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Effect of pelleting temperature and probiotic supplementation on growth performance and immune function of broilers fed maize/soy-based diets

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ABSTRACT

The aim of the present experiment was to examine the effect of pelleting temperature and a probiotic supplementation based on three *Bacillus subtilis* strains on growth performance and the immune function of broilers fed maize/soy-based diets. The experimental design was a 2×3 factorial arrangement of treatments evaluating two levels of probiotic supplementation (without or with 1.5×10^5 cfu/g feed) and three pelleting temperatures (75, 85 and 90 °C). Each treatment was fed *ad libitum* to 8 pens of 22 male broilers, in both the starter (1–21 day) and the grower (22–42 day) phases. On day 21 and 42, 6 birds per treatment were randomly selected to measure blood immunoglobulin M (IgM) and immunoglobulin A (IgA), production of reactive oxygen intermediates (ROI) and duodenal secretory IgA. Data were analysed by two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of statistical analysis software (SAS). During the starter phase (1–21 day), pelleting temperature had no effect (P>0.05) on broiler performance. However, probiotic supplementation tended to reduce feed intake (P=0.055) compared to unsupplemented diets with no effect (P>0.05) on weight gain or feed conversion. During the finisher phase (22–42 day) and over the entire period (1–42 day) pelleting temperature at 85 °C reduced (P<0.05) weight gain compared to those fed diet pelleted at 75 or 90 °C. Probiotic supplementation reduced (P<0.05) feed intake and improved (P<0.05) feed conversion. No interactions (P>0.05) were observed for any of the measured performance parameters during the overall period. There was no effect (P>0.05) of dietary treatments on the measured blood IgM, IgA and ROI. Probiotic supplementation and pelleting temperatures (85 and 90 °C) increased (P<0.05) the concentration of secretory IgA in the duodenum on day 21. On day 42 an interaction (P<0.01) between probiotic and pelleting temperature was observed for the duodenal concentration of secretory IgA. In probiotic supplemented diets, pelleting temperature at 90 °C reduced the concentration of secretory IgA compared to diets pelleted at 75 or 85 °C. In conclusion, the results of this study suggest that increased pelleting temperature and probiotic supplementation stimulate duodenal secretory IgA production during the starter phase (1–21 day). Furthermore, over the entire period (1–42 day), probiotic supplementation based on three *Bacillus subtilis* strains improved feed conversion ratio in broilers fed maize/soy diets, regardless of the pelleting temperature used.

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1. Introduction

In the manufacture of poultry feeds, pelleting is the most common form of thermal treatment (Amerah et al., 2011a). The benefits of pelleting on weight gain, feed intake and feed efficiency in broilers are well documented (Calet, 1965; Douglas et al., 1990; Jensen, 2000) and have been attributed *inter alia* to higher feed density, improved digestibility, increased nutrient intake, reduced feed wastage and decreased energy spent for eating (Calet, 1965; Jensen, 2000). There is a wide range of conditioning temperature and retention time combinations used in the commercial feed milling (McCracken, 2002). At current industry practices, the conditioner temperatures in some feed mills may reach 90 °C, with the feed industry tending to move to even higher and harsher feed processing to control feed borne pathogens such as *Salmonella* (Jones and Richardson, 2004; Doyle and Erickson, 2006).

The commercially available additives should be able to tolerate the industry trend moving toward harsher feed processing practices. Probiotics based on spore-former *Bacillus* species are heat stable, have extended shelf-life at room temperature, survive the low gastric pH and most of the entire dose of ingested bacteria can reach the small intestine intact (Cutting, 2010; Lee et al., 2010a). These qualities have increased the interest in *Bacillus* species in the poultry industry. Several modes of action have been proposed in the literature, such as, controlling the pathogenic bacteria, modulating immune responses, competing for adhesion receptors in the gut epithelium with toxin-producing bacteria (Nava et al., 2005; Cutting, 2010; Lee et al., 2010a) and altering metabolism by increasing digestive enzyme activity (Jin et al., 2000).

Although probiotics based on *Bacillus* species are known for being resistant to high temperatures, there is a scarcity in data of their ability to survive typical steam-pelleting conditions used by the poultry feed industry. Interactions of *Bacillus* supplementation with different pelleting temperatures on broiler performance and immune response are also unknown, considering the possible physical and chemical changes caused by the different conditioning temperatures (McCracken, 2002; Svihus et al., 2005). Therefore, the purpose of this study was to examine the effects of pelleting temperature and the supplementation with a probiotic based on three *Bacillus subtilis* strains on growth performance and the immune function of 42-day-old broilers fed maize/soy-based diets.

