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Effect of Sugarcane Bagasse and Sunflower Hull on Productive Performance, Egg Quality, Tibia Characteristics, and Cecal Microbial Population of Laying Hens Raised in High Stocking Densities

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Abstract

This study evaluated the effects of different sources of insoluble fiber at different cage densities on egg performance, egg quality, tibia characteristics, and cecal microbial population of 70-week-old Hy-Line W-36 for 10 weeks. A total of 120 laying hens were allocated to six treatments, including control (CTL) and two sources of insoluble fiber (sugarcane bagasse (SB) and sunflower hulls (SFH) in 5 % of diet) at two stock densities (3 and 5 hens per cage) with five replicates. Dietary inclusion of SB decreased average daily feed intake (ADFI) and egg production (EP), but improved egg weight (EW), egg mass (EM) and feed conversion ratio (FCR) compared to the birds fed SFH. High stock density reduced ADFI, EP, and EW, but increased FCR. Egg quality parameters such as Haugh unit, shell strength, shell thickness, percentage of shell, percentage of yolk, percentage of white and yolk color, and tibia characteristics of laying hens were not affected by insoluble fiber, stock density, or their interactions. High stock density–challenged hens had higher plasma corticosterone levels, but lower feather scores compared to the normal stock density hens ($P < 0.05$. Besides, subjecting hens to high stocking density decreased populations of *Lactobacillus* spp. and increased the cecal population of *E. coli* compared to the control group ($P < 0.05$). The inclusion of SFH and SB in layer diets, however, increased populations of *Lactobacillus spp*. and decreased the cecal population of coliforms and *E. coli* compared to birds in the CTL group. Therefore, dietary inclusion of insoluble fiber, especially sugarcane bagasse, can be a useful method to improve productive performance and cecal microbial population of old laying

Introduction

The stocking density is an important factor affecting the welfare of laying hens. An area in the range of 432 to 555 cm^2 per cage is recommended by the American Egg Producers Association (United Egg Producers, 2016). In order to reduce the cost of housing, however, the farmers need to rear their hens at a higher density . It has been shown that high stocking density can reduce the layers' production performance and can negatively affect the quality of the tibia of laying hens (Zhang *et al*., 2018). Also, egg production, egg weight, shell quality, and feed intake can be negatively affected by the cage or floor density (Jalal *et al*., 2006). (Campbell *et al*., 2017). Increased blood corticosterone concentration is often associated with chronic stress in the birds in particular during high stock density. Excess corticosterone affects bone mass by enhancing osteoclast proliferation and reducing bone mineral density (Kang *et al*., 2016). Feather cover is affected by various factors that include stocking density. Abnormal behavior such as feather pecking can occur due to high stock density (Enneking *et al*. 2012; Fidan & Nazligul, 2013). Various scoring methods have been used to measure the amount of feather loss from the body of a hen. A feather score provides a

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numerical equivalent of a subjectively identified amount of plumage cover. Several studies conducted using different scoring methods have shown that hens with better plumage conditions produce more eggs (Yngvesson *et al*., 2004; Su *et al*., 2006). Other studies examining the relationship between feather score and feed consumption have found that hens with better feather cover consume less feed (Jensen *et al*., 2005; Su *et al*., 2006). When the bird is busy with activities such as feeding and drinking, the chances of feather pecking incidences decreases. The incidences of pecking behavior could decrease as a result of the consumption of nutritional factors that increase the duration of time spent feeding. It was shown insoluble fiber (IF) accumulates in the gizzard and slows the feed passage rate, at least in the proximal part of the GIT (Mateos *et al*., 2012). Moreover, it has been shown dietary inclusion of IF, such as rice hulls, can improve egg production of laying hens (Incharoen & Maneechote, 2013). The potential benefits of fiber depend on type and the quantity and quality offiber source all of which could affect microorganism population at the end part of the digestive tract (Amerah *et al*., 2009). It was shown that fiber has a stimulating effect on the production of lactate and butyrate in the small and large intestines by reducing the number of coliform bacteria (Mateos *et al*., 2002). Carbohydrate fermentation reduces protein fermentation and reduces the number of entero-bacteria in feces. The hypothesis tested in this study was that the inclusion of IF can reduce the negative effect of high stock density on the productive performance of layers. To our knowledge, the effects of dietary inclusion of IF in high stock density conditions have not been studied in the past. The present study, therefore, was designed to investigate the effect of different sources of IF on performance, egg quality, blood biochemistry, corticosterone concentration levels, bone tibia characteristics, and the cecal microbial population of laying hens reared at high stock density.

