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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^{TM}) in broiler breeder diets at two levels, two dietary non-starch polysaccharides (NSPs) levels and *in ovo* injection of Biacid^{TM} on their progenies performance, respectively. 240 broiler breeders of Ross 308 strain were fed 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 m*l* of Biacid^{TM} 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^{TM} 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^{TM} did not show significant effects in this regards ($p \geq 0.05$). Offspring's abdominal fat was neither affected by broiler breeders' rations nor *in ovo* injection of Biacid^{TM} ($p \geq 0.05$). Biacid^{TM} 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^{TM} increased the primary IgG, primary IgT and secondary IgG responses ($p<0.05$). The interaction of the effects of Biacid^{TM} and high NSPs in broiler breeders' ration and also *in ovo* injection of Biacid^{TM} affected progenies' weight gain, feed intake, feed conversion ratio and European production index significantly ($p<0.05$). It seems that using Biacid^{TM} 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 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

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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 for example, are known to increase voluntary feed intake as well as stimulating nutrient digestion and exhibiting anti- bacterial properties (Lee *et al*., 2004; Kamatou *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 Buttin, 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^{TM} 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^{TM} or lack of it in breeder rations, high NSPs in breeder rations and *in ovo* injection of Biacid^{TM} 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^{TM} consist of citric acid, calcium butyrate, calcium lactate, calcium fumarate, cinnemaldehyde, thymol and carvacrol. The density of Biacid^{TM} is 600–800 kg/m^3 and pH is 4–4.5.

An *in ovo* injection of Biacid^{TM} 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 and were fed for 12 weeks. Twelve hens and 2 roosters were put in each pen. Group 1 were fed with diets without Biacid^{TM} and NSPs, diet of second group was without Biacid^{TM} and contained high NSPs, diet of third group contained Biacid^{TM} and without NSPs and the fourth group was fed with diet contained Biacid^{TM} 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

Table 1. **Experimental treatments**

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

Table 2. **Broiler breeder rations formulations and nutrients**

	Hen Diets				
	With high NSPs		Without NSPs		
Ingredients (%)	With Biacid^{TM}	Without Biacid^{TM}	With Biacid^{TM}	Without Biacid^{TM}	Rooster Diet
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
$NaHCO_3$	—	—	—	—	0.1
DL- Methionine	0.032	0.032	—	—	
Concentrate 2.5%*	2.5	2.5	2.5	2.5	2
Biacid^{TM}	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, 6.18% 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 D_3, 100 IU vitamin E, 3.3 mg vitamin K, 2 mg thiamine, 9 mg riboflavin, 12 mg pantothenic acid, 40 mg niacin, 5 mg pyridoxine, 0.03 mg cobalamin, 2 mg folic acid, 0.2 mg biotin, 50 mg Fe, 10 mg Cu, 125 mg Zn, 100 mg Mn, 1.8 mg I, 0.4 mg Se, 600 mg choline chloride, 300 FTU phytase, 70 units β xylanase and 100 units β glucanase per kg of diet.

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 m*l* of Biacid^{TM} and the other group was injected by 0.5 m*l* of distilled water during trans-

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				
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_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.

ferring from setter to hatchery in day 18th of incubation. Dilution rate of Biacid^{TM} for injection was 1% and injection was done in amniotic fluid by using 2 m*l* syringe with needle 22G (Salahi *et al*., 2011). The average weight of the eggs was 66.1±4 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 *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:

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

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

μ: 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

ε_{ijkm}: error

Results

The results of this sturdy (Table 4) indicated that including Biacid^{TM} 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^{TM} did not show significant effects in this regard ($p\geq 0.05$). Using Biacid^{TM} and high levels of NSPs in broiler breeders' ration affected embryo mortality significantly ($p<0.05$) in such a way that including Biacid^{TM} 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^{TM} 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^{TM} 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 hicks can be decreased by using Biacid^{TM} in broiler breeders' ration (Table 4) whereas *in ovo* injection of Biacid^{TM} did not show significant effects in this regard ($p\geq 0.05$). Results mentioned in Table 4 indicated that using Biacid^{TM} 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^{TM} did not have significant effects on progenies' carcass yield ($p\geq 0.05$). Offspring's abdominal fat was neither affected by Biacid^{TM} and high amounts of NSPs in broiler breeders' ration nor *in ovo* injection of Biacid^{TM} significantly ($p\geq 0.05$).

Biacid^{TM} 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^{TM} 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^{TM} 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^{TM} only increased secondary responses of IgG significantly ($p<0.05$).

Interaction of the effects of Biacid^{TM} and high level of NSPs in broiler breeders' ration and also *in ovo* injection of Biacid^{TM} 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^{TM} led to decrease the weight gain in treatments 7 and 8, *in ovo* injection of Biacid^{TM} 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^{TM} in the diets of broiler breeders with the lack of NSPs

