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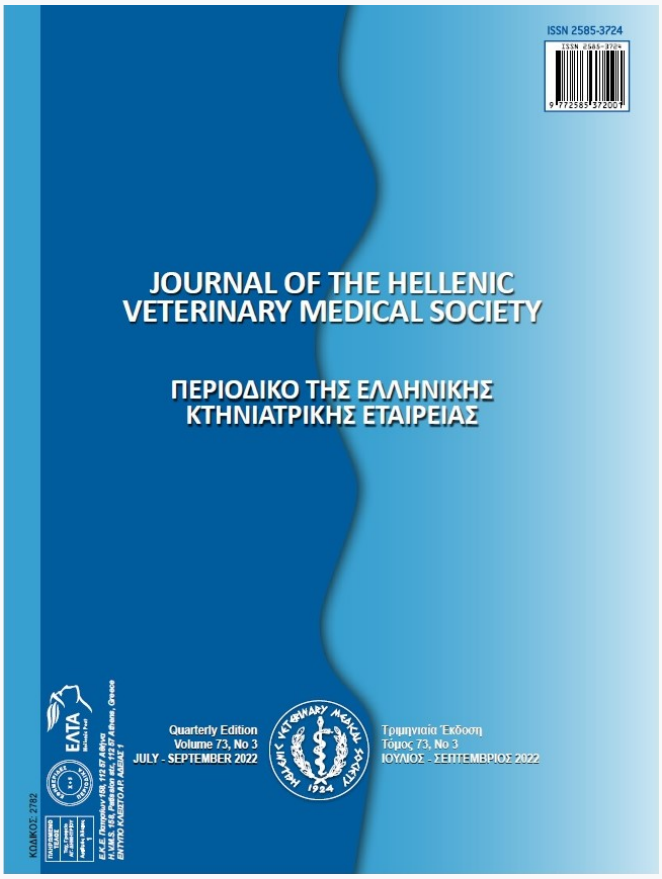

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Research article
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Replacing soybean meal with sunflower meal in laying hens rations and its effects on cecal volatile fatty acids profile and intestinal microbial colonization

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ABSTRACT: This experiment was performed to evaluate the effects of replacing different levels of soybean meal with sunflower meal (with and without enzyme) on cecal volatile fatty acid profile and intestinal microbial colonization in laying hens. In this experiment, 360 laying hens (1530 ± 20 g) from the age of 47 to 57 weeks, in 9 treatments and five replications (8 hens in each replicate) were performed as a 3 × 3 factorial experiment in a completely randomized design for ten weeks. The results showed that the replacement of 30 and 60 percent sunflower meal and enzyme (100 and 200 gr/ton) has significantly improved the concentration of n-valeric acid on the cecum of laying hens (P< 0.05). Simultaneously, increasing 30 percentage of sunflower meal or 100 of an enzyme enhanced isovaleric acid, n-butyric acid, isobutyric acid, propionic acid, and acetic acid (P< 0.05). Reduction in intestinal log *E. coli* was discovered for samples treated with 30 and 60 percent of sunflower (P<0.05), but for log baglus that treated with 30 and 60 percent of sunflower showed higher (P<0.05). The main effects of the enzyme had no significant impact on the cecal microbial population (P>.0.05) while, the effect of meal source and enzyme additive interactions on the tested parameters was significant (P<.0.05). Hence, sunflower meal could be used as an alternative protein source in laying hens ration to improve cecal VFA profile and reduce cecal E. coli population. Substitution of sunflower meal in laying hens is recommended.

Keywords: Cecal VFA profile, Laying hens, Microbial population, Soybean meal, Sunflower meal