2. Materials and methods

2.1. Birds and housing

A total of 1.056 one-day-old Ross 308 male broiler chicks were obtained from a commercial hatchery, weighed and assigned on the basis of body weight to 48 floor pens. Chicks were allotted into 6 treatment groups consisting of 8 replicates with 22 birds per replicate at the Broiler Floor Pen Unit of Imasde Agroalimentaria in the Veterinary University of Murcia, Spain. The floor pens were located in an environmentally controlled room with 18 h of fluorescent illumination per day, automated electric heating and forced ventilation. Each pen was exactly similar in layout (1.58 m × 1.16 m), with one bell drinker and one feed hopper per pen. The floor was cemented and covered in a 5 cm deep layer of fresh wood shavings. Room temperature was maintained at 32 ± 1 °C during the first week of the study and gradually decreased to 22 °C by the end of the fourth week, temperature that was maintained until the end of the trial. All procedures used in this research were in compliance with the European guidelines for the care and use of animals in research (Directive 2010/63/EU).

2.2. Diets and conduct of the trial

The basal diets, starter (1–21 day) and finisher (22–42 day) were based mainly on maize and soybean meal (Table 1). Diets were formulated to meet the Ross 308 strain recommendations (Ross, 2007), except for energy, for broilers. Two levels of probiotic supplementation (without or with 1.5×10^5 cfu/g feed) and three pelleting temperatures (75, 85 and 90 °C) were used in a 2 × 3 factorial arrangement. For that purpose, the basal diet was divided into two equal batches. The first batch was pelleted at three different temperatures: 75, 85 and 90 °C by adjusting the steam flow rate. The second batch was supplemented with the probiotic based on three *B. subtilis* strains (BS8, 15AP4 and 2084; Enviva Pro™ 202 GT, Danisco Animal Nutrition, Marlborough, UK) at a rate of 1.5×10^5 cfu/g diet and was then similarly pelleted at the three different temperatures: 75, 85 and 90 °C. Non-probiotic containing feeds were made before probiotic-containing feeds. The feed was pelleted in a BUHLER (DFCP-65909-S, Bühler AG, Uzwil, Switzerland) pellet press. The pellet diameter was 2.5 mm in starter and 3.0 mm in finisher feeds, and 1.6 mm lenght. Feed samples used for further analyses were collected at the beginning, middle, and end of each pelleting process. Pelleting temperature was measured continuously at the outlet (close to the exit point) of the pellet press using an infrared thermometer (Société L.TELLIER, Mod. N3124, Argenteuil, France). The enumeration of *B. subtilis* probiotic in the feed samples was conducted using 22 g from each diet. Briefly, feed samples were serially diluted with peptone, and then the number of colonies was counted following 48 h incubation at 32 °C on tryptic soy agar. The three strains of the probiotic product were identified by colony morphology.

Each dietary treatment was fed *ad libitum* to eight replicate pens. For each pen, body weight and feed intake were recorded at 21 and 42 days of age. Mortality was recorded daily. Any bird that died was weighed and feed conversion ratios were calculated by dividing total feed intake by weight gain of live plus dead birds.

Table 1
Composition and calculated analysis (g/kg as fed) of the basal diet.[a]

Ingredient	Starter 1–21 day	Finisher 22–42 day
Maize	528	555
Soybean meal, 440 g/kg CP	306	206
Fullfat soy bean, extruded	100	170
Lard (HQ-1.5 acidity)	19.3	27.7
DL-Methionine, 880 g/kg	3.30	2.67
L-Lysine HCl 500 g/kg	3.22	1.51
L-Threonine, 980 g/kg	0.77	0.31
Calcium carbonate	15.7	14.3
Monocalcium phosphate	15.6	13.6
Salt	4.33	4.11
Vitamin & Mineral Premix[b]	4.00	4.00
Calculated chemical composition[c]		
AME_n, MJ/kg diet	12.24	12.97
Lysine	13.2	11.2
Methionine + cysteine	9.6	8.6
Threonine	8.9	7.8
Tryptophan	2.5	2.4
Ash	65.5	59.5
Calcium	10.0	9.0
Total phosphorus	7.3	6.7
Sodium	1.8	1.7
Analysed chemical composition		
Crude protein	231	201
Crude fibre	39	35
Ether extract	62	81
Starch	360	391
Neutral detergent fibre	114	111