Materials and Methods

Animal ethics statement

All the procedures undertaken in the study were approved by the Animal Ethics Committee at the Agricultural Sciences and Natural Resources University of Khuzestan, Ahvaz, Iran. All efforts were made to minimize animal suffering.

Chemical analysis of insoluble fiber

Sunflower hull (SFH), and sugarcane bagasse (SB) were obtained from Isfahan sunflower seed processing (Iran), and Imam Khomeini Agro-industrial (Shooshtar, Iran), respectively. The insoluble fiber was analyzed according to AOAC (2000) methods (Table 1). Neutral (NDF) and acid detergent fiber (ADF) samples were determined sequentially as described by Van Soest *et al.* (1991).

Table 1. Chemical composition[1] (g/kg) of dietary fiber sources

Parameter	SFH[2]	SB[3]
Dry matter	923.0	548.0
Ash	30.3	45.4
Crude protein	48.0	14.5
Ether extract	46.0	4.0
Crude fiber	484.0	487.0
NDF[4]	693.0	830.0
ADF[5]	468.0	630.0
NFC[6]	183.0	106.0
NFE[7]	392.0	449.0
AME_n (Kcal/kg)[8]	339	315
Total dietary fiber[9]	801.0	726.9
Insoluble dietary fiber[9]	700.0	725.0
Soluble dietary fiber[9]	101.0	1.9

[1]All values on a DM basis unless stated otherwise.
[2]SFH; Sunflower hulls, [3]SB; Sugarcane bagasse, [4]NDF; Neutral Detergent Fiber, [5]ADF; Acid Detergent fiber, [6]Non-Fiber-Carbohydrate (NFC) = 1000-(CP + Ash + EE + NDF), [7]Nitrogen free extract NFE = 1000- (CP + Ash + CF + EE).
[8]Calculated values (Janssen, 1989), [9]Data from Knudsen (2014), and Kheravii *et al*. (2017)

Birds, Diets, and General Experimental Procedure

The present study was performed in the Poultry Research Station of Agricultural Sciences and Natural Resources University of Khuzestan (Mollasani, Iran). A total of 120 Hy-Line W-36 leghorn hens (at 70 wk old) were used in a 2×3 factorial arrangement. The treatments were 2 cage densities (3 and 5 hens per cage; 38 by 38 cm), and 3 diets with 5 replicates. Diets consisted of a basal diet as a control and basal diet supplemented with fiber sources (SB and SFH, in 5% of thediet). The fiber sources were ground by a hammer mill with a 2-mm screen before their inclusion in the diets. The first 2 wk (71 and 72 wk of age) was the adaptation period. The main trial period lasted for a total of 8 wk, from 73 to 80 wk of age. The basal experimental diet (Table 2) was formulated based on Hy-Line W-36 recommendations (Hy-Line International, 2007). Using a micromixer, the diets were mixed. The chemical composition of the samples was analyzed according to AOAC (2000). A windowless space with artificial light (16L:8D) and temperature and humidity of 20-23°C and 45% ± 10%, respectively, was used for housing the birds throughout the experiment. Feed and water were provided ad libitum.

Table 2. Ingredients and chemical composition of basal diet during the experiment (as fed on basis)[1]

Ingredients composition	(g/kg, unless stated otherwise)
Corn	618.9
Soybean Meal (440 g CP/kg)	190.0
Vegetable oil	35.0
Fish Powder (600 g CP/kg)	25.0
Di-calcium phosphate	12.5
Oyster shell powder	53.0
Limestone	56.0
Mineral premix[2]	2.5
Vitamin premix[3]	2.5
D-L methionine	1.1
Salt	3.5
Calculated analysis	
Metabolism energy (Kcal/kg)	2900
Crude protein	153.8
Methionine + Cystine	6.5
Methionine	3.8
Lysine	8.7
Arginine	10.3
Calcium	46.0
Available Phosphorus	4.0
Sodium	1.7
Analyzed values	
Dry matter	919.2
Crude protein	154.7
Crude fiber	27.1
Ether extract	62.4

[1]The experimental diets resulted from the inclusion of 5% of SB or SFH at the expense (wt:wt) of the whole basal diet.
[2]Minerals provided per kg of diet: Fe (50 mg), Zn (50 mg), Se (0.2 mg), Co (0.2 mg), Cu (10 mg), Mn (100 mg), I (1 mg), choline chloride (250 mg).
[3]Vitamins provided per kg of diet: vitamins A, 10000 IU; D_3, 5000 IU; E 50 IU; K_3, 2 mg; B_1, 2 mg; B_2, 4 mg; B_3, 30 mg; B_5, 20 mg; B_6, 2 mg; B_9, 2 mg; B_{12}, 0.01 mg; C, 1 mg; 100 g; inositol, 10 mg.