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INTRODUCTION

The use of sunflower meal in poultry diets can be beneficial because of the effect of fiber on lowering cholesterol and the complication of cannibalism (Baghban-Kanani et al., 2019). According to Shi et al. (2012), there are two hypotheses to explain that sunflower meal lowers plasma cholesterol and egg yolk: 1) Dietary fiber indirectly inhibits cholesterol synthesis 2) Dietary fiber increases the secretion of cholesterol into the bile. Research has shown that the use of high-fiber feed (insoluble non-starch polysaccharides) in poultry diets can improve the production performance of poultry by increasing the digestibility of nutrients and the intestinal microbial population (Walugembe et al., 2015). Dietary crude fiber provides the energy needed by gastrointestinal bacteria, especially the colon, and facilitates their growth. This increase in bacterial growth can improve the digestibility of crude fiber in the diet. The high metabolism of bacteria leads to increased production of volatile fatty acids such as lactic acid, butyric acid, and propionic acid at the end of the digestive system, which can lower the pH of the intestine and even the gills and increase the villi of the small intestine and consequently increase the level of intestinal absorption (Scholz-Ahrens et al., 2007; González-Alvarado et al., 2008; Jiang et al., 2017; Nogal et al., 2021). Research has shown that low pH in the end parts of the digestion system increases the bioavailability of minerals, especially calcium, zinc, and magnesium (Philipp Schuchardt and Hahn, 2017; Corte-Real and Bohn, 2018; Gibson et al., 2018; Rogaska et al., 2018). It has also been shown that this decrease in pH can inhibit the growth of protons in the cell membrane of the harmful microbial flora of the gastrointestinal tract, such as *E. coli* and Salmonella (Aprisal and Husmaini, 2020; Roy et al., 2021), by destroying their motility. As a result, it improves intestinal health in poultry (Alloui et al., 2013). Secondary structures negatively affect poultry performance, as intestinal digestion decreases and high intestinal viscosity adversely affects the gut's immune response and microbial proliferation. To solve this problem, some exogenous enzymes such as protease, phytase, avizyme, ß-mannanase, and β-gluconase could be added to poultry diets to help in the digestion of fibers and to decline their negative impact on poultry productivity and health (Saeed et al., 2019). Hence, we hypothesized that replacing sunflower meal in the diet of laying will improve gastrointestinal health and calcium absorption. Additional investigation of cecal volatile fatty acids profile would further our understanding on development of the cecal microflora. Therefore, this study aimed to evaluate the replacement of different levels of soybean meal with sunflower meal (with and without enzyme) on cecal volatile fatty acids profile and intestinal microbial colonization in laying hens.

MATERIAL AND METHODS

The animal welfare committee of Islamic Azad University (Maragheh Branch) approved the animal care protocol used in this experiment (no. 1319-IAU. 01.25.2017). A total of 360 laying hens (1530 ± 20 g) from the age of 47 to 57 weeks in 9 treatments and 5 replications (8 hens in each replicate) were divided into treatments in a 3 × 3 factorial arrangement consisting of sunflower meal (0, 30, and 60) and enzyme additive (0, 100 and 200 gr/ton) in a completely randomized design for ten weeks. The hens had access to *ad libitum* feed and water, and the lighting program was 16 hours of light and 8 hours of darkness. The hens were fed a practical ration, adjusted to the recommended nutrient requirements of the 36 Hy-Line strains (Management Guide, 2020). To examine the cecal microbial population at the end of the experimental period, two birds from each replicate (close to the average body weight) were randomly selected and anesthetized using sodium thiopental. Samples were taken from the cecum, transferred to the laboratory in containers containing ice. Conventional culture medium was used to count Lactobacillus bacteria at 37 °C for 48 hours under anaerobic conditions and count Escherichia coli bacteria at 37 °C for 24 hours under aerobic conditions (Pang and Applegate, 2007). Cecal VFAs were determined with gas chromatography (Agilent 7890A, Agilent Technologies, Santa Clara, CA) following Shen et al. (2009).

The data were analyzed using the general linear model procedure of SAS (SAS Institute, Inc., Cary, North Carolina, USA). Data were log-transformed before analyzing in case of unequal variances (Palangi, 2021). The linear model was:

$$Y_{ijk} = \mu + A_i + B_j + AC_{ij} + BC_{jk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = a dependent variable, μ = overall mean, A_i = the effect of sunflower meal replacement, B_j = the effect of Multi-enzyme, AB_{ij} = the interaction of factors A and B, and ε_{ijkl} = the residual deviation of the observation from the effects in the model. Tukey's test at the 5% level of probability was used to compare means.

RESUTLS

Cecal microbial population

The effects of replacing soybean meal with sunflower meal (with and without enzyme) in laying hens rations on cecal microbial population are shown in Table 1. As can be seen in Table 1, the use of sunflower meal was affected the Bacillus and E. coli population ($P<0.05$). So that increasing the sunflower meal leads to increasing the Bacillus and reducing the E. coli population.

