction effects, major effects not listed in the table.

Table 6. Interaction of experimental factors on performance of offspring

| Treatment | Live Weight (g) | Total Feed Intake (g) | FCR | European Production Index |
|-----------|-----------------|-----------------------|-----|--------------------------|
|          | Day 7           | Day 42                |     |                          |
| 1 (control) | 176.38e       | 2032.40e              | 3759.20e | 1.85b            | 239.20e |
| 2         | 189.70d        | 2185.00d              | 3757.60d | 1.72c            | 276.40b |
| 3         | 170.54f        | 1969.60f              | 3601.20f | 1.82b            | 234.40h |
| 4         | 153.86g        | 1825.00g              | 3574.60g | 1.95a            | 190.00d |
| 5         | 211.40b        | 2419.20b              | 4120.40b | 1.70c            | 318.60a |
| 6         | 214.66a        | 2517.20a              | 4265.60a | 1.69c            | 334.00e |
| 7         | 199.18c        | 2235.80c              | 3814.00c | 1.70c            | 285.40b |
| 8         | 197.38c        | 2207.80d              | 3761.80c | 1.70c            | 281.80b |
| SEM       | 0.24           | 2.84                  | 8.68   | 0.003             | 3.79    |

| P values  | Biacid™        | <0.0001              | <0.0001    | <0.0001         |
|           | NSPs           | <0.0001              | <0.0001    | <0.0001         |
|           | In ovo injection | <0.0001    | 0.03       | 0.0003          |
|           | Biacid™ × NSPs | 0.0008              | 0.01       | 0.09            |
|           | In ovo injection | 0.0008        | 0.0042     | 0.35            |
|           | NSPs × In ovo injection | 0.0008      | 0.0042     | 0.35            |
|           | Biacid™ × NSPs × In ovo injection | 0.0008    | 0.0042     | 0.35            |

a, b, c, d, e, f and g: Different alphabetic words referred as existence of significant difference between treatments (p<0.05)
Results mentioned in Table 6 indicate that lowest FCR in offspring belonged to treatments 5, 6, 7 and 8 which contained Biacid™ in their breeders’ diets ($p<0.05$). *In ovo* injection of Biacid™ while broiler breeder diet did not contain Biacid™ and high NSPs level (treatment 2) did not result in significant improvement in FCR in comparing with treatments 5, 6, 7 and 8 ($p \geq 0.05$). Highest FCR in offspring belonged to treatment 4 which the breeders’ diet contained high NSPs without using Biacid™ ($p<0.05$) and was not *in ovo* injected with Biacid™. *In ovo* injection of Biacid™ while broiler breeder diet contained high NSPs without using Biacid™ (treatment 3) resulted in significant improvement in FCR in comparing with treatments 4 ($p<0.05$). Treatments 5 and 6 which contained Biacid™ and lacked NSPs in their breeders’ diets had the highest European production index of offspring ($p<0.05$) while *in ovo* injection of Biacid™ did not show significant effects in this regard (Table 6). Lowest European production index in offspring belonged to treatment 4 which the breeders’ diet contained high NSPs without using Biacid™ ($p<0.05$) and was not *in ovo* injected with Biacid™. *In ovo* injection of Biacid™ while broiler breeder diet contained high NSPs without using Biacid™ (treatment 3) improves European production index significantly, in such a way that it will be as the same as control group (Table 6).

**Discussion**

Based on the results of this study, high NSPs diets in broiler breeders not only decrease their production performance, but also reduce the performance of their offspring. Many researches have been done regarding the effects of rations containing high NSPs on performance of poultry that indicating high levels of NSPs have antinutritional characteristics which lead to decrease the performance and bioavailability of the nutrients (Garcia et al., 2003; Moharrery and Mohammadpour, 2005; Rebel et al., 2006; Khodambashi et al., 2013; Liu et al., 2013; Masey O’Neill et al., 2014). Researches have shown that most of essential oils stimulate digestion process, so they can increase the poultry performance via increasing the bioavailability of nutrients (Lee et al., 2004; Kamatou et al., 2005; Mounchid et al., 2005; Oussalah et al., 2007; Santurio et al., 2007; Rusenova and Parvanov, 2009; Bravo et al., 2014). Increased protein availability as a result of increasing the bioavailability of nutrients in broiler breeders results in increasing the weight of produced day old chicks (Rebel et al., 2006; Mohiti-Asli et al., 2012; Van Emous et al., 2013, 2015). Feed intake rate in broilers is linearly affected by day old chick’s weight (Rebel et al., 2006; Van Emous et al., 2013, 2015).

