

Management factors resulting in a severe reduction in feed intake–induced spiking mortality syndrome in young broiler chicks

C. Li,^{*} S. Schallier,[†] C. Lamberigts,[†] J. Lesuisse,[†] N. Everaert,[‡] W. Merckx,[§] and J. Buyse^{†,1}

^{*}State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, 100193 Beijing, P. R. China; [†]Laboratory of Livestock Physiology, Department of Biosystems, KU Leuven, 3001 Leuven, Belgium; [‡]Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, University of Liège, B-5030 Gembloux, Belgium; and [§]Engineering and Technology Group, Pilot Facilities Science, TransFarm KU Leuven, 3360 Lovenjoel, Belgium

ABSTRACT This study aimed to induce spiking mortality syndrome (SMS) in 10-day-old broiler chicks by changing feed particle size (crumble feed to pellet feed) and/or feed source location (from a small feeder at the pen's center to a large feeder at the front of the pen), followed by full day feed deprivation of all broiler chicks on day 11. In total, 396-day-old male Ross 308 broiler chicks were randomly assigned to 4 treatments (Con: without change in feed particle size and feed source location; Par: changing crumble feed to pellet feed on day 10; Loc: changing feed source location on day 10; LocPar: changing both feed particle size and feed source location on day 10). Each treatment consisted of 9 replicate pens with 11 chicks each. Each treatment was applied at 09:00 on days 10 and 11. On both days, chicks with SMS were identified based on clinical symptoms (down in sternal or lateral recumbency, hyperventilation). Plasma glucose, 3, 3', 5-triiodothyronine (T_3), thyroxine (T_4) concentrations, insulin, and liver glycogen concentrations of chicks

without (normal) and with SMS were measured. Proportional organ and digestive tract including content weights were recorded. Broiler behavior was assessed hourly from 08:30 to 17:30 on day 10. On day 10, the Par, Loc, and LocPar groups spent significantly less time feeding and more time lying down compared with the Con group. On days 10 and 11, SMS clinical signs were observed around 2.5 to 3.5 h after the initiation of treatments, and the Loc group had the most SMS morbidity level. Spiking mortality syndrome chicks had significantly less digestive tract contents compared with Normal chicks on day 10. Spiking mortality syndrome was induced successfully with the treatments, according to their significantly reduced plasma glucose, insulin, T_3 and T_4 concentrations as well as liver glycogen content. A significant correlation between plasma glucose and liver glycogen was observed in SMS chicks. In conclusion, management factors inducing the reduction or absence of feed intake on day 10 or day 11 can trigger the occurrence of SMS in young broiler chicks.

Key words: spiking mortality syndrome, feed particle size, feed location, feed deprivation, broiler chick

2020 Poultry Science 99:4939–4946

<https://doi.org/10.1016/j.psj.2020.06.032>

INTRODUCTION

Spiking mortality syndrome (SMS) is a sudden increase in broiler chick mortality which happens around 10 to 18 D of age. With approximately 0.5 to 2% of birds affected, SMS leads to major economic losses in the broiler industry (Davis et al., 1995; Davis and

Vasilatos-Younken, 1995; Karki et al., 2008; Dinev and Kanakov, 2011). The syndrome is characterized by low morbidity but high mortality and the broilers who suffer from SMS mainly show hypoglycemic symptoms (Davis et al., 1995; Kumari et al., 2016). They suffer from low insulin concentration, whereas their blood glucose concentration can be as low as 17 mg/dL, in contrast to 240 mg/dL for healthy chicks (Dinev and Kanakov, 2011). Spiking mortality syndrome appears to be a multifactorial condition, with factors such as mycotoxins in the feed, arenavirus-like particle infection in the pancreas, intestines, and/or liver (Goodwin et al., 1993), lack of dark period inducing reduced level of melatonin, and induced stress contributing to the syndrome

© 2020 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received January 5, 2020.

Accepted June 16, 2020.

¹Corresponding author: johan.buyse@kuleuven.be

(Karki et al., 2008). Most experts suggest that the most likely cause of SMS is an arenavirus-like particle infection together with stress or starvation, inducing an endocrine disturbance, which results in severe hypoglycemia (Davis et al., 1995, 1996). However, the arenavirus-like particle has not yet been identified, and the mechanisms involved in the hypoglycemia are not yet elucidated (Hassanzadeh, 2009).

Spiking mortality syndrome can be experimentally induced by a short period of feed deprivation after viral infection (as shown by tissue or fecal-urate homogenates taken from broilers experiencing SMS as shown by Davis et al., 1996). Flock supervisors also reported that this syndrome often occurs after chicks had been out of feed for a short period or after they had been stressed by physical exertion (Davis et al., 1995). The SMS manifestation period coincides with the change from starter to grower feed which normally occurs around 10 to 14 D in the field, sometimes combined with a change in feed particle size (crumble to pellet feed). All those factors may cause a reduction in feed intake of broilers. During one of our own broiler trials in 2015, a spike in SMS occurred after weighing our broilers and changing feeder type and location on day 10. When the trial was repeated (Lesuisse et al., 2018), the same thing happened after those routine practices on the same day. Chicks with minor SMS clinical signs recovered quickly with immediately eliminating the feed-related stressor by putting the original feeder back to its original location. Apparently, weighing and feed changing induced external stress for the chicks, and stress has been addressed in the SMS-related studies (Karki et al., 2008). Therefore, the stress related to the feed change and its resulting starvation may be a cause of SMS in broiler chicks.