Production Performance

Hens' body weight was measured at 70 and 80 wk of age. Feed residues were collected and weighted weekly to estimate the ADFI (g/hen/day). Moreover, eggs from each replicate were counted and weighted daily to calculate the egg production (g/kg), egg weight (g/hen/day), and egg mass (g/hen/day). The feed conversion ratio was calculated as follows: FCR = feed intake/egg mass and these parameters were reported for the total period of the experiment.

Egg Quality Measurements

Every 28-d period, egg quality parameters were Measured . For analyzing egg quality, all eggs produced during the last 2d of each period were collected and haugh unit (HU), yolk color (YC), shell thickness (ST), and shell strength (SS) were measured. An egg multi-tester (EMT-5200, Robotmation Co., Ltd. Tokyo, Japan) was used to evaluate YC and HU. A micrometer screw gauge was used to determine the eggshell thickness and mean values of 3 points (air cell, equator, and sharp end) were considered as thickness. Shell breaking strength was determined by a mechanical resistive device (Karl Kolb GmbH & Co., Dreieich, Germany).

Blood Biochemicals

Blood samples were collected from the brachial vein of 3 birds per replicate on d 56 of the trial. Plasma was separated after centrifugation at 4500 g at 4°C for 10 min and frozen at −20°C until further analysis. The concentrations of glucose, low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol, and triglycerides (TG) were measured using kits (Zist Shimi, Tehran, Iran) and an autoanalyzer (Autolab PM 4000; Medical System, Rome, Italy). Plasma corticosterone levels were determined by the ELISA method described by De Jong *et al.* (2001).

Cecal Microbial Population

At the end of the experiment (at d 56 of the trial), two birds per replicate euthanized by cervical dislocation were selected and caecal digesta (1 g) from each bird were aseptically transferred into 9 mL of sterile saline solution and serially diluted. *Lactobacilli* were enumerated on de Man– Rogosa–Sharpe agar and *E. coli* and *coliform* were counted on MacConkey agar after incubation at 37°C for 48 h in an anaerobic chamber and 24 h in an aerobic chamber, respectively (Masouri *et al*., 2017). All samples were plated in duplicate.

Tibia bone characteristics

At the end of the experimental period (at d 56 of the trial), 10 birds per treatment (2 birds of each replicate) were randomly selected, weighed, and sacrificed by cervical dislocation. The left tibia bone was excised and frozen at –20°C. After thawing, the bones were individually weighted, and proximal length (cm), lateral cortex thickness (cm), and proximal head thickness (cm) at both the femoral and metatarsal sides were measured by a digital caliper, (Guz *et al*., 2019). The volume of bones was determined by placing the bone in a graded cylinder containing a certain amount of water assuming that the specific gravity of the water at room temperature was one gram per cubic centimeter. The dry matter of sheared tibia pieces was determined overnight at 105°C, and ashes were in a muffle furnace (Isotemp; Fisher Scientific, Pittsburgh, PA) at 600°C for 2h (Scheideler, 1993).

Feathering score

All birds were given feathering scores for back, wing, and tail using a score of 1 to 5 (i.e. 1, <25% coverage; 2,25-50% coverage; 3, 50-75% coverage; 4,>75% coverage and 5,complete coverage) at the end of the experiment (Lai *et al*, 2010).

Statistical analysis

The standard ANOVA test (SAS Institute, 2008) was done according to a 2×3 factorial arrangement of the treatments. Significant differences between the treatments were determined using Tukey's test at $P < 0.05$.

Results

Performance

There was not any mortality during the experiment. The effects of dietary fiber source supplementation on performance traits of hens exposed to high-stocking density are shown in Table 3. No interactions between the fiber source and density were detected for ADFI, EP, EW, and FCR of laying hens. Dietary inclusion of SB significantly ($P < 0.05$) decreased ADFI and EP, but improved EW and FCR compared to the birds fed SFH. As presented, subjecting hens to high stocking density reduced ADFI, EP, EW, and increased FCR ($P < 0.05$).

