ISSN : 0974 - 7435 Volume 11 Issue 10

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(10), 2015 [376-384]

Analysis of fermented alfalfa meal nutrients and the effects on growth performance and cecal microbial population in *Gushi* geese

Haicheng Yin*

College of Biological Engineering, Henan University of Technology, Zhengzhou, Henan Province 450001, (P.R.CHINA)

100 Lianhua Street, Zhengzhou New and High-tech Industrial Development Zone, Zhengzhou 450001, (CHINA)

ABSTRACT

The nutritional value of alfalfa meal (AM) can be improved by solid-state fermentation (SSF), which provided an alternative ingredient for replacing soybean meal (SBM) in geese feed. The experiment assessed the quality of the fermented alfalfa meal (FAM) and its feasibility of replacing SBM with the FAM in diet on the growth and cecal microbial population of *Gushi* geese. Six isoenergetic (metabolizable energy 11.0 MJ/kg) and isoproteic (crude protein 15.3%) diets were formulated, in which 0 (control), 20, 40, 60, 80 and 100% of SBM were replaced by FAM. A total of 300 healthy geese (with the initial weight 392 g) were randomly assigned to six treatment groups with five replicates of 10 geese (5 males and 5 females). Geese were fed ad libitum for 35 days. The results showed that the FAM was lower in MD, NCF, ADF and NDF, higher in CP, CF, Ash and NE, and approximately equal in Ca and P, and supplemented protease compared to AM. The geese fed the diets with 40% or lower SBM substitution levels had no significantly effects final body weights (FBW) and average day gain (ADG) ($P<0.05$), whereas 60% or higher caused poor growth record of geese compared to the control diet. The average daily feed intake (ADFI) and feed/gain ratio (F/G) were higher than other groups ($P<0.05$) with a 40% or higher substitution level. The numbers of *Bifidobacterium* and *Lactobacillus* in the cecal digesta of geese fed with FAM increased ($P<0.05$), whereas no effect were found in the 20% and 40% SBM substitution level compared with the control ($P>0.05$). On the contrary, the *Coliforms* counts of cecum decreased with the increasing inclusion of FAM, while this counts were significantly reduced in geese fed the diets with a 60% or higher SBM substitution level ($P<0.05$). Results of above suggest that fermentation changed the nutritional characteristics of alfalfa meal, and a certain percentage FAM in feed could promote the growth, by increasing beneficial microbiota, and effecting cecum microbiome of *Gushi* geese.

© 2015 Trade Science Inc. - INDIA

KEYWORDS

Fermented alfalfa meal;
Nutritional characteristic;
Gushi geese;
Growth performance;
Cecal microbiome.

INTRODUCTION

Alfalfa is often applied to animal feed because its high protein and minerals contents[37]. Several studies have conducted that the inclusion of moderate amounts of alfalfa meal (AM) in diet of experimental animals improved their health[33, 37], reduce physiological stress[14] and result in increased their intake feed and improved growth performance[10]. However, Kass et al. (1980) reported that poor palatability of AM reduces feed intake of swine and thus influence its growth performance. The negative effects of AM are also probably related to its higher fiber level[32, 6]. Renteria-Flores et al. (2008) reports the feed intake reduces as the fiber level of AM increases in a swine diet. However, the negative aspects could be changed by adjust to the supplementation levels or with fermention.

Bacillus subtilis (*B. subtilis*) is one of the probiotics and used in feeds[31, 6, 7]. Previous study suggested that the use of solid-state fermentation (SSF) with *B. subtilis* may increase the content of crude protein, improve the nutritive value and enhances palatableness of forage grass when it is used in poultry feed[21, 5, 34]. The used of *B. subtilis*-fermented forage grass showed a positive influence on growth and bacterial ecology in chick feeds[9]. Yan et al. (2010) reported that in fermented alfalfa (silage) can occur in diets without any negative effects on growth and physiological functions of Boer goat. To date, however, there has been a dearth of information to suggest which fermented alfalfa meal (FAM) is more beneficial on *Gushi* geese. The herbivorous waterfowl, *Gushi* goose is a local breed in *Gushi* County, Henan Province (China), characterized by white and square body. The objective of the present study was to evaluate the effect of *Bacillus subtilis* on fermentation of alfalfa meal, and replacement ratio of soybean meal by FAM on growth performance and cecal microbial population in *Gushi* geese.