This study attempted to induce SMS in young broilers by changing feed particle size and/or feed source location on day 10 and fasting the broilers on day 11. Behavioral observations are the most intuitive and direct way to assess the general condition of animals (Gonyou, 1994). Chicks with SMS portray clear clinical symptoms, namely a large proportion of time spent in sternal or lateral recumbency and hyperventilation (Dinev and Kanakov, 2011). Therefore, in the current study, we investigated the condition of the chicks using production, behavioral, and physiological variables. We hypothesized that factors resulting in feed intake reduction cause SMS in young broilers.

MATERIALS AND METHODS

The present research was approved by the ethical committee for the experimental use of animals of the KU Leuven under the accession number of P208/2015.

Animals and Experimental Design

The trial used a total of 396 one-day-old healthy male Ross broiler chicks, obtained from a local hatchery (Belgabriod, Merksplas, Belgium). The chicks were

randomly assigned to 1 of 4 treatments: Con, without any change in feed particle size and feed location; Par, changing feed particle size on day 10; Loc, changing feed location on day 10; LocPar, changing both feed particle size and feed location on day 10. Each treatment consisted of 9 replicate pens with 11 chicks each. Changing feed particle size refers to changing crumble feed (spherical particles with 3 mm in diameter) to pellet feed (cylindrical particles with 3 mm in circumference). In terms of feed source location, small feeders at the pen's center were replaced by large feeders at the front of the pen. Each treatment was induced at 09:00 on day 10. On day 11, also from 9:00 on, all 4 groups were feed deprived by blocking their access to the feed. After the treatments, chick behavior was observed and recorded hourly. Chicks displaying typical SMS clinical symptoms (down in sternal or lateral recumbency and hyperventilation) (day 10, $n = 15$; day 11, $n = 13$) and some randomly selected Normal chicks (day 10, $n = 16$; day 11, $n = 16$) were sacrificed for sampling. Specifically, on day 10, of the 16 Normal chicks, 6, 6, and 4 were from the Con, Par, and Loc group, respectively. Meanwhile, of the 15 SMS chicks, 1, 9, and 5 were from the Par, Loc, and LocPar group, respectively.

Husbandry and Management

Temperature schedules followed the management guide of Ross 308 broilers (Aviagen, 2014). Between day 0 and day 11, the photoperiod was 23L:1D. The pen (120 × 55 × 70 cm) floors were covered with new wood shavings as bedding material. A waterline with 4 drinking nipples was situated at the back of each pen. The chicks were introduced to the pens the day after hatch, and to make sure all the chicks have access to water, a small round water trough (16 cm in diameter) was provided at the back-left corner of each pen. The water troughs were cleaned and refilled daily. A round feeder (26 cm of diameter) was situated at the pen's center. The front of each pen was fenced by the backside of a big metal feeder (55 × 20 × 100 cm). When changing the feed source location on day 10, the round feeder at the center was removed, and the big metal feeder at the pen's front was utilized. The starter diet was provided during the whole experimental period, and the nutritional composition was identical for crumble and pellet feed (both 3 mm in diameter, refer to Lesuisse et al., 2017 for the detailed diet composition).

Measurements

Between day 0 and day 7, body weight was recorded at pen level to calculate the body weight gain (BWG) per chick. Feed intake (FI) per pen was followed, and the feed conversion ratio (FCR) was calculated based on the following formula: $FCR = FI/BWG$. On days 10 and 11, SMS chicks and randomly selected Normal chicks were dissected after quick decapitation. Before dissection, blood samples were collected by puncturing the vena cutanea ulnaris. Body weight, organ weights

(liver, pancreas, bursa, and thymus), and weights of different parts of the digestive tract including its contents (crop, gizzard, intestine, and ceca) were recorded. Proportional organ weights (as % of body weight) were calculated for data analysis. Plasma was obtained by centrifuging blood for 15 min at 1,500 *g* at 4°C and was stored at -20°C until analysis. Plasma glucose concentrations were measured using a commercially available kit (LabAssay Glucose Wako kit, Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin concentrations were determined using an ELISA (Mouse Insulin ELISA, Mercodia, Uppsala, Sweden), according to the manufacturer's instructions. The absorbance was measured at 450 nm (VICTOR³™ 1420 Multilabel plate reader, Perkin Elmer, Waltham, MA). For the measurements of plasma 3, 3', 5-triiodothyronine (**T**₃) and thyroxine (**T**₄) concentrations, the method of radioimmunoassay described by Darras et al. (1996) was followed. The Wallac 1277 GammaMaster gamma counter (Pharmacia, Stockholm, Sweden) was used to measure the radioactivity of the precipitates. The antisera for **T**₃ and **T**₄ were purchased from Byk-Belga (Brussels, Belgium). Liver glycogen concentrations were determined by using the method described by Bennett et al. (2007). The VICTOR³™ 1420 Multilabel plate reader (Perkin Elmer) was used to measure the absorbance at 490 nm.