Table 3. Effect of fiber source on performance of high stocking density-challenged laying hens during 73–80 wk of age

Cage stocking density	Fiber sources[1]	Egg weight (g)	EP[2] (%)	EM[2] (g/d)	ADFI[4] (g/d/bird)	FCR[5]
	CTL	65.96	86.77	57.23	106.97	1.87
3	SB	65.72	84.05	55.23	104.24	1.88
	SFH	63.78	78.00	49.75	111.11	2.23
	CTL	61.87	80.75	49.96	101.25	2.02
5	SB	60.82	78.12	47.51	98.39	2.07
	SFH	61.16	71.96	44.01	108.82	2.47
SEM [6]		0.819	4.990	3.920	2.44	0.064
Stocking density						
	3	65.15[a]	82.94[a]	54.03[a]	107.44[a]	1.98[b]
	5	61.28[b]	76.94[b]	47.14[b]	102.82[b]	2.18[a]
SEM		0.309	0.990	0.929	0.120	0.032
Fiber sources						
	CTL	63.92[a]	83.76[a]	53.54[a]	104.11[ab]	1.94[b]
	SB	63.27[ab]	81.08[a]	51.30[a]	101.31[b]	1.97[b]
	SFH	62.47[b]	74.98[b]	46.84[b]	109.96[a]	2.34[a]
SEM		0.437	1.414	1.230	0.720	0.045
Probability						
Stocking density		<0.0001	<0.0001	<0.0001	<0.0001	<0.0002
Fiber sources		0.0034	<0.0001	<0.0001	0.0154	<0.0001
Interaction[6]		0.144	0.559	0.625	0.599	0.468

[a-c] Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] Egg production: (total produced egg/days) ×100.
[3] Egg mass = (egg production × egg weight)/100.
[4] ADFI: Average daily feed intake.
[5] Feed conversion ratio = feed intake/ egg mass.
[6] *SEM*: Standard error of the mean.
[7] Interaction = Stocking density × Fiber sources.

Egg quality traits

The effects of dietary fiber source supplementation on egg quality traits of hens exposed to high-stocking density during the first (73–76 wk of age) and second (78–80 wk of age) periods are shown in Tables 4 and 5, respectively. Haugh unit, Shell strength, Shell thickness, percentage of shell, percentage of yolk, and percentage of white and yolk color (YC) of laying hens were not affected by insoluble fiber, stock density, or the interaction.

Table 4. Effect of fiber source on egg quality measurements of high stocking density-challenged laying hens during 73–76 wk of age

Cage stocking density	Fiber sources[1]	Haugh unit	Shell strength (kg/cm^2)	Shell Thickness (mm)	Shell (%)	Yolk (%)	White (%)	Yolk color
	CTL	83.81	2.16	0.352	8.68	26.14	63.65	7.90
3	SB	84.10	1.81	0.349	8.49	25.95	63.92	7.95
	SFH	84.85	2.12	0.359	8.99	26.05	63.98	7.85
	CTL	83.88	2.02	0.343	8.46	26.04	63.81	7.95
5	SB	83.45	1.97	0.342	9.01	26.25	63.44	7.65
	SFH	84.02	2.07	0.340	8.92	26.40	63.42	7.85
SEM[2]		0.63	0.10	0.05	0.23	0.48	0.67	0.16
Stocking density								
	3	84.25	2.03	0.353	8.72	26.04	63.85	7.90
	5	83.78	2.02	0.342	8.79	26.23	63.55	7.81
SEM		0.32	0.05	0.04	0.12	0.25	0.35	0.09
Fiber sources								
	CTL	83.86	2.09	0.348	8.58	26.08	63.75	7.92
	SB	83.78	1.89	0.346	8.76	26.09	63.68	7.80
	SFH	84.45	2.09	0.350	8.96	26.21	63.70	7.85
SEM		0.45	0.07	0.03	0.17	0.32	0.49	0.13
Probability								
Stocking density		0.14	0.88	0.08	0.35	0.55	0.25	0.38
Fiber sources		0.36	0.23	0.91	0.34	0.64	0.98	0.49
Interaction[3]		0.78	0.61	0.71	0.33	0.95	0.74	0.69

[a-c] Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] *SEM*: standard error of the mean.
[3] Interaction = Stocking density × Fiber sources.

Table 5. Effect of fiber source on egg quality measurements of high stocking density-challenged laying hens during 77–80 wk of age