MATERIALS AND METHODS

FAM Preparation and chemical composition

The alfalfa of the same batch used for the study was harvested at the flowering stage in June 20, 2014. Harvested alfalfa was rinsed twice under distilled water to remove its dirt respectively, drained through a strainer at 30 °C for 24 h, and then ground (0.2 mm). The main chemical compositions of the forages are analyzed for dry matter (DM), crude protein (CP), crude fat (CF), Ash, ADF, NDF, NFC, calcium, phosphorus and NE by the Association of Official Analytical Chemists (2007). The AM was used after SSF using the *B. subtilis* ACCC 01746.

The *B. subtilis* ACCC 01746 was kindly provided by Dr. Guan (College of Bioengineering, Henan University of Technology). The strain was precultured on a potato dextrose agar and later cultured in culture broth medium (containing yeast extract 0.25%, K_2HPO_4 0.25%, KH_2PO_4 0.4% at pH 7.0) at 35°C for 24 h. The per two milliliter culture (1.52×10^8 cfu/ml) was diluted using sterile distilled water and sterilized AM (1 kg) were spray inoculated and SSF in large sterile plastic bag at 35°C and pH 7.0 for 7-day. The FAM were dried and refrigerated until mixed in the diets. The treatment was completed in triplicate. The chemical compositions of FAM were analyzed according to the method described above. The nutrients in FAM compared with AM are shown in TABLE 1.

Approximate location for TABLE 1

Diet formulation

The six approximately isoprotein (crude protein, 15.3%), isoenergetic (metabolizable energy, 11.0 MJ/kg) diets were formulated by replacing 0 (con-

TABLE 1 : Chemical compositions determined for AM and FAM[1] (%)

Diet (%)	AM	FAM[2]
DM	89.6	44.3
CP	19.20	22.18
CF	2.60	2.84
Ash	7.78	8.12
NFC	27.4	25.7
ADF	40.8	32.4
NDF	50.2	37.6
Ca	2.05	2.09
P	0.44	0.48
NE (Mcal/kg)	1.20	1.51

[1]On a dry matter basis; [2]Per milliliter 1.52×10^8 cfu/g *Bacillus subtilis*

FULL PAPER

TABLE 2 : The ingredients and composition of the experimental diets (air-dry basis, %)

Ingredients	SBM substitution level					
	0%	20%	40%	60%	80%	100%
Corn	55.9	55.7	55.4	55.2	55	54.8
Fishmeal	5	5	5	5	5	5
Wheat bran	14	14	14	14	14	14
SBM	10	8	6	4	2	-
FAM	-	4.3	8.6	12.9	17.2	21.5
Soybean oil	5.02	4.45	3.9	3.34	2.4	1.33
α-Cellulose	4.55	3.71	2.85	1.81	0.85	-
CaH_2PO_4	0.9	0.6	0.4	0.3	0.2	0.1
NaCl	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	2.56	2.17	1.78	1.5	1.5	1.5
Met	0.2	0.2	0.2	0.19	0.19	0.18
Lys	0.17	0.17	0.17	0.16	0.16	0.14
Choline chloride	0.3	0.3	0.3	0.3	0.3	0.3
Compound promix[a]	1.2	1.2	1.2	1.1	1	0.95
Nutrient levels (%)[b]						
Metabolizable energy (MJ/kg)	11.02	11.01	11.01	11.00	11.02	11.02
Crude protein	15.40	15.40	15.39	15.39	15.39	15.38
Crude fiber	6.15	6.15	6.14	6.15	6.14	6.13
Calcium	0.81	0.79	0.80	0.80	0.81	0.81
Total phosphorus	0.51	0.51	0.52	0.53	0.53	0.54
Lysine	0.79	0.79	0.80	0.80	0.79	0.78
Met	0.41	0.41	0.40	0.40	0.39	0.39