Behavioral Observations

On day 10, broiler behavior in each pen was observed on site with 10 scan samples at the beginning of each observational hour (the pens were sequentially scanned for 10 rounds) from 08:30 to 17:30 (10 observational sessions), which makes in total 100 times scans per pen. The behavioral observation was conducted by 1 experienced person during the whole experimental period. Treatments were carried out at 09:00; therefore, the observational session of 08:30 on day 10 was taken as the basal condition of the chicks. Chicks with SMS were identified, and morbidity was recorded based on the clinical symptoms, that is sternal or lateral recumbency and hyperventilation (Dinev and Kanakov, 2011). Behavioral parameters such as feeding, drinking, sitting, lying, standing, walking, object pecking, preening, foraging, and dust bathing were evaluated based on the description of Bokkers and Koene (2003) and recorded as the percentage of chicks showing each activity. The behavioral ethogram is listed in Table 1. For each behavioral parameter, the average percentages of 10 times scan sampling during each observational session were used for further analysis.

Statistical Analysis

All data were analyzed with JMP Pro. 12 software (SAS Institute Inc., Cary, NC). One-way ANOVA was performed on the production and physiological parameters. Proportional organ weights and SMS morbidity were arcsine square-root transformed to meet the

Table 1. Ethogram of observed behavior.

Behavior	Description
Feeding	With head above or in the feeder
Drinking	Pecking at a drinking nipple or drinking out of the bell shape drinker
Preening	Grooming of own feathers with the beak
Object pecking	Object pecking (including feather pecking of other chicks, litter pecking, and wall pecking) while standing, walking, or sitting
Sitting	Sitting with hocks resting on ground without any other activities
Standing	Standing on straight legs without doing any other behaviors
Walking	Locomotion with a normal speed or with quick steps
Lying down	With head flat on the bedding or with head tucked under a wing.
Dust bathing	Performed with fluffed feathers while lying, head rubbed on floor, wings opened, scratching at ground

assumption of normality and were then analyzed with the method of one-way ANOVA. The behavioral data were angular transformed as well, and repeated measure ANOVA was used to examine the effects of treatment, time, and their interaction. If an interaction between treatment and time interaction was observed, one-way ANOVA was used to obtain further statistical information. When *P* values were smaller than 0.05, the differences were considered to be statistically significant. A tendency toward significance was defined as a *P*-value between 0.05 and 0.1. Data are presented as mean ± SEM.

RESULTS

Production and Behavioral Results

Because the treatment started on day 10, chicks assigned to the 4 treatments during week 1 had similar BWG, FI, and FCR (*P* > 0.05, Table 2). On day 10, the Loc and LocPar chicks consumed significantly less feed compared with the Con chicks (*P* = 0.030, Table 2). For the behavioral results on day 10 (Figure 1), significant differences were found for feeding, object pecking, sitting, and lying down (*P* < 0.05). In general, before treatment, no difference in behavior was observed between the 4 groups (*P* > 0.05). After treatment, the Con chicks spent significantly more time feeding compared with the Par and LocPar chicks (*P* < 0.05). For some time points, the same could be said when comparing the Con to the Loc group (09:30, 12:30, 13:30, and 14:30). For object pecking, at 09:30 and 10:30, the Loc chicks pecked significantly more than the Con and Par chicks (*P* < 0.05). For sitting behavior, a significant difference was only found at 12:30 between the Con group and the Loc and LocPar groups, who spent less time sitting down (*P* < 0.05). At the same time point (12:30), the LocPar chicks lied down significantly more often compared with the other 3 groups (*P* < 0.05). No differences were seen

Table 2. Performance of broilers during the first week and their feed intake and spiking mortality syndrome (SMS) morbidity on the 10th day.

Age	Parameters	Con		Par		Loc		LocPar		P-values
		Value	SEM	Value	SEM	Value	SEM	Value	SEM	
Week 1	BWG (g)	135.2	3.7	126.0	3.7	124.3	3.5	128.1	3.1	0.168
	FI (g)	145.6	3.6	139.4	3.7	139.7	3.6	141.3	2.4	0.548
	FCR	1.1	0.0	1.1	0.2	1.1	0.0	1.1	0.0	0.815
Day 10	FI (g)	2.0 ^a	0.4	1.4 ^{ab}	0.1	1.0 ^b	0.2	1.1 ^b	0.2	0.030
	SMS morbidity (%)	1.0 ^b	1.0	1.5 ^b	1.1	15.2 ^a	5.9	7.1 ^{ab}	2.5	0.026

^{a,b}Different superscripts within a row stand for treatment means have significance ($P < 0.05$).