Cage stocking density	Fiber sources[1]	Haugh unit	Shell strength (kg/cm^2)	Shell Thickness (mm)	Shell (%)	Yolk (%)	White (%)	Yolk color
	CTL	84.20	1.95	0.359	8.95	26.03	63.87	7.30
3	SB	84.55	2.02	0.357	8.70	26.60	63.89	7.38
	SFH	85.09	2.14	0.377	9.13	26.35	64.20	7.45
	CTL	83.65	1.85	0.354	8.67	26.55	63.90	7.57
5	SB	83.86	1.84	0.362	8.60	26.29	63.80	7.40
	SFH	83.92	1.94	0.364	8.83	26.05	64.10	7.36
SEM[2]		0.70	0.10	0.05	0.28	0.50	0.65	0.15
Stocking density								
	3	84.61	2.04	0.364	8.92	26.32	63.99	7.37
	5	83.81	1.88	0.360	8.70	26.30	63.93	7.44
SEM		0.34	0.08	0.03	0.12	0.23	0.32	0.08
Fiber sources								
	CTL	83.93	1.90	0.356	8.78	26.29	63.87	7.43
	SB	84.20	1.93	0.359	8.65	26.45	63.84	7.39
	SFH	84.51	2.04	0.370	8.97	26.19	64.15	7.40
SEM		0.50	0.08	0.09	0.18	0.36	0.44	0.12
Probability								
Stocking density		0.05	0.12	0.76	0.45	0.92	0.85	0.24
Fiber sources		0.20	0.06	0.07	0.50	0.85	0.84	0.95
Interaction[3]		0.91	0.30	0.19	0.95	0.80	0.98	0.74

[a-b] Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] *SEM*: Standard error of the mean.
[3] Interaction = Stocking density × Fiber sources.

Blood parameters

As shown in Table 6, no interactions between the fiber source and density were detected for plasma corticosterone levels. While the high stocking density–challenged hens had higher plasma corticosterone levels compared with those in normal density cages ($P < 0.05$). There was a significant interaction between the fiber source and density related to glucose concentration ($P < 0.05$); dietary inclusion of SFH decreased plasma glucose levels in high stocking density–challenged hens and also those kept in normal stocking cages. High-stocking-density hens exhibited significantly greater plasma glucose levels than normal stocking-density hens ($P < 0.05$). There was no significant interaction between fiber source and density for TG, cholesterol, HDL, and LDL. Plasma concentrations of TG, however, decreased in SFH group compared to the other treatments ($P < 0.05$).

Table 6. Effect of fiber source on blood parameters of high stocking density-challenged laying hens at the end of the experiment

Cage stocking density	Fiber sources[1]	Corticosterone (ng/mL)	Glucose (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	CTL	530.50	232.50[a]	1412.25	134.75	27.97	39.75
3	SB	527.75	235.25[a]	1444.50	130.25	26.60	35.50
	SFH	548.25	209.00[b]	1385.00	125.00	28.92	28.25
	CTL	556.50	239.75[a]	1467.00	137.75	24.27	39.25
5	SB	546.75	239.50[a]	1462.25	128.25	36.55	31.00
	SFH	553.75	215.00[b]	1395.25	138.25	34.72	36.80
SEM [2]		9.28	4.44	25.30	7.56	7.61	2.36
Stocking density							
	3	535.50[b]	225.58[b]	1413.91	130.00	27.83	34.50
	5	552.33[a]	231.41[a]	1441.50	134.75	31.85	35.68
SEM		2.64	1.22	17.65	3.78	1.23	1.18
Fiber sources							
	CTL	543.50	236.12[a]	1439.62[a]	136.25	26.12	39.50
	SB	537.25	237.37[a]	1453.38[a]	129.25	31.57	33.25
	SFH	551.00	212.00[b]	1390.12[b]	131.62	31.82	32.52
SEM		3.73	3.14	14.89	5.34	1.17	1.66
Probability							
Stocking density		<0.0001	0.003	0.306	0.068	0.069	0.506
Fiber sources		0.061	<0.0001	<0.0007	0.101	0.070	0.060
Interaction[3]		0.231	<0.0001	0.060	0.271	0.150	0.069

[a-c] Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] *SEM*: Standard error of the mean.
[3] Interaction = Stocking density × Fiber sources.

Feather Scores

The effects of dietary fiber source supplementation on feather scores of high-stocking density-challenged hens in the wing, back, and tail are shown in Table 7. No interactions between the fiber source and density were detected for feather scores of laying hens. Birds kept in high stocking density cages had lower feather scores in the wing, back, and tail ($P < 0.05$) compared with those in normal density cages. Although dietary inclusion of fiber had no marked effects on feather scores of layers at the end of the experiment.

Characteristics of tibia

The effects of dietary fiber sources on tibia characteristics of high-stocking density-challenged hens are presented in Table 8. No interactions between the fiber source and density were detected for tibia weight, proximal length, diaphysis thickness, volume, and outer and inner diameter of the diaphysis. Also, compared to birds kept in normal-density cages, those kept in high stocking density conditions had lower tibia dry matter and ash ($P < 0.05$). Furthermore, layers of the SFH diet group had higher ash of tibia compared to the other treatments ($P < 0.05$).