The effects of sunflower meal × multi-enzyme, on Bacillus and E. coli population in all replacement ratios were significant ($P<0.05$). Interestingly, the main effects of the enzyme on the measured parameters were not significant ($P >0.05$). While the 0% sunflower meal × 200 gr/ton multi-enzyme interactions showed the highest Bacillus population, the highest E. coli population were observed for 0% sunflower meal × 0 gr/ton multi-enzyme interactions.

Cecal volatile fatty acids

The effects of replacing soybean meal with sunflower meal in laying hens rations on cecal volatile fatty acids are shown in Table 2. Cecal VFA composition significantly improved using sunflower meal ($P<0.05$), the 30% replacement level had the largest increase in the composition of VFA profiles. The addition of 100 gr/ton enzyme also had the greatest effect on the VFA profile ($P<0.05$). Interactions of using 30% sunflower pulp with 100 gr/ton of the enzyme had the highest amount of VFA.

DISCUSSION

Cecal microbial population

Various experiments have shown that diet composition (Feng et al., 2020; Guo et al., 2020), type and amount of NSP (Yang et al., 2020; Farahat et al., 2021), intestinal viscosity (Sjofjan et al., 2019), grain type (Wu et al., 2017), antibiotics (Levesque et al., 2017; Cai et al., 2018), probiotics (Qorbanpour et al., 2018; Xu et al., 2019), prebiotics (Teng and Kim, 2018), organic acids (Yang et al., 2019), and other diet-related factors affect the intestinal microbial population. Insoluble polysaccharides affect the intestinal microflora and reduce the incidence of intestinal problems such as enteritis (Zhou et al., 2018; Ge et al., 2021; Tejeda and Kim, 2021), but the beneficial effects of these compounds on the small intestinal microflora and their mechanism are not exactly known. Various factors such as host, interactions of microorganisms, and feed affect the microbial population of the cecum (Kers et al., 2018). Unabsorbed nutrients at the end of the small intestine are potential substrates for microbes in the cecum of the gastrointestinal tract. Dietary changes affect the accessibility of the substrate and the composition of the bacterial species at the end of the gastrointestinal tract. Sunflower meal increases intestinal health by acidifying the upper parts of the gastrointestinal tract and leads to a natural barrier against pathogenic bacteria sensitive to an acidic environment, including E. coli. Lactobacillus are considered beneficial microorganisms due to their ability to inhibit the growth of pathogenic bacteria (Shokryazdan et al., 2017; Alizadeh et al., 2020). Therefore, increasing the population of these bacteria due to high levels of sunflower meal is also one of the beneficial effects of this protein source in laying hens. The main reasons for an increase in useful intestinal bacteria such as bacillus may be related to the amount of dietary fiber as the amount of fiber in sunflower meals in contrast to soybean meal is high, so the production of bacteria like bacillus be more. This kind of bacteria can improve the intestinal microbial population. Some research shows the beneficial effects of intestinal microbial composition on health and performance (Roberts et al., 2015; Tarabees et al., 2020; Alwaleed et al., 2021).

Cecal volatile fatty acids

Short-chain fatty acids, such as acetate, butyrate, and propionate, are produced during carbohydrate fermentation, while branched-chain fatty acids are a product of protein fermentation in the gastrointestinal tract (Ndazigaruye et al., 2019). Changes in microbial

Table 1. The effect of replacing soybean meal with sunflower meal (with and without enzyme) in laying hens rations on cecal microbial population

Trt	Sunflower meal					Multi-enzyme					Interaction of sunflower meal and multi-enzyme									
	0	30	60	SEM	P value	0	100	200	SEM	P value	0×0	0×100	0×200	30×0	30×100	30×200	60×0	60×100	60×200	SEM	P value
Bacillus	5.76^{b}	6.39^{a}	6.22^{ab}	0.864	0.0257	6.09	5.76	6.50	0.1624	0.0107	5.20^{b}	5.03^{b}	7.04^{a}	6.61^{a}	5.91^{ab}	6.65^{a}	6.48^{ab}	6.36^{ab}	5.82^{ab}	0.2814	0.0003
Ecoli	6.18^{a}	5.25^{b}	5.38^{b}	0.1450	<0.0001	5.75	5.39	5.68	0.1250	0.1132	6.92^{a}	5.45^{b}	6.09^{ab}	5.19^{b}	5.21^{b}	5.34^{b}	5.13^{b}	5.40^{b}	5.60^{b}	0.2166	0.0041