[a] Each basal diet was split into two fractions, without and with probiotic. Each fraction was then divided into three fractions and pelleted at 75, 85 or 90 °C.
[b] Provided per kilogram of diet: vitamin A: 10,000 IU; vitamin D_3: 2000 IU; vitamin E: 30.00 mg; vitamin K_3: 2.0 mg; vitamin B_1: 2.0 mg; vitamin B_2: 5.0 mg; vitamin B_6: 3.0 mg; vitamin B_{12}: 10.0 μg; calcium pantothenate: 10.0 mg; nicotinic acid: 40.0 mg; biotin: 100.0 μg; folic acid: 1.0 mg; choline chloride: 400 mg; Mn (MnO): 70.0 mg; Zn (ZnO): 80.0 mg; I (IK): 2.0 mg; Fe ($FeCO_3$): 60.0 mg; Cu ($CuSO_4 \cdot 5H_2O$): 8.0 mg; Co ($CoCO_3 \cdot 7H_2O$): 0.20 mg; Se (Na_2SeO_3): 0.148 mg.
[c] Based on FEDNA (2010) values for feed ingredients.

2.3. Pellet durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen Pellet Tester, TekPro Ltd., Norfolk, UK) using the method described by Amerah et al. (2008). The pellet samples (100 g) were circulated pneumatically through a closed pipe for 30 s before being passed through a 3-mm sieve. The pellet durability index was calculated as the relative proportion by weight of pellets retained on the 3-mm sieve.

2.4. Haematological parameters

Blood was collected on day 21 and 42 from the wing vein of 6 randomly selected birds per treatment (one per pen, from 6 randomly selected pens per treatment) and analysed for the following parameters: IgM, IgA and production of reactive oxygen intermediates (ROI). Serum IgA and IgM were quantified by commercial ELISA systems using specific reagents (chicken IgG, IgM and IgA ELISA quantitation sets, Bethyl Laboratories Inc., Montgomery, TX, USA). The production of reactive oxygen intermediates (ROI) was determined as described by Hangalapura et al. (2005).

2.5. Duodenal secretory IgA, immunohistochemistry

On day 21 and 42, six birds per treatment were randomly selected (one per pen, from 6 randomly selected pens per treatment) and euthanised by cervical dislocation to determine the secretory IgA in the duodenum. The avidin–biotin–peroxidase complex technique was used for the detection of IgA secretory cells in duodenum (Fu et al., 2010). Sections were counterstained with Mayer's haematoxylin, dehydrated and mounted. Five fields of duodenum mucosa lamina propria were examined in each section at high magnification (200×) and the number of IgA+ cells were counted (Fu et al., 2010).

2.6. Jejunal digesta viscosity

On day 21 and 42, the jejunum of the six birds per treatment used to determine the secretory IgA were carefully excised. The contents of the jejunum (defined as the length of gut from the end of the duodenal loop to Meckel's diverticulum)

Table 2
Target pelleting temperatures and measured temperatures ($n = 4$).

Target temperature (°C)	Measured temperatures	
	Starter	Finisher
75	74.2 ± 1.8	75.8 ± 0.1
85	85.0 ± 1.0	85.4 ± 0.2
90	87.4 ± 0.4	90.2 ± 1.1

were allowed to drain into suitable containers. Viscosity of jejunal digesta supernatant was measured using a Brookfield viscometer (model DV-II+LV, Brookfield Viscometers Ltd., Essex, UK) at 20 °C.

2.7. Chemical analysis

Feeds were analysed for moisture by oven drying (930.15), ash by incineration (942.05), protein by Kjeldahl (984.13) and ether extract by Soxhlet fat analysis (920.39) as described by the AOAC International (2000). Neutral detergent fiber was determined as described by Van Soest et al. (1991). Starch content of ingredients and feeds was measured enzymatically using α-amylase glucosidase (method 996.11, AOAC International, 2000). Starch gelatinisation was determined by enzymatic hydrolysis as a proportion of total starch, as described by Medel et al. (1999).

The relative viscosity of the feed samples was measured at 20 °C using a Brookfield viscometer in a model digestion system according to Bedford and Classen (1993) and the results are expressed relative to the viscosity of water. The amounts of total and soluble pentosans were determined according to the method of Rouau et al. (1994).

2.8. Statistical analysis

For performance parameters, pen means served as the experimental unit for statistical analysis. For haematological parameters, immunohistochemistry and jejunal viscosity, individual birds were considered as the experimental unit. All analyses were performed with IBM SPSS Statistics 19.0 software. Results were expressed as treatment means with their pooled SEM. The data were analysed by 2-way ANOVA with probiotic supplementation and pelleting temperature as the fixed factors. When a significant interaction effect was found, Scheffe *post hoc* tests were performed. A probability value of $P<0.05$ was described to be statistically significant, although P-values between 0.05 and 0.10 are shown and described as a trend.