Abbreviations: BWG; body weight gain per chick; Con, without feed particle size and feed source location changing; FCR, feed conversion ratio; FI, feed intake per chick; Loc, changing feed source location on day 10; LocPar, changing both feed particle size and feed source location on day 10; Par, changing crumble feed to pellet feed on day 10.

n = 9.

for the other behaviors, and therefore, these data are not shown. Based on the observations of the clinical symptoms, SMS occurred 3.5 h and 2.5 h after treatment introduction on days 10 and 11 respectively. The SMS morbidity level of the Loc (15.2%) group was significantly higher compared with the Con (1.0%) and Par (1.5%) group, and the LocPar (7.1%) group have an intermediate level of morbidity. On day 11, the total SMS morbidity was 21.2%. Aside

from the sacrificed chicks for dissection, there was no mortality during the entire experimental period.

Proportional Organ and Digestive Parts With Content Weights

On day 10, no differences were found in terms of proportional organ weights of the liver, pancreas, bursa, and thymus between SMS and normal chicks ($P > 0.05$,

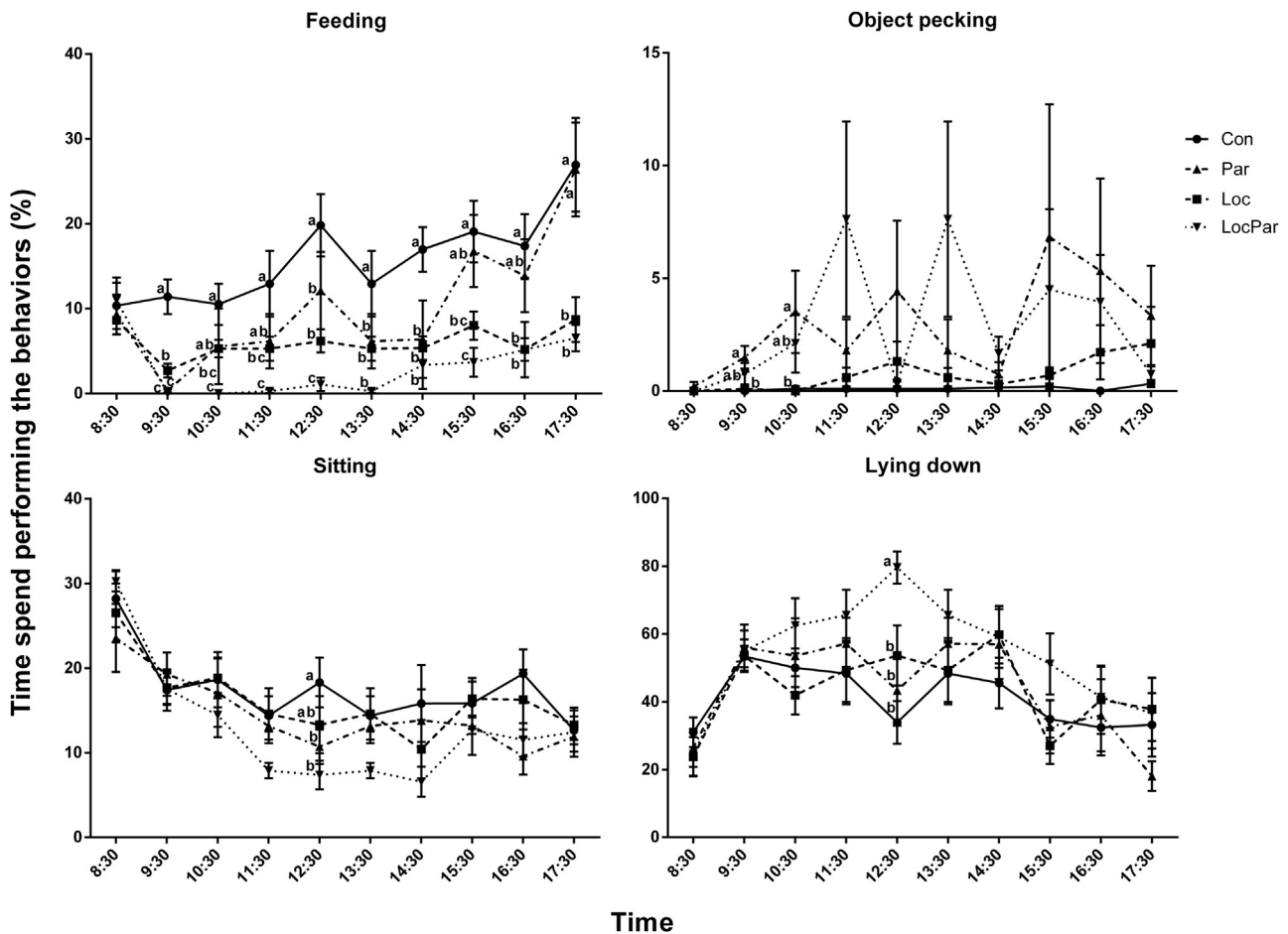


Figure 1. Percentage of time spent on different behaviors at each observational time point for the broilers on day 10. ^{a,b,c}Means within each time point, treatments with different superscripts have significant treatment effects ($P < 0.05$). Abbreviations: Con = without change in feed particle size and feed source location; Loc = changing feed source location on day 10; LocPar = changing both feed particle size and feed source location on day 10; Par = changing crumble feed to pellet feed on day 10

Table 3. The proportional organ weight and different digestive parts with content weight of broilers with (SMS) or without (normal) spiking mortality syndrome on day 10 and day 11.