[a] The compound premix provides per kg of diet: Cu 10 mg, Fe 60 mg, Mn 80 mg, Zn 60 mg, I 1.5 mg, Se 0.3 mg; vitamin A 10000 IU, vitamin D 2500 IU, vitamin E 25 IU, vitamin K 1.0 mg, vitamin B_1 2.0 mg, vitamin B_2 8.0 mg, vitamin B_6 2.5 mg, vitamin B_{12} 0.01 mg, vitamin PP 30.0 mg, vitamin B_5 15.0 mg, vitamin B_9 0.5 mg, biotin 0.15 mg; [b] Analysed values

trol), 20, 40, 60, 80 and 100% soybean meal with graded inclusion levels of FAM (0, 4.3%, 8.6%%, 17.2% and 21.5% TABLE 2). Soybean oil and α-cellulose were added to reach the equal level of crude fat and fiber in all diets, and Met and Lys were supplemented to meet the essential requirements for the geese. All ingredients were mixed, homogenized, and then pelleted (3-mm diameter) with a granulator (QRLS-150, Luoyang machinery Co., Ltd., China). All pellet diets were dried at 35°C for about 4h in a ventilated oven to approximately 7% moisture, and stored in sealed bags at 4 °C until used.

Approximate location for TABLE 2

Geese and management

Three hundred 7-day-old male and female *Gushi* geese were purchased from *Gushi* Sanmu farming Co., Ltd (Henan Province, China). At the start of the experiment, geese were acclimated to the control diet for 3 days and randomly distributed into 6 dietary treatments with 5 replicates (10 geese, 5 males and 5 females per replicate). At the end of the acclimation period, geese were weighted separately (average initial weight 392 g), housed and equipped with respective an open trough feeder and provided water via a trough in an environmentally controlled room from day 11 to 45. The room temperature for 15-day-old formulate containing (28-30)°C, 25-day-old containing (26-27)°C, 35-day-old containing (24-25)°C and 35 days after the temperature from 24°C to normal atmospheric temperature. During the entire feeding trial, the air relative humidity was maintained at 65 to 70 %. At 8 pm every day, uneaten feed were collected and weighed to calculate the total feed intake.

Analysis of protease activities

At different time points, a 2 g FAM sample was taken out from the SSF-culture. The sample was put into 100 ml lactic acid buffer solution for acid protease (pH 3.0), phosphate buffer solution for neutral protease (pH 7.0) and borate buffer solution (pH 10.5) for alkaline protease, stirred and incubated for 5 min at 25 ÚC, and then centrifuged at 5,000 g at 4°C for 10 min. The supernatant was used the enzyme activity test. The method of Folin phenolwas used to measure the activity of protease[19]. The protease activity, expressed as U/g (dwb), was calculated by dividing units of neutral protease activity by the dry matter mass in the original sample. One unit of enzyme activity is defined as the amount of 1 mL enzyme solution required to produce an increase of 1 μg tyrosine under the defined conditions.

Growth response

The feeding trial was terminated and the geese were starved for 12 h at 45-day-old. The geese were then individually weighed to gain information on growth. The geese's feed intake of each dietary treatment was counted. The final body weights (FBW) the average day gain (ADG, g), average daily feed intake (ADFI, g) and feed/gain ratio (F/G) were determined from these data.

Cecal microflora population

The total anaerobic bacteria, *Lactobacilli*, *Coliform bacteria* and *Bifidobacterium* were cultured on the Plate-count agar, MRS-agar, MacConkey-agar and BBL-agar (purchased from the Nanjing Jiancheng Insititute of Bioengineering, China), respectively, according to the method of Xia et al. (2004) and Wang et al. (2009). The cecal chyme from each goose (in triplicate, 1 g per sample) was determined and diluted ten times with sterilized PBS and vortexed for 5 min. All digesta samples were thoroughly mixed with butter-fields phosphate buffer dilution solution and further diluted to a factor of 10^{-8}. The sterile Petri dishes containing selective media was inoculated with 0.1 ml sample and incubated for analysis of total colony count, total anaerobic bacteria at 30°C for 72 h, *Lactobacilli* at 37°C for 72 h, *Coliform bacteria* 37°C for 24 h and *Bifidobacterium* at 37°C for 48 h. All of the bacteria number which were calculated as $\log_{10}{}^{CFU}$ (colony-forming units, CFU) per g of chyme for statistical analysis.

Statistical analysis

All experimental data were subjected to analysis of variance using SAS (1996). The differences among means for the six dietary treatments were compared by Duncan's multiple range tests. The significance of differences was considered with $P<0.05$. General linear and quadratic models were performed on growth, enzyme activities and cecal microflora population against dietary substitution level of SBM by FAM.