SEM: Standard Error Means

Table 2. The effect of replacing soybean meal with sunflower meal (with and without enzyme) in laying hens rations on cecal VFA composition

TRT		nVA	iVA	nBA	iBA	PA	AA
Sunflower							
0		0.381^{b}	1.013^{b}	3.595	1.067^{b}	7.659	6.382^{c}
30		0.786^{a}	1.409^{a}	4.225	2.735^{a}	7.329	25.390^{a}
60		0.710^{a}	0.977^{b}	3.383	0.983^{b}	7.733	15.009^{b}
SEM		0.1150	0.0269	0.3586	0.0544	0.1798	0.4469
P Value		0.0400	<0.0001	0.2386	<0.0001	0.2521	<0.0001
Enzyme							
0		0.375^{b}	0.979^{b}	3.084^{b}	0.997^{b}	7.643^{b}	8.799^{c}
100		1.029^{a}	1.573^{a}	5.125^{a}	3.107^{a}	9.576^{a}	26.416^{a}
200		0.274^{b}	0.847^{c}	2.989^{b}	0.681^{c}	5.503^{c}	11.486^{b}
SEM		0.1149	0.0269	0.3586	0.0544	0.1798	0.4469
P Value		<0.0005	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
Sunflower × Enzyme							
0	0	0.526^{b}	1.246^{b}	2.233^{bc}	1.276^{b}	7.146^{d}	9.616^{c}
0	100	0.000^{c}	0.736^{ef}	2.976^{bc}	0.898^{bc}	6.716^{de}	3.666^{e}
0	200	0.616^{b}	1.056^{bed}	4.576^{b}	1.026^{bc}	9.116^{c}	5.866^{de}
30	0	0.000^{c}	0.616^{fg}	1.754^{bc}	0.698^{c}	3.206^{g}	11.346^{c}
30	100	2.360^{a}	3.126^{a}	9.882^{a}	7.506^{a}	17.046^{a}	56.106^{a}
30	200	0.000^{c}	0.486^{g}	1.036^{c}	0.000^{d}	1.736^{h}	8.476^{cd}
60	0	0.600^{b}	1.076^{bc}	4.266^{b}	1.016^{bc}	12.576^{b}	5.436^{de}
60	100	0.726^{b}	0.856^{de}	2.256^{bc}	0.916^{bc}	4.966^{f}	19.476^{b}
60	200	0.806^{b}	1.000^{ed}	3.356^{bc}	1.016^{bc}	5.656^{ef}	20.116^{b}
SEM		0.1992	0.0467	0.6211	0.0942	0.3114	0.7742
P Value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

nVA: n-valeric acid, iVA: isovaleric acid, nBA: n-butyric acid, iBA: isobutyric acid, PA: propionic acid, and AA: acetic acid; TRT: Treatments SEM: Standard Error Means

flora reflect changes in VFA composition. Gram-positive organisms were assumed to be involved in fermenting polysaccharides into VFA, especially acetate and butyrate (Seon et al., 2014). VFA is responsible for reducing E. coli, and significant negative correlations were observed between the numbers of Enterobacteriaceae (*E. coli*) and acetate in the ceca (Zhu et al., 2015). VFAs may play a key role in developing microflora in the ceca of laying hens. Still, it is not well understood the relationship and the mechanisms of reducing *E. coli* cecal colonization and cecal VFA. Furthermore, van der Wielen et al. (2000) investigated that the increased concentrations of butyric acid were related to decreased amounts of E. coli. Therefore, the high concentration of butyric acid produced in the ceca of hens may play an important role in the mechanism that inhibits *E. coli* population. Fibers are the main source for fermentation and production of new substances such as volatile fatty acids. Fibers migrate to the large intestine and are fermented. One of the main products in this region is volatile fatty acids. These acids have some advantages, such as improving intestinal pH and decreasing it. Reducing intestinal pH has different advantages with health, immune, and production (Sun et al., 2020; Kim et al., 2020).

CONCLUSION

In conclusion, supplementation of sunflower meal in laying hens diets can stimulate the activity of beneficial cecal microbial population and result in increased production of VFA. The results indicate that sunflower meal has the potential to improve immune status of laying hens.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

Protocol (no. 1319-IAU. 01.25.2017) was ap-

proved by the experimental animal ethics committee of Maragheh Islamic Azad University.

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