3. Results

3.1. Feed characterisation

The determined crude protein, crude fibre, ether extract, starch and neutral detergent fibre concentration in the experimental diets are presented in Table 1. The determined values were slightly higher than calculated values which may be explained by higher nutrient values than expected in the ingredients used. Pelleting temperatures measured at the end point of the pelleting process are shown in Table 2. Reached temperatures at that point were very close to target values. The recovery count of probiotics in the feed samples was over 90% in all treatments compared to each treatment pre-pellet count (Table 3).

The influence of pelleting temperature on pellet durability index, gelatinised starch (g/kg of total starch), soluble pentosans and total pentosans concentration, and *in vitro* viscosity in the starter and finisher diets is presented in Table 4. It seems there is no effect of pelleting temperature on gelatinised starch. Soluble pentosans were doubled when the mash diet was pelleted in the starter feeds. On average, pelleting increased *in vitro* viscosity of the diet by 35% in the starter and 44% in the finisher diets compared to the mash feeds.

Table 3
Determined probiotic recovery.

Pelleting temperature (°C)	Target (log cfu/g)	Measured in pre-pellet (log cfu/g)		Measured in pellet (log cfu/g)		Recovery, % of pre-pellet feed	
		Starter	Finisher	Starter	Finisher	Starter	Finisher
75	5.18	5.70	5.43	5.18	5.18	90.8	95.3
85	5.18	5.50	5.30	5.08	5.11	92.4	96.5
90	5.18	5.50	5.51	5.11	5.11	93.0	92.9

Table 4
Influence of conditioning temperature on pellet durability, gelatinised starch (g/kg of total starch), soluble pentosans (g/kg), total pentosans (g/kg) and *in vitro* viscosity (cPs) in the starter and finisher diets.[a]

Starter phase						Finisher phase				
Conditioning temperature (°C)	Pellet durability index (%)[b]	Gelatinised starch	Soluble pentosans	Total pentosans	*In vitro* diet viscosity	Pellet durability index (%)[b]	Gelatinised starch	Soluble pentosans	Total pentosans	*In vitro* diet viscosity
75	48.1 ± 2.3	305	0.35	44.3	3.61	13.4 ± 0.2	316	0.17	42.1	3.03
85	62.1 ± 1.4	301	0.34	44.6	3.66	19.4 ± 2.2	315	0.17	41.6	3.25
90	62.3 ± 0. 9	312	0.34	45.6	3.78	22.1 ± 1.8	313	0.26	43.6	3.26
Mash (pre-pelleting)	–	n.m	0.17	44.5	2.73	–	n.m	0.17	39.7	2.44

[a] Chemical analyses were done in duplicates, the values are average of the two measurements.
[b] Pellet durability index measurement was conducted using six replicate samples. n.m, not measured.

3.2. Performance

The influence of treatments on the performance of broilers is summarised in Table 5. During the starter phase (1–21 day), probiotic supplementation tended to reduce (P=0.055) feed intake compared to unsupplemented diets with no effect on weight gain or feed conversion ratio. Pelleting temperature had no effect (P>0.05) on broiler performance. During the finisher phase (22–42 day) and over the entire period (1–42 day), pelleting temperature at 85 °C reduced (P<0.05) weight gain compared to those fed diet pelleted at 75 or 90 °C. Probiotic supplementation reduced (P<0.05) feed intake and improved (P<0.05) feed conversion. No interactions (P>0.05) were observed for any of the measured performance parameters during the overall period.

The main effects of pelleting temperature and probiotic supplementation had no effect (P>0.05) on broiler mortality (Table 5). However, a tendency (P=0.07) for an interaction was observed. Probiotic supplementation at 75 °C tended to reduce the mortality percentage compared to the unsupplemented diet at the same pelleting temperature.

Table 5
Influence of probiotic supplementation and pelleting temperature on the weight gain (g), feed intake (g), .feed conversion ratio (g/g) and mortality (%) of broilers.[a]