Table 4. **Main effects of experimental factors on performance**

	Hatchability (%)	Embryo mortality (%)	Weight of DOCs[1] (%)	Carcass yield (%)	Abdominal fat (%)
Biacid^{TM} in b.b[2] ration					
Not applied	70.4^{b}	15.2^{a}	41.0^{b}	66.1^{b}	2.45
Applied	82.9^{a}	7.6^{b}	47.4^{a}	67.9^{a}	2.41
SEM	1.09	1.21	0.08	0.13	0.02
NSPs in b.b ration					
Without	80.2^{a}	9.3^{b}	45.9^{a}	67.6^{a}	2.41
High	73.1^{b}	13.5^{a}	42.5^{b}	66.4^{b}	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^{TM}	76.7	11.1	44.1	67.0	2.42
SEM	1.09	1.21	0.08	0.13	0.01
P values					
Biacid^{TM}	<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$^{TM}\times$NSPs	0.76	0.79	0.01	0.39	0.50
Biacid$^{TM}\times$*In ovo* injection	0.74	0.71	0.007	0.18	0.04
NSPs$\times$*In ovo* injection	0.02	0.07	0.01	0.004	0.78
Biacid$^{TM}\times$NSPs$\times$*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

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.b[2] ration						
Not applied	4.6^{b}	2.7^{b}	1.8^{b}	7.7^{b}	4.6^{b}	3.1^{b}
Applied	5.1^{a}	3.0^{a}	2.0^{a}	8.6^{a}	5.1^{a}	3.4^{a}
SEM	0.01	0.01	0.02	0.01	0.01	0.01
NSPs in b.b ration						
Without	5.0^{a}	3.0^{a}	2.0^{a}	8.5^{a}	5.1^{a}	3.3^{a}
High	4.6^{b}	2.7^{b}	1.9^{b}	7.8^{b}	4.5^{b}	3.2^{b}
SEM	0.01	0.01	0.02	0.01	0.01	0.01
***In ovo* injection**						
Distilled water	4.8^{b}	2.8^{b}	1.9	8.1	4.8^{b}	3.3
Biacid™	4.9^{a}	2.9^{a}	1.9	8.1	4.9^{a}	3.3
SEM	0.01	0.01	0.02	0.01	0.01	0.01
P values						
Biacid™	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
NSPs	<0.0001	<0.0001	0.01	<0.0001	<0.0001	0.001
In ovo injection	0.01	0.0004	0.66	<0.0001	0.0009	0.05
Biacid™×NSPs	0.76	0.15	0.29	0.53	0.07	0.62
Biacid™×*In ovo* injection	0.01	0.31	0.23	<0.0001	0.12	0.01
NSPs×*In ovo* injection	0.04	0.004	0.79	0.001	0.02	0.0006
Biacid™×NSPs×*In ovo* injection	0.47	0.26	0.24	0.13	0.63	0.17

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

Table 6. **Interaction of experimental factors on performance of offspring**

	Live Weight (g)		Total Feed	FCR	European Production Index
	Day 7	Day 42	Intake (g)		
Treatment					
1 (control)	176.38^{e}	2032.40^{e}	3759.20^{c}	1.85^{b}	239.20^{c}
2	189.70^{d}	2185.00^{d}	3757.60^{c}	1.72^{c}	276.40^{b}
3	170.54^{f}	1969.60^{f}	3601.20^{d}	1.82^{b}	234.40^{c}
4	151.50^{g}	1825.00^{g}	3574.60^{d}	1.95^{a}	190.00^{d}
5	211.40^{b}	2419.20^{b}	4120.40^{b}	1.70^{c}	318.60^{a}
6	214.66^{a}	2517.20^{a}	4265.60^{a}	1.69^{c}	334.00^{a}
7	199.18^{c}	2235.80^{c}	3814.00^{c}	1.70^{c}	285.40^{b}
8	197.38^{c}	2207.80^{d}	3761.80^{c}	1.70^{c}	281.80^{b}
SEM	0.24	2.84	8.68	0.003	3.79
P values					
Biacid™	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NSPs	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
In ovo injection	0.03	0.0017	0.35	0.70	0.88
Biacid™×NSPs	<0.0001	0.0042	<0.0001	<0.0001	0.84
Biacid™×*In ovo* injection	0.0008	0.01	0.09	0.80	0.53
NSPs×*In ovo* injection	<0.0001	<0.0001	0.0031	<0.0001	0.0023
Biacid™×NSPs×*In ovo* injection	<0.0001	<0.0001	0.01	<0.0001	0.04

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

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).

Results mentioned in Table 6 indicate that lowest FCR in offspring belonged to treatments 5, 6, 7 and 8 which contained Biacid^{TM} in their breeders' diets ($p<0.05$). *In ovo* injection of Biacid^{TM} while broiler breeder diet did not contain Biacid^{TM} and high NSPs level (treatment 2) did not result in significant improvement in FCR in comparing with treatments 5, 6, 7 and 8 ($p\geq0.05$). Highest FCR in offspring belonged to treatment 4 which the breeders' diet contained high NSPs without using Biacid^{TM} ($p<0.05$) and was not *in ovo* injected with Biacid^{TM}. *In ovo* injection of Biacid^{TM} while broiler breeder diet contained high NSPs without using Biacid^{TM} (treatment 3) resulted in significant improvement in FCR in comparing with treatments 4 ($p<0.05$). Treatments 5 and 6 which contained Biacid^{TM} and lacked NSPs in their breeders' diets had the highest European production index of offspring ($p<0.05$) while *in ovo* injection of Biacid^{TM} 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^{TM} ($p<0.05$) and was not *in ovo* injected with Biacid^{TM}. *In ovo* injection of Biacid^{TM} while broiler breeder diet contained high NSPs without using Biacid^{TM} (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 (Alverdy *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^{TM} 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^{TM}) 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 Immerseel *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^{TM} in broiler breeders' rations. Moreover, *in ovo* injection of Biacid^{TM} can be an alternative for using Biacid^{TM} 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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