RESULTS

Chemical composition alfalfa meal and fermented alfalfa meal

The chemical compositions of the AM and FAM are shown in TABLE 1. There were obviously differences in chemical compositions between AM and FAM. The AM naturally had much higher DM content than FAM, 89.6% and 44.3% respectively. FAM was lower in NCF, ADF and NDF, higher in CP, CF, Ash and NE, and approximately equal in Ca and P compared to AM.

Protease activities in different fermentation periods

As can be seen in Figure 1, enzyme activities increased gradually from 12 to 36 h for acid protease and alkaline protease and from 12 to 24 h for neutral protease, at which it were maximal, at 304.45, 121.33 and 418.15 U/g; it then slowly decreased with time, respectively.

Approximate location for Figure 1

Growth performance and feed utilization

Geese fed the control diet (0% SBM substitution level) showed the highest FBW and ADG (TABLE 3). When FAM replaced 60% or more from SBM, FBW and ADG were significant lower compared with the other groups, however no significant differences were observed at or less 60% SBM substitution level ($P<0.05$). Compared to the control treatment, there were significant increases in ADFI

FULL PAPER

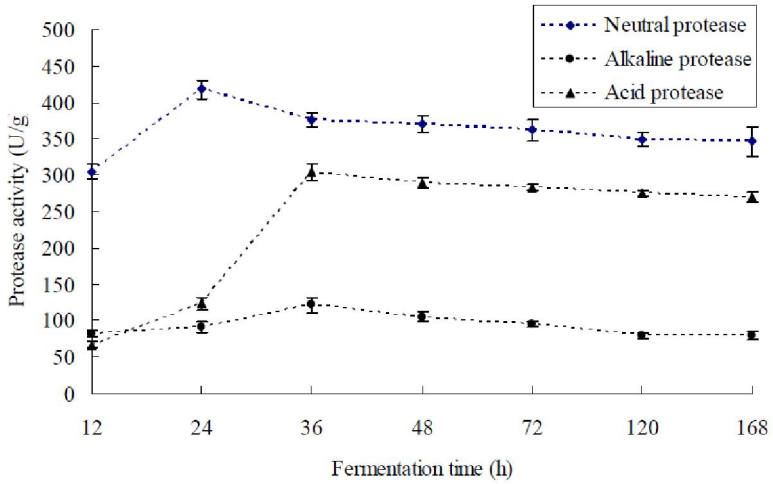

Figure 1 : Protease activities changes at different fermentation times

TABLE 3 : Effects of replacing SBM with FAM on growth and feed utilization of *Gushi* geese[1, 2] (gÿN=5)

SBM substitution level	IBW	FBW	ADG	ADFI	F/G
0%	392.36	1912.76a	43.44a	144.01c	3.12d
20%	391.19	1898.30a	43.06a	146.83bc	3.41cd
40%	392.54	1870.27ab	42.23ab	149.76b	3.55c
60%	393.22	1841.89b	41.41b	153.52ab	3.71b
80%	390.37	1766.57c	39.32b	161.11a	4.10ab
100%	394.41	1687.66d	36.95c	156.64ab	4.24a
SEM[3]	9.37	20.28	1.54	4.51	0.07
P-value	0.998	<0.001	<0. 011	0.014	0.006

Note : [1]Values are means of three replicates; [2]a, b: Means with different superscripts at a column are significant differences (P<0.05); [3]Pooled standard error of mean.

of geese fed diets with 40% or more substitution level (P<0.05), however no significant differences in the fed the diets with 20% substitution level (P > 0.05). F/G show similar tendency.

Approximate location for TABLE 3

Cecal microflora composition

The number of total anaerobic bacteria in the cecal digesta of geese decreased with increasing dietary FAM levels, however no significant differences were observed in these groups fed FAM substitution of SBM compared to the control group (TABLE 4). Geese fed the diet supplemented with 60% or higher had significantly higher (P<0.05) cecal *Lactobacillus* than fed the control diet; whereas, the number of *Lactobacillus* in cecal chyme of geese fed 20% and 40% SBM substitution levels were not significantly different than fed the control diet. The number of *Bifidobacterium* in cecal chyme of geese showed a similar trend as with the number of *Lactobacillus* TABLE 4. The number of *Coliforms* in cecal chyme of geese decreased with increasing dietary FAM substitution of SBM levels (TABLE 4). No significant differences in number of *Coliforms* were observed in the group fed 40% or lower substitution of SBM compared to the control treatment; however, when the substitution level was 60% or more, number was significantly lower than the control group (P<0.05).