1–21 day					22–42 day			1–42 day			
Probiotic supplementation	Pelleting temperature (°C)	Weight gain	Feed intake	Feed conversion	Weight gain	Feed intake	Feed conversion	Weight gain	Feed intake	Feed conversion	Mortality (%)
–	75	972	1256	1.293	1858	3556	1.919	2830	4811	1.702	13
–	85	948	1239	1.307	1727	3444	1.996	2675	4683	1.751	11
–	90	946	1250	1.320	1841	3532	1.921	2788	4781	1.716	6.8
+	75	945	1219	1.290	1831	3445	1.883	2776	4664	1.680	5.7
+	85	943	1222	1.294	1803	3419	1.898	2747	4641	1.690	8.5
+	90	961	1251	1.302	1852	3421	1.853	2812	4672	1.662	11
SEM (n = 8)[b]		10.7	10.9	0.011	32	39	0.030	31	38	0.018	2.5
Main effects											
Probiotic supplementation											
–		956	1248	1.307	1809	3510a	1.945a	2764	4759a	1.723a	10
+		950	1231	1.296	1829	3428b	1.878b	2778	4659b	1.677b	8.5
SEM[b]		6.2	6.3	0.006	19	22	0.017	18	22	0.010	1.5
Pelleting temperature (°C)											
75		959	1237	1.291	1844a	3500	1.901	2803a	4738	1.691	9.4
85		946	1230	1.301	1765b	3431	1.947	2710b	4662	1.720	9.7
90		953	1250	1.312	1846a	3476	1.887	2800a	4727	1.689	9.1
SEM[b]		7.6	7.7	0.008	23	28	0.021	22	27	0.012	1.8
Probabilities											
Probiotic supplementation		NS	0.055	NS	NS	*	*	NS	**	**	NS
Pelleting temperature		NS	NS	NS	*	NS	NS	**	NS	NS	NS
Probiotic × temperature		NS	NS	NS	NS	NS	NS	NS	NS	NS	0.07

NS, not significant.
"a and b" means in a column not sharing a common letter are significantly different (P<0.05).
[a] Each value represents the mean of eight replicates (22 birds per replicate).
[b] Pooled standard error of the mean (n = number of replicates per treatment).
* P<0.05.
** P<0.01.

Table 6
Influence of probiotic supplementation and pelleting temperature on the duodenal secretory IgA (sIgA, number of IgA$^+$ cells) and blood IgM and IgA (μg/ml) and production of reactive oxygen intermediates (ROI, optical density at 690 nm) of broilers.

21 day						42 day			
Probiotic supplementation	Pelleting temperature (°C)	sIgA	IgM	IgA	ROI	sIgA	IgM	IgA	ROI
–	75	30	211	144	0.156	229bc	712a	399	0.053
–	85	86	197	142	0.137	231bc	437bc	209	0.030
–	90	83	241	137	0.180	262bc	520abc	253	0.039
+	75	97	238	181	0.181	273ab	533abc	180	0.034
+	85	108	217	199	0.192	324a	669ab	240	0.010
+	90	112	247	154	0.139	211c	422c	320	0.016
SEM (n = 6)[a]		15	30	36	0.041	18	83	52	0.019
Main effects									
Probiotic supplementation									
–		66b	216	141	0.158	241	556	287	0.040
+		106a	234	178	0.171	270	542	247	0.020
SEM[a]		8.8	17	21	0.024	11	48	31	0.011
Pelleting temperature (°C)									
75		64b	224	162	0.169	251	622	289	0.044
85		97a	207	171	0.165	278	553	225	0.020
90		97a	244	145	0.159	237	471	287	0.027
SEM[a]		11	21	25	0.029	13	59	38	0.014
Probabilities									
Probiotic supplementation		**	NS	NS	NS	0.07	NS	NS	NS
Pelleting temperature		*	NS	NS	NS	0.10	NS	NS	NS
Probiotic × temperature		NS	NS	NS	NS	**	*	NS	NS

NS, not significant.
"a–c" means in a column not sharing a common letter are significantly different (P<0.05).
[a] Pooled standard error of the mean (n = number of replicates per treatment).
* P<0.05.
** P<0.01.

3.3. Immune parameters

The effect of treatments on the duodenal secretory IgA (sIgA) and blood IgM, IgA and production of ROI is summarised in Table 6. On day 21 neither pelleting temperature nor probiotic supplementation had effect on IgM, IgA and ROI. However, probiotic supplementation and pelleting at 85 or 90 °C increased (P<0.05) sIgA by 61% and 51%, respectively. On day 42 a significant interaction was observed for the duodenal concentration of sIgA. In probiotic supplemented diets, pelleting temperature at 90 °C reduced the concentration of sIgA compared to diets pelleted at 75 or 85 °C. In addition, pelleting temperature at 90 °C reduced (P<0.05) serum IgM compared to 85 °C in the diets supplemented with the probiotic.

3.4. Jejunal digesta viscosity

The effect of treatments on the jejunal digesta viscosity is summarised in Table 7. Neither pelleting temperature nor probiotic supplementation had any effect on digesta viscosity at 21 or 42 day of age.

4. Discussion

The recovery of the probiotic was over 90% compared to mash in all treated groups, confirming the ability of probiotics based on *Bacillus* to tolerate high temperatures under typical steam-pelleting conditions used by the poultry feed industry.