Cecal microbial population

The effects of diet treatments on cecal microbial populations in birds are shown in Table 9. There is no interaction between the fiber source and density for the cecal population of *E. coli*, *Lactobacillus spp.*, or *coliforms*. Subjecting hens to high stocking density decreased populations of *Lactobacillus spp.* and increased the cecal population of *E. coli* ($P < 0.05$) compared to the normal density group. The inclusion of SFH and SB in layer diets increased populations of *Lactobacillus spp.* and decreased the cecal population of *coliforms* and *E. coli* compared to birds in the CTL group ($P < 0.05$).

Table 7. Effect of fiber source on feather scores of high stocking density-challenged laying hens at the end of the experiment

Cage stocking density	Fiber sources[1]	Wing	Tail	Back
	CTL	4.70	4.98	4.90
3	SB	4.80	4.70	4.94
	SFH	4.34	4.36	4.46
	CTL	3.00	3.14	3.18
5	SB	3.68	4.12	3.88
	SFH	3.34	3.32	3.68
SEM [2]		0.231	0.240	0.256
Stocking density				
	3	4.61[a]	4.68[a]	4.77[a]
	5	3.34[b]	3.51[b]	3.58[b]
SEM		0.115	0.120	0.128
Fiber sources				
	CTL	3.85	4.06	4.04
	SB	4.24	4.41	4.41
	SFH	3.84	3.84	4.02
SEM		0.163	0.170	0.181
Probability				
Stocking density		<0.0001	<0.0001	<0.0001
Fiber sources		0.257	0.128	0.160
Interaction[3]		0.072	0.087	0.174

[a-c] Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] *SEM*: Standard error of the mean.
[3] Interaction = Stocking density × Fiber sources.

Table 8. Effect of fiber source on tibia characteristics of high stocking density-challenged laying hens at the end of the experiment

Cage stocking density	Fiber sources[1]	Weight (g)	Proximal length (mm)	Outer diameter of diaphysis (mm)	Inner diameter of diaphysis (mm)	Diaphysis thickness (mm)	Volume (cm^3)	dry matter (%)	Ash (%)
	CTL	5.75	113.87	6.46	4.22	2.22	7.76	74.94	49.86
3	SB	5.45	113.00	6.59	4.50	2.07	7.38	73.95	49.77
	SFH	5.60	113.14	6.82	4.41	2.38	7.45	71.97	50.81
	CTL	5.78	114.34	6.42	4.07	2.32	7.74	71.85	45.08
5	SB	5.41	113.35	6.69	4.46	2.21	7.35	70.87	45.65
	SFH	5.76	114.50	6.47	3.95	2.48	7.89	70.18	50.46
SEM		0.22	0.82	0.15	0.35	0.24	0.26	0.94	1.39
Stocking density									
	3	5.60	113.33	6.62	4.37	2.21	7.53	73.62[a]	50.14[a]
	5	5.65	114.06	6.52	4.16	2.33	7.66	70.97[b]	47.06[b]
SEM [2]		0.12	0.40	0.06	0.56	0.14	0.14	0.46	0.69
Fiber sources									
	CTL	5.76	114.11	6.41	4.14	2.27	7.77	73.41	47.47[b]
	SB	5.42	113.18	6.60	4.48	2.14	7.36	72.38	47.69[b]
	SFH	5.68	113.83	6.61	4.20	2.44	7.68	71.09	50.62[a]
SEM		0.16	0.58	0.11	0.10	0.19	0.20	0.67	0.97
Probability									
Stocking density		0.67	0.18	0.35	0.07	0.45	0.54	0.02	0.03
Fiber sources		0.30	0.51	0.46	0.25	0.63	0.37	0.08	0.03
Interaction[3]		0.98	0.93	0.50	0.70	0.10	0.83	0.43	0.21

[a-b] Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] *SEM*: Standard error of the mean.
[3] Interaction = Stocking density × Fiber sources.

Table 9. Effect of fiber source on the cecal microbial population (log cfu g^{-1}) of high stocking density-challenged laying hens at the end of the experiment

Cage stocking density	Fiber sources[1]	*Lactobacillus*	*Escherichia coli*	*coliform*
	CTL	9.34	9.36	8.68
3	SB	9.40	8.96	8.56
	SFH	9.41	8.49	8.47
	CTL	8.92	9.37	8.93
5	SB	9.42	8.45	8.38
	SFH	9.40	9.37	8.37
SEM [2]		0.082	0.089	0.345
Stocking density				
	3	9.38^{a}	8.94^{b}	8.57
	5	9.24^{b}	9.17^{a}	8.56
SEM		0.016	0.022	0.026
Fiber sources				
	CTL	9.13^{b}	9.36^{a}	8.80^{a}
	SB	9.41^{a}	8.70^{b}	8.47^{b}
	SFH	9.40^{a}	8.93^{b}	8.42^{b}
SEM		0.058	0.055	0.065
Probability				
Stocking density		<0.001	<0.001	0.162
Fiber sources		0.014	<0.001	<0.001
Interaction[3]		0.130	0.081	0.100