Approximate location for TABLE 4

DISCUSSION

Chemical composition and protease activity

As can be seen in TABLE 1 and Figure 1, the FAM was lower in NCF, ADF and NDF, higher in CP, CF and NE, and contenting higher protease activity compared to AM. Microbial fermentation[11] is a superior approach to improve the nutritive value

TABLE 4 : The number of cecal microflora composition of geese fed the diets with different levels of FAM (log_{10} (CFU/g))[1, 2]

SBM substitution level	*Caecum*			
	Total anaerobic bacteria	*Lactobacillus*	*Coliforms*	*Bifidobacterium*
0%	6.19	6.86b	7.65a	5.26b
20%	6.11	6.90b	7.10ab	5.28b
40%	6.06	7.11ab	7.11abc	5.63ab
60%	6.01	7.56a	6.85bc	5.92a
80%	5.95	7.47a	6.28bc	6.04a
100%	5.90	7.42a	6.05c	6.13a
SEM[3]	0.24	0.38	0.18	0.24
P-value	0.483	0.017	0.034	0.032

Note : [1] Values are means of three replicates; [2] a, b: Means with different superscripts at a column are significant differences ($P<0.05$); [3] Pooled standard error of mean

of forage grass, not only can produce microbial enzymes to increase its beneficial factors, but also can accumulate some other beneficial metabolites, which are thought of the main factor that affect the quality of FAM. The increased contents of protein may be due in part to the decreased carbohydrate content after fermentation. So, through SSF, microorganism can improve the quality of AM, and cause the level of protein to increase, cellulose degradation to produce disaccharides and monosaccharides that contributes to the animal absorption. McAllister et al. (1998) confirmed that there was an enhancement in ADG and ADFI of feedlot steers fed diet containing alfalfa hay fermented.

Growth performances

The fermented alfalfa products are nutritionally superior to unfermented alfalfa. This reason for the FAM is that fermentation can increase protein levels, essential amino acids, bioactive peptides and enzymes[29], and enhance fiber digestivity[39]. Previous studies confirmed that alfalfa haylage increased milk production of lactating dairy cows[17] and growth performance of feedlot steers[18], whereas a diet with 2-6% the ensilage alfalfa improved growth performance of *Yangzhou* goslings and geese[9, 41]. In the present study, diets with 4.3-8.6% FAM inclusion levels had no reduce the growth of *Gushi* geese compared to the control diet. Replacing SBMs with fermented products at a reasonable level does not affect the nutritional composition or even advantageous to improve the digestibility and utility ratio of feedstuff, as has been suggested in different species of poultry fed diets supplemented with the fermented plant protein feed, including broiler chickens[37, 40] and *Landaise* geese[42]. These results could be attributed to the improvement of the nutritional quality of fermented products[29]. In addition to this, the high vitamin and mineral concentrations could allow them to be more superior for bird diets. However, the replacing up to 60% of dietary SBM by FAM led to poor growth of geese. A high forage grass level can adversely affect the palatability or digestion and absorption of nutrients in animal feeds[12].

Many authors reported that *B. subtilis* can improve growth performance and enhance feed efficiency in laying hens and broilers[28, 24]. Similarly, the beneficial effects of SSF with *B. subtilis* were also confirmed in Sen et al. (2011) and Lee et al. (2014) was consistent with the study. Improved ADG of geese fed diets supplemented with *B. subtilis* products have also been reported by Chen et al. (2013). The better growth of geese fed the diets supplemented with 8.6% or less FAM was associated with relatively higher F/G radio in the present study.

Cecal microbiota

Cecal microbiota plays an important role for geese. It is common knowledge that numerous bacteria in caecum are capable of digesting and supplying many nutritious components. The present study found the *Bifidobacterium* and *Lactobacillus* number increased, i.e. confirmed beneficial effects of

FULL PAPER

dietary inclusion of FAM. Dietary FAM are directly associated with beneficial effects in preventing colonization by pathogens in intestine, such as *E. coli* and so on[20]. A similar potential of alfalfa silage to suppress pathogenic bacteria has been confirmed[16]. The cecal microbiota differences are also consistent with previous reports for cows feed alfalfa silage[39]. The effect of FAM on the change of beneficial and harmful microorganisms in cecum is most likely due to the supplementation with FAM. In this study, alfalfa in initial flowering stage was harvested, which was fermented with *Bacillus bacteria*. The increased and/or decreased the caecal microflora found in geese fed the FAM are in line with the results of other studies involving both broilers and weanling pig fed diets supplemented with fermented products with *Bacillus bacteria*[30, 15]. Studies have shown that the *Bacillus bacteria* as probiotics could increase beneficial microorganisms[24] and decrease pathogenic microorganisms[10, 1].