Gelatinised starch as percent of total starch did not change with pelleting temperature in the current study. Abdollahi et al. (2010b) reported a significant increase in gelatinised starch content with higher conditioning temperatures in maize-based diets (16.0, 16.9 and 19.9% gelatinised starch at 60, 75 and 90 °C, respectively). However, the amount of gelatinised starch reported by Abdollahi et al. (2010b) was approximately half to what was observed in the current study which may be explained by the differences in the pelleting process between the two studies or the methodology used for analysis of starch gelatinisation. The degree of starch gelatinisation depends on the amount of added moisture and moisture level may limit the degree of gelatinisation (Moritz et al., 2001). In their review, Svihus et al. (2005) reported that during steam conditioning and pelleting, only between 10 and 20% of starch is usually gelatinised.

Table 7
Influence of probiotic supplementation and pelleting temperature on jejunal digesta viscosity (cPs).

Main effects	21 day	42 day
Probiotic supplementation		
–	2.74	2.75
+	2.56	2.83
SEM[a]	0.16	0.19
Pelleting temperature (°C)		
75	2.78	2.47
85	2.63	2.79
90	2.55	2.58
SEM[a]	0.19	0.23
Probabilities		
Probiotic supplementation	NS	NS
Pelleting temperature	NS	NS
Probiotic × temperature	NS	NS

NS, not significant.
[a] Pooled standard error of the mean.

In the current study, over the entire period (1–42 day) birds that received feeds pelleted at 75 and 90 °C were heavier than those fed 85 °C diets. In maize based diets, similar results were observed by Abdollahi et al. (2010a,b) who reported that conditioning temperature at 60 and 90 °C improved body weight gain of 21-day-old broilers compared to those fed diet conditioned at 75 °C. These researchers explained their finding by two possible explanations. Firstly, the higher lubricating effect caused by higher moisture when feed was conditioned at 90 °C reducing the negative effects of the die friction heat on heat-labile nutrients. Secondly, they speculated that conditioning at 90 °C compared to 75 °C seem to overcome the negative effects of high temperatures on nutrient availability and restore performance due to higher pellet quality. However, in the current study, pellet durability measurement cannot explain the later hypothesis. Other measurements such as pellet hardness, as a parameter of pellet quality, may have explained these results. In contrast, Kirkpinar and Basmacioglu (2006) reported that pelleting maize-based diet at 65 °C resulted in higher weight gain compared to those pelleted at 75 and 85 °C.

Probiotic supplementation reduced feed intake and improved feed conversion with no effect on weight gain throughout the trial, indicating that the probiotic indeed exerted some beneficial effect on broilers. Previous studies examining the effect of probiotics showed inconsistent effect on broiler performance. Amerah et al. (2011b) and Amerah and Gracia (2011) showed similar response using the same three strains of the *B. subtilis* on performance in maize and wheat based diets. Zhou et al. (2010) found that feeding *Bacillus coagulans* at two levels improved body weight and feed conversion ratio in Guangxi Yellow chickens. Similarly, Cavazzoni et al. (1998) reported that feeding *B. coagulans* strain as probiotic to broiler chickens improved body weight and feed conversion ratio, an effect comparable to virginiamycin. Also, Jin et al. (1998) showed that addition of a single *Lactobacillus acidophilus* I-26 or a mixture of 12 *Lactobacillus* cultures to broiler diets significantly improved body weight gain and feed conversion ratio in broilers from 0 to 6 weeks. However, other researchers have found no or minimal effect of probiotic supplementation on broiler performance (Watkins and Millar, 1983; Willis et al., 2007), which may be related to differences in the type of probiotic used, dose, method of preparation, type of diet, sanitary status of the animals, age, *etc.* There are several proposed modes of action which have been extensively discussed in the literature (Patterson and Burkholder, 2003; Nava et al., 2005; Lee et al., 2010a). Some of these mechanisms include, balance of intestinal microflora, immunomodulation, competition for adhesion sites and production of antimicrobial agents (Patterson and Burkholder, 2003; Lee et al., 2010a).