$^{a\text{-}c}$ Means with different letters within the same column differ significantly ($P < 0.05$).
[1] CTL: Control, SB: Sugarcane bagasse, SFH: Sunflower hull.
[2] *SEM*: Standard error of the mean.
[3] Interaction = Stocking density × Fiber sources.

Discussion

There was no report in the literature on the possible effects of insoluble fiber on hens experiencing stress caused by high stock density. Hence, the present study was aimed to investigate the effect of dietary supplementation of insoluble fiber on the performance, egg quality traits, blood parameters, cecal microbial population, characteristics of the tibia, and feather scores of laying hens reared under high stock density.

Performance

Our study revealed that there was no interaction between dietary insoluble fiber and stocking density related to performance parameters in hens. Also, ADFI, EP, and EW were significantly higher in birds kept at normal density cages than those kept at high stock density. Similarly, FCR was significantly improved in layers placed at normal-density cages compared to those at high stock density. Consistent with our findings, Sarica *et al*. (2008) also observed that EP was significantly decreased at higher stocking densities. Stress, reduced feeding area, and cannibalism occurrence (Jalal *et al*., 2006), all resulting from high-density conditions, could be the reasons for the decrease in EP. It has also been reported that decreasing the stocking density can improve feed intake, weight gain, and FCR in broilers (Abudabos *et al*., 2013). The movement of the birds at high stock density may restrict. Therefore, access to feeders and drinkers may be difficult in birds under high stock density than those under normal stock density. Thus, decreased feed intake could result in lowered EP and poor FCR at high stock density.

Dietary inclusion of SB decreased ADFI and EP, but improved FCR compared to the birds fed SFH. It has been reported that sugarcane bagasse as a source of insoluble fiber increased the number of intestinal *Bacillus spp*. bacteria. Also, as a prebiotic, it can be improved the intestinal health and performance of broiler chickens (Kheravii *et al*. 2017). Perhaps the reason for the improved feed conversion ratio in SB-consuming birds is the lower feed intake of these birds compared to other fiber sources. It was assumed that the spongy forms of insoluble fiber in digesta allowed enzymes to penetrate easier. Therefore, the surface area and accessibility of nutrients to enzyme activity are increased, resulting in increased absorption and retention of nutrients and improved growth performance of birds. It has been shown that insoluble fiber sources pass through the gastrointestinal tract more rapidly towards the end of the gastrointestinal tract, thereby increasing feed intake (Hetland *et al*. 2003). And this might explain an increased feed intake in birds fed SFH.

Egg quality traits

Egg quality parameters did not influence either by the addition of fiber sources or by the stock density. A similar observation was reported regarding the role of insoluble fiber in egg quality parameters (Abdollahi *et al*., 2021). Likewise, Samli (2006) reported yolk weight, albumin weight, shell thickness, and shell weight of 22-week-old laying hens did not affect by adding 0.5, 5, and 10% rice bran to the diet. In line with what was reported by Sarica *et al*., (2008), and Jalal *et al*., (2006), we also observed that the stock density did not affect egg quality traits. In contrast to our findings, Jahanian & Mirfendereski (2015) reported a thicker eggshell in hens subjected to high stocking density.

Blood parameters

Compared to the control group, plasma glucose levels in the high-stocking density group were significantly greater which is in accordance with previous reportss(Mirfendereski & Jahanian, 2015). Even though plasma glucose can be an early indicator of the stress condition, caution must be taken because blood glucose levels can also be affected by other factors, including hormone secretion and nutrition levels.

Similar to previous reports (Mirfendereski & Jahanian, 2015), we also found that subjecting hens to high stock density can increase blood corticosterone concentration compared to the normal density. Similarly, Cheng & Muir (2004) also reported that laying hens kept in single-bird cages (525 cm^2/bird) show lower plasma corticosterone levels compared to those in 10-bird cages (419 cm^2/bird).