CONCLUSION

In conclusion, the study showed that, it is tempting to suggest that the beneficial FAM supplemented to *Gushi* geese diets improved growth, serum antioxidant enzyme, digestion enzymes and positively involved in modifying the cecal microflora.

ACKNOWLEDGEMENTS

This works were supported by *Gushi* Sanmu farming Co., Ltd (Henan Province, China) and Grant Agreement from Henan Provincial Natural Science Fund Committee (no.132102110026, China).

REFERENCES

[1] Anas Abdelqadera, Abdur-Rahman Al-Fataftaha, Gürbüz Das; Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, Eggshell quality, Intestinal morphology and microflora composition of laying hens in the late phase of production, Anim.Feed Sci.Technol., **179**, 103-111 **(2013)**.

[2] AOAC International, Official Methods of Analysis, 17th Edition, Gaithersburg, MD, **(2007)**.

[3] C.S.Bai, Z.Yu, C.J.Wang; Effects of different additives on the fermentation quality of alfalfa silage, Chinese Journal of Animal Nutrition (in Chinese), **21**, 755-762 **(2009)**.

[4] J.Boguhn, H.Kluth, M.Rodehutscord; Effect of total mixed ration composition on fermentation and efficiency of ruminal microbial crude protein synthesis in vitro, J.Dairy Sci., **89**, 1580-1591 **(2006)**.

[5] L.M.Cao, M.Goto, M.Ohshima; Variations in the fermentation characteristics of alfalfa silage of different harvest times as treated with fermented juice of epiphytic lactic acid bacteria, Grassland Science (in Chinese), **47**, 583-587 **(2002)**.

[6] P.R.Cheeke, J.S.Powley, H.S.Nakaue, G.H.Arscott; Feed preference responses of several avian species fed alfalfa meal, High-and low-saponin alfalfa, and quinine sulfate, Can.J.Anim.Sci., **63**, 707-710 **(1983)**.

[7] K.L.Chen, W.L.Kho, S.H.You, R.H.Yeh, S.W.Tang, C.W.Hsieh; Effects of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* Mixed fermented feed on the enhanced growth performance of broilers, Poult.Sci., **88**, 309-315 **(2009)**.

[8] W.Chen, X.Z.Zhu, J.P.Wang, Z.X.Wang, Y.Q.Huang; Effects of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* fermented liquid feed on growth performance, Relative organ weight, Intestinal microflora, and organ antioxidant status in Landes geese, J.Anim.Sci., **91**, 978-985 **(2013)**.

[9] G.Chiang, W.Q.Lu, X.S.Piao, J.K.Hu, L.M.Gong, P.A.Thacker; Effects of feeding solid-state fermented rapeseed meal on performance, Nutrient digestibility, Intestinal ecology and intestinal morphology of broiler chickens, Asian-Aust., J.Anim.Sci., **23**, 263-271 **(2010)**.

[10] J.Y.Choi, P.L.Shinde, S.L.Ingale, J.S.Kim, Y.W.Kim, K.H.Kim, I.K.Kwon, B.J.Chae; Evaluation of multimicrobe probiotics prepared by submerged liquid or solid substrate fermentation and antibiotics in weaning pigs, Livest.Sci., **138**, 144-151 **(2011)**.

[11] K.J.Hong, C.H.Lee, S.W.Kim; *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals, J.Med.Food, **7**, 430-435 **(2004)**.

[12] J.C.Hsu, T.W.Lu, P.W.S.Chiou, B.Yu; Effects of different sources of dietary fibre on growth performance and apparent digestibility in geese, Anim.Feed Sci.Technol., **60**, 93-102 **(1996)**.

[13] M.L.Kass, P.J.Soest.Van, W.G.Pond, B.Lewis, R.E.McDowell; Utilization of dietary fiber from alfalfa by growing swine, 1. Apparent digestibility of

diet components in specific segments of the gastrointestinal tract, J.Anim.Sci., **50**, 175-191 **(1980)**.