Items	Day 10					Day 11				
	Normal, n = 16		SMS, n = 15		P-values	Normal, n = 16		SMS, n = 13		P-values
	Value	SEM	Value	SEM		Value	SEM	Value	SEM	
Liver	33.9	0.7	34.7	1.1	0.537	32.2	0.9	30.0	0.8	(0.062)
Pancreas	4.8	0.2	5.5	0.3	0.335	4.4	0.2	4.3	0.2	0.579
Bursa	1.8	0.1	1.7	0.1	0.606	2.0	0.1	2.0	0.1	0.896
Thymus	3.6	0.2	3.6	0.3	0.951	3.1	0.1	3.8	0.2	0.017
Crop with content	3.1	0.9	0.6	0.6	0.028	–	–	–	–	–
Gizzard with content	17.7	1.0	13.5	1.1	0.009	12.0	0.9	10.7	1.0	0.354
Intestine with content	34.3	2.6	21.6	3.0	0.003	11.4	1.3	11.6	1.5	0.893
Ceca with content	10.9	1.1	8.6	0.4	(0.065)	10.0	0.9	8.0	0.9	(0.093)

Abbreviation: SMS, spiking mortality syndrome.

A significant difference was considered with $P < 0.05$. A tendency toward difference was considered with $0.05 < P < 0.10$. “–” means that the data were not available, and the analysis was not run.

Table 3). However, on day 11, SMS chicks had a significantly higher proportional thymus weight ($P = 0.017$, **Table 3**) but tended to have a lower proportional liver weight ($P = 0.062$, **Table 3**) compared with that of normal chicks. On day 10, the SMS chicks had significantly lighter proportional weights of filled crops ($P = 0.028$), gizzards ($P = 0.009$), and intestines ($P = 0.003$) (**Table 3**). There was also a tendency toward lighter proportional ceca (with content) in the SMS chicks compared with normal chicks ($P = 0.065$ on day 10, $P = 0.093$ on day 11, **Table 3**). No differences were found regarding the proportional gizzard and intestine with content weights ($P > 0.05$, **Table 3**). Owing to a sampling error, the crop weights were not recorded on day 11.

Physiological Measurements

On both day 10 and 11, SMS chicks had significantly lower plasma glucose and liver glycogen concentrations compared with normal chicks (glucose: $P_{\text{day 10}} < 0.001$, $P_{\text{day 11}} = 0.004$; glycogen: $P_{\text{day 10}} < 0.001$, $P_{\text{day 11}} = 0.002$, **Table 4**). In SMS chicks, there was a significant linear correlation detected between plasma glucose and liver glycogen concentrations (day 10: $Y = 0.020 \times X - 0.151$, $R^2 = 0.488$, $P = 0.008$; day

11: $Y = 0.004 \times X + 1.315$, $R^2 = 0.524$, $P = 0.012$, **Figure 2**). No such linear correlation was observed in normal chicks on both sampling days ($P > 0.05$, **Figure 2**). There were 2 outliers in the SMS chicks on day 10. After omitting the 2 outliers from analysis, we obtained a similar equation for day 10 ($Y = 0.009 \times X + 0.900$, $R^2 = 0.853$, $P < 0.001$, **Figure not shown**) compared with the SMS chicks on day 11.

In addition, SMS chicks showed significantly lower plasma insulin concentrations (day 10, $P < 0.001$; day 11, $P = 0.020$) and T3 concentrations (day 10, $P = 0.003$; day 11, $P = 0.010$) compared with normal chicks (**Table 4**). Plasma T₄ concentrations tended toward lower levels for SMS chicks on day 10 ($P = 0.079$) and were significantly lower on day 11 ($P < 0.001$) in comparison with the normal chicks (**Table 4**).

DISCUSSION

This study's aim was to investigate whether a change in feed particle size and/or feed source location, followed by feed deprivation, could induce SMS in young broilers. All experimental treatments were designed to eventually lead to a reduction in feed intake of broilers. Based on

Table 4. The plasma glucose, 3, 3', 5-triiodothyronine (T₃), thyroxine (T₄), insulin, and liver glycogen concentrations of chicks with (SMS) and without (normal) spiking mortality syndrome on day 10 and day 11.