The probiotic stimulated the duodenal sIgA on day 21 regardless of the pelleting temperature, but this effect was only observed at 85 °C on day 42. Under the influence of the gut microflora, the tissue structure of the chicken gut continues to develop until 3–4 weeks post-hatching, hand-in-hand with the gradually maturing mucosal immune system (Uni et al., 1995; Patterson and Burkholder, 2003). In the current study, the increased amount of duodenal sIgA that was observed at day 21, but not at day 42, suggests that feeding probiotic induces earlier maturation of the humoral immune responses in the duodenal mucosa and that effect is evened out upon the maturation of the immune system. Consistent with these observations, *Bacillus* spp. feeding has been previously shown in 22-day-old chickens to induce changes in immune response, including changes in mucosal lymphocyte populations and induction of cytokines IL-2, IL-4, IL-6 and IL-10 from intra-epithelial lymphocytes that are associated with IgA class switching in B-cells (Lee et al., 2010b, 2011). Since blood IgA or IgM concentrations were not altered on day 21 and were altered in an inconsistent manner on day 42, the probiotic and pelleting temperature seem to have mainly impact on mucosal antibody response in this experimental setting. Interestingly, on day 42, probiotic supplementation at 90 °C reduced sIgA and IgM compared to the supplemented groups at 75 °C or 85 °C. These results may be explained by the chemical changes at 90 °C which may directly or indirectly influenced mucosal immunity. Another possible explanation is that the pelleting process at 90 °C made probiotic less immunostimulant. To draw any conclusions on these results, further studies are required.

Another interesting finding in the current study is that the higher pelleting temperature at 85 and 90 °C stimulated sIgA on day 21. These results may be explained by chemical changes caused by higher pelleting temperatures (McCracken, 2002) causing stimulation in the immune response. To authors' knowledge, no previous study examined the effect of pelleting temperature on the mucosal secretory IgA response. Further research is needed to understand the mechanism behind this stimulation.

5. Conclusions

Probiotic supplementation reduced feed intake by 2.1% and improved feed conversion ratio by 2.3% at 42 day, regardless of pelleting temperature. Moreover, probiotic increased duodenal sIgA concentration on day 21 suggestive of stimulation of mucosal antibody response. In addition, birds receiving feeds pelleted at 75 and 90 °C were heavier than broilers fed 85 °C diets over the entire period. Furthermore, this trial showed that the mucosal immunity can be influenced by the pelleting temperature of the feed, and that probiotics based on *Bacillus* tolerate high temperatures under typical steam-pelleting conditions used by the poultry-feed industry.

References

Abdollahi, M.R., Ravindran, V., Wester, T.J., Ravindran, G., Thomas, D.V., 2010a. Influence of conditioning temperature on the performance, nutrient utilisation and digestive tract development of broilers fed on maize- and wheat-based diets. Br. Poult. Sci. 51, 648–657.

Abdollahi, M.R., Ravindran, V., Wester, T.J., Ravindran, G., Thomas, D.V., 2010b. Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize- and sorghum-based diets. Anim. Feed Sci. Technol. 162, 106–115.

Amerah, A.M., Gracia, M.I., 2011. Influence of Three *Bacillus subtilis* Strains Combination on the Performance, Intestinal Morphology and Blood Parameters of Broilers Fed Wheat-Based Diet. International Poultry Scientific Forum, Atlanta, Georgia.

Amerah, A.M., Gilbert, C., Simmins, P.H., Ravindran, V., 2011a. Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. World's Poult. Sci. J. 67, 29–46.

Amerah, A.M., Jansen van Rensburg, C., Plumstead, P.W., 2011b. Effect of feeding diets containing a probiotic or antibiotic on broiler performance and litter water-soluble phosphorus. Poult. Sci. 90 (E-Suppl. 1), 15.

Amerah, A.M., Ravindran, V., Lentle, R.G., Thomas, D.G., 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87, 2320–2328.

AOAC International, 2000. Official Methods of Analysis, 17th ed. AOAC International, Gaithersburg, MD.

Bedford, M.R., Classen, H.L., 1993. An in vitro assay for prediction of broiler intestinal viscosity and growth when fed rye-based diets in the presence of exogenous enzymes. Poult. Sci. 72, 137–143.

Calet, C., 1965. The relative value of pellets versus mash and grain in poultry nutrition. World's Poult. Sci. J. 21, 23–52.

Cavazzoni, V., Adami, A., Castrovilli, C., 1998. Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Br. Poult. Sci. 39, 526–529.

Cutting, S.M., 2010. *Bacillus* probiotics. Food Microbiol. 28, 214–220.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJL 276, 33–79, 20.10.2010.

Douglas, J.H., Sullivan, T.W., Bond, P.L., Struwe, F.J., Baier, J.G., Robeson, L.G., 1990. Influence of grinding, rolling, and pelleting on the nutritional-value of grain sorghums and yellow maize for broilers. Poult. Sci. 69, 2150–2156.

Doyle, M.P., Erickson, M.C., 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult. Sci. 85, 960–973.