[14] K.L.Landers, Z.R.Howard, C.L.Woodward, S.G.Birkhold, S.C.Ricke; Potential of alfalfa as an alternative molt induction diet for laying hens: egg quality and consumer acceptability, Bioresour.Technol., **96**, 907-911 **(2005)**.

[15] S.H.Lee, S.L.Ingale, J.S.Kim, K.H.Kim, Anushka Lokhande, E.K.Kim, I.K.Kwon, Y.H.Kim, B.J.Chae; Effects of dietary supplementation with Bacillus subtilis LS 1-2 fermentation biomass on growth performance, Nutrient digestibility, Cecal microbiota and intestinal morphology of weanling pig.Anim.Feed Sci.Technol., **188**, 102-110 **(2014)**.

[16] H.L.Liu; The effect of epiphytic lactic acid bacteria on gramineous grass silage fermentation characteristics and dairy cows performance, Inner Mongolia: Inner Mongolia Agricultural University (in Chinese), **(2008)**.

[17] D.D.Maulfair, A.J.Heinrichs; Eating behavior, Ruminal fermentation, and milk production in lactating dairy cows fed rations that varied in dry alfalfa hay and alfalfa silage content. Livest.Sci., **151**, 179-187 **(2013)**.

[18] T.A.McAllister, R.Feniuk, Z.Mir, P.Mir, L.B.Selinger, K.L.Cheng; Inoculants for alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers, Livest.Prod.Sci., **53**, 171-181 **(1998)**.

[19] C.E.Mcdonald, L.L.Chen; Lowry modification of the Folin reagent for determination of proteinase activity, Anal.Biochem., **10**, 175-177 **(1965)**.

[20] A.T.Niba, J.D.Beal, A.C.Kudi, P.H.Brooks; Bacterial fermentation in the gastrointestinal tract of non-ruminants: Influence of fermented feeds and fermentable carbohydrates, Trop.Anim.Health.Pro., **41**, 1393-1407 **(2009)**.

[21] A.Pandey, C.R.Soccol, D.A.Mitchell; New developments in solid-state fermentation, Part 1. Bioprocesses and products, Process Biochem., **35**, 1158-1169 **(2000)**.

[22] J.A.Renteria-Flores, L.J.Johnston, G.C.Shurson, D.D.Gallaher; Effect of soluble and insoluble fiber on energy digestibility, nitrogen retention, and fiber digestibility of diets fed to gestating sows., J.Anim.Sci., **86**, 2568-2575 **(2008)**.

[23] SAS Institute; SAS User's Guide: Statistics, Version 7.0 ed. SAS Institute Inc., Cary, NC, **(1996)**.

[24] S.Sen, S.L.Ingale, Y.W.Kim, J.S.Kim, K.H.Kim, C.Khong, J.D.Lohakare, E.K.Kim, H.S.Kim, I.K.Kwon, B.J.Chae; Effect of supplementation of *Bacillus subtilis* LS 1-2 grown on citrus-juice waste and corn-soybean meal substrate on growth performance, nutrient retention, caecal microbiologyand small intestinal morphology of broilers, Asian-Aust.J.Anim.Sci., **24**, 1120-1127 **(2011)**.

[25] S.Sen, S.L.Ingale, Y.W.Kim, J.S.Kim, K.H.Kim, J.D.Lohakare, E.K.Kim, H.S.Kim, M.H.Ryu, I.K.Kwon, B.J.Chae.; Effect of supplementation of *Bacillus subtilis* LS 1–2 to broiler diet on growth performance, Nutrient retention, Caecal microbiology and small intestinal morphology, Res.Vet.Sci., **93**, 264-268 **(2012)**.

[26] Y.H.Shi, C.Z.Wang, M.L.Chen, H.X.Yao, D.D.Yuan, X.K.Wang; Effects of alfalfa meal on growth performance, Antioxidant and immune values of Sichuan white geese. Pratacultural Science (in Chinese), **28**, 841-847 **(2011)**.

[27] I.R.Sibbald; Passage of feed through the adult rooster rooster its crowing at dawn heralds each new day. [Western olklore: Leach, 329] See : Dawn rooster symbol of maleness. [Folklore: Binder, 85] See : Virility. oult.Sci., **58**, 446-459 **(1979)**.

[28] M.Skřivan, M.Marounek, I.Bubancova, M.Podsedníček; Influence of limestone particle size on performance and egg quality in laying hens aged 24-36 weeks and 56-68 weeks, Anim.Feed Sci.Technol., **158**, 110-114 **(2010)**.