Items	Day 10					Day 11				
	Normal, n = 16		SMS, n = 15		P-values	Normal, n = 16		SMS, n = 13		P-values
	Value	SEM	Value	SEM		Value	SEM	Value	SEM	
Glucose (mg/dL)	260.0	7.1	160.3	22.1	<0.001	228.3	5.9	169.8	19.1	0.004
Glycogen (mg/dL)	6.8	1.2	1.9	0.4	<0.001	3.7	0.6	1.4	0.3	0.002
Insulin (ng/dL)	47.0	2.3	38.2	0.5	<0.001	38.6	0.6	36.7	0.5	0.020
T3 (ng/mL)	2.0	0.3	0.8	0.3	0.003	1.2	0.2	0.5	0.1	0.010
T4 (ng/mL)	6.4	1.0	3.7	1.1	(0.079)	9.5	1.3	3.3	0.6	<0.001

Abbreviation: SMS, spiking mortality syndrome.

A significant difference was considered with $P < 0.05$. A tendency toward difference was considered with $0.05 < P < 0.10$.

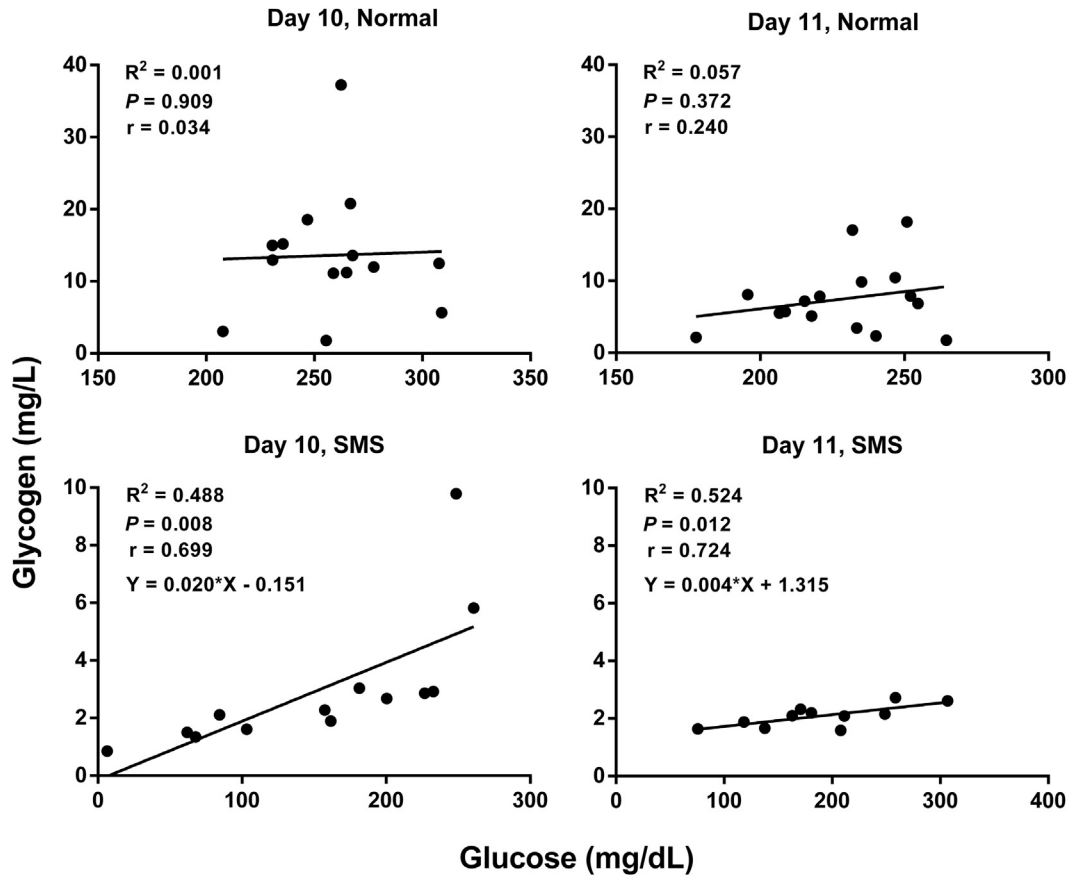


Figure 2. The correlation between plasma glucose and liver glycogen concentration of broilers with (SMS) or without (normal) spiking mortality syndrome. $P < 0.05$ stands for statistically strong correlation. Abbreviation: SMS, spiking mortality syndrome.

the clinical symptoms and physiological parameters, SMS was successfully induced but with different levels of morbidity for each treatment.

Production and Behavioral Performances

No significant differences were observed between the treatments for BWG, FI, and FCR as all treatments received the same diet and management during the first week. This confirms that the chicks were randomly divided into their groups and that they all started at the same biological baseline when treatment was introduced. After changing feed particle size and/or feed source location on day 10, the reduced FI of Loc and LocPar chicks and intermediate values of Par chicks corresponded to the reduced time spent feeding compared with Con chicks. Immediately after changing feed particle size and/or feed source location, there was a clear reduction in feeding behavior. After a period of acclimatization, the Loc chicks showed increased feeding behavior. However, starting from 12:30, differences between treatments became pronounced, which could be explained by the onset of SMS syndrome. After 15:30, the feeding behavior of the Loc chicks returned to the same levels as the Con chicks. When changing only the particle size, but not the feeder, we observed a decrease in feeding behavior in the Par chicks, but there was no FI difference between the Par

and Con chicks. This is in agreement with the research of [Portella et al. \(1988\)](#), who observed that changing particle abruptly from crumble to pellet did not adversely affect overall feed consumption. In addition, chicks need less time to consume the same amount of feed by eating pellet feed in comparison with eating crumble feed ([Jensen et al., 1962](#); [Portella et al., 1988](#)). Hence, although there was, in general, less feeding time of the Par chicks in our experiment, this explains why the overall feed intake stayed the same as that of the Con chicks.