In our study, plasma concentrations of TG and glucose decreased in the SFH group compared to the other treatments. These results are in line with those reported by Boazar *et al* (2021). They observed that the mash diets and inclusion of fibers (SFH, cellulose, sugar-beet pulp, and wheat bran) can reduce the TG concentration of broiler chickens. Mohiti-Asli *et al*. (2012) showed that plasma concentration of cholesterol decreased in broiler breeders fed 3% cellulose. The decreased TG concentrations in serums of SFH-fed hens may be related to the role of fiber in bile salts deconjugation, so the absorption of fat in the gut reduce and the body fat (liver fat) is used for metabolic needs, and reduces the concentration of TG in the serum.

Feather Scores

Insoluble fibers were very effective in preventing or stopping cannibalism in laying hensThis is because birds fed a diet containing high insoluble fiber spend more time feeding than those fed a low fiber diet and thus appear calmer. Although in our experiments, the effect of fiber was not significant on feather score, the high stocking density–challenged hens had lower feather scores in the wing, back, and tail compared with those in normal density cages. These results are in accordance with Sarica *et al*. (2008) study. They reported plumage damage and feather loss on all body parts of hens that were kept in a more restricted area. Likewise, Morrissey *et al*. (2014) showed that feather pecking decreased in broiler breeders fed fibrous diluted diets. It was also shown that a wider space allowance can lead to healthy hens feeling no pain and not showing any abnormal behavior such as pecking on other animals' body surfaces (Bright *et al*., 2006). A higher level of feather loss is another important observation in birds allowed a restricted space. Interestingly, in contrast to our findings, Asensio *et al*., (2020) reported that a fibrous diet can improve the wing-feather score of laying hens at 22 wk compared with those fed the control diet.

Characteristics of tibia

As an indicator of bone strength (Rath *et al*., 2000) and also an important organ in the growth and production of laying hens, the tibia can be negatively affected when birds are rearing in a limited space (Zhang *et al*., 2018; Wang *et al*., 2020). Our findings confirm this, as in our experiments high stock density–challenged hens had a lower tibia dry matter and ash (as compared with those in normal density cages. This could be in part due to a lower serum parathyroid hormone concentration in layers under high stocking density, which can impair the absorption of calcium and reduce the tibia ash (Wang *et al*. 2020). In addition, we hypothesized that there could be greater skeletal development in the hens fed the insoluble fiber diet than in the control group. This hypothesis was fulfilled in the hens fed SFH diet that showed a higher amount of tibia ash compared to the other treatments. The inclusion of insoluble fiber, which can lower the diet density, can accelerate the passage time of the digesta through the digestive tract, especially in the jejunum and ileum. The less time the digesta spends in the jejunum and ileum can decrease the paracellular absorption of Ca and P (Adedokun & Adeola, 2013), which, in turn, may impair bone mineral deposition and ash content.

Cecal microbial population

Low numbers of *Lactobacillus sp.* and high numbers of *E. coli* were observed in the cecum of the high stock density–challenged hens, compared with those kept in normal stocking cages, However, no bacterial population was significantly affected by the interaction of insoluble fiber and stock density. This data suggests that stress from high stock density can disturb the gut microbial population, which is in agreement with the findings of Kridtayopas *et al* (2019). They reported a lower-than-control count of *Lactobacillus sp.* and *Clostridium sp.* and a higher-than-control count of *E. coli* in the gastrointestinal tract of the high stock density group.

In our study, dietary inclusion of SB and SFH resulted in an increase in beneficial bacteria and a decrease in *E. coli* in the cecum. Similar findings in birds fed SFH were reported by Pourazadi *et al*. (2020) for the cecal population of *Lactobacillus spp*. An increase in the population of this bacterial strain is usually considered to be beneficial to the host because they can outcompete and prevent the colonization of pathogens such as *E. coli*. It was reported that the presence of *Lactobacillus spp*. in the ileum and *Bifidobacterium spp*. in the ileum and caeca increased and the presence of ileal and cecal *E. coli* and *Clostridium spp*. reduced by dietary supplementation of lignocellulose in broiler chickens (Bogusławska-Tryk *et al*., 2015). Kheravii *et al*. (2017) showed that counts of *Bacillus spp*. in the ileum of broilers can increase by dietary inclusion of sugarcane bagasse at 20 g/kg. One implication of the current and previous studies is that the effect of dietary fiber on the gastrointestinal microbiota in poultry can vary, depending on the source and composition of the fiber, which needs to be further characterized in future studies.

Conclusion

These data indicate that high stock density negatively influenced performance, increased serum corticosterone, decreased feather score, and cecal population of *Lactobacillus spp*. of laying hens. Dietary inclusion of insoluble fiber at 5% improved laying performance and cecal microbial population, effects that in general were more pronounced with SB than with SFH.

Conflict of interest

The authors declare no conflict of interest.

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