[29] H.Sun, J.W.Tang, X.H.Yao, Y.F.Wu, X.Wang, J.Feng; Improvement of the nutritional quality of cottonseed meal by *Bacillus subtilis* and the addition of papain, Int J.Biochem.Cell.Biol., **14**, 563-568 **(2012)**.

[30] H.Sun, J.W.Tang, X.H.Yao, Y.F.Wu, X.Wang, J.Feng; Effects of dietary inclusion of fermented cottonseed meal on growth, Cecal microbial population, small intestinal morphology, and digestive enzyme activity of broilers, Trop.Anim.Health.Prod., **45**, 987-993 **(2013)**.

[31] G.W.Tannock; Molecular assessment of intestinal microflora, Am.J.Clin.Nutr., **73**, 410-414 **(2001)**.

[32] P.A.Thacker, I.Haq; Nutrient digestibility, performance and carcass traits of growing-finishing pigs fed diets containing graded levels of dehydrated lucerne meal, J.Sci.Food.Agr., **88**, 2019-2025 **(2008)**.

[33] R.Timmer; The influence of diets containing different fiber sources on parameters of the bacterial flora in the gastro-intestinal tract of geese, In: United Kingdom Branch of the Worlds Poultry Science Asso-

ciation. Proceedings of 9th European Symposium on Poultry Nutrition: European Poultry Conference, United Kingtom: Glasgow, 81-88 **(1994)**.

[34] M.Vijayakumar; Solid state fermentation of oil cakes and wheat flour and evaluation of the products in shrimp feed, M.F.Sc. (Mariculture) Dissertation, Central Institute of Fisheries Education, Mumbai, India, 85, **(2003)**.

[35] Z.Y.Wang, S.R.Shi, M.J.Xu et al.; 16S r2ÿNA-based analysis of bacterial diversity in the microbialflora of the geese intestinal tract, J.Anim.Feed.Sci., **18**, 531-540 **(2009)**.

[36] M.S.Xia, C.H.Hu, Z.R.Xu; Effect of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broiler, Poult.Sci., **83**,1868-1875 **(2004)**.

[37] S.Y.Xia,0C.Z.Wang, X.B.Yan,0Y.H.Shi,0W.N.Fan; Effects of substitution of fermented protein feed for soybean meal on growth performance, Nutrient digestibility and intestinal microflora of broiler chickens, Chinese Journal of Animal Nutrition (in Chinese), **22**, 352-357 **(2010)**.

[38] X.B.Yan, Q.W.Liu, C.Z.Wang, Y.X.Guo, D.F.Li; Effect of feeding with fresh alfalfa forage on growth performance and blood physiological biochemical indexes of Boer goat, ACTA Agrestia Sinica (in Chinese), **18**, 456-461 **(2010)**.

[39] T.Zhang, L.Li, X.F.Wang, Z.H.Zeng, Y.G.Hu, Z.J.Cui; Effects of *Lactobacillus buchneri* and *Lactobacillus plantarum* on fermentation, aerobic stability, bacteria diversity and ruminal degradability of alfalfa silage, World,J.Microbiol Biotechnol., **25**, 965-971 **(2009)**.

[40] X.H.Zhang, F.L.Cao, Z.Y.Sun, W.W.Yu, L.G.Zhao, G.B.Wang, T.Wang; Effect of feeding *Aspergillu sniger*-fermented *Ginkgo* biloba-leaves on growth, Small intestinal structure and function of broiler chicks, Livest.Sci., **147**, 170-180 **(2012)**.

[41] X.C.Zhu, D.C.Zhang, H.N.Sun, Z.M.Cai, Z.Y.Wang, H.M.Yang; Effects of different fiber source diets on growth performance and gastrointestinal development of yangzhou geese aged from 1 to 4 weeks, Chinese Journal of Animal Nutrition (in Chinese), **26**, 760-767 **(2014)**.

[42] X.Z.Zhu, L.L.Liu, Z.X.Wang, Y.Q.Huang, X.Y.Wang, Y.Y.Jiang, W.Chen., Effects of fermented feed on growth performance, fatty liver performance, intestinal mucosa structure and organ tissue ATPase activity of Landaise geese, Chinese Journal of Animal Nutrition (in Chinese), **25**, 2668-2674 **(2013)**.