As far as we know, we are the first to investigate the SMS problem of young broilers from a behavioral perspective. By observing their behavior once per hour, we found that the clinical syndrome of SMS started to appear after 2.5 (day 11) to 3.5 (day 10) hour of treatment induction, especially in the Loc and LocPar chicks. This corresponds to the time when LocPar chicks lied down the most and sat down the least. In other studies, the chicks started to show SMS 45 min after inducing stress by spraying the chicks with cold water ([Davis et al., 1996](#)). Poultry farmers also indicated that SMS occurred in young broilers that were without feed for a short period ([Davis et al., 1995](#)). In our study, compared with the aforementioned studies, it took longer before the first chick showed clinical sign of SMS. It is likely that the nature and severity of the stress-inducing feed intake reduction determine the speed of SMS manifestation.

Proportional Organ and Digestive Parts With Content Weights

No differences in proportional weights of the immune organs were found on day 10. However, on day 11, the proportional weight of the thymus of the SMS chicks was significantly higher than that of the normal chicks. It was expected that the SMS chicks would have smaller proportional immune organs than the normal chicks, as there could be a regression of thymus and bursa of Fabricius in SMS chicks (Davis et al., 1995; Karki et al., 2008; Hassanzadeh, 2009; Dinev and Kanakov, 2011). However, in the present experiment of day 10, no thymus regression was observed in the SMS chicks. A possible explanation could be that the SMS chicks were killed and dissected immediately after showing the first clinical symptoms, which did not leave enough time for the organs to regress. On day 11, the higher proportional thymus weight of the SMS chicks comparison to the normal chicks might be because of their reduction in feed gain, resulted in a smaller general body weight. The lack of difference in the proportional weight of the liver and pancreas between the 2 groups on both day 10 and day 11 is in contrast with the findings of Mendelson et al. (1995). Again, organ weights do not seem to be affected yet if the chicks are killed immediately after showing the first symptoms of SMS.

The reduced weights of crop, gizzard, and intestines including their content of SMS chicks on day 10 are in correspondence with the study of Dinev and Kanakov (2011) in which chicks with SMS had almost no feed in their digestive system. The lack in difference in ceca including content weight can be explained by them only being emptied twice a day and therefore respond more slowly to the presence of the SMS (Svihus, 2014). The absence of difference in organ weight on day 11 is not surprising as both groups were without food for an equal amount of time. We also see that these weights are lower than the respective weights of the previous day.

Physiological Parameters

The reduced plasma glucose concentration of SMS chicks is in line with several studies (Goodwin et al., 1993; Davis et al., 1995b; 1996; Hassanzadeh, 2009; Dinev and Kanakov, 2011). Additionally, there was a much larger variation in plasma glucose concentration among the chicks suffering from SMS. A possible explanation is that the decrease in glucose concentration might depend on the severity of SMS. Because the glucose concentration in the plasma of the SMS group was significantly lower, the chick's glycogen reserves were used to try to bring the plasma glucose concentration back to normal levels, resulting in a reduced hepatic glycogen concentration for the SMS chicks. There was a clear positive correlation between glucose and glycogen concentration on both days for chicks suffering from SMS but not for normal chicks, which concurs with maintaining glucose levels corresponding to the use of glycogen. Reduced insulin and glucose levels in SMS

chicks make sense, as the islets of Langerhans are not stimulated to produce insulin to lower blood glucose levels (Bouman et al., 2008). In addition, the lower concentrations for both T3 and T4 of SMS chicks may indicate a reduced metabolism (Darras et al., 1996, 2009; Koibuchi and Chin, 2000).

In conclusion, changing the feed particle size and feed source location at 10 D of age and fasting at 11 D of age can induce SMS under our experimental conditions. The experimental treatments, mainly changing feed source location and fasting, resulted in a reduction in feed intake. Clinical symptoms of SMS manifested 2.5 to 3.5 h after the introduction of treatments, and during this period, the flock were lying down the most frequently. In addition, the plasma concentrations of glucose, T3, T4, insulin, and liver concentration of glycogen were in line with the clinical symptoms of SMS. Therefore, management practices should ensure sufficient feed intake during this critical period of young broilers.

ACKNOWLEDGMENTS

The diligent assistance of laboratory technician Daniel Vermeulen, animal caretaker André Respen, and thesis student Karlijn Van Endert are greatly appreciated.

Conflict of Interest Statement: The authors did not provide any conflict of interest statement.