The results of this study showed that high NSPs diets in broiler breeders end in decreasing the immune response of their progenies. Researches have indicated that high NSPs diets impair the balance of gut microflora that can affect gut morphology, nutrition, pathogens activity and immune system (Alveryd et al., 2005; Antongiovanni et al., 2007; Oussalah et al., 2007; Deriu et al., 2008; Isabel and Santos, 2009; Horosova et al., 2012; Khodambashi et al., 2013; Masey O’Neill et al., 2014). Increasing the gut microbial fermentation results in increasing the litter humidity and providing more substrates for pathogens that negatively affected the immune system (Khodambashi et al., 2013; Masey O’Neill et al., 2014) and suppressing maternal immunity (Van Emous et al., 2013, 2015). Moreover, researches have indicated that high NSPs diets result in metabolic stress that lead to increasing the corticosterones. The corticosterones suppress the immune system of poultry which lead to decreasing the maternal immunity and hence immune responses of their offspring (Rebel et al., 2006; Oussalah et al., 2007; Isabel and Santos, 2009; Van Emous et al., 2015). On the other hand, researches showed that essential oils have stress relief activity (Lee et al., 2004; Isabel and Santos, 2009; Karadas et al., 2013). Including Biacid™ in broiler breeder diets increased the immune response of their progenies. Researches have indicated that essential oils have antioxidant characteristics and stimulate feed intake and digestion possesses that lead to decrease the substrates of undesirable microorganisms of the gut microflora. On the other hand, they have antimicrobial activity and hence they can enhance the immune system and health status of poultry (Mimica et al., 2003; Lee et al., 2004; Kamatou et al., 2005; Mounchid et al., 2005; Oussalah et al., 2007; Santurio et al., 2007; Rusenova and Parvanov, 2009; Shapiro et al., 2013). Results obtained from this study showed that using blend of essential oils and volatile fatty acids (Biacid™) in broiler breeder diets can enhance the production performance of them and their offspring. Various researches have been conducted on the effects of essential oils have shown that most of these components stimulate both feed intake and digestion process, so they can increase the poultry production performance via increasing the bioavailability of nutrients (Lee et al., 2004; Kamatou et al., 2005; Mounchid et al., 2005; Oussalah et al., 2007; Santurio et al., 2007; Rusenova and Parvanov, 2009; Bravo et al., 2014). Moreover, several studies have been done on metabolic effects of VFAs have indicated that VFAs can enhance the poultry performance via improving cell proliferation and stimulating protein synthesis (Zhonghong and Yuming, 2006; Antongiovanni et al., 2007; Kim and Paik, 2007; Khodambashi et al., 2013; Milbradt et al., 2014).

The correct combination of essential oils can exhibit greater responses and in other word they express synergy (Lee et al., 2004; Prabuseenivasan et al., 2006; Deriu et al., 2008; Isabel and Santos, 2009; Horosova et al., 2012; Karadas et al., 2013; Shapiro et al., 2013; Bravo et al., 2014). Moreover, several studies have shown that VFAs have antibacterial activity (Dibner and Buttin, 2002; Antongiovanni et al., 2007; Paul et al., 2007; Isabel and Santos, 2009; Khodambashi et al., 2013; Milbradt et al., 2014). Unlike inorganic acids, VFAs are easily absorbed through the cell wall of bacteria and damage the structure of DNA in the cells’ nuclei which lead to disrupting bacterial multiply, so cell death can accrue (Van Immersel et al., 2006; Isabel and Santos, 2009; Milbradt et al., 2014). Coating VFAs can preserve and extend their bacteriostatic activity in the intestine and ceca of poultry via slower and gradual release.
of them (Zhonghong and Yuming, 2006; Antongiovanni et al., 2007; Al-Zenki et al., 2009; Isabel and Santos, 2009; Zirelbeke and Belguom, 2013). A number of nutrigenomics researches imply on influences of breeder rations on progeny performance, hence it would be expected that manipulation of breeder rations lead to better performance and improved health status in their offspring (Johanna et al., 2006; Rebel et al., 2006; Koedijk et al., 2010; Van Emous et al., 2015). Many researches have been conducted in recent years regarding the various aspects of nutrigenomics in poultry and other animals indicating that nutrition can influence the performance through affecting the gene expression (Gazala et al., 2003; Friedman and Bar-Shair, 2005; Johanna et al., 2006; Rebel et al., 2006; Winzenberg et al., 2006; Koedijk et al., 2010; Walker et al., 2014). It is shown in poultry that higher levels of PepT1 in chicken’s gut facilitate the peptide uptake which leads to improve the performance. In this regard, nutrigenomics studies imply that gene expression of PepT1 can be affected by nutrition, mainly by VFAs in the feed (Koedijk et al., 2010). There are other experiments indicating that broiler breeder nutrition can affect the progenies’ performance. For example, study of the effect of different levels of Zn ion in broiler breeder diet on performance of the offspring showed that Zn deficiency in broiler breeder diet decrease the progenies’ growth rate and immune responses, whereas supplementing of Zn in broiler breeder diet led to increase the immune response and vitality of the offspring (Walker et al., 2014). Another study in this regard showed that supplementing of vitamin A in broiler breeder diet enhanced the live level of vitamin A in the liver of progenies and also increased the fat oxidation rate in fetus and offspring (Gazala et al., 2003).

Conclusions

Based on the results of this study, negative effects of high levels of NSPs in broiler breeders’ ration on their progenies’ performance can be decreased by using Biacid™ in broiler breeders’ rations. Moreover, in ovo injection of Biacid™ can be an alternative for using Biacid™ in broiler breeders’ rations whenever in ovo injection of it has significant effect, although it will be depended on economical situations and appropriate facilities.

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