FEDNA, 2010. Guidelines of the Spanish Foundation for Development of Animal Nutrition for the formulation of compound feeds. C de Blas, P García & GG Mateos, Ed. Fundación Española para el Desarrollo de la Nutrición Animal. ETSIA (Madrid Polytechnical University), Spain.

Fu, J.L., Wang, Z.H., Li, G.Z., Wang, Y.R., Liu, P., 2010. Decreased IgA+ plasma cells and IgA expression in acute liver necrosis mice. World J. Gastroentero. 14, 3827–3833.

Hangalapura, B.N., Nieuwland, M.G.B., De Vries Reilingh, G., Buyse, J., Van Den Brand, H., Kemp, B., Parmentier, H.K., 2005. Severe feed restriction enhances innate immunity but suppresses cellular immunity in chicken lines divergently selected for antibody responses. Poult. Sci. 84, 1520–1529.

Jensen, L.S., 2000. Influence of pelleting on the nutritional needs of poultry. Asian-Aust. J. Anim. Sci. 13, 35–46.

Jin, L.Z., Ho, Y.W., Abdualah, N., Jalaludin, S., 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. Poult. Sci. 77, 1259–1265.

Jin, L.Z., Ho, Y.W., Abdualah, N., Jalaludin, S., 2000. Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. Poult. Sci. 79, 886–891.

Jones, F.T., Richardson, K.E., 2004. *Salmonella* in commercially manufactured feeds. Poult. Sci. 83, 384–391.

Kirkpinar, F., Basmacioglu, H., 2006. Effects of conditioning temperature of phytase supplemented broiler feed on tibia mineralisation, calcium and phosphorus content of serum and performance. Czech. J. Anim. Sci. 51, 78–84.

Lee, K.W., Lillehoj, H.S., Siragusa, G.R., 2010a. Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. J. Poult. Sci. 47, 106–114.

Lee, K.W., Lee, S.H., Lillehoj, H.S., Li, G.X., Jang, S.I., Babu, U.S., Park, M.S., Kim, D.K., Lillehoj, E.P., Neumann, A.P., Rehberger, T.G., Siragusa, G.R., 2010b. Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. Poult. Sci. 89, 203–216.

Lee, K.W., Lillehoj, H.S., Lee, S.H., Jang, S.I., Babu, U.S., Lillehoj, E.P., Neumann, A.P., Siragusa, G.R., 2011. *Bacillus subtilis*-based direct-fed microbials augment macrophage function in broiler chickens. Res. Vet. Sci. 91, 87–91.

McCracken, K.J., 2002. Effects of physical processing on the nutritive value of poultry diets. In: McNab, J.M., Boorman, K.N. (Eds.), Poultry Feeds, Supply, Composition and Nutritive Value. CAB International, New York, pp. 301–316.

Medel, P., Salado, S., de Blas, J.C., Mateos, G.G., 1999. Processed cereals in diets for early-weaned piglets. Anim. Feed Sci. Technol. 82, 145–156.

Moritz, J.S., Beyer, R.S., Wilson, K.J., Cramer, K.R., 2001. Effect of moisture addition at the mixer to a maize-soybean-based diet on broiler performance. J. Appl. Poult. Res. 10, 347–353.

Nava, G.M., Bielke, L.R., Callaway, T.R., Castañeda, M.P., 2005. Probiotic alternatives to reduce gastrointestinal infections: the poultry experience. Anim. Health Res. Rev. 6, 105–118.

Patterson, J.A., Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. J. Poult. Sci. 82, 627–631.

Ross, 2007. Ross 308 Broiler: Nutrition Specification. June 2007. Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

Rouau, X., El-Hayek, M.L., Moreau, D., 1994. Effect of an enzyme preparation containing pentosanases on the bread-making quality of flours in relation to changes in pentosan properties. J. Cereal Sci. 19, 259–272.

Svihus, B., Uhlen, A.K., Herstad, O.M., 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. Anim. Feed Sci. Technol. 122, 303–320.
Uni, Z., Noy, Y., Sklan, D., 1995. Post hatch changes in morphology and function of small intestines in heavy and light strain chicks. Poult. Sci. 74, 1622–1629.
Van Soest, P.J., Robertson, J.B., Lewis, A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Watkins, B.A., Millar, B.F., 1983. Competitive gut exclusion of avian pathogens by *Lactobacillus acidophilus* in gnotobiotic chicks. Poult. Sci. 62, 1772–1779.
Willis, W.L., Isikhuemben, O.S., Ibrahim, S.A., 2007. Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poult. Sci. 86, 1856–1860.
Zhou, X., Wang, Y., Gu, Q., Li, W., 2010. Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. Poult. Sci. 89, 588–593.