REFERENCES

- Aviagen. 2014. Ross 308 Broiler Management Guide. Aviagen Incorporated, Huntsville, AL.
- Bennett, L. W., R. W. Keirs, E. D. Peebles, and P. D. Gerard. 2007. Methodologies of tissue preservation and analysis of the glycogen content of the broiler chick liver. *Poult. Sci.* 86:2653–2665.
- Bokkers, E. A., and P. Koene. 2003. Behaviour of fast- and slow growing broilers to 12 weeks of age and the physical consequences. *Appl. Anim. Behav. Sci.* 81:59–72.
- Bouman, L. N., J. A. Bernards, and H. W. G. M. Boddeke. 2008. *Medische Fysiologie. Bohn stafleu van Loghum/Springer, Houten, The Netherlands.* ISBN 9789031346752.
- Darras, V. M., S. P. Kotanen, K. L. Geris, L. R. Berghman, and E. R. Ku. 1996. Plasma thyroid hormone levels and iodothyronine deiodinase activity following an acute glucocorticoid challenge in embryonic compared with posthatch chickens. *Gen. Comp. Endocrinol.* 104:203–212.
- Darras, V. M., S. L. Van Herck, S. Geysens, and G. E. Reyns. 2009. Involvement of thyroid hormones in chicken embryonic brain development. *Gen. Comp. Endocrinol.* 163:58–62.
- Davis, J. F., A. E. Castro, J. C. De la Torre, H. J. Barnes, J. T. Doman, M. Metz, H. Lu, S. Yuen, P. A. Dunn, and M. N. Teng. 1996. Experimental reproduction of severe hypoglycemia and spiking mortality syndrome using field-derived and embryo-passaged preparations. *Avian Dis.* 40:158–172.
- Davis, J. F., A. E. Castro, J. C. De La Torre, C. G. Scanes, S. V. Radecki, R. Vasillatos-Younken, J. T. Doman, and M. Teng. 1995. Hypoglycemia, enteritis, and spiking mortality in Georgia broiler chickens: experimental reproduction in broiler breeder chicks. *Avian Dis.* 39:162–174.
- Davis, J. F., and R. Vasillatos-Younken. 1995. Markedly reduced pancreatic glucagon levels in broiler chickens with spiking mortality syndrome. *Avian Dis.* 39:417–419.
- Dinev, I., and D. Kanakov. 2011. Spiking mortality syndrome in broiler chickens clinical and morphological examinations of the cases recorded in Bulgaria. *Acta Vet. (Beogr).* 61:49–55.
- Gonyou, H. W. 1994. Why the study of animal behavior is associated with the animal welfare issue. *J. Anim. Sci.* 72:2171–2177.

- Goodwin, M. A., D. L. Hill, M. A. Dekich, and M. R. Putnam. 1993. Multisystemic adenovirus infection in broiler chicks with hypoglycemia and spiking mortality. *Avian Dis.* 37:625–627.
- Hassanzadeh, M. 2009. New approach for the incidence of ascites syndrome in broiler chickens and management control the metabolic disorders. *Int. J. Poult. Sci.* 8:90–98.
- Jensen, L. S., L. H. Merrill, C. V. Reddy, and J. McGinnis. 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poult. Sci.* 41:1414–1419.
- Karki, K., P. Manandhar, T. R. Neupane, S. Manandhar, and P. Koirala. 2008. A preliminary clinical laboratory investigation of endemic spiking mortality syndrome of broiler chickens in Nepal. *Vet. World* 1:329.
- Koibuchi, N., and W. W. Chin. 2000. Thyroid hormone action and brain development. *Trends Endocrinol. Metab.* 11:123–128.
- Kumari, A., U. K. Tripathi, P. Boro, S. Sulabh, M. Kumar, and R. Nimmanapalli. 2016. Metabolic disease of broiler birds and its management: a review. *Int. J. Vet. Sci. Anim. Hus.* 1:15–16.
- Lesuisse, J., C. Li, S. Schallier, J. Leblois, N. Everaert, and J. Buyse. 2017. Feeding broiler breeders a reduced balanced protein diet during the rearing and laying period impairs reproductive performance but enhances broiler offspring performance. *Poult. Sci.* 96:3949–3959.
- Lesuisse, J., S. Schallier, C. Li, A. Bautil, B. Li, J. Leblois, J. Buyse, and N. Everaert. 2018. Multigenerational effects of a reduced balanced protein diet during the rearing and laying period of broiler breeders. 2. Zootechnical performance of the F1 broiler offspring. *Poult. Sci.* 97:1666–1676.
- Mendelson, C., H. B. Nothelfer, and G. Monreal. 1995. Identification and characterization of an avian adenovirus isolated from a 'spiking mortality syndrome' field outbreak in broilers on the Delmarva Peninsula, USA. *Avian Pathol.* 24:693–706.
- Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle size preference by broilers. *Can. J. Anim. Sci.* 68:923–930.
- Svihus, B. 2014. Function of the digestive system. *J. Appl. Poult. Res.* 